29th International

Carbohydrate Symposium

14th – 19th July 2018

Faculdade de Ciências, Universidade de Lisboa

Book of Abstracts

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ICS 2018

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COM O ALTO PATROCÍNIO
DE SUA EXCELÊNCIA

UNDER THE HIGH PATRONAGE OF THE
PRESIDENT OF THE PORTUGUESE REPUBLIC

O Presidente da República

IUPAC ENDORSEMENT

INTERNATIONAL UNION OF
PURE AND APPLIED CHEMISTRY

WELCOME

The Organizing Committee cordially welcomes all participants and accompanying persons to the Universidade de Lisboa, Faculdade de Ciências for the 29th European Carbohydrate Symposium (ICS 2018). This conference, held under the high patronage of the President of the Portuguese Republic, and IUPAC endorsement, aims at stimulating new emerging areas in glycosciences, with contributions in topics covering recent advances in carbohydrate chemistry, biology and biotechnology.

The ICS Iberian Day honours the scientific brotherhood amongst the Portuguese and Spanish communities, and will certainly foster new collaborations with carbohydrate-based solutions for societal needs.

The organisation of a workshop dedicated to the young researchers, by initiative of CARBOMET, is indeed a unique occasion for discussion of their newest findings, encouraging scientific networking.

With the contribution of international experts covering areas of industrial application, this symposium clearly demonstrates the role of glycosciences in pharmaceuticals, personalised medicine, food and nutrition, as well as in new materials and biorenewables.

We hope you will enjoy this symposium, held in the beautiful and culturally rich city of Lisbon!

Amélia Pilar Rauter, Chair ICS2018

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The Organizing Committee is grateful for donations by the following entities:

CarboMet

Division of Carbohydrate Chemistry
U.S. Advisory Committee for International Carbohydrate Symposia (USACICS)
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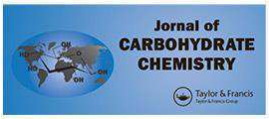
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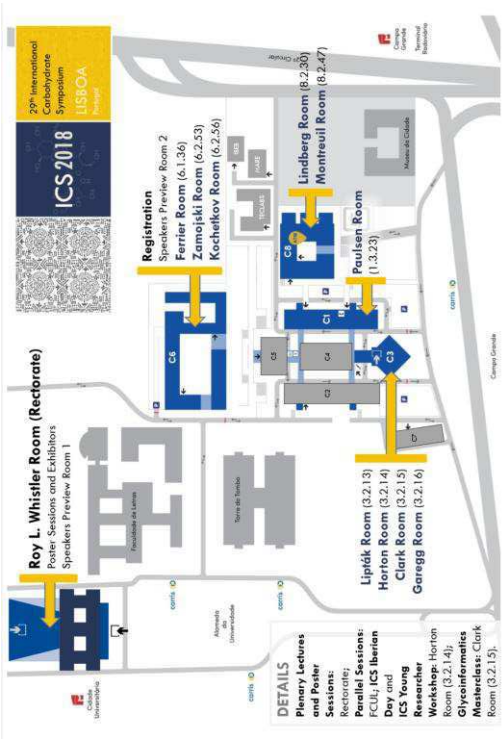
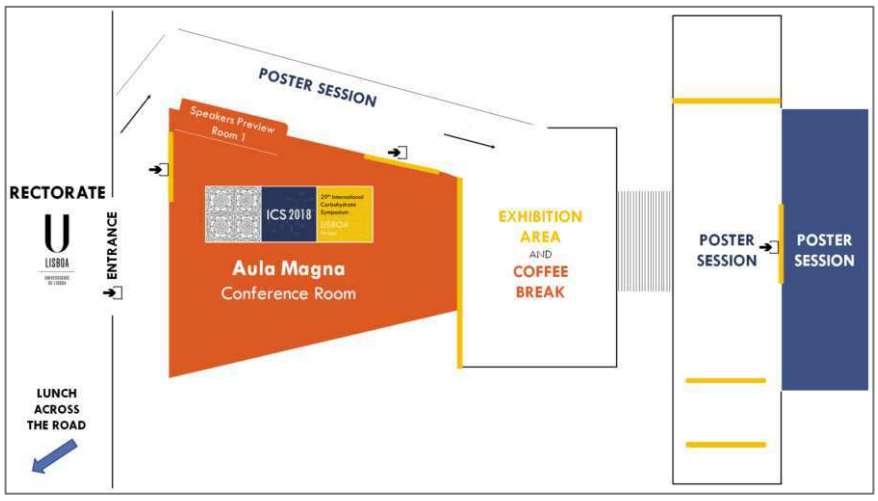
GENERAL INFORMATION

Meeting Venue

The Meeting will take place at Faculdade de Ciências, Universidade de Lisboa (buildings C1, C3, C6 and C8) and at the Rectorate of Universidade de Lisboa, located in Cidade Universitária, Alameda da Universidade. Both are easily accessed from Lisbon airport, either by taxi (5 -10 min, ca 10€) or by Metro ("Cidade Universiária" subway station, yellow line, for the Rectorate, and "Campo Grande" for the Faculdade de Ciências.



Inside the Rectorate, the Confecerence Hall AULA MAGNA (Roy L. Whistler Room), speakerspreview room and exhibition, poster sessions and coffee break areas will be accordingly signposted, as illustrated in the map below.



Lunches

On Saturday (ICS Iberian Day) and Sunday (ICS Young Researcher Workshop), lunches will be at Faculdade de Ciências, while from Monday to Thursday, lthey will be served at the University Canteen, a few minutes walking distance from Rectorate. Lunches are included in the registration fee, and we kindly ask all participants to present their lunch tickets to the staff.

Wifi Access

A temporary login for the wireless Academic Network (eduroam) has been created. Please use the following credentials:

Network: eduroam
Guest User Name: carbohydrate@fc.ul.pt
Password: ics2018

Official Language

English is the Official Language of ICS 2018.

Insurance

All conference attendees are advised to arrange private travel insurance. The conference organizers and committee accept no liability for personal accidents or damage to property. The organizing committee of ICS2018 reserves the right to amend and/or alter the conference program without prior notice.

Time Zone

The time zone in Lisbon is GMT.

Water

Tap water in Portugal is drinking water!

Electricity

The voltage in Lisbon is the local current is 220 AC and the connection is made by a two-pin plug. Travellers from the USA will require a voltage converter. Travellers from the UK will require a plug adapter and this is best bought in the UK as they are hard to find in Lisbon (can try at the Lisbon airport).

Currency, Banks and Post Offices

The national currency in Portugal is Euro. Banks are open from Monday to Friday between 8.30 am and 3 pm. Post offices are usually open between 8.30 am and 6 pm. Exchange houses operate everyday between 9 am and 1 pm and from 2 pm to 7 pm.

Shopping

Shops are opened from Monday to Friday, between 9 am and 7 pm, in some cases with lunch break from 1 pm to 3 pm. On Saturdays, shops open only in the morning, from 9 am to 1pm. The exception is the Shopping Centres that do a 10 am to 10 or 11 pm stretch 7 days a week. The large Supermarkets also stay open until 9 or 10 pm, 7 days a week.

Climate

During July, the temperature in Lisbon is on average 23°C (Low of 18°C and Max of 28°C), and can eventually rain.

SOCIAL PROGRAMME

CONCERT AT THE BASILICA OF MAFRA NATIONAL PALACE

TUESDAY, THE 17TH OF JULY

Departure of buses starts at 19h15 from the venue Faculdade de Ciências, direction Mafra. The concert starts at 21h00.

The organ concert will be played at the Basilica of the Palace of Mafra, which six pipe organs are widely known around the world. They are a unique organ ensemble, originally conceived to play together by the two most important Portuguese organ builders, António Cerveira and Joaquim Fontanes. They were completed in 1807 and restored several times along the centuries. Made of "pau-santo" wood, they bear applications in golden bronze executed by the Neapolitan sculptor Carlo Amatucci, representing flowers, columns and capitals and also diverse musical instruments, like trumpets and violins.

Conserved until today, the six pipe organs are open to the public. With a remarkable number of works specially composed for this unique instrument, its musical versatility reflects the genius of major names of Portuguese organ mastery.

GENERAL SCIENTIFIC INFORMATION

Registration

The registration will take place in the atrium of building C6 (white building), at Faculdade de Ciências da Universidade de Lisboa.

Presentation Preview Room

Speakers are kindly asked to contact the organizing committee (Ana Marta de Matos, Rafael Nunes, João Barros, Maria Teresa Blázquez-Sánchez, Rita Gonçalves Pereira) at the preview room of Faculdade de Ciências for the afternoon sessions and at the Rectorate preview room for the morning sessions, 24 h before presentation. If the speakers use a Mac computer, previewing is also advised.

Posters

Posters will be displayed in the selected halls of the Rectorate. Authors are requested to display their own posters on the boards on Sunday before the opening session. Material to attach posters will be available. Three poster sessions are scheduled on the 16th, 17th and 19th of July from 14h30 to 15h30. Authors are requested to stay near their posters during all sessions in order to be able to answer any questions asked by the participants and by the evaluation panel, in charge of selecting the posters to be awarded with Poster Prizes. Authors are requested to remove their posters on Thursday afternoon, after the poster session.

AWARDS AND PRIZES

The Roy L. Whistler International Award in Carbohydrate Chemistry

The Roy L. Whistler International Award in Carbohydrate Chemistry was established in 1984 to recognize scientists who have made contributions of excellence in carbohydrate chemistry and biochemistry, and with promise of continuing significant contributions.

We are honoured to announce that Prof. Dr. David Crich will receive this award at the Opening Ceremony of the 29th International Carbohydrate Symposium on the 15th of July 2018.

Prof. Dr. David Crich, the recipient of the Roy L. Whistler Award in 2018

The ICO Young Investigator Award

This award has been recently created aiming to recognize young researchers for their outstanding contributions in carbohydrate chemistry and glycobiology, encouraging them to pursue the excellence.

The first Award was given to Dr. Christian Lizak (ETH, Switzerland) by the occasion of the 27th International Carbohydrate Symposium, and Dr. Benjamin M. Swarts (Central Michigan University, USA) was the recipient of this Award in 2016.

We are now delighted to announce

Dr Nuria Martinez Sáez
Utrecht Institute for Pharmaceutical Sciences
Utrecht University

as the winner of the ICO Young Investigator Award in 2018.

ChemPubSoc Europe Prizes

The ChemPubSoc Europe will award four Poster Prizes:

| *Chemistry – A European Journal* Poster Prize
| *European Journal of Organic Chemistry* Poster Prize
| *ChemBioChem* Poster Prize
| *Angewandte Chemie* Poster Prize

Organic Biomolecular Chemistry Poster Prizes

OBC will award four Poster Prizes:

| OBC Poster Prize: Organic Synthesis
| OBC Poster Prize: Carbohydrates for Medicine
| OBC Poster Prize: Vaccines
| OBC Poster Prize: Chemical Biology

Journal of Carbohydrate Chemistry Poster Prizes

The JCC will award four Poster Prizes:

| *Journal of Carbohydrate Chemistry* Poster Prize: Novel Synthesis
| *Journal of Carbohydrate Chemistry* Poster Prize: Biomedical Oligosaccharides
| *Journal of Carbohydrate Chemistry* Poster Prize: Cyclodextrines
| *Journal of Carbohydrate Chemistry* Poster Prize: Chemical Glycosylation

Carbohydrate Research Prizes

Carbohydrate Research will award four Poster Prizes and one Flash Prize:

| Carbohydrate Research Poster Prize: Analysis
| Carbohydrate Research Poster Prize: Chemical Synthesis
| Carbohydrate Research Poster Prize: Glycans and Glycoconjugates
| Carbohydrate Research Poster Prize: Carbohydrate Processing Enzymes, Biomolecular Structure and Function
| Carbohydrate Research Best Flash Presentation ICS2018

Pharmaceuticals Poster Prize

Carbohydrate Polymers Poster Prize

Tokyo Chemical Industry Prizes

Tokyo Chemical Industry will award two Prizes:

Tokyo Chemical Industry Oral Communication Prize

Tokyo Chemical Industry Flash Presentation Prize

CarboSynth Oral Communication Prize

World Scientific Flash Presentation Prizes

Two WS Flash Presentation Prizes

Megazyme Prize for the Best Flash Presentation

J. Pereira da Cruz Innovation Award

SPECIAL ISSUES DEDICATED TO ICS 2018

Pure and Applied Chemistry

Speakers are invited to submit a paper to the issue of Pure and Applied Chemistry dedicated to ICS2018. Manuscript submission and review will proceed following the journal policy.

Marine Drugs

Participants are encouraged to submit the title and abstract to the guest editor (Prof. Amelia Pilar Rauter) of their proposed contribution to the Marine Drugs special issue dedicated to carbohydrates and related areas of research

Pharmaceuticals

Carbohydrates play a major role in a diversity of processes relevant to health and disease. Among the topics covered in the 29th International Carbohydrate Symposium (ICS2018), a special attention is given to recent advances and emerging trends in carbohydrate research towards medical applications, including carbohydrates in therapeutics and diagnosis, glycosylation and disease, carbohydrates in inflammation and disease, glycosciences and personalized medicine and carbohydrate vaccines. The growing knowledge on the importance of carbohydrates in health and in disease progress, driving new therapeutic strategies, is the motto for this Special Issue. Contributions arising from the presentations at the Symposium within the context of the previously mentioned topics are welcome (Guest editor: Dr Nuno Xavier).

SCIENTIFIC PROGRAMME

SCIENTIFIC PROGRAMME

ICS IBERIAN DAY

Time	Saturday, July 14
09:00	**OPENING CEREMONY** L. Carriço, J. Jimenéz-Barbero, J. Cañada, A. Galvão, M. Coimbra, A. P. Rauter
09:20	***Chaired by J. Jimenéz-Barbero, A. P. Rauter***
	ID-IL1 C. Ortiz-Mellet | Universidad de Sevilla, Spain [Carbohydrate for Medicine and Diagnosis]
09:40	**ID-IL2 P. Videira** | Universidade Nova de Lisboa, Portugal [Glycosciences and Personalized Medicine]
10:00	**ID-OC1 A. T. Carmona** | Universidad de Sevilla, Spain [Carbohydrates for Medicine and Diagnosis]
10:15	**ID-FL1 J. Morais da Conceição** | Faculdade de Farmácia, Universidade do Porto, Portugal [Glycosciences and Personalized Medicine]
10:20	**ID-FL2 H. Coelho** | CIC Biogune, Spain [Inflammation and Disease]
10:25	**ID-OC2 J. Barros** | Faculdade de Ciências, Universidade de Lisboa, Portugal [Carbohydrates for Medicine and Diagnosis]
10:40	**ID-IL3 O. Boutureira** | Universitat Rovira i Virgili, Spain [Carbohydrates for Medicine and Diagnosis]
11:00	Coffee Break
11:30	***Chaired by P. Videira, J. Cañada***
	ID-IL4 A. Llebaria | IQAC-CSIC, Spain [Carbohydrates for Medicine and Diagnosis]
11:50	**ID-FL3 A. Peixoto** | IPO, Porto Research Center, Portugal [Glycosylation and Disease]
11:55	**ID-FL4 G. Cutrone** | Universidad de Almeria, Spain [Carbohydrates for Medicine and Diagnosis]
12:00	**ID-FL5 S. Leal** | Universidade do Minho, Portugal [Carbohydrates for Medicine and Diagnosis]
12:05	**ID-FL6 J. Guzmán-Caldentey** | CIB, Spain [Inflammation and Disease]
12:10	**ID-IL5 A. Canales** | Universidad Complutense de Madrid, Spain [Glycans]
12:30	Lunch
14:00	***Chaired by J. M. Garcia Fernandez, N. M. Xavier***
	ID-IL6 N. Reichardt | CICbiomaGUNE, País Basco, Spain [Glycosylation and Disease]
14:20	**ID-FL7 I. Kalograiaki** | Centro de Investigaciones Biológicas, CSIC, Spain [Natural Glycoconjugates]
14:25	**ID-FL8 A. G. Santana** | Instituto General de Quimica Organica CSIC, Spain [Carbohydrates for Medicine and Diagnosis]

14:30	**ID-FL9 M. N. Dominguez** | Biological Research Center, Spain [Synthesis]
14:35	**ID-IL7 R. Ventura** | ITQB, Portugal [Synthesis]
14:55	**ID-FL10 F. Queda** | Universidade Nova de Lisboa, Portugal [Synthesis]
15:00	**ID-FL11 M. González-Cuesta** | Universidad de Sevilla, Spain [Synthesis]
15:05	**ID-IL8 M. T. Blázquez-Sánchez** | Faculdade de Ciências, Universidade de Lisboa, Portugal [Synthesis]
15:25	**ID-FL12 E. Coelho** | Universidade de Aveiro, Portugal [Carbohydrates and Nutrition]
15:30	**ID-IL9 V. Luque** | Universidad de Extremadura, Spain [Synthesis]
15:50	**ID-FL13 A. Fortuna** | Faculdade de Ciências, Universidade de Lisboa, Portugal [Synthesis]
15:55	**ID-FL14 R. Nunes** | Faculdade de Ciências, Universidade de Lisboa, Portugal [Glycoinformatics]
16:00	**ID-IL10 C. Nunes** | Universidade de Aveiro, Portugal [Glycomaterials]
16:20	**ID-OC3 B. Trastoy** | CIC Biogune, Spain [Biomolecule Structure]
16:35	**ID-IL11 M. M. Marques** | Universidade Nova de Lisboa, Portugal [Synthesis]
16:55	Coffee Break
17:25	***Chaired by N. Reichardt, F. Marcelo***
	ID-OC4 D. Solís | Instituto de Química Física Rocasolano, CSIC, Spain [Carbohydrates for Medicine and Diagnosis]
17:40	**ID-FL15 A. C. Diniz** | Universidade Nova de Lisboa, Portugal [Biomolecule Structure]
17:45	**ID-OC5 P. Nieto** | Universidad de Sevilla, Spain [Biomolecule Structure]
18:00	**ID-FL16 H. Duarte** Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup/i3S), Portugal [Glycosylation and Disease]
18:05	**ID-FL17 J. L. de Paz** | Instituto de Investigaciones Químicas IIQ, CSIC [Glycans]
18:10	**ID-OC6 J. A. Ferreira** | Portuguese Institute of Oncology of Porto, Portugal [Carbohydrates for Medicine and Diagnosis]
18:25	**ID-IL12 R. Hurtado** | University of Zaragoza, Sapin [Biomolecule Structure]
18:45	**ID-IL13 A. M. de Matos** | Faculdade de Ciências da Universidade de Lisboa, Portugal [Carbohydrates for Medicine and Diagnosis]
19:05	CLOSURE S. Penadés, M. Martin Lomas, J. Jimenéz Barbero, J. Cañada, J. C. Palácios, D. Wessel, M. Coimbra, N. M. Xavier, A. P. Rauter
19:30	SOCIAL PROGRAM Ensemble "3 Bairros": **Ricardo Gama** Portuguese guitar, **João Correia** Classic guitar, **Guilherme Madeira** Voice **Margarida Soeira** Fado Singer

ICS Young Researcher Workshop

Time	Sunday, July 15	
08:30	**OPENING SESSION** S. Flitsch, A. P. Rauter, N. M. Xavier	REGISTRATION
08:45	YRW-PL1 Sabine Flitsch |UK	
	CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS	
09:30	YRW-FL1 M. Gintner| Austria	
09:35	YRW-FL2 A. Dahlqvist |Sweden	
09:40	YRW-FL3 S. Achilli | France	
09:45	YRW-FL4 S. Balicki |Poland	
09:50	YRW-FL5 R. Ikono |Indonesia	
	Break	
	SYNTHESIS	
10:00	YRW-FL6 C. J. Crawford | Ireland	
10:05	YRW-FL7 T. Tamura | Japan	
10:10	YRW-FL8 D. Willén | Sweden	
10:15	YRW-FL9 R. Gonçalves-Pereira | Portugal	
10:20	YRW-FL10 D. A. Ahiadorme | Russian Federation	
	Coffee Break	
	VACCINES	
10:45	YRW-FL11 B. Didak | France	
10:50	YRW-FL12 N. Trattnig | Austria	
10:55	YRW-FL13 C.-Y. Chen | Taiwan	
	GUT MICROBIOTA	
11:00	YRW-FL14 P. García-Vello | Spain	
11:05	YRW-FL15 V. Correia | Portugal	
11:10	YRW-F16 I. D. Young | UK	
	GLYCOMATERIALS	
11:15	YRW-FL17 G. Tegl | Canada	
11:20	YRW-FL18 M. Delbianco | Germany	
	GLYCANS/ NATURAL GLYCOCONJUGATES	
11:25	YRW-FL19 M. Pallach | Italy	
11:30	YRW-FL20 I. Izzo | Germany	
	GLYCOSYLATION AND DISEASE	
11:35	YRW-FL21 A. Almeida | Australia	
11:40	YRW-FL22 R. Marchetti | Italy	

	GLYCOINFORMATICS
11:45	YRW-FL23 E. Crisman |Spain
	ANALYSIS
11:50	YRW-FL24 P. Danby | Canada
11:55	YRW-FL25 V. J. Somovilla | The Netherlands
12:00	YRW-PL2 Ten Feizi | UK
12:45	Lunch

GLYCOINFORMATICS MASTERCLASS*

Time	Sunday, July 15	
08:30	**WELCOME INTRODUCTION** S. Perez | France	REGISTRATION
08:40	**S. Perez** |CNRS-Univ-Grenoble-Alpes, FR *A Traveler-Guide to Carbohydrates in the Cyber-Space*	
09:00	**J. Koca** |CEITEC, Brno, CZ *Chemo-and Structural GlycoInformatics*	
09:45	**R. Woods** | University Georgia, Athens, USA *3D Building & Displaying Complex Carbohydrates*	
10:30	Coffee Break	
10:45	**R. Marchetti** | Univ-Napoli, IT *Integrated Use of Databases in Structural Investigations of Complex Carbohydrates*	
11:30	**K. Aoki Kinoshita** | Soka University, JP *Guide to Using Glycomics Databases*	
12:45	**F. Lisacek** | SIB-Univ. Geneva, CH *Portal of GlycoInformatics*	
13:15	Break	
	RECENTLY DEVELOPED TOOLS AND DATABASES	
13:25	**F. Bonnardel** | CNRS-Grenoble, FR / SIB Geneva, CH *UniLectin: A structure-based database*	
13:30	**O. Clerc** | Univ Lyon, CNRS, FR *MatrixDB*	
13:35	**J. Birch** | DTU, Copenhagen, DK *EPS-DB: Exopolysaccharide Structures & Properties Database*	
13:40	**M. Montenegro** | FUSFV, Petroli, BR *CycloMolder*	
13:45	**S. Perez** | CNRS-Univ-Grenoble-Alpes, FR *Polys-Glycan Builder*	
13:50	**J. Koca** | CEITEC, Brno, CZ *LiteMol*	
13:55	**CONCLUDING REMARKS** S. Flitsch | United Kingdom	
14:00	Lunch	

*The course will be available on https://www.glycopedia.eu/ after the symposium.

ICS2018 Schedule

Roy L. Whistler Room (Aula Magna – Rectorate)

Time	Sunday July15	Monday July 16	Tuesday July17	Wednesday July 18	Thursday July 19
8:30	REGISTRATION	PL1 J. Magnani USA	PL6 Y. Kajihara Japan	PL11 A. Imberty France	PL15 P. Seeberger Germany
9:15		PL2 J. Jiménez-Barbero Spain	PL7 T. Lowary Canada	PL12 T. Endo Japan	PL16 D. Vocadlo Canada
10:00		IL1 S. C. Hung Taiwan	IL2 X. Chen China	IL3 J. Rojo Spain	IL4 M. Brimble New Zealand
10:30		Coffee Break	Coffee Break	Coffee Break	Coffee Break
11:00		PL3 Z. Guo USA	PL8 B. Ernst Switzerland	PL13 N. Pohl USA	PL17 P. Rudd Ireland
11:45		PL4 C. Nativi Italy	PL9 M. Sollogoub France	PL14 B. Turnbull UK	PL18 P. Kosma Austria
12:30		PL5 R. Field UK	PL10 T. Lindhorst Germany	Lunch	PL19 G. Marel The Netherlands
13:15		Lunch	Lunch		Lunch
14:30		POSTER SESSION 1	POSTER SESSION 2	PARALLEL SESSIONS	POSTER SESSION 3
		PARALLEL SESSIONS	PARALLEL SESSIONS	PARALLEL SESSIONS	VACCINES
15:30		PARALLEL SESSIONS	PARALLEL SESSIONS	PARALLEL SESSIONS	IL50 S. Sucheck USA
16:00		PARALLEL SESSIONS	PARALLEL SESSIONS	PARALLEL SESSIONS	YIL10 G. Bernardes Portugal
16:30	OPENING CEREMONY	PARALLEL SESSIONS	PARALLEL SESSIONS	PARALLEL SESSIONS	IL51 L. Mulard France
17:15	Roy L. Whistler Award	PARALLEL SESSIONS	PARALLEL SESSIONS	PARALLEL SESSIONS	CLOSING LECTURE
17:30	Roy L. Whistler Lecture D. Crich	PARALLEL SESSIONS	PARALLEL SESSIONS	EXCURSION	PL20 B. Yu China
18:15	ICO Young Researcher Award	PARALLEL SESSIONS	PARALLEL SESSIONS	EXCURSION	CLOSURE
18:30	ICO Young Researcher Lecture N. Martinez-Saez	PARALLEL SESSIONS	PARALLEL SESSIONS	EXCURSION	
19:15	CONCERT	PARALLEL SESSIONS	PARALLEL SESSIONS	EXCURSION	
20:00	WELCOME RECEPTION	PARALLEL SESSIONS	PARALLEL SESSIONS	EXCURSION	DEPARTURE TO GALA DINNER

Labels

PL | IL | YIL | SESSION TITLE

Horton Room (3.2.14 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			SYNTHESIS
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL8 E. McGarrigle Ireland
15:00			IL9 L. Somsák Hungary
	SYNTHESIS	SYNTHESIS	
15:30	IL5 A. Demchenko USA	IL7 S. Castillón Spain	
15:45			
16:00	OC1 V. Wittmann Germany	OC7 C. Galan UK	
16:15	OC2 C. Gauthier Canada	OC8 H. Dong China	
16:30	OC3 Y. Maki Japan	OC9 T. Nokami Japan	
16:45	OC4 E. Scanlan Ireland	OC10 V. B. Krylov Russian Federation	
17:00	Coffee Break	Coffee Break	EXCURSION
17:30	IL6 N. Jayaraman India	OC11 M. I. Matheu Spain	
17:45		OC12 C. De Meo USA	
18:00	OC5 L. Kononov Russian Federation	OC13 G. Despras Germany	
18:15	OC6 R. Giuliano USA		

Labels:

SESSION TITLE | **OC** | **IL**

Clark Room (3.2.15 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL13 I. Carvalho Brazil
	CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS	CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS	IL14 B. Vauzeilles France
15:30	IL10 P. Andreana USA	IL11 U. Nilsson Sweden	
16:00	OC14 J. Ramos-Soriano SRSC Award - Spain	OC21 N. E. Nifantiev Russian Federation	
16:15	OC15 D. Kwan Canada	OC22 M. Harvey The Netherlands	
16:30	OC16 Y. Blériot France	OC23 T. M. Wrodnigg Austria	
16:45	OC17 A. Borg Austria	OC24 S. Nomura Japan	
17:00	Coffee Break	Coffee Break	
17:30	YIL1 P. Bojarova Czech Republic	IL12 K. Tanaka Japan	EXCURSION
18:00	OC18 J. Lukasiewicz Poland	OC25 A. A. Joseph Israel	
18:15	OC19 O. Lopez Spain	OC26 P. Greimel Japan	
18:30	OC20 M. Artola The Netherlands	OC27 V. Ferro Australia	
18:45	FL1 N. Pellegrini France	OC28 D. Goyard France	

Labels:

SESSION TITLE	OC	IL	YIL	FL

Garegg Room (3.2.16 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			SYNTHESIS
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL18 A. Gomez Spain
15:00			IL19 A. Marra France
	SYNTHESIS	SYNTHESIS	EXCURSION
15:30	IL15 S. Hotha India	IL17 P. Murphy Ireland	
16:00	OC29 S. Vincent Belgium	OC34 D. Takahashi Japan	
16:15	OC30 A. Martin Spain	OC35 P. G. Wang USA	
16:30	YIL2 N. M. Xavier Portugal	YIL3 X.-W. Liu Singapore	
17:00	Coffee Break	Coffee Break	
17:30	O31 M. Blaukopf Austria	OC36 U. Westerlind Germany	
17:45	OC32 C. Pedersen Denmark	OC37 J. L. Blanco Spain	
18:00	OC33 F. Lamiable-Oulaidi New Zealand	OC38 R. Okamoto Japan	
18:15	IL16 C. Marino Argentina		

Labels:

SESSION TITLE	OC	IL	YIL

Lipták Room (3.2.13 - FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			SYNTHESIS
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL24 S. Jarosz Poland
15:00			OC52 C. C. Wang Taiwan
15:15			OC53 C. Stanetty Austria
	GLYCOINFORMATICS BRIDGING THE GAP	CARBOHYDRATE IN LIGNOCELLULOSIC BIOMASS VALORIZATION	EXCURSIONS
15:30	IL20 F. Lisacek Switzerland	IL22 G. Cravotto Italy	
16:15	OC39 A. Franconetti Spain	OC45 D. Mangan Ireland	
16:30	OC40 L. Masgrau-Fontanet Spain	OC46 R. Galhano Portugal	
16:45	OC41 O. Grant USA	OC47 I. van Munster UK	
17:00	Coffee Break	Coffee Break	
17:30	IL21 S. Martin-Santamaria Spain	IL23 F. Gírio Portugal	
18:00	OC42 C. Rovira Spain	OC48 C. Romanò Denmark	
18:15	OC43 W. Plazinski Poland	OC49 A. Lopes Portugal	
18:30	OC44 R. Woods USA	OC50 M. Martínez-Sanz Spain	
18:45		OC51 A. C. Fernandes Portugal	

Labels:

SESSION TITLE | **OC** | **IL**

Ferrier Room (6.1.36 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			BIOMOLECULE STRUCTURE
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL29 F. Corzana Spain
15:00			IL30 Y. Queneau France
	BIOMOLECULE STRUCTURE	BIOMOLECULE STRUCTURE	EXCURSION
15:30	YIL4 F. Marcelo Portugal	YIL5 A. Titz Germany	
16:00	OC54 G. Nestor Sweden	IL27 S. Fushinobu Japan	
16:15	OC55 M. Schubert Austria		
16:30	IL25 A. Planas Spain	OC59 S. Sonnino Italy	
16:45		OC60 J. Young USA	
17:00	Coffee Break	Coffee Break	
17:30	IL26 M. Frank Sweden	IL28 A. Silipo Italy	
18:00	OC56 J. Caims Thailand	OC61 A. Varrot France	
18:15	OC57 D. O. Ribeiro Portugal	OC62 U. Naezhda Russian Federation	
18:30	OC58 A. Clarke Canada	OC63 S. Barbirz Germany	
18:45	FL2 I. Rœder Norway	FL3 I. Calloni Spain	

Labels:

SESSION TITLE	OC	IL	YIL	FL

Zamojski Room (6.2.53 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS/BIOMOLECULE STRUCTURE
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	OC78 E. Bokor Hungary
14:45			YIL7 A. Palma Portugal
15:15			OC79 E. Laigre France
	GLYCOSCIENCES AND PERSONALIZED MEDICINE	BIOMOLECULE STRUCTURE	
15:30	YIL6 C. Rademacher Germany	IL32 E. Parker New Zealand	
16:00	OC64 Y. Liu UK	OC70 M. Wimmerová Czech Republic	
		GLYCANS	
16:15	OC65 J. Cramer Switzerland	OC71 A. Kitchenkov Russian Federation	
16:30	OC66 S. Manabe Japan	FL4 Y. Manabe Japan	
16:35		FL5 A. Zykwinska France	
16:40		FL6 J.M. Madern The Netherlands	EXCURSION
16:45	IL31 E. Osinaga Uruguai	OC72 E. Fadda Ireland	
17:00		OC73 D. Wander The Netherlands	
17:15	Coffee Break	Coffee Break	
	ANALYSIS	OC74 K. Ding China	
17:45	OC67 J. Tamura Japan		
18:00	OC68 N. Guerrini Italy	OC75 M. H. Clausen Denmark	
18:15	OC69 L. Betschart Canada	OC76 M. D. Bandara USA	
18:30		OC77 I.M. Ryzhov Russian Federation	

Label

SESSION TITLE	OC	IL	YIL	FL

Kochetkov Room (6.2.56 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			NATURAL GLYCOCONJUGATES
14:30	POSTER SESSION 1 Rectorate	Photo	OC89 D. Varon Silva Germany
14:45		POSTER SESSION 2 Rectorate	OC90 Y. Fujimoto Japan
15:00			OC91 H. Bakker Germany
15:15			OC92 K. Totani Japan
	VACCINES	VACCINES	EXCURSION
15:30	IL33 R. Adamo Italy	IL35 P. Kovac USA	
16:00	OC80 K. Fukase Japan	OC86 L.-A. Barel France	
16:15	OC81 C. Pereira Germany	OC87 G.-G. Desjardins Canada	
16:30	IL34 N. Ravenscroft South Africa	IL36 S. Oscarson Ireland	
17:00	Coffee Break	Coffee Break	
17:30	OC82 G. Stefanetti USA	IL37 M. Monteiro Canada	
17:45	OC83 P. Henriques Italy		
18:00	OC84 P. Wang USA	OC88 A. H. Röder Germany	
18:15	OC85 M. M. Kuttel South Africa	FL7 L. Del Bino Italy	
		FL8 T. Hogervorst The Netherlands	

Labels:

SESSION TITLE | **OC** | **IL**

Lindberg Room (8.2.30 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			SYNTHESIS
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL41 M. Fridman Israel
15:00			OC104 H. Ando Japan
15:15			FL12 P. Abronina Russian Federation
15:20			FL13 F. Portier France
15:25			FL14 S. Krauter Austria
	GLYCOSYLATION AND DISEASE	GLYCOSYLATION AND DISEASE	
15:30	YIL8 E. Goddard-Borger Australia	YIL9 J. Rodrigues Portugal	
16:00	OC93 S. A. K. Jongkees The Netherlands	OC100 R. O'Flaherty Ireland	
16:15	OC94 R. Azevedo Portugal	OC101 T. Suzuki Japan	
16:30	IL38 Y. Zhang France	IL39 C. Reis Portugal	
17:00	Coffee Break	Coffee Break	
	INFLAMMATION AND DISEASE		EXCURSION
17:30	OC95 E. M. Sánchez-Fernández Spain	IL40 S. Williams Australia	
17:45	OC96 O. Schwardt Switzerland		
18:00	OC97 W. Römer Germany	OC102 R. Ashmus USA	
18:15	OC98 S. Rabbani Switzerland	FL9 S. Mereiter Portugal	
18:20		FL10 T. Wang USA	
18:25		FL11 A. Tamburrini Italy	
18:30	OC99 S. Neelameghan USA	OC103 S. I. Shoda Japan	

Labels:

SESSION TITLE	OC	IL	FL	YL

Paulsen Room (1.3.23 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS
14:30	POSTER SESSION 1 Rectorate	Photo	OC120 K. McReynolds USA
		POSTER SESSION 2 Rectorate	OC121 F. Garcia-Martin Japan
15:00			FL18 F. Berni The Netherlands
			FL19 S. Lamandé-Langle France
			FL20 M. Kiss Hungary
			TCI CHEMICALS
15:15			OC122 N. Yuasa Japan
	GLYCOMATERIALS	POLYSACCHARIDE BIOTECHNOLOGY	EXCURSION
15:30	IL42 B. Christensen Norway	OC113 B. Svensson Denmark	
15:45		OC114 S. Colliec-Jouault France	
16:00	OC105 C. Crucho Portugal	OC115 V. Ferrières France	
16:15	OC106 P. Sahariah Iceland	OC116 J. Ertl Germany	
16:30	OC107 Y. Guo UK	OC117 M. Czemierska Poland	
16:45	OC108 R. Estevez Spain		
17:00	Coffee Break	Coffee Break	
		MARINE GLYCANS	
17:30	OC109 J. Xie France	IL43 J. Mano Portugal	
17:45	OC110 F. Pfrengle Germany		
18:00	FL15 I. Mo Norway	OC118 M. Bardor France	
18:05	FL16 A. Rebelo Portugal		
18:10	FL17 E. Castanheira Portugal		

18:15	OC111 J. C. Muñoz-García UK	OC119 E. Ficko-Blean France	
18:30	OC112 A. Rolland-Sabaté France		

Labels:

SESSION TITLE	**OC**	**IL**	**FL**

Montreuil Room (8.2.47 – FCUL)

Time	Monday July 16	Tuesday July17	Wednesday July 18
			SYNTHESIS
14:30	POSTER SESSION 1 Rectorate	POSTER SESSION 2 Rectorate	IL48 P. Tyler New Zealand
15:00			IL49 R. Daniellou France
	CARBOHYDRATES AND NUTRITION	GUT MICROBIOTA	ANALYSIS
15:30	IL44 M. J. Hederos Denmark	IL46 M. Pintado Portugal	FL29 A. Malik - Germany FL30 L. Medve – Italy FL 31 E. Stender - Denmark
16:00	OC123 A. Ranjan India	OC128 H. Stalbrand Sweden	
16:15	OC124 V. Kren Czech Republic	OC129 A. Laws UK	
16:30	IL45 M. Coimbra Portugal	OC130 P. Rahfeld Canada	
16:45		OC131 F. Di Lorenzo Italy	
17:00	Coffee Break	Coffee Break	
	SYNTHESIS	OC132 A. Shimoyama Japan	
17:30	OC125 A. Ishiwara Japan		
17:45	OC126 D. Gamenara Uruguay	OC133 M. A. Hachem UK	
		NATURAL GLYCOCONJUGATES/GLYCANS	EXCURSION
18:00	FL21 A. Pardo Germany	IL47 J. Voglmeir China	
18:05	FL22 E. Stepanova Russian Federation		
18:10	FL23 T. Hansen The Netherlands		
18:15	OC127 I. Perez-Martin Spain		
18:30	FL24 O. Pitirollo Italy	OC134 P. Quentin France	
18:35	FL25 A. Sau UK		
18:40	FL26 J. Enotarpi The Netherlands		
18:45	FL27 N. Yagami Japan	FL28 M. Guberman Germany	

Labels:

SESSION TITLE | OC | IL | FL

DETAILED PROGRAMME

Detailed Programme

Saturday, July 14 | ICS Iberian Day | Horton Room (3.2.14)

08:00 Registration

09:00 Opening Ceremony | L. Carriço, J. Jiménez-Barbero, J. Cañada, A. Galvão, M. Coimbra, A. P. Rauter

MORNING SESSION 1 | *Chaired by J. Jiménez-Barbero and A. P. Rauter*

09:20	ID-IL1	Carbohydrates for Medicine and Diagnosis	*Sp^2 iminosugars: opportunities for new glycotherapies* **C. Ortiz-Mellet** Universidad de Sevilla, Spain
09:40	ID-IL2	Glycosciences and Personalized Medicine	*Glycoimmunology: craking sugars to develop new immunotherapies* **P. Videira** Universidade Nova de Lisboa, Portugal
10:00	ID-OC1	Carbohydrates for Medicine and Diagnosis	*Discovery of potent glycosidase inhibitors by in situ analysis of a library of Hybrid molecules generated via click chemistry* **A. T. Carmona** Universidad de Sevilla, Spain
10:15	ID-FL1	Glycosciences and Personalized Medicine	*Hydroxypropyl-B-cyclodextrin and B-cyclodextrin as tablet direct compression fillers* **J. M. Conceição** Faculdade de Farmácia, Universidade do Porto, Portugal
10:20	ID-FL2	Inflammation and Disease	*Co-administration of anti-microbial peptides helps in the activity of FP7 glycolipid (TLR4 antagonist)* **H. Coelho** CICBiogune, Spain
10:25	ID-OC2	Carbohydrates for Medicine and Diagnosis	*Synthesis of N-acetylgalactosamine mimetics tackling multiple targets: hemorragic virus and Alzheimer's disease* **J. Barros** Faculdade de Ciências, Universidade de Lisboa, Portugal
10:40	ID-IL3	Carbohydrates for Medicine and Diagnosis	*Halogens and chalcogens in chemical glycobiology* **O. Boutureira** Universitat Rovira I Virgilli, Spain

11:00 Coffee Break

MORNING SESSION 2 | *Chaired by P. Videira and J. Cañada*

11:30	ID-IL4	Carbohydrates for Medicine and Diagnosis	*Glycosphingolipid and analogues as Immunostimulants of Nkt cells* **J. Llebaria** IQAC-CSIC, Spain
11:50	ID-FL3	Glycosylation and Disease	*Hypoxia enhances the malignant nature of bladder cancer cells and concomitantly antagonizes protein O-glycosylation extension* **J. Peixoto** IPO, Porto Research Center, Portugal
11:55	ID-FL4	Carbohydrates for Medicine and Diagnosis	*Post-synthetic strategy for superficial Carbohydrate-coating of mesosporous Mil-100(Fe) NANOMOFs as biocompatible nanocarriers* **G. Cutrone** Universidad de Almeria, Spain
12:00	ID-FL5	Carbohydrates for Medicine and Diagnosis	*Bacterial cellulose surface modification for microfluidic applications* **S. Leal** Universidade do Minho, Portugal
12:05	ID-FL6	Inflammation and Disease	*Toll-like receptor 4. Computational Chemistry studies on its modulation* **J. Guzmán-Caldentey** CIB, Spain
12:10	ID-IL5	Glycans	*Lanthanide-chelating carbohydrate conjugates to detect carbohydrate-Protein interactions* **A. Canales** Universidad Complutense de Madrid, Spain

12:30 Lunch

AFTERNOON SESSION 1 | *Chaired by J. M. Garcia Fernandez and N. M. Xavier*

14:00	ID-IL6	Glycosylation and Disease	*Development of glycomimetics for dendritic cell targeting* **N. Reichardt** CICbiomaGUNE, País Basco, Spain
14:20	ID-FL7	Natural Glycoconjugates	*Insights in the recognition of listeria cell wall teichoic acids by phage endolysins using NMR* **I. Kalograiaki** Centro de Investigaciones Biológicas, CSIC, Spain
14:25	ID-FL8	Carbohydrates for Medicine and Diagnosis	*A fast NMR-assisted combinatorial method for optimizing nucleic acid binders* **A. G. Santana** Instituto General de Quimica Organica, CSIC, Spain
14:30	ID-FL9	Synthesis	*Obtaining the first thioglycoligase from the GH3 family* **M. N. Dominguez** Biological Research Center, Spain
14:35	ID-IL7	Synthesis	*Carbohydrate synthesis for solving Biological problems* **R. Ventura** ITQB, Portugal
14:55	ID-FL10	Synthesis	*Bacterial cell wall surrogates from chitosan: a new recognition system* **F. Queda** Universidade Nova de Lisboa, Portugal
15:00	ID-FL11	Carbohydrates for Medicine and Diagnosis	*Cyclodextrin-based molecular vector theranostics* **M. González Cuesta** Universidad de Seville Spain
15:05	ID-IL8	Synthesis	*Synthesis of C-glycosyl molecular entitiesas a strategy for the treatment of amyloid and infectious diseases* **M. T. Blázquez-Sánchez** Faculdade de Ciências, Universidade de Lisboa, Portugal
15:25	ID-FL12	Carbohydrates and Nutrition	*Contribution of non-enzymatic trans-glycosylation reactions to honey oligosaccharides origin and diversity* **E. Coelho** Universidade de Aveiro, Portugal
15:30	ID-IL9	Synthesis	*Eco-friendly synthesis and antiproliferative activity of carbohydrate-derived heterocycles* **V. Luque-Agudo** Universidad de Extremadura, Spain
15:50	ID-FL13	Synthesis	*Synthesis of new types of potentially bioactive furanosyl nucleoside and nucleotide analogues* **A. Fortuna** Faculdade de Ciências, Universidade de Lisboa, Portugal
15:55	ID-FL14	Glycoinformatics	*In silico design of halogenated carbohydrate mimetics as potential halogen--bonding ligands* **R. Nunes** Faculdade de Ciências, Universidade de Lisboa, Portugal
16:00	ID-IL10	Glycomaterials	*Functional polysaccharide-based materials for food and biomedical applications* **C. Nunes** Universidade de Aveiro, Portugal
16:20	ID-OC3	Biomolecule Structure	*Enzymatic tools to glycoengineer antibodies* **B. Trastoy** CIC Biogune, Spain
16:35	ID-IL11	Synthesis	*From chitosan to Nag-Nam surrogates* **M. M. Marques** Universidade Nova de Lisboa, Portugal

16:55 Coffee Break

AFTERNOON SESSION 2 | *Chaired by N. Reichardt and F. Marcelo*

17:25	ID-OC4	Carbohydrates for Medicine and Diagnosis	*Adaptable microarray approach for rapid evaluation of bacterial anti--adhesives* **D. Solís** Instituto de Química Física Rocasolano, CSIC, Spain
17:40	ID-FL15	Biomolecule Structure	*Molecular recognition of glycans by lectins of the immune system* **A. C. Diniz** Universidade Nova de Lisboa, Portugal
17:45	ID-OC5	Biomolecule Structure	*Chondroitin sulfate derivatives and dendrimers and their binding to midkine and langerine by NMR* **P. Nieto** Universidad de Sevilla, Spain
18:00	ID-FL16	Glycosylation and Disease	*Unravelling ErbB2 glycosylation signature in gastric cancer cells* **H. Duarte** Institute of Molecular Pathology and Immunology of the University of Porto (Ipatimup/i3S), Portugal
18:05	ID-FL17	Glycans	*Synthesis of chondroitin sulfate dendrimers and binding to growth factor midkine* **J. L. de Paz** Instituto de Investigaciones Químicas, IIQ, CSIC
18:10	ID-OC6	Carbohydrates for Medicine and Diagnosis	*Exploring sialyl-Tn expression in microfluidic-isolated circulating tumor cells: a biomarker for precision oncology applications* **J. A. Ferreira** IPO, Porto Research Center, Portugal
18:25	ID-IL12	Biomolecule Structure	*The interdomain flexible linker of the polypeptide GalNAc transferases dictates their long-range glycosylation preferences* **R. Hurtado-Guerrero** University of Zaragoza, Spain
18:45	ID-IL13	Carbohydrates for Medicine and Diagnosis	*The story behind the discovery of nature- inspired sugar conjugates with therapeutic potential against Diabetes and Alzheimer's disease* **A. M. de Matos** Faculdade de Ciências, Universidade de Lisboa, Portugal

19:05 **Closure** | J. Cañada,M. Coimbra, A. P. Rauter, A. Galvão
A tribute to S. Penadés, M. Martin Lomas, J. Jimenéz-Barbero and J. C. Palácios

19:30 **Social Program** | Ensemble "3 Bairros"
Ricardo Gama - Portuguese Guitar
João Correia – Classic Guitar
Guilherme Madeira – voice
Margarida Soeiro – Fado Singer

Sunday, July 15 | Horton Room (3.2.14)

ICS YOUNG RESEARCHER WORKSHOP

08:30 **Opening Ceremony | S. Flitsch, N. M. Xavier, A. P. Rauter**

Chaired by N. M. Xavier

08:45 YRW-PL1 *Analysis of carbohydrates using fragment based hyphenated mass spectrometry*
S. Flitsch
The Manchester Institute of Biotechnology, United Kingdom

CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS

09:30 YRW-FL1 *A novel and concise synthesis of legionaminic acid and 4-epi-legionaminic acid*
M. Gintner
Department of Organic Chemistry, University of Vienna, Austria

09:35 YRW-FL2 *C-glycosides – Novel galectin inhibitor scaffolds*
A. Dahlqvist
Centre for Analysis and Synthesis, Lund University, Sweden

09:40 YRW-FL3 *DC-SIGN, DC-SIGNR and LSECtin: potential selective interaction with positional antennae isomers*
S. Achilli
Institut de Biologie Structurale, Isère (FR) Grenoble, France

09:45 YRW-FL4 *The mechanisms of anticoagulant activity of the Glycoconjugates isolated from Conyza 4anadensis L. by the ultrasounds-assisted extraction*
S. Balicki
Department of Organic and Pharmaceutical Technology, Wrocław University of Science and Technology, Poland

09:50 YRW-FL5 *Development of a novel hybrid chitosan-nano TiO_2 sponge for alveolar bone tissue regeneration*
R. Ikono
Department of Bionanotechnology, Nano Center Indonesia, Indonesia

SYNTHESIS

10:00 YRW-FL6 *Synthesis of capsular polysaccharide fragments of the fungal pathogen Cryptococcus neoformans and their potential applications as FRET probes*
C. J. Crawford
School of Chemistry, University College Dublin, Ireland

10:05 YRW-FL7 *Synthesis of XylB1-4Rbo, partial structure of O-mannosyl glycan*
T. Tamura
Department of Agricultural Science, Tottori University, Japan

10:10 YRW-FL8 *Synthetic exploration of xylose analogs*
D. Willén
Centre for Analysis and Synthesis, Lund University, Sweden

10:15 YRW-FL9 *Synthesis of novel D-glucuronamide-based triazole nucleosides as potential anti-Alzheimer agents*
R. Gonçalves Pereira
Faculdade de Ciências, Universidade de Lisboa, Portugal

10:20 YRW-FL10 *Concentration-dependent reactivity and stereoselectivity glycosylation: why a reaction may stop at some concentrations?*
D. A. Ahiadorme
N.K. Kochetkov Laboratory of Carbohydrate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Federation

10:25 Coffee Break

VACCINES
Chaired by A. Palma

10:45 YRW-FL11 *Selection of novel C-type lectin ligands through new scanning method*
B. Didak
GLYcoDiag, Chevilly, France

10:50 YRW-FL12 *Synthesis and vaccine potential of bacterial mimetics against HIV-1*
N. Trattnig
Department of Chemistry, University of Natural Resources and Life Sciences, Austria

10:55 YRW-FL13 *Facial synthesis of Globo H analogs conjugated vaccines and their immunogenicity studies*
C.-Y. Chen
Genomic Research Center, Academia Sinica, Taiwan

GUT MICROBIOTA

11:00 YRW-FL14 *Decoding Akkermansia muciniphila lipopolysaccharides*
P. García-Vello
Department of Chemical Sciences, University of Naples Federico II

11:05 YRW-FL15 *Assessing human microbiota systems for glycan recognition in the gut*
V. Correia
UCIBIO@REQUIMTE, FCT/NOVA, Portugal

11:10 YRW-FL16 *Understanding the molecular mechanism underpinning the immunomodulatory function of levan exopolysaccharide*
I. D. Young
The Gut Health and Food Safety Programme, Quadram Institute, UK

GLYCOMATERIALS

11:15 YRW-FL17 *A GH 20 thioglycoligase as versative catalyst for glycoconjugate synthesis*
G. Tegl
Department of Chemistry, University of British Columbia, Canada

11:20 YRW-FL18 *Well-defined oligo and polysaccharides as ideal probes for structural studies*
M. Delbianco
Biomolecular Systems Department, Max Planck Institute of Colloids and Interfaces, Germany

GLYCAN/GLYCOCONJUGATES/GLYCOSYLATION AND DISEASE

11:25 YRW-FL19 *Structural and immunological features of Acetobacter pasteurianus CIP103108 lipopolysaccharide*
M. Pallach
Department of Chemical Sciences, University of Naples Federico II, Italy

11:30 YRW-FL20 *Synthesis of cyclic peptoid-based iminosugar clusters*
I. Izzo
University of Salerno, Italy

11:35 YRW-FL21 *In-depth glycomics/glycoproteomics evaluation of tumour-associated carcinoembryonic antigen using state-of-art mass spectrometry*
A. Almeida
Institute for Glycomics, Griffith University, Australia

11:40 YRW-FL22 *Unravelling N-glycans recognition by SigLecs*
R. Marchetti
Department of Chemical Sciences, University of Naples Federico II, Italy

GLYCOINFORMATICS/ANALYSIS

11:45	YRW-FL23	*Molecular recognition of sialic acids by immunity receptors: computational approaches* **E. Crisman** Department of Structural and Chemical Biology, CIB-CSIC, Spain
11:50	YRW-FL24	*Unsaturate cyclitols as glycoside mimics: spontaneous and enzymatic hydrolysis* **P. Danby** Department of Chemistry, University of British Columbia, Canada
11:55	YRW-FL25	*Profiling HA-NA-balance by means of an HPLC-MS-Based Method* **V. J. Somovilla** Department of Chemical Biology and Drug Discovery, Utrecht University, The Netherlands
12:00	YRW-PL2	*Beam search arrays for (O)-glycan ligand discovery* **T. Feizi** Department of Medicine, Imperial College London, United Kingdom
12:45	Lunch	

ICS 2018

Sunday, July 15 | Roy L. Whistler Room (Rectorate)

16:30	**Opening Ceremony**
	J. M. Pinto Paixão - Vice-Rector of the Universidade de Lisboa
	Z. Witczak – ICO President
	C. Brett – IUPAC Vice-President/President-Elect
	A. Soares da Silva - President of the Portuguese Chemical Society
	L. Carriço - Director of Faculdade de Ciências da Universidade de Lisboa
	Z. Barroso – Conselho de Escola, Faculdade de Ciências da Universidade de Lisboa
	F. Nicotra - President of IUPAC Division of Organic and Biomolecular Chemistry
	S. Williams – ICO Secretary
	H. Burrows – Editor of Pure and Applied Chemistry
	Z. Guo - Editor of Carbohydrate Research
	J. Jimenéz-Barbero - Director CIC bioGUNE
	A. P. Rauter – President of Departamento de Química e Bioquímica and Chair of ICS2018
	Choir LACTVCVLAE VOCES *Portuguese composers: Joly Braga Santos, Miguel Jesus and Paulo Lourenço*
17:15	Roy L. Whistler International Award in Carbohydrate Chemistry
17:30	Roy L. Whistler International Award in Carbohydrate Chemistry Lecture *Chaired by Z. Witczak*
	R. Saccharides, Pseudosaccharides and their Mimetics **David Crich** Department of Chemistry, Wayne State University, Michigan, USA
18:15	ICO Young Researcher Award
18:30	ICO Young Researcher Award Lecture *Chaired by A. P. Rauter*

The antigen structural insights for engineering MUC1-glycopeptides that offer a new via in cancer-fighting
Nuria Martínez-Saéz
Department of Chemical Biology and Drug Discovery, Utrecht University, The Netherlands

19:15 Concert | Symphonic Orchestra of the Higher School of Applied Arts – Castelo Branco Polytechnic Institute by Maestro Osvaldo Ferreira

20:00 Welcome Reception

Monday, July 16

Roy L. Whistler Room (Rectorate)

Chair: M. Martin Lomas

08:30 PL1 *Three Different Glycomimetic Drugs in Clinical Trials for Inflammatory Disease and Cancer*
J. L. Magnani
GlycoMimetics Inc, Research Department, USA

09:15 PL2 *Breaking the limits in investigating Glycan-Protein Interactions by using NMR*
J. Jiménez-Barbero
CIC bioGUNE, Spain

10:00 IL1 *Probing Specific Cell-Surface Heparan Sulfate-Protein Interactions*
S.-C. Hung
Genomics Research Center, Academia Sinica, Taiwan

10:30 Coffee Break

Chair: R. Schmidt

11:00 PL3 *Synthesis and Biological Studies of GPIS and GPI-Anchored Proteins*
Z. Guo
Department of Chemistry, University of Florida, USA

11:45 PL4 *Constrained glycomimetics of mucin antigens*
C. Nativi
Department of Chemistry, University of Florence, Italy

12:30 PL5 *Glycosciences and the Algae*
R. Field
Department of Biological Chemistry, John Innes Centre

13:15 Lunch

14:30 **POSTER SESSION 1**

PARALLEL SESSIONS

Horton Room (3.2.14) - FCUL
SYNTHESIS

Chair:C. Galan

15:30 IL5 *From Stereocontrolled Glycosylation to Automated Oligosaccharide Synthesis*
A. V. Demchenko
Department of Chemistry and Biochemistry, University of Missouri – St. Louis, USA

16:00 OC1 *Convergent Synthesis of N-Glycopeptides from Thioaspartic Acid-Containing Peptides*
V. Wittmann
Department of Chemistry, University of Konstanz, Germany

16:15 OC2 *Synthetic O-Antigens Related to Pathogenic Burkholdderia Species Enable to decipher the Minimal Binding Epitopes*
C. Gauthier
INRS-Institut Armand-Frappier, Canada

16:30 OC3 *Semisynthesis of Complex-Type Biantennary Oligosaccharides Containing Lactosamine Repeating Units from a Biantennary Oligosaccharide Isolated a Natural Source*
Y. Maki
Department of Chemistry, Osaka University, Japan

16:45 OC4 *Chemical Synthesis of Glycopeptides and Glycoproteins Using Acyl-Thiol-Ene Mediated 'Click' Ligation*
E. Scanlan
Department of Chemistry, Trinity College Dublin, Ireland

17:00 Coffee Break

Chair: L. Somsák

17:30 IL6 *Unsaturated Sugar Synthons for Newer Chemical Glycosylations and Bioconjugations*
N. Jayaraman
Department of Organic Chemistry, Indian Institute of Science, India

18:00 OC5 *Reactivity and Stereoselectivity in Glycosylation: Emphasis on Structure of Glycosyl Acceptor and Concentration of Reagents*
L. O. Kononov
N.K. Kochetkov Laboratory of Carbohydrate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Federation

18:15 OC6 *C-Glycosides in the Synthesis of the Phytotoxin Diplopyrone*
R. Giuliano
Department of Chemistry, Villanova University, USA

Clark Room (3.2.15) - FCUL
CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS

Chair: J. Cañada, B. Ernst

15:30 IL10 *Entirely Carbohydrate Immunogens for Anti-Tumor mAb Development*
P. Andreana
Department of Chemistry and Biochemistry, University of Toledo, USA

16:00 OC14 *Glycodendritic Systems as Tools in Biomedicine*
J. Ramos-Soriano
Instituto de Investigaciones Químicas (IIQ), cic-Cartuja, CSIC-Universidad de Sevilla, Spain

16:15 OC15 *A new approach to identifying fucosyltransferase inhibitors as potential cancer therapeutics*
D. Kwan
Department of Biology, Concordia University, Canada

16:30 OC16 *Recent Advances in the Synthesis and Biology of 2-Acetamido Iminosugars C-Glycosides*
Y. Blériot
Department of Chemistry, IC2MP, France

16:45 OC17 *Mechanistic Analysis on a UDP-Glucuronic Acid 4-Epimerase*
A. Borg
Institute of Biotechnology and Biochemical Engineering, Graz University of Technology, Austria

17:00 Coffee Break

Chair: P. Kosma

17:30	YIL1	*Multivalent Glycoconjugates: Galectin Ligands with a Therapeutic Potential* **P. Bojarova** Institute of Microbiology, Czech Academy of Sciences, Czech Republic
18:00	OC18	*Identification of Structural Modifications that Determines Diversity of Klebsiella Pneumoniae O1, O2, and O3 O-Antigens and Are Important for Future Monoclonal Antibody-Based Diagnostics and Therapy* **J. Lukasiewicz** Department of Immunochemistry, Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Poland
18:15	OC19	*Tuning the activities of iminosugars* **O. López** Department of Organic Chemistry, University of Seville, Spain
18:30	OC20	*Rational Design of New Glycosidase Inhibitors: Cyclic Sulfates as Conformational Traps to Selectively Inhibit Glycosidases* **M. Artola** Department of Bio-organic Synthesis (LIC), Leiden University, The Netherlands
18:45	FL1	*Investigating Exo-Glycals Reactivity - Recent Progress and Applications* **N. Pellegrini** Faculté des Sciences et Technologies, UMR 7053 L2CM

Garegg Room (3.2.16) - FCUL
SYNTHESIS

Chair: S. Oscarson

15:30	IL15	*Gold-catalysed synthesis of giant oligosaccharides of mycobacterial cell wall* **S. Hotha** Indian Institute of Science Education and Research, Pune, India
16:00	OC29	*Exo-glycals: novel synthetic approaches and biochemical applications* **S. Vincent** Department of Chemistry, University of Namur, Belgium
16:15	OC30	*Modoluating reactivity in cyclodextrins by radical C-H functionalization* **A. Martin** Departmento de Síntesis de Productos Naturales, Instituto de Productos Naturales y Agrobiologia del CSIC, Spain
16:30	YIL2	*Structurally Innovative Nucleoside and Nucleotide Analogs of Potential Therapeutic Interest* **N. M. Xavier** Faculdade de Ciências, Universidade de Lisboa, Portugal
17:00	Coffee Break	

Chair: Y. Queneau

17:30	OC31	*Synthesis of 1,5-Anhydro-D-glycero-D-gluco-heptitol Derivatives as Potential Inhibitors of Bacterial Heptose Biosynthetic Pathways* **M. Blaukopf** Department of Chemistry, University of Natural Resources and Life Sciences-Vienna, Austria
17:45	OC32	*Self-promoted Stereospecific N-Glycosylation* **C. Pedersen** Department of Chemistry, University of Copenhagen, Danmark
18:00	OC33	*DNA methyltransferase 1 inhibitors: design & synthesis based on transition state structure*

F. Lamiable-Oulaidi
Ferrier Research Institute, Victoria University of Wellington, New Zealand

18:15 IL16 *Strategies for the synthesis of furanose containing molecules as useful tools in glycobiology*
C. Marino
Department of Organic Chemistry, University of Buenos Aires, Argentina

Lipták Room (3.2.13) - FCUL
GLYCOINFORMATICS BRIDGING THE GAP

Chair: S. Perez

15:30 IL20 *Glycomics@ExPASy: A web portal to explore the multiple facets of carbohydrates*
F. Lisacek
Department of Proteome Informatics Group, SIB Swiss Institute of Bioinformatics, Switzerland

16:15 OC39 *Interaction between carbohydrate-based capping agents and gold nanoparticles: A theoretical perspective*
A. Franconetti
Department of Chemistry, Universitat Autònoma de Barcelona, Spain

16:30 OC40 *Computational studies of carbohydrate active enzymes*
L. Masgrau-Fontanet
Department of Chemistry, Universitat Autònoma de Barcelona, Spain

16:45 OC41 *Molecular modeling tools for Glyco-Research*
O. Grant
Department of Complex Carbohydrate Research Center, University of Georgia, USA

17:00 Coffee Break

Chair: M. Frank

17:30 IL21 *Computational approaches to toll-like receptor 4 modulation*
S. Martin-Santamaria
Department of Structural & Chemical Blology, Center for Blological Research CIB-CSIC, Spain

18:00 OC42 *Unusual catalytic itineraries of glycosidases unveiled from QM/MM metadynamics simulations*
C. Rovira
Department of Inorganic and Organic Chemistry, University of Barcelona, Barcelona

18:15 OC43 *Furanose solution conformations: Insights from multiscale molecular simulations*
W. Plazinski
Department of Adsorption, Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Poland

18:30 OC44 *rapid methods for generating 3D structures of glycoproteins*
R. Woods
Department of GA, University of Georgia, USA

Ferrier Room (6.1.36) – FCUL
BIOMOLECULE STRUCTURE

Chair: S. Sonnino,

15:30 YIL4 *Structural Insights into mucin glycosylation and recognition*
F. Marcelo

Faculdade de Ciências e Tecnologia, Univ. Nova de Lisboa, Portugal

16:00 OC54 *Uniformly 13C-labeled carbohydrates for probing carbohydrate-protein interactions by NMR spectroscopy*
G. Nestor
Department of Molecular Sciences, Swedish University of Agricultural Sciences, Sweden

16:15 OC55 *A secondary structure element in fucosylated glycoepitopes*
M. Schubert
Department of Biosciences, University of Salzburg, Austria

16:30 IL25 *Understanding specificity of chitin and peptidoglycan deacetylases: structure, function, and engineering*
A. Planas
Bioengineering Department, University Ramon Llull, Spain

17:00 Coffee Break

Chair: J. M. Garcia Fernandez

17:30 IL26 *Molecular simulation of carbohydrate binding*
M. Frank
Biognos AB, Sweden

18:00 OC56 *Effect of human GBA2 pathogenic mutations on TxGH116 sugar binding and hydrolysis*
J. Cairns
Institute of Science, School of Chemistry, Suranaree University of Technology, Thailand

18:15 OC57 *Unraveling family 50 CBMs of clostridium thermocellum: structural-functional characterization of a new LYSM domain*
D. O. Ribeiro
Department of UCIBIO@REQUIMTE, FCT-NOVA, Portugal

18:30 OC58 *Structural and mechanistic basis for the O-acetylation of peptidoglycan by the virulence factors PatB of neisseria gonorrhoeae and OatA from both Staphylococcus aureus and Streptococcus pneumoniae.*
A. Clarke
Department of Molecular & Cellular Biology, University of Guelph, Canada

18:45 FL2 *Efficient enzymes for the production of sialic acids*
I. Ræder
Department of Chemistry, UiT-The Arctic University of Norway, Norway

Zamojski Room (6.2.53) – FCUL
GLYCOSCIENCES AND PERSONALIZED MEDICINE

Chair: S. Flitsch

15:30 YIL6 *Fragment-based design of mammalian carbohydrate receptor ligands*
C. Rademacher
Department of Medicine Imperial College London, UK

16:00 OC64 *The unique polysialic acid binding property of human adenovirus 52 revealed by glycan microarray analysis - future implications*
Y. Liu
Department of Medicine, Imperial College London, UK

16:15 OC65 *Properties and Application of Hydroxyl Groups in Glycomimetics and Other Marketed Drugs*
J. Cramer
Department of Pharmaceutical Sciences, University of Basel, Switzerland

16:30 OC66 *Homogeneous antibody-drug conjugate preparation via glycan linkage*
S. Manabe
Synthetic Cellular Chemistry Laboratory, RIKEN, Japan

16:45 IL31 *Polypeptide-galnac-transferases as prognostic and predictive cancer biomarkers*
E. Osinaga
Laboratory of Glycobiology and Tumor Immunology, Institut Pasteur of Montevideo, Uruguai

17:15 Coffee Break

ANALYSIS

Chair: M. Coimbra

17:45 OC67 *Analytical study of chondroitin sulfate in deer antler*
J. Tamura
Department of Life & Environmental Agricultural Sciences, Tottori University, Japan

18:00 OC68 *NMR and chemometrics in the characterization of heterogeneous polysaccharide drugs*
N. Guerrini
NMR Center, Istituto di Ricerche Chimiche e Biochimiche G. Ronzoni, Italy

18:15 OC69 *A fluorogenic probe for monitoring of thioglycosidase activity*
L. Betschart
Department of Chemistry, UBC Vancouver, Canada

Kochetkov Room (6.2.56) – FCUL

VACCINES

Chair: V. Bencomo

15:30 IL33 *Glycoconjugate vaccines: Approaches for preparation and recent trends*
R. Adamo
Department of Antigen Design, GSK Vaccines, Italy

16:00 OC80 *Synthesis and immunological evaluation of self-adjuvanting anticancer vaccine candidates using N-modified tri sialyl-Tn antigen*
K. Fukase
Department of Chemistry, Graduate School of Science, Osaka University, Japan

16:15 OC81 *Next generation of glycoconjugate vaccines*
C. Pereira
Department of Chemistry, Vaxxilon Deutschland GmbH, Germany

16:30 IL34 *Using NMR spectroscopy to facilitate the development and licensure of glycoconjugate vaccines*
N. Ravenscroft
Department of Chemistry, University of Cape Town, South Africa

17:00 Coffee Break

Chair: M. Monteiro

17:30 OC82 *The induction of protective antibodies by a glycoconjugate vaccine to prevent francisella tularensis infection*
G. Stefanetti
Department of Microbiology and Immunobiology, Harvard Medical School, USA

17:45 OC83 *A structural explanation of the importance of acetylation in Neisseria meningitidis serogroup A capsular polysaccharides to raise functional antibodies*

P. Henriques

Department of Vaccine chemistry, GSK Vaccines, Italy

18:00 OC84 *Development of structurally defined immunostimulatory vaccine adjuvants*
P. Wang
Department of Chemistry, University of Alabama at Birmingham, USA

18:15 OC85 *Comparison of capsular polysaccharide conformations in Streptococcus group B serotype III and Streptococcus pneumoniae serotype 14: implications for immunogenicity*. **M. M. Kuttel**
WESTERN CAPE , University of Cape Town , South Africa

Lindberg Room (8.2.30) – FCUL
GLYCOSYLATION AND DISEASE

Chair:C. Reis

15:30 YIL8 *Sulfoglycolysis: Mechanistic and structural insights into a novel metabolic pathway*
E. Goddard-Borger
Department of Chemistry, University of Melbourne, Australia

16:00 OC93 *Macrocyclic peptide inhibitors of carbohydrate-active enzymes*
S. A. K. Jongkees
Department of Chemical Biology and Drug Discovery, Utrecht University, The Netherlands

16:15 OC94 *CD44-Glycoprofilling: Establishing the molecular basis for targeted therapeutics in bladder cancer*
R. Azevedo
Experimental Pathology and Therapeutics Group, Research Centre, Portuguese Institute of Oncology of Porto (IPO-Porto), Portugal

16:30 IL38 *Chemoenzymatically synthesized glycosphingolipids toward cancer therapy*
Y. Zhang
Department of Chemistry, Sorbonne University, France

17:00 Coffee Break

INFLAMMATION AND DISEASE

Chair: S. Williams

17:30 OC95 *sp^2-iminosugar glycolipids as p38α MAPK activators: Drug candidates for diabetic retinopathy*
E. M. Sánchez-Fernández
Department of Organic, Faculty of Chemistry, University of Seville, Spain

17:45 OC96 *SIGLEC-8 - A promising target for asthma*
O. Schwardt
Department of Molecular Pharmacy, University of Basel, Switzerland

18:00 OC97 *Bacterial lectins as master manipulators of host cell physiology*
W. Römer
Faculty of Biology, University of Freiburg, Germany

18:15 OC98 *Conformational switch of the bacterial adhesin FIMH in the absence of the regulatory domain – Engineering a minimalistic allosteric system*
S. Rabbani
Department of Molecular Pharmacy, University of Basel, Switzerland

18:30 OC99 *Inhibition of O-glycan biosynthesis using hexosamine analogs*
S. Neelameghan
Department of Chemical & Biological Engineering, State University of New York-Buffalo, USA

Paulsen Room (1.3.23) – FCUL
GLYCOMATERIALS

Chair: Y. Ito

15:30	IL42	*Polysaccharide engineering: Lateral and terminal modification* **B. Christensen** Department of Biotechnology and Food Science, NTNU-Norwegian University of Science and Technology, Norway
16:00	OC105	*Silica glyconanoparticles for nanomedicine* **C. Crucho** CQE, Instituto Superior Técnico, Universidade de Lisboa, Portugal
16:15	OC106	*Synthetically modified chitosan as efficient antimicrobial agents* **P. Sahariah** Faculty of Pharmaceutical Sciences, University of Iceland, Iceland
16:30	OC107	*Dissecting multivalent lectin-carbohydrate interactions using polyvalent multifunctional glycan-quantum dot* **Y. Guo** Department of Food Science and Nutrition, University of Leeds, UK
16:45	OC108	*D-Galactose derived beta-amino acids and their peptides: Synthesis and structural studies* **R. Estevez** Center for Research in Biological Chemistry and Molecular Materials, University of Santiago de Compostela, Spain
17:00	Coffee Break	

Chair: D. Gammon

17:30	OC109	*Synthesis and properties of photoswitchable glycomacrocycles* **J. Xie** Department of chemistry, ENS Paris-Saclay, France
17:45	OC110	*Chemo-enzymatic synthesis of artificial xylan polysaccharides with defined substitution patterns* **F. Pfrengle** Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Germany
18:00	FL15	*Polysaccharide Block Copolymers* **I. Mo** Department of Biotechnology and Food Science, Norwegian Univeristy of Science and Technology, Norway
18:05	FL16	*Optimisation of a Carbohydrate-Functionalised Collagen-based System for Ventral Mesencephalic Cells Delivery* **A. Rebelo** Centre for Research in Medical Devices (CURAM), National University of Ireland Galway, Ireland
18:10	FL17	*Smart hybrid glyco nanocarriers for theranostic* **E. Castanheira** Centro de Química-Física Molecular, Universidade de Lisboa, Portugal

18:15 OC111 *Structural insights on the mechanism of gelation of cellulose hydrogels by a combination of solution and solid state NMR*
J. C. Muñoz-García
School of Pharmacy, University of East Anglia, UK

18:30 OC112 *Structural features and properties of high-molar mass dextrans bio-designed in vitro using enzymes*
A. Rolland-Sabaté
UMR SQPOV, INRA, France

Montreuil Room (8.2.47) – FCUL
CARBOHYDRATES AND NUTRITION

Chair: J. Defaye

15:30 IL44 *Processes towards the production of human milk oligosaccharides*
M. J. Hederos
Department of Downstream Process, Glycom R&D, Denmark

16:00 OC123 *Milk oligosaccharides as therapeutic agents*
A. Ranjan
Department of chemistry, CMP COLLEGE, UNIVERSITY OF ALLAHABAD, India

16:15 OC124 *Deglycosylation of rutin with novel diglycosidase rutinosidase: Novel concept of "solid state biocatalysis"*
V. Kren
Laboratory of Biotransformation, Institute of Microbiology of the Czech Academy of Sciences, Czech Republic

16:30 IL45 *Non-enzymatic transglycosylation reactions in foods: the example of honey, coffee, and starch*
M. Coimbra
Department of chemistry, University of Aveiro, Portugal

17:00 Coffee Break

SYNTHESIS

Chair: F. Bolaños

17:30 OC125 *L-arabinofuranosylated probes for the analysis of novel L-arabinofuranosidases from Bifidobacterium longum*
A. Ishiwata
Synthetic Cellular Chemistry Lab., RIKEN, Japan

17:45 OC126 *On the road to an organocatalytic approach to Neplanocin A: synthesis of rare sugars and glycomimetics*
D. Gamenara
Organic Chemistry Department, Facultad de Química. Universidad de la República (UdelaR), Uruguay

18:00 FL21 *A hybrid approach to sialylated glycan synthesis: combination of automated solid-phase and enzymatic synthesis*
A. Pardo
Biomolecular Systems, Max Planck Institute for Colloids and Interfases, Germany

18:05 FL22 *Length matters: one extra methylene group in glycosyl acceptor can dramatically change the reactivity pattern of glycosyl donor*
E. Stepanova

Research School of Chemistry & Applied Biomedical Sciences, Tomsk Polytechnic University, Russian Federation

18:10 FL23 *Capturing the conformational behaviour and stereoselectivity of oxocarbenium ions*

T. Hansen

Leiden Institute of Chemistry, The Netherlands

18:15 OC127 *Intramolecular cine substitution on carbohydrates*

I. Perez-Martin

Síntesis de Productos Naturales, Instituto de Productos Naturales y Agrobiología, CSIC, Spain

18:30 FL24 *Shape-controlled gold nanoparticles functionalized with synthetic oligorhamnoses of group a streptococcus*

O. Pitirollo

Department of chemistry, University of Milan, Italy

18:35 FL25 *Palladium-catalyzed stereoselective synthesis of deoxyglycosides*

A. Sau

School of Chemistry, University of Bristol, UK

18:40 FL26 *Synthesis of E. faecalis diheteroglycan oligosaccharides*

J. Enotarpi

LIC bio-organic synthesis, Leiden University, The Netherlands

A novel synthetic approach toward diverse b-glyceroglycolipids based on a neighboring free 1,2-trans-selective glycosylation

N. Yagami

18:45 FL27 Department of Applied Bioorganic Chemistry, Gifu University, Japan

Tuesday, July 17

Roy L. Whistler Room (Rectorate)

Chair: M. Chmielevski

08:30	PL6	*N-glycans on proteins* **Y. Kajihara** Osaka University, Department of Chemistry, Japan
09:15	PL7	*Synthesis as an enabling technology for understanding bacterial glycan biosynthesis and function* **T. Lowary** Canadian Glycomic Network (GlycoNet), Univ. of Alberta, Canada
10:00	IL2	*Chemical Labeling and Quantitative Analysis of Protein O-GlcNAcylation* **X. Chen** College of Chemistry, Peking University, China
10:30	Coffee Break	

Chair: J. Jiménez Barbero

11:00	PL8	*Carbohydrate-lectin interactions – what makes them unique?* **B. Ernst** Department of Pharmaceutical Sciences, University of Basel, Switzerland
11:45	PL9	*Taylor-made cyclodextrins for bio-inspired applications* **M. Sollogoub** IPCM, Sorbonne Université, France
12:30	PL10	*Optoglycomics: Photoswitchable Glycoconjugates to Explore Carbohydrate Recognition* **T. Lindhorst** Department of chemistry, Christiana Albertina University of Kiel, Germany
13:15	Lunch	

PARALLEL SESSIONS

Horton Room (3.2.14) - FCUL
SYNTHESIS

Chair: V. Wittmann

15:30	IL7	*Playing around with glycosylation: from strategy to green processes* **S. Castillón** Department of Química Analítica i Química Orgánica, University Rovira i Virgili, Spain
16:00	OC7	*Catalytic stereoselective synthesis of deoxyglycosides* **C. Galan** School of Chemistry, University of Bristol, UK
16:15	OC8	*Fe(acac)3, an Inexpensive and Green Catalyst for Regioselective Acylation of Carbohydrates* **H. Dong** School of Chemistry and Chemical Engneering, Huazhong University of Science and Technology, China
16:30	OC9	*De Novo Synthesis of Partially Fluorinated Carbohydrates* **T. Nokami** Department of Chemistry and Biotechnology, Tottori University, Japan
16:45	OC10	

Pyranoside-into-Furanoside rearrangement – winding road from side reaction to new synthetic method
V. B. Krylov
The Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of organic chemistry, RAS, Russian Federation

17:00 Coffee Break

Chair: S. Castillón

17:30 OC11 *Synthesis of polyfluorinated alfa-galactosyl ceramide analogues and biological implications*
M. I. Matheu
Analytical Chemistry and Organic Chemistry, University Rovira i Virgili, Spain

18:00 OC12 *Novel Methodologies in Sialylation Reactions*
C. De Meo
Department of Chemistry, Southern Illinois University Edwradsville, USA

18:15 OC13 *Design, synthesis and properties of shape-switchable glycomacrocycles*
G. Despras
Otto Diels Institute of Organic Chemistry, Christian Albrechts University, Germany

Clark Room (3.2.15) - FCUL
CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS

Chair: M. Sollogoub

15:30 IL11 *Design, synthesis, and development of lectin ligand mimetics into the clinic*
U. Nilsson
Department of Chemistry, Centre for analysis and Synthesis, Sweden

16:00 OC21 *Reinvestigation of fine epitope specificity of EB-A2 antibody used in the immune detection of galactomannan of aspergillus fumigatus: molecular basis of false positive signals*
N. E. Nifantiev
Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of organic chemistry, RAS, Russian Federation

16:15 OC22 *Synthetic Schistosoma mansoni glycan coated gold nanoparticles and their use in diagnostic and vaccine applications*
M. Harvey
Department of Bio-organic synthesis, Universiteit Leiden, The Netherlands

16:30 OC23 *Amadori rearrangement products as ligands for mannoside-specific lectins*
T. M. Wrodnigg
Institute of Organic Chemistry, Graz University of Technology, Austria

16:45 OC24 *Selective cell targeting by synergistic interactions of peptide and glycans*
S. Nomura
Biofunctional Synthetic Chemistry Laboratory, RIKEN, Japan

17:00 Coffee Break

Chair: T. Lowary

17:30 IL12 *Therapeutic in vivo synthetic chemsitry*
K. Tanaka
Biofunctional Synthetic Chemistry Laboratory, RIKEN, Japan

18:00 OC25 *Towards catalytic antibiotics*
A. A. Joseph
Schulich Faculty of Chemistry, Technion- Israel Institute of Technology, Israel

18:15 OC26 *GPR55 Ligands: From Endocanabinoids to Lysolipids*
P. Greimel

Brain Science Institute, RIKEN, Japan

18:30	OC27	*Glycosylations of Simple Acceptors with 2-O-Acyl L-Idose or L-Iduronic Acid Donors Reveal Only a Minor Role for Neighbouring Group Participation* **V. Ferro** School of Chemistry and Molecular Biosciences, The University of Queensland, Australia
18:45	OC28	*Multivalent glycomimetics with affinity and selectivity toward fucose-binding receptors from emerging pathogens* **D. Goyard** Department of molecular chemistry, CNRS/UGA, France

Garegg Room (3.2.16) - FCUL
SYNTHESIS

Chair: R. Field

15:30	IL17	*Epimerisations of glycosyl thiols and glycomimetic synthesis* **P. Murphy** National Univ. of Ireland, Ireland
16:00	OC34	*Boronic acid catalyzed regio- and 1,2-cis-stereoselective glycosylation of unprotected sugar acceptors via SNi-TYPE MECHANISM* **D. Takahashi** Applied Chemistry, Keio University, Japan
16:15	OC35	*Access to Synthetic Glycomes through Enzymatic Synthesis* **P. G. Wang** Department of Chemistry, Georgia State University, EUA
16:30	YIL3	*Dual Glycosylation: A Minimalist Approach for Oligosaccharide Synthesis* **X.-W. Liu** Nanyang Technological University, Singapore
17:00	Coffee Break	

Chair: M. Brimble

17:30	OC36	*Mucin peptide microarrays in studies of protein-carbohydrate binding events* **U. Westerlind** Department of Chemistry, Umeå University/ Leibniz Institute for Analytical Sciences, Germany
17:45	OC37	*Modular synthesis of multifaceted cyclooligosaccharides based on trehalose building blocks* **J. L. Blanco** Department of Organic Chemistry, University of Seville, Spain
18:00	OC38	*Total Synthesis of O-GalNAcylated Antifreeze Glycoprotein Toward Elucidation of the Functional Role of O-GalNAcylation* **R. Okamoto** Department of Chemistry, Osaka University, Japan

Lipták Room (3.2.13) - FCUL
CARBOHYDRATE IN LIGNOCELLULOSIC BIOMASS VALORIZATION

Chair: J. Bordado

15:30	IL22	*Combined cavitational and enzymatic treatments for the valorization of lignocellulosic feedstock, from lab to pilot scale* **G. Cravotto**

Department of Drug Science and Technology, University of Turin, Italy

16:15 OC45 *Novel assays for the measurement of glucuronyl esterase and α-glucuronidase, two enzymes involved in lignin and hemicellulose hydrolysis*
D. Mangan
Department of Research, Megazyme, Ireland

16:30 OC46 *Development of a Chemical Platform for the production of added-value products from forest residues*
R. Galhano
CERENA, IST, Portugal

16:45 OC47 *Modifying lignocellulose to enable valorization: how fungi do it*
I. van Munster
School of Chemistry, University of Manchester, UK

17:00 Coffee Break

Chair: L. Roseiro

17:30 IL23 *Biotechnology of polysaccharides for biofuels and bio-based products*
F. Gírio
LNEG - Laboratório Nacional de Energia e Geologia, IP, Portugal

18:00 OC48 *Synthesis of branched oligosaccharides related to the pectic rhamnogalacturonan I polysaccharide*
C. Romanò
DTU Kemi, Denmark Technical University, Denmark

18:15 OC49 *Deep eutectic solvents as tools for fractionation and valorization of wood macromolecular components*
A. Lopes
Department of chemistry, University of Aveiro/ciceco - Aveiro institute of materials, Portugal

18:30 OC50 *Valorization of acuatic biomass for the extraction of lignocellulosic polysaccharides*
M. Martínez-Sanz
Food Safety and Preservation Department, Institute of Agrochemistry and Food Technology, Spain

18:45 OC51 *One-pot synthesis of nitrogen compounds from biomass resources*
A. C. Fernandes
Department of chemistry, CQE, IST, Portugal

Ferrier Room (6.1.36) – FCUL
BIOMOLECULE STRUCTURE

Chair: A. Planas

15:30 YIL5 *Bacterial lectins as targets for diagnostics and therapy*
A. Titz
Helmholtz Centre for Infection Research, German

16:00 IL27 *Structural biology of glycan-degrading enzymes from beneficial human gut microbe bifidobacteria*
S. Fushinobu
Department of Biotechnology, The University of Tokyo, Japan

16:30 OC59 *Synthesis of II3Neu5Ac-[6-3HGal]Gg4-N3*
S. Sonnino
Department of Medical Biotechnology and Translational Medicine, University of Milan, Italy

16:45 OC60 *Enabling Glycoscience Research with Improved Data Representation of Carbohydrates in the Protein Data Bank*
J. Young
RCSB Protein Data Bank, Rutgers, The State University of New Jersey, EUA

17:00 Coffee Break

Chair: C. Maycock

17:30 IL28 *Understanding the role of glycoconjugates as key molecules for cell survival and communication*
A. Silipo
University of Naples Federico II, Italy

18:00 OC61 *Selective recognition of O-methylated glycans by fungal tectonin, a defense efector*
A. Varrot
CERMAV, CNRS, France

18:15 OC62 *Sulfated polysaccharides of marine origin: new insights on structural features and biological properties*
U. Nadezhda
Laboratory of Glycoconjugate Chemistry, N.D. Zelinsky Institute of Organic Chemistry, Russian Federation

18:30 OC63 *Bacteriophage-like exopolysaccharide hydrolases control biophysical properties of biofilm in plant pathogens*
S. Barbirz
Physical Biochemistry, University of Potsdam, Germany

18:45 FL3 *Neisseria meningitidis a capsular polysaccharide and carbasugar mimetic: conformational studies*
I. Calloni
Chemical Glycobiology Lab, CIC bioGUNE, Spain

Zamojski Room (6.2.53) – FCUL

BIOMOLECULE STRUCTURE

Chair: O. Renaudet

15:30 IL32 *Twisting tails and curious channels – the phosphoribosyltransferases*
E. Parker
Ferrier Research Institute, Victoria University of Wellington, New Zealand

What we can learn from multibladed beta-propeller lectins?
M. Wimmerová

16:00 OC70 Masaryk University, Czech Republic

GLYCANS

16:15 OC71 *Click reactions in chitosan chemistry*
A. Kritchenkov
Laboratory of natural polymers, Institute of Macromolecular Compounds of Russian Academy of Sciences, Russian Federation

16:30 FL4 *Synthesis of complex type N-glycans*
S. Manabe
Department of Chemistry, Osaka University, Graduate School of Science, Japan

16:35 FL5 *Investigation of growth factor - glycosaminoglycan-mimetic marine exopolysaccharide interactions by atomic force microscopy*
A. Zykwinska
EM3B, Ifremer, France

16:40 FL6 *Synthesis, stability, reactivity, stereoselectivity and computational study of 4-thio furanosides*
J.M. Madern
Department of Bio-organic Synthesis, Leiden Institute of Chemistry, The Netherlands

16:45 OC72 *The role of core fucosylation on the dynamics of IgG1-type complex N-glycans: An MD study of sequence-to-structure relationship in complex carbohydrates*
E. Fadda
Department of Chemistry, Maynooth University, Ireland

17:00 OC73 *Doxorubicin and aclarubicin: shuffling antracyclin glycans for improved cytotoxic agents*
D. Wander
Department of Bio-Organic Synthesis, Leiden University, The Netherlands

17:15 Coffee Break

Chair: P. Murphy

17:45 OC74 *RN1, a novel galectin-3 inhibitor impedes pancreatic cancer cell growth in vitro and in vivo via blocking galectin-3/EGFR/ERK/Runx1 signaling pathway*
K. Ding
Shanghai Institute of Materia Medica, Chinese Academy of Sciences, China

18:00 OC75 *Chemical biology of plant cell wall glycans*
M. H. Clausen
Department of Chemistry, Technical University of Denmark, Denmark

18:15 OC76 *Solution and solid-phase automated synthesis of human milk oligosaccharides*
M. D. Bandara
Department of Chemistry and Biochemistry, University of Missouri St. Louis, USA

18:30 OC77 *Glyco-engineering: site-specific ligation of RBC glycocalyx with blood group antigen*
I.M. Ryzhov
Laboratory of carbohydrates, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Russian Federation

Kochetkov Room (6.2.56) – FCUL
VACCINES

Chair: R. Adamo

15:30 IL35 *Conjugate Vaccines from Synthetic and Bacterial Carbohydrates Using Squaric Acid Chemistry*
P. Kovac
NIDDK, LBC, National Institutes of Health, USA

16:00 OC86 *Divergent chemoenzymatic synthesis of shigella flexneri pentasaccharides for vaccine development*
L.-A. Barel
BSC, Institut Pasteur, France

16:15 OC87 *Capsular polysaccharides of streptococcus suis: potential as vaccine antigens*
G.-G. Desjardins
Faculty of Veterinary Medicine, University of Montreal, Canada

16:30 IL36 *Identification of immunologically protecting epitopes in heterogeneous microbial polysaccharides for the development of glycoconjugate vaccines*
S. Oscarson
Centre for Synthesis and Chemical Biology, University College Dublin, Ireland

17:00 Coffee Break

Chair: K. Fukase

17:30	IL37	*The design of a platform for a multi-valent diarrheal vaccine* **M. Monteiro** Department of Chemistry, University of Guelph, Canada
18:00	OC88	*Fluorinated Antigen-Mimetics For Glycoconjugate Vaccine Development* **A. H. Röder** Department of Chemistry, Ludwigs-Maximilian University, Germany
18:15	FL7	*Regioselective glycosylation of galactose and its application to the synthesis of group B Streptococcal capsular polysaccharide fragments* **L. Del Bino** Glycoconjugate Synthesis and Analitics Lab, GSK Vaccines, Italy
18:20	FL8	*Trifunctional mannoside conjugates to explore crosstalk between CLRS and TLRS and their effect on antigen presentation* **T. Hogervorst** Department of Bio-organic Synthesis, Leiden Institute of Chemistry, The Netherlands

Lindberg Room (8.2.30) – FCUL
GLYCOSYLATION AND DISEASE

Chair: A. Molinaro

15:30	YIL9	*Sulfoglycolysis: Mechanistic and structural insights into a novel metabolic pathway* **J. Rodrigues** Department of Chemistry, University of Melbourne, Australia
16:00	OC100	*Complete Spatial Characterisation of N-Glycans in an Adult Rat Brain* **R. O'Flaherty** Leinster, NIBRT, Ireland
16:15	OC101	*Biology of NGLY1: Not just taking care of junks* **T. Suzuki** Glycometabolic Biochemistry Laboratory, RIKEN, Japan
16:30	IL39	*Glycosylation in cancer: molecular functions and clinical implications* **C. Reis** Glycobiology in Cancer, Ipatimup / i3S - University of Porto
17:00	Coffee Break	

Chair: P. Rudd

17:30	IL40	*Sulfoglycolysis: Mechanistic and structural insights into a novel metabolic pathway* **S. Williams** Department of Chemistry, University of Melbourne, Australia
18:00	OC102	*Development of novel fluorescence-quenched probes for monitoring lysosomal glycosidase activity in live cells* **R. Ashmus** Department of Chemistry, Simon Fraser University, Canada
18:15	FL9	*The molecular and functional impact of oncogenic O-glycan truncation on CD44* **S. Mereiter** Glycobiology in Cancer, i3S/IPATIMUP - University of Porto, Portugal
18:20	FL10	*Glycosyl nitrates in synthesis: from selectively protected building blocks to oligosaccharides* **T. Wang** Chemistry and Biochemistry, University of Missouri-St. Louis, USA

18:25 FL11 *One-pot synthesis of pseudo-thiodisaccharides through aziridine opening reactions*
A. Tamburrini
Department of Chemistry, Università degli Studi di Milano, Italy

18:30 OC103 *New aspect of glycosidase-catalyzed transglycosylation involving 1,2-anhydro sugar intermediate*
S. I. Shoda
Department of Biomolecular Engineering, Tohoku University, Japan

Paulsen Room (1.3.23) – FCUL
POLYSACCHARIDE BIOTECHNOLOGY

Chair: B. Christensen

15:30 OC113 *Complexation of Alginates and Lactic Acid Bacteria Hetero-exopolysaccharides with Milk Proteins and Structural Analysis of Beta-lactoglobulin Binding Sites*
B. Svensson
Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark

15:45 OC114 *Anti-metastatic properties of a marine bacterial exopolysaccharide-based derivative designed to mimic glycosaminoglycans*
S. Colliec-Jouault
RBE Ressources Biologiques et Environnement, Ifremer, France

16:00 OC115 *New laminarin-conjugates: Bioprotection and molecular tools to study penetration in plant leaves*
V. Ferrières
ISCR, CORINT, Univ Rennes, ENSCR, ISCR, UMR CNRS 6226, France

16:15 OC116 *Product-oriented chemical surface modification of a levansucrase (SACB) via an ENE-type reaction*
J. Ertl
Institute of organic chemistry, University of Würzburg, Germany

16:30 OC117 *Flocculating activity of exopolysaccharides from actinobacteria*
M. Czemierska
Department of Biochemistry, M. Curie-Sklodowska University, Poland

17:00 Coffee Break

MARINE GLYCANS

Chair: T. Baasov

17:30 IL43 *Marine-derived polysaccharides in hybrid building blocks for tissue engineering*
J. Mano
CICECO - Dept. Chemistry, University of Aveiro, Portugal

18:00 OC118 *Chlamydomonas reinhardtii as a green microalgae to understand evolution of the N-glycosylation pathways in microalgae*
M. Bardor
Glyco-MEV Lab, UNIROUEN, Normandie University, France

18:15 OC119 *Carrageenan catabolism is encoded by a complex regulon in marine heterotrophic bacteria*
E. Ficko-Blean
CNRS/UPMC Roscoff Biological Research Station, France

Montreuil Room (8.2.47) – FCUL

GUT MICROBIOTA

Chair: H. Overkleeft

15:30 IL46 *Prebiotic carbohydrate polymers from byproducts towards healthier gut microbiota*
M. Pintado
Department of Downstream Process, Glycom R&D, Denmark

16:00 OC128 *Surface exposed glycoside hydrolases of human gut bacteria conferring galactomannan dietary fibre catabolism*
H. Stalbrand
Department of Biochemistry and Struct. Biol., Lund university, Sweden

16:15 OC129 *Characterisation of exopolysaccharides produced by Lactobacillus fermentum LF2 and their potential applications as immune modulators*
A. Laws
Chemical Sciences, University of Huddersfield, UK

16:30 OC130 *Screening the human gut microbiome for carbohydrate modifying enzymes to generate universal donor blood*
P. Rahfeld
Department of chemistry, University of British Columbia, UK

16:45 OC131 *Gut microbiota lipopolysaccharides: reverting the concept from bad to good*
F. Di Lorenzo
Department of Chemical Sciences, University of Naples Federico II, Italy

17:00 Coffee Break

Chair: H. Overkleeft

17:30 OC132 *Characterization of novel lipooligosaccharide from gut symbiotic bacteria and chemical synthesis of its active principle lipid A*
A. Shimoyama
Department of Chemistry, Graduate School of Science, Osaka University, Japan

17:45 OC133 *Differential bacterial capture and transport preferences facilitate co-growth on dietary xylan in the human gut*
M. A. Hachem
Department of Biotechnology and Biomedicine, Technical University of Denmark, Denmark

NATURAL GLYCOCONJUGATES

Chair: A. French, L. Bento

18:00 IL47 *Promiscuities in Sialic Acid Biosynthesis*
J. Voglmeir
College of Food Science and Technology, Nanjing Agricultural University, China

18:30 OC134 *The arabinofuranosidase CtAraf51: A versatile biocatalyst for the synthesis of novel glycofuranoconjugates*
P. Quentin

18:45 FL28 *Automated Glycan Assembly of Lewis Antigens*
M. Guberman
Biomolecular Systems Department, Max Planck Institute of Colloids and Interfaces, Germany

Wednesday, July 18

Roy L. Whistler Room (Rectorate)

Chair: H. Kamerling

08:30	PL11	*Exploring fungal and bacterial lectome: 1000 ways to bind your sugars* **A. Imberty** Centre de Recherches sur les Macromolécules Végétales (CERMAV), France
09:15	PL12	*Processing of O-mannosyl glycan and its pathological role in muscular dystrophy* **T. Endo** Tokyo Metropolitan Institute of Gerontology, Japan
10:00	IL3	*Multivalent neoglycoconjugates as tools to interfere with the immune system* **J. Rojo** Instituto de Investigaciones Químicas, CSIC Sevilla, Spain
10:30	Coffee Break	

Chair: S. Penadés

11:00	PL13	*Challenges of machine-assisted carbohydrate synthesis and analysis* **N. Pohl** Joan and Marvin Carmack Chair, Indiana University Bloomington, Department of Chemistry, USA
11:45	PL14	*Re-engineering bacterial toxin lectins for synthetic glycobiology* **B. Turnbull** School of Chemistry-Univ. of Leeds,UK
12:30	Lunch	
14:30	**POSTER SESSION 1**	

PARALLEL SESSIONS

Horton Room (3.2.14) - FCUL
SYNTHESIS

Chair: E. Scanlan

14:30	IL8	*Applications of organocatalysts in carbohydrate chemistry* **E. McGarrigle** School of Chemistry, University College Dublin, Ireland
15:00	IL9	*New syntheses and new effects of glycomimetics* **L. Somsák** Department of Organic Chemistry, University of Debrecen, Hungary

Clark Room (3.2.15) - FCUL
CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS

Chair: Y. Blériot

14:30	IL13	*Synthesis and evaluation of fluorescent Trypanosoma cruzi GPI anchors in support of drug discovery for Chagas' disease* **I. Carvalho** Department of Pharmaceutical Sciences, University of Sao Paulo, Brazil
15:00	IL14	*Detecting live pathogens using carbohydrate-based metabolic probes* **B. Vauzeilles** ICMMO/ICSN, CNRS, France

Garegg Room (3.2.16) - FCUL
SYNTHESIS

Chair: N. Jayaraman

14:30	II18	*Carbohydrates and fluorescent probes. New avenues in chemistry and glycochemistry* **A. Gomez** Department of Bio-Organic Chemistry, Instituto de Química Orgánica General, CSIC Madrid, Spain
15:00	IL19	*Metal-free click ligations for the synthesis of multivalent sugars and iminosugars* **A. Marra** Institut des Biomolécules Max Mousseron, University of Montpellier, France

Lipták Room (3.2.13) - FCUL
SYNTHESIS

Chair: H. P. Wessel

14:30	IL24	*Stereoselective Synthesis of Sugar Mimetics from Simple Monosaccharides* **S. Jarosz** Institute of Organic Chemistry, Polish Aacdemy of Sciences, Poland
15:00	OC52	*Chemistry on protection and functionalization of free sugars* **C. C. Wang** Institute of Chemistry, Academia Sinica, Taiwan
15:15	OC53	*The acyloxyallylation of (un)protected tetroses revealing a pronounced diastereodivergence and a fundamental difference in the performance of the mediating metal* **C. Stanetty** Institute of Applied Synthesis, TU Wien, Austria

Ferrier Room (6.1.36) – FCUL
BIOMOLECULE STRUCTURE

Chair: C. Unverzagt

14:30	IL29	*The use of fluoroproline in MUC1 antigen enables efficient detection of antibodies in patients with cancer* **F. Corzana** Department of Chemistry, University of La Rioja, Spain
15:00	IL30	*Amphiphiles in glycosciences: from biobased to biological chemistry* **Y. Queneau** ICBMS, University of Lyon, France

Zamojski Room (6.2.53) – FCUL
CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS/BIOMOLECULE STRUCTURE

Chair: T. Feizi

14:30	OC78	*First syntheses of 2-C-glucopyranosyl pyrimidines* **E. Bokor** Department of Organic Chemistry, University of Debrecen, Hungary
14:45	YIL7	*Targeting protein-carbohydrate interactions in polysaccharide biodegradation: the power of carbohydrate microarrays* **A. Palma** Department of Chemistry, UCIBIO-NOVA University of Lisbon, Portugal
15:15	OC79	*Glycodendrimer array: an efficient tool to screen multivalent glycoconjugates towards lectins*

E. Laigre

Département de Chimie Moléculaire, Université Grenoble Alpes, France

Kochetkov Room (6.2.56) – FCUL
NATURAL GLYCOCONJUGATES

Chair: J. Vliegenthart

14:30	OC89	*Synthesis and Characterization of Homogeneous Glycosylphosphatidylinositol Anchored Glycoproteins* **D. Varon Silva** Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Germany
14:45	OC90	*Synthesis and immunomodulatory activities of glycoconjugates containing lyso-phosphatidyl inositol* **Y. Fujimoto** Department of Chemistry, Keio University, Japan
15:00	OC91	*C-Mannosylation of Thrombospondin Repeats* **H. Bakker** Department of Clinical Biochemistry, Hannover Medical School, Germany
15:15	OC92	*Endomannosidase Triages Misfolded Glycoprotein in the Endoplasmic Reticulum* **K. Totani** Department of Materials and Life Science, Seikei University, Japan

Lindberg Room (8.2.30) – FCUL
SYNTHESIS

Chair: I. Maya

14:30	IL41	*Glycosylation as a strategy for de-toxification of aminoglycoside antibiotics* M. Fridman School of Chemistry, Raymond and Beverly Sackler, Faculty of Exact Sciences, Tel Aviv University, Israel
15:00	OC104	*A novel method for the complete stereo-control of α-sialylation* **H. Ando** G-CHAIN, Gifu University, Japan
15:15	FL12	*A new reaction of switchable ring contraction in selectively protected pyranosides with retention of aglycon* **P. Abronina** Laboratory of carbohydrates, N. D. Zelinsky Institute of Organic Chemistry of the Russian Academy of Sciences, Russian Federation
15:20	FL13	*A versatile approach to molecular design of monovalent and multivalent C-glycoconjugates as ligands of bacterial lectins* **F. Portier** Physicochemistry and self-assembly of glycopolymers, CERMAV-UGA, France
15:20	FL14	*Synthesis of secondary cell wall polymer fragments of Paenibacillus ALVEI* **S. Krauter** Department of Chemistry, University of Natural Resources and Life Sciences Vienna, Austria

Paulsen Room (1.3.23) – FCUL
CARBOHYDRATES FOR MEDICINE AND DIAGNOSIS

Chair: C. Marino

14:30 OC120 *The synthesis of multivalent glycodendrimers as anti-viral agents*
K. McReynolds
Department of Chemistry, California State University, Sacramento, USA

14:45 OC121 *MUC1 glycopeptides recognition profiling by macrophage galactose lectin to target dendritic cells*
F. Garcia-Martin
Faculty of Advanced Life Science, Hokkaido University, Japan

15:00 FL18 *Synthesis of well-defined teichoic acids fragments and their evaluation through microarray technology*
F. Berni
Leiden Institute of Chemistry, University of Leiden, The Netherlands

15:05 FL19 *Recent development in glyco-rgd conjugates: synthesis, biological evaluation and pet application*
S. Lamandé-Langle
Faculté des Sciences et Technologies, UMR 7053 L2CM, France

15:10 FL20 *Nanomolar inhibition of human OGA by 2-acetamido-2-deoxy-D-glucono-1,5-lactone hydrazone derivatives*
M. Kiss
Department of Organic Chemistry, University of Debrecen, Hungary

TCI CHEMICALS

15:15 OC122 *Três setas; Glycan, Enzyme, Binding protein. TCI boosts Glycoscience research by the unique reagents*
N. Yuasa
Department of Glycotec, Tokyo Chemical Industry, Japan

Montreuil Room (8.2.47) – FCUL
SYNTHESIS

Chair:P. Kovac

14:30 IL48 *Enzyme Transition State Analysis and Inhibitor Design*
P. Tyler
Ferrier Research Institute, Victoria University of Wellington, New Zealand

15:00 IL49 *Glycoside hydrolases as efficient biocatalysts: from fundamental research to cosmetic applications*
R. Daniellou

15:30 FL29 *Synthesis and structural study of glycosylphosphatidyl-inositols fragments in monolayers*
D. Willén
Max Planck Institute of Colloids and Interfaces, Germany

FL30 *C-Type lectin targeting with glycomimetics: a screening in microarray-format*
L. Medve
University of Millan, Italy

	FL31	*Structural insight into mannuronic acid specificity of a polysaccharide Lyase family 6 alginate lyase from human gut Bacteroides* **E. Stender** Technical University of Denmark, Denmark

Thursday, July 19

Roy L. Whistler Room (Rectorate)

Chair: F. Nicotra

08:30	PL15	*Synthetic carbohydrate materials* **P. Seeberger** Max Planck Institute of Colloids and Interfaces, Germany
09:15	PL16	*Chemical Biology Tools to Perceive and Perturb Glycans and Carbohydrate Processing Enzymes in Living Systems* **D. Vocadlo** Department of Chemistry and Molecular Biology & Biochemistry, Simon Fraser University, Canada
10:00	IL4	*Synthesis of bioactive glycopeptides and neoglycopeptides using solid phase peptide synthesis, native chemical ligation, click chemistry and enzymatic glycosylation* **M. Brimble** Department of Chemistry, University of Auckland, New Zealand
10:30	Coffee Break	

Chair: N. Nifantiev

11:00	PL17	*Deciphering the Glycoproteome: a step towards understanding the complexity of biological systems* **P. Rudd** National Institute for Bioprocessing, Research and Training and University College, Dublin, Ireland
11:45	PL18	*Bacterial higher-carbon sugars-key compounds in immune recognition* **P. Kosma** Universität für Bodenkultur Wien, Division of Organic Chemistry, Austria
12:30	PL19	*On Phosphates and Sugars* **G. Marel** Leiden Institute of Chemistry, Leiden University, The Netherlands
13:15	Lunch	
14:30	**POSTER SESSION 3**	

VACCINES

Chair: P. Seeberger

15:30	IL50	*Enhancing Antigenicity of Synthetic Saccharide-Based Antigens by Targeting Fcgamma Receptors* **S. Sucheck** Department of Chemistry and Biochemistry, University of Toledo, USA
16:00	YIL10	*Glycans in diagnostics and vaccines* **G. Bernardes** IMM, Faculdade de Medicina, Univ. de Lisboa, Portugal & Dep. of Chemistry-Univ. of Cambridge, UK
16:30	IL51	*Synthetic carbohydrate-based conjugates as promising vaccine candidates against Shigella: from concept to first-in-human study* **L. Mulard** Department of Chemistry of Biomolecules, Institut Pasteur, France

CLOSING LECTURE

Chair: Z. Guo

17:30 PL20 *Total Synthesis of Pregnane Glycosides to Decipher Their Biological Activities*
B. Yu
Shanghai Institute of Organic Chemistry, China

18:15 **Closure** | Z. Witczak, S. Williams, J. Pereira da Cruz, J. Jiménez-Barbero, Z. Guo, M. Brimble, H. Burrows, A. P. Rauter

Presentation of future Carbohydrate Meetings
OC/FL/Poster Prizes
Lyric Recital
Soprano – Alexandra Bernardo
Piano – Duarte Pereira Martins

20:30 **Gala Dinner** | Casino do Estoril

ICS IBERIAN DAY

INVITED LECTURES

sp^2 IMINOSUGARS: OPPORTUNITIES FOR NEW GLYCOTHERAPIES

Carmen Ortiz Mellet

Department of Organic Chemistry - University of Sevilla, C/ Profesor García González 1, 41012, Sevilla, Spain, mellet@us.es

The unmet potential of carbohydrates to engender molecular diversity and control the function of other biomolecules through conjugation or molecular recognition makes sugars essential players in cell life and clear-cut sensors of cell state alterations. A straight consequence is that any deregulation in carbohydrate metabolism generally translates into severe disease conditions, ranging from metabolic disorders such as diabetes or lysosomal storage disorders to neurodegeneration or cancer development. Sugar mimetics capable to interfere with and modulate enzymatic routes and binding events involving oligosaccharides and glycoconjugates bear thus a high therapeutic promise. Since their discovery in the 1960's, sugar-like polyhydroxylated alkaloids of the iminosugar family have largely dominated research in this field, inspiring much synthetic work and becoming essential utensils in glycobiology. Disappointingly, the efforts to apply the fundamental knowledge accumulated over the years on the biological activities of iminosugars to drug design and therapies have met little success after several clinical trials failures. The inability of iminosugars to properly mimic the acetal-type bond characteristic of glycosides, therefore missing essential structural information for enzyme or receptor recognition, and their strong hydrophilic nature often result in poor selectivity and pharmacokinetics, which seriously handicap their translation into the clinics. We found that replacing the amine-type endocyclic nitrogen of iminosugars into a pseudoamide-type nitrogen (sp^2-iminosugars) significantly enhances the stability and drugability of the prototypes, further enabling tuning of their affinity towards complementary receptor/enzyme partners. Interestingly, sp^2-iminosugars can be subjected to the typical transformations of monosaccharides, including glycosylation reactions; in other words, they are true carbohydrate chemical mimics from which glycoside functional mimetics with unprecedented selectivity profiles can be accessed. The concept has been applied to the development of drug candidates against several lysosomal storage disorders with strong neurological implications such as Gaucher, GM_1-gangliosidosis or Fabry diseases [1,2]. Further developments include anti-inflammatory [3], anticancer [4] and antileishmanial derivatives [5]. The biochemical mechanisms underlining their therapeutic potential will be discussed.

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GLYCOIMMUNOLOGY: CRACKING SUGARS TO DEVELOP NOVEL IMMUNOTHERAPIES

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[c] REQUIMTE, CQFB, Faculty of Science and Technology/UNL, Caparica, Portugal
[d] Glycosciences Laboratory, Department of Medicine, Imperial College London, Northwick Park and St. Marks Campus, Watford Road, Harrow, Middlesex HA1 3UJ, UK
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Immune response allied to the recognition of cancer-specific antigens has motivated the development of targeted immunotherapies. Our group has been particularly interested in understanding how glycans expressed at cell surface can interfere with immunity. One of our interests has been the sialyl-Tn (STn), which is expressed in several types of cancer and absent in normal healthy tissues [1]. STn antigens are associated with tumor invasiveness and metastasis and we showed that STn+ cancer cells are prone to cause immune tolerance, by downregulating the activity of innate and adaptive immune cells [2]. Therefore, either STn and sLeX are tumor specific antigens and potential targets for immunotherapy. Our group has recently developed a novel anti-STn monoclonal antibody (mAb), the L2A5, using hybridoma technology with high specificity against sialylated structures (STn included) and reactivity against STn-expressing cells lines. Results obtained from ELISA, WB and glycan arrays demonstrated that L2A5 mAbs were reactive and susceptible to sialidase treatment when using molecules and samples enriched in STn. Flow cytometry assays also showed that these antibodies were capable of binding glycosylated molecules in cancer cell lines overexpressing STn. Compared with existing antibodies, the L2A5 mAbs react with higher intensity sialylated antigens present in bladder or colorectal tumour tissues but absent in healthy tissues. L2A5 can be used for diagnostic purposes and has potential therapeutic applications. The other antigen of our interest is sialyl-LewisX (SLe^X), which is overexpressed in a cancer tissue and a prototype ligand of selectins. We observed that primary breast cancer cells express sLe^X and showed E-selectin reactivity and can adhere to the endothelial E-selectin under blood flow conditions. Abrogation of fucosylation dramatically reduces sLe^X and E-selectin reactivity and cell proliferation. MS and bioinformatics analysis of the targeted glycoproteins, narrowed down to two most clinically relevant species in breast cancer identified as key E-selectin ligands. The co-expression of sLe^X-carrier proteins was confirmed in clinical breast cancer tissue samples. These results suggest a key role of sLe^X -and respective carriers in metastasis development and draws our attention to this glycoprotein as an anti-cancer target.
In this talk other glycans which have been of interest for the group due to their immunoregulatory role and potential targets for immunotherapy will also be presented.

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HALOGENS AND CHALCOGENS IN CHEMICAL GLYCOBIOLOGY

Omar Boutureira

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Chemical Glycobiology has experienced an impressive growth in the last decade as a result of the discovery of the role of carbohydrates in relevant recognition processes, yet the use of seleno and fluoro glycoconjugates to study such events is still in its infancy. Selective incorporation of Se/F into biomolecules allows simultaneous modulation of their electronic, lipophilic and steric parameters, all of which can influence their biological function. Moreover, these elements have been widely employed as structural, functional and mechanistic probes for the study of biological processes by several cutting-edge non-invasive molecular imaging techniques.

In this abstract we present a survey of synthetic methods developed to access novel fluoro/trifluoromethyl and seleno carbohydrate probes and their application as reagents in chemical site-selective protein modification protocols [1,2], selective carbohydrate reporters for protein-binding events [3], and modulators of oxidative/nitrosative stress-mediated vascular damage.

Acknowledgements

Financial support from the Spanish Government-MINECO, the national agency of investigation-AEI (RYC-2015-17705), and the European Regional Development Fund is acknowledged.

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GLYCOSPHINGOLIPID AND ANALOGUES AS IMMUNOSTIMULANTS OF NKT CELLS

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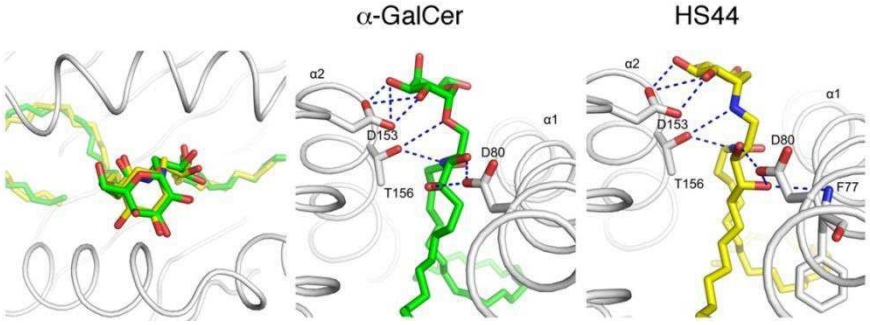
The immune system is organized into two lines of defense involving specific tissues and cell types: the innate immunity acting in a non-specific manner and adaptive immunity, able to release a specific response for a given molecular antigen. Among all this complex system, a hybrid type of cells emerged: the Natural Killer T cells (NKT), which singularly combine molecular features of Natural Killer cells (innate immunity) and T cells (adaptive immunity) incorporating a characteristic T cell receptor (TCR), typically found in T lymphocytes. NKT cells can stimulate immune response by releasing both Th1 pro-inflammatory cytokines and Th2 inflammatory cytokines. This distinctive immunoregulatory profile has pointed NKT cells as target of interest in different disease immunotherapeutics, from type 1 diabetes to cancer. Singularly, NKT cell TCRs recognize in restricted manner glycolipid antigens presented by the MHC class I-like molecule CD1d. Although the putative endogenous glycolipid ligand has been elusive, α-galactosylceramide (αGalCer) a synthetic analogue of a glycolipid isolated from a marine sponge was identified as a powerful NKT cell activator. αGalCer contains a galactose ring alpha-linked to a phytoceramide skeleton with a C26 *N*-acyl chain and is of pivotal importance in NKT biology. However, αGalCer presents serious drawbacks: induces NKT cell overstimulation leading to an unresponsiveness state, offers a poor selectivity (both Th1 and Th2 cytokines are simultaneously released after αGalCer NKT cell stimulation) and clinical trials have been deceptive. Therefore, improved NKT ligands are needed and the search of alternative synthetic analogues is an interesting and challenging goal. We have designed non-glycosidic analogues of immunomodulatory lipids by attaching a series of aminocyclitols to phytoceramide lipid backbone [1,2]. These compounds display potent in vitro and in vivo activity on invariant natural killer T (iNKT). The biological tests of the compounds resulted in interesting activities [3,4] and structural studies show a similar binding mode of the aminocyclitol lipids to the receptor proteins.



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LANTHANIDE-CHELATING CARBOHYDRATE CONJUGATES TO DETECT CARBOHYDRATE-PROTEIN INTERACTIONS

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N-glycans are ubiquitous in nature and functionalize glycoproteins. Protein glycosylation is required for proper biological and biophysical function and often, alterations in glycosylation are related to diseases. [1]

Complex glycosylation patterns containing multiantennary N-glycans are typically found in mature glycoproteins. However, the structural characterization of these glycans is rather challenging. Usually, NMR and X-ray diffraction techniques fail to provide specific answers on the structure and molecular recognition features due to the intrinsic attributes of the glycan. The properties of the glycosidic bond and especially the presence of 1-6 linkages endow a large flexibility to the molecule. This feature often precludes the crystallization of complex carbohydrates and/or hampers the detection of enough electron density for most of the glycan part in the X-ray diffraction analysis of glycoproteins. Thus, any advance in this area is of fundamental value.

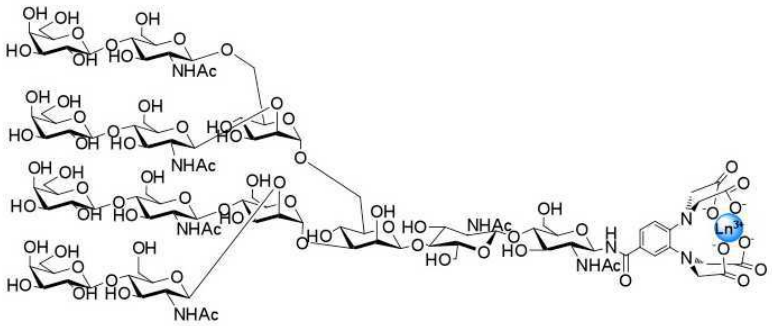

Fig. 1 - Tetraantennary N-glycan characterized in this work.

As a promising approach, carbohydrates conjugated to lanthanide-binding tags have recently revealed high potential toward this aim [2,3]. In its vicinity, a complexed paramagnetic ion induces significant chemical shift changes of the NMR signals of the glycan due to dipolar interactions involving the unpaired electron of the metal.
These pseudocontact shifts (PCS) depend on the distance between each proton and the metal (PCS are proportional to 1/r3) [4]. This methodology has first been applied to the study of small oligosaccharides (di-, tri- and tetrasaccharides) [5-9], then to N-glycans [2,3]. Proceeding from this experimental basis, we herein extend this concept to the level of high-degree branching and long chain N-glycans. In addition, the molecular recognition properties of these complex glycans were characterized in detailed thanks to the unprecedented resolution obtained in the spectra [10].

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DEVELOPMENT OF GLYCOMIMETICS FOR DENDRITIC CELL TARGETING

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Dendritic cells (DCs) are considered to be the most efficient antigen presenting cells and targeting DC surface receptors has become an attractive approach for improving antigen uptake in vaccinations or for modulating immune responses in the treatment of autoimmune diseases or allergies. Here we show our results on C-type lectin screening using glycan arrays and on targeting of dendritic cells via C-type lectins receptors, a group of cell surface carbohydrate binding proteins, with neoglycoproteins functionalized with full length synthetic N-glycans [1,2,3]. In addition, an on-chip synthesis strategy for the preparation of a collection of over 200 N-glycan mimetics on microarray slides is presented that provided a number of strong leads for the targeting of C-type lectins with increased selectivity.

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CARBOHYDRATE SYNTHESIS FOR SOLVING BIOLOGICAL PROBLEMS

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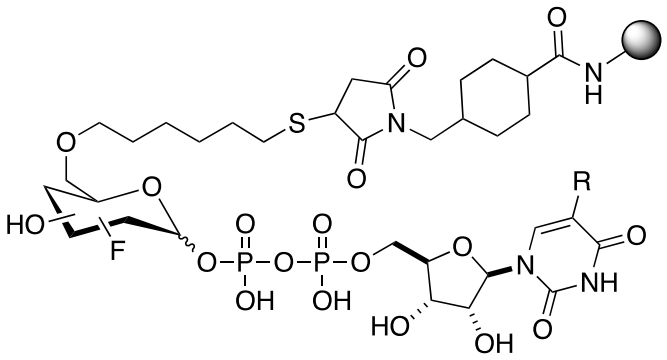
Trypanosoma brucei (*T. brucei)* is a protozoan parasite carried by the tsetse fly that causes African sleeping sickness, a serious neglected disease with inadequate methods of treatment. *T. brucei* requires several UDP-Gal-dependent glycosyltransferases (GTs) to synthesise its range of glycoproteins, several of them remain unidentified, being potential new and specific drug targets [1,2].

We have recently described the synthesis of immobilised UDP-galactose and its C-4 fluorinated galactose derivative [3], as affinity-based probes to identify new GTs.

The synthesis of several new analogues of the natural substrate, UDP-Gal, modified on the sugar, on the amine base or the nature of the glycosidic bond, and attached to a solid support, will be presented (Fig. 1).



R = H, aromatic, heteroaromatic ring

Fig. 1.

Acknowledgments

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C-GLYCOSYLATION AS A KEY TRANSFORMATION TOWARDS AMYLOID AND INFECTIOUS DISEASE DRUG LEADS

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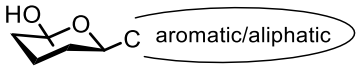
Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal; mblazquez@fc.ul.pt

C-glycosides are resistant to enzymatic and acidic hydrolysis, when compared to their *O*-glycoside counterparts, and have become attractive molecular entities as potential therapeutic agents [1,2].

A diversity of *C*-glycosyl molecular entities will be synthesized from scaffolds given in Fig. 1. Compounds type A embody glycones differing in structure, in particular in their deoxygenation pattern and configuration. Aromatic and aliphatic aglycones linked by a C-C bond will be investigated, with the purpose of evaluating their biological activity. Polyphenols have been chosen as aromatic moiety since they have the ability to prevent toxic amyloid aggregates formation [3] and due to the biological properties of their C-glycosyl derivatives remains unexplored. In this work, we will disclose synthetic approaches for the total synthesis of such compounds.

Our previous findings have demonstrated that alkyl deoxy glycosides are potent antimicrobials [4-6] and some of these molecular entities have shown (Rauter et al. unpublished results) anti-amyloidogenic properties. Aiming at the access to a library of such alkyl deoxy *C*-glycosides, we present now the strategy followed for the synthesis of these molecular entities, covering a new, simple and efficient approach to build the alkyl chain starting from a C-allyl glycoside precursor.



Structure A

Fig. 1. General structure of the *C*-glycosyl molecular entities

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ECO-FRIENDLY SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF CARBOHYDRATE-DERIVED HETEROCYCLES

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Our research group has explored the use of eco-friendly methodologies for the synthesis of heterocycles derived from carbohydrates, which have subsequently been evaluated for their potential biological activity.

On the one hand, "on water" conditions [1] have been used to study the reactivity of a couple of 2-nitro-D-glycals. It is interesting to know how these conditions affect the reactivity of this class of compounds, since these substances do not exhibit the typical reactivity of electron-deficient nitroolefins. In the same way, a study about the influence of the stirring method on “on water” reactivity has also been carried out. In the specific case of the reactions between furans and these 2-nitro-D-glycals, it has been determined that the stirring allows to direct the transformation towards the formation of some products or another (Michael acyclic adducts/C3 substituted derivatives, Fig. 1a). Finally, a reaction mechanism based on experimental facts has been proposed to explain these results [2].

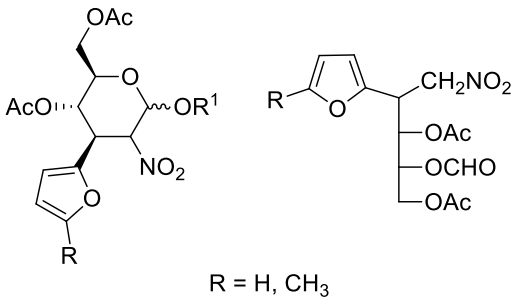

Fig. 1a

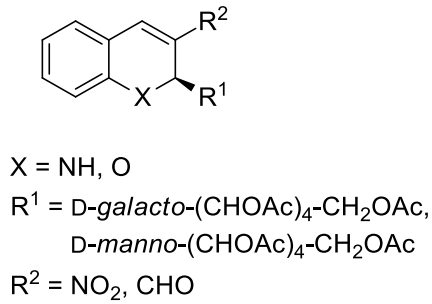

Fig. 1b

By other side, 3-nitro and 3-formyl-1,2-dihydroquinolines and 3-nitro and 3-formylquinolines, as well as 3-nitro-2*H*-chromenes with a carbohydrate fragment bound to C2 (Fig. 1b) have been synthesized by reactions carried out in a minimal amount of solvent and with neutral alumina as catalyst. These processes are not only of interest because of the asymmetry induced by the chain itself, but because these types of compounds exhibit antiproliferative activity. Thus, it has been evaluated against a panel of six tumor cell lines, showing GI_{50} values moderate and, in some specific cases, better than those of the pharmacological standards used as reference (unpublished results).

Acknowledgements

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FUNCTIONAL POLYSACCHARIDE-BASED MATERIALS FOR FOOD AND BIOMEDICAL APPLICATIONS

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Polysaccharides are widely distributed in nature and are easily available, which render them interest due to their functional and sustainable characteristics for food and biomedical applications.

The polysaccharides have been exploited to develop edible and biodegradable films to extend shelf-life and improve quality of food while reducing packaging waste. For example, chitosan has the advantage to be antioxidant and antimicrobial agent. However, to compete with synthetic polymers it needs to meet the requirements of cost-effective materials ensuring the mechanical and gas barrier characteristics for food packaging, which can be achieved by chemical modification. The covalent bonding of a crosslinker, as genipin, enhances the resistance to the typical acidity of food while assigning mechanical properties [2]. Moreover, the grafting to chitosan of some functional molecules can still improve its antioxidant and hydrophobicity character [3]. Also, the addition of nanofillers improves the mechanical and barrier properties and can attribute electrical conductivity and magnetic properties to chitosan derived materials for active and intelligent packaging. Electrical conductivity is a required property for the processing of food at low temperature using electric fields or for sensors application that detect contaminants in foods or monitor changes in packaging conditions or integrity[4].

The majority of polysaccharides from marine organisms are still unexplored, although their great interest for biomedical fields, such as drug delivery and tissue engineering, due to their biocompatibility, biodegradability, and ability to form hydrogels/films. Owing to their structural complexity, there is a strong need for basic research able to identify the structural characteristics that can be responsible to the reported activities, allowing the exploitation of brand new functional materials composed/derived from polysaccharides [1]. Polysaccharides from marine organisms, namely algae and microalgae, may be remarkable for the biomedical field due to the presence of sulfate groups, which do not occur in polysaccharides from other origins. Sulfation seems to be essential to confer antioxidant, immunostimulatory, anti-inflammatory, and anti-viral properties to the polysaccharides. These polysaccharides can be used for coating magnetite particles, allowing to have a biocomposite for hyperthermia treatment in cancer. In addition, this material has the potential to be used for a controlled release of molecules with diagnostic capability, a theranostics approach.

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FROM CHITOSAN TO NAG-NAM SURROGATES

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Peptidoglycan (PGN), a major component of the bacterial cell wall, is made of repeating N-acetylglucosamine (NAG) – N-acetylmuramic (NAM) disaccharide units (red), linked via [NAG-(β-1,4)-NAM] linkage, with stem peptides (black) attached to the D-lactyl (Lac) moiety of each NAM (Figure 1) [1]. The synthesis and composition of the PGN is associated with expression of bacterial resistance to different antibiotics and with a variety of host/bacteria interactions.

The determination of the role of PGN in host disease has been hampered by the lack of pure and homogeneous polymerized PGN [2]. A major limitation encountered in these studies is the limited availability of pure PGN fragments obtained through purification procedures from natural sources. To circumvent this bottleneck, we and others have been developing strategies for the synthesis of muropeptides from glucosamine residues [3,4].

Our group has been focusing the synthesis of NAG-NAM oligosaccharides using raw chitosan, the soluble commercially available derivative of chitin [5,6]. Herein, innovative approaches for the synthesis of oligosaccharides related to peptidoglycan will be presented. We have developed two different approaches: one relying on the use of high molecular weight chitosan and other on the use of peracetylated chitobiose. The oligosaccharides prepared by these routes are biologically relevant to the recognition of receptors and enzymes of both bacterial cell wall and innate immune system. In particular, the strategy combining chemical and enzymatic approaches have become an extremely attractive option, relatively to the traditional orthogonal synthesis. This route provides a novel and simple route for an easy access to bacterial cell wall fragments – biologically important targets.

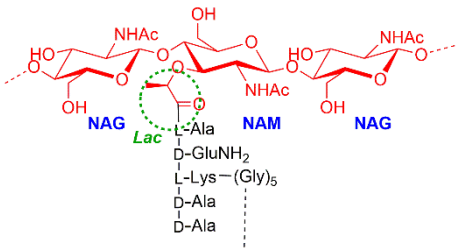

Fig. 1- Structure of the *S.aureus* PGN (a Lys-type PGN).

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THE INTERDOMAIN FLEXIBLE LINKER OF THE POLYPEPTIDE GalNAc TRANSFERASES DICTATES THEIR LONG-RANGE GLYCOSYLATION PREFERENCES

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The polypeptide GalNAc-transferases (GalNAc-Ts), that initiate mucin-type O-glycosylation, consist of a catalytic and a lectin domain connected by a flexible linker. In addition to recognizing polypeptide sequence, the GalNAc-Ts exhibit unique longrange N- and/or C-terminal prior glycosylation (GalNAc-O-Ser/Thr) preferences modulated by the lectin domain. Here we report studies on GalNAc-T4 that reveal the origins of its unique N-terminal long-range glycopeptide specificity, which is the opposite of GalNAc-T2. The GalNAc-T4 structure bound to a monoglycopeptide shows that the GalNAc-binding site of its lectin domain is rotated relative to the homologous GalNAc-T2 structure, explaining their different long-range preferences. Kinetics and molecular dynamics simulations on several GalNAc-T2 flexible linker constructs show altered remote prior glycosylation preferences, confirming that the flexible linker dictates the rotation of the lectin domain, thus modulating the GalNAc-Ts' long-range preferences. This work for the first time provides the structural basis for the different remote prior glycosylation preferences of the GalNAc-Ts.

THE STORY BEHIND THE DISCOVERY OF NATURE-INSPIRED SUGAR CONJUGATES WITH THERAPEUTIC POTENTIAL AGAINST DIABETES AND ALZHEIMER'S DISEASE

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Presently, 415 million adults are estimated to live with diabetes mellitus, 91% of whom are type 2 diabetic patients. Arising as a consequence of obesity, unhealthy diets and lack of physical activity, type 2 diabetes (T2D) is the clinical diagnosis for insulin resistance and associated glucose intolerance, being commonly referred to as the non-insulin-dependent type of diabetes. These patients are also up to 73% more likely to be diagnosed with dementia, including Alzheimer's disease (AD) – characterized, among other events, by neurovascular dysfunction and brain accumulation of amyloid β oligomers (Aβo) triggering oxidative stress, neuroinflammation and neuronal death [1].

Starting from a multitarget antidiabetic isoflavone isolated from *Genista tenera* [2], we have developed a small library of CNS-targeted flavone analogues and their *C*-glucosyl derivatives, which have shown to inhibit acetylcholinesterase (AChE), and to rescue human neuroblastoma (SHSY-5Y) cells from both H_2O_2- and $A\beta_{1-42}$-induced neurotoxicity. Moreover, some of these molecules were also moderate α- and β-glucosidase inhibitors, thus displaying therapeutic potential against T2D and AD, which frequently go hand-in-hand. In this communication, we will present the studies behind the establishment of the flavone prototype for chemical modification, and disclose the bioactivity and pharmacokinetic properties of the generated compound library. In addition, the adopted strategies for structural optimization will be presented and discussed, focusing on the ability of these molecules to directly bind to Aβo and potentially prevent their interaction with Aβo receptors, including the cellular prion protein (PrP^C).

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ORAL COMMUNICATIONS

DISCOVERY OF POTENT GLYCOSIDASE INHIBITORS BY *IN SITU* ANALYSIS OF A LIBRARY OF HYBRID MOLECULES GENERATED *VIA* CLICK CHEMISTRY

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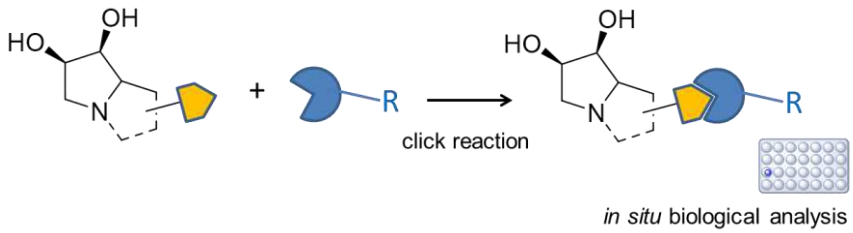
Glycosidases, enzymes that catalyze the hydrolysis of the glycosidic bond of oligosaccharides and glycoconjugates, are involved in a wide range of biological processes from metabolism to cell-cell and cell-virus recognition. The inhibition of these enzymes is therefore an important target in order to find new therapeutic agents against diseases such as diabetes, viral infections, lysosomal storage disorders and tumor metastasis [1]. Iminosugars, carbohydrate mimics with the endocyclic oxygen replaced by a nitrogen atom, constitute the most important family of glycosidase inhibitors and numerous efforts have been devoted to the synthesis of potent and selective inhibitors [2]. The inhibition properties of glycosidase inhibitors may be increased when an aromatic/aliphatic moiety is attached to the iminosugar backbone, as it has been observed in the case of α-fucosidase inhibitors [3a]. Click chemistry followed by *in situ* biological evaluation appears as an interesting approach for the rapid identification of an optimal moiety attached to an iminosugar core. We have recently applied a combinatorial strategy based on the generation of libraries of pyrrolidine-triazole hybrid molecules *via* copper-catalyzed azide-alkyne cycloaddition (CuAAC) that, in combination with their *in situ* biological screening, has allowed us the rapid and efficient identification of potent glycosidase inhibitors [3]. We report the extension of this methodology to the (thio)urea-forming click reaction for the discovery of glycosidase inhibitors based on bicyclic iminosugars. Using this strategy, we have prepared a pyrrolizidine-scaffolded iminosugar showing α-galactosidase inhibition in the nanomolar range.



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SYNTHESIS OF *N*-ACETYLGALACTOSAMINE MIMETICS TACKLING MULTIPLE TARGES: HEMORRAHGIC VIRUS AND ALZHEIMER'S DISEASE

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The human macrophage galactose C-type lectin (MGL) is a key physiological receptor expressed on the surface immature dendritic cells (DCs) and macrophages. MGL recognizes the mucin-like envelope glycoprotein of filovirus like Ebola, Marburg or Influenza and promotes the virus entry and infectivity. MGL binds with high affinity to the terminal *N*-acetylgalactosamine (GalNAc) residues, like the Tn antigen (α-GalNAc-Ser/Thr) of mucin-like glycoproteins (GP) in a Ca^{2+} - dependent manner [1,2]. Therefore, the design of MGL inhibitors could help to block the virus entry and represents a new strategy for developing more effective antiviral drugs.

In addition to potential applications as antivirals, GalNAc and other monosaccharides, such as *N*-acetylglucosamine and mannose *O*-glycosylated, when attached to Ser/Thr side chain of a prion protein (PrP) via an α-glycosidic linkage, have been demonstrated to promote the inhibition of amyloidogenesis in Alzheimer's disease (AD). This study also showed that the same event does not occur with galactose. This suggests that the hydroxy group in the axial position of C2 and the *N*-acetylamino group in the equatorial position of C2 are important in the interaction between sugar and peptide, inhibiting amyloid formation [3]. Those interactions are potentially related to the enhanced electric environment created by either the amide or the hydroxy group on axial position at C2.

In the present work, we have synthetized GalNAc mimetics, including phenyl selenogalactosides bearing amide moieties and related groups at position 2, that could bind the MGL receptor more effectively than GalNAc itself, therefore competing to the interaction between the MGL and the GP of Ebola or Marburg filovirus. The potential of the synthesized molecules to act as protein-protein interaction inhibitors (PPIIs) will be discussed, particularly focusing on their ability to directly bind to $A\beta_{1-42}$ small oligomers, widely accepted as the most neurotoxic type of Aβ aggregates [4]. Ultimately, this project aims to achieve a wider understanding of the applicability of sugar mimetics in the disruption of $A\beta_{1-42}$-PrP aggregation, which has been increasingly described as a central element in the pathophysiology of AD [4].

Acknowledgments

The European Union is gratefully acknowledged for the support of the project entitled "Diagnostic and Drug Discovery Initiative for Alzheimer's Diseases" (D3i4AD), FP7-PEOPLE-2013-IAPP, GA 612347.

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ENZYMATIC TOOLS TO GLYCOENGINEER ANTIBODIES

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Streptococcus pyogenes EndoS is a bacterial endoglycosidase that specifically hydrolyzes the β-1,4-di-*N*-acetylchitobiose core glycan linked to residue Asn297 of human immunoglobulin G (IgG) antibodies [1]. This renders antibodies incapable of eliciting host effector functions through either complement or Fc γ receptors, providing the bacteria with a survival advantage. On account of this antibody-specific modifying activity, EndoS is being developed as a promising injectable therapeutic for autoimmune diseases that rely on autoantibodies. Additionally, EndoS is a key enzyme used in the chemoenzymatic synthesis of homogenously glycosylated antibodies with tailored Fc γ receptor-mediated effector functions [2, 3]. Despite the tremendous utility of this enzyme, the molecular basis of EndoS specificity for, and processing of, IgG antibodies has remained poorly understood.

We showed that EndoS is composed of five distinct protein domains, including glycosidase, leucine-rich repeat, hybrid Ig, carbohydrate binding module, and three-helix bundle domains, arranged in a distinctive V-shaped conformation [4]. Our data suggest that the substrate enters the concave interior of the enzyme structure, is held in place by the carbohydrate binding module, and that concerted conformational changes in both enzyme and substrate are required for subsequent antibody deglycosylation. The EndoS structure presented here provides a framework from which novel endoglycosidases could be engineered for additional clinical and biotechnological applications.

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ADAPTABLE MICROARRAY APPROACH FOR RAPID EVALUATION OF BACTERIAL ANTI-ADHESIVES

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Bacterial adhesion is a key step for initiating infection that frequently involves specific recognition of host glycans by microbial adhesins. This is the case for uropathogenic *Escherichia coli* (UPEC), which exploits FimH, a prevalent mannose-specific adhesin, for infecting the human urothelium. Different monovalent and multivalent compounds have been synthesized and evaluated as microbial anti-adhesives [1]. We here report on the development of a versatile microarray approach for evaluation of bacterial adhesion and efficiency of FimH-targeted compounds. A straightforward set-up was established, allowing to directly monitor the binding of fluorescently labeled *E. coli* UTI89 (UPEC clinical isolate) to custom-designed mannan microarrays. Three novel fullerene hexakis-adducts bearing 12 peripheral mannose residues grafted onto the C_{60} core by different spacers were tested in competition assays as representative compounds. The IC_{50} values obtained for the fullerenes evidenced a clear multivalency effect, with up to 18-fold enhancement of the relative inhibitory potential per mannose residue compared to methyl α-D-mannopyranoside, in line with previous studies [2,3]. Differences among fullerenes were detected, pointing to a noticeable impact on compound activity of the precise structure and length of the spacer used for linking mannose to the fullerene core. Comparison of array-derived results obtained for the archetypal mannose-binding lectin concanavalin A with data obtained by isothermal titration calorimetry analyses of ConA-mannofullerene interactions fully validated the utility of the arrays for evaluating the inhibitory potential of anti-adhesive compounds.

Acknowledgements

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CHONDROITIN SULFATE DERIVATIVES AND DENDRIMERS AND THEIR BINDING TO MIDKINE AND LANGERINE BY NMR

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The biological activity of Midkine (MK), a cytokine involved in neurogenesis and tumorigenesis, and Langerine (Lg) a C-lectin related with the early steps response of immune system are both regulated by its binding to glycosaminoglycans (GAG) as heparin or chondroitin sulfate (CS**)** [1,2].

We have studied the interaction between MK and several synthetic tetrasaccharides with diverse sequences mimicking CS, sulfation pattern and monosaccharide composition. We have found that the introduction of aromatics substituents in the GAG increases the affinity for MK [3].

We have also studied the influence of the attachments disaccharides to dendrimers (Figure 1) with diverse number of copies and geometries in the enhancement of the binding affinities in their interaction with Lg.[1] We have found that the characteristics of the core are also playing some role.

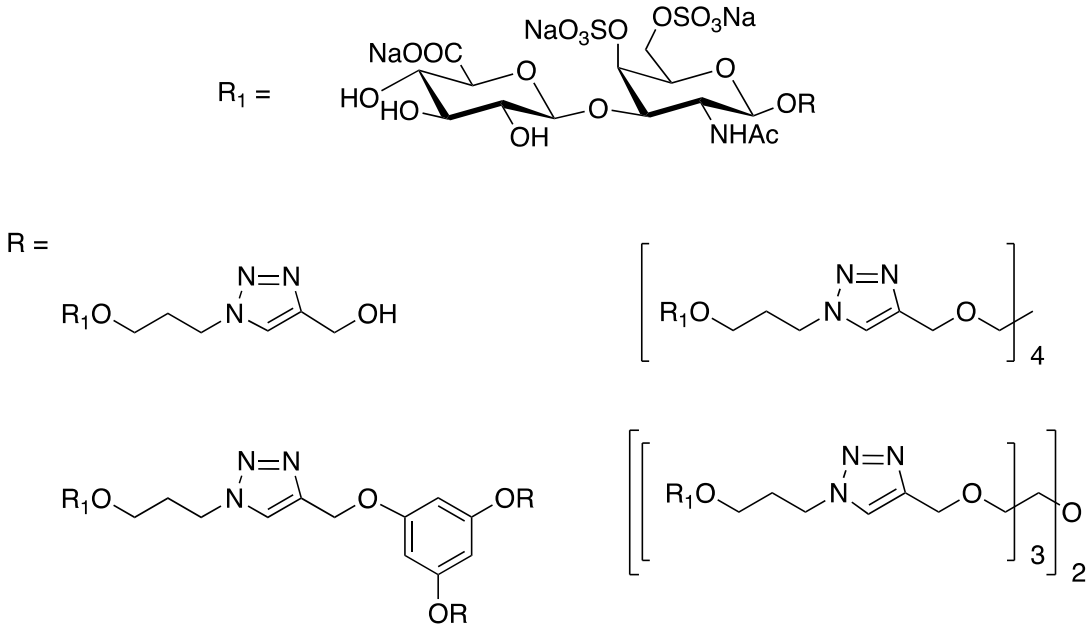

Fig. 1.

Acknowledgments

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EXPLORING SIALYL-TN EXPRESSION IN MICROFLUIDIC-ISOLATED CIRCULATING TUMOUR CELLS: A NOVEL BIOMARKER FOR PRECISION ONCOLOGY APPLICATIONS

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Circulating tumour cells (CTC) originating from the primary tumour, lymph nodes and distant metastasis hold tremendous potential for liquid biopsies by providing a molecular fingerprint for disease dissemination and its temporal evolution through the course of disease management [1,2]. CTC enumeration, classically defined based on surface expression of epithelial cell adhesion molecule (EpCAM) and absence of pan-leukocyte marker CD45, has shown to correlate with clinical outcome[1]. However, existing approaches introduce bias in the subsets of captured CTC, which may be excluding biologically and clinically relevant subpopulations [1]. Here we explore the overexpression of the membrane protein O-glycan sialyl-Tn (STn) antigen in advanced bladder and colorectal tumours, but not in blood cells, to propose a novel CTC isolation technology. Using a size-based microfluidic device, we show that the majority of CTC (>90%) isolated from the blood of metastasized bladder and colorectal cancers express the STn antigen, supporting its pancarcinomic link with metastization. More importantly, STn+CTC counts were significantly higher compared to EpCAM-based detection in colorectal cancer, providing a more efficient cell-surface biomarker for CTC isolation. Exploring this concept, we have built a glycan affinity-based microfluidic device for selective isolation of STn+CTC and propose an enzymatic-based strategy for the recovery of viable cancer cells for downstream biomedical research. Finally, clinically relevant cancer biomarkers (transcripts and mutations) in bladder and colorectal tumours, were identified in microfluidic-isolated cells, confirming their malign origin and highlighting the potential of this technology in the context of precision oncology.

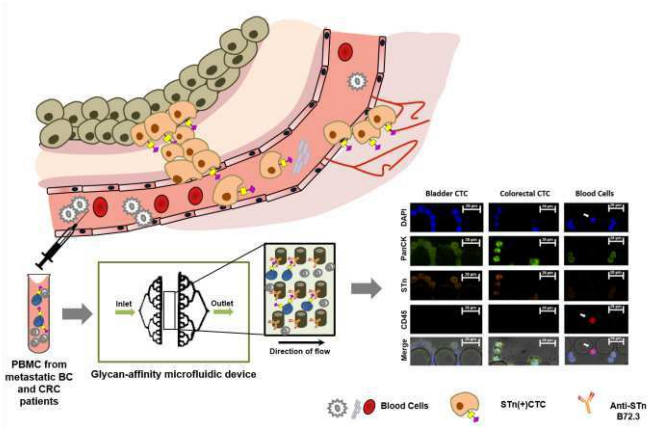

Fig. 1 - Schematic representation of the experimental procedure for glycan-affinity microfluidic device and STn expression in bladder and colorectal CTC.

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FLASH COMMUNICATIONS

HYDROXYPROPYL-β-CYCLODEXTRIN AND β-CYCLODEXTRIN AS TABLET DIRECT COMPRESSION FILLERS

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Background: Cyclodextrins (CDs) are cyclic carbohydrates with hydrophilic outer surface and hydrophobic central cavity [1]. These compounds are used excipients to formulate solid, semi-solid and liquid dosage forms [1, 2]. In tablet formulations, CDs can be used with different pharmaceutical applications such as [1]: i) to enhance drug dissolution and bioavailability; ii) to modify/control the release of drugs; iii) to increase drug stability; iv) to mask the bitter taste of drugs; v) to minimize adverse drug reactions; vi) to act as a tablet excipient; and vii) to act as an osmotic pump agent.
Objectives: The purpose of this publication was to study the ability of hydroxypropyl-β-cyclodextrin (HP-β-CD; Roquette) and β-cyclodextrin (β-CD; Roquette) as tablet direct compression fillers. In this way, CDs several parameters were evaluated: i) the flow properties (angle of repose, flow time, Carr index and Hausner ratio); ii) the compaction behavior (forces, energies, plasticity index and force/time compression profiles); and iii) the influence on carbamazepine release characteristics (dissolution rate and disintegration time) from uncoated tablets. In addition, these properties of the CDs were compared with others direct compression fillers (Tablettose® 100, Pearlitol® 300 DC, Emcompress® Premium and Vivapur® 102) and co-processed excipients (Avicel® HFE-102 and Cellactose® 80).
Methods: In a first phase, samples were analyzed by Scanning Electron Microscopy (SEM), and the Martin diameter and the moisture content were determined. Afterwards, the materials flow properties were evaluated according to the European Pharmacopoeia 9. Tablets, with a target weight of 500 mg and a hardness value of 70 N, were prepared by direct compression using an instrumented alternative tableting machine (Dott.Bonapace, model CPR-6) with 11 mm diameter punches. Eight formulations (batches of 100 g) were studied and the tablets physical characterization was performed. Carbamazepine (100 mg per tablet) and sodium stearyl fumarate (Pruv®; 0.5%, w/w) were used as a model of a poorly soluble drug and as an antiadherent lubricant respectively. *In vitro* drug dissolution studies were carried out during 4 hours and the carbamazepine concentration was determined by spectrophotometry at 288 nm. Additionally, tablet disintegration tests were also conducted.
Results: All materials presented a moisture content less than or equal to 8% and suitable/good flow properties, except carbamazepine which showed poor characteristics (cohesive powder). Tablets with uniform aspect and suitable physical properties were obtained, but it was not possible to prepare tablets from Vivapur® 102 and Avicel® HFE-102. Moreover, tablets presented a friability value greater than 1.0%. SEM images for all materials and force/time compression profiles were recorded. A drug calibration curve was obtained ($y = 45.761x + 0.0049$; $R^2 = 0.9998$) and *in vitro* dissolution tests showed at 240 minutes a mean carbamazepine release from 15±5 to 113±6%. Disintegration times of the tablets were less than or equal to 18 minutes, except Emcompress® which presented a value greater than 4 hours. HP-β-CD presented a faster and greater dissolution rate and a shorter disintegration time (about half) than β-CD.
Conclusions: HP-β-CD and β-CD can be used as tablet direct compression fillers. Considering the flow characteristics, the compaction behavior and the influence on drug release characteristics, HP-β-CD showed better properties than β-CD.

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CO-ADMINISTRATION OF ANTI-MICROBIAL PEPTIDES HELPS IN THE ACTIVITY OF FP7 GLYCOLIPID (TLR4 ANTAGONIST)

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TLR4 is expressed at the surface of innate immune cells (macrophages, dendritic cells) and specifically recognizes bacterial endotoxins, i.e., lipopolysaccharide (LPS), the main molecular component of gram-negative bacteria cell walls [1]. This activation by LPS is the basis of inflammatory and innate immune response to invading pathogens in humans. Modulation of innate immunity receptors by agonists and antagonists with synthetic small molecules able to modulate the biological activity of represent a powerful tool to study the TLR4 receptor system and are of great pharmacological interest as antisepsis and anti-inflammatory agents (antagonists) or as vaccine adjuvants (agonists) [2,3].

This study describes the effect of co-administration of antimicrobial peptides (AMPs) and synthetic glycolipid FP7 (TLR4-antagonist) [4], active in inhibiting inflammatory cytokine production caused by TLR4 activation and signaling [4]. The co-administration of two LPS-neutralizing peptides (a cecropin A-melittin hybrid peptide and a human cathelicidin) enhances by an order of magnitude the potency of FP7 in blocking the TLR4 signal. This work focused on the characterization of the interaction between the synthetic TLR4 antagonist FP7, and two synthetic antimicrobial peptides, CA(1-8)M(1-18) and LL37, by nuclear magnetic resonance (NMR) and Transmission Electron Microscopy (TEM). Our experiments showed that peptide addition changes the aggregation state of FP7, promoting the formation of larger micelles (Fig. 1). It suggests a correlation between the aggregation state of lipid A-like ligands and the type and intensity of the TLR4 response [5].

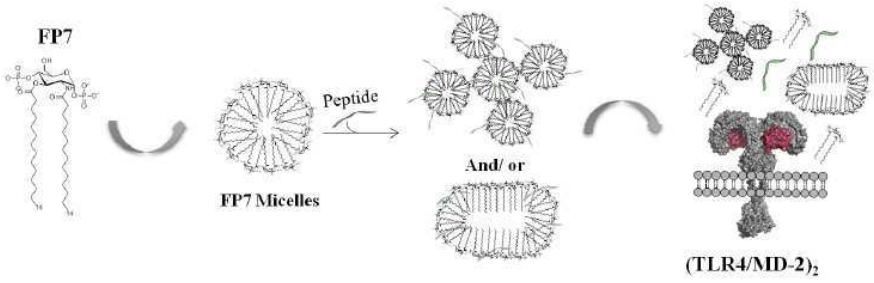

Fig. 1- Squematic representation the interactions of FP7 glycolipid with anti-microbial peptides.

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HYPOXIA ENHANCES THE MALIGNANT NATURE OF BLADDER CANCER CELLS AND CONCOMINANTLY ANTAGONIZES PROTEIN O-GLYCOSYLATION EXTENSION

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Invasive bladder tumours express the cell-surface Sialyl-Tn (STn) antigen, which stems from a premature stop in protein *O*-glycosylation. The STn antigen favours invasion, immune escape, and possibly chemotherapy resistance, making it attractive for target therapeutics [1,2]. However, the events leading to such deregulation in protein glycosylation are mostly unknown. Since hypoxia is a salient feature of advanced stage tumours [3], we searched into how it influences bladder cancer cells glycophenotype, with emphasis on STn expression. Therefore, three bladder cancer cell lines with distinct genetic and molecular backgrounds (T24, 5637 and HT1376) were submitted to hypoxia. To disclose HIF-1α-mediated events, experiments were also conducted in the presence of Deferoxamine Mesilate (Dfx), an inhibitor of HIF-1α proteasomal degradation. In both conditions all cell lines overexpressed HIF-1α and its transcriptionally-regulated protein CA-IX. This was accompanied by increased lactate biosynthesis, denoting a shift toward anaerobic metabolism. Concomitantly, T24 and 5637 cells acquired a more motile phenotype, consistent with their more mesenchymal characteristics. Moreover, hypoxia promoted STn antigen overexpression in all cell lines and enhanced the migration and invasion of those presenting more mesenchymal characteristics, in an HIF-1α-dependent manner. These effects were reversed by reoxygenation, demonstrating that oxygen affects *O*-glycan extension. *O*-glycosylation extension antagonization was further confirmed by a new approach termed Cellular *O*-Glycome Reporter/Amplification (CORA). Glycoproteomics studies highlighted that STn was mainly present in integrins and cadherins, suggesting a possible role for this glycan in adhesion, cell motility and invasion. The association between HIF-1α and STn overexpressions and tumour invasion was further confirmed in bladder cancer patient samples. In conclusion, STn overexpression may, in part, result from a HIF-1α mediated cell-survival strategy to adapt to the hypoxic challenge, favouring cell invasion (Fig. 1). In addition, targeting STn-expressing glycoproteins may offer potential to treat tumour hypoxic niches harbouring more malignant cells.

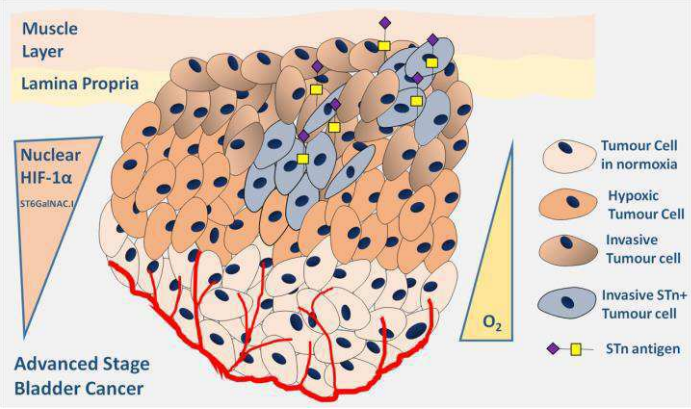

Fig.1 - Schematic representation of advanced stage hypoxic tumours showing increased STn expression.

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POST-SYNTHETIC STRATEGY FOR SUPERFICIAL CARBOHYDRATE-COATING OF MESOPOROUS MIL-100(Fe) NANOMOFs AS BIOCOMPATIBLE NANOCARRIERS

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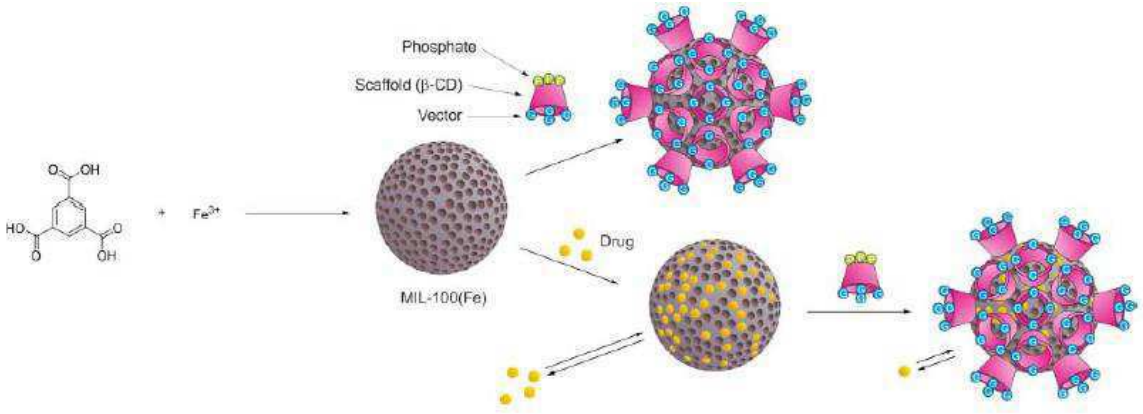
The increase of multidrug resistance (MDR) in bacteria makes urgent an improvement of the antibiotics bioavailability and biodistribution. The remarkable capability of bacteria to adapt and survive, and the poor intracellular transport of these substances, reduce the efficiency of many antibiotics treatments.
To overcome this problem a promising solution appears to be the development of nanodevices able to deliver the drugs directly into infected cells. Nanosized metal-organic frameworks (nMOFs) are an emerging new class of porous structures that can be used as efficient carriers. MIL-100(Fe) (MIL standing for Material from Institute Lavoiser) nMOFs are built up from iron(III) octahedral trimers and trimesate linkers (1,3,5-benzene tricarboxylate). Its interconnected porous structure reaches unprecedented loadings (within the 20-70 wt% range) [1]. The use of nMOFs in targeted drug delivery (TDD) requires their superficial functionalization with a delivery vector that interacts with specific biological receptors located at the cells surface. For instance, mannose-receptors, such as lectins DCSIGN and FimH, are part of the complex signaling system used by different bacteria to infect cells. In this communication we present a post-synthetic strategy for superficial coating of MIL-100(Fe) directly in water following a "green" method, taking advantage of the ability of phosphate groups to coordinatively bind the Fe(III) sites on the nMOFs surface [2,3]. We report the synthesis of a series of phosphorylated carbohydrate scaffolds such as β-cyclodextrin (β-CD) or dextran (DXT) derivatives containing simultaneously phosphate groups as anchoring moieties, and motifs of mannose, tetra(ethylene glycol) (TEG), or large chains of poly(ethylene glycol) (PEG). Selected scaffolds are biocompatible, non-toxic, stable in biological conditions, very easy to modify through their large number of hydroxyl groups, and can improve bioavailability and solubility of the drug delivery device. Mannose residues are intended to act as biological vectors, while TEG and PEG chains are expected to stabilize and increase the biocompatibility of the systems. The phosphated conjugates were tested as self-assembled coating building blocks for previously drug-loaded MIL-100(Fe) nMOFs. The shell formation and stability are studied by isothermal titration calorimetry (ITC), and the effect of the shell on the drug release kinetics was also estimated.



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BACTERIAL CELLULOSE SURFACE MODIFICATION FOR MICROFLUIDIC APPLICATIONS

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Research focused on the design of skin-on-chip systems has been growing, driven by the particular demands of skin research and models. These systems aim to enable in vitro investigation of skin functions and interactions, to study normal physiology, model pathological environments and explore drug discovery and substances testing applications, in a simple, ready-to-use, yet realistic platform. Several materials have been studied and proposed for the design of skin-on-chip systems. However, polydimethylsiloxane (PDMS) is still one of the most used materials. As the technology evolves, diverse technical challenges arise and PDMS reveals its disadvantages, such as complex fabrication processes, hindering scaling up and mass production [1].

Modified bacterial cellulose films are herein presented as substrates for the design of microfluidic platforms. For the successful application of this naturally hydrophilic material, it must be modified to be able to resist wetting by diverse organic liquids. To achieve the desired surface properties, two methods were studied, namely oxygen plasma treatment and organosilane vapour deposition [2]. Developed bacterial cellulose (BC) materials were thoroughly characterized to evaluate the choice of modification methodologies and to assess their feasibility to be used as substrates for lab on chip designs, namely wettability and surface properties through contact angle measurements, SEM, FTIR and XPS and standard biological tests (ISO/EN 10993).

Results demonstrate that the chosen methods have proven effective in obtaining bacterial cellulose membranes with attractive properties for the envisaged applications. The particular combination of both oxygen plasma treatment and silane vapour deposition resulted in a highly hydrophobic BC surface (132,6º±7,2) with promising biological performance.

These results contribute to the establishment of bacterial cellulose for lab-on-chip applications, further highlighting the use of natural materials and techniques to produce low-cost chips able to support the growth of a skin equivalent, which will serve as a model for fundamental and applied studies.

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TOLL-LIKE RECEPTOR 4. COMPUTATIONAL CHEMISTRY STUDIES ON ITS MODULATION

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Toll-like receptors (TLRs) are pattern recognition receptors involved in the innate immunity. In particular, TLR4 binds to lipopolysaccharides (LPS), a membrane constituent of Gram-negative bacteria, and together with MD-2 protein, forms a heterodimeric complex which leads to the activation of the innate immune system response [1]. TLR4 activation has been associated with certain autoimmune diseases, noninfectious inflammatory disorders, and neuropathic pain, suggesting a wide range of possible clinical settings for application of TLR4 antagonists, while, TLR4 agonists would be useful as adjuvants in vaccine development and in cancer immunotherapy [2].

Regarding TLR4 modulation we have undertaken computational studies on the binding mode of different peptide TLR4 modulators by means of docking and MD simulation. Several agonist and antagonist peptides were studied to bind different pockets of the TLR4/MD2 system, thus proposing a mechanism for their biological activity. Also, the binding mode of a natural lipopolysaccharide with some unusual carbohydrates, and some natural phospholipids has been addressed accounting for their biological agonist activity (Fig. 1).

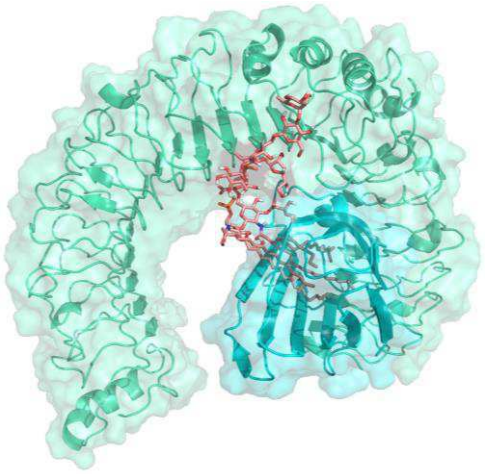

Fig. 1 - LPS in complex with TLR4/MD2 after docking and MD simulation.

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INSIGHTS IN THE RECOGNITION OF *LISTERIA* CELL WALL TEICHOIC ACIDS BY PHAGE ENDOLYSINS USING NMR

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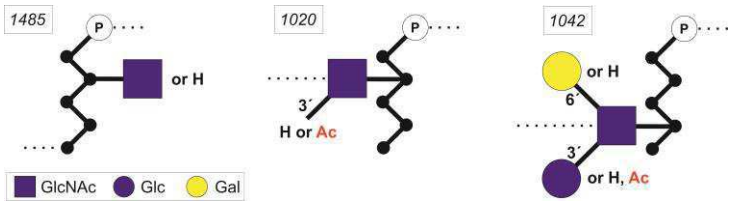
Wall teichoic acids (WTAs) are the most abundant and diverse natural glycopolymers found on the cell wall of Gram-positive bacteria including *Listeria monocytogenes*, whose antigenic pattern has been long correlated with virulence[1]. WTA structure often defines recognition by bacteriophage endolysins, enzymes that are able to provoke bacterial lysis via their catalytic domain. Susceptibility to this action depends on the target selectivity of their cell wall binding domain, frequently a carbohydrate-binding domain (CBD). Despite recent advances in glycan analysis, insights in the structure-function relationship of *L. monocitogenes* WTAs remain challenging to obtain due to their extraordinary heterogeneity in terms of the polymer length, GlcNAc/ribitol connectivity, mutable *O*-acetylation and/or further hexose substitution of the GlcNAc unit[2]. In order to elucidate the structural specificity of CBDs for *Listeria* WTA, the interaction of three *Listeria*-specific CBDs (CBDP35, CBD025, CBD500) with a panel of native and mutant WTAs was pursued by Saturation Transfer Difference (STD) NMR. A complete set of NMR experiments including ^{1}H-^{13}C HSQC, TOCSY, ROESY and DOSY aided to determine the relative composition, degree of *O*-acetylation and Rbo-GlcNAc connectivity of intact glycopolymers belonging to distinct antigenic patterns (1485, 1020 and 1042) and appreciate their disparity in monomeric units. Recognition by CBDs was therefore correlated to these features. It was observed that, in the comb-like 1485 polymer, the *α*-pendant GlcNAc unit lacking *O*-acetylation was strongly engaged by the P35 receptor, whereas the deficiency of Glc substitution at position 3´ for the backbone-integrated *β*-GlcNAc was critical to attain CBD025 binding. The contribution of *O*-acetylation and hexose substitution in CBD500 binding was determined by STD NMR using a panel of 1042 polymer mutants, and further supported by surface plasmon resonance studies. Besides, isothermal titration calorimetry performed on selected WTA-CBD pairs confirmed target selectivity of CBDs and provided thermodynamic views. This is the first report providing insights into the interactions between *Listeria*-specific CBDs and WTA, paving the way for further studies on the structure-function of these bacterial glycopolymers and the application of endolysins as chemotherapeutics.



Acknowledgements

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A FAST NMR-ASSISTED COMBINATORIAL METHOD FOR OPTIMIZING NUCLEIC ACID BINDERS

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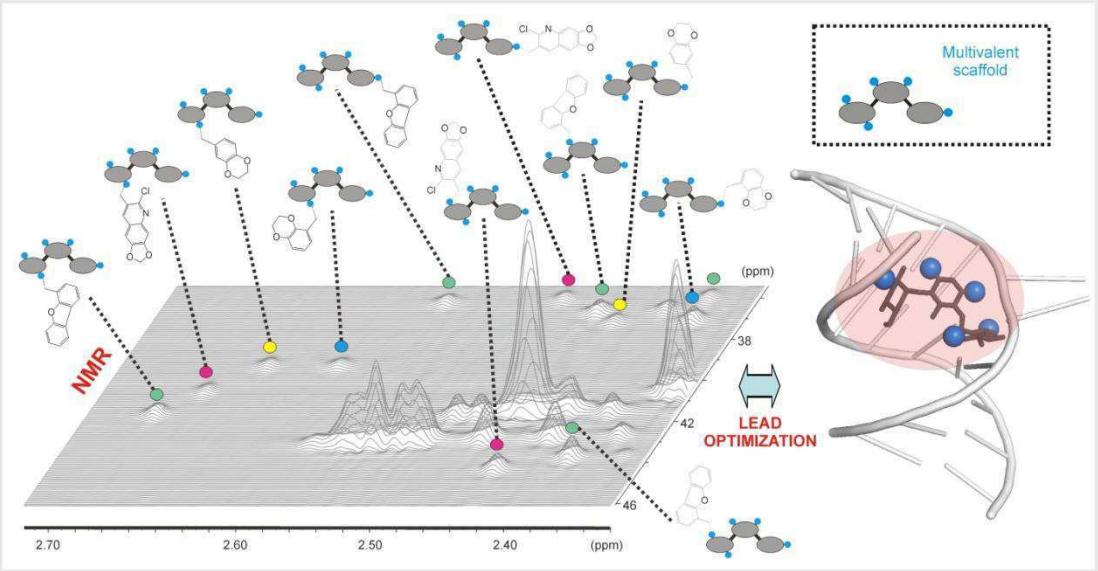
Development of strong and selective binders from promiscuous lead compounds represents one of the most expensive and time-consuming tasks in drug discovery. Herein we present a novel fragment-based combinatorial strategy for the optimization of multivalent polyamine scaffolds as DNA/RNA ligands. Our protocol provides a quick access to a large variety of regioisomer libraries that can be tested for selective recognition by combining microdialysis assays with simple isotope labeling and NMR experiments. To illustrate our approach, 20 small libraries comprising 100 novel kanamycin-B derivatives have been prepared and evaluated for selective binding to the ribosomal decoding A-Site sequence. Contrary to the common view of NMR as a low-throughput technique, we demonstrate that our NMR methodology represents a valuable alternative for the detection and quantification of complex mixtures, even when integrated by highly similar or structurally related derivatives, a common situation in the context of a lead optimization process. Furthermore, this study provides valuable clues about the structural requirements for selective A-site recognition [1].



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OBTAINING THE FIRST THIOGLYCOLIGASE FROM THE GH3 FAMILY

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Thioglycoligases are mutated glycosyl hydrolases in which the residue responsible of the general acid/base catalysis has been replaced by an inert amino acid. In the absence of this catalytic assistance, the mutants require an activated glycosyl donor in order to form the enzyme-substrate intermediate and a strong nucleophile which can work as an acceptor without base catalysis (Fig. 1). This requirement made that the scope of these mutants was considered limited, although so far the research on thioglycoligases has comprised only few enzymes from GH families 1, 2, 13, 31, 35, 51 and 89, all of them displaying maximal activity at pH values close to neutral [1]. In this sense, GH3 glycosidases would be an interesting source to explore the potential of highly acidic thioglycoligases. Moreover, this family included interesting (hemi)cellulolytic activities such as β-glucosidase and β-xylosidase and several examples of transglycosylases.

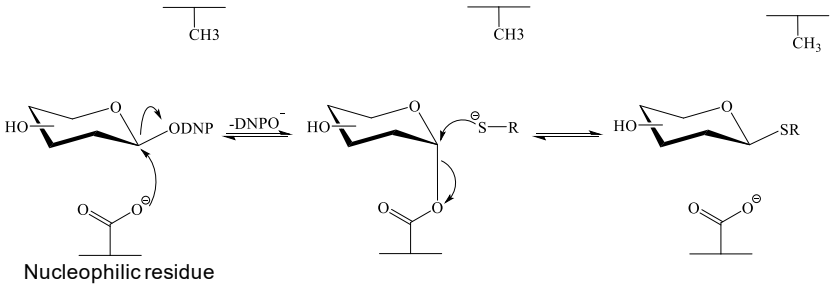

Figure 1. Mechanism of thioglycoligase mutants

BxTW1 is a fungal GH3 β-xylosidase from the fungus *Talaromyces amestolkiae* combining remarkable activity at low pH and a broad range of transxylosylation acceptors [2]. Mutants at the catalytic proton donor residue have been obtained and compared. Among them, replacement by alanine led to the highest production yield of glycosides. The mutant catalyzed the formation of tiophenyl β-D-xylopyranoside transferring a xylose unit from *p*-nitrophenyl β-D-xylopyranoside to thiophenol, a typical acceptor of thioglycoligases. In the same way, assays in the presence of increasing concentrations of sodium azide and formiate displayed the expected profiles of rescued activity.

Further characterization revealed that other nucleophiles can also act as glycosylation acceptors with high yields. This is the first report on a GH3 thioglycoligase and future studies are expected to deepen its properties.

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BACTERIAL CELL WALL SURROGATES FROM CHITOSAN: A NEW RECOGNITION SYSTEM

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Peptidoglycan (PGN) is the major component of the bacterial cell wall, and is composed of alternating β-(1,4) linked N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) residues, cross-linked by short peptide bridges. PGN is recognized by invertebrate and vertebrate innate immune system (IIS) and is capable of inducing an innate immune response [1]. Due to the biological relevance of PGN several research groups have contributed to the development of muropeptide synthesis. Our research group have been dedicated to the preparation of glucosamine building blocks and NAG-NAM disaccharides [2-3]. During our research on PGN recognition by molecular patterns on IIS, we came across with the structural similarity of chitin/chitosan and the carbohydrate skeleton of bacterial PGN, murein [2]. Thus we have embarked on the synthesis of PGN of different molecular weight from chitosan, taking advantage of its β-(1,4) glycosidic linkage, through selective chemical modifications of the naturally abundant biopolymer. Herein we will present our recent developments on the quest for an artificial bacterial PGN, starting with commercial chitosan through chemoselective modifications and enzymatic recognition.

Acknowledgments

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UNRAVELLING NEW BINDING MODES IN MULTIVALENT GLYCOSIDASE INHIBITION WITH PILLAR[5]ARENE GLYCO(MIMETIC)ROTAXANES

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The saccharidic portion in glycocojugates is involved in different binding-recognition processes that trigger or inhibit a wide variety of biological phenomena. Carbohydrate-recognizing (lectins) and carbohydrate-processing (glycosidases, glycosyltransferases) proteins can act as the complementary reader partners. The multivalent presentation of carbohydrates to protein receptors is often essential to reach highly selective carbohydrate-protein interactions. Over the past few years, several glycosidases have been found to be also responsive to multivalent presentations of binding partners (*multivalent inhibition effect*) [1], a phenomenon first discovered for iminosugar-type inhibitory species (*inhitopes*) and recently demonstrated for multivalent carbohydrate constructs (*glycotopes*) [2,3], definitely blurring the boundaries between lectin and glycosidase recognition [3]. Deepening in this notion, our next interest was to investigate the effect of molecular size and flexibility in the inhibitory strength of inhitope/glycotope heteromultivalent prototypes and in the corresponding binding modes. To this end, here we present the synthesis of pillar[5]arene glyco(mimetic)rotaxanes (Fig. 1) and their evaluation against a series of glycosidases purposely chosen to cover different glycone/nonglycone site geometries and mono or multimeric quaternary structures. The combination of microplate-supported competitive crosslinking assays, using reporter lectins, and computational experiments provide new hints on the basis of the multivalent inhibitory effect.

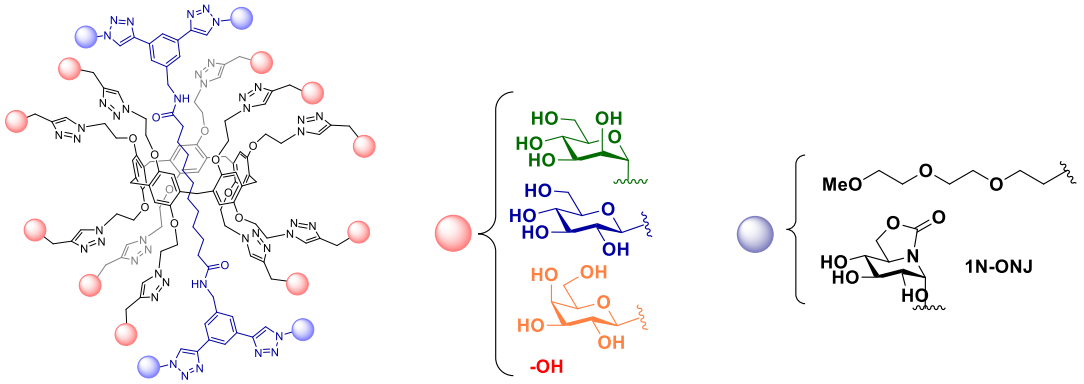

Fig.1- Schematic representation of rotaxane heteroglycoclusters constructed on a pillar[5]arene platform designed to explore dual lectin-glycosidase binding modes.

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CONTRIBUTION OF NON-ENZYMATIC TRANSGLYCOSYLATION REACTIONS TO THE HONEY OLIGOSACCHARIDES ORIGIN AND DIVERSITY

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Honey is essentially a very concentrated aqueous solution of fructose and glucose, composed also by a complex mixture of other carbohydrates [1]. Some of the carbohydrates present in honey are attributed to the α-glucosyl transferase activity of invertase, able to transfer α-glucosyl residues to other carbohydrate moieties. Similarly to the invertase transglucosylation activity that explains the presence of α-glucose linked sugars [2,3], the presence of fructooligosaccharides (FOS) may be attributed to the transfructosylation activity of enzymes from microorganisms found in honey [4]. Nonetheless, there is still no explanation for the origin of the oligosaccharides present in honey holding different combinations of glycosidic linkages and configurations.

The present work hypothesizes that non-enzymatic transglycosylation reactions could occur in honey, promoted by honey maturation conditions, such as high sugar concentrations in acidic media, inducing condensation of carbohydrates [5]. In order to validate this hypothesis, six concentrated model solutions (water content of 20%) containing sucrose + glucose, and sucrose + fructose were prepared using water and diluted citric acid at pH 4.0 and 2.0. The model solutions were kept at 35 ºC, mimicking the average temperature inside the beehive. Electrospray ionization mass spectrometry analysis allowed to monitor the carbohydrates reactions along the time. The occurrence of non-enzymatic oligosaccharide synthesis, with a degree of polymerization up to 6, was observed after 5 months. These compounds were fractionated by ligand-exchange/size-exclusion chromatography and methylation analysis was performed to determine the oligosaccharides glycosidic linkages. It was observed a glycosidic linkage composition similar to that of honey oligosaccharides. Terminally-linked glucose residues together with lower amounts of (1→2)-, (1→3)-, (1→4)- and (1→6)-linked glucose, were found in higher amounts. Concerning fructose, terminally-linked fructose was the most abundant residue, followed by (2→1)- and (2→6)-linked fructose. The identification of the oligosaccharides was further elucidated combining the results obtained by glycosidic linkage analysis and oligosaccharide derivatization as alditol acetates. The FOS inulobiose and blastose were identified in all model solutions, whereas 1-kestose and neokestose were present in all samples except in solutions at pH 2.0. Moreover, maltose, isomaltose, sophorose, gentiobiose, cellobiose, panose, and inulotriose were also identified in some model solutions. Some of these oligosaccharides such as 1-kestose, neokestose, panose[6], inulobiose, inulotriose and blastose [7] are reported to have prebiotic effect. In conclusion, honey oligosaccharides may be resulting from non-enzymatic reactions without the intervention of enzymes, contributing to honey oligosaccharides diversity.

Acknowledgments

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SYNTHESIS OF NEW TYPES OF POTENTIALLY BIOACTIVE FURANOSYL NUCLEOSIDE AND NUCLEOTIDE ANALOGUES

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Nucleoside and nucleotide analogues are important groups of molecules in medicinal chemistry, due to their ability to display a variety of biological effects, such as anticancer [1], antiviral [1] and cholinesterase inhibitory properties [2,3]. Such molecules are capable to mimic their natural counterparts, acting by inhibition of nucleotide-dependent enzymes, interfering with nucleic acid synthesis and cell cycle progress [1].

Hence, we were motivated to explore the synthesis of original nucleoside and nucleotide-like molecules, namely isonucleotides comprising a phosphate group and an N-heteroaromatic moiety linked to a position other than C-1 of the sugar ring, and nucleoside mimetics having a guanidine moiety as a surrogate of a purine nucleobase, via 3,5-bis functionalization of furanose scaffolds. Also, neutral and relatively stable isostere moieties for a phosphate group were included in the structures. The synthetic strategies for the access to the target molecules included azide-alkyne 1,3-dipolar cycloaddition, sugar azidation, guanidinylation, phosphorylation or Arbuzov-type reaction. In this communication, the synthetic work will be presented and discussed. Preliminary results on the biological evaluation of the newly synthesized compounds, namely their antiviral potential, will be also disclosed.

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IN SILICO DESIGN OF HALOGENATED CARBOHYDRATE MIMETICS AS POTENTIAL HALOGEN-BONDING LIGANDS

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The molecular recognition of carbohydrates by proteins is characterized by the presence of classical hydrogen bonds stabilizing binding together with an important contribution from other intermolecular interactions conferring high specificity [1]. The design of glycomimetic ligands has been increasingly exploited as a strategy towards the generation of selective modulators of protein-carbohydrate binding events. Those modulators are valuable tools in the framework of chemical glycobiology [2] or carbohydrate-based drug discovery [3].

While a diversity of functional groups has been successfully introduced in carbohydrate templates [2], the use of halogens has been largely neglected in glycomimetic design, except for fluorine. However, heavier halogens (X = Cl, Br, or I) can establish highly directional, R–X···B interactions with Lewis bases (B), known as halogen bonds (XB) [4]. These unusual intermolecular interactions, where halogens behave as electrophilic species, are explained by the anisotropic distribution of the electron density in covalently bound halogens, due to polarization along the R–X covalent bond. This leads to the existence of an electropositive site at their outermost region, named σ-hole [5]. XB-mediated molecular recognition phenomena are widespread across biological systems, namely in protein-ligand complexes, and have already inspired many applications in medicinal chemistry [6], amongst other fields.

In the search for novel glycomimetics with the potential to modulate carbohydrate-protein recognition *via* XB interactions, we performed a quantum mechanical study on the XB donor propensity of model halogenated sugar derivatives. In particular, we systematically introduced halogen substituents at different positions of pyranosyl units and subsequently computed the respective molecular electrostatic potential surface maxima. This procedure allowed us to map the chemical space of halogenated sugars in terms of their potential to act as XB interaction partners with XB acceptor species commonly found in proteins (i.e. oxygen, sulfur, nitrogen, or aromatic systems). The results encourage further *in silico* optimization towards the design and synthesis of new halogen-bonding glycomimetic ligands.

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MOLECULAR RECOGNITION OF GLYCANS BY LECTINS OF THE IMMUNE SYSTEM

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Glycosylation is one of the most important post-translational modifications of proteins and lipids. Glycans cover 95% of all cell surface proteins and have an important role in many cellular processes [1]. Aberrations of the glycosylation at cell-surface is a hallmark of autoimmune diseases and have an important role in the regulation and development of cancer [2]. Lectins from the immune system are a family of receptors, which recognize the truncated O-glycans present on tumour cells by their carbohydrate recognition domain [1]. The recognition of the cancer-specific tumour associated glycans epitopes by lectins of the immune system has a high impact in cancer immune surveillance and metastasis. Recent evidence shows that aberrant glycosylation inhibits antitumour immune responses and facilitates immune escape by the tumour [1, 3]. Consequently, the interactions between the glycan epitopes present in cancer and lectins from the immune system are highlighted as novel immune checkpoints and attractive targets for new immunotherapies [3]. Therefore, structural insights with atomic resolution of the glycan/immune lectins complexes are essential for a rational design of potential glycan-based therapies, namely drugs and vaccines. Herein, we will report our main advances on the structural characterization of key glycan/immune lectins complexes by NMR spectroscopy assisted with molecular modeling protocols and sustained by molecular biology protocols.

Acknowledgments

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UNRAVELLING ErbB2 GLYCOSYLATION SIGNATURE IN GASTRIC CANCER CELLS

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Introduction: The abnormal expression and activation of the human epidermal growth factor receptor 2 (ErbB2) represent central molecular events underlying the neoplastic transformation of the gastric tissue [1]. Despite the extracellular domain of this cancer-relevant receptor tyrosine kinase (RTK) being a well-known target for extensive glycosylation, its detailed glycosylation profile and the molecular mechanisms through which it actively tunes ErbB2 towards malignancy in gastric cancer (GC) cells remain elusive [2, 3].

Materials and Methods: The expression of relevant glycosyltransferase-coding genes, and the expression and activation of the ErbB receptors were assessed in four GC cell lines. Further glycan characterization was performed in NCI-N87 GC cells, an *in vitro* model of ErbB2 overexpression and hyperactivation. Using NCI-N87 whole cell lysates, ErbB2 was immunoprecipitated and validated by MALDI/TOF-TOF tandem mass spectrometry. Receptor's glycosylation was confirmed by Peptide-*N*-Glycosidase F digestion and characterized using a panel of carbohydrate-binding lectins and monoclonal antibodies (mAbs). The expression of genes controlling the biosynthesis of cancer-associated glycans in association to ErbB2 status were studied. Expression and activation of ErbB2 were assessed in ErbB2-overexpressing cells submitted to in vitro deglycosylation and mAb-mediated glycan blocking.

Results and Discussion: Cellular- and receptor-specific glycan profiling of ErbB2-overexpressing NCI-N87 cells disclosed an intricate glycosylation pattern harboring the tumor-associated sialyl Lewis a (SLe^a) antigen. The expression of SLe^a and key enzymes integrating its biosynthetic pathway showed to be strongly upregulated in this GC cell line. An association between the expression of *ERBB2* and *FUT3*, a central gene in SLe^a biosynthesis, was additionally established in GC patients. Furthermore, cellular deglycosylation and CA 19.9 antibody-mediated blocking of SLe^a drastically disrupted both receptor's expression and activation in NCI-N87 cells.

Conclusion: Our results show that the disclosed glycosylation profile of ErbB2 in GC cells has a major functional impact on receptor's biology with potential clinical applications. Furthermore, NCI-N87 cell model constitutes an appealing *in vitro* system to study glycan-mediated regulation of ErbB2 in GC [4].

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SYNTHESIS OF CHONDROITIN SULFATE DENDRIMERS AND BINDING TO GROWTH FACTOR MIDKINE

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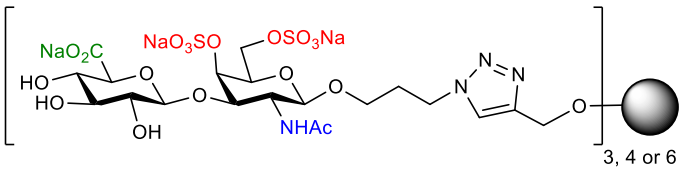
Chondroitin sulfate (CS) is a very heterogeneous polysaccharide that is involved in numerous biological processes, such as cancer, neuroregeneration and inflammation. The biological functions of this biopolymer are due to the interactions between specific oligosaccharide sequences and a wide range of proteins. For this reason, there is a great demand for the synthesis of homogeneous CS oligosaccharides. However, the preparation of long sequences is still a complicated task. In this context, the development of glycomimetics that retain, or even increase, the biological activity of natural sequences and, at the same time, can be easily prepared in comparison with the natural oligosaccharides is highly desirable.

Here, we report the preparation of novel dendrimers bearing CS-E disaccharide units [1]. Using a fluorescence polarization competition assay we have demonstrated that these multivalent monodisperse systems interact with midkine, a relevant heparin-binding growth factor involved in cancer and cell development, in the low micromolar range. Our results highlight the potency of these disaccharide-displaying dendrimer systems as interesting CS mimetics, demonstrating that the multivalent presentation of the sugar epitopes on the dendritic scaffold strongly enhanced their protein recognition.



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ICS YOUNG RESEARCHER WORKSHOP

PLENARY LECTURES

ANALYSIS OF CARBOHYDRATES USING FRAGMENT BASED HYPHENATED MASS SPECTROMETRY

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Ion mobility mass spectrometry (IM-MS) is increasingly used for the successful identification of isobaric stereoisomers of glycoconjugates, but has the drawback that it largely relies on authentic standards for verification of structure, which limits its applications in *de novo* sequencing. Here we present a IM-MS based strategy that uses a fragment-based approach, in which ion mobility is used for the characterization of ions generated by gas phase fragmentation. Given the structural redundancy in glycan building blocks, the need for standards is dramatically reduced.

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BEAM SEARCH ARRAYS FOR (*O*)-GLYCAN LIGAND DISCOVERY

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Microarrays of sequence-defined glycans which we introduced in 2002 [1] have revolutionized identification of glycan sequences in bio-recognition processes in health and in infective and non-infective disease. Our neoglycolipid (NGL)-based microarray system [2] includes glycans (natural or synthesized chemically) conjugated to a lipid molecule, and this is also applicable to glycolipids as well as the glycans derived from them. This affords the flexibly to clinch the role of carbohydrate and ceramide in recognition [3]. The lipid-linked glycans are arrayed in a liposomal formulation that behaves as a planar membrane, mimicking the cell surface. The lipid-linked glycans can be incorporated into live cells to evaluate the biological significance of binding data. The increasing repertoire of glycans enable discoveries of unpredicted details of glycan-binding specificity [2,4].

The special feature of this platform is the provision for generating 'designer' microarrays from targeted glycomes [5,6], and thus the naturally occuring ligands can be pinpointed for isolation and characterization.

I will discuss a recent advance, the *Beam Search* approach, which a robust and efficient means of pinpointing ligands within heterogeneous glycan populations. This approach which incorporates robotic arraying of glycans in the course of fractionation with monitoring by mass spectrometry, has been documented for the highly heterogeneous *O*-glycan population on an epithelial glycoprotein, and has revealed novel components as ligands (receptors) for rotaviruses [7]. The *Beam Search* approach is applicable to glycome recognition studies in a wide range of biological settings to give insights into glycan recognition structures in natural microenvironments. I will discuss applications and illustrate the important complementarity with the increasing repertoire of sequence-defined glycans in microarrays.

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FLASH COMMUNICATIONS

A NOVEL AND CONCISE SYNTHESIS OF LEGIONAMINIC ACID AND 4-*EPI*-LEGIONAMINIC ACID

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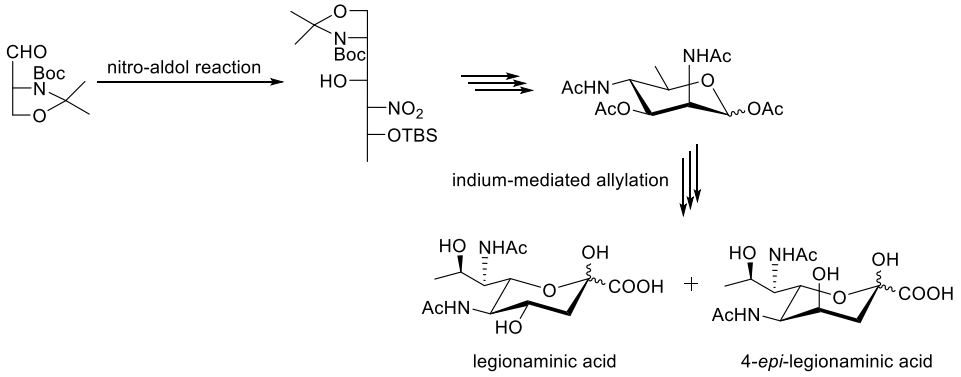
Legionaminic acid and 4-*epi*-legionaminic acid are assumed to play a crucial role in the virulence of *Legionella pneumophila*, the causative agent of Legionnaires' disease [1]. Bearing in mind that these nonulosonic acids are ideal target motifs for the development of vaccines and pathogen detection [2], we hereby present a novel and cost-efficient *de novo* synthesis of legionaminic acid and 4-*epi*-legionaminic acid. Starting from simple D-serine the C-9 backbone is built up by two C-C-coupling key reactions. First, the protected D-serine motif is elongated utilizing a highly stereoselective nitro-aldol reaction [3] to give the C-6 precursor of the desired relative configuration. Second, an indium-mediated allylation [4] is used to further elongate the carbon backbone and introduce a masked α-keto acid functionality. Experimental details of our synthetic approach will be presented.



Scheme 1 - Key reactions applied for carbon backbone elongation.

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C-GLYCOSIDES – NOVEL GALECTIN INHIBITOR SCAFFOLDS

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Galectins form a distinct family in the broader category of carbohydrate binding proteins. Fifteen mammal galectins are known, ten of which are found in humans. Three different subgroups of galectins exist; dimeric (galectins -1, 2, -5, -7, -10, -11, -13, -14 and -15), tandem (galectins -4, -6, -8, -9 and -12) and chimera (galectin-3). Dimeric or “prototype” galectins are homodimers with two identical carbohydrate recognition domains (CRDs), tandem galectins are heterodimers with two nonidentical CRDs and the sole chimera galectin, galectin-3 has one CRD attached to a collagen-like tail and the ability to form multimer binding complexes [1]. A key function of galectins in vivo is the organization of cell surface protein; the galectins bind to glycans on the proteins forming clusters via crosslinking. One effect of clustering of receptors is increased signalling. Proteins crosslinked in this fashion by galectins include vascular endothelial growth factor receptor (VEGFR) and integrins, galectins a role in modulating interactions between a cell and its surroundings [2,3].
Previous galectin inhibitor scaffolds have mainly been disaccharides like N-Acetyllactosamine or thiodigalactoside [4], but recently C-glycosides have been shown to be promising new galectin inhibitor scaffolds. We have developed new C-glycoside monogalactoside based scaffolds that offer galectin affinities down to the low micromolar range and selectivities up to and including complete selectivity, tunable to target either galectin-1 or galectin-3. Our new scaffolds retain only the parts of galactose necessary for galectin recognition while adding non-carbohydrate-like structures that enhance both affinity and selectivity towards one particular galectin. C1-arylheterocycle galactopyranosides can tuned towards galectin-1 or galectin-3 selectivity depending on the choice of heterocycle. A wide variety of different heterocycles are accessible through a common intermediate using modern catalytic methods. C1-(aryltriazolyl)methyl galactopyranosides are galectin-1 selective, and accessible through a new diastereoselective hydroboration that gives 2-deoxyheptuloses in excellent yields and with excellent diastereoselectivities.

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DC-SIGN, DC-SIGNR AND LSECtin: POTENTIAL SELECTIVE INTERACTION WITH POSITIONAL ANTENNAE ISOMERS

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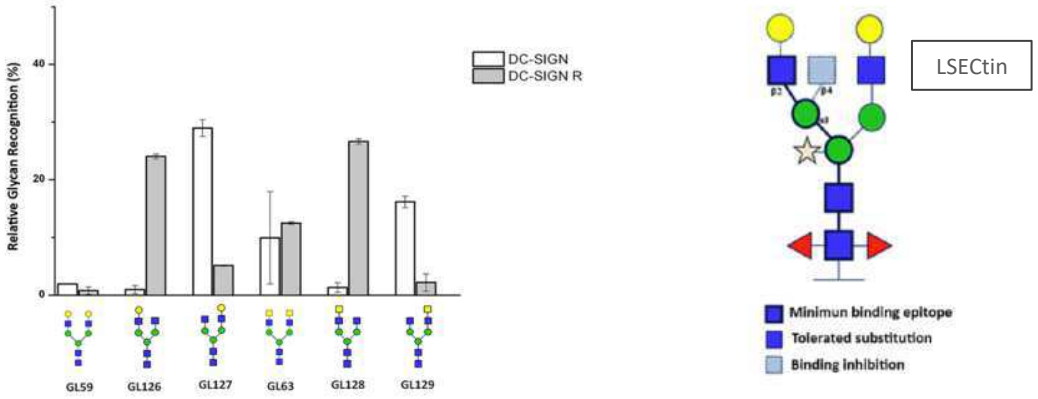
C-type Lectin Receptors (CLRs) are Ca^{2+}-dependent glycan binding proteins expressed, for example, on the surface of the professional sentinels of the innate immune system, the Dendritic Cells (DCs). CLRs recognize pathogens or damaged cells by interacting with specific glycan features and this encounter is the necessary step for the activation of the adaptive immune system [1]. CLRs are an attractive target to tailor the response of immunity and molecules selective to each CLR have to be identified for both fundamental research, as they could help the understanding of the lectin functions, and the applicative development of immunotherapy. Here, we explore context-depended binding of three CLRs involved in viral recognition, namely DC-SIGN, DC-SIGNR and LSECtin. Various combination of N-glycans decorated with GalNAcβ1,4GlcNAc and GalNAcβ1,4[Fucα1,3]GlcNAc motifs, commonly found in the parasite *Schistosoma mansoni*, were synthetized and their interaction with fluorescently labeled CLRs were assessed using glycan microarray technique.



a) b)

Fig. a) Relative glycan recognition (%) for DC-SIGN and DC-SIGNR b) Minimum binding epitope for LSECtin

Interestingly, the structural asymmetry between isomers led to different interactions with DC-SIGN, DC-SIGNR and LSECtin. Despite having the same composition of antennae, glycans showed a selectivity dependency to the relative position of their antennae, highlighting, for the first time, that CLR recognition is sensitive to the context of glycan presentation. In conclusion, we have identified three C-type lectins of the immune system that bind selectively to pairs of positional N-glycan isomers [2]. These findings open the door for further improvement on selectivity and affinity of the identified epitopes.

Acknowledgements

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THE MECHANISMS OF ANTICOAGULANT ACTIVITY OF THE GLYCOCONJUGATES ISOLATED FROM *CONYZA CANADENSIS* L. BY THE ULTRASOUNDS ASSISTED EXTRACTION

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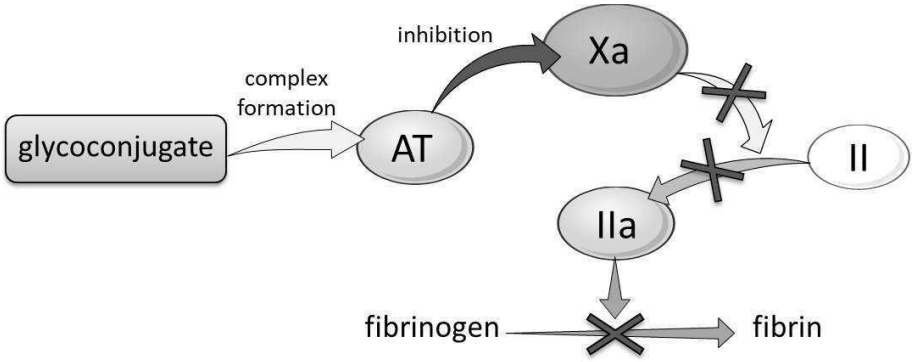
The canadian horseweed (*Erigeron canadensis* L.) is a common, annual herbal plant grows especially in the northern climate zone [1]. Flowering parts of *E. canadensis* are very often utilized in folk medicine [2], due to presence of various compounds, such as essential oils, tannins and flavonoids [3,4]. The preparations from horseweed are used as diuretic, tonic, antifungal and astringent agents. Our research team have obtained and investigated from this plant the polyphenolic glycoconjugates, according to the already described multi-step isolation process [5], with application of the ultrasounds assisted extraction (UAE) in the alkaline environment. The hot water extraction, even in alkaline condition, consumes time and solvents, therefore usage of UAE, due to the specific phenomenon such as microcavitation forces, can radically decrease time of the whole process.



The examined macromolecules from *E. canadensis* exhibited *in vitro* anticoagulant activity on human blood plasma, what was assessed in aPTT and PT tests. These *E. canadensis* conjugates were able to inhibit mainly the activity of Factor Xa when they were mediated by antithrombin, where such mechanism of action is typical for the highly sulphated glycosaminoglycans.

Acknowledgments

This work is supported by Wrocław University of Science and Technology, Wrocław, Poland. Part of analyzes was made on the instruments purchased by the Project „WroVasc – Integrated Cardiovascular Center", co-financed by the European Regional Development Fund, within Innovative Economy Operational Program, 2007-2013 realized in Regional Specialist Hospital, Research and Development Center in Wrocław.

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DEVELOPMENT OF A NOVEL HYBRID CHITOSAN-NANO TiO_2 SPONGE FOR ALVEOLAR BONE TISSUE REGENERATION

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Many studies have revealed that, in periodontal tissue treatment, for instance, in periodontitis or tooth implant replacement -while it can replace most of the functions of natural tooth, usually is not followed by a proper alveolar bone regeneration [1,2]. In this study, we used chitosan that has long been used in many dental applications [3] as sponge, hybridized with TiO2 nanoparticles. TiO_2 nanoparticles were added with 2 main functions: (1) it promotes bone tissue regeneration [4-5], (2) upon UV exposure, the hydrophilicity improves, thus promotes better bone regeneration [6]. Sponge was prepared by mixing chitosan acetate solution with nano TiO_2 at different concentrations, before subsequently lyophilized. Chitosan-nano TiO_2 hybrid sponge with approximately a diameter of 1 cm was successfully synthesized. Observation through SEM showed interconnected pores with an equal distribution of nano TiO_2 at the surface of sponge. Sponge was then observed for its anti-bacterial capability, and showed superior anti-bacterial capability towards dental specific bacteria, *Streptococcus* mutans. In conclusion, the chitosan-nano TiO_2 hybrid sponge showed a preliminary result as a good candidate for alveolar bone tissue regeneration. Future works will include cell culture on the sponge to see the bone regeneration capability of the sponge.

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SYNTHESIS OF CAPSULAR POLYSACCHARIDE FRAGMENTS OF THE FUNGAL PATHOGEN *CRYPTOCOCCUS NEOFORMANS* AND THEIR POTENTIAL APPLICATIONS AS FRET PROBES

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Cryptococcus neoformans is an encapsulated yeast, which exists ubiquitously in the environment. It is responsible for 957,900 cases of cryptococcal meningitis annually, resulting in approximately 624,700 deaths [1]. Infection occurs in immunocompromised individuals, e.g. HIV/AIDS, and those on immunosuppressive drugs, e.g. chemotherapy. The capsule of *C. neoformans* consists of three distinct components, the focus of our investigation is the glucuronoxylomannan (GXM) polysaccharide polymer. The typical structure of the GXM is of a linear (1→3)-α-D-mannopyranan backbone bearing β-D-xylopyranosyl, β-D-glucopyranosyluronic acid and 6-*O*-acetyl substituents [2].
Synthesis of structures relating to the GXM will be presented, with a particular focus on problems encountered during the final hydrogenolysis step. Potential applications of these structures will also be discussed, including further derivatisation of the oligosaccharides for use as Förster Resonance Energy Transfer (FRET) probes. These FRET probes will be used to determine the activity of 18B7, a catalytic monoclonal antibody that hydrolyses both synthetic and native GXM polysaccharides [3].

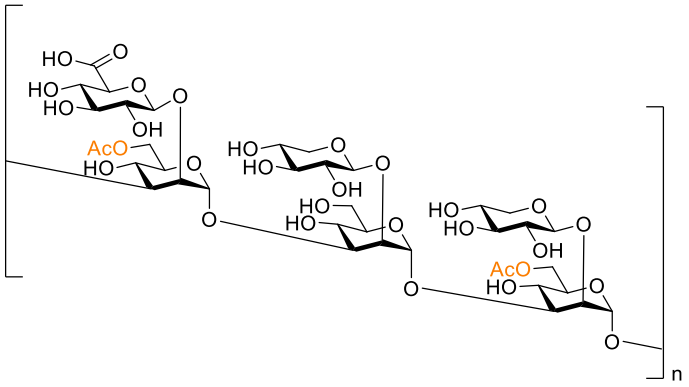

Fig. 1 - The GXM capsule does not contain a distinct repeating unit, instead repeating motifs have been identified. Shown above is the M2 motif of the GXM of *C. neoformans*.

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SYNTHESIS OF Xylβ1-4Rbo, PARTIAL STRUCTURE OF *O*-MANNOSYL GLYCAN

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O-Mannosyl glycan, one of the glycan part of α-dystroglycan, is classified into three subclasses; core M1, M2 and M3. Recently, the total structure of the core M3 has been reported (Fig. 1) [1]. We paid attention to the two types of Xylose (Xyl) residues in the core M3 of *O*-Mannosyl glycan. The Xyl at the reducing end β-links to *O*-4 of ribitol (Rbo) [2], while the Xyl in the repeating region has α-linkage to *O*-3 of glucuronic acid (GlcA) [3]. In addition, GlcA at the reducing terminal has a α1-4 linkage to Xyl different from the repetitive GlcA-Xyl. TMEM5 which is a specific xylosyltransferase, is only active for Rbo at the non-reducing side residue of the core M3 [2]. These facts prompted us to synthesize the Xylβ1-4Rbo for analytical purpose and for elucidation of the biosynthesis.

Suitably protected Rbo compound masked with TBDPS at *O*-5 was synthesized. To this was coupled a xlyosyl chloride in the presence of AgOTf and collidine in CH_2Cl_2 to give the β-linked Xyl-Rbo. All the protecting groups were removed to afford the desired Xylβ1-4Rbo(**1**). ^{1}H and ^{13}C NMR data of compound(**1**) supported the structure already reported [2].

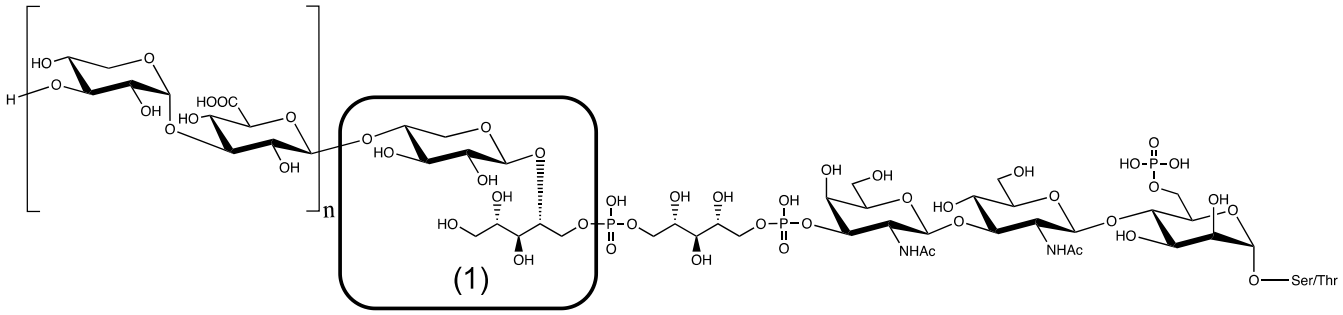

Fig. 1 - Structure of core M3 of O-Mannosyl glycan.

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SYNTHETIC EXPLORATION OF XYLOSE ANALOGS

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Glycosaminoglycans (GAGs) and proteoglycans are major components in the extracellular matrix, and are involved in important processes such as cell-cell interactions, growth factor uptake and uptake of biomolecules. A key substrate in the biosynthesis of these chains is the carbohydrate xylose, which is the substrate for the enzyme β4GalT7. It has been shown that a xylose with a naphthol aglycon can function either as a primer of GAGs or as an inhibitor depending on the modification of the carbohydrate. It is thus interesting to synthesize modified xylosides to investigate the biosynthesis of GAGs and how these affect cells.

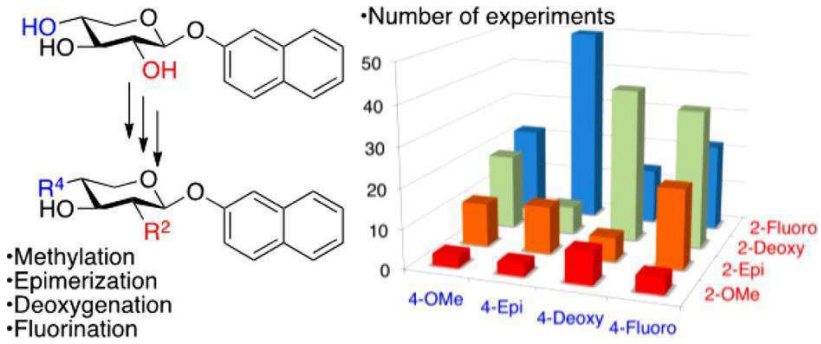

Fig. 1 - Main aspects of the doubly modified xylose investigation

To probe the active site of β4GalT7, several doubly modified naphthoxylosides were synthesized.[1] Superficially simple compounds were shown to require complex synthetic routes (Fig. 1). Even though there are a lot of methods for synthesizing carbohydrates, double modifications of pentoses turns out to be quite problematic and present challenges due to conformational, steric and stereoelectronic effects. The effect of replacing endo and exocyclic oxygen was also explored.[2] There is a profound effect in priming ability when replacing the oxygens for sulfur, increasing the ability of the substrate by approximately 15 times due to conformational change (Fig. 2).

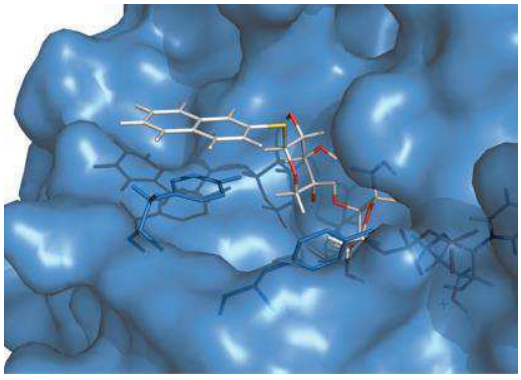

Fig. 2 - Docking complex of enzyme and dithio-modified xyloside

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SYNTHESIS OF NOVEL D-GLUCURONAMIDE-BASED TRIZOLE NUCLEOSIDES AS POTENTIAL ANTI-ALZHEIMER AGENTS

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The synthesis of analogues of nucleosides occupies a relevant place in medical chemistry due to the diversity of biological activities that there compounds are prone to exhibit. Some of these molecules display anticancer and antiviral activities, among which several examples reached clinical application [1]. We have previously showed the potential of nucleosides from D-glucuronamide derivatives [2] and of 6'-isonucleosides [3, 4], including a triazole derivative [3], to inhibit cholinesterases. These enzymes are relevant therapeutic targets for Alzheimer's disease, a neurodegenerative pathology that is the sixth leading cause of death worldwide.

Within the context of our interest in the access of novel nucleoside analogs embodying rather unexploited glycosyl units and motivated by our previous results, the work reported herein was focused on the synthesis of novel furanosyl nucleoside analogs, based on D-glucuronamide templates and embodying anomerically-linked triazole moieties, as potential cholinesterase inhibitors. For their synthesis, glucofuranurono-6,3-lactone was used as precursor for the access to various *N*-dodecyl 1-azido glucofuranuronamides, which were further engaged in a 1,3-dipolar cycloaddition with an alkyne. Some of the newly synthesized molecules showed selective and potent inhibition of acetylcholinesterase along with low cytotoxicity in human fibroblasts and in a neuronal cell line, rendering them promising lead compounds for further development.
In this communication both the synthetic work and the results of the biological evaluation will be presented and discussed.

Acknowledgements: 'Fundação para a Ciência e Tecnologia' is acknowledged for funding (projects IF/01488/2013/CP1159/CT0006 and UID/MULTI/00612/2013).

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CONCENTRATION-DEPENDENT REACTIVITY AND STEREOSELECTIVITY OF GLYCOSYLATION: WHY A REACTION MAY STOP AT SOME CONCENTRATIONS?

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Considerable influence of concentration of reactants on their reactivity and stereoselectivity of glycosylation were reported in a number of cases [1]. We found that stereoselectivity of glycosylation of $HOP(O)(OBu)_2$ with 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl bromide (**1**) in the presence of i-Pr_2NEt in MeCN at 20 °C unusually depends on concentration (0.001—0.2 M) of **1** [2], switching from unselective reaction to almost complete stereoselectivity below a critical [3] concentration (C = 0.02 M) that was detected by polarimetry (Fig. 1). Apparently, this concentration separates two ranges of concentration with different structures of the reaction solution (including the structure of supramers of reactants) [3]. Detailed studies revealed that this reaction is rather complex. A fast reaction between **1** and **2** (0–1 min) is followed by a considerably slower reaction (1–120 min). Remarkably, at $C \le 0.02$ M more than 90% of **1** is consumed within 1 min and then conversion of **1** slowly reaches 100% after 1–2 h implying higher reactivity of supramers of **1** in this concentration range; at $C > 0.02$ M conversion of **1** never exceeds 80% suggesting the presence of much less reactive supramers. Intriguingly, although anomerization of both glycosyl bromide **1** and glycosyl phosphate **2** was shown (by NMR and polarimetry) to be possible under the conditions used, the α/β ratio of initially (0–1 min) formed **2** almost does not change within 2 h of monitoring for all concentrations studied. Analysis of data obtained suggests that the observed α/β ratio of **2** is determined within the first minute of reaction by the structure of supramers of **1** present at a particular concentration. Importantly, at $C > 0.02$ M considerable part of molecules of **1** seem to be buried inside tight [4] supramers, which lowers their apparent reactivity, while at $C > 0.02$ M most of molecules of **1** are incorporated in more loose supramers hence more accessible for reaction with nucleophile. This work was supported by the Russian Science Foundation (Project No. 16-13-10244).

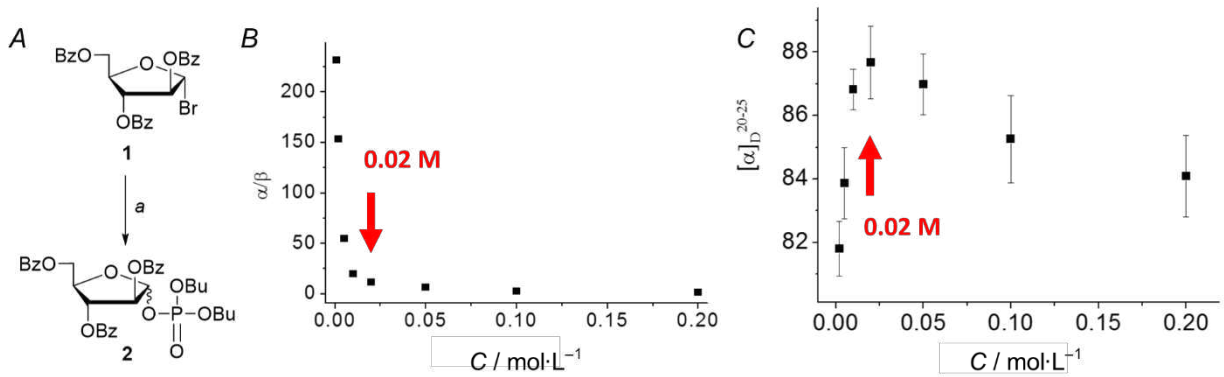

Fig. 1- ***A.*** *Reagents and conditions*: *a*. $HOP(O)(OBu)_2$, i-Pr_2NEt, MeCN, 20 °C, 2 h. Concentration dependences of stereoselectivity of glycosylation (***B***) and optical rotation of solution of glycosyl donor **1** in MeCN measured 15 min after dissolution (***C***). Red arrows designate critical concentration (0.02 M).

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SELECTION OF NOVEL C-TYPE LECTIN LIGANDS THROUGH NEW SCANNING METHOD

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C-type lectins bind glycans in calcium dependent manner and represent the biggest group among lectins. During the years, this definition is changed to all glycan binding proteins which contain one or more C-type lectin carbohydrate recognition domains, regardless of whether calcium is necessary for the binding of glycan or not. They are involved in a wide range of important biological phenomena from cell signaling, cell adhesion, differentiation and maturation of cells to sensing of danger signals, innate immunity and are crucial in inflammatory responses.

C-type lectin carbohydrate recognition domain has very low affinity for ligands which is overcome by multivalent ligand presentation at the surface of cells or pathogens. Based on distance spacing between glycans, ligands may be optimized to allow efficient targeting of one specific C-type lectin. In order to mimic the oligosaccharides on cell surface or on surfaces of viruses and bacteria, we focused our research on synthesis of multivalent molecules based on shapes glycomimetics, dendrons and/or neoglycoproteins.

To fulfill our results, we are using a GLYcoPROFILE®, technique developed in our laboratory for fast and easy screening of glycobiological interactions between lectins and glycans. With this technology, we are determining glycan signatures on molecules or cells with plant, recombinant bacterial and finally recombinant human lectins. By use of lectin array, we are able to compare IC50 concentrations of inhibitors in order to find the strongest ligand for certain lectin.

Our results of design and synthesis of dendrons and neoglycoproteins show that they are useful tools for studying sugar-protein interactions. Glycoprofiles on DC SIGN, Langerin, Dectin-2 and MGL of our multivalent structures, lead us to identification of new, better and more specific ligands for those C-type lectins.

SYNTHESIS AND VACCINE POTENTIAL OF BACTERIAL MIMETICS AGAINST HIV-1

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Gp120, present on the surface of the human immunodeficiency virus (HIV), harbors clusters of oligomannose structures that are recognized by several broadly neutralizing antibodies and thus constitutes an important target for HIV-1 vaccine development [1]. Lipooligosaccharide (LOS), isolated from the bacterium *Rhizobium radiobacter* Rv3, closely resembles these oligomannose structures present on gp120 and immunization of wild-type mice with heat-killed bacteria elicited serum antibodies, which bound to monomeric gp120 (**Fig. 1, a**) [2,3].

To determine the antigenic potential of mannose mimetics that contain features of the viral and bacterial structures, a library of novel oligomannose based glycoconjugates harboring a viral D3-arm surrogate was synthesized. One of these conjugates (**Fig. 1, b**) revealed efficient binding to several HIV broadly neutralizing antibodies and immunization of Hu-Ab transgenic rats resulted in promising neutralizing activity against a selection of tier 2 HIV strains [4].

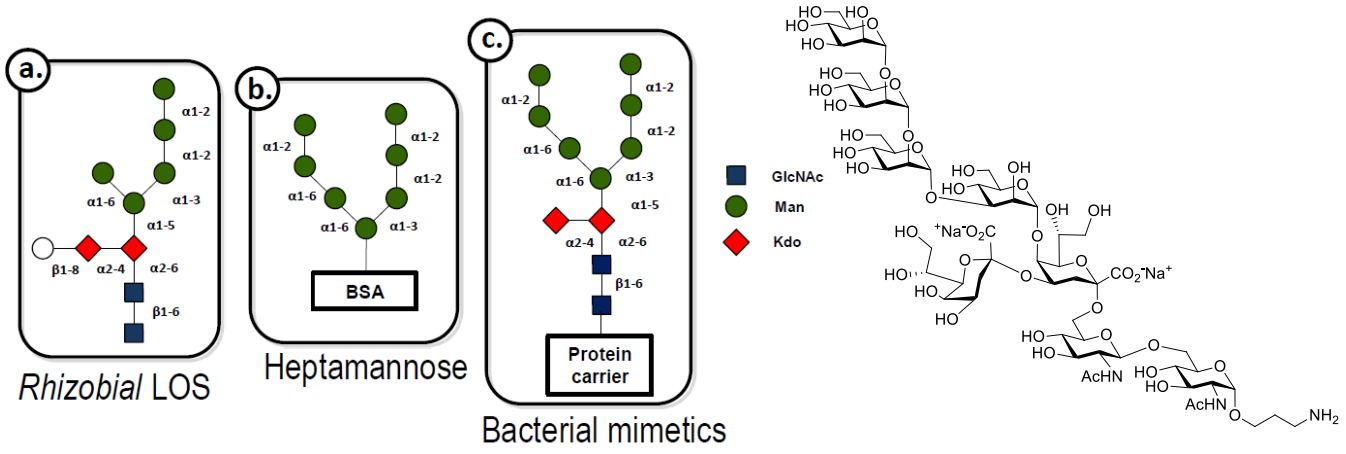

Fig. 3 - a) LOS from *Rhizobium radiobacter* Rv3; b) Lead antigen in binding studies; c) Targeted bacterial mimetics and octasaccharide spacer derivative

Based on these results the synthesis towards bacterial mimetics, containing the core of *rhizobial* LOS ($Kdo_2GlcNAc_2$), was performed (**Fig.1, c**).[5] Kdo residues were efficiently introduced using an α-selective isopropylidene protected Kdo fluoride donor. Elongation at the unreactive 5-OH position of Kdo was achieved by the design of a highly reactive, orthogonally protected mannose trichloroacetimidate donor. Subsequent coupling with a mannotriosyl donor gave the α-(1→3) extended octasaccharide in a good yield, which was then globally deprotected to give the central LPS core fragment of *Rhizobium radiobacter* Rv3 LOS.

Acknowledgments: FWF-grant P 26919 (to PK) and NIH grant 1R0AI134299-01 (to RP)

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FACIAL SYNTHESIS OF Globo H ANALOGS CONJUGATED VACCINES AND THEIR IMMUNOGENICITY STUDIES

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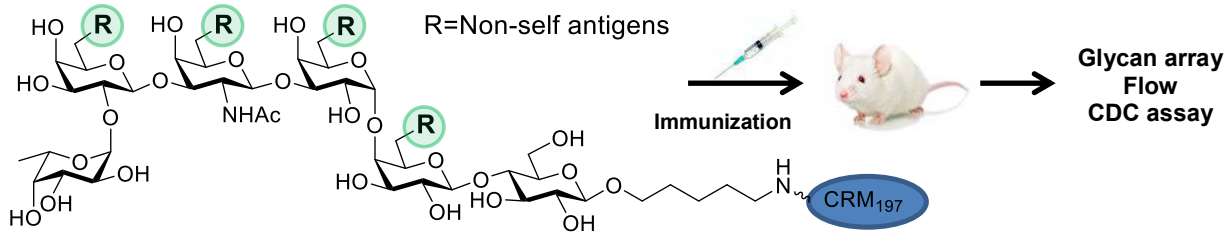
Globo H is a potential cancer vaccine due to it is over-expressed on many types of cancer cell but rarely expressed on normal tissue. Globo H based cancer vaccines have been used in the clinical trials including breast, ovarian and prostate cancers. However, the low immunogenicity of Globo H based cancer vaccine is the major challenge in clinical trial. Previous report indicated that non-self antigens modified carbohydrate-based vaccines induce stronger immunogenicity. To elicit stronger immunogenicity of Globo H conjugated vaccines, we synthesized different Globo H analogs with non-self antigens modifications. The modifications were installed at the C'6 of Gal or/and GalNAc on Globo H by chemoenzymatic methods. We applied the glycosyltransferases to install different modified monosaccharide into Globo H. Although some modified monosaccharides were not suitable donors for LgtD galactose transferase, we adopted galactose oxidase which showed excellent regio-selectivity to oxidize the non-reducing end galactose of lactose, GB4, and GB5. The terminal C'6 oxidized oligosaccharides can be converted into different derivatives in few steps and elongated to Globo H analogs by glycosyltransferases. These Globo H analogs were conjugated with carrier protein diphtheria toxoid cross-reactive material (CRM) 197 (DT) as the vaccine candidates for immunization studies. In this study, we have synthesized series of Globo H analogs conjugated vaccines in efficient steps. In the future, we will utilize the glycan array to examine the antibody titer from the immunized mice and explore which modification and modified position can provide strong immunogenicity for future vaccine development.



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DECODING *AKKERMANSIA MUCINIPHILA* LIPOPOLYSACCHARIDES

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Akkermansia muciniphila is a commensal non-pathogenic bacterium that inhabits the mucus layer of mammals' guts. This mucus layer is a physical defense that covers and protects the intestinal epithelium against pathogens and chemicals. Mucus contains antibacterial peptides and antibodies; even so, this non-pathogenic bacterium is one of the few that successfully inhabits there [1,2]. Its integrity depends on diverse homeostatic regulatory mechanisms where microbiota-host interactions play a crucial role [3]. Akkermansia participates as a mucin degrader, breaking down the main glycoprotein of the mucus layer. This way, it is a fundamental actor in the renovation and well being of the mucus layer [4].

Also, there is diverse evidence of its capacity to perform important changes beyond the intestine. In metabolic diseases such as obesity and diabetes, Akkermansia population decreases, producing thinning mucus layer and inflammatory processes [5,6]. But, its subsequent artificial increase shows an improvement on the whole host´s metabolic profile [2]. However the procedure of those interaction are still to be clarify.

As gram-negative bacteria, Akkermansia possesses lipopolysaccharides (LPS) as mayor components of its exterior membrane, which protects the bacteria and interacts with the environment and the host [7]. Recent studies suggest that LPS can produce relevant changes on the intestinal epithelium permeability and integrity, although the mechanisms are not clear [8].

Identifying the composition and particularities of Akkermansia LPS is crucial to understand the details of this mutualism relation, also its capacity to live in the mucus layer and to participate in metabolic disorders. Through a multi-technique approach comprising chemical analyses and diverse spectroscopy techniques, the chemical composition and structure of *Akkermansia muciniphila* lipopolysaccharides was determined.

Globally, our results shed light on the molecular basis of the role of LPS on the mutualism between humans and *A. muciniphila.* Nevertheless, future *in vitro* and *in vivo* studies, should be warranted to progress in the understanding on this special mutualism relation.

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ASSESSING HUMAN MICROBIOTA SYSTEMS FOR GLYCAN RECOGNITION IN THE GUT

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The human gut microbiota is a highly carbohydrate-active microbial community with a broad capacity to metabolize dietary and host-derived glycans. This is a key feature to keep human nutritional balance, modulate the immune system or promote disease. Thus, understanding carbohydrate recognition in the gut is of utmost importance for human health [1].

Abundant microbiota strains, such as *Bacteroides spp*, exhibit extensive sets of substrate-specific genes that allow bacteria to cope with nutrient fluctuation. These genes are grouped in polysaccharide-utilization loci (PULs). Each PUL encodes all the necessary elements for the recognition and degradation of a specific glycan, including a cohort of starch-utilization system (Sus)-like proteins and modular carbohydrate-active enzymes (CAZymes) associated with carbohydrate-binding modules (CBMs) [2]. Non-catalytic CBMs and SusD-like proteins are thought to enhance and mediate specific glycan targeting and recognition, reflecting the PUL specificity itself. As the microbiome (full collection of all microbiota genes) data piles up, there's an urgent need to develop and apply high-throughput approaches to study these recognition systems at a functional and structural level.

In this communication, we walk you through our integrative strategy to characterize novel human microbiome glycan-recognition systems, combining glycan microarray technology [3,4] and X-ray crystallography [5,6]. We have focused on glycan recognition by PULs proteins from representative strains of the gut microbiota, *Bacteroides thetaiotaomicron* and *Bacteroides ovatus*, which have adapted to target mammalian- or dietary-derived glycans, respectively. The designed strategy includes: bioinformatic sequence analysis and production of a recombinant-protein library (over 100 different putative glycan binding domains); high-throughput screening analysis of glycan-binding using microarrays comprising a diverse range of mammalian-, plant- or pathogen-derived glycan probes; and structural characterization of newly identified protein-oligosaccharide interactions using X-ray crystallography. The use of alternative glycan microarrays aims to cover the glycan structural diversity found in the gut, in a miniaturized format.

The successful implementation of this combined approach impacts on the understanding of the microbiome metabolic capabilities, as well as the microbial strategies to cooperate and interact with the human host. In future, this strategy can be extended to other microbial strains to help unravel the holistic effect of glycan recognition in the gut and pinpoint target pre- and probiotics for novel biopharmaceutical solutions.

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UNDERSTANDING THE MOLECULAR MECHANISM UNDERPINNING THE IMMUNOMODULATORY FUNCTION OF LEVAN EXOPOLYSACCHARIDE

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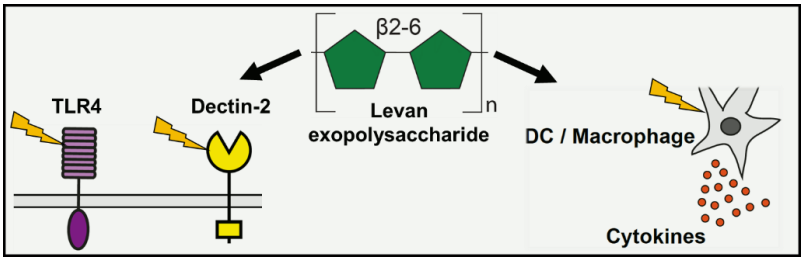
Polysaccharides (PS) found in edible plants, fungi, and bacteria have been shown to modulate immune function in humans [1], but their underlying molecular mechanisms are unclear. Here, we investigated the interaction of bacterial levan, a high molecular weight β-(2, 6) fructofuranose exopolysaccharide reported to have immunomodulatory activity [3], with immune cell pathogen recognition receptors including toll-like receptors (TLRs) and C-type lectin receptors (CLRs) found on myeloid cells.

Using a high-throughput cell-based reporter assay expressing specific CTRLs and TLRs, we found that crude levan bound to Dectin-2, a mannose-specific lectin found on dendritic cells (DCs) responsible for anti-fungal and anti-mycobacterial immunity, and TLR4. However, the binding was significantly reduced following extensive purification of levan by gel permeation chromatography, use of lipid removal agent, and chemical treatment to remove lipopolysaccharide (LPS), a potent TLR4 ligand. Similar results were seen using an enzymatically-synthesised (ES) levan. Force spectroscopy measurements using atomic force microscopy confirmed that ES levan did not bind to human or mouse Dectin-2 proteins. To further assess the immunomodulatory properties of LPS-free levan and identify other potential receptors, we used murine bone marrow-derived dendritic cells (BMDCs). We showed that crude levan induced cytokine production in BMDCs in a TLR4-dependent manner whereas purified and ES levans did not induce cytokine production in BMDCs.



Overall, we demonstrated that the DC-mediated immune response of levan *in vitro* was largely dependent on the presence of LPS. As levan is produced by commensal gut bacteria and is in food [3], it may be that modulation of immune function is mediated by intestinal epithelial cells.

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A GH 20 THIOGLYCOLIGASE AS VERSATILE CATALYST FOR GLYCOCONJUGATE SYNTHESIS

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Carbohydrate mimetics, resistant towards enzymatic hydrolysis, have proven useful as inhibitors and therapeutics. Thioglycosides turned out stable glycosides with similar structural and physicochemical properties compared to their O-glycoside counterparts. Chemical synthesis of such compounds is often cumbersome considering the known issues in the control of stereochemistry and numerous protections/deprotection steps. Enzymatic synthesis of thioglycoside linkages in oligosaccharides could overcome these issues and is therefore of immense value. Several thioglycoligases are reported to date, covering both, the formation of α and β glycosidic linkages [1,2,3].

Herein, we present the first thioglycoligase derived from the mutation of the catalytic acid/base of *Streptomyces plicatus* N-acetyl-β-hexosaminidase (SpHex E314A). SpHex is a retaining GH family 20 *exo*-hexosaminidase cleaving β-1,4 glycosidic linkages between GlcNAcs via substrate assisted catalysis [4]. PNP-GlcNAc and GlcNAc oxazoline were chosen as reactive glycosyl donors and tested against a series of (3,4,6-) deoxythio sugars as glycosyl acceptors. The SpHex variant catalyzed β-thioglycosidic linkage formation with all tested deoxythio sugars of Glu, Gal, Man, GlcNAc, GalNac and ManAc in high yield. Enzyme mediated GlcNAc transfer to various substituted (O-)phenols spanning a broad pKa range gave insights into the donor reactivity. SpHex E314A also catalyzed the reverse reaction hydrolyzing different thioglycosides revealing a 50% decrease in the catalytic efficiency compared to the wildtype enzyme. The remarkable capacity of the SpHex thioglycoligase was successfully expanded to other bioactive acceptors, including cysteine and cysteine bearing oligopeptides, opening a new synthesis path towards GlcNAcylated peptide mimetics.
In summary, we demonstrate that SpHex E314A is a versatile thioglycoligase showing a remarkable thio acceptor promiscuity which renders this variant a potent candidate for generating metabolically stable glycoconjugates.

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WELL-DEFINED OLIGO AND POLYSACCHARIDES AS IDEAL PROBES FOR STRUCTURAL STUDIES

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Polysaccharides are the most abundant organic materials in nature, yet correlations between their three-dimensional structure and macroscopic properties have not been established [1]. Automated glycan assembly (AGA) enables the preparation of well-defined oligo- and polysaccharides resembling natural as well as unnatural structures [2]. A collection of related compounds, modified at specific positions of the chain, is presented. These synthetic glycans are ideal probes for the fundamental study of polysaccharides, shedding light on how the modification patterns affect the polysaccharides properties (i.e. three dimensional shape and aggregation behavior). Molecular modelling simulations and NMR analysis show that different classes of polysaccharides adopt fundamentally different conformations, drastically altered by single-site substitutions [3].

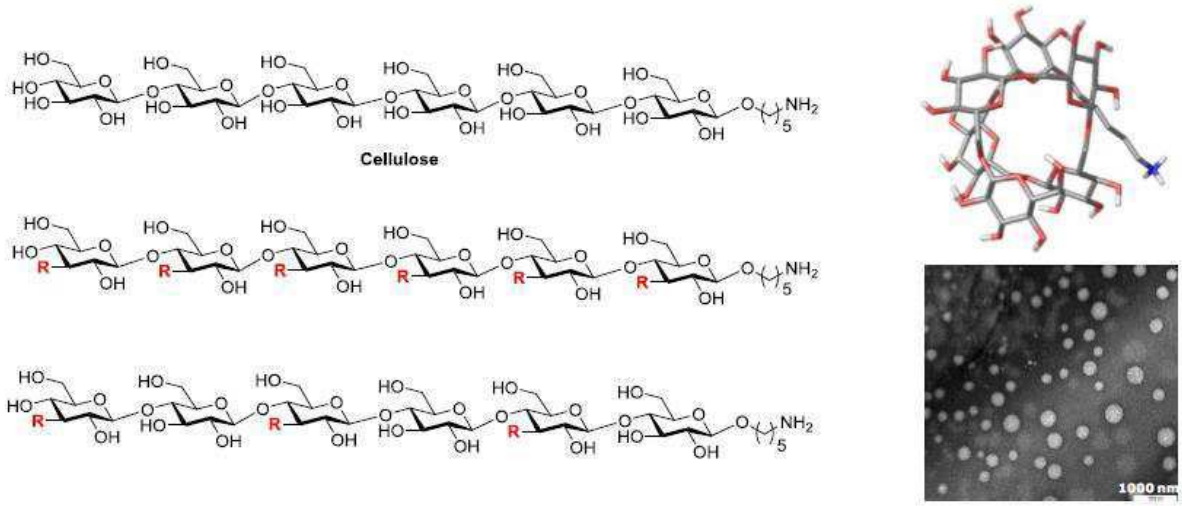

Fig. 1 - Synthetic cellulose oligomers with different modification patterns. Molecular dynamic simulations and oligosaccharide-based nanoparticles (NPs).

The aggregation behavior of these synthetic materials is strongly affected by the single chain conformation and the modification patterns; spherical particles as well as linear fibers are observed. The information gained through this study will guide the development of novel carbohydrate-based biomaterials with tunable properties (e.g. NPs for biomedical applications).

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STRUCTURAL AND IMMUNOLOGICAL FEATURES OF *Acetobacter pasteurianus* CIP103108 LIPOPOLYSACCHARIDE

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The lipopolysaccharide (LPS), the main component of Gram-negative bacteria outer membrane (OM), covering around 75% of its surface, is crucial for bacterial survival, contributes significantly to the integrity and stability of the OM, making up an excellent barrier against external milieu stress factors. Moreover, the LPS is one of the key molecules involved pathogen-host interactions, is a highly conserved structure, targeted by innate immune system cells and thus recognized as Pathogen Associated Molecular Patterns (PAMP). More precisely, the lipopolysaccharides are targeted by the TLR4/MD-2 receptorial complex [1], formed by a transmembrane receptor, the Toll-like receptor-4 (TLR4), and a soluble protein, the MD-2. If the TLR4/MD-2 driven activation of the innate immune response is beneficial to combat the infection, its over-stimulation leads to sepsis and finally life threating septic shock. Hence, studies on TLR4/MD-2 modulators able to antagonize the pathway are crucial [2].

Among the three different domains composing the lipopolysaccharide, the outermost O-polysaccharide, a glycolipid portion anchoring the LPS into the OM, called lipid A and core oligosaccharide linking both moieties, the immunostimulatory properties of the molecule lies in the lipid A. Variations in the LPS structure can lead to dramatical changes of its properties, including inhibition of TLR4/MD-2 activation by potent agonistic molecules; hence, the search of LPS molecules possessing inhibitory activity is a high interest topic [3].

Here, I will present of the isolation, purification and structural characterization of the LPS produced by the acetic acid bacterium *Acetobacter pasteurianus* CIP103108.
A. pasteurianus is one of organisms used in production process of Japanese black rice vinegar called *kurozu*, which consumption is believed to carry significant health benefits. The structure was identified using combination of chemical and spectral data including MS and NMR. The LPS showed low immunostimulatory activity, being able to lower the NF-kB activation by agonistic *E. coli* LPS.

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SYNTHESIS OF CYCLIC PEPTOID-BASED IMINOSUGAR CLUSTERS

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N-propargyl-α-peptoids (**1**-**3**, Fig.1), functionalized with *N*-alkyl 1-deoxynojirimycin (DNJ) residues **7** led to a series of 6- to 10-valent DNJ derivatives (Fig.1).[1] From the azide-armed trivalent iminosugar dendron **8** and cyclic peptoids **1**-**6**, a second generation of 18- to 48-valent DNJ glycoclusters was formed.[2] The modular approach to synthesize cyclic peptoids has been crucial to access higher valencies and to obtain α mannosidase inhibitors (in the nM range) displaying the largest inhibitory multivalent effects ever reported.[2] Moreover this study provided decisive insights into the molecular mechanisms underlying the outstanding affinity enhancements observed over the corresponding monovalent model (up to 5 orders of magnitude).[3]

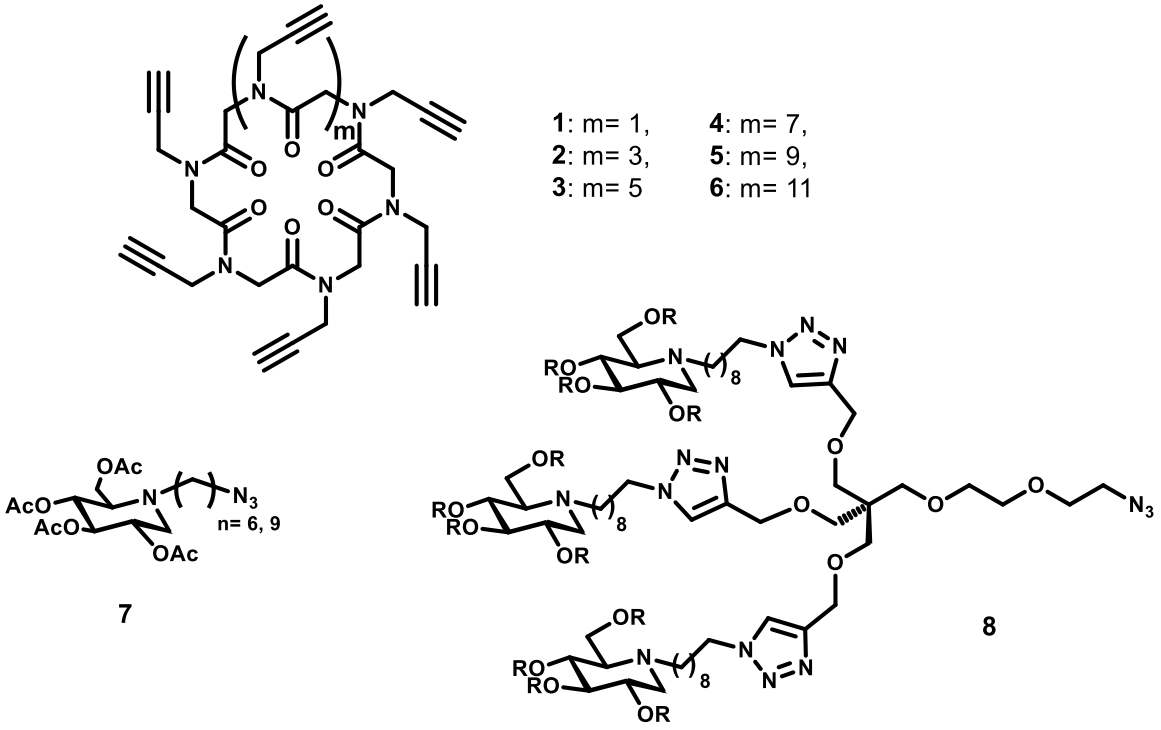

Fig.1. 6-to 16-*N*-propargyl-α-cyclic peptoids (**1**-**6**), monovalent DNJ-derivative (**7**) and its trivalent analogue (**8**).

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IN-DEPTH GLYCOMICS/GLYCOPROTEOMICS EVALUATION OF TUMOUR ASSOCIATED CARCINOEMBRYONIC ANTIGEN USING STATE-OF-ART MASS SPECTROMETRY

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Background & Aim

The Carcinoembryonic Antigen (CEA) glycoprotein is a FDA approved tumour marker strongly associated with tumour progression and metastasis. Even though *N*-glycans attribute to 60 % of the CEA molecule and are distributed over its 28 predicted *N*-glycosylation sites, current knowledge on the specific glycan structures in health and disease is surprisingly scarce.

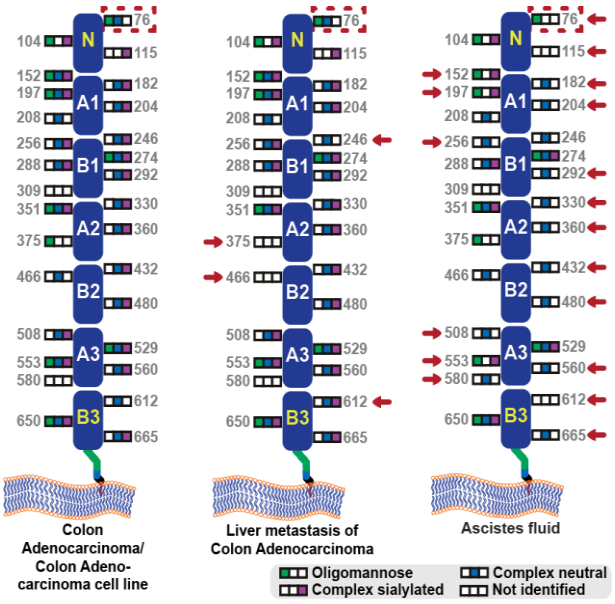
To fill in this gap, we performed a detailed glycomic & glycoproteomic assessment of specific glycosylation signatures of CEA originated from different human body sources (tissue and fluids) that can provide novel opportunities **to improve current CEA diagnostics**.

Methods

We developed a detailed glycomics and glycoproteomics workflow and investigated CEA purified from human colon adenocarcinoma (cell line and tissue), liver metastasis of colon adenocarcinoma and ascites fluid. Glycopeptides obtained by pronase digestion were analysed in a dual LC-setup using reversed-phase and porous-graphitized-carbon nano-LC-ESI-MS/MS within a single run to determine site-specific glycosylation patterns. A comprehensive *N*-glycomics assessment was achieved by applying porous-graphitized-carbon nano-LC-ESI-MS/MS glycomics.

Results and Conclusions

With our glycoproteomics approach we identified that 27 out of the 28 predicted *N*-glycosylation sites are glycosylated. The different CEAs exhibited **distinct source and site-specific, site occupancy glycosylation patterns**. To our surprise, we also identified **a novel 29th site of *N*-glycosylation** that is located within **a non-canonical sequence** motif in the functionally important N-domain of CEA.



With **over 270 individual *N*-glycans** detected by our glycomics approach CEA exhibited, to the best of our knowledge, the most heterogeneous and comprehensive glycome map of a single glycoprotein to date. **Distinct glycosylation differences** such as *N*-glycan branching, degree of sialylation and level of bisecting *N*-glycans were found between the CEA's from different body sources. Antennae fucosylation, such Lewis x and Lewis b/y determinants were found among all CEAs, however with significant abundance differences between the different sources. CEA is also a **carrier of sialyl-Lewis x**, an important Lewis blood group antigen involved in cell-cell recognition and metastasis. Thus, the ability to specifically detected and better understand CEA glycosylation opens novel opportunities to study the function of CEA glycosylation and its involvement in cancer pathogenesis.

UNRAVELLING N-GLYCANS RECOGNITION BY SIGLECS

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Siglecs are a family of I-type lectins, cell surface transmembrane receptors that function to recognize ubiquitous sialic acid epitopes on cell surface glycoconjugates, predominantly expressed by innate immune cells. Siglecs are increasingly recognized for their role in helping immune cells to distinguish between "self" and "non-self", dampening autoimmune responses and controlling inflammation in response to various pathogens [1]. Conversely, several feared pathogens, including membrane-enveloped viruses and bacteria, such as group B streptococci and *Campylobacter jejuni*, can decorate themselves with sialylated ligands that mimic "self'' and have the potential to elude or subvert the host immune responses, thus promoting successful bacterial colonization. Furthermore, the increase in the expression of certain sialylated glycans has been linked to metastatic cell behaviours such as invasion and enhanced cell survival (Fig. 1) [2].
Given that Sialic acid-Siglec interactions have been associated with a broad spectrum of diseases, ranging from autoimmunity to neurodegeneration and cancer, strategies to tune these interactions could have great therapeutic potential.

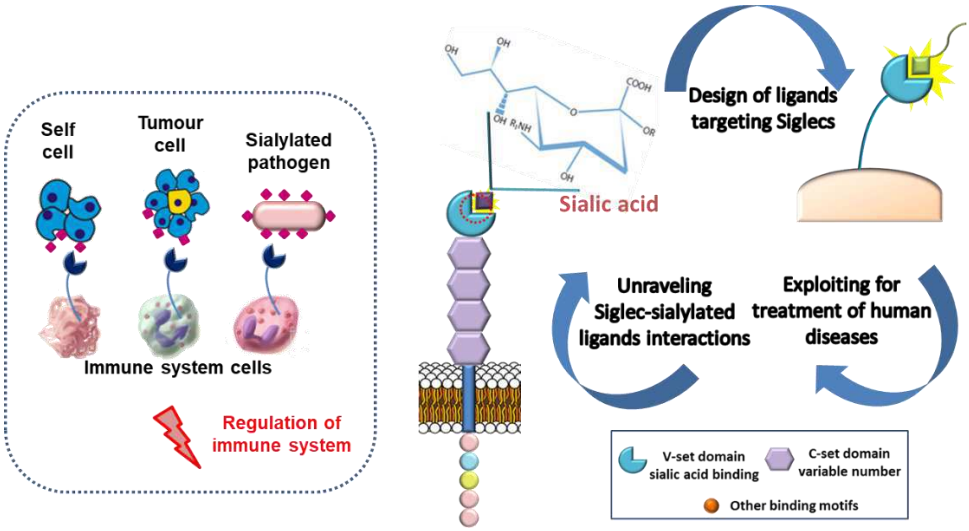

Figure 1. Siglecs and the regulation of immune system.

Within this frame, we investigated the interactions between different Siglecs and their natural and synthetic [3] substrates with the aim to exploit their properties for treatment of human diseases. Advanced ligand-based NMR techniques, taken together with Molecular Modelling studies [3], allowed us to get crucial information on binding mechanisms which may open a route for the design of novel glycomimetics for therapeutic targeting of the Siglecs – sialylated glycans axis.

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MOLECULAR RECOGNITION OF SIALIC ACIDS BY IMMUNITY RECEPTORS: COMPUTATIONAL APPROACHES

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Sialic acids are negatively charged nine-carbon carbohydrates predominantly found at the terminal portion of glycan chains and responsible of modulating a wide range of biological processes, which confers them a critical role in many health disorders [1]. Some of them are related to the immune system, mainly by acting as self and non-self markers through the specific recognition by immunity proteins such as Siglec-2 and factor H (FH). Siglec-2 (Sialic acid-binding immunoglobulin-like lectin-2), also known as CD22, is a glycoprotein exclusively expressed at the surface of B-cells.[2] There, it acts as an inhibitor of BCR signaling through the recognition of *cis* sialoglycans and the formation of homo-nanoclusters that establish a threshold of antigen binding necessary for B-cell activation [2]. This immunosuppressive nature of CD22 makes it an interesting target for autoimmune diseases and B-cell-derived malignancies. FH is a plasma protein that acts as the main regulator of the complement alternative pathway by discerning between self and non-self surfaces. FH is able to exhibit a higher binding affinity for surface-bound C3b when some polyanions such as some sialic acids are present by the simultaneous recognition of both molecules [3]. Since C3b binding to cell surfaces is unspecific, FH is essential to avoid complement action on self-cells. In fact, some pathogens such as *Neisseria meningitidis* take advantage of this complement regulation mechanism by expressing lipoproteins that mimic host carbohydrates, recruiting FH and avoiding complement action [3].

We here report the computational studies that we have performed to deepen into the atomic details for sialic acid recognition by CD22 and FH. Conformational analysis, docking and MD simulations have been combined to provide further knowledge about: i) the *cis* divalent binding of sialylated glycans to two CD22 molecules, and ii) the recognition of sialic acid by FH and FH-related proteins.

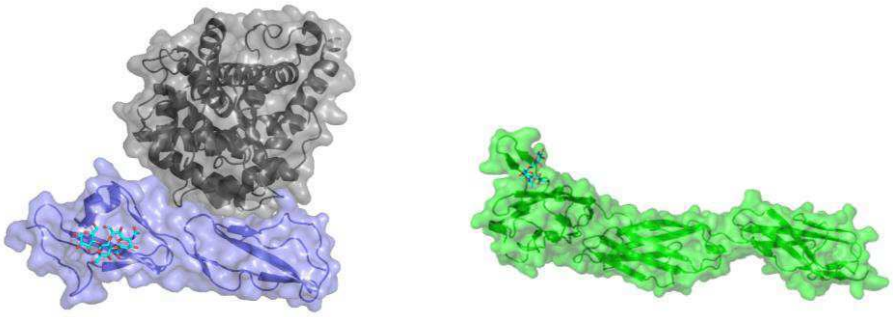

Fig. 1 - Left: X-ray crystallographic structure of FH (blue) in complex with C3d (grey) and 3'sialyllactose (cyan) (PDB code 4ONT). Right: X-ray crystallographic structure of CD22 Ig domains 1-3 in complex with 6'sialyllactose (PDB code 5VKM).

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UNSATURATED CYCLITOLS AS GLYCOSIDE MIMICS: SPONTANEOUS AND ENZYMATIC HYDROLYSIS

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Recently, Mahmud and coworkers reported the identification of a "pseudo-glycosyltransferase" as part of the biosynthetic pathway responsible for producing Validamycin A (a fungicide that targets trehalase) [1]. This pseudo-GT has structural and sequence similarity to known glycosyltransferase trehalose-6-phosphate synthase however it catalyzes transfer of a C-7 unsaturated cyclitol (valienol) moiety rather than a glycoside. Mechanistically this similarity suggests that, in the absence of oxocarbenium–ion character at the transition state, this pseudo-GT may instead exploit an allylic cation to achieve transition state stabilization through delocalization. Since glycoside hydrolases (GHs) also employ a mechanism with an oxocarbenium ion-like transition state, we anticipated that some GHs may be able to cleave pseudo-glycosidic linkages of valienol substrates and do so through an allylic cation.

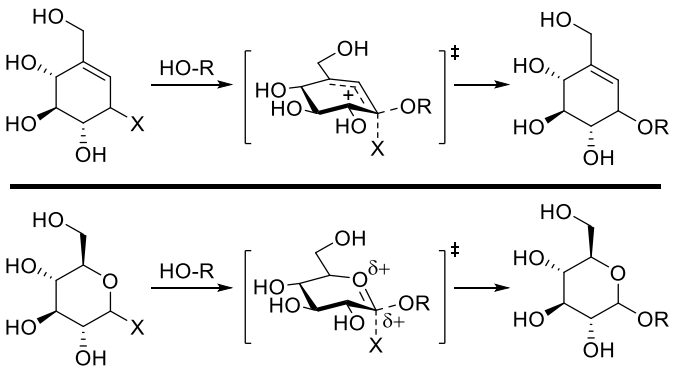

Fig. 1. (Top) Putative allylic cation TS for valienyl ether cleavage. (Bottom) Traditional glycoside hydrolysis via oxocarbenium ion-like TS.

Valienol derivatives were prepared using known methods [2] and elaborated to incorporate activated, chromogenic leaving groups at the C-1 position. We first established the rates of spontaneous solvolysis of these valienol substrates for direct comparison to the analogous glycosides. Remarkably the rates of solvolysis for valienols and glycosides were found to be nearly identical. Given that these intrinsic reactivities were similar we next screened glycoside hydrolases from the GH1 family. We identified several enzymes capable of cleaving the valienyl ether linkage. Further characterization revealed that these active enzymes completed cleavage by a mechanism nearly identical to that expected for the native glycosides but with evidence that supports allylic cation formation in place of the traditional oxocarbenium ion [3]. We anticipate that these newly identified 'cyclitolases' can be further evolved for increased and novel activities.

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PROFILING HA-NA-BALANCE BY MEANS OF AN HPLC-MS-BASED METHOD

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Influenza A Virus (IAV) is a global threat, due to both the risk of a new pandemic occurring when a new virus strain emerges and the lack of a universal vaccine. World Health Organization estimates that influenza virus causes about 3–5 million cases of severe illness, and a mortality rate of 290,000–650,000 deaths yearly [1]. The virus infects the human host through the respiratory tract causing acute respiratory diseases.

Influenza A virus has two major envelope glycoproteins: hemagglutinin (HA) and neuraminidase (NA). HA binds to sialic acid moieties of glycoconjugates of the host respiratory cells to initiate infection, whereas NA facilitates the release of the viral progeny from infected cells by cleaving sialosides. The virus attachment and release from sialoside receptors is determined by a balance between hemagglutinin (HA) and neuraminidase (NA) [2]. The molecular determinants that mediate these interactions are still poorly understood. An extensive range of HA binding studies have been carried out to profile the preferred glycan structures of these glycoproteins [3]. However, a method to determine the kinetics of the sialidase activity of NA still lacks in the virologist toolbox to fully understand the evolution of viruses.

Herein, we show a new HPLC-MS-based method using C^{13}-labelled Sialic acid as an internal standard to measure the Neuraminidase activity. The combination of Hydrophilic Liquid Interaction Chromatography (HILIC) together with the high accuracy and sensibility of Mass Spectrometry provide us with the technical requirements to measure the kinetics of two different NA, N1 (H1N1), N9 (H7N9) against different oligosaccharides (Fig. 1). This work unravels the structural features that favor the sialidase activity enabling us to shed some light on the virus evolution trajectory.

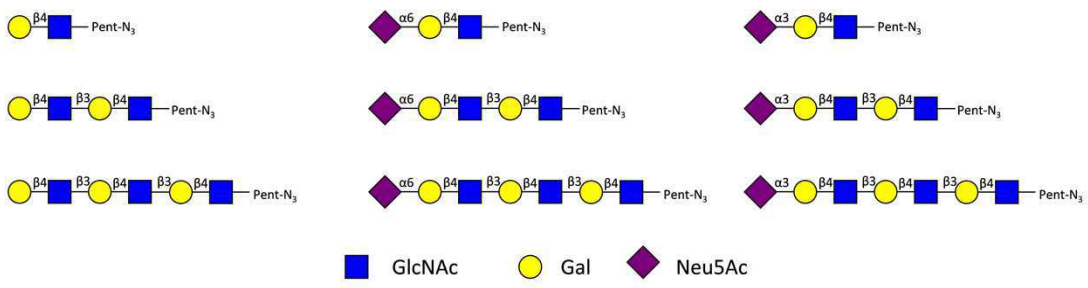

Fig. 1 - Non-sialylated, 2,6-sialylated and 2,3-sialylated oligosaccharides used in the sialidase activity experiments by MS of different Neuraminidases.

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INTERNATIONAL CARBOHYDRATE SYMPOSIUM

AWARD LECTURES

SACCHARIDES, PSEUDOSACCHARIDES, AND THEIR MIMETICS

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This lecture will cover a selection of recent and largely unpublished studies from the Crich laboratory, with the conformation of the hexopyranoside side chain and its influence on the reactivity of glycosyl donors and on the activity of pharmacologically active substances as common theme. More specifically, the lecture will address aspects of i) side chain conformation analysis, ii) the reactivity and selectivity of glycosyl donors, with particular emphasis on the bacterial sialic acids, and iii) the design, synthesis and evaluation of antibacterial agents and glycosidase inhibitors.

TN ANTIGEN STRUCTURAL INSIGHTS FOR ENGINEERING MUC1-GLYCOPEPTIDES THAT OFFER A NEW VIA IN CANCER-FIGHTING

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Tn antigen (α-O-GalNAc-Ser/Thr) is one of the most specific human tumour-associated antigens. In general, it is presented in cancer cells as a part of modified glycoproteins, such as mucin 1 (MUC1). This glycoprotein is strongly over-expressed in various tumours, displaying truncated carbohydrates that expose the Tn antigen to the immune system.[1] Consequently, this antigen has found widespread use as a biomarker and as a potential therapeutic target against cancer.

The key structural features of the Tn antigen recognition by anti-MUC1 antibodies were revealed in our group by analyzing the first crystal structure of a MUC1-like glycopeptide in complex with the SM3 antibody.[2] This study provided experimental evidence for distinct presentations of the Tn-carrying serine (Tn-Ser) and threonine (Tn-Thr) and the implications that these changes have in the context of recognition by the antibodies. The exhaustive analysis of the structure revealed that while the antigen Tn-Thr exhibited the same conformation in the SM3-bound state than in solution, the antibody recognized a high energy conformer of the Tn-Ser determinant. Moreover, the β-methyl group of the threonine is engaged in a CH-π interaction with a tyrosine of the antibody. In light of these findings, we have designed engineered MUC1 derivatives with modifications at Cβ and/or Cα of the threonine to tune the interactions with anti-MUC1 antibodies. One of these unnatural Tn mimetics was utilized to design a cancer vaccine glycopeptide, showing that the presentation and dynamics of the sugar moiety displayed by the MUC1 derivative are critical for a strong immune response.[3] In addition, the afore-mentioned X-ray structure showed that the non-terminal proline residue of the of the MUC1 recognition epitope APDT(α-O-GalNAc)RP is key in the stabilization of the antibody/antigen complex. In fact, the replacement of this amino acid by the unnatural (4*S*)-4-fluoro-L-proline provokes a clear enhancement in the affinity to anti-MUC1 antibodies and, in turn, in the detection of these antibodies in the plasma of prostate cancer patients.[4]

These findings can contribute to the design of new non-natural MUC1-based vaccines with improved immunological response against cancer and pave the way to the structure-based design of synthetic antigens with improved potential value in tumour diagnosis and detection.

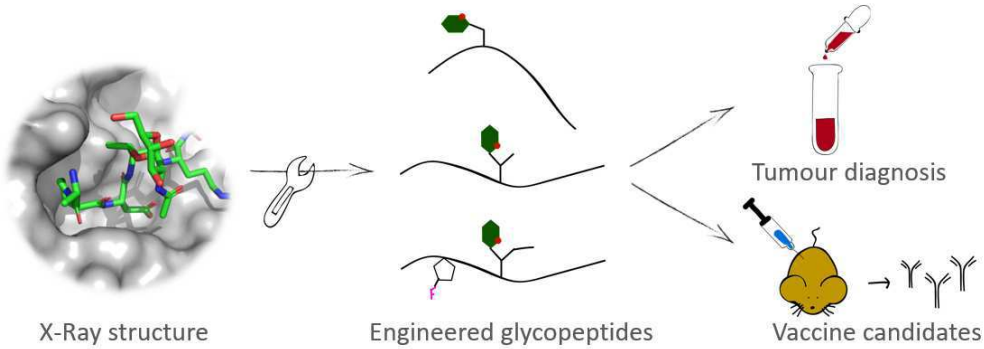

Figure1. Rational design of novel MUC1-glycopeptides for cancer detection or treatment.

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PLENARY LECTURES

THREE DIFFERENT GLYCOMIMETIC DRUGS IN CLINICAL TRIALS FOR INFLAMMATORY DISEASE AND CANCER

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All cells in Nature are coated with carbohydrates that contain dense structural information used in recognition processes among cells, bioactive molecules, and pathogens and their products. Due to this ubiquitous expression and functional properties, Glycobiology represents a relatively untapped source of novel therapeutics. The functional carbohydrates, sialyl Lea and sialyl Lex were originally discovered as tumor markers and once identified as ligands for E-selectin were shown to facilitate extravasation from the bloodstream and function in inflammatory disorders and cancer metastasis. A shared trisaccharide core structure required for binding E-selectin identified an epitope for drug design. Using empirical techniques such as NMR (both transfer NOE and STD) and X-ray crystallography, the bioactive conformation of sialyl Lea/x in the CRD of E-selectin was determined. Modifications to stabilize this conformation (reduce entropy costs, ΔS) and add second site interactions to increase the enthalpy (ΔH) of binding resulted in the rational design of potent glycomimetic antagonists with greatly improved pharmacokinetics and drug-like properties. Rivipansel was designed to bind all three selectins (E, P and L) and showed efficacy in several animal models of inflammatory disease with particularly strong effects in preventing vaso-occlusive crisis in a sickle cell mouse model. Rivipansel was advanced into clinical trials to treat sickle cell patients in crisis entering the hospital in 22 sites in the United States and Canada. The study was double-blinded and placebo-controlled and treatment with Rivipansel resulted in the consistent reduction of multiple clinical endpoints including the need for narcotic pain relief, the duration of the crisis, and the length of hospital stay. Pfizer licensed Rivipansel and is currently conducting the Phase 3 clinical trial at worldwide clinical sites.

GMI-1271 is a glycomimetic compound that was designed to specifically and more potently inhibit E-selectin (K_D = 450 nM). Acute myelogenous leukemia (AML) cells are known to infiltrate the bone marrow where they bind to E-selectin which is constitutively expressed in these microdomains and protects them from the effects of standard chemotherapy treatments. Survival studies in mice engrafted with either syngeneic AML cells, patient AML blasts or a human AML cell line all show significant improvement in survival of mice treated with the combination of GMI-1271 with chemotherapy compared to chemotherapy alone. E-selectin binding to AML cells induced chemoresistance through the well-known NFκB pathway. Treatment GMI-1271 broke this chemoresistance and in animal models resulted in continuous mobilization of AML cells into the bloodstream over a 25 hour period with no mobilization of normal hematopoietic stem cells (HSC) observed. GMI-1271 was advanced into human clinical trials in combination with standard chemotherapy (MEC; mitoxantrone, etoposide, cytarabine) for treatment of patients with relapsed or refractory AML. After a single course of induction treatment with GMI-1271 a higher positive response rate (CR/CRi) was observed compared to historical controls. There was a remarkable low incidence of mucositis with only one grade 3/4 event. Biomarker analysis revealed that patients with AML blasts expressing the E-selectin ligand (sialyl Lex) were more likely to achieve complete remission providing strong evidence of clinical proof of concept of the mechanism of action of GMI-1271 in AML patients.

GMI-1359 is a glycomimetic compound that inhibits both E-selectin and the chemokine receptor, CXCR4. It was rationally designed to have balanced efficacy for E-selectin and CXCR4. Both of these targets function in sequestering cancer cells in the bone marrow where they escape the effects of chemotherapy. GMI-1359 was tested in survival models of mice engrafted with human solid tumors that metastasize to or reside in the bone (breast, pancreatic, osteosarcoma). In all cancer models tested, the combination of GMI-1359 with standard chemotherapy significantly improved survival over chemotherapy alone. GMI-1359 is currently being evaluated in a Phase 1 single-dose escalation trial in healthy volunteers. In this trial, volunteer participants receive a single injection of GMI-1359, after which they are evaluated for safety, tolerability, PK and pharmacodynamics.

BREAKING THE LIMITS IN GLYCAN RECOGNITION BY USING NMR

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Molecular recognition by specific targets is at the heart of the life processes. In recent years, it has been shown that the interactions between proteins (lectins, enzymes, antibodies) and carbohydrates mediate a broad range of biological activities, from fertilization and tissue maturation, to pathological processes. The elucidation of the mechanisms that govern how sugars are accommodated in the binding sites of these receptors is currently a topic of interest. Thus, unravelling the structural and conformational factors and the physicochemical features that rule the interactions of these molecules is of paramount interest. This presentation is focused on the application of state-of-the-art NMR methods both from the ligand and receptor's perspective to study molecular recognition processes between a variety of receptors of biomedical interest and glycans. Lectins, antibodies, and enzymes, both wild type and mutants, have been used as receptors with the final aim to know and to evaluate the relative importance of polar (hydrogen bonding, electrostatic interactions) and non polar (van der Waals, CH-π) forces in the recognition process. As recent examples, key details of glycan recognition will be shown, with special emphasis in the application of novel ^{19}F- and paramagnetic-NMR methods.

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SYNTHESIS AND BIOLOGICAL STUDIES OF GPIS AND GPI-ANCHORED PROTEINS

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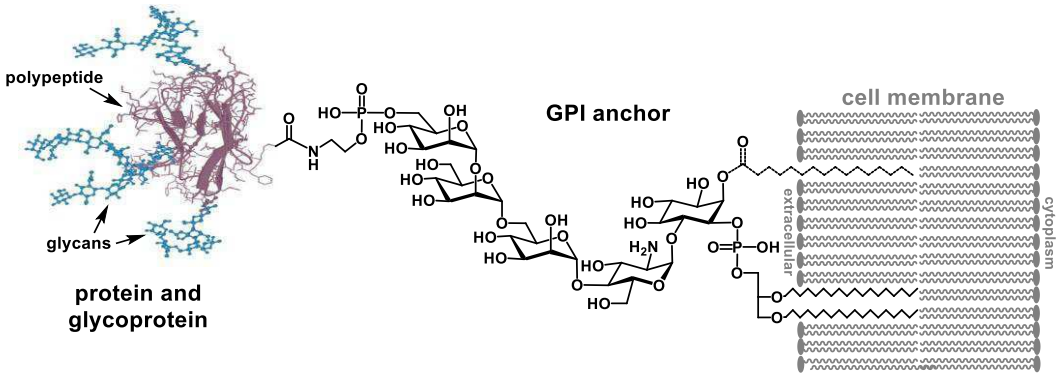
Many surface proteins are anchored onto the cell membrane via glycosylphosphatidylinositols (GPIs), a group of complex glycolipids sharing the following conserved core structure: $H_2NCH_2CH_2OP(O)(OH)$-6-α-Man-(1→2)-α-Man(1→6)-α-Man-(1→4)-α-GlcNH_2(1→6)-inositol-1-P(O)(OH)-glycerolipid [1]. GPIs and GPI-anchored proteins play a critical role in various biological and pathological events [2]. To study these events, it is essential to have access to GPIs and GPI-linked proteins in homogeneous and structurally defined forms, which represents a significant synthetic challenge [3].



Our research aims at developing new methods for the synthesis of natural GPIs, GPI-anchored peptides, glycopeptides and proteins, and their derivatives, as well as investigating GPI biology by using synthetic GPIs and GPI conjugates. In this regards, we have developed a convergent synthetic strategy for GPIs utilizing phospholipidated pseudodisaccharides as common key intermediates and applied the strategy to various GPIs and related analogs [4]. With *para*-methoxybenzyl group as a permanent protecting group for hydroxyl groups, the strategy was also employed to synthesize GPIs with unsaturated lipids and other functionalities [5]. Moreover, we have developed some chemical and enzymatic methods for the synthesis of GPI-linked peptides, glycopeptides, and proteins [6] The synthetic molecules have been used to probe GPI-bacterial toxin interactions, GPI-cell membrane interactions, GPI structure-activity relationships, and GPI-anchored proteomics [7].

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CONSTRAINED GLYCOMIMETICS OF MUCIN ANTIGENS

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Mucins are biomarkers for adenocarcinomas, are involved in malignant pathways and validated immune- targets. The immuno-dominant epitope of MUC1, the most studied cancer mucin, is a repetitive esapeptide presenting the saccharidic antigens: alpha-Tn, TF or STn (generally known as Tumor Associated Antigens -TACAs). These antigens are exclusively formed on cancer cells and almost absent on normal cells [1].
MUC1-based immunotherapy has been explored for the treatment of breast cancer, colon and pancreas but with scarce success for native TACAs are self-like, generally unstable in vivo and not efficiently presented to the immune system which remains silent [2].
Non self-like mimetics of alpha-Tn, TF or STn with higher stability and a better presentation when linked to the MUC1 esapeptide or to an immunogenic carrier-protein might address for a better immuno-recognition and for a more effective response [3]. We recently designed and synthesized constrained mucin mimetics to perturb the self tolerance [4]. Thanks to their saccharidic nature and structures, strictly related to native TACAs, their possible appearance of xeno-antigen determinants is minimized and a specific recognition in vivo by the immune system was observed.
The synthesis of alpha-Tn, TF and STn mimetics, the Tn-mimetic multivalent presentation, in vitro and in vivo tests will be discussed [5-7].

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GLYCOSCIENCE AND THE ALGAE

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The algae are an enormous, diverse collection of organisms, ranging from single celled, micron diameter microorganisms to seaweeds that are many metres in length. While there is a general appreciation that macroalgae are sources of polysaccharides - alginates and fucoidans, for instance - the glycobiology of the algae in general remains largely unexplored. This presentation will highlight aspects of the carbohydrate and natural products capability of the freshwater microalgae Euglena gracilis, which have been enabled by transcriptomics and follow up biochemical studies. Further studies on the glycoscience of the harmful-algal-bloom causing Prymnesium parvum and its' lytic virus will also be presented, along with our efforts to provide practical solutions for early detection of blooms and their management.

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N-GLYCANS ON PROTEINS

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Chemical syntheses of glycoproteins having homogeneous oligosaccharides were performed in order to understand the function of oligosaccharides.

The glycoprotein quality control (GQC) system in the endoplasmic reticulum (ER) effectively uses chaperone-type enzymes and lectins such as UDP-glucose:glycoprotein glucosyltransferase (UGGT), calnexin, calreticulin, protein disulfide bond isomerases (ERp57 or PDIs), and glucosidases to generate natively folded glycoproteins from nascent glycopolypeptides. However, the individual processes of the GQC system at the molecular level are still not well understood.

We chemically synthesized a series of several homogeneous glycoproteins bearing M9-high-mannose type oligosaccharides (M9-glycan), such as erythropoietins (EPO), interferon-β and interleukin 8 and their misfolded counterparts, and used these glycoprotein probes to better understand the GQC process [1-3]. Analyses by LC-MS showed that the synthetic misfolded glycoproteins refolded to their native forms through folding intermediates, indicating that there was a relationship between glucosylation and the glycoprotein refolding processes. Experiments using these probes demonstrated that the GQC system isolated from rat liver acts in a catalytic cycle regulated by the fast crosstalk of glucosylation/deglucosylation in order to accelerate the refolding of misfolded glycoproteins.

In order to understand the role of sialyloligosaccharides (sialyglycan) of secreted glycoproteins, we selected EPO as a target molecule and designed an efficient synthetic strategy for the chemical syntheses of five homogeneous EPO glycoforms varying in glycosylation position and the number of human-type biantennary sialyloligosaccharides [4,5]. All EPO glycoforms showed biological activity dependent on glycosylation number and their position *in vivo*.

This presentation will discuss the function of sialyglycan and M9-glycans on proteins.

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SYNTHESIS AND AN ENABLING TECHNOLOGY FOR UNDERSTANDING GLYCAN FUNCTION AND ASSEMBLY

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Synthetic glycoconjugates are essential biological probes. This seminar will focus on ongoing investigations on the synthesis of complex glycans and their used in understanding the assembly and function of glycans in biology. Emphasis will be on glycans of microbial origin, including those from mycobacteria.

CARBOHYDRATE-LECTIN INTERACTIONS – WHAT MAKES THEM UNIQUE?

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Over the last two decades, a wealth of physiological and pathophysiological functions related to carbohydrate-lectin interactions have been uncovered. However, only a fraction of these discoveries have led to new therapeutic concepts [1]. The reasons are manifold: first, carbohydrates are generally regarded as highly demanding lead structures because of their notoriously insufficient pharmacodynamic (PD) properties, as well as their nondrug-like pharmacokinetic (PK) profiles. In addition, lectins typically exhibit solvent-exposed, extended binding sites, and are therefore often considered to be undruggable targets. However, an improved understanding of the principles controlling carbohydrate-lectin interactions have recently led to a number of promising preclinical and clinical candidates, e.g. for the therapy of inflammation [2], cancer [3], and viral and bacterial infections [4].

In this lecture, the various pharmacodynamic and pharmacokinetic drawbacks traditionally associated with lectin targets will be discussed, using specific examples from selectins, siglecs, and bacterial lectin targets.

In the second part, solutions to these PK/PD drawbacks will be presented, exemplified by approaches leading to glycomimetics with nanomolar affinity as well as drug-like pharmacokinetic properties.

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TAYLOR-MADE CYCLODEXTRINS FOR BIO-INSPIRED APPLICATIONS

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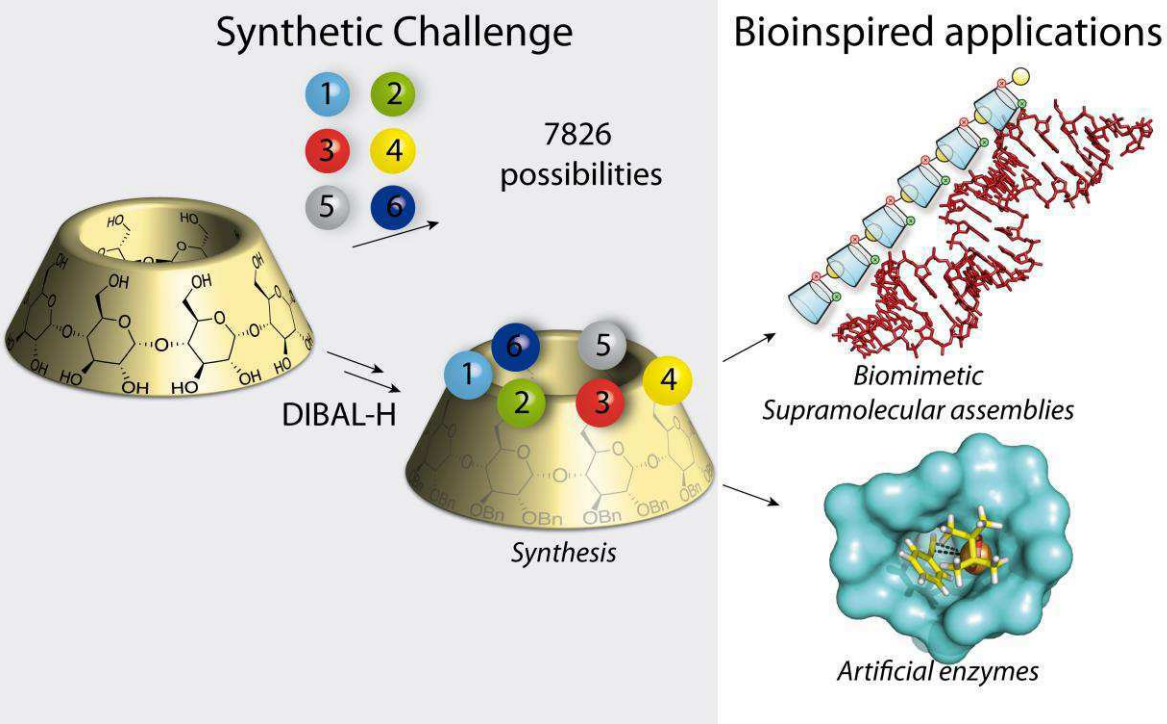
Site-selective functionalization of complex molecules, which consists in targeting only one position out of many similar ones, is a particularly demanding challenge. Concave molecules such as cyclodextrins desperately need efficient and regioselective poly hetero-functionalization methods to expand their field of applications, but this task is highly difficult because of their high symmetry. As an illustration, there are 7826 ways to arrange six functions on the primary rim of α-cyclodextrin and 117655 ways to arrange seven functions on the primary rim of b-cyclodextrin. We delineated several strategies to access polyhetero-functionalized cyclodextrins [1]. The access to such complex structures allows applications in a wide range of areas including biomimetic supramolecular assemblies [2] and catalysis [3] that will be illustrated.



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OPTOGLYCOMICS: PHOTOSWITCHABLE GLYCOCONJUGATES TO EXPLORE CARBOHYDRATE

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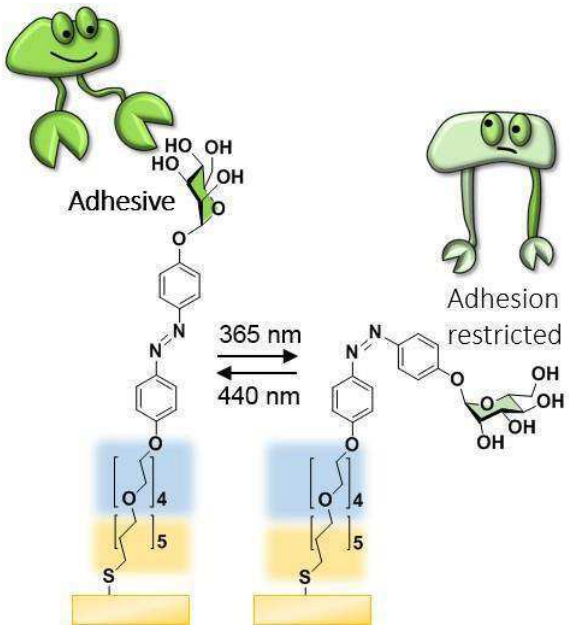
All cells are covered by a sweet molecular layer of particular complexity, dimension and biological significance. This extracellular compartment is called a cell's glycocalyx. Molecular recognition of glycocalyx constituents such as glycoconjugates and their interaction with specialized proteins are fundamental to cell biology. An important part in the orchestration of carbohydrate recognition on the cell surface is apparently played by relative orientation of sugar epitopes. In order to investigate the relevance of sugar orientation, we have started a program involving sugars & light, to "switch" carbohydrate orientation on surfaces, such as for the control of bacterial adhesion (cf. Figure). We suggest to name this Photosensitive approach to glycomics "optoglycomics".



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LECTINS FROM BACTERIA AND FUNGI: THERAPEUTICAL TARGETS AND RESEARCH TOOLS

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Lectins are frequently involved in infection by pathogens: human lectins act as receptors for micro-organisms polysaccharides, while pathogens receptors bind to human glycoconjugates. In the latter case, lectins mediate the endocytosis of many bacteria, viruses and toxins that stick to specific glycolipids on the cell membrane. Thus lectins constitute strategic elements for cell adhesion and cell entry. Furthermore, the multivalency of lectin is proposed to play a role in their strong avidity for glycosylated cell surfaces and also in their ability to affect membrane dynamics by clustering glycosphingolipids. The role of lectins in membrane invagination indicates that they could also play a role in internalization of intracellular pathogens.
Because of their fine specificity towards human epitopes, lectins can also be used as diagnostic tools or in quality control for verifying the glycosylation of therapeutic glycoproteins. Recombinant lectins offer better reproducibility and availability than the ones purified from plants, fungi or invertebrates Furthermore, they can be modified, thereby producing neolectins with controlled specificity and valency.

PROCESSING OF O-MANNOSYL GLYCAN AND ITS PATHOLOGICAL ROLE IN MUSCULAR DYSTROPHY

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Glycosylation is the most common post-translational modification of proteins. The past decade of research on glycan function has revealed the etiology of a growing number of human genetic diseases including muscular dystrophy with aberrant glycan formation.

O-Mannosyl glycan formation is important in muscle and brain development. α-Dystroglycan (α-DG) is a high-glycosylated surface membrane protein. The main glycan of α-DG was found to be O-mannosyl glycan, which is required for binding with various ligands including laminin to form stable complexes in the membrane. We identified and characterized glycosyltransferases, protein O-mannose ß1,2-N-acetylglucosaminyltransferase (POMGnT1) and protein O-mannosyltransferase 1 (POMT1) and its homolog, POMT2 are involved in O-mannosyl glycan formation. Then POMGNT1 is found to be responsible for muscle-eye-brain disease (MEB) and POMT1/2 are for Walker-Warburg syndrome (WWS). MEB and WWS are congenital muscular dystrophies with brain malformation and structural eye abnormalities, and are named as dystroglycanopathies. The common biochemical feature of dystroglycanopathy is abnormal glycosylation of α-DG. In just the last few years, the entire structure of O-mannosyl glycan has been identified. The tandem ribitol-phosphate and GlcA-Xyl repeat structures are unique, and the former structure is essential for the latter structure formation [1]. The tandem ribitol-phosphate structure was shown to be synthesized through the sequential enzymatic actions of fukutin and FKRP as ribitol-phosphate transferases and LARGE synthesizes multiple GlcA-Xyl repeats. Fukutin, FKRP, and LARGE are causative for different dystroglycanopathies. Finally, we found that fukutin forms a complex with POMGnT1, and the POMGnT1-fukutin complex is important to form a platform that requires further glycosylation of the GlcA-Xyl repeat by LARGE [2].

Details of molecular pathology of dystroglycanopathies are not fully understood and structures and processing of O-mannosyl glycans are highly complicate [3]. I will focus on the relation between aberrant glycosylation of α-DG and dystroglycanopathies. Possible regulatory mechanism of O-mannosyl glycan synthesis will be also discussed.

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THE INTERWOVEN CHALLENGES OF CARBOHYDRATE SYNTHESIS AND ANALYSIS

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Many advances in understanding the role of carbohydrates in biological systems are stalled by the lack of diverse and chemically well-defined glycan structures and methods to quickly and definitively identify such structures. Because these problems of carbohydrate synthesis and analysis are interrelated, we have taken a systems approach to tackling these challenges. This talk will provide an overview of the current challenges in the development of solution-phase-based automated oligosaccharide protocols [1, 2], with a particular focus on the use of light fluorous tags and including access to monosaccharide building blocks, and the development of robust *de novo* carbohydrate sequencing workflows [3,4]. A new open-source approach to building automated synthesizers and computational approaches [5] will also be briefly discussed.

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RE-ENGINEERING BACTERIAL TOXIN LECTINS FOR SYNTHETIC GLYCOBIOLOGY

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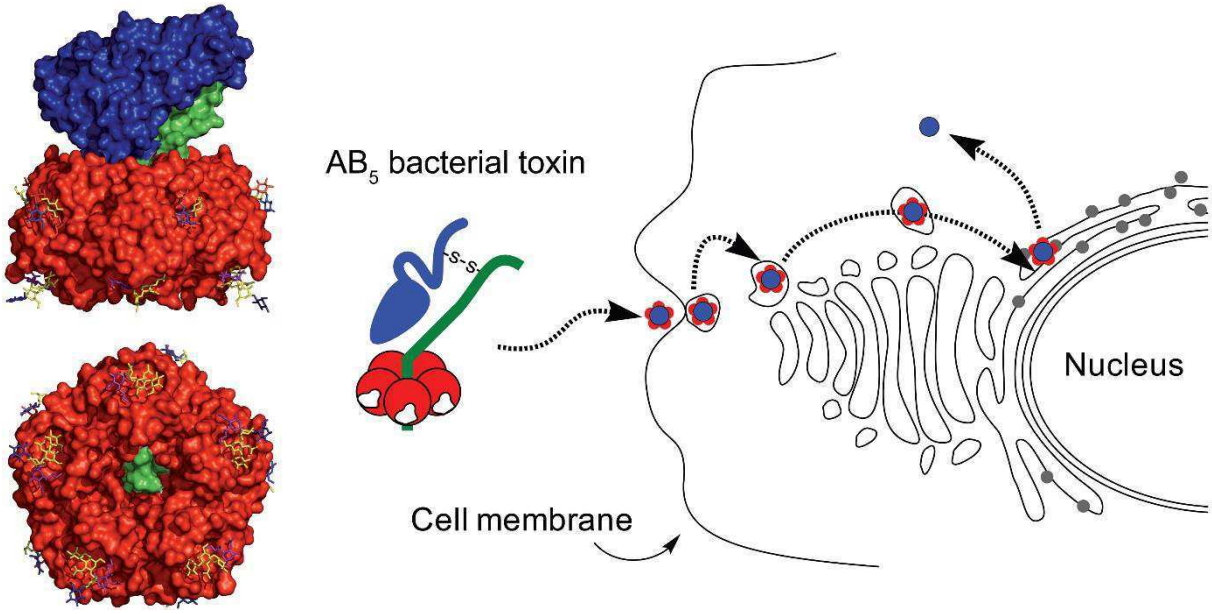
In the post-genomic era, synthetic biology has provided tools for the precise (re)design of genes and proteins to create novel biological systems that are revolutionising both medicine and materials science. These developments coincide with our rapidly growing understanding of glycobiology and the physical properties of the glycocalyx that control the flow of information to the cell membrane. We are thus poised to exploit “Synthetic Glycobiology” to control diverse cell surface processes through reengineering both the glycocalyx, and the proteins with which it interacts.

In this presentation I will describe several strategies that we have been developing for the redesign and modification of bacterial toxins for diverse applications as multivalent inhibitors, targeted delivery systems for directing molecular cargoes into motor neurones and as building blocks for constructing more complex architectures of lectins, some of which gain novel emergent properties including vesicular fusion.



MATERIALS MADE OF SYNTHETIC POLYSACCHARIDES

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Pure glycans are key to establishing structure-function relationships for this class of biopolymers. Automated glycan assembly (AGA) [1,2] allows now for the rapid assembly of polysaccharides using a commercial synthesizer [3]. Access to diverse glycans including those with cis-linkages [4] and as long as 50-mers [5] enables materials science investigations. These synthetic polysaccharides can be combined much like “molecular LEGO” [6] to create even larger oligosaccharide assemblies to address fundamental questions of carbohydrate structure, folding and material science applications. The synthetic work is combined with molecular modelling and physical methods to characterize carbohydrate structure.

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CHEMICAL BIOLOGY TOOLS TO PERCEIVE AND PERTURB GLYCANS AND CARBOHYDRATE PROCESSING ENZYMES IN LIVING SYSTEMS

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In this presentation I will discuss selected recent advances from the Laboratory of Chemical Glycobiology at Simon Fraser University, focusing on our interests in the development of chemical biology tools to probe and perturb glycans and carbohydrate processing enzymes in cells and in vivo. Topics to be discussed will include studies on the function of the two enzymes that regulate levels of the intracellular O-GlcNAc modification, the creation of new inhibitors of these enzymes that can be used in vivo, and the use of these tools to uncover physiological roles for this modification in nutrient sensing. I will also describe our recent research on the creation and use of new tools to monitor the activity of glycoside hydrolases in living cells, with particular focus on the lysosomal enzyme glucocerebrosidase (GCase), which is a major genetic risk factor for Parkinson Disease (PD).

Their use, alongside chemical biology probes for monitoring O-GlcNAc levels in cells and in vivo, for fundamental research into understanding the functional roles of O-GlcNAc in cell and mammalian physiology will be summarized. Finally, recent work on the synthesis and optimization of live cell imaging agents that allow quantitative measurement of the activity of glycosidases in live cells will be outlined. Specific examples exemplifying our work at the chemistry biology interface will be selected from our studies illustrating the use of these tools for examining the roles of O-GlcNAc in nutrient sensing, identifying the protective roles of O-GlcNAc in animal models of Alzheimer Disease (AD), evaluating the effects of altered O-GlcNAc on cellular proteostasis, describing the role of O-GlcNAc in regulating transcription, creating antibody-free methods for mapping O-GlcNAc to chromatin, and developing screening methods to identify modulators of enzymes including glucocerebrosidase (GCase), which is a key risk factor involved in Parkinson Disease (PD).

DECIPHERING THE GLYCOPROTEOME: A STEP TOWARDS UNDERSTANDING THE COMPLEXITY OF BIOLOGICAL SYSTEMS

Pauline M Rudd[a],[b] Roisin O'Flaherty[a] Radka Saldova[a]

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Biological systems are mosaics of interrelated systems, pathways and molecules. Organisms are required to perform effectively in their customary environment and also to have the flexibility to adapt to changing circumstances. This clear in all species but is highly refined in human adaptation where evolution, the environment and culture together provide multiple levels of fine tuning that is required for both day-to-day and long term survival of individuals and groups. At a molecular level, DNA, proteins and carbohydrates provide diversity at different points during the biosynthesis of glycoproteins, enabling the levels and fine structures of the molecules to be controlled. These three key components of matter are themselves subject to many modulators including lipids, metabolites and the transcriptome. We have designed an automated glycoanalytical platform capable of quantitatively interrogating the N-glycosylation of large numbers (upwards of 10K) of glycoprotein samples and linking these data with metadata from the same samples derived from other –omics including genomics, transcriptomics, lipidomics, metabolomics and proteomics. The aim is to link big data sets from individuals to better understand pathways and systems to identify key tipping points that lead to disease.

BACTERIAL HIGHER-CARBON SUGARS – KEY COMPOUNDS IN IMMUNE RECOGNITION

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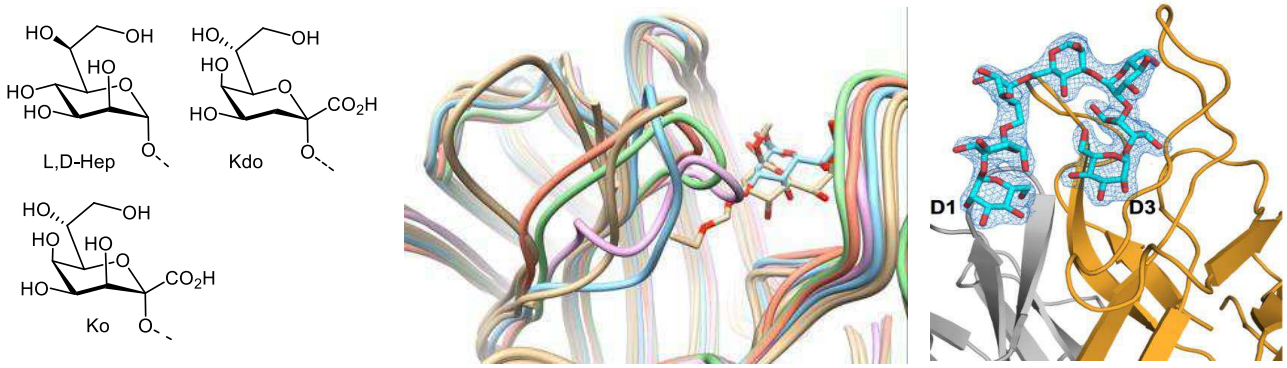
Infections by Gram-negative bacteria present an increasing threat to global human health due to an exponential increase of resistance against multiple antimicrobial drugs. [1] Non-mammalian higher carbon sugars occurring in the outer leaflet of the outer membrane in Gram-negative bacteria, however, are unique and relatively conserved structural entities in bacterial lipopolysaccharides (LPS) comprising L-glycero-D-manno-heptose (L,D-Hep), 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) and D-glycero-D-talo-oct-2-ulosonic acid (Ko) as examples. Inhibition of the underlying biosynthetic pathways leading to avirulent strains opens new perspectives to combat bacterial infections. In addition, these sugars are prime candidates to be recognized as non-self antigens in the interaction with the adaptive and innate immune system. Examples will be illustrated for a library of germline-encoded, anti-Kdo antibodies detailing the molecular features seen in highly specific versus promiscuous binding motifs and providing structural evidence for selection of CDRH3 conformations by native and artificial Kdo ligands.

Based on the structure of the LPS core region from Rhizobium radiobacter Rv3, a panel of oligomannoside-containing neoglycoconjugates has recently been synthesized, which trigger a broadly neutralizing anti-HIV antibody response in humanized transgenic rats [2,3]. The lead compound acts as a bacterial mimetic of Man9GlcNAc2, binding to neutralizing antibodies of the PGT family in a near identical conformation. This way, however, the evasion of immuno-silent epitopes can be overruled by the bacterial analogs, enabling promising future options for anti-HIV vaccine development.



Acknowledgment: Financial support from FWF (grant P26919).

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ON PHOSPHATES AND SUGARS

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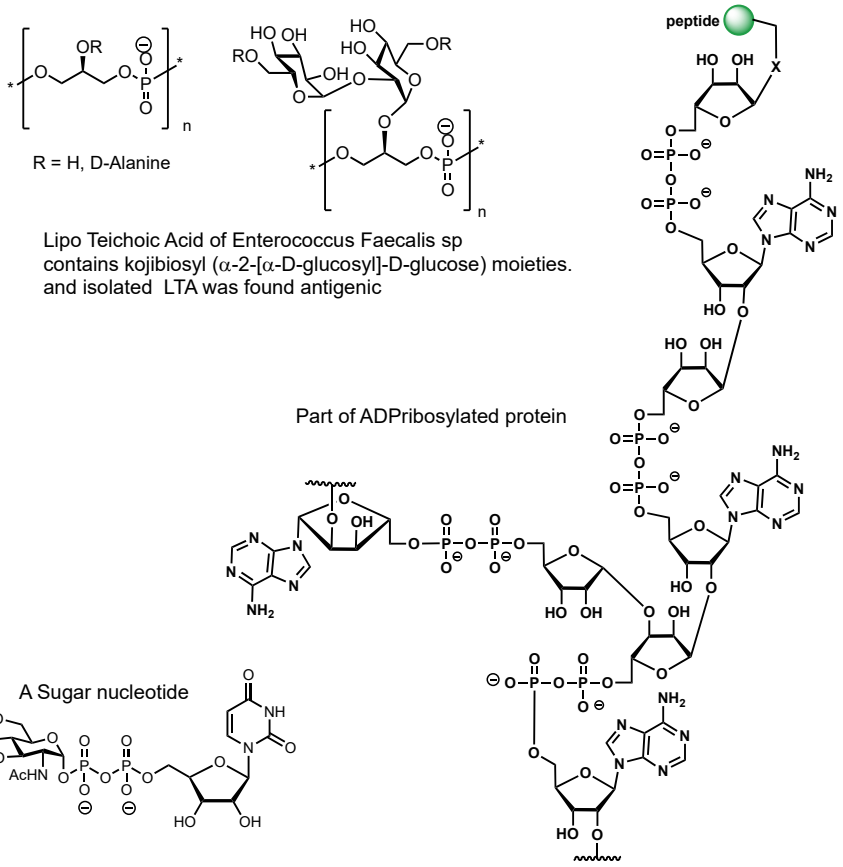
Besides nucleic acids, many biologically active polymers can be regarded as phosphodiesters. A lot of synthetic methods developed for the assembly of oligonucleotides can be used for the synthesis of these phosphodiesters. The synthesis in solution and on solid support of teichoic acids will be discussed. Teichoic acids [1] are structurally diverse glycosylated poly(alditolphosphates), that occur in the cell wall of Gram-positive bacteria and the prepared oligomers can be used to assess their immunological properties.



Pyrophosphates are another important structural element of several biomolecules. A general procedure to construct sugar-nucleotides, the sugar donors of glycosyl transferases, will be discussed. This procedure can also be applied for the introduction of pyrophosphate moieties in ADP-ribosylated peptides [2]. Adenosine diphosphate ribosylation (ADP-ribosylation) is a post-translational modification of proteins in which specific enzymes transfer one or more ADP-ribosyl (ADPr) residues from NAD+ to nucleophilic amino acid side chains. ADP-ribosylation plays a regulatory role in important processes such as DNA repair. The synthesis of fragments of ADP-ribosylation polymers will be discussed.

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INVITED LECTURES

PROBING SPECIFIC CELL-SURFACE HEPARAN SULFATE-PROTEIN INTERACTIONS

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Glycans are exceptionally diverse and complex that deciphering the functions embedded within the glycome is a substantial challenge. The multiple regio- and stereoselective permutations for linking several monosaccharide units and the modifications that may follow chain assembly allowed these complex sugars to hold structural information densities that surpass DNA or proteins. With biosynthetic pathways that are regulated rather than template-driven, the sugars are usually expressed as an array of related structures that may possess subtle differences in activity. Several biological processes involve glycans, yet understanding their ligand specificities is impeded by their inherent diversity and difficult acquisition. Generating synthetic sugar libraries for bioevaluations forms the core of chemoglycomics approaches to unravel glycan structural information. The one-pot strategies to prepare a variety of cell-surface carbohydrates will be presented. Affinity screening and further 3-dimensional structural analysis of these synthesized sugars with proteins involving in infectious diseases to provide key insights at the molecular level will be also highlighted.

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CHEMICAL LABELING AND QUANTITATIVE ANALYSIS OF PROTEIN O-GlcNAcylation

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O-GlcNAc (O-linked N-acetylglucosamine) modification is a non-canonical form of protein glycosylation, which occurs intracellular and is dynamically regulated. In mammalian cells, more than a thousand cytoplasmic, nuclear, and mitochontrial proteins are post-translationally modified with O-GlcNAc, which regulates many important biological processes. OGT and OGA are two enzymes responsible for dynamically cycling O-GlcNAc on and off the modified proteins. How O-GlcNAc affects protein stability remains to be investigated at the proteome level. In this talk, I will present the development of a time-resolved quantitative proteomic strategy to analyze the turnover dynamics of O-GlcNAcylated proteins. We found that not all protein O-GlcNAcylation events were reversible. Interestingly, a subset of O-GlcNAcylated proteins are hyper-stable, exhibiting minimal removal of O-GlcNAc or degradation of protein backbones. The hyper-stable population included three core proteins of box C/D small nucleolar ribonucleoprotein complexes (snoRNPs), fibrillarin (FBL), NOP56, and NOP58. Our studies showed that O-GlcNAcylation stabilized these proteins and regulated snoRNP assembly. Blocking O-GlcNAcylation on FBL altered the 2'-O-methylation of ribosomal RNAs, and impaired cancer cell proliferation and tumor formation in vivo. Our work reveals stable O-GlcNAc as an important regulatory mechanism for stabilizing proteins.

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MULTIVALENT NEOGLYCOCONJUGATES AS TOOLS TO INTERFERE WITH THE IMMUNE SYSTEM

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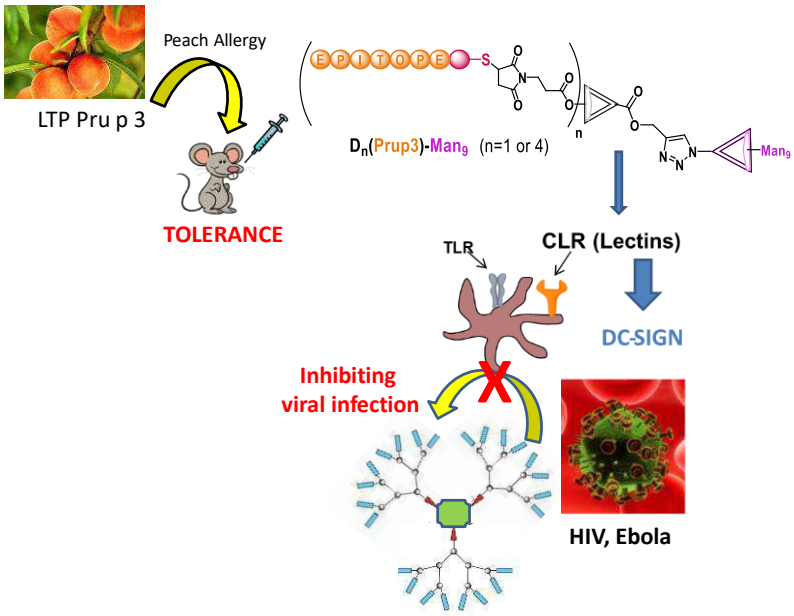
DC-SIGN (Dendritic Cell-Specific ICAM-3 Grabbing Non-Integrin) is a C-type lectin expressed mainly on the surface of Dendritic Cells (DCs), one of the most relevant Antigen Presenting Cells (APCs) in the immune system. This lectin recognizes highly glycosylated conjugates that contains fucose or mannose units. Our group has developed different glycodendritic structures to target DCs through DC-SIGN with the aim to interact with this lectin for different applications in biomedicine.

We have explored different multivalent presentations of mannoses using a variety of central scaffolds to construct glycodendritic molecules capable to inhibit viral infection mediated by DC-SIGN such as those caused by HIV or Ebola virus. These multivalent systems have shown EC_{50} in the μM to pM range, inhibiting the infection processes [1]. Also, we have combined in a single well-defined structure a multivalent presentation of 9 mannose units together with peptide epitopes, in particular a T epitope of the Pru p 3 LTP, to create synthetic vaccines for immunotherapy in allergy diseases [2]. These systems have been tested in vitro and in vivo demonstrating the capacity to protect mice against peach allergy anaphylaxis. Moreover, one of the systems has protected mice against anaphylaxis even 5 weeks after treatment inducing tolerance.



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SYNTHESIS OF BIOACTIVE GLYCOPEPTIDES AND NEOGLYCOPEPTIDES USING SOLID PHASE PEPTIDE SYNTHESIS, NATIVE CHEMICAL LIGATION, CLICK CHEMISTRY AND ENZYMATIC GLYCOSYLATION

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Novel chemical methods to access homogeneous samples of glycopeptides and glycoproteins have developed rapidly in recent years owing to an increased need to investigate both their roles in molecular biology and their potential as therapeutic agents. Glycopeptide and glycoprotein mimetics with non-native linkages, so-called neoglycopeptides and neoglycoproteins respectively, are often readily accessible and usually available as a single neoglycoform.

This lecture will showcase some of our work on the application of the copper(I)-catalyzed azide-alkyne "click" cycloaddition (CuAAC) for the chemical synthesis of neoglycopeptides and neoglycoproteins [1]. Execution of the neoglycopeptide "click chemistry" on solid phase and its use in combination with other ligation methods such as native chemical ligation, widens the scope and complexity of the neoglycopeptide constructs that can be accessed via chemical methods. The synthesis of neoglycopeptide mimics of erythropoietin and the pancreatic hormone amylin [2] will be described. In the latter example combination of click chemistry with enzymatic glycosylation provided a powerful method to access neoglycopeptides decorated with complex carbohydrate moieties.

The synthesis of the natural glycoactive bacteriocin Glycocin F will also be described. Glycocin F is produced by *Lactobacillus plantarum* KW30 glycosylated with two β-linked *N*-acetyl-D-glucosamine (GlcNAc) sugars, and exhibits rapid and reversible bacteriostasis on susceptible cells. The role of certain structural features of glycocin F have not been studied to date. The synthesis of various modified glycocin F analogues using solid-phase peptide synthesis (SPPS) and native chemical ligation (NCL) will be described [3], Access to the synthetic analogues enabled us to probe the role of different structural features of this glycopeptide. Our results indicate that the bacteriostatic activity of glycocin F is controlled by the glycosylated interhelical loop, while the glycosylated flexible tail appears to be involved in localizing the peptide to its predominate cellular target.

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FROM STEREOCONTROLLED GLYCOSYLATION TO AUTOMATED OLIGOSACCHARIDE SYNTHESIS

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From the building blocks of nature to disease-battling therapeutics and vaccines, carbohydrates have had a profound impact on evolution, society, economy, and human health. Numerous applications of these essential biomolecules in many areas of science and technology exist, most of which can be found in the area of therapeutic-agent and diagnostic-platform development. Although carbohydrates are desirable for the biological, pharmaceutical and medical communities, these molecules are very challenging targets for chemists because of the need for functionalization, protecting and leaving group manipulations, controlling anomeric stereoselectivity, etc. The development of practical and general methods for chemical glycosylation [1] and oligosaccharide synthesis [2] represent demanding areas of research.

At the core of this presentation is the development of new methods, strategies, and technologies for chemical glycosylation and oligosaccharide assembly. These tools will be discussed in light of recent results related to the H-bond-mediated aglycone delivery [3], regenerative glycosylation [4], and HPLC-assisted automated synthesis [5]. The effectiveness of methods developed will be illustrated by the synthesis of medicinally relevant oligosaccharides and conjugates thereof [6]. This work has been generously supported by the National Institutes of Health (USA).

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UNSATURATED SUGAR SYNTHONS FOR NEWER CHEMICAL GLYCOSYLATIONS AND BIOCONJUGATIONS

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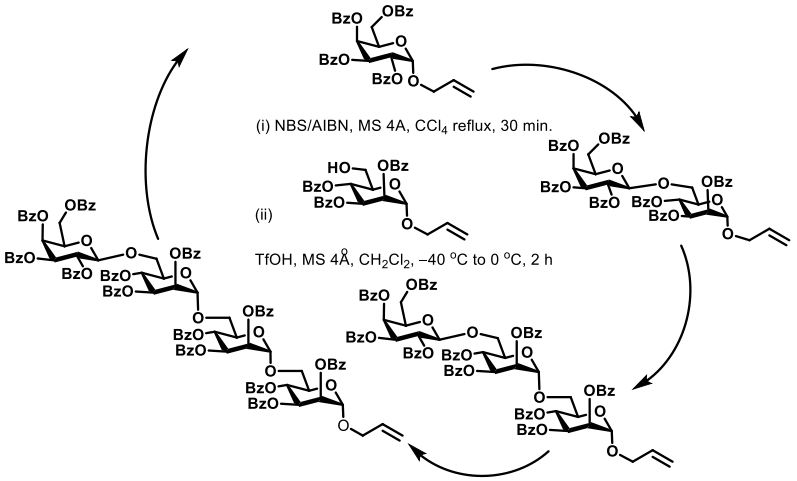
Presence of an unsaturation in a monosaccharide offers rich possibilities to introduce defined modifications. Both *endo*- and *exocyclic* unsaturations are valuable for chemical modifications, examples are 1,2-unsaturated sugars, allyl- and pentenyl glycosides. 2,3-Unsaturated thioglycosides are excellent synthons to implement 1,3-migrations, leading to the formation of 3-deoxy-3-thio-sugars. 3-Deoxy-3-thio-sugars undergo rearrangement and additions reactions, in the form of Pummerer rearrangement and Michael addition reactions upon appropriate modifications. Generation of 2,3-unsaturated vinylsulfoxides of the sugar, followed by reactions with amine is an excellent source for bioconjugation, for example, amino-moiety of an aminoacid or a peptide, including such reactions being performed under aqueous environment. In addition to nitrogen nucleophiles, reactions with carbon, oxygen and sulfur nuclephiles are facile on sugar vinyl sulfoxides.

Pertaining to *exocyclic* unsaturations, *O*-allyl glycosides are prime examples as reactive synthons for reactions, such as, allylic carbon activations. A free-radical induced allylic halogenation is a facile reaction, the resulting allylic halide is subjected to an *endocyclic* oxygen induced elimination, leading to the formation of a glycosyl cation. Facile formation of the glycosyl cation is taken advantage to conduct a glycosylation reaction. The reaction occurs in near quantitative yields, thereby emerging to be a new glycosylation methodology and adds to the repertoire of glycosylation techniques. An allylic glycoside donor can be activated and reacted to another glycoside acceptor in a 'latent-active' manner, such that the newly-formed glycoside, for example, a disaccharide from two allylic glycoside monosaccharides, could be extended further to a trisaccharide by reaction with an allylic glycoside acceptor. Optimal reaction conditions are identified so as to extend the reactions up to tetra- and pentasaccharides, in a linear fashion of the synthesis (Figure 1). Aspects of synthesis and studies initiated from unsaturated sugars will be presented.



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PLAYING AROUND WITH GLYCOSYLATION: FROM STRATEGY TO GREEN PROCESSES

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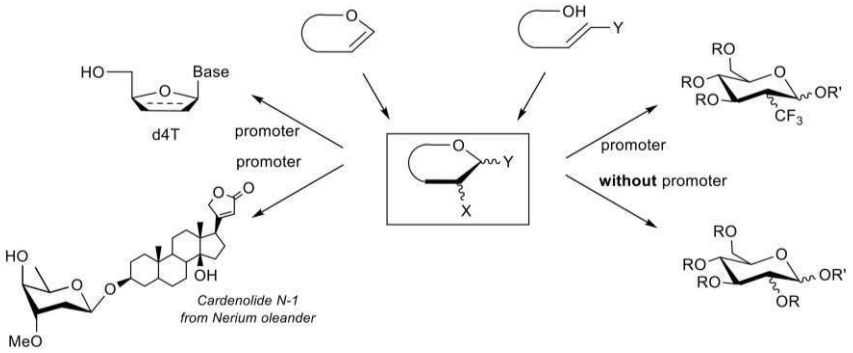
Glycosylation is a key reaction in the synthesis of relevant natural products such as nucleosides, glycosides and oligosaccharides, glycolipids, glycopeptides and glycoproteins [1]. Since the establishment of its foundation by Michael and Fischer at the end of XIX century, a large number of glycosylation methods have been described [2]. Nevertheless, the complexity and diversity of glycoconjugates continuos to demand efficient synthetic methods. All elements contributing to the glycosylation reaction affect the yield and selectivity of the process: glycosyl acceptor and donor, protecting and leaving groups, promoter, solvent, temperature, etc. However, the conjunction leaving group/activator-promoter is a key issue for the success of the reaction and hence, there have been continuous efforts to develop novel leaving groups and new promoter/catalyst pairs in order to enhance the glycosylation efficiency. Moreover, the presence of appropriate functionalities at position 2 of the glycosyl allow to control the selectivity of process. In consequence, the synthesis of 2-deoxy-glycoconjugates is a challenge that still requires attention.

In this communications we will present different aspects of the glycosylation reaction (See Scheme) developed in our laboratory [3] related with a) the stereoselective of synthesis of 2-deoxy-nucleosides or 2-deoxy-glycosides from glycals by reaction with electrophilic halogen (I) or a chalcogen (Se), b) synthesis of non-common 2-deoxy-2-iodoglycosides, c) application in the synthesis of natural products containing 2-deoxy-glycosides of gulo and talo configurations, d) synthesis of 2-deoxy-2-trifluoromethyl glycosides and comparison with the 2-deoxy-2-fluoro-deoxyglycosides, and e) our attempts about glycosylation in the absence of promotor.



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APPLICATIONS OF ORGANOCATALYSTS IN CARBOHYDRATE CHEMISTRY

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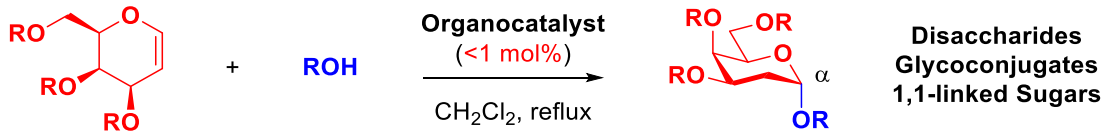
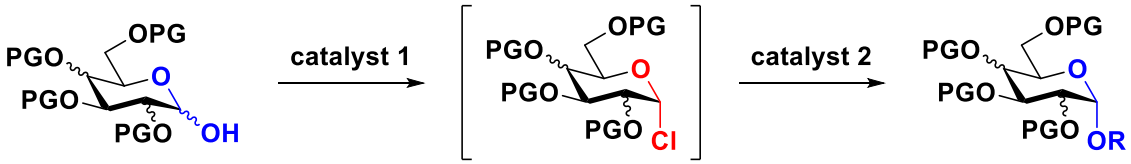
This talk will describe our group's efforts to develop organocatalyzed reactions for use in carbohydrate chemistry [1]. We have developed a simple cheap organocatalyst that is effective at 0.1 mol% for the stereoselective synthesis of 2-deoxygalactosides. This methodology has been applied on a gram scale and enables the synthesis of 1,1'-linked trehalose type disaccharides, as well as the glycosylation of steroids and amino acids. We have investigated the mechanism of this reaction and these results will be described [2].



We have systematically investigated the use of organocatalysts in stereoselective and regioselective glycosylations of a range of carbohydrate-based diols. High stereoselectivities were observed while regioselectivities varied from 1:1 to 7.3:1. The effect of protecting groups, solvent, catalyst structure and temperature on the regioselectivity will be described [3].

We will describe the use of a phosphorus-based organocatalyst for the synthesis of glycosyl halides, and its application to a dual-catalytic one-pot synthesis of disaccharides from hemiacetals [4].



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NEW SYNTHESES AND NEW EFFECTS OF GLYCOMIMETICS

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The universal role of glycans in the vast majority of biological phenomena became a scientific commonplace during the past 2-3 decades. Glycans have been shown ubiquitous to all cells in nature and essential in all known forms of life. Due to the intrinsic complexity of such molecules and the difficulties in chemical syntheses of glycosides (such as variable ring size, necessity of complicated protection-deprotection strategies, issues of stereoselectivity in the formation of glycosidic linkages) it is still a challenge to get natural glycans by pure chemical means. In addition, the sensitivity of the glycosidic bond to acidic and especially enzymatic hydrolysis represents another obstacle to the wide use of glycans as biological probes. These problems raise an ever increasing demand toward glycomimetics, i. e. molecules that resemble natural glycans' structure and/or biological function. Such compounds may be obtained by simpler synthetic pathways, are stable to hydrolysis, offer several derivatization possibilities, and can be used as glycobiological tools as well as leads for drug design. Studies of glycomimetics have already resulted in several marketed carbohydrate based medications.

This presentation will survey our recent results in the syntheses of *C*-glycosyl heterocycles and sugar-spirocycles as glycogen phosphorylase inhibitors as well as the application of anhydro-aldose tosylhydrazones and thiol-ene couplings to obtain glycomimetic compounds. In addition, recent findings about unprecedented biological effects of glycogen phosphorylase inhibitors will also be presented.

ENTIRELY CARBOHYDRATE IMMUNOGENS FOR ANTI-TUMOR mAb DEVELOPMENT

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The overarching goal for our research is to develop a cancer immunotherapeutics that target tumor-associated-carbohydrate-antigens (TACAs) found on malignant cells. In the process of carcinogenesis, certain glycosyltransferases are over-expressed, which lead to different glycosylation patterns than those of normal cells. For example, the Thomsen-nouveau (Tn) antigen is expressed abundantly on human breast, ovarian and colon cancers. These abnormal glycosylations on tumor cell surfaces provide significant opportunities for researchers to develop carbohydrate-based anticancer therapeutics.

The strategy used for most carbohydrate-based cancer vaccines entails immunogenic proteins as carriers to cross over into the cellular arm of the immune system because of the inherent T-cell independent nature of TACSs. There are both advantages and disadvantages for this strategy. We have been working with the unique zwitterionic capsular polysaccharide PS A1 to find alternative pathways avoiding protein carriers and at same time retain both a cellular and humoral immune response. PS A1 is found on *Bacteroides fragilis'* cell wall, consisting of a tetrasaccharide-core repeating unit carrying an electrostatic charge character on adjacent monosaccharides able to induce a specific and selective immune response similar to that noted for exogenous proteins. This lecture will describe the synthesis of Tn-PS A1 conjugates and their application in developing mAbs for furthering an understanding in glycoimmunology.

DESIGN, SYNTHESIS, AND DEVELOPMENT OF LECTIN LIGAND MIMETICS INTO THE CLINIC

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Galectins are glycoconjugate-binding lectins that via cross-linking of galactopyranose containing glycans are believed to influence e.g. glycoprotein trafficking, localization, residence times, which in turn may impact glycoprotein functions in cell proliferation, inflammatory processes, tumor growth, and tumor metastasis [1-3]. Consequently, this has sparked a growing interest in developing galectin inhibitors as research tools and possibly drug leads [4,5]. Herein we will present our efforts towards discovery and development of high-affinity, selective, and orally available galectin-3-inhibiting compounds [6,7] and their use in studying the role galectin-3-glycan interactions in cancer and fibrosis [8,9]. Furthermore, validation of galectin-3 as a target in fibrosis and key aspects of IND-enabling investigations and phase 1b/IIa clinical trials will be presented.

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THERAPEUTIC IN VIVO SYNTHETIC CHEMSITRY

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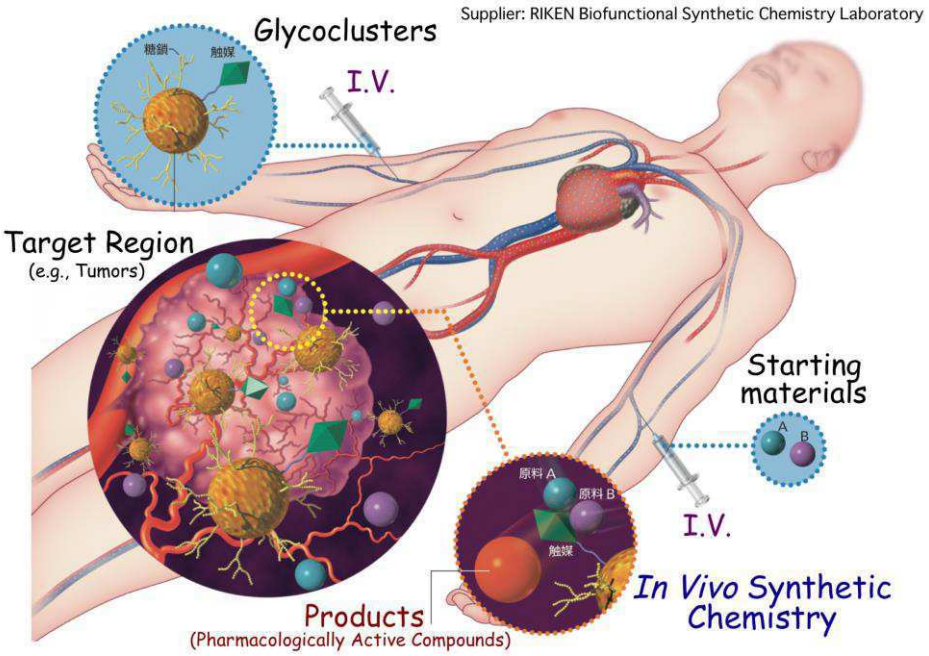
We challenge to develop a system where bioactive compounds can be synthesized within living animals, which we refer to as “Therapeutic In Vivo Synthetic Chemistry”. Main benefit of this approach is that a cascade of organic transformations can be directly executed at target regions within the body during predefined times to generate a bioactive molecule that elicits a localized biological effect. This strategy entirely circumvents the off-target and peptide instability problems associated with currently applied drugs. We realized first examples of (i) transition-metal catalyzed transformation in mice and (ii) synthetic transformation of a toxic endogenous product to diagnostic and therapeutic molecules selectively on oxidative stress-associated diseases in mice and even human patients. A part of our strategies has already met with successful outcome in clinical trials and could be applied to pharmaceutical fields and hospitals.



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SYNTHESIS AND EVALUATION OF FLUORESCENT *TRYPANOSOMA CRUZI* GPI ANCHORS IN SUPPORT OF DRUG DISCOVERY FOR CHAGAS' DISEASE

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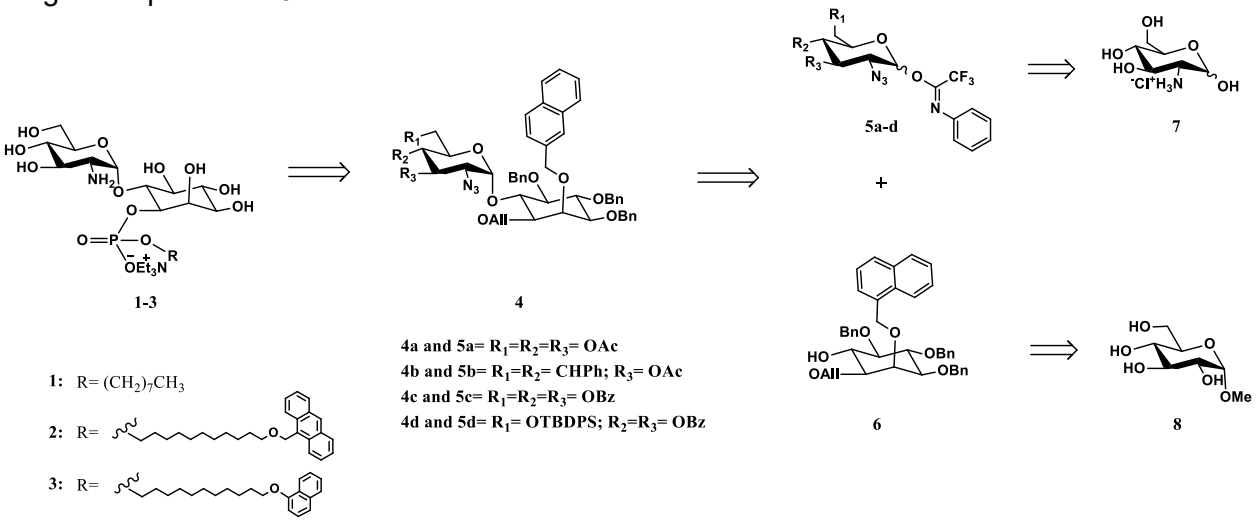
American trypanosomiasis (Chagas disease) is a life-threatening infection caused by the protozoan parasite*Trypanosoma cruzi* (*T. cruzi*). It is estimated that over 10 000 people die every year, and more than 25 million people are at risk of acquiring the disease [1]. Therapeutic arsenal is still scarce and only effective in accute phase, calling attention to the search for new compounds and more selective parasite targets. Glycosylphosphatidylinositol (GPI) anchors are molecules which comprises a conserved glycan core with a phosphoethanolamine linker and a phospholipid chain. These molecules support diverse proteins in the outer leaflet of cell membrane of all eukaryotes. GPI anchors are mainly abundant in protozoan parasites, such as trypanosomatids, and their structure can be found attached to complex phosphosaccharides, to glycoproteins and as surface glycolipids. More specifically in *T. cruzi*, GPIs anchor mucins and *trans*-sialidase in the parasite's cell surface are related to invasion and survival of parasites in host cells [2]. The high density of GPI structures at all life-cycle stages of protozoan parasites suggests that the GPI biosynthetic pathway might be an interesting target for the development of anti *T. cruzi* drugs. In this context, our research group is interested in the synthesis of GPI anchor parts that may inhibit *T. cruzi* GPI pathway. Moreover, in collaboration with Prof. Rob Field at John Innes Centre, compounds **1-3** were synthesised as probes to track GPI anchors biosynthesis in microssomal membranes of *Euglena gracilis*, a protozoan algae which can be used as a non pathogenic model to study GPI anchor pathway [3]. To achieve compounds **1-3**, four different donors (**5a-d**) were synthesized from D-glucosamine hydrochloride [4]. In parallel, the myo-inositol derivative (**6**) was obtained from methyl-α-D-glucopyranoside (**8**) in 10 steps [5]. A study of *O*-glycosylation reactions between donor compounds **5a-d** and acceptor **6** was performed to evaluate the best conditions to achieve the required α-linked pseudo-disaccharide (**4a-d**). Then, regioselective introduction of the phospholipid chain gave the target compounds **1-3**.



Compounds **1-3** will be tested using E.gracilis microsomes to elucidate the biosynthesis pathways and support the development of potential parasites GPI inhibitors.

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DETECTING LIVE PATHOGENS USING CARBOHYDRATE-BASED METABOLIC PROBES

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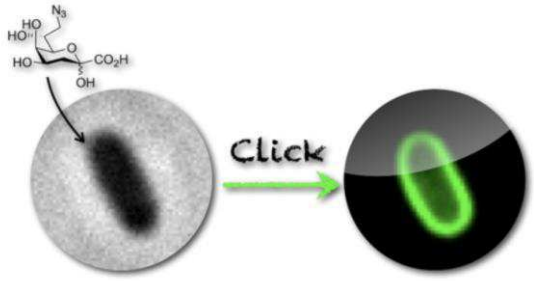
In the pre-antibiotic era, bacterial infections could have serious consequences, and some epidemic outbreaks could prove dramatic. During the 20th century, the discovery of these molecules considerably impacted our life conditions. Some bacteria remain however difficult to treat or to detect, and the development of resistant strains, combined with their rapid diffusion within our globalized societies, have considerably reduced our antibiotic arsenal. Epidemic outbreaks can regularly have severe sanitary, but also economic impact. Rapid detection and identification of these bacteria remains therefore a major challenge.

We are developing an approach to address this question, relying on metabolic labeling of the bacterial cell surface. As an example, the external membrane of Gram-negative bacteria is covered by a dense lipopolysaccharide layer (LPS) which is involved in cell integrity, but also in the virulence of some strains.

Our recent work [1] has shown that, when metabolically active, Gram-negative bacteria, can specifically incorporate a chemically modified, *azide*-containing monosaccharide within their LPS. This bioorthogonal reporter group can then be used to "reveal" labeled bacteria, using a click-chemistry ligation method. This strategy allows for rapid detection of live pathogenic bacteria.



This lecture will present our major and most recent results in this field, including the development of a specific labeling method for *Legionella pneumophila*, a bacterium responsible for legionnaire's disease [2], and a method for the concentration of bacterial samples [3].

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STRATEGIES FOR THE SYNTHESIS OF FURANOSE CONTAINING MOLECULES AS USEFUL TOOLS IN GLYCOBIOLOGY

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Furanosic sugars, as D-Gal*f* and D-Ara*f*, are constituents of a number of key glycoconjugates from bacteria, fungi, and parasites, being many of them pathogenic. The furanose form of galactose and arabinose is absent in mammals, hence the interest in the metabolism of such structures as a chemotherapy target. The availability of glycoconjugates, oligosaccharides and glycomimetics containing D-Gal*f* and D-Ara*f* units are valuable tools for understanding the mechanisms of action of the related enzymes.

The synthesis of furanosyl containing molecules requires practical synthetic procedures of sugar derivatives in the furanosic configuration, free from the pyranosic forms, as precursors of furanosic units in the target molecules. Furthermore, efficient glycosylation methodologies and the consequent availability of furanosyl donors, as well as conveniently substituted derivatives as glycosyl acceptors, are required. Some of the contributions made to these different aspects of the synthesis of D-Gal*f* containing molecules, as well as of other furanosic sugars will be described.

EPIMERISATIONS OF GLYCOSYL THIOLS AND GLYCOMIMETIC SYNTHESIS

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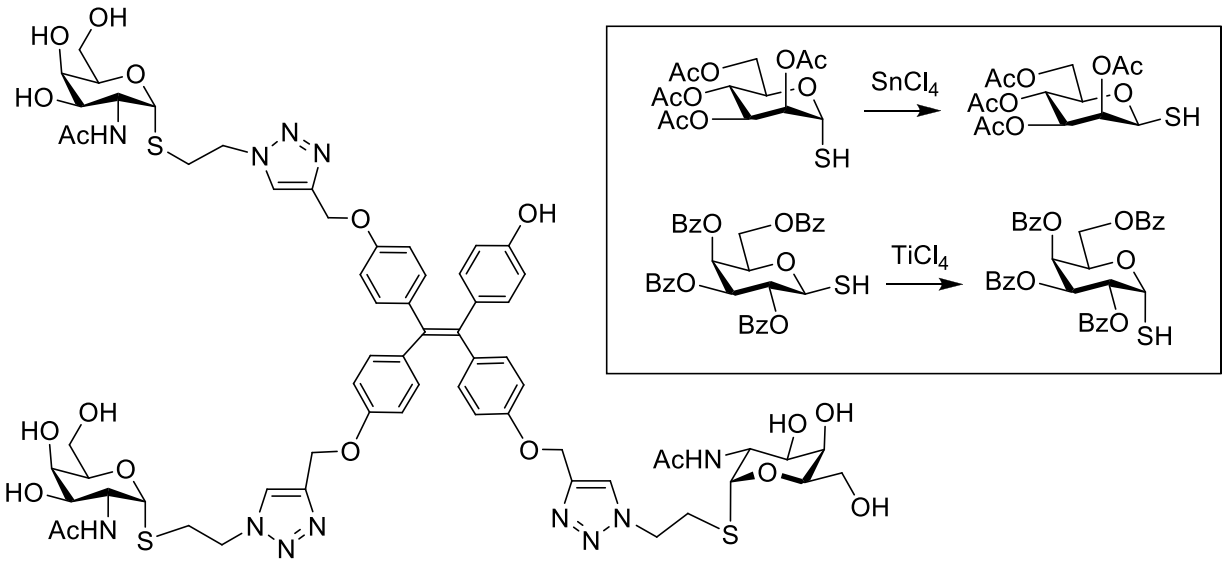
Glycosyl thiols are widely used in stereoselective S-glycoside synthesis, including the preparation of glycomimetics. The results of a study on the Lewis acid promoted epimerization reactions of such thiols, in both directions will be presented [1]. Equatorial to axial epimerization was attained using $TiCl_4$ whereas $SnCl_4$ led to axial-to-equatorial epimerization. The latter included the stereoselective preparation of β-D-mannopyranosyl and β-L-rhamnopyranosyl thiols from their α-precursors. NMR Spectroscopy has been used in mechanistic studies and reveals major differences in species formed in solution from the respective Lewis acids. Evidence will be presented that complex formation explains the preference for equatorial thiols in reactions promoted by $SnCl_4$. Alternatively, $TiCl_4$ shifts the equilibrium towards 1,2-cis thiols through the promotion of 1,3-oxathiolane formation.

The epimerization/anomerisation reactions have recently been studied for xylopyranosides, arabinopyranosides and fucopyranosides. Applications of glycosyl thiols and thioglycosides in glycomimetic synthesis will be included, such as the generation of potent glycocluster ligands for macrophage galactose C-type lectin, which are demonstrated to be useful in a histochemical context even in the presence mixed lectins [3]. Other applications will be included in the presentation.



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SYNTHESIS OF GIANT OLIGOSACCHARIDES OF *MYCOBACTERIUM TUBERCULOSIS* GLYCOCALYX BY GOLD-CATALYZED GLYCOSIDATIONS

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The glycocalyx of *Mycobacterium tuberculosis* comprises arabinose and galactose in furanosyl form along with other saccharides in pyranosyl form [1]. Two major structural constituents of the cell wall of M. tuberculosis are identified as Lipoarabinomannan and Arabinogalactan [2]. A decade long effort in the group culminated in excellent methods for the stereoselective synthesis of 1,2-*cis* and 1,2-*trans* furanosides [3] Quite recently, we discovered a novel glycosidation protocol by the synergistic action of gold and silver salts on stable glycosyl carbonates [4]. Combining the bioinspired stereoselective synthesis of 1,2-*cis*-/*trans*- furanosides and the new glycosidation method, we accomplished the synthesis of a heneicosasaccharide containing 19 Ara*f* residues and 2 Gal*f* residues, and a pentacosasaccharide containing 23 Ara*f* residues and 2 Ara*f* residues which are of mycobacterial origin [5]. The synthesis of highly branched, complex and challenging hencontapentasaccharide of Lipoarabinomannan is currently underway in the group.

An account of these results will be presented.

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CARBOHYDRATES AND FLUORESCENT PROBES. NEW AVENUES IN CHEMISTRY AND GLYCOCHEMISTRY

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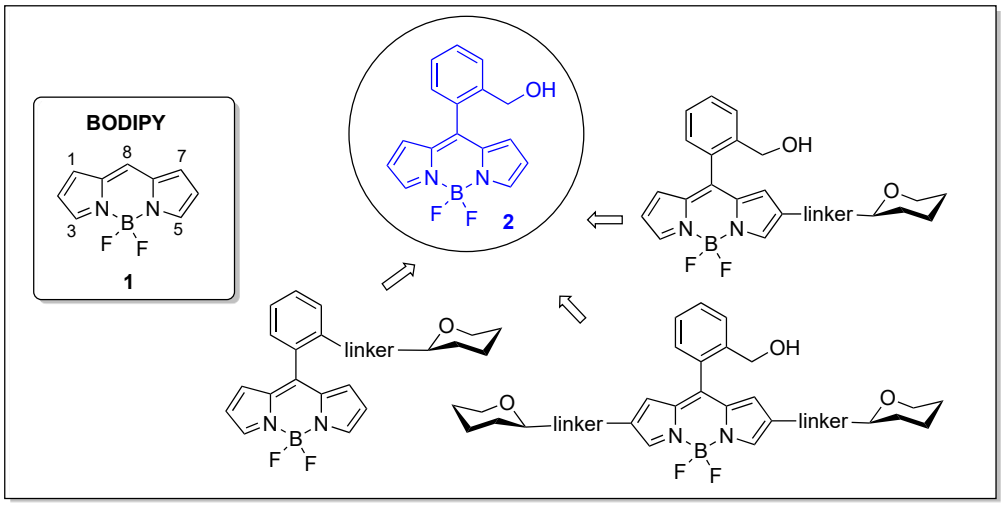
The difluoroboron dipyrromethene (4,4-difluoro-4-bora-3a,4a-diaza-sindacene, BODIPY, **1**) fluorophores have emerged as a class of small molecules with remarkable properties for a variety of biological applications, ranging from biological labelling, fluorescence imaging, chemical sensing, to cancer therapy [1]. These properties include large molar absorption and extinction coefficients, narrow emission bandwidths, high fluorescence quantum yields, long fluorescence lifetimes, relatively high photochemical stability, and high cellular permeability and compatibility [2]. Besides these notable properties -at least- two aspects related to the use of BODIPYs for biomedical applications still demand further attention: *i)* enhanced water-solubility and *ii)* adjustable bioconjugatable properties that would allow their attachment to structurally diverse target molecules and/or ligands

In this context, we have recently turned our attention to the preparation of ortho-substituted 8-*C*-aryl BODIPY derivatives, e.g. **2** [3]. In these compounds, the ortho-substituent plays a key dual role, *i)* it provides the handle for derivatization and/or conjugation to the target molecule and *ii)* it impedes the rotation of the phenyl moiety about the 8C-aryl bond, which results in an improved fluorescence quantum yield by reducing rotational radiative-relaxation. On the other hand, we [4] and others [5] have shown that "combining" carbohydrates and BODIPY dyes results in "glycoprobes" [6] with improved water-solubility and with a targeted imaging and theranostic ability. Altogether, considering the practical importance of carbohydrate-BODIPY hybrids (see Scheme) and the synthetic challenges involved in their preparation, we are currently exploring different and complementary approaches for their preparation.



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METAL-FREE CLICK LIGATIONS FOR THE SYNTHESIS OF MULTIVALENT SUGARS AND IMINOSUGARS

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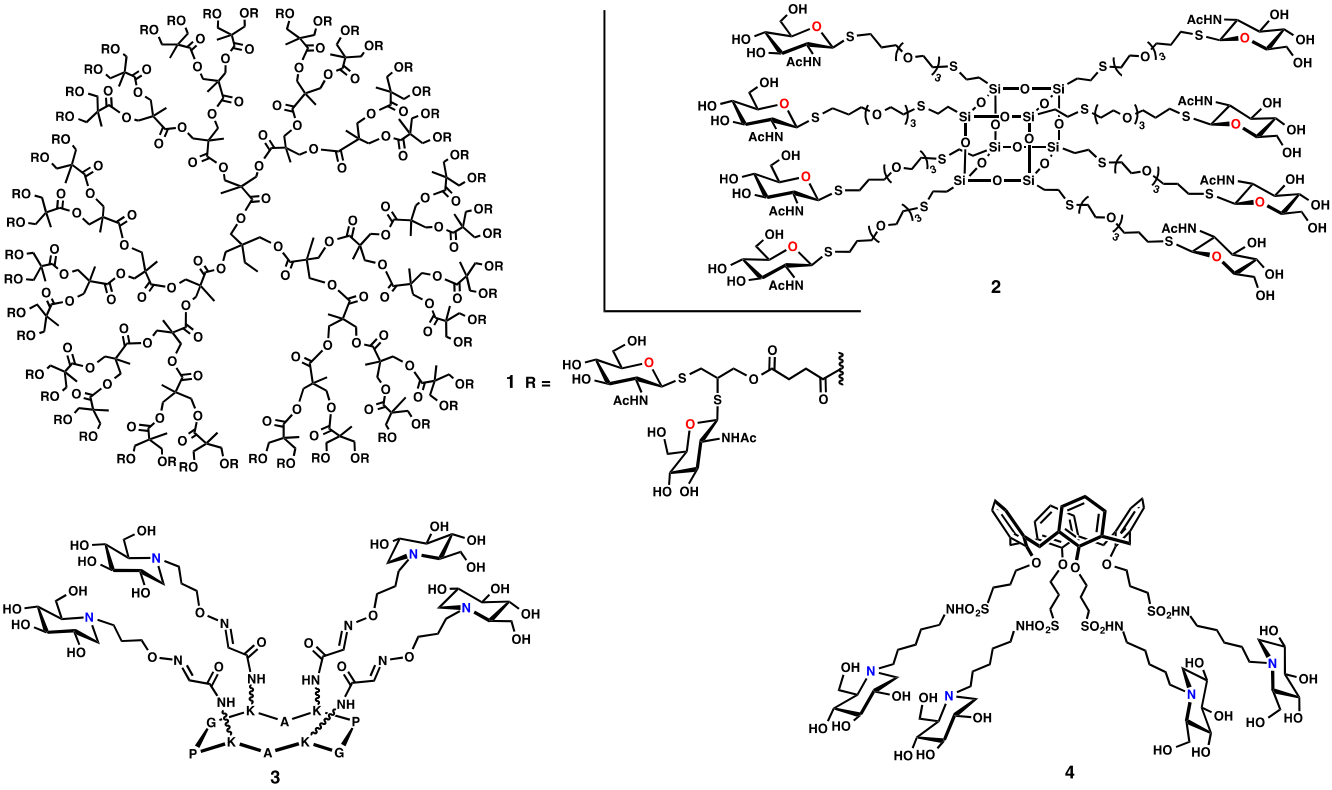
Multivalent sugars [1] and multivalent iminosugars [2] are widely used as probes for the carbohydrate-lectin recognition studies and as strong, selective inhibitors of glycosidases, respectively. Most of these compounds have been prepared by means of the copper-mediated azide-alkyne cycloaddition (CuAAC). Unfortunately, the CuAAC has a serious drawback, namely the possible contamination of the cycloadducts by significant amounts of toxic copper ions. Thus, to envisage pharmacological applications of the sugar and iminosugar clusters, metal-free click ligations are required. We have recently developed very efficient chemoselective glycoconjugation reactions in the absence of metal catalysts such as the photoinduced radical thiol-ene [3] (TEC) and thiol-yne [4] coupling (TYC), the oxime [5] ligation, and the sulfonyl fluoride-based [6] (SuFEx) coupling, using various molecular platforms (calixarenes, silsesquioxanes, cyclopeptides, dendrimers) to prepare multivalent sugar (e.g. **1**-**2**) and iminosugar architectures (*e.g.* **3**-**4**).



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Glycomics@ExPASy: A WEB PORTAL TO EXPLORE THE MULTIPLE FACETS OF CARBOHYDRATES

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Glycomics@ExPASy the glycomics tab of the Swiss Institute of Bioinformatics server (www.expasy.org/glycomics) was launched in 2016 to centralise web-based glycoinformatics resources developed within an international network of glycoscientists [1]. The philosophy of our toolbox is to be {glycoscientist AND protein scientist}–friendly with the aim of popularising (a) the use of bioinformatics in glycoscience and (b) the relation between glycobiology and protein-oriented bioinformatics resources. The scarcity of bridging data led us to design tools as interactive as possible based on database connectivity in order to facilitate data exploration and support hypothesis building.

The current set of resources is mostly built on top of curated or experimental data relative to glycan structures, glycoproteins and glycan-binding proteins. The information is provided to the user with dedicated services embedded in web applications. Query tools support compositional, substructure, epitope queries to match structures and related features. We aim at implementing modular, interoperable and reusable applications. To ensure the consistency of these on-line resources and the compliance with external data sources, the same standards, nomenclatures and ontologies are applied.

The architecture of our resources is shaped to reflect the situation at the cell surface where glycan-mediated protein-protein interactions take place. Our implementation promotes easy navigation correlating glycan structures to binders or glycoproteins to diseases. Recent technological improvement in glycomics and glycoproteomics is bringing these fields closer to larger scale studies. Consequently Glycomics@ExPASy data and tool collection is steadily growing and now encompasses glycome profiling [2], glyco-epitope mapping [2], protein glyco-site characterization [3] and mass spectrometry data analysis for glycan [4] or glycopeptide identification [5].

Our project as part of a wider initiative within an international network is intended as a major step towards interconnecting isolated efforts within the broad and interdisciplinary field of glycobiology and as such, meant to boost fast progress in modern Glycoscience.

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COMPUTATIONAL APPROACHES TO TOLL-LIKE RECEPTOR 4 MODULATION

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Toll-like receptor 4 (TLR4), as a pattern recognition receptor (PRR), perceives the presence of both damage-associated and pathogen-associated molecular patterns, *e.g.* bacterial lipopolysaccharides (LPSs). Together with MD-2 protein, its dimerization initiates the activation of innate immune system signaling pathways (Figure 1A) [1], thus being a promising therapeutic target.

We have applied a combination of several computational techniques (docking calculations, virtual screening techniques and drug reprofiling approaches, NMA, and MD and CG simulations) to characterize the architecture of TLR4/MD-2/ligand complexes, the TLR4 agonist/antagonist mechanism of newly designed glycolipids (Figure 1B) [2], non LPS-like modulators [3,4], natural LPS and membrane insertion mechanism (Figure 1C). All these molecules present fascinating binding abilities and hold promising therapeutic properties.

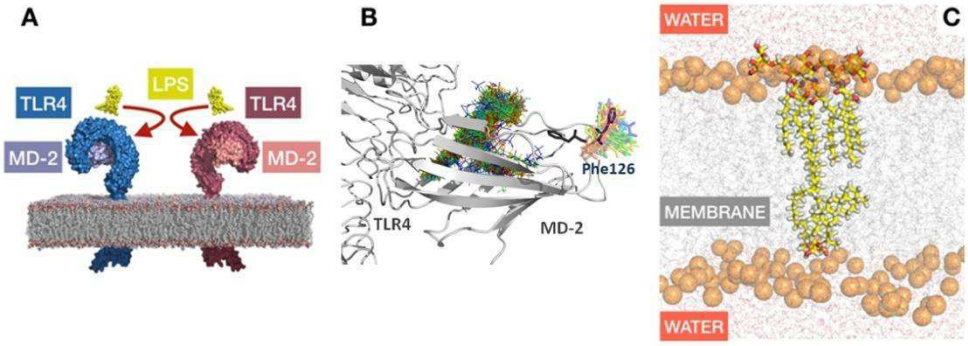

Fig. 1

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COMBINED CAVITATIONAL AND ENZYMATIC TREATMENTS FOR THE VALORIZATION OF LIGNOCELLULOSIC FEEDSTOCK, FROM LAB TO PILOT SCALE.

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This communication reports some of the results achieved within the BBI project US4GREENCHEM[1]. A consortium that unifies the broadest expertise and most extensive experience available in research and industrial development in the targeted fields, i.e. biomass pre-treatment, hydrolysis, biocatalysis design, extraction and conversion of lignocellulose derived compounds and biorefinery design. The aim of the project is to apply only “green” technologies for the conversion of lignocellulosic biomass (mainly wheat straw) into sustainable biorefinery for chemicals and fuels. Cost-effective and environmentally friendly biomass valorization depends on the development of highly efficient physical treatments for delignification, leading to an improved enzymatic hydrolysis. After lab scale tests with different ultrasonic devices and hydrodynamic cavitation units, the pretreatment was scaled up to pilot reactors, namely a rotor/stator generator of hydrodynamic cavitation (volume range 20-40 L) and a flow 25 kHz multi-transducer sonochemical apparatus (volume range 50-100 L) which could effectively disrupt the lignocellulosic matrix. The optimized alkaline cavitational treatments gave an efficient wheat straw delignification and higher sugars yields in the following enzymatic hydrolysis. The optimized enzyme cocktails developed by VTT was used with a substrate consistency of 100 g/L with about 70% improvement in saccharification yields compared with commercial reference enzymes cocktail. The synergistic technologies combination of cavitational and enzymatic treatments showed to be competitive with the classic process of steam explosion.

Acknowledgements: This project has received funding from the Bio Based Industries Joint Undertaking under the European Union´s Horizon 2020 research and innovation programme under grant agreement No 669055.

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BIOTECHNOLOGY OF POLYSACCHARIDES FOR BIOFUELS PRODUCTION AND BIO-BASED PRODUCTS

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Fossil fuels depletion and climate change, in addition to the current global energy demands require the search for suitable bio-based products and biofuels from renewable sources. This work shall focus on biochemical-based technologies for lignocellulosic biomass (LCB) conversion paying attention to recent advances on biomass deconstruction techniques and their integration on enzymatic hydrolysis, fermentation technology and chemical conversion reactions towards biofuels and bio-based products.

It is well known that LCB recalcitrance hampers the economic viability of most advanced technologies for bio-based products and next generation biofuels. The development of disruptive methodologies for LCB pre-treatment based on low-temperature (120-140ºC) processes with a low energy requirement and use of non-hazardous catalysts and/or green solvents to avoid the use of mineral acids are a key breakthrough for high-added-value bioproducts valorisation of all LCB fractions. These new technologies should also benefit of better energy-efficient separation and recovery of the three main LCB fractions. Complementary, the use of on-site production of enzymes using inexpensive pre-treated lignocellulosic solids provides an attractive integrated solution for the conversion of lignocellulosic feedstock into biofuels and chemicals.

While cellulose is breakdown to cellobiose and/or glucose for gasoline substitutes blends (eg. ethanol, iso-butanol), or for biochemicals specialities, hemicellulose breakdown is mostly targeted for building blocks as intermediary molecules for different value chains. Lignin, a non-sugar moiety-based polymer, can be either used for energy purposes (combustion) or in a better biorefinery integration, upgraded to higher-added value biomaterials.

An outlook of current biotech developments for valorization of the main lignocellulosic biomass components: cellulose, hemicellulose and lignin shall be discussed.

STEREOSELECTIVE SYNTHESIS OF SUGAR MIMETICS FROM SIMPLE MONOSACCHARIDES

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The concise approach to bicyclic sugar mimetics: imino- and carbasugars from simple monosaccharides will be presented. First method is based on the fragmentation of sugar allyltin derivatives (*e.g.* **5**, prepared from D-glucose in ca. 10 steps; route ***a*** in Fig.1) into dienoaldehyde **1** (exclusively *E*-configuration across the internal double bond). This aldehyde was converted into bicyclic carbasugars (*e.g.* **3** or **4b**). Alternatively, it was transformed into bicyclic iminosugars: **2** or **4a** [1,2]. The configuration at the ring junction is fixed, which results from the mechanism of the cyclization of intermediates (*e.g.* **1a**).

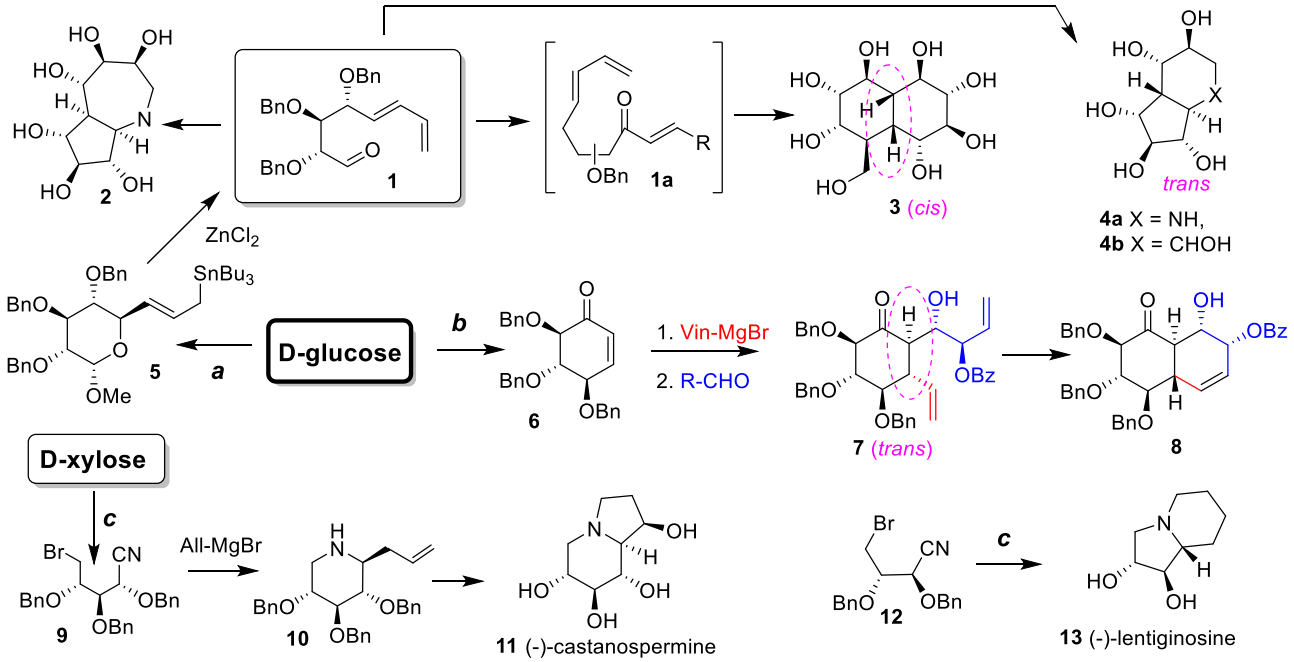

Fig. 1 - Preparation of sugar mimetics (imino- and carbasugars) from simple monosaccharides.

The problem of obtaining the alternative isomers of decalin was solved by an introduction of both substituents in the *trans*-relation before cyclization (route ***b***).

Dienoaldehyde **1** was used also as a precursor of 'odd' iminosugars such as **2** and **4a** [2]. We have also elaborated another, alternative to 'allyltin approach', method to bicyclic iminosugars which is based on the application of sugar-derived bromonitriles. Thus, and efficient synthesis of unnatural (-)-castanospermine and natural (-)-lentiginosine was realized in good yield and stereoselectivity [2,3].

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UNDERSTANDING SPECIFICITY OF CHITIN AND PEPTIDOGLYCAN DEACETYLASES: STRUCTURE, FUNCTION, AND ENGINEERING

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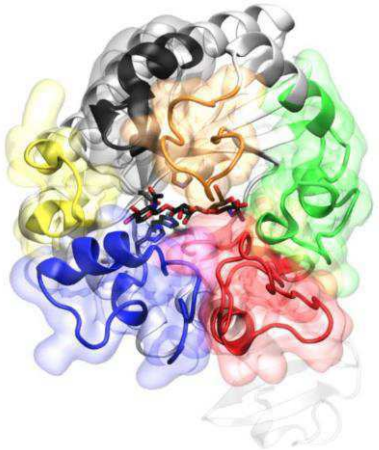
Carbohydrate esterases family 4 (CE4 enzymes) includes chitin and peptidoglycan deacetylases, acetylxylan esterases, and poly-N-acetylglucosamine deacetylases that act on structural polysaccharides, altering their physicochemical properties, and participating in diverse biological functions. Chitin and peptidoglycan deacetylases are not only involved in cell wall morphogenesis and remodeling in fungi and bacteria, but they are also used by pathogenic microorganisms to evade host defense mechanisms.

Chitin de-N-acetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and chitooligosaccharides (COS). The deacetylation pattern exhibited by CDAs and related carbohydrate esterase (CE4) enzymes active on COS is diverse, some being specific for a single position, others showing multiple attack. Since biological activities associated with COS seem to be largely dependent not only on the degree of acetylation but also on the pattern of acetylation, a major challenge is to understand how CDAs and related CE4 enzymes specifically define the distribution of GlcNAc and GlcNH2 moieties in the oligomeric chain. Based on the first 3D-structure of a CDA in complex with substrates [1], we proposed the “subsite capping model” by which the deacetylation pattern exhibited by different CDAs is governed by a series of variable and flexible loops that shape the binding site cleft of CE4 enzymes.



Here we report an update of our work on the structure, specificity and engineering of CE4 enzymes active of COS [1-5], including chitin and peptidoglycan deacetylases, with the goal of understanding the determinants of substrate specificity and engineering novel deacetylation patterns. The search of new enzyme by genome-wide bioinformatics and the engineering of substrate specificity, either by structure-guided and directed evolution approaches [6] will extend the toolbox of selective enzymes for the biotechnological production of tailored and sequence-defined COS to evaluate their biological functions and develop novel biotech and biomedical applications.

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MOLECULAR SIMULATION OF CARBOHYDRATE BINDING

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When carbohydrates meet their macromolecular receptor, a multitude of conditions need to be met before a 'successful' binding event can occur. Binding efficiency and affinity is likely to be dependent on how well the conformation of the carbohydrate is complementary to the shape and properties of the binding site (lock-and-key principle). However, is it known that carbohydrates as well as parts of protein receptors (e.g. loops or side chains of amino acids at the protein surface) can be very flexible. In this case - before a stable complex can be formed either conformational changes need to occur while the ligand is transiently bound to the receptor (induced fit) or ligand and receptor randomly need to meet when their shapes transiently fit (conformational selection) [1]. Consequently, knowledge of the dynamics of the receptor as well as the carbohydrate ligand is essential for understanding the binding specificity e.g. of a lectin. Knowledge of receptor dynamics can also open up new opportunities for drug design. For example, the detection of transient dynamic binding pockets in the Siglec-7 receptor has led to the successful design of high-affinity glycomimetic inhibitors [2].

In nature, carbohydrates very often occur as glycoconjugates. In this case the carbohydrate moiety is part of a larger biomolecular structure or molecular assembly with the important consequence that it cannot orientate freely anymore when meeting with the receptor. Consequently, the 'presentation' of the carbohydrate (epitope) will also be a limiting factor in receptor binding; for example whether a virus can bind successfully to a glycolipid embedded in a membrane. It has been experimentally shown that Norovirus particles bind to isolated Lewis-X molecules, but binding no longer occurs when the Lewis-X moiety is part of a glycolipid embedded in a membrane. Using molecular modeling and simulation it could be shown that in the membrane environment the critical fucose epitope of Lewis-X is no longer accessible to the virus [3]. A further increase in complexity of the factors that determine carbohydrate binding arises from the recent discovery that also transient interactions can influence specificity in virus binding [4,5].

With the advances in computer technology it has become feasible to simulate the dynamics of even large molecular systems routinely on the microsecond timescale. This helps to better interpret existing experimental results and can lead to valuable new ideas for planning the next round of experiments. Here I will present applications of a novel high-throughput platform for analysis of MD simulations, which allows detailed investigation of the dynamics of binding events as well as transient interactions that influence binding affinity.

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STRUCTURAL BIOLOGY OF GLYCAN-DEGRADING ENZYMES FROM BENEFICIAL HUMAN GUT MICROBE BIFIDOBACTERIA

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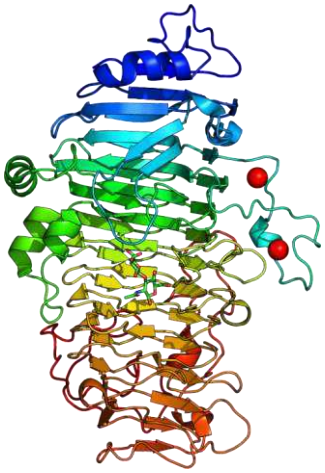
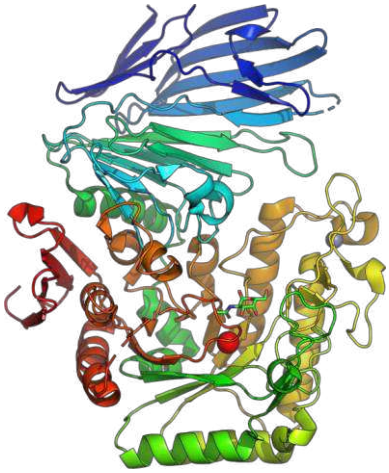
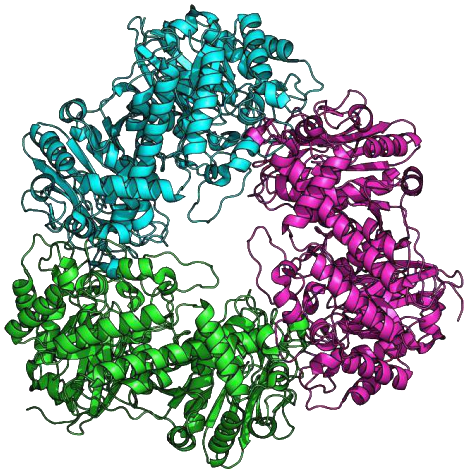
Bifidobacterium is a well-known representative genus of health-promoting probiotics in human gut microbiota. As digestible carbohydrates such as starch are scarce in their habitat, bifidobacteria possess various glycosidases, transporters, and metabolizing enzymes for utilizing indigestible carbohydrates and glycoconjugates present in diets and human gastrointestinal tract. Our group has been studying structural bases of the enzymes and proteins involved in the unique carbohydrate degradation systems discovered from bifidobacteria. I will present our recent crystallographic works on novel glycoside hydrolase structures of families GH136, GH129 and GH42, which act on human milk oligosaccharides, mucous glycoproteins and plant glycoconjugates.



GH136
lacto-*N*-biosidase
(LNBase)
from *Bifidobacterium longum* ☐



GH129
α-*N*-acetylgalactosaminidase
(NagBb)
from *Bifidobacterium bifidum* ☐



GH42
α-L-arabinopyranosidase
(*Bl*Arap42B)
from *Bifidobacterium animalis*
subsp. *lactis* ☐

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UNDERSTANDING THE ROLE OF GLYCOCONJUGATES AS KEY MOLECULES FOR CELL SURVIVAL AND COMMUNICATION

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Carbohydrates are rarely simple molecules; they occur either alone or covalently linked to proteins and lipids (glycoconjugates). This ubiquitous occurrence translates in many roles and functions such as structurally rigid and plastic elements, energy storage in living cells, interactions between viruses, bacteria and the surfaces of mammalian cells and molecular recognition for intracellular trafficking.
Innate immunity is the first line of defence against invading microorganisms in vertebrates and the only in invertebrates and plants and therefore plays a crucial role in the early recognition and subsequent triggering of a pro-inflammatory response to invading pathogens. This mechanism relies on recognition of evolutionarily conserved structures on microbes, termed microbe-associated molecular patterns (MAMPs), through a limited number of germ line-encoded pattern recognition receptors. MAMPs are characterized by being invariant among entire classes of microbes, essential for their survival, and distinguishable from "self". Microbial glycoconjugates such as lipopolysaccharide and peptidoglycan act as MAMPs in eukaryotic/bacteria interactions. Besides their general architectural principle, a number of subtle chemical variations are at the basis of the dynamic host-guest recognition that in case of pathogens is followed by the innate response and in case of symbiosis is followed by its suppression. Therefore, the structural study of such glycoconjugates involved as virulence or beneficial factors in animal or plant interactions is a pivotal pre-requisite for the comprehension at molecular level of the innate immune mechanisms [1-3].
Likewise, sialic acid binding immunoglobulin (Ig)-like lectins (Siglecs) bind sialic acids which are the most prevalent residues located at the terminal position of (*N*- or *O*-) glycan structures on cell surface glycoproteins and glycolipids found on all mammalian cells. Given their capability to recognize a common structural element of the mammalian glycome, the Siglecs are nowadays recognized for their role in helping immune cells to distinguish between “self” and “non-self”. In contrast to others immune cell proteins, which recognize MAMPs and damaged cells (DAMPs), indeed, the Siglecs detect peculiar ligands that are determinants of “self”, referred to as self-associated molecular patterns (SAMPs). The Siglecs influence almost every cell in the immune system and modulate a plethora of immune responses which are of relevance both in health and disease,
In this communication, I will show some examples of isolation, structure determination of complex glycoconjugates [1-3] and their contribute to the cell survival as well as their interaction with their receptor(s) [4-5].

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THE USE OF FLUOROPROLINE IN MUC1 ANTIGEN ENABLES EFFICIENT DETECTION OF ANTIBODIES IN PATIENTS WITH CANCER

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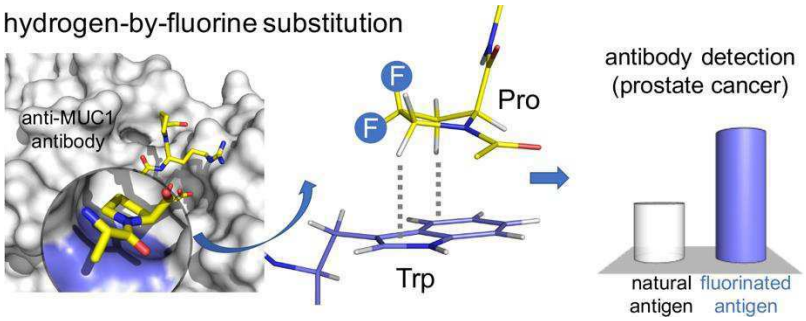
MUC1 is a glycoprotein overexpressed in most types of cancer [1]. This overexpression is associated with elevated concentrations of antibodies against MUC1 in the blood of patients [2].

In this talk, a structure-based design of a new generation tumor-associated glycopeptides with improved affinity against two anti-MUC1 antibodies is described [3]. These unique antigens feature a fluorinated proline residue, such as a (*4S*)-4-fluoro-L-proline or 4,4-difluoroproline, at the most immunogenic domain (see Figure). Binding assays using bio-layer interferometry reveal 3-fold to 10-fold affinity improvement with respect to the natural glycopeptides. According to X-ray crystallography and MD simulations, the fluorinated residues stabilize the antigen-antibody complex by enhancing key CH/π interactions. Interestingly, a notable improvement in detection of cancer-associated anti-MUC1 antibodies from serum of patients with prostate cancer is achieved with the non-natural antigens, which proves that these derivatives can be considered better diagnostic tools than the natural antigen for this type of cancer.



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AMPHIPHILES IN GLYCOSCIENCES: FROM BIOBASED TO BIOLOGICAL CHEMISTRY

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Glycoamphiphilicity is the property common to all biological glycolipids and synthetic carbohydrate-based surfactants, associated with innumerable applications and biological roles and functions. While glycolipids are components of the biological membrane where key biological processes take place and are essential to life, surfactants are commodity chemicals used in a wide range of situations of the everyday life, requiring constant progress in terms of sustainability and performance. The physicochemical properties of glycoamphiphiles are dictated, one on hand, by non-covalent interactions between their highly hydroxylated moieties, and, on the other hand, between their hydrophobic appendages. Considering the huge range of structure accessible by combining carbohydrates and lipids, there is a fascinating subtlety in the relations between structure and properties, with consequences on the preparative and applicative viewpoints [1,2].

The topic will be illustrated by results on the synthesis and the structure-property relationships of carbohydrates-based surfactants and emulsifiers [3-7], and by recent investigations on the amphiphilic character of acyl steroid glycosides (ASGs) [8]. A focus will be made on α-CAG (6-*O*-tetradecanoyl cholesteryl α-D-glucopyranoside), an acyl steroid glucoside found in the membrane of pathogenic bacteria *H. pylori*. Comparing α-CAG with a series of analogues, we show how much both the carbohydrate and the lipid moieties influence its membrane behavior, with significant differences in the way lipids redistribute and form domains within model membrane Langmuir monolayers.

Overall these examples show how much glycoamphiphilicity is a peculiar and important property with relevance to both the green and biological sides of glycosciences.

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POLYPEPTIDE-GALNAC-TRANSFERASES AS PROGNOSTIC AND PREDICTIVE CANCER BIOMARKERS

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The synthesis of *O*-linked glycosylation is started in the Golgi apparatus by the covalent linkage of an α-N-acetylgalactosamine residue (GalNAc) to the hydroxyl group of Ser/Thr residues. This reaction is catalyzed by the family of UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase (GalNAc-Ts, EC 2.4.2.41) composed at least by 20 members in humans. GalNAc-Ts have been found to be differentially expressed in malignant tissues compared to correspondent normal ones. We found GalNAc-T6 expression significantly higher in breast cancer cells comparing with normal or benign mammary cells [1]. Using a RT-PCR assay, we identified *GALNT6* expression in bone marrow samples related to poor clinical outcome in lymph node–negative breast cancer patients [2]. Its expression level was also associated with the presence of venous invasion and pTNM staging in patients with gastric carcinoma [3]. In contrast, we have shown that GalNAc-T6 expression is an independent predictor for better overall survival in colon cancer patients, especially in those with advanced disease [4]. In a human neuroblastoma model we found that *GALNT13* was the most strongly up-regulated gene in metastatic neuroblasts compared with primary tumor xenograft [5]. In the same study, we demonstrated that *GALNT13* expression at diagnosis in bone marrow of neuroblastoma patients was a strong predictor of poor clinical outcome. Conversely we found that the brain specific gene *GALNT9* is present in neuroblasts derived from primary tumor but absent in bone marrow metastatic ones. In a cohort of 122 neuroblatoma patients, *GALNT9* expression in primary tumor was associated with higher overall survival, independently of the standard risk-stratification covariates [6]. On the other hand, we recently found GalNAc-T13 expression in human non–small cell lung cancer (NSCLC). We produced a specific monoclonal antibody (MAb T13.5) which was used to assess the expression profile of the GalNAc-T13 protein in primary tumors from a well-defined population of 443 surgically resected NSCLC patients with 7 years follow-up. We found that the enzyme was expressed more frequently in lung adenocarcinomas than squamous cell carcinomas. GalNAc-T13 expression correlated significantly with worse overall survival in adenocarcinoma patients treated with neoadjuvant chemotherapy. These data suggest that GalNAc-T13 could be a novel marker associated to chemoresistance in lung adenocarcinomas. We have also identified and partially characterized nine splice variants of GalNAc-T13, which adds further complexity to the GalNAc-T family [7].

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TWISTING TAILS AND CURIOUS CHANNELS – THE PHOSPHORIBOSYLTRANSFERASES

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Phosphoribosyltranferases (PRTs) catalyse the transfer of the sugar, ribose 5-phosphate to a nitrogenous base. We have examined catalysis by two PRT enzymes, both of which play key roles in amino acid biosynthesis, and have been identified as new targets for antimicrobial therapeutics.

ATP phosphoribosyltransferase (ATP-PRT) catalyses the first step of histidine biosynthesis resulting in the transfer of a phosphoribosyl unit to ATP. We have carried out kinetic isotope effect measurements to determine the mechanism of this reaction for ATP-PRT enzymes the pathogens *Campylobacter jejuni* and *Mycobacterium tuberculosis*. We are currently using this information to design transition state analogues as inhibitors of this enzyme.

Anthranilate phosphoribosyltransferase (An-PRT) catalyses the formation of phosphoribosyl anthranilate in the biosynthetic pathway for tryptophan. Our results with substrate analogues and inhibitors of An-PRT from *Mycobacterium tuberculosis* reveal how a substrate binding channel both protects an enzyme-bound reactive intermediate and acts as an Achilles heel by providing a mechanism for inhibition.

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GLYCOCONJUGATE VACCINES: APPROACHES FOR PREPARATION AND RECENT TRENDS

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Cell surface carbohydrates have been proven optimal targets for vaccine development. Conjugation of polysaccharides to a carrier protein triggers a T-cell dependent immune response to the glycan moiety[1]. Licensed glycoconjugate vaccines are produced by chemical conjugation of capsular polysaccharides to prevent meningitis caused by meningococcus, pneumococcus and *Haemophilus influenzae* type b. However, other classes of carbohydrates (O-antigens, exopolysaccharides, wall/teichoic acids) represent attractive targets for developing vaccines [1].

This lecture will give an overview of the surface carbohydrates targeted for vaccine development and the technological approaches for obtaining glycoconjugate vaccines. Focus will be given to (Figure 1):

(a) *production of the carbohydrate component* by classic semisynthetic approaches and the use of chemically or enzimatically produced carbohydrates. In this context, structure-based selection of the carbohydrate epitopes on the basis of interaction studies with protective antibodies is provinding a powerful tool for the design of modern vaccines [2].
(b) *novel conjugation methods* such as site-selective chemical/enzymatic methods, glycoengineering, incorporation of unnatural amino acids, enabling the preparation of more homogeneous glycoconjugates and the exploitation of pathogen related proteins as carrier for the glycan [3, 4].
(c) *Multicomponent constructs* targeting receptors responsible for modulation of the immune response in order to control the quality and magnitude of the response.

Synergies among these methodologies hold the potential to contribute in the understanding of the mechanism of action of this class of biomolecules and design more efficacious vaccines.

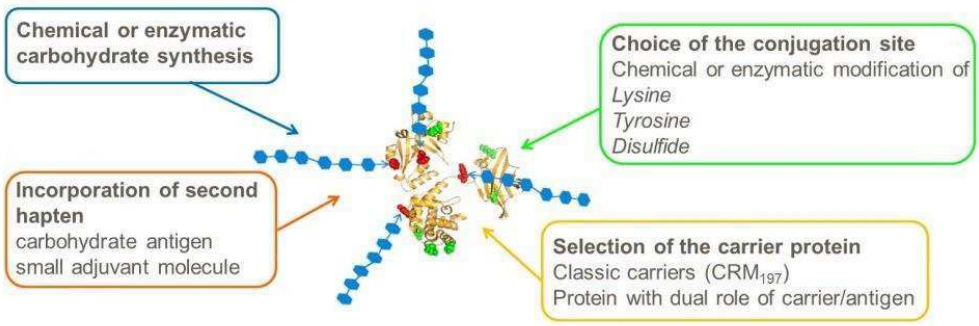

Fig. 1 – Factors contributing to the immunogenicity of glycoconjugate vaccines.

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USING NMR SPECTROSCOPY TO FACILITATE THE DEVELOPMENT AND LICENSURE OF GLYCOCONJUGATE VACCINES

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Glycoconjugate vaccines are the most effective way to protect against bacterial diseases caused by encapsulated pathogens. Following the success of *Haemophilus influenzae* type b (Hib) vaccines, multivalent conjugate vaccines against meningococcal and pneumococcal bacteria and *Salmonella typhi* have been licensed with many other vaccines in development. Conjugates are structurally diverse and complex comprised of polysaccharide or oligosaccharide fragments, linked through a variety of coupling chemistries to different carrier proteins. The traditional strategy of preparing conjugates from polysaccharides [Fig. 1, routes (i) to (iii)] has been extended to O-antigens and now includes synthetic and biosynthetic approaches [routes (iv) and (v)].

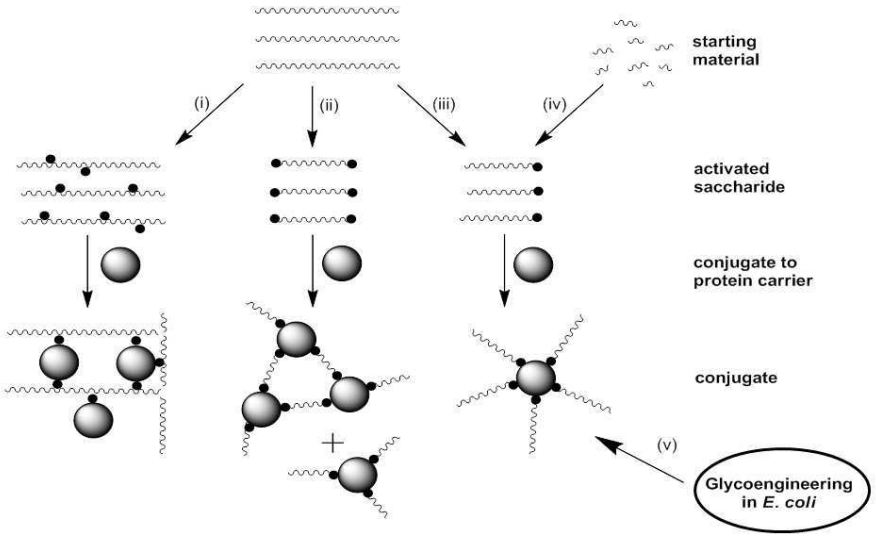

Fig. 1 - Current and new strategies for the preparation of glycoconjugate vaccines.

NMR spectroscopy is used to determine the structure of carbohydrate antigens, however, its use in the vaccine industry was initially only as an alternative method to serology for polysaccharide identity testing. This has changed, firstly when it was shown that pneumococcal polysaccharide identity of the 23 valent vaccine can be tested by 1D NMR analysis instead of the multiple immunochemical and chemical assays specified in regulatory documents [1]. Secondly, in the absence of a reliable potency assay, physicochemical methods of characterization especially NMR were employed during development of Hib conjugate vaccines [2]. The sensitivity of high field NMR spectroscopy and cryoprobe technology together with HR-MAS have made NMR spectroscopy an essential structural tool for vaccine development and licensure [3]. Its application to the identification of new antigens and vaccine targets, polysaccharide quantification (using qNMR) and the tracking of carbohydrate antigen structure from the surface of the pathogen through intermediates to the conjugate vaccine will be presented.

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CONJUGATE VACCINES FROM SYNTHETIC AND BACTERIAL CARBOHYDRATES USING SQUARIC ACID CHEMISTRY

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Neoglycoconjugates are a rapidly expanding class of probes in the life sciences. Some of such substances are used as experimental vaccines. Squaric acid chemistry is an efficient way to conjugate amine-containing carbohydrates to amino group-containing carriers by the single-point attachment model, yielding well-defined constructs. We have been using this method to make neoglycoconjugates from synthetic carbohydrate antigens that mimic structure of O-specific polysaccharides (O-SP) and recombinant protein carriers for a number of years. Purified O-PS-core (OPSC) fragments of some bacterial lipopolysaccharides (LPS) can also be conjugated in this way. We will show that when squaric acid chemistry is used to link a one amino group-containing OPSC to proteins, e.g. those isolated from *Vibrio* or *Shigella* pathogens, the reaction is simple to perform, easy to control, and introduction of spacers into either carrier or carbohydrate antigens is not necessary. Progress of the conjugation can be monitored, the finished product – a neoglycoconjugate vaccine – can be well characterized, and the carbohydrate–protein ratio in the conjugate can be predetermined. The conjugation efficiency with both synthetic oligosaccharides and bacterial polysaccharide is high, ~80 and ~50%, respectively. Experimental results will be presented, which arguably invalidate objections to using squaric acid chemistry in conjugate vaccine development because of the assumed strong antigenicity/immunogenicity of the squarate epitope present in the neoglycoconjugates prepared in this way.

IDENTIFICATION OF IMMUNOLOGICALLY PROTECTING EPITOPES IN HETEROGENEOUS MICROBIAL POLYSACCHARIDES FOR THE DEVELOPMENT OF GLYCOCONJUGATE VACCINES

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Cryptococcus neoformans is an opportunistic fungi which is a major problem when infecting immuno-compromised persons, e.g. AIDS and organ transplant patients [1]. The fungi is surrounded by capsular polysaccharides (CPSs) important for virulence of which the major one is referred to as the GXM (GlcA, Xyl, Man) CPS. This polysaccharide has a partly defined structure, an α-D-Man-(1$\rightarrow$3)-α-D-Man backbone with β-linked D-Xyl and D-GlcA residues attached to the 2-positions and acetates to the 6-positions but the substitution pattern is heterogeneous and the native polysaccharide is a mixture of a number of structural motifs, their ratio changing with each batch of polysaccharide [2]. The GXM polysaccharide has been conjugated to a carrier protein to form glycoconjugate vaccine candidates, which have been evaluated in mice immunization experiments. The conjugates have proven to be strongly immunogenic but with variable efficacy of protection against *C. neoformans* infections, probably due to the CPS heterogeneity and thus different CPS structures being present in the vaccines injected [3,4]. Monoclonal mAbs have been produced that are protective but there is no information available of their carbohydrate specificity [5]. Since the native polysaccharide due to its heterogeneity can't be utilized for the determination of antibody specificity or be used as part of a (functional) glycoconjugate vaccine we are pursuing an approach using (well-defined) synthetic GXM part structures [6,7,8]. Synthetic methods have been developed that have allowed the construction of a library of GXM oligosaccharides ranging in size from disaccharides up to octadecasaccharides [9,10,11]. This compound library has been printed to form a GXM glycan microarray which is screened using the above mentioned mAbs. Structures recognized by neutralizing mAbs are conjugated to a carrier protein to form glycoconjugate vaccine candidates, which are being evaluated in mice immunization experiments. Results from the chemical synthesis, the array printing and screening, and the mice immunization experiments will be presented and discussed.

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THE DESIGN OF A PLATFORM FOR A MULTI-VALENT DIARRHEAL VACCINE

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Campylobacter jejuni, Enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* sp. are major causes of bacterial diarrhea worldwide. Approaches to *C. jejuni* and *Shigella* vaccines include conjugate vaccines in which *C. jejuni* capsular polysaccharides and *Shigella* lipopolysaccharides are conjugated to proteins. In the case of ETEC vaccines, most current approaches are based on recombinant proteins that are involved in virulence. Here, the creation of a multi-pathogen vaccine using ETEC proteins as conjugating partners for *C. jejuni* and *Shigella* polysaccharides is described. Three vaccines were synthesized in which two *C. jejuni* polysaccharides were conjugated to two recombinant ETEC adhesins based on CFA/I (CfaEB) and CS6 (CssBA), and LPS from *Shigella flexneri* was also conjugated to CfaEB. The glycoconjugates were made by first activating the polysaccharides through TEMPO-mediated oxidation [1] (selective/stoichiometric oxidation of primary alcohols) followed by ligation to the ETEC proteins by reductive amination or carbodiimide chemistry. The vaccines were immunogenic in mice as monovalent, bivalent and trivalent formulations. Importantly, functional antibodies capable of inducing hemaglutination inhibition (HAI) of a CFA/I expressing ETEC strain were induced in all vaccines containing CfaEB. These data suggest that conjugate vaccines could be a platform for a multi-pathogen, multi-serotype vaccine against the three major causes of diarrheal disease worldwide.

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CHEMOENZYMATICALLY SYNTHESIZED GLYCOSPHINGOLIPIDS TOWARD CANCER THERAPY

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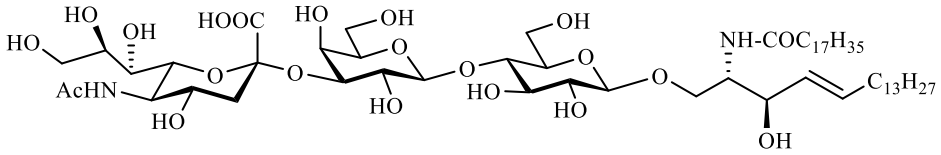
Glycosphingolipids (GSLs) are components of all animal cell membranes and are involved in many cellular functions including proliferation, adhesion, motility, and differentiation [1]. Ganglioside GM3 (NeuAcα3Galβ4Glcβ1Cer), the first and simplest member in the metabolic series of a GSLs family containing sialic acids (*N*-acetyl- and *N*-glycolyl-neuraminic acids and their *O*-acyl derivatives), is known as one of the most abundant tumor-associated carbohydrate antigens on several types of tumors [2]. Glycosphingolipid structures, and their changes associated with biological functions, have been the central focus of our studies, since structural change is the starting point for understanding biological significance, and enzymatic/genetic mechanisms.



Structure of GM3

We discuss here the design, synthesis and biological evaluation of several novel GM3 analogues [3] aiming at development of cancer treatment.

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GLYCOSYLATION IN CANCER: MOLECULAR FUNCTIONS AND CLINICAL IMPLICATIONS.

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Alterations of glycosylation are common on the cell surface during malignant cell transformation and are associated with cancer progression and poor prognosis of the patients. These glycosylation modifications have been shown to impact the biology of cancer cells, as well as the tumour microenvironment, the process of metastasis formation and cancer progression [1].

The characterisation of the glycosylation modifications occurring in cancer is of high interest and represents a source of biomarkers for cancer detection, patient stratification and therapeutic intervention. This presentation reports the application of glycomics and glycoproteomics for: (a) the characterisation of the effects of increased expression of terminal sialylated structures in cancer cells, and (b) the evaluation of the activation of tyrosine kinase receptors, HER2 (ErbB2) MET (HGFR, Hepatocyte growth factor receptor) and RON (Macrophage-stimulating protein receptor), in human gastric cancer cells [2,3,4,5], that lead to the activation of downstream intracellular signalling pathways and induction of cancer cell aggressive phenotypes [3,4]. We finally discuss the application of the expression and detection of these aberrant glycan structures in cancer extracellular vesicles [6] and circulating glycoproteins as biomarkers in cancer for diagnosis purposes and patient stratification [7].

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SULFOGLYCOLYSIS: MECHANISTIC AND STRUCTURAL INSIGHTS INTO A NOVEL METABOLIC PATHWAY

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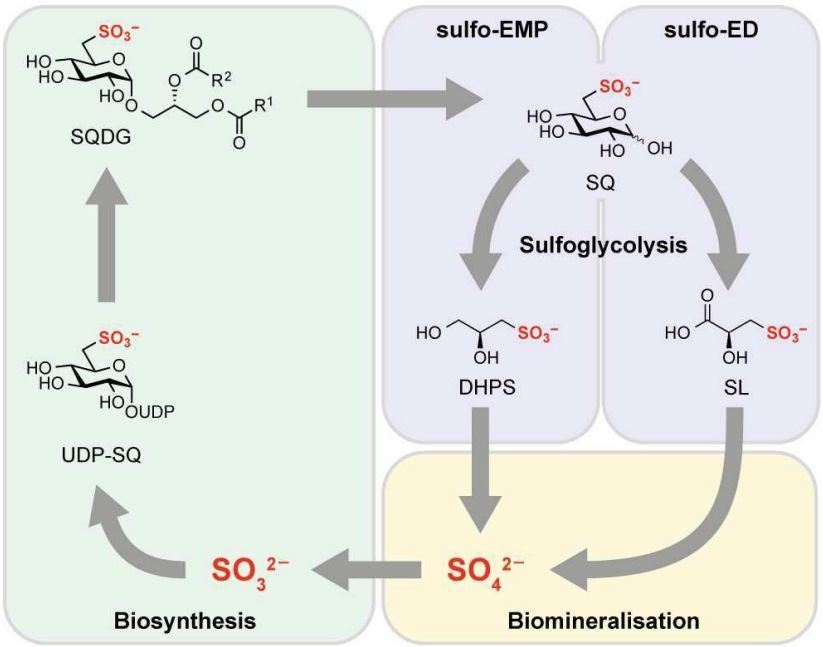
In 1959, Andrew Benson used newly available radioactive ^{35}S as a tracer to discover a novel sugar sulfolipid, sulfoquinovosyl diacylglycerol (SQDG) [1]. This compound was subsequently shown to be produced by essentially all photosynthetic organisms, and to play a role in membrane function of photosynthetic organelles and their associated photoproteins [2]. An estimated 10 billion tonnes of the head-group sugar sulfoquinovose (SQ) is produced annually, amounts commensurate with the amino acids cysteine and methionine. The biosynthesis of SQ is well-established, yet only recently was the first sulfoglycolytic pathway discovered (in *E. coli*) capable of degrading this important sugar [3]. In this talk I will discuss our efforts to identify new enzymes as part of this pathway and to develop a detailed molecular understanding of sulfoglycolysis catalysis.



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GLYCOSYLATION AS A STRATEGY FOR DETOXIFICATION OF AMINOGLYCOSIDE ANTIBIOTICS

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Aminoglycoside antibiotics (AGs) perturb with the fidelity of bacterial translation by binding to the A-site decoding region of the prokaryotic ribosome. These antibiotics are highly effective in treating severe pulmonary infections, especially those frequently occurring in cystic fibrosis patients. Unfortunately however, the antibacterial efficacy of AGs is overshadowed by the side effects that accompany systemic treatment with these antibiotics. Development of strategies to overcome this major obstacle is therefore of high priority.

Several mechanistic studies have evaluated the relative contributions of perturbation with the fidelity of the mammalian cell mitochondrial and cytoplasmic translation to AG-induced toxicity. We proposed a unique strategy to reduce this toxicity while also evading the action of AGs-deactivating enzymes. To achieve this goal we exploited the phylogenetic differences in the nucleic acid bases that form the A-sites of bacterial, mitochondrial, and mammalian cytoplasmic ribosomes to generate AGs with higher specificity for the bacterial translation machinery, establishing chemical modifications that will reduce the toxicity of these important antibiotics. In a preliminary study we demonstrated that attachment of a *β-O*-linked D-ribofuranose at the C-5 position of AGs with an equatorial amine at the C-2'-position significantly contributed to recognition of the bacterial A-site and to reduction in the inhibition of eukaryotic translation [1]. To cope with antibiotics resistance caused by bacterial enzymes that deactivate AGs through a variety of chemical modifications, we designed and synthesized C-5 ribosylated AGs derived from semi-synthetic scaffolds derived from apramycin and geneticin (G-418). Both these antibiotics have broad spectrum activity against bacteria with resistance to other AGs. This led to compounds that displayed over two orders of magnitude improved selectivity to prokaryotic translation as well as potent antibacterial activity.

Regioselective ribosylation proved a promising rational strategy to reduce the effect that AGs have on mammalian cell translation. This study therefore offers a robust chemical modification that can guide drug designers to reduce the toxicity that has prevented the broad use a unique and important class of carbohydrate-baes antibiotics.

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POLYSACCHARIDE ENGINEERING: LATERAL AND TERMINAL MODIFICATION

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Polysaccharides have been utilized by the humanity for thousands of years, first as parts of natural food and wood-based products. In modern times polysaccharides have been purified and modified in different ways to enhance their functionalities. Today polysaccharides are key ingredients in foods, pharmaceuticals and biomaterials, etc. The various developmental steps in polysaccharide science and technology may conveniently be divides into seven generations: 1] Plant extracts 2] Animal extracts 3] Chemical derivatives of plant and animal extracts 4] Microbially produced glycans 5] Enzymatically polymerized glycans 6] Chemically synthesized glycans and 7] Macromolecular assembly of glycan modules.

The lecture will briefly cover these seven types, but will concentrate on the three last three, focusing by examples [mainly alginates [1-3], chitosans, 1,3-β-glucans [4]] the differences between terminal and lateral modifications. Recently initiated development of new hybrid glycans based on terminal module assembly [5, 6] will be described. Such glycans may contribute to new types of utilization of recalcitrant biomass, and also to supplement or even substitute oil-based polymeric materials.

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MARINE-DERIVED POLYSACCHARIDES IN HYBRID BUILDING BLOCKS FOR TISSUE ENGINEERING

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The possibility of regenerating organs and tissues would bring new possibilities of improving current treatments or find solutions for untreatable situations, thus having an enormous impact in the quality of life of patients. Tissue Engineering has been integrating principles of engineering, chemistry, materials science, biology and health sciences in order to develop regenerative-based therapeutic strategies combining stem cells and biomaterials. From the different sources of biomaterials, polysaccharides have been proposed to produce matrices able to interact favourably with cells. Due to their hydrophilic nature and richness in chemically active groups, such polymers can be used to produce a variety of structures fabricated using aqueous-based or other environmental-favourable procedures. Examples are shown in the modification of polysaccharides and processing of devices into different sizes and shapes (e.g. hydrogels, multilayered coatings and membranes, particles) with structural and functional characteristics suitable to be used in tissue engineering and regenerative medicine applications. In particular, distinct strategies involving bioinspired approaches and nano/micro-technologies will be shown to prepare hybrid soft systems containing cells, in the form of microparticles, microgels or thin films, that could give rise to biomedical devices using bottom-up strategies.

PROCESSES TOWARDS THE PRODUCTION OF HUMAN MILK OLIGOSACCHARIDES

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Human milk oligosaccharides (HMOs) is the family name of the natural carbohydrates found in mother's milk building up the fourth most abundant component after lactose, lipids and proteins. Over 200 different HMOs have been isolated with more than 130 complete HMO structures elucidated.[1] HMOs can be attributed to several positive health effects in infants e.g. stimulation of growth of good gut bacteria, reduction of pathogens in the intestinal microbiota by acting as soluble decoy receptors for pathogens, and promote development and maturation of the immune system.[2] In addition, HMOs offer health benefits to adults and hence are of high application relevance as food and/or nutraceutical ingredients.[3] Consequently, development of commercial viable and industrial applicable technologies of this diverse class of compounds has been under attention for long time, and finally the code has been cracked.

Glycom has developed a powerful industrial microbial fermentation process and an efficient downstream- and purification process to access HMO end-products with excellent purity suitable for a wide range of nutrition applications including infant formula. Here we present insights into the processes implemented and currently running in the commercial production of several HMOs. The presentation will focus on the downstream- and purification processes including crystallization of HMOs derived from whole cell *in-vivo* microbial fermentation processes. Furthermore, examples of Glycom's *in vitro* enzymatic technology that gives access to even the more complex HMOs (Figure 1), thus mimicking more closely the natural diversity found in mother's milk, will be presented.

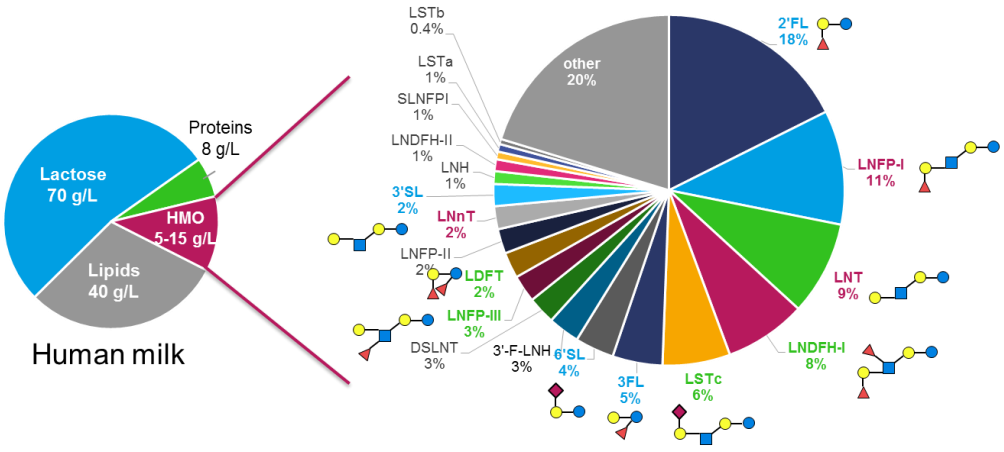

Fig. 1. Abundance of individual HMOs in human milk (g/L).

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NON-ENZYMATIC TRANSGLYCOSYLATION REACTIONS IN FOODS: THE EXAMPLE OF HONEY, COFFEE, AND STARCH

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Transglycosylation reactions allow the transfer of a sugar residue from a glycoside to another one. Glucosyltransferases are enzymes able to promote the biochemical transfer of sugar residues from a glycosyl donor to a glycosyl acceptor, originating new glycosylated compounds. The glycosyl donor is usually an activated nucleotide sugar, as in polysaccharides biosynthesis, although sucrose is the donor of fructose residues in fructans and galactinol (Gal-inositol) is the donor of galactose in oligosaccharides of the raffinose family.

Transglycosylation reactions can also occur without any enzymatic activity when dry oligosaccharides are heated. New oligo and polysaccharides have been formed by dry-heating treatments of mannotriose [1], arabinotriose [2], and their mixtures [3,4], allowing to identify the formation of roasting derived chimeric polysaccharides in coffee samples [3]. In addition, transglycosylation reactions also promote the modification of hydroxylated compounds such as the chlorogenic acids present in coffee [5], modulating the properties of polysaccharides and conferring them higher antioxidant properties. These reactions are catalyzed by the acidic medium of the foods and their extension is inhibited by the presence of peptides through the Maillard Reaction [6].

Baked products are, similarly to coffee, also submitted to high temperatures in low water content environments, as those occurring in ovens. Structural modifications caused by dry heating of amylose and amylopectin also include non-enzymatic transglycosylation reactions with formation of new glucosidic linkages, preferentially 1,6-Glc*p*, and residues in β-anomeric configuration, resulting in lower digestibility [7]. The formation of resistant starch and non-digestible carbohydrates should be responsible for beneficial effects in human intestinal tract.

Honey, although not submitted to the high temperatures of roasted foods, have an acidic and low water activity medium, which for long periods is favorable to transglycosylation reactions that promote the formation of oligosaccharides. GC-MS and Electrospray ionization mass spectrometry analysis of model solutions of honey samples confirm the occurrence of reducing and non-reducing fructo- and gluco-oligosaccharides up to a degree of polymerization of 6 holding different combinations of glycosidic linkages and anomeric configuration, explaining the relevance of these reactions in low water activity foods.

Acknowledgements: Thanks are due to FCT/MEC for the financial support of QOPNA (FCT UID/QUI/00062/2013) through national funds, co-financed by the FEDER, within the PT2020 Partnership Agreement.

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PREBIOTIC CARBOHYDRATE POLYMERS FROM BYPRODUCTS TOWARDS HEALTHIER GUT MICROBIOTA

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The importance of human gut microbiota in maintaining host health is well-known and in the past few decades, the consumer's awareness for healthier foods has increased. There are several strategies to stimulate the proliferation of beneficial intestinal bacteria, including the consumption of prebiotics. Prebiotics, as is referred in Gibson et al. (2017), is "a substrate that is selectively utilised by host microorganisms conferring a health benefit". Currently, there is a range of prebiotic carbohydrates on the market, most of them isolated from plant polysaccharides such as inulin and fructooligosaccharides (FOS), but there is an increasing interest in the development of new prebiotics, with added functionality. Establishment of a bio-based and green society allows to take actions to exploit high value resources that can be converted into valuable biological ingredients. Conversion of food processing waste and by-products into valuable ingredients has been identified as a timely challenge for food research and development associated with numerous applications of carbohydrate polymers. In this sense, chitosan, extracted from crustaceous exoskeletons is a polysaccharide that presents a structure very similar to prebiotic glucooligosaccharides. The main difference is the presence of amino groups, which are the cause of antimicrobial effect of chitosan.

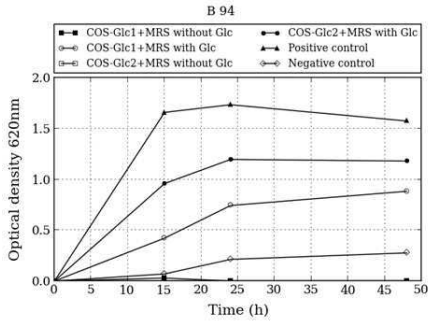

Fig. 1. Growth curves of Bifidobacterium lactis B94 in media containing MRS broth (with and without Glc as carbon source), supplemented with 0.5% (w/v) of COS-Glc1 or 0.5% (w/v) of COS-Glc2.

Chitooligosaccharide (COS) derivatives obtained by the Maillard reaction and enzymatic hydrolysis showed potential prebiotic effects, inducing changes in both the pattern of generated metabolic products and the count of Bifidobacterium (see Figure 1), which might contribute to a healthy intestinal environment. Another example studied in our group was Brewer's spent yeast, a natural byproduct from the brewing industry that may be a source of polysaccharides with bioactivity. The polysaccharides from autolyzed spent brewer´s yeast composed by a complex of glycogen-like polysaccharide, mostly 1,4-Glucans linkages (78% molar) showed ability for increasing probiotic species growth acting as carbon sources, and under colon conditions, leads mainly to the production of acetic, propionic and butyric acids proving positive effects on intestinal health.

During this presentation other examples of studies developed in our research group encompassing different ingredients obtained from byproducts containing polysaccharides with potential prebiotic activity will be presented. Studies of prebiotic potential of vegetable flour obtained from industry byproducts or xylooligosaccharides from wine bagasse will be presented.

The impact of each studied ingredient on probiotic strain or faecal bacterial dynamics and their metabolic activity will be discussed and their contribution towards a healthy intestinal environment as well.

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PROMISCUITIES IN SIALIC ACID BIOSYNTHESIS

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N-acetylneuraminic acid (Neu5Ac) is synthesized in nature by a class of enzymes called Neu5Ac aldolases, which catalyze the aldol addition of ManNAc and pyruvate. In a previous study we reported the substrate promiscuity of a bacterial Neu5Ac aldolase from *Dyadobacter fermentas*, which allowed to generate sialic acids from pyruvate and a wide range range of sugars such as D-ManNAc, D-mannose, D-glucose, D-galactose, L-fucose, D-arabinose, L-arabinose and D-rhamnose. Given the close homology between Neu5Ac aldolases in bacteria and animals, we examined if recombinantly expressed human Neu5Ac aldolase shows similar substrate promiscuities. The results and implications of these findings will be presented.

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ENZYME TRANSITION STATE ANALYSIS AND INHIBITOR DESIGN

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Transition state analysis of enzyme-catalyzed reactions provides a geometric and electrostatic map of the transition state. This can be used to design and synthesize compounds with stereoelectronic similarity to the transition state that are powerful enzyme inhibitors. Using this approach we have developed inhibitors of a number of enzymes with anti-cancer, antibiotic and anti-viral applications.[1-3]

A perspective of transition state analysis and inhibitor design applied across a range of enzymes will be presented along with some interesting and surprising results we have encountered offering lessons for future applications.

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GLYCOSIDE HYDROLASES AS EFFICIENT BIOCATALYSTS: FROM FUNDAMENTAL RESEARCH TO COSMETIC APPLICATIONS

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Carbohydrates play an important part in a vast array of biological processes and therefore glycomimetics are currently becoming a powerful class of novel therapeutics. Amongst them, thioglycosides, in which a sulfur atom has replaced the glycosidic oxygen atom, are tolerated by most biological systems. Their major advantages rely in the fact that they adopt similar conformations than the corresponding *O*-glycosides and especially that they prove to be less sensitive to acid/base or enzyme-mediated hydrolysis. Besides the synthetic methodologies developed throughout the years by organic chemists, the presence of natural *S*-glycoconjugates was recently assessed and lead to the discovery of some glycosyltransferases involved in such rare biocatalytic processes.[1] In parallel, the increases of knowledge on the mechanism and the structure of glycoside hydrolases have conducted to the development of original catalysts with greatly improved synthetic properties for thioglycosidic linkages.[2] However biocatalyzed procedures of thioglycosylation still represent an emerging area.[3] Herein, we will discuss our recent findings in this tremendous field, and more especially, the results we recently obtained in the chemo-enzymatic synthesis of carbohydrates for cosmetic applications.

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ENHANCING ANTIGENICITY OF SYNTHETIC SACCHARIDE-BASED ANTIGENS BY TARGETING FCγ RECEPTORS

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Improving antigenicity of bacterial, viral, and tumor antigens is of general importance for improving protective antibody and T-cell responses. We have designed a rhamnose (Rha)-cholesterol conjugate which acts as an immunoadjuvant and an antibody recruiting molecule (ARM). Rha-cholesterol is capable of recruiting naturally occurring anti-Rha antibodies and is easily formulated into liposomes in combination with lipidated antigens. The resulting liposomes can be used to target antigens to Fcγ receptors (FcγR) on antigen presenting cells (APC). The targeting mechanism results in the enhancement in both CD4+ (1,2) and cytotoxic C8+ T-cell priming (3).

To complement this technology, we designed a conjugatable azide-containing synthetic glycopeptide antigen that contains a peptide sequence from the tumor marker MUC1 and contained the tumor-associated carbohydrate antigen (TACA) α-*N*-acetyl galactosamine (GalNAc). We synthesized an azide-conjugatable form of the immunoadjuvant and Toll-like receptor ligand Pam_3CysSK_4 (Pam_3CysSK_4-DBCO) using a mixed solid and solution phase synthesis. Pam_3CysSK_4-DBCO was conjugated to N_3-TSAPDT(GalNAc)RPAPGSTAPPAHGV (MUC1-TACA) using a Cu-free strain-promoted azide-alkyne cycloaddition (SPAAC). The resulting Pam_3CysSK_4-linker-MUC1-TACA molecule was formulated into liposomes using the Rha-cholesterol ARM and the immune responses against MUC1-TACA were evaluated in groups of C57BL/6 mice. Some groups of mice received affinity-purified human anti-Rha antibodies by adoptive antibody transfer. Mice that received both the Rha liposomal vaccine and human anti-Rha antibody showed higher humoral and cellular immunogenicity to MUC1-TACA compared to the control group of mice. The Primed C8+ T-cells were capable of tumor cell killing.

Other important saccharide antigens of interest to our lab include bacterial antigens such those found on the lipopolysaccharide (LPS) of *P. aeruginosa*. Cystic fibrosis patients and immunosuppressed populations are highly susceptible to *P. aeruginosa* infections. This combined with rising drug resistance creates a need to consider vaccination as part of the management of theses infections. We will briefly describe the synthesis of trisaccharide and a tetrasaccharide antigens from the outer core domain of *P. aeruginosa* lipopolysaccharide LPS using a novel hydroquinone-based reducing end capping group (4). The resulting antigens can potentially be used as components of a vaccine.

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SYNTHETIC CARBOHYDRATE-BASED CONJUGATES AS PROMISING VACCINE CANDIDATES AGAINST *SHIGELLA*: FROM CONCEPT TO FIRST-IN-HUMAN STUDY

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Shigellosis, or bacillary dysentery, caused by the enteroinvasive bacteria *Shigella*, remains one of the top diarrheal diseases in children under five.[1] Species/serotype diversity and geographical distribution strongly support the need for a multivalent vaccine against *S. flexneri*. Epidemiological data suggest that protection against re-infection is mainly achieved by antibodies specific for the O-antigen (O-Ag) moiety of the lipopolysaccharides (LPS). In the search for a highly immunogenic *Shigella* vaccine able to generate protective immunity in young children, we have engaged into the development of immunogens consisting of synthetic fragments of the putative O-Ags covalently linked *via* single point attachment to carrier proteins as a possible alternative to detoxified *Shigella* LPS-protein conjugates.

A multidisciplinary strategy interfacing medicinal chemistry and structure-based vaccinology was implemented. It consists firstly in the identification of sets of “protective” epitopes by use of a diversity of well-defined synthetic oligosaccharides representing fragments of the O-Ag of interest. Protein conjugates of the most promising oligosaccharides are then evaluated for their immunogenicity in mice. SF2a-TT15, a tetanus toxoid (TT) conjugate encompassing a synthetic hapten corresponding to three basic repeating units of the O-Ag from *S. flexneri* 2a (SF2a), the most prevalent *Shigella* serotype, was designed accordingly.[2] In preclinical studies, SF2a-TT15 has been shown to induce anti-LPS bactericidal antibodies. A GMP batch was produced and a first-in-human, single-blinded, observer-masked randomized, dose escalation, placebo-controlled study was conducted to assess safety and immunogenicity in healthy adult volunteers.[3]

With the first rationally designed synthetic oligosaccharide conjugate vaccine candidate in hand for the most prevalent *Shigella* serotype, this presentation primarily provides an overview of our strategy for a broad coverage *Shigella* vaccine. Emphasis is on hapten selection, vaccine design, safety and immunogenicity data following first use in human. Moreover, the presentation reports progress on a synthetic carbohydrate-based vaccine against *S. flexneri* 3a, another prevalent serotype. The improved synthesis, structural and antigenicity data of oligosaccharides representative of the O-Ag are exposed and the immunogenicity in mice of selected *S. flexneri* 3a synthetic oligosaccharide-protein conjugates are discussed. Lastly, the first promising bivalent glycovaccine candidate against *Shigella flexneri* is introduced.

This work was supported by the European Commission Seventh Framework Program (FP7/2007-2013) under Grant agreement No. 261472-STOPENTERICS and the Institut Pasteur Vaccinology Program (GPF Vaccinologie) under Grant agreement FlexBiVac.

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YOUNG SCIENTIST INVITED LECTURES

MULTIVALENT GLYCOCONJUGATES: GALECTIN LIGANDS WITH A THERAPEUTIC POTENTIAL

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Galectin-3 (Gal-3) is a soluble human lectin that takes an important part in cancer-related processes such as metastasis, tumorigenesis or angiogenesis. Like all galectins, Gal-3 binds b-galactoside-terminated glycans; however, we have recently discovered that the terminal *N*,*N*´-diacetyllactosamine (GalNAcβ1,4GlcNAc, LacdiNAc) epitope is a highly selective ligand for Gal-3 in contrast to the similarly frequent galectin-1.[1,2] In mammals, this epitope is rarely present in some N-and O-linked glycoproteins and it also occurs in connection with several types of cancer. The C-terminal carbohydrate recognition domain (CRD) of Gal-3 contains a binding groove composed of several subsites. Whereas its conserved part binds galactose-terminated disaccharides, longer oligosaccharide ligands of a suitable structure/length form additional interactions with its non-conserved part.[3] Gal-3 may also interact with the substituents at the glycan non-reducing end. Though it is present as a monomer in solution, Gal-3 is able to form oligomers upon contact with multivalent ligands. Therefore, presentation of glycans on multivalent carriers brings a considerable enhancement in binding affinity to Gal-3 due to cluster glycoside effects.

We have thoroughly studied the affinity of Gal-3 to a series of complex oligosaccharides based on Lac(di)NAc to examine Gal-3 binding specificity in all parts of its binding groove. The glycans were chemically conjugated to two types of multivalent carriers, namely bovine serum albumin, producing neo-glycoproteins,[4] and water soluble *N*-(2-hydroxypropyl) methacrylamide (HPMA) based copolymers aimed for *in vivo* applications.[5] Furthermore, multivalent attachment of a model LacdiNAc disaccharide to the polymer carrier through several linkers was examined to identify the most profitable conjugation strategy. The affinities of prepared compounds were determined in ELISA-type assays. The kinetics of binding was measured by surface plasmon resonance using our novel method of Gal-3 immobilization *via* biotin-neutravidin AviTag (Fig. 1).[3] The binding affinity constants K_d of the best conjugates were found to be in sub-nanomolar range. The biomedical potential of selected conjugates was tested *in vitro* using cultures of Gal-3-positive cancer cells.

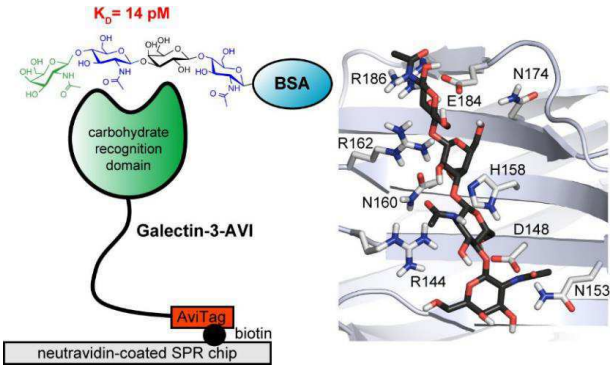

Fig. 1. The strongest Gal-3 ligand in the series: surface plasmon resonance setup and molecular modeling.[3]

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STRUCTURALLY INNOVATIVE NUCLEOSIDE AND NUCLEOTIDE ANALOGS OF POTENTIAL THERAPEUTIC INTEREST

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The development of novel nucleosides, nucleotides and analogs/mimetics is a relevant subject in organic and in medicinal chemistry, due to their ability to display a variety of biological properties.[1,2,3] Various compounds of these types are effective anticancer or antiviral drugs, acting through interference with nucleic acid biosynthesis.[1] However, some drawbacks limit their use, such as low oral bioavailability and the acquisition of resistance by cancer cells or by viruses against their action. The propensity of these types of molecules to show antimicrobial effects and to inhibit cholinesterases has also been described.[2,3] Therefore, the design and synthesis of novel bioactive nucleoside/nucleotide-like structures that may exhibit distinctive mechanisms of action as well as the focus on other or rather less studied potential therapeutic uses for these types of compounds is highly encouraging.

In this context, in this communication the synthesis and biological evaluation of novel nucleosides based on rather unexploited glycosyl units, 5'/6'-isonucleosides and nucleotide analogs embodying potential neutral bioisostere moieties for a phosphate system is presented. The groups of molecules synthesized included furanosyl and pyranosyl nucleosides constructed on 5- or 6-azido glucosyl and D-glucuronamide templates, nucleotide analogs possessing phosphoramidate or sulfonamide groups, including isonucleosides, and comprised purine, uracil and triazole derivatives as N-heteroaromatic motifs. The synthetic strategies involved N-glycosylation, sugar azidation, “click” 1,3-dipolar cycloaddition, Mitsunobu coupling or Staudinger-type reaction as key steps.

Some molecules were shown to display potent antiproliferative activities in cancer cells and to act via pathways that may be valuable to circumvent chemotherapy resistance. Enzymatic assays on relevant therapeutic targets revealed low micromolar inhibitors of acetylcholinesterase, while showing low neurotoxicity. Their GI_{50} or K_i values were similar or close to those of standard drugs, qualifying them as promising lead compounds for cancer or for Alzheimer’s disease. In addition, some nucleosides showed their ability to inhibit an essential ATP-dependent non-structural enzyme for Zika-virus replication, indicating their potential interest as anti-flavivirus agents.

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DUAL GLYCOSYLATION: A MINIMALIST APPROACH FOR OLIGOSACCHARIDE SYNTHESIS

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The diverse presence as well as their very specific bio-responses of glycoconjugates found in all living species requires scientists to synthesize and display the precise structure of these complex oligosaccharides and glycoconjugates for various studies in glycoscience. Multitudinous approaches to make glycosidic bonds in a highly stereoselective manner have speedingly emerged for the last two decades. Nonetheless, an efficient and universal method to confidently synthesize various glycoconjugates have yet been established, especially one that is applicable for large scale and structural diversity.

This lecture will present our recent advances in carbohydrate chemistry and glycobiology with emphasis on conceptually new strategies and tactics to illustrate our contribution to this field. Specifically, this talk will cover the acceptor-controlled glycosylation and protection-less glycosylation for oligosaccharide synthesis, and dual native chemical ligation (dNCL) for glycoprotein synthesis. I will present to you an easy access to peptidoglycans, which are used for metabolic labeling of bacteria.

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STRUCTURAL INSIGHTS INTO MUCIN GLYCOSYLATION AND RECOGNITION

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Mucin glycoproteins, involved in fundamental biological processes in health and disease, have multiple sites of glycosylation decorated with complex *O*-GalNAc glycans with distinct core structures. In particular, abnormal glycosylation of mucins is hallmark of cancer which make mucin-based tumor-associated carbohydrate antigens (TACAs) attractive targets for the development of glycan-based cancer therapies [1].

Mucins glycosylation is initiated by a large family of enzymes named (UDP)-N-acetyl-α-D-galactosamine(GalNAc):polypeptide transferases (GalNAc-Ts) [2]. Misregulation in expression/function of GalNAc-Ts yield mucin-based TACAs, key players in cancer cell growth and tumour immune surveillance [3]. In addition, lectins from immune cells and antibodies interact with mucin-based TACAs strongly modulating immune responses [4].

In this communication it will be reported the application of NMR methods to follow the glycosylation process of mucins by GalNAc-Ts enzymes, unveiling new structural, conformational and dynamic insights at atomic level of *O*-glycosylation [5]. As well, new structural insights into TACAs recognition by lectins from immune system and antibodies, uncovered by NMR protocols and assisted with molecular dynamics simulations, will be described [6,7].

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CARBOHYDRATE-BINDING PROTEINS AS TARGETS FOR ANTI-INFECTIVES AND DIAGNOSTICS: ESKAPE PATHOGEN PSEUDOMONAS AERUGINOSA AND ITS LECTINES

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Pseudomonas aeruginosa causes a substantial number of nosocomial infections and is the leading cause of death of cystic fibrosis patients. This Gram-negative bacterium is highly resistant against antibiotics and further protects itself by forming a biofilm. Moreover, a high genomic variability among clinical isolates complicates therapy.

Its lectin LecB, a carbohydrate-binding protein, is a virulence factor and necessary for adhesion and biofilm formation.[1] We analyzed the sequence of LecB variants in a library of clinical bacterial isolates and demonstrate that it can serve as a marker for strain family classification. LecB from the highly virulent model strain PA14 presents 13% sequence divergence with LecB from the well characterized PAO1 strain. Despite several amino acid variations at the carbohydrate binding site, glycan array analysis showed a comparable binding specificity for both variants.[2]

Based on the crystal structures of the lectin with its glycan ligands, we dissected the contributions of individual functional groups to protein binding in a biophysics-guided approach. This knowledge was then used for the development of small and drug-like glycan-based molecules as LecB inhibitors as future anti-biofilm compounds in chronic *P. aeruginosa* infections.[3-7] Multiparameter optimization yielded potent anti-biofilm compounds for both strain types and oral availability in mice.[8]

Thus, the different LecB sequences serve as marker for strain classification, but due to comparable ligand selectivity, LecB is a highly promising target for anti-virulence therapies, addressing members from both *P. aeruginosa* families, PAO1 and PA14.

In contrast, LecA binds galactosides with much lower affinity hampering therapeutic intervention at this target. Therefore, we have developed the first covalent inhibitor of a lectin and employed this LecA-specific irreversible inhibitor for LecA-dependent biofilm imagining of *P. aeruginosa*.[9]

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FRAGMENT-BASED DESIGN OF MAMMALIAN CARBOHYDRATE RECEPTOR LIGANDS

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In a multicellular organism, mammalian receptors recognizing carbohydrate structures mediate many important aspects of life such as self/non-self-differentiation, cell adhesion and migration. In particular, carbohydrate binding proteins expressed by cells of the innate immune system have gained increased interest for their restricted expression pattern and for offering the opportunity to modulate cellular functions. However, chemical probes that specifically address these receptors are sparse and carbohydrates as their natural ligands only offer limited affinity and specificity. Hence, the development of small molecules capable of binding to carbohydrate receptors and consequently modulating their biological function is an important task.

Unfortunately, carbohydrate recognition sites are rather flat and featureless, often solvent exposed and highly hydrophilic, thus being less accessible for drug-like molecules. We used fragments (MW 150 – 250 Da) of such drug-like molecules to probe the surface of several carbohydrate binding proteins for the availability of binding sites suitable for modulating of lectin function. Fragment screening was performed using several orthogonal methods such as NMR, SPR, and flow cytometry and hits were evolved into micromolar binders for targets from the C-type lectin family (Langerin, DC-SIGN). Lessons learned about lectin structure and dynamics, as well as the development of chemical probes and their immune cell modulation will be covered in this presentation [1-3].

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TARGETING PROTEIN-CARBOHYDRATE INTERACTIONS IN POLYSACCHARIDE BIODEGRADATION: THE POWER OF CARBOHYDRATE MICROARRAYS

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Biodegradation of plant and fungal cell-wall polysaccharides by bacteria is central to life sustainability and has an enormous potential for industrial and human health applications. Bacteria have evolved different strategies to efficiently target the carbohydrate-active enzymes (CAZymes) to the substrate, including: 1) assembly at the cell surface of a multi-protein complex, the cellulosome[1], displayed by the archetypal cellulolytic bacteria *Clostridium thermocellum* and *Ruminococcus flavefaciens* FD-1; 2) co-location and co-regulation of contiguous genes in polysaccharide utilization loci (PULs), displayed by the bacteria that colonize the human gut[2]. A common feature is that the CAZymes have a modular architecture in which the catalytic domains are appended to non-catalytic carbohydrate-binding modules (CBMs)[3]. With their specificities and substrate-targeting functions, CBMs play a pivotal role in polysaccharide biodegradation, potentiating the enzyme catalytic efficiency.

The CBM sequences identified with putative carbohydrate-binding activities and deposited in the CAZy database[4] have been exponentially growing. This is paralleled by the information derived from microbial genomics, metagenomics and transcriptomics studies. However, most await structural-functional characterization. The development of carbohydrate microarrays in the recent years has revolutionized the study of carbohydrate-protein interactions, addressing the need for high-throughput and systematic arraying of carbohydrate-probe libraries to identify the specificities and biological roles of carbohydrate-binding proteins[5-7]. In this communication, I will present our research focused on CBMs from two archetypal cellulolytic bacteria, *C. thermocellum* and *R. flavefaciens* FD-1, that colonize the soil and the rumen of mammals, respectively. Our integrative approach combines the development of carbohydrate microarrays[6,7] with biochemical and structure-function studies. We have amplified all the CBMs ('CBMomes') of these bacteria assigned to different CAZY families and located into different modular architectures in their genomes, and have screened in total 88 CBMs of *C. thermocellum* and 60 of *R. flavefaciens* FD-1 using carbohydrate microarrays composed of a diverse range of plant- and fungal-related carbohydrate-probes. The results show that the two bacteria express CBMs with different carbohydrate-binding specificities, reflecting to some extent the different polysaccharides that the bacteria may encounter in their habitat. This comparative analysis provides experimental evidence for the way that the different ecological niches modulate the selection of CAZyme modules with distinct carbohydrate ligand specificities. The structure-function studies give insight into the functionality of CBMs from complex polysaccharide utilization systems at the molecular level, toward understanding the mechanisms of polysaccharide utilization by different bacteria.

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INSIGHTS INTO THE ROLE OF TRYPTOPHAN IN C-MANNOSYLATION

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Abstract not provided by the author

NEW INSIGHTS INTO THE ROLE OF PROTEIN GLYCOSYLATION IN PROTOZOAN PARASITES

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Abstract not provided by the author

GLYCANS IN DIAGNOSTICS AND VACCINES

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Abstract not provided by the author

ORAL COMMUNICATIONS

CONVERGENT SYNTHESIS OF *N*-GLYCOPEPTIDES FROM THIOASPARTIC ACID-CONTAINING PEPTIDES

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The development and application of chemoselective ligation reactions is a field of research with growing importance. In this context, thiocarboxylic acids (thioacids) exhibit a unique and diverse chemistry. Via an oxidative mechanism, they can react with amines to form amides [1].Thus, they were not only used for the synthesis of larger peptides via ligation of fragments [2] but also for the synthesis of native *N*-glycopeptides [3-4]. In previous work, we applied thioacids for the preparation of glycosylated and fluorescently labeled amino acids [5]. The main challenge for the synthesis of *N*-glycopeptides using thioacids is the incorporation of thioaspartic acid into peptides via solid-phase peptide synthesis (SPPS).

Here, we report the synthesis of thioaspartic acid-containing decapeptides and their application to the convergent synthesis of *N*-glycopeptides (Fig. 1A). Tripeptides of the general type shown in Fig. 1B serve as building blocks for the incorporation of thioaspartic acid via Fmoc SPPS. A C-terminal pseudoproline motif suppresses racemization during fragment coupling and hinders aspartimide formation [6-7]. After global deprotection, the thioaspartic acid-containing peptides can be chemoselectively ligated with unprotected glycosylamines derived from mono- and oligosaccharides to form *N*-glycopeptides.

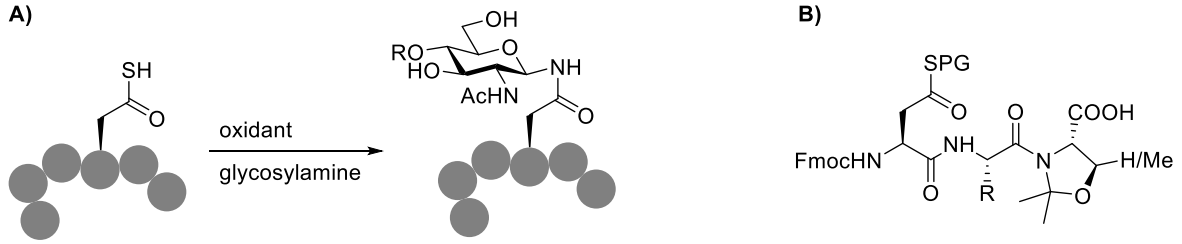

Fig. 1 - Convergent synthesis of *N*-glycopeptides using thioacids.

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SYNTHETIC O-ANTIGENS RELATED TO PATHOGENIC *BURKHOLDERIA* SPECIES ENABLE TO DECIPHER THE MINIMAL BINDING EPITOPES

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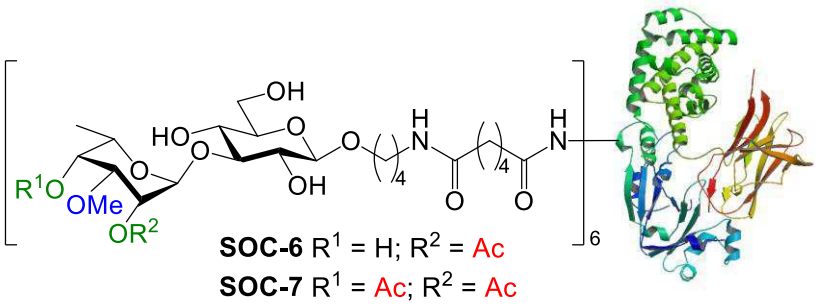
Burkholderia pseudomallei (*Bp*) and *Burkholderia mallei* (*Bm*), the etiologic agents of melioidosis and glanders, respectively, cause severe diseases in both humans and animals and are potential bioterrorism agents[1,2]. *Bp* and *Bm* lipopolysaccharides (LPS) are important virulence factors and major targets of the immune response [3]. Herein, we describe the synthesis of oligosaccharides featuring all of the reported acetylation/methylation patterns associated with *Bp* and *Bm* LPS O-antigens (OAgs) [4]. Using enzyme-linked immunosorbent assay, surface plasmon resonance and saturation transfer difference-nuclear magnetic resonance, we show that passively protective LPS-specific monoclonal antibodies strongly interact with the 6-deoxy-L-talose residue found at the terminal end of the OAgs [4]. Mice immunized with terminal disaccharide-CRM197 constructs (SOC-6 and SOC-7, see Figure) produced high-titer antibody responses that crossreacted with *Bm*-like OAgs [4]. These semi-synthetic LPS-based constructs thus stand as potential vaccines and diagnostics against *Burkholderia* related diseases.



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SEMISYNTHESIS OF COMPLEX-TYPE BIANTENNARY OLIGOSACCHARIDES CONTAINING LACTOSAMINE REPEATING UNITS FROM A BIANTENNARY OLIGOSACCHARIDE ISOLATED FROM A NATURAL SOURCE

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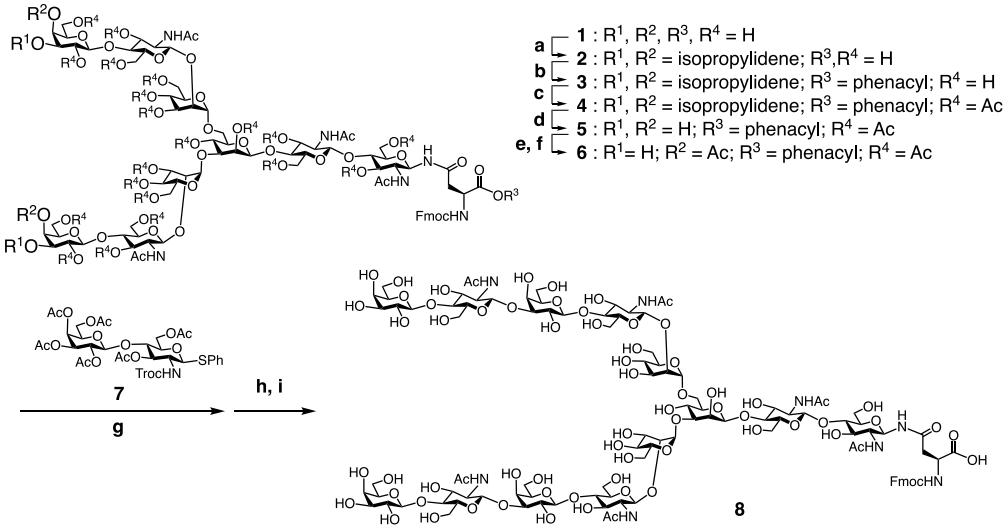
Poly-*N*-acetyllactosamine (Poly-LacNAc) structures on nonreducing terminals of oligosaccharides is one of the major structural features observed on complex-type oligosaccharides. This poly-LacNAc structures play important roles in many biological processes such as cell-cell adhesions; however, a detail of relationships between poly-LacNAc structures and chemical properties/biological activities of glycoproteins is still not clear.

To promptly access desired complex-type oligosaccharides, we have been developing novel semisynthetic strategies based on complex-type biantennary oligosaccharide **1** [1]. Oligosaccharide **1** can be isolated from hen egg yolk in large quantities [2], and then we can use oligosaccharide **1** as a starting material to synthesize varieties of complex-type oligosaccharides.

Here, we describe a facile semisynthetic strategy to convert the biantennary oligosaccharide **1** into a LacNAc-extended complex-type biantennary oligosaccharide **8** via ten-step chemical sequences. First, Gal-3,4-OH of oligosaccharide **1** was protected by isopropylidene groups. Subsequently, a carboxylic acid and other hydroxy groups were protected by phenacyl ester and acetyl groups. After acidic cleavage of the isopropylidene groups, we used orthoester to obtain appropriate oligosaccharyl acceptor **6** having two free Gal-3-OH. Next, we examined glycosylation reactions of acceptor **6** with disaccharyl thioglycoside **7** and then obtained a desired LacNAc-extended oligosaccharide. Finally, deprotection steps successfully yielded complex-type biantennary oligosaccharide **8** containing LacNAc repeating units [3].



Scheme. Synthesis of LacNAc-Extended Complex-Type Biantennary Oligosaccharide.
Reagents and conditions: (a) TsOH, acetone/DMF, 37 °C; (b) 2-bromoacetophenone, $^{i}Pr_2NEt$, DMF, 2 h; (c) DMAP, Ac_2O/pyridine, 2 h; (d) aq TFA, 0 °C, 5 min; (e) $CH_3C(OCH_3)_3$, TsOH, CH_2Cl_2, 20 min; (f) aq AcOH, 20 min; (g) donor **7**, NIS, TfOH, CH_2Cl_2, molecular sieves 4A, 0 °C, 1 h; (h) Zn, AcOH/ Ac_2O, 1 h; (i) 5 M NaOH/ MeOH, 15 min, then FmocOSu, $NaHCO_3$, CH_3CN/ H_2O, 2 h.

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CHEMICAL SYNTHESIS OF GLYCOPEPTIDES AND GLYCOPROTEINS USING ACYL-THIOL-ENE MEDIATED 'CLICK' LIGATION

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Thiol-ene ligation has emerged as a highly efficient 'click' reaction with diverse applications in glycoscience and chemical biology [1] The high yield, mild reaction conditions and complete regioselectivity have enabled site specific elaboration of peptides and proteins with a range of biomolecules including carbohydrates [2]. The methodology is fast and is compatible with oxygen, buffers and an aqueous environment.

We have explored a novel thiol-ene ligation strategy involving thio-acyl radicals (Acyl-Thiol-ene) for the rapid chemical synthesis of *N*-linked glycopeptides and glycoproteins [3]. Using an sequential, one-pot, ligation/acyl-transfer/desulfurisation approach we have prepared a range of glycopeptides and glycoproteins with native linkages [4]. The Acyl-Thiol-ene ligation is suitable for both peptide ligation and late stage *N*-glycosylation, rendering it an extremely useful methodology for glycoprotein synthesis. This approach represents a highly efficient strategy for accessing homogeneous glycoconjugates suitable for biological evaluation.

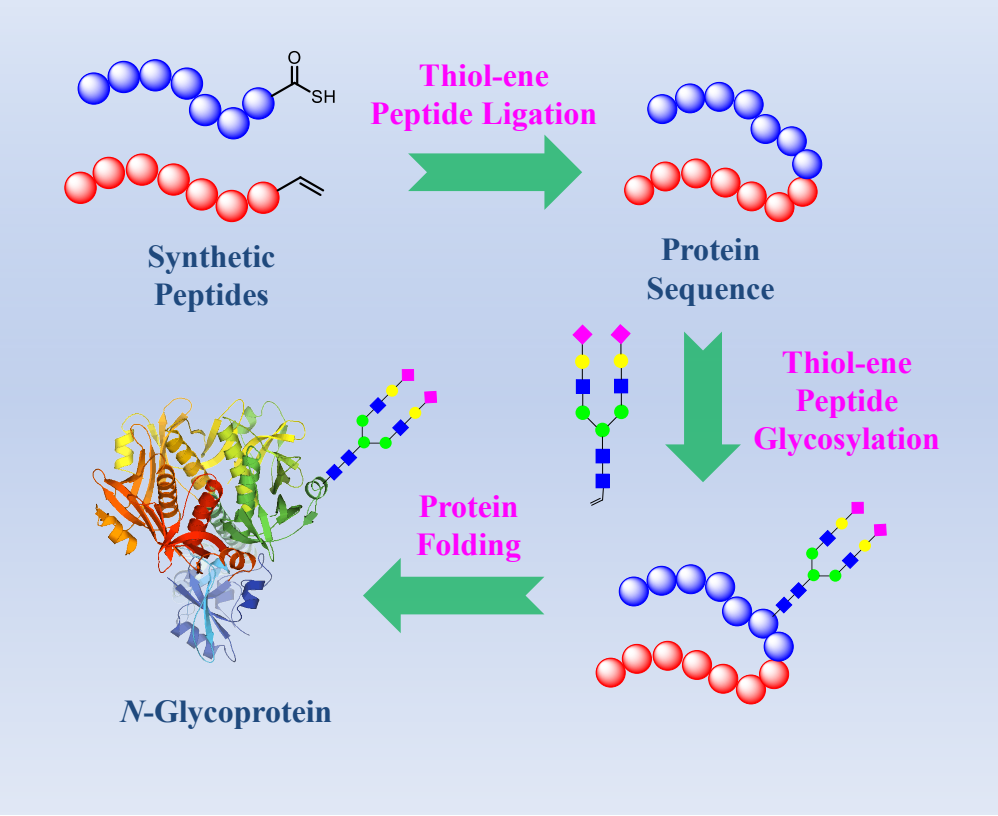

Fig. 1 - General strategy for chemical synthesis of glycoproteins using Acyl-Thiol-ene (ATE) ligation

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REACTIVITY AND STEREOSELECTIVITY IN GLYCOSYLATION: EMPHASIS ON STRUCTURE OF GLYCOSYL ACCEPTOR AND CONCENTRATION OF REAGENTS

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Considerable influence of concentration of reagents on the outcome of glycosylation has been reported in a number of cases, both the product yield and stereoselectivity as well as the reactivity of the glycosyl donor being affected by dilution of the reaction mixture. In most publications, results of the glycosylations at only two concentrations – “dilute” (0.001—0.005 mol/L) and “regular” (0.05 mol/L) – were usually compared. A more detailed analysis of the influence of concentration on glycosylation outcome, performed in a wide concentration range [1-3], revealed that concentration changes may affect, for example, stereoselectivity in a rather complicated manner. Depending on a system, stereoselectivity may dramatically increase upon dilution [2], experience a maximum [3] or depend on concentration of reagents in a more complex way [1]. Worthy of note is recently disclosed bimodal concentration-dependent reactivity pattern of a glycosyl donor [3], which is a special case of a more general phenomenon of existence of clearly defined concentration ranges, separated by critical concentrations,[4] where the solute has distinct chemical properties [1-7] (see the review [8]).

Although concentration is becoming increasingly realized as an important factor in glycosylation [3], there is a lack of commonly accepted understanding of the reasons behind. Current theories of reactivity of glycosyl donors and stereoselectivity of glycosylation pay attention mostly to the details of the glycosylation reaction mechanism and molecular structures of glycosyl donor [9] and, more rarely [10], glycosyl acceptor. In our opinion, rational discussion of the influence of concentration of reagents and the nature of glycosyl acceptor [5] on glycosylation outcome may benefit from using an approach, recently proposed by us [1-8], which explicitly accounts for the structure of a reaction solution and is based on the hypothesis that in many cases the real reactive species in solution are non-covalently-bonded supramolecular aggregates, “supramers”, rather than isolated molecules of reagents. An overview of the published [1-3, 5-7] and novel data will be presented, which suggests that the reaction solution structure cannot be ignored when analyzing the results of glycosylation. This work was supported by the Russian Science Foundation (Project No. 16-13-10244).

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C-GLYCOSIDES IN THE SYNTHESIS OF THE PHYTOTOXIN DIPLOPYRONE

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Diplopyrone is a phytotoxin isolated from the fungus *Diplodia mutila* and reported by Evidente and coworkers in 2003. *D. mutila* is considered to be responsible for cork oak decline in parts of Europe where the disease has large and negative economic and environmental impacts. The pyranopyran core structure found in diplopyrone occurs in many other natural products that encompass a range of biological effects including antibiotic activity. Viewing diplopyrone as a "C-glycoside problem" opens up multiple synthetic routes that are based on carbohydrate starting materials. We have been exploring carbon-carbon bond forming reactions that utilize glycosyl sulfones, nitriles, and vinyl glycosides in our efforts to synthesize enantiomeric, (-)-diplopyrone from D-glucose and D-galactose. We have successfully synthesized the enantiomer of the structure that was originally proposed for diplopyrone. Our carbohydrate-based synthesis is completely stereoselective and amenable to the preparation of analogs for studies of phytotoxic and antibiotic activity.

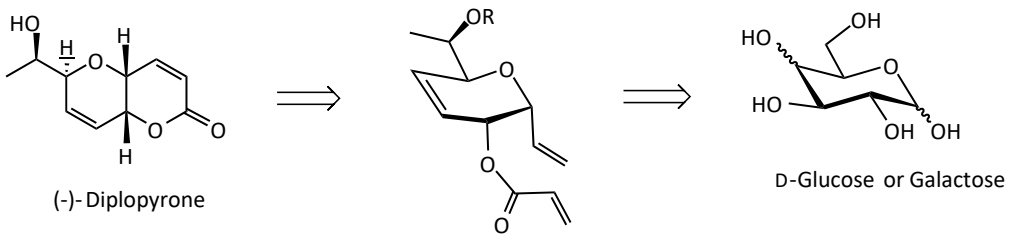

Fig. 1

CATALYTIC STEREOSELECTIVE SYNTHESIS OF DEOXYGLYCOSIDES

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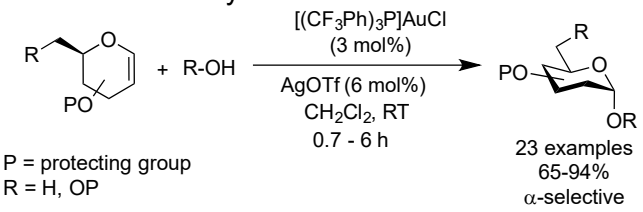
The ability to perform *O*-glycosylation reactions in a catalytic and stereoselective manner is one of the main remaining challenges in carbohydrate chemistry. Biologically relevant chiral acetals such as deoxy-hexoses are prominent components of natural products [1], and present a significant synthetic challenge because of the lack of substituents at C-2 to direct the nucleophile approach. Thus, efforts, from our group have been devoted to the development of mild and catalytic methods for their stereoselective synthesis [2]. Recent years have seen a steady increase in the application of transition metal catalysis to oligosaccharide synthesis [3], since the careful choice of ligand/transition metal combination can offer significant improvements over traditional methods in terms of atom economy, high yields and control of anomeric selectivity.



Herein, we report the first example of Au(I) direct activation of glycals to yield α-deoxyglycosides. Products resulting from the *syn* addition of a proton and oxygen from the nucleophile across the carbon-carbon double bond are formed when [(*p*CF_3Ph)$_3$P)AuCl] and AgOTf are used as the glycosylation promoter. This mechanistically interesting reaction is mild and widely applicable to a range of glycal donors and nucleophile acceptors with excellent yields and high selectivity for the α-anomer and is tolerant of most common protecting groups. We exemplify the generality and versatility of the approach in the stereoselective synthesis of a series of disaccharides, glycosyl-amino acids and other glycoconjugates [4].

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$Fe(acac)_3$, AN INEXPENSIVE AND GREEN CATALYST FOR REGIOSELECTIVE ACYLATION OF CARBOHYDRATES

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Regioselective protection strategy remains a central challenge in carbohydrate chemistry, for the preparation of value-added carbohydrate chemicals and the synthesis of complex oligosaccharides. Although organotin reagents were identified as the best reagents for the selective protection [1], they have to be limited or banned now due to their inherent potential toxicity. The reduced amounts of organotin reagent as a catalyst [2] and an organoboron reagent (Taylor's catalyst) as a catalyst [3] have been successfully used for the selective protection. Recently, an $Fe(dibm)_3$ catalyst was reported by us to lead high regioselectivities for the alkylation of carbohydrates. [4] The iron-based reagents are usually non-toxic and environmentally benign. The present work showed an inexpensive and commercial available catalyst, $Fe(acac)_3$. This Fe(III)-based catalyst could catalyze regioselective acylation of 1,2- and 1,3-diols and glycosides containing a *cis*-vicinal diol (Figure 1). The catalytic process is proposed to proceed through a cyclic dioxolane-type intermediate formed by diol with iron(III) species under base condition. The same product patterns as for dibutyl tinoxide-mediated approaches were obtained. Therefore, Fe(III) reagents as green catalysts proved to have a great potential to be widely used in regioselective protection of carbohydrates.

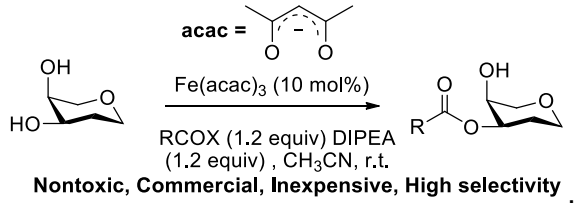

Fig. 1 - $Fe(acac)_3$-catalyzed regioselective acylation of carbohydrates containing a *cis*-vicinal diol.

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DE NOVO SYNTHESIS OF PARTIALLY FLUOTINATED CARBOHYDRATES

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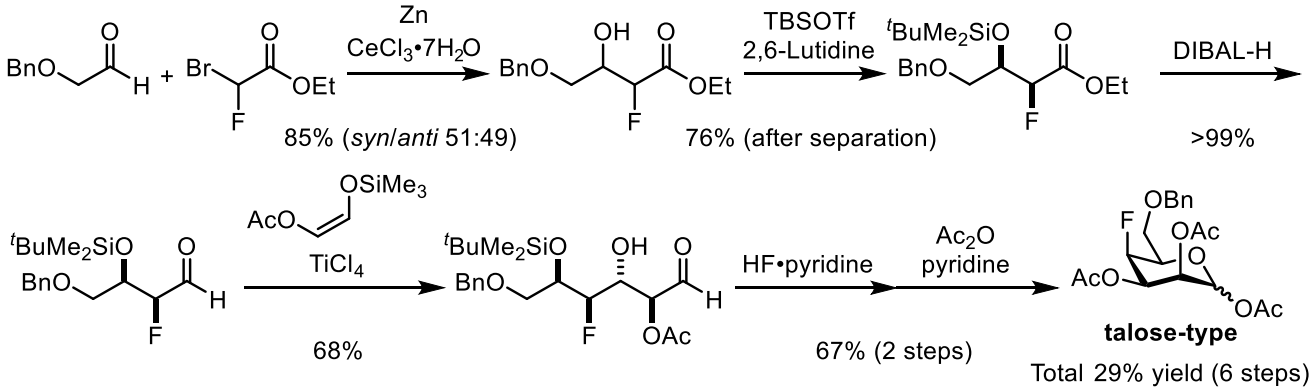
Fluorine atoms work as a stable bioisoster of other functional groups such as a hydroxyl group and ether. Therefore, introduction of fluorine atoms into natural products has been paid much attention because of their effects on biological activity [1]. Moreover, a partially fluorinated carbohydrate such as ^{18}F fluorodeoxyglucose has already been used for positron emission tomography (PET) in clinics [2]. Conventional syntheses of fluorinated carbohydrates rely on fluorinating reagents which can introduce fluorine atoms into organic molecules [3]. There are several drawbacks in this strategy. For example, the precursor requires multiple steps to prepare and fluorinating reagents are expensive. Therefore, a synthetic strategy which enables preparation of complex fluorinated molecules from a small fluorinated compound is highly desirable.

We have interested in *De Novo* synthesis which is a powerful method to make carbohydrates including rare sugars. McMillan and co-workers have already achieved a short step synthesis via direct aldol reaction using an organocatalyst and the subsequent Mukaiyama aldol reaction [4]. Thus, we envisioned that partially fluorinated carbohydrates would be a target molecule of *De Novo* synthesis from a small achiral molecule. Ethyl 2-bromo-2-fluoroacetate was used as a stable fluorinated molecule and Reformatsky reaction and the subsequent ^{t}BuMe$_2$Si (TBS) protection and DIBAL reduction afforded the substrate for Mukaiyama aldol reaction. Optimization of reaction conditions such as Lewis acid, solvent, and temperature enables us to obtain the precursor of partially fluorinated carbohydrate as a single diastereomer. Further transformation including deprotection of TBS group and acylation of free hydroxy groups afforded the talose-type product in 29% overall yield (6 steps). Preparation of a chiral intermediate enables us to access optically active fluorinated sugars. Synthesis of other-types of partially fluorinated carbohydrates has also achieved by changing reaction conditions of Mukaiyama aldol reaction.



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PYRANOSIDE-*INTO*-FURANOSIDE REARRANGEMENT – A WINDING ROAD FROM A SIDE REACTION TO A NEW SYNTHETIC METHOD

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Great interest to natural furanoside-containing compounds has challenged the development of preparative methods for their synthesis. Herein we report new results achieved using a novel reaction in carbohydrate chemistry, namely a pyranoside-*into*-furanoside (PIF) rearrangement permitting transformation of selectively O-substituted pyranosides into the corresponding furanosides [1,2], including studies of its mechanism, synthetic scopes and limitations and most recent synthetic applications. The discovered process, which has no analogy in organic chemistry, includes acid-promoted per-O-sulfation of pyranosides which is accompanied by ring rearrangement into the furanoside form followed by solvolytic O-desulfation. Some interesting conformational effects occurring in exhaustively sulfated monosaccharides were observed during the mechanistic investigations [3]. Their possible role in governing the PIF transformation is discussed in this communication. The reported reaction was shown to be a very useful tool for the preparation of furanoside-containing complex oligosaccharides, which was demonstrated by syntheses of oligosaccharides related to bacterial and fungal cell-wall polysaccharides.[4-8]

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SYNTHESIS OF POLYFLUORINATED α-GALACTOSYL CERAMIDE ANALOGUES AND BIOLOGICAL IMPLICATIONS

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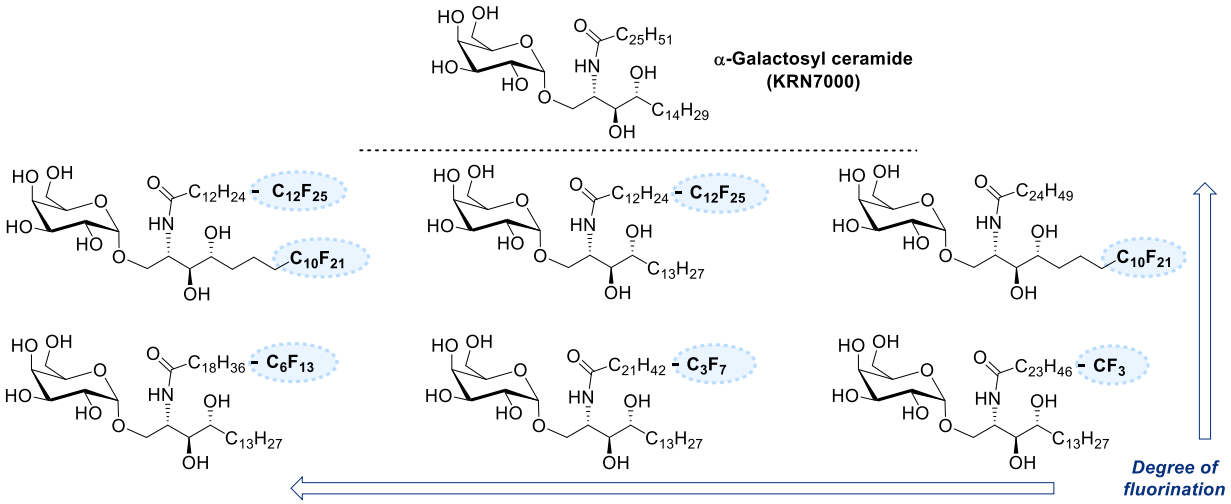
α-Galactosyl ceramide (KRN7000) is a synthetic glycosphingolipid developed as anticancer drug candidate, which upon association with CD1d protein activates NKT cells. This event leads to the release of different cytokines which modulate a TH1 response (antitumoral and antimicrobial functions) or a T_H2 response (against autoimmune diseases). Unfortunately, the simultaneous secretion of both cytokines limits the therapeutic potential of KRN7000 as they can antagonize the biological functions of each type alones. For that reason, the synthesis of new KRN7000 analogues with a more biased T_H1/T_H2 profile is an area of special interest [1].

It has been suggested that T_H1 response is certainly favoured by stabilization of the KRN7000-CD1d-NKT complex [2]. In this regard, it has been recently demonstrated that perfluorinated chains produce stronger interactions with hydrophobic cavities of proteins than its hydrocarbon counterparts [3].

In this communication, we will report the synthesis of set of KRN7000 analogues bearing different perfluoroalkyl chains at the ceramide moiety, with the aim of increasing the stability of the complex to obtain a selective T_H1 response. Biological implications based on binding affinity towards mouse CD1d protein as well as human iNKT cell stimulation experiments will be also discussed.



Acknowledgements

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NOVEL METHODOLOGIES IN SIALYLATION REACTIONS

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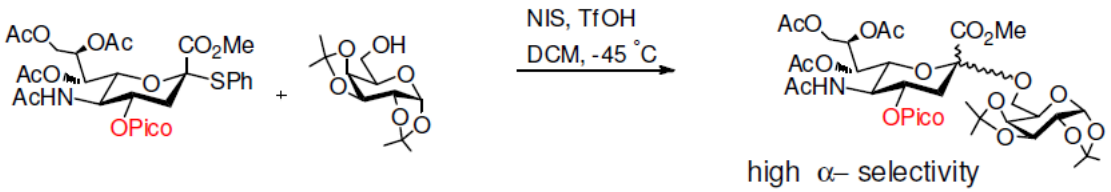
N-Acetylneuraminic acid is expressed at the terminal position of glycoconjugates in cell membranes [1,2].
The synthesis of sialic acid containing glycoconjugates (sialosides) is important for understanding its biological functions and for designing therapeutics. On the other hand, the lack of efficient chemical methodologies for the high-yielding and stereoselective synthesis of these targets is 'bottleneck' in oligosaccharide synthesis. Despite the tremendous advancement in the last decades, little is known about the effect of protecting groups at C-4 and at the glycerol chain (C-7, C-8 and C-9). As a part of ongoing research effort dedicated to investigating O-protecting groups, herein we report the investigation of *O*-picoloyl groups. All aspects of picoloyl group chemistry including regioselective introduction and effect on the outcome of sialylation reactions in synergy with excess of triflic acid [3].



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DESIGN, SYNTHESIS AND PROPERTIES OF SHAPE-SWITCHABLE GLYCOMACROCYCLES

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Macrocycles are challenging synthetic targets that display unique properties stemming from their circular shape [1]. Carbohydrates are cheap and highly relevant building blocks for achieving macrocycles because they are chiral, polyfunctional, and their restricted conformational freedom imparts rigidity to the macrocyclic backbone, thereby resulting in a well-defined molecular shape [2].

Enabling the conformational switching of glycomacrocycles upon the influence of external stimuli is an attractive means of selectively modulating their molecular and supramolecular features. This approach can be implemented by the incorporation of one or several photoresponsive azobenzene hinges into the macrocyclic backbone [3]. Indeed, light is a spatiotemporally resolved and non-invasive stimulus, and the azobenzene photoswitch offers excellent and widely tunable photochromic properties.

By using different synthetic strategies and molecular designs, we were able to build a set of different photoswitchable macrocyclic architectures displaying unique properties. On the one hand, the azobenzene *trans*/*cis* photoisomerization drives a large and reversible geometrical change in the overall structure. On the other hand, a transfer of chirality operates from the sugar units to the azobenzene, hence resulting in a spatially directed photoswitching [3,4]. Such a reciprocal transmission of information between the carbohydrates and the azobenzene(s) through the cyclic structure opens up a new way for the conception of smart molecular systems with applications in supramolecular chemistry, chiroptical devices or nanomachines.

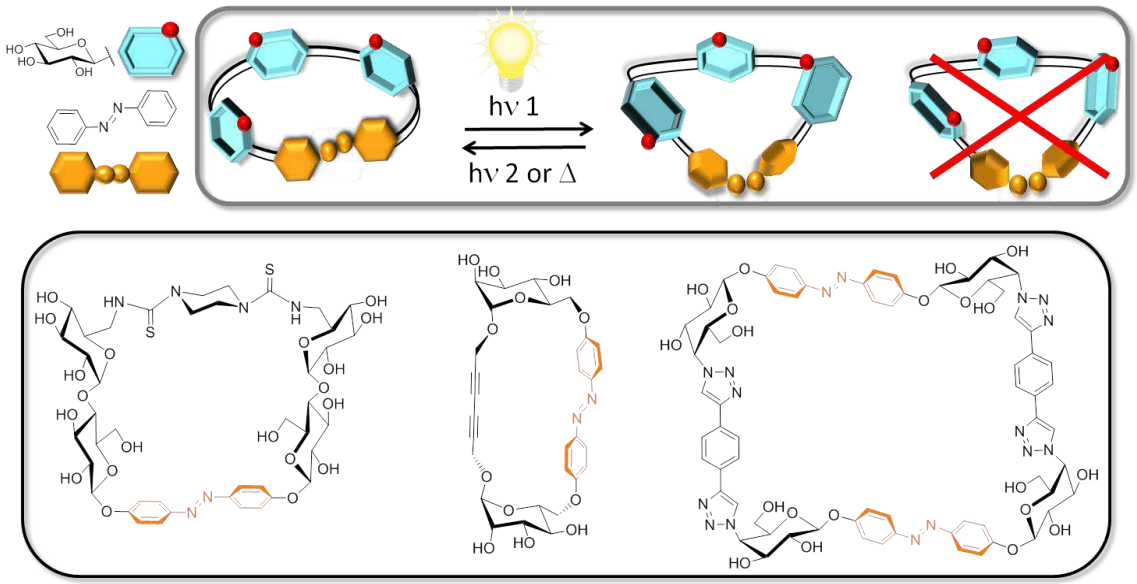

Fig. 1 – Top: shape switching of glycomacrocycle and unidirectional photoisomerization of the azobenzene hinge. Bottom: examples of structures prepared by our group.

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GLYCODENDRITIC SYSTEMS AS TOOLS IN BIOMEDICINE

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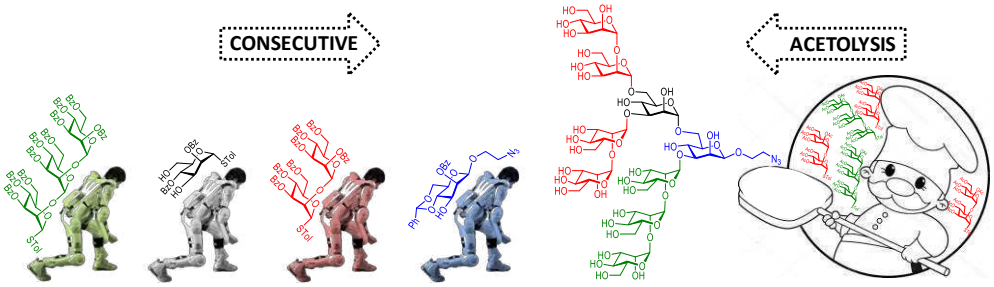
DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin) is a C-type lectin present on the surface of immature dendritic cells and plays a key role in the immune system and viral infections. The main carbohydrate ligand recognized by DC-SIGN is the high-mannose glycan, $(Man)_9(GlcNAc)_2$, with the mannosyl nonasaccharide (Man_9) being the main epitope to interact with this receptor. Our research group is interested in the interaction, both blocking and internalization, of the multivalent sugar-decorated glycoconjugates with this lectin due to the enormous relevance in many biological applications. In this work, we proposed the use of glycodendritic systems as tools in biomedicine, in particular:

Glycodendritic systems functionalized with peptides (glycodendripeptides) and several copies of mannose with potential biomedical activities as a proof of concept to develop a new class of synthetic vaccines. We have demonstrated (both in vitro and using a mouse model) the potential application of these glycodendropeptides as synthetic vaccines against food allergy[1].

The total synthesis of Man_9 and other complex mannose oligosaccharides has been explored; however, the complexity of this kind of branched glycan structures prevents the accessibility to large amounts required to address biological studies. In this work, novel convergent, fast, straightforward, high yield and large amount accessible synthesis of the nonasaccharide Man_9, using two different strategies. In the first approach, we used a “consecutive strategy”, developed recently in Dr. Javier Rojo group,[2] to obtain the key intermediates in few synthetic steps reducing the number of purification chromatographies. The second approach is based on obtained these key intermediates directly from the acetolysis of dry baker’s yeast, a starting material very accessible at a very low cost[3].



Finally, we reported the synthesis of a new hexakis adduct of [60]fullerene substituted with twelve cyclooctyne, maleimide or mixture moieties to further carry out copper-free reactions (SPAAC and thiol-maleimide reactions). This strategy to achieve clicked adducts of [60]fullerene is of special interest for biological applications[4].

Acknowledgements. We would like to acknowledge MINECO for financial support (CTQ2011-23410 and CTQ2014-52328-P) and for a FPI fellowship to Javier Ramos-Soriano.

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A NEW APPROACH TO IDENTIFYING FUCOSYLTRANSFERASE INHIBITORS AS POTENTIAL CANCER THERAPEUTICS

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In cancers, increased fucosylation (attachment of fucose sugar residues) on cell-surface glycans—resulting from the abnormal upregulation in the expression of specific fucosyltransferase enzymes (FUTs)—is one of the most important types of glycan modifications associated with malignancy. Fucosylated glycans on cell surfaces are involved in a multitude of cellular interactions and signal regulation in normal biological processes. For example, sialyl Lewis^X is a fucosylated cell-surface glycan that is abnormally abundant in some cancers where it has been implicated in facilitating metastasis, allowing circulating tumour cells to bind to the epithelial tissue within blood vessels and invade into secondary sites by taking advantage of glycan-mediated interactions.

To identify inhibitors of FUT enzymes as potential cancer therapeutics, we have developed a novel high-throughput assay that makes use of fluorogenically labeled oligosaccharides as probes of fucosylation. These probes, which consist of methylumbelliferyl glycosides, are recognized and hydrolyzed by specific glycoside hydrolase enzymes to release fluorescent methylumbelliferone, yet when the probes are fucosylated prior to treatment with the glycoside hydrolases, hydrolysis does not occur and no fluorescent signal is produced. We have demonstrated that this assay can be used to measure the inhibition of FUT enzymes by small molecules, since blocking fucosylation will allow glycosidase-catalyzed hydrolysis of the labeled oligosaccharide to produce a fluorescent signal.

Employing this assay, we have screened a focused library of small molecules for inhibitors of a human FUT enzyme involved in the synthesis of sialyl LewisX, and demonstrate that our approach can be used to identify potent FUT inhibitors from compound libraries in microtitre plate-format.

RECENT ADVANCES IN THE SYNTHESIS AND BIOLOGY OF 2-ACETAMIDO IMINOSUGARS C-GLYCOSIDES

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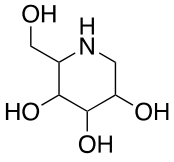
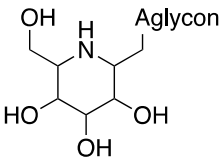
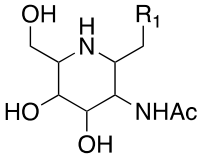
Iminosugars represent one of the most promising classes of sugar analogs [1]. Their C-glycosides allow incorporation of stereochemically defined aglycon moieties to access glycoconjugate analogs [2]. The main challenge associated with this family of compounds is the development of robust and general routes to introduce structural diversity at a late stage to accelerate the discovery of biologically relevant molecules [3]. Our group is exploring synthetic pathways [4] to introduce structural diversity at C-1, C-2 and C-6 of six-membered iminosugars, in order notably to access GlcNAc analogs [5] of therapeutic relevance. Our most recent results obtained on this topic in terms of chemistry and biological activity will be presented.



Iminosugar



Iminosugar C-glycoside



GlcNAc analogs

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MECHANISTIC ANALYSIS ON A UDP-GLUCURONIC ACID 4-EPIMERASE

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Short-chain dehydrogenases/reductases (SDRs) are a superfamily of enzymes which catalyze $NAD(P)^+$/NAD(P)H dependent oxidations and reductions on a wide range of substrates [1]. Within the SDR family, UDP-glucuronic acid 4-epimerases convert UDP-D-glucuronic acid to its epimer UDP-D-galacturonic acid, an important precursor for the synthesis of cell-wall polysaccharides in plants and bacteria [2]. The catalytic mechanism of UDP-glucuronic acid 4-epimerases is yet unknown and crystal structures of these enzymes have not been reported. Here, we show the biochemical characterization and crystallization of a novel UDP-glucuronic acid 4-epimerases (Glc-epi), in order to decipher its mechanism of action. The crystal structure of Glc-epi was solved in its Michaelis-Menten complex together with the natural substrate UDP-D-glucuronic acid and cofactor NAD^+ at 1.3 Å resolution. Based on the crystallographic information and mutagenesis studies, we have investigated the importance of active site amino acid residues and their influence on the catalytic mechanism. In order to gain more detailed understanding on the epimerases in general, the product UDP-D-galacturonic acid and a fluorinated inhibitor have been synthesized as mechanistic probes for crystallographic and catalytic studies. Enzymatic synthesis of these activated sugars is presented in order to highlight the power of biosynthesis in production of nucleotide sugars.

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IDENTIFICATION OF STRUCTURAL MODIFICATIONS THAT DETERMINES DIVERSITY OF *KLEBSIELLA PNEUMONIAE* O1, O2, AND O3 O-ANTIGENS AND ARE IMPORTANT FOR MONOCLONAL ANTIBODY-BASED DIAGNOSTICS AND THERAPY

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Klebsiella pneumoniae causes severe nosocomial infections, bloodstream infections, pneumonia, meningitis, and sepsis, mainly in individuals with impaired immune system. The pathogen is at the forefront of Gram-negative bacteria causing sepsis and belongs to ESKAPE group of pathogens characterized by resistance to last resort antibiotics, especially by the carbapenem-resistance. Since available therapies against *Klebsiella* infections are not sufficient, new therapeutic strategies have to be developed and may use O-antigen (lipopolysaccharide, LPS built of the O-specific polysaccharide, O-PS) as a target molecule. Contrary to capsular antigens (approx. 78 K-serotypes), *K. pneumoniae* O-PS regions seem to have limited variability – seven main O-serotypes and a few variants were identified to date [1]. Further insights into diversity of main surface antigens of *Klebsiella*, especially LPS O-PS*,* is necessary for developing efficient O-antigen based therapy.

Herein, we sum up our results of studies on modifications or subserotypes discovered for O1, O2, and O3 *K. pneumoniae* O-serotypes, what contradicts a view about limited diversity of *K. pneumoniae* O-antigens. A new galactan type was previously identified for O1 and O2 serotypes and called D-galactan-III (modified galactan-I substituted by terminal D-α-Gal) [1, 2]. We also reported that the O3 serogroup, previously considered to have uniform O-antigen built up of pentasaccharide repeating unit of mannan, is represented by three different subtypes (O3, O3a, O3b) differing in the number of mannose residues within the O-PS repeating units. Two new subserotypes were identified: O3a (tetrasaccharide repeating unit of the O-PS) and O3b (trisaccharide repeating unit of the O-PS) and denoted in the same way as *E. coli* O9 subserotype (O9a). All structural modifications identified (O1, O2, and O3, O3a, O3b) were supported by analyses of the genes encoding each O-PS. Substitution of →3)-α- D-Gal*p* by a branching terminal α-D-Gal*p* in the galactan-III was dependent on the presence of the *gmlABC* operon encoding additional glycosyltransferase. The novel variants of O3 strains (O3a and O3b) carry mutations in the WbdA mannosyltransferases, which can explain the observed structural differences.

Structural analyses were performed for wild strains and isogenic mutant pairs using sugar and methylation analysis, MALDI-TOF mass spectrometry as well as classical and High-Resolution Magic Angle Spinning (HR-MAS) NMR.

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TUNING THE ACTIVITIES OF IMINOSUGARS

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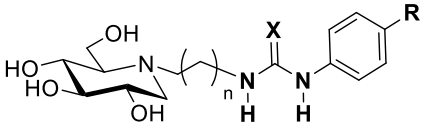
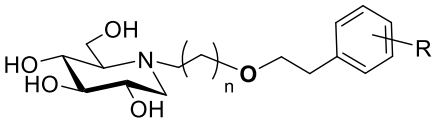
Iminosugars, that is, sugar mimetics where the endocyclic oxygen has been replaced by a nitrogen atom behave as inhibitors of glycosidases, pivotal enzymes involved not only in carbohydrate metabolism, but also in cell wall formation, lysosomal storage disorders, diabetes, cancer, and microbial infections [1]. Accordingly, iminosugars are currently considered as attractive drug candidates, and their modification is a relevant area within Medicinal Chemistry research [2-5].

Herein we report the synthesis and evaluation of novel 1-deoxynojirimycin derivatives, decorated with both, chalcogenide motifs (**1**), and substituted aryl fragments (**2**), leading to fine-tuned activities, as selective β-glucosidase, or acetylcholinesterase inhibitors. Modulation of such enzymes can be of interest for the treatment of Gaucher's or Alzheimer's diseases, respectively.



1

X = O, S, Se
R = Halogen, alkyl, OAlkyl



2

R = H, OH, OMe, -O-CH_2-O-

We thank the Dirección General de Investigación of Spain (CTQ2016-78703-P, CTQ2011-28417-C02-01, CEI10/00018), the Junta de Andalucía (FQM134), the CONACYT (México), the European Regional Development Fund (FEDER) (501100008530), and the EU Research Potential (FP7-REGPOT-2012-CT2012-31637-IMBRAIN) for financial support

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RATIONAL DESIGN OF NEW GLYCOSIDASE INHIBITORS: CYCLIC SULFATES AS CONFORMATIONAL TRAPS TO SELECTIVELY INHIBIT GLYCOSIDASES

Marta Artola,[a] L. Wu,[b] C. Hedberg,[a] C. de Boer,[a] L. Raich,[c] J. D. Codée,[a] G. A. van der Marel,[a] J. M. F. G. Aerts,[d] C. Rovira,[c] G. J. Davies[b] and H. S. Overkleeft.[a]

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Cyclophellitol and cyclophellitol aziridine conformers are potent, irreversible retaining glycosidase inhibitors that covalently target glycosidases by conformationally mimicking the emerging half-chair oxocarbenium ion transition state.[1,2] The Koshland double displacement mechanism employed by these enzymes proceeds through an oxocarbenium ion-like transition state involving the formation of a covalent glycosyl-enzyme intermediate.[3]

We have developed a conceptually new class of glycosidase inhibitors, named “Cyclosulfates”, bearing a cyclic sulfate as an electrophilic trap affording different conformational glycomimetics. As for example, α-*glu*-cis-cyclosulftate **1** adopts a $^4C_1$ different from the $^4H_3$ half chair transition state conformation of natural glycosyl, cyclophellitol or cyclophellitol aziridine, and covalently inhibits α-glucosidases in an potent and selective manner by mimicking the Michaelis conformation.[4] (Figure 1). Variation of the configuration of the cyclophellitol core, exploration of cyclosulfate bioisosteres and trans cyclic sulfates as well as the implementation of this approach to disacharide or furanose analogues has been a valuable strategy for the generation of diverse effective covalent and competitive glycosidase inhibitors. To further validate this new class of inhibitors, we have performed metadynamics simulations, 3-D crystal structure analysis in complex with different glycosidases, *in vitro* and *in situ* activity based protein profiling, as well as *in vivo* studies in Zebrafish embryo.

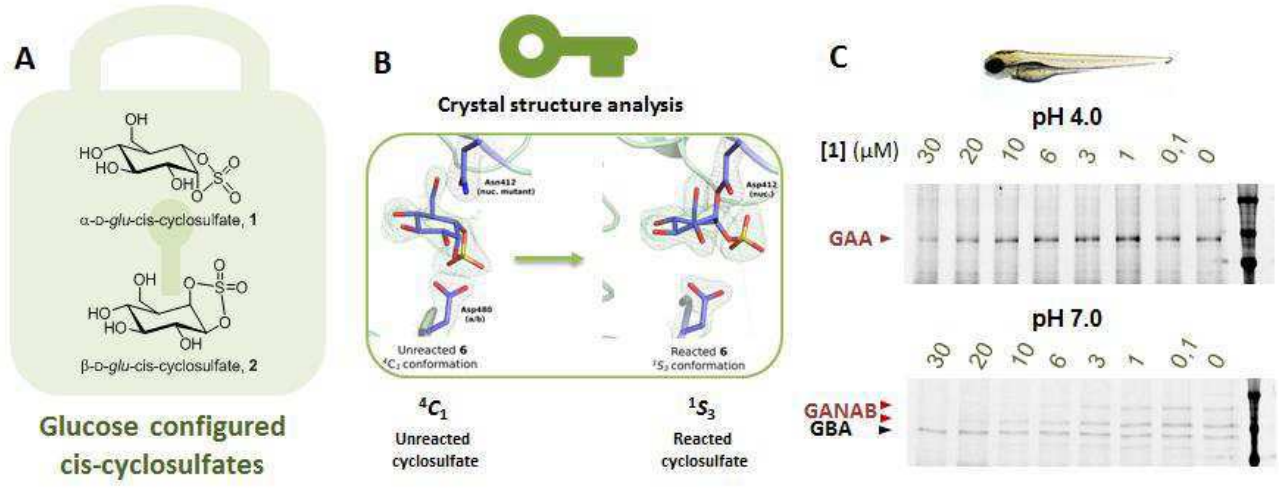

Figure 1. Rational design of new conformational glycosidase inhibitors by using cyclic sulfates as electrophilic trap. A. Chemical structures of new glucosidase inhibitors cyclosulfates **1** and **2**. B. Crystal structure analysis of α-*glu*-cis-cyclosulfate **1** inhibition of α-glucosidases. C. Selective inhibition of α-glucosidases in Zebrafish embryo.

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REINVESTIGATION OF FINE EPITOPE SPECIFICITY OF EB-A2 ANTIBODY USED IN THE IMMUNE DETECTION OF GALACTOMANNAN OF ASPERGILLUS FUMIGATUS: MOLECULAR BASIS OF FALSE POSITIVE SIGNALS

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Over the past decades fungal diseases have become a serious clinical problem stimulating the development of new diagnostics. ELISA-based methods were shown to be widely applicable in laboratory practice. But certain diagnostics of this type are accompanied by false-positive signals because of cross-reactivity of used antibodies to challenge the development of instruments for assessment of antibody specificities. This is of importance for antibodies against fungal cell-wall polysaccharides which can be heterogenic and hold the epitopes which are common for several pathogens. That is why the native fungal polysaccharides can be not applicable for assessment epitope structure of anti-polysaccharide antibodies.

The use of GlycoArray built up of synthetic oligosaccharide of defined structure and representing the fragments of fungal polysaccharides permits the precise characterization of antibody specificity. This will be reported on the example of epitope assessment for EB-A2 mAb used in ELISA based assay for detection of galactomannan of *Aspergillus fumigatus*. This complex antigenic polysaccharide is built up of mannan core chain with attached oligogalactofuranosyl side chains [1,2]. The synthesis of corresponding oligosaccharides was based on recently described pyranoside-*into*-furanoside ("PIF") rearrangement [3-6]. Target oligosaccharides which represent different galactomannan fragments were obtained as aminopropyl glycosides which were further transformed into corresponding biotinylated conjugates and loaded onto streptavidin coated plates to produce thematic GlycoArray.

Assaying of EB-A2 mAb on thus designed GlycoArray revealed that this mAb recognizes in fact not the tetra- but the disaccharide epitope which is common for a number of fungal and bacterial species to show the molecular basis of widely protocolled false positive results in the use of EB-A2 mAb based diagnostics. This discovery challenges the obtaining of more specific antibodies to increase the accuracy of galactomannan determination. This work is in the progress [7].

Present study was supported by RSF grant 14-23-00199.

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SYNTHETIC *SCHISTOSOMA MANSONI* GLYCAN COATED GOLD NANOPARTICLES AND THEIR USE IN DIAGNOSTIC AND VACCINE APPLICATIONS

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Schistosomiasis, a major neglected tropical disease, is caused by parasitic *Schistosoma* worms. It has become clear that a vaccine against schistosomiasis should be part of an effective elimination strategy [1]. Our goal is to explore specific glycan antigens as a potential vaccine candidate against schistosoma infection. Analysis of the glycans from *Schistosoma mansoni* revealed a subset of sugars containing a unique and specific alpha-(1→2)-difucose element that is antigenic during schistosoma infection. Here we present a modular synthetic route towards a library of alpha-(1→2)-difucose rich schistosoma glycans and their conjugation to gold nanoparticles for diagnostic and vaccination purposes [2]. We showed that these gold nanoparticles could be recognized by monoclonal antibodies and IgG present in infected human sera by using an ELISA based diagnostic assay.

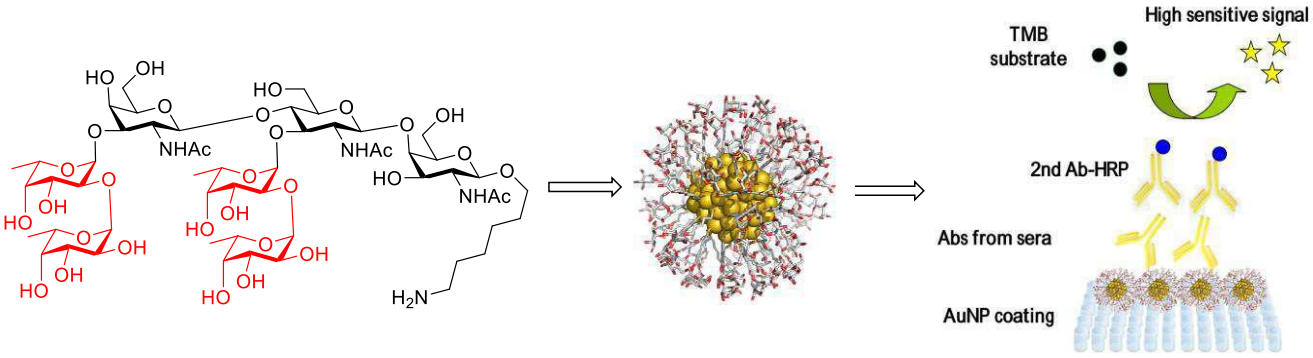

Fig. 1 - Example of a glycan bearing the unique alpha-(1→2) difucose element (red) and their conjungation to a gold nanoparticle. These particles were screened against monoclonal antibodies and sera from infected humans.

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AMADORI REARRANGEMENT PRODUCTS AS LIGANDS FOR MANNOSIDE-SPECIFIC LECTINS

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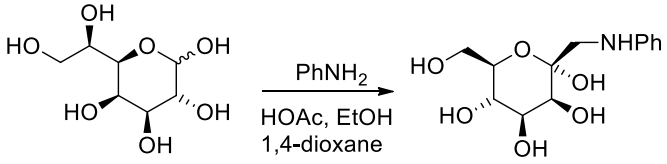
Lectins are highly versatile carbohydrate-complexing proteins with an impressive structural and functional diversity. [1,2] They occur in all types of organisms, where they are required for highly divers types of biological events, such as signaling, cell recognition, and cell adhesion. In this respect, the study of structure and function of lectins has become an important research field called functional lectinomics.[3,4]

Lectin ligands can be considered as biochemical tools and as small molecule drugs, which should ideally be stable against hydrolytic enzymes, typically present in a cellular environment. This cellular stability is provided by *C*-glycosyl-type glycoconjugate tools, because *C*-glycosidic linkages, other than the common natural *O*- and *N*-glycosides, are insensitive to hydrolysis.



1 **2**

1-(*N*-Phenyl)amino-1-deoxy-α-D-*manno*-hept-2-ulose (**2**), a D-*manno*-configured *C*-glycosyl-type compound derived from the Amadori rearrangement of D-*glycero*-D-*galacto* aldoheptose (**1**) [5] and a multivalent analogue thereof were synthesised and have been evaluated as ligands for mannoside-specific lectins of various sources. Synthetic details and analysis of the compared to methyl α-D-mannoyranoside with a series of mannoside-specific lectins (four plant, one bacterial and two human recombinant C-type lectins) will presented.

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SELECTIVE CELL TARGETING BY SYNERGISTIC INTERACTIONS OF PEPTIDE AND GLYCANS

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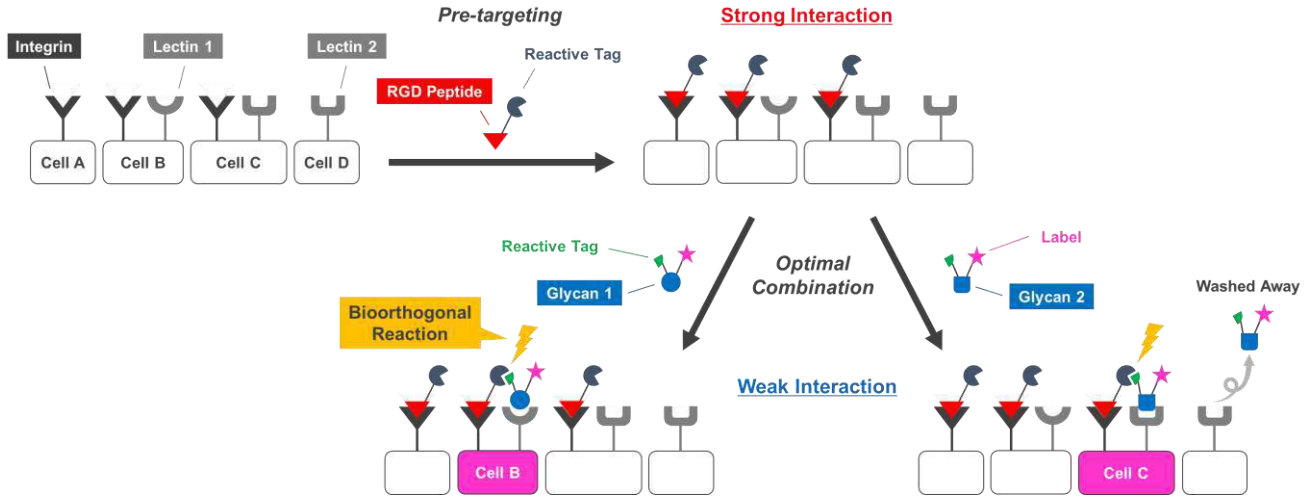
In the field of molecular imaging, the target selectivity is one of the most important factors in determining the degree of imaging contrast. Many studies have focused on developing ligands with high-affinity to surface receptors of interests. However, it is sometimes difficult to find specific surface receptor to the target. In addition, the “strongly” interacting ligands to the target, e.g., cancer cells, often give high background, when the same receptor can also be expressed on the other cells (even to lesser extent), hence resulting in poor imaging contrast.

We developed an entirely unexplored idea that is based on a pre-targeting strategy by a) simultaneously recognizing two receptors using “high”- and “low”-affinity ligands and b) ligating them directly on the target cell surface. Glycan ligands with fluorescent label bound to the cell surface, even with mM binding affinity levels, can be tightly anchored by the pre-targeted “high”-affinity peptide ligand on the cell surface. The advantage of using low-affinity glycan ligands is that excess of the labeled glycans can be washed off from the cells. Through this approach, unspecific fluorescence background can be minimized. In situ ligation of “weakly” interacting glycans to the pre-targeted “strongly” interacting peptide on target cell surfaces is the key to the success of exploiting the utility of “weak” interaction; Because the interaction of pre-linked peptide/glycan conjugates is dominated by the strong interaction of the peptide.

In this work, we demonstrated this strategy to differentiate various cancer cell lines by utilizing peptide and various different glycan structures. The method was also applied to selectively imaging the target cancer in vivo.

Strategy of this work



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TOWARDS CATALYTIC ANTIBIOTICS

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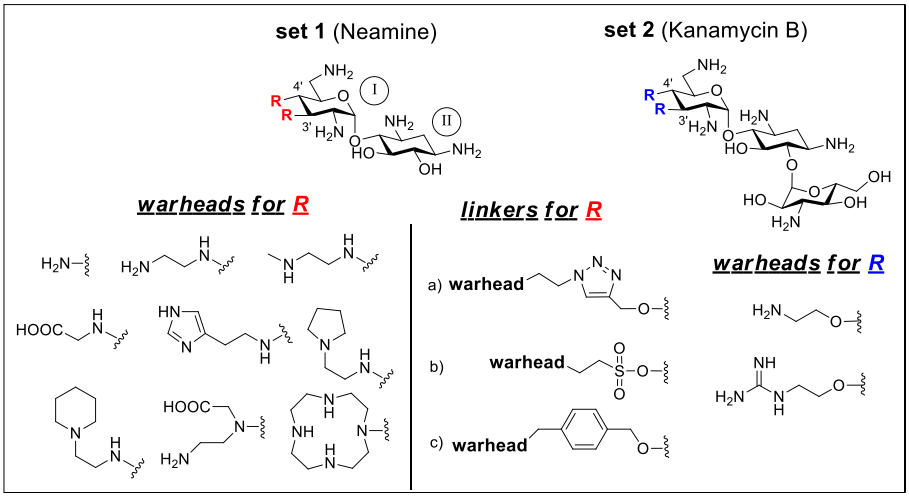
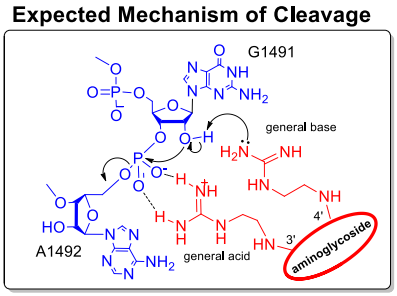
The key function and abundance of the bacterial ribosome make it an obvious target for antibacterial agents; and indeed, a large number of clinically used antibiotics exert their antibacterial activity by targeting this ribonucleoprotein machinery. Aminoglycosides are broad-spectrum antibiotics that selectively target the bacterial ribosome leading to a series of miscoding and translation inhibition events, finally resulting in bacterial death. Unfortunately, the prolonged clinical use of aminoglycosides has resulted in rapid evolvement of resistant bacterial strains that severely restrict their use. Over the last few decades many synthetic analogs of natural aminoglycosides have been synthesized. These analogs demonstrated similar activity to their parent scaffold or a rather poor antibacterial activity. Moreover, when these derivatives were introduced to clinical use, the appearance of resistant bacteria was rather fast. This dire public health concern has revived an interest in the discovery and development of novel strategies that can address the problem of growing antibacterial resistance.





One such potential strategy is the development of catalytic antibiotics that will be able to induce a fast and irreversible inactivation of their target. The possible benefits include: activity at lower dosages and subsequent elimination of side effects, activity against drug-resistant bacteria, and reduced potential for generating new resistance. Using high resolution structures of aminoglycosides bound to their ribosomal binding site, we were able to rationally design a series of new structures with a potential to specifically bind and catalytically cleave a highly conserved region within the ribosomal decoding site RNA; therefore, resulting in a fast inactivation of translation machinery. These compounds contain Neamine and Kanamycin-B scaffolds, substituted at positions 3' and 4' of ring I with various linkers as potential “catalytic warheads”. The rational for the design of two vicinal warheads at positions 3' and 4' was the high potential of their cooperative action when one functions as the general base and the second as the general acid. The introduced two vicinal warheads were expected to induce massive structural changes into the binding A-site so to allow more easily reach the desired low energy transition state for the cleavage of the phosphodiester bond of rRNA. The design principles along with the synthesis and preliminary biological evaluation of the target structures will be discussed.

GPR55 LIGANDS: FROM ENDOCANABINOIDS TO LYSOLIPIDS

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G-protein coupled receptor 55, GPR55, is highly expressed in the brain and peripheral nervous system, including dorsal root ganglion (DRG) sensory neurons, and immune system. It was initially suggested to be the surmised third cannabinoid receptor, due to its mild activation by cannabinoid ligands [1]. Later, its homology to lysophosphophatidic acid (LPA) receptors was recognized and lyso-phosphatidylinositol was reported as a potent ligand [2]. Recently, we demonstrated [3] that lyso-phosphatidyl-β-D-glucoside (lyso-PtdGlc) is an endogenous ligand of GPR55 that regulates targeting of sensory neurons during development. GPR55 upregulation has been reported in a variety of cancers, while GPR55 knock out mice show abnormal responses to inflammation and mechanical stimuli, suggesting a role of GPR55 in neuropathic pain and inflammatory processes. Furthermore, GPR55 has been linked to a variety of physiological and pathological processes, such as synaptic transmission, obesity, bone development and gastrointestinal functions. Utilizing various large pharmacological libraries, a series of synthetic GPR55 agonists and antagonists with varying specificity have been identified in order to facilitate further studies on the precise biological role of GPR55 and its signaling cascade [4].

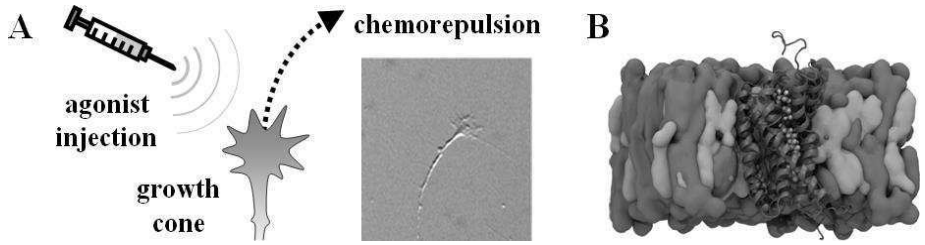

Fig. 1 - Growth cone turning assay (A) and molecular dynamics simulation (B) of GPR55.

Utilizing our phosphorus(III) based approach, we have established synthetic access to lyso-PtdGlc and prepared a variety of synthetic lyso-PtdGlc analogues. The biological activity of our synthetic analogues was evaluated using our previously developed functional assay, based on primary cultured DRG sensory neurons endogenously expressing GPR55 (Fig. 1A). Additionally, we investigated the biophysical properties of PtdGlc, compared it with phosphatidylinositol and validated our lyso-PtdGlc force field parameters. To overcome the lack of structural information of GPR55, we performed molecular dynamics simulations with our GPR55 homology model in the presence of natural and synthetic lysolipid ligands (Fig. 1B). The dynamics simulation was in good agreement with our functional assay results.

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GLYCOSYLATIONS OF SIMPLE ACCEPTORS WITH 2-*O*-ACYL L-IDOSE OR L-IDURONIC ACID DONORS REVEAL ONLY A MINOR ROLE FOR NEIGHBOURING GROUP PARTICIPATION

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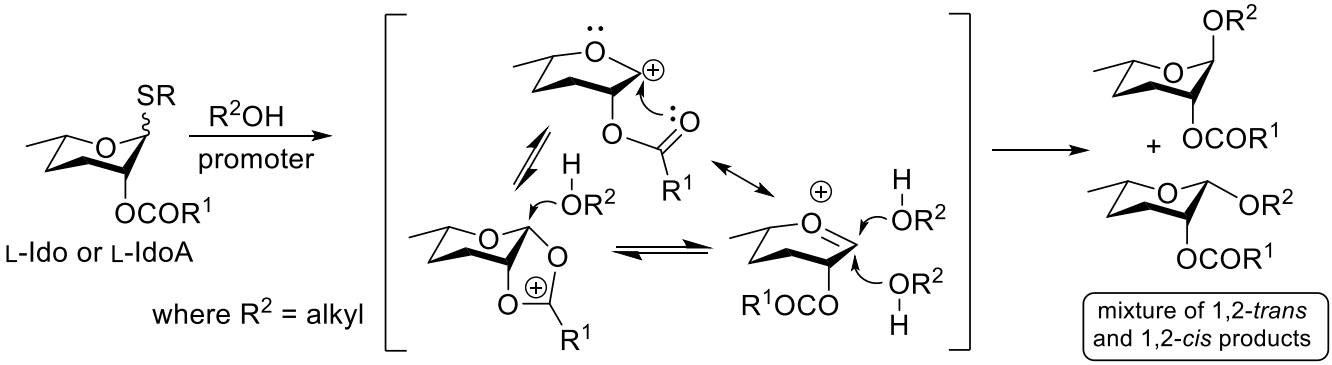
Several L-idose and L-iduronic acid glycosyl donors (mostly thioglycosides but also halides and trichloroacetimidates) with acyl protecting groups at the C-2 position were prepared and evaluated in glycosylation reactions with simple acceptors. In glycosaminoglycan oligosaccharide syntheses in the literature,[1] the presence of C-2 acyl protecting groups in L-ido-configured glycosyl donors generally results in exclusive formation of 1,2-*trans* glycosidic linkages, a finding that has typically been attributed to neighbouring group participation. However, glycosylations of simple alcohols with L-ido-configured donors (particularly thioglycosides), reported here, generally displayed incomplete stereocontrol and gave mixtures of the 1,2-*trans* and 1,2-*cis* products, suggesting that neighbouring group participation has lesser importance in these reactions. Glycosyl donors and reaction conditions were identified that gave improved, but not exclusive, selectivity for the desired α-L-anomer (1,2-*trans*) as the major product. Interestingly, glycosylations under the same reaction conditions with more complex monosaccharide acceptors gave exclusively the expected 1,2-*trans* products. The role of neighbouring group participation in these glycosylations was explored with density functional theory (DFT) calculations, which revealed that the non-stereoselective addition of the acceptor alcohol to the intermediate oxocarbenium ion is competitive with the stereospecific addition of the acceptor to the acyloxonium ion intermediate.



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MULTIVALENT GLYCOMIMETICS WITH AFFINITY AND SELECTIVITY TOWARD FUCOSE-BINDING RECEPTORS FROM EMERGING PATHOGENS

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Bacterial and fungal pathogens involved in lung infections in cystic fibrosis patients utilize a particular family of glycan -binding proteins, characterized by the presentation of six fucose-binding sites on a ring-shaped scaffold (Fig. **2**). These lectins are attractive targets for anti-infectious compounds that could interfere in the recognition of host tissues by pathogens.

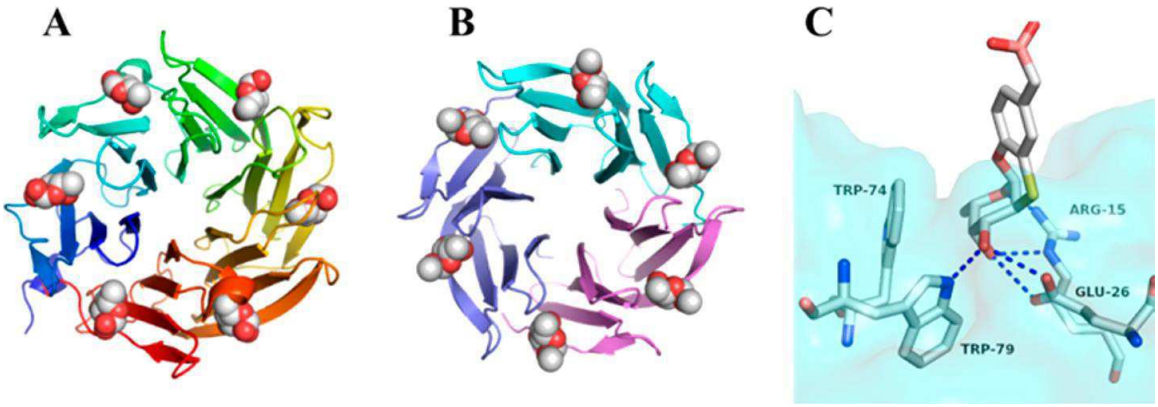

Fig. 2 - Crystal structures of (A) BambL and (B) AFL, complexed with fucose or fucoside. (C) Model of monovalent fucomimetic in intramonomeric site of BambL

The design of a cyclopeptide-based hexavalent structure allowed for the presentation of six fucose residues [1]. The synthetic hexavalent compound displays a suitable geometry resulting in high avidity binding by lectins from *Aspergillus fumigatus* and *Burkholderia ambifaria*. Replacing the fucose residue with a conformationally constrained fucomimetic (**Fig. 3**) does not alter the affinity and provides fine specificity with no binding to other fucose-specific lectins [2].

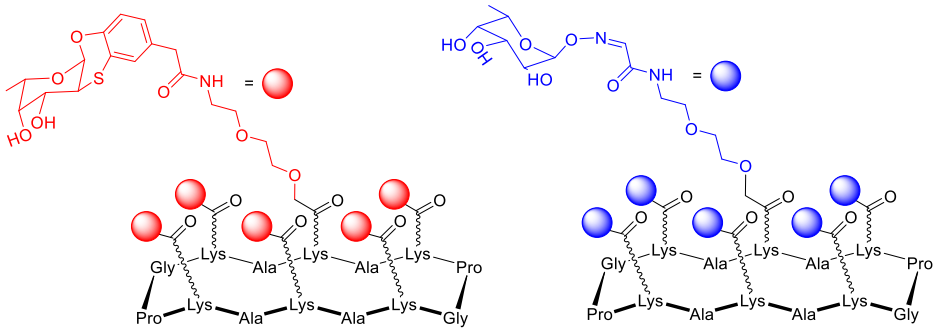

Fig. 3 - Structure of hexavalent fucomimetic and fucose-based glycoclusters

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EXO-GLYCALS: NOVEL SYNTHETIC APPROACHES AND BIOCHEMICAL APPLICATIONS

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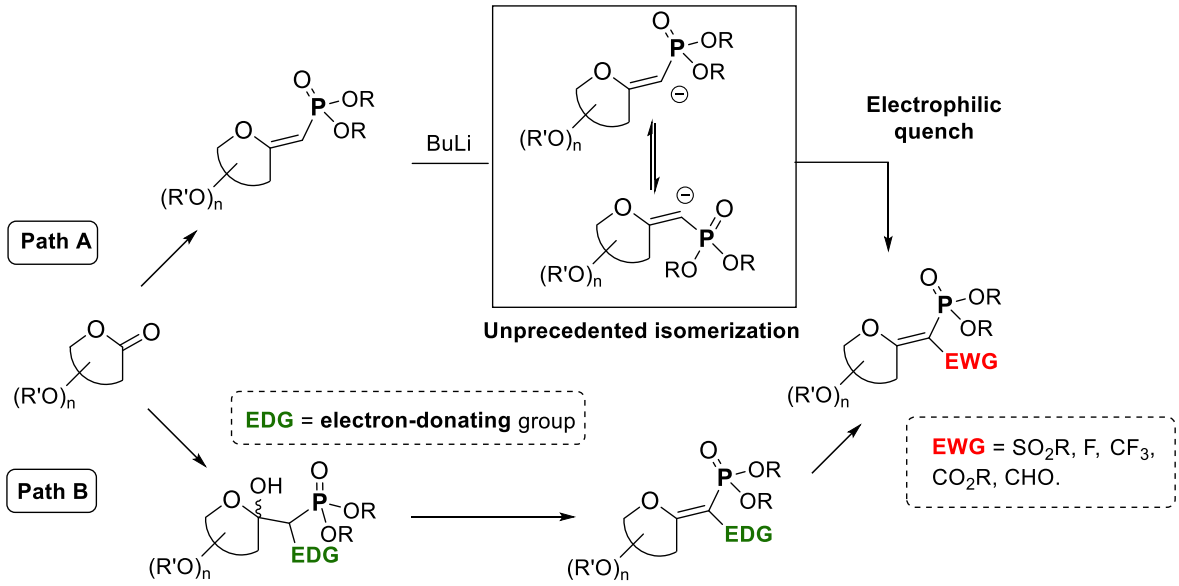
Our laboratory is interested in the mechanistic and inhibition studies of essential enzymes involved in the bacterial cell wall biosynthesis of important human pathogens such as *Mycobacterium tuberculosis*. Within this context, we have developed novel synthetic methodologies for the preparation of fluorinated [1] and electron-deficient *exo*-glycals [2].



In particular, we developed the two synthetic pathways depicted in the scheme above. In path A, we discovered a novel isomerization [3] allowing us to generate unprecedented tri- and tetrasubstituted *exo*-glycals. Some of the final molecules were coupled to uridine monophosphate (UMP) to generate nucleotide-sugars analogues designed as enzyme inhibitors or inactivators.

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MODULATING REACTIVITY IN CYCLODEXTRINS BY RADICAL C-H FUNCTIONALIZATION

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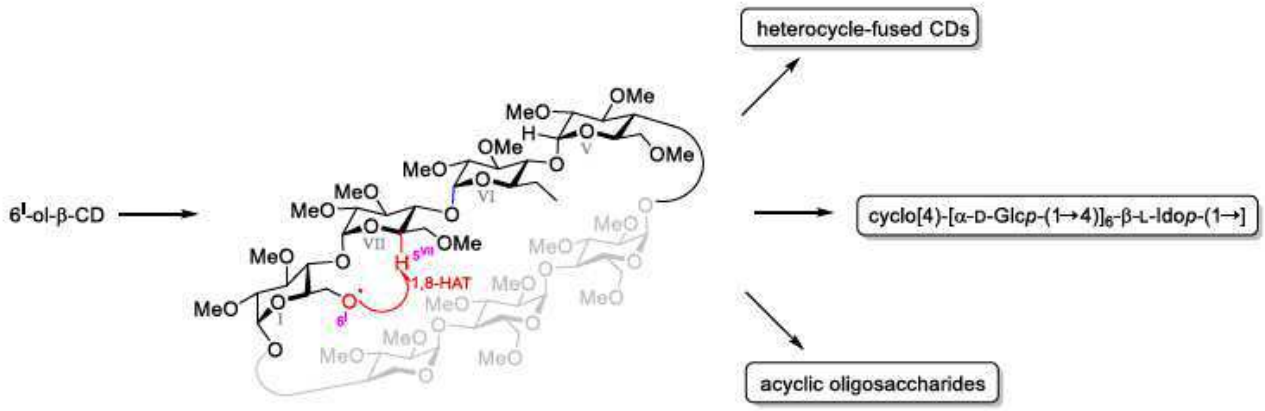
The ability of cyclodextrins (CDs) to encapsulate biomolecules in their internal cavity and its potential application as nanocarriers has stimulated an intense research to modify and improve their chemophysical properties through chemical transformations [1]. But interestingly, in the methodologies applied to generate these modifications, radical reactions are practically unknown.

Taking in account our previous investigations based on an intramolecular 1,8-hydrogen atom transfer (1,8-HAT) reaction between the two pyranose units in Hex*p*(1→4)-Hex*p* disaccharides (e.g., β-maltose) [2], herein we will show our most recent work by extension of this methodology of remote funcionalization to more complex carbohydrate such as CDs [3]. The well-suited disposition of the glucose units in these macrostructures favors that the 6'-*O*-yl radical abstract preferentially the H5 of the vicinal unit to generate a radical at C5 whose fate will depend on the reaction conditions, and especially on the reagents used to form the alkoxyl radical.



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SYNTHESIS OF 1,5-ANHYDRO-D-*GLYCERO*-D-*GLUCO*-HEPTITOL DERIVATIVES AS POTENTIAL INHIBITORS OF BACTERIAL HEPTOSE BIOSYNTHETIC PATHWAYS

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A threat to global health is presently associated with the increase of multidrug-resistant bacteria, for several of which common antibiotics are not effective anymore [1]. Novel approaches, such as the antivirulence concept, are therefore urgently needed to identify bacterial targets and to develop appropriate compounds with effective and specific modes of action. Various isomers of seven carbon sugars termed heptoses play crucial roles in gram negative bacteria. L-*glycero*-D-*manno*-heptose has been found to link the inner and outer core region of lipopolysaccharide (LPS) which is located on the outer membrane of the cell envelope. The biosynthesis of heptoses and their nucleotide-activated forms has been elucidated in great detail. The first enzymatic steps are catalyzed by the sedoheptulose-7-phosphate isomerase GmhA and anomeric kinases HldE and HddA. Blocking these enzymes leads, for example, to deep rough type bacterial mutants of the Re chemotype with impaired barrier functions, which are rapidly cleared from serum by the immune system.

Therefore inhibitors of the LD-heptose biosynthetic pathway hold promise as broad-band drug candidates, which would not harm mammalian enzymes, would not damage the gut microbiome, and could serve as potent antivirulence agents. Previously, the group of Vincent has carried out the preparation of a library of D-*glycero*-D-*manno*-heptose 7-phosphates with manifold structural variations at the exocyclic side chain [2]. Notably a D-*gluco* derivative was found to inhibit both GmhA and HldE with IC_{50} values in the low micromolar region whose motif we set out to further explore.

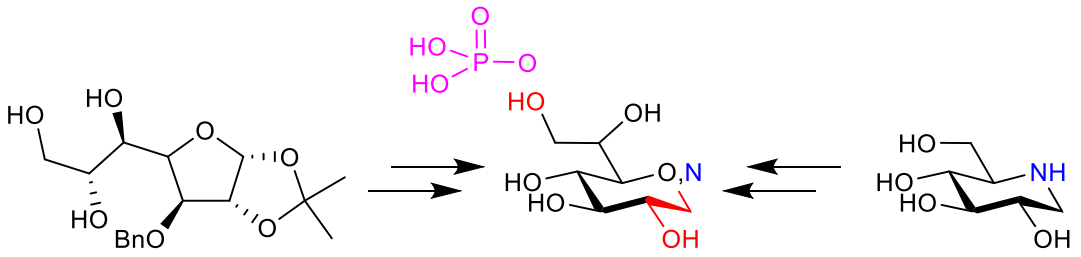

Fig. 4 -Synthetic approach towards 1,5-anhydro heptitol derivatives

A series of 1,5-anhydro-D-*glycero*-D-*gluco*-heptitol derivatives (Fig. 4) have been prepared from 3-O-benzyl-1,2-O-isopropylidene-D-*glycero*-D-*gluco*-heptofuranose *via* conversion into anomeric bromide and thiophenyl derivatives, followed by glycal formation and reductive desulfurization, respectively. Global deprotection of the protected intermediates afforded the 1,5-anhydro derivatives of the D-*glycero*-D-*gluco*- and 1,2-dideoxy-D-*altro*- configuration as well as the 1,5-anhydro-2-deoxy-D-*altro*-hept-1-enitol. In addition, the 7-O-phosphorylated D-*glycero*-D-*gluco*-heptose and its 1,5-anhydro analogue were prepared in good yields utilizing phosphoramidite chemistry. A novel heptitol analogue based on a 1-deoxynojirimycin scaffold was also elaborated. These inhibitors have now provided further insight into the binding motive of these two enzymes and contributed to the further design of more potent inhibitors.

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SELF-PROMOTED STEREOSPECIFIC *N*-GLYCOSYLATION

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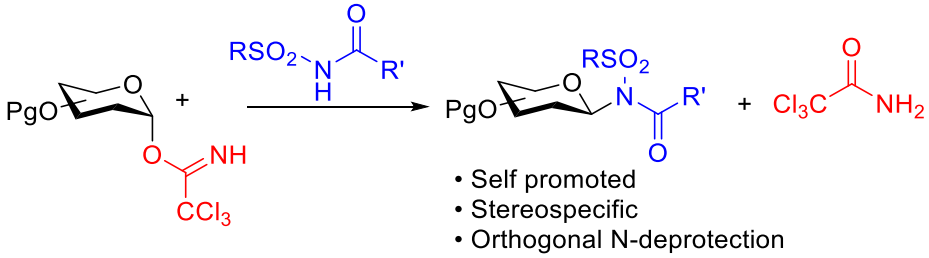
Glycosyl trichloroacetimidates have been used as glycosyl donors together with carbamate protected sulfonamides for the synthesis of *N*-glycosides. No additional catalyst or promoter was added for the glycosylations to take place. Axial trichloroacetimidates are stereo-specifically substituted, whereas the selectivity drops when having equatorial trichloroacetimidates. NMR studies suggest that the decrease in stereo-specificity is due to an anomerisation of the trichloroacetimidiate. The *N*-carbamate and the *N*-sulfonyl groups were further shown to be orthogonal protective groups of the *N*-glycoside.



DNA METHYLTRANSFERASE 1 INHIBITORS: DESIGN & SYNTHESIS BASED ON TRANSITION STATE STRUCTURE

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DNA methylation is an epigenetic mark that cells use to silence gene expression [1]. It occurs at "CpG sites" grouped in islands and largely located at gene promoter regions. DNA methylation is involved in normal key processes such as: cell cycle, immune response or X-chromosome inactivation. DNA methyltransferases (DNMTs) are the enzymes responsible for DNA methylation in mammalian genome [2]. DNMTs catalyse the formation of 5-methyl-2'-deoxycytidine units within DNA using S-adenosyl-L-methionine (SAM) as the methyl donor (Fig.1).

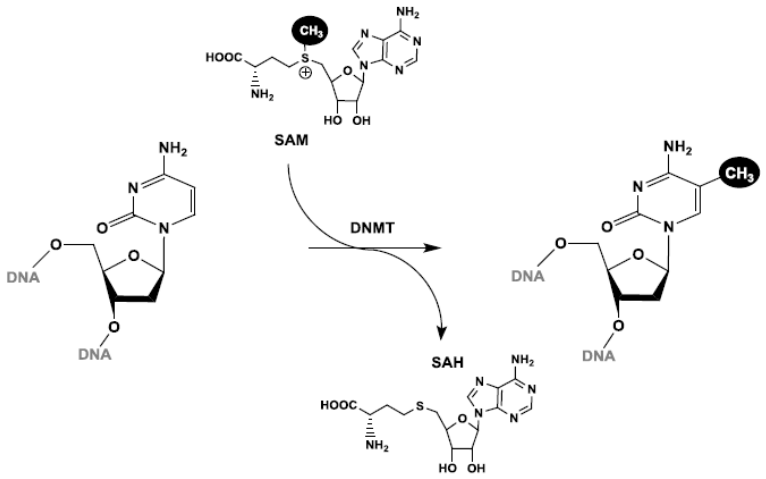

Fig.1 - Schematic representation of DNA methylation on 2'-deoxycytidine

While hypomethylation is associated with gene expression, hypermethylation on "CpG islands" induces gene silencing often leading to carcinogenesis [3]. DNMT1 inhibitors represent an important therapeutic target for the treatment of cancers [4]. The current DNMT inhibitors [5] on the market being mutagenic and cytotoxic, indicate a strong need for new specific and powerful inhibitors.

Transition state (TS) structure elucidation had proven to be a powerful tool for the design of potent enzyme inhibitors [6] The TS structure of an enzyme can be experimentally determined through a combination of kinetic isotope effect measurements and quantum chemical computations. DNMT1 TS structure [7] has been solved and has led us to the design of target–specific DNMT1 inhibitors.

I will present the DNMT1 inhibitor structures synthesised and their inhibitory activities.

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BORONIC ACID CATALYZED REGIO- AND 1,2-*cis*-STEREOSELECTIVE GLYCOSYLATION OF UNPROTECTED SUGAR ACCEPTORS VIA S_Ni-TYPE MECHANISM

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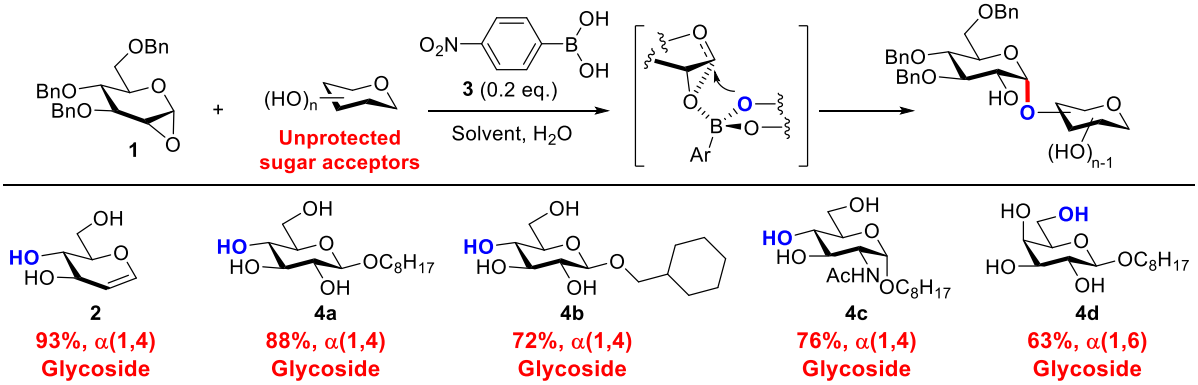
1,2-*cis*-Glycosides are frequently found in various biologically active natural products and glycoconjugates. Therefore, to explore the precise roles of these glycosides, efficient syntheses of these 1,2-*cis*-glycosides have been paid considerable attention. Based on the background, complex oligosaccharides and glycoconjugates possessing 1,2-*cis*-glycosides have been synthesized in a highly stereoselective manner. However, these synthetic schemes were highly dependent on protecting group strategies to control the regio- and stereoselectivities of each glycosylation step, leading to a decrease in step and atom economies of the overall process. Therefore, regio- and 1,2-*cis*-stereoselective chemical glycosylation without any protecting groups have been highly desirable. In this context, we have recently developed regio- and 1,2-*cis*-stereoselective glycosylations using diol sugar acceptor-derived boronic esters [1]. Herein we wish report a novel regio- and 1,2-*cis*-stereoselective glycosylations of 1,2-anhydro donors and unprotected sugar acceptors using boronic acid catalyst.

Initially, we selected 1,2-anhydro donor **1** and D-glucal (**2**) as a glycosyl donor and an unprotected sugar acceptor, respectively, and investigated glycosylations of **1** and **2** using several boronic acid catalysts under various reaction conditions. The results showed, for the first time, that the glycosylation of **1** and **2** using *p*-nitrophenylboronic acid (**3**) catalyst (0.2 eq.) in the presence of an excess amount of water proceeded effectively to give a corresponding α(1,4) glycoside in 93% yield with high regio- and excellent stereoselectivity. In addition, regio- and stereoselective glycosylations of several 1,2-anhydro donors and several unprotected sugar acceptors proceeded effectively to provide the corresponding 1,2-*cis*-glycosides in high yields with good to high regioselectivity and excellent stereoselectivity. Next, the reaction mechanism of this glycosylation was analyzed by measurement of the ^{13}C KIE and DFT calculations. The mechanistic study using ^{13}C KIE indicated that the present glycosylation can be regarded as a highly dissociative concerted S_Ni or S_N1 reaction with an extremely short-lived intermediate. Furthermore, computational analysis clearly supported the feasibility of the highly dissociative concerted S_Ni mechanism and high regioselectivity of this glycosylation [2].



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ACCESS TO SYNTHETIC GLYCOMES THROUGH ENZYMATIC SYNTHESIS

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As one of the four fundamental classes of macromolecules that comprise living systems, glycans were found to play diverse roles in a wide range of biological processes. Understanding the structures and functions of glycans is central to understanding biology. However, glycans found in nature possess an inherited diversity, complexity, and in most cases low abundance, makes it extremely hard to access structurally defined glycans in sufficient amounts. Theoretically, due to the variable and multiple connectivity, five monosaccharides can form a pentasaccharide with over half a billion possible structures. On the other hand, in any real biological system, much smaller numbers of glycans than the theoretical number may exist since glycans are synthesized by glycosyltransferases (or other enzymes) with limited sugar to sugar connection variations. For example, it is estimated 20,000 to 30,000 glycan backbone structures may cover most mammalian glycomes. Thus, the perspective is that in the next decade majority of biologically important glycan backbone sequences will be synthesized by separationand chemo-enzymatic synthesis. Post-glycosylational modification on the glycan backbone sequences will further produce the diversity and complexity of glycans.

In mammalian glycomes, *O*-glycans are linked to Ser/Thr in glycoproteins and have diverse structures and glyco-sequences. By a core synthesis – enzymatic extension (CSEE) strategy, we achieved the complexity and diversity of *O*-glycans/glycopeptides/glycoproteins with most natural structural diversity. Starting with 8 chemically prepared *O*-GalNAc core structures and 2 *O*-Man cores, we diversified the glycans with specific glycosyltransferases to over 100 *O*-GalNAc glycans and over 40 *O*-Man glycans. For *O*-glycopeptide/glycoprotein synthesis, the chemically prepared core glycans were incorporated into glycopeptides/glycoproteins by solid-phase peptide synthesis, and the glycans were further enzymatically extended. The *O*-glycans and the *O*-glycopeptides/glycoproteins serve as standards for analysis and for drug screening and discovery.

MUCIN PEPTIDE MICROARRAYS IN STUDIES OF PROTEIN-CARBOHYDRATE BINDING EVENTS

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Mucins are densely glycosylated proteins that populate the cell-surface of epithelial tissues [1]. The extracellular tandem repeat peptide regions rich on proline, threonine and serine residues characterize the mucins. By display of *O*-glycans often organized in a multivalent fashion, the mucins and mucin like glycoproteins are involved in a plethora of cell-surface binding events [2]. Glycans on mucins often act as ligands for invading pathogens, studies of such interactions are useful for characterization of microbes and viruses as well as to develop new anti-adhesive drugs. Mucins also play a critical role in cancer progression and through aberrant glycosylation tumor-cell adhesion and anti-adhesion events can be modulated. By chemical synthesis of well-defined glycan and glycopeptide probes we aim to identify and map the functions of mucins and their interacting binding partners involved in cancer and infection processes.

In recent years we have developed efficient total synthesis strategies to construct over 300 different mucin *O*-glycopeptides modified with short tumor-associated glycan structures and more complex elongated mucin core structures [3-5]. Using enzymes, the elongated core structures were further diversified by fucosylation, sialylation and polyLacNAc. The synthetic glycopeptides have been immobilized on biocompatible hydrogel slides that display the glycopeptides in a multivalent mode. Microarray analysis to evaluate binding epitopes of antibodies directed against tumor-associated mucin glycopeptide antigens [5-7] and lectins (galectins) that are involved in tumor progression have been preformed. Analysis of virus and bacterial lectin recognition is currently in progress. Our recent findings will be described at the ICS in Lisbon.

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MODULAR SYNTHESIS OF MULTIFACETED CYCLOOLIGOSACCHARIDES BASED ON TREHALOSE BUILDING BLOCKS

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Nanometric sized rigid molecular entities (molecular nanoparticles, MNPs) have proven particularly favorable for gene vector design. In most cases, the synthetic approaches take advantage of the two-face dissymmetrical character (Janus) of the native MNP platform, e.g. cyclodextrins or calixarenes, to incorporate functional elements for nucleic acid complexation, compaction and delivery with a precise spatial orientation [1]. An alternative convergent strategy based on covalent assemblage of pre-functionalized α,α'-trehalose building blocks led to a novel family of amphiphilic cyclooligosaccharides termed cyclotrehalans (CTs) [2]. The possibility of domain-by-domain molecular vector construction, in combination with the versatility and ease of manipulation of the precursors, offers unprecedented opportunities for structure/self-assembly/biological activity relationship studies. Beyond the bifacial corset, the synthesis of multifaceted architectures with three equally or distinctively functionalized regions (star, Mickey Mouse or Hecate cyclooligosaccharides; see Fig. 1) will be presented.

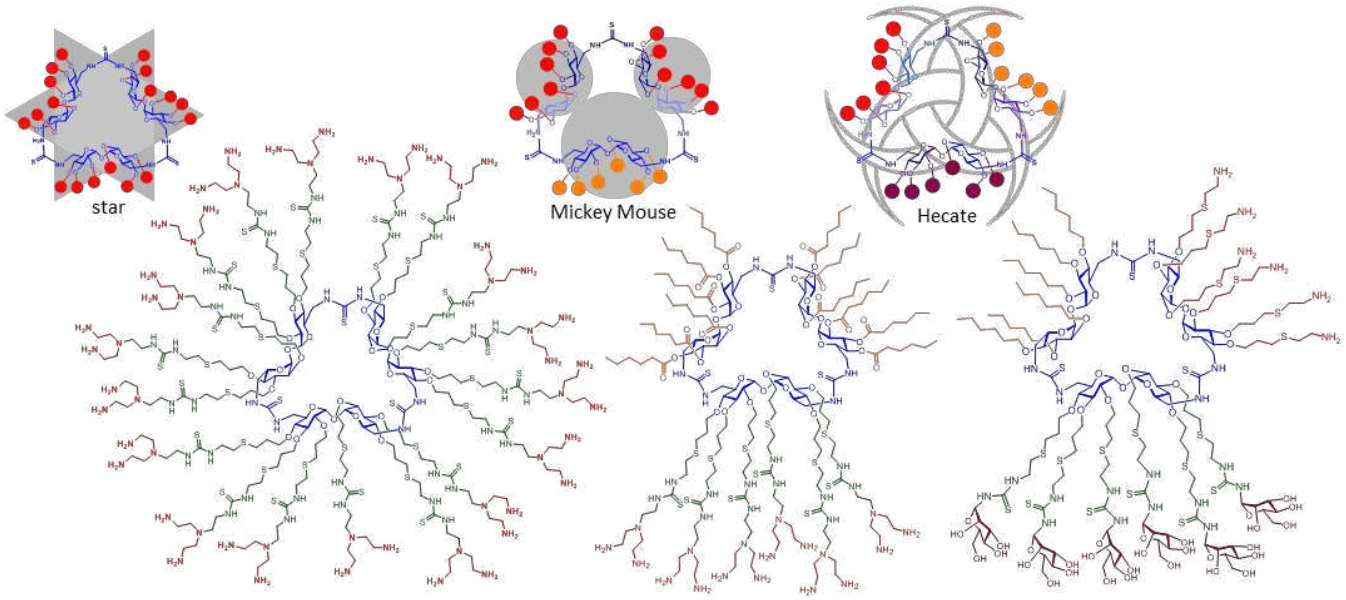

Fig. 1– Examples of star, Mickey Mouse and Hecate cyclotrehalanes with cationic/lipophilic/glycotope domains for nucleic acid complexation, compaction and delivery.

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TOTAL SYNTHESIS OF *O*-GalNAcYLATED ANTIFREEZE GLYCOPROTEIN TOWARD ELUCIDATION OF THE FUNCTIONAL ROLE OF *O*-GalNAcYLATION

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Mucin type *O*-glycosylation is one of the major protein glycosylation that frequently found in eukaryotes. This type *O*-glycosylation is initiated from *N*-acetylgalactosamine (GalNAc) and result in diverse glycan structures. Nevertheless, the role that this core monosaccharide residue plays at the molecular level is still unclear due to the lack of structurally-defined form of functional O-GalNAcylated glycoproteins.

To uncover the general function of *O*-GalNAc, we focus on a unique mucin type *O*-glycoprotein antifreeze glycoprotein (AFGP) as a probe of *O*-GalNAcylated glycoprotein. AFGP consists of triglycopeptide repeat: $[Ala\text{-}Thr\text{-}Ala]_n$ (n = 4-50) where the Thr residue is *O*-glycosylated with a D-Galβ1-3-D-GalNAcα1. It has been suggested that the core *O*-GalNAc residue is involved in the antifreeze activity of AFGP [1]. However, the role of the O-GalNAcylation to the activity of AFGP has been unclear as is the case of other mucin type *O*-glycoproteins.

In this context, we carried out the total synthesis of homogeneous *O*-GalNAcylated AFGP (AFGP(GalNAc)). The synthesis was achieved by using a peptidyl-*N*-pivaloylguanidine that is an “unreactive” peptide in peptide coupling reactions, but is interconvertible with peptide-α-thioester that is a “reactive” peptide derivative. The unique switchable reactivity of peptidyl-*N*-pivaloylguanidine allowed us to perform an efficient sequential glycopeptide coupling strategy and obtain various lengths of homogeneous *O*-GalNAcylated AFGP including 120 amino acids length having 40 *O*-GalNAcylation sites (Figure) [2]. Evaluation of the antifreeze activity of the synthetic AFGP(GalNAc) revealed that *O*-GalNAcylation is an essential *O*-glycosylation for both structural and functional basis of AFGP to exhibit antifreeze activity. Upon these results, we also synthesized partially GalNAc deleted AFGP(GalNAc) analogues to detail the role of *O*-GalNAcylation. Spectroscopic analysis of the AFGP(GalNAc) derivatives suggest that *O*-GalNAcylation affect on the dynamics of peptide backbone correlating with the antifreeze activity.

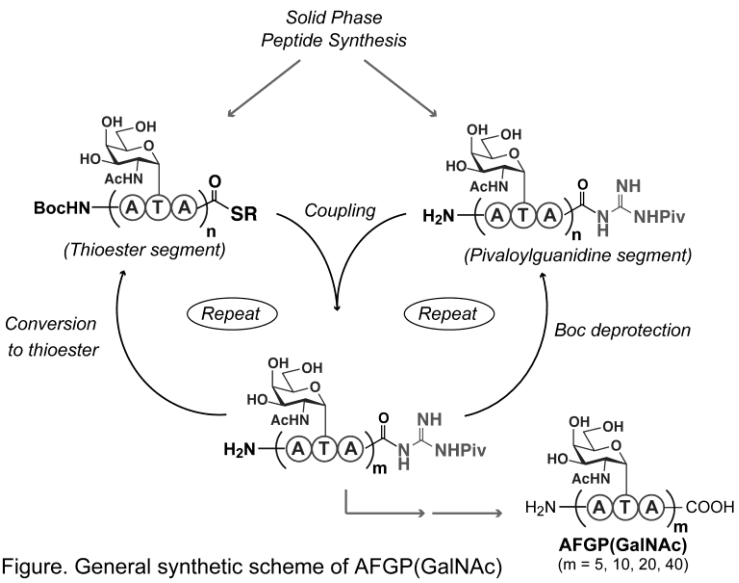

Figure. General synthetic scheme of AFGP(GalNAc)

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INTERACTION BETWEEN CARBOHYDRATE-BASED CAPPING AGENTS AND GOLD NANOPARTICLES: A THEORETICAL PERSPECTIVE

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Gold nanoparticles have gained attention in the last decade due to their multiple applications from environmental and medical points of view as well as their easy controlled synthesis [1]. It is known that stabilizing or capping agents are used prevent the aggregation of these nanoparticles. In this context, different polymers have been applied to stabilize different metallic cores such as Au and Pt, among others. In particular, the polysaccharide chitosan have emerged as promising capping tool due to its features as polyelectrolyte which provides an effective stabilization of negative charged nanoparticles [2] as well as non-toxic, biodegradable and biocompatible properties. Despite this fact, the interaction of these polymers on the nanoparticles surface remains unclear.

The large size of this kind of systems makes unapproachable for quantum calculations whereas, molecular dynamics simulations have been only applied for studying chain conformations together with conformational flexibility of the glycosidic linkage of chitosan. However, new avenues have arisen thanks to the application of Dissipative Particles Dynamics (DPD). These simulation models are based on particles that represent coarse-grained (CG) portions of the system under study. These low resolution models are rapidly growing in science as a consequence of eliminating details and providing access to length and time scale inaccessible for other kinds of models [3].

Herein, to the best of our knowledge we report the first study about the interaction between chitosan and gold nanoparticles using CG models (Fig. 1). In order to obtain feasible interactions between coarse-grained particles, DFT calculations at M06-2X/LanL2DZ level of theory were also carried out by using simple models. The obtained theoretical binding energies were correlated with a_{ij} parameters of DPD. The results reveal that chitosan effectively involves and prevent the aggregation of gold NPs owning different sizes. Moreover, the results bring to light a different situation for NPs synthesized from reduction with citrate and NPs directly synthesized from chitosan.

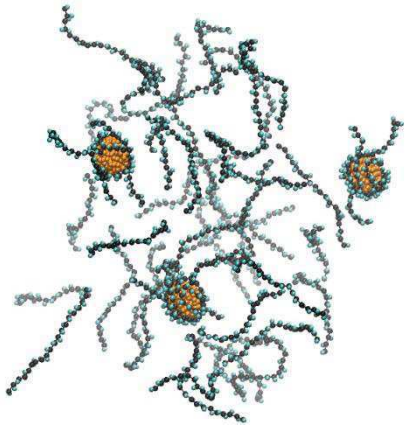

Fig. 1

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COMPUTATIONAL STUDIES OF CARBOHYDRATE ACTIVE ENZYMES

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In the last years, our group has applied molecular dynamics simulations and quantum mechanics molecular mechanics (QM/MM) methods to help in the elucidation of the reaction mechanism followed by retaining glycosyltransferases to catalyze the formation of a new glyosidic bond [1].

Recently, we have turned our attention to the application of such expertise in the study of other carbohydrate active enzymes, in particular, of wild-type and engineered glycosidases (GHs). GHs are mainly hydrolytic (H) enzymes but, under certain conditions, they may work in the 'reverse' mode for glycan synthesis (transglycosylation, T). Mutation of glycosidases has produced a series of tranglycosidases showing enhanced T/H ratio [2]. In another approach, GHs have been converted by mutation onto glycosynthases [3]. The molecular basis for the effect of such mutations are not completely understood yet, and some aspects may be improved.

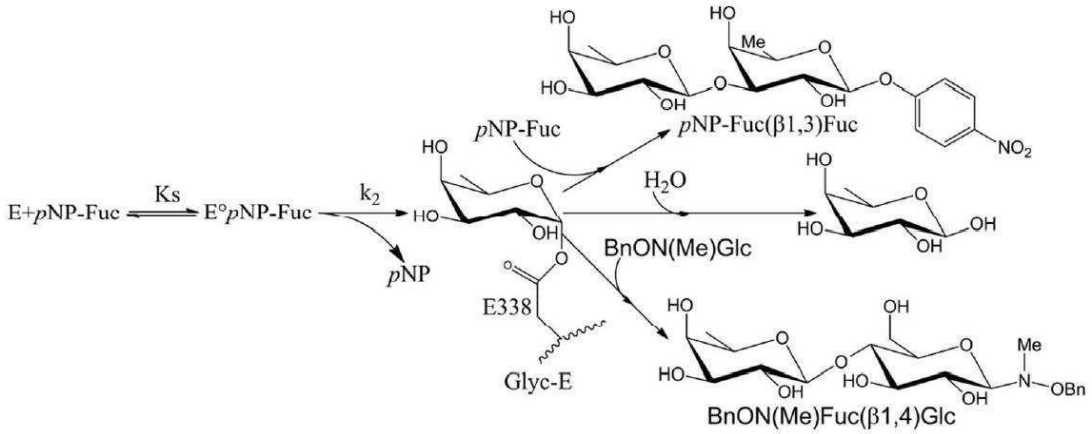

Fig. 1 – from ref. 2.

Our aim is to provide a molecular level interpretation of such experiments and to assist in the design of new biocatalysts for glycan synthesis. We have started with the study of CAZy family GH1 *Thermus thermophillus* β-glycosidase (TtβGly), as it has been shown to significantly increase its transglycosylation activity in several mutants (Y284P, N282T, F401S) [2]. In this communication we will present and compare the results obtained for the hydrolysis and transglycosylation reactions catalyzed by the wild-type and the Y284P enzymes.

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MOLECULAR MODELING TOOLS FOR GLYCO-RESEARCH

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Glycans play myriad roles in the pathogenesis of a wide range of human diseases, including initial adhesion of pathogens to host cell-surface glycans, as well as shielding of pathogen cell surface proteins from the host immune system. Well established examples include influenza virus adhesion to glycans in human respiratory epithelia, and HIV viruses coating their cell-surface proteins in human glycans to escape the immune response. Research in this area could be driven by, or benefit from, complementary molecular modeling techniques, and yet such tools require expertise to be judiciously employed and adapted to specific research questions.

In this work we show how both currently available and tailor-made molecular modeling tools can complement lab-based research. In the case of human influenza A virus, the haemagluttinin protein's binding preference for multiantennary glycans was established, and a tailor-made modeling program provided a structure-based rationale for the observed specificy pattern amongst multiantennary glycans of varying size. More broadly useful software has been developed to study densly glycosylated glycoproteins such as hiv SOSIP trimers; a webtool (to be available at glycam.org/gp) has been developed that generates 3D structures of glycosylated proteins. The tool attaches user defined glycans to user selected residues (Asn, Ser, Thr or Trp) and then uses a genetic algorithm to adapt each glycan's orientation to fit amongst the protein structure and other glycans.

A suite of such webtools has been made available on glycam.org along with a series of scenarios that teach the user how these webtools can be employed and adapted to their own research questions.

UNUSUAL CATALYTIC ITINERARIES OF GLYCOSIDASES UNVEILED FROM QM/MM METADYNAMICS SIMULATIONS

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We will here present recent examples of the application of first principles QM/MM simulations to unravel catalytic mechanisms of glycosidases [1]. First, simulations on a β-mannosidase show that the lysozyme fold can be co-opted to catalyze the hydrolysis of different polysaccharides in a mechanistically distinct manner [2]. Second, computational design of experiments uncover the conformational itinerary for GH125 α-mannosidases [3]. Third, we will report the mechanism of a novel engineered β-glucosidase that allows β-stereoselective catalysis for glycoside synthesis, showing that the once unusual S_Ni-like mechanism is more widespread than was early assumed [4].

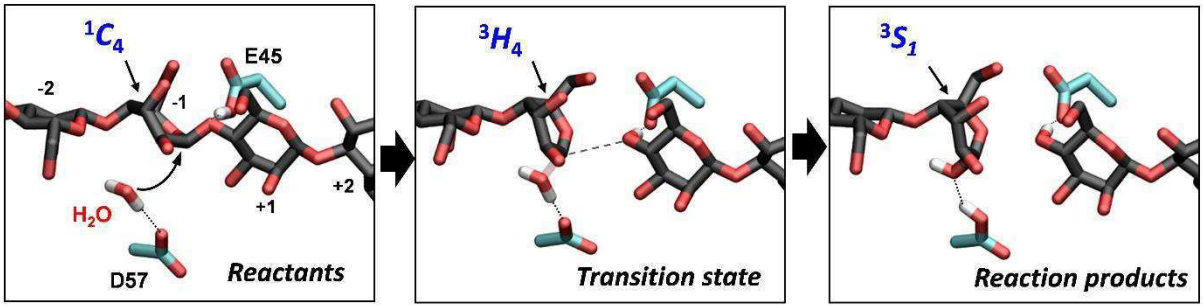

Fig. 1

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FURANOSE SOLUTION CONFORMATIONS: INSIGHTS FROM MULTISCALE MOLECULAR SIMULATIONS

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Furanoses are carbohydrates that contain five-membered rings produced from the cyclization of linear aldoses or ketoses. Furanose moieties are an important component of many biologically-relevant compounds including DNA, RNA and saccharides present in plants as well as those creating bacterial cell walls. Due to their biological significance, it is crucial to understand the details of structure and dynamics of this class of carbohydrates. This can be achieved by means of computational methods, either based on empirical, molecular-mechanics force fields or more exact and computationally-demanding, *ab initio*-based potentials.

We have applied the multiscale molecular modeling approach to study one of the most important conformational degree of freedom in furanoses, i.e. the inherent flexibility of their five-membered rings.

In the first stage of the study we have applied the hybrid QM/MM approach to study the conformational properties of the prototypic furanose molecule, i.e. tetrahydrofuran (THF) immersed in aqueous solution. We have found that the free energy profiles associated with the process of puckering of the five-membered ring (pseudorotation) of THF differ substantially from those obtained during the static energy scans routinely performed during the *ab initio* calculations. These differences have been ascribed to the influence of water molecules which presence leads to a change in the distribution of atomic charges within the THF molecule; subsequently, such change disfavors the low-energy envelope conformers dominating in gas phase (E_O and ^OE) and promotes the alternative, twist conformers ($^3T_2$ and $^2T_3$) instead. The energetic magnitude of this effect has been estimated as equal to ~2.4-3.4 kJ/mol (depending on the type of *ab initio* potential) and is expected to exist in all furanose rings. Moreover, the pseudorotation phase has been found to be correlated with the corresponding amplitude which suggests that the degree of deviations of the ring shape from the ideal planar structure depends on the puckering properties of the considered furanose (understood as the preference for the given pseudorotation phase).

In the second stage of the study, we have used the above-described results (in combination with other elements of the parameterization procedure such as an extensive validation against available experimental data in solution) to design the classical, biomolecular force-field capable to accurately simulate the structural and dynamic properties of various furanoses interacting with solvent. We have used the GROMOS functional form to create the set of parameters covering any furanose-based saccharide with restriction to compounds of the elements C, O, H and presenting only single bonds (for example: all aldopentoses, aldohexoses, tetroses, ketohexoses, etc. as well as their deoxy derivatives). The results systematize the influence of the ring substituents on the conformational flexibility of furanose rings and confirm that the two-state model is oversimplified with respect to the observed behavior of numerous furanoses.

RAPID METHODS FOR GENERATING 3D STRUCTURES OF GLYCOPROTEINS

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The role of glycans in modulating the antigenicity [1], immunogenicity [2] and function [3] of glycoproteins is well established, but fundamentally not well understood. The development of optimal vaccines against pathogens, such as influenza A or HIV, depends on understanding the relationship between the structure of the glycans at each site on the protein, the location of the glycan on the protein surface, and the ability of the glycans to shield the protein from immune surveillance. Conversely, the activity of biological therapeutics is most highly modulated [4] by variations in the glycosylation, and yet the 3D structural reasons for these variations are not known.

Methods, such as mass spectrometry, are widely used for determining the glycan structures at each site in a glycoprotein, and crystallography or NMR spectroscopy are commonly employed for deriving the 3D structure of the protein, however the glycans are poorly resolved in most crystal structures of glycoproteins. Indeed, no experimental method is able to routinely resolve the structures of the glycans in large highly glycosylated proteins, such as hemagglutinin, gp120/160, erythropoietin, etc. Recently cryo-electron microscopy has shown promise in this regard, because it can provide a 3D structure of the volume of the glycoprotein, but the 3D structures of the constituent glycans must still be derived by modeling.

The challenge for modeling glycoproteins stems from the facts that 1) glycans may adopt many conformations, 2) the linkage to the protein may also adopt multiple conformations, and 3) collisions between arbitrarily-placed glycans may be difficult to resolve by traditional methods such as energy minimization of molecular dynamics.

Here we introduce two approaches that enable 3D structures of glycoproteins to be rapidly generated, and show that they are consistent with experimental observations based on statistical surveys of known structures. This tool will significantly aid in developing structure-activity relationships relating protein glycosylation to biological activity.

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NOVEL ASSAYS FOR THE MEASUREMENT OF GLUCURONYL ESTERASE AND α-GLUCURONIDASE, TWO ENZYMES INVOLVED IN LIGNIN AND HEMICELLULOSE HYDROLYSIS

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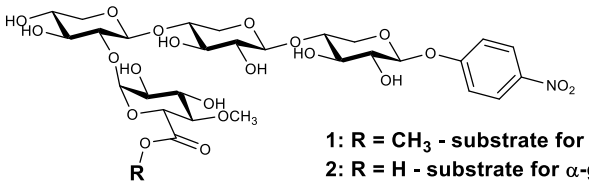
Following the successful development of enzyme-coupled colorimetric assays for the measurement of a range of *endo*-acting glycosyl hydrolases including *endo*-1,4-β-glucanase [1], *endo*-1,4-β-xylanase [2], our research activity has been focused on two additional classes of glycosyl hydrolases: namely glucuronoyl esterase (GE) and α-glucuronidase.

Glucuronoyl esterases (GE) are a recently discovered class of enzymes and were first reported in 2006 [3]. These enzymes are involved in lignin hydrolysis and are capable of hydrolysing the ester linkages between lignin polyphenols and the glucuronoxylan present in the hemicellulose fraction of lignocellulosic biomass.[4] Given that these linkages are believed to contribute to the recalcitrant nature of lignocellulose, the discovery of new and improved glucuronyl esterases is an important target for the biofuel industry. A number of synthetic substrates to measure GE activity have previously been described in the literature.[5] Building on these seminal studies, we prepared substrate **1**: the methyl ester of a 4-nitrophenyl-linked aldotriouronic acid. This substrate, when combined with a GH67 α-glucuronidase from *Geobacillus stearothermophilus* and a GH43 β-xylosidase from *Selenomonas ruminantium*, form the basis of a colorimetric assay for glucuronoyl esterases.

α-Glucuronidases are a class of enzymes able to hydrolyse the uronic acids decorating the xylan backbone in hemicellulose.[6] While a commercial enzymatic assay kit for the measurement of α-glucuronidase activity has been available for a number of years, we sought to develop a new colorimetric assay that would further simplify the measurement of this enzyme.[7] Substrate **2** containing a free carboxylic acid group and a 4-nitrophenyl chromophore was readily prepared and was combined with the β-xylosidase described above to form the basis for the colorimetric assay of α-glucuronidase.

The preparation of these synthetic substrates will be discussed briefly along with the development of the required enzyme coupled assay formats and their application to the measurement of glucuronyl esterases and α-glucuronidase.



1: R = CH_3 - substrate for glucuronoyl esterases (GE)
2: R = H - substrate for α-glucuronidases

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DEVELOPMENT OF A CHEMICAL PLATFORM FOR THE PRODUCTION OF ADDED-VALUE PRODUCTS FROM FOREST RESIDUES

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The world's compliance with petroleum as feedstock is on the edge of a breakdown. The industrial and technological advances achieved over the years, regarding well-being and economic stability of modern societies, depends on secure and affordable energy and commodities. A new paradigm and economic models, involving greener processes and raw materials, are vital to assure the livability of the planet for the generations to come. Concepts such as biorefineries, circular economy, and neutral emission cycles play a vital and crucial role in the conversion of waste into valuable resources. Such processes will assist to achieve the targeted low-carbon economy, aimed by the European Commission. Presently, biomass is faced as a viable option to contribute to the development of alternative renewable resources. Wastes arising from end-of-life products and those generated by the industrial sector should also be considered. This "new" feedstock includes products containing, e.g., oils [1], carbohydrates [2], crops residues [3], algae [4], lignocellulosic feedstock/residues [5] along with others without any value. Livestock sector can also be considered a source of raw material [6]. Up to now, the preference has fallen mainly on lignocellulosic biomass due to its abundance [7–9].

In this scenario, underpinned by a transition to renewable energy sources and materials, the development of a Technological Platform for the Production of Energy and chemicals, by upgrading wastes and biomass, is envisaged. Such platform should integrate the circular economy concept along with sustainable and regenerative processes, in opposition to the pernicious impact of petrol exploitation.

In a nutshell, a platform that integrates some unit operations to upgrade different biomasses and wastes, heading the attainment of eco-sustainable products is aimed. The central and critical process involved is the Thermochemical Liquefaction. The liquefaction of lignocellulosic materials has already been applied to several residues allowing the production of polyols, foams, sugars, fuels, adhesives, antioxidants.

Usually, the extraction of fractions enriched in reducing sugars, from biomass, is conducted by enzymatic and biological processes. However, such processes are costly and time-consuming, leading most of the times to low yields. Thus the liquefaction process may be equated as an alternative solution, once the raw material is liquefied in high yields, affording valuable products, in particular carbohydrates. The work to be disclosed will be focused on the composition of the carbohydrates fraction as well as on its putative applications such as substrates for fermentation processes to produce bio-diesels, polyhydroxyalkanoates (PHAs), pigments or as valuable chemicals.

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MODIFYING LIGNOCELLULOSE TO ENABLE VALORIZATION: HOW FUNGI DO IT.

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Fungi are able to degrade lignocellulose in plant biomass, and we already exploit their enzymes on industrial scale to produce simple sugars from agricultural lignocellulose waste. We have poor knowledge of the effect of fungi and their enzymes on the actual insoluble complex substrate, while such understanding underpins exploitation of enzymes to make designer polysaccharide materials, effective degradative enzyme cocktails and to engineer fungal production strains.

We investigated how exposure and accessibility of polysaccharides and lignin on the surface of lignocellulose particles changes over time during cultivation with the industrially relevant fungus *Aspergillus niger*, with the aim to increase understanding of how this fungus and its degradative enzymatic machinery interacts with and modifies lignocellulose.

Analysis of time-staged degradation of lignocellulose, (performed by immunohistochemistry, lignocellulose fractionation followed by ELISAs and epitope detection chromatography employing antibodies specific for carbohydrate epitopes), showed differential degradation of xylan, xyloglucan, pectin and mixed-linkage beta-glucan polysaccharides. Furthermore, degradation of specific types of polysaccharides was not always linked to presence of known corresponding degradative enzymes. For example, no reductions of the glucuronic acid decorations on xylan were detected during first phase of growth despite the presence of high levels of xylanases and accessory enzymes. This suggests that degradation of this polysaccharide may be limited by interference of other lignocellulose compounds, or by lack of enzyme activity and/or absence of essential accessory enzymes.

Concluding, we identify here for the first time a time-staged modification and degradation of lignocellulose by an ascomycete fungus. Our results highlight that a full understanding of fungal and enzymatic lignocellulose degradation requires a combination of enzyme biochemical data with identification of modifications in real, complex lignocellulose materials.

SYNTHESIS OF BRANCHED OLIGOSACCHARIDES RELATED TO THE PECTIC RHAMNOGALACTURONAN I POLYSACCHARIDE

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Pectin is one of the major plant cell wall components. It is constituted by highly complex and heterogeneous polysaccharides, which contribute to the modulation of several physiological processes such as cell growth and differentiation, cell-cell adhesion and cell wall support, and defense mechanisms.[1,2] Rhamnogalacturonan I (RG-I) is one of the structural domains of pectic polysaccharides, along with homogalacturonan, rhamnogalacturonan II, xylogalacturonan, and apiogalacturonan. Structurally diverse and complex, RG-I polysaccharides have a backbone consisting of [→2)-α-L-Rha*p*-(1→4)-α-D-GalA-(1→] disaccharide repeats, with numerous branching side-chains of galactans, arabinans, or arabinogalactans, generally found at the C-4 position of L-rhamnose residues.[3]

Given the structural complexity of RG-I polysaccharides, well-defined synthetic RG-I related oligosaccharides are necessary for investigating enzymes involved in pectin biosynthesis and degradation, as well as for aiding structural analyses of the peptic polysaccharides. While several syntheses of fragments corresponding to the RG-I backbone have been carried out, large structures with a branched backbone resulting from chemical synthesis have not been previously reported.[4] Herein, we present the synthesis of a heptasaccharide backbone by means of a [4+3] glycosylation and late-stage oxidation approach, followed by the installation of galactan side-chains of different length to furnish novel branched RG-I fragments (Figure 1).

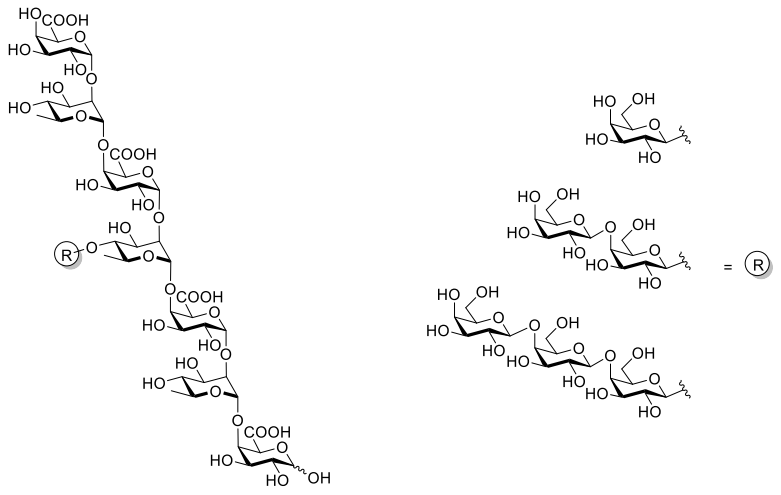

Fig. 5- Branched-backbone RG-I fragments

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DEEP EUTECTIC SOLVENTS AS TOOLS FOR FRACTIONATION AND VALORIZATION OF WOOD MACROMOLECULAR COMPONENTS

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The current need to move from a fossil-derived economy to a sustainable bio-based and circular economy is highly demanding. The utilization of lignocellulosic biomass as a renewable source towards the production of fuels, chemicals and materials in the frame of the biorefinery concept is a promising solution that has been widely explored in recent years [1]. To reach such goal, biomass deconstruction must be efficiently achieved and separation of main macromolecular components, namely cellulose, hemicelluloses and lignin should be a target. However, besides an efficient biomass separation, the application of cheaper, greener and more sustainable technologies with low environmental impact are highly required. In this context, biomass processing using deep eutectic solvents (DES) is one of the most promising technologies due to their low cost, green credentials and special features of that allow selective fractionation of biomass components [2]. Additionally, when aqueous solutions of DES are used instead of the pure counterparts and apart from considerable cost reduction, improvements in the efficiency of the processes might be improved.

The present work describes the use of DES aqueous solutions for the selective dissolution of hemicelluloses and lignins, first using models compounds and then applying the best conditions to *E. globulus* wood fractionation. The developed strategies demonstrate that DES aqueous solutions are promising solvents to apply in biomass processing and the selective extraction properties of these systems could allow an integrated fractionation of cellulose, hemicellulose and lignin for further valorization in the frame of the biorefinery concept.

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VALORIZATION OF ACUATIC BIOMASS FOR THE EXTRACTION OF LIGNOCELLULOSIC POLYSACCHARIDES

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The vast majority of plastics currently produced worldwide are derived from virgin fossil feedstocks. Due to the growing concerns about the negative impact of plastic materials on the environment and the steady depletion of fossil fuels, great efforts are currently being focused on the development of biodegradable plastics from renewable resources, also known as bio-based polymers. Lignocellulosic biomass is one of the most abundantly available raw material on Earth and can be extracted from multiple sources, such as forestry and agro-industrial wastes. Sourcing it from waste or underutilised resources that do not compete with the food chain is particularly interesting and in line with the principles of circular economy. In this work, we propose the valorization of aquatic biomass waste materials, as an alternative to land biomass, for the extraction of lignocellulosic fractions and the production of bio-based food packaging materials.

Two different aquatic resources were compared: 1) residues of the marine plant *Posidonia oceanica*, accumulated in the sea shores of the Mediterranean Sea; and 2) residues generated from the removal of the invasive aquatic plant *Arundo donax* from freshwater environments. Different lignocellulosic fractions were produced by applying a sequential extraction protocol and characterized. The purification of cellulose after the consecutive extraction steps, leading to increased crystallinity and thermal stability, was confirmed. Subsequently, the different fractions were used to generate pure lignocellulosic films, presenting superior water barrier and mechanical performance than benchmark biopolymers, such as starch. Although the removal of lignin and hemicelluloses yielded a consistent improvement in the transparency, mechanical and water vapor barrier properties, the presence of other type of impurities had a positive impact on the properties of the films. The produced lignocellulosic fractions were also incorporated as additives into starch to produce biocomposite films with enhanced properties by melt compounding. Pure cellulose was seen to provide the greatest improvements in the mechanical performance of the films. However, similar water vapor permeability reductions were achieved with the least purified fractions.

These results demonstrate the potential of lignocellulosic fractions extracted from aquatic biomass waste for the development of sustainable bio-based materials for food packaging applications, while providing an added value to these residues.

ONE-POT SYNTHESIS OF NITROGEN COMPOUNDS FROM BIOMASS RESOURCES

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Conversion of carbohydrates into valuable nitrogen compounds represents an interesting possibility for the sustainable synthesis of biologically active amines, which has been achieved with limited success.

Amines derived from furfural (furfurylamines) have diverse applications, including in the preparation of polymers, biologically active compounds and as intermediates in the synthesis of pharmaceuticals such as antiseptic agents, antihypertensives and diuretics (e.g. furosemide). Aminophosphonates are also an important class of compounds with diverse biological activities and potential to be employed as enzyme inhibitors, antibiotics and antitumor agents.

In continuation of our work on the synthesis of amines [1] and α-aminophosphonates, [2] here we report a novel and efficient methodology for one-pot conversion of xylose and xylan into several furfurylamines and α-aminophosphonates catalyzed by $HReO_4$ in good overall yields and chemoselectivity (Figure 1).

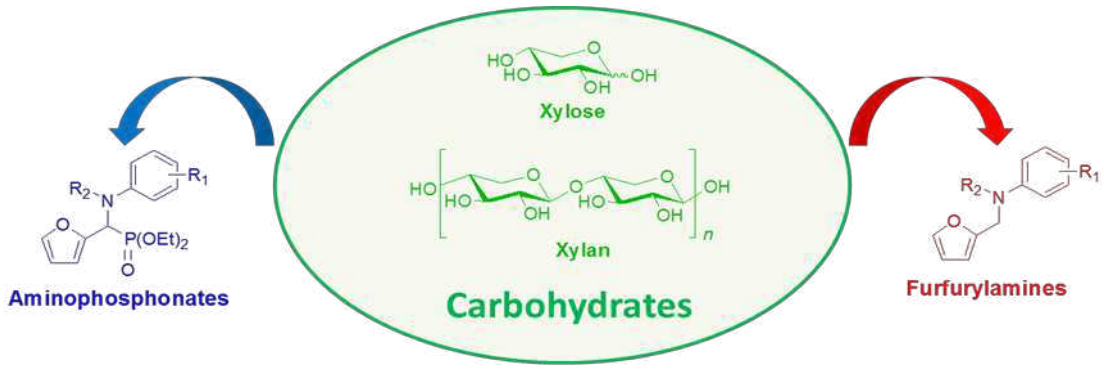

Fig.1 – One-pot conversion of xylose and xylan into furfurylamines and aminophosphonates

Acknowledgements: The authors thank the project UID/QUI/00100/2013 and the Portuguese NMR Network (IST–UTL Center) for providing access to the NMR facilities. Vera Isca thanks FCT for grant and ACF (IF/00849/2012) acknowledges FCT for the “Investigador FCT” Program.

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CHEMISTRY ON PROTECTION AND FUNCTIONALIZATION OF FREE SUGARS

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Chemical synthesis is one of the most practical approaches to access pure oligosaccharides with different functionalities. However, it is subject to multiple synthetic steps and complicated purifications. Here, an efficient one-pot synthetic strategy was established to produce a variety of sugar derivatives, including carbohydrate 6-phosphates, especially biologically important trehalose mono-6-phosphate and glucosamine 6-phosphate easily within only 2 steps in excellent yields [1,2,3]. In addition, the CH_3CN-promoted HMDS trimethylsilylation enabled the subsequent homogenous chemoselective *N*-functionalisation on amino sugars in one step without any purification [4]. Recently, we have integrated this silylation method into microwave chemistry and developed a microwave-assisted one-step synthesis of 1,6-anhydrosugars with their pyranose or furanose form controllable. The same strategy was also employed in glucosamine, of which the differentiation of *O*3 and *O*4 could be achieved by using the hydrogen bonding between *N*2 and *O*4. Eventually, the microwave-assisted intramolecular anomeric protection (iMAP) of glucosamine was established to synthesize 1,6-anhydrogalactosamine and 1,6-anhydroallosamine in concise steps. (Figure 1) [5].

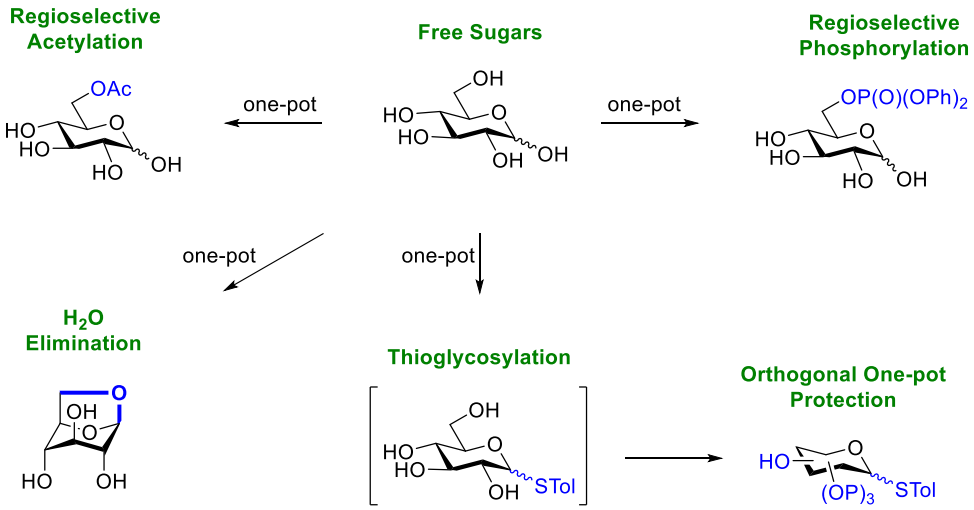

Fig. 1 - A summary of our achievements on the chemistry of free sugars.

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THE ACYLOXYALLYLATION OF (UN)PROTECTED TETROSES REVEALING A PRONOUNCED DIASTEREODIVERGENCE AND A FUNDAMENTAL DIFFERENCE IN THE PERFORMANCE OF THE MEDIATING METAL

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The acyloxyallylation of unprotected aldoses was first demonstrated more than a decade ago as an elegant two-carbon homologation of reducing sugars (upon ozonolysis) [1], however, its application in real case syntheses remained scarce. Following up on such a successful show-case (short and scalable synthesis of the important bacterial sugar L-*glycero*-D-*manno*-heptose [2]) and to address several pending questions about this attractive transformation, we engaged in an in depth methodological re-investigation. The epimeric tetroses L-erythrose and D-threose in unprotected and protected aldehyde form were successfully applied to the indium and also zinc mediated acyloxyallylation, the latter being a first for an unprotected sugar. The investigation largely benefited from the choice of these more exotic starting materials as it allowed unambiguous identification of all hexose-products (authentic reference materials) as well as the reliable quantification of all diastereomers formed, even at very low proportions.

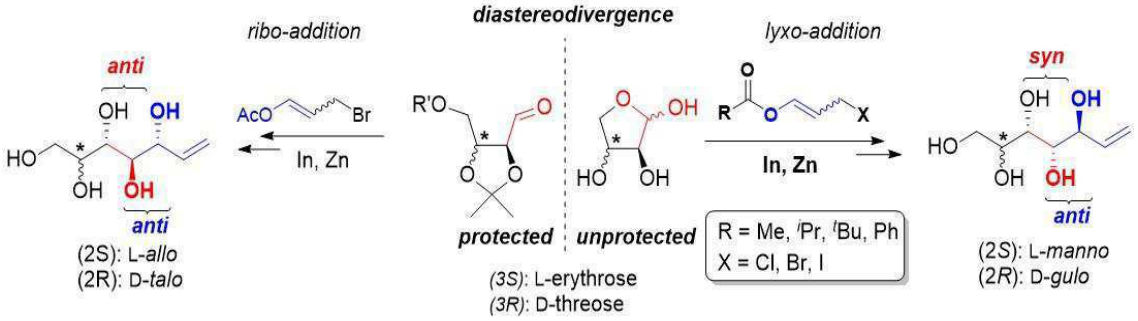

Fig. 1 – Diastereodivergence in the acyloxyallylation of protected and unprotected tetroses

The observed diastereoselectivities indicate a strong substrate control (stereochemistry at *O*2) and the influence of the reagent's structure on the selectivity was investigated in great detail based on a GC-based quantitative analysis. A strong facial diastereodivergence between related protected and unprotected structures was demonstrated (Fig. 1) and an unexpected, pronounced principle difference in performance between indium and zinc was revealed. This unexpected and rather rare difference in these two mediating metals supports our mechanistic hypotheses about the diastereodivergence as well as observed diastereoselectivities [3].

This consolidated knowledge allows for more refined predictions and we hope will inspire more people to consider acyloxyallylation as a synthetic tool, within and beyond the realm of carbohydrate chemistry. Applications based on acyloxyallylation towards further short synthetic solutions to currently rare and exotic sugars are currently being developed in our lab.

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UNIFORMLY ^{13}C-LABELED CARBOHYDRATES FOR PROBING CARBOHYDRATE-PROTEIN INTERACTIONS BY NMR SPECTROSCOPY

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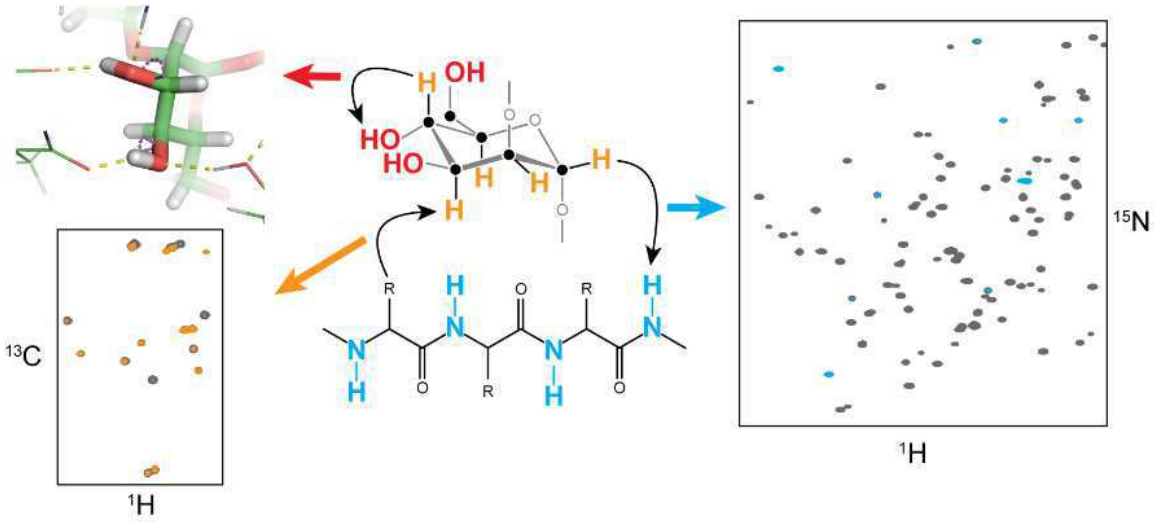
Carbohydrates used in structural studies by NMR are rarely ^{13}C-labeled, despite severe resonance overlap in their ^{1}H NMR spectra. This is caused by the lack of easily accessible isotope-labeled material, preventing exploitation of the ^{13}C spectral dispersion in 3D or higher order NMR experiments.

In this presentation, we demonstrate the power of using a uniformly ^{13}C-labeled trimannoside to delineate the carbohydrate-protein interface by tailored isotope-filtered experiments [1]. Our approach is applicable to systems that exhibit slow exchange on the NMR time scale, which therefore are not amenable to trNOE or STD experiments, commonly used in the fast exchange regime. As model system we selected the complex of a ^{13}C-labeled Manα(1–2)Manα(1–2) Manα*O*Me trisaccharide, bound to cyanovirin-N (CV-N). NOE-based mapping of carbohydrate-protein contacts established that Manα(1–2)Manα(1–2)Manα *O*Me is bound more intimately with its two reducing-end mannoses into the domain A binding site of CV-N than with the non-reducing end unit. Using different isotope labels on the glycan and the protein our approach provides a versatile means for simultaneously mapping binding interfaces on both a carbohydrate and its protein binding partner.



We also identified four carbohydrate hydroxyl protons that form hydrogen bonds with CV-N backbone carbonyl oxygens [2]. Their resonances, surprisingly, were observable in the room-temperature spectra of the complex and were assigned via scalar couplings from the adjacent sugar ring protons. Intra- and intermolecular NOEs involving these hydroxyl protons permitted the determination of their orientation and hydrogen-bonding patterns. Further studies on similar mannoside/CV-N complexes also revealed such hydroxyl proton resonances, highlighting the general applicability of this novel approach for characterizing hydrogen bonding in carbohydrate-protein interactions.

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A SECONDARY STRUCTURE ELEMENT IN FUCOSYLATED GLYCOEPITOPES

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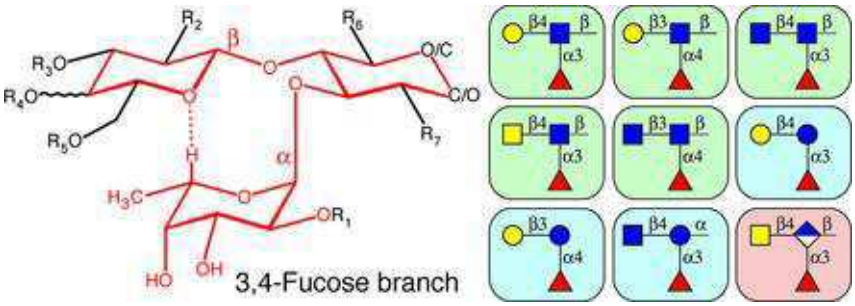
A heavily debated issue regarding protein-carbohydrate recognition processes is the degree of flexibility or rigidity of oligosaccharides. Combining NMR structure determination based on extensive experimental data together with density functional theory (DFT) and database searches, we have identified a set of trisaccharide motifs that adopt a superimposable architecture [1].



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Central to the three-dimensional arrangement of the trisaccharide motifs is a non-conventional C-H···O hydrogen bond. This structural arrangement is present in numerous classes of oligosaccharides found in a variety of organisms ranging from bacteria to mammals. They include Lewis blood group antigens but also unusual glycoepitopes from amphibians and marine invertebrates. The trisaccharide motifs can be summarized with the consensus X-β1,4-[Fucα1,3]-Y and X-β1,3-[Fucα1,4]-Y. We believe that this arrangement represents the first secondary structure element in oligosaccharides that is supported by experimental nuclear Overhauser effects, chemical shifts and ab initio calculations. The wide spectrum of possible modifications of this scaffold points towards a large variety of glycoepitopes that nature generated using the same underlying three-dimensional structure.

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EFFECT OF HUMAN GBA2 PATHOGENIC MUTATIONS ON *Tx*GH116 SUGAR BINDING AND HYDROLYSIS

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Thermoanaerobacterium xylanolyticum glycoside hydrolase family GH116 □-glucosidase, *Tx*GH116, is a structural model for the GH 116 family, including human GBA2 glucosylceramidase.[1] Defects in human GBA2 are responsible for cases of hereditary spastic paraplegia (HSP), autosomal recessive cerebellar ataxia (ARCA) with spasticity and Marinesco-Sjögren-like syndrome.[2,3,4] The GBA2 mutations causing these defects have negligible activity upon expression in cell culture,[5] but some of the corresponding mutations appear to have milder effects when reproduced in *Tx*GH116. For instance, the TxGH116 R786H mutation is expected to weaken the binding of the glucosyl 6-OH in the -1 subsite, consistent with its 87-fold increase in K_M for 4-nitrophenol □-D-glucoside with only 4-fold increase in k_{cat} compared to wild type. Despite this apparent weaker binding of substrate, the R786H mutant was more sensitive to glucose inhibition, with a competitive K_i of 1.48 mM compared to 3.95 mM for wild type TxGH116. At same time, it displayed less efficient hydrolysis of disaccharides, but similar or higher rates of hydrolysis of longer oligosaccharides compared to wild type. The structure of the R786H mutant in complex with 2-fluoroglucoside showed relatively weak density compared to wild type. The structures of TxGH116 catalytic residue mutants in complexes with oligosaccharides showed that R786 contributes to the +1 subsite glucosyl residue binding as well as the -1 site. Mutation of another residue in the +1 subsite, Y62A, had a similar effect of increasing the apparent K_i to 1.0 mM, although it had little effect on disaccharide hydrolysis. Although the other disease related active site mutation, D508H, caused an 8800-fold decrease in k_{cat}/K_M compared to wild type GH116, the other mutations, F347V, R544W, and G599R, showed relatively little effect on kinetics and only slight effects on the stability of TxGH116, suggesting that the mesophilic human GBA2 is more sensitive to these mutations compared to the thermophilic TxGH116.

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UNRAVELING FAMILY 50 CBMs OF *CLOSTRIDIUM THERMOCELLUM:* STRUCTURAL-FUNCTIONAL CHARACTERIZATION OF A NEW LYSM DOMAIN

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Clostridium thermocellum is an anaerobic thermophilic bacterium present in hot springs and soil that is highly efficient in the degradation of plant cell-wall polysaccharides[1]. This process is crucial for life sustainability and the degradation products are resources for nutritional and biotechnological applications[1,2]. The genome of *C. thermocellum* codifies a high number of modular Carbohydrate Active enZymes (CAZymes), usually associated with non-catalytic carbohydrate-binding modules (CBMs). Given their carbohydrate-binding specificity, CBMs exhibit an important substrate-targeting function, enhancing the CAZymes activity and promoting the cellulolytic capabilities of bacteria[2]. In the bacterial genome, functional CBMs not associated with an enzyme are also identified. Examples of these CBMs are the Lysin Motifs (LysM), which display binding to peptidoglycan and chitin and have important roles in signalling and symbiosis between bacteria and plants, as well as in bacterial spores morphogenesis[3,4].

Numerous putative CBM sequences were identified in *C. thermocellum* and deposited in the CAZy database[5], and await structure-function characterization. Aiming to study such putative sequences, an initial screening of 65 *C. thermocellum* CBMs assigned to different CAZy families was performed using a carbohydrate microarray containing representative plant and fungal cell-wall carbohydrates. As a result of this analysis, CBMs assigned to family 50 were identified as highly specific for β-1,4-linked-*N*-acetyl-glucosamine (GlcNAc). To narrow-down the ligand-specificity of *C. thermocellum* family 50 CBMs (C*t*CBMs 50), further analysis was carried out using a sequence-defined microarray based on the NGL-technology[6], comprising lipid-linked oligosaccharide probes with diverse sequences and degree of polymerisation (DP). The analyses confirmed the binding-specificity restricted to β-1,4-GlcNAc oligosaccharides, with a minimum DP requirement of 3 residues. Structural characterization was pursued for a *Ct*CBM50, identified as a LysM domain, and its oligosaccharide ligands, by X-ray crystallography. The mode of binding to GlcNAc- and peptidoglycan-sequences with different DPs was also assessed by molecular docking and molecular dynamics simulations using the X-ray structure. The structure-function information derived elucidate the role of this LysM domain family in *C. thermocellum* and reveal important insight of biotechnological interest.

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STRUCTURAL AND MECHANISTIC BASIS FOR THE O-ACETYLATION OF PEPTIDOGLYCAN BY THE VIRULENCE FACTORS PatB OF *Neisseria gonorrhoeae* AND OatA FROM BOTH *Staphylococcus aureus* AND *Streptococcus pneumoniae*.

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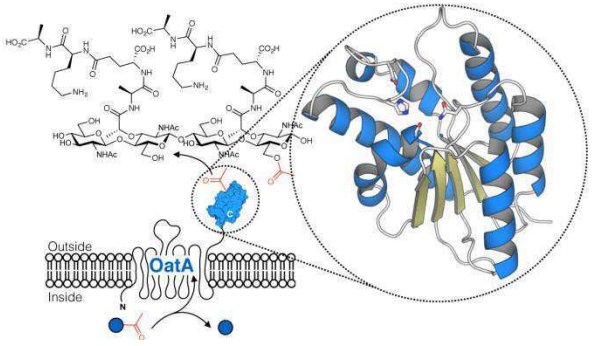
The O-acetylation of the essential bacterial cell wall polymer peptidoglycan (PG) is known to occur in a large number of bacteria including many important human pathogens, such as *Staphylococcus aureus*, species of *Enterococcus, Helicobacter pylori, Campylobacter jejuni*, and *Neisseria gonnorrhoeae*.[1] This modification to the C-6 position of *N*-acetylmuramoyl residues in PG inhibits the action of muramidases (lysozymes) of innate immune systems in a concentration dependent manner, and it totally precludes the activity of the lytic transglycosylases, bacterial autolysins that are involved with the insertion of flagella, pili, and secretion/transport systems, as well as the general biosynthesis and turnover of the PG sacculus. We have characterized two distinct two-component systems for PG O-acetylation in Gram-positive and Gram-negative bacteria, respectively. In Gram-negative bacteria, such as *N. gonorrhoeae*, an integral membrane protein, PG *O*-acetyltransferase (Pat) A, is proposed to translocate acetate from cytoplasmic pools of acetyl-CoA through the cytoplasmic membrane to the periplasm for its transfer to PG by PatB.[2] With *S. aureus, S. pneumoniae* and other Gram-positive pathogens, a single protein, *O*-acetyltransferase (OatA), appears to be a fusion of PatA and PatB to catalyze both the translocation and transfer of acetyl groups for PG O-acetylation.[3]

We present the first biochemical characterization and X-ray crystal structures of *N. gonorrhoeae* PatB and the C-terminal catalytic domains of OatA from *S. pneumoniae* (*Sp*OatA$_C$) and *S. aureus* (*Sa*OatA$_C$) The kinetic parameters for various acetyl donors and acceptors were determined for each *O*-acetyltransferase using a chromogenic assay coupled with MS analysis. These data indicated that each uses a ping-pong, bi-bi catalytic pathway for acetyl transfer to acceptor sugars. A novel PG-based substrate was used to delineate the unique specificities for the OatA$_C$ enzymes which account for their different temporal activities in PG metabolism. The structure of all three enzymes adopts an α/β hydrolase fold comparable to SGNH esterases, and a Ser-His-Asp catalytic triad was identified within the active site grooves on the surface of each enzyme. However, unique oxyanion loop-orientations compared to other SGNH esterases were found. Site-specific replacements confirmed the identification of these catalytic residues. The structure of *S. pneumoniae* OatA$_C$ was also determined in complex with a mechanism-based inhibitor covalently bound to the catalytic Ser. A mechanism of action is proposed for these PG *O*-acetyltransferases involving the formation of an acetyl-enzyme intermediate prior to the acetylation of *N*-acetylmuramoyl residues in PG. Structural insights provide an explanation as to why these enzymes would function strictly as *O*-acetyl-transferases *in vivo* rather than as esterases.



Pathway for PG O-acetylation in Gram-positive bacteria and structure of *Sp*OatA$_C$.

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SYNTHESIS OF II^{3}Neu5Ac-[*6*-^{3}H*Gal*]Gg$_4$-N$_3$

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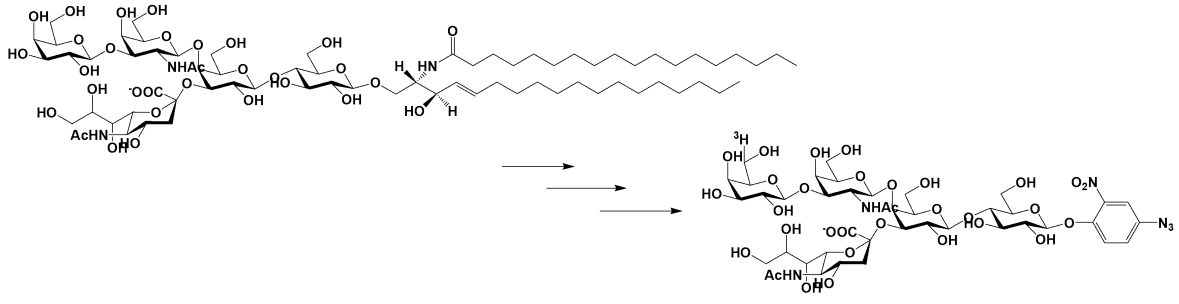
Several data suggest a specific role of ganglioside GM1 in neuronal differentiation and development, but the molecular mechanism of these processes is still unknown. The involvement of GM1 ganglioside in the process of neurite production has been reported for many years. But, recently we showed that the sole oligosaccharide portion of GM1 is responsible for the process of neurite elongation. To continue our studies, we developed a process to synthesize tritium labelled and photoactivable GM1 oligosaccharide

Starting from natural GM1, we obtained the oligosaccharide portion by ozonolysis, than we introduced tritium on external galactose of the oligosaccharide chain.



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ENABLING GLYCOSCIENCE RESEARCH WITH IMPROVED DATA REPRESENTATION OF CARBOHYDRATES IN THE PROTEIN DATA BANK

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The Protein Data Bank (PDB) is the single global repository for experimentally determined three-dimensional structures of biological macromolecules and their complexes with ligands. The Worldwide Protein Data Bank (wwPDB) is the international collaboration that manages the PDB archive according to the FAIR Principles: Findability, Accessibility, Interoperability, and Reusability. PDB archive now holds more than 138,000 experimentally determined structures of biological macromolecules with approximately 8% carbohydrate containing structures, which are all publicly accessible without restriction.

Carbohydrates play key roles in generation of energy, cell signaling, recognition of markers, and in the formation of various structural components of cellular and extracellular matrix. Understanding the structure and organization of carbohydrates is critical to comprehending their biological roles in health and disease. It is impossible to overstate the importance of 3D molecular structure in advancing the development of therapeutic agents and in generating insight into biological processes. The PDB is the preeminent repository for all such data generated for biomolecules, and implements rigorous acceptance criteria for protein structures. However, the complex nature of carbohydrates, which exhibit stereo-isomers, anomeric configurations, and branched chains place unique demands data storage that were not envisioned when the PDB was created for proteins.

There are unique challenges in storing carbohydrate data in such a way that coordinates can be searched easily for structure retrieval. Although three-letter residue names have been used for carbohydrates that are adequate for most situations, no standard representation has emerged. The present lack of a robust representation for carbohydrates in the PDB limits the integration of 3D structural information with other carbohydrate databases and resources. As a result, glycoscientists and other experts cannot fully utilize the rich structural information available for these structures.

This presentation will describe overall project planning of the carbohydrate remediation including communication plan with glycoscience and PDB communities. The goal of the carbohydrate remediation project is to enable Findability, Accessibility, Interoperability and Reusability for carbohydrates in the PDB archive with consistent standard representations that can be easily translated to other representations commonly used by glycobiologists. This will be accomplished by (1) standardizing sugar nomenclature following IUPAC/IUBMB; (2) providing a uniform polymer representation for polysaccharides with linear descriptor(s); (3) adopting community software for reliable carbohydrate identification, assignment of standard nomenclature, and detection of intra/inter-molecular connectivity between monosaccharides and other molecules/proteins; (4) providing intra- and inter-molecular connectivity at atom level explicitly.

SELECTIVE RECOGNITION OF O-METHYLATED GLYCANS BY FUNGAL TECTONIN, A DEFENSE EFECTOR

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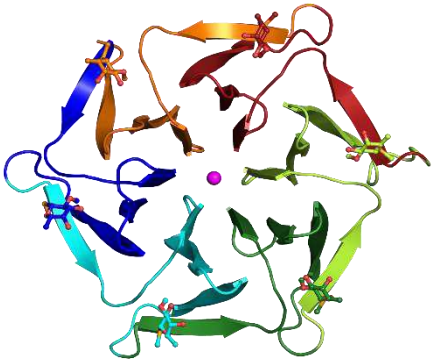
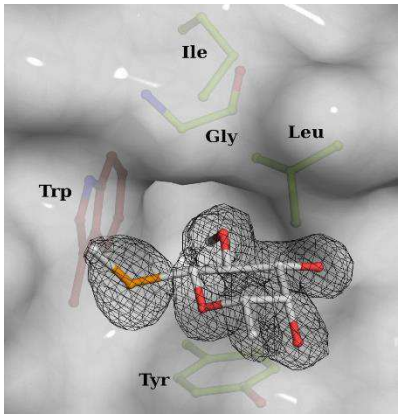
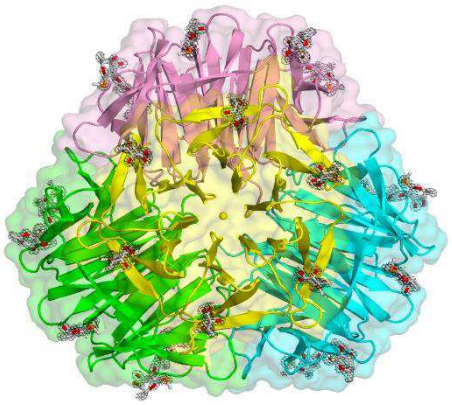
Innate immunity is the first line of defense against pathogens and predators. Lectin-carbohydrate interactions play a major role in the discrimination between self and non-self necessary to initiate a immune response. Tectonin 2 from the mushroom *Laccaria bicolor* (Lb-Tec2) is considered a protein effector of the fungal defense against bacteria and nematodes upon binding to O-methylated glycans as non-self epitopes.[1] These glycoepitopes appear as conserved targets in the tectonin superfamily. Here, we present the crystal structures of Lb-Tec2 in complex with two O-methylated monosaccharides unraveling a six-bladed □-propeller exhibiting one highly conserved binding site per blade and the molecular basis for this unique specificity to date. Furthermore, we uncovered the formation of a ball-







shaped tetramer with 24 binding sites distributed at its surface, resembling a small virus capsid.

Structural analysis led to the identification of a methylation recognition motif that was found in the sequence of many tectonins from bacteria to human. It could be used as a prediction tool. Our results support a key role of tectonins in innate defense based on a distinctive novel type of lectin-glycan interaction conserved through evolution.[2]

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SULFATED POLYSACCHARIDES OF MARINE ORIGIN: NEW INSIGHTS ON STRUCTURAL FEATURES AND BIOLOGICAL PROPERTIES

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Sulfated polysaccharides fucoidans from brown seaweeds and fucosylated chondroitin sulfates (FCS) from sea cucumbers demonstrated a wide spectrum of biological activities including anticoagulant, antithrombotic, antitumor, anti-inflammatory ones. The level of the biological effect strongly depends on a structure of the polysaccharides. Types of glycoside bonds, degree and pattern of sulfation, number of branches, molecular weight were found to be important parameters which could determine the properties of the polysaccharides.

Recently a number of new structural features of sulfated polysaccharides were discovered. Particularly it was shown that FCS could include not only the repeating unit of type **I** (Figure 1), but also the fragments of types **II-V** [1-3]. Therefore, structural variations were determined in positions of fucosyl branches (at O-3 of GlcA or O-6 of GalNAc) and in pattern of sulfation of Fuc (Fuc2,4SS,Fuc3,4SS or Fuc4S), GlcA (GlcA3S or GlcA2,3SS) and GalNAc (GalNAc4,6SS or GalNAc4S) residues.

Several structurally different sulfated polysaccharides, as well as synthetic related oligosaccharides were studied on the ability to inhybit blood coagulation, thrombous formation, angiogenesis, adhesion of cancer cells to platelets monolayer. The results of the study will be presented.

This work was supported by RSF grant 14-13-01325.

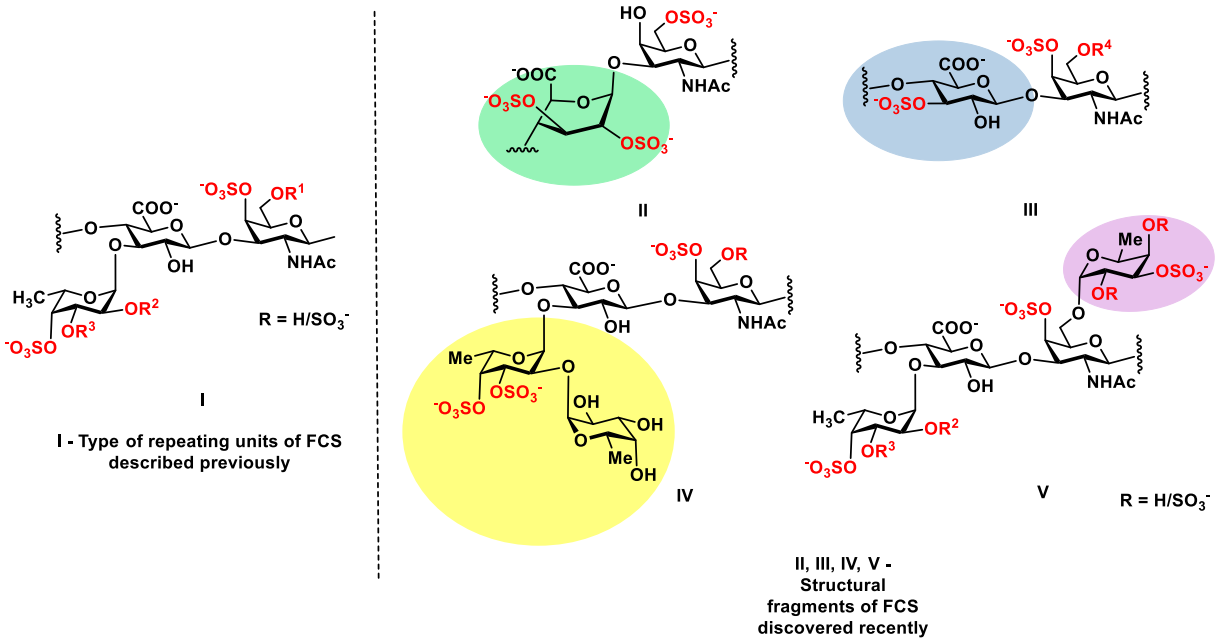

Fig. 1. Structural features of fucosylated chondroitin sulfates.

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BACTERIOPHAGE-LIKE EXOPOLYSACCHARIDE HYDROLASES CONTROL

BIOPHYSICAL PROPERTIES OF BIOFILM IN PLANT PATHOGENS

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Stewartan and amylovoran are anionic heteropolysaccharides produced by the Gram-negative bacteria *Pantoea stewartii* and *Erwinia amylovora*, respectively. They represent the major exopolysaccharide (EPS) biofilm component of these plant pathogens. Stewartan and amylovoran secretion causes Stewart's wilt in corn and fireblight in apple and pear trees with severe economic consequences. Within the stewartan and amylovoran biosynthetic pathways we have biochemically and structurally characterized two proteins of so far unknown function for EPS synthesis.[1] Wcef from *P. stewartii* and Amsf from *E. amylovora* are native 240 kDa trimers of right-handed parallel beta-helices with structural similarity to bacteriophage tailspike proteins (TSP).[2] Wcef and Amsf are hydrolases that rapidly reduce the high viscosity of the stewartan and amylovoran matrices, respectively. Consequently, they produce oligosaccharides, however, at much lower velocities compared to the respective bacteriophage enzymes.[3] Fluorescence correlation spectroscopy, isothermal titration calorimetry and surface plasmon resonance analyses revealed characteristic biophysical properties of these exopolysaccharide interacting proteins distinct from enzymes active in solution that are needed for maintaining permeability and viscosity in the biofilm matrix.

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THE UNIQUE POLYSIALIC ACID BINDING PROPERTY OF HUMAN ADENOVIRUS 52 REVEALED BY GLYCAN MICROARRAY ANALYSIS - FUTURE IMPLICATIONS

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Neoglycolipid (NGL)-based microarray system is an advanced glycan array platform that is available to the international scientific community for recognition studies of diverse carbohydrate binding systems (http://www.imperial.ac.uk/glycosciences) [1]. The clustered and flexible presentation of non-covalently immobilized lipid-linked probes printed in a liposomal formulation on a nitrocellulose matrix renders the NGL system powerful in providing information on the molecular basis of the virus-host interactions, including those of fastidious viruses [2,3]. The probe library of the NGL-based system is one of the largest worldwide and currently contains almost 900 sequence-defined lipid-linked oligosaccharide probes, including major types of mammalian sequences as well as glycans derived from fungal, bacterial and plant polysaccharides [4]. Some of the probes, such as those derived from polysialic acids and glycosaminoglycans, are under-presented in other screening array platforms.

In this communication, I will highlight our recent observations in a collaborative study with the groups of Niklas Arnberg (Umeå, Sweden) and Thilo Stehle (Tübingen, Germany) on host-glycan-recognition by human adenovirus 52 (HAdV-52). Human adenoviruses are common human pathogens associated with gastrointestinal, ocular and respiratory infections. HAdV-52, first isolated in 2003 from a small outbreak of gastroenteritis, is one of only three known HAdVs equipped with both a long and a short adhesive fiber protein. Having shown that the knob domain of HAdV-52 long fiber (52LFK) binds to the coxsackie- and adenovirus-receptor (CAR) and that the knob domain of the short fiber (52SFK) binds to sialic acid-containing glycoproteins [5], we report here new findings from glycan microarray analysis and cellular studies, that the 52SFK recognizes α-2,8-linked polysialic acid sequences as high affinity ligands [6]. Structural analyses have provided insights into an unusual mode of polysialic acid binding and suggest a novel mechanism by which HAdVs enter target cells. To our knowledge, this is the first time polysialic acid is identified as the preferred cellular receptor for a human pathogenic virus. As polysialic acid has been found to be expressed at high levels in brain and lung cancers, where its presence is associated with poor prognosis, our findings have implications for future designs of vectors for gene therapy of these cancers.

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PROPERTIES AND APPLICATION OF HYDROXYL GROUPS IN GLYCOMIMETICS AND OTHER MARKETED DRUGS

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Drug–receptor binding relies on a combination of hydrophilic and hydrophobic interactions. While attractive hydrophobic forces primarily depend on shape complementarity of the ligand and receptor binding sites, polar interactions, based on complementary electrostatic contacts between fully or partially charged moieties, are mostly mediated by functional groups that contain heteroatoms.[1] The hydroxyl group is the most prevalent polar function in carbohydrate-derived drugs and a common feature of other drug molecules. The high directionality and strongly polar nature of interactions of OH groups can be both, highly advantageous, as well as deleterious for the affinity of a biologically active compound. Due to the high energetic cost for its desolvation, a hydroxyl group can be detrimental for binding affinity when it is placed in a position where it cannot favorably contribute to the binding process.[2,3] As a result, great care has to be exercised when the inclusion of hydroxyl groups is considered during the design of a pharmacological lead molecule. Nevertheless, a great number of marketed drugs contain this functional group. An analysis of the ChEMBL database shows that 35% of all marketed drugs contain at least one hydroxyl group. When the dataset of approved drugs is analyzed with regard to the origin of the respective pharmacophore structure, it becomes evident that drugs derived from natural products are far more likely to contain one or multiple OH groups. A study of the X-ray crystallographic binding mode of drug molecules in complex with their respective target reveals that hydroxyl groups are often utilized to mimic the features of a natural ligand or occupy the position of a conserved water molecule. In other cases, the addition or removal of OH groups can allow a targeted modification of pharmacokinetic properties or influence the pathway of drug metabolism in the liver.

Because of their unique physico-chemical properties, the introduction of hydroxyl groups into the molecular scaffold of a drug molecule can have a dramatic effect on its properties. A detailed analysis of the applications of this functional group in marketed drugs can help medicinal chemists to make more informed decisions during lead optimization.

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HOMOGENEOUS ANTIBODY–DRUG CONJUGATE PREPARATION VIA GLYCAN LINKAGE

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Antibodies usually have an *N*-glycan moiety at the Asn297 in the Fc region. Glycans of antibodies are usually heterogeneous, and non-human-type glycans have immunogenicity. It is also known that the glycan structure of antibodies influences antibody-dependent cell-mediated cytotoxicity (ADCC).

Antibody–drug conjugates (ADCs) are monoclonal antibodies known to have therapeutic effects against cancer. The cyctotoxic agents were conjugated to antibodies. To expand the safety-margin, homogeneous ADCs are required. As the conjugation-site is specific when glycan is used for conjugation,homogeneous ADCs can be obtained with homogeneous glycan structure.

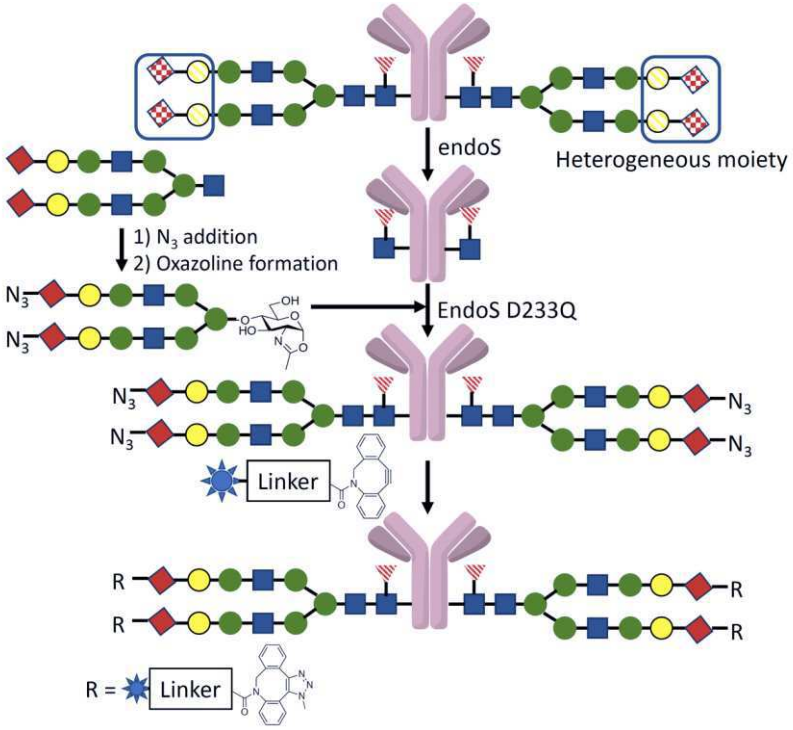

Fig. 1 Reaction scheme of glycan conjugated ADC synthesis.

We used trastuzumab as a model antibody, because it internalizes to cellinside effectively. Heterogeneous glycans were removed by using endoS to obtain GlcNAc-carrying trastuzumab. In this step, the heterogeneous glycans were removed. Homogeneous human-type glycan from egg-yolk was functionalized with an azide group. After oxazoline preparation, glycan was added by using endoS D233Q to the glycan-truncated trastuzumab. This reaction was carried out under slightly acidic conditions to suppress the side-reaction between oxazoline and the amino group at Lys residues. Next, a cytotoxic monomethyl auristatin E(MMAE) with a cathepsin cleavable linker was added by the Cu-free Huisgen reaction. These processes were monitored by HILIC column chromatography. MS analyses after proteolytic digestion showed high homogeneity of the prepared ADC.

The ADC showed significant toxicity against high Her2-expressing cells such as N-87, OE-19, and SK-BR-3. The IC50 of ADC against SK-BR-3 cells was 0.3 nM *in vitro*, which was the same as that of MMAE. However, the ADC did not show toxicity against low Her2-expressing cells such as MKN-45 and MCF-7.

In conclusion, we successfully prepared a cytotoxic, homogeneous glycan-conjugated ADC.

ANALYTICAL STUDY OF CHONDROITIN SULFATE IN DEER ANTLER

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Deer antler at an early growing stage (velvet antler) has been used as valuable traditional Chinese medicine for tonic and pain-reliever. The demand of the velvet antler in Korea and China is still increasing. However, it is obscure what is really active in the velvet antler for these effects. We have precisely analyzed the velvet antler and isolated much amount of chondroitin sulfate (CS).

We have already reported the amounts and sulfation patterns of CS in several tissues of marine resources containing sharks and squids[1-3]. Based on the analytical strategies we have elucidated that the velvet antler contained higher density of CS in dry-defatted tissue compared to many fishes including sharks. CS exists in the top part of the velvet antler, while the ossified bottom part contains less amount of CS. It is noteworthy that the sulfation patterns of CS change along with the extention of the antler. CS might deeply relate to the fast ossification of the deer antler. We also clarified that the contents and the sulfation patterns of CS in the velvet antlers of *Cervus nippon* had no significant difference between wild deers in Japan and farm-raised deers in Korea.

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NMR AND CHEMOMETRICS IN THE CHARACTERIZATION OF HETEROGENEOUS POLYSACCHARIDE DRUGS

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Polysaccharides are ubiquitous in animals and plants, where they play important roles in diverse physiological situations. They often comprise a heterogeneous mixture of polymers, varying in size and substitution pattern, whose composition can change between batches. One of the best-known examples of a polysaccharide drug is heparin. Heparin, which is included in the World Health Organisation's "Essential Drugs List".[1] originates from animal sources, is used to prevent the formation of blood clots and is one of the world's most widely-sold polysaccharide drugs.

Heparin chains can vary in length and comprise various combinations of differently sulfated disaccharide building blocks, making heparin an extremely heterogeneous mixture. Stringent quality control of these drugs during the production process is critical. However, determining their quality is more complex than with traditional small molecule pharmaceuticals. Nuclear magnetic resonance (NMR) spectroscopy is the leading technique in the characterization of complex polysaccharide compounds, does not require prior separation of the constituent components and provides a broad range of information, ranging from a chemical fingerprint to structural constraints.

Here, we discuss the current state-of-the art in the use of qualitative and quantitative mono- and bi-dimensional NMR spectroscopy coupled with chemometric techniques to validate that a product meets the required specifications, as well as to establish the structural equivalence of the generic product with the originator. [2]

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A FLUOROGENIC PROBE FOR MONITORING OF THIOGLYCOSIDASE ACTIVITY

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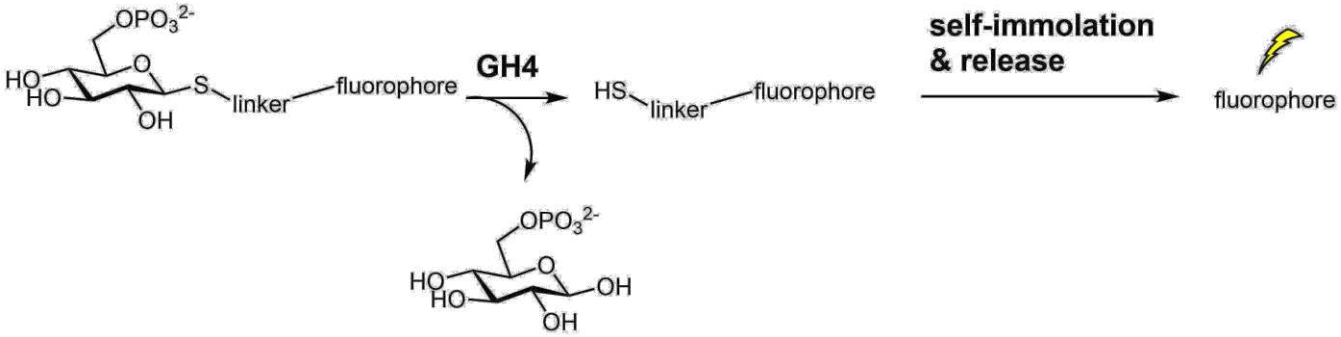
Glycoside hydrolase family 4 (GH4) of the CAZY classification system[1] features an unusual mechanism, involving a complex sequence of redox and elimination/addition events.[2] Our research program has a longstanding interest in discovering and elucidating glycosidase mechanisms[3] that deviate from the classical Koshland model.[4] However, the discovery, detection and study of GH4 enzymes is complicated by a number of factors: In stark contrast to other GH families, a broad range of substrate specificities exists within GH4, which cannot be easily predicted. Moreover, when screeningfor new GH4 enzymes by activity,[5] their detection is obfuscated by the interference of other GH families, which process the same type of substrates, but are much more prevalent.



In this work, we demonstrate how the unique ability of GH4 to process unactivated thioglycosides[6] was used for the development of a specific fluorogenic probe molecule. To this end, a fluorogenic probe was connected to a carbohydrate substrate via an alkyl thiol linker. Upon cleaving the glycoside, the linker self-immolates and releases the reporter molecule. The glycosidase activity can then be monitored by detection of a fluorescence signal. Design and validation of this new type of fluorogenic probe will be discussed as well as applications in screening for new enzymes. With this newly developed type of probe we anticipate to discover new GH families.

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WHAT WE CAN LEARN FROM MULTIBLADED β-PROPELLER LECTINS?

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Lectins are ubiquitous carbohydrate-binding proteins, which play a key role in various processes including cell-cell communication and host-pathogen interaction, but also serve as a valuable tool for medicine and life sciences research. Carbohydrate-mediated recognition plays an important role in the ability of pathogenic bacteria to adhere to the surface of the host cell in the first step of their invasion and infectivity. Lectin-carbohydrate interactions are usually characterised by a low affinity for monovalent ligands that is balanced by multivalency resulting in high avidity for complex glycans or cell surfaces.

Contribution is focused on structure-function studies of several examples of microbial lectins participating in the host-pathogen interaction as well as lectins from bacteria that may be involved in nematobacterial complexes highly pathogenic for a broad range of insects. Importance of CH-π interactions in protein-carbohydrate binding will be also discussed.

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CLICK REACTIONS IN CHITOSAN CHEMISTRY

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Success in synthetic organic chemistry has undoubtedly favored significant progress in the field of chemical modification of chitosan and copolymerization of the related polymeric materials. For example, the use of such reagents as dicyclohexyl carbodiimide has made possible the application of methods of peptide chemistry for chitosan conjugation with carboxylic acids, whereas the development of the approach of phthalimide and benzylidene amino group protection has permitted selective transformations at this or other functional groups of the polymer. These changes have extended the potential of chitosan chemistry, but the diversity of derivatives of this polymer has been restricted by the limitations of the classical organic synthesis methods using traditional polymer-analogous transformations consisting of the formation of chitosan ethers, esters, and amides. The concept of click chemistry has now provided chitosan chemistry with powerful synthetic methods. In particular, the azide-alkyne cycloaddition catalyzed by copper ions as the first approach in chitosan click chemistry led to the development of methods for synthesis of new chitosan derivatives. The metal-free click reactions (cycloaddition of azides to electrophilic alkynes and other highly active dipolarophilic systems, Diels-Alder reaction, and thiol-ene addition) also offer mild conditions for the preparation of chitosan derivatives with a number of attractive physicochemical and biological characteristics and have the critical advantage that no purification of the obtained polymer from metal ions is necessary. Thus, the application of methods of the modern click chemistry for chitosan derivatization opens up attractive and straightforward ways for controlled, selective, and technically convenient synthesis of biocompatible and biodegradable polymers with specified structures and properties [1].

This report provides the first systematic information on the use of click reactions in chitosan chemistry for the preparation of novel polymers with attractive physicochemical and biological properties, including novel results of our scientific group. The reactions of copper-catalyzed azide-alkyne cycloaddition and the click reactions of chitosan derivatives occurring in the absence of salts or metal complexes will be discussed in detail. The data on the pre-click modification of chitosan (i.e., the introduction of azide function, alkyne fragment, highly dipolarophilic moieties, and thiol group into the polymer) will be presented. Particular attention will be given to the application of new chitosan derivatives obtained by click modification.

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THE ROLE OF CORE FUCOSYLATION ON THE DYNAMICS OF IGG1-TYPE COMPLEX *N*-GLYCANS: AN MD STUDY OF SEQUENCE-TO-STRUCTURE RELATIONSHIP IN COMPLEX CARBOHYDRATES

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The Fc glycosylation of human immunoglobulins G1 (IgG1) is essential for their activity and the specific nature of the Fc glycoforms is known to modulate the IgG1 effector function. In this framework, the effects of core α(1-6)-fucosylation is particularly interesting, because it greatly affects the antibody-dependent cell-mediated cytotoxicity (ADCC) function, with obvious repercussions in the design of therapeutic antibodies. Previous studies have shown that, while core α(1-6)-fucosylation does not affect significantly the protein structure within the IgG1-Fc region, it may modulate the *N*-glycan conformational propensity. In this work we report the results of a systematic study, based on extensive molecular dynamics (MD) simulations, on the effect of core α(1-6)-fucosylation on the structure and dynamics of unbound, and increasingly larger, complex biantennary N-glycoforms: from chitobiose to the sialylated *N*-glycan species found in the Fc region of human immunoglobulin G1 (IgG1). Extensive conformational sampling shows that while core fucosylation does not affect significantly the intrinsic dynamics of the isolated (unbound) *N*-glycans, it can affect the conformational propensity of the glycan when bound to the antibody. Moreover, we also find that galactosylation of the α(1-6) arm shifts dramatically the glycan conformational equilibrium from an outstretched to a folded conformation. In this presentation we will discuss how this change in conformational propensity can ultimately affect the molecular recognition and FcγRIII binding of fucosylated and non-fucosylated species.

DOXORUBICIN AND ACLARUBICIN: SHUFFLING ANTRACYCLIN GLYCANS FOR IMPROVED CYTOTOXIC AGENTS

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Doxorubicin is one of the Topoisomerase II inhibitors that are used for the treatment of various types of cancer, including leukaemia and non-Hodgkin lymphoma. According to its typical mechanism of action, intercalation occurs into the DNA, their target topoisomerase is trapped, thereby generating DNA double-strand breaks and ultimately cell death. As effective and popular as this drug is, its usage is hugely limited by the cumulative cardiotoxicity it brings along.

It was recently shown that these anthracyclines are able to induce histone eviction from chromatin [1]. Amongst the consequences are a marked delay in DNA repair and diverse epigenetic changes. This additional activity of the anthracyclines may explain the difference in potency and side-effects between these drugs and structurally different Topo II inhibitors.

We have developed a flexible methodology that allows for the preparation of mono-, di- and trisaccharide analogues of doxorubicin and aclarubicin (a related anthracycline trisaccharide). Structural variation in the target compounds has been achieved by swapping and shuffling of the sugar sequence and varying the alkylation pattern on the amine functionality. The saccharides can be assembled by iterative stereoselective couplings using thiophenyl donors, which can then be coupled to the desired aglycon using Yu's ortho-alkynyl benzoate donor methodology [2].

A combined cell-biology and bio-informatic pipeline allows us to gain more insight in the biological and cytotoxic properties of the designer antracyclines, in search of better chemotherapeutic agents with diminished side-effects.

This has so far led to the discovery of a doxorubicin-aclarubicin hybrid structure that lacks the classical DSB mechanism, yet maintains its histone evicting property and cytotoxicity. Moreover, its cardiotoxic property has shown to be abolished in vivo in mice. Efficacy studies on human AML xenografts in mice are currently ongoing with the hope of ultimately providing non-cardiotoxic anthracycline anti-cancer treatment for humans.

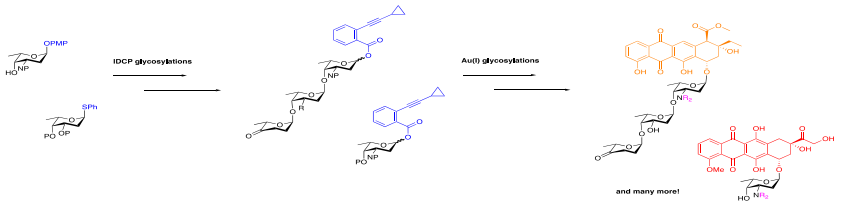

Fig. 1 – Retrosynthesis towards doxorubicin and aclarubicin analogs/hybrids.

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RN1, A NOVEL GALECTIN-3 INHIBITOR IMPEDES PANCREATIC CANCER CELL GROWTH IN VITRO AND IN VIVO VIA BLOCKING GALECTIN-3/EGFR/ERK/RUNX1 SIGNALING PATHWAY

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Galectin-3 (Gal-3) implicated in pancreatic ductal adenocarcinoma (PDAC), and its candidacy as a therapeutic target has been evaluated. Gal-3 is highly expressed pancreatic tumor and commonly associated with poor prognosis. We report that RN1, an arobinogalectan purified from the flower of Panax notoginseng may bind to Gal-3 and suppresses its expression. In addition, RN1 markedly inhibits PDAC cells growth in vitro & in vivo and in patient-derived xenografts (PDXs). Mechanism study suggests that RN1 binds to epidermal growth factor receptor (EGFR) and Gal-3, thereby blocking the interaction between Gal-3 and EGFR and inactivates ERK phosphorylation to further impede Runx1 (transcription factor of Gal-3) function. Silence of Runx1 by RN1 suppresses Gal-3 expression and interrupts Gal-3-associated signaling pathways, including the EGFR/ERK/Runx1, BMP/smad/Id-3 and integrin/FAK/JNK signaling pathways. In addition, RN1 can also bind to bone morphogenetic protein receptors (BMPR1A and BMPR2) and block the interaction between Gal-3 and the BMPRs. The results suggest that RN1 is a novel Gal-3 inhibitor which may be a potential new drug candidate for human PDAC treatment via targeting multiple functional proteins and their signaling pathways.

CHEMICAL BIOLOGY OF PLANT CELL WALL GLYCANS

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Plant cell walls are structurally complex and contain a large number of diverse carbohydrate polymers. These plant fibers are a highly valuable bio-resource and the focus of food, energy and health research. We are interested in studying the interplay of plant cell wall carbohydrates with proteins such as enzymes [1–2], cell surface lectins, and antibodies [3–5]. However, detailed molecular level investigations of such interactions are hampered by the heterogeneity and diversity of the polymers of interest. To circumvent this, we target well-defined oligosaccharides with representative structures [6–9] that can be used for characterizing protein-carbohydrate binding. The presentation will highlight chemical syntheses of plant cell wall oligosaccharides from the group and provide examples from studies of their interactions with proteins.

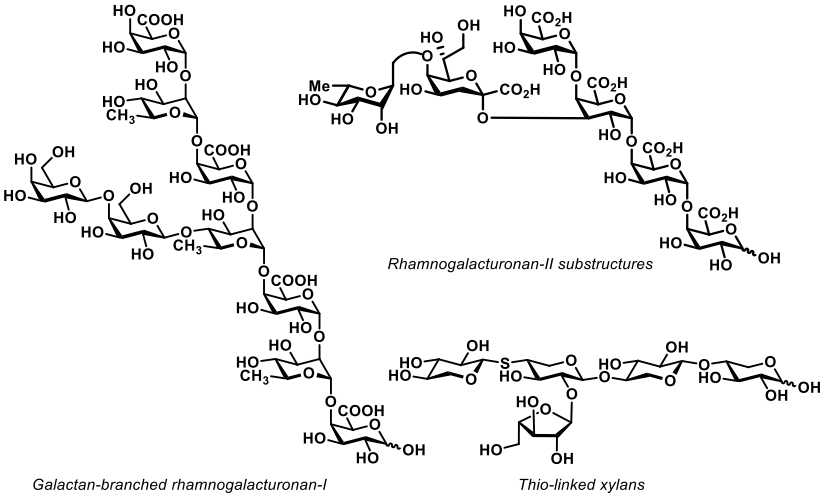

Fig. 1 – Examples of synthetic targets

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SOLUTION AND SOLID-PHASE AUTOMATED SYNTHESIS OF HUMAN MILK OLIGOSACCHARIDES

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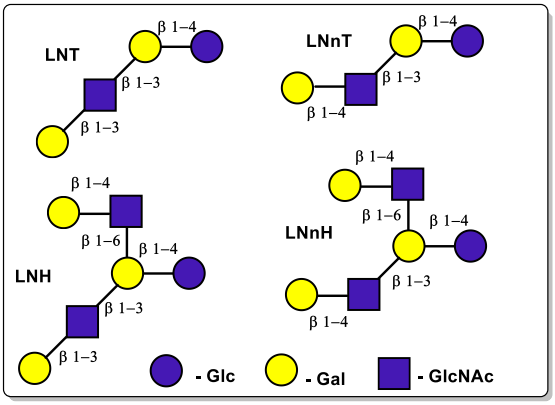
Human milk oligosaccharides (HMOs) are a family of structurally related glycans that are highly abundant in human milk. Oligosaccharide fraction is the third largest solid component in human milk after lactose and lipids.[1] There is an accumulating evidence that HMOs can provide significant benefits to the breast-fed infants.[2] However understanding of the HMO functions is still incomplete due to the lack of individual HMOs in sufficient quantities. We believe that the availability of a library of pure HMOs will significantly enhance our ability to study these compounds.



Reported herein is the synthesis of common core HMOs[3] including Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LNnT), Lacto-N-hexaose (LNH) and Lacto-N-neohexaose (LNnH). We have been developing new methods for streamlined access to orthogonally protected monosaccharide building blocks and expeditious strategies for the oligosaccharide assembly. Synthesis of HMOs was carried out both in solution and using HPLC-based automated synthesizer developed by our labs.

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GLYCO-ENGINEERING: SITE-SPECIFIC LIGATION OF RBC GLYCOCALYX WITH BLOOD GROUP ANTIGEN

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ABO histo-blood group antigens are widely distributed throughout the human body. Determination of human ABO group is critical in predicting the clinical outcomes of both blood transfusion and organ transplantation. Agglutination of human RBCs is the most practical method of ABO typing, however, the precise mechanism and stoichiometry of agglutination are still poorly understood mainly because of heterogeneity of A and B antigens on RBC and clonality and different subtypes of antibodies.

Recently developed KODE technology implying kodecytes – blood group O RBCs modified with Function-Spacer-Lipid (FSL) constructs allows to overcome the problem of ABO antigen heterogeneity. Kodecytes retain all the features of natural RBC, and at the same time such parameters as overall density of the glycans, the distance between antigen sites, and their distance from the membrane bilayer can be controlled and varied.

Five FSLs 1–5 (Figure 1) with A (type 2) antigen were used in the study reported here. FSL 1 contained short 2 nm spacer. FSL 2 had rigid oligopeptide spacer with 7 nm length comparable with glycocalyx thickness. FSL 3 had no spacer between cholesterol fragment and glycan. FSLs 4 and 5 were multivalent, in 4 antigen determinants were significantly (>10 nm) spaced from each other and 5 had very short distance between tetrasaccharides, not suitable for bi- or trivalent docking of the antibody.

A series of experiments on agglutination of natural A1 RBCs and kodecytes prepared using solutions with different concentrations of FSLs 1-5 with monoclonal anti A antibodies and polyclonal O sera were carried out. Results obtained elucidated influence of FSL steric properties (spacer length, valence, distance between antigenic determinants) and their density on the RBC surface on their agglutinating ability. Also new mechanistic details of the interaction between FSLs on the cell surface and antibodies were revealed. In another set of experiments kodecytes were compared with blood group O RBCs chemically glycosylated with Atetra (type 2)-Ad-NHS ester (in this case antigen was attached to amino groups of proteins in peripheral glycocalix). Chemically modified cells demonstrated better agglutination ability, than kodecytes demonstrating, that glycoproteins are of higher importance for antibody binding.

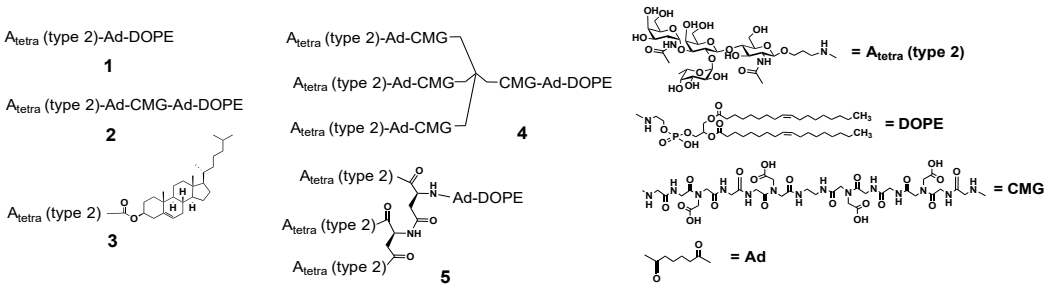

Fig. 1

Acknowledgements: The work was supported by grant # 14-50-00131 of the Russian Science Foundation.

FIRST SYNTHESES OF 2-*C*-GLUCOPYRANOSYL PYRIMIDINES

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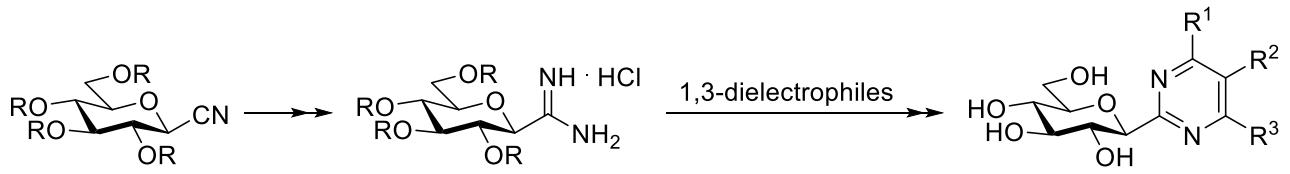
C-Glycosyl heterocycles are hydrolytically stable sugar conjugates with various biological activities. The continuous interest in the syntheses of such molecules is strongly related to efforts aimed at finding new glycomimetics for drug design. In this context, *C*-glycofuranosyl N-heterocycles as analogues of nucleosides and nucleotides have been widely studied [1,2]. *C*-Glycopyranosyl derivatives of five-membered heterocycles have also received great attention, while six-membered heterocyclic *C*-glycopyranosyl compounds are scarcely represented [3]. For instance, 2-*C*-glycopyranosyl pyrimidines are practically unknown compounds, only one derivative, a 2-(2'-deoxy-β-D-ribopyranosyl)pyrimidine obtained as a by-product could be located in the literature [4]. These preliminaries prompted us to investigate the syntheses of this type of molecules.



1,3-dielectrophiles: malonic acid and methylenemalonic acid derivatives, β-ketoesters, α,β-unsaturated β-chloroketones, ynones, vinamidinium salts

For the formation of pyrimidines the Pinner type cyclisation of carboxamidines with 1,3-dicarbonyl derivatives is a reliable method [5,6] as it was demonstrated earlier in the syntheses of 2-glycofuranosyl-pyrimidines, as well [7,8]. Thus, we turned to this convenient route and systematically studied the syntheses of diversely substituted 2-(β-D-glucopyranosyl)pyrimidines by reactions of either isolated or *in situ* obtained *C*-(β-D-glucopyranosyl)formamidines [9] with a wide range of 1,3-dielectrophiles.
In the presentation details of this extensive synthetic work will be reported.

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GLYCODENDRIMER ARRAY: AN EFFICIENT TOOL TO SCREEN MULTIVALENT GLYCOCONJUGATES TOWARDS LECTINS

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Lectins play key roles in a large variety of biological processes. These proteins are characterized by their ability to recognize carbohydrates, inducing a highly specific interaction when these structures are presented as clusters [1]. Therefore, the development of synthetic multivalent glycoconjugates is of utmost importance for diagnostic and therapeutic application. However, the development of active compound is still faced to difficulties since no general rules for the design have emerged so far and it often requires the synthesis of libraries of structures in sufficient quantity for biological investigation. Our aim is to develop a microarray-based approach to screen and identify in a miniaturised environment the best valency and ligand geometry to achieve high affinity towards lectin. To demonstrate to proof of concept, we have selected the hexameric Helix pomatia agglutin lectin (HPA) that binds specifically GalNAc moieties [2,3]. Preliminary studies in our group have highlighted a significant interaction of HPA with tetravalent cluster displaying GalNAc [4]. To increase the affinity of our ligands for HPA, several tetra- and hexadecavalent glycoconjugates were synthesized using scaffolds with diverse architecture, valency and spacers. These compounds have been immobilized covalently on glass slide and clear differences of interaction have been observed (Figure 1). Here we present these experiments and the result of the screening.

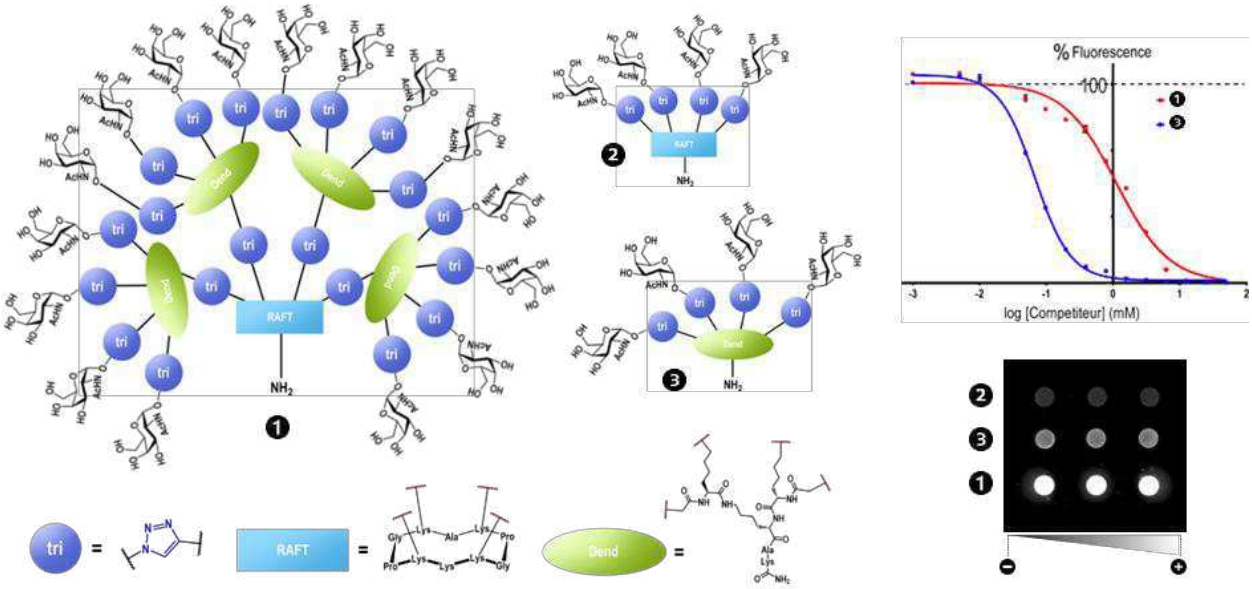

Fig. 1 - Interaction between multivalent glycoconjugates and HPA screened by microarray.

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SYNTHESIS AND IMMUNOLOGICAL EVALUATION OF SELF-ADJUVANTING ANTICANCER VACCINE CANDIDATES USING *N*-MODIFIED TRI SIALYL-TN ANTIGEN

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Tumor cells are distinguished from normal cells by cell surface displays of aberrant levels and types of carbohydrates, called tumor-associated carbohydrates antigens (TACAs). Sialyl-Tn (STn) is richly expressed on a number of tumors, but it is rarely observed on normal tissues. The STn-keyhole limpet hemocyanin (KLH) conjugate was developed as a therapeutic vaccine (Theratope®) for metastatic breast cancer. Unfortunately, this STn-based vaccine failed in phase III of the clinical trial in 2003 [1]. Thus, to improve the immunological response for anti-STn immunization, we have been developing the methods for modulating the innate and adaptive immune responses. Here, we have elaborated the new neoglycoconjugates. The native and *N*-propionyl triSTn were used as the antigens of tumor MUC1, since *N*-propionyl sialic acid proved to enhance the immunogenicity of STn. [2]. We previously showed TLR2 ligands induce potent immunostimulating and antitumor activity [3]. The antigens were conjugated with the T cell epitope and the TLR2 ligand (lipopeptide ligand, Pam_3CSK_4) to construct the antitumor vaccines **1** and **2** *via* copper click chemistry and thioether formation (Figure 1). The results of *in vivo* immunization indicated both of vaccine **1** and **2** were able to induce anti-triSTn IgG and IgM antibody titers. Moreover, the IgG antibody stimulated by vaccine **2** have a higher specificity to triSTn against mono-STn compared with the one stimulated by vaccine **1**.

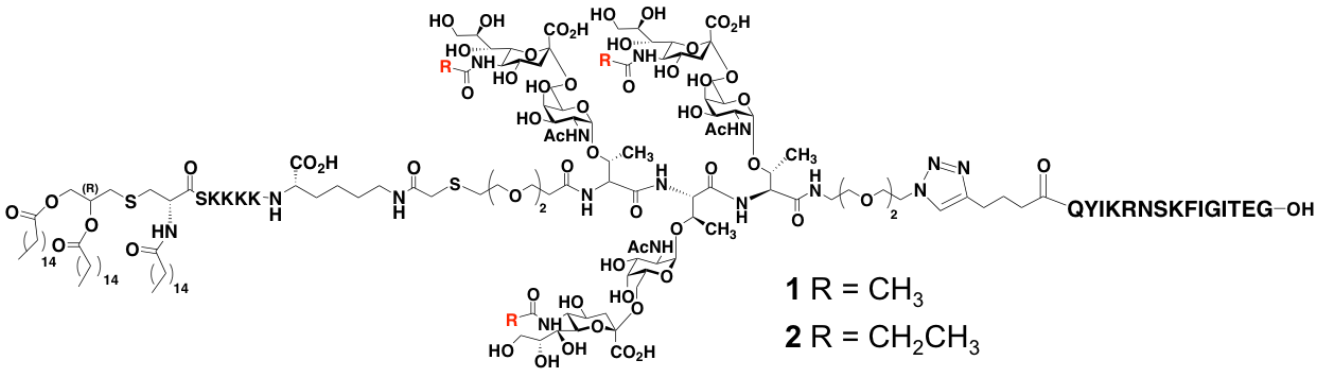

Fig 1 – Structure of self-adjuvanting triSTn anticancer vaccine **1** and **2**.

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NEXT GENERATION OF GLYCOCONJUGATE VACCINES

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The introduction of glycoconjugate vaccines has led to great advances in the prevention of infections including those caused by *H. influenzae*, *S. pneumoniae* and *N. meningitidis*. A new glycoconjugate vaccine against *S. typhi* has also been recently launched. With the current increase in antimicrobial resistance and the advent of superbugs and with few antibiotics in clinical development, innovative vaccines against these pathogens is becoming of central importance. At present, poly- or oligosaccharide components of glycoconjugate vaccines are biologically sourced, and glycoconjugate vaccines are complex to manufacture resulting in high costs. Some of these vaccines may also have drawbacks including stability, reproducibility of different batches and suboptimal efficacy issues. Using organic syntheses, Vaxxilon has developed a technology platform enabling quick discovery of glycan antigens and assess synthetic, homogenous epitopes at reduced costs. The technology is based on the rational design to make structural changes on a molecular level to the natural repeating unit. The resulting shorter synthetic oligosaccharides have several advantages - such as introducing structural modifications, consistency between the batches, better solubility, efficient conjugation consistency, no loss of protective epitopes and better yields. The ease of purification leads to better analytical control of the antigen and conjugate vaccine allowing manufacturing of the vaccine at commercial levels e.g. without cumbersome sterile filtration issues. Overall the approach of Vaxxilon is one of the emerging novel vaccine technologies leading to well characterized, highly stable, potent oligosaccharide antigens being part of improved or new glycoconjugate vaccines for potentially any bacterial pathogen including those for which the conventional approach has failed to deliver.

THE INDUCTION OF PROTECTIVE ANTIBODIES BY A GLYCOCONJUGATE VACCINE TO PREVENT *FRANCISELLA TULARENSIS* INFECTION

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Francisella tularensis, the causative agent of tularaemia, has been classified as a category A bioterrorism agent because it is readily aerosolized and exhibits a high degree of infectivity and lethality in humans. An attenuated live vaccine strain (LVS) has been developed but has not been licensed because of an incomplete understanding of the basis for its attenuated virulence and associated side effects. There have been significant research efforts aimed at elucidating *Francisella* pathogenesis and identifying components for rational vaccine design. Although *F. tularensis* is a facultative intracellular pathogen, studies from several laboratories have demonstrated that humoral immunity is also important for protection [1]. Lipopolysaccharide (LPS) of *F. tularensis* plays a significant role in evasion of host immune response and its O-antgen (OAg) is considered a potential target for vaccination. However, O-antigen based glyconjugate vaccine tested so far have failed to confer protection against intransal LVS infection in mice, the most relevant challenge route [1,2]. The acid hydrolysis of LPS is the preferred method for OAg extraction. We discovered that *F. tularenisis* OAg was susceptible to the conditions used so far for OAg extraction producing a reduced molecular size of 20kDa (LMW-OAg) compared to the native 80kDa (HMW-OAg). An optimized milder hydrolysis resulted in the extraction of the OAg from LPS retaining its full size. When tested as OAg-based glycoconjugate vaccines, using tetanus toxoid (TT) as carrier protein, HMW-OAg-TT conferred better protection than LMW-OAg-TT. To further investigate the role of OAg size in the immunogenicity provided by the vaccine, we generated a *F. tularensis* mutant with an increased OAg size (220 kDa, VHMW-OAg) by modification of the chain length regulator gene *wzz.* We first generated a *F. tularensis* ∆wzz1 mutant unable to regulate the OAg size, and then we transformed the ∆wzz1 mutant strain with a plasmid that carries a wzz gene from the closely related *F. novicida,* responsible for generating a very high molecular weight OAg, *F. tularensis* ∆*wzz1/wzz2*^{Fn}. The immunization with VHMW-OAg-TT conjugate provided a marked increase in protection compared to the glycoconjugates made with the smaller O-antigen sizes, despite a low level of IgG titer. A comparison of the humoral response produced by the immunization with the different conjugate vaccines revealed an increased presence of antibodies against OAg conformational epitopes [3]. in the VHMW-OAg-TT serum. An investigation on the specific features that make conformational antibodies relevant in controlling the *F. tularensis* infection and their importance in developing glycoconjugate vaccines against intracellular pathogens is currently ongoing.

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A STRUCTURAL EXPLANATION OF THE IMPORTANCE OF ACETYLATION IN *NEISSERIA MENINGITIDIS* SEROGROUP. A CAPSULAR POLYSACCHARIDES TO RAISE FUNCTIONAL ANTIBODIES

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Meningococcus remains a devastating cause of meningitis epidemics and sepsis, with more than one million cases resulting in approximately 135,000 deaths reported annually worldwide with a fatality rate of 20% in developing countries [1]. *Neisseria meningitidis* serogroup A (MenA) is a Gram-negative encapsulated bacterium responsible for epidemic meningitis in the sub-Saharan region of Africa, termed meningitis belt [2]. Multivalent conjugate vaccines containing also MenA capsular polysaccharide are now available in western countries, and their efficacy in preventing infection in humans is well established. In a surveillance study monitoring the introduction of vaccination with the conjugate vaccine MenAfriVac (specific for serogroup A) in the meningitis belt, a 57% of decline in incidence was recorded [3]. MenA capsular polysaccharide (CPS) consists of (1→6) linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units with O-acetylation predominantly at position 3 [4]. Two parameters influence the immunogenicity of MenA conjugates: the oligomer length and the acetylation level [5]. Moreover, MenA glycoconjugate vaccines have a poor hydrolytic stability [6]. Although a conjugate vaccine containing MenA oligosaccharides with an average chain length of six was immunogenic in adults [7], the minimal antigenic determinant of MenA polysaccharide is still unknown. Therefore molecular understanding antigen-antibody interactions is crucial to reveal the minimal structural requirements for the vaccines immunological activity and the rationale for a more stable MenA vaccine based on glycan mimetics.

Herein, we applied an integrated approach to screen a MenA oligosaccharide library of fragments by competitive ELISA and surface plasmon resonance (SPR) in order to select a minimal epitope lead fragment for further mapping of the molecular determinants of interaction at atomic detail by Saturation Transfer Difference (STD)-NMR. Competitive ELISA and SPR revealed that an oligomer containing 6 repeating units (DP6), has kinetic rates of dissociation (4,19E-3 s-1) converging with that of longer fragments. Upon de-O-acetylation of MenA minimal epitope, the kinetics of dissociation increase 1000 fold, revealing that these moieties play a pivotal role in recognition. A quantitative evaluation of the STD effect for the sugar proton signals of DP6 showed that the strongest enhancement was observed for the O-acetyl group (100%) located at C3 followed by the acetamide positioned at C2, indicating that these moieties are in close contact with the functional monoclonal antibody, experiencing the highest transfer of saturation. Furthermore, STD signals were also observed for proton signals at positions C6 and C5, showing that this could also play a role in the stabilization of the complex in the binding pocket. Screening of different length fragments by these techniques in combination with inhibition of serum bactericidal activity is currently ongoing to determine the polysaccharide antigenic determinant and the impact of acetylation in interactions with protective antibodies.

MenAfrivac is a registered trademark of Serum Institute of India Private Ltd.

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DEVELOPMENT OF STRUCTURALLY DEFINED IMMUNOSTIMULATORY VACCINE ADJUVANTS

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Recent efforts in developing new vaccines against cancers and infectious diseases have relied heavily on subunit antigen constructs. Since the refined and homogeneous antigens are often less immunogenic, it is necessary to use immune adjuvants to enhance the ability of vaccines to elicit strong and durable immune responses to specific antigens. However, the choice of adjuvants for human vaccines is severely limited, and currently, only three adjuvants have been approved for use in the United States. There still need adjuvants that are capable of stimulating a mixed Th1/Th2 response and antigen-specific CTL production that are necessary for vaccines against intracellular pathogens (*e.g.*, HIV, TB, and malaria) and cancers. Discovery and development of potent new adjuvants has emerged as a critical frontline effort in modern vaccine field [1]. Having promising lead compounds is the crucial first step toward successful development of synthetic vaccine adjuvants. We use the naturally occurring saponins isolated from the tree bark of Quillaja saponaria (QS) Molina as the leads [2,3], and our design, synthesis, and immunological evaluation have led to promising new adjuvant candidates [4,5].

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COMPARISON OF CAPSULAR POLYSACCHARIDE CONFORMATIONS IN *STREPTOCOCCUS AGALACTIAE* SEROTYPE III AND *STREPTOCOCCUS PNEUMONIAE* SEROTYPE 14: IMPLICATIONS FOR IMMUNOGENICITY.

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Streptococcus agalactiae (Group B Streptococcus) is a primary cause of neonatal sepsis and meningitis, particularly in infants born to carriers. *Streptococcus pneumoniae* is another cause of serious infections in young infants, including meningitis and pneumonia. For these bacteria, the capsular polysaccharide (CPS) is essential for bacterial virulence and vaccination with CPS-protein conjugates potentially provides effective serotype-specific protection. Ten serotypes of Group B Streptococcus have been identified, with serotype III (GBSIII) currently the most prevalent [1]. Over 90 serotypes of *Streptococcus pneumoniae* have been identified; prior to the introduction of conjugate vaccines, Serotype 14 (Pn14) was the most common cause of invasive pneumococcal disease in children [2]. GBSIII is present in conjugate vaccines undergoing clinical trials. The branched CPSs of GBSIII and Pn14 are very similar, differing only in that GBSIII has a terminal sialic acid on the branches. Our new molecular modelling studies provide a rationale for conflicting evidence on the minimal epitopes for GBSIII and Pn14 [3,4]. Simulations of 3- and 6RU oligosaccharide representatives of the GBSIII and Pn14 CPs show a well-defined conformational epitope at the branch point that is absent in the unbranched oligosaccharide. Further, we find that, aside from the branch point, the GBSIII/Pn14 backbone is very flexible with no well-defined conformation. The simulation conformations are supported by NMR NOEs measured for the GBSIII polysaccharide. This work suggests that antibody families recognising the stable, common branch points for GBSIII and Pn14 should show

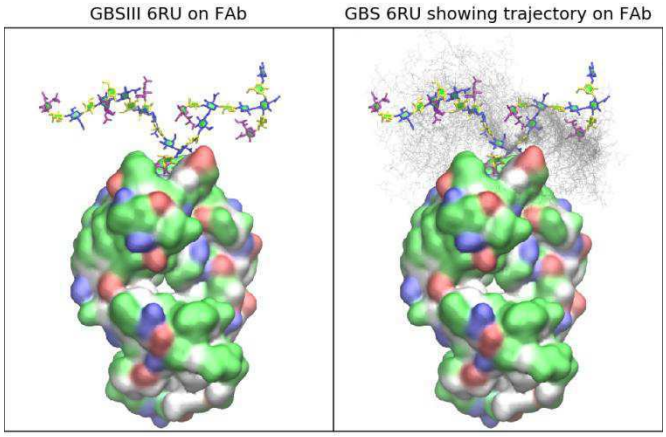

Fig. 1 – Overlay of 6RU of GBSIII on the FAb crystal structure [4], with a single representative conformation (left) and a range of simulation conformations (right). Residues are coloured according to type: Glc/GlcNAc – blue, Gal -yellow and sialic acid –purple.

mutual cross-protection and efficacy. However, antibodies families recognising the terminal sialic acid in GBSIII would have no cross-reactivity with Pn14. Further, antibody families binding the highly flexible backbone are likely to be ineffective at killing. This work suggests a common GBSIII/Pn14 bacterial strategy for evasion of the host immune system, combining a flexible backbone with more stable branches presenting human-mimic epitopes.

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DIVERGENT CHEMOENZYMATIC SYNTHESIS OF *SHIGELLA FLEXNERI* PENTASACCHARIDES FOR VACCINE DEVELOPMENT

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Enteroinvasive gram-negative bacteria from the *Shigella flexneri* (SF) species are of particular concern and a prime target for vaccine development [1]. Most SF serotypes exhibit closely related O-antigens (O-Ag) that are a main target of protection against reinfection. SF O-Ag backbones of interest comprise a unique repeating unit made of four residues (ABCD). Serotype specificity is determined by site-selective *O*-acetylation and α-D-glucosylation (E) (Fig. 1) [2]. The chemo-enzymatic conception of glycobricks, easy-to-assemble into biologically active molecules mimicking the O-Ag from various serotypes, is the keystone of an ongoing strategy to a synthetic carbohydrate-based *Shigella* vaccine [3].

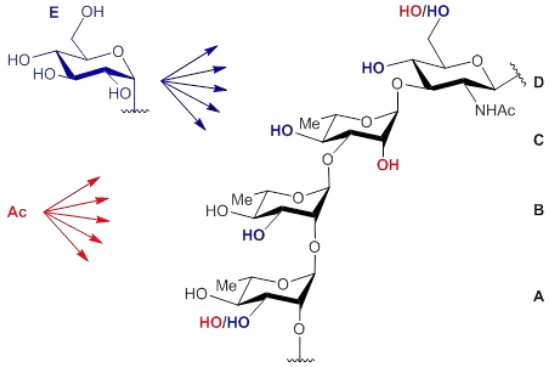

Fig 1 - Tetrasaccharide repeating unit (ABCD) of the SF O-Ag common backbone, and sites of serotype-specific O-acetylation (Ac) and α-D-glucosylation (E).

A pioneering divergent synthesis of a selected set of glycobricks is under study. It is based on the regio- and stereo-specific enzymatic α-D-glucosylation of a lightly protected ABCD-like tetrasaccharide scaffold (ABC'D'). The careful design of the selected ABC'D' fulfilling criteria such as (i) light protection pattern compatible with glucansucrase binding site topology, (ii) easy access, and (iii) possible elongation at either end will be exposed. The different strategies implemented to synthesize the selected acceptor will be discussed. Focus will be on the most promising synthesis, which has been optimized and scaled up to prepare multigram amounts of the target ABC'D'.

A step further has been reached with the regio- and stereo-specific glucansucrase-mediated conversion of ABC'D' into two pentasaccharides, both of interest as SF glycobricks as defined based on NMR analysis. This is, to the best of our knowledge, a novel breakthrough. These promising outcomes and openings thereof in the context of vaccine development will be presented.

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CAPSULAR POLYSACCHARIDES OF *STREPTOCOCCUS SUIS*: POTENTIAL AS VACCINE ANTIGENS

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Streptococcus suis is an encapsulated bacterium and one of the most important swine pathogens and a zoonotic agent for which no effective vaccine exists. To date, 35 serotypes have been described based on capsular polysaccharide (CPS) antigenic diversity. Globally, the predominant *S. suis* serotypes isolated from clinical cases in pigs are, in decreasing order, types 2, 9, 3, 1/2, 7, 8, 4, 22, 5, and 1 [1]. So far, we have reported the repeating unit structures for the CPSs of types 2 [2], 14 [3], 1 and 1/2 [4], and 9 [5]. The CPSs of types 2 and 14 are known to be poor activators of antigen-presenting cells (APC): stimulation with these CPSs induces secretion of CCL2 and CCL3 only, with no production of IL-1β, IL-6, TNF, IL-12p70 or IL-10 [6]. Additionally, types 2 and 14 CPSs are non-immunogenic in mice with undetectable levels of anti-CPS antibodies [7].

Our main objective was to pursue the structural elucidation of *S. suis* CPSs' repeating units in order to study their potential as vaccine antigens. Following chemical and NMR analyses, we report for the first time the CPS structures for types 3, 7, and 8 of *S. suis*. Quality controls for the purified CPSs confirmed the absence of significant contamination by proteins (< 0.7 % w/w) and nucleic acids (< 0.5 % w/w). To study the ability of purified CPSs to stimulate APCs *in vitro*, bone-marrow derived dendritic cells (DCs) were stimulated for 24 h with purified CPSs of types 1, 1/2, 3, 7, 8, and 9, and cytokine production in the supernatant was measured by ELISA. All tested CPSs induced secretion of CCL2 at the same levels as those reported for CPS type 2, indicating that the production of this chemokine is independent of the CPS composition and structure. Interestingly, type 3 CPS also induced significant production of IL-6 and TNF, while other tested CPSs failed to induce pro-inflammatory cytokine production by DCs. These results suggest that type 3 CPS might possess immunogenic/stimulatory properties. *In vivo* immunogenicity testing of the purified CPSs of types 3, 7, 8, and 9 is currently being performed. Mice are being immunized with 2 doses at 21-day interval. Serum samples are being collected weekly to follow the kinetics of CPS-specific antibody production by ELISA. Final antibody titration and isotyping will be performed at 42 days post-immunization. Preliminary results indicate that type 3 CPS induces a strong anti-CPS antibody response in mice. Finally, in the case of non-immunogenic CPSs such as type 2, a glycoconjugate vaccine, made from CPS coupled to tetanus toxoid by reductive amination, has been found to be immunogenic in mice and protective in pigs against this serotype. Lastly, CPSs of *S. suis* possess great potential as vaccine antigens for both immunogenic CPSs (such as type 3) or as a glycoconjugate vaccine for non-immunogenic CPSs (such as type 2).

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FLUORINATED ANTIGEN-MIMETICS FOR GLYCOCONJUGATE VACCINE DEVELOPMENT

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Synthetic glycoconjugate vaccines have been in the focus of modern vaccine development for years, by offering high reproducibility, homogeneity, as well as a possibility for tailor-made epitope design. [1] Since the stability of carbohydrates in biological systems is a point of continuous attention, glycomimetics have gained recent attention to enhance the biostability of crucial glycotopes *in vivo*. [2] Incorporation of fluorinated glycans into a given antigenic structure is a promising approach to meet this challenge. Fluorosugars are thougth to sustain the properties of their natural counterparts while enhancing the stability of the glycosidic linkage against enzymatic degradation.[3] Recently, it was shown that fluorinated analogs of tumor-associated antigens do not compromise antibody recognition properties and the corresponding semi-synthetic vaccines furnished antibodies cross-reactive to the native structures.[4] With this in mind, we became interested in applying this concept to synthetic glycotope mimetics of clinically relevant pathogens. Herein, we present the synthesis of distinct libraries of fluorinated antigen mimetics of *Streptococcus pneumoniae* type *14*, *Leishmania donovani* and *Neisseria meningitidis* type *Y* for the construction of fully synthetic vaccines.

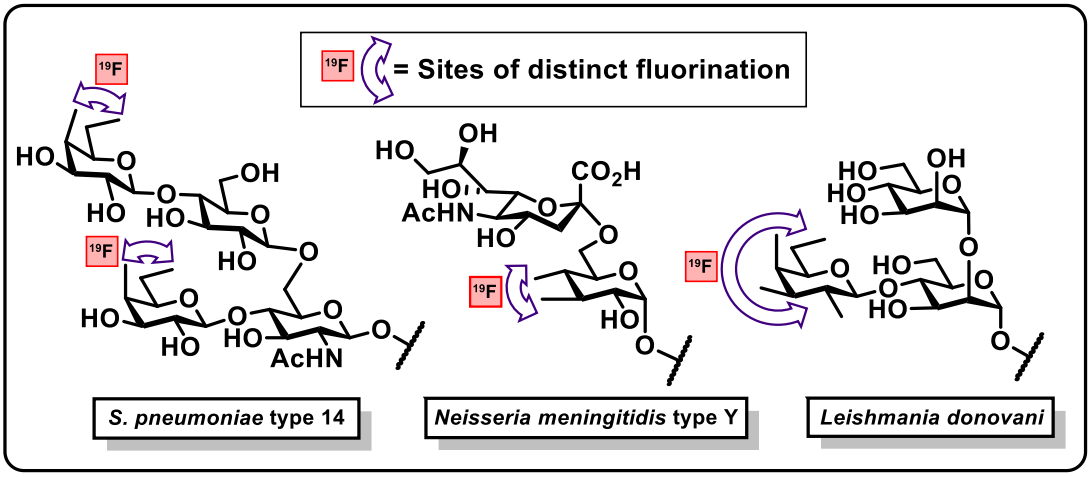

Fig 6 - Schematic depicition of fluorinated antigen mimetics.

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SYNTHESIS AND CHARACTERIZATION OF HOMOGENEOUS GLYCOSYLPHOSPHATIDYLINOSITOL ANCHORED GLYCOPROTEINS

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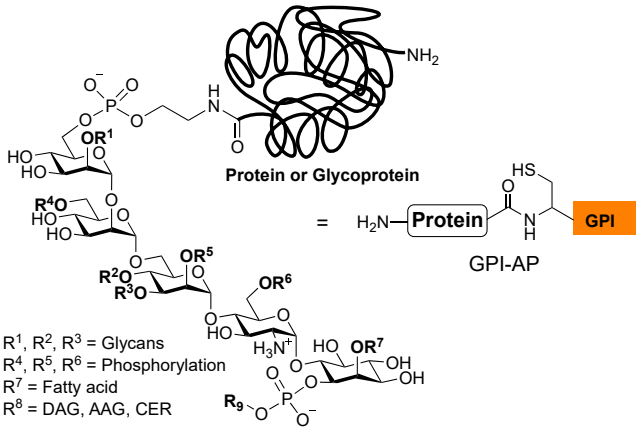
Glycosylphosphatidylinositol anchors (GPIs) are complex glycolipids anchoring eukaryotic proteins and glycoproteins to the outer leaflet of the cell membrane. GPIs are posttranslationally added to the C-terminus of proteins and contain a conserved core glycan structure and lipids that are modified in a cell-specific manner.[1] GPI-anchored proteins (GPI-APs) are functionally diverse, participate in different cellular processes such as the regulation of the immune system, and play an important role during infections by protozoan parasites. The difficult access to pure and homogeneous GPI-APs from natural sources hampers the investigations to disclose the role of GPIs and their modifications in these and other processes as well as in the function and structure of the attached proteins.



Recently, we have established different strategies to obtain GPI glycolipids. In this report will be presented the synthesis and characterization of GPI-anchored glycoproteins using a combination of synthetic GPI anchors,[2] synthetic glycopeptides and expressed proteins. We report the challenges and developments to obtain an optimized strategy for the synthesis of GPI-anchored glycopeptides and their application in the synthesis of GPI-anchored glycoproteins containing both, O- and N-glycosylations. Furthermore, we describe the production of fully modified proteins using the *Npu* DnaE split intein ($Int^{N/C}$),[3] and the results of the characterization of the effect of the GPI glycolipids in the conformation of the anchored proteins and in the interaction with model membranes.

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SYNTHESIS AND IMMUNOMODULATORY ACTIVITIES OF GLYCOCONJUGATES CONTAINING LYSO-PHOSPHATIDYL INOSITOL

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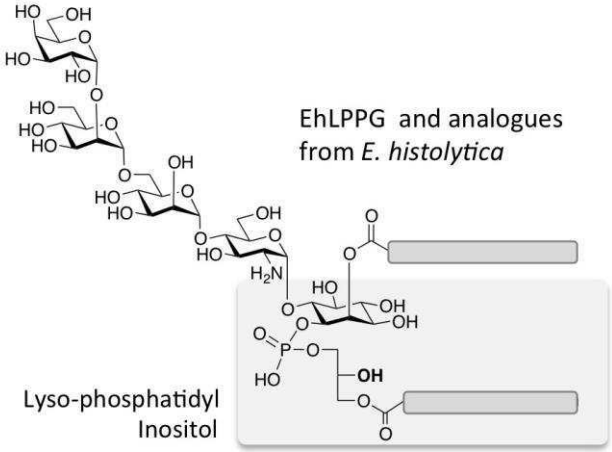
On surface of immune cells, various glycolipids are recognized by innate immune receptors and also by lipid antigen-presenting molecules, which lead to activate the immune system. Among these glycolipids, lyso-phosphatidyl inositol-containing glycolipids show particular activities. For instance, a protozoan, *Entamoeba histolytica*, has lipopeptidophosphoglycan (EhLPPG), which has glycosyl-phosphatidylinositol (GPI) anchor type glycan structure and also contains lyso-phosphatidyl inositol moieties, EhPIa and EhPIb. These compounds have been shown as natural killer T (NKT) cell stimulators via CD1d restricted manner. The structures of EhPIa and EhPIb contain unusual features, such as 1) characteristic long chain fatty acid with length of carbon number 28 or 30 with an unsaturated bond connected to lyso-glycerol at sn-1 position; and 2) additional palmitoyl groups at 2-position of inositol for EhPIb. In the present study, we synthesized these EhLPPG fragment structures and also related GPI anchor structures to clarify their detailed imunomodulatory activities.

For the synthesis of the EhLPPG fragment structures, we employed a protecting strategy utilizing allyl and allyloxycarbonyl groups for permanent protection of hydroxyl groups, with avoiding the usage of frequently-used benzyl-type protection, because of unsaturated fatty acids in the compounds. The allyl and alloc groups can be orthogonally cleaved under mild condition with transition metal catalyst in the high polar solvent. We also developed regioselective phosphorylation reaction of *myo*-inositol utilizing chiral phosphorylating reagents bearing (*R*)-BINOL as a chiral auxiliary, and afforded inositol monophosphate in good yield and desired selectivity. We also applied Ni catalyzed alkyl-alkyl cross coupling reaction for the synthesis of the long-chain fatty acids. Based on these newly developed methods, the first total syntheses of EhLPPG unit structure and EhPIa,b bearing different types of long fatty acids and these diastereomers of glycerol moiety were achieved. We then elucidated the NKT cell stimulatory activity of the synthesized compounds and observed various cytokine induction activities including IFN-γ, IL-4, IL-13, and IL-17 in human PBMCs. Some of the compounds also showed considerable immunostimulatory effects targeted against *Leishmania major*, a representative species responsible for cutaneous leishmaniasis (CL). Treatment led to a marked reduction in the number of intracellular Leishmania parasites in vitro, and ameliorated CL in a mouse model.



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C-MANNOSYLATION OF THROMBOSPONDIN REPEATS

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Protein C-mannosylation is the only known type of glycosylation involving a C-C linkage. The canonical reaction is the modification of the first tryptophan of a WXXW motif. C-mannosyltransferases use Dol-P-Man as donor substrate. Although C-mannosylation is known for over twenty years now, its function is still poorly understood. Since we identified C. elegans DPY-19 as a C-mannosyltransferase in 2013[1], new experimental possibilities are available. Surprisingly, four DPY-19 homologs were found to be present in mammals, of which one was just found to be mutated in the most common form of globozoospermia, which results in infertility. This protein (DPY19L2) is expressed exclusively in sperm and, although expected to be a C-mannosyltransferase, no activity has been attributed to it. We could, however, show that mammalian DPY19L1 and DPY19L3 are C-mannosyltransferases having different specificities. This was established by generating CRISPR-Cas knockouts in CHO cells of the different putative C-mannosyltransferases, both single and multiple, resulting in cells with reduced or no C-mannosylation. We could show that the mouse UNC-5 netrin receptor containing elongated C-mannosylation motifs (WXXWXXW) could be mannosylated on all three tryptophans and that DPY19L1 was modifying the first two tryptophans, whereas DPY19L3 specificity mannosylated the third tryptophan, thereby not using the established WxxW motif as substrate[2].

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ENDOMANNOSIDASE TRIAGES MISFOLDED GLYCOPROTEIN IN THE ENDOPLASMIC RETICULUM

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Protein glycosylation contributes to protein folding, stability, localization and interaction. The glycoprotein quality control in the endoplasmic reticulum (ER) is essential for their folding, sorting and degradation. A central quality control machinery of the nascent glycoprotein is the calnexin/calreticulin cycle (Figure 1). This cycle monitors glycan structure and assists and checks the protein folding. However, the mechanisms that release the misfolded glycoproteins from the cycle remain unclear.

Here we unearthed a novel ER-endo-α-mannosidase (ER-EM) activity comprising carboxylesterase 1D and functions as driving force for the releasing step. We found in precise *in vitro* assays using structurally defined synthetic substrates that ER-EM allosterically acts on glucosyl high-mannose substrates with a hydrophobic aglycone, representing misfolded glycoprotein. Interestingly, the aglycone specificity of ER-EM was identical to a folding sensor enzyme UGGT in the calnexin/calreticulin cycle. ER-EM generates deglucosylated glycan ligands that exit the cycle. These findings demonstrate that ER-EM triages glycoproteins in the calnexin/calreticulin cycle depending on the aglycone hydrophobicity, releasing misfolded glycoproteins and enabling their degradation.

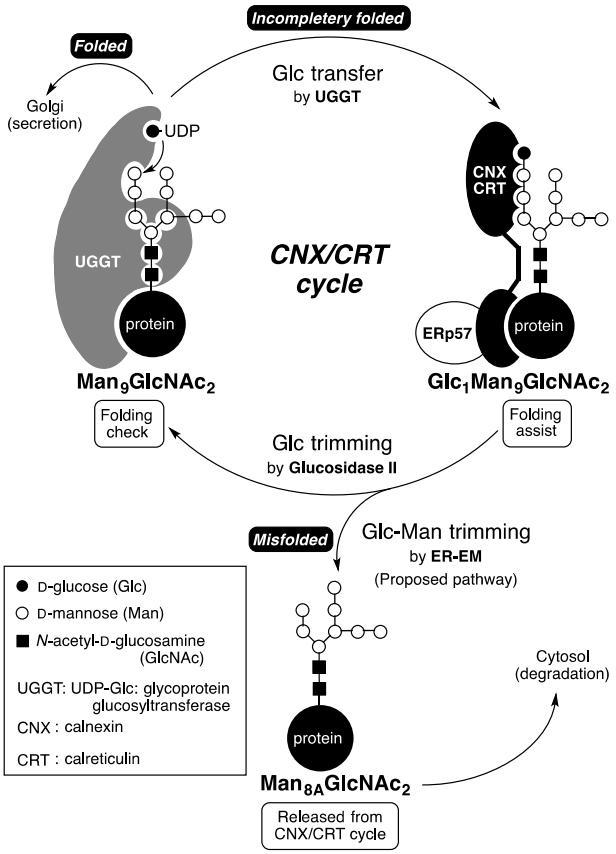

Figure 1. Calnexin/calreticulin cycle and a proposed function of ER endo-α-mannosidase.

MACROCYCLIC PEPTIDE INHIBITORS OF CARBOHYDRATE-ACTIVE ENZYMES

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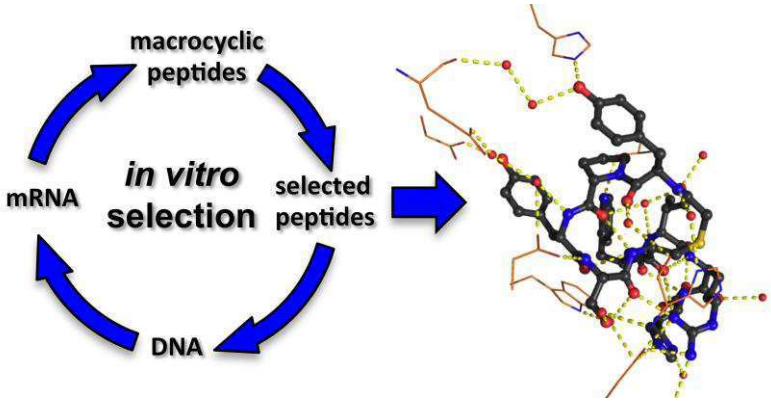
Many diseases arise from errors in carbohydrate processing. These diseases range from malicious activity of pathogens or inherent errors in metabolism, misrecognition of self or non-self by the immune system, to removing a barrier to tumour spreading. In many such cases, targeting the enzymes that carry out the carbohydrates processing provides opportunities to restore correct functioning, provided the protein can be targeted selectively and with high affinity.

The traditional 'small-molecule' approach to deriving inhibitors of carbohydrate-active enzymes often suffers from a lack of selectivity. This arises from the low affinity such proteins often have for their substrates, and the similarity of transitions states in the most common mechanisms employed. An alternative approach is provided by the use of small macrocyclic peptides. These are able to provide an ideal interaction surface for high affinity binding to both shallow binding surfaces as well as deeper binding pockets, while also allowing selectivity between different proteins with very similar binding surfaces. For other challenging applications such as protein-protein interactions, discovery of new macrocyclic peptide ligands with such desirable characteristics has been made possible through the use of mRNA display and genetic code reprogramming, generating and screening libraries on the order of 10^{13} diversity that include multiple non-canonical amino acids [1]. I will describe the first application of these technologies to carbohydrate active enzymes [2], with the ultimate aim of simplifying the development of selective and potent inhibitors of this broad class of biological catalysts.

In order to target binding of peptides to the active site of the enzyme, and thereby increase the chances of deriving competitive inhibitors, a highly potent motif from a natural product inhibitor of pancreatic α-amylase [3] was adapted for display in a peptide library, using a combination of genetic code reprogramming and post-translational modification [4]. While this did not directly yield a peptide scaffold able to present the complete natural product motif, highly potent peptide inhibitors were nonetheless found. The peptides arising from this work achieve low nanomolar potency for human pancreatic α-amylase using short interacting motifs presented in diverse tertiary structure elements. Further modification to reintroduce a part of the natural product lead to picomolar affinity [5]. On-going structural characterisation is revealing the key elements for these unique peptide-protein interactions, and providing clues for how this strategy can in future be applied to more diverse enzymes.



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CD44-GLYCOPROFILLING: ESTABLISHING THE MOLECULAR BASIS FOR TARGETED THERAPEUTICS IN BLADDER CANCER

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Bladder Cancer (BC) presents one of the highest recurrence rates amongst solid tumours and constitutes the second deadliest disease of the genitourinary tract [1]. The introduction of molecular models for disease management and effective targeted therapeutics remains a challenging aspect due to significant inter and intra-tumour molecular heterogeneity [2]. Nevertheless, CD44, a heavily *O*-glycosylated membrane protein involved in cell-cell interactions, cell adhesion and migration [3] has been suggested to play a critical role in bladder cancer progression and dissemination [4], holding potential for targeted therapeutics. However, the gene encoding for CD44 generally undergoes significant alterative splicing, which results in many functionally distinct isoforms of variable molecular weights and glycosylation sites [5]. Nevertheless, the nature of these isoforms in bladder cancer are yet to be fully disclosed. Building on these insights, this work aims to highlight clinically relevant CD44 isoforms with potential for targeting more aggressive clones. Particular emphasis is also given to the identification of cancer-associated *O*-glycans envisaging the molecular rational for designing highly specific cancer ligands. Accordingly, it was observed that CD44 is increased in the urine of bladder cancer patients in relation to healthy controls. This effect is more pronounced for advanced stages of the disease, particularly upon muscle invasion, mimicking CD44 expression in bladder tumours. Moreover, a targeted approach by RT-qPCR demonstrated that superficial bladder cancer cell model 5637 and non-invasive bladder tumours overexpress high molecular weight CD44 isoforms (CD44v3-10high, CD44v8-10high, CD44slow phenotype). Conversely, T24 and HT1376 cell lines derived from muscle invasive tumours and invasive lesions predominantly overexpress lower molecular weight isoform CD44s (CD44v3-10low, CD44v8-10low, CD44shigh phenotype). In addition, chemoresistant clones from T24 cells challenged with cisplatin also overexpressed CD44s. Likewise, bladder tumours from patients with invasive tumours presented a similar phenotype, supporting CD44s association with more aggressive phenotypes. Glycomics and glycoproteomics studies involving T24 cell line further demonstrated the expression of CD44 glycosylated with sialyl-Tn (CD44-STn) and di-sialyl-T (dST) antigens, previously associated with poor prognosis. In parallel, immunohistochemistry and *in situ* proximity ligation assays confirmed the existence of CD44-STn and CD44-dST in muscle invasive tumours. In conclusion, CD44s, possibly modified with cancer-associated STn and dST glycans, holds potential to selectively target more aggressive bladder cancer lesions and chemoresistant clones, setting the molecular rational for ligands design. Future studies should now focus on disclosing the functional impact of CD44 remodeling towards lower molecular weight isoforms, accompanying transition from superficial to invasive lesions.

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sp²-IMINOSUGAR GLYCOLIPIDS AS p38α MAPK ACTIVATORS: DRUG CANDIDATES FOR DIABETIC RETINOPATHY

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Almost two decades ago, the sp²-iminosugar term was introduced to designate a distinct family of glycomimetics characterized by the presence of a pseudoamide-type functionality in place of the endocyclic acetal oxygen in monosaccharides. Since then, interesting biological properties have been described for these compounds, including pharmacological as chaperones for lysosomal storage diseases [1], antiparasitic [2] or anticancer agents [3]. Whereas in the first case the target is a glycosidase, the results were not consistent with this assumption in the two other cases. Instead, a glycolipid-like structure was found critical, probably through interfering in the innate immune response cascade. Indeed, representatives showing antitumoral properties did also display anti-inflammatory activity in the context of diabetic retinopathy (DR), the most frequent complication of type 2 diabetes mellitus [4]. For instance, the bicyclic nojirimycin-carbamate **1**, bearing a sulfone-linked pseudoanomeric lipophilic substituent (**1**, Fig. 1), was able to revert the inflammatory phenotype induced by bacterial lipopolysaccaride (LPS) in microglia and in mouse retinal explants. Biochemical and computational studies support that sp²-iminosugar glycolipids (sp²-IGLs) emulate the capacity of phosphatidylinositol ether lipid analogues (PIAs) or the phospholipid perifosine to modulate the p38α mitogen-activated protein kinase (MAPK) after binding at an allosteric site, inducing the expression of the anti-oxidant enzyme Heme oxygenase-1 (HO-1) and drastically decreasing the levels of proinflammatory cytokines [5].

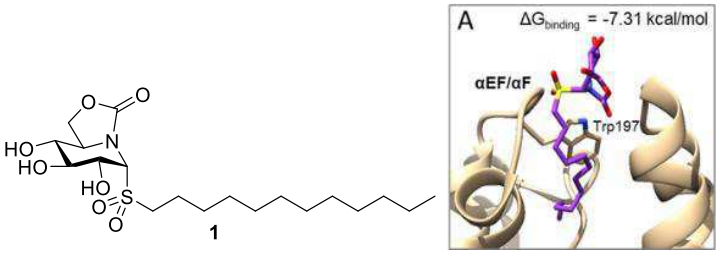

Fig. 1 - Predicted binding mode of the sp²-iminosugar glycolipid **1** to p38α MAPK.

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SIGLEC-8 – A PROMISING TARGET FOR ASTHMA

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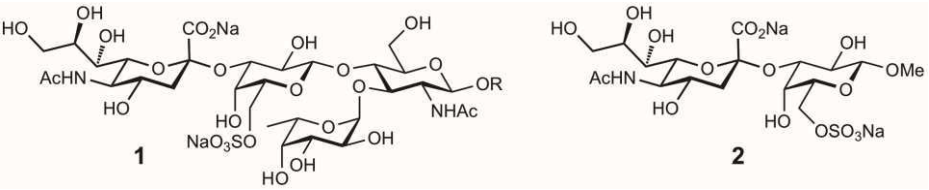
Sialic acid-binding immunoglobulin-like lectins (siglecs) are members of the immunoglobulin family. They are predominantly found on cells of the immune system. To date, 15 siglecs have been identified [1]. Among them is Siglec-8, which is expressed on human eosinophils and mast cells and regulates their survival [2, 3]. Crosslinking Siglec-8 with specific glycan ligands or antibodies induces apoptosis [3, 4]. In some diseases, like asthma or chronic rhinosinusitis, an excessive amount of eosinophils is produced, causing an inflammatory response. Thus, targeting Siglec-8 provides a unique opportunity for controlling such allergic reactions.

Using a glycan array, the tetrasaccharide 6'-sulfo-sLe^x (**1**) was identified as a ligand of Siglec-8 [5]. Its binding mode to Siglec-8 in solution was established by an NMR investigation [6]. However, tetrasaccharide **1** exhibits poor drug-like properties and its synthesis is cumbersome and of high complexity. Therefore, we synthesized a number of mono- and oligosaccharides derived from the scaffold of **1** and determined their affinities for Siglec-8.



The disaccharide **2** was identified as a potent ligand displaying a 2.2-fold higher affinity than the natural ligand **1**. Modifications made to this structure, like removal of the sulfate group or switching from α2-3 to α2-6 linkage, led to a significant reduction or almost complete loss of binding to Siglec-8. These results suggest that the scaffold of **2** contains the essential epitope of 6'-sulfo-sLe^x (**1**) required for Siglec-8 binding, thus opening the perspective for the synthesis of novel families of Siglec-8 ligands.

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BACTERIAL LECTINS AS MASTER MANIPULATORS OF HOST CELL PHYSIOLOGY

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The bacterium *P. aeruginosa* emerged as a major human opportunistic pathogen during the past decades, maybe as a consequence of its resistance to antibiotics and disinfectants. It is able to cause severe infections of the respiratory and urinary tract, skin and eye, predominantly in immune compromised patients. *P. aeruginosa* has a vast range of virulence factors, amongst them, the two bacterial lectins LecA and LecB, which, by virtue of their sugar binding, contribute to the pathogens' host specificity and adherence to host cells. Whereas LecA is galactophilic, LecB displays a very high affinity to L-fucose.

Recently, we have demonstrated that the interactions of LecA with the host cell glycosphingolipid Gb3 were sufficient to induce the engulfment of the bacterium, independent of actin polymerization. The absence of one of these two factors led to a reduction of invasiveness of the bacterium by around 70 % [1]. Moreover, the interaction of LecA with Gb3 led to the phoshphorylation of the adaptor protein CrkII, independently of Abl kinase [2]. In collaboration with research groups from Switzerland and France, we have identified a sugar complex from galactoside-conjugated arrays that prevents LecA from docking onto its host cell receptor by binding to the bacterial protein with great precision. In tests conducted in cell culture, we could reduce the cellular invasion of human lung epithelial cells by *P. aeruginosa* by more than 80 % when using the inhibitor in nano-molar concentrations [3, 4].

We have also discovered a so far unknown role of the *P. aeruginosa* lectin LecB in bacterial virulence. LecB alone was sufficient to attenuate migration and proliferation of human lung epithelial cells and to induce transcriptional activity of NF-κB. These effects are characteristic of impaired tissue repair. Moreover, we found a strong degradation of both plasma membrane-associated and cytosolic β-catenin, which was partially recovered by the proteasome inhibitor Lactacystin. The resulting loss of cell-cell contacts and the reduced expression of the β-catenin targets c-myc and Cyclin D1 are also known to negatively influence tissue repair [5]. LecB was also identified as potent inducer of the differentiation of the acute myeloid leukemia cell line THP-1. During LecB-induced differentiation, a functional autophagy as well as a low β-catenin level was essential. High β-catenin expression stabilized proliferation and inhibited autophagy resulting in low differentiation ability. Remarkably, induced by LecB, β-catenin was degraded, autophagy became active and differentiation took place [6].

A better understanding of the mode of action of natural lectins together with glycoreceptors may encourage their future use as tools in therapeutic approaches. Tailor-made lectins with controlled valency and specificity [7] were designed based on natural examples and are currently investigated in basic as well as in applied research.

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CONFORMATIONAL SWITCH OF THE BACTERIAL ADHESIN FIMH IN THE ABSENCE OF THE REGULATORY DOMAIN – ENGINEERING A MINIMALISTIC ALLOSTERIC SYSTEM

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For many biological processes such as ligand binding, enzymatic catalysis, or protein folding, allosteric regulation of protein conformation and dynamics is fundamentally important. One example is the bacterial adhesin FimH, where the C-terminal pilin domain exerts negative allosteric control over binding of the N-terminal lectin domain to mannosylated ligands on host cells. When the lectin and pilin domains are separated under shear stress, the FimH–ligand interaction switches in a so-called catch-bond mechanism from low to high affinity state. So far, it has been assumed that the pilin domain is essential for the allosteric propagation within the lectin domain that would otherwise be conformationally rigid. To test this hypothesis, we generated mutants of the isolated FimH lectin domain and characterized their thermodynamic, kinetic, and structural properties using ITC, SPR, NMR and X-ray techniques. Intriguingly, some of the mutants mimicked the conformational and kinetic behaviors of the full-length protein and, even in absence of the pilin domain, conducted the crosstalk between allosteric sites and the mannoside binding pocket. Thus, these mutants represent a minimalistic allosteric system of FimH, useful for further mechanistic studies and antagonist design.

INHIBITION OF O-GLYCAN BIOSYNTHESIS USING HEXOSAMINE ANALOGS

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Robust metabolic strategies to inhibit O-linked glycosylation are currently lacking. We tested the efficacy of a panel of N-acetylgalactosamine (GalNAc) analogs to reduce cellular O-glycosylation biosynthesis and leukocyte adhesion to the selectin family of adhesion molecules. Among the compounds tested, GalNTGc, a C-2 sulfhydryl substituted GalNAc was metabolically active. The culture of HL-60 promyelocytic leukocytes with this hexosamine analog reduced cell-surface sialyl Lewis-X expression by 50-80%. Such treatment also reduced cell adhesion to L- and P-selectin bearing substrates by 50-75% ex vivo in microfluidics based flow chamber studies, and it reduced P-selectin dependent HL-60 binding to activated platelets under hydrodynamic shear. In mechanistic studies, GalNTGc dramatically increased the binding of VVA-lectin to HL-60s by >10-fold, indicating the truncation of O-glycan biosynthesis. Consistent with this, the molecular mass of mucinous proteins, PSGL-1 and CD43, was also reduced by 20-25%. Mass spectrometry based glycome profiling confirm profound inhibition of O-glycan elaboration upon treatment with GalNTGc, with negligible change in N-linked glycosylation. Studies with Maleimide-FITC labeling of GalNTGc treated cells suggests low levels of direct GalNTGc incorporation into cellular glycoconjugates, with this carbohydrate and its derivatives being noted on cellular O-glycans, N-glycans, and carbohydrates attached to glycosphingolipids. When bone marrow cells from donor animals were cultured with GalNTGc for 2-days prior to infusion into recipients, GalNTGc was observed to reduce granulocyte migration to sites of inflammation in a murine peritonitis model. Directly feeding GalNTGc to mice over 4-days prior to the induction of peritonitis also reduced granulocyte homing to sites of inflammation by ~50%. Thus, GalNTGc is a pharmacologically active compound that may reduce selectin mediated leukocyte adhesion in vivo. More significantly, it is a robust and specific O-linked glycosylation inhibitor that could be useful for a range of applications.

COMPLETE SPATIAL CHARACTERISATION OF *N*-GLYCANS IN AN ADULT RAT BRAIN

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INTRODUCTION: The cardinal role of *N*-glycans for the nervous system is brought forth by congenital glycosylation diseases, resulting in different neuropathological symptoms like mental retardation, seizures and epilepsy. Previous studies have reported the conservation of *N*-glycan processing in rodents[1]. However, there is a lack of spatial resolution of these glycan profiles as the tissue samples were homogenized intact for these studies. Since the biosynthesis of *N*-glycans undergoes a stringent spatio-temporal regulation within the tissue, we hypothesize that the spatial resolution of *N*-glycans isolated from striatum and substantia nigra can give an insight into their involvement in regulation of striatal and nigral cues for establishment and pathophysiological degeneration of neural circuitry in Parkinson's disease.

EXPERIMENTAL METHODS: Sprague–Dawley rats were used in this study (n=35). Intact brains from adult rats were snap frozen and lyophilized for glycan analysis. Micron biopsy punching was used to isolate the striatal and nigral tissues from whole brains. *N*-glycans were released from samples using the high-throughput method described by Royle *et al* [2]. The *N*-linked glycans were released using peptide *N*-glycanase F, labelled with 2-AB, resolved and identified using HILIC-UPLC, WAX-UPLC, array of exoglycosidase digestions and LC-MS.

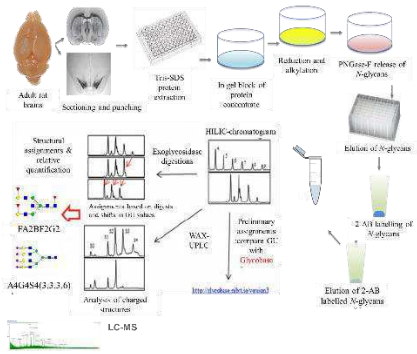

Fig. 1 – Study outline for isolation, release, identification and quantitation of spatially resolved *N*-glycans from adult rat brains

RESULTS, DISCUSSION AND CONCLUSION: This is the first report elucidating the types and structures of neutral and charged *N*-glycan species found in striatum and substantia nigra of adult rat brains.It will serve as a foundation for identifying the 'brain-type' glycans and their modulation in neurodegenerative disorders.

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BIOLOGY OF NGLY1: NOT JUST TAKING CARE OF JUNKS

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The cytoplasmic peptide:*N*-glycanase (PNGase) is the enzyme widely conserved throughout eukaryotes [1]. This enzyme is involved in the degradation of misfolded/non-functional glycoproteins destined for the degradation process called ERAD (ER-associated degradation). In 2012, a patient harboring mutations of PNGase gene (*NGLY1*) was first reported. Symptom of these patients includes developmental delay, multifocus epilepsy, involuntary movement and liver dysfunction. From this report, it is clearly suggested that the cytoplasmic PNGase play a pivotal role in normal human development.

We analyzed *Ngly1*-deficient mice and found that they are embryonic lethal in C57BL/6 (B6) background [2]. Surprisingly, the additional deletion of *Engase,* encoding another cytosolic deglycosylating enzyme called ENGase (endo-β-*N*-acetylglucosaminidase), resulted in the partial rescue of the lethality of the *Ngly1*-deficient mice. Additionally, we also found that a change in the genetic background of B6 mice, produced by crossing the mice with an outbred mice strain (ICR) could rescue the embryonic lethality of *Ngly1*-deficient mice [2]. Viable *Ngly1*-deficient mice in a B6 and ICR mixed background, however, showed a very severe phenotype reminiscent of the symptoms of *NGLY1*-deficiency subjects. Again, many of those defects were strongly suppressed by the additional deletion of *Engase* in the B6 and ICR mixed background.

We also showed that in *Ngly1*-KO cells, ERAD process was compromised[3]. Interestingly, not only delayed degradation but also the deglycosylation of a model substrate was observed in this cell. The unexpected deglycosylation was found to be mediated by ENGase. Surprisingly, the ERAD dysregulation in *Ngly1*-KO cells were restored by the additional KO of *Engase* gene [3]. These observations collectively suggest that the ENGase represents one of the potential therapeutic targets for this genetic disorder. In this symposium, we will overview our most recent progress on our *NGLY1*-research, and also introduce our efforts to develop drugs for *NGLY1*-deficiency.

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DEVELOPMENT OF NOVEL FLUORESCENCE-QUENCHED PROBES FOR MONITORING LYSOSOMAL GLYCOSIDASE ACTIVITY IN LIVE CELLS

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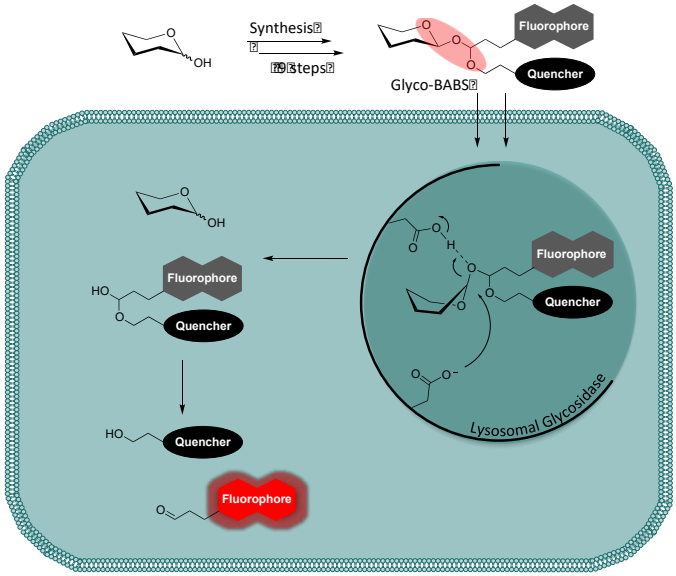
α-Galactosidase A (GalA) and β-Glucocerebrosidase (GCase) are two lysosomal glycoside hydrolases that process globotriaosylceramide and glucosylceramide. Deficiencies of these glycosidases, which are commonly linked to mutations of their encoding genes *GLA* and *GBA1*, lead to the accumulation of their respective substrates and the development of the lysosomal storage disorders Fabry disease (FD) and Gaucher disease (GD) [1]. These mutations ultimately lead to the expression of unstable forms of the enzymes, which do not reach the lysosome [2]. As a result, the levels of active enzyme within the lysosome are reduced. Current therapeutic strategies to increase enzyme activity in the lysosome involve enzyme replacement therapy and chaperone therapy [3]. A challenge associated with optimizing and advancing these therapies is to quantitatively measure enzyme activity within the lysosome of live cells.

Fluorescence-quenched substrates are a powerful tool for monitoring the activity of diverse hydrolytic enzymes (i.e. *endo*-glycosidases) in their native cellular environment. The pocket-shaped active sites of *exo*-glycosidases (i.e. GalA) are, however, more sterically demanding making the development of such probes more difficult. We have recently proposed a broadly applicable strategy to address this problem by attaching the fluorophore and quencher in the aglycone through a bis-acetal functionality [4]. Cleavage of the bis-acetal based substrate (BABS) probe would liberate a hemiacetal that would spontaneously breakdown into two fragments. Here we demonstrate the bis-acetal functionality is amenable to α-galactose and β-glucose residues for the preparation of two novel substrate probes. These probes show highly efficient fluorescence-quenching (>99.9%) and, more importantly, were able to selectively monitor enzyme activity within the lysosome of live cells. In addition to improve our understanding of the enzymes' activity in a cellular environment, these new probes could facilitate the discovery of novel small-molecule chaperones.



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NEW ASPECT OF GLYCOSIDASE-CATALYZED TRANSGLYCOSYLATION INVOLVING 1,2-ANHYDRO SUGAR INTERMEDIATE

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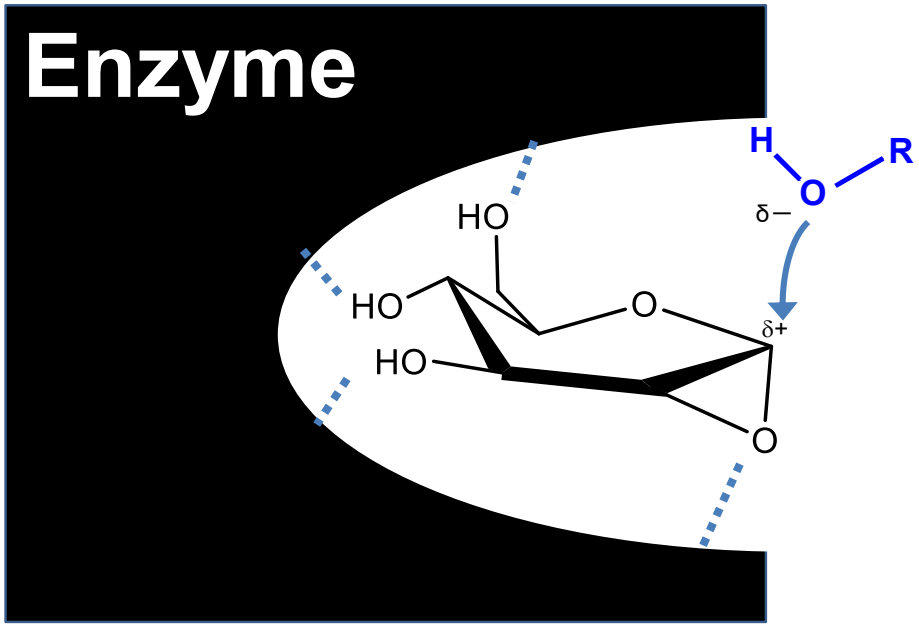
Glycosidase-catalyzed transglycosylation has become an attractive methodology in the synthetic field of carbohydrate chemistry. Cyclic sugar derivatives are considered to be potentially efficient glycosyl donors for enzymatic glycosylation, because the torsional strain by a condensed ring structure lowers the activation barrier between the glycosyl donor and the transglycosylated product. For example, sugar oxazolines have extensively been employed for *N*-acetylglucosaminidase-catalyzed transglycosylations. We envisioned that 1,2-anhydro sugars having a bicyclic structure where a six-membered ring and three-membered oxirane ring are fused could be recognized by a glycosidase and behave as a glycosyl donor for enzymatic tansglycosylation.

We have already succeeded in the protection-free one-step preparation of sugar oxazolines starting from various *N*-acetyl-2-amino sugars by using formamidinium dehydrating agents in aqueous solution.[1] Recently, we applied this methodology to gluco-type sugars and reported the first synthesis of unprotected 1,2-anhydro sugars in aqueous media. These results prompted us to investigate the possibility of 1,2-anhydro sugars as a potentially useful substrate for glycosidase-catalyzed transglycosylation.

After screening various commercially available endo-glucanases by using 1,2-anhydrocellobiose, we found that endo-β-1,4-glucanase I from *Aspergillus aculeatus* showed high transglycosylating ability toward cellobiose derivatives, giving rise to the corresponding cellotetraose derivatives as transglycosylated product. This is the first glycosidase-catalyzed transglycosylation reaction using 1,2-anhydro sugars as a donor substrate. Based on these results, the "*1,2-eposide mechanism*" for transglycosylation reaction catalyzed by glycosidases has been proposed.



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A NOVEL METHOD FOR THE COMPLETE STEREO-CONTROL OF α-SIALYLATION

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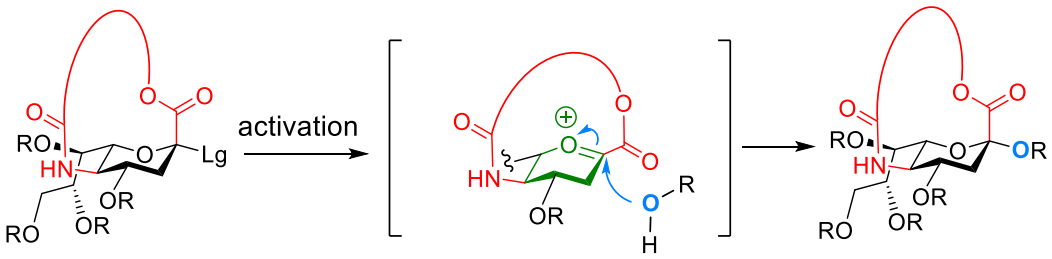
Due to their significant biological profile and challenging structural diversity, numerous efforts have been made toward the synthesis of sialic acid containing glycans. Since our research group developed a practical strategy for α-sialylation that exploited nitrile solvent participation [1,2], we have achieved the synthesis of complex architectures of neuritegenic gangliosides found in echinoderms [3], and we also have developed a series of fluorescent gangliosides and revealed specific interplays between gangliosides and nano-sized domain in the cell membrane by single molecule imaging technique [4]. Despite continuous efforts, the synthesis of sialic acid containing glycans remains demanding and time-consuming primary due to the difficulty in α-sialylation. In the present study, we have developed a novel method for the complete α-selective sialylation by employing a macrocyclized sialic acid donor as the synthetic equivalent of a bridgehead oxocarbenium cation (anit-Bredt oxocarbenium cation).

To confirm whether the bridgehead oxocarbenium cation of sialic acid was amenable to glycosylation, we examined the glycosidation of macrocyclized sialyl donors varied at the ring size. It was clearly shown that the α-thioglycosides of macrocyclized sialic acids were reactive to NIS-TfOH at low temperature to produce α-glycosides with a glycosyl acceptor as the single isomer in all the cases. The examination of glycosylation of various coupling partners revealed the broad substrate scope of the α-sialylation. We could produce glycosidic linkages of sialic acid found in natural glycans, such as sialyl-α(2,6)Glc (Gal or GalNAc) -α(2,3)Gal, in high yields. This method also allowed to directly install sialic acids to oligosaccharyl acceptors in a regioselective manner, giving mono- and disialyl oligosaccharides in high yields. Furthermore, by embedding a 2-dichloroethoxycarbonyl moiety in the tethering part, the macrocyclic moiety become selectively cleavable under reductive conditions to retrieve a free amino group at the C5 position, thereby providing a pivotal access to *N*-acetyl, *N*-glycolyl and *N*-glycosylglycolyl congeners of sialic acid. More importantly, we found that macrocyclization ameliorated the reactivity of the C8 hydroxyl group. The glycosylation of the 8-OH of the macrocyclized sialyl acceptor gave sialyl-α(2,8)sialic acid in an excellent yield.

α-Exclusive sialylation via anti-Bredt oxocarbenium ion



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SILICA GLYCONANOPARTICLES FOR NANOMEDICINE

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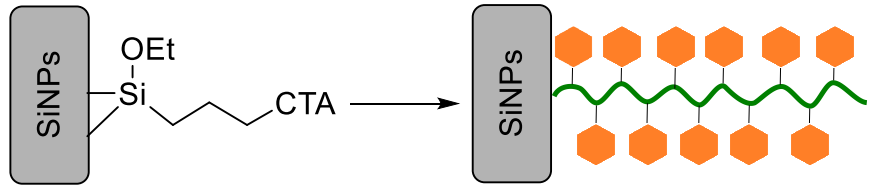
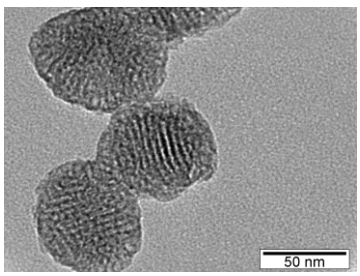
In a 1964 Disney musical classic, Mary Poppins sang "A spoonful of sugar helps the medicine down…" and this is not far from the truth. In fact, sugars are ubiquitous in nature and intervene in a wide range of biological functions [1]. During the last decade, there have been many attempts to integrate sugars in nanomaterials. Glyconanoparticles were used as carriers with high affinity and binding specificity due to the multivalent interactions between surface sugar ligands and targeted receptors [2]. Here we develop a new theranostic platform for controlled drug release, based in silica nanoparticles with a glycopolymer shell, featuring tunable cell targeting and blood circulation behavior. In addition, by incorporating highly bright perylenediimide (PDI) fluorescent dyes in the silica structure, the particles combine therapeutic and diagnostic (theranostic) functionalities. To accomplish this, we used the "grafting from" method, where the glycopolymer grows from the particle surface by reversible addition-fragmentation chain transfer (RAFT) polymerization of the corresponding acrylate-modified sugars. We selected galactose and mannose due to their affinity for specific lectins present in the surface of cancer cells. Silica nanoparticles with controlled morphology and diameter under 100 nm were modified at the external surface with (3-aminopropyl)triethoxysilane (APTES) in order to obtain surface amine groups. This surface modification was used to couple the chain transfer agent (CTA) for RAFT polymerization for the grafting of the glycopolymeric chain. Solution NMR was used for the identification and quantification of the covalently bound ligands, a method recently developed in the group [3]. This multifunctional carrier is expected to be highly specific towards the microenvironment of some tumors. In addition, it will control the release of therapeutic agents transported in the pores of mesoporous silica nanoparticles upon sugar degradation by the targeted cells. The synergetic combination of silica nanoparticles and glycopolymers is expected to provide a very strong platform for the development of precision drug delivery and theranostics.



CTA: chain transfer agent

SiNPs: Stöber or MSNs

Sugar



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SYNTHETICALLY MODIFIED CHITOSAN AS EFFICIENT ANTIMICROBIAL AGENTS

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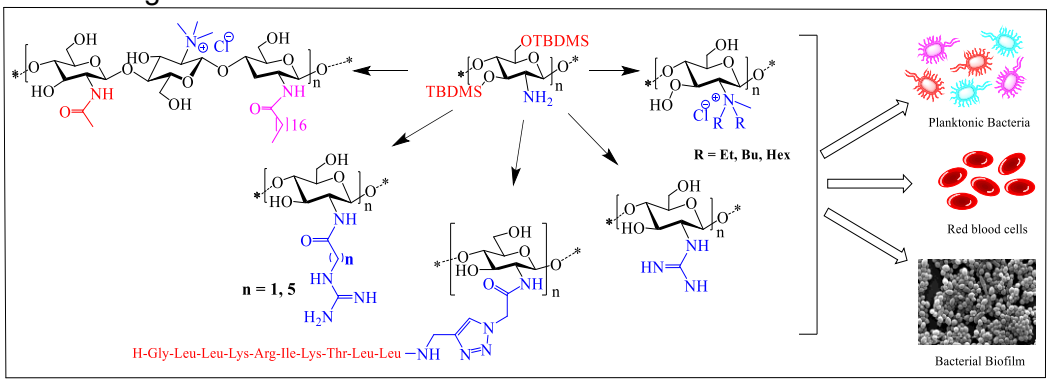
Chitosan is a linear biopolymer with many interesting properties like biocompatibility, non-toxicity, biodegradability, including antimicrobial activity. In recent years, it has been shown that chemical modification of chitosan can lead to significant improvement of its antimicrobial effect. The aim of this study was therefore, to selectively modify the amino group of chitosan by various cationic and lipophilic moieties to obtain derivatives having improved antimicrobial activity and aqueous solubility than chitosan itself. The aim was also to investigate the effect of average molecular weight (Mw) of chitosan on their antimicrobial properties.

The synthesis was performed by using tertiarybutyldimethylsilyl protected chitosan as a precursor and the various modifications like trimethylation, guanidinylation, quarterization, multiple functionalization and attachment of peptides was carried out at the 2-amino position of chitosan in a controlled manner [1-3]. Acidic hydrolysis of chitosan and its derivative was performed to obtain a series of compounds have a wide Mw range. The antibacterial efficacy of the derivatives towards planktonic bacteria was assessed against a panel of clinically important bacterial strains like *S. aureus, E. coli, P. aeruginosa* and *E. faecalis*, while their hemocompatibility was determined against human red blood cells. The results were then used to develop an overall structure-activity relationship for these biomaterials [4]. For further assessment of the antibacterial efficacy of these materials, some of the chitosan derivatives were treated against bacterial biofilms. Preformed biofilms of *S. aureus* when treated with different polymer concentrations showed biofilm eradication. Furthermore, the effect of the chitosan derivatives on biofilms of *S. aureus* was visualized by imaging the biofilms via confocal laser scanning microscopy using live/dead staining.



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DISSECTING MULTIVALENT LECTIN-CARBOHYDRATE INTERACTIONS USING POLYVALENT MULTIFUNCTIONAL GLYCAN-QUANTUM DOT

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Multivalent protein-sugar interactions are widespread in biology and play a vital role in viral/bacterial infection, cell-cell communication and regulation of immune response [1]. They initiate the first contact between pathogens and target cells. While individual protein-sugar interactions are typically weak and not biologically functional, by coating their surface with arrays of specific glycans, viruses can simultaneously bind to multiple cell surface multimeric viral receptors (lectins) to greatly enhance the affinity, allowing them to gain cell entry which ultimately leads to infection. Synthetic glycoconjugates can block such interactions and prevent infection with potency critically depending on the spatial-/orientation-match between the multivalent binding partners [2]. The lack of structural information for many important cell surface lectins have hampered such development, due to challenges in solving the structure of such flexible, complex, and multimeric cell surface membrane proteins.

To address this challenge, we are pursuing a polyvalent multifunctional nanoparticle (PMN) strategy to fully exploit multivalency and unique chemical/physical properties of nanoparticles. Herein we report that fluorescent quantum dots (QDs) displayed with a dense array of simple sugars (*e.g.* mannose, manα-1,2-man) are powerful tools to dissect multivalent lectins (DC-SIGN/R) -sugar interactions underpinning the HIV/Ebola infections [3]. 1st, we report a highly-efficient cap-exchange method to prepare dense glycan-coated QDs, allowing us to quantify the multivalent binding between DC-SIGN/R (a pair of closely related, almost identical tetrameric receptors) [3] and QD *via* a ratio-metric FRET readout strategy [4]. We find that a polyvalent display of such glycans on the QD not only enhances its DC-SIGN binding affinity by 4-6 orders of magnitude, but also endows >100 fold multivalent binding selectivity for DC-SIGN over DC-SIGNR, [4a] despite their almost identical tetrameric structures [3]. We attribute this selectivity to the different orientation of their four glycan-binding-domains: they point uprightly in DC-SIGN, but side-ways in DC-SIGNR, making the later unable to bind multivalently to one QD. 2nd, we verify the binding-site orientation difference by exploiting the QD's high contrast in TEM imaging: binding of DC-SIGN gives isolated QDs but binding of DC-SIGNR gives aggregated QDs. 3rd, we find the QDs potently inhibit pseudo-Ebola virus infection of DC-SIGN expressing cells with sub-nM IC_{50} values, matching well with their DC-SIGN binding affinity measured by FRET (*e.g.* 0.7 v.s. 0.6 nM) [4]. This result suggests that our FRET based binding affinity method can be potentially used to predict glycan-nanoparticle inhibition potency of virus infection of target cells [4b]. Finally, the development of glycan-gold nanoparticles as potent anti-viral reagents will also be presented and discussed [4c].

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D-GALACTOSE DERIVED β-AMINO ACIDS AND THEIR PEPTIDES: SYNTHESIS AND STRUCTURAL STUDIES

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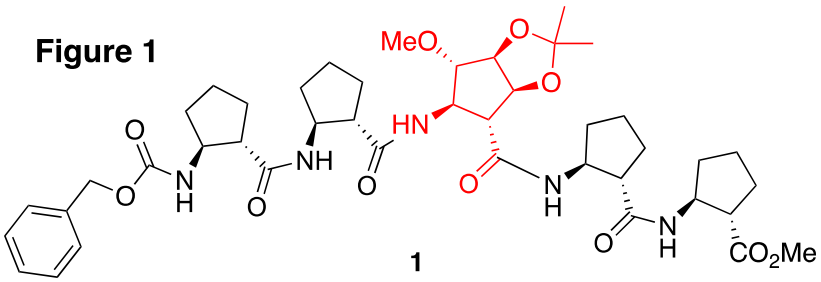
Peptides play a key role in Biology. However, their use as therapeutic agents suffer from their poor metabolic stability, transport properties and limited structural diversity, which is related to the limited number of secondary structures and backbones resulting from the small number of proteinogenic amino acids. Accordingly, much work has been carried aimed at the synthesis of non-natural amino acids. This constitutes the first step for the development of peptidomimetics that may act as new drugs and overcome the pharmacological limitations of proteins [1].

Among the non-natural amino acids, cyclic β-amino acids became particularly interesting as stabilizers of peptide conformations, a property that has been related to the high tendency of their homo-polymers to fold in rigid secondary structures in short peptide sequences [2].

As a part of an ongoing project aimed at the synthesis of polyhydroxylated cycloalkane β-amino acids,[3] we will present the following results:

- A D-galactose based syntheses of the polysubstituted cyclopentane β-amino acid (red residue) required for the preparation of β-peptide **1**.
- A protocol for the incorporation of this β-amino acid into β-peptide **1**.
- The folding properties of β-peptide **1**.

Figure 1



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SYNTHESIS AND PROPERTIES OF PHOTOSWITCHABLE GLYCOMACROCYCLES

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Macrocyclic compounds represent a unique class of molecules because of their natural existence and unique structural, physicochemical, and biological properties as well as their potential applications in nanotechnology, biology, and drug delivery [1]. Development of sugar-based macrocycles is of particular interest for chemical, supramolecular, analytical, and biological applications [2]. Recently, photoresponsive "intelligent" molecular systems based on photochromic molecules have been shown to be extremely promising for spatial and temporal control over various chemical and biological processes [3]. Photochromic molecules can be reversibly isomerized by light into isomers featuring different structural and/or electronic properties. There is increasing use of the reversible photoisomerisation property to control the conformation as well as the activity of nucleic acids, peptides, proteins or ionic canals [4]. Incorporation of photochromic moiety like azobenzene, stilbene, spiropyrane or diarylethene in biomolecules has allowed modulating the bioactivity in a spatiotemporal manner. We have decided to develop photochromic glycomacrocycles to investigate their photochromic properties and potential applications. Through a one-pot *O*-alkylation mediated macrocyclization approach [5], we have successfully prepared four 16- or 17-membered azobenzene-based glycomacrocycles (Fig. 1). These macrocycles can be reversibly isomerized between *E*- and *Z*-isomers with excellent photostability and thermal stability for both isomers. A chirality transfer from the sugar to the azobenzene moiety has been observed for the four macrocycles. One of them is able to form organogels with multi stimuli-responsive behavior upon exposure to environmental stimuli including thermal, photo, and mechanical responses. A temperature-dependent helical inversion, tunable by a repeated heating-cooling procedure, has also been demonstrated. These promising results should stimulate the design and synthesis of new photoswitchable glycomacrocycles for various applications.

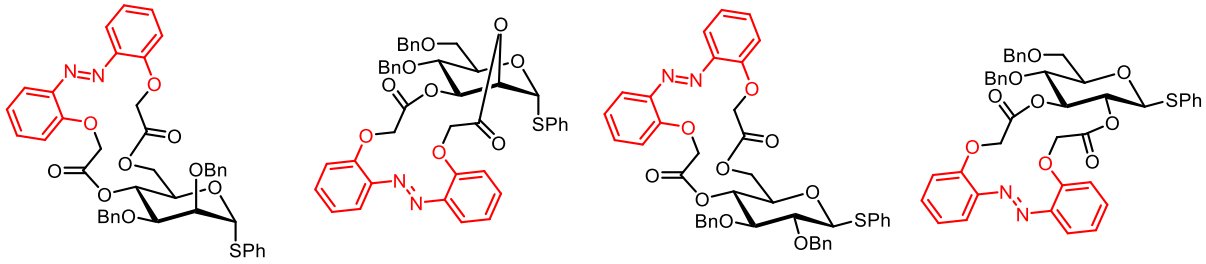

Fig. 1 - Structure of synthesized photoswitchable glycomacrocycles

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CHEMO-ENZYMATIC SYNTHESIS OF ARTIFICIAL XYLAN POLYSACCHARIDES WITH DEFINED SUBSTITUTION PATTERNS

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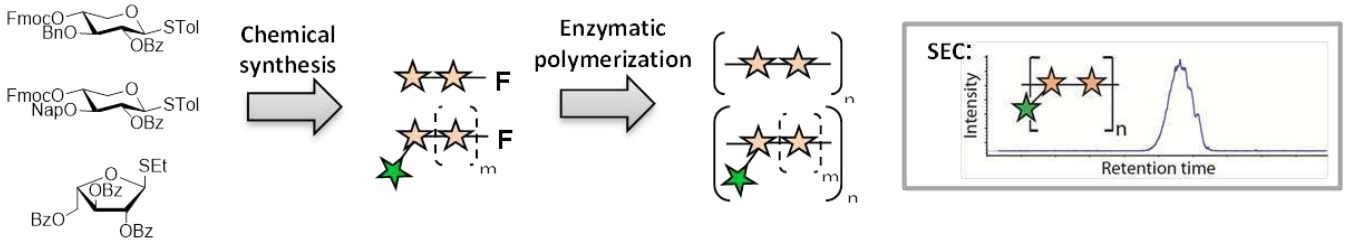
Cellulose and xylan are the two most abundant polysaccharides in plant biomass. Together, they form the cell walls in materials such as wood and straw. While cellulose is widely used for the production of sustainable and eco-friendly materials, xylan has only recently begun to be explored for the same purposes. The heterogeneity of natural xylan polysaccharides complicates investigations into the molecular basis of xylan-cellulose interactions, which determine strength and digestibility of the cell wall, and are thus key to the development of improved methods for biomass exploitation [1].

Well-defined molecular tools mimicking natural xylan polysaccharides have great potential for studying structure and properties of xylan, including its interaction with cellulose. We present here the chemo-enzymatic synthesis of a series of xylan polysaccharides with perfectly regular patterns of arabinose substitution. Our approach is based on the chemical synthesis of arabinoxylan oligosaccharide building blocks [2-4] that are subsequently polymerized using a xylan glycosynthase enzyme [5]. The obtained polysaccharides contain polysaccharide chains with molecular masses up to 80 kDa (606 monosaccharides). Due to the defined nature of the polysaccharide substitution patterns, specific properties were observed for particular members of the prepared arabinoxylan collection rather than the simple linear correlation between e.g. crystallinity and degree of substitution reported previously for arabinoxylans obtained from natural sources [6].



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STRUCTURAL INSIGHTS ON THE MECHANISM OF GELATION OF CELLULOSE HYDROGELS BY A COMBINATION OF SOLUTION AND SOLID STATE NMR

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Cellulose hydrogels are highly hydrated tangled fibrillar networks capable of interacting with interstitial water at different levels. The polymers are physically or chemically cross-linked, providing the network structure and physical integrity. The high water content allows the excellent biocompatibility of these materials. The interaction of the fibrils with water and the solvent distribution within the network are critical factors for the mechanical strength of hydrogels [1] and their ability to control important processes such us drug release.

Cellulose is a linear homo-polysaccharide consisting of β-(1-4) linked D-glucose residues. It is the most abundant renewable resource on earth, and will probably become the main chemical resource in the future [2]. In particular, plant cell walls (PCW) are the world's most abundant type of biomaterials and have applications including dietary fibre and structuring the components in foods, raw materials for biorefining and as a source of polymers for gum and stabilisers. Due to the increasing demand for environmentally friendly and biocompatible products, together with the presence of numerous hydroxyl groups in cellulose, a wide range of cellulose-based hydrogels can be easily developed to mimic PCW over a broad range of applications [3].

We present a combination of solid state and novel solution state NMR methods, based on saturation transfer difference (STD) NMR and water-polarization transfer experiments, to provide structural and dynamics insights on the impact of temperature and the presence of different hemicelluloses on the mechanism of gelation of cellulose hydrogels. By looking at cellulose-bound water and manipulating water magnetization transfer to cellulose fibrils, we are able to unveil the changes in hydrogel network architecture and water structure and dynamics giving rise to the macroscopic properties of cellulose-based hydrogels.

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STRUCTURAL FEATURES AND PROPERTIES OF HIGH-MOLAR MASS DEXTRANS BIO-DESIGNED IN VITRO USING ENZYMES

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Due to their abundance, carbon-neutrality and low cost, polysaccharide-based materials are seen as interesting alternatives to polymers derived from fossil resources for producing a wide range of materials. α-glucans are widely employed in industry for their functional properties, for instance xanthan, gellan or dextran exhibit texturing or film-forming properties and are exploited in a broad range of applications such as controlled release system and wound healing tissue engineering [1]. In particular dextrans synthesized with different dextransucrase mutants have shown interesting mechanical properties for potential application in bioplastics, which depend on their structure [2]. However, their mechanical properties are still insufficient for most non-food uses. A deeper knowledge of the structure properties relationships of dextrans is thus needed in order to explore their potential for applications as bioplastics. The aim of this work was to develop dextrans with new structures in order to modulate their mechanical properties by a tailoring approach.

For this purpose, a series of new dextrans with different molar masses and controlled architecture (α (1 → 2) and α (1 → 3) branched dextrans) were synthetized using both dextransucrases and branching enzymes. The solubility, macromolecular characteristics, crystalline structure, rheological properties, film forming ability and thermal and mechanical properties of these new biopolymers have been studied compared to those of commercial dextrans [3,4]. Their macromolecular structure and size were determined by ^{1}HNMR and by asymmetrical flow field flow fractionation (AF4) coupled with multi-angle laser light scattering (MALLS). Crystallinity was determined by wide-angle X-ray scattering (WAXS) and thermal properties by differential scanning calorimetry. Dextrans films were prepared by casting and their mechanical properties were determined using a humidity-controlled dynamic mechanical analysis.

These new dextrans exhibited branching rates ranging from 0 to 37% with average molar masses and size ranging from 10^4 to 10^9 g.mol^{-1} and from 10 to 600 nm respectively. Their physico-chemical properties were shown to be dependent on the molar mass, the type and the degree of branching. In particular, the mechanical properties of the films, especially the storage modulus, are governed by the linkage type and the degree of branching whereas the crystallinity of these dextrans is linked to the degree of branching. These results open new perspectives for the design of biosourced materials for applications in bioplastics.

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COMPLEXATION OF ALGINATES AND LACTIC ACID BACTERIA HETERO-EXOPOLYSACCHARIDES WITH MILK PROTEINS AND STRUCTURAL ANALYSIS OF β-LACTOGLOBULIN BINDING SITES

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Polysaccharide–protein complexes are key in texture formation typical of certain dairy and fermented foods. We investigated 2 alginates (ALGs) that are common food ingredients and hydrocolloids with different mannuronic acid/guluronic acid ratio, and 7 hetero-exopolysaccharides (HePSs) of known repeat unit structure from different lactic acid bacteria strains. The study focused on their interaction with the major milk and whey proteins β-casein, κ-casein, native and heat-denatured β-lactoglobulin (βlg) as analyzed by using surface plasmon resonance analysis, isothermal titration calorimetry, dynamic light scattering, analytical ultracentrifugation and small angle X-ray scattering.[1,2,3] This revealed distinct differences among the ALGs and among the HePSs with regard to binding affinity and binding capacity as well as biophysical characteristics of the formation of soluble aggregates. Furthermore, to gain insight into structural determinants in such complexes, we produced and purified both alginate oligosaccharides (AOSs) with degree of polymerization of 3 (DP3) generated from ALGs using a *Sphingomonas* sp. lyase and oligosaccharide repeat units of DP4 and DP5 from HePSs of *Streptococcus thermophilus* LY03 and *Lactobacillus delbrueckii* sp. *bulgaricus* 291, respectively, obtained by mild acid hydrolysis. The oligosaccharide structures were determined by ^{1}H and ^{13}C NMR spectroscopy and their binding sites in isotope-labelled recombinant βlg were identified by chemical shift perturbation of backbone resonance peaks in the ^{1}H,^{15}N-HSQC NMR spectrum and for the AOSs also by X-ray crystallography. This provided the first identification of carbohydrate binding sites in the βlg structure. The NMR structure showed two binding areas on the surface of βlg for the HePS oligosaccharides and AOSs at pH 2.65; one of these being oligosaccharide-specific and the other shared. Only one binding site was found for AOSs at pH 4.0, while at pH 3.0, a bound monosaccharide unit was seen in the crystal structure at a different site from which AOSs of DP3 and DP4 were modelled. Generally these oligosaccharides serving as polysaccharide models show K_d values in the low millimolar range. The findings on oligosaccharide binding will be discussed in the light of the behavior of the corresponding individual polysaccharide–βlg complexes.

This work was supported by the Danish Research Council for Independent Research | Technical and Production Sciences to the project “HEXPIN” and the Strategic Research Council to the project “StrucSat” as well as by parts of PhD stipends from Technical University of Denmark (to EGPS, JB, CK)

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ANTI-METASTATIC PROPERTIES OF A MARINE BACTERIAL EXOPOLYSACCHARIDE-BASED DERIVATIVE DESIGNED TO MIMIC GLYCOSAMINOGLYCANS

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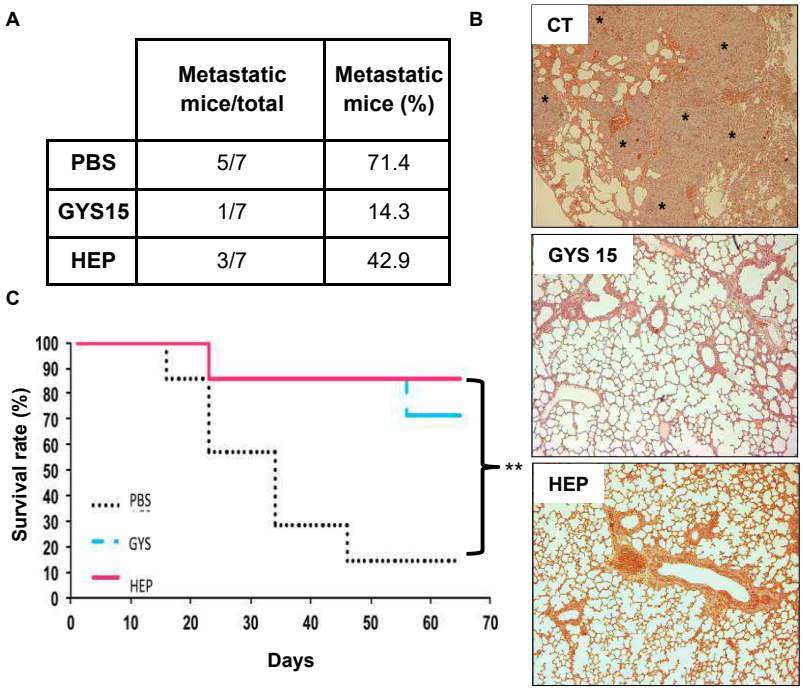
Osteosarcoma is the most frequent malignant primary bone tumor characterized by a high potency to form lung metastases. In this study, the effect of three oversulfated low molecular weight marine bacterial exopolysaccharides (OS-EPS) with different molecular weights (4, 8 and 15 kDa) were first evaluated *in vitro* on human and murine osteosarcoma cell lines. Different biological activities were studied: cell proliferation, cell adhesion and migration, matrix metalloproteinase expression. This *in vitro* study showed that only the OS-EPS 15 kDa derivative (GYS15) could inhibit the invasiveness of osteosarcoma cells with an inhibition rate close to 90%. Moreover, this derivative was potent to inhibit both migration and invasiveness of osteosarcoma cell lines; had no significant effect on their cell cycle; and increased slightly the expression of *MMP-9*, and more highly the expression of its physiological specific tissue *inhibitor TIMP-1*. Then, the *in vivo* experiments showed that the GYS15 had no effect on the primary osteosarcoma tumor induced by osteosarcoma cell lines but was very efficient to inhibit the establishment of lung metastases *in vivo* (see Figure). These results can help to better understand the mechanisms of GAGs and GAG-like derivatives in the biology of the tumor cells and their interactions with the bone environment to develop new therapeutic strategies. [1]



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NEW LAMINARIN-CONJUGATES: BIOPROTECTION AND MOLECULAR TOOLS TO STUDY PENETRATION IN PLANT LEAVES.

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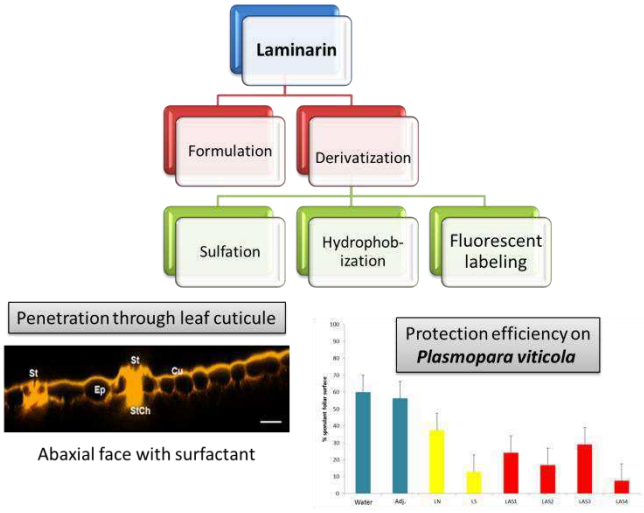
Laminarin, a β-(1→3)-glucan produced by *Laminaria digitata*, is known for its ability to act as bioprotector and/or resistance inducer in grapevine against the downy mildew.[1] Some of its derivatives, for instances the persulfated ones,[2,3] are much more efficient than the native polysaccharides. Unfortunately, these efficiency is significantly limited both in standardized or real field conditions. One major explanation is probably a limited penetration through the leaf cuticle following spray application.

In order to overcome such drawbacks, we have investigated two complementary options:

1. We first focused our attention on formulation and studied various surfactants as penetration enhancers. We have established that penetration rate of sulfated laminarin was much higher on the stomateous abaxial surface of the leaf than on the adaxial surface;[3]

2. We secondly designed and prepared partially hydrophobized laminarins in order to favor laminarin-conjugate interactions with lipid components constituents of the leaf, and so increase biodisponibility and penetration of the active saccharide. Various chemical links between the polysaccharide and the aliphatic chains were considered. For better comparison with previous derivatives of laminarin, the less hydrophobic polysaccharides were further sulfated and fluorescent label compounds were also prepared as molecular tools for studying their diffusion pattern in *Arabidopsis thaliana* and grapevine.



Chemical synthesis, analytical data, biological results will be described underlying possible impact of structural modulations on physicochemical properties and resistance enhancement.

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PRODUCT-ORIENTED CHEMICAL SURFACE MODIFICATION OF A LEVANSUCRASE (SACB) VIA AN ENE-TYPE REACTION

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Chemical protein modification evolved into an indispensable tool for selective protein functionalization and enables rational engineering of biocatalysts. Herein we describe the tyrosine specific chemical modification of a levansucrase from *Bacillus megaterium* (*Bm*-LS) via an Ene-type reaction. *Bm*-LS produces exclusively small fructooligosaccharides with a degree of polymerization (DP) of 2 - 20 fructose units. Depending on their size fructan polymers (levan) provide a broad variety of applications in the chemical, health, cosmetic and food industries.[1] For the production of high molecular weight (HMW) levan, initial interactions between growing oligosaccharides and enzyme need to be stable enough to prevent early substrate release.[2] Site-directed mutagenesis of residues close to the active site has already been proved successful for altering the fructan size towards shorter products.[3] In contrast structural manipulation of fructosyltransferases to foster the synthesis of HMW polymer is, to the best of our knowledge, unexplored. We presumed that strategic modification of side chains in the outer periphery of the central cavity might have an impact on promoting the polysaccharide elongation. Based on solvent accessibility and participation in polymer elongation the residue Y196 was selected for chemical bioconjugation in a two-step process. Tyrosine specific modification with a luminol derivative possessing an alkyne group, was followed by copper(I)-catalysed azide-alkyne cycloaddition with four different azides, including 1-Azido-1-deoxy-β-D-glucopyranoside (1AzGlc). (Figure 1) The second modification step resulted in a dramatic shift from small oligosaccharide formation to the production of HMW levan. We suggest that the change in product specificity is induced by generating new intra polar contacts and introducing a steric hindrance, which may impede early protein-oligosaccharide dissociation events. Results with other modified tyrosine residues introduced at exposed positions (D248Y, E314Y and F445Y) in the periphery of the binding pocket show similar shifts in the product profile. This supports the previous assumptions that creating more polar contacts sustains the elongation of nascent oligosaccharides.

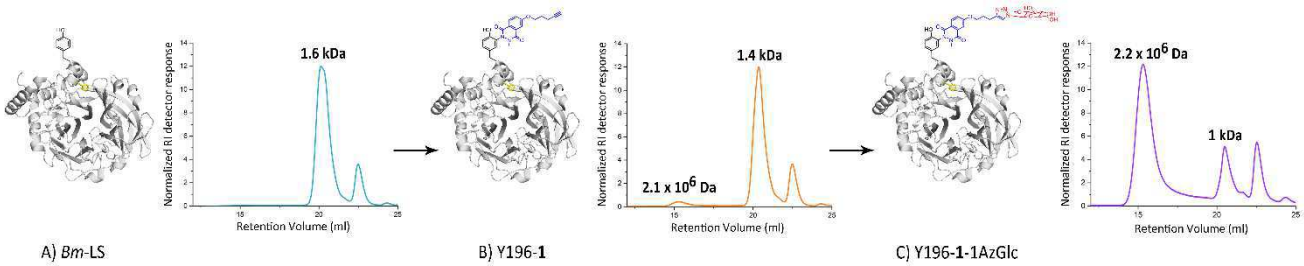

Fig. 1. Effect of a two-step chemical modification of Y196 of *Bm*-LS. A) Unmodified *Bm*-LS, B) Y196-1, C) Y196-1-1AzGlc. The protein engineering strategy is displayed in the left panels. Gel permeation chromatograms of the product size (in Da) are shown in the right panels.

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FLOCCULATING ACTIVITY OF EXOPOLYSACCHARIDES FROM ACTINOBACTERIA

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Actinobacteria are Gram-positive microorganisms commonly found in soil, water and compost. A wide range of tolerance for some environmental conditions allowed them to exist in various extreme habitats like deserts, glaciers of Antarctica, caves and oceans [1]. Actinobacteria synthesize biologically active substances about antibacterial, antifungal, antiviral and anticancer activities. Through the synthesis of many enzymes, Actinobacteria are able to decompose organic matter by degradation of lignin, cellulose and chitin. They can also bind nitrogen and, in symbiosis with plants, convert ammonia into nitrates that are better absorbed by organisms [2, 3]. Moreover, these microorganisms produce exopolysaccharides (EPS) with high biotechnological potential. Basic features of the natural EPS, such as a biodegradability, a high efficiency, a non-toxicity and a lack of secondary pollution, determine their role as a potential alternative to substitute chemical substances applied in industry [4]. Exopolysaccharides found various applications in processes such as wastewater treatment, heavy metals removal and fermentation. Particularly valuable feature of EPS is their capacity to purify the water by flocculation process. This phenomenon occurs in presence of active groups of exopolysaccharides, which react with particles suspended in solutions, causing their aggregation and settlement. The efficiency of flocculation depends on distinct factors and is strictly correlated with properties of EPS used in the process. At present, many scientists are searching for new microbial exopolysaccharides with high flocculating activity to improve already used applications and to discover other potential fields for their exploitation.

In the present study, bacterial strain belonging to Actinobacteria has been tested due to exopolysaccharide synthesis. The optimum culture conditions of *Rhodococcus opacus* FCL1069 were deter*rm*ined using different variants of culture, such as carbon and nitrogen sources, salts addition and pH value of the medium. Supernatants obtained from culture broths were tested for the flocculating activity in the presence of kaolin and calcium chloride suspension. Additionally, the receiving of the exopolysaccharide from culture broth was optimised due to the production and the purification parameters e.g. the day of extraction, *inoculum* size, the type and volume of the precipitating agent added, the mixing time after precipitation and the total precipitation time of the exopolysaccharide. Optimal parameters of production and precipitation process were determined based on the amount of exopolysaccharide obtained in each variant.

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CHLAMYDOMONAS REINHARDTII AS A GREEN MICROALGAE TO UNDERSTAND EVOLUTION OF THE N-GLYCOSYLATION PATHWAYS IN MICROALGAE

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Despite the biological and physiological significance, along with knowledge regarding the *N*-glycosylation processing in Eukaryotes, little attention has been paid to this biosynthetic pathway in microalgae even if they are interesting organisms spread in different phyla of the tree of life. Moreover, microalgae emerged recently as potential cell bio-factories for the production of biopharmaceuticals for which glycosylation represent a critical quality attribute.

In order to characterize the *N*-glycosylation pathways in microalgae, we took advantage of the recent genomic sequencing of the diatom *Phaeodactylum tricornutum* and the green microalgae, *Chlamydomonas reinhardtii* and identify a set of putative orthologs involved in the different key steps of the *N*-glycan biosynthesis and maturation. Moreover, functional characterization of the glycoenzymes such as the *N*-acetylglucosaminyltransferase I (GnT I) allow us to identify that *P. tricornutum* is driven through a GnT I dependent pathway [1] which is not the case for *C. reinhardtii*. Indeed, *C. reinhardtii* proteins arise from a GnT I-independent Golgi processing of oligomannosides giving rise to $Man_5GlcNAc_2$ substituted eventually with one or two xylose(s) [2; 3]. Complementation of *C. reinhardtii* with heterologous GnT I was investigated by expression of GnT I cDNAs originated from Arabidopsis and the diatom *Phaeodactylum tricornutum*. No modification of the *N*-glycans was observed in the GnT I transformed cells. Consequently, the structure of the $Man_5GlcNAc_2$ synthesized by *C. reinhardtii* was reinvestigated. Mass spectrometry analyses combined with enzyme sequencing showed that *C. reinhardtii* proteins carry linear $Man_5GlcNAc_2$ instead of the branched structure usually found in eukaryotes. Moreover, characterization of the lipid-linked oligosaccharide precursor demonstrated that *C. reinhardtii* exhibit a $Glc_3Man_5GlcNAc_2$ dolichol pyrophosphate precursor. We propose that this precursor is then trimmed into a linear $Man_5GlcNAc_2$ that is not substrate for GnT I. Furthermore, cells expressing GnTI exhibited an altered phenotype with large vacuoles, increase of ROS production and accumulation of starch granules, suggesting the activation of stress responses likely due to the perturbation of the Golgi apparatus [4].

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CARRAGEENAN CATABOLISM IS ENCODED BY A COMPLEX REGULON IN MARINE HETEROTROPHIC BACTERIA

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Macroalgae contribute substantially to primary production in coastal ecosystems. Their biomass, mainly consisting of polysaccharides, is cycled into the environment by marine heterotrophic bacteria using largely uncharacterized mechanisms. Carrageenans are a major component of the cell wall of red macroalgae. These sulfated polysaccharides have been widely used in various industries as gelling and texturizing agents. Carrageenans and their derived oligosaccharides have promising potential as bioactives (antivirals, anticoagulants, immunomodulators, etc.) presumably due to their resemblance to animal sulfated glycosaminoglycans. Ecologically, these polymers are an enormous source of nutrients in coastal ecosystems, particularly for heterotrophic marine bacteria that are key players in the recycling of organic matter in the oceans. We have determined the complete catabolic pathway for carrageenans, in the marine heterotrophic bacterium Zobellia galactanivorans, using a combination of biochemical, crystallographic, transcriptomic, genetic and bioinformatics techniques [1]. This research provides a major advancement towards understanding the molecular mechanisms governing the carbon cycle in coastal ecosystems. It is also a breakthrough for blue biotechnology and in particular the valorization of algal biomass.

Carrageenan catabolism relies on a multifaceted carrageenan-induced regulon, including a non-canonical polysaccharide utilization locus (PUL) and genes distal to the PUL, including a susCD-like pair. The carrageenan utilization system is well conserved in marine Bacteroidetes but modified in other phyla of marine heterotrophic bacteria, including the absence of the susCD-like pair. The core system is completed by additional functions that might be assumed by non-orthologous genes in different species. This complex genetic structure is the result of multiple evolutionary events including gene duplications and horizontal gene transfers. These results allow for an extension to the definition of bacterial PUL-mediated polysaccharide digestion.

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THE SYNTHESIS OF MULTIVALENT GLYCODENDRIMERS AS ANTI-VIRAL AGENTS

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Glycodendrimers are a versatile class of biopolymers with diverse biological applications. In our lab, we are focused on the development of glycodendrimers as anti-viral agents, with our current focus on HIV-1. In nature, many cell-surface proteins are presented in multiple copies, or multivalently. Glycodendrimers are also multivalent in structure, which can lead to stronger binding to a target protein than one would expect from a one to one interaction. HIV-1 presents its proteins, gp41/gp120 on the viral surface as a heterotrimeric complex, with gp41 membrane bound and gp120 electrostatically associated with gp41. Our glycodendrimers are designed as multivalent inhibitors of gp120 binding to the host cell. The objective our work is to design and synthesize diverse glycodendrimer structures to serve as potential anti-HIV agents.

This presentation will focus on the development of our glycodendrimers as anti-HIV agents, beginning with our early inhibitors that incorporated the commercially available dendrimer core, poly(amidoamine), or PAMAM [1]. These inhibitors served as our proof of concept molecules. Building on what we learned in this initial set of compounds, we have developed and synthesized a variety of hydrophilic core structures, incorporated microwave chemistry where possible, and eliminated the use of protecting group chemistry on the sugars. This strategy allows us to create large glycodendrimers in an efficient, high yielding manner. Once synthesized, our glycodendrimers are evaluated for anti-HIV activity through the use of two separate biological assays. The results of these assays will also be discussed.

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MUC1 GLYCOPEPTIDES RECOGNITION PROFILING BY MACROPHAGE GALACTOSE LECTIN TO TARGET DENDRITIC CELLS

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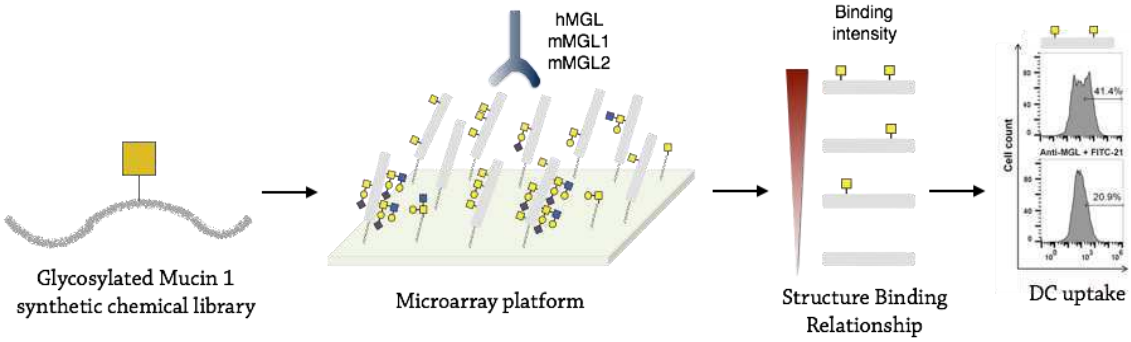
Lectin–carbohydrate interactions have essential roles on the modulation of the immune system. In literature, many examples focused on how single carbohydrate moieties bind to the immune system receptors. However, in the surface of cells many glycans are linked to proteins. In here, we hypothesize the contribution of both glycan and protein backbone on the binding to lectins. In this regard, here we confined our attention on macrophage galactose lectin (MGL), present on dendritic cells (DCs) and macrophages, and it can bind to galactose (Gal) and *N*-acetylgalactosamine (GalNAc). As potential ligand, we chose mucin 1 (MUC1) glycoprotein as it expresses altered truncated glycans as terminal Gal and GalNAc during malignant processes. These structural differences have placed MUC1 as a prioritized objective of study to create an efficient cancer vaccine.

In the present study, we employ a MUC1-based glycopeptide microarray to characterize the specificities of three MGL (Clec10a) orthologs: human MGL (hMGL), murine MGL1 (mMGL1) and MGL2 (mMGL2). This research work covers (i) synthetic chemical library of 35 compounds, (ii) evanescent-field fluorescence microarrays as a high-throughput screening approach to detect lectin ligands with high reproducibility[1] and (iii) internalization assays on DCs.

As result, by using our microarray platform we were able to monitor, under equilibrium conditions, the similar recognition profile of hMGL and mMGL2 with natural mimetic MUC1 glycoforms. Herein, we have shown the so far unprecedented role of mucin-based peptides on the recognition by lectins of GalNAc moieties expressed in tumour-altered mucins. On this account, we have demonstrated the relevance of the sugar site in the MUC1-mMGL1 binding. In addition, for three orthologs, we have also proved a positive direct correlation the bivalency effect and the binding affinity. To assess the utility of the glycopeptide binders of the MGL orthologs for MGL targeting, we performed uptake assays with fluorescein-MUC1 using murine DCs. The diglycosylated MUC1 peptide was highly internalized in an MGL-dependent fashion, thus showing the utility for bivalent GalNAc to target MGL.[2]

In conclusion, the MGL-dependent uptake of MUC1-derived glycopeptides into murine DCs highlights the potential of MGL-based DC targeting which may be used to design novel anticancer vaccines with an enhanced effectiveness.



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TRÊS SETAS ; GLYCAN, ENZYME, BINDING PROTEIN. TCI BOOSTS GLYCOSCIENCE RESEARCH BY THE UNIQUE REAGENTS

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TCI provides over 28,000 reagents, of which, there are over 1,000 glyco-related reagents. Three unique groups of Glycoscience products are provided by TCI. The first group consists of glycan and glycan-derivatives, which are chemically synthesized and isolated from natural sources. The second group consists of enzymes; an *endo*-*N*-Acetyl-β-$_D$-glucosaminidase named Endo-M and its mutants are unique enzymes produced by TCI. The third group includes antibodies and lectins; we deal with many anti-Glyco antibodies and fucose-specific lectins. With these “três setas”, we have constantly been developing various products to support researchers in the field of Glycoscience. In this presentation, we will talk about the latest developments in synthetic glycans, enzymes and detection probes, lectins, and antibodies.

MILK OLIGOSACCHARIDES AS THERAPEUTIC AGENTS

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Milk is naturally occurring component and complete food for neonates as well as adults. It contains proteins, fat and carbohydrates including oligosaccharides which have varied biological activities viz. antioxidant, anticancer, lipid lowering and post heparin lipolytic activities.

Human milk oligosaccharides (HMOS) have been described to act as prebiotics and as potent inhibitors of bacterial adhesion to intestinal epithelial receptors. Moreover, as they are not digested and are absorbed only in a small proportion in the gastrointestinal tract, they may play a role in the local intestinal immune system of the breast-fed infants, thus acting in the prevention of infectious disease. Milk oligosaccharide also helps in mineral absorption, immunomodulators, tumor marker and sialylated milk for brain development.

BIOCATALYTIC PRODUCTION OF QUERCETIN WITH RUTINOSIDASE: NOVEL CONCEPT OF "SOLID STATE BIOCATALYSIS".

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Glycosylation can significantly alter the physicochemical and biological properties of small molecules like vitamins, antibiotics, flavors, and fragrances. Enzymes are excellent tools in selective modification of natural products [1].

Quercetin occurs in human food (e.g. apples, onions) in the form of glycosides. Quercetin is utilized as a phytochemical remedy for diseases like diabetes/obesity, circulatory dysfunction, inflammation, mood disorders, and can be used as efficient and affordable radio protectant. Quercetin is prepared by acid hydrolysis of rutin. Boiling in a strong acid leads to a partial decomposition of all reactants. We discovered and cloned novel diglycosidase – rutinosidase, able to cleave entire rutinose (α-L-rhamnosyl-(1$\rightarrow$6)-D-glucose) from rutin [2]. This enzyme has also very strong transglycosylation activity enabling also glycosylation of phenolic aglycones [3]. Existing enzymatic methods request completely dissolved rutin and their productivity is limited by low rutin solubility (10 g/L); the use of co-solvents (EtOH, DMSO) damage enzymes lowering final yields and extending reaction times, and afford mixtures (rhamnose + glucose).

Our new method allows working with rutin in aqueous suspensions of rather high concentrations (up to ca 300 g/L, ca 0.5 M) without any co-solvents yielding precipitated product – quercetin - that could be easily filtered and purified just by washing at the filter. The filtrate contains still active enzyme, which could be reused. As a side product, a rare sugar rutinose is produced in a high purity that could be eventually obtained by crystallization from concentrated filtrate. This rare sugar can be now produced in kilogram amounts for affordable price, which opens its application e.g. in cosmetics.

We present here a novel concept of "Solid State Biocatalysis" enabling extremely high space-time-yield.

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L-ARABINOFURANOSYLATED PROBES FOR THE ANALYSIS OF NOVEL L-ARABINOFURANOSIDASES FROM BIFIDOBACTERIUM LONGUM

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The L-arabinofuranosylated oligosaccharide has been found in the indigestible dietary fibers such as plant cell wall glycoconjugates. In the non-reducing terminal end of glycan in the glycoconjugates such as hydroxyproline-rich glycoproteins (HRGPs), β-L-arabinofuranoside (β-L-Ara*f*) is existed. The *O*-glycan linked to Hyp in HRGP is oligo-Larabinofuranoside (L-Araf_{1-4}) including β-L-Ara*f* which is hydrolyzed by L-arabinofuranosidases (L-Ara*f*ases) [1] isolated from the human gastrointestinal bacterium, *Bifidobacterim longum*, including a novel β-L-Ara*f*ase, HypBA1 (GH127). The other β-L-arabinofuranosylated motifs have been also found in plant arabinan whose structures might be also cleaved by other unknown β-L-Ara*f*ases [2]. To analyze the catalytic mechanism of the GH127 β-L-Ara*f*ase, we would like to present our synthetic study of various L-arabinofuranosylated probes.

The stereoselective synthesis of non-reducing terminal β-L-Ara*f*-linked di- and trisaccharide motifs of various plant glycans [3,4] as well as PNP β-L-Ara*f* for the substrate of β-L-Ara*f*ase [5] have been achieved stereoselectively by the application of NAP-ether mediated intramolecular aglycone delivery [6]. We also prepared the glycosyl amine derivatives for the probes in order to carry out the biological and the structural biological studies β-L-Ara*f*ases. Although the amination of hemiacetal gave the mixture with the di- and triglycosylated amine structures, peracetylated L-arabinofuranosylazide as the monoglycosylamine equivalent could be prepared selectively which was used as the intermediate to afford the L-arabinofuranosylamine derivatives stereoselectively through Staudinger reaction followed by acylation.

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ON THE ROAD TO AN ORGANOCATALYTIC APPROACH TO NEPLANOCIN A: SYNTHESIS OF RARE SUGARS AND GLYCOMIMETICS

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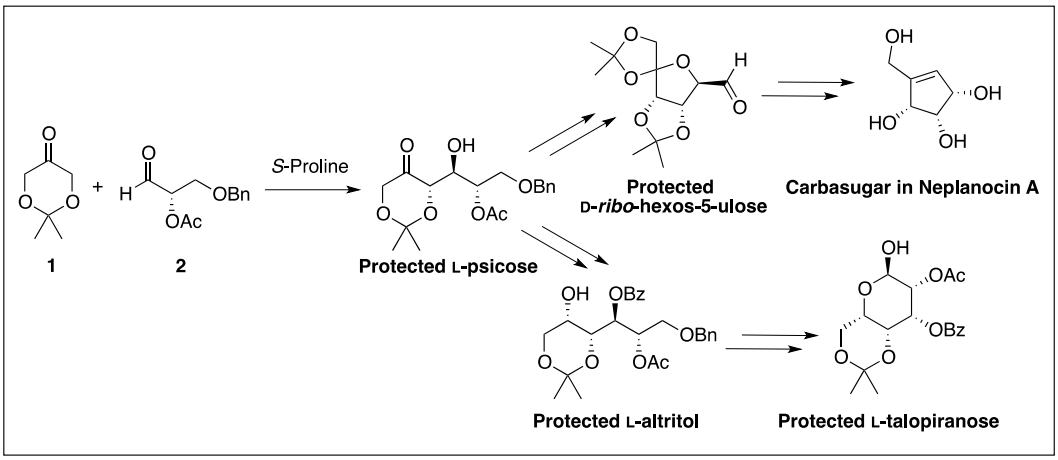
The high stereoselectivity, together with the possibility of obtaining enantiomerically pure products is an attractive feature in organocatalyzed reactions [1]. In addition, these processes are not only economic (mostly having lower energetic requirements and using less expensive inputs) but also, due to their high selectivity, have the advantage of generating less wastes.

Modified sugars are important moieties present in a large variety of bioactive compounds, such as nucleoside analogues, and have shown antiviral, antimicrobial, antifungal, and other outstanding biological activities [2].

In this work we describe the advances in our organocatalytic approach to the synthesis of the carbasugar in Neplanocin A, and the preparation of the rare sugars L-psicose, L-altritol, L-talose [3], and the oxidized derivative D-*ribo*-hexos-5-ulose [4].



The key step in the designed synthetic sequence is the *S*-proline-catalyzed aldol reaction of dioxanone **1** and the orthogonally diprotected L-glyceraldehyde derivative **2**, which confers an outstanding versatility to the synthetic route. Compound **2** was synthesized from L-gulono-γ-lactone, as previously described [5]. Protected L-psicose derivative was obtained, as a single diastereomer in 70% yield, using *S*-proline as catalyst. From this key intermediate, protected L-altritol and L-talopiranose derivatives were prepared in 44% and 18% yield respectively [3]. On the other hand, the oxidized sugar *ribo*-hexos-5-ulo-5,2-furanose derivative was obtained in 38% yield [4]. Finally, the last steps in route to the carbasugar in Neplanocin A, were an olefination through a Wittig reaction, selective deprotection of the acetonide groups, olefination of the resulting ketone in the opened form, and finally a RCM reaction.

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INTRAMOLECULAR CINE SUBSTITUTION ON CARBOHYDRATES

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The reactions of C-radical systems with a β-ester group are considered of great interest since migration resulting products could be obtained. These processes involve a cation-radical intermediate which could be trapped inter- or intramolecularly by a nucleophile in an *ipso* or *cine* way (Fig. 1).

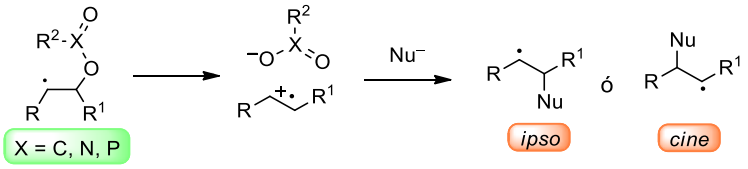
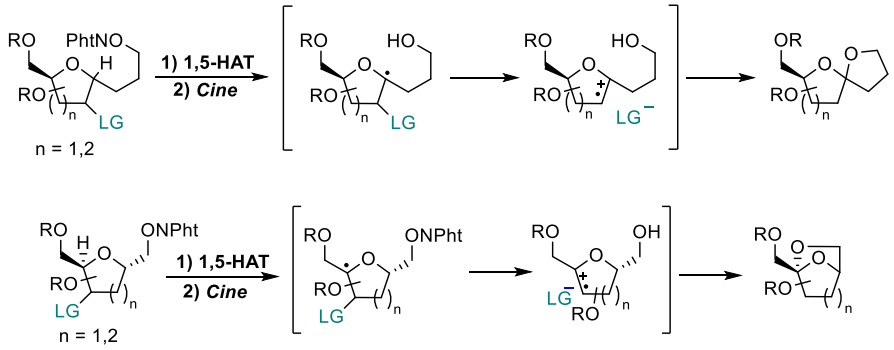


Fig. 1

In this sense, intramolecular processes offer the possibility to synthesize interesting cyclic products from acyclic structures [1].

However, to this moment only a few examples of intramolecular *cine* substitution applied to cyclic starting materials has been reported [2], and no studies of the probable influence of the stereoelectronic nature of the leaving group (LG) over the mechanism, have been developed.
Herein, we describe an intramolecular *cine* substitution on conformationally restricted carbohydrate systems to show some light over the stereoelectronic requirements of the LG to promote the *cine* mechanism against others.

We have studied the evolution of 1-glycosyl and 4-glycosyl radicals, with proper adyacent LGs, resulting from a 1,5-Hydrogen Atom Transfer (HAT) process to obtain spiroacetalic and dioxabicyclic compounds respectively.



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SURFACE EXPOSED GLYCOSIDE HYDROLASES OF HUMAN GUT BACTERIA CONFERRING GALACTOMANNAN DIETARY FIBRE CATABOLISM

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Galactomannans are a type of hemicellulose commonly found as seed storage polysaccharides (1), used as food thickeners (2). Thus they are a part of our diet and can be utilised by several different human gut bacteria. The studied Bifidobacteria express single surface exposed beta-mannanases (3,4). *Bacteroides ovatus*, on the other hand, expresses several glycoside hydrolases from a polysaccharide utilisation locus (PUL) (a gene cluster) (5) and the enzymes act in a sequential manner (6). These enzymes are two glycoside hydrolase family 26 (GH26) β-mannanases, BoMan26A and BoMan26B, and a GH36 α-galactosidase, BoGal36A (6,7). We show that this PUL is essential for *B. ovatus* galactomannan utilisation. The two β-mannanases was characterised, including solving crystal structures of BoMan26A and BoMan26B, contributing to a model of the combined function of the enzymes and binding proteins of this galactomannan PUL (7). Fluorescence microscopy showed that BoMan26B is exposed on the surface of *B. ovatus* and makes the initial endo-attack on galactomannan. BoMan26B has an exceptional capability to depolymerize highly substituted guar galactomannan, explained by the open and extended active site cleft visible in the crystal structure. BoMan26A has distinctly different properties and is located in the perisplasm. Since BoMan26A is severely restricted by galactose side groups carried by galactomannans, it acts in synergy with the periplasmic alpha-galactosidase BoMan36A and releases mainly mannobiose (7). A crystal structure of BoMan26A revealed a similar structure to the exo-mannobiohydrolase CjMan26C from Cellvibrio japonicus (8), with conserved -1 and -2 subsites, and a narrow active site cleft with a loop closing the active site beyond subsite -2 (7). The narrow active site cleft of BoMan26A explains the different mode of attack compared to BoMan26B. Phylogenetic analysis place BoMan26A and BoMan26B in different clades of family GH26. Based on the structure-function analysis of the enzymes and binding proteins, we propose a scheme of galactomannan utilization conferred by this *B. ovatus* PUL. The outer membrane associated BoMan26B initially acts on galactomannan, producing comparably large oligosaccharide fragments.

Galactomanno-oligosaccharides are imported and further processed in the periplasm: degalactosylated by BoGal36A and subsequently hydrolysed into mainly mannobiose by BoMan26A (7). Our model for galactomannan catabolism by *B. ovatus* may be valid for other bacteria, since several bacteroidetes appear to have a similar PULs including predicted genes for two GH26 mannanases.

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CHARACTERIZATION OF A NOVEL EXOPOLYSACCHARIDE PRODUCED BY *BIFIDOBACTERIA BREVE* JCM7017

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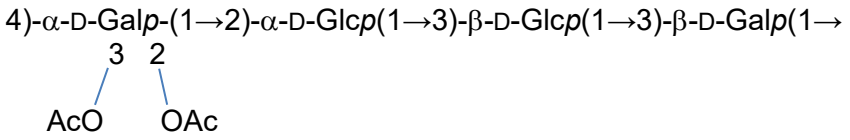
Bifidobacterium is an important bacterial genus and in the first few months of life it constitutes an significant component of the microbiota of the gastrointestinal tract of healthy humans[1,2]. There has been much recent interest in specific strains of bifidobacteria as their presence in the gut microbiota has been linked to a range of health benefits[3] and a number of strains are considered as probiotic organisms[4,5]. In order to exert a probiotioc effect bacteria need to be able to colonise a host. Comparative genomic studies have suggested that the synthesis of exopolysaccharides (EPS) is one strategy adopted by bacteria to enhance their ability to colonise hosts. A number of different *Bifidobacteria breve* strains have been found in human milk, in the adult gut and in the human vagina. At the same time genomic studies have identified that *B breve* strains have the capacity to synthesis EPS but todate the structures of these polysaccharides are not known.

In our work we have developed a fermentation media supporting the growth of *B. breve* JCM7017 in batch culture and have isolated and purified an EPS. The results of monomer analysis, linkage analysis and absolute sugar determination were combined with a detailed 1D and 2D-NMR investigation to determine the structure for the EPS.

The EPS form *B breve* JCM7017 has a novel structure: the repeating unit is composed of a linear tetrasaccharide in which one of monomers is highly decorated and carries two adjacent acetyl groups and the EPS also contains a branch. The analysis of the EPS was complicated by the fact that the repeating unit structure changed over a period of a couple of months at room temperature (or several hours at the elevated temperatures used to record NMRs). Our NMR studies suggest that the branch is connected to the main chain by a labile bond and is easily lost. After dialysis, the resulting repeating unit, minus the branch, has the following structure:



Questions still remain about the nature of the branch, the point of linkage and type of linkage of the branch to the main chain. Evidence providing answers to these questions will be discussed.

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SCREENING THE HUMAN GUT MICROBIOME FOR CARBOHYDRATE MODIFYING ENZYMES TO GENERATE UNIVERSAL DONOR BLOOD

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Blood transfusion is an indispensable part of the health care system, saving many thousands of lives annually. Although significant improvements in the collection and use of blood have been made over the years, there are always shortages in the supply of blood. O-type red blood cells lack the A or B carbohydrate antigens, thus they are non-antigenic for the vast majority of people, so O-type blood can be used as a universal donor. Consequently there is always a demand for group O-type blood units due to emergency situations. A solution to this problem could lie in the enzymatic conversion of A, B or AB blood groups into universal donor red blood cells. However, no economically reasonable enzymes that catalyze this process are currently available. To facilitate larger scale conversion of A/B-type RBCs into universal donor blood, novel glycosyl hydrolases able to effectively cleave all subtypes of A and B antigens are urgently needed.

The human gut mucus layer harbors mucins, glycoproteins presenting O-glycan structures like the A and B blood antigens, which are foraged by present microorganisms, an ideal source of yet unknown carbohydrate-active enzymes. To avoid classical cultivation biases a human gut metagenomic fosmid library was created and screened for activity against A and B blood-type related substrates. The functional metagenomic screening yielded 5 hits, 3 from known A antigen cleaving GH109 family and 2 uncharacterized carbohydrate-active enzymes showing a unique mechanism to convert the A blood antigen into H (O-type). Serum-type conversion tests with those enzymes on A^+-Type RBC showed a very high and very specific A-cleavage activity, outcompeting any known A antigen cleaving enzyme.

Further characterizations of the enzymes and their serum-type conversion efficiency are necessary. So far, we are a step closer towards an efficient enzymatic production of universal donor blood from A-Type Blood.

GUT MICROBIOTA LIPOPOLYSACCHARIDES: REVERTING THE CONCEPT FROM BAD TO GOOD

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The Gut Microbiota (GM) is an essential actor in the modern concept of human health driving many host physiological and pathological processes, including immune system modulation. Accumulating evidences highlighted that studies of the immune system during health or disease cannot ignore our GM.[1] Initial sensing of microbes by the host immunity is mediated by the recognition of microbial-associated molecular patterns, such as lipopolysaccharides (LPS), which are highly conserved among bacteria, thus shared by both commensals and pathogens. The LPS structure strongly influences the biological effects on the host immune system. Defined LPS structures can act as potent agonists on the immune receptors whereas other can operate as antagonists reducing or inhibiting the cytokine production otherwise induced by toxic LPSs.[2,3] Thus, a crucial question to address is how the immune system distinguishes between permanently established commensals LPS and pathogens LPS.
The elucidation of the structure and the immunological activity of LPS isolated from gut microbes will bear new advances in the medicinal chemistry and in the field of search of new molecules able to antagonize pathogens LPS effect, as well as of GM LPS-inspired molecules able to prevent uncontrolled host immune response against our microbiota. This will also shed light on the structure-activity relationship of LPS itself, which is an open question in immunology field. In particular, this will improve the knowledge of the still poorly investigated GM world, giving insights in the host-microbe interaction mechanisms both at intestinal and systemic level furnishing, in parallel, information about the elicitation/modulation of immune response triggered by pathogens and commensals LPS, thus improving the overall knowledge of the IS.

In this communication, I will show some very recently elucidated GM LPS structures and their immunological properties that revealed to express unique and interesting features. Among others, I will discuss about the structure and activity of LPS from *Bacteroides vulgatus* mpk, a commensal bacterium whose beneficial effects on health were clearly demonstrated.[4,5] *B. vulgatus* mpk LPS showed a lipid A as a heterogeneous mixture of *mono*-phosphorylated tetra- and penta-acylated species and a unique saccharide moiety. The full structure of such an LPS and of all the LPSs that will be presented, was defined by employment of a multi-technique approach comprising wet chemistry, NMR spectroscopy and mass spectrometry.
The evaluation of the immunological properties of the *B. vulgatus* mpk LPS highlighted a very weak agonistic activity on bone marrow derived dendritic cells (BMDCs) and the capability to convert intestinal dendritic cells into a tolerant and tolerogenic phenotype thus mediating the maintenance of intestinal homeostasis. This effect is mainly due to the LPS peculiar chemical structure and I clarify the phenomenon both at a molecular and biological level.

Insights gained from the structural and molecular analysis of *B. vulgatus* mpk LPS and, in general, of GM LPSs might help to chemically design novel inflammation-silencing drugs as a potential alternative therapeutic approach for the treatment of inflammatory disorders.

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CHARACTERIZATION OF NOVEL LIPOOLIGOSACCHARIDE FROM GUT SYMBIOTIC BACTERIA AND CHEMICAL SYNTHESIS OF ITS ACTIVE PRINCIPLE LIPID A

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Lipopolysaccharides (LPS) are the major glycoconjugates in outer membrane of Gram-negative bacteria and activate innate immunity to induce strong inflammation. The terminal glycolipid called lipid A is the active principle of LPS. Low inflammatory LPS, lipid A, and their derivatives have been expected as immunoadjuvants for vaccines and agents for immunotherapy.

Alcaligenes sp. have been known as opportunistic pathogens. Kiyono *et al.* showed that *Alcaligenes faecalis* inhabits dendritic cells in human gut Peyer's patch, which plays an important role in immune response. [1]

In this study, we isolated LPS from *A. faecalis* and found that its component is lipooligosaccharide (LOS) with shorter oligosaccharide. Interestingly, *A. faecalis* LOS was found to be promising immune adjuvant, since it showed very low toxicity and weak inflammatory activity but high potency of antibody induction. These results suggested *A. faecalis* LOS is an important regulator for the gut immunity.

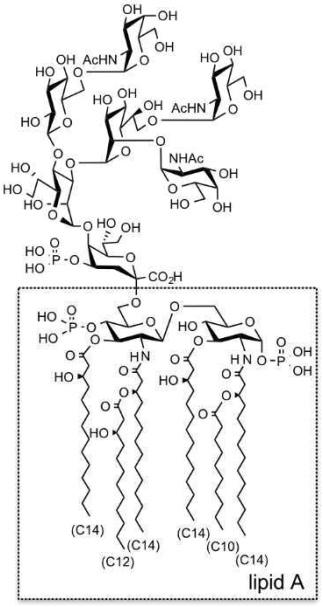

Fig. 1. *A. faecalis* LOS/lipid A

We then determined the chemical structure of LOS by using NMR and MS to be a glycolipid composed of nona-saccharide and multiple fatty acids (molecular weight: ca. 3,000, Fig. 1). Furthermore, we synthesized *A. faecalis* lipid A from D-glucosamine hydrochloride via key intermediate **1**[2] (Fig. 2). After the acylation of disaccharide intermediate, two phosphate groups were simultaneously introduced into **2**, affording **3** in a good yield. All protecting groups were removed to accomplish the synthesis of *A. faecalis* lipid A. Synthesized *A. faecalis* lipid A showed moderate immune stimulating activity in comparison with *E. coli* LPS.

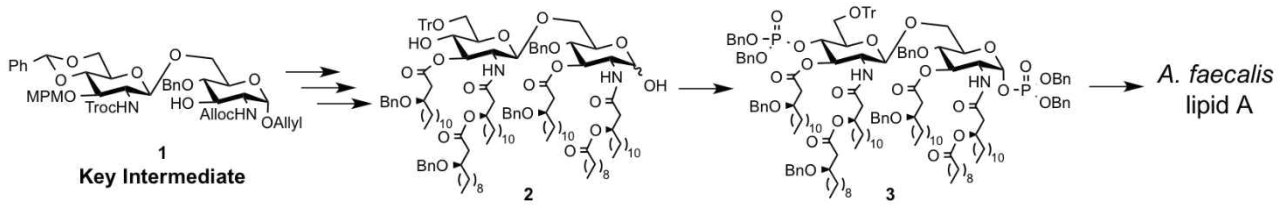

Fig. 2. Synthetic strategy of *A. faecalis* lipid A

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DIFFERENTIAL BACTERIAL CAPTURE AND TRANSPORT PREFERENCES FACILITATE CO-GROWTH ON DIETARY XYLAN IN THE HUMAN GUT

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Metabolism of dietary glycans is pivotal in shaping the human gut microbiota. The mechanisms that promote competition for glycans amongst gut commensals, however, remain unclear. *Roseburia intestinalis*, an abundant butyrate-producing Firmicute, is a key degrader of the major dietary fiber xylan. Despite the association of this taxon to a healthy microbiota, insight is lacking into its glycan utilization machinery. Here, we investigate the apparatus that confers *R. intestinalis* growth on different xylans.

R. intestinalis displays a large cell-attached modular xylanase that promotes multivalent and dynamic association to xylan via four xylan-binding modules. This xylanase operates in concert with an ATP-binding cassette (ABC) transporter to mediate break-down and selective internalization of xylan-fragments[1]. The transport protein of *R. intestinalis* prefers oligomers of 4−5 xylosyl-units[1], whereas the counterpart from a model xylan-degrading *Bacteroides* commensal targets larger ligands[2]. While *R. intestinalis* and the *Bacteroides* competitor co-grew in a mixed culture on xylan, *R. intestinalis* dominated on the preferred transport substrate xylotetraose.

These findings highlight the differentiation of capture and transport preferences as a possible strategy to facilitate co-growth on abundant dietary fibers and may offer a unique route to manipulate the microbiota based on glycan-transport preferences in therapeutic interventions to boost distinct taxa.

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THE ARABINOFURANOSIDASE CtAraf51: A VERSATILE BIOCATALYST FOR THE SYNTHESIS OF NOVEL GLYCOFURANOCONJUGATES

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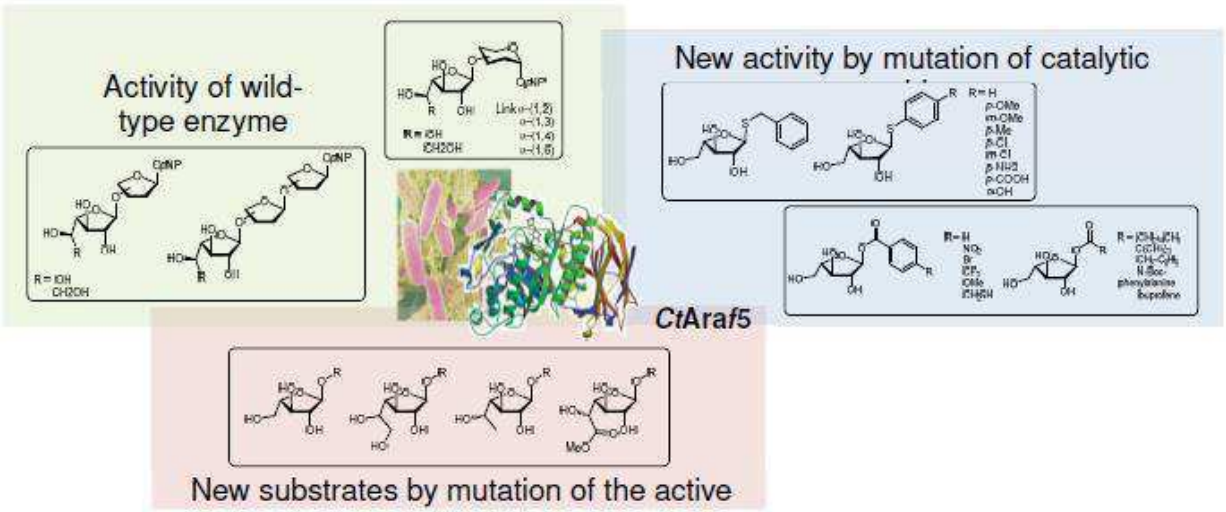
Galactofuranoconjugates, while xenobiotic in mammals, are crucial constituents of the glycocalix in cell walls of pathogenic microorganisms like Micobacterium, Aspergillus and Leishmania. The expression of these patterns, in these microorganisms, makes them molecular targets for the development of tools for the fight or the prevention of the associated diseases. Some strategies were described to synthetize such scaffolds but they usually involved conventional carbohydrate chemistry (multi-step synthesis, protecting group, toxic solvent or metallic promoters).

These works describe a new strategy using a glycosylhydrolase available in the laboratory, the α-arabinofuranosidase from Rumini Clostridium Thermocellum (CtAraf51) for the synthesis of novel glycofuranoconjugates from unprotected sugars in aqueous solvent. CtAraf51 was already described to catalyse the breakdown of osidic bonds but also, in certain condition, the reverse reactions (transglycosylation and self-condensation) [1,2]. Thanks to molecular biology and studies of the active site, we managed some mutations to increase further the versatility of the enzyme in terms of substrates and reaction.



The conversion of CtAraf51 in thioligase was performed by mutation of the acid/base residue [3]. This mutation allowed to quench the hydrolytic activity of water and to favour the reaction of others nucleophiles like thiophenol, benzylmercaptan or carboxylic acid [4]. With this mutated enzyme a large range of thioarabinofuranoses and acylarabinofuranoses were synthetized. In addit the docking studies of the active site revealed that three residues responsible of a steric clash with bulkier substrates (galactofuranose, fucofuranose and methyl ester galactofuranuronate). Selective mutations of these residues allowed to improve the kinetics parameters for the new substrates and to extend the above reaction to them.

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FLASH COMMUNICATIONS

INVESTIGATING *EXO*-GLYCALS REACTIVITY RECENT PROGRESS AND APPLICATIONS

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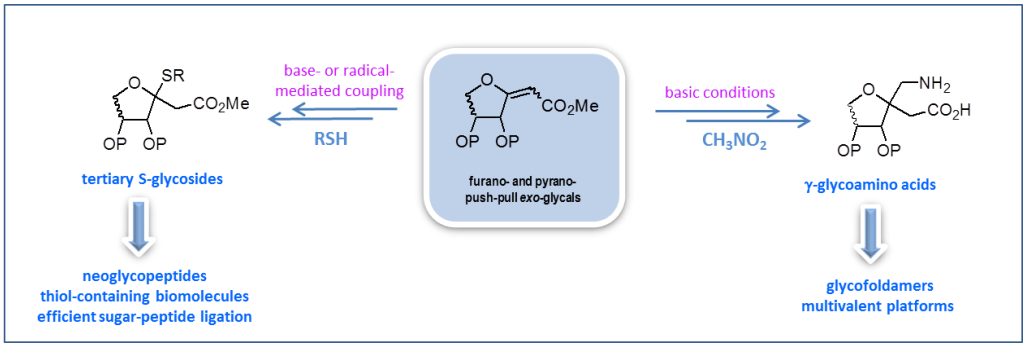
Exo-glycals or *C*-glycosylidene compounds having an exocyclic double bond at the anomeric centre are readily available by Wittig olefination of carbohydrate lactones with stabilized phosphoranes.[1] Investigating the chemistry of this class of unsaturated carbohydrates possessing an interesting push-pull substitution of the double bond, characterized by an electron-withdrawing and an electron-donating group, is a challenging and innovative task.[2]

For original access to new compounds of interest in the field of carbohydrates, *exo*-glycals can be valuable substrates in the well-known Michael addition. This prompted us to investigate the formation of a carbon-sulfur bond at the quaternary center by the addition of various thiols derivatives. The hydrothiolation of the trisubstituted double bond was performed with different thiols, thio-sugars, and thiol containing amino acids and peptides under radical and basic conditions. This opens the way to an efficient sugar-peptide ligation method and permits the preparation of tertiary *S*-glycosides, complex neoglycopeptides and thiol-containing biomolecules.[3]

Exo-glycals can also be valuable substrates for Michael additions of nitroalkane anion to access new interesting bis *C*,*C*-glycosyl compounds. These can be subsequently converted into the corresponding anomeric γ-amino acids and incorporated in glycopeptides for folding properties studies (glycofoldamers)[4,5] and multivalent platforms synthesis.[6]



Both approaches and their scopes and limitations will be presented and their applications will be discussed.

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EFFICIENT ENZYMES FOR THE PRODUCTION OF SIALIC ACIDS

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We aim to bring forward efficient enzymes for the production of sialic acids. Today, the largest market for sialic acid is for use in the production of anti-viral drugs, but the largest future demand will most likely be as a nutritional additive, in particular to infant milk formulas, and for cosmeceutical and pharmaceutical applications. We are optimizing enzymes for production of high-value sialic acid starting from sources containing *N*-acetylglucosamine (GlcNAc), the building block of chitin. Chitin is an abundant biomass found in for example crustacean shells. One of the enzymes is an *N*-acetylneuraminic acid lyase (sialic acid aldolase or NAL). This enzyme has unique properties, also facilitating the use of this enzyme alone for production of sialic acid from the cheaper substrate GlcNAc. Alternatively, the aldolases can be used in combination with with *N*-acetylglucosamine 2-epimerases (AGEs) for production of sialic acid (Figure 1). These processes are compared. Our ultimate goals are to bring forward enzymes that can utilize GlcNAc or chitin hydrolysates for the production of sialic acids. We are also investigating the possibility for using the enzymes in production of sialic acid derivatives with potential application as second-generation anti-infuenza drugs. Different synthesized substrates are being tested with our enzymes. Protein three-dimensional structures are determined using X-ray crystallography in order to understand the chemical reactions and rational design of the enzymes to produce modified sialic acids.

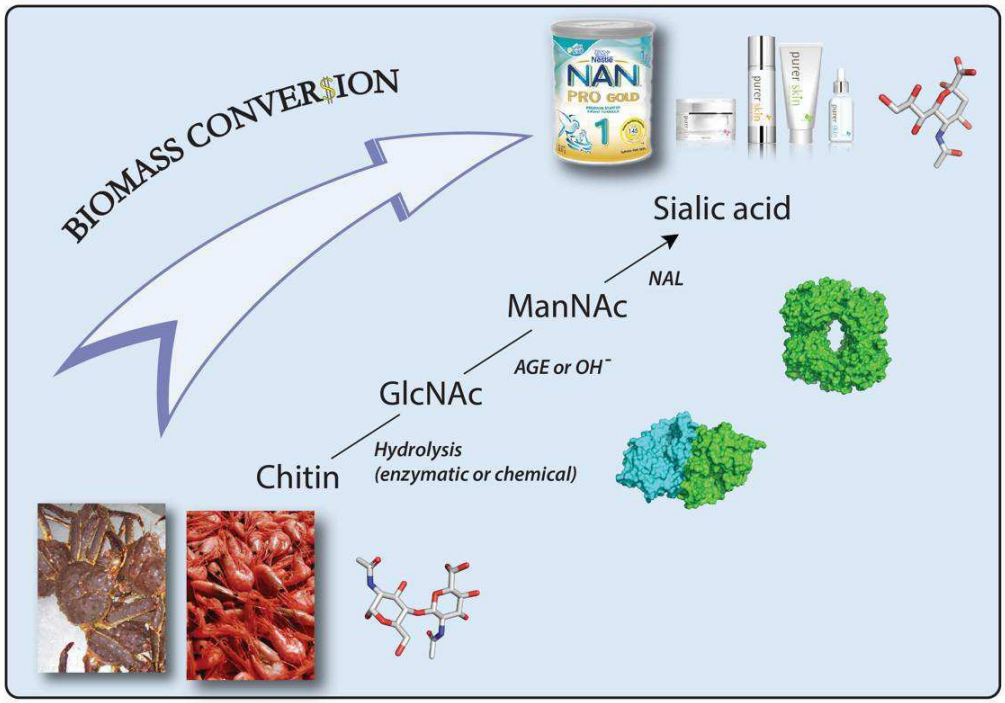

Fig. 1. The process of biomass conversion starting from cheap chitin and ending with expensive sialic acid that have many application areas. Enzymes that can be used in production and sugar intermediates are indicated. Sialic acid has great potential as a nutritional and cosmeceutical additive, and is used as a base for synthesis of anti-viral drugs.

NEISSERIA MENINGITIDIS A CAPSULAR POLYSACCHARIDE AND CARBASUGAR MIMETIC: CONFORMATIONAL STUDIES

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Neisseria meningitidis serogroup A (MenA) is an aerobic diplococcal Gram-negative bacterium responsible for epidemic meningitis disease, especially in the Sub-Saharian region of Africa[1]. The carbohydrate capsule (CPS), which covers the bacteria cell surface, has been identified as the primary virulence factor of Men A. Consequently, there is an increasing interest in making use of the CPS or mimetics thereof as potential vaccines against meningitis disease. Structurally, CPS consists of (1→6)-linked 2-acetamido-2-deoxy-α-D-mannopyranosyl phosphate repeating units, predominantly O-acetylated at 3-OH (80%)[2]. This polysaccharide suffers from chemical liability in water.[3] Thus, the design and synthesis of novel and hydrolytically stable structural analogues of MenA CPS is of paramount importance. In particular, herein, the structural features of the natural compound have been analyzed and compared to those of its carba-analogue,[4] where the endocyclic oxygen has been replaced by a methylene moiety. The hypothesis behind this research relies on the assumption that structure and function are intrinsically correlated in biomolecules. Therefore, in order to identify the best candidate that mimics the molecular recognition behaviour of the natural counterpart, it is essential to perform detailed structural studies. It is well-established that to predict the conformational behaviour of a given molecule, it is necessary to be able to estimate the relative energy of the different possible geometries in a reliable manner. Thus, the lowest energy structure has been calculated using a combination of *ab-initio* techniques and molecular dynamics simulations. The predicted results have been compared and validated using NMR experiments[5,6]. We believe that these results provide key information in order to determine if the analogue could better mimic the natural structural features and, concomitantly, to achieve the stimulation of the immune response in host-pathogen interactions.

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SYNTHESIS OF COMPLEX TYPE *N*-GLYCANS

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Asparagine-linked oligosaccharides (*N*-glycans) on glycoproteins have high diversity and complexity and are involved in a variety of important physiological events, such as protein quality control, cell-cell recognition, adhesion, and signal transduction. To reveal the biological function of *N*-glycans, we have investigated the chemical syntheses of the complex type *N*-glycans such as core fucose containing *N*-glycan and bisecting glucosamine containing *N*-glycan (Figure 1).

We first investigated β-mannosylation, α-sialylation and *N*-glycosylation under microfluidic conditions. Utilization of microflow reactor suppressed the side reaction and enhanced the stereoselectivity of glycosylations even in large scale owing to the precise reaction temperature control.

We then synthesized *N*-glycan containing core fucose and sialic acid. In our synthetic strategy, glycosyl-Asn structure was first synthesized by *N*-glycosylation, and the oligosaccharide chains were then elongated. This strategy facilitates the synthesis of various Asn-containing *N*-glycans, which can be used for the synthesis of glycopeptides or other bioconjugates. We employed convergent synthetic route: two non-reducing end donors were connected to the reducing-end acceptor at the blanched mannose. After the thorough investigation, these key mannosylations were achieved in high yields when ether was used as a solvent. We also discovered that protection of the amide group of NHAc at sialic acid significantly improves the reactivity of glycosylation even at the position apart from sialic acid residues. This diacetyl protection strategy enabled the efficient glycosylation with sialic acid containing fragments. After the construction of dodecasaccharide, global deprotection gave the desired core fucose containing *N*-glycan.[1]

We also synthesized the bisecting GlcNAc containing *N*-glycan via the similar convergent synthetic route. Stereoselective α-mannosylation at the 3 and 6 position at blanching mannose was achieved after the investigation of protection pattern at mannose residue in the glycosyl donor.

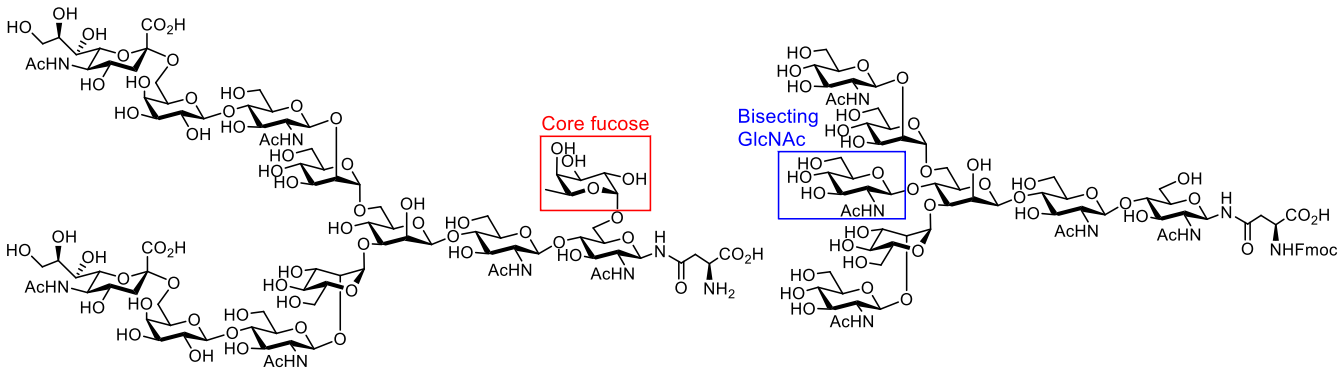

Fig. 1 Core fucose containing *N*-glycan and bisecting GlcNAc containing *N*-glycan

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INVESTIGATION OF GROWTH FACTOR - GLYCOSAMINOGLYCAN-MIMETIC MARINE EXOPOLYSACCHARIDE INTERACTIONS BY ATOMIC FORCE MICROSCOPY

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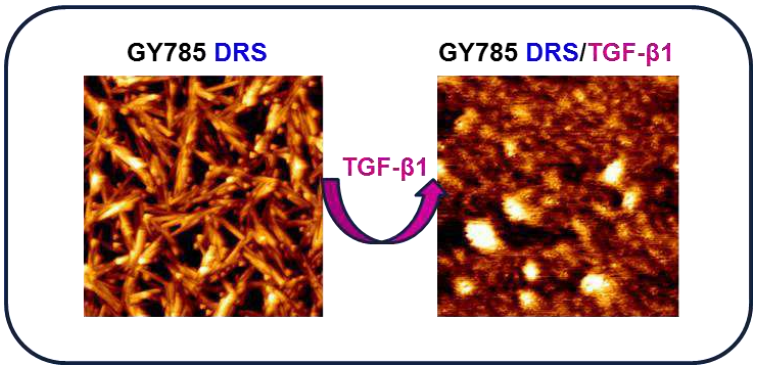
Sulfated polysaccharides, such as glycosaminoglycans (GAG) regulate numerous important biological activities through their interactions with growth factors. Investigating this interaction becomes therefore the key to understand the structure-function relationship of GAG. Oversulfated derivatives prepared from the marine GY785 exopolysaccharide (EPS) have already shown their ability to stimulate the chondrogenic differentiation of human adipose-derived mesenchymal stem cells in the presence of Transforming Growth Factor-β1 (TGF-β1).[1-3] In the present study, in order to elucidate the structure-function relationship of the GAG-mimetic derivatives prepared from marine GY785 EPS, two approaches based on AFM were applied. The affinity between naturally sulfated derivative of low sulfate content (GY785 DR) or chemically oversulfated derivative (GY785 DRS) and TGF-β1 was explored in both imaging and single-molecule force spectroscopy modes. On the one hand, the derivatives and the growth factor were incubated in aqueous solutions and resulting co-assemblies were imaged. On the other hand, the interaction between both derivatives and TGF-β1 was probed by measuring the adhesion forces at single-molecule level. The number of measured interactions and the interaction strength were both higher for the oversulfated derivative compared to the naturally sulfated one. These results clearly emphasize the involvement of sulfate groups in the protein binding and open new ways to tune cellular processes by designing macromolecules with adjustable sulfate charge density.



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SYNTHESIS, STABILITY, REACTIVITY, STEREOSELECTIVITY AND COMPUTATIONAL STUDY OF 4-THIO FURANOSIDES

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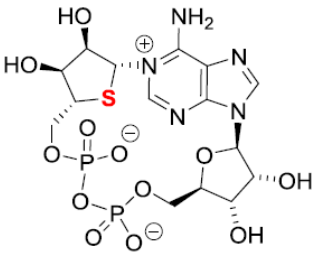
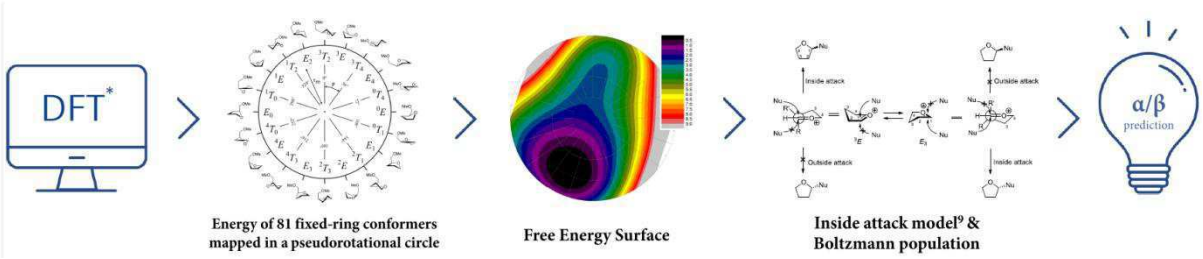
4'-thio analogues of DNA and RNA nucleotides have been studied for their potential anti-viral[1], anti-biotic[2,3] and anti-cancer[3,4] properties. Kinetic studies regarding the acid-catalyzed hydrolysis of 4'-thio nucleosides show a large **decrease in reactivity** compared to the natural 4'-oxo nucleosides.[5] Other examples of **stabilized biomimics** include a 4'-thio analogue of cADPR (figure) with similar biological activity, yet stable enough to be studied,[6] and a 4'-thio nucleotide containing DNA strand which was successfully transcribed by T7 RNA polymerase.[7] Surprisingly the stability of these analogues is still poorly understood. And 5-thio pyranosides actually show a large **increase in reactivity** compared their 5-oxo pyranoside counterparts.[8] Using an approach combining computational and experimental studies[10] the reactivity, stereoselectivity and stability of 4-thio furanosides has been investigated.



Cyclic 4'-thio ADPR



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REGIOSELECTIVE β(1→3) GLYCOSYLATION OF GALACTOSE AND ITS APPLICATION TO THE SYNTHESIS OF GROUP B STREPTOCOCCAL CAPSULAR POLYSACCHARIDE FRAGMENTS

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Despite substantial progresses in the prevention of group B Streptococcus (GBS) disease with the introduction of intrapartum antibiotic prophylaxis, this pathogen remains a leading cause of neonatal infections. Capsular polysaccharide conjugate vaccines have been tested in phase I/II clinical studies, showing promise for further development.[1] Ten serotypes of GBS have been identified on the basis of variation in the sugar composition of the capsular polysaccharide.
The elucidation of polysaccharide epitopes is relevant for understanding the mechanism of action of glycoconjugates and designing synthetic carbohydrate-based vaccines. Recently an X-ray/NMR based approach was applied to determine the epitope of GBS PSIII, which resulted composed of six sugar residues included within two repeating units, paving the way towards the use of synthetic structures for vaccine development.[2]

The repeating units of GBS serotypes Ia, Ib and III share similarities in their sugar composition, such as the NeuNAcβ(1–›3)Gal branch and the GlcNAcβ(1–›3)Gal motif. A flexible and convergent synthetic route could offer access to GBS PSIa, Ib and III defined fragments. We envisaged the regioselective glycosylation of galactose 3-OH to form the disaccharide motif GlcNAcβ(1–›3)Gal as a key step for the synthesis of fragments from the three serotypes. Reported syntheses of this disaccharide, which is recurrently expressed both by bacteria and mammalian cells as well as in milk oligosaccharides,[3,4] employ a galactose acceptor bearing a temporary protective group on position 4, which has to be removed before further elongation of the oligosaccharide. We designed an alternative synthetic approach to disaccharide GlcNAcβ(1–›3)Gal, exploiting the well-recognized higher reactivity of Gal 3-OH compared to the 4-OH in order to achieve a regioselective glycosylation.

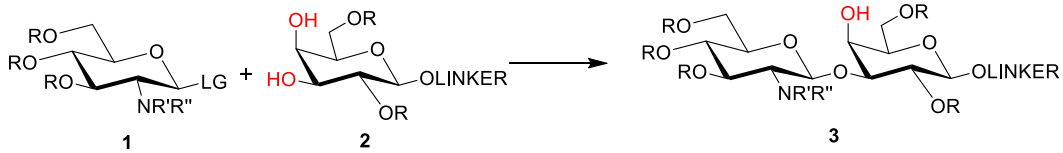

Figure 1. Regioselective approach to disaccharide **3**

After screening a number of building blocks with a different pattern of protective groups, conditions to achieve a regioselective glycosylation at 3-OH of galactose were identified. Unprotected Gal 4-OH was therefore available for a second glycosylation, allowing the synthesis of longer oligosaccharides. This innovative synthetic design was successfully applied to the synthesis of oligosaccharide fragments from different serotypes of GBS capsular polysaccharide, which will be used to support epitope mapping studies and to gain molecular insights into interactions of oligosaccharides with mAbs.

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TRIFUNCTIONAL MANNOSIDE CONJUGATES TO EXPLORE CROSSTALK BETWEEN CLRS AND TLRS AND THEIR EFFECT ON ANTIGEN PRESENTATION

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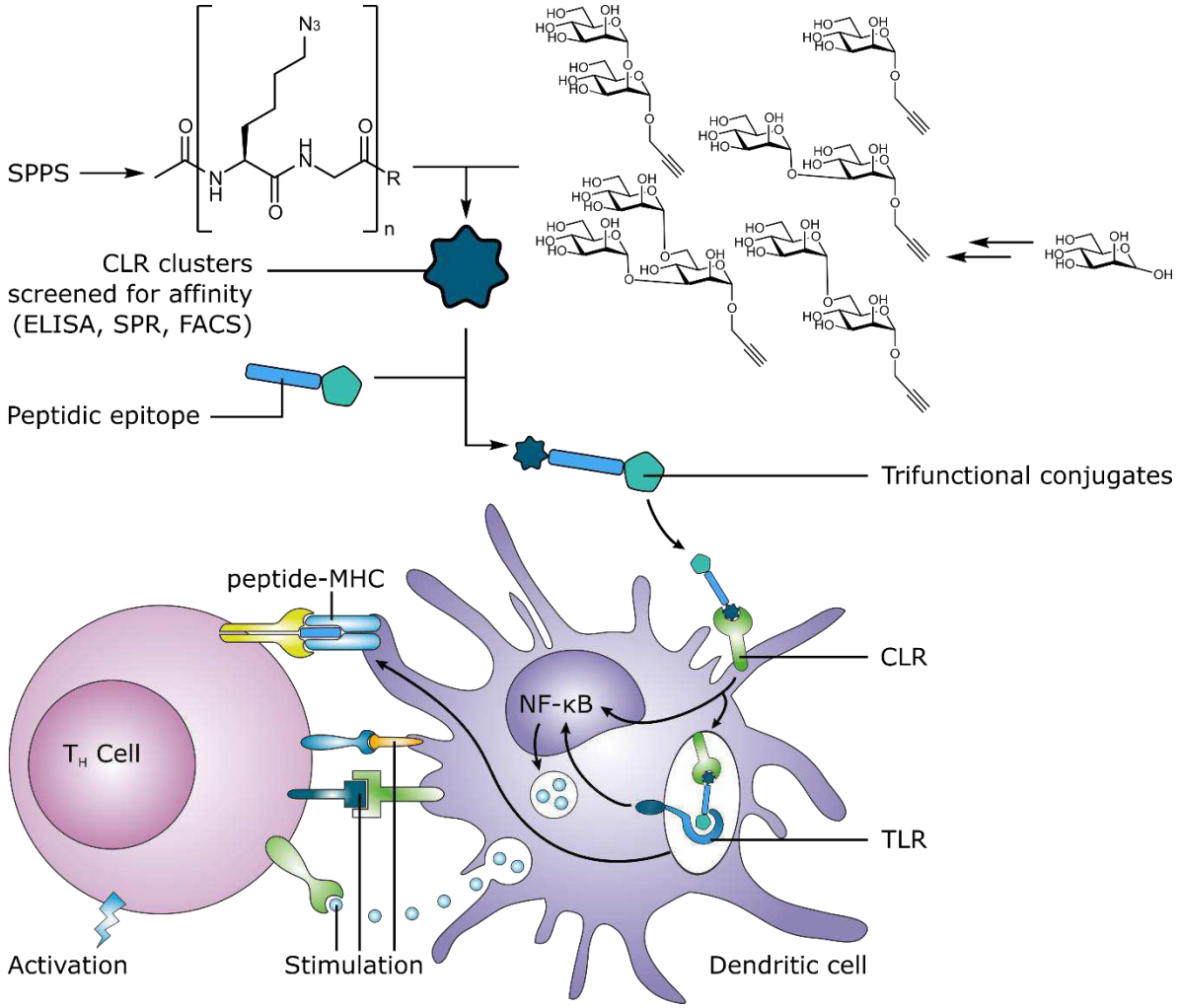
The (adaptive) immune response is a complex system that we have studied with well-defined synthetic single molecules. Trifunctional conjugates are designed to trigger activation and immune modulatory systems simultaneously. Here we study the crosstalk between C-type lectin receptors (CLRs) and Toll-like receptors (TLRs) which can lead to a synergistic enhancement in antigen presentation.[1]



A small library of clusters consisting of *O*-mannosides in increasing valency was synthesized and evaluated for their binding affinity to DC-SIGN and langerin, using enzyme-linked immuno sorbent assay, surface plasmon resonance and flow cytometry. The obtained data were applied for the design and synthesis of a library of well-defined conjugates containing the most relevant mannose clusters, a melanoma epitope and a TLR ligand. These trifunctional conjugates were tested for their ability to activate antigen presenting cells, the level of cross-presentation, and T cell activation by quantification of degranulation surface markers and T cell cytokine excretion. Is there synergy?

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THE MOLECULAR AND FUNCTIONAL IMPACT OF ONCOGENIC O-GLYCAN TRUNCATION ON CD44

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CD44 is a highly heterogeneous glycoprotein that is frequently overexpressed during gastric carcinogenesis and cancer progression. The central role of CD44 in various cellular processes, such as receptor tyrosine kinase activation and cellular adhesion to hyaluronan and selectins, suggests a driving role in the malignant cellular phenotype. CD44 has over 41 described mucin-type *O*-glycosylation sites and is therefore among the highest *O*-glycosylated non-mucin proteins in humans. *O*-glycan structures are frequently truncated in gastric cancer [1,2] and we have previously reported that CD44 is an important carrier of truncated *O*-glycan structures such as sialyl Tn[3,4].

In an attempt to understand the functional impact of *O*-glycan truncation on CD44 we have comprehensively analyzed gastric cancer cell line models that have been glyco-engineered to express truncated *O*-glycans. We have induced the truncation of *O*-glycans through the overexpression of various sialyltransferases or the abrogation of elongation pathways [4-6]. Here we demonstrate that the aberrant *O*-glycan termination by different means affects CD44 molecular features in a highly consistent way. Firstly, we show that CD44 is indeed a major carrier of truncated *O*-glycans and that this *O*-glycan alteration severely alters its molecular mass. Furthermore, we show that the truncation of *O*-glycans is accompanied by profound functional alterations of CD44 affecting its colocalization with receptor tyrosine kinases. This promoted colocalization is accompanied by increased activation of the respective receptors in the gastric cancer cells and more aggressive cellular phenotypes. In addition, one of the major functions of CD44, the binding of hyaluronan, is significantly enhanced by the shortened *O*-glycan structures. Finally, we corroborate our findings in tumors of gastric cancer patients.

Altogether, our results shed new light on the molecular mechanisms of how the truncation of *O*-glycans confers a more malignant cancer phenotype. Our data suggests for the first time that the cellular function of CD44, such as hyaluronan binding and the promotion of receptor tyrosine kinase activation, is modulated in gastric cancer through alterations in the glycosylation machinery, suggesting new biomarkers and therapeutic strategies.

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GLYCOSYL NITRATES IN SYNTHESIS: FROM SELECTIVELY PROTECTED BUILDING BLOCKS TO OLIGOSACCHARIDES

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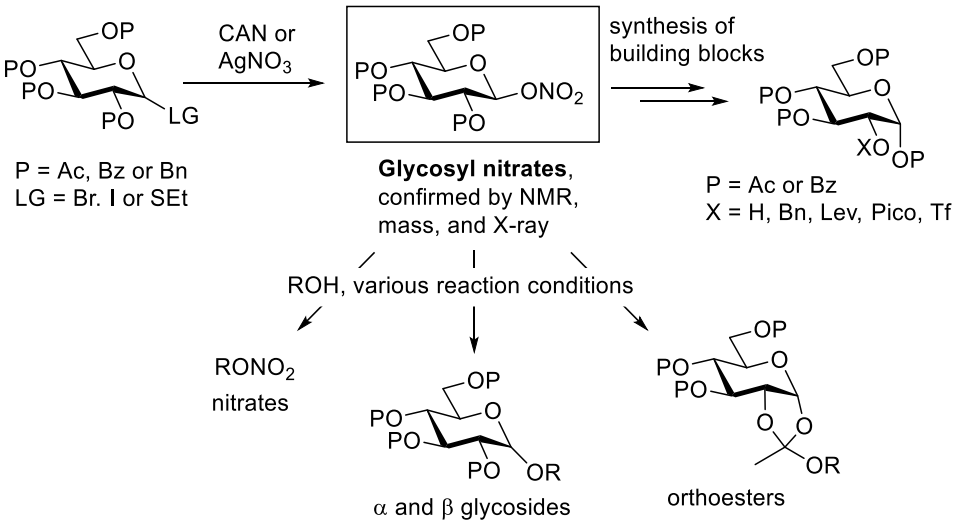
The differentiation between the trans-diequatorial C-2 / C-3 hydroxyl group pair in the D-gluco series is one of the most challenging tasks in regioselective protection of carbohydrates. In attempt to refine a CAN-mediated synthesis of 1,3,4,6-tetra-O-acetyl-α-D-glucopyranose (2-OH glucose)[1] earlier reported by our group,[2] we unexpectedly discovered that this reaction proceeds via the intermediacy of glycosyl nitrates. Improved mechanistic understanding of this reaction led to the development of a more versatile synthesis of 2-OH glucose from a variety of precursors. The isolated yield of the final product was increased to 75%.[3] Also demonstrated was the conversion the 2-OH glucose into a variety of building blocks differentially protected at C-2, a position that it generally hard to protect regioselectively in the glucopyranose series.

Meanwhile, we also observed that glycosyl nitrates can act as effective glycosyl donors in the presence of a variety of promoters. In addition, the synthesis of 1,2-orthoesters and sugar nitrates at the non-anomeric positions has been demonstrated. These new synthetic approaches will be discussed in the context of the synthesis of challenging oligosaccharides.



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ONE-POT SYNTHESIS OF PSEUDO-THIODISACCHARIDES THROUGH AZIRIDINE OPENING REACTIONS

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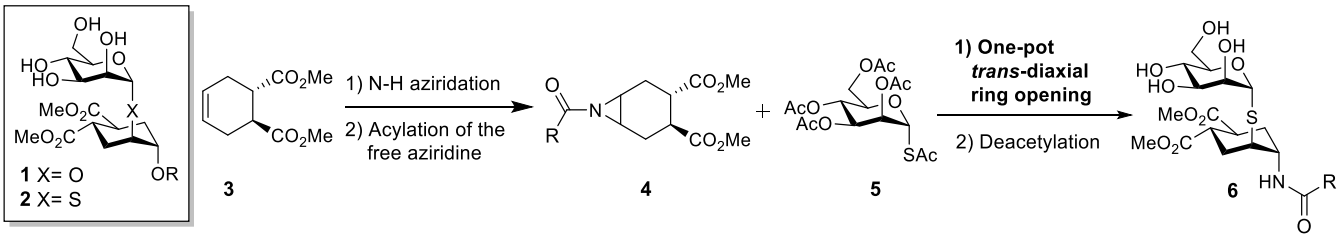
Glycomimetics are under active investigation as antagonists of medically relevant lectins implicated in cell-cell communication and pathogen recognition events. Indeed, carbohydrate mimics can be designed as competitors of natural lectin ligands and they often show improved drug-like characteristics and stability to enzymatic degradation.

Our group has been developing selective DC-SIGN ligands based on the structure of the pseudo-1,2-dimannoside **1**.[1] Recently we have also reported an efficient synthesis of the corresponding pseudo-*thio*-1,2-dimannoside **2**.[2] Since they are tolerated by most biological systems, thioglycosides and derivatives are attracting significant interest in the development of new therapeutics. In particular compound **2** was found to share with **1** both the conformational behavior and the DC-SIGN binding activity, but displayed an improved resistance to enzymatic hydrolysis by α-mannosidase (jack bean), thanks to the presence of the sulfur-linkage.

We now report conditions for the one-pot ring-opening reaction of aziridine **4** by α-mannosyl thiolate, generated *in situ* by a selective *thio*-deacylation of **5**. Starting from the enantiomerically pure olefin **3**, the free aziridine was prepared[3] and coupled *in situ* with various functionalized or un-functionalized acylating agents, to give *N*-acylaziridines of general structure **4**. Finally, the pseudo-*thio*-disaccharide **6** was obtained as a single isomer by an exclusively *trans*-diaxial opening of **4** by the α-thiolate of **5**, followed by deacylation of the sugar moiety.

All the synthetic and mechanistic aspects will be discussed, as well as preliminary data about the scope of the approach. Furthermore, the affinity of the *thio*-glycomimetics **6** towards DC-SIGN, as determined by SPR analysis, will be presented.



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A NEW REACTION OF SWITCHABLE RING CONTRACTION IN SELECTIVELY PROTECTED PYRANOSIDES

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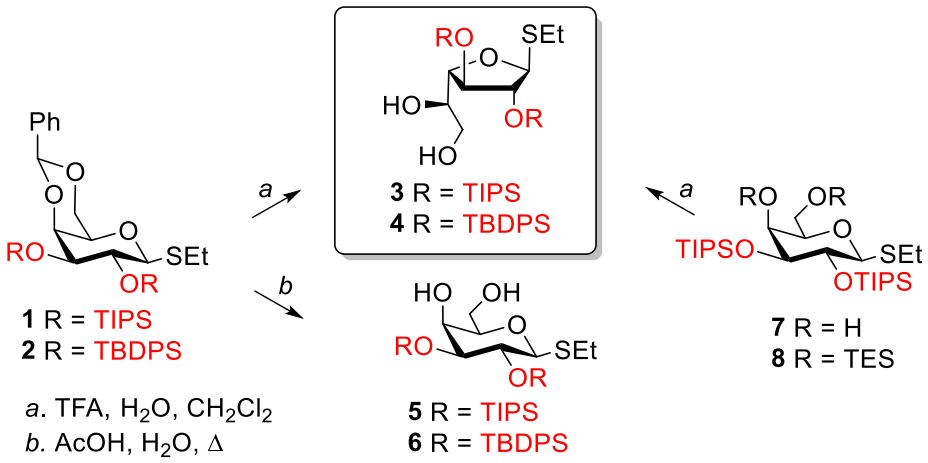
Synthesis of furanose-containing oligosaccharides is an important area of current research because furanoses are frequent constituents of polysaccharides of pathogenic microorganisms, and several methods to access furanosyl building blocks have been reported [1].

Bulky silyl substituents in a glycosyl donor are known to significantly alter the geometry of the pyranose ring, which affects outcome of glycosylation [2]. We used stereocontrolling effect of a single TIPS group in a glycosyl donor to achieve efficient 1,2-*cis*-glycosylation [3,4]. Here, we report the ability of bulky silyl groups in the molecules of thioglycoside glycosyl donors to favor contraction of the pyranose ring with retention of aglycon under mild acidic conditions.

We found that the choice of acid used for removal of 4,6-*O*-benzylidene group determines the size of the ring in the product formed. Heating of ethyl 1-thio-β-D-galactopyranosides (**1**, **2**) containing TIPS or TBDPS groups at O-2 and O-3 with aqueous acetic acid gave the normally expected diols (**5**, **6**) in pyranose form. Conversely, treatment of **1** or **2** with aqueous trifluoroacetic acid in CH_2Cl_2 led to the corresponding partially protected ethyl 1-thio-β-D-galactofuranosides (**3**, **4**) with the same anomeric configuration (70–80% yield). Similar results were obtained for diol **7** or derivative **8** with acid-labile TES groups at O-4 and O-6. A possibility to switch the direction of the reaction by choice of an acid may present an advantage in the divergent synthesis of selectively protected glycosyl donors for further use in glycosylation. Scope and limitations of the discovered rearrangement will be discussed. This work was supported by the Russian Foundation for Basic Research (Project No. 16-03-00755).



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A VERSATILE APPROACH TO MOLECULAR DESIGN OF MONOVALENT AND MULTIVALENT C-GLYCOCONJUGATES AS LIGANDS OF BACTERIAL LECTINS

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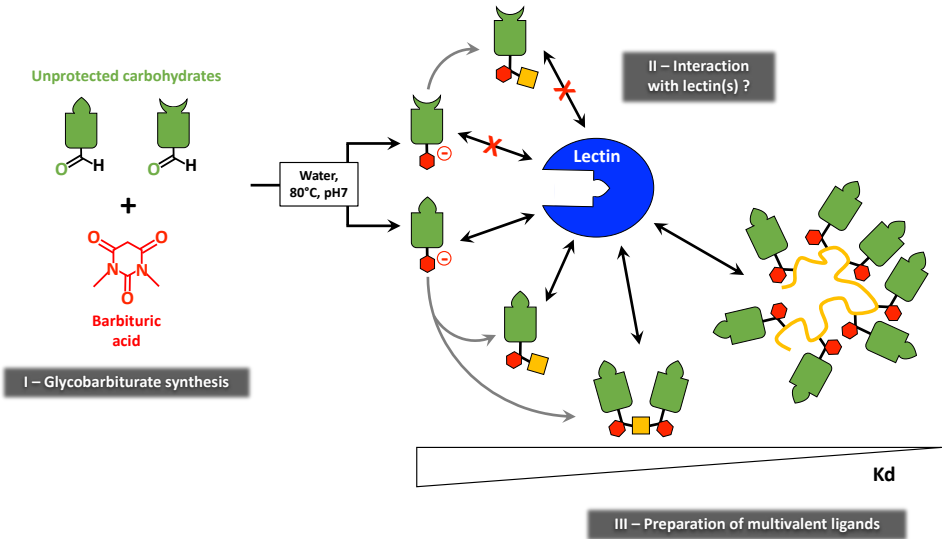
Glycocomponents participate to a wide range of crucial functions within living organisms. They constitute a chemical source of energy (e.g.: glucose), play a structural role (e.g.: cellulose, chitin, hyaluronic acid…) or are involved in specific molecular recognition events (e.g.: glycocalyx). In glycobiology, there is a need to develop chemical tools allowing the access to glycomimetics or (neo)glycoconjugates in order to treat or/and diagnose disease associated to carbohydrate-recognizing proteins (lectins, glyco-enzymes, growth factors, etc.). Thus, glycochemist community has developed various, but still limited, chemoselective ligations to the anomeric hydroxyl group at the reducing end of carbohydrates. One of most interesting is certainly the preparation of *C*-glycosides.

Due to their C-C glycosidic bond, *C*-glycosides have the great advantage to be hydrolytically stable analogues of the natural *O*-glycosides. Indeed, they can be used as glyco-enzymes inhibitors [1]/substrates [2] or as ligands of lectins [3].

Among the numerous reactions already developed to produce *C*-glycosides, Knoevenagel condensation offers several strong advantages. This reaction is chemo- and stereo-selective (essentially β-glycosidic bond), directly performed from unprotected carbohydrates and occurs in water at mild conditions. Surprisingly, few works have been carried out on this reaction and particularly on the condensation of carbohydrates with barbituric acids [4].



The presentation will discuss about an enlarged study of the scope of this reaction using carbohydrates interacting with lectins coming from opportunistic pathogens. Thermodynamic studies of lectin−*C*-glycosyl barbiturates interactions have been determined by isothermal titration calorimetry. Structure-binding relationships have been extracted in order to evaluate the impact of the barbiturate moiety (e.g.: steric hindrance, presence of a negative charge…). Finally, after a detailed analysis of the best carbohydrate ligands for the lectin PA1L, we have developed a convenient and original method to prepare multivalent ligands (from glycoclusters to glycopolymers) for affinity enhancement.

Acknowledgments: This work was supported by CNRS, Université Grenoble Alpes and the Labex ARCANE (ANR-11-LABX-0003-01).

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SYNTHESIS OF SECONDARY CELL WALL POLYMER FRAGMENTS OF *PAENIBACILLUS ALVEI*

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Surface-layer proteins of *Paenibacillus alvei* are non-covalently bound to the cell envelope through the pyruvylated ManNAc residue of the bacterium's secondary cell wall polymer (SCWP) [→3)[4,6-*O*-(pyruvyl)]-ManNAc-(β1→4)-GlcNAc-(β1→] [1]. In order to elucidate molecular details of the binding interactions and the biosynthetic steps involved in the pyruvyl transfer, the synthesis of target compounds **7** and **8** was performed. Synthesis of the disaccharide-backbone was elaborated from **1** and **2** leading to the NPTFA glucosyl donor **3** and the activated glucosyl acceptor **4**, respectively. Subsequent glycosylation then gave the β-(1→4)-linked disaccharide **5** in good yields. Inversion of the *gluco* configuration at position 2 and introduction of the 2-NHAc moiety gave access to the central intermediate **6** allowing for global deprotection with or without selective pyruvylation (Fig. 1).

In addition, the enzymatic pathway was investigated *in vitro* using 11-phenoxyundecyl-diphosphoryl GlcNAc as acceptor [2], coupled with sequential reactions of TagA as a UDP-β-D-ManNAc:GlcNAc-lipid carrier transferase and UDP-ManNAc, followed by treatment with CsaB as a pyruvyltransferase and PEP as donor substrate [3]. The isolated reaction product **9** was fully characterized by MS and NMR.

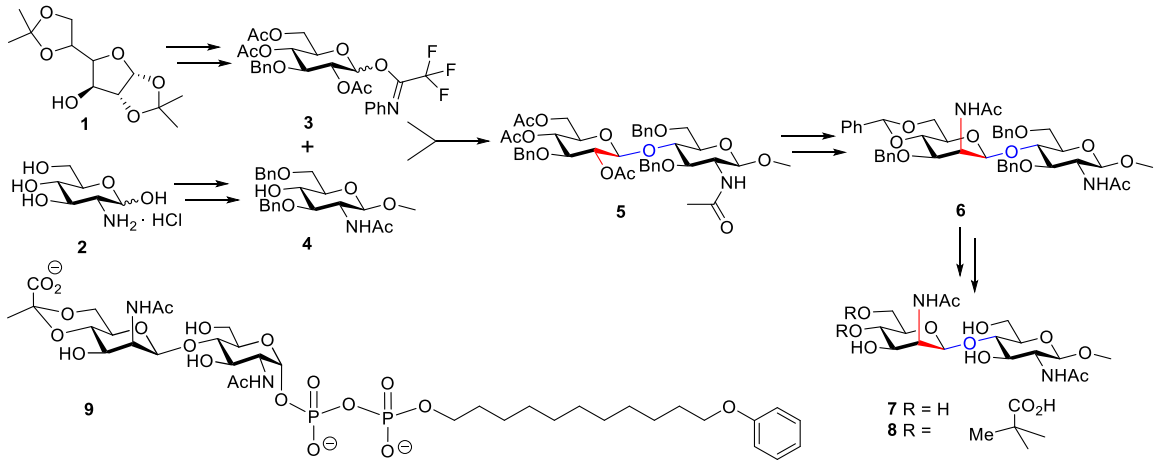

Fig. 1. Synthetic route to the disaccharide repeat of SCWP and structure of the enzymatic pyruvylation product **9**.

Acknowledgment: Financial support from Austrian Science Fund (FWF, grant P27374-B22 to CS).

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POLYSACCHARIDE BLOCK COPOLYMERS

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More effective exploitation of recalcitrant polysaccharides and the need for supplementing or even replacing synthetic (oil based) polymers by carbon neutral resources, have initiated research to prepare and explore new block copolymers partly or solely composed of poly- or oligosaccharides [1]. By terminal coupling of polysaccharides, the intrinsic properties of the biopolymer are retained, as opposed to the traditional lateral grafting where the functional groups are modified [2,3]. By taking advantage of the intrinsic chemical and physical properties of the native polysaccharides, it is possible to induce chain-chain interactions by using different switches (*e.g.* pH, ionic strength, temperature etc.) to form self-assembling structures [2].

As a first step, we have conjugated the reducing end of different polysaccharides (chitin, chitosan, alginate and dextran) to bifunctional hydrazides (NH_2-NH-CO-) or oxyamines (NH_2-O-). The rate and equilibrium yields were determined by real time NMR. Schiff bases (E- and Z- oximes) were observed to be the major products for oxyamines, whereas the hydrazides predominantly formed cyclic *N*-pyranosides. In a second step, we reduced the products to form stable conjugates. We observed that the Schiff bases were readily reduced in contrast to the more stable cyclic *N*-pyranosides. The equilibrium yields, the type of products formed, and the reaction rates were strongly dependent on pH and concentration of hydrazide/oxyamine. However, the reaction was independent of the degree of polymerization of the oligosaccharides. We have further prepared and characterized block copolymers composed of oligomers conjugated to both sides of the bifunctional hydrazides/oximes as a proof of principle for the preparation of polysaccharide block copolymers.

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OPTIMISATION OF A CARBOHYDRATE-FUNCTIONALISED COLLAGEN-BASED SYSTEM FOR VENTRAL MESENCEPHALIC CELLS DELIVERY

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Parkinson's Disease (PD) is a neurodegenerative disorder primarily characterised by the death of dopaminergic neurons in the substantia nigra, which relates to different movement disorders [1]. However, currently there are only symptomatic therapies available and none of these systems address the specific pathophysiology of the disease [2]. Hydrogels have shown potential as a vehicle for delivery of cells into the brain, namely ventral mesencephalic (VM) embryonic cells (recently used in PD clinical trials), protecting them from the host environment and maintaining their viability [3]. One biomaterial of interest is collagen since it has high compatibility with neural tissue [3] and is suitable for cell encapsulation. Additionally, the therapeutic potential of collagen can be further expanded by functionalising it to address a specific aspect of PD pathology, such as the glyco-signature and differently regulated sugars upon onset of the disease. The goal of this project is to develop and optimise a glycan-conjugated collagen-based hydrogel that will target the differently expressed carbohydrates in PD models, while encapsulating embryonic VM cells to replace the lost neurons.

In this study, collagen microgels were fabricated using different concentrations of polymer (2 and 3 mg/ml) and crosslinker (4S-StarPEG at 0.2 and 0.4mM) and their properties studied, such as their stability, complex viscosity and encapsulation profiles (Figure 1. A-C). Afterwards, the collagen was conjugated with different glycans (glucose, galactose, 3'-siallylactose, 2'-fucosyllactose) in order to expose gluco-, galacto-, 3'-sialylgalacto-, and 2'-fucosylgalactose. The functionalised compounds were analysed (Figure 1. D-F). These functionalised systems were used to fabricate gels and their biological properties assessed.

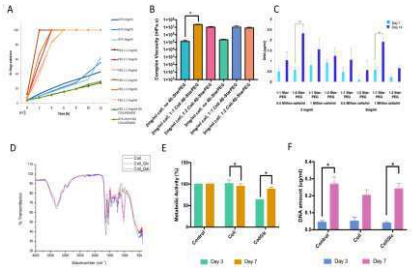

Fig. 1 - Characterisation studies of the compounds used. **A.** Degradation of the gels was assessed through Coomassie Brilliant Blue assay. 3mg/ml collagen microgels show higher crosslinking efficiency and stability. **B**. Complex viscosity of the collagen gels shows high stability in all crosslinked gels. **C.** Cell proliferation of VM cells inside collagen microgels assessed through the PicoGreen® assay. VM cells inside collagen microgels proliferate in all groups up to two weeks post-encapsulation. **D.** Spectra of the functionalised collagen with glucose and galactose, where the peaks between 1000 and 1200cm^{-1} highlight the binding between the collagen and the glycans. **E & F** Cytotoxicity assays performed to assess the effect of the gels coll-glc on primary VM cells extracted from rat embryos.

The collagen microgels have high crosslinking efficiency and stability and were suitable to successfully encapsulate embryonic VM rat cells, showing high viability up to two weeks in 2mg/ml collagen and 0.4mM 4S-StarPEG microgels. In parallel, glycan expression was analysed in pre-clinical models of PD to choose the optimal for use in future *in vivo* studies.

Acknowledgements: EU Horizon 2020 Programme (H2020-MSCA-ITN-2015) under the Marie Skłodowska-Curie Innovative Training Network and Grant Agreement No. 676408 and SFI, co-funded under the European Regional Development Fund, Grant Number 13/RC/2073.

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SMART HYBRID GLYCO NANOCARRIERS FOR THERANOSTIC

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Hepatic cancer was declared the second deadliest cancer worldwide by the world health organization in 2015, and therefore there is a large interest on the development of a theranostic approach for this disease. Bionanotechnology applied to medicine have reached a point where drug delivery systems can be controlled by external or internal stimuli reducing drug side effects and improving bioavailability, solving the requirement for high drug concentration on cancer cells, while avoiding toxic effects in healthy tissues [1].

In this communication we will present the development of a hybrid nanocarrier that may be recognized by the asialoglycoprotein receptor in human hepatocellular carcinomas, and release therapeutic agents on cancer cells by a pH stimulus. To achieve this objective, we develop multifunctional mesoporous silica nanoparticles (MSNs) coated with a stimuli-responsive polymer containing biomimetic glycopolymers. MSNs with controlled pore structure and small size offer several advantages, including high loading capacity and the ability to protect the guest molecules in the systemic circulation. In addition, incorporation of a highly bright dye in the pore structure will allow a live track of the nanoparticles using optical imaging techniques [2]. MSNs with diameter under 100 nm were coated with a copolymer shell of a pH-responsive inner block and a glycopolymer outer block, using a “grafting from” methodology by controlled radical polymerization. A tertiary amine-based acrylate monomer was selected due to the pKa values similarity with the pH of cancer cells and tissues. In acidic medium (extracellular tumor or endosomes) the tertiary amine is protonated leading to a structural modification of the polymer chains, from a globule conformation (normal tissues, higher pH) to an extended coil conformation [3]. This transition will be used for the controlled release of the drug from the pore structure of the MSNs. Last but not least, regarding the sweet part of this work, we selected a galactose-based polymer, to target tumorous hepatic cells due to the presence of hepatic asialoglycoprotein receptor, a type C-lectin that binds reversibly and specifically to galactose and *N*-acetylgalactosamine [4].

This platform will provide both diagnostic and therapeutic (theranostic) functionalities and is a promising approach to achieve controlled release with site-specific delivery of drugs to different tumors by changing the sugar recognition moiety.

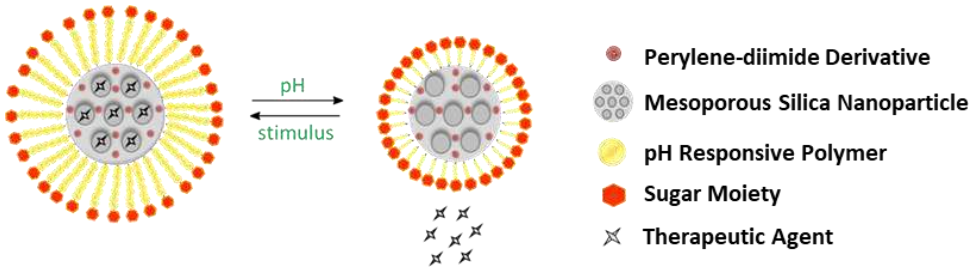

Fig. 1 – Smart hybrid glyco nanocarrier general scheme.

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SYNTHESIS OF WELL-DEFINED TEICHOIC ACIDS FRAGMENTS AND THEIR EVALUATION THROUGH MICROARRAY TECHNOLOGY

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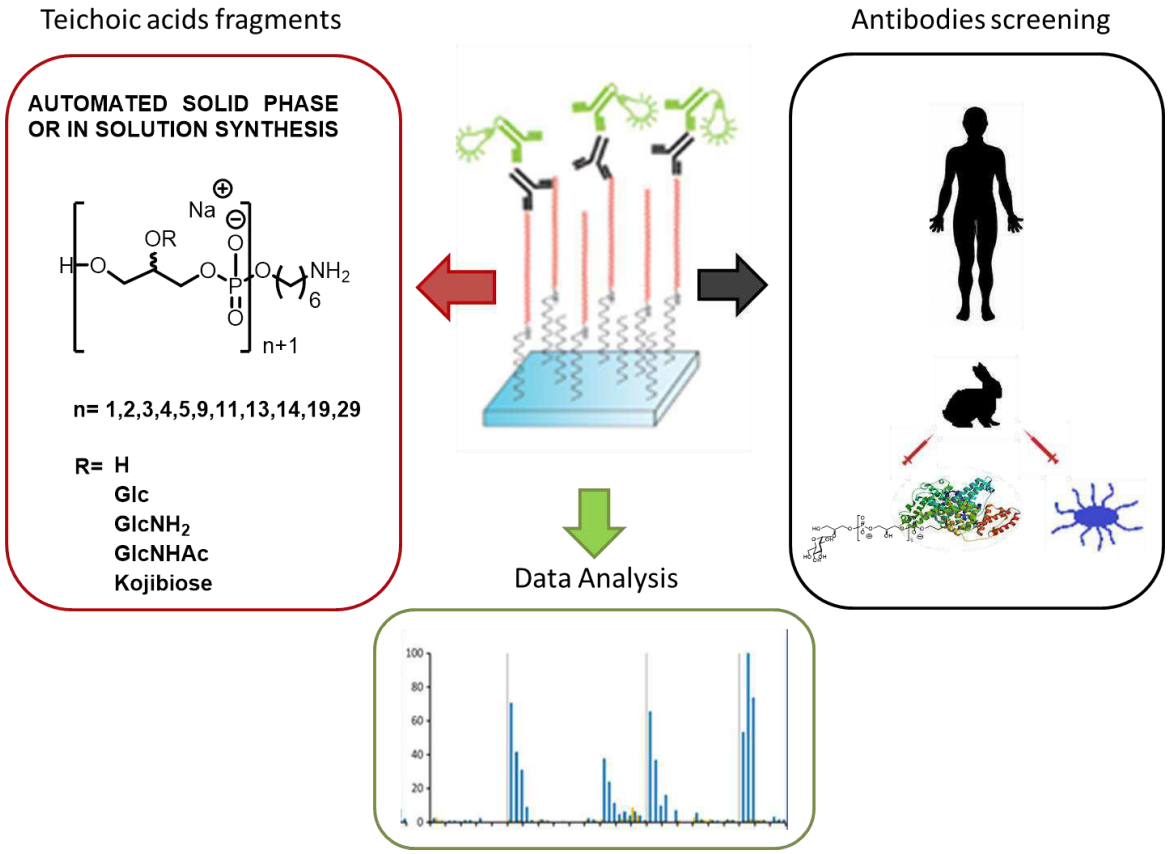
Staphylococcus aureus, Enterococcus faecalis and *Enterococcus faecium* are Gram-positive bacteria that are able to establish coexistence within the human body from the first hours of our lives. Unfortunately they've also been identified as the major cause of nosocomial and community acquired bacterial infections and in the last decades they showed an increased antibiotic resistance. Teichoic acids (TA) are the main structural element of the bacterial cell wall that is shared among the bacterial species mentioned above and therefore these TAs have been selected as possible universal antigen candidates for a broader protection in vaccination strategies.[1] Here we present the generation of a synthetic library of well-defined glycerolphosphate fragments varying for the number of repeating units and the position of different carbohydrate appendages (α-kojibiosyl, α-glucosyl, α-glucosamine and *N*-acetyl α-glucosamine residues) along the chain. In order to investigate and understand the interaction of TAs with the immune system, a structurally diverse TA-microarray platform has been generated.[2] Differences in binding recognition using different sera sources (immunized rabbits, healthy volunteers, infected patients) are revealed at molecular level (See Figure below).



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RECENT DEVELOPMENT IN GLYCO-RGD CONJUGATES: SYNTHESIS, BIOLOGICAL EVALUATION AND PET APPLICATION

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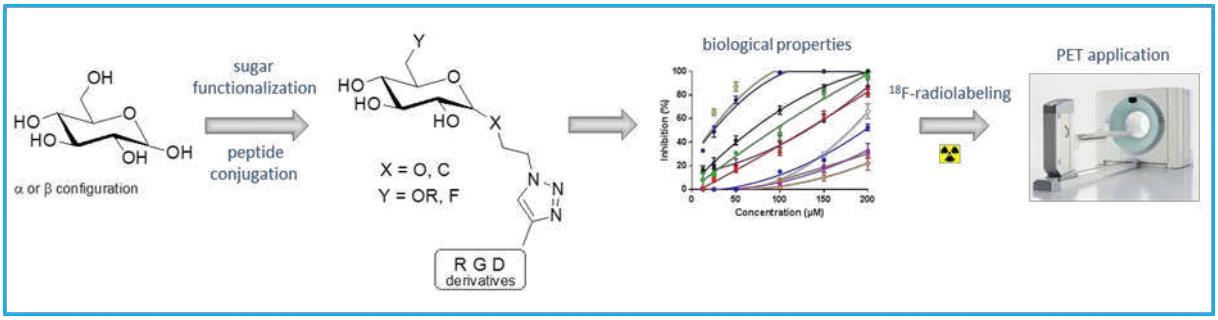
Integrins are cell-adhesion molecules involved in angiogenesis signaling pathways and overexpressed on many cancer cells.[1] The most extensively studied integrins are the integrin $\alpha_v\beta_3$, which represents a highly specific biomarker in particular to monitor cancer progression and the $\alpha_{IIb}\beta_3$ integrin that lies across the plasma membrane of human platelets. The arginine-glycine-aspartic acid amino acid sequence (RGD) is one of the first examples of short peptides which bind integrins. Glycosylation of biomolecules and more particularly peptides for example is known to enhance their bioavailability, pharmacokinetic, and in vivo clearance properties.[2]

In this context and in connection with our researches on the development of glycosylated tracers for PET (Positon Emission Tomography) imaging,[3] we developed the synthesis of new types of glyco"RGD" built with a *C*-glycosidic bond.[4] As powerful tool in the chemical ligation, the CuAAC was selected to conjugate glycosides derivatives and RGD peptides containing a cysteine residue. Biological evaluation of the new conjugates was investigated (ADP-induced platelet aggregation and effects on endothelial cells). Promising biological properties were obtained for these glyco-RGD derivatives. These results prompted us to envision the use of these derivatives as tools for diagnosis purposes in PET imaging.



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NANOMOLAR INHIBITION OF HUMAN OGA BY 2-ACETAMIDO-2-DEOXY-D-GLUCONO-1,5-LACTONE HYDRAZONE DERIVATIVES

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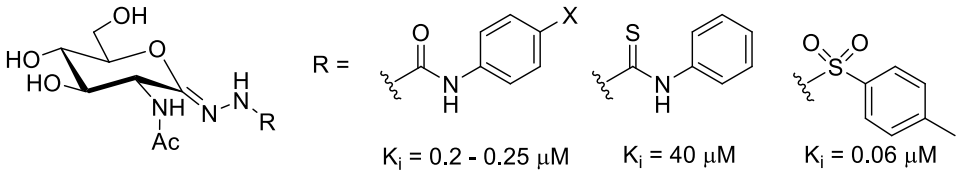
O-GlcNAcylation is a dynamic post-translation modification by adding and removing a single β-*N*-acetylglucosamine (GlcNAc) moiety to and from serine/threonine residues of nucleocytosolic and mitochondrial proteins. The dynamic cycling of *O*-GlcNAc modification on numerous, functionally diverse set of proteins is catalyzed by two enzymes: *O*-linked β-*N*-acetylglucosamine transferase (OGT) and β-*N*-acetylglucosaminidase (OGA) in response to cellular signals or cellular stages, similarly to phosphorylation. Indeed, in many cases, phosphorylation and *O*-GlcNAcylation target the same serine/threonine residues in a reciprocal manner. Hyperphosphorylated tau proteins are observed in human brains with Alzheimer Disease showing noticeably lower levels of *O*-GlcNAc than the levels found in healthy brains. In a rodent model, the pharmacological inhibition of OGA resulted in an increased *O*-GlcNAc level and consequently slowed down the progression of neurodegeneration.[1] Human OGA is a promising therapeutic target in diseases where aberrant low level of *O*-GlcNAc is experienced.

Full length human OGA isomorph has been heterologously expressed* and an expression and isolation strategy was developed to obtain soluble, stable and active wild type enzyme for inhibition studies. Environmental factors were also revealed that greatly affected OGA stability. 2-Acetamido-2-deoxy-D-glucono-1,5-lactone hydrazones were synthesized by adaptation of a literature procedure.[2] Kinetic studies using either 4-nitrophenyl-*N*-acetyl-β-D-glucosaminide as a chromophore substrate or a fluorescent artificial substrate (4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide) revealed some of the synthetized molecules to be potent competitive inhibitors of OGA. Details of the synthesis, enzyme expression and kinetic studies will be shown in the presentation.



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A HYBRID APPROACH TO SIALYLATED GLYCAN SYNTHESIS: COMBINATION OF AUTOMATED SOLID-PHASE AND ENZYMATIC SYNTHESIS

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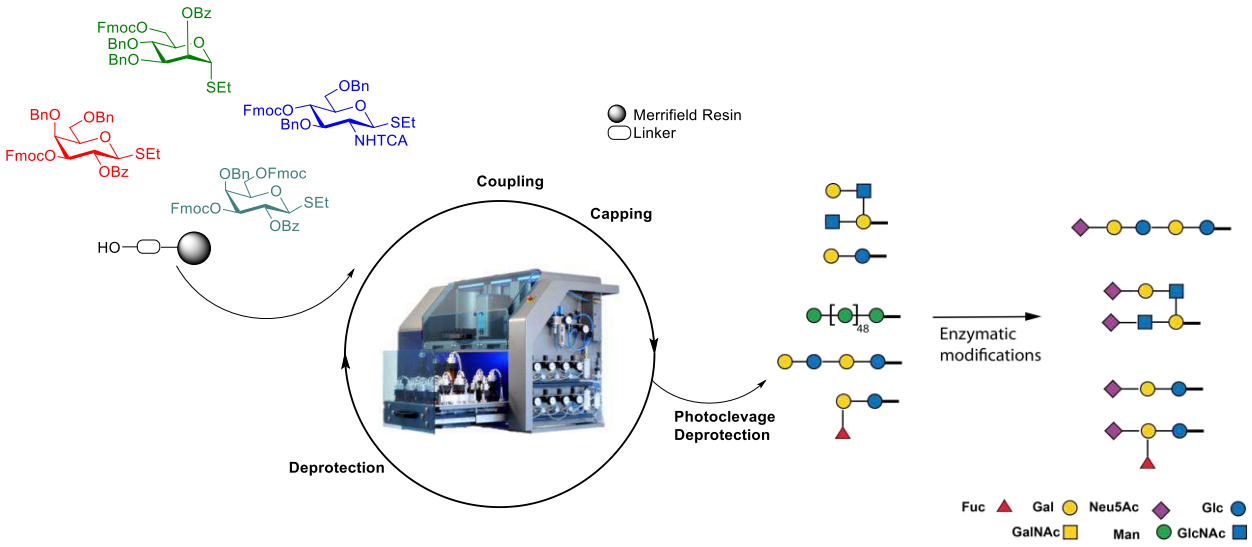
Synthesis of complicated oligosaccharides is a very time-consuming and challenging problem for many research groups. Solid Phase Automated Glycan Assembly (AGA) has been demonstrated to be capable of producing such molecules in fraction of time compared to traditional approaches, e.g. a 50-repeating units of Mannosides can be obtained in one week [1]. For exquisite control of regio- and stereo-selectivity, difficult motifs such as sialylated glycans require the use of enzymatic glycosyltransferases. In this work, we combine the power of AGA to provide rapid access to large library of N-glycan precursors, which are suitable substrates for enzymatic modification. Asymmetric as well as non-linear structures including α (2,3)-sialylated [2] and α (2,8) linkages are now available for further biological studies



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LENGTH MATTERS: ONE EXTRA METHYLENE GROUP IN GLYCOSYL ACCEPTOR CAN DRAMATICALLY CHANGE THE REACTIVITY PATTERN OF GLYCOSYL DONOR

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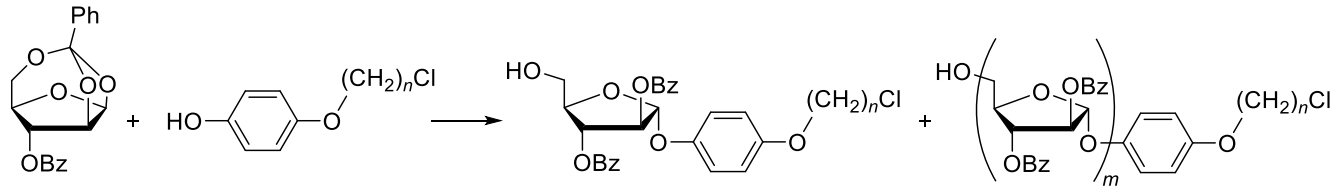
Arabinofuranose orthoesters have been widely used in the synthesis of arabinofuranosides related to mycobacterial arabinans [1]. Direct access to selectively protected arabinofuranose building blocks with removable 4-(2-chloroethoxy)phenyl (CEP) pre-spacer aglycon, useful in the block synthesis of neoglycoconjugates [1], from orthoester **1** is surprisingly difficult since, unlike clean reaction with alcohols [2,3], nucleophilic opening ($SnCl_4$, CH_2Cl_2, –25 °C) of **1** with 4-(2-chloroethoxy)phenol (CEP-OH, **2a** [4]) leads mainly to $\alpha(1\rightarrow5)$-linked arabinofuranose oligomer-homologs **4a** [5] rather than to the expected monosaccharide glycoside **3a**. This unfortunate feature of **2a** seems to be related to its limited solubility. Noteworthy, its homolog CPP-OH (**2b**) [4] with one extra methylene group is featured by substantially higher solubility in comparison with that of **2a**. Structure of solutions of **2a** and **2b** in CH_2Cl_2 was studied by combination of NMR and IR spectroscopy, and dynamic light scattering (DLS). At low concentration (0.02 M) both phenols form solutions in which solute molecules are linked by weak hydrogen bonds (δ_H(OH) 4.54 ppm, ν(OH) 3585 cm^{-1}) to form similar non-covalently-bonded supramolecular aggregates (supramers [6]) of **2a**,**b** with OH groups apparently being *buried* inside, which lowers their nucleophilicity. Accordingly, orthoester **1** reacts with these supramers of **2a**,**b** similarly, oligomerisation being the dominant reaction pathway. At higher concentrations (0.1–1.2 M), which are inaccessible for CEP-OH (**2a**) due to its low solubility in CH_2Cl_2 (0.02 M at –25 °C), CPP-OH (**2b**) forms solutions that are featured by the presence of supramers (detectable by DLS), in which individual molecules are linked with stronger hydrogen bonds (δ_H(OH) 5.20 ppm, ν(OH) 3429 cm^{-1}), with OH groups apparently being *exposed* hence possessing higher nucleophilicity. Accordingly, nucleophilic opening of **1** with **2b** proceeds faster and consistently gives high yields (70–77%) of monosaccharide glycoside **3b** with CPP aglycon in wide range of concentrations of **2b** (0.1–1.2 M) and molar ratios of reagents (**1**:**2b** = 1:2.5 – 1:8). The results obtained may be relevant for glycosylation reactions involving other nucleophiles including carbohydrate alcohols also capable of forming hydrogen-bonded supramers of variable structure hence different reactivity. This work was supported by the Russian Science Foundation (Project No. 16-13-10244).



1 | **2a** *n* = 2 (CEP-OH), **2b** *n* = 3 (CPP-OH) | **3a** *n* = 2, **3b** *n* = 3 | **4a** *n* = 2, *m* ≥ 2, **4b** *n* = 3, *m* ≥ 2

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CAPTURING THE CONFORMATIONAL BEHAVIOUR AND STEREOSELECTIVITY OF OXOCARBENIUM IONS

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The broad application of synthetic oligosaccharide constructs in glycoscience and other fields is largely hampered by the lack of control on the stereochemical outcome of glycosylation reactions. Oxocarbenium ions are key reactive intermediates in the glycosylation reaction. As a result of the fleeting nature of these high-energy intermediates, it is currently impossible to study them directly under relevant classical glycosylation conditions. *In silico* we disclose the three-dimensional structural preference of a set of oxocarbenium ions (more than 25 examples) by mapping their conformational energy landscape (CEL). In combination with experimental results, we confirm the crucial impact of the conformational behaviour of the oxocarbenium ion on its stereoselectivity.

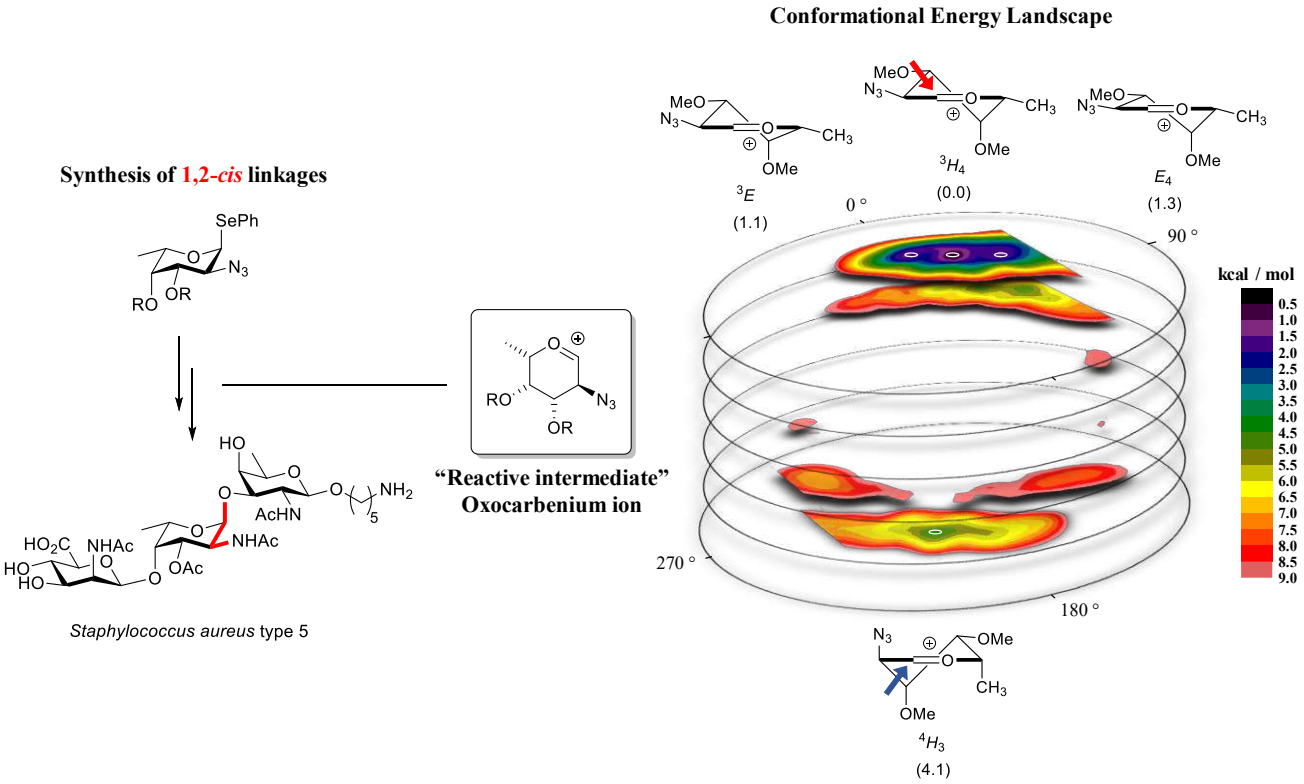

Figure 1. Mapping the conformation and reactivity of glycosyl oxocarbenium ions to understand the role of these reactive intermediates in the assembly of complex oligosaccharides [1].

One example of many is depicted in Figure 1, in which the oxocarbenium ion excellently explains the stereochemistry of the product in the assembly of *S. aureus* type 5 trisaccharide. A key finding based on the CEL maps is the tremendous preference of fully substituted per-*O*-benzylated protected glycosyl oxocarbenium ions to form 1,2-*cis* linkages, which are to date by far the most complicated linkages to synthesise. This outcome was confirmed by experimental model glycosylations and will be a crucial finding for future glycosylation approaches.

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SHAPE-CONTROLLED GOLD NANOPARTICLES FUNCTIONALIZED WITH SYNTHETIC OLIGORHAMNOSES OF GROUP A STREPTOCOCCUS

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Group A streptococcus (GAS) causes a large range of diseases from skin infections to dangerous post infections, like acute rheumatic fever and rheumatic heart disease. These rheumatic disorders are the major cause of heart diseases in children and adolescents in developing countries, responsible for about 350.000 deaths per year [1]. The Lancefield group A carbohydrate (GAC), consisting of α-L-Rha*p*(1→3)-α-L-Rha*p*(1→2)-β-D-Glc*p*NAc-(1→3) repeats is highly conserved and expressed in all the GAS serotypes, and has been proposed as a target for glycoconjugate vaccine development. However, recently it has been hypothesized that GlcNAc residues could provoke cross-reactive antibodies relevant to immunopathogenesis of rheumatic diseases [2], while structures containing only polyrhamnose backbone promote the opsonophagocytosis of multiple GAS strains [3].
In this scenario, we decided to synthesize oligorhamnoses of different chain length, (tetra and hexa-rhamnose) and to explore the effect of their multivalent presentation on anti-polyrhamnans or GAS polysaccharide antibody recognition.
Gold nanoparticles (AuNPs) offer great potential for many applications due to their biocompatibility, low toxicity, chemical stability, and plasmonic features. Moreover, some examples of carbohydrate antigens conjugated to AuNPs showing high *in vitro* immunogenicity have been already reported [4].
In this work tetra and hexa-rhamnose fragments, structurally related to the polyrhamnose backbone of GAS polysaccharide, were synthesised. In addition, hydrolytically stable Glyco-PEG$_{5000}$-AuNPs with spherical or anisotropic shape (star-like) were prepared by following a reported protocol [5] which afforded oligorhamnose fragments covalently coated on AuNP surface. These glyco-AuNPs were fully characterized and the antigen density was accurately evaluated as number of antigen chains per AuNP, by using a combination of high performance anion exchange chromatography (HPAEC-PAD) and light scattering techniques. Competitive ELISA tests will be performed in order to verify the effect of oligorhamnoses multivalency on nanoparticles of different shape/size on the inhibition of anti-polysaccharides antibodies.

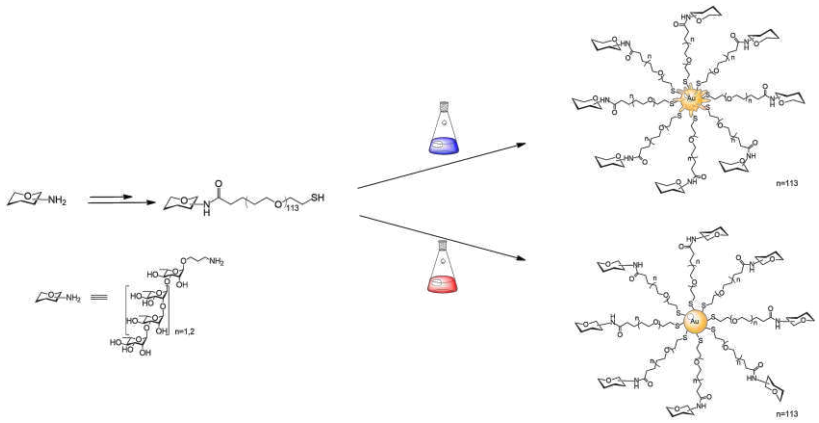

Fig. 1 - Synthesis of Glyco-PEG$_{5000}$-AuNPs. Tetra- and hexa-rhamnoses were functionalized with PEG$_{5000}$ and then linked to AuNPs.

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PALLADIUM-CATALYZED STEREOSELECTIVE SYNTHESIS OF DEOXYGLYCOSIDES

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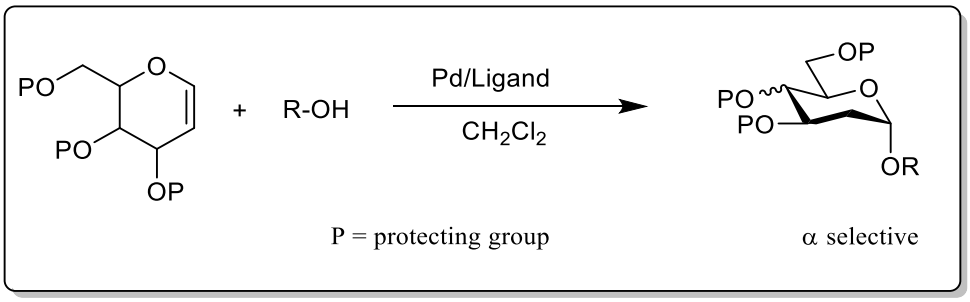
The chemical synthesis of complex carbohydrates generally involves the coupling of a fully protected glycosyl donor bearing a leaving group at its anomeric center, with a suitably protected glycosyl acceptor (R-OH). In many instances, these reactions lead to a mixture of two stereoisomers. To this day, the stereoselective synthesis of glycosides remains one of the biggest challenges in carbohydrate chemistry [1,2].

Herein we report the development of a catalytic and practical approach for the α-stereoselective synthesis of deoxyglycosides using Pd-catalysis. The method is mild and tolerant of a wide range of protecting groups on both the alcohol (primary and secondary) and glycal substrates [3]



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SYNTHESIS OF *E. FAECALIS* DIHETEROGLYCAN OLIGOSACCHARIDES

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Enterococcus faecalis is a gram positive bacterium that inhabits the gastrointestinal tracts of humans and other mammals. It is responsible wound infections, urinary tract infections and endocarditis especially in immunocompromised patients in nosocomial enviroments [1]. Multiple strains have shown a growing resistance towards antibiotics such as vancomycin leading to the need of alternative strategies, such as vaccination. The capsular polysaccharide of strains C and D is composed of β-D-glucopyranose-(1-6)-β-D-galactofuranose repeating units (Fig. 1) which can be decorated with a lactic acid on the C3-OH and acetyl groups on the C5-OH of the galactofuranose [2]. It has been observed that this antigen is recognised by antibodies raised against these bacteria [1,2] .

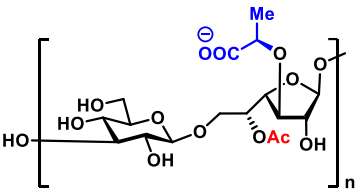
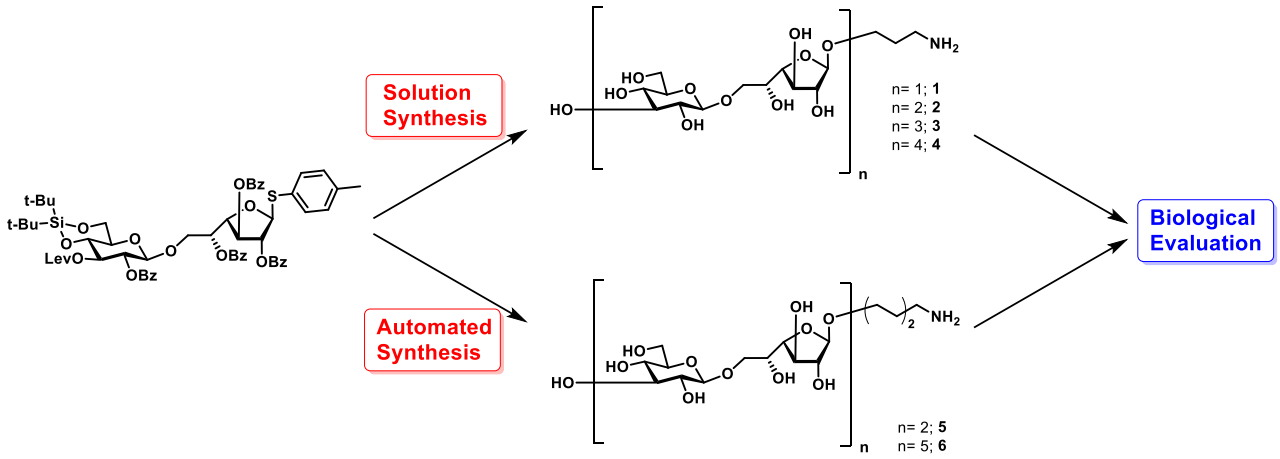


Fig. 2 - Diheteroglycan repeating unit

The goal of this project is to assemble a library of well-defined fragments of the capsular polysaccharide and test these for their antigenicity (scheme 1). The in solution synthesis allowed to obtain fragments up to 4 repeating units, with good overall yields, which have been conjugated to BSA, as a carrier protein, to interrogate their biological activity. In addition, an automated fashion synthesis has been developed to achieve even longer fragments in a streamlined oligomerization.



Scheme 1 - Overview of synthesis and application

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A NOVEL SYNTHETIC APPROACH TOWARD DIVERSE β-GLYCEROGLYCOLIPIDS BASED ON A NEIGHBORING FREE 1,2-*trans*-SELECTIVE GLYCOSYLATION

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The stereoselective construction of glycosidic bond is of the greatest importance to achieve the efficient synthesis of glycan molecules. Generally, to obtain 1,2-*trans*-selectivity the neighboring participation of acyl group has been most widely used. However, the method is not always suitable for the synthesis of ester substituted glycoconjugates, which are represented by β-glyceroglycolipids. Recently, we have developed a neighboring participation independent 1,2-*trans*-selective glycosylation method[1]. We herein describe the newly developed glycosylation reaction and its application to the synthesis of β-glyceroglycolipids.

The C2 and C3 hydroxyl groups of a glucoside derivative were protected cyclically as six to eight membered fused ring to afford the bicyclo-glucosyl donors, which were then subjected to glycosidation at low temperature (-80~ -40 ºC) under conventional reaction conditions. In all cases, high 1,2-*trans*-selectivity was provided together with high yield of glycosidated outcome. Among the cyclic substituents examined, *o*-xylylene group was found most useful in light of stereoselectivity and chemical stability. Next, to facilitate the cleavage of *o*-xylylene group, we installed a naphthalenedimethyl (NAPDM) group, which was cleavable under acidic condition. The NAPDM substituted glucosyl donor exhibited comparable 1,2-*trans*-selectivity to *o*-xylylene donor and NAPDM group was selectively cleaved with TFA in toluene.

Having the NAPDM donor in hand, we next investigated the synthesis of β-glyceroglycolipids. To achieve efficient glycosylation of a glycerol moiety, we developed *p*-nitrobenzylidene-protected glycerol acceptor as an acid-stable acceptor. The glycosylation of both glucose or galactose donor proceeded 1,2-*trans*-selectively (α/β = 1/4 ~ 1/7) in high yields without migration of the acetal moiety. In the following glycosylations to assemble disaccharides, excellent 1,2-*trans*-selectivities were again obtained. Next, the *p*-nitrobenzylidene group was removed in one step, which was followed by the introduction of fatty acids to afford β-glyceroglycolipid frameworks in high yields. Finally, global deprotection completed the synthesis of β-glyceroglycolipids.

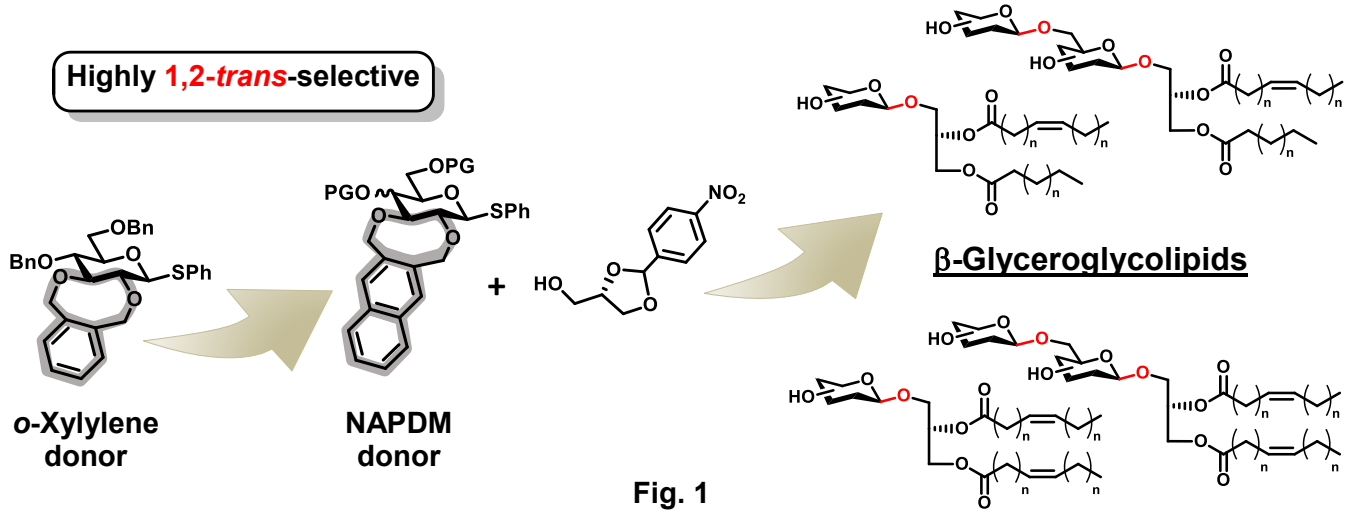

Fig. 1

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AUTOMATED GLYCAN ASSEMBLY OF LEWIS ANTIGENS

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Lewis antigens play an important role in a wide variety of physiological and pathological processes. They have been identified as oncofetal antigens, implicated in viral and bacterial infections, and found to be involved in cell-signaling processes [1]. Access to these antigens is key to the research of their role in infectious processes and their application in cancer diagnostic and treatment [2].

Lewis antigen syntheses have been mainly limited to total syntheses of single structures. Assembly strategies range from traditional solution-phase to automated synthesis [3]. This work introduces a general method for obtaining a whole series of Lewis type-I and type-II chain glycans (Fig. 1). By means of Automated Glycan Assembly (AGA) and a selection of a minimum set of orthogonally protected building blocks, a combinatorial approach allowed assembling of all intended glycans with excellent stereoselectivity. The use of a resin-bound linker allows avoiding time-consuming intermediate purification steps [4]. Complex glycans like trifucosylated nonasaccharide KH-1 were assembled with this methodology in overnight reactions. Following cleavage from the solid support and global deprotection, glycans bearing a C5-aminolinker at their non-reducing end were obtained. Thereby, rapid access to conjugation-ready Lewis antigens for their further application in biological studies can be achieved by this general approach.

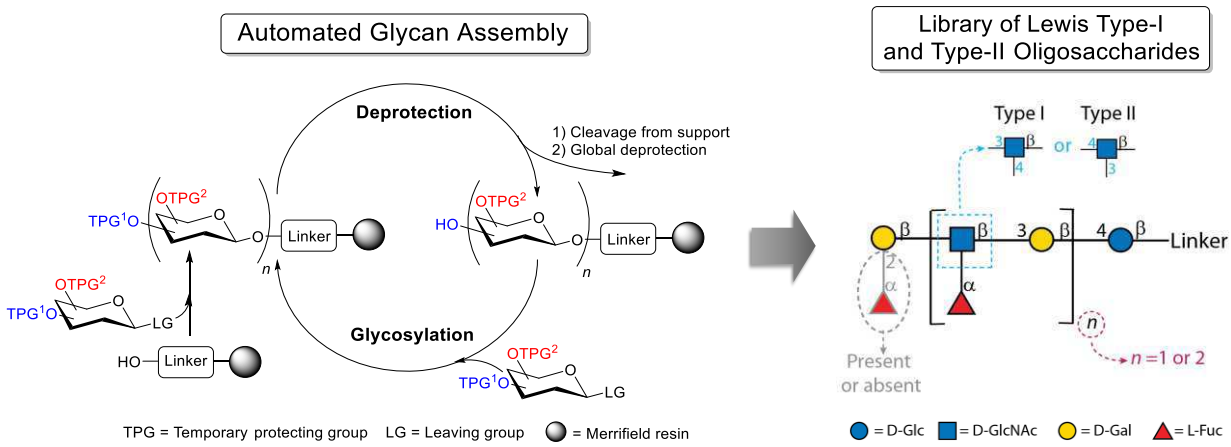

Fig. 1 - General approach for Automated Glycan Assembly of Lewis antigens.

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SYNTHESIS AND STRUCTURAL STUDY OF GLYCOSYLPHOSPHATIDYL-INOSITOLS FRAGMENTS IN MONOLAYERS

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Glycosylphosphatidylinositols (GPIs) are complex glycolipids that are ubiquitous in eukaryotic cells. GPIs are found as a post-translational modification at the C-terminus of proteins or as free glycolipids displayed on the outer leaflet of the cell membrane. [1] The structure of all known GPIs contains a conserved pseudopentasaccharide glycan core and a phospholipid. GPIs and GPI-anchored proteins are involved in diverse biological and pathological events. The lipid chains present on GPIs are highly variable and may interact through van der Waal interactions in the formation of microdomains. However, the presence of the hydrophilic oligosaccharide head group in GPI distinguishes it from other phospholipids by providing flexibility and additional hydrogen bonding that can dominate the lipid interactions in model membranes. [2]

Studies on the structural arrangement of GPIs in the membrane could provide better insight into the relationship between structural membrane arrangement and biological function. [3] In order to evaluate the correlation between GPI composition and structural arrangement of GPI in model membranes, we report here the results of a comparative study of GPI-fragments having different lipid compositions. We disclose the synthesis of GPI-fragments bearing linear and branched lipid chains and the results of their affinity towards the formation of lipid rafts in monolayers at the water/air interface. The monolayer structure parameters were determined by using Grazing Incidence X-Ray Diffraction (GIXD) measurements using the tilt angle of the alkyl chains to determine the change in the packing mode and lattice. GIXD patterns and contour plots of monolayer depicted different monolayer structure that depends on the lipid composition and the polar head group. Additional to the GIXD results, the importance of the lipid chains in the biophysical properties of GPIs and their participation in protein sorting and membrane organization will be discussed.

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C-TYPE LECTIN TARGETING WITH GLYCOMIMETICS: A SCREENING IN MICROARRAY-FORMAT

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The C-type lectin receptor family members (CLRs) serve as pattern recognizing receptors (PRRs) and detect pathogen- and altered self-associated molecular fingerprints. These recognition events are fundamental in immunological responses, from microbial adhesion, through inflammatory signaling, to directing T-lymphocyte differentiation. Targeting CLRs has a great potential for medical purposes, since the immune response could be steered in therapeutically favourable directions. Yet, several CLRs remain to be described in details, with regards to their function, structure and carbohydrate-binding characteristics, and very few selective CLR antagonists have been described, so far.

Recently, we reported a small library of mannose-based glycomimetic antagonists[1] for one of the best-known CLRs, the dendritic cell receptor DC-SIGN, which was singled out because of its involvement in viral and bacterial infections (HIV-1, Ebola, Dengue, *M. tuberculosis*).[2] It is anticipated that other Man-specific CLRs will also interact with these glycomimetics and that the aglycon moieties incorporated in the structure will be able to tune their activity by enhancing affinity towards the proteins and/or leading to selective receptor-binding. Therefore, the library diversity was expanded to include 40 mannosylated glycomimetic ligands (**1**) with the help of chemoinformatic tools. Due to overlapping carbohydrate-specificity based on structural similarities in the CRD, mannose-binding CLRs often bind also fucosylated epitopes. Thus, 11 β-Fuc ligands (**2**) were also added to the library, to further expand its chemical space. (**Fig.1.**)

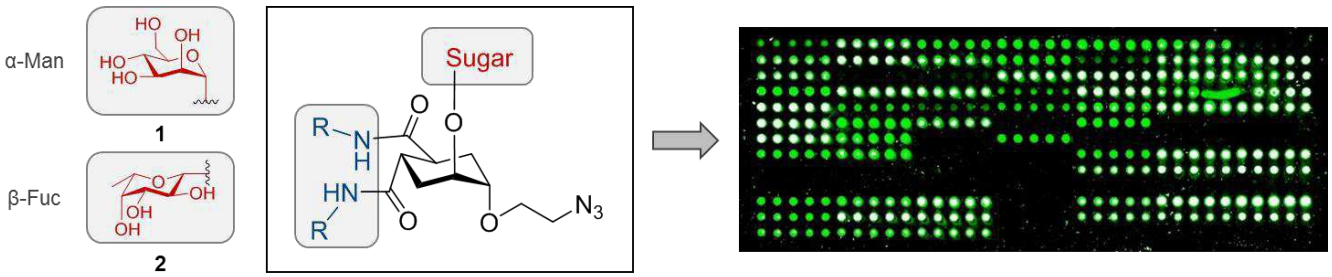

Fig.1.

All the glycomimetics were equipped with a functionalized tether, which allowed the preparation of glycomimetic microarrays that were then interrogated with fluorescently labeled hCLRs. The relative affinity of glycomimetic ligands towards various lectins on-chip provided a selection of potential high-affinity and selective binders and the results were further elucidated by complementary interaction assays (Surface Plasmon Resonance, SPR) and computational studies. Potential lead structures were identified that could be starting points for highly selective, glycan-based immunotherapeutics in the treatment of cancer, autoimmune diseases and allergy.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 642870 (Immunoshape)

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Structural insight into mannuronic acid specificity of a polysaccharide Lyase family 6 alginate lyase from human gut *Bacteroides*

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Alginate is an anionic polysaccharide that is the main component of the cell wall of brown algae. Its unique gelling, stabilizing, and viscosity modulating properties mean that it is extensively used as a food additive in the Western world and an estimated 38,000 tons are produced annually for human consumption.[1] Alginate is a co-block polysaccharide consisting of β-D-mannuronic acid (M) and α-L-guluronic acid (G) arranged in polyM, polyG or polyMG blocks. Alginate was thought indigestible in humans, but recent work has demonstrated that it can be degraded by human gut *Bacteroidetes.*[2] However, very little is known about the enzymes involved in the degradation of alginate in the human gut. Alginate can be depolymerized by alginate lyases, which are essential for alginate utilization by marine *Bacteroidetes.*[3] Here, we report the crystal structure solved to a resolution of 1.9 Å and biochemical characterization of an alginate lyase (*Bcel*PL6) from human gut *Bacteroides cellulosilyticus* belonging to polysaccharide lyase family 6. The enzyme is exo-acting and produces unsaturated di- and trisaccharides. *Bcel*PL6 is very specific and incapable of degrading polyG but does not distinguish between alginate and polyM. This specificity has not previously been characterized in polysaccharide lyase family 6.[4] The enzyme has a melting point of 63.1°C as analyzed by DSC, is a monomer and adopts a parallel β-helix fold with a calcium ion acting as the neutralizing ion of the C5 carboxyl group in the +1 subsite. The conserved K249 and R270 acts as catalytic residues. The unusual specificity of the *Bcel*PL6 may be associated with a narrower positive active site as compared to that of related structures.[5] Overall, these results provide insight into the structural basis of substrate specificity in polysaccharide lyase family 6. This is to our knowledge the first structural characterization of an alginate lyase from human gut microbiota.

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POSTER PRESENTATIONS

POSTERS | S – Synthesis

REGIO- AND STEREOSELECTIVE SYNTHESIS OF MATRIGLYCAN TETRASACCHARIDE

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Matriglycan is a repeating disaccharide region of α-dystroglycan (αDG) which has an interaction with laminin in skeletal muscle forming a bridge structure from basement membrane to cytoskeleton [1]. Recently, total structure of the glycan part of αDG including matriglycan has been clarified [2]. The repeating structure of matriglycan is -3)αXyl(1-3)βGlcA(1- linking to O-4 of βXyl at the reducing terminal [3]. We have started to synthesize the oligosaccharide of matriglycan to clarify the exact length which may interact with laminin. Suitably protected repeating disaccharide unit, αXyl(1-3)GlcA is useful to perform the oligomerization. However, it was not easy stereoselectively to synthesize the α-xyloside due to the equilibrium of xylose residue between 1C and C1 conformations. This problem was clearly resolved by employing the intramolecular aglycon delivery (IAD) strategy [4]. We have regio- and α-selectively obtained the desired αXyl(1-3)GlcA unit which was converted to the corresponding disaccharide donor and acceptor. These disaccharides were coupled to each other to give the desired tetrasaccharide, dimer of -3)αXyl(1-3)βGlcA(1-, regio- and β-selectively (Fig. 1). These results opened the way to the regio- and stereoselective oligomerization of the αXylβGlcA unit for medical use.

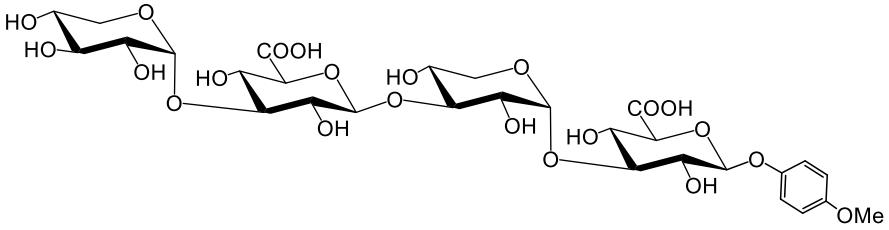

Fig.1 – Dimeric Xyl-GlcA in matriglycan

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ACCESS TO GALACTOFURANOCONJUGATES FOUND IN PATHOGENIC MICROORGANISMS: COMPARAISON OF A CHEMICAL AND A CHEMO-ENZYMATIC APPROACH TO TACKLE REGIOSELECTIVE GALACTOFURANOSYLATION

Laurent Legentil,* Quentin Pavic, Olivier Tasseau, Sylvain Tranchimand and Vincent Ferrières

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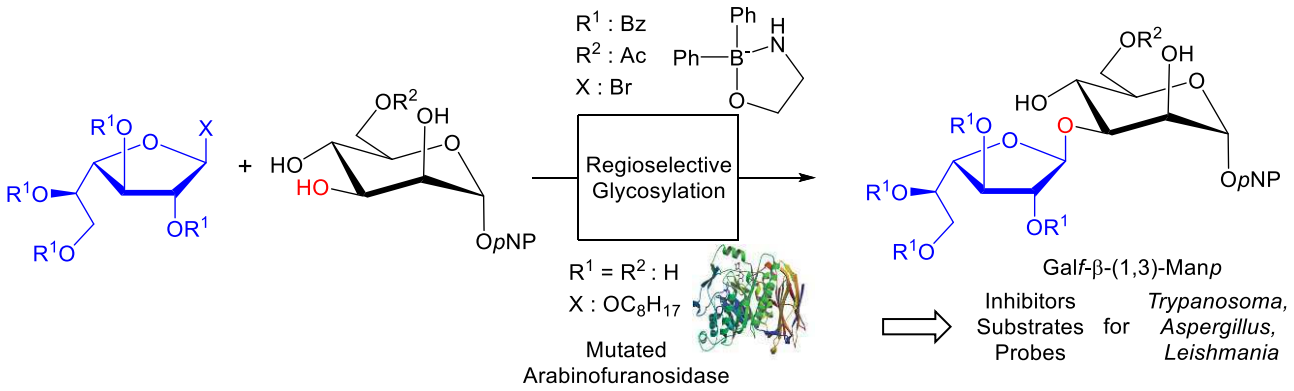
Galactofuranoconjugates constitute an original feature of many microorganisms cell wall when compared with mammals. Such motifs are particularly abundant in the glycocalix of microorganisms that are known pathogens.[1] Interestingly a common pattern, the β-D-Gal*f*-(1→3)-α-D-Man*p*, was identified in *Trypanosoma*, *Aspergillus* and Leishmania [2]. These microorganisms are responsible of deadly infections like Chagas disease, aspergillosis or leishmaniosis. Original scaffolds that mimic such disaccharide are therefore a unique target for the development of inhibitors, substrates or molecular probes linked with the biosynthesis, catabolism or degradation of the native galactofuranoconjugate.

Different methods are now available to easily synthesize such scaffolds but they generally require multi-steps synthesis and protecting group manipulations to isolate selectively the corresponding furanose-pyranose disaccharide [3]. In our ongoing research on galactofuranosyl derivatives, we have explored two different strategies that relied on regioselective galactofuranosylation of *p*-nitrophenyl mannopyranoside using either a diphenylborinate as inducer of regioselectivity or a mutated arabinofuranosidase as biocatalyst (Scheme 1). Those strategies have the advantage to be straightforward with the minimum of protecting groups on both acceptors and donors.



Scheme 1

For the first strategy, high regioselectivity was obtained when the primary alcohol was masked on the *p*NP mannoside. The reaction however suffered from the formation of orthoesters. Interestingly computational calculation supported the strong dependence between the nucleophilicity of the acceptor and the selectivity of the reaction [4]. For the second strategy, thanks to docking studies, crucial amino-acids that hampered the recognition of the galactofuranosyl motif into the catalytic pocket of the enzyme were identified. Gratifyingly, the mutation of a spotted amino-acid allowed to increase both the efficiency of the enzyme against this non-natural substrate, and the regioselectivity of the reaction for the (1→3)-linkage. Such improvement was further explained by molecular dynamics approaches.

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STEPS TOWARDS THE SYNTHESIS OF GROUP B *STREPTOCOCCUS* TYPE II (GBS II) REPEATING UNIT

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Group B *streptococcus* (GBS) is a gram positive bacterium, which causes neonatal invasive diseases as well as severe infections in the elderly and immune compromised patients [1]. On the basis of the capsular polysaccharide (CPS) and protein antigens ten serotypes of GBS have been identified (Ia, Ib, II through IX) [2]. Among these, type II GBS is one of the predominant GBS serotypes and responsible for 13% of early onset diseases. Although the structure of GBS II was elucidated in 1983, to date no synthetic scheme to obtain its repeating unit has been reported in the literature [3]. The repeating unit of GBS type II is composed of **α-Neu5Ac (2-3)-β-D-Gal-(1-4)- β-D-GlcNAc(1-3)-[-β-D-Gal-(1-6)]-β-D-Gal-(1-4)-β-D-Gal-(1-3)-β-D-Glc** (Fig. 1).

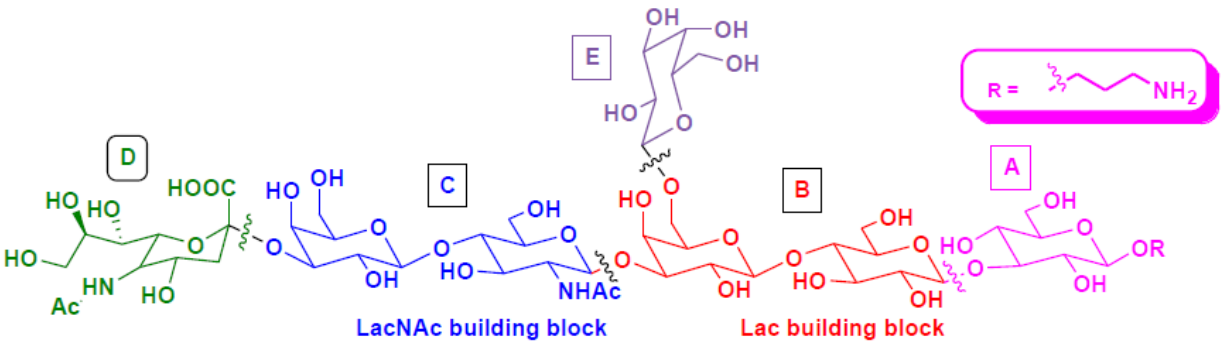

Fig. 1 – Structure and retrosynthetic fragmentation of GBS type II.

In the present communication, we show our results of an unprecedented approach to the synthesis of this heptasaccharide, based on the disconnections highlighted in Fig. 1. Suitably protected lactosamine **C** and lactose derivatives **B** will be pivotal building blocks in our endeavor. The synthetic GBS II fragments will be used for glycan array and structural studies with specific monoclonal antibodies, followed by their immunochemical characterization.

Acknowledgements: This project has received funding from the H2020-MSCA-ITN-2015 “Glycovax’’ under grant agreement No 675671.

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SYNTHESIS AND OPTIMIZATION OF HEXAVALENT GLYCODENDRIMERS AS ANTIVIRAL AGENTS

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A study into the efficient synthesis of novel glycodendrimers has been conducted. The ability of glycodendrimers to exhibit the multivalent effect makes them prime candidates to possess bioactive properties. Multivalency plays a vital role in numerous interactions between proteins and receptors. The synthesis thus far has proven highly successful, producing confirmed yields of 84%, 97%, 93%, and 53% (two step) for the initial 5 step dendrimer synthesis. All synthetic steps were conducted by novel and efficient techniques such as using aqueous bases, and the use of an CEM MARS 5 laboratory grade microwave whenever possible. To complete the pathway, a innovative synthetic strategy was employed to chemoseletively couple the hexavalent dendrimer with 5 oligosaccharides to create 5 novel glycodendrimers. It was possible to accomplish the glycosylation of the dendrimer core without the use of protecting group chemistry or harsh conditions. Overall, by using these methods, the reaction yields have increased compared to traditional reactions, and waste production has been minimized. The goal of the project ultimately is to make each novel glycodendrimer polyanionic through sulfation. It is hypothesized that the sulfated glycodendrimers generated will have significant antiviral characteristics, all while maintaining low cytotoxicity.

SYNTHESIS OF OCTAVALENT GLYCODENDRIMERS AS POTENTIAL ANTI-VIRAL AGENTS

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Dendrimers are known to be integral tools in a wide variety of biomedical applications. These globular molecules consist of three main parts: A central core, linker branch units, and terminal groups. Our laboratory focuses on novel synthetic techniques to produce a variety of glycodendrimers. These glycodendrimers will be made using unprotected sugars and a minimum number of reaction steps. The current work focuses on a nine-step synthetic pathway starting with an eight-step synthesis to achieve the octavalent core, followed by an additional chemoselective coupling reaction with the sugar.

Currently, work is being done to synthesize octavalent glycodendrimers. First, a linker was synthesized using a two-step process. Here, ethylene glycol underwent a Michael addition to give a t-butyl protected linker, resulting in a 38% yield. This linker then underwent a mesylation reaction on the hydroxyl end, resulting in a 96% yield. Next, this was added to a divalent core under basic refluxing conditions, which was carried forward to deprotection, revealing the carboxy-terminated tetravalent-core. This is followed by a Fischer esterification and displacement of the ester with a diamine resulting in the amine terminated tetravalent core. Next, further branching and deprotection will occur in a two-step process leading to the complete octavalent carboxy core. The final steps of the synthesis will feature the addition of a final linker in an amide coupling, deprotection of the terminal group, followed finally by the chemoslective coupling with unprotected sugars, yielding the completed glycodendrimers. These will then be sent out for testing to examine their ability as possible anti-viral properties.

TOTAL SYNTHESIS OF LANDOMYCIN E FOR ANTI-PROLIFERATION STUDIES

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Landomycins are a family of angucyclins, which have a tetracyclic aglycone core that is connected to a deoxyoligosaccaharide fragment. Landomycin E is a member of the landomycin family obtained by biosynthetic manipulation, which exhibits promising cytotoxic activities against various breast cancer cell lines [1, 2]. In our work, hydroquinone and 3,5-dimethylphenol are employed as starting materials to construct the A-D rings of the landomycinone core via Dötz annulation and protecting group assisted aryl-aryl coupling (Fig. 1) [3, 4]. Sequential glycosylations of the landomycinone core with appropriate dexoyglycosyl building blocks enable the synthesis of landomycin E.

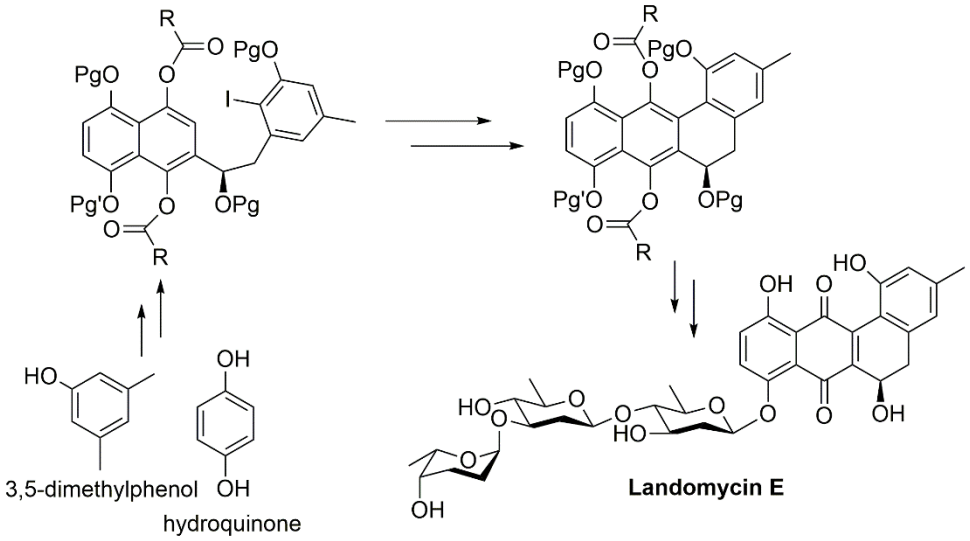

Fig. 1 – Flow chart of synthesis toward Landomycin E.

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BIOTRANSFORMATION OF AROMATICS FOR THE PREPARATION 2*C*-METHYL-D-RIBOSE AND THE SUGAR RESIDUE OF HYGROMYCIN A

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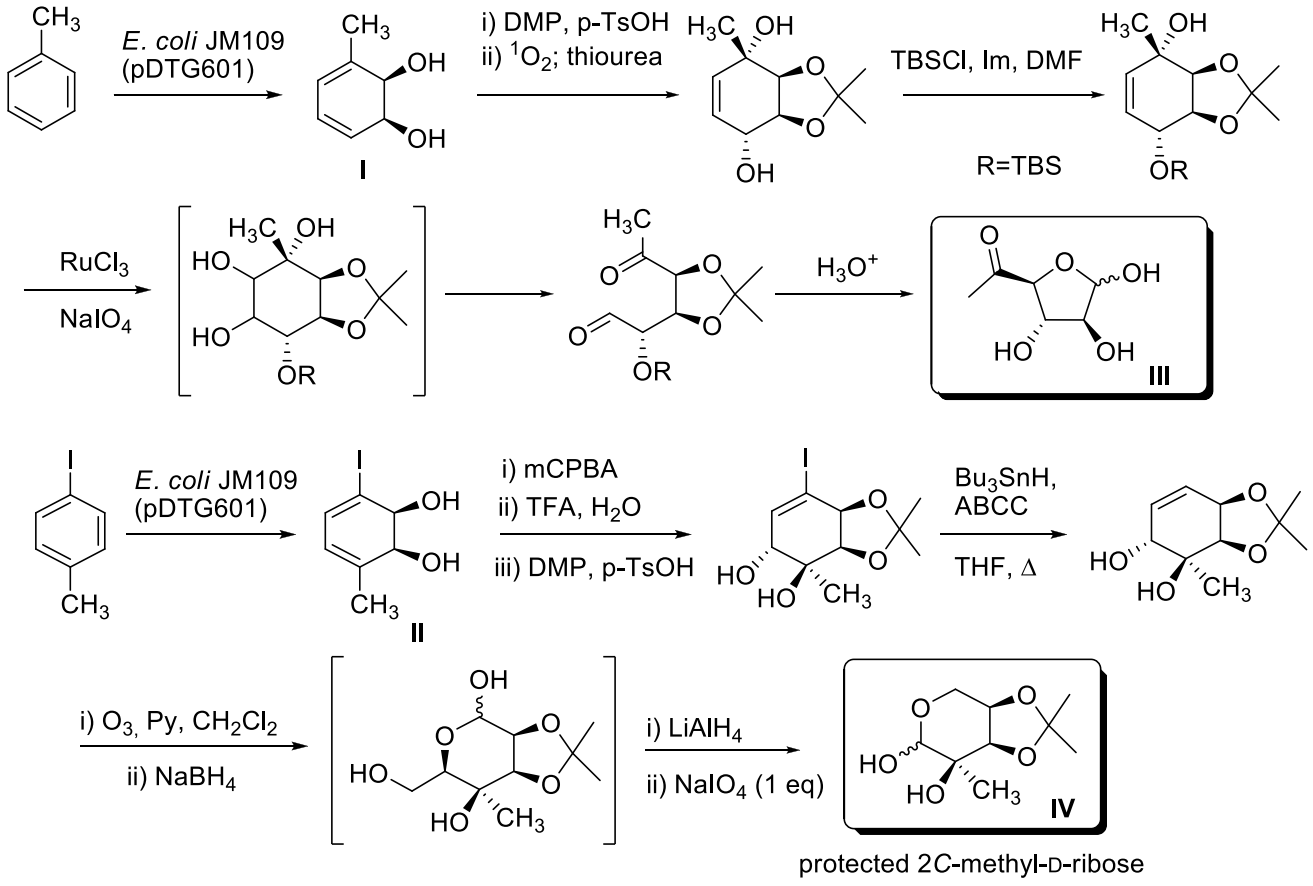
The enzymatic dihydroxylation of aromatics has become an established methodology for enantioselective synthesis. The outstanding synthetic potential associated to the high optical purity and dense functionalization of the *cis*-diols resulting of the cited biotransformation has allowed the use of these diols as synthons for the preparation of several classes of compounds, such as terpenes, sugars, alkaloids, and miscellaneous natural products [1]. In our laboratory, we produce these diols through a *E. coli* JM109 (pDTG601)-mediated biotransformation [2], and employ them in several synthetic schemes.

In this work we describe the use of the cis-diols **I** and **II**, derived from the biotransformation of toluene and *p*-iodotoluene, respectively, for the concise preparation of the sugar residue of Hygromycin A (6-deoxy-5-keto-D-*arabino*-hexofuranose, **III**) [3], and 2*C*-methyl-D-ribose (**IV**), as shown below.



protected 2*C*-methyl-D-ribose

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PROBING THE MECHANISM OF NICKEL TRIFLATE-MEDIATED 1,2-*CIS*-2-AMINO GLYCOSYLATION WITH *N*-SUBSTITUTED TRIFLUOROMETHYLBENZYLIDENEAMINO PROTECTED DONORS

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One the key constituents in naturally occurring oligosaccharides and polysaccharides are 2-aminosugars.[1] These saccharide bearing 2-aminosugar moieties are essential to mediate the interactions between cells, including heparin sulfate (for use to prevent blood clotting and heart disease), tumor-associated mucin antigens (for use as cancer vaccine therapy), and heparan sulfate (for studies of cancer metastasis) [2-5]. Use of the commercially available, nickel triflate, produces saccharides bearing these 2-aminosugars in excellent yields and high α-selectivity under an operationally simple and mild conditions [6-7]. It has been shown to promote formation of 2-aminosugars in the gram-scale permitting biomedical researchers to obtain adequate amounts for further studies of cancer and heart diseases [7]. We have recently began to probe the reaction mechanism through the use of expermination, computation caluctions, low-temperature NMR, and kinetic studies.

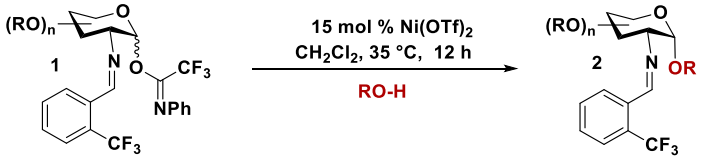

Fig.1 – Nickel-triflate mediated glycosylation for the formation on 1,2-cis-2-aminoglycosides.

Through these mechanistic studies we have concluded that the reaction is in fact mediated by the slow release of triflic acid as a "hidden Brønsted acid" from the nickel triflate precatalyst [9] After donor activation, the reaction then goes through a glycosyl triflate intermediate, which was detected by low-temperature NMR [10-11]. Through computation calculations we found that it is more favorable for the thermodynamically stable α-glycosyl triflate to anomerize to the more reactive β-glycosyl triflate and undergo nucleophilic attack on the α-face [11].

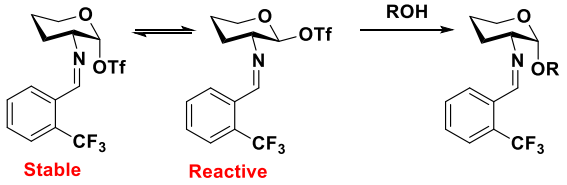

Fig. 2 – Anomerization of glycosyl triflate intermediate to achieve 1,2-*cis*-2-aminoglycosides.

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TMSOTf-CATALYZED PER-*O*-SILYLATION: STREAMLINED REGIOSELECTIVE ONE-POT FUNCTIONALIZATION OF CARBOHYDRATES AND CONCISE SYNTHESIS OF D-GALACTOSAMINE AND D-ALLOSAMINE DERIVATIVES

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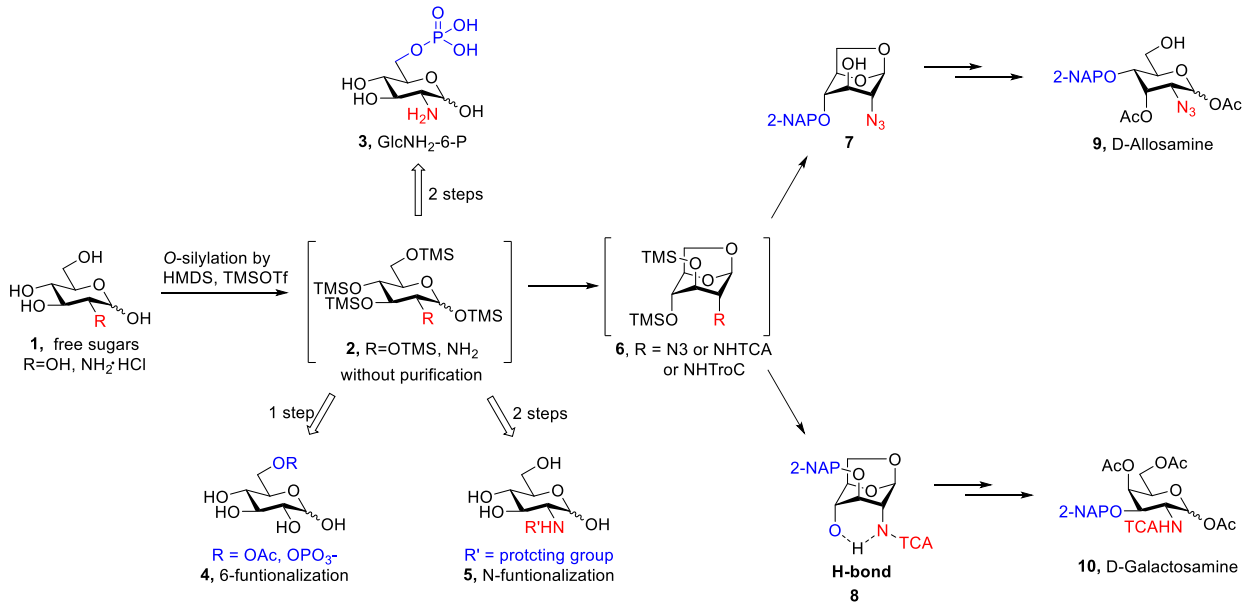
Based on the efficient TMSOTf catalyzed HMDS silylation[1], a highly regioselective one-pot functionalization of carbohydrates was established. The following reaction could be performed smoothly without any purification and further afforded 6-*O*-funtionalized sugar **3** [2], *N*-functionalized sugar **4** [3] and glucosamine 6-*O*-phosphate **5** [3] under concise synthetic steps with high regioselectivity [4]. In addition, the TMSOTf catalyzed HMDS silylation of glucosamine followed by further TMSOTf catalyzation in-situ generated 1,6-anhydrosugar **6** [5], of which *O*3 and *O*4 secondary hydroxyl group could be differentiated by the presence/ absence of intramolecular hydrogen bonding [6]. Eventually, the rare D-allosamine **9** and D-galactosamine **10** derivatives were obtained in concise steps.



Scheme 1. The one-pot synthetic strategy of free sugars.[1-3, 5]

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SYNTHESIS OF D-GALACTOSAMINE AND D-ALLOSAMINE DERIVATIVES VIA A MICROWAVE-ASSISTED PREPARATION OF 1,6-ANHYDROGLUCOSAMINE

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We report an intramolecular anomeric protection (iMAP) of glucosamine, which conducts a microwave-assisted intramolecular reaction and facilitates the concise transformation of 1,6-anhydroglucosamine into 1,6-anhydrogalactosamine and 1,6-anhydroallosamine. Our iMAP method simultaneously avoids both the *O*1 and *O*6 protection. Because of the hydrogen bonding between *N*2 and *O*4, we were able to well differentiate the *O*3 and *O*4 of 1,6-anhydroglucosamine by the *N*2 functionality. By the epimerization of *O*4 and *O*3, we obtained the galactosamine and allosamine derivatives respectively.

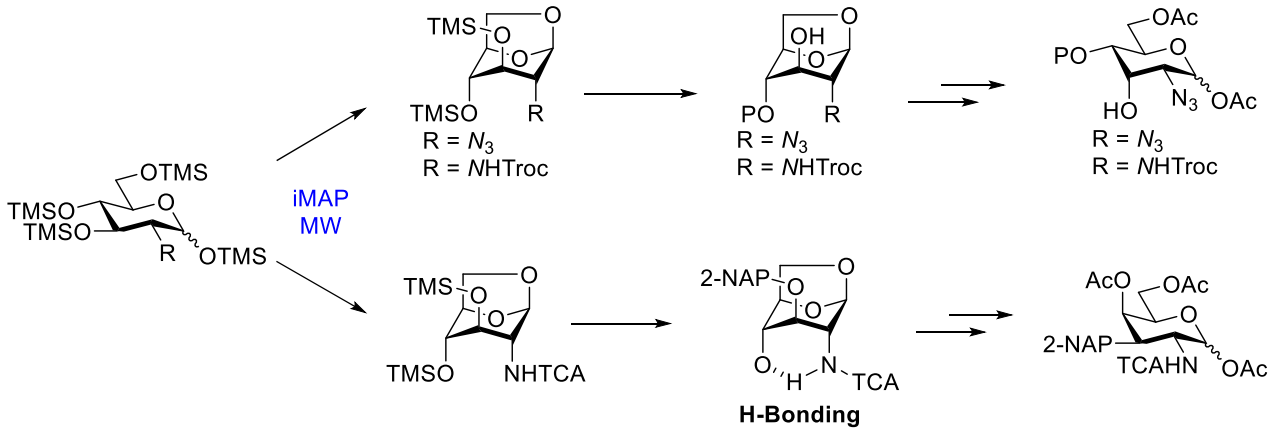

Fig. 1 – The synthetic strategy of D-galactosamine and D-allosamine derivatives

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IN VIVO GOLD CATALYZED REACTION USING GLYCOCLUSTER AS ORGAN SELECTIVE METAL CARRIER

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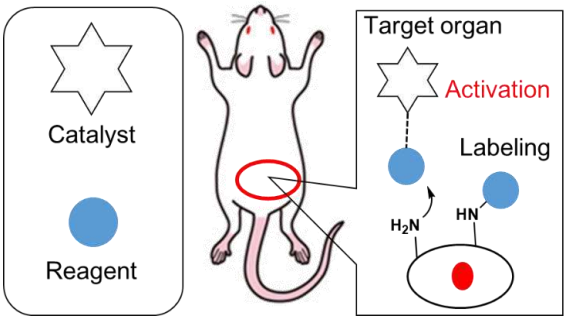
Conventional disease therapy using bioactive small molecules often causes adverse side effects to healthy organs. To date, numerous research groups are actively exploring methods to avoid these problems. The approach used by our group to address this challenge is based on the use of metal catalyzed reactions that are localized to targeted tissue.

Previously, our group successfully developed “Glycoclusters”, which were synthesized via the introduction of approximately ten complex glycan molecules onto protein surface lysines via the “RIKEN-click reaction”. It was discovered that *in vivo* biodistribution of these glycoclusters could be controlled by changing the structure of the glycan. Using these glycoclusters as metal carriers, we envisioned their use in metal-catalyzed reactions that can be localized to specific organs *in vivo*. Our approach is as follows. First, the metal catalyst is conjugated to the glycocluster. Following injection into mice, these glycoclusters preferentially accumulated to the liver or intestine depending on the glycan type. Once the glycocluster-catalyst accumulates to targeted organs, a second injection is then made with a near-infrared probe that can be activated for ligation to nearby membrane surface proteins via metal catalyst. In the control mice that were not injected with glycocluster-catalyst, fluorescence was detected throughout the entire body. However, in mice injected with liver-targeting glycocluster-catalyst, whole-body imaging showed selective liver labeling. In the case with mice injected with intestine-targeting glycocluster-catalyst, imaging instead shows selective intestine labeling. As a result of this work, we have succeeded in performing the first literature example of an organ-selective, metal catalyzed reaction *in vivo*. With this methodology, we are currently adapting it for an antitumor therapy where *in vivo* amine-conjugation has shown promising results in animal models.



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SYNTHESES OF OLIGOVALENT S/Se-CONTAINING GLYCOCONJUGATES BASED ON A QUINOLINE CENTRAL SCAFFOLD

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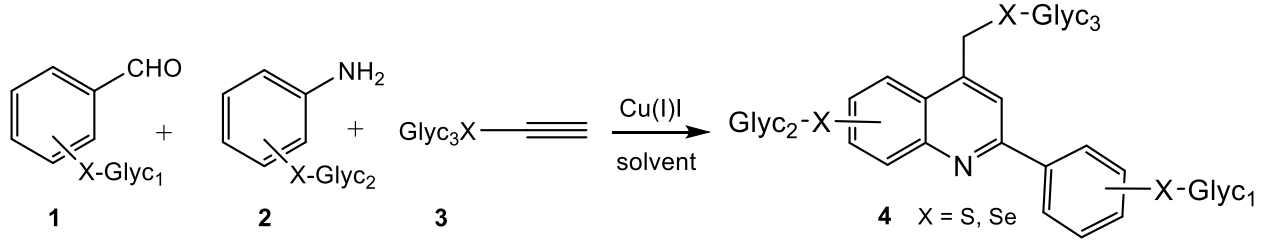
Increasing evidence is highlighting the involvment of lectins in various diseases. Lectin inhibitors represent a promising approach to block such pathological processes. We have previously recorded specific binding of oligovalent aromatic disulfide glycosides to plant lectins (such as concanavalin A or VAA) as well as to human galectins [1, 2] with affinities surpassing that of the cognate sugar. Further glycoconjugates with *S/disulfide/Se* glycosidic bonds to central naphthalene scaffolds were found to inhibit the binding of various lectins to tumor cell lines and in assays with animal tissue sections [3]. In addition, aromatic disulfidogalactosides displayed remarkable inhibitory activities against *Trypanosoma cruzi*, the etiologic agent of Chagas's disease [4].
Here we describe the synthesis of a set of novel oligovalent glycoconjugates wherein a central quinoline unit is decorated by multiple carbohydrate moieties; with $Glyc_n$ being various mono- or disaccharide units (Glc, Gal, Man, GlcNAc, Lac, LacNAc, etc.). Taking advantage of a one-pot threee-component [5] reaction the target compounds (**4**) are obtained from S/Se-glycosylated precursors (**1-3**):



These derivatives are expected to display favorable bioactivities in terms of lectin binding or antiparasitic efficiency via the quinoline nucleus, itself a known pharmacophore, the presence of several carbohydrate units to boost multivalency and resistance to hydrolysis via substituting of oxygen by *S/Se* in the glycosidic bonds.

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TOTAL SYNTHESIS OF RESIN GLYCOSIDE MURUCOIDINS VIA INTERRUPTED PUMMERER REACTION MEDIATED GLYCOSYLATIONS

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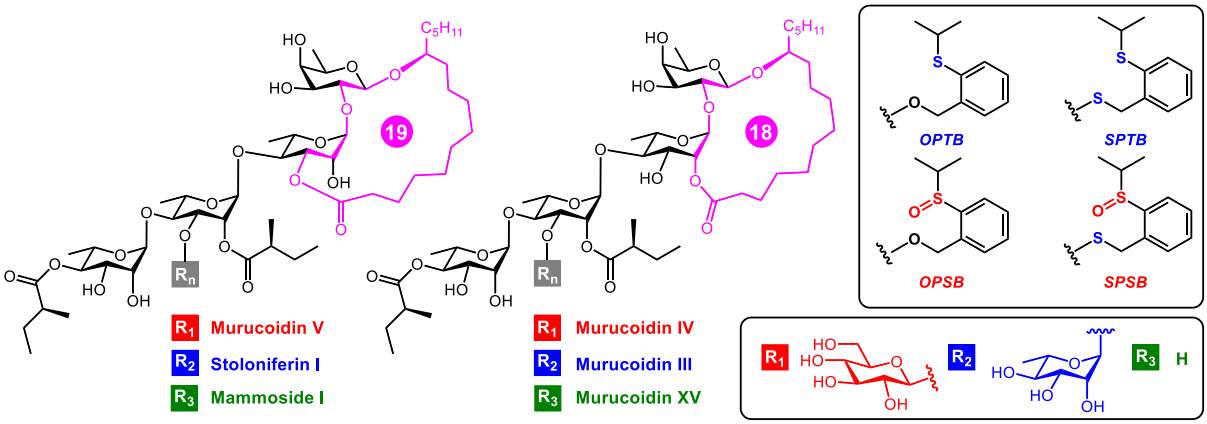
Latent *O/S*-glycosides, *O/S*-2-(2-propylthiol)benzyl (PTB) glycosides, were converted into the corresponding active glycosyl donors, O/S-2-(2-propylsulfinyl)benzyl (PSB) glycosides, by a simple and efficient oxidation. Treatment of PSB donors and various acceptors with triflic anhydride provided the desired glycosides in good to excellent yields. Meanwhile, the mechanism studies revealed that the active O/S-benzyl glycosides were activated *via* an interrupted Pummerer reaction. A series of resin glycosides were synthesized efficiently through this approach.



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SYNTHESIS OF NOVEL QUINOLIZIDINE AND CONIDINE IMINOSUGARS AS GH31 α-GLUCOSIDASE INHIBITORS

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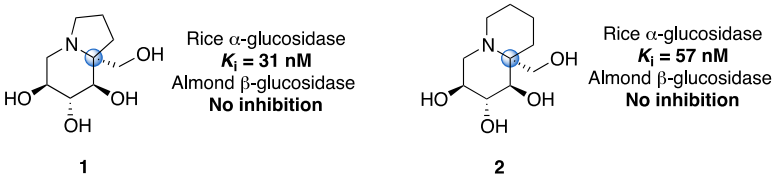
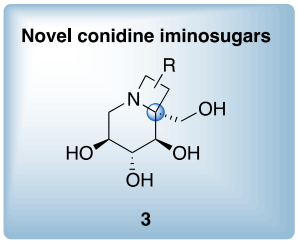
Iminosugars are carbohydrate mimetics bearing a nitrogen atom in place of the endocyclic oxygen atom. Due to their stability *in vivo* and their activity as glycosidase inhibitors or activators, they are recognized as among the most promising drug candidates for the treatment of diverse diseases such as diabetes, viral infections and lysosomal storage disorders [1].
Recently, our group has identified a series of iminosugars (i.e. compounds **1** and **2**) exhibiting a quaternary center in α-position to the nitrogen atom, that proved to be excellent inhibitors (nanomolar K_i) of α-glucosidases of the GH31 family [2] with unprecedented selectivity [3] To study the structure-activity relationship of these iminosugars and better understand their mode of interaction with glycosidases, we engaged in the synthesis of conidine analogues **3**. Only a few polyhydroxylated conidines have been described in the literature, with no example bearing a substituent at the ring junction, and their biological activities have been underinvestigated [4].





In this communication, we will present the synthesis of new conidines **3** and our latest results on the evaluation of D-*gluco*-configured bicyclic iminosugars bearing a quaternary center at the ring junction as glycosidase inhibitors. Their structure-activity/selectivity relationship will be discussed in detail, based on their capacity to inhibit glycosidases and on crystallographic studies.

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INVESTIGATION OF THE GLASER COUPLING FOR THE SYNTHESIS OF SHAPE-SWITCHABLE GLYCOAZOBENZENE MACROCYCLES

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Carbohydrate-based macrocycles are of growing interest due to their potential applications and synthetic and structural complexity [1]. The benefit of working with cycles containing carbohydrates lies in their stereoisomeric diversity and multifunctionality, which leads to a large structural variety while maintaining conformational restrictions. To reversibly control molecular features of macrocycles by external stimuli, we designed shape-switchable macrocycles, expecting a fundamental change in multiple properties upon switching between two defined structural shapes. To achieve this, azobenzene glycoconjugates were used as building blocks for the design of unprecedented glycoazobenzene macrocycles [2]. The photoresponsive azobenzene hinge allows for switching the molecular shape of the glycomacrocycles by irradiation, effecting reversible E→Z→E isomerization of the azobenzene N=N double bond. This leads to vast changes in chirality and shape of the designed rings, thus making them interesting molecules for a variety of applications.

In order to explore alternative molecular designs and broaden our synthetic repertoire in the formation of glycomacrocycles, we have replaced azobenzene glycosides with glycoazobenzenes derivatives in which the azobenzene moiety is conjugated at the primary alcohol function of the sugar ring. Accordingly, we present the use of the copper(I)-catalyzed Glaser coupling as an effective reaction for the ring-closure of these photoresponsive glycomacrocycles (Fig. 1). The various synthesized novel shape-switchable molecules possess different interesting properties which change upon irradiation, like their photochemical and chiroptical behavior, solubility and aggregation.

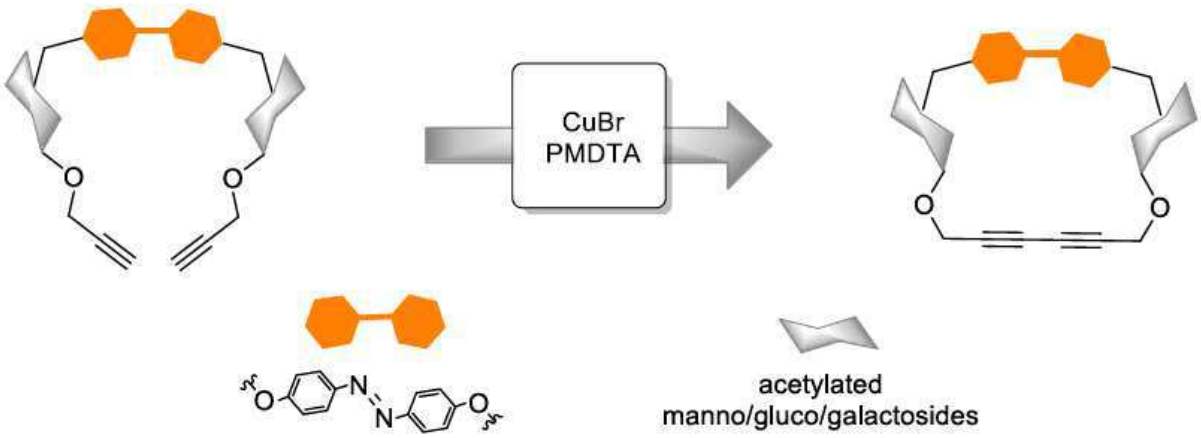

Fig. 1 - Cu(I) is used in an intramolecular Glaser coupling to prepare various glycoazobenzene macrocycles. PMDTA: N,N,N′,N″,N″-pentamethyldiethylenetriamine.

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DYSPROSIUM(III) TRIFLATE-CATALYZED SOLVENT-FREE PER-*O*-ACETYLATION AND ANOMERIC DE-*O*(*S*)-ACETYLATION OF CARBOHYDRATES

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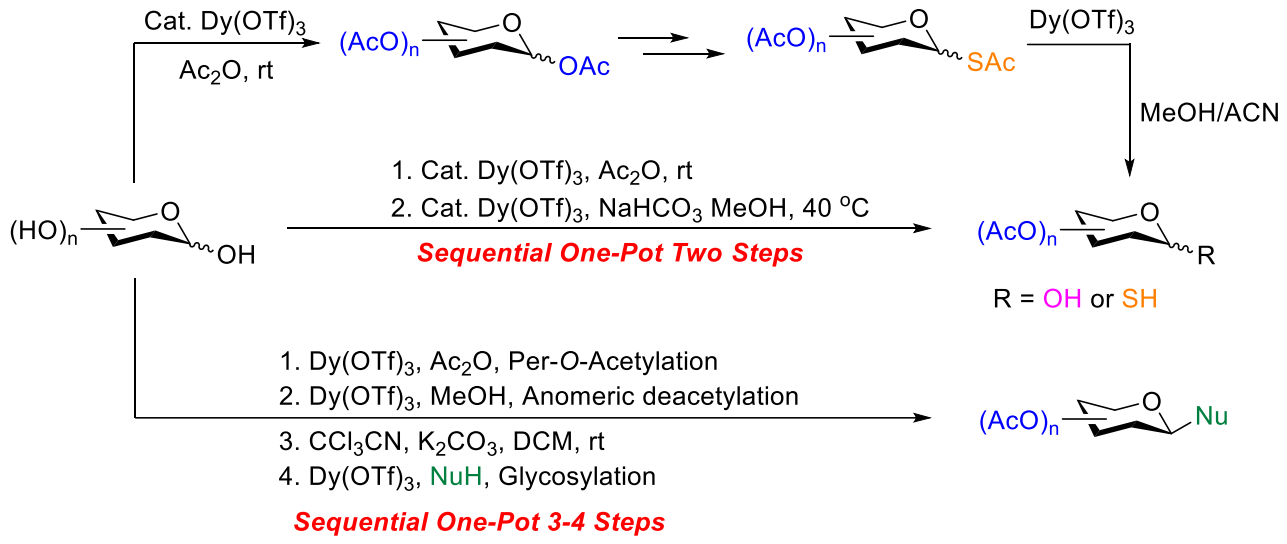
Peracetylated and their derived hemiacetal of carbohydrates are valuable building blocks for the preparation of complex oligosaccharides and glycoconjugates. Here, we report the use of $Dy(OTf)_3$ as a dual catalyst for preparation of peracetylated carbohydrates with a stoichiometric amount of acetic anhydride under solvent-free condition, and extend to the synthesis of per-*O*-acetylated hemiacetal *via* one-pot two steps method. Furthermore, the obtained hemiacetals can be successfully transformed into trichloroimidates followed by glycosylation catalyzed by $Dy(OTf)_3$. Moreover, the combination of MeOH and MeCN was an ideal solvent system for the chemoselective *S*-deacetylation of glycosyl 1-thioacetate. Notably, $Dy(OTf)_3$ is an environmentally friendly catalyst, and a recycling strategy was successfully employed for the sequential one-pot two step strategy.



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REAGENT CONTROLLED STEREOSELECTIVE SYNTHESIS OF α-GLUCANS

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The development of a general glycosylation method that allows for the stereoselective construction of glycosidic linkages is a tremendous challenge. Because of the differences in steric and electronic properties of the building blocks used the outcome of a glycosylation reaction can greatly vary when switching form one glycosyl donor-acceptor pair to another. We developed a strategy to install *cis*-glucosidic linkages in a fully stereoselective fashion that is under direct control of the reagents used to activate a singly type of donor building block. The activating reagents are tuned to the intrinsic reactivity of the acceptor alcohol to match the reactivity of the glycosylating agent with the reactivity of the incoming nucleophile. A protecting group strategy is introduced that is based on the sole use of benzyl-ether type protecting groups to circumvent changes in reactivity as a result of the protecting groups. For the stereoselective construction of the α-glucosyl linkages to a secondary alcohol, a per-benzylated glusosyl imidate donor is activated with a combination of trimethylsilyltriflate and DMF, while activation of the same imidate donor with trimethylsilyl iodide in the presence of triphenylphospine oxide allows for the stereoselective *cis*-glucosylation of primary alcohols. The effectiveness of the strategy is illustrated in the modular synthesis of a linear α-(1-2)-tetrasaccharide, α-(1-3)-nonasaccharide and a *Mycobacterium tuberculosis* nonasaccharide, composed of an α-(1-4)-oligoglucose backbone bearing different α-glucosyl branches.

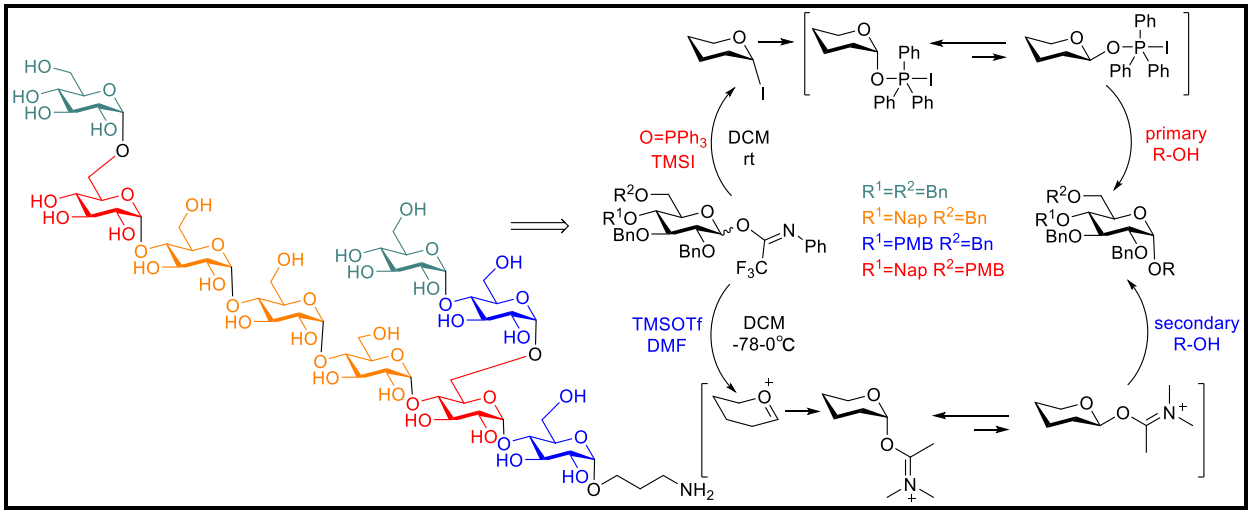

Fig. 1 – Branched α-glucan and proposed glycosylation mechanism

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CHEMICAL SYNTHESIS OF FRAGMENTS OF THE MULTIANTENNARY GROUP-SPECIFIC POLYSACCHARIDE OF GROUP B *STREPTOCOCCUS*

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Group B *Streptococcus* (GBS) is a Gram-positive bacterium that can act either as a commensal organism or as a pathogen in humans [1]. It remains the most common cause of neonatal infections of the blood (septicemia) and of the brain (meningitis) [2]. A major component of the GBS cell wall is a complex multiantennary carbohydrate, termed the Group B-specific antigen (GBC) by Rebecca Lancefield. Although the structure has been known for a long time, the role of this unique carbohydrate remains poorly understood [3]. To facilitate studies that may unravel the function of the GBC we set out to assemble the structure by chemical synthesis.

The GBC is composed of rhamnose rich oligosaccharides interspaced with glucitol phosphates. We initially focused on the assembly of the termini of the multiantennary structure as depicted in Fig. 1. The tridecasaccharide **1** is a challenging branched oligsaccharide constituted by four different monosaccharides: α-L-rhamnose, α-D-galactose, β-D-glucosamine and glucitol. The synthesis of the tridecasaccharide **1** follows a convergent [5+8] coupling strategy employing a pentasaccharide phosphoramidite **3** and a branched octasaccharide with a free galactosyl C-6-OH **4**.

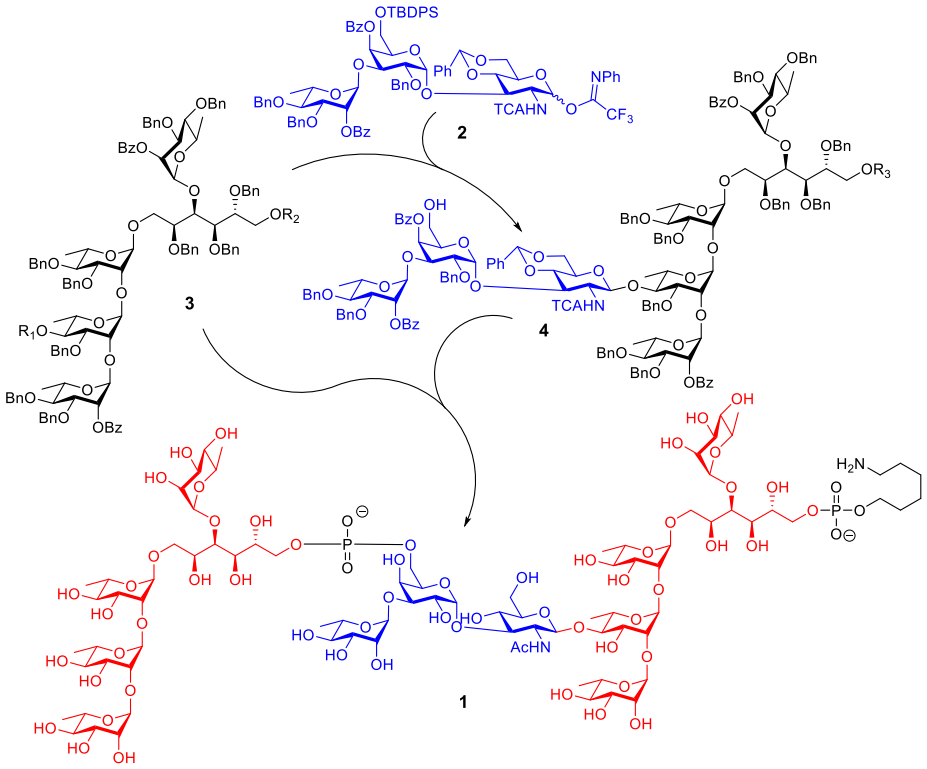

Fig. 1 – Chemical synthesis strategy to generate the CPS fragments

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MALTOSE AS A SCAFFOLD MOLECULE FOR THE SYNTHESIS OF HETEROMULTIVALENT GLYCOCLUSTERS

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Cell surfaces are covered with complex glycoconjugates formaing a thick sweet layer called the cell's glycocalyx. Glycocalyx constituents play a fundamental role in biology, i.a. in cell recognition and cell adhesion processes. The biological meaning of glycocalyx glycoconjugates is "decoded" by the interaction with specific proteins, called the lectins. The interaction between lectins and their carbohydrate ligands is fine-tuned by multivalency effects, which have been extensively reviewed [1]. Synthetic multivalent glycomimetics are valuable tools in multivalency studies [1].

For the design of multivalent glycomimetics, the choice of a suitable scaffold molecule is important. We have shown earlier, that carbohydrate scaffolds are favorable structures for the construction of multivalent oligosaccharide mimetics.[2] Carbohydrates provide a defined and rigid conformation, possess a distinct stereochemistry, and in addition, their multifunctionality offers various possibilities for flexible derivatization. We have shown earlier, that mannose can be turned into multivalent scafoold glycosides of an AB_4-type, serving in the preparation of homomultivalent glycomimetics [3, 4].

Here we show the synthesis of a new orthogonally functionalized ABC-type scaffold based on maltose, which can be diversely derivatized into heteroglycoclusters (Fig. 1). Maltose was modified at the anomeric position and at the 6- and 6'-position, respectively. The anomeric functionalization with an alkyne allows the attachment of a linker for immobilization on a surface (e. g. polystyrene, gold) or other further derivatization. The primary positions were derivatized as orthogonally protected amino groups allowing sequential modification by peptide coupling in order to achieve heteroglycoclusters (Fig. 1). Testing of the ligand properties of the synthesized maltose-based heteroglycoclusters and evaluation of "heterocluster effects" [5] is planned in bacterial adhesion studies.

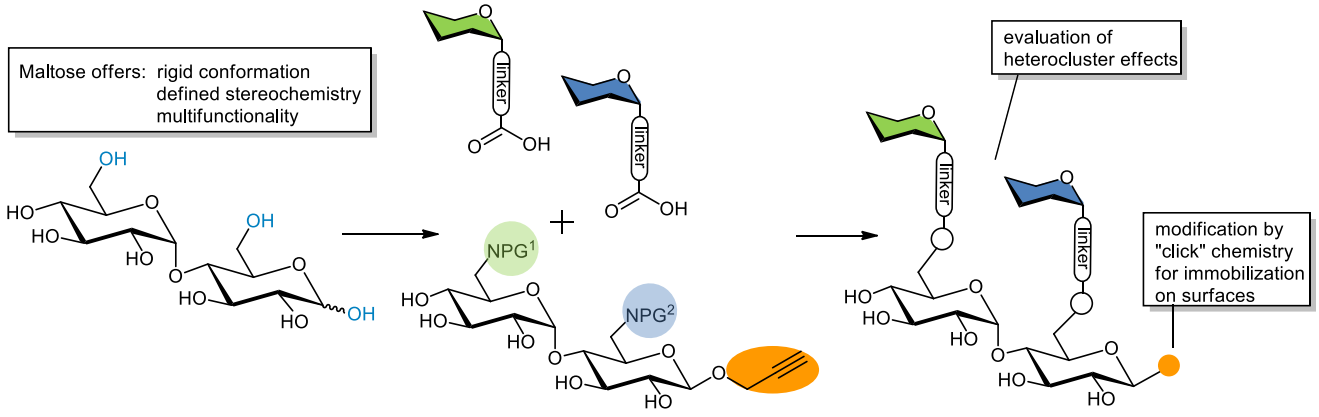

Fig. 1 – Maltose-based multifunctional scaffold for functionalization with various carbohydrate ligands.

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SYNTHESIS OF PYRAZOLIDIN-3-ONES WITH POTENTIAL ACTIVITY FOR THE TREATMENT OF BIPOLAR DISORDER

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Bipolar disorder, also known as manic-depressive illness, is an incapacitating, chronic, and serious neuropsychiatric disease in which the patient has recurrent episodes of two opposing mood states, mania and depression. The available medical treatment for this disorder currently is not the most appropriate. The most used therapeutic approach is a mixture of antidepressants and antipsychotics, in which the first is used to treat depressive crisis and the second ones to treat manic episodes. Another worldwide drug used to control this disease is Lithium Carbonate which has serious side effects [1-4]. Because of the lack of efficacy of the treatments available and the various side effects, it is crucial to design new molecules with potential for the treatment of this illness [5-7].

Carbohydrate derivatives have gained ground in the pharmaceutical industry over the last few years because of the interesting pharmacological activities that they have shown for the treatment of several illnesses, including neurological diseases, and their biocompatibility [7,8]. Therefore, the aim of this work is to design and synthesize carbohydrate derivatives, namely pyrazolidin-3-ones, with potential interest in the treatment of bipolar disorder and further *in vitro* biological evaluation.

The synthesis of the pyrazolidin-3-ones started with a commercially available compound, 1,2:5,6-Di-O-isopropylidene-α-D-glucofuranose. Several reactions were performed until an aldehyde group at the position 5 of the furanosidic ring was obtained. Then, an α,β-unsaturated ester by a Wittig reaction was prepared, which is the final precursor to the reaction in which the ring is closed to afford the pyrazolidin-3-one system.

The *in vitro* cytotoxicity assays performed shows that the compounds present no relevant cytotoxicity in normal human dermal fibroblasts and on the neuronal N27 cell line and therefore other biological evaluations to assess their potential interest as anti-bipolar agents can be considered.

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STEREOSELECTIVE β,β-1,1' GLYCOSYLATION TOWARDS ORTHOGONALLY PROTECTED DIGLUCOSAMINE DERIVATIVES

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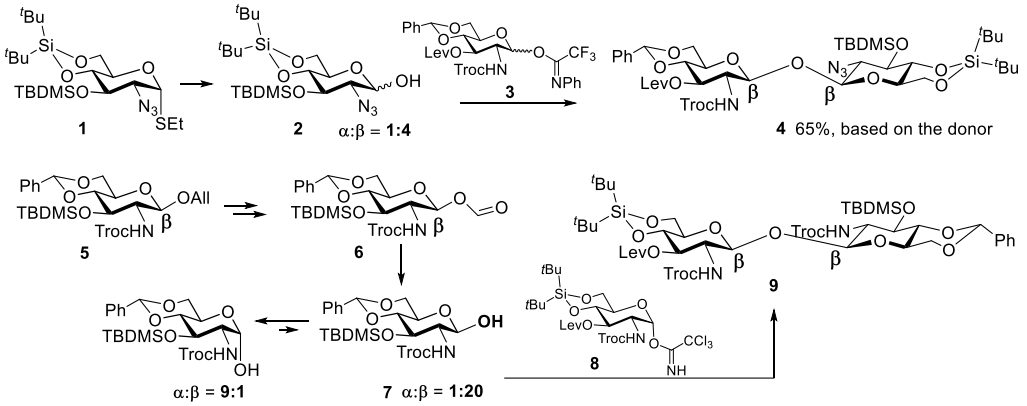
Toll-like Receptor 4 (TLR4)-mediated pro-inflammatory signaling plays a crucial role in the pathogenesis of numerous chronic and acute inflammatory and autoimmune diseases which highlights the potential of addressing TLR4 as therapeutic target. Besides, TLR4 provides a useful link between the innate and adaptive immunity, which fosters the development of TLR4 agonists as potential vaccine adjuvants. Rational pharmacological manipulation of TLR4 complex is not well established owing to both species-specificity and unpredictable effects on the TLR4 activation imposed by subtle variations in the structure of a natural TLR4 ligand Lipid A. We have recently demonstrated the advantages of application of the non-reducing β,α- and α,α-linked disaccharides as scaffolds for artificial TLR4 ligands [1-3]. According to our SAR studies, Lipid A mimetics based on the β,β-1,1'-linked diglucosamine scaffold would provide powerful TLR4 agonists. The development of synthetic routes to nonsymmetric orthogonally protected β,β-1,1'-linked disaccharides is challenging due to a possible formation of four diastereomeric products. In our novel approach, the β-stereoselectivity was ensured by application of tortionally locked (via 4,6-*O*-DTBS- or 4,6-*O*-benzylidene acetal protecting group) *N*-carbamate GlcN-imidate donor, whereas the stereochemistry on the side of the glycosyl acceptor was established by two different approaches.

In the first approach, NBS-mediated hydrolysis of the 2-azido protected thioglycoside **1** afforded lactol **2** with high excess of β-anomer (α:β=1:4). In the subsequent TMSOTf-promoted glycosylation of hemiacetal **2** by imidate donor **3**, the superior reactivity of the β-configured anomeric OH- group in acceptor **2** allowed for a high-yielding nonsymmetric β,β-1,1' glycosylation. In the second approach, β-allyl glycoside **5** was isomerized to the corresponding 1-propenyl ether which was further subjected to ozonolysis to give formyl glycoside **6** of the same anomeric configuration. Hydrolysis of the formyl group under mild basic conditions ensured the formation of β-hemiacetal **7** with retention of β-configuration. TMSOTf-promoted glycosylation of β-hemiacetal acceptor **7** by imidate donor **8** under conditions suppressing anomerization of the lactol acceptor furnished β,β-1,1'-linked disaccharide **9** in excellent yield.



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CONTROLLED CHEMOENZYMATIC SYNTHESIS OF HEPARAN SULFATE

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Heparan sulfates are heavily *O*-/*N*-sulfated linear polysaccharides ubiquitously expressed on cell surface and in the extracellular matrix. As result of its extreme number of positional isomers, the study of HS has been seriously hindered by a lack of efficient methodologies to prepare diverse libraries of HS oligosaccharides. Enzyme-mediated synthesis of HS is very promising, but limited to enzyme specificities, only highly sulfated heparin-like structures with repetitive sequences can be obtained. Recently we discovered that a 6-*O*-methyl group on a GlcNS residue not only prevented sulfation of the protected hydroxyl group by 6-OST, but also blocked epimerization/2-*O*-sulfation of the reducing-side GlcA by C5-epi and 2-OST, providing unique opportunities to control enzymatic epimerization and sulfation of HS backbones [1]. However, the unremovable methyl ether permanently shut down the disaccharide unit, -GlcNS6Me-GlcA-, to modification by 6-OST, C5-Epi and 2-OST, seriously limiting the synthetic flexibility. And the unnatural methyl ether group is likely to interfere binding between the HS analogs and HS-binding proteins.

In this study, an efficient chemical approach to pre-install removable 6-*O*-acetate groups site-specifically on synthetic heparason oligosaccharides was developed. The 6-*O*-acetate group was used for selective modification of heparan sulfate backbones by 6-OST, C5-epi and 2-OST, after which it can be removed upon will to abrogate the selectivity to allow access to universally modified HS molecules. The flexibility of the controlled enzymatic approach enabled by the removable acetate group was demonstrated by convergent synthesis of 5 HS hexasaccharides varying in *N*-/*O*-sulfation & epimerization from just one precursor (**Fig. 1**). The methodology is expected to aid the preparation of large HS libraries for structure-activity relationship study.

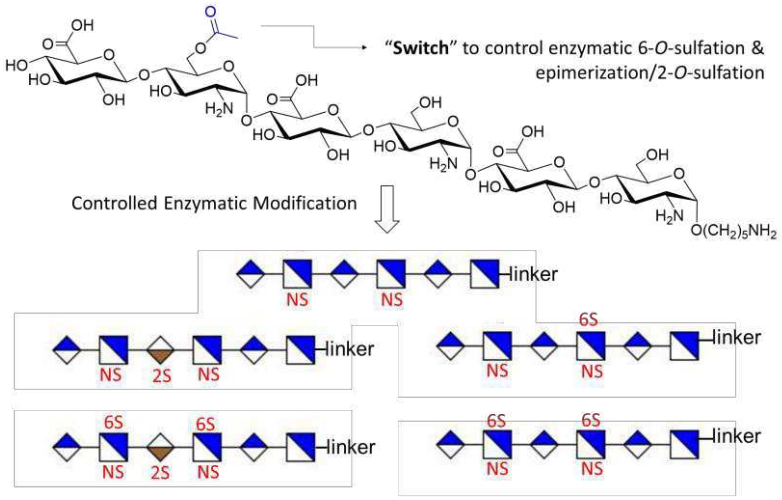

Fig. 1 6-OAc for controlled chemoenzymatic synthesis of HS

Acknowledgements: This work was financially supported by the National Institute of General Medical Sciences (Research Resource for Integrated Glycotechnology P41GM103390 to G.-J.B.).

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SYNTHESIS OF OLIGOSACCHARIDE STRUCTURES FROM THE LIPOPOLYSACCHARIDE OF MORAXELLA CATARRHALIS TOWARDS THE DEVELOPMENT OF GLYCOCONJUGATE VACCINES

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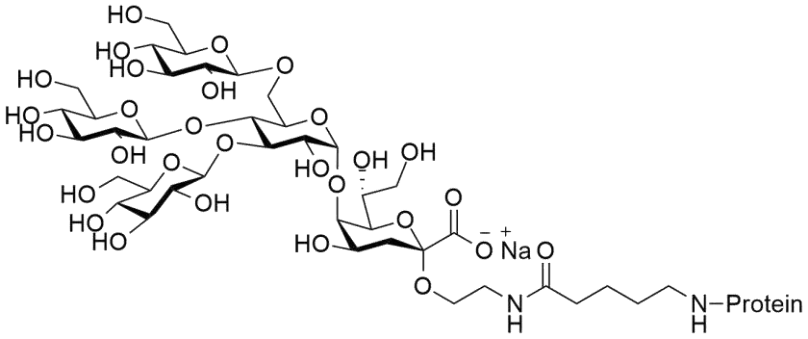
Moraxella catarrhalis is an unencapsulated gram-negative bacterium. It has been recognized as a major pathogen in a number of respiratory diseases, such as otitis media, and chronic obstructive pulmonary disease (COPD). This pathogen has shown varying degrees of resistance to all current marketed drugs. There is still no licensed vaccine against M. catarrhalis. Glycoconjugate vaccines have been introduced as a powerful weapon against infectious bacterial diseases. The development of a vaccine against M. catarrhalis infection is crucial to gain control of these respiratory diseases. The structure of the cell-surface lipopolysaccharide (LPS) of M. catarrhalis has been elucidated. In order to investigate the immunobiological properties of the LPS and to develop glycoconjugate vaccines, chemical synthesis of partial core structures are of interest. We now present recent progress of the synthesis of pentasaccharide, which contains a core tetrasaccharide and a Kdo-moiety. These structures will be conjugated to a carrier protein through the spacer-moiety to produce vaccine candidates.



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SYNTHESIS OF DEACETYLATED STRUCTURES OF *CRYPTOCOCCUS NEOFORMANS* CAPSULAR POLYSACCHARIDES

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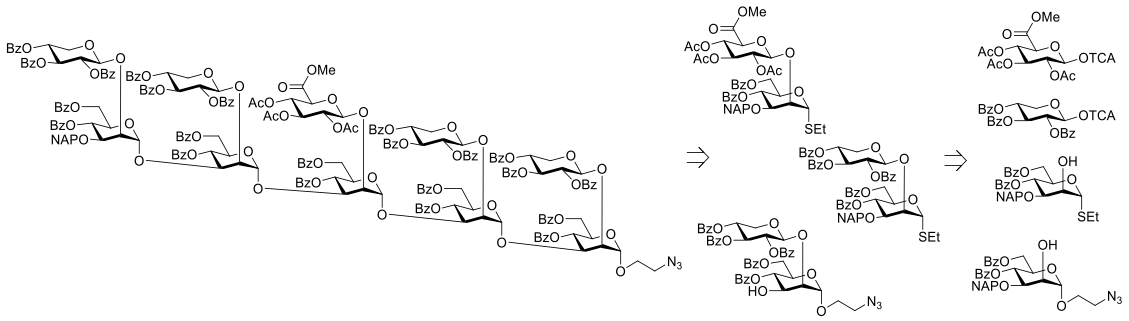
Cryptococcus neoformans is an opportunistic fungal pathogen that causes life-threatening diseases especially in immunocompromised individuals (*e.g.* HIV positive patients) [1]. *C. neoformans* is surrounded by a thick layer of capsular polysaccharides (CPS), which is an important virulence factor and is composed primarily of glucuronoxylomannans (GXM). GXM has been found to elicit both protective and non-protective antibodies due to their heterogeneity [2,3]. Therefore well-defined fragments of the polysaccharide are required, in order to investigate the immunobiological properties and to develop glycoconjugate vaccines [4]. One of the features of GXMs is the presence of acetyl groups on the 6 position of the mannose units [5]. A new, improved synthetic pathway is envisioned towards the assembly of GXM structures, aimed at investigating the role of the acetyl groups in the immunological response. The presence of permanent ester groups limits the use of basic reaction conditions in fear of ester hydrolysis. Hence a faster, more efficient route to the synthesis of GXM fragments is possible when these moieties are not present. The synthesis of disaccharide building blocks that can be assembled, using coupling conditions previously optimized in the group [6], to build *C. neoformans* CPS structures of different lengths, is presented.



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P-AMINO LIGANDS FROM IMINOSUGARS: NEW READILY AVAILABLE AND MODULAR LIGANDS FOR THE ASYMMETRIC PD-CATALYZED ALLYLIC SUBSTITUTION REACTION

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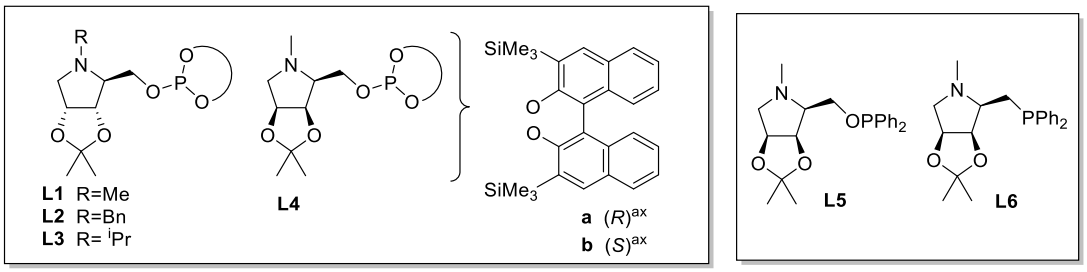
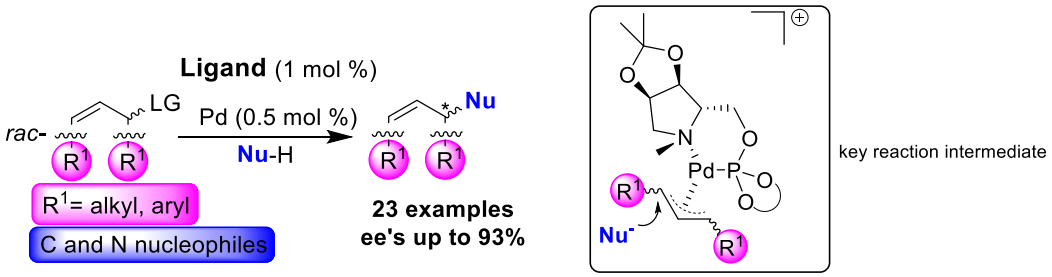
The synthesis of a new type of amino-phosphite/phosphinite/phosphine ligands containing a protected pyrrolidine-3,4-diol moiety is presented. These ligands are obtained in enantiomerically pure form, from readily available sugars. They thus contain the advantages of carbohydrates in terms of selection of the stereogenic carbons, polyfunctional groups able to modulate the electronic and sterical properties, and the general good stability of carbohydrate derivatives [1-3].



They constitute a new type of P,N-ligands that have been used in the asymmetric Pd-catalyzed allylic substitution reaction of acyclic and cyclic substrates with varied steric requirements, using different C- and N-nucleophiles. The results indicate that for carbohydrate derived amino-phosphite ligands and linear substrates, high enantioselectivity in the reactions requires an *R*-configuration of the binaphthyl moiety, while for cyclic substrates both enantiomers of the alkylated products are obtained by simply setting out the configuration of the binaphthyl phosphite moiety.



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SYNTHESIS OF THE PELLICLE REPEATING UNITS OF *LACTOCOCCUS LACTIS* STRAINS FOR INVESTIGATION OF LACTOCOCCAL PHAGE INFECTION

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Lactococcus lactis, a gram-positive bacteria, is widely used by the dairy industry due to its innate ability to produce lactic acid via lactose fermentation. The bacteria is encased in a strain specific polysaccharide pellicle, typically composed of hexasaccharide repeating units bridged by phosphodiester bonds [1]. These pellicles function as protective barriers against host phagocytosis, however lactococcal bacteriophages have been found to bind to these carbohydrate moieties via Receptor Binding Proteins (RBPs). Infection can lead to delayed fermentation, alteration of product quality and, in severe cases, loss of product; all resulting in considerable economic loss [2]. Understanding the recognition and binding processes involved in lactococcal bacteriophage infection is key to the prevention and control of its occurrence.

A common core trisaccharide, derived from the repeating units of *L. lactis* pellicles, was chemically synthesised and used to acquire structural information on the RBP/receptor site-specific binding.[3] Studies suggested that the phage-host adsorption mechanism involves two separate interactions; an initial non-specific interaction of the common core trisaccharide, followed by an additional strain-specific interaction of the remaining components. Herein, we report the block synthesis of pellicle repeating units corresponding to three distinct *L. lactis* strains (Fig. 1). The synthesised structures will be used to experimentally verify the suggested adsorption mechanism.

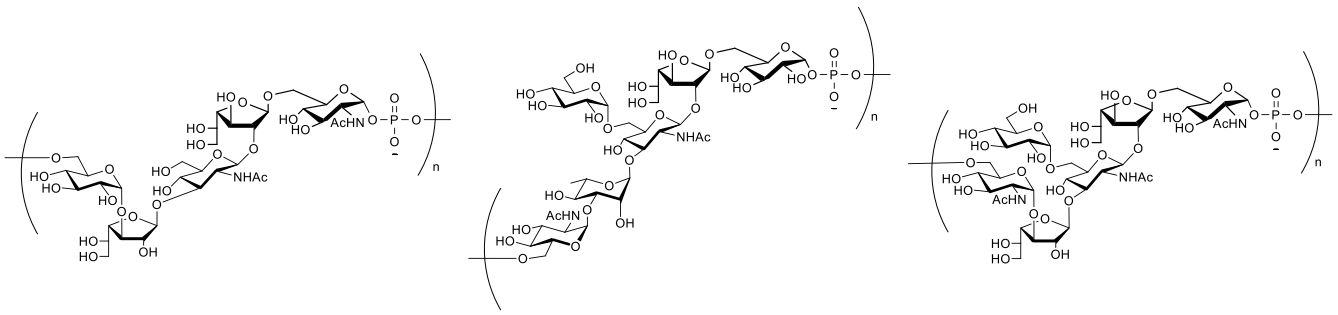

Fig. 1 - Pellicle repeating units related to L.lactis strains 3107, MG1363 and SMQ-388 (from left to right)

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TOWARDS THE SYNTHESIS OF LEWIS B STRUCTURES TO INVESTIGATE BINDING PREFERENCES OF *HELICOBACTER PYLORI*

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Helicobacter pylori is a gram-negative bacterium found in the stomach and is a major cause of gastritis, peptic ulcers and stomach cancer [1]. The bacteria utilises a lectin called the Blood-group Antigen Binding Adhesin (BabA) to bind to Lewis b (Le^b)-type structures displayed on the surface of host cells (Fig. 1) [2]. Recently, a crystal structure of the carbohydrate-binding domain of BabA has been obtained while bound to a synthetic Lewis b structure [3]. *H. pylori* strains are classified as either "generalists" or "specialists" depending on their antigen binding preferences and analysis of the crystal structure has revealed the reason behind this. In a recent publication, it was found that by varying the pH of the environment of BabA, it was possible to transform a generalist into a specialist and vice-versa [4].

The synthesis of the Le^b hexasaccharide along with A- and B-Le^b will allow variable pH studies to be performed and observe how the binding preferences of BabA changes. A linear synthesis towards the Le^b structures will be presented. The synthesis begins with a D-lactose-derived acceptor which is obtained *via* a 3',4'-*endo*-*O*-benzylidene group. Attachment of the non-reducing end D-Gal residue, with an orthogonal temporary protecting group at the 3-position, allows extension of the structure to form the A- and B-Le^b variants.

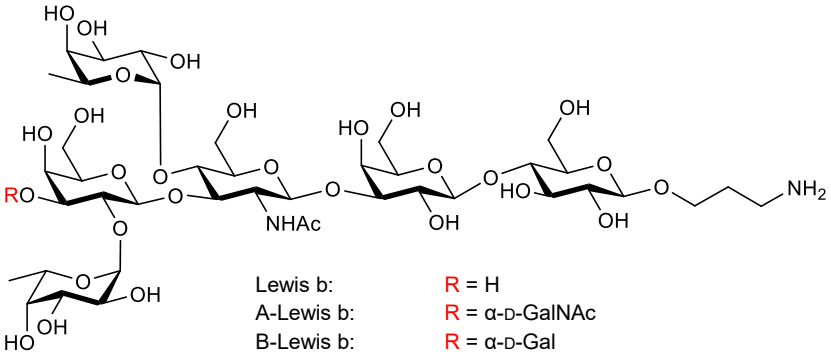

Fig. 1 – Lewis b structures attached to an amino-propyl linker.

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A NOVEL THIOESTER-MEDIATED APPROACH FOR THE SYNTHESIS OF GLYCOPEPTIDES AND GLYCOPROTEINS

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Chemical modification of proteins is a powerful tool for the investigation of biological systems and the development of therapeutic conjugates. Particularly, the facile generation of homogeneous *O*- and *N*-linked glycoprotein libraries is essential for the development of novel glycopeptide-based therapeutics [1]. To synthesize peptide carrying complex *O*- and *N*- carbohydrate structures application of segment condensation methods are essential. To date, Native Chemical Ligation (NCL) represents the gold standard for the thioester-mediated, chemoselective peptide ligation; despite its efficacy and widespread use, NCL still has certain limitations, such as the essential requirement of an *N*-terminal cysteine (Cys) at the ligation site [2]. Although several synthetic advancements were reported over the years [3] the development of efficient alternatives to NCL is an expanding area of research. The recent emergence of dehydroalanine aminoacid (ΔAla) and its successful application for protein modification [4] prompted us to develop a novel ligation methodology based on the ΔAla chemical reactivity. ΔAla is a highly electrophilic moiety, which can rapidly react with sulphur nucleophiles to generate alkyl cysteine analogues. Chemical of ligation (ΔAla) with a specific glycosylated amino thioacid furnishes a thioester intermediate, which spontaneously undergoes to an *S*-to-*N* acyl shift forging a native peptide bond (Fig. 1). The novel methodology enriches the state-of-the-art in glycoprotein ligations and undoubtedly represents a valid alternative disconnection within the overall NCL strategy.

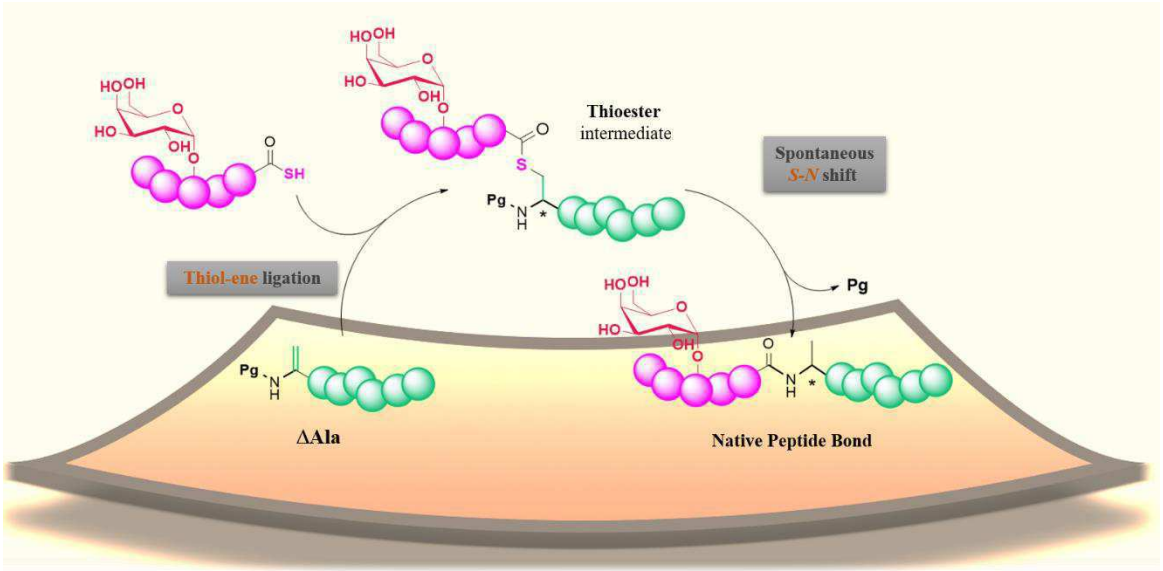

Fig. 1 - Schematic overview of ΔAla-mediated chemical ligation.

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STEREODIRECTING EFFECTS OF DEOXOFLUORINATION AT C-3 AND C-4 OF 2-AZIDO-2-DEOXY-HEXOPYRANOSYL DONORS

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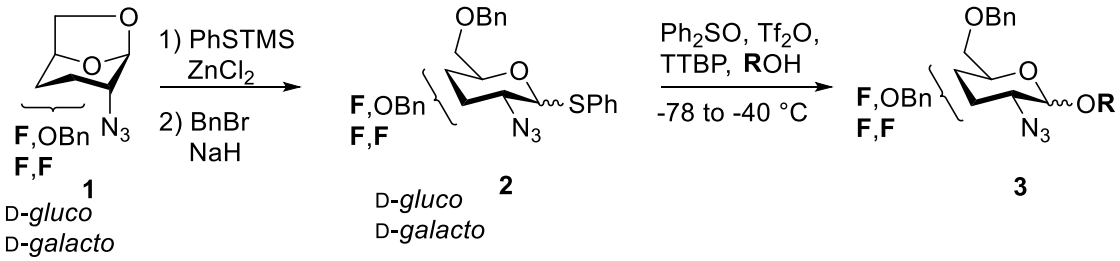
Replacement of a hydroxyl group in carbohydrates by fluorine (deoxofluorination) is a useful approach to modulate the properties of the target carbohydrate molecule because fluorine introduction has a minimal steric impact but results in a variety of other effects, including destabilization of an oxocarbenium ion during glycosylation, alterations in hydrogen bonding pattern, increased lipophilicity or stabilization of unfavorable conformations. Moreover, interaction of fluorinated carbohydrates with biomacromolecules can be studied by ^{19}F NMR which is an excellent tool for this purpose due to an absence of fluorine in biomolecules and solvents, high ^{19}F NMR sensitivity and a wide range of ^{19}F chemical shift [1].

Amino sugars are ubiquitous in nature. Both α and β linked D-glucosamine and D-galactosamine derivatives are essential components of cell-surface and extracellular glycoconjugates which participate in processes like tumour metastasis or an immune response [2]. To synthesize oligosaccharides containing deoxofluorinated analogues of D-glucosamine and D-galactosamine, the corresponding deoxofluorinated 2-amino-2-deoxyhexopyranosyl donors need to be prepared and their reactivity and stereoselectivity in glycosylation examined. We have previously described the synthesis of 3-fluoro, 4-fluoro and 3,4-difluoro analogues of 1,6-anhydro-2-azido-2-deoxy-D-gluco- and galactopyranoses **1** [3]. They have been converted to the corresponding deoxofluorinated phenyl 1-thioglycoside donors **2** in which the amino group is masked as an azide. Here we present the results obtained in glycosylation of a range of model carbohydrate acceptors with donors **2** using diphenyl sulfoxide/triflic anhydride (Ph_2SO/Tf_2O) activation [4]. Discussion of the stereoselectivity and comparison with non-fluorinated donors is included [5].



ROH = carbohydrate acceptor
TTBP = 2,4,6-tri-*tert*-butylpyrimidine

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SYNTHESIS OF A DIVERSITY OF FRAGMENTS FROM THE *SHIGELLA SONNEI* POLYSACCHARIDE ANTIGENS

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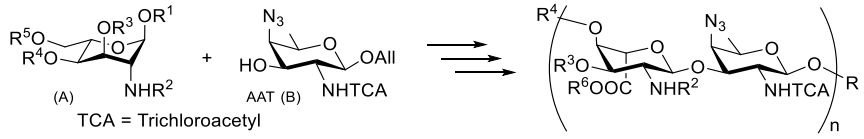
Shigellosis is a major public health concern worldwide and one of the top causative agents of death in children under 5 years of age. Epidemiological data still call for an effective vaccine against *Shigella*, especially against *Shigella sonnei* [1]. A *S. sonnei* polysaccharide-protein conjugate vaccine was immunogenic in children younger than three years but failed to induce protection in the one to three year cohort [2]. Otherwise, a synthetic-based carbohydrate conjugate vaccine against *S. flexneri* 2a [3] was demonstrated to be strongly immunogenic in human (see abstract by L. A. Mulard). This attractive strategy is now extended to *S. sonnei* as a possible alternative to the use of polysaccharides purified from bacterial cell culture.

The *S. sonnei* O-antigen and capsular polysaccharide feature a zwitterionic disaccharide repeat encompassing two rare monosaccharides, a 2-acetamido-2-deoxy-L-altruronic acid (residue A) and a 2-acetamido-4-amino-2,4,6-trideoxy-D-galactopyranose (AAT, residue B) 1,2-*trans* linked to one another. In order to identify suitable *S. sonnei* haptens, we engaged in the synthesis of oligosaccharides that have the {β-D-Fuc*p*NAc4N-(1→}$_{0/1}$[4)-α-L-Alt*p*NAcA-(1→3)-β-D-Fuc*p*NAc4N-(1→]$_n${4)-α-L-Alt*p*NAcA}$_{0/1}$ core sequence $(B_{0/1}(AB)_nA_{0/1}$, n ≥ 1) [4]. The synthesis of the AB repeating unit, chain elongation and aglycon (R) selection need careful consideration of the orthogonal protecting groups, taking into account the presence of the uronic moiety at position 6_A and of the amino moiety at position 4_B, while keeping in mind that residues A and B both have a 2-acetamido function.



This presentation will report the synthesis of selected targets using a post glycosylation oxidation strategy in combination with imidate chemistry. In particular, suitable building blocks and glycosylation chemistries were identified and validated up to the $(AB)_2$ tetrasaccharide. Additional optimization of protecting/masking group combinations, especially at the A residue ($R^{1\text{-}6}$), compatible with chain elongation and full deprotection will be discussed. Orthogonally protected precursors to residues A and B were obtained from L-glucose and D-glucosamine, respectively.

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SYNTHESIS OF C-3 AND C-4 DEOXYFLUORINATED 2-AZIDO-2-DEOXYHEXOPYRANOSYL DONORS

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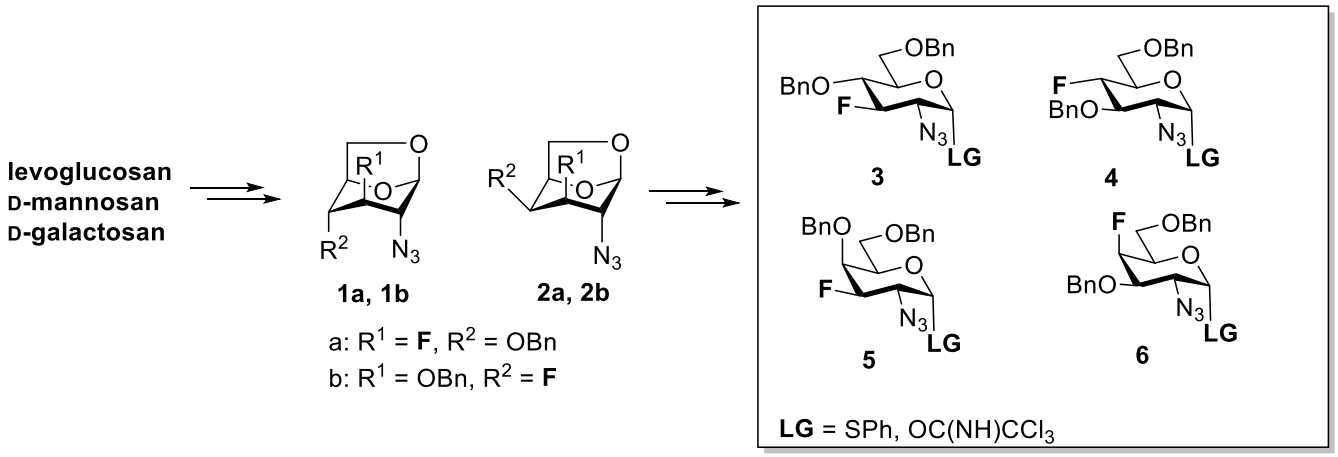
Fluorinated mono- and oligosaccharides can effectively mimic biologically or pharmaceutically important carbohydrates. Replacement of a hydroxyl for fluorine (deoxyfluorination) causes minimal steric disturbance but can confer desirable changes in stereoelectronic properties, lipophilicity, metabolic stability, hydrogen bonding pattern, or interactions with proteins. Fluorinated carbohydrates have found use as radiotracers, covalent inhibitors of glycosidases, probes of carbohydrate-lectin interactions, or unnatural carbohydrate analogues for metabolic glycoengineering. While deoxyfluorination of monosaccharides has been extensively studied [1] synthesis of fluorinated oligosaccharides remains underexplored. Central to preparation of fluorinated oligosaccharides is the synthesis of fluorinated glycosyl donors and their use in glycosylation.

We have developed a synthesis of C-3 and C-4 deoxyfluorinated D-glucosamine and D-galactosamine from levoglucosan, D-mannosan and D-galactosan [2] The key intermediates were deoxyfluorinated 2-azido-2-deoxy-1,6-anhydro-β-D-hexopyranoses **1a-b** and **2a-b**. Here we present an extension of our work to the synthesis of deoxyfluorinated 2-azido-2-deoxy-hexopyranosyl donors **3**-**6** from 1,6-anhydrohexopyranoses via intermediates **1** and **2**. Phenyl 1-thioglycosides and anomeric trichloroacetimidates were chosen as the target glycosyl donors because they are widely used and highly versatile [3]. The amino group was masked as an azide to permit stereocontrolled formation of α-linked glycosides (1,2-*cis* glycosylation) [4].



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INNOVATIVE THERAPEUTICS STRATEGIES FOR WILSON DISEASE

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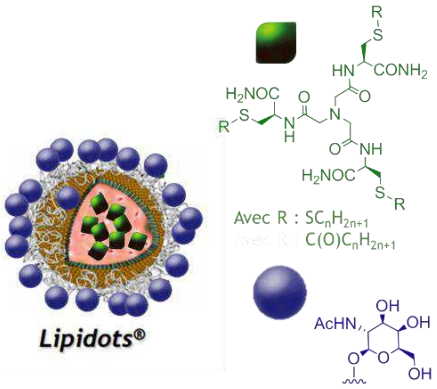
Wilson disease is a genetic disorder which induces a toxic copper overload mainly in the liver. Because current copper chelating therapies present many side effects, intrahepatocyte copper(I) chelators has been previously developed in the SYMMES laboratory as promising drug candidates. These prodrugs are based on bioinspired cysteine-based chelators, functionalized with N-acetylgalactosamine residues, known ligands of the asialoglycoprotein receptors (ASGPr) leading to their hepatocyte incorporation [1].
In this project, we propose an alternative strategy taking advantage of nanoparticles. Lipidots®, biocompatible and stable lipid-based nanoparticles developed by the DTBS team, show a great interest for drug delivery of lipophilic drugs [2]. Then, lipophilic derivatives of the efficient and selective copper chelator NTA($CysNH_2$)$_3$ are encapsulated in the lipid core of the Lipidots®. The surface is functionalized by N-acetyl-galactosamine residue to effectively target ASGPr. The ability of these functionalized nanoparticles to enter the hepatocytes has been evaluated by flow cytometry.



This research was supported by Région Rhone Alpes ARC1 Santé and Labex Arcane (Grant ANR-11-LABX-0003-01). The author thanks Glyco@Alps for financial support for the participation at the ICS 2018

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SYNTHESIS OF ANOMERIC SPIRO-FUSED D-FRUCTOSE OXAZOLINES

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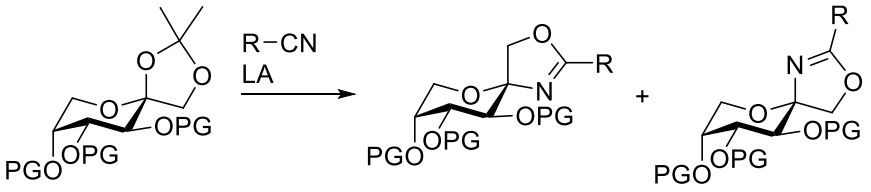
The design of new ligands for metal catalyzed stereo selective reactions is an ongoing field in organic chemistry. Several highly efficient ligands are derived from carbohydrates [1]. An important aspect for the design of chiral ligands is molecular rigidity of the backbone. Spiro-fusion of bicyclic compounds provides rigid molecules. Due to this spiro-fusion has already been applied in the construction of ligands for stereo selective catalysis [2-4]. 2-Oxazolines are heterocyclic compounds which are part of a lot of important classes of privileged ligands like bis(oxazoline) (BOX) or phosphinooxazoline (PHOX) ligands.[5] An often used way to build up oxazolines is the Ritter reaction of diols or amino alcohols with nitriles under acidic conditions [6, 7].

Here we present the synthesis of anomeric spiro-fused D-fructose oxazolines **2** via Ritter reaction of the 1,2-isopropylidene protected fructose derivative **1** with a Lewis acid and a nitrile. The reaction proceeds in fair to high yield (up to 86 %). The oxazolines are obtained as mixtures of the α- and β-anomer which can be separated by column chromatography. The α/β-ratio depends on the substitution pattern of the fructose derivative **1**. We determined the configuration at the anomeric center by X-ray crystallography. Mechanistic considerations regarding the synthesis and the molecular structure of the oxazolines will be presented. The spiro oxazolines **2** can be used as building blocks for the synthesis of ligands for metal catalyzed stereo selective reactions.



1 **2-α** **2-β**

PG: protective group, LA: Lewis acid

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SYNTHESIS AND PROPERTIES OF CRYPTANDS WITH SUCROSE SCAFFOLD

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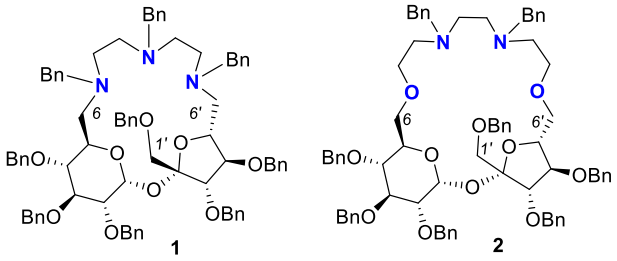
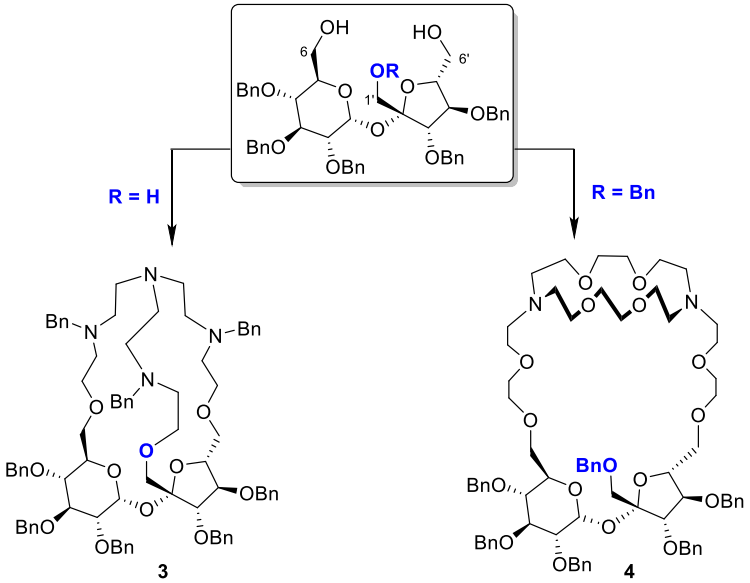
We are engaged in the synthesis of macrocyclic derivatives with sucrose scaffold. The results obtained by us up to date indicate that sucrose can be an efficient chiral platform for the receptors able for enantioselective recognition of chiral guests. [1]



Recently we have proposed convenient methods for the synthesis of crown and aza-crown ether analogs with sucrose scaffold (*e.g.* compounds **1** and **2**). We have demonstrated that such receptors have good affinity towards ammonium cations. [2] We expect, based on our previous results, that sucrose cryptands (*e.g.* compounds **3** and **4**) having much more rigid structure should possess better complexing properties towards chiral ammonium cations. As is reported, cryptands have much higher affinity towards ammonium cations than the corresponding aza-crown analogs. [3]



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CHIRAL CYCLOTRIVERATRYLENE-BASED MOLECULAR CONTAINERS WITH SUCROSE UNIT

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Chiral recognition of biologically important compounds by synthetic receptors, being the main trend in supramolecular chemistry, plays a significant role in pharmaceutical and biological research [1,2].

Macrocyclic derivatives with sucrose scaffold can be applied as receptors for the complexation of chiral guests [3]. On the other hand, cyclotriveratrylene and its derivatives are well-known molecular receptors for metals, fullerens, ammonium salts, and small organic compounds [4,5].

This communication will present the synthesis pathway to new chiral molecular containers composed of both building blocks. Sucrose, in a three-step synthesis, was converted into 2,3,3',4,4'-penta-*O*-benzyl derivative **1**, which was subsequently transformed into triiodide **2**. The combination of the cyclotriveratrylene unit **3** with triple alkylating agent **2** afforded two chiral molecular containers ***P*-4** and ***M*-4**.

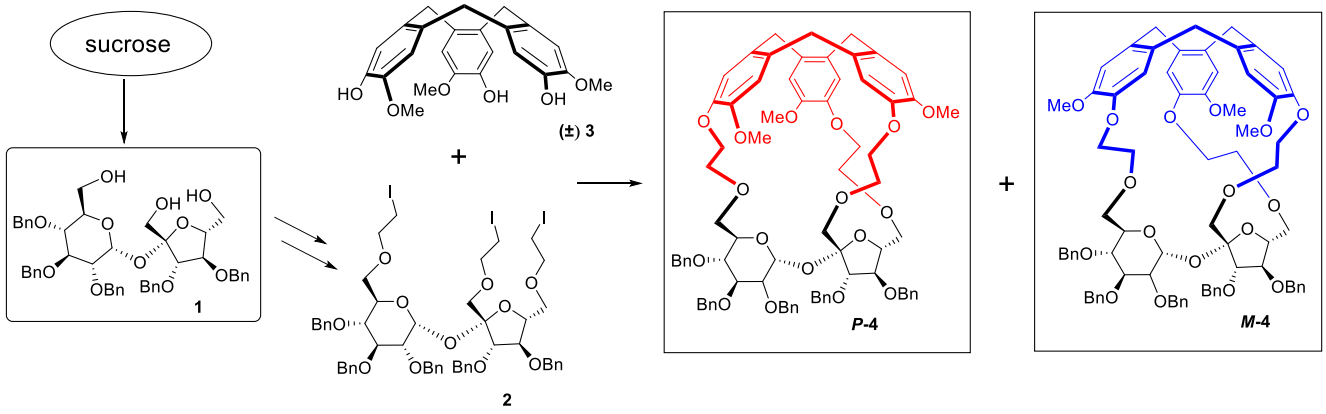

Fig. 1. Synthesis of chiral molecular containers based on sucrose.

These receptors are the first molecular containers obtained from sucrose and cyclotriveratrylene moiety.

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SURFACTANTS OF BIOLOGICAL ORIGIN: THE ROLE OF Mo(VI) AND MICROWAVES IN THE SYNTHESIS OF XYLAN-BASED NON-IONIC SURFACTANTS

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Surfactants of biological origin are attracting increasing attention because they represent eco-friendly alternative to their synthetic counterparts. These compounds can be tailored for many applications to achieve new materials with desired physico-chemical properties. Owing to their excellent surfactant properties, higher stability at extremes of pH, temperature and salinity, they are gaining interest in the market for detergent and cosmetic applications with high ecological profile [1].

In the current context of sustainability, there is a growing demand in developing effective catalysts for the production of functional materials based on xylan. Recently, solid heteropolyacids have received a considerable attention as they exhibit high catalytic activity and selectivity. In continuation of our efforts on the application of heterogeneous catalysts, especially molybdates, we wish to demonstrate that phosphomolybdic acid, supported on silica gel ($PMoA/SiO_2$), is an efficient catalyst for the synthesis of the natural non-ionic surfactants. The microwave-assisted $PMoA/SiO_2$-catalyzed, glycosylation of unprotected D-xylose and D-lyxose, obtained after tandem Mo(VI)-catalyzed xylan hydrolysis-epimerization reaction, provides alkyl xylosides and lyxosides in short reaction times [2]. A homologous series of amphiphilic alkyl pentosides varying in chain structure (C_8–C_{14}) was prepared in very good yields (38−73%). New catalytic approach using the reusable heterogeneous $PMoA/SiO_2$ catalyst provides benefits in terms of yields, environmental safety, operational simplicity, and thus opens new perspectives for the rational hemicellulose biomass utilization.

The application of this approach for the synthesis of new family of non-ionic amphiphiles makes the method attractive because of their potential use in biomedical and pharmaceutical chemistry.

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THE TRANSFORMATION OF BETULIN CORE

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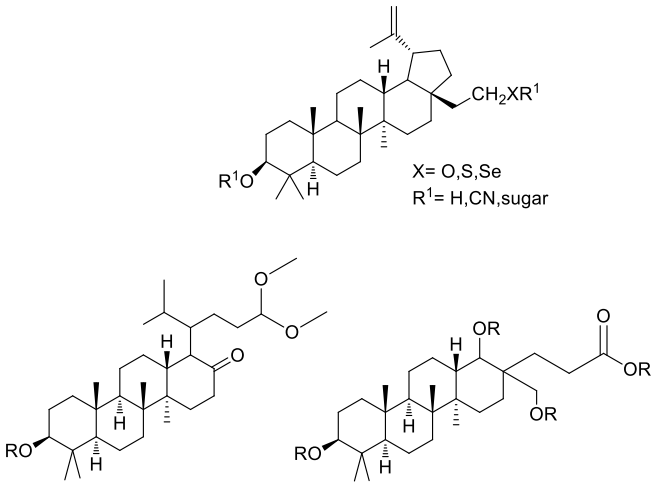
Betulin, widely widespread in nature, belongs to the lupane triterpenes. The richest source of betulin is the outer layer of a birch bark. Betulin and its derivatives show high biological activity; *eg.* cytotoxicity, anti-inflammatory and antiviral effects. The broad spectrum of biological activity, low toxicity and high availability of betulin attract attention of the pharmaceutical and cosmetic industries.
In our laboratory we have developed synthesis of new modified lupane triterpenoids and lupane saponins. In vitro studies indicated that the presence of oxygen atom located on the C-17 side chain is necessary for the cytotoxicity of the studied compounds. Large number of tested derivatives enabled us to relate their cytotoxicity and structure. As a result, preliminary determination of active sites in the structure of the studied lupanes was possible. This encouraged us to undertake broader research into the influence of betulin E-ring modification on the biological activity. Modifications included replacing the oxygen atom in the side chain with sulfur or selenium atoms as well as the opening of the E ring. As we expect, it could enhance anticancer effect. Details will be presented during the conference.



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SYNTHESIS AND IMMUNE FUNCTION OF *CAMPYLOBACTER JEJUNI* LIPID A

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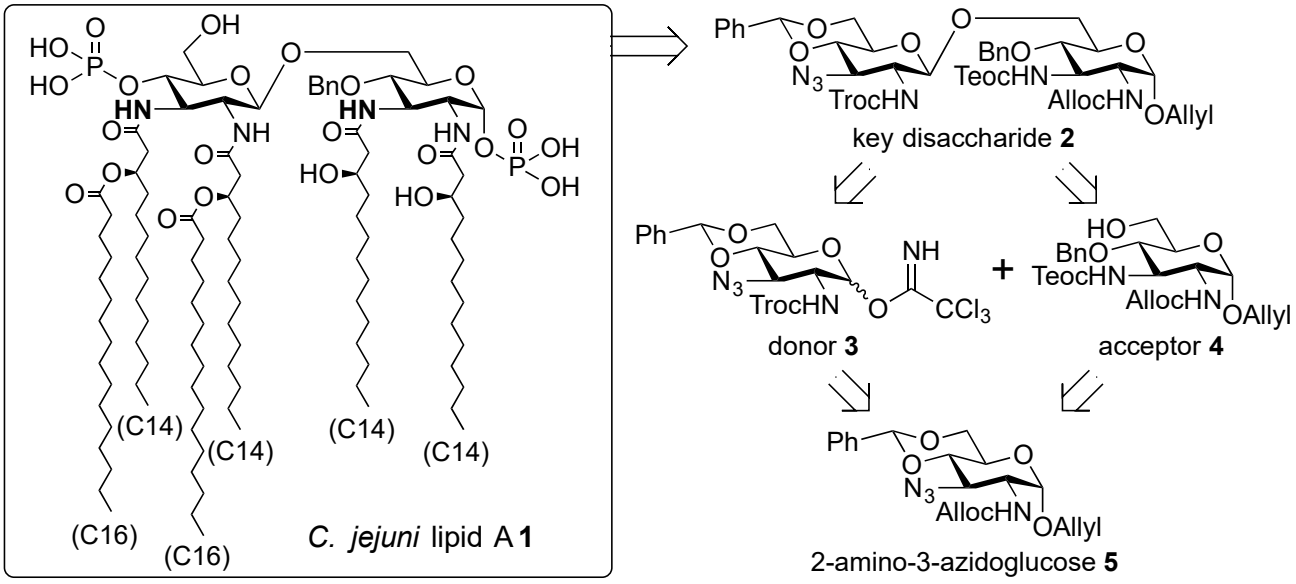
Gram-negative bacterium *Campylobacter jejuni* (*C. jejuni*) produces lipooligosaccharide (LOS), which consists of glycolipid "lipid A" and oligosaccharide [1]. Since the terminal part of the oligosaccharide has molecular homology with gangliosides, *C. jejuni* infection induces cross-reactive antibodies to cause autoimmune diseases such as Guillain-Barré syndrome in a small number of patients [2]. However, *Helicobacter pylori* possessing the same oligosaccharide hardly causes autoimmune diseases [3]. We therefore hypothesized that the lipid A is also a dominant factor for autoimmune diseases.

Unlike general lipid As composed of glucosamine disaccharide skeleton, *C. jejuni* lipid As exist as congeners which have unique disaccharide skeletons composed of 2,3-diaminoglucose and/or glucosamine and had never been synthesized [4]. In this work, we achieved the first synthesis of *C. jejuni* lipid A **1** possessing 2,3-diaminoglucose disaccharide skeleton by the following strategy (Scheme 1).

We synthesized 2-amino-3-azidoglucose **5** as a common monosaccharide intermediate by azidation of glucosamine via sequential S_N2 inversion. Glycosyl donor **3** and acceptor **4** prepared from intermediate **5** were then coupled to give a key disaccharide **2**, which can be used for the synthesis of various *C. jejuni* lipid As. Acyl and phosphate groups were then successively introduced to **2**. Final deprotection afforded *C. jejuni* lipid A **1**. NF-κB-inducing activity of **1** was also evaluated by using HEK-Blue™ TLR4 cells.



Scheme 1. Retrosynthesis of *C. jejuni* lipid A

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DISACCHARIDE THIOLS FOR THE SYNTHESIS OF S-LINKED GLYCOSAMINOGLYCAN ANALOGUES

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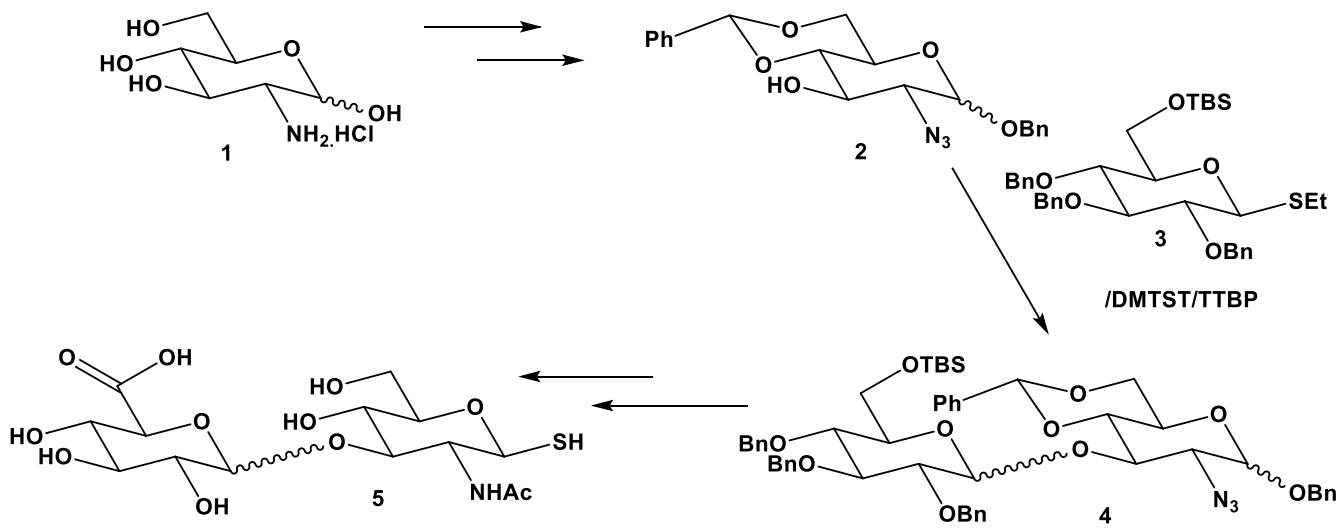
Glycosaminoglycans (GAGs) are an important group of biomolecules and are unbranched polysaccharides consisting of repeating disaccharide moieties. One GAG, heparin, is polysulfated with alternating D-glucosamino and either D-glucuronic or L-iduronic acid units and is used as an anticoagulant drug [1]. As part of our work on heparin analogues in which the disaccharide units are S-linked rather than O-linked, we are attempting to synthesize the model disaccharide thiol **5**. The planned route to **5** is shown in the Scheme. Glucosamine hydrochloride **1** has already been converted into intermediate **4** and work on the conversion of **4** into **5** is underway. If **5** can be synthesized and used successfully to make a heparin analogue, the synthesis will be modified in order to obtain a single diastereoisomer of **5** with a β-glycosidic bond.



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GLYCOSAMINOGLYCAN MIMETICS FROM MARINE BACTERIAL EXOPOLYSACCHARIDES AND INNOVATIVE CONCEPTS FOR THEIR STRUCTURAL ELUCIDATION

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Polysaccharides such as glycosaminoglycans (GAG) are attractive for their biological activities (heparin, hyaluronic acid) or/and physicochemical properties (gellan, xanthan). Extraction of these molecules from animal tissue is the main issue (difficulty of extraction, possibility of contamination…).
In research of new bioactive molecules, bacteria from marine origin constitute a considerable source of innovative molecules. In particular, exopolysaccharides (EPS) produced by these bacteria become a renewable source of biocompatible and biodegradable molecules. The GY785 EPS, produced by the deep-sea hydrothermal vent strain *Alteromonas infernus*, is an anionic branched high molecular weight heteropolysaccharide with a nonasaccharide repeating unit. The low molecular weight derivatives of this EPS have previously shown to display some interesting GAG-like properties (Fig. 1) [1, 2].
However, two questions remain unanswered: what is the accurate structure of the obtained derivatives? And how do the modifications affect the biological properties of the derivatives?
To answer to these questions, oligosaccharidic fragments that are a constitutive part of the GY785 EPS will be obtained by either enzymatic degradation [3] or organic synthesis. The organic synthesis of a pentasaccharide fragment of the EPS repeating unit is currently in progress and will be described during this presentation.

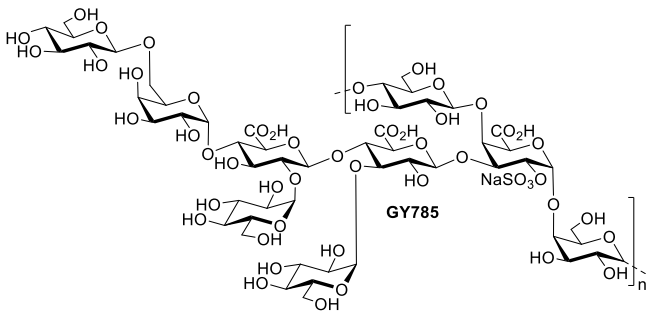

Fig. 1 - Exopolysaccharide GY785

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SYNTHESIS AND ACTIVITIES OF THE FUNCTIONAL STRUCTURES OF A GLYCOLIPID ESSENTIAL FOR MEMBRANE PROTEIN INTEGRATION

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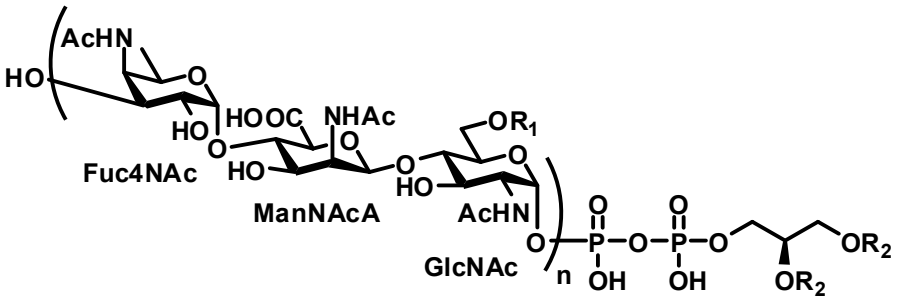
MPIase is the first known glycolipid that is essential for membrane protein integration in the inner membrane of *E. Coli* [1]. We synthesized the minimal unit of MPIase, a trisaccharyl pyrophospholipid termed mini-MPIase-3, and its derivatives. Integration assays revealed that the chemically synthesized trisaccharyl pyrophospholipid possesses significant activity, indicating that it includes the essential structure for membrane integration. Structure-activity relationship studies demonstrated that the length of glycan and the 6-*O*-acetyl group on *N*-acetylglucosamine contribute to efficient integration. Furthermore, anchoring in the membrane by a lipid moiety was essential for the integration. However, addition of phosphorylated glycans devoid of the lipid moiety modulates the integration activity of MPIase embedded in liposomes, suggesting the interaction between phosphorylated glycans and substrate proteins in aqueous solution. The prevention of protein aggregation required the 6-*O*-acetyl group on *N*-acetylglucosamine, a phosphate group at the reducing end of the glycan, and a long glycan chain. These results verified the integration mechanism in which a membrane protein is captured by a glycan of MPIase, which maintains its structure to be competent for integration, and then MPIase integrates it into the membrane by hydrophobic interactions with membrane lipids. Since the amount of natural MPIase available for analysis is limited and it contains structural heterogeneity, precisely designed synthetic derivatives are promising tools for further elucidation of its membrane protein integration mechanism.



MPIase : n=9-11, R_1=Ac or H, R_2=C16:1, C16:0, C18:1, C18:0 etc.
mini-MPIase-3 : n=1, R_1=Ac, R_2=C14:0
mini-MPIase-3 (6-OH) : n=1, R_1=H, R_2=C14:0

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MOLECULAR CHARACTERIZATION OF MANNOSE 2-EPIMERASE

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Carbohydrate epimerization is an important reaction in the carbohydrate metabolism. Acylglucosamine 2-epimerase (AGE) and cellobiose 2-epimerase (CE), which catalyze the 2-epimerization of *N*-acetylglucosamine and cellobiose, respectively, share an $(\alpha/\alpha)_6$-barrel catalytic domain and form AGE superfamily. Runsl_4512 from *Runella slithyformis* is a member of AGE superfamily, but shows low sequence identity with any characterized enzymes. In this study, Runsl_4512 and its homologue Dfer_5652 from *Dyadobacter fermentans* were biochemically characterized.

Recombinant Runsl_4512 with the C-terminal His-tag was produced in *Escherichia coli* and purified to homogeneity by Ni-affinity column chromatography. It produced D-mannose and D-glucose from D-glucose and D-mannose, respectively, while it did not act on Manβ1-4Man, *N*-acetylglucosamine, and D-fructose, which are substrates of the other known AGE superfamily members. Consequently, we concluded that Runsl_4512 is a novel carbohydrate epimerase, D-mannose epimerase (ME), catalyzing the interconversion between D-mannose and D-glucose. Recombinant Dfer_5652 prepared in the same manner as Runsl_4512 also showed ME activity. The k_{cat}/K_m values of Runsl_4512 and Dfer_5652 for the D-mannose epimerization were 3.90 and 3.48 $min^{-1}mM^{-1}$, respectively. D-Mannose production level by Runsl_4512 reached approximately 30%. The equilibrium constant was consistent well with that of the reactions of CE with β1-4disaccharides [1].

In the time course analysis of the reaction of Runsl_4512 by ^{1}H-NMR, formations of β-D-glucose and β-D-mannose from D-mannose and D-glucose, respectively, were observed as the initial reaction products. The signals of 2-H of D-mannose and D-glucose were not detected in the ^{1}H-NMR analysis of the equilibrated reaction product produced in D_2O, and the signals of deuterium were detected at the corresponding chemical shifts of 2-H of D-mannose and D-glucose in ^{2}H-NMR. This result indicates that 2-H of the substrate was abstracted by the general base catalyst of the enzyme, and a deuterium was added by the general acid catalyst. This proton abstraction-addition mechanism is consistent with the mechanism postulated in the CE and AGE reactions [2,3]. His274 and His404 of Runsl_4512 were predicted to be the catalytic residues based on structural comparison of Runsl_4512 with the AGE superfamily enzymes.

Comparison of the amino acid sequences of MEs and CEs suggested that MEs have an insertion including 23 amino acid residues into the loop region connecting the seventh and eighth α-helices of the catalytic domain. As this loop forms the binding site of non-reducing-end sugar residue in CEs, this long loop of ME might be important for the monosaccharide specificity.

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IS UGT74B1 A PURE *S*-GLYCOSYLTRANSFERASE?

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Thioglycosides (***S*-glycosides),** in which a sulfur atom has replaced the glycosidic oxygen atom of carbohydrates, are tolerated by most biological systems. Their major advantages rely on the fact that they adopt similar conformations than the corresponding *O*-glycosides and especially that they prove to be **less sensitive to acid/base or enzyme-mediated hydrolysis** [1,2]. So far, few examples of natural *S*-glycosides were reported in the literature. Until recently, plant **glucosinolates** were the only group of *S*-glycosides that were characterized in Nature. These compounds are found in *brassicae* and are involved in the plant protective strategy, called "mustard oil bomb", that occurs after their hydrolysis and subsequent degradation in toxic sulfur-containing compounds (thiocyanates, isothiocyanates, …).

Arabidopsis thaliana ***S*-glycosyltransferase UGT74B1** [3] catalyzes the formation of *S*-glucosidic bond in the aromatic glucosinolates biosynthetic pathway, using UDP-glucose as sugar donor and thioxydroximate as thiol acceptor.

Fig. 1

UGT74B1 has previously proved to be an efficient catalyst able to be used in the **first chemo-enzymatic synthesis of desulfoglycosinolates analogues** bearing other sugar moieties than glucose [4]. Using a range of acceptors, UGT74B1 could catalyze *S*-glycosylation, as well as *O*-glycosylation, yet with a much lower efficiency. However, the specificity for thiol vs. alcohol was **correlated** by Brønsted analysis not only to the nature of the atom, but also to the **chemical properties of the acceptor** (*eg*. p*K*a). This was experimentaly demontrasted by 'tuning' the pKa of a range of alcohol acceptors that led to an increase of *O*-glycosylation rates to a level close to *S*-glycosylation. Thus, UGT74B1 substrate specificity (*S*- *vs. O*- acceptor) is mostly dictated by the acidity of the nucleophile, and not by the nature of the atom to be glycosylated.

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PREPARATION OF SUGAR 1-PHOAPHATES BY ANOMERIC KINASES COMBINED WITH THE ATP-REGENERATION SYSTEM USING PYRUVATE AS THE ENERGY SOURCE

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Sugar 1-phosphates are important compounds in the sugar metabolism and the sugar chain-synthesis. They are also used in the enzymatic preparation of oligosaccharides by phosphorylases. Several sugar 1-phosphates can be synthesized from monosaccharides and ATP by anomeric kinases. Anomeric kinases are often inhibited severely by high concentration of ATP, their phosphate donor, making it impossible to synthesize the compounds at practical concentrations from monosaccharides and ATP. We previously demonstrated the gram-scale preparation of sugar 1-phosphates from monosaccharides and phosphoenolpyruvate (PEP) by the combined actions of anomeric kinase and pyruvate kinase [1]. The ATP-regeneration system allows the syntheses at the practical concentrations. However, this reaction consumes equimolar of PEP, an expensive compound, with the generation of sugar 1-phosphate.

We designed the practical ATP-regeneration system with the consumption of pyruvate, which is not so expensive as PEP or ATP. The system consists of four enzymatic reactions as follows. (1) Pyruvate oxidase converts pyruvate, phosphate, and O_2 into acetyl phosphate, CO_2, and H_2O_2. (2) Anomeric kinase produces sugar 1-phosphate from monosaccharide and ATP. (3) Acetate kinase converts acetyl phosphate and ADP into acetate and ATP. (4) Catalase disproportionate H_2O_2 to 1/2 O_2 and H_2O. Totally, monosaccharide, pyruvate, phosphate, and 1/2 O_2 are converted into sugar 1-phosphate, CO_2, acetate, and H_2O by the concerted actions of the four enzymes in one pot. The reactions were performed with 0.40 M pyruvate, 0.20 M monosaccharide, 0.22 M phosphate, 0.5 mM ATP, and four enzymes in petri dishes with the depth of 0.5 cm at 30 °C under oxygen atmosphere. We examined four monosaccharides (Gal, Man, GlcNAc and GalNAc) to produce sugar 1-phosphates. The recombinant enzymes, galactokinase and *N*-acetylhexosamine 1-kinase from *Bifidobacterium longum* [2], were used to produce Gal1P and Man1P/GlcNAc1P/GalNac1P, respectively. Those of the reaction yield based on the monosaccharides reached approximately 90 % after the reaction for 180 hr. The resultant sugar 1-phosphates were purified through two electrodialysis steps using membranes with different cut-off sizes. Finally, the compounds were crystallized as dipotassium or biscyclohexylammonium salts.

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STUDIES TOWARD THE SYNTHESIS OF *BACTEROIDES FRAGILIS* α-GALACTOSYLCERAMIDE (α-GalCer$_{Bf}$)

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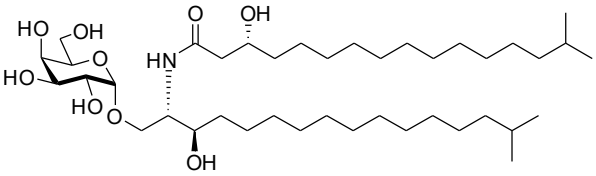
Invariant Natural Killer T (iNKT) cells contribute to the innate immune response. Through T-cell receptors (TCR) expressed on the surface, iNKT cells recognize glycolipid antigens when presented by the antigen-presenting molecule CD1d on antigen presenting cells. The best-known lipid antigen for iNKT cells is the α-galactosylceramide KRN7000, a purely synthetic molecule, which can mediate a variety of pro-inflammatory and immuno-regulatory functions [1].

Bacteroides fragilis (*Bf*) is a member of the human gut microbiota and produces an inseparable mixture of glycolipids termed α-GalCer$_{Bf}$. These natural products have a striking structural similarity to KRN7000, leading to speculation that they may be CD1d-restricted iNKT cell antigens. Two independent studies have presented conflicting reports on the activity of α-GalCer$_{Bf}$, with one showing that α-GalCer$_{Bf}$ stimulated iNKT cells *in vitro* [2], while the other showed it inhibited iNKT activation by KRN7000 [3].

In this project, we are undertaking the synthesis of one pure component of α-GalCer$_{Bf}$, namely α-GalCer$_{Bf\text{-}716}$ and some analogues. Our proposed approach requires three fragments: a chiral 3-hydroxy iso-fatty acid, a sphinganine alcohol, and a galactosyl donor. They can be linked together by glycosylation and an amide coupling. Acquisition of pure α-GalCer$_{Bf\text{-}716}$ will allow more detailed examination of its effects upon CD1d-restricted T cells and provide a better understanding of the effects of the *Bacteroides fragilis* glycolipids on the human immune system.

KRN7000



α-GalCer$_{Bf\text{-}716}$

Fig. 1

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SELENOISOPEPTIDE CHEMICAL LIGATION TOWARD CHEMICAL SYNTHESIS OF HOMOGENEOUS UBIQUITINATED GLYCOPROTEIN CONTAINING DISULFIDE BOND

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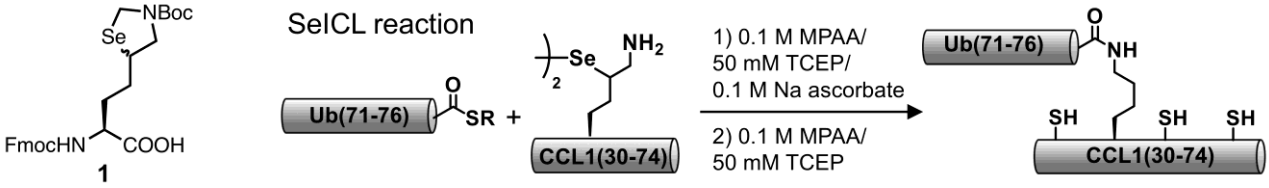
We have been studying the glycoprotein quality control system in the endoplasmic reticulum (ER) using chemically synthesized homogeneous misfolded glycoproteins [1]. Misfolded glycoproteins which could not gain native structure through this refolding process are directed toward ER associated degradation (ERAD) pathway, and finally degraded in the cytosol with ubiquitin-proteasome system. To analyze the degradation process of misfolded glycoproteins, we decided to synthesize homogeneous ubiquitinated glycoprotein as a probe. Isopeptide chemical ligation (ICL), which utilizes δ-mercapto-lysine residue at the ubiquitination site, is frequently used for chemical ubiquitination of proteins. However, ICL requires desulfurization of δ-mercapto-lysine residue after ligation. Many glycoproteins contain disulfide bonds and protection of the sidechain of cysteines are required for selective desulfurization of the δ-mercapto-lysine residue. To avoid this tedious synthetic step, we designed selenoisopeptide chemical ligation (SeICL), which utilize δ-seleno-lysine residue instead of δ-mercapto-lysine residue. We envisioned that selective deselenization can be achieved in the presence of free sulfhydryl groups of cysteine residues.

First, we examined the chemical synthesis of suitably protected δ-seleno-lysine derivative for solid phase peptide synthesis (SPPS). Fmoc-δ-seleno-L-lysine derivative **1** was synthesized in 14 steps from commercially available δ-DL-hydroxy-DL-lysine via enzymatic optical resolution using L-aminoacylase [2]. We selected CC motif chemokine 1 (CCL1) as a model glycoprotein. CCL1 consists of 74 amino acids and one N-glycan. We decided to introduce high-mannose type N-glycan because glycoproteins degraded through ERAD pathway contains this type of N-glycan. Full-length CCL1 glycosylpolypeptide was divided into three segments; N-terminal peptide-α-thioester, middle glycopeptide-α-thioester, and C-terminal peptide containing δ-seleno-lysine residue. Ubiquitin-α-thioester was synthesized by three-segment ligation strategy. Ubiquitin(1-27)-α-thioester and ubiquitin(28-45)-α-thioester were synthesized by Boc-SPPS, whereas ubiquitin(46-76)-α-hydrazide was synthesized by Fmoc-SPPS. One-pot three segment ligation gave ubiquitin(1-76)-α-hydrazide. To determine the reaction condition of SeICL, we examined ligation between ubiquitin(71-76)-α-thioester and CCL1(30-74, δ-Se-Lys42). Ligation reaction proceeded smoothly in the presence of 0.1 M 4-mercaptophenylacetic acid (MPAA), 50 mM tris(2-carboxyethyl)phosphine (TCEP), 0.1 M sodium ascorbate. Selective deselenization proceeded in the presence of 50 mM TCEP, 0.1 M MPAA without any desulfurization. SeICL reaction using full-length ubiquitin-α-thioester is now under examination. We believe that homogeneous ubiquitinated glycoprotein can be synthesized efficiently using the SeICL reaction.



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THE DIRECT CONNECTION OF ANOMERIC CENTRES

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Carbon bridged trehalose-analogues gained considerable interest, due to their potential medical application based on reversible glycosidase inhibition [1-3]. In contrast to these bicyclic sugar moieties linked by a carbon spacer, carbohydrates featuring the direct connection of the anomeric centres are rare and only a few examples are known in the literature [4], [5]. In this work, we extend the scope of anomeric coupling reactions, represented by four synthetic strategies.

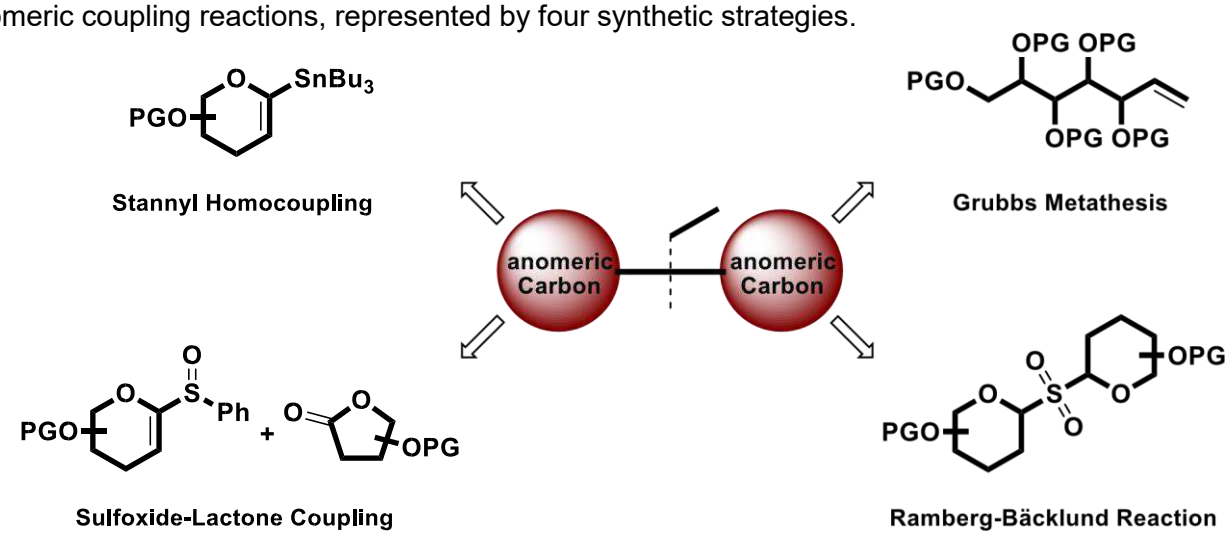

Fig. 1

We apply nucleophilic glycal derivates as versatile precursors. Thus, 1-tributylstannyl glycals perform homocoupling reactions under Stille conditions to furnish glycal dimers, whereas phenylsulfinyl glycals attack carbohydrate lactones in the present of a strong lithium base. The Grubbs metathesis of terminal hept-1-enitols depicts another alternative, providing long-chain dodeco-6-enitols. Furthermore, sulfon bridged dipyranosids convert to dimeric *exo*-glucals via a Ramberg-Bäcklund reaction.

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SYNTHETIC 4-*C*-FORMYL BRANCHED OCTOSES WITH STRUCTURAL ANALOGY TO BRADYRHIZOSE

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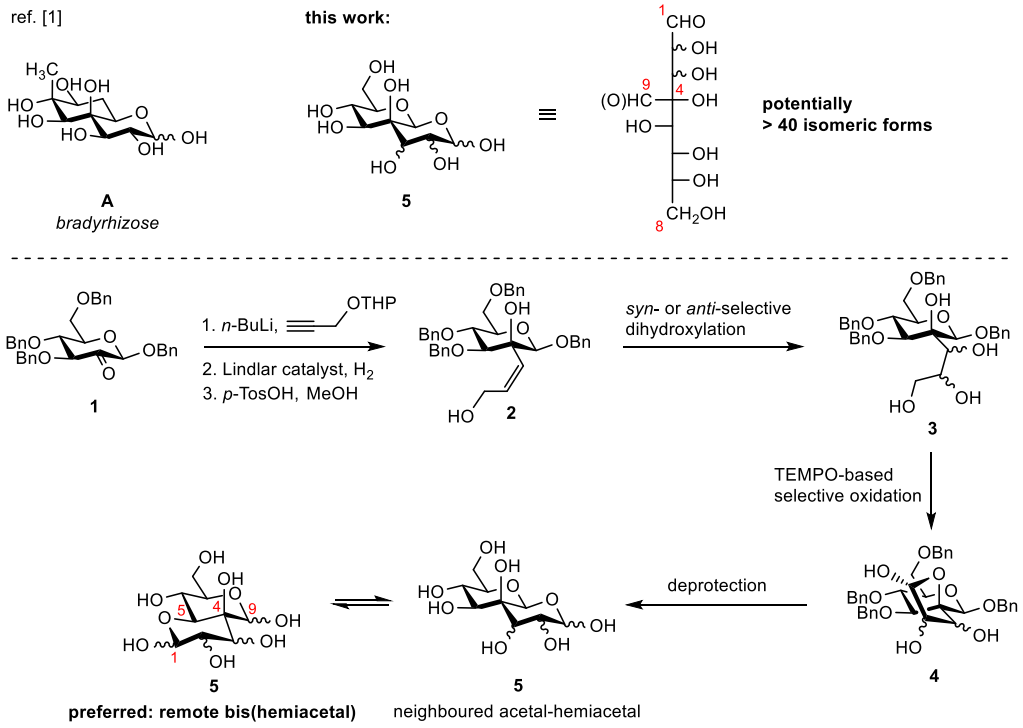
Bradyrhizose **A**, an unusual inositol derived annulated sugar, was isolated and identified to be the only monosaccharide component of the lipopolysaccharides of *Bradyrhizobium* sp. BTAi1, Gram-negative soil bacteria capable of nitrogen-fixation and establishing symbiosis with the legume plant *Aeschynomene indica* [1]. These interesting biological aspects and the structural complexity of the free hemiacetal **A**, featuring diverse ring-closing isomers (pyranoses and spiro-furanoses), have prompted us to synthesize formyl-branched octoses of type **5**. In these molecules, substitution of the carbasugar moiety in **A** for a conventional sugar (β-mannoside) gives rise to a vastly increased number of potential isomeric forms. Here, we describe the stereodefined synthesis of compounds **5** with variable configurations of the side chain stereogenic centers. Based on our earlier work including 2-*keto* glucosides [2], a synthetic series containing alkynylation of ketone **1**, partial hydrogenation and deprotection gave allyl alcohol **2** as a versatile substrate for stereoselective dihydroxylations of the side chain. A synthetically challenging selective primary alcohol oxidation of tetrols **3** gave spirofuranosides **4** and, after deprotection, 4-*C*-formyl octoses **5**.



A detailed NMR analysis of the complex isomeric mixtures of **5** in aqueous solution revealed the remote bis(hemiacetal) form to be highly preferred over the bradyrhizose-related neighboured acetal-hemiacetal form. Thereby, the work described here gives insight in synthetic challenges and structural features of complex higher carbohydrate systems of potential biological interest.

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SYNTHESIS OF DIULOSES VIA TRANSITION METAL CATALYSIS

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Diuloses are alpha-dicarbonyl compounds which occur as intermediates in the Maillard-reaction [1]. These substances determine color and flavor of the food and are under investigation as cancerogen [2]. On the other hand, the roots of *Psacalium Peltatum* (a diulose-containing mexican plant [3]) are used as natural medicine against rheumatism and type 2 diabetes [4]. The synthesis and possible application of this carbohydrate-class are currently under investigation.

Here, we present two synthetic routes that use different transition metals in important steps. Starting from inexpensive carbohydrate-derivates, the terminal alkene was obtained in six steps. The following Grubbs-metathesis afforded the cross-coupled product in quantitative yield. After dihydroxylation and oxidation, a diulose has been isolated and characterised. As shown by Menzel *et al*.[5], the enantioselective dihydroxylation and following oxidation appears to be difficult. To avoid these problems, we developed an alternative synthetic route. Instead of adding lithiated alkynes to aldehyds, we applied a Rhodium-catalyzed coupling in order to enhance the stereoselectivity [6]. Next, Palladium-catalyzed oxidation of the triple bond offered the corresponding diketons [7].

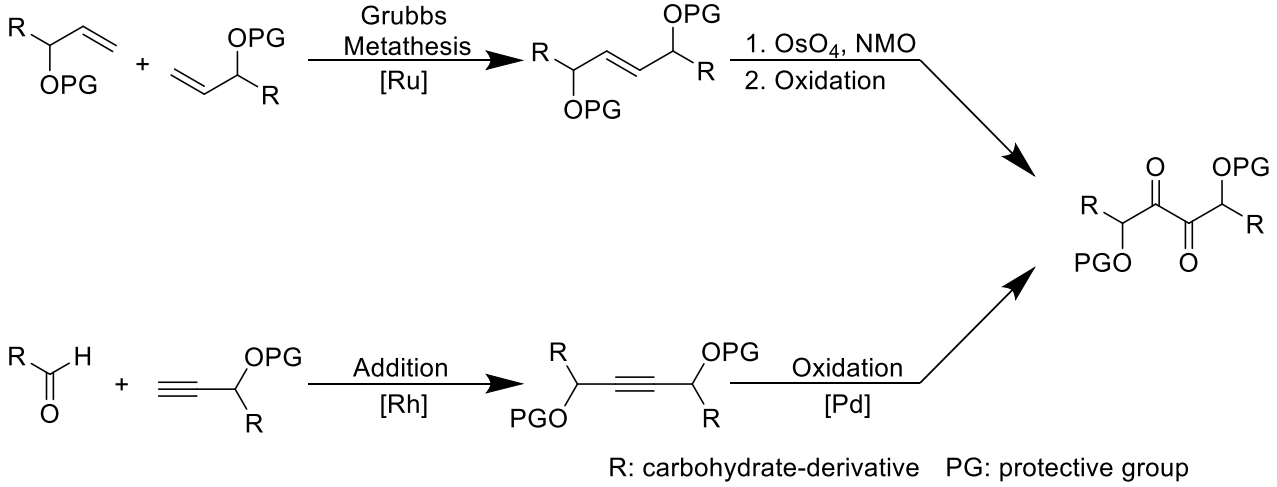

Fig. 1

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CATALYTIC APPEL SYNTHESIS OF GLYCOSYL CHLORIDES

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Glycosyl chlorides are useful donors and precursors in oligosaccharide synthesis. Commonly used methods for synthesis of glycosyl chlorides from hemiacetals require stoichiometric amounts of reagents, additional additives or heating [1,2]. Herein, we report new method to synthesise glycosyl chlorides using catalytic Appel conditions [3,4]. Triphenylphosphine oxide which is generally a stoichiometric waste product in conventional Appel reactions is used as a catalyst in our method.

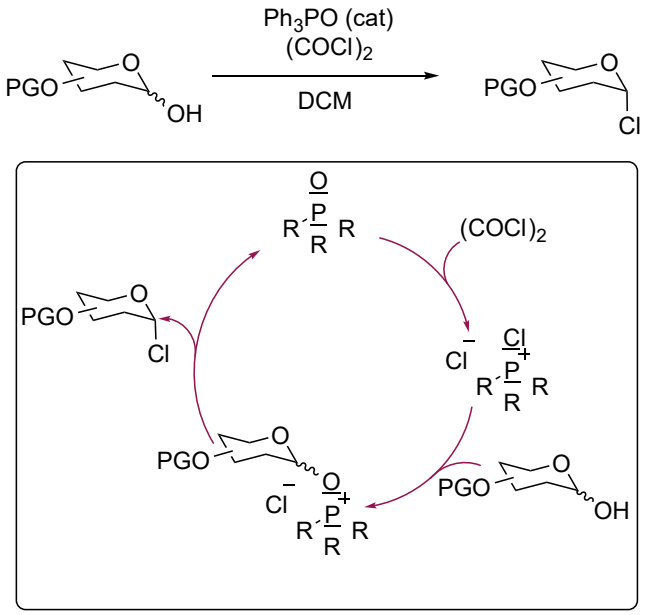

Fig. 1 - Catalytic Synthesis of Glycosyl Chloride. Inset- Catalytic Appel Cycle.

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CHARACTERISATION OF A BACTERIAL GALACTOKINASE WITH HIGH ACTIVITY AND BROAD SUBSTRATE TOLERANCE FOR CHEMOENZYMATIC SYNTHESIS OF 6-AMINOGALACTOSE-1-PHOSPHATE AND ANALOGUES

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Glycosyl phosphates are important intermediates in many metabolic pathways and are substrates for diverse carbohydrate active enzymes. There is a need to develop libraries of structurally similar analogues that can be used as selective chemical probes in glycomics. Here we explore chemoenzymatic cascades for the fast generation of glycosyl phosphate libraries without protecting group strategies. The key enzyme is a new bacterial galactokinase (LgGalK) cloned from *Leminorella grimontii* which was produced in *E. coli* and shown to catalyse 1-phosphorylation of galactose. LgGalK displayed a broad substrate tolerance, being able to catalyse the 1-phosphorylation of a number of galactose analogues, including 3-deoxy-3-fluorogalactose and 4-deoxy-4-fluorogalactose, which are first reported substrates for wild-type galactokinase. LgGalK and galactose oxidase variant M_1 were combined in a one-pot two-step system to synthesise 6-oxogalactose-1-phosphate and 6-oxo-2-fluorogalactose-1-phosphate, which were subsequently utilised to produce a panel of 30 substituted 6-aminogalactose-1-phosphate derivatives by chemical reductive amination in a one-pot three-step chemoenzymatic process.

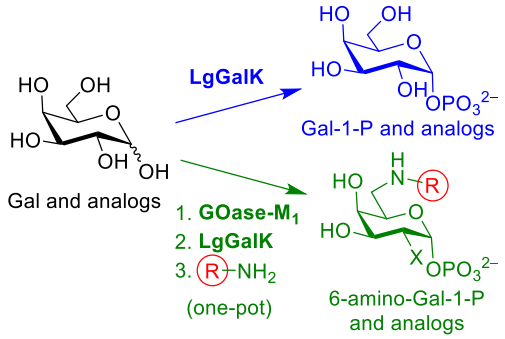

Fig. 1

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SYNTHESIS OF *CAMPYLOBACTER JEJUNI* (*C. JEJUNI*) NCTC 11168 (HS:2) CPS - TETRASACCHARIDE FRAGMENT

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The human pathogen *Campylobacter jejuni* (Gram-negative bacterium) is the leading cause of food-borne bacterial gastroenteritis, causing more foodborne illnesses worldwide than Sa*lmonella*, *Shigella* and Listeria combined[1]. *C. jejuni* produces a species specific capsular polysaccharide (CPS) that is an important virulence factor involved in colonization, serum resistance and invasion (key roles in the interaction between the organism, host and the environment). The HS:2 serotype CPS possesses a tetrasaccharide repeating unit (below) with a number of unusual motifs; it poses a considerable synthetic challenge[2-6]. In the interest of developing chemistry that could be used to assemble fragments of this CPS, we have targeted this tetrasaccharide for synthesis. Our synthetic approaches as well as the challenges involved in the synthesis will be discussed in detail.

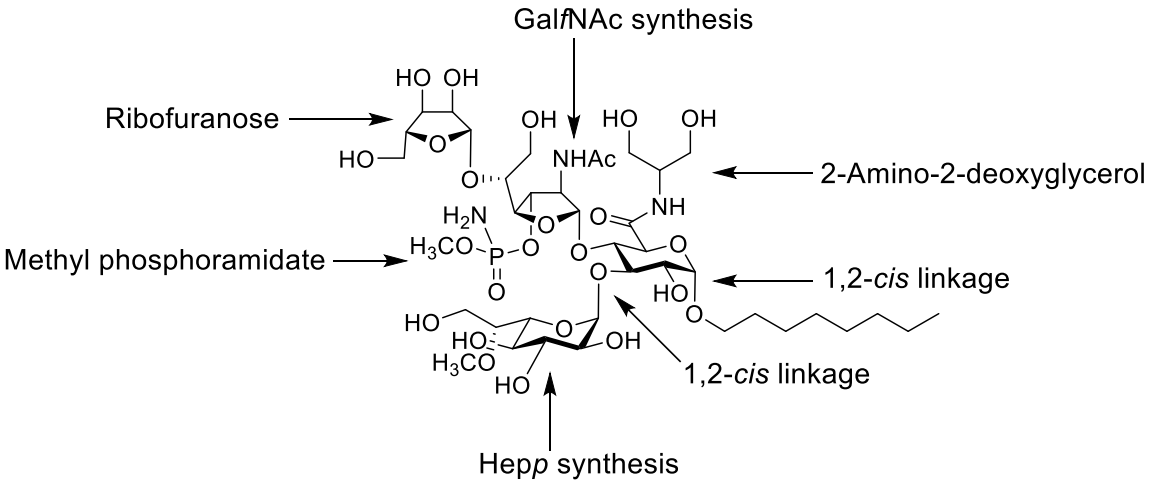

Fig. 1

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SYNTHESIS OF THE REPEATING UNIT OF GROUP B STREPTCOCCUS SEROTYPE IV CAPSULAR POLYSACCHARIDE USING GLYCONEER AUTOMATED GLYCAN SYNTHESIZER

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Specific recognition of bacterial polysaccharides is the basis of their application for developing conjugate vaccines. Oligosaccharides for vaccine production are typically obtained by fragmentation of polysaccharides extracted from bacterial cultures. This method provides complex mixtures of oligosaccharides with different chemical identities. Chemical synthesis allows the preparation of chemically pure samples of target oligosaccharides and therefore allows a variety of protein-carbohydrate interaction methodologies to be applied.

Automated solid-phase synthesis of carbohydrates is an ideal strategy for the preparation of large libraries of oligosaccharides. It makes use of differentially protected monosaccharide building blocks (BB) to generate the target structures. Each BB can be used in different automated protocols to generate different structures. The optimization of the individual couplings is still an ongoing process whose success ultimately determines the pertinence of the automated strategy.

This project target is a library of oligosaccharide fragments related to type IV Group B Streptococcus polysaccharide by using the Glyconeer® as an automated solid-phase oligosaccharide synthesizer. The library is to be used in structure-immunogenicity relationship studies in order to map the carbohydrate-protein interactions and define the minimal epitope. The repeating unit of the target polysaccharide is known [1]. Following a retrosynthetic approach, BBs were designed and synthesized. Our progress in optimizing the individual coupling reactions on the Glyconeer® to assemble the final target is presented.

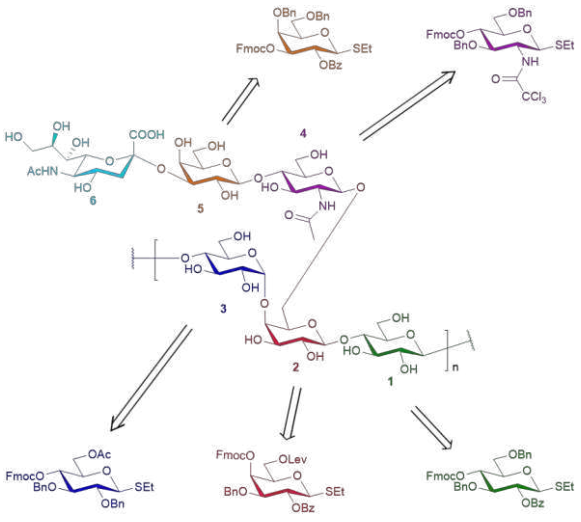

Fig. 1

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OXIDATIVE ACTIVATION OF C-S BONDS WITH AN ELECTROPOSITIVE NITROGEN PROMOTER ENABLES ORTHOGONAL GLYCOSYLATION OF ALKYL OVER PHENYL THIOGLYCOSIDES[1]

Ester Jiménez-Moreno,[a] Annabel Kitowski,[a],[b] Míriam Salvadó,[a],[c] Jordi Mestre,[c] Sergio Castillón,[c] Gonzalo Jiménez-Osés,[d]* Omar Boutureira,[c]* and Gonçalo J. L. Bernardes[a],[b]*

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Carbohydrates represent one of the largest groups of key biomolecules since they are involved in many essential biological processes [2]. For a better understanding of their roles in biological systems, as well as for the development of carbohydrate-based therapeutics and vaccines [3], it is key to access chemically defined oligosaccharides. Thus, efforts have been devoted to the development of efficient methods that allow their controlled synthesis [4].

Here, we present a method for the selective activation of thioglycosides that uses the N^+-thiophilic reagent O-mesitylenesulfonylhydroxylamine (MSH) as a promoter. The reaction proceeds via anomeric mesitylensulfonate intermediates, which could be isolated and fully characterized by placing a fluorine atom at the C-2 position. In the presence of a soft Lewis acid, glycosylation reaction proceeds at ambient temperature with good yields. We further demonstrate that it is possible to orthogonally activate S-ethyl in the presence of S-phenyl donors enabling the design of sequential glycosylation strategies.

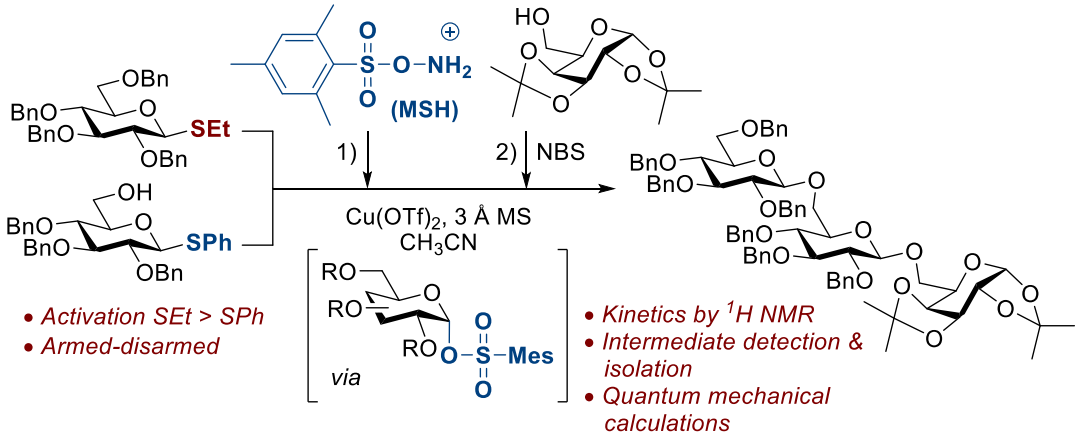

Fig. 1

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SYNTHESIS OF UNIQUE CARBOHYDRATE HEMIKETALS FROM ORTHO-HYDROXYAROMATIC ALDEHYDES AND DIHYDROLEVOGLUCOSENONE (DHLG)

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The reaction of dihydrolevoglucosenone **1**, (DHLG), Cyrene^{R} with aromatic aldehydes **2-5** in the presence of a base produces *exo*-cyclic enones [1,2]. It seemed interesting to study aromatic aldehydes containing additionally a hydroxyl group in the ortho-position. As anticipated the reaction proceeding smoothly to form expected aldol condensation products **6-10** followed by the reaction involving the carbonyl and aromatic hydroxyl group. The resulting product are rare carbohydrate hemiketals derived from aromatic OH-group.

Four products (**6-10**) formed starting from o-hydroxybenzaldehydes are presented. The reaction mechanism, NMR and crystal data products are discussed.

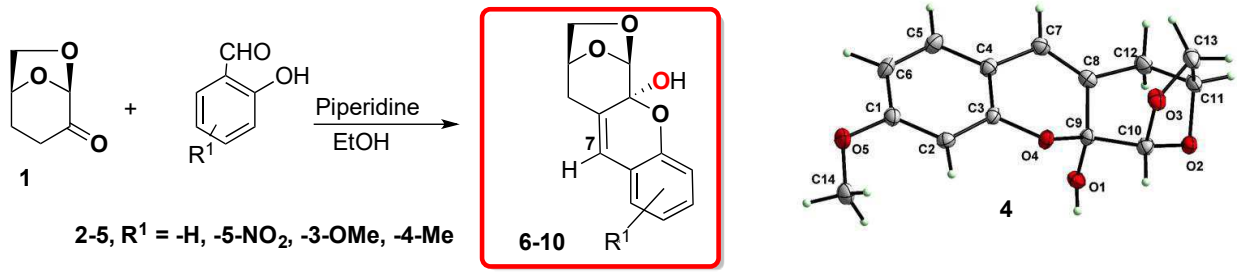

Fig. 1

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CARBOHYDRATE *EXO*-CYCLIC ENONES AS INTERMEDIATES TO SPIRONO-γ-LACTONES

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The reaction of dihydrolevoglucosenone **1** (DHLG) Cyrene^R with aromatic aldehydes in the presence of a base produces *exo*-cyclic enones [1] as a main (and usually only) product. However, when 2-pirydylaldehyde was used, we noticed formation of entirely different product **2** in a good 48 % yield. The NMR data and crystal structure differed significantly from those of typical *exo*-cyclic enone **3**. The crystal structure unambiguously established the formation of a spironolactone structure. Interestingly, the product derived from the reaction of two aldehyde molecules with one molecule of DHLG. We are in the process of studying the mechanism leading to the formation of this unexpected product. Two plausible mechanisms are presented and discussed.

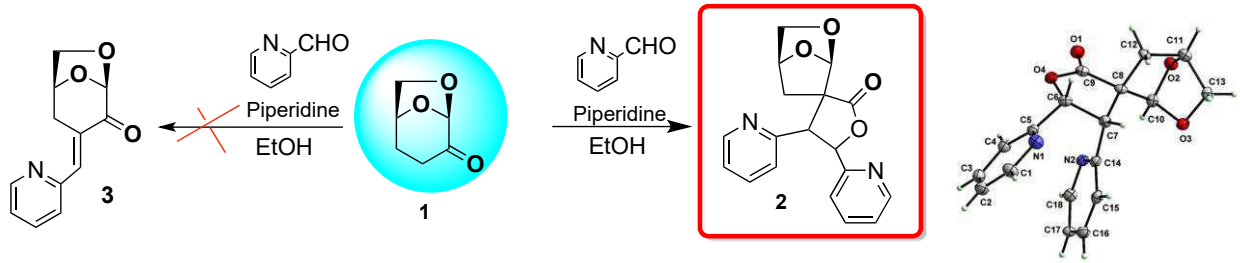

Fig. 1

References

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SYNTHESIS OF α- AND β-ANOMERS OF METHYL 2-AZIDO-2-DEOXYRIBOFURANOSIDE

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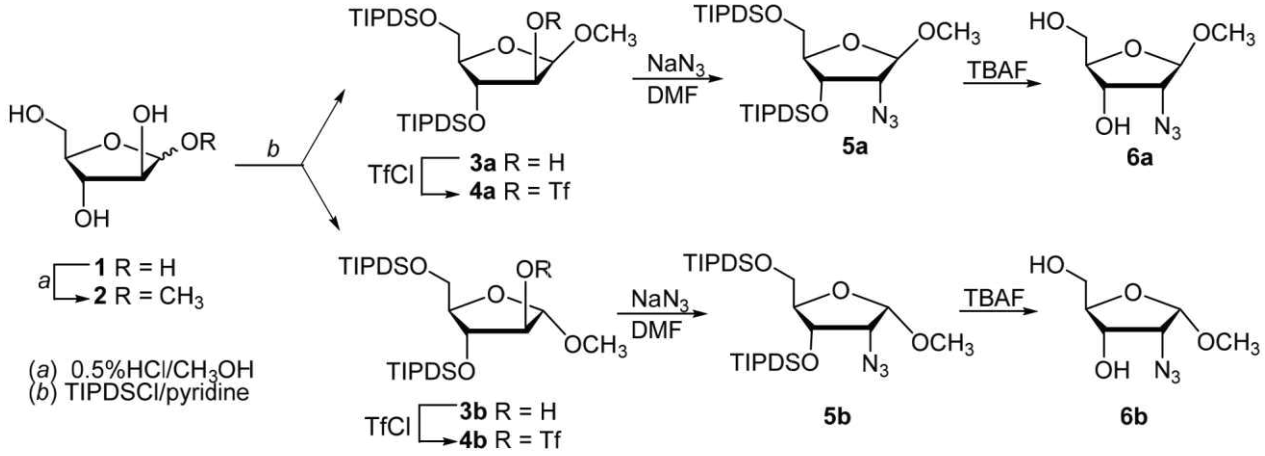
We are interested to study formation and subsequent reactions of N-centered aminyl radicals upon radiation-mediated one-electron attachment to azido nucleosides (e.g., AZT) [1,2]. In order to eliminate interaction of the intermediary aminyl radicals with nucleoside bases, we were in need of azido-modified pentofuranoses as an abasic model compounds to investigate H-atom abstraction pathways by RNH• from the sugar moieties.[2] Surprisingly, we found only one report for the synthesis of 2-azido-2-deoxy-Dribose which is based on detaching the sugar moiety from 2′-azido-2′-deoxyuridine by reaction of the latter with 15% hydrazine hydrate. The 2-azido-2-deoxy-D-ribose was prepared in 32% overall yield from uridine as a mixture of α/β anomers of pyranose and furanose forms [3]. Synthesis of methyl 2-azido-2deoxy-α-D-lyxofuranoside was developed from D-xylose (5 steps, 24% yield) [4].
Herein, we report a convenient and efficient method for the synthesis of α and β anomers of methyl 2azido-2-deoxy-D-ribofuranosides (**6a** and **6b**) from D-arabinose **1**. The four-step protocol requires only one column chromatography purification providing single α and β anomers of methyl 2-azido-2deoxyribofuranosides in 86% (**6a**) and 57% (**6b**) overall yields. Shortly, silylation of methyl Darabinofuranosides **2** with TIPDSCl gave a separable mixture of 3,5-di-O-TIPDS protected α and β anomers of **3a** and **3b**. Triflation (TfCl/DMAP/DCM) of **3a** provided 2-*O*-triflate ester **4a** in excellent yield and purity. Displacement of triflate from crude **4a** with NaN_3 in DMF followed by removal of the silyl protection groups from **5a** with TBAF afforded methyl 2-azido-2-deoxy-β-D-ribofuranoside **6a** in 86% overall yield from **3a** without necessity of purification of triflate **4a** and protected azide **5a**. Subjection of α-arabinofuranoside **3b** to the same sequence yielded 2-azido-2-deoxy-α-D-ribofuranoside **6b** in 57% overall yield from **3b**.



Prehydrated electron attachment to **6** produced the transient azide anion radical (RN3•⁻) which reacts via rapid N_2 loss at 77 K, forming the nitrene anion radical (RN•⁻), which upon rapid protonation formed neutral aminyl radical. RNH•. The ESR studies with **6** established thermally-activated intermolecular Hatom abstraction by RNH• from methoxy group at C1.

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MICROWAVE-ASSISTED SIMULTANEOUS *O*,*N*-SULFATION IN THE SYNTHESIS OF HEPARIN-LIKE OLIGOSACCHARIDES

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The synthesis of well-defined fragments of heparin (HP) and heparan sulfate (HS) is often hampered by difficult post-assembly manipulations on the fully elaborated oligosaccharides. In particular, the *O*- and *N*-sulfation steps, usually perfomed separately, require long reaction times and often meet with low yield due to problems associated with the incompletion of the reaction and the purification of highly sulfated products, thus representing a bottle-neck in the synthesis. We report herein an effective microwave-assisted protocol for the simultaneous *O*,*N*-sulfation of HP/HS-like saccharides. Complete *O*- and *N*-sulfation were attained when using SO_3·NEt_3 complex in a solvent mixture of NEt_3/pyridine at 100 °C (MW heating) for 15 min, thus facilitating the purification process. Easy to implement, per-*O*,*N*-sulfation of mono- to penta-saccharides with two to eight reactive sites were performed effectively in short reaction times and excellent yields. Under smooth deprotection conditions, the resulting per-*O*,*N*-sulfated saccharides were fully deprotected in high yields (>88%), providing saccharides pertinent for the synthesis of HP/HS-like fragments, including Fondaparinux Sodium. Moreover, we developed a microwave-assisted protocol for the one-pot selective *O*-sulfation/*N*-acetylation on disaccharide, which could be applied to synthesize members of the GAG family bearing *N*-acetyl groups.

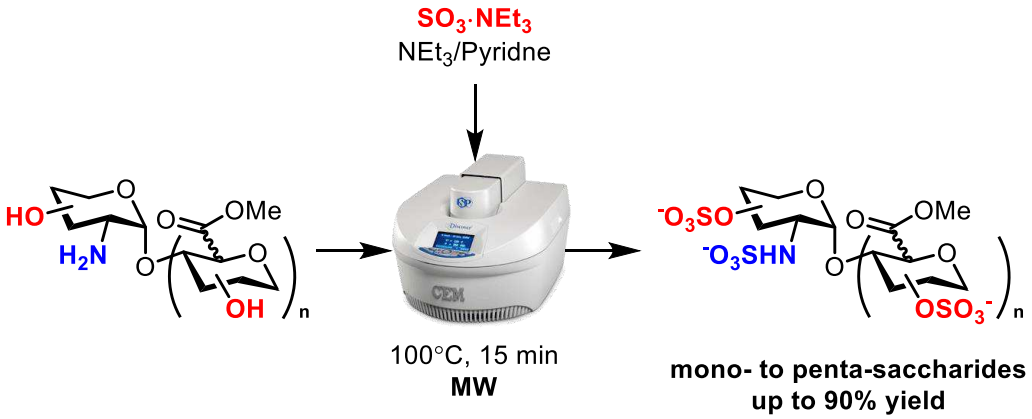

Fig. 1

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USES OF I-TAGS FOR GLYCOSYLTRANSFERASE SUBSTRATES

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Oligosaccharides are the most diverse group in nature and involved in a myriad of biological processes, ranging from fertilisation, immunity to pathogenic attack [1]. Carbohydrates offer great potential as a pharmaceutical target, however only limited carbohydrate-based drugs have been developed. This is mainly due to the lack of efficient methods to obtain structurally defined samples by either chemical synthesis or isolation, for biological screening.

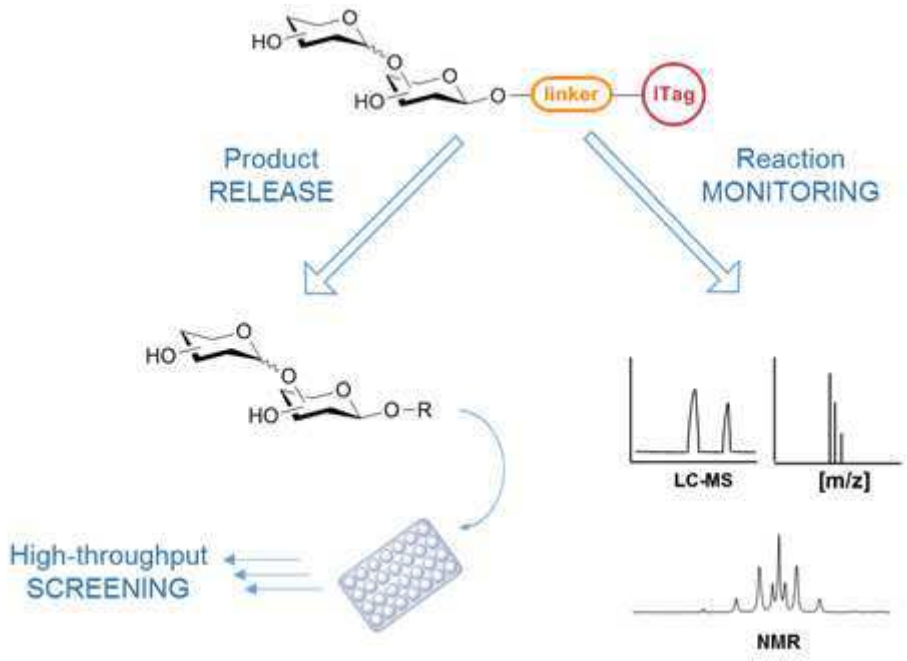

Fig. 1 - Use of ITags for enzyme monitoring [2,3].

Recently, our team developed the use of ionic liquid Tags (I-Tags) for chemical and enzymatic oligosaccharide synthesis, as soluble support. These I-Tags allow us to monitor enzymatic reaction progress by mass spectrometry (MS) techniques and facilitate product isolation, as a purification handle [2,3].

Herein, the synthesis of novel I-Tag labels will be reported and their application in enzymatic transformations involving glycosyltransferases will be also shown.

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SYNTHESIS OF TREHALOSE 6-PHOSPHATE AND ITS DERIVATIVE USING TREHALOSE 6-PHOSPHATE PHOSPHORYLASE

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Trehalose 6-phosphate (Tre6*P*: α-D-Glc*p*-(1↔1)-α-D-Glc*p*6*P*), the intermediate of trehalose biosynthesis, has profound regulatory functions on plant metabolism, growth, and development. However, availability of Tre6*P* is limited, because no efficient production system of Tre6*P* has been established thus far. Tre6*P* synthesis through fermentation by yeast was published, but the yield of Tre6*P* was low [1]. Therefore, in this study, we developed the efficient enzymatic synthesis of Tre6*P* using trehalose 6-phosphate phosphorylase (EC 2.4.1.216) from *Lactococcus lactis* ssp. *lactis* (LlTrePP). In *L. lactis*, LlTrePP catalyzes the phosphorolysis of Tre6*P*, which is synthesized when trehalose is transported into cells by phosphotransferase system, to generate β-glucose 1-phosphate (β-Glc1*P*) and glucose 6-phosphate (Glc6*P*) [2]. Through a reverse reaction of phosphorolysis (synthesis), Tre6*P* and its derivative were synthesized.

Recombinant LlTrePP was produced in an *Escherichia coli* BL21 (DE3) transformant. From the cells harvested from 1 L of culture fluid, 19 mg of LlTrePP was purified by nickel-affinity column chromatography. Substrate specificity of LlTrePP was investigated by measuring its activities towards 17 acceptor candidates (10 mM) in the presence of 10 mM β-Glc1*P*. LlTrePP showed activity towards Glc6*P* and mannose 6-phosphate (Man6*P*). The reaction velocity for Glc6*P* was 253 μmol/min/mg, and that for Man6*P* was 0.134 μmol/min/mg (0.053% of the value for Glc6*P*) [3].

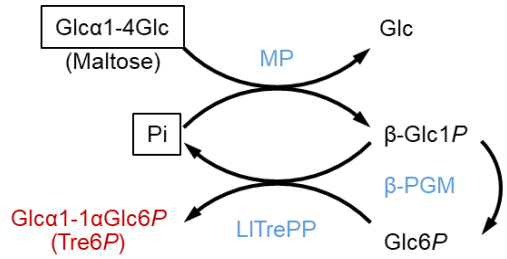

Fig. 1 - Reaction scheme of Tre6*P* synthesis from maltose by LlTrePP, MP, and β-PGM

In Tre6*P* synthesis, 91 mM Tre6*P* was produced from 100 mM β-Glc1*P* and 100 mM Glc6*P* at 30°C for 36 h (yield, 91%). By using maltose phosphorylase (MP), β-Glc1*P* was provided from maltose and inorganic phosphate (Pi) (Fig. 1). Through the one-pot reaction for 28 h, 65 mM Tre6*P* was synthesized from 100 mM maltose and 100 mM Glc6*P* in the presence of 20 mM Pi (yield, 65%). β-Phosphoglucomutase (β-PGM) was supplemented to convert β-Glc1*P* to Glc6*P* (Fig. 1). It enabled to use maltose as a sole carbohydrate source. The highest concentration of Tre6*P* (35 mM) was obtained from 100 mM maltose with 50 mM Pi in a 72-h reaction (yield, 70%), although 12 mM Glc6*P* remained. By reducing Pi concentration to 20 mM, β-Glc1*P* and Glc6*P* were reduced and 20 mM Tre6*P* was obtained after a 72-h reaction. The amount of Tre6*P* was stoichiometric with respect to the amount of Pi. Produced Tre6*P* was easily separated from residual maltose and glucose by gel-filtration column chromatography. Compared with the synthesis from β-Glc1*P* and Glc6*P*, residual sugar phosphates were reduced in the reaction from maltose and Pi, and it facilitated purification. From 100-mL reaction system with 10 mmol maltose and 2 mmol Pi, 2 mmol Tre6*P* was prepared as a dipotassium salt.

Suitable acceptor substrates of LlTrePP were Glc6*P*, and, at a low level, Man6*P*. From 246 μmol β-Glc1*P* and 246 μmol Man6*P*, 205 μmol product was obtained. The product was purified through electrodialysis with a yield of 51% based on substrates (126 μmol). By ESI-MS and NMR, the reaction product was determined to be a new sugar phosphate, α-D-Glc*p*-(1↔1)-α-D-Man*p*6*P* [3].

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A NOVEL SYNTHETIC ROUTE TO CONTROLLED STEREOSELECTIVE GLYCOSYLATIONS

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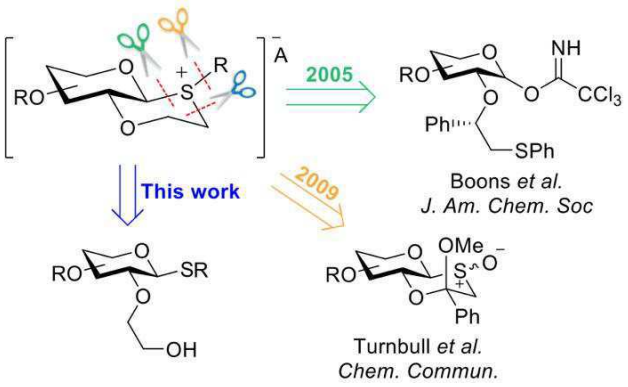
The major challenge in the chemical synthesis of oligosaccharides is the stereoselective synthesis of the glycosidic bond. 1,2-*trans* glycosides can be synthesized with high selectivity by the use of neighboring group participation of a C-2 acyl group and is applicable to gluco- and manno-type sugars. In contrast, the stereoselective synthesis of 1,2-*cis* glycosides remains more challenging. By utilizing neighboring group participation using C-2 chiral auxiliaries, the stereoselective synthesis of 1,2-*cis* gluco [1]- and manno [2]-type sugars could be achieved. These reactions proceed *via* an intermediate sulfonium ion, which is depicted in scheme 1. This intermediate can be prepared by making three disconnections, of which two have already been explored [1,3].



Scheme 1. Retrosyntheses of the *β*-sulfonium ion.

Using a thioglycoside precursor, *β*-sulfonium ion intermediates could by prepared utilizing an unexplored disconnection. The synthetic route to this glycoside donors allows for a late stage introduction of the C-2 auxiliary, giving more control over the outcome of the stereoselectivity using a single glycosyl donor. Using low-temperature NMR studies, the activation of the glycosyl donor, as well as the formation of the *β*-sulfonium ion were investigated. Subsequently, the influence of the thioaryl moiety, as well as the benzyl protecting groups on the stereoselectivity was explored.

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SYNTHESIS AND STUDIES OF GLYCOSIDIC BOND EXPANDED CYCLIC OLIGOSACCHARIDES

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Cyclic oligosaccharides, such as, cyclodextrins and cycloinulo-oligosaccharides arise immense interest due to their value addition in both fundamental studies and applications. Modification at the glycosidic bond is an interesting approach to derive new, un-natural oligosaccharides that offer altered conformations and molecular recognition properties. In this direction, we advanced a query through incorporation of an additional methylene moiety in between the glycosidic linkages, so as to form glycosidic bond expanded cyclic oligosaccharides [1-3]. Incorporation of the additional methylene in between the glycosidic oxygen and *C*-4 methine carbon leads to the macrocycles being larger in the ring size when compared to the native cyclodextrins constituted with similar number of monosaccharide residues. This increased molecular cavity sizes, with attendant increase in amphiphilic character, lead to enhancement of binding affinities with chosen ligands, in comparison to native cyclodextrins. We wish to present our recent work on synthesis and studies of glycosidic bond expanded cyclic pentasaccharide. Synthesis was performed in a step-wise manner, first to a linear pentasaccharide, followed by a cycloglycosylation to afford the target cyclic pentasaccharid [4]. Molecular dynamics modeling shows that the 30-membered macrocyclic pentasaccharide is a distorted ellipsoid, the lower and upper rims are occupied by secondary and primary hydroxyl groups, respectively. Following synthesis, the microenvironment of the cyclic pentasaccharide was assessed through isothermal titration calorimetric evaluation upon complexation with 1-aminoadamantane (AMT) in aqueous solution, which shows the formation of ~1:2 host-to-guest complex and a binding affinity of 10,500 (±425) M^{-1}. Synthesis and studies of cyclic oligosaccharides, with particular emphasis to a cyclic pentasaccharide, will be presented.

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SYNTHESIS OF NOVEL TRIAZOLE-DERIVED GLYCOPEPTIDES AS ANALOGS OF α-DYSTROGLYCAN MUCINS

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α-Dystroglycan (α-DG) mucins are essencial for maintenance of the structural and functional stability of the muscle fiber, besides being expressed in other tissues such as neural and epithelial. They are constituted by enriched serine/ threonine peptide sequences linked to the tetrasaccharide motif Neu5Acα2–3Galβ1-4GlcNAcβ1-2Manα-*O*-Ser/Thr. When hypoglycosylated, α-DG mucins are directly involved in pathological processes such as dystroglycanopathies (Congenital Muscular Dystrophies-CMDs) and epithelial tumors, due to defective interactions with extracellular matrix proteins, such as laminin [1]. Thus, α-DG mucins are considered valuable targets for development of new therapeutic and diagnostic strategies against CMDs and cancer. Within this context, this work presents the synthesis of the novel 1,2,3-triazole-derived glycopeptides NHAcThrVal[αGlcNAc-1-triazol-2Manα]ThrIleArgGlyOH(**1**) and NHAcThrVal[Gal-β1,4-αGlcNAc-1-triazol-2Manα]ThrIleArgGlyOH (**2**) as analogs of α-DG mucins (Fig. 1).

The synthesis of glycopeptides **1** (23**%**) and **2** (12%) were carried out by solid phase assembly [2], involving sequential couplings of the Fmoc-protected amino acids Arg, Ile, Thr, Val, or the 1,2,3-triazole-derived glycosyl amino acids αGlcNAc-1-*O*-triazol-2Manα-ThrOH (**3**, 72%) and Gal-β1,4-αGlcNAc-1-*O*-triazol-2Manα-ThrOH (**4**, 35%), which were previously obtained by Cu(I)-assisted 1,3-dipolar azide-alkyne cycloaddition reactions (CuAAC) between the azide-glycosyl amino acid αManN_3-FmocThrOBn (**5**) and the corresponding alkyne-functionalyzed sugars 2'-propynyl-αGlcNAc (**5**) and 2'-propynyl-Gal-β1,4-αGlcNAc (**6**) [3]. After cleavage from resin (TFA/ TIPS), *N*-acetylation (Py/ Ac_2O) and *O*-deacetylation (NaOMe) reactions, the obtained glycopeptides **1** and **2** were purified by RP-HPLC and characterized by RMN and ESI-MS analyses.

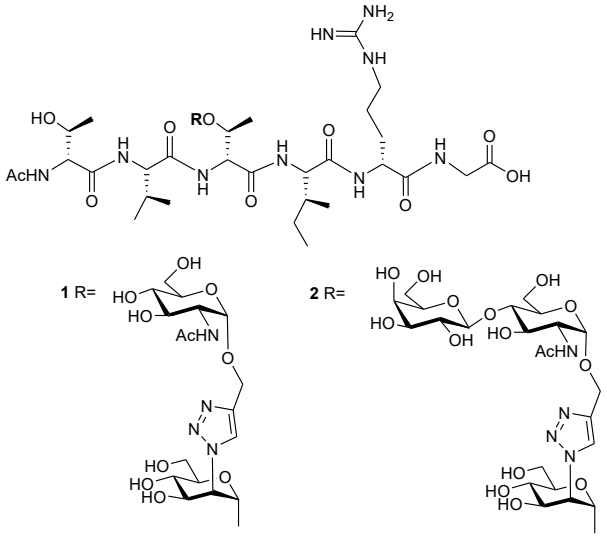

Fig. 1.- Chemical structures of glycopeptides **1** and **2** as analogs of α-DG mucins.

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HYBRID *N*-GLYCANS AS LIGANDS FOR HIV-1 BROADLY NEUTRALIZING ANTIBODIES

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The development of HIV vaccine has been hampered by the extraordinary mutability and genetic diversity of the virus, particularly the substantial sequence diversity of gp120 and gp 41 envelope glycoproteins existing in more than 2,000 HIV variants. The highly diverse glycans on HIV spikes are commonly considered as immunologically silent self-antigens; however, the discovery of highly potent broadly neutralizing antibodies (bNAbs) from HIV patients targeting surface glycans has raised a major question about the origin of their antigens. Recent epitope mapping studies of the bNAb PG9 indicated a requirement of a properly spaced high mannose and a complex type glycan connected by a short peptide spacer. We have recently discovered that a 1:1 mixture of Man_5 and sialyl biantennary glycan with well-defined distance and without the peptide spacer is well recognized by PG9 with high avidity, and thus proposed that a hybrid glycan with oligomannose and complex-type arm could be the proper ligand of PG9 [1] To verify this proposition, we first designed and chemo-enzymatically synthesized a series of unusual hybrid type *N*-glycan structures, which may exist on HIV surface glycoproteins through the host-guided *N*-glycosylation pathway. The synthetic hybrid glycans were then used to prepare glycan arrays for the binding study of PG9 and several other highly potent bNAbs, including PG16, PGT121, PGT128-3C, 2G12, VRC13, VRC-PG05, VRC26.25, VRC26.09, PGDM1400, 35O22, and 10-1074. Our results demonstrated that PG9 and some other bNAbs bind with strong avidity (sub-nanomolar Kd) to certain hybrid structures, suggesting that these unusual glycans may serve as epitopes for the design of vaccines against HIV [2].

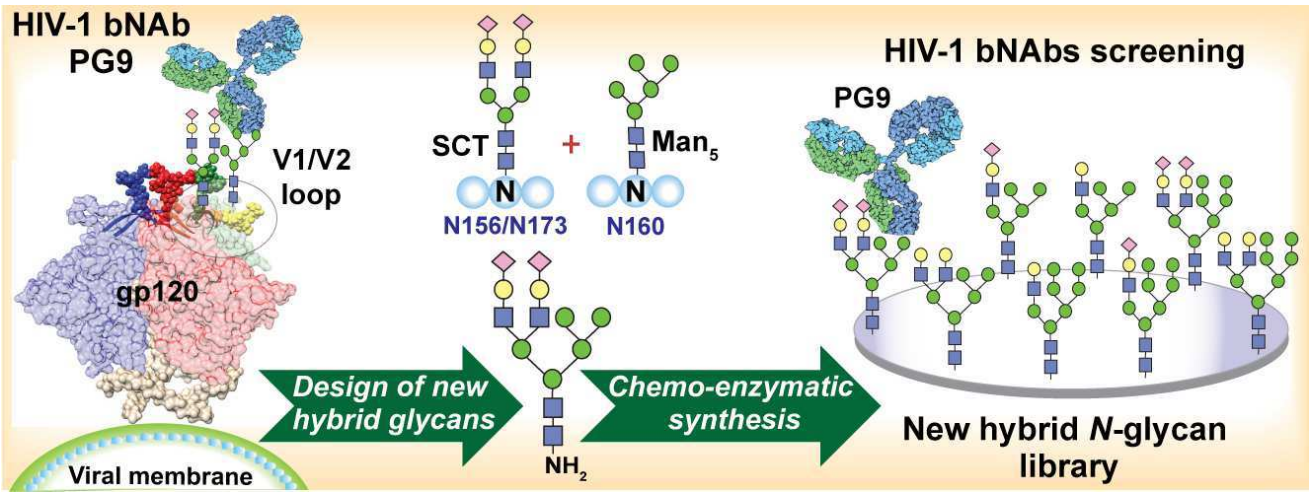

Fig. 1

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SYNTHESIS OF PHOSTONES AND PHOSTINES BY ALCOXYL RADICAL FRAGMENTATION (ARF)

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Bioisosterism is a useful approach to induce biological activity, for that reason the search for new bioisosteric groups is an important issue. Phostones [1] and phostines (also called 1-phospha-sugars) have been studied in drug discovery as bioisosteres of furanose or pyranose ring, in which the anomeric carbon atom is replaced by the phosphate or phosphite moiety. Phostones (phosphonosugars) have gained considerable attention mainly due to they are potential inhibitors of glycosidases. Phostines (phosphinosugars) are consider as glycomimetics and have shown anticancer activities.

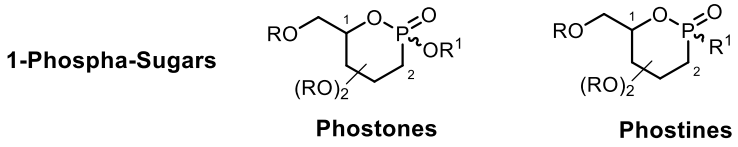

Fig. 1

We design two different approaches (A and B) to obtain phostones and phostines in few steps and good yields starting from carbohydrates using Alkoxyl Radical Fragmentation [2] as key step and Arbuzov [3] reaction to introduce phosphorus. When we apply ARF to carbohydrates, an alkoxy radical in anomeric position is formed, β-fragmentation takes place and generates a formate group at the anomeric carbon and a C2-radical. Substituent located in the C2-radical play a decisive role in the final product.

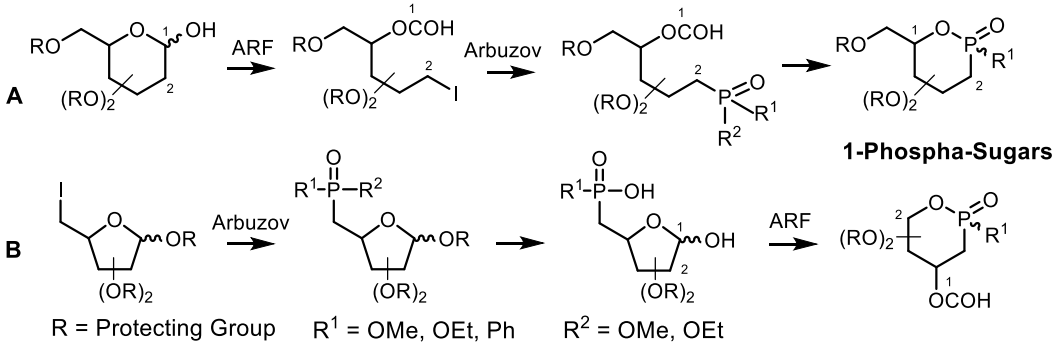

Fig. 2

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ALLYLIC HALOGENATION MEDIATED GLYCOSYLATION: APPLICATION TO THE SYNTHESIS OF XYLO-OLIGOSACCHARIDES

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Allyl glycosides are powerful in glycosylation reactions, either as a protecting group or as an activating moiety through their conversion to the corresponding vinyl glycosides. In a glycosylation reaction, the allyl-moiety can thus be either latent moiety or as moiety which can be transformed to an active vinyl ether moiety suitable for activation and further glycosylation. Allyl-moiety is highly reactive to various reactions. A reaction of our interest is the radical mediated allylic halogenation reaction. A bromine radical, formed through either a photochemical activation or a radical initiator, undergoes a facile reaction at the allylic carbon and forms as an allylic halide. We desired that an allylic halide of a glycoside could be a reasonable approach in order to generate active glycosyl cation. In effect, it was feasible to generate a glycosyl cation from allylic halide of a glycoside, which when subjected to reaction with a glycosyl acceptor, including a latent allyl glycoside acceptor, leads to the formation of a glycosylation product [1]. This new glycosylation methodology, tested initially with a number of allyl glycoside donors and suitable allyl glycoside acceptors, was extended to the preparation of xyloside oligomers. Upon suitable protection of *C*-2 and *C*-4 hydroxyl functionalities, glycosylation of allyl glycoside donor with an allyl glycoside having *C*-3 hydroxyl functionality as the acceptor was conducted and glycosylated xyloside was obtained. Details of the application of the new methodology to prepare xyloside oligosaccharides will be presented.

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β-SELECTIVE XYLULOFURANOSYLATION USING A 3,4-*O*-XYLYENE-PROTECTED DONOR

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Glycosides of xylulofuranose (Xul*f*) are found in polysaccharides from different microorganisms. Natural xylulose glycosides are present in both α and β-anomeric configurations. Both *α*-Xul*f* and *β*-Xul*f* residues are found in the capsular polysaccharides from *Campylobacter jejuni* [1], whereas *β*-Xul*f* residues occur in N-linked glycans of chloroviruses [2] and the lipopolysaccharide *O*-chain of *Yersinia enterocolitica* [3].

The chemical synthesis of Xul*f*-containing oligosaccharides is important to provide materials for investigations that will lead to the identification of new drug targets and vaccines for treating and preventing diseases. However, there has been almost no work on developing methods to these glycosidic linkages. The three main challenges in the chemical synthesis of Xul*f*-containing oligosaccharides are: (1) the high price of commercially available xylulose; (2) the stereoselectivity of xylulofuranosylation reactions; (3) identification of the anomeric configuration in the products.

This presentation will focus on how we addressed these three challenges. In particular, it will report the preparation of xylulose thioglycoside donors **1**–**4** from arabinose, the study of stereoselective xylulofuranosylation with various acceptors, and the determination of anomeric configuration for xylulofuranosides. We have also applied this methodology to synthesize the pentasaccharide repeating unit of lipopolysaccharide O-antigen from *Y. enterocolitica* (**5**).

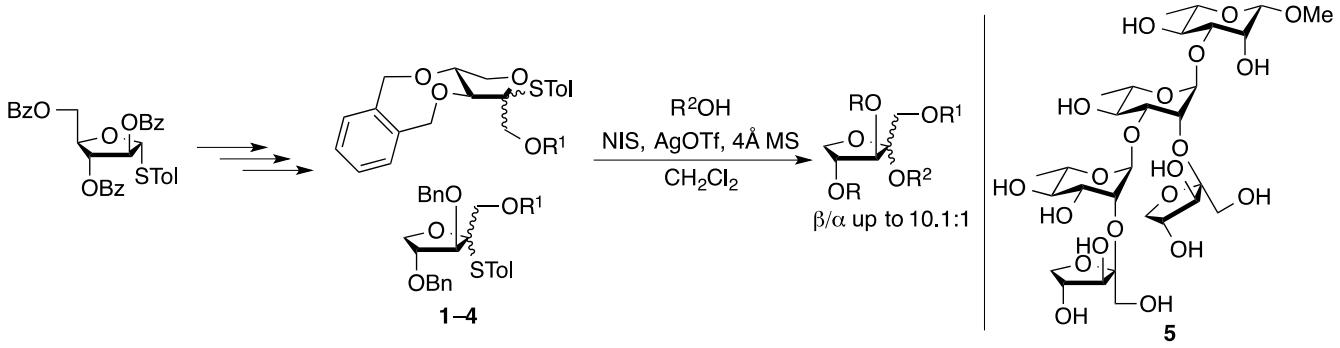

Fig. 1

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APPLICATION OF BORONIC-ACID-CATALYZED GLYCOSYLATION TO LATE-STAGE MODIFICATION OF NATURAL GLYCOSIDES AND OLIGOSACCHARIDE SYNTHESIS

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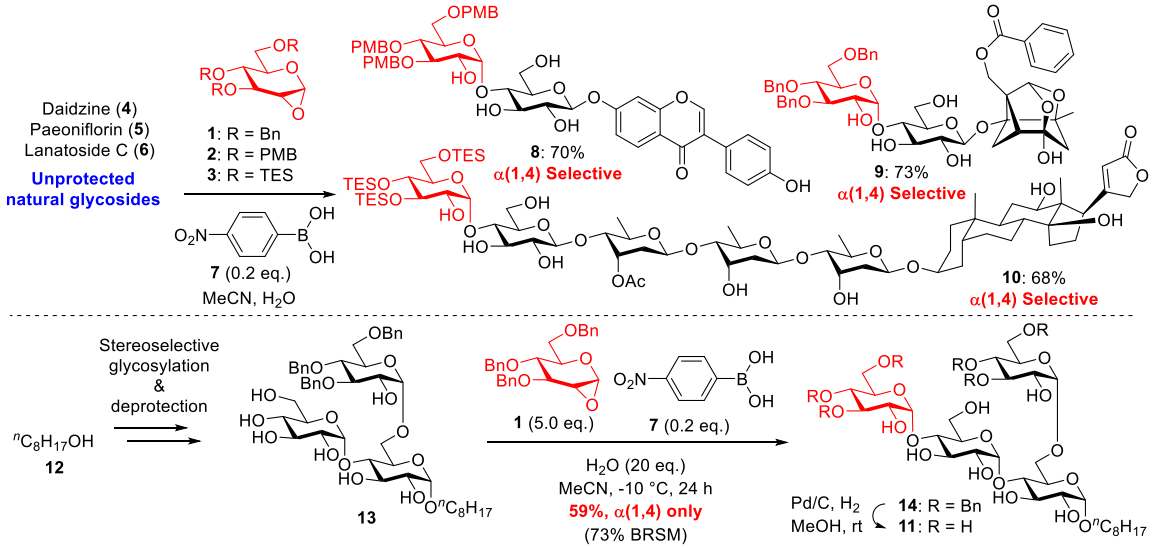
The late-stage modification of biologically active natural glycosides has led to the development of a large amount of novel therapeutic agents. Therefore, intensive effort has been directed toward the development of catalyst-controlled regioselective functionalization [1] of natural glycoside. Particularly, from a standpoint of glycodiversification, a regio- and stereoselective glycosylation method has been desirable because the biological activity of natural glycosides is often influenced by the anomeric configuration [2]. Therefore, several regio- and 1,2-*trans*-stereoselective glycosylations [3] of natural glycosides have been developed, however, no regio- and 1,2-*cis*-stereoselective glycosylation of natural glycoside has been reported. In this context, we have recently developed a boronic acid catalyzed regio- and 1,2-*cis*-stereoselective glycosylation of unprotected sugar acceptors. Herein we wish report its application to a direct regio- and 1,2-*cis*-stereoselective glycosylation of unprotected natural glycosides and the synthesis of branched α-glucan **11**.

Initially, we chose 1,2-anhydro sugar **1** and daidzine (**4**) as a glycosyl donor and a natural glycoside, respectively, and investigated the glycosylation of **4** with **1** under various conditions. As the results, the glycosylation proceeded efficiently to provide α(1,4) glycoside **8** in 70% yield with high regio- and stereoselectivities. Furthermore, when paeoniflorin (**5**) and lanatoside C (**6**) were employed as natural glycosides, the corresponding α(1,4) glycosides **9** and **10** were obtained in good yields with high regio- and stereoselectivities. Next, we applied the present glycosylation method to the synthesis of branched α-glucan **11**. Trisaccharide **13** was efficiently synthesized from octanol (**12**) and 1,2-anhydro sugars with excellent stereoselectivities utilizing boronic-acid-catalyzed and borinic-acid-catalyzed glycosylations [4]. The glycosylation of **13**, which possesses seven free hydroxyl groups, with **1** using boronic acid catalyst **7** in the presence of water gave α(1,4) glycoside **14** with excellent regio- and stereoselectivities. Finally, deprotection of the benzyl groups gave branched α-glucan **11** [5].



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PROGRESS TOWARDS SYNTHESIS OF ANTIFREEZE GLYCOLIPIDS FROM THE ALASKAN BEETLE *UPIS CERAMBOIDES*

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One of the strategies by which cold-blooded organisms evolved to survive in subzero ecosystems is the production of antifreeze substances. Until 2009, the only substances thought to be capable of inducing antifreeze activity are either proteins or glycoproteins. This perception was overturned with the discovery of an antifreeze protein-free glycolipid from the freeze tolerant Alaskan beetle *Upis ceramboides* [1].

The structure of this glycolipid was elucidated by Duman and co-workers and shown to consist of β-Man*p*-(1→4)-β-Xyl*p* disaccharide repeating units, with the possibility of a covalently-linked lipid component. Though the structure of the glycan was confirmed via chemical synthesis by three groups [2-4], several key questions remain with regards to the chain length of the glycan needed for antifreeze activity, the identity of the lipid and its mode of linkage to the glycan as well as the mechanism of antifreeze activity.

Gaining insights into such questions therefore requires a library of chemically well-defined xylomannan derivatives. In this poster, I will present my recent progress towards the synthesis of a library of 15 xylomannan molecules sharing the general structure **1** with a varying number of repeating units and lipid chains. It was envisioned that all can be accessed via convergent assembly using disaccharide repeating units as key building blocks.

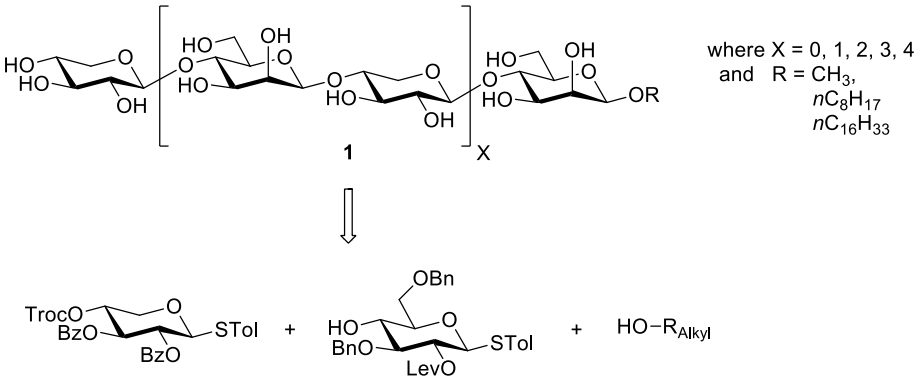

Fig. 1

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CHIRAL PHOSPHORIC ACID-CATALYZED SITE-SELECTIVE GLYCOSYLATION AND ITS APPLICATION TO AMINOGLYCOSIDE SYNTHESIS

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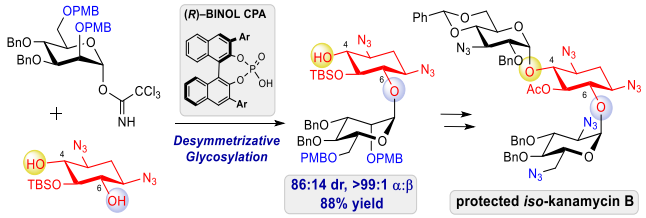
Aminoglycosides are a group of carbohydrate-based antibiotics, which function through binding to specific sites in prokaryotic ribosomal RNA (rRNA) and affecting the fidelity of protein synthesis [1]. While certain aminoglycosides such as paromomycin or gentamycin are FDA-approved drugs for the treatment of bacterial infections, their extensive clinical use has been curtailed by the inherent toxicity and the rapid increase of aminoglycoside resistant strains of bacteria [2]. Therefore, the design and synthesis of new aminoglycosides with improved pharmacological properties represents an important challenge. The majority of aminoglycosides contain an achiral (*meso*) 2-deoxystreptamine (2-DOS) subunit that carries glycone at either the C4 and C5 or C4 and C6 hydroxy groups. While the most concise way to desymmetrize 2-DOS is through the direct glycosylation, only enzymatically controlled glycosylations at the C4 position of 2-DOS are known [3]. However, the biosynthetic method is limited to specific substrates. Therefore, a non-enzymatic method for desymmetrizative glycosylation of 2-DOS is highly desired.

Based on our experiences and observation along with previous literature reports [4-6], BINOL based chiral phosphoric acids (CPA) were envisioned to catalyze such desymmetrization in order to build key disaccharides for aminoglycoside synthesis. Indeed, the BINOL-CPA could catalyze the mannose-derived trichloroacetimidates to undergo α-selective glycosylation, which is required for the synthesis of natural aminoglycosides, towards 2-DOS *meso* derivative. In addition, our experiments show that the chirality of CPA dictates the outcome of the glycosylation reactions, and the use of enantiomeric CPAs results in either C4-glycosylated (67 : 33 d.r.) or C6-glycosylated (86 : 14 d.r.) 2-DOS [7].

These disaccharides could be further used for the assembly of various aminoglycoside antibiotics and their isomeric forms with inversed C4/C6 connectivity. Through protecting group manipulation from the disaccharides intermediate, both *natural*-neamine derivative, and its isomeric form, *iso*-neamine derivative were synthesized. Moreover, the stereoselective synthesis of disaccharide offered a viable route to convert this compound to isomeric kanamycin B, which has the reverse connectivity of the sugars at the C4 and C6 position compared to natural kanamycin B. The direct functionalization of kanamycin B to form such derivative represents a significant challenge, while the desymmetrizative functionalization of 2-DOS would provide a more straightforward access to aminoglycosides of this type. Upon subsequent glycosylation followed by protecting group manipulation, *iso*-kanamycin B derivative was afforded, and this is a new type of aminoglycoside that has not been reported in the literature [8]. This example demonstrates the potential application of our methods in divergent synthesis of complex molecules in the context of drug discovery.



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SYNTHESIS OF GALACTOFURANOSE-CONTAINING GLYCOLIPIDS AS PROBE FOR MYCOABACTERIAL GALACTAN BIOSYNTHESIS PATHWAY

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Tuberculosis is a worldwide public health problem. The disease is caused by *Mycobacterium tuberculosis*, which produces an unusual cell wall compared to other bacteria. The major structural component of the cell wall is made up of the unique mycolyl–arabinogalactan (mAG) complex, peptidoglycan, and various lipids. The mAG complex has a galactan moiety composed of a long chain of ~35 galactofuranose (Gal*f*) residues that are connected to an α-Rha*p*-(1→3)-α-Glc*p*NAc disaccharide to peptidoglycan. The production of the galactan is essential for mycobacterial viability and therefore compounds that inhibit its biosynthesis are potential drugs for treating these infections.
Recent studies have supported a model of galactan biosynthesis in which the entire structure is assembled by the action of two bifunctional galactofuranosyltransferases, GlfT1 and GlfT2, which operate in the cytoplasm. GlfT1 transfers the first two Gal*f* residues to an undecaprenyl α-Rha*p*(1→3)-α-Glc*p*NAc disaccharide pyrophosphate intermediate inserted into the cell membrane. After that, the bifunctional galactosyltransferase GlfT2 installs 5- and 6-linked Gal*f* residues to produce a polyprenol-linked Gal*f* polymer. Following its synthesis, the Gal*f* polymer is transferred by a flippase from the cytosolic to the periplasmic side of the plasma membrane, where it is further elaborated and eventually transferred to peptidoglycan. To date, this flippase has been poorly characterized. This presentation will focus on the synthesis of long chain galactan-containing phospholipids as probes for this enzyme.

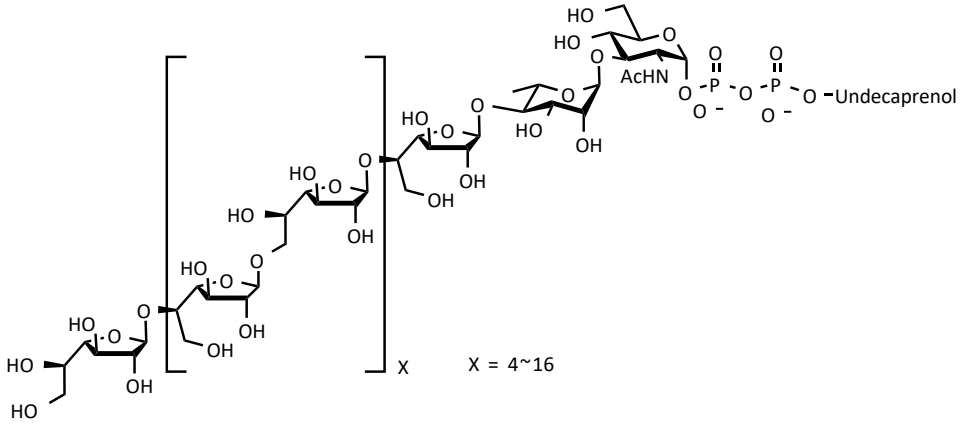

Fig. 1

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1,2-*CIS*-β-STEREOSELECTIVE GLYCOSYLATIONS USING MANNURONO-2,6-LACTONES

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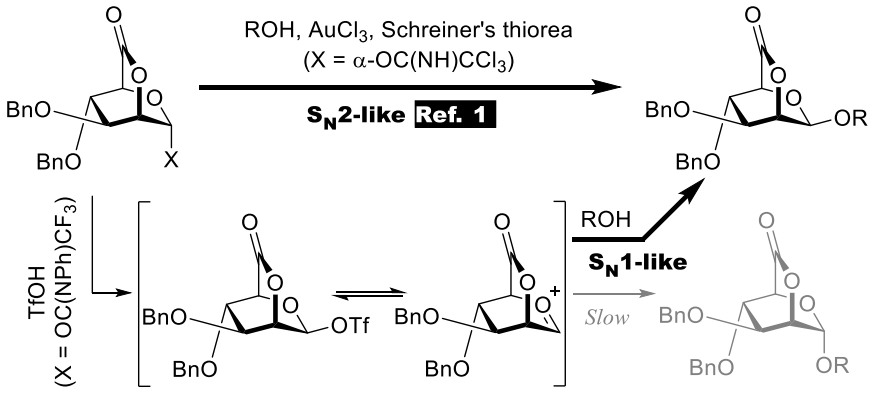
1,2-*cis*-β-glycosidic linkages (e.g. β-mannosidic linkages) are found in many natural products and play vital roles in animals and plants. However, their chemical constructions are not so easy tasks [1]. In our laboratory, novel methodologies employing glycosyl donors bearing a mannurono-2,6-lactone moiety have been reported, whose conformations are restricted to $^{5}S_{1}$–$^{2,5}B$–$^{2}S_{O}$. The *O*-glycosylation reaction using α-trichloroacetimidate as a leaving group proceeded with an excellent β-stereoselectivity in a stereoinverted manner at C-1 position, through S_N2-like machanisms, when activated by a combined catalyst of $AuCl_3$ and Schreiner's thiourea [2,3]. Contrastingly, the *C*-glycosylations using diphenylphosphate as a leaving group exhibited extremely high β-stereoselectivity *via* S_N1-like reactions. The latter indicates that the glycosyl cation bearing a 2,6-lactone moiety has an inherent β-direction, although it has been left to elucidate whether it arises sterically or stereoelectronically. These results encouraged us to investigate β-stereoselective *O*-glycosylation reactions through S_N1 mechanisms. We believe that S_N1 reactions are practically advantageous, because both anomers can be employed as donors. More importantly, the efficiency of the S_N1 reaction should be independent of the reactivities of the acceptors according to the definition of the term S_N1, which grants wider substrate scope to our methodology.

We found that *N*-phenyltrifluoroacetimidates congener afforded β-mannosides with high stereoselectivities, irrespective of the stereochemistry at C-1 position of the donor, which suggests the reaction proceeded in the S_N1 mechanisms. In addition, we found some side-reactions affording α-glycosides to eliminate and managed to suppress the side-reactions by mechanism-based optimization of the conditions. Furthermore, we have also investigated the substrate scope of the glycosylation, especially regarding *O*-3 and *O*-4 substituents, because the diastereomeric outcome in β-mannosylations reported so far are frequently affected by the substituent patterns in glycosyl donors. As a result, even when the donors are employed with a bulky substituent, such as TBS group, at the *O*-3 or with another sugar moiety at *O*-4 of the donor, the reaction exhibited high β-stereoselectivity. These results indicated that this methodology might be applicable to convergent oligosaccharide syntheses. Overall, we think that mannurono-2,6-lactone structure can be a promising solution for constructing 1,2-*cis*-β-glycosidic linkages.



Scheme 1. Glycosylations using mannurono-2,6-lactones.

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SYNTHESIS OF SUBSTRATES FOR MONITORING THE HYDROLYZING ACTIVITY OF ENDO-α-MANNOSIDASE

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Endo-α-mannosidases are classified as family of GH99 enzymes and hydrolyze the α1-2 mannosidic linkage between mono-, di-, or tri-glucose-substituted mannose residues [1]. The proposed hydrolysis mechanism is unique in that it proceeds through an α1,2-anhydro sugar intermediate [2]. Recently, we demonstrated the transglycosylation activity of endo-α-mannosidase toward α1-2 linked oligo-mannosides [3]. The biological role of this enzyme is believed to provide an alternative pathway for the removal of glucose residues in the *N*-glycan processing in the Golgi apparatus. Endo-α-mannosidase activity was also observed in the endoplasmic reticulum (ER) fraction, and possible additional biological roles have gained increasing attention.

To elucidate the functions of endo-mannosidase in the ER, a novel enzyme assay system based on fluorescence-quenching was developed using a tetrasaccharide probe labeled with *N*-methylanthraniloyl (NMA) as a reporter dye at the non-reducing end and 2,4-dinitrophenyl (DNP) as a quencher at the reducing end (NMA-Glc1Man3-DNP). To synthesize the tetrasaccharide probe, we prepared the Manα1-2Manα1-2Man skeleton via one-pot mannosylation using a bifunctional mannose derivative with a thiophenyl group at the anomeric position and a hydroxyl group at the C-2 position [4]. Upon activation of the thiophenyl group, the prepared molecule undergoes self-mannosylation to generate α1-2 linked oligo-mannoside derivatives. Subsequently, glucose residues were introduced to the non-reducing end of mannose residue at the mannotriose derivative and the tetrasaccharide (Glc1Man3) derivative was afforded. The tetrasaccharide derivative was labeled with the NMA group at the non-reducing end and DNP at the reducing end. Using this probe, photophysical properties could be quantified and the hydrolysis reaction of endo-α-mannosidase was monitored.

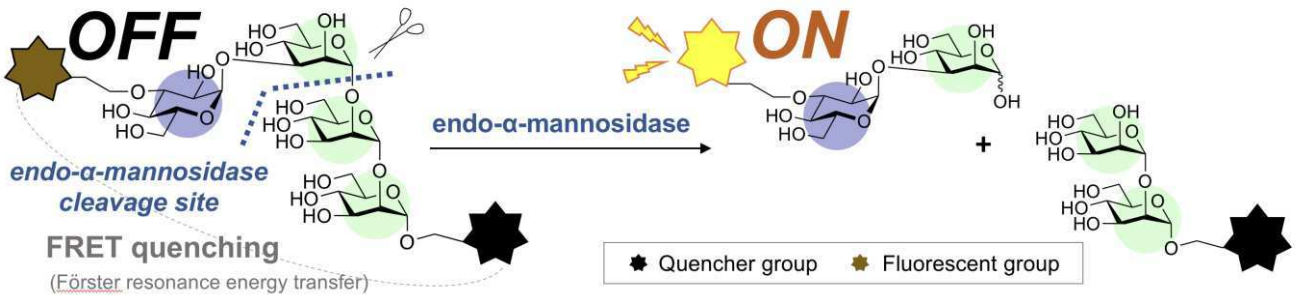

Fig. 1

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CHEMO-ENZYMATIC SYNTHESIS OF GLYCOPEPTIDES CARRYING T-, ST-, OR STN-ANTIGEN: PROFILING REACTIVITY OF C1GALT1, ST3GAL1 AND ST6GALNAC1

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Mucin-type *O*-glycosylations reside in most epithelial tissues and play a key role in biological processes. It is well known that structural and compositional changes in mucin glycans strongly relate to malignancy or other diseases. As tumor-associated antigens (TAAs), Tn- (GalNAcα1-Thr/Ser), T-(Galβ1,3GalNAcα1-Thr/Ser), ST-(Neu5Acα2,3Galβ1,3GalNAcα1-Thr/Ser) and STn-(Neu5Acα2,6GalNAcα1-Thr/Ser) antigens are frequently observed in malignant cells. Thus, mucin glycopeptides carrying TAAs have been used for drug development such as development of antibody, vaccine and so on. In this presentation, we show chemo-enzymatic synthesis of glycopeptides carrying T-, ST- or STn-antigen. Throughout the enzymatic reaction, we attempted to characterize each glycosyltransferase. On the synthesis, five GalNAc glycopeptides were chemically synthesized by Fmoc-SPPS under microwave irradiation and the further sugar elongation was accomplished by glycosyltransferases (Fig. A and B). Five substrates used in this study consist of the same amino acids, while these have a different reaction site. For enzymatic sugar-elongation, three glycosyltransferases [C1GalT (from *Drosophila melanogaster*), ST3Gal1 (from human) and ST6GalNAc1 (from human)] were successfully expressed using a methylotrophic yeast, *Ogataea minuta*. ST3Gal1 was active over five T-antigen glycopeptides in similar conversion yield; however, C1GalT and ST6GalNAc1 showed activity in a substrate-dependent manner. In particular, ST6GalNAc1 barely worked on substrates with a GalNAc-Ser residue. To overcome this problem, substrates bearing T-antigen were utilized for ST6GalNAc1 reaction. ST6GalNAc1 could convert T-antigen on serine residues to α2,6-sialylated products in improved yield. *In vitro* enzymatic synthesis could help simply characterizing of glycosyltransferases as a synthetic tool but also understating the *in vivo* biosynthesis of mucin *O*-glycans.

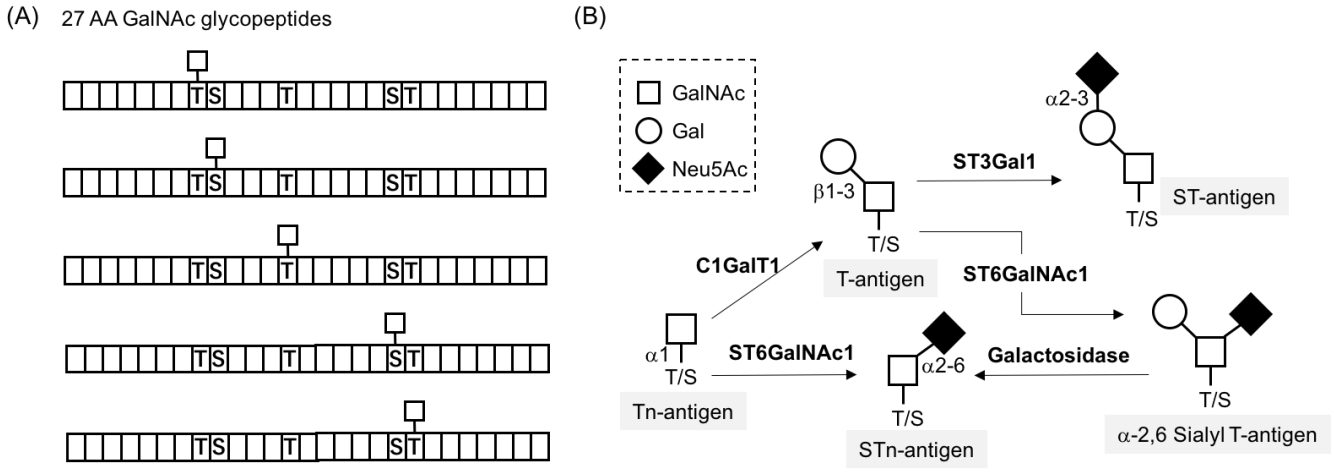

Fig. 1

NEW LOW-MOLECULAR CONJUGATES OF ANTITUMOR DRUGS WITH DERIVATIVES N-ACETYL-D-GALACTOSAMINE

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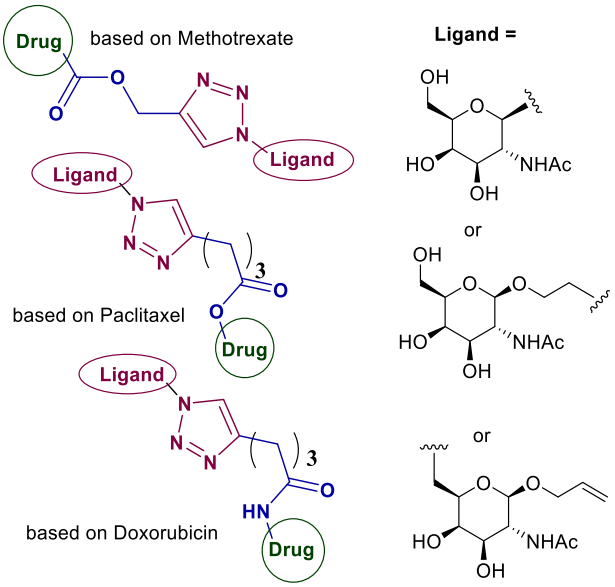
Hepatocellular carcinoma is the most common liver tumor, the result of malignant degeneration of hepatocytes. In terms of the number of deaths, hepatocellular carcinoma ranks 2nd in the world among all malignant tumors according to the World Health Organization [1]. Existing drugs for nowadays have several disadvantages, such as: 1) low selectivity 2) a wide range of side effects 3) low efficiency. Therefore, there is an urgent need to find new highly effective antitumor agents for the treatment of liver diseases. The using of targeted delivery strategy would help to avoid these shortcomings.

The asialoglycoprotein receptor is an excellent target for targeted delivery to hepatocytes of galactose-based derivatives [2]. In this work, three novel N-acetyl galactosamine ligands which show the highest affinity for ASGP-R have been synthesized. These ligands were also conjugated with antitumor drugs (paclitaxel, doxorubicin, methotrexate), which are used in clinical practice [3,4].



The reaction of [3 + 2] copper-catalyzed azide-alkyne cycloaddition (CuAAC) was used for the ligand and drug fragments conjugation. Subsequently, all the resulting conjugates were tested for cytotoxicity. Cytotoxicity was determined by the standard MTS method on the HepG2 cell line. Also, the method of fluorescent microscopy was used for the characterization of doxorubicin conjugates. The obtained results indicated the accumulation of free doxorubicin in the cell nucleus. In the case of doxorubicin conjugates, accumulation in the cytoplasm was observed. Some of the synthesised compounds exhibited cytotoxicity comparable to free drugs.

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SYNTHETIC STRATEGIES FOR SERINE/THREONINE-RHAMNOSYLATED GLYCOPEPTIDES

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Protein glycosylation is a ubiquitous posttranslational modification which plays important roles in many biological processes. Yet glycoproteins have had modest impact in glycobiological research, presumably due to synthetic challenges, i.e., extensive protection/deprotection strategies and the stereoselective formation of glycosidic linkages. In 2013 Lassak *et al.* discovered arginine-rhamnosylation as a new strategy for activation of the translation elongation factor EF-P. Assuming that EF-P arginine rhamnosylation is probably not unique, it would be desirable to identify other proteins bearing similar modifications. Herein we present efficient synthetic routes to serine/threonine-rhamnosylated haptens, which allow the generation of specific antibodies for detection of yet undiscovered proteins bearing serine/threonine-rhamnosylated motifs. The challenging stereoselective synthesis of α- and β-rhamnosylated glycopeptides could successfully be addressed by novel and efficient methodologies.

FULLY ENZYMATIC SYNTHESIS OF MONO- AND DIACYL TREHALOSE CONJUGATES

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Trehalose-lipid conjugates have gained increased interest for their potential applications: in bioremediation technologies and oil/petroleum industry, as biosurfactants; in biomedical/healthcare industry, as antimicrobial and therapeutic agents [1]. However, their preparation still requires multi-step synthesis [2-3] since lipase-mediated esterifications are not efficient enough, affording only mono-[4] or diester derivatives but in a long reaction time [5].

The issues we have addressed in this study are: i) the development of an efficient enzymatic access to 2,2,2-trifluoroethyl esters, and ii) the control of the transesterification process towards the selective preparation of diacyl and/or monoacyl trehalose, thus opening access to unsymmetrical derivatives. This approach was applied to a set of linear or branched, saturated or unsaturated acids, as well as to extracted membrane mycolic acids. Microwave assistance as well as a well-defined amount of acyl donor were required to efficiently perform the desired synthesis [6].

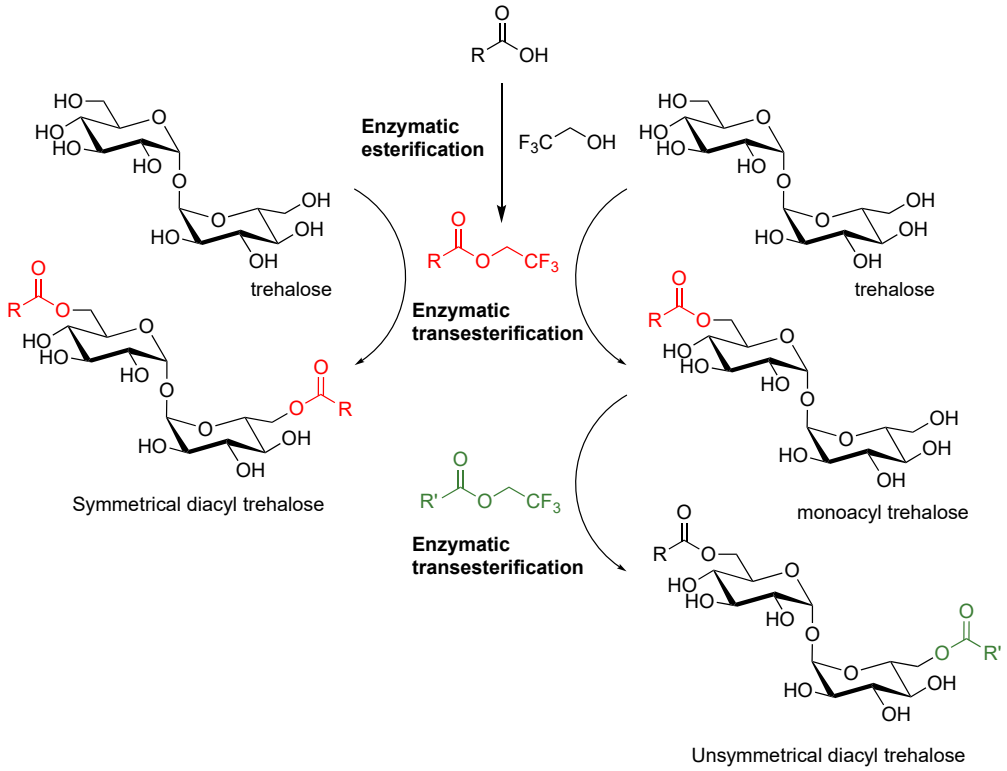

Fig. 1 - Lipase mediated acylation of trehalose.

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EXAMINATIONS OF α-SIALYLATION USING A MACRO-BICYCLIC GLYCOSYL DONOR

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The chemical synthesis of the sialoglycans is one of the most challenging subjects in the field of carbohydrate chemistry due to characteristic structure of the sialic acids. Although there are several efficient methods for highly stereoselective sialylation, some of which exploit the nitrile solvent effect and/or C5-modified sialic acid donors, their stereoselectivity frequently vary depending on the structures of coupling partners as it has also been seen in other glycosidation reactions that proceed via S_N1 pathway. In this study, we attempted a novel stereocontrol of sialylation by using a macro-bicyclic α-sialyl donor, in which the C1 and C5 positions were tethered with a proper alkyl chain. We envisaged that the bicyclic oxocarbenium ion generated from the sialic acid donor could direct the nucleophilic attack of the coupling partner exclusively to α-face by the steric hindrance of macro-cyclic moiety on the β-face (Fig. 1).

According to our previous report, a 1,5-lactamized sialic acid donor, having a bicyclo[2.2.2] system, was not capable of generating the oxocarbenium ion at the bridgehead anomeric position, following the Bredt's rule [1]. First, we examined a proper tether length between C1 and C5 that allows to form oxocarbenium ion at the anomeric position. A C5-NH_2 intermediate was conjugated with various tether parts, which then underwent macro-cyclization reactions to provide bicyclic donors. The examination of glycosidation of the sialyl donors in CH_2Cl_2 revealed a suitable design of the tether part to proceed α-sialylation. We then investigated the reaction substrate scope. Furthermore, the optimized method had been successfully applied on the synthesis of gangliosides, and also α(2,8)-linked oligo-sialc acids. Here, the details of these results are presented.

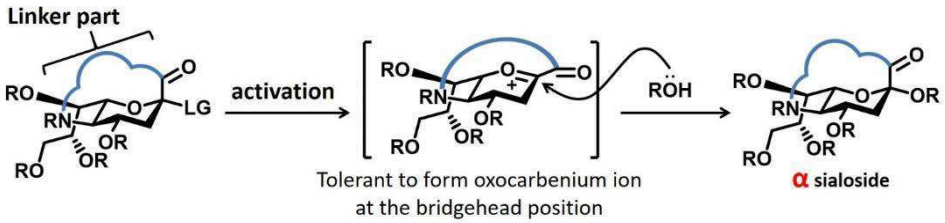

Fig. 1

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FURANOID CARBASUGARS AS GH 20 HEXOSAMINIDASE INHIBITORS AND POTENTIAL PHARMACOLOGICAL CHAPERONS

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Lysosomal storage diseases (LSDs) are a series of metabolic disorders of lysosomal enzymes caused by mutations of specific genes, responsible for their biosynthesis. Roughly, 25 % of these enzymes are involved in the chemical manipulation of *N*-acetyl-D-glucosaminyl or *N*-acetyl-D-galactosaminyl residues from degradation-bound polysaccharides and glycoconjugates [1].

Thus, a set of new potential hexosaminidase inhibitors have been synthesized starting from commercially available *N*-acetyl-D-glucosamine **Figure 1**. The new furanoid carbasugar structures are inhibitors of *N*-acetyl-β-D-hexosaminidases and have been synthesized via a 2+3 cycloaddition-based approach [2-4]. In addition, these compounds are potential pharmacological chaperones for Tay Sachs and Sandhoff diseases related mutants of lysosomal hexosaminidases.

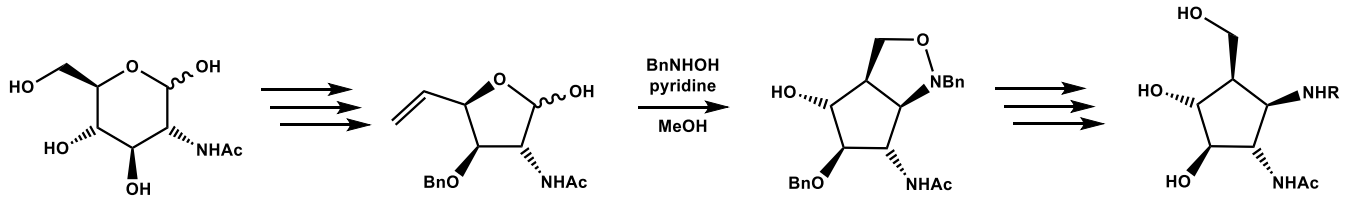

Fig. 2

Synthetic details as well as preliminary biological data will be presented.

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SYNTHESIS OF HAEMOPHILUS INFLUENZAE TYPE A OLIGOSACCHARIDES FOR VACCINE DEVELOPMENT

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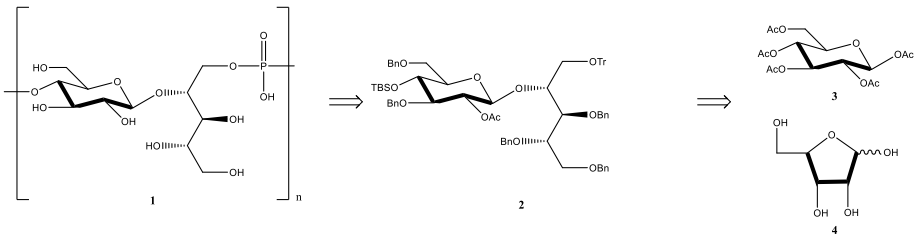
Haemophilus influenzae (Hi) is a human pathogen causing severe diseases, i.e.meningitis, sepsis and otitis, especially affecting young children. According to the structure of the capsular polysaccharide (CPS), the Gram-negative bacteria Hi can be classified into 6 serotypes (a-f), where Hia and Hib are the most virulent strains. Before the introduction of effective based on protein-conjugated polysaccharide vaccines, H. influenzae infections were dominated by Hib strains and were the leading cause of bacterial meningitis in children in the U.S. However, a massive vaccination campaign in the 1990s nearly eliminated Hib disease in some populations and raised some concerns that other strains may emerge as causes of disease. In recent years an increasing rate of infections caused by Hia have been reported in North and South America and Australia and, currently, Hia causes up to 10 % of Haemophilus infections. Furthermore, Hia infections exhibit antimicrobial resisitance to commonly used pharmaceutical agents, making treatment more challenging [1]. The capsular polysaccharide of Hia is a polymer of the disaccharide repeating unit 4-β-D-Glc-(1→4)-D-ribitol-5-(PO_4→). Although there is a structural similarity between Hia and Hib CPS, no cross-protection has been observed to type a by immunization with Hib conjugate vaccines [2]. On the other hand, no vaccine specifically targeting Hia is currently available or under development. We therefore anticipated that synthetic oligomers such as **1** (n= 1 -5) could be valuable tools to identify a protective epitope within Hia CPS (**Scheme 1**). In this communication we focus on the synthesis of the disaccharide **2**, obtained from a glucose donor and a ribitol acceptor which is a crucial and versatile building block for the assembly of the phosphodiester-linked oligomer **1**. The most suitable protecting groups for the donor and acceptor have been carefully investigated to achieve a convenient glycosylation reaction affording disaccharide **2**.



Scheme 1. Retrosynthetic strategy to achieve oligomers of Hia CPS.

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STEREOSELECTIVE SYNTHESIS OF TRIMANNOSIDE-BRANCHES IN HIGH-MANNOSE GLYCAN ~REACTIVITY TUNING WITH MIXED SOLVENT SYSTEM~

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In endoplasmic reticulum (ER) glycoprotein quality control, tribranched high-mannose glycans are function as secretion and degradation signals. These signals are produced by regioselective trimming of a terminal mannose residue in the each branch structures by α-1, 2-mannosidases in ER. [1] In this study, we synthesized three trimannosides having different innermost glycosidic linkage (Fig. 1) to understand regioselectivity of the α-1, 2-mannosidases in ER.

In the A branch synthesis, to optimize the effect of neighboring group participation, we selected to use toluene as reaction solvent having a low dielectric constant. The disaccharide synthesis successfully provided the α-product in 94% yield. However, the following trisaccharide synthesis (Fig. 2) provided lower yield (33%, Table 1) due to the bulkiness of the acceptor. To improve the reactivity, we mixed toluene with CH_2Cl_2 which is a nonpolar solvent having higher dielectric constant than toluene. Trisaccharide synthesis was carried out CH_2Cl_2/Toluene (1:1), resulted in greatly improved yield (80%, Table 1) with slight decrease of stereoselectivity (α/β = 87:13). This result suggests that the reactivity was improved by increasing the dielectric constant of solvent. To improved stereoselectivity, we used CH_2Cl_2/Toluene (1:2), which resulted in improved stereoselectivity (α/β = 93:7) with good yield (84%, Table 1). From these results, we found that stereoselectivity and yield can be tuning by adjusting the ratio of mixed solvent. Since similar tendencies were observed in B and C branch synthesis, adjusting the ratio of the mixed solvent (CH_2Cl_2/Toluene) might be useful for other substrates.

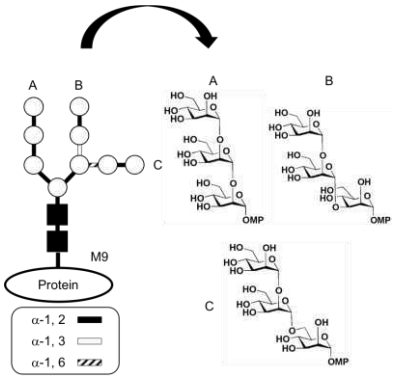

Fig 1. Three types of trimannoside synthesized in this study

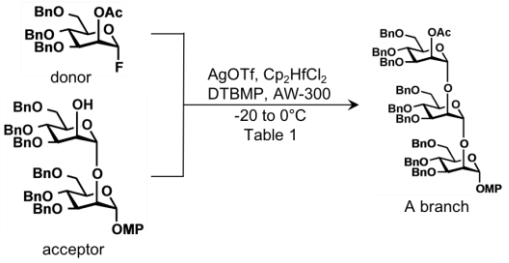

Fig 2. Trisaccharide synthesis (A branch)

Table 1. Investigation of the ratio of mixed solvent in A branch synthesis

Entry	Solvent	Time	Yield
1	Toluene	7 h	33% (α/β ≧ 95:5)
2	Toluene/CH_2Cl_2 = 1:1	1.5 h	80% (α/β = 87:13)
3	Toluene/CH_2Cl_2 = 2:1	1.5 h	84% (α/β = 93:7)

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CYCLOPENTANOID *N*-ACETYL-β-D-HEXOSAMINIDASE INHIBITORS

P. Weber,[a],* B. M. Pabst,[b] E. Paschke,[b] A. E. Stütz,[a] M. Tschernutter,[b] W. Windischhofer,[b] A. Wolfsgruber[a] and T. M. Wrodnigg[a]

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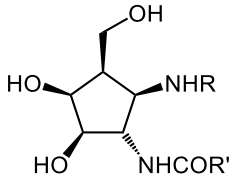
Based on successful synthetic approaches to basic β-D-*galacto* configured hydroxymethylcyclopentane derivatives, we have recently also prepared highly potent inhibitors **1** of human lysosomal β-D-hexosaminidases featuring the same scaffold [1]. Focusing on structural variability, we have now also gained access to the D-*gluco* configuration as well as to varying *N*-substituents on both vicinal amino functions. New results on these synthetic targets will be presented.



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USE OF SUPERACID CONDITIONS TO HIGHLIGHT UNPRECEDENTED TRANSIENT INTERMEDIATES IN GLYCOCHEMISTRY

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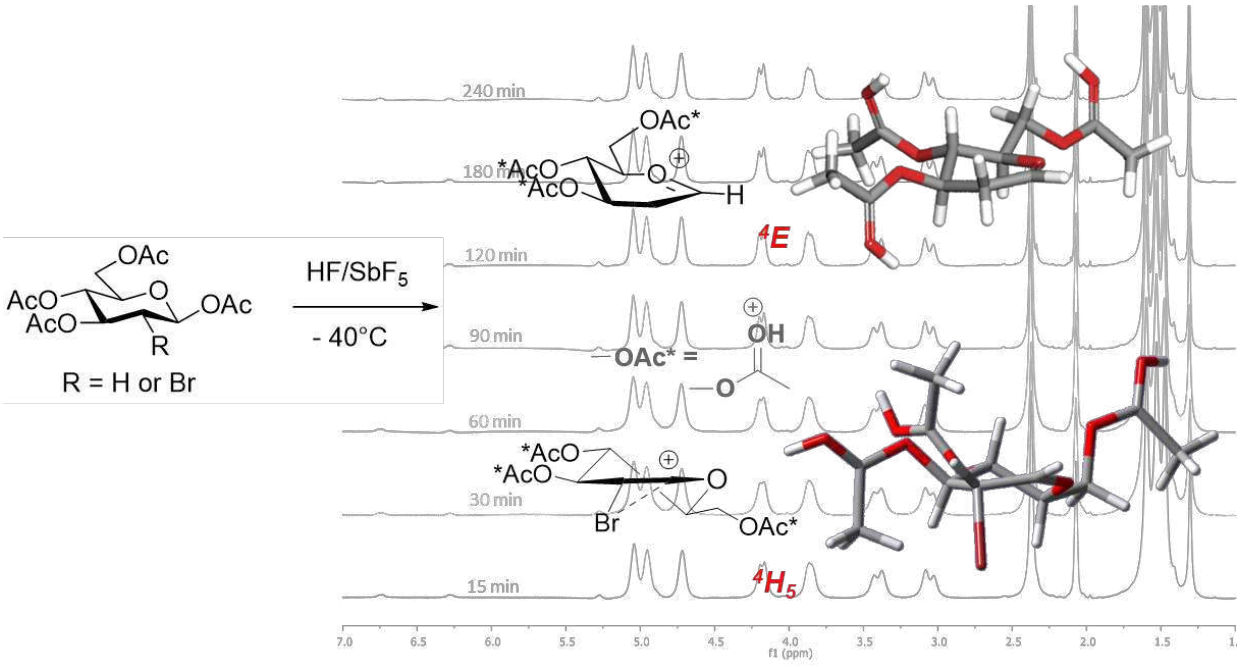
Glycosylation is the central reaction in glycoscience but details of its mechanism are still not totally understood. Thus, oxocarbenium and dioxalenium ions are considered to be key intermediates in the glycosylation reaction, but observation of these intermediates remains a challenging project that could be used to rationalize the stereochemical outcome of glycosylation reactions.

Several groups [1-3] are currently involved in the observation of the oxocarbenium ion using different methods, but the extremely short life of this intermediate makes its experimental observation particularly difficult. Our approach consists of generating these intermediates in superacid media. [4] It allowed their study by low-temperature *in situ* NMR supported by calculations giving access to the preferential conformation of these unprecedented transient intermediates. [5] The most recent results of this promising strategy will be presented.



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SYNTHESIS OF GLYCOCONJUGATED AZA-BODIPYS FOR PHOTODYNAMIC THERAPY

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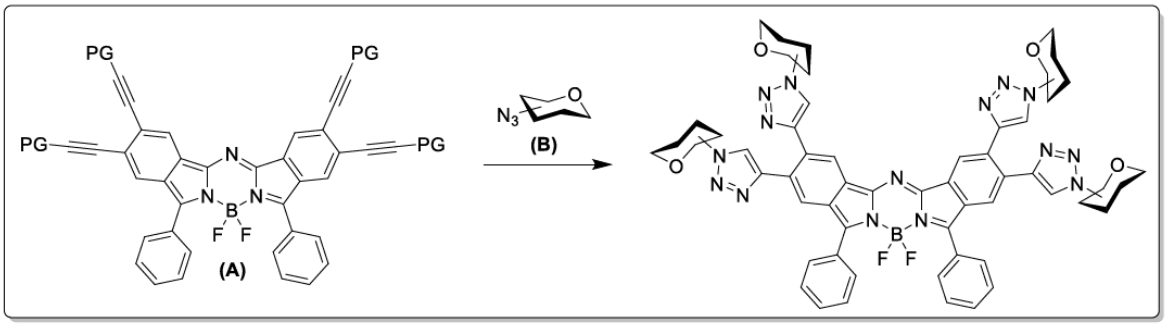
Photodynamic therapy has been of great interest over the past years. Our workgroup has previously published a number of promising third-generation photosensitizers, such as Phthalocyanines[1,2] and Porphyrazines [3].

Given that annulated Aza-BODIPYs offer excellent photophysical properties,[4] we established an accessible synthetic route to glycoconjugated Aza-BODIPYs. For this we used a triazole linkage to connect the benzoannulated Aza-BODIPY core (A) with the carbohydrate moiety (B).

Using this strategy, it is possible to generate a wide variety of glycoconjugated Aza-BODIPYs by variation of the applied carbohydrate-azides.



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TOTAL SYNTHESIS, STRUCTURAL ANALYSIS AND BIOLOGICAL STUDIES OF ZWITTERIONIC SP1 OLIGOSACCHARIDES

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Zwitterionic polysaccharides (ZPSs) are found on the surface of *Bacteroides fragilis* and *Streptococcus pneumoniae* and they exhibit unique immunomodulatory properties. ZPSs are the only known carbohydrate antigens to induce an immune response by a T cell-dependent pathway. To understand the mechanism of their immunomodulatory activity, well-defined oligosaccharides of ZPSs are needed. The synthesis of ZPSs oligosaccharides represents a major challenge because of the presence of rare monosaccharide constituents (such as trideoxy-diaminogalactose residues), the 1,2-*cis*-glycosidic bonds, and the presence of both positive and negative charges in the molecules. Here, we report the first total synthesis of a Sp1 ZPSs monasaccharide and dodecasaccharide, representing three and four repeating units of the capsular polysaccharide of *S. pneumoniae* type 1. We also present structural studies (Molecular Modeling, NMR and CD) and the first biological evaluation on these ZPS oligosaccharides.

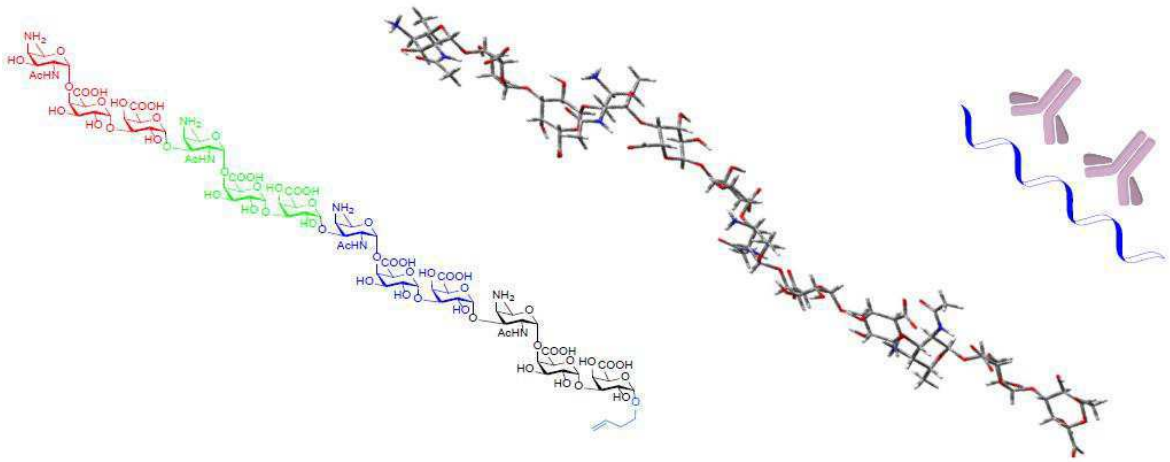

Fig 1. The structure of the Sp1 dodecasaccharide containing four repeating units. The nona- and dodecasaccharide adopt a helical structure, which has been shown to be crucial for the capsular polysaccharide 1 specific antibodies recognition.

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SYNTHESIS OF HETEROCICLIC COMPOUNDS USING SUGAR DERIVATIVES AS STARTING MATERIAL

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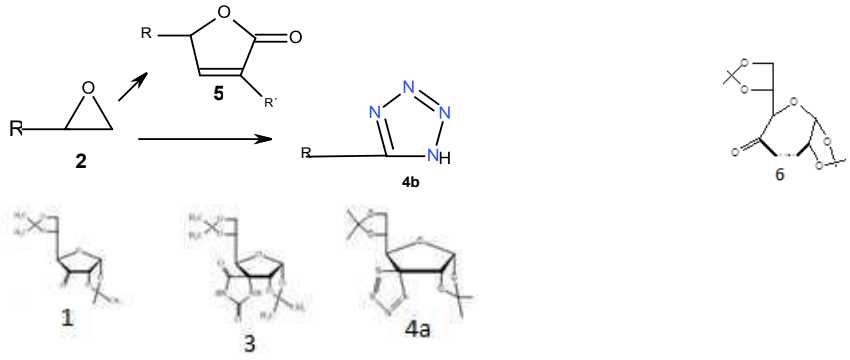
Heterocyclic compounds occupy a prominent place among organic compounds, are essential and important drugs; It play an important role in the metabolism of all living cells. Hydantoins, tetrazoles, lactones and lactams stand out because of its potential as a prototype for the development of new drugs. Therefore, the development of new methods for its preparation constitutes an interesting challenge for organic chemists in general and in particular, the carbohydrates field.

In this work we report the synthesis of hydantoin derivative type **3**, Tetrazole derivative type **4a** and **4b**, lactone derivative type **5** and lactam derivative type **6** (Figure 1).

Tetrazole **4a**, lactam **6** and Hydantoin **3** derivatives were prepared using Keto sugar type **1** as starting material using the Schmidt[2] and Bucherer-bergs procedures respectively; lactone **5** and tetrazole **4b** derivatives were prepared using sugar epoxide type **2** as starting material, applying A.P Rauter[1] and Farkas[3] procedures respectively.

All the compounds were obtained in good yields and their structures were elucidated by chemicals tests and its IR spectra's.



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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF MANNOSE-BASED GLYCODENDRIMERS AS POTENT DC-SIGN ANTAGONISTS FOR DENGUE INFECTION

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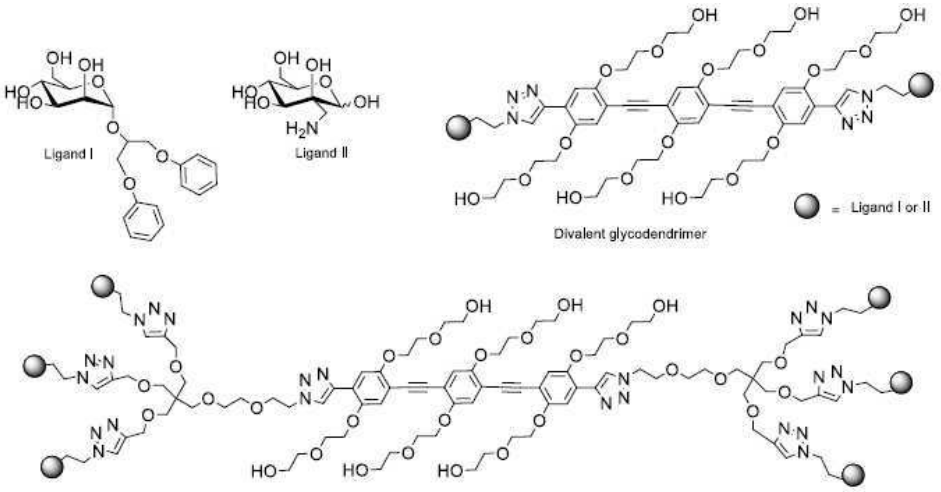
DC-SIGN is a C-type lectin expressed on dendritic cells. It contains four Ca2+- dependent carbohydrate recognition domains that specifically recognize complex mannose glycans present on the surface of invading microorganisms. For some pathogens, including viruses like HIV, Ebola or Dengue,[1] this recognition event contributes to infection transmission, making DC-SIGN a very interesting target for the design of antiviral agents.
Dengue virus infection (DENV) is currently expanding worldwide since it is present in more than 128 countries in the world.[2] DENV is the most prevalent mosquito-borne viral disease causing clinical syndromes in humans. As there is no available vaccine or treatment, DENV infection has become a major international public health concern and the search for anti-dengue treatment is of extreme importance and it is an active field of research.
Among the DC-SIGN antagonists reported in the literature, two mannose derivatives have drawn our attention (Ligand I [3] and Ligand II [4]), which are two of the most potent monovalent mannose derived DC-SIGN antagonists that are reported so far.
The well-known weak binding affinity which characterizes carbohydrate-protein interactions, can be overcome by multivalent presentation. Based on the previous experience of our group, herein we discuss the synthesis of the two ligands I and II functionalized with an azido linker and their conjugation to a rod-core system [5] in order to prepare both hexa- and di-valent glycodendrimers as DC-SIGN antagonists.



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PROBING THE FUNCTIONAL ROLE OF *O*-GALNACYLATION ON ANTIFREEZE GLYCOPROTEIN BY USING CHEMICALLY SYNTHESIZED ANALOGUES

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Antifreeze glycoprotein (AFGP) is an *O*-glycoprotein that displays antifreeze activity through depression of the freezing point of water. AFGP consists of an Ala-Thr-Ala glycotripeptide repeat, and the Thr residue is *O*-glycosylated with a D-Galβ1-3-D-GalNAcα1 (Gal-GalNAc). GalNAc is a core sugar structure of AFGP and contributes to the induction of antifreeze activity of this glycoprotein[1]. Many papers have discussed that the antifreeze activity is exhibited by adsorbing AFGP on ice surfaces. However, the function of *O*-glycosylations with GalNAc is still unclear (or remains unclear) .

To elucidate how *O*-glycosylations regulate antifreeze activity, herein we carried out the chemical synthesis of homogeneous *O*-GalNAcylated AFGP as well as partially *O*-GalNAc deleted AFGP analogues. The synthesis was achieved by Boc solid phase peptide synthesis (Boc SPPS) and subsequent peptide coupling reactions that we have established recently [2]. Based on this synthetic strategy, we have succeeded in the synthesis of a series of AFGP analogues consisting of 30 amino acids residues (AFGP30, Figure 1). These analogues included a fully *O*-GalNAcylated form of AFGP30 and partially *O*-GalNAlylated forms of AFGP30s containing 1~4 GalNAc deletion. Evaluation of antifreeze activities of these analogues revealed that there was clear correlation of the antifreeze activity and numbers of *O*-GalNAcylation. We have introduced ^{15}N-labeled Ala in these AFGP30 analogues to detail the dynamics of their protein backbone. This NMR analysis revealed that GalNAc deletion increased the flexibility of protein backbone. These results suggest that *O*-GalNAcylation induce inherent rigid conformation, which induce antifreeze activity of AFGP.

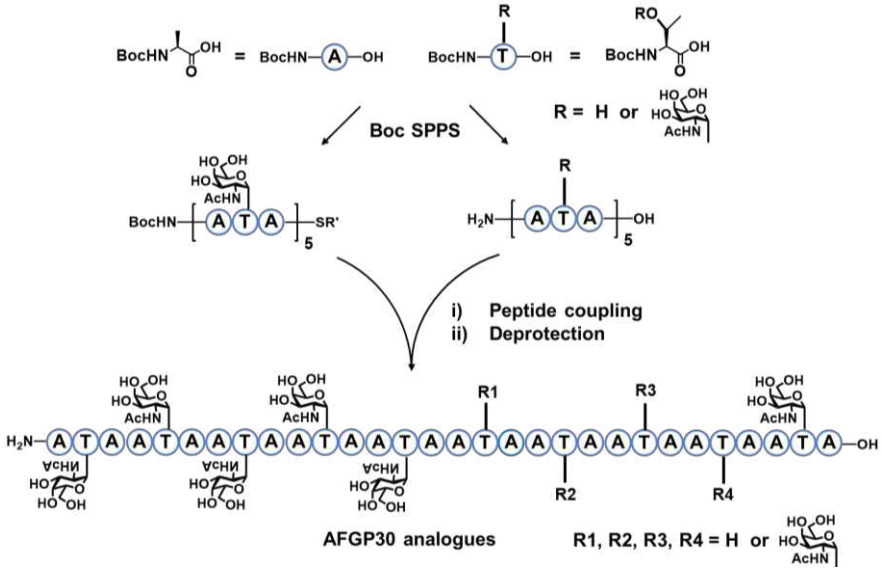

Figure 1. Synthesis of AFGP30 analogues for elucidation of role of GalNAc

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THE *N*-HETEROCYCLIC CARBENE CONTROLLED DEHOMOLOGATION OF ALDOSES

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Despite carbohydrates being Nature's largest chiral pool, only a comparably small set of representatives, is readily available to date. In our research, we focus on the development of new synthetic methodology for the interconversion of abundant sugars to more exotic ones by targeting the reactivity of the aldehyde moiety.[1,2] In our current study, we investigate the organocatalytic anomeric activation with *N*-heterocyclic carbenes (NHCs) as promising highly carbonyl selective reagents (see Figure 3).

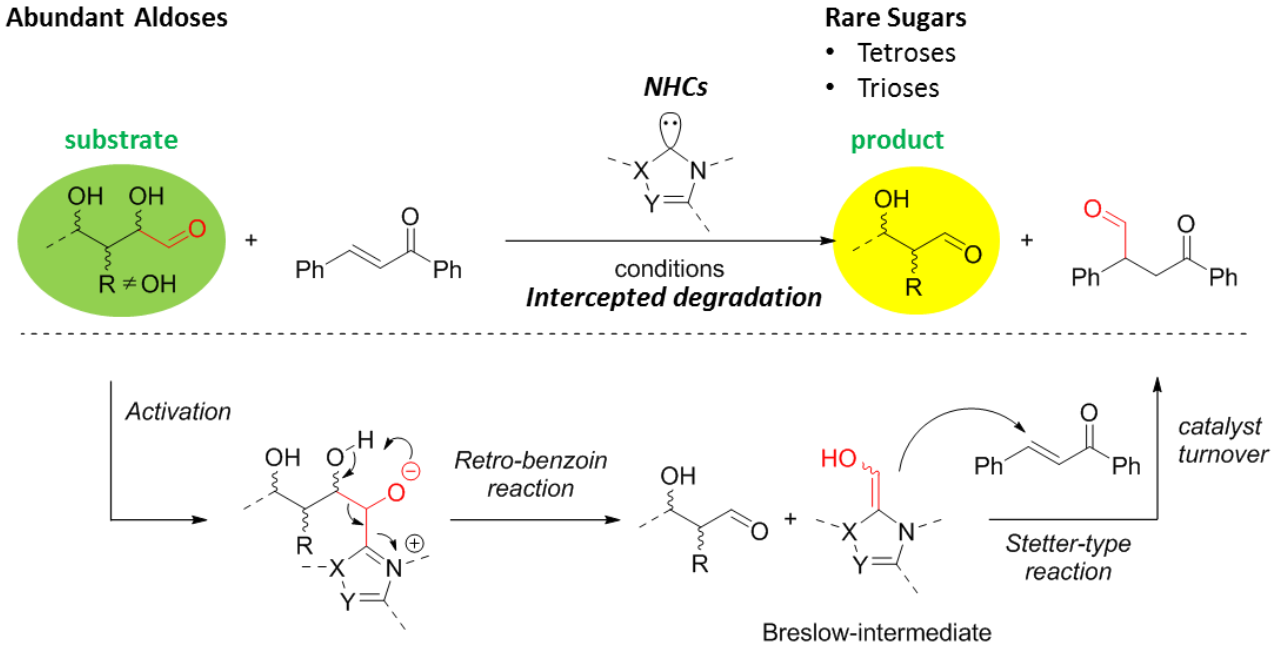

Figure 3 NHC controlled dehomologation of aldoses

Building on the recently reported sacrificial degradation of aldoses[3], we set out to develop a methodology for an intercepting degradation (by replacing specific OH groups) to allow for the isolation of defined sugar derivatives with shortened chain length.

Upon successful proof of concept with 3-*O*-Bn-D-glucose, we are currently investigating this new and complex transformation. We are optimizing for ideal reaction conditions as well as governance of both the catalyst's and the sugar substrates' structure. Aiming for an efficient screening, we developed a robust and quantitative method for the analysis of the observed mixtures of reducing sugars which is based on solid phase extraction (SPE), derivatization and calibrated GC-analysis. Therewith, we could achieve time resolved analysis of the reaction targeting to pin down kinetic as well as thermodynamic influences.

We discovered a crucial follow-up reaction, which can be suppressed or even enhanced by the right combination of catalyst and substrate and also identified parameters, which need to be controlled to allow for fast and clean conversions. We are *en route* to better understand the selective interaction between NHCs and aldose's aldehyde function of which we expect great gain of knowledge and application for within the realm of synthetic carbohydrate chemistry and beyond.

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LARGE SCALE SYNTHESIS OF THE NATIVE GPR55 AGONIST LYSO-PHOSPHATIDYL-β-D-GLUCOSIDE

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G protein-coupled receptor 55 (GPR55) was initially identified as the missing third canabinoid receptor. GPR55 is highly expressed in brain and peripheral nervous system and moderately activated by endocannabinoids. Additionally, GPR55 signalling has been associated with neuropathic pain and inflammation. Recently, GPR55 was demonstrated to play an important role during neuronal development due to its involvement in the guidance of extending neuronal growth cones of nociceptive axons[1]. In contrast to initial reports, the endogenous ligand for GPR55 has been identified as lyso-phosphatidyl-β-D-glucoside (LysoPtdGlc).[1] To further functional studies on the LysoPtdGlc-GPR55 signaling axis, access to sufficient amounts of synthetic LysoPtdGlc is required. However, current synthetic procedures [2,3] rely predominantly on chemoenzymatic approaches providing only microgram scale amounts.

In the present study, we report the first complete chemical synthesis of LysoPtdGlc. To overcome difficulties in maintaining a high enantiomeric purity of the key intermediate, β-glucopyranosyl hydrogenphosphonate, during scale-up, we developed a flow microreactor based synthetic approach. Next, potential loss of enantiomeric purity during subsequent coupling reaction with mono- and diacylglycerol building blocks due to ester migration was avoided by utilizing an acetal protected glycerol building block. Subsequent deprotection by hydrolysis and fatty acid ester introduction preserved the enantomeric purity and provided a practical synthetic access to LysoPtdGlc in high yield. Utilizing our synthesized material, we are now focusing our studies on the LysoPtdGlc-GPR55 interaction and signalling axis.

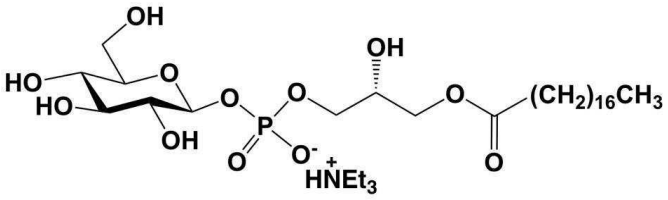

Fig 1. Structure of lyso-phosphatidyl-β-D-glucoside (LysoPtdGlc).

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EN ROUTE TO A FULLY SYNTHETIC CARBOHYDRATE-BASED VACCINE AGAINST *LEISHMANIA DONOVANI* CONTAINING FLUORINATED EPITOPES

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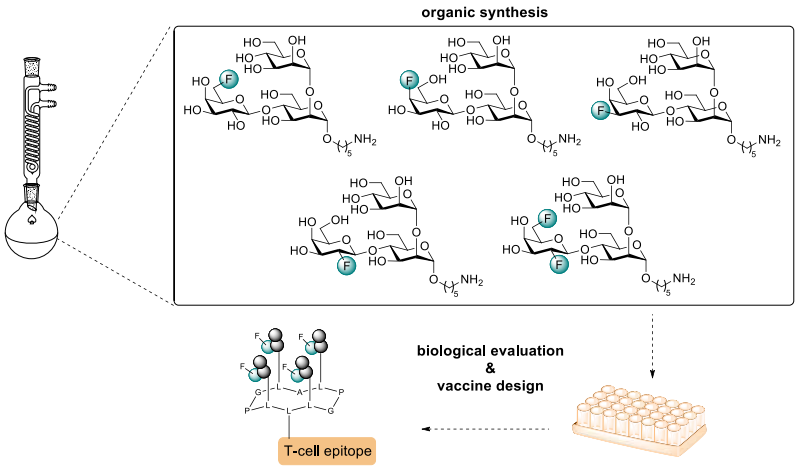
Owing to their structural uniqueness and dense distribution on the surface of pathogenic organisms, carbohydrate antigens are interesting target compounds for vaccine development.[1] A major drawback of carbohydrate-based vaccines is their insufficient immunogenicity and limited bioavailability which often leads to poor immune responses.[1] Strategic fluorination of glycans is an increasingly employed approach to overcome these limitations and several fluorinated carbohydrate-based vaccine candidates have been reported to date.[2] We have recently started to apply this concept to a proven leishmanial antigenic epitope. The species of the protozoan parasite Leishmania affect people in more than 88 countries of the world and lead to estimated 20000 to 50000 deaths per year.[3] Several vaccination approaches have already targeted structural characteristics of the leishmanial cell surface Lipophosphoglycan (LPG),[4] but incorporation of fluorine into the LPG capping structure has not been tackled before. Herein, we present the synthesis of novel fluorinated trisaccharides derived from the capping structure of *Leishmania donovani* in order to investigate the impact of fluorination on antibody recognition and vaccination efficacy.



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CHEMICAL SYNTHESIS OF *N*-RHAMNOSYLATED GLYCOPEPTIDES PROMOTE THE DISCOVERY OF NOVEL GLYCOSYLATIONS

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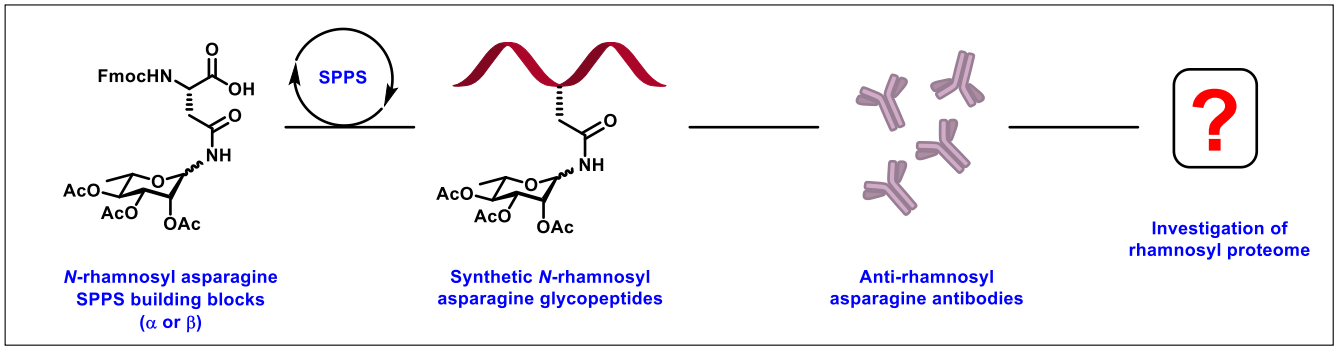
The previously discovered posttranslational arginine rhamnosylation is essential for elongation factor P (EF-P) dependent rescue of polyproline stalled ribosomes in clinically relevant species such as *Pseudomonas aeruginosa* or *Neisseria meningitidis* and represents the first example of protein *N*-rhamnosylation in bacteria.[1,2] Considering that glycosylation is a universal strategy to alter structural and functional properties of proteins, one can assume that *N*-rhamnosylation might be more widespread in nature. Nevertheless, almost nothing is known about this novel type of *N*-linked protein glycosylation so far. In this regard, synthetic rhamnosyl glycopeptide haptens are valuable tools to further investigate the rhamnosyl proteome in bacteria.[3,4] Since *N*-glycosylation is commonly found on asparagine, we herein present a novel synthetic route towards α- and β-rhamnosyl asparagine glycopeptide haptens for the generation of highly specific anti-rhamnosyl asparagine antibodies. This in turn allows the detection of protein mono-rhamnosylation in diverse species and lays the foundation to discover novel and yet unknown glycosyl transferases as a prerequisite to engineer glycosynthases with novel substrate specificities.



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STEREOELECTRONIC EFFECTS IN HYDROXYLATED SATURATED HETEROCYCLES

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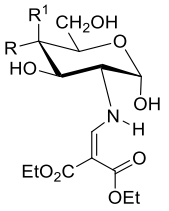
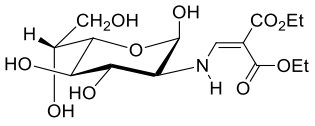
Six-membered hydroxylated saturated heterocycles show a plethora of steric and stereoelectronic effects: Perlin effect, anomeric and exoanomeric effects, reverse anomeric effect, gauche (or 1,3-diaxial) interactions, etc.[1] An interesting case occurs in some derivatives of 2-amino-2-deoxyaldoses: while their enamines (**1,2**) exist prevalently as α-anomers, their iminic counterparts (**3,4**) display β-configuration at the anomeric center. Both behaviors have been widely exploited in synthetic carbohydrate chemistry, as the anomeric configuration can easily be preserved through such derivatives. So far, however, no satisfactory explanation has been provided.[2]



1 R=OH, R^1=H
1 R=H, R^1=OH



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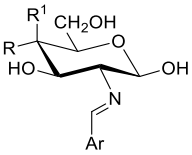
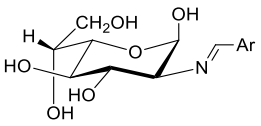


3 R=OH, R^1=H
3 R=H, R^1=OH



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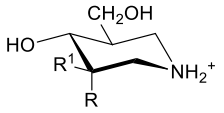
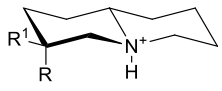
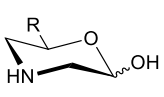
The preferential β-configuration in imines could most likely be traced to previous studies by Bols *et al*. on polyhydroxylated piperidines,[3] which show significant activity as glycosidase inhibitors. The pK_a of such heterocycles depends on the axial or equatorial orientation of the hydroxyl group (e.g., **5**-**6**, ΔpK_a ~0.8; **7**-**8**, ΔpK_a ~1.3).



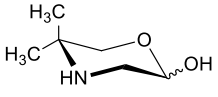
5 R=OH, R^1=H, pK_a=9.2
6 R=H, R^1=OH, pK_a=8.4



7 R=OH, R^1=H, pK_a=9.9
8 R=H, R^1=OH, pK_a=8.6



9 R=H
10 R=CH_3
11 R=CH_2OH



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The stereoelectronic effect accounting for the changes in basicity observed by Bols and coworkers, could also be extrapolated, at least in part, to imines from 2-amino-2-deoxyaldoses, because the distance and spatial arrangement between the anomeric hydroxyl and the iminic nitrogen are similar to those of nitrogen and hydroxyl groups in piperidine derivatives. To this end, we have synthesized some model compounds (*e.g*. morpholines **9-12**), studying their conformational preferences in the solid state (X-ray diffraction) and in solution (NMR), together with theoretical DFT calculations [M062X/6-311 ++G(d,p)] in both gas-phase and taking into account solvent effects (SMD methods and discrete solvation). Stereoelectronic effects have also been analyzed through second-order stabilizing interactions (NBO analysis).

We thank the financial support from the Junta de Extremadura and Fondo Europeo de Desarrollo Regional, Grant GR15022.

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STEREOELECTRONIC EFFECTS IN GLYCOSYL ISOTHIOCYANATES

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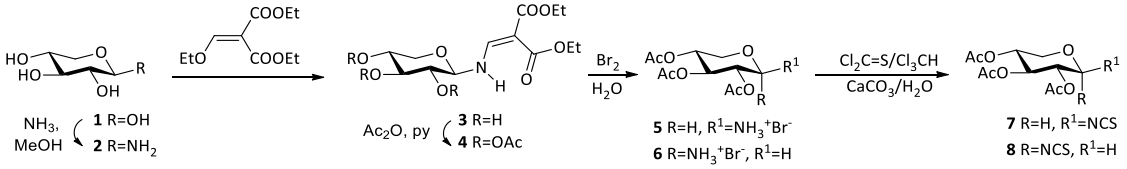
Among the steric and stereoelectronic effects exhibited by saturated six-membered hydroxylated heterocycles, the anomeric, *exo*-anomeric and inverse anomeric effects have been widely studied. It is well established that polar groups (OH, OR, halogens, etc.) at the glycosidic position usually adopt an axial arrangement (anomeric effect), which contrasts with amino functionalities preferring an equatorial disposition (inverse anomeric effect).[1] The anomeric behavior of both isocyanate and isothiocyanate groups, often described as "pseudohalogens", has been scarcely studied. To bridge this gap, we have synthesized and evaluated in detail α and β anomers of 2,3,4-tri-*O*-acetyl-D-xylopyranosyl isothiocyanate (**7**, **8**) according to the scheme outlined below.



During deprotection of the amino group, partial anomerization (**5**:**6**, α/β ratio = 1:3) could be observed. The conformational preferences of compounds **5**-**7** has been evaluated experimentally in the solid state (X-ray diffractometry) and in solution (NMR), and theoretically through DFT calculations at the M06-2X/6-311++G(d,p) level in the gas phase and simulating solvent effects (SMD method). The possible stereoelectronic effects have been estimated by an NBO analysis of second-order stabilizing interactions. The β-anomer (**6**) shows in solution a $^4C_1$ conformation, while the α-anomer (**5**) exists in equilibrium between the latter and a predominant $^1C_4$ conformer. This behavior is consistent with an "inverse anomeric effect" of the ammonium group, similar to that shown by other nitrogen derivatives with or without charge.[2] In contrast, the conformational behavior of anomers **7** and **8** shows an opposite trend. The β-configured isothiocyanate (**7**) exhibits a $^4C_1$ conformation, identical to that found in the solid state (X-ray data), while the α-anomer (**8**) shows a balance between $^4C_1$ and $^1C_4$ conformations. These data agree with those obtained by theoretical calculations, which indicate that $^1C_4$ conformer of **8** is more stable than its $^4C_1$ counterpart (ΔΔG ~1.5 kcal/mol, both in the gas-phase and solvents, i.e. $CHCl_3$ and EtOH). The NBO analysis shows appreciable stabilizing interactions LPO → $\sigma^*_{C1\text{-}N}$ in both **7** ($^4C_1$) and **8** ($^1C_4$) (~15 kcal/mol). The above results suggest that the isothiocyanate group exerts a significant anomeric effect.

We thank the financial support from the *Junta de Extremadura and Fondo Europeo de Desarrollo Regional* (Grant GR15022).

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SYNTHESIS OF WATER SOLUBLE, GLYCOCONJUGATED PHTHALOCYANINES

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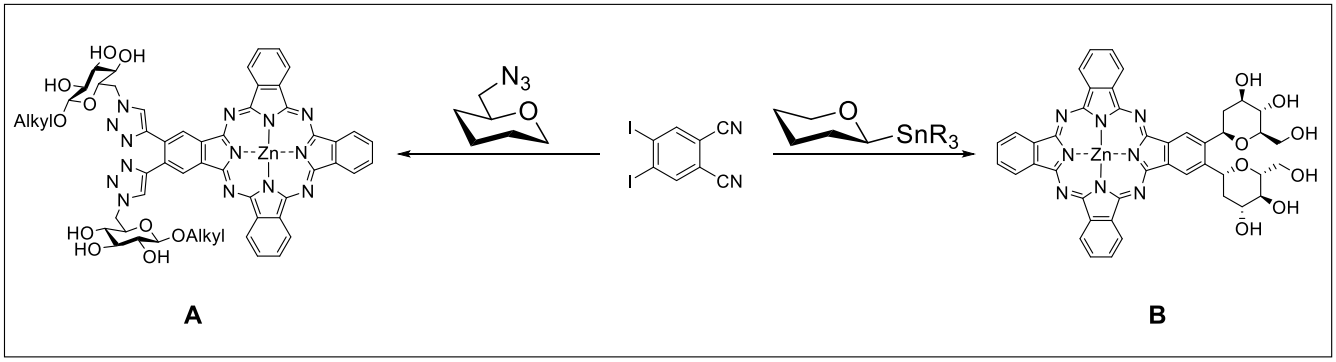
Glycosylated and glycoconjugated phthalocyanines (PCs) have found application in Photodynamic Therapy for the treatment of cancer. In the present work, we present two different approaches for the synthesis of such compounds.[1] Their structure consists of a phthalocyanine core with 1,2,3-triazole-linked (**A**) or directly C-C-linked glycosides (**B**). The carbohydrate moiety is thought to support the selective delivery to the tumour tissue.[2]



Disubstituted glycoconjugated phthalonitriles were used as precursors for tetramerization affording the phthalocyanine macrocycles. Towards **A**, azido pyranosides and towards **B**, stannyl pyranosides were used as the carbohydrate precursors. It is very important to understand the aggregation behaviour of such photosensitizers in solution, since the type of aggregation heavily affects their photophysical properties. As a matter of fact, we observed very different aggregation behaviours for **A** and **B**. Therefore, we precisely studied the aggregation behaviour of the PCs by high temperature NMR spectroscopy, MALDI-Tof mass spectrometry, UV-Spectroscopy and CD-Spectroscopy in different media.

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SYNTHESIS OF A TETRASACCHARIDE AND A PENTASACCHARIDE CONSTITUENTS OF *Trypanosoma cruzi* MUCINS

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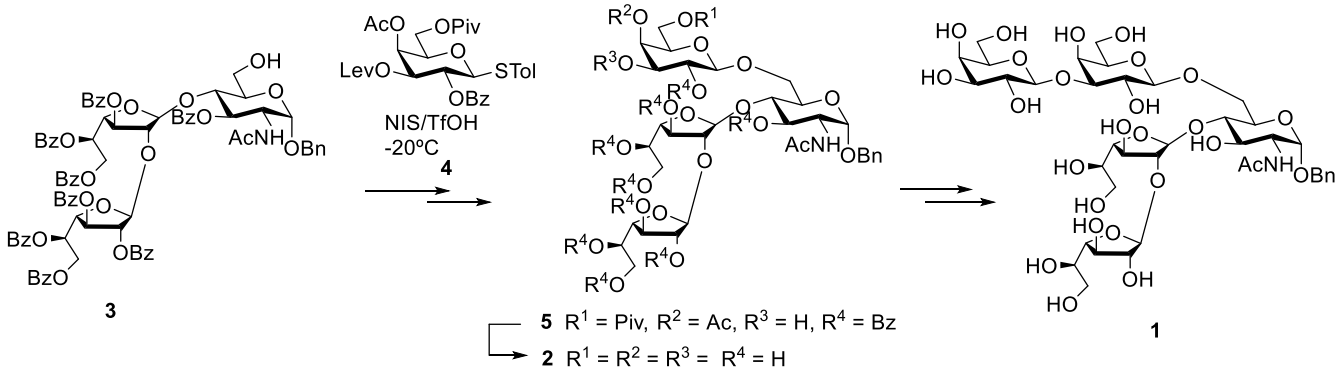
Trypanosoma cruzi, the etiological agent of Chagas disease, is a protozoan parasite with a complex life cycle that alternates between hematophagous triatomine vectors and mammals, including humans. The surface of *T. cruzi* is covered by a dense glycocalix and its composition is characteristic of each differentiation stage. The mucin-like glycoproteins are major components in the protozoan surface. The oligosaccharides in the mucins are *O*-linked to the protein by a α-GlcNAc unit. They are derived from two cores, Gal*p*(β1→4)-GlcNAc or Gal*f*(β1→4)-GlcNAc which are further branched by Gal*f* or Gal*p*. The terminal β-Gal*p* residues are acceptors of sialic acid in the trans-sialidase reaction, which is involved in the invasion of the host cells. The presence of β-Gal*f* in the oligosaccharide mucins is a remarkable feature restricted to certain parasite strains in the insect stage, the non-infective epimastigotes and the highly infective metacyclic trypomastigotes. We have been developing methods of synthesis of the Gal*f*-containing oligosaccharide family with the aim to correlate their structure with the ability to act as substrates in the *trans*-sialidase reaction.[1,2] Moreover, these synthetic oligosaccharides could be used as tools for elucidating their biological role and for biosynthetic studies.

We now present the synthesis of pentasaccharide **1** and tetrasaccharide **2** as benzyl glycosides. The alditol of **1**, β-D-Gal*p*(1→3)-β-D-Gal*p*(1→6)-[β-D-Gal*f*(1→2)-β-D-Gal*f*(1→4)]-D-GlcNAc-ol was isolated from the Tulahuen strain by reductive β-elimination. Previously, the synthesis of **1** was performed by a [3+2] convergent strategy with moderate yield. In this case, a sequential strategy was followed by the use of trisaccharide **2** with an internal Gal*f* as acceptor and thiogalactopyranoside **4** as donor to give the corresponding tetrasaccharide with excellent yield. Deprotection of the orthogonal Lev group gave **5.** The differences of both strategies will be discussed.



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SYNTHESIS OF D-GALACTOSAMINE AND D-ALLOSAMINE DERIVATIVES VIA A MICROWAVE-ASSISTED PREPARATION OF 1,6-ANHYDROGLUCOSAMINE

Pin-Hsuan, Liao and Cheng-Chung, Wang

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We report an intramolecular anomeric protection (iMAP) of glucosamine, which conducts a microwave-assisted intramolecular reaction and facilitates the concise transformation of 1,6-anhydroglucosamine into 1,6-anhydrogalactosamine and 1,6-anhydroallosamine. Our iMAP method simultaneously avoids both the *O*1 and *O*6 protection. Because of the hydrogen bonding between *N*2 and *O*4, we were able to well differentiate the *O*3 and *O*4 of 1,6-anhydroglucosamine by the *N*2 functionality. By the epimerization of *O*4 and *O*3, we obtained the galactosamine and allosamine derivatives respectively.

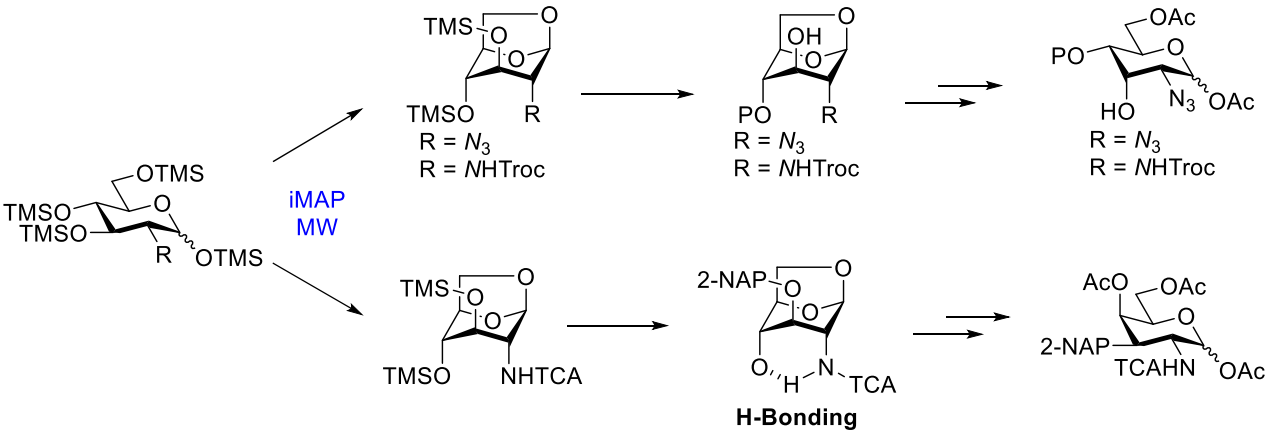

Figure 1. The synthetic strategy of D-galactosamine and D-allosamine derivatives

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NOVEL ROUTE TO 2-DEOXY GLYCOSYL STANNANES TROUGH REDUCTION OF D-GLUCALS

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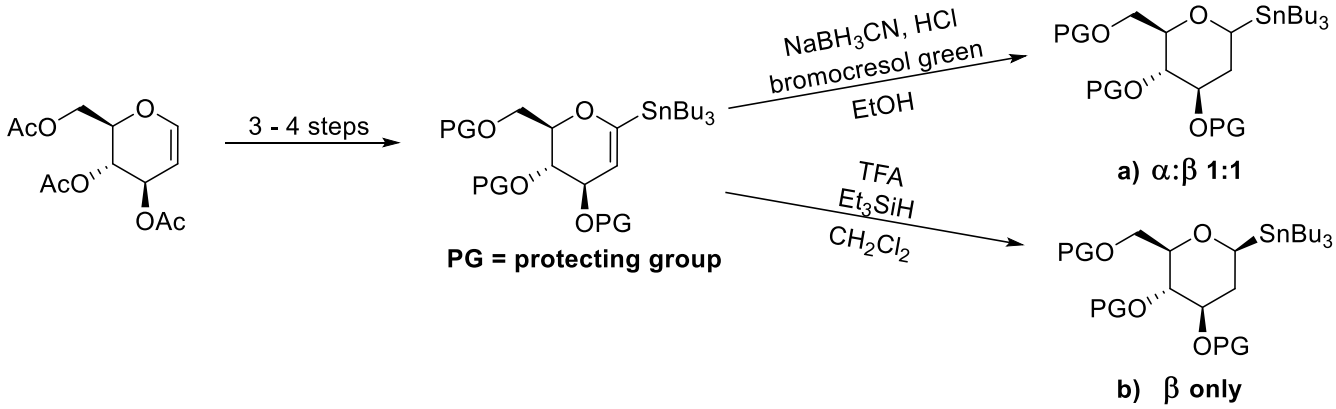
Aryl *C*-glycosides are present in many natural products. Due to their biological activity combined with their stability towards enzymatic and chemical hydrolysis they attract considerable interest in research and drug development. One example is the class of pluramycins which show antibacterial and antitumor activity.[1] The key structural motif of aryl *C*-glycosides is the direct *C*-*C* bond between an (poly-) aromatic aglycon and a glycon. A common strategy to create the *C*-*C* linkage is the transition-metal-mediated cross-coupling of metalated glycals with aryl halides like the Stille Cross-Coupling.[2] *Walczak* and coworkers recently reported a variant of this reaction by coupling configurationally stable sp^3-hybridized glycosyl stannanes with aryl halides in a stereoselective manner.[4] Hereby the access to both anomers of the stannane is crucial. Therefore, we investigated a new approach to the synthesis of 2-desoxy glycosyl stannanes by reduction of the stannylated glucals.



Our synthesis starts with the commercially available tri-*O*-acetyl-D-glucal, which we converted into the protected *C*-1 stannylated glucal. In literature, the reduction of vinyl stannanes is described as rather troublesome.[6] On this account we tested the reduction with $NaBH_3CN$ (**a**) which was already successfully reported for coupled glucals as educts.[5] Under mild acidic conditions we generated a 1:1 mixture of both anomers in low yields. Since the pH was vital in this reaction we used bromocresol green as an indicator. A second approach was made with Et_3SiH as the reduction agent (**b**) in the presence of a Lewis or Brønsted acid. Interestingly enough, only the beta anomer of the product was found under these conditions. Overall these two methods provide an alternative, mild route for the synthesis of 2-deoxy glycosyl stannanes circumventing the use of the rather instable 2-deoxy glycosyl chlorides which are commonly used as precursors for the 2-deoxy glycosyl stannanes.[4,7]

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TMSOTF-CATALYZED PER-*O*-SILYLATION: STREAMLINED REGIOSELECTIVE ONE-POT FUNCTIONALIZATION OF CARBOHYDRATES AND CONCISE SYNTHESIS OF D-GALACTOSAMINE AND D-ALLOSAMINE DERIVATIVES

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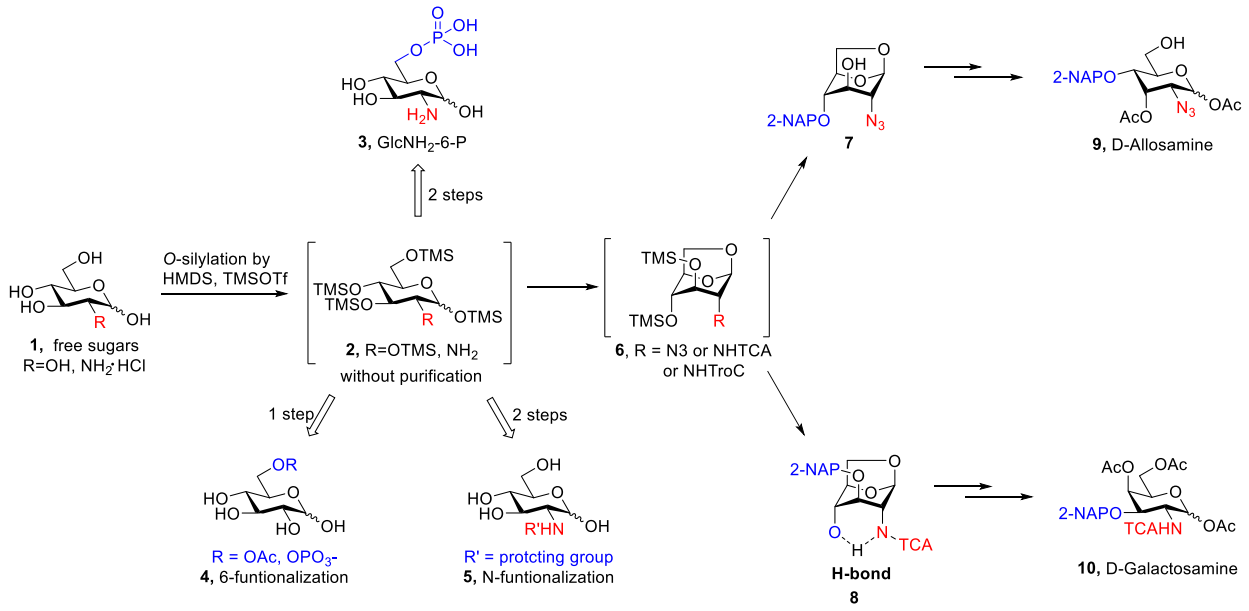
Based on the efficient TMSOTf catalyzed HMDS silylation, [1] a highly regioselective one-pot functionalization of carbohydrates was established. The following reaction could be performed smoothly without any purification and further afforded 6-*O*-funtionalized sugar **3** [2], *N*-functionalized sugar **4** [3] and glucosamine 6-*O*-phosphate **5**[3] under concise synthetic steps with high regioselectivity. [4]. In addition, the TMSOTf catalyzed HMDS silylation of glucosamine followed by further TMSOTf catalyzation in-situ generated 1,6-anhydrosugar **6** [5], of which *O*3 and *O*4 secondary hydroxyl group could be differentiated by the presence/ absence of intramolecular hydrogen bonding[6]. Eventually, the rare D-allosamine **9** and D-galactosamine **10** derivatives were obtained in concise steps.



Scheme 1. The one-pot synthetic strategy of free sugars.[1-3, 5]

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SYNTHESIS OF 4-AMINO-4-DEOXY-L-ARABINOSE TRANSFERASE INHIBITORS

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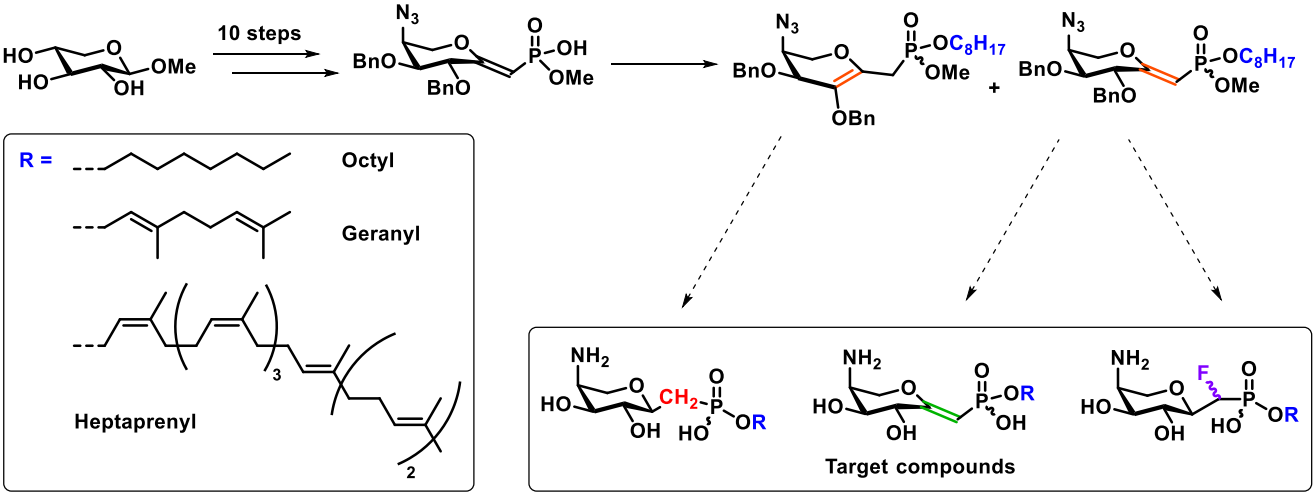
Burkholderia cepacia complex is a group of 17 opportunistic pathogens which have been linked to severe infections in cystic fibrosis and other immunocompromised conditions.[1] The core region of *Burkholderia* lipopolysaccharide is highly conserved across the genus and contains 4-amino-4-deoxy-L-arabinose (Ara4N) units located at the inner Kdo residue.[2] Ara4N also binds to the phosphate groups of lipid A and its presence has been suggested to account for diminished interaction with cationic agents and thus to be a major cause of antibiotic resistance.[3] Based on rational design, our synthetic endeavours aim at a series of plausible Ara4N transferase inhibitors capable of blocking the biosynthetic pathway leading to the formation of activated Ara4N species.[4] Specifically, we seek to inhibit the transfer of Ara4N residues both to *Burkholderia* lipopolysaccharide core and lipid A *in vitro*. Preliminary synthetic results towards *C*-glycosidic substrate and transition state analogues will be presented.



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SYNTHETIC STUDIES TO ACCESS CARBANUCLEOSIDES TARGETING ADENOSINE RECEPTORS

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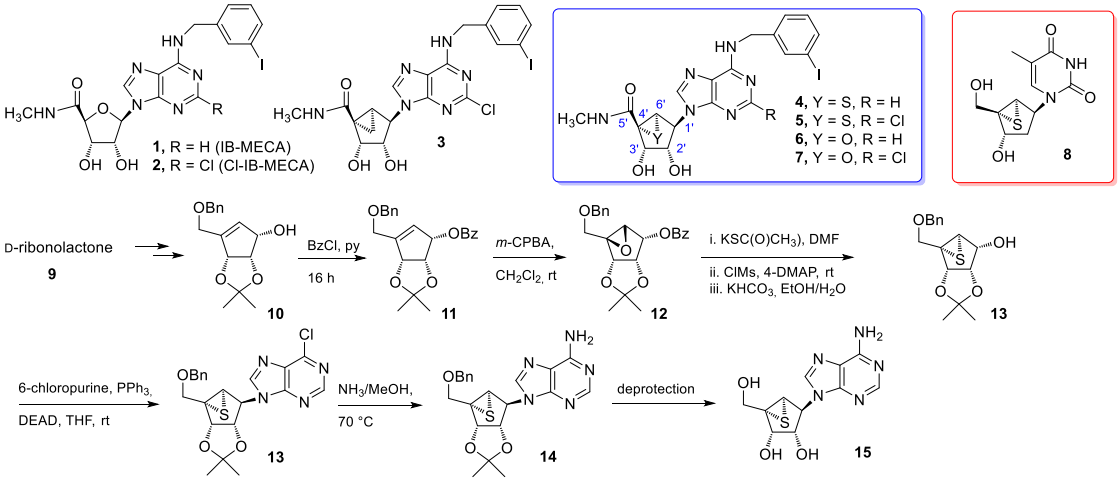
The aglycone of a specific nucleoside unit exists in a dynamic equilibrium between Northern geometry and the respective antipodal Southern conformation according to the pseudorotational cycle defined by Altona.[1] On the other hand, biological activity of nucleosides is toughly influenced by sugar conformation, which is modulated not only by the anomeric effect but also by the nature and the stereochemistry of the functional groups present in the sugar moiety affecting shaping and puckering of the ring. Hence, this conformation does control and facilitate reactions between nucleosides and specific enzymes involved in the sequence of activation leading to active metabolites triphosphates. However, the difficult of relating the favored sugar conformation in solution by an enzyme is its flexibility not found in the crystal state. For example, the crystal structure of dideoxycytidine is observed in the Southern geometry, whereas in solution is found in close to 80% in the Northern conformation. Nevertheless, cyclopentenyl nucleosides that are conformationally locked into either the *N*- or the *S*-geometry have been employed to study the preferred conformation for molecular recognition,[2] bearing in mind that a three-membered ring (cyclopropane, epoxide, thiirane) fused to a cyclopentane moiety, in an appropriate position, proved to be a suitable pseudosugar that fixes the conformation of a carbocyclic nucleoside in one of the extreme antipodes.

On the other hand, compounds **1** and **2** are potent A_3 adenosine receptors (AR) agonists, whereas conformationally rigid carbanucleoside **3** and other closely related drugs exhibited beneficial gain in binding affinity toward A_3AR.[3] Compounds **4**–**7** represents our target molecules based on the minor size of the group that fixes nucleoside conformation (thiirane or epoxide group versus a cyclopropyl group) would be favorable for molecular recognition. Our experience indicates that thiirane derivatives are stable compounds such as **8**.[4] The synthetic efforts aimed at obtaining **4**–**7** and simplified derivatives, particularly those having different oxidation state at C-5' will be discussed. The strategy in which we are working on is illustrated in the Scheme.



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SYNTHESIS OF POTENTIAL *T. CRUZI* GPI ANCHORS INHIBITORS

FOR TREATMENT OF CHAGAS' DISEASE

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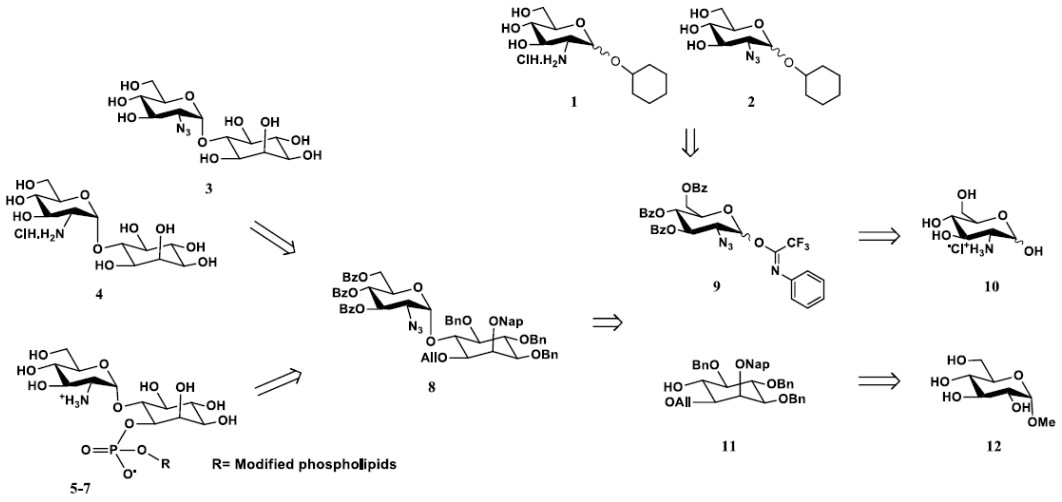
Trypanosoma cruzi is a protozoan parasite and causative agent of Chagas disease. It affetcs over 7 million people worldwide and is responsible for more than 10000 deaths every year. [1] Treatment for this illness is scarce and not well tollerated by many patients, requiring attention for the search and discovery of new and more selective drugs. GPI anchors are molecules that contain a conserved glycan core linked to a phosphatidylinositol moiety and a phosphoethanolamine residue which supports proteins on the cell membrane of all eukaryotes. In *T. cruzi*, GPIs are responsible for the ancorage of mucins and *trans*-sialidases that are important tools for invasion and survival of the parasite in host cells. [2] High density of GPIs along all protozoan parasites' life-cycle suggest that GPI biosynthetic pathway may contain interesting targets to be explored for the development of anti *T. cruzi* molecules. Our research group is interested in synthesis of potential *T. cruzi* GPI pathway inhibitors. Thus, a series of carobydrate-based molecules which may mimic GPI anchors parts were synthesised in parallel using orthogonal protection/ deprotection. According to the retrosynthesis, commercial D-glucosamine hydrochloride **10** [4] was converted to the glycosyl donor **9** in 4 steps. Moreover, methyl-α- D-glucopyranoside **12** was used to achieve the *myo*-inositol acceptor **11** in 10 steps. [5] *O*-glycosylation between **9** and **11** gave intermediate **8** in 65% yield, which was later subjected to overall deprotection (compounds **3-4)**. Products **5-7** were obtained after selective deprotection of *O*-allyl group of compound **8** and attachment of phosphate groups containing linear 8 or 11 carbon chains as modified lipids (compounds **5-7**). A study of *O*-glycosylation reactions was also performed between **9** and commercial cyclohexanol to give simplified compounds **1-2**, after deprotection of benzoyl groups, in order to assess the impact of the *myo*-inositol core in the the enzyme activity. All derivatives were obtained in good or satisfactory yields and will be subjected to assays in enzymes related to *T. cruzi* GPI anchor biosynthesis (Phosphatidylinositol - ceramide synthase, α-acetylglucosamine transferase, α-GlcNAc-de-N-acylase).



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MULTIVALENT α-GAL NEOGLYCOCONJUGATE FOR IMMUNOLOGICAL STUDIES IN CHAGAS DISEASE

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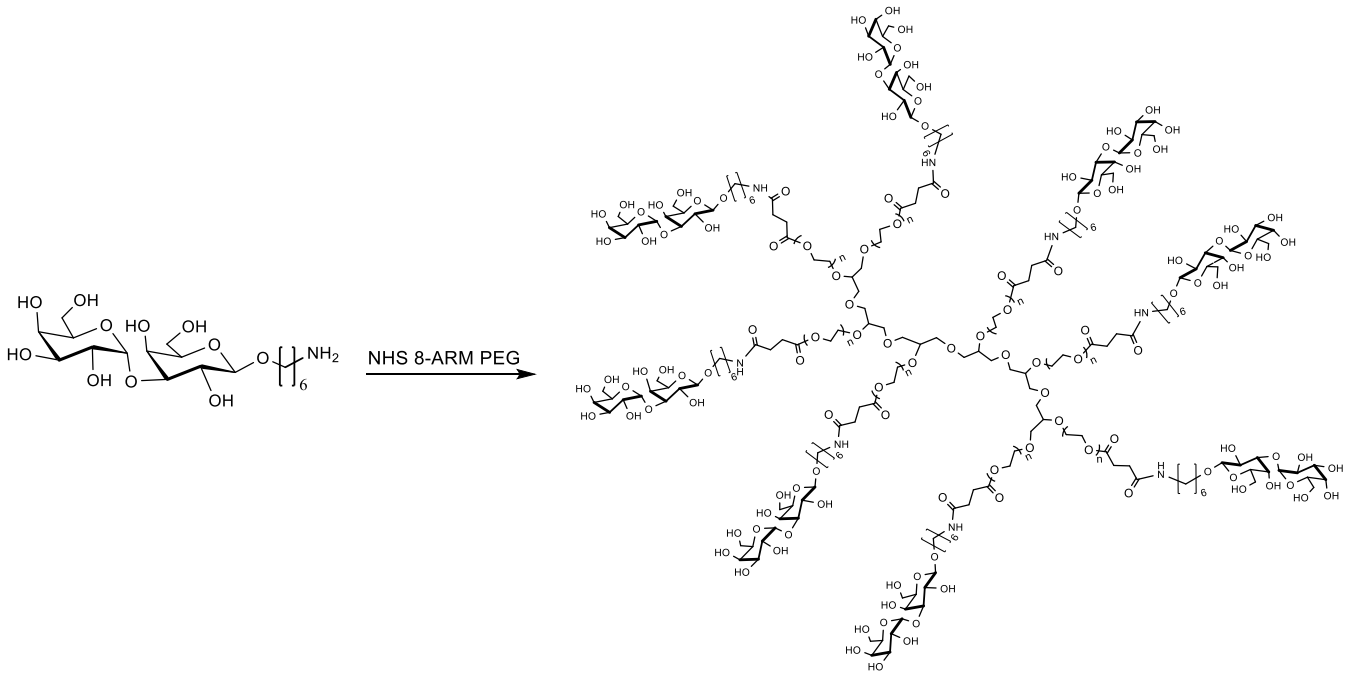
Synthetic oligosaccharides and their glycoconjugates are useful tools for the diagnostic of several diseases and for immunological studies. On the other hand, the introduction of multivalency by incorporation of an antigenic structure into a multiarm scaffold increases the density of the epitopes, similarly to the presentation that glycoconjugates often have in biological systems. This increment favors the binding specificity to serum antibodies.

Carbohydrate PEGylation using poly(ethylene glycol) (PEG) scaffolds have been used for many application in chemotherapy and drug delivery to prolong the *in vivo* circulation half-life, and to enhance the solubility and stability of conjugates.[1,2] We have previously proved that lactose analogs, inhibitors of trans-sialidase from *Trypanosoma cruzi*, linked to a multi ARM PEG improved their bioavailability retaining the inhibitory properties.[3]

Herein we describe a straightforward synthesis of disaccharide 6-aminohexyl α-D-Gal*p*(1→3)β-D-Gal*p,* which is the minimal antigenic unit present in trypomastigote mucins of chronic chagasic patients, and its conjugation via an amino coupling with *N*-hydroxysuccinimide activated octa-ARM PEG platform (NHS 8-ARM PEG, MW 40000). In this way an octavalent homogeneous PEG-derivative with high molecular weight containing 8 units of the synthetic carbohydrates antigen was afforded. The substitution of all the arms was confirmed by analysis of the NMR spectra. This multivalent neoglycoconjugate is a good candidate for immunological studies.



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AUTOMATED SYNTHESIS OF HEPARAN SULFATE: POTENTIAL THERAPEUTICS FOR ALZHEIMER'S DISEASE

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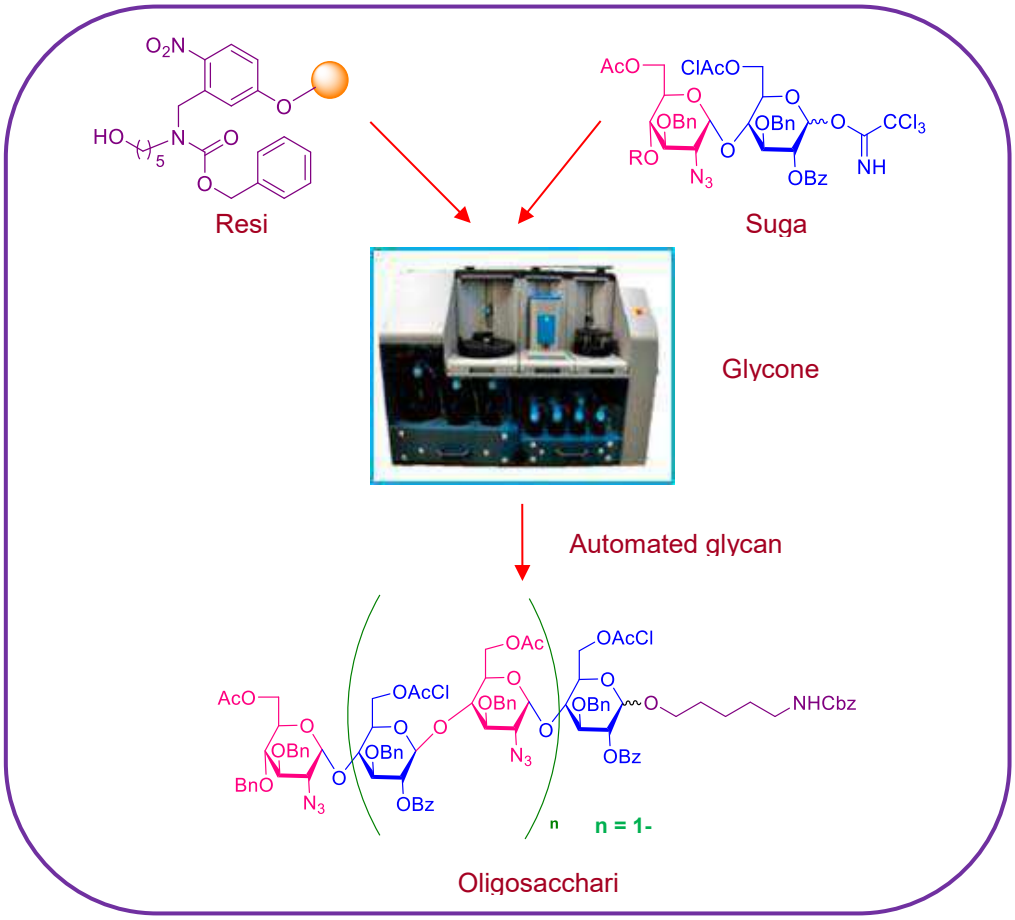
Heparan sulfates (HS) are a class of linear polysaccharides that function as dynamic biological regulators of the functions of diverse proteins. Through attachment to core proteins, it is present as a glycoprotein on mammalian cell surfaces and in the extracellular matrix. HS was identified as the first natural regulator of the cleavage of the amyloid precursor protein by β-secretase.[1] This cleavage is a critical step in the formation of amyloid plaques present in the brains of Alzheimer's patients. The ability to synthesize discrete HS oligomers of octasaccharide size and larger would offer potential therapeutics for Alzheimer's disease. [2] The state-of-the-art in automated oligosaccharide synthesiser, Glyconeer[3] allows solid phase synthesis of HS oligomers, which eliminates the need for purifying intermediates and simplifies the removal of excess reagents.



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EFFECTS OF THE PICOLOYL SUBSTITUENT IN CHEMICAL SIALYLATIONS

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Sialic acid refers to a family of carbohydrates frequently found in the terminal positions of biological glycoconjugates. The derivative of sialic acid found most commonly in mammals, N-acetylneuraminic acid (Neu5Ac), exists predominantly in alpha-glycosidic configurations. Chemical glycosylations with Neu5Ac donors tend to suffer from poor yields and stereoselectivities. It was discovered recently that the picoloyl substituent can have a dramatic influence on the outcome of chemical sialylations. In an effort to understand the effects of the picoloyl functionality on chemical sialylations further, several picoloylated Neu5Ac derivatives were synthesized for systematic comparison. Herein we describe the synthesis of these donors and the outcome of the resulting chemical glycosylations.

TOTAL SYNTHESIS OF LINCKOSIDES A AND B, THE REPRESENTATIVE STARFISH POLYHYDROXYSTEROID GLYCOSIDES

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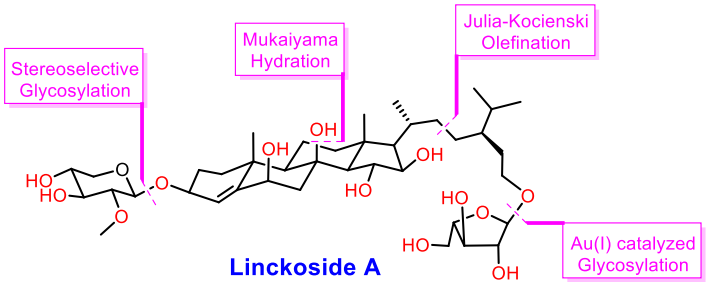
Linckosides A and B were identified from starfish *linckia laevigata* by Ojika *et al* in 2002. [1] They possessed promising neuritogenic activities and a synergistic effect with nerve growth factor. We reported the first synthetic approach to these two molecules, which features introduction of 8β-OH via Mukaiyama hydration, installation of the side chain by Julia-Kocienski olefination and Au(I) catalyzed β-selective glycosylation.[2]



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HYDROXYPROPYL CELLULOSE AS PREPARATION OF PROCESSABLE HIGHLY CONDUCTIVE POLYANILINE

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Preparation of highly conducting and processable polyaniline was carried out in the presence of anionic surfactant AOT (di-2-ethyl hexylsulfosuccinic acid sodium salt) together with hydroxypropylcellulose(HPC) as processable highly conductive polyaniline. The good environmental and electrical stability of PANI seems to be very promising conducting polymer. Several types of acid and dopants can be used to synthesize highly conductive PANI with conductivity as high as 300S/cm. These were achieved by so-called 2-steps method: first PANI in emeraldine salt (ES) form was synthesized and then dedoped into emeraldine base (EB) form; secondly the EB powder was redoped with dopant (CSA, DBSA and etc) and dissolved in m-cresol and cast into film.[1-3] This method is cost and time consuming as compared to our method, where the PANI is prepared in the presence of dopant(AOT) and then “in-situ” secondary doped by direct addition of m-cresol in the PANI solution.[4]

We are prompted to investigate the effect of secondary doping on the electrical conductivity of the resultant AOT-doped PANI-HPC composite synthesized through emulsion polymerization. Hydroxypropylcellulose was played an important role on purification and crystallization of polyaniline solid film to make AOT-doped PANI-HPC composite highly conductive. Conductivity of AOT-doped PANI-HPC composite film before and after secondary doping with m-cresol. The secondary doping induces polymer backbone conformation from folded coil-like to expanded coil-like and the highest conductivity obtained was over 500S/cm. The secondary doping induces polyaniline backbone conformation from “folded coil-like” to “expanded coil-like”. The more polaron presence along polymer backbone directly influences the efficiency of counterion-exchange interactions during secondary doping as implied by relation of conductivity to % loading of m-cresol.

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STRUCTURE-BASED DESIGN OF GALACTOPYRANOSIDE MALONYL ESTERS AS GALECTIN-8*N* INHIBITORS WITH SINGLE DIGIT µM AFFINITY

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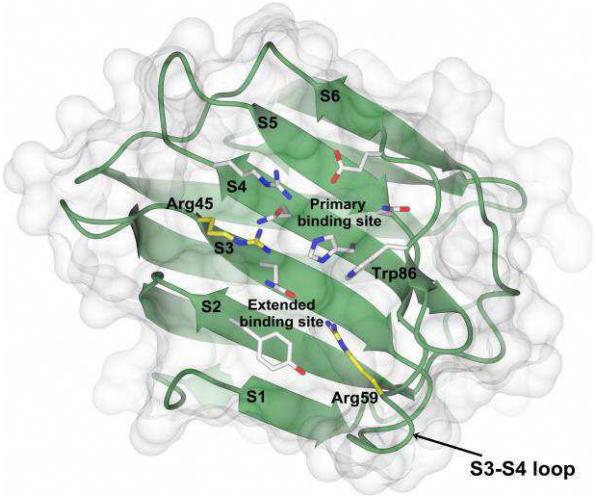

Galectin-8 is a β-galactoside recognising protein that contains two carbohydrate recognition domains (*N* and *C*-CRD) in tandem, linked by a variable length amino acid linker [1]. Galectin-8 plays an important role in rheumatic, autoimmune and inflammatory disorder. [2,3] Recently, it was found as a potential target for osteoporosis. [4] High binding affinity of galectin-8 with anionic oligosachharides is probably due to its *N*-terminal carbohydrate binding site. [5] The X-ray crystallography study explains that the high affinity with galectin-8*N* is due to presence of unique amino acid residue Arg59, which is present on unique S3-S4 loop. In light of such hypothesis, Phenylmalonyl methyl-β-D-galactopyranoside esters have been designed by targeting amino residues of the galectin-8*N* extended binding site (shown in figure). These designed ligands were synthesized and evaluated for *in vitro* binding affinity by isothermal titration calorimetry. This structure based drug design led to discovery of monosaccharide galactose based inhibitors with a single digit µM affinity with galectin-8*N*. Isothermal titration calorimetry and molecular modelling study suggest that phenyl malonyl moiety gives affinity enhancing effect to galactose based inhibitors.

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FROM GLYCALS TO 2-DEOXY-C-GLYCOSIDE ANTIMICROBIALS

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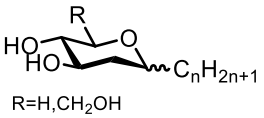
Sugar-based surfactants are a versatile class of compounds, due to their low toxicity and biodegradation [1], with the potential of interacting with membranes, leading to several biological and medicinal applications [1,2]. The self-organization properties of these molecules depends on the nature of the sugar and the hydrophobic tail length, which therefore determine their application [3].

Previous work in our group has proven that the biological activity of surfactant deoxy *O*-glycosides can be tuned by structural changes depending on glycone structure, in particular its deoxygenation pattern and anomeric configuration [2]. In this context, the synthesis of alkyl 2-deoxy-*C*-glycosides is presented for further structure/activity relationship studies. A simple and efficient methodology for their synthesis has been developed in our group, based on the transformation of 2-deoxy allyl *C*-glycosides via metathesis reaction. This reaction will be applied to 2-deoxy allyl *C*-glycosides prepared from commercially available as well as synthesized glycals, namely D-xylal, D-glucal and D-galactal. The general structure **A** of the target compounds is depicted in Fig. 1. The results obtained will be presented and discussed.



Structure A

Fig. 1 - General structure **A** of alkyl 2-deoxy-*C*-glycosides

Acknowledgements: Support of this work was provided by the European Union´s Seventh Framework Programme (FP7-PEOPLE-2013-IAPP, GA 612347). Fundação para a Ciência e a Tecnologia is also acknowledged for the support of the projects MULTI/UID 0612/2013.

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TOWARDS THE SYNTHESIS OF ALKYL 6-DEOXY C- AND S-FUCOSIDES TO EXPLORE THEIR ANTIMICROBIAL ACTIVITY

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Carbohydrate surfactants are characterized by their amphiphilic properties: a hydrophilic sugar moiety and a hydrophobic tail which facilitate their interaction with the lipid bilayer of cell membranes, therefore inducing biological activity [1]. There are numerous examples of carbohydrate-based surfactants acting as antibiotics, namely lauroyl and palmitoyl glycosides showing potent antimicrobial activity against several bacteria [2].

In our research group, the synthesis of long chain alkyl 2-deoxy and 2,6-dideoxy *O*-glycosides has been accomplished and their antimicrobial activity has been tested [3,4]. In this communication we present a new synthetic approach to alkyl *C*- and *S*-glycosides and their 2-deoxy counterparts (See Fig. 1).

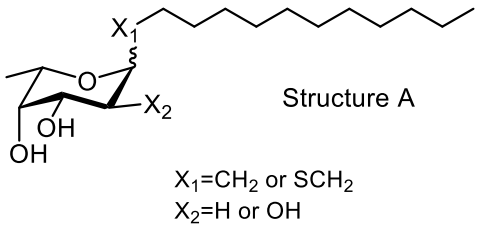

Fig. 1 - General structure **A** for the alkyl C- and S-fucosides

Acknowledgements: Support of this work was provided by the European Union´s Seventh Framework Programme (FP7-PEOPLE-2013-IAPP, GA 612347). Fundação para a Ciência e a Tecnologia is also acknowledged for the support of the projects MULTI/UID 0612/2013.

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SYNTHESIS OF POTENTIAL ANTIMICROBIALS BASED ON DODECYL C- AND S-L-RHAMNOSIDES AND THEIR 2-DEOXY ANALOGUES

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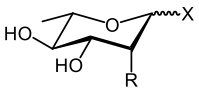
Bacterial resistance to the available antibiotics requires new and efficient problem-solving approaches. Sugar-based surfactants as biocompatible molecules with low levels of toxicity are indeed good candidates to be explored. In our group, we have developed new molecular entities belonging to this family of compounds and exhibiting some of them potent antimicrobial activity [1,2].

In this context we present the synthesis of new *C*- and *S*-rhamnosides linked to a dodecyl chain and their corresponding 2-deoxy L-arabinoside analogues, which general structure is depicted in Fig. 1. L-Rhamnose has been used as starting material for the preparation of both the *S*- and the *C*-glycosides, the latter synthesized via a metathesis reaction.



X=$C_{12}H_{25}$ or $SC_{12}H_{25}$
R=H or OH

Structure A

Fig. 1 - General structure **A** for the alkyl C- and S-glycosides

Acknowledgements: Support of this work was provided by the European Union´s Seventh Framework Programme (FP7-PEOPLE-2013-IAPP, GA 612347). Fundação para a Ciência e a Tecnologia is also acknowledged for the support of the projects MULTI/UID 0612/2013.

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SYNTHESIS OF C-GLYCOSYL POLYPHENOLS AND STD-NMR INTERACTION STUDIES WITH AN AMYLOID POLIPEPTIDE

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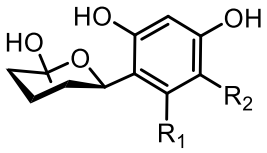
Polyphenols have shown numerous biological activities and health benefits for treatment of diabetes and Alzheimer´s including the capacity to inhibit the accumulation of β-amyloid fibrils [1-2]. Previous work in our group has disclosed a C-glucosyl flavonoid to interact with amyloid oligomers preventing aggregates formation [3].

In this work, we present the synthesis of new C-glycosy polyphenols in an attempt to enhance the bioactivity of previous reported leads [3] by modification of both the sugar moiety and the phenol residue. Scaffold **X** depicted in Fig. 1 has been designed as a common fragment and decorated with different saccharides. Additionally, different positions of the phenol fragment have been substituted (R1 and R2).

Finally, STD-NMR interactions studies of the C-glycosyl polyphenols with Islet amyloid polypeptide are presented.



Scaffold **X**

Fig. 1 – Scaffold **X** for the generation of C-glycosyl polyphenols

Acknowledgements: Support of this work was provided by the European Union´s Seventh Framework Programme (FP7-PEOPLE-2013-IAPP, GA 612347). Fundação para a Ciência e a Tecnologia is also acknowledged for the support of the projects MULTI/UID 0612/2013.

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Building Small Molecule Carbohydrates at a Public PUI

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Much of the work in the carbohydrate synthesis field centers on building large, complex saccharides or glycoconjugates for a multitude of purposes. Some of these projects can take up the entirety of a graduate student's career and make use of the instrumentation and high quality facilities available at large institutions. On the other hand, students at primarily undergraduate institutions (PUI's) rarely have access to these types of resources. Smaller sugar-containing structures, such as benzophenone glycosides and some resins, are therefore well within the reach of undergraduates, even at schools with little instrumentation and high student turnover. Presented here is the work accomplished in under a year by several undergraduate students at the University of Minnesota, Morris. UMM is a small public liberal arts college in central Minnesota serving undergraduates from a diverse set of backgrounds including first generation students and underrepresented minorities. The work accomplished in this time frame indicates the potential for viable and useful research at small public liberal arts colleges.

Figure 1: Examples of benzophenone glycosides contatinig both C- and O- glycosidic bonds.

Small molecule compounds can have impressive activity despite their size[1]. Synthesis of these compounds may be completed in relatively good yield in several steps using common carbohydrate methods. So far, sophomore-level students in second-semester organic chemistry lab have made natural-product-like *O*-benzophenone glycosides (blue lines, Figure 1) of mannose and rhamnose using commercially available benzophenones, with glucose and galactose analogues forthcoming. Other senior students have synthesized non-natural benzophenones via Grignard coupling reactions followed by an oxidation and will be able to couple sugars to the novel benzophenones soon. Work on the *C*-glycosides (red lines, Figure 1)) is forthcoming within the next year. Small libraries of these compounds will be tested for their bioactivity.

Rhamnose-containing resins like merremin E have long alkyl chains rather than aromatic cores that modulate their solubility and their uptake by cells. Normally, rhamnose-containing oligosaccharides are obtained from marine sources[2] and most follow a certain connectivity pattern. The bonds in antibiotic-resistance reversing merremin E and the other resins isolated in the past few years have a different and as yet unpublished connectivity. Furthermore, merremin E and related resins have a rare D-fucose moiety that provides an interesting challenge to undergraduate workers. The majority of the pieces en route the 3+2 synthesis of the merremin E resin, including the three rhamnose building blocks and the alkyl chain, have been have been completed in the space of one year by a single undergraduate researcher.

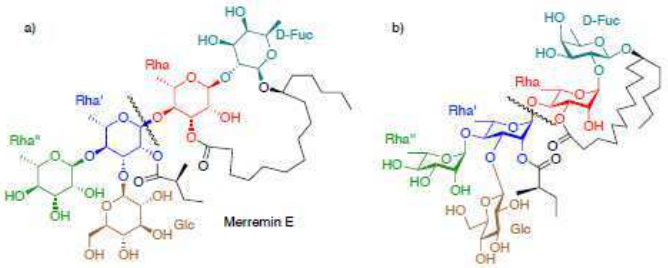

Figure 2: Structure of the merremin E resin. Colors indicate differing building block structures.

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SYNTHESIS OF NOVEL ARABINASE FRET PROBES

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Tuberculosis is a serious disease caused by the bacterium *Mycobacterium tuberculosis.* This bacterium displays a unique pathology, being resistant to chemotherapeutic treatment, and being able to modulate the host's immune response. These characteristics have been attributed, in part, to several arabinofuranose (Ara*f*)-containing glycans located in the cell wall, namely arabinogalactan (AG) and lipoarabinomannan (LAM). Crucial to cell wall growth and repair in *M. tuberculosis*[1] are glycosidases that cleave glycosidic bonds in complex cell wall sugars. Comparatively little is known about glycosidases present in *M. tuberculosis*. In particular arabinases, enzymes able to cleave arabinose chains such as those found in AG and LAM are very poorly studied. A major limiting factor in studying arabinases is the lack of suitable assays with which to measure enzymatic activity and specificity[2].

This project focuses on synthesizing several AG fragments that are coupled at their reducing and nonreducing termini to a chromophore and a quencher molecule, respectively, to form a FRET pair (Figure 1). Enzymatic hydrolysis of the molecule will spatially separate the chromophore from the quencher molecule effectively "switching it on"[3]. This concept will allow for immediate detection of arabinase activity, as well as giving insight into substrate specificity. Five targets were selected for synthesis, which are major structural units found in AG: α-(1→5) linked chains of varying lengths, a branched α-(1→3) fragment, and the β-(1→2) fragment known commonly as Ara_6. Total synthesis of these targets and their use in a novel arabinase assay will be presented.

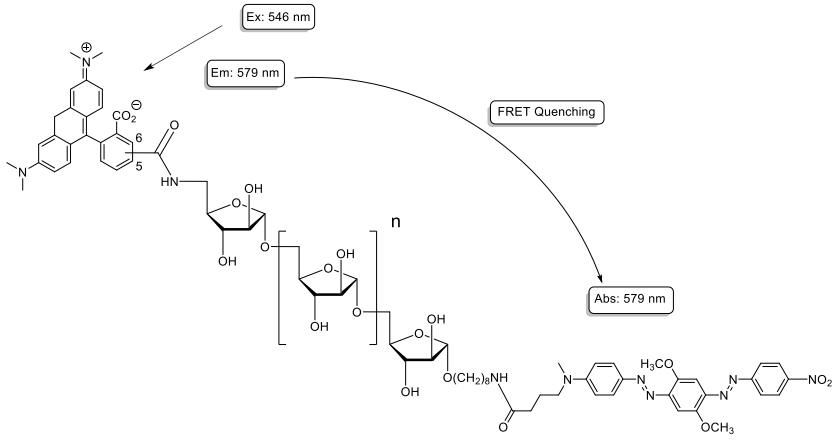

Figure 1. Design of arabinase FRET probe.

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POSTERS | A – Analysis

CARBOHYDRATE MICROARRAYS CONTAINING GLYCOSYLATED FLUORESCENT PROBES ARE POWERFUL TO DETECT GLYCOSIDASE ACTIVITIES

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Cellular glycans are produced mainly in the form of glycoconjugates by the action of glycosyltransferases and glycosidases. It is known that genetic deficiencies of lysosomal glycosidases cause lysosomal storage disorders. Moreover, inhibitors of glycosidases have been developed as therapeutic agents to treat viral infections, diabetes and cancer. Because of biological and pathological significance of glycosidases, it is of great importance to develop new methods to rapidly assess their catalytic activities. In this study, we prepared carbohydrate microarrays containing glycosylated fluorescent probes by immobilizing five glycosylated near-infrared (NIR) probes appended by the hydrazide group onto epoxy modified glass slides. When the microarrays were incubated with glycosidases, the fluorescence intensity was increased by removal of the sugar moiety from glycosylated near-infrared (NIR) probes, the event which can be measured by using microarray scanner. In addition, the microarrays were also successfully applied to determination of IC50 values of inhibitors of glycosidases. We demonstrated the usefulness of the present carbohydrate microarrays for profiling glycosidase activities and measurements of IC50 values of inhibitors of glycosidases.

UNUSUAL CONFORMATIONAL EQUILIBRIA IN A COMPLEX, SALICIN-TYPE GLYCOCONJUGATE

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Glycoconjugates are structurally complex molecules and are involved in a number of important biological processes, such as cell–cell interactions, cell–matrix interactions, detoxification, etc. Because of the structural complexity, one can expect that glycoconjugates exhibit a multifaceted conformational equilibrium when dissolved in various solvents.
The investigated glycoconjugate is constituted by two glucopyranoses (Glc) linked via quinazolinone-like structure. The three-dimensional structure of glycocojugate has been studied in dimethylsulfoxide (DMSO) and water by means of high-resolution NMR and DFT calculations. High-resolution ^{1}H, ^{13}C and two-dimensional NMR data showed that two stable forms are present in solution at room temperature. These two forms differ in geometry at the glycosidic linkages connecting the aromatic rings and the glucose residues. DFT calculations, at the ωB97XD/6-311++G(2d, 2p) level of theory and the smd model to account for solvents (DMSO, water), enabled detailed molecular geometry description of both forms. 2D NMR NOESY spectra disclosed dipolar interactions among protons on the aromatic rings and OH protons at the glucose residues that are in agreement with the theoretical model obtained by DFT results.

A REVISION ON THE NUMBER AND STRUCTURES OF LIPOCHITIN OLIGOSACCHARIDES NODULATIONS SIGNALS FROM *RHIZOBIUM TROPICI* CIAT 899

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[b] Department of Microbiology, Faculty of Biology, University of Seville
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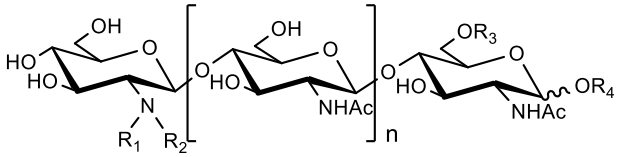
Rhizobia are nitrogen-fixing bacteria that are able to invade the roots of leguminous plants and trigger the formation of the nodule that contains the nitrogen-fixing microsymbiont. During the symbiotic process, the host plant roots secrete flavonoids that induce the rhizobial nodulation genes (*nod, nol* and *noe* genes). These genes have been shown to be involved in the synthesis and secretion of bacterial nodulation signals called Nod factors or lipo-chitin oligosaccharides (LCOs). The nodulation factors consist of a backbone of three to five GlcNAc residues, bearing an amide-bound fatty acyl residue on the non-reducing terminal GlcN residue. This basic structure has structural variations that determine the host-specificity. We have described the structures of the LCOs produced by *Rhizobium tropici* CIAT899 [1]. In this paper we identified 16 LCOs having the following structures:



R_1= Fatty Acid R_3 = S / H
R_2 = Me / H R_4 = Hex / H

Fig. 1. General structure of the LCOs isolated from *R. tropici* CIAT899.

In this communication, we report on the identification of the LCOs from *R. tropici* CIAT899 using the new facilities of the Mass Spectrometry Service of our University. In particular, a Thermo Scientific liquid chromatography system consisting of a quaternary UHPLC Dionex Ultimate 3000 SD connected to a quadrupole-orbitrap Q-exactive hybrid mass spectrometer (ThermoFisher Scientific) with a HESI ionization probe.

We have identified 61 nodulations factors thanks to the higher sensibility of the mass spectrometer. Moreover, the possibility to determine the exact mass of pseudomolecular and fragment ions allowed us to resolve certain ambiguities in the assignation of the structures (such as V($C_{20:1}$) / V($C_{18:2}$-OH, NMe)). Some of the structures of the LCOs correspond to the previously described but, in addition, we have identified new structures bearing substituents such as $C_{18:2}$, or $C_{20:3}$, as well as some LCOs with three glucosamine residues.

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STRUCTURAL AND GENETIC STUDIES ON THE O-ANTIGENS OF *ESCHERICHIA ALBERTII*, A RECENTLY RECOGNIZED CLOSE RELATIVE OF *ESCHERICHIA COLI*

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Escherichia albertii is a new emerging diarrhoeagenic pathogen of humans and birds, which caused sporadic infections and outbreaks in different countries. It was first isolated from five Bangladeshi children with diarrhea and initially identified as *Hafnia alvei*. In 2003, it was re-classfied as *E. albertii*, a new enteric bacterial species within the genus *Escherichia,* which also includes *Escherichia coli* and *Escherichia fergusonii*. Recently, a substantial proportion of strains earlier identified as enteropathogenic and enterohemorrhagic *E. coli* have been shown to be *E. albertii*. In this work, we established O-polysaccharide structures and characterized O-antigen gene clusters of representative strains of all seven *E. albertii* molecular types (and potential O-serotypes) that have been recognized recently (H. Wang et al., *Front. Microbiol.* 2017, 8, 1857).
The O-polysaccharides of *E. albertii* O1-O7 were studied by sugar analysis, selective solvolysis with CF_3SO_3H (for O1) or CF_3CO_2H (for O4 and O5), Smith degradation (for O2), and one- and two-dimensional ^1H and ^{13}C NMR spectroscopy. As a result, the following new O-polysaccharide structures of *E. albertii* O1, O2, O4, and O5 were elucidated:

O1 →4)-β-D-Man*p*NAc3NAcA-(1→4)-β-D-Glc*p*N(MeC=NH)3NAcA-(1→3)-α-D-Glc*p*NAc-(1→

O2 →3)-β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→3)-α-L-Fuc*p*-(1→3)-β-D-Glc*p*NAc-(1→
4 4
↑ |
1 ~70% *OAc*
~70% α-L-*Fucp*

O4 →2)-α-Rha*p*-(1→2)-α-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→

O5 →3)-α-D-Glc*p*NAc-(1→4)-β-D-Gal*p*6Ac-(1→6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*NAc-(1→

The O-polysaccharide structures of *E. albertii* O3 and O6 were found to be identical to those of *E. coli* O181 and O3, respectively, except for the lack of O-acetylation in *E. albertii* O3. The structure of the O-polysaccharide of *E. albertii* O7 (named that time *H. alvei*) has been established earlier (R. Eserstam et al., *Eur. J. Biochem*. 269 (2002) 3289-3295). The linear part of the O7 repeating unit is identical to that of *E. coli* O124 and O164 (R. Stenutz et al., *FEMS Microbiol. Rev.* 30 (2006) 382-402).
Functions of genes in the O-antigen gene clusters of the strains studied were tentatively assigned by comparison with sequences in available databases and were consistent with the O-polysaccharide structures established.

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DIRECT EXPERIMENTAL CHARACTERIZATION OF GLYCOSYL CATIONS BY SPECTROSCOPY

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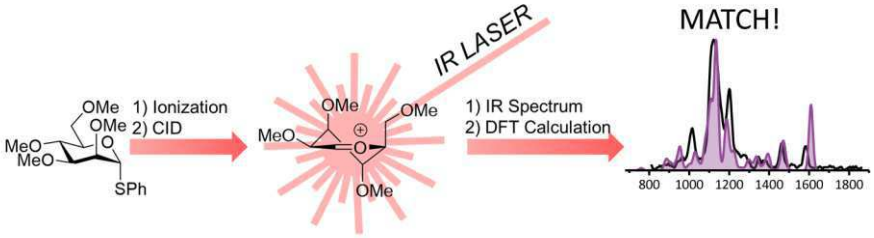

Glycosyl cations are crucial intermediates formed during enzymatic and chemical glycosylation. The intrinsic high reactivity and short lifetime of these reaction intermediates make them very challenging to characterize using spectroscopic techniques. Herein, we report the use of collision induced dissociation tandem mass spectrometry to generate glycosyl cations in the gas phase followed by infrared ion spectroscopy using the FELIX infrared free electron laser. The experimentally observed IR spectra were compared to DFT calculated spectra enabling the detailed structural elucidation of elusive glycosyl oxocarbenium and dioxolenium ions [1].

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CHARACTERIZATION OF BACTERIAL EXOPOLYSACCHARIDES PRODUCED BY *LACTOBACILLUS FERMENTUM*2

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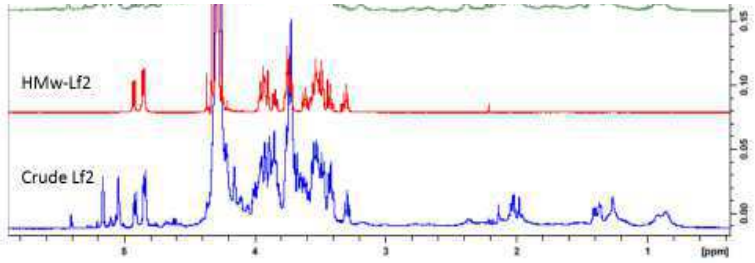
Lactic acid bacteria (LAB) are believed to be probiotic organisms which, when consumed, provide potential health benefits. They generate exopolysaccharides (EPS) which are widely used in the dairy industry especially for their thickening properties[1]. Researchers have also suggested that EPS, produced by LAB, show antitumor and cholesterol lowering activities[2]. They may act as protective agents against pathogens[3] and also improve intestinal barrier function[4]. Our work is focused on characterizing the EPS produced by *Lactobacillus fermentum* 2 (Lf2- isolated as a non-starter culture in Cremoso cheese, producing a high yield of EPS (~2 g/L[5]). NMR spectroscopy, size exclusion chromatography, HPAEC-PAD analysis, monomer and linkage analysis employing GCMS have been used to analyse the EPS structure.

NMR analysis of the crude EPS (Fig. 1-bottom spectrum) shows the presence of more than one polysaccharide. Preparative size exclusion chromatography (S-500 HR) separated two different polysaccharides; high molecular weight (HMw – Fig. 1-middle spectrum) and medium molecular weight (MMw- Fig. 1-top spectrum). For MMw EPS, monomer analysis suggests variable amounts of glucose, galactose and small amounts of amino sugars. Linkage analysis indicated that a complex mixture of differently linked sugars are present including: a terminal hexose, a 1,2-linked hexose, a 1,3-linked hexose, a 1,4-linked hexose and a 1,6-linked hexose. The biological activity of the EPS is currently under investigation and our preliminary results will be discussed.

Fig. 1. Overlaid spectra of Crude, HMw and MMw EPS from Lf2



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APPLICATIONS OF GALACTOSE OXIDASE FOR THE CHEMOENZYMATIC LABELLING OF GLYCOPROTEINS

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Galactose oxidase (GOase) is a radical copper-dependent enzyme that catalyzes the oxidation of the C6-OH of Galactose with strict regioselectivity. GOase has been widely used in biotechnology, its applications span from chemical synthesis to whole cell labelling (1,2). Here we describe the development of GOase mutants with extended substrate range for the oxidation of monosaccharides and glycoconjugates beyond galactosides. Oxidation of the constituent terminal monosaccharides of selected glycoproteins was first examined via a high throughput liquid phase screening method. This was also supported by NMR and high resolution mass spectrometry data. Using bio-orthogonal labelling techniques of the aldehyde generated in glycoproteins, we are able to selectively biotinylate glycoproteins (1). We demonstrate that GOase mutants enable a biorthogonal labelling strategy for glycoconjugates bearing a range of terminal glycosides.

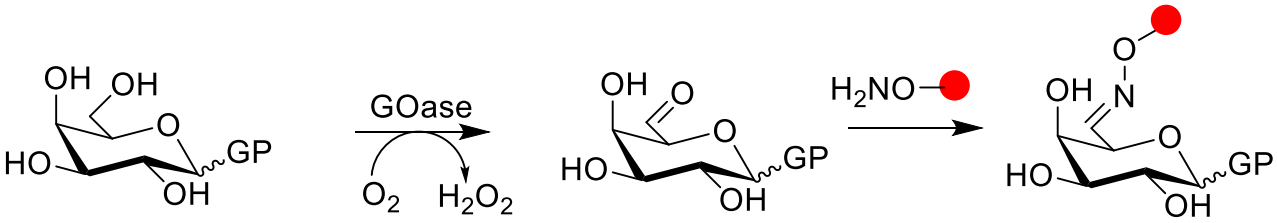

Fig. 1. Approach used by Rannes et al. for the chemoenzymatic labelling of glycoproteins with terminal Galactose residues (1).

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THE INTERACTION BETWEEN MANNOSE AND DC-SIGN. A NEW BINDING MODE DETECTED BY ^{19}F-NMR EXPERIMENTS AND MD SIMULATIONS

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Dendritic cell-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) is a transmembrane tetrameric calcium-dependent lectin, expressed by macrophages and dendritic cells, which specifically recognizes highly-glycosylated patterns at the surface of pathogens and host glycoproteins [1]. In particular, DC-SIGN plays a key role in the dissemination and survival of viral infections, including the human immunodeficiency virus (HIV) [2]. DC-SIGN binds HIV with high affinity through a glycoprotein present on the envelope of the virus, gp120, which displays abundant N-linked high mannose oligosaccharides.

To gain insight into the features of the recognition process, in this work we describe a strategy that is both useful as a general screening method to detect weak binders and to infer what positions of the ligand are key to the binding process. Using a set of monofluorinated monosaccharides, in which every hydroxyl group is substituted one by one by fluorine, we are able to trace the crucial interactions between DC-SIGN and the interacting ligand molecules from a mixture of 26 species all at once, by applying a ^{19}F transverse relaxation rate (R_2) approach [3,4]. Additionally, molecular dynamics simulations (MD) have been conducted to extend the understanding of the recognition process in solution and support the experimental results.

The combined analysis of NMR and computational data indicates the existence of a new binding mode for mannose at DC-SIGN's binding site, which has not been previously described by any other technique until date.

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SIALOLOME EXAMINATION OF DIFFERENT CELL TYPES BY A COMBINATION OF LASERMICRODISSECTION AND LC-MS

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Sialic acids are a family of 9-carbon α-keto acids forming the terminal building blocks of numerous glycoproteins and glycolipids in vertebrates. Structural diversity of these C9 sugars arises by modification with different functional chemical groups like acetyl- or sulfate-residues. Usually, sialic acids are present as monomers but they can also be linked to each other by members of the α2,8-sialyltransferase (ST8Sia) gene family to form sialic acid di-, tri-, oligo- and polymers. Due to their exposed position sialic acids are involved in many important biological processes like opsonization-processes by the immunesystem or regulation of differentiation. Intriguingly, pathological events, e.g. development of cancer, come often along with a rearrangement of the sialolome. By analyzing whole organs or tissues, these changes in the sialylation pattern can easily be missed, since a mixture of different cell types is analyzed. The variations within a cell type of interest, a defined cell population and/or functional subunits can now be detected by a powerful combination of laser microdissection together with HPLC-FD and/or HPLC-ESI-MS approaches. Selected examples using the outlined strategy will be presented for distinct biological questions.

DETECTION OF CARBOHYDRATE-CARBOHYDRATE INTERACTION USING SUGAR-CONJUGATED MAGNETIC BEADS

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Cell adhesion among mouse embryonic cells is supposed to due to their weak interaction between the cell-surface carbohydrates.[1] Since detection of the weak interaction events are technically challenging, simple assay system for the carbohydrate-carbohydrate interaction will be necessary for understanding significant glycan-matching. We have reported magnetic beads-assisted mild enrichment procedure for weak-binding lectins.[2] Based on this system, herein we proposed novel detection procedure for the carbohydrate-carbohydrate interaction using sugar-conjugated magnetic beads.

To evaluate our procedure, we selected to use galactose as a conjugated glycan, because galactose is relatively well present at the non-reducing end of the cell-surface glycolipids. Before analysis of the carbohydrate-carbohydrate interaction, we synthesized galactose-conjugated Dynabeads as well as glycine-conjugated beads for negative control. Interaction analysis of the galactose-conjugated beads with target *p*NP-□-D-glucopyranosides was carried out in the system as shown in Fig. 1. After sedimentation of the galactose-beads, concentration of the residual *p*NP-□-D-glucopyranosides around the surface of the mixture was analyzed by treating with □-glucosidase, resulted in significant decrease of the concentration. The resulting data indicated that galactose on the beds will interact with *p*NP-□-D-glucopyranoside. In this study, comprehensive analysis of various carbohydrate-carbohydrate interactions will be also presented.

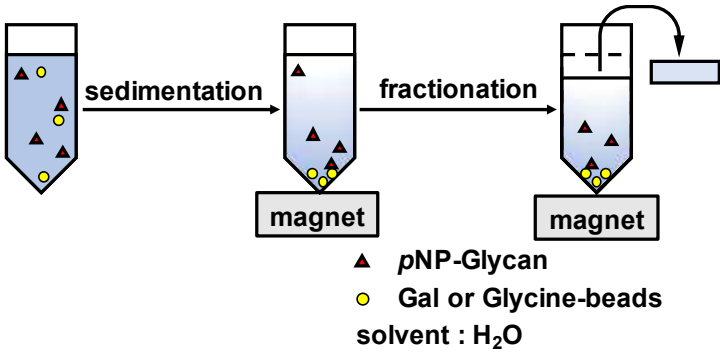

Fig. 1 Detection system

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PEPTIDE SPECIFICITY ANALYSIS OF PEPTIDE: N-GLYCANASES USING SYNTHETIC CHITOBIOSE-PENTAPEPTIDES

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A series of peptide: N-glycase (PNGase) is a deglycosylating enzyme for N-glycoprotein. Although glycan specificity of the enzymes have been reported, their peptide specificities are still unclear. In this study, we analyzed peptide specificity of several PNGases by using synthetic chitobiose-pentapeptides as substrates.

We selected to use three types of PNGase, namely bacterial PNGase F, plant PNGase A, and yeast cytoplasmic PNGase. We carried out peptide specificity analysis of the PNGases by using synthetic substrates having systematic series of amino acid sequences composed of hydrophobic leucine and hydrophilic serine (Figure 1). The reactions were analyzed by ultra-performance liquid chromatography combined with electrospray ionization mass spectrometry. We found that all three types of PNGases had higher activity for the hydrophobic synthetic chitobiose-pentapeptide substrates. However, precise specificities of the PNGases were diverse. Yeast cytoplasmic PNGase showed similar activity for all chitobiose-pentapeptide substrates. In contrast, plant PNGase A showed moderate specificity for the substrates. PNGase F showed more tight specificity for the substrates. These results indicate PNGases from different origins have significantly independent peptide specificities for the synthetic chitobiose-pentapeptide substrates (Figure 2)[1]. Although this study demonstrated for the limited peptide sequences, the broad specificity of yeast cytoplasmic PNGase suggest the PNGase can permit various sequence of glycopeptides that will contribute glycoprotein degradation process.

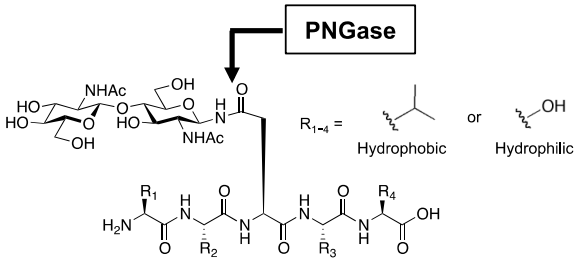

Fig. 1. Synthetic substrates used in this study.

Fig. 2. Peptide specificies of PNGases.

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GLYCAN STANDARDS AS KEY TOOLS FOR ROBUST AND RELIABLE ANALYSIS OF GLYCOPROTEINS

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For most therapeutic glycoproteins the glycosylation patterns greatly influence clinical performance of the drug product, particularly its *in vivo* safety and efficacy profile.[1] In biological tissues glycosylation patterns can also correlate with the state of health or disease of the individual.[2] Given this, there is increasing interest in accurately characterizing glycosylation, for example monitoring glycosylation patterns of biopharmaceutical therapeutics throughout the product lifecycle as well as in glycan biomarker discovery for medical diagnostics.

Robust analytical strategies are required to meet the challenge of accurately and reliably characterizing glycosylation. A key component in a well-designed analytical strategy is the inclusion of glycan or glycopeptide standards. This can improve glycan characterisation, allow quantitation, and provide vital information on analytical performance. The standards can include system suitability standards, process standards, reference standards, and/or quantitative standards. This poster focuses on the uses of well-characterized glycan and glycopeptide standards to support analysis of glycosylation patterns. We will demonstrate which standards can be used for best practice during the analysis of sialic acids, monosaccharides, N-glycans and/or O-glycans.

Finally, we will highlight the use of quantitative glycan standards to both determine the absolute amount of an analyte in a sample and also to quantify the efficiency of a process. As a case study, we will show how quantitative sialyl glycopeptide and glycan Man-8 standards can be implemented as positive process controls to ensure reliable data is obtained in sialic acid analysis and monosaccharide analysis.

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EFFECTIVE MASS SPECTROMETRY-BASED METHODS FOR LARGE-SCALE ANALYSIS OF PROTEIN GLYCOSYLATION

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Glycosylation is one of the most common and essential protein modifications in cells. It often determines protein folding, trafficking and stability, and regulates many cellular events, especially cell-cell communication, cell-matrix interactions, and cellular response to environmental cues[1]. Glycoproteins contain a wealth of information related to cellular developmental and diseased statuses[2,3], and aberrant protein glycosylation is directly related to human disease, including cancer and infectious diseases[4]. Global analysis of protein glycosylation is critical in understanding glycoprotein functions and identifying glycoproteins as biomarkers and drug targets. However, due to the low abundance of many glycoproteins and heterogeneity of glycans, it is extraordinarily challenging to comprehensively analyze glycoproteins in complex biological samples.

Currently mass spectrometry (MS)-based proteomics provides a unique opportunity to globally analyze protein glycosylation. However, effective enrichment is imperative prior to MS analysis. Previously, boronic acid was demonstrated to have great potential in universally enriching glycopeptides for the global analysis of protein glycosylation because of its reversible covalent interactions with glycans[5]. However, the method suffers from relatively weak interactions; therefore, low-abundance glycoproteins are not effectively enriched. In this work, we develop a new method to more effectively enrich glycopeptides, especially those of low-abundance, by greatly enhancing the interactions between boronic acid and glycopeptides[6]. First, different boronic acid derivatives are tested, and benzoboroxole is found to be highly effective to enrich glycopeptides due to dramatically strengthened interactions. Second, based on the common features of a glycan containing multiple monosaccharides and one sugar bearing several hydroxyl groups, benzoboroxole conjugated dendrimer beads can synergistically interact with glycopeptides. The experimental results demonstrate that conjugating benzoboroxole to a dendrimer significantly increases the enrichment efficiency, even for glycopeptides only containing O-GlcNAc (N-acetyl glucosamine).

The method is applied for the global analysis of glycoproteins in yeast (*S. cerevisiae*), mouse brain tissue, and human cells (MCF7, HEK 293T and Jurkat). The reversible nature of the interactions allows us to analyze intact O-glycopeptides with glycan structure information. These results demonstrate that the new method is universal and highly effective in enriching glycopeptides, especially from low-abundance glycoproteins that are normally of greater biological importance. The current results also provide valuable information regarding glycoproteins in yeast and human cells to biological and biomedical research communities. Without sample restrictions, the current method can be applied to many other samples for glycoprotein analysis.

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GLYCOSYLATION CHANGES IN CANCER DETERMINED BY LECTIN-BASED PROTEIN MICROARRAY

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Determination of glycosylation status of glycoconjugates, mainly glycoproteins, may reveal the trend in changes of glycan composition which occur due to disease, aging, life style or other reason. Many studies suggested that altered glycosylation of proteins is one of the cancer-related markers and information on glycosylation status can significantly increase the informative value of glycoprotein biomarkers [1,2]. In this study, we focused to the screening of the differences in glycan composition in serum samples from patients with various type of cancer (mainly colorectal cancer, breast cancer and ovarian cancer) before their medical treatment and 3 months after. The serum samples were first depleted to remove abundant proteins, as albumin and IgG. For the glycoprofiling we have developed the lectin-based protein microarray platform enabling high-throughput glycan – lectin recognition [3]. The samples were spotted into arrays on microarray slide and incubated with a panel of 16 chosen biotinylated lectins. The detection was performed after incubation with fluorescent conjugate of streptavidin using microarray scanner. The same approach was used for the measurement of samples from control group of healthy individuals. Determined differences in signal intensities showed statistically significant changes in glycosylation for the patients' samples. The most evident differences were observed for the samples from patients with ovarian cancer. In this case, the samples taken before medical treatment showed increased sialylation (interactions with SNA and WGA), fucosylation (PhoSL, AAL) and mannosylation (ConA), as well increased reactivity with RCA, in comparison with samples taken after medical treatment. For the samples from group of healthy controls were not observed any differences. The used method is suitable for high-throughput screening analysis of differences in glycosylation pattern in various types of samples containing glycoproteins.

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A LEWIS ACID BASE-MEDIATED GLYCOSYLATION REACTION

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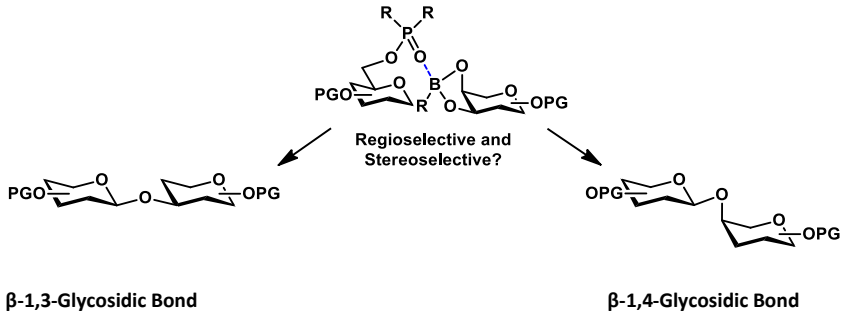
Oligosaccharides are involved in numerous medical disorders and have applications in drugs and vaccines.[1] In nature, enzymes stereoselectively link unprotected monosaccharides together. In the lab, however, in the absence of enzymes, glycosylations proceed with little or no regio- or stereocontrol as each monosaccharide bears multiple reactive hydroxyl (OH) groups. A major challenge is enabling a reaction at a single hydroxyl group (regioselectivity) and controlling which epimer is formed at the anomeric position (stereoselectivity).

To address this challenge, we hypothesised that a substrate bearing a Lewis base could guide a monosaccharide-linked boronic ester and thus undergo a stereo- and regiospecific reaction, forming a disaccharide. The Lewis basic group co-ordinates with the boronic ester to facilitate the delivery of the acceptor to the face of the glycosyl donor proximal to the Lewis basic group. This is a substrate-controlled reaction and the aim of this project is to control the regio- and stereo-selectivity of a new glycosidic bond by using a Lewis base, a phosphine oxide, attached to a glycosyl donor, which will co-ordinate to and activate a boronate ester derived acceptor (see below). Glycosyl donors bearing Lewis basic groups such as pyridine N-oxide or diphenylphosphine oxide on carbon-6 have been synthesised. Monosaccharide acceptors, containing a Lewis acidic boronic ester are reacted with the donors to form new disaccharides. The lewis base should bind to the Lewis acid boronic ester and make the alkoxide substituents more nucleophilic.[2][3] Based on Taylor's work,[2] we hypothesised that the equatorial alkoxide group would react selectively.



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CONFORMATIONS OF *SHIGELLA FLEXNERI* 7A AND 7B O-SPECIFIC POLYSACCHARIDE

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Approximately one in ten deaths of children under five are due to diarrhoeal or dysentery related diseases. Several pathogens are responsible for almost all of these illnesses, however infection by the Shigella bacterium is the most common. Annually, shigellosis causes over half a million deaths globally and it remains endemic in south Asia and sub-Saharan Africa. There are over 40 different serotypes of *Shigella* based on the organism's O-specific polysaccharide (OPS), although a small number are responsible for nearly all such disease.[1] The polysaccharide capsule is the main virulence factor and the target of conjugate shigella vaccines currently in development.

Similar polysaccharide antigen structures may elicit similar immune responses and thus provide cross protection against disease caused by related strains. Previous studies have shown that conformational analyses of oligosaccharide fragments for bacterial polysaccharide repeating units can provide insight into vaccine cross protection between serotypes.[2] We are currently undertaking a systematic and incremental computational approach to explore the OPS repeating unit conformations of *Shigella flexneri* serotypes 7a and 7b (Figure 1). The repeating unit of both serotypes consist of a tetrasaccharide backbone [→2)-α-L-Rha*p*^{III}-(1→2)-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→3)-β-D-Glc*p*NAc(1→] with α-D-Glc*p*-(1→2)-α-D-Glc*p* at O4 of GlcNAc. The only difference between them is that serotype 7b has O-acetylation on the O2 of Rha^{I}.

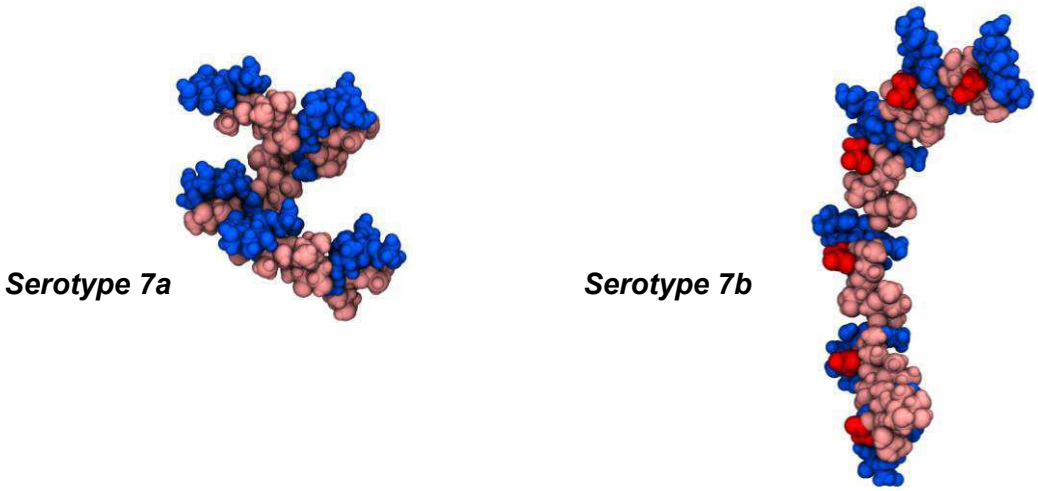

Fig.1. Preliminary six repeating unit O-specific polysaccharide models of *Shigella flexneri* serotypes 7a and 7b. Glc and GlcNAc residues are blue, Rha residues are pink and the O-acetyl group is red.

Potential of mean force (PMF) calculations were performed in aqueous solution to identify low energy conformations of each of the glycosidic linkages by rotation around the ϕ and φ dihedral angles. The lowest energy dihedral angles, as identified by the PMF calculations, were used to construct the repeating unit oligosaccharides of two serotypes. Initial MD simulations of the oligosaccharides in solution indicate that O-acetylation may result in notable conformational differences between the O-specific polysaccharides of serotypes 7a and 7b.

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THE ANOMER PREFERENCES FOR GLUCURONIC AND GALACTURONIC ACID AND DERIVATIVES

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Chemical reactivity at the anomeric centre is influenced by factors including protecting groups,[1] stereochemical orientation of substituents and conformational preferences of the ring and its substituents. Previous studies within the group reveal that the presence of a carboxylic acid (or derivative) at the C-5 position leads to considerable increase in the rate of $TiCl_4$ and $SnCl_4$ induced anomerisation.[2] Such reactions show an increased preference of the axial anomer and in some cases, higher for the galacturonic acids and galactopyranoses compared to their glucuronic acids and glucopyranose analogues. It has not been clear whether there exists an intrinsically increased preference in uronic acids for the axial anomer compared to analogous pyranoses. The aim of this research is to determine anomer equilibrium in the absence of Lewis acids. The effect of acylation and reduced polarity of solvation of the monosacharides are also explored. The effect of more electron-withdrawing substituents on the anomeric ratio was also investigated. Here, the hydroxyl substituent was replaced with a fluorine atom at the 2, 3, 4, and 6 positions individually for both glucose and galactose, and their anomeric ratio was measured at equilibrium.
In addition, we needed to consider the influence of relaxation times when obtaining ^{1}H-NMR spectra of the monosaccharides in determining anomeric ratios as proton relaxation is strongly influenced by anomeric configuration.[3] The rates of relaxation for α and β anomers differ quite considerably,[3] therefore, to quantify the anomeric ratio with 100% certainty the relaxation time for both anomers must be calculated and then applied when obtaining spectra. The results of these investigations will be presented.

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SCIENCE MEETS ART: INVESTIGATION ABOUT THE YELLOWING OF REFINED WHITE SUGAR CUBES USED IN AN CONTEMPORARY ART WORK

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Table sugar, sucrose, can have an unusual use in the visual arts as a playful vehicle to express and explore serious issues [1]. It is present in the painting, sculpture, video, photography, installations and performances of contemporary artists, all over the world, and especially since the 60's of the twentieth century.

The research presented here is about the use of table sugar as a raw material in the production of contemporary work of art "Metrópole"(2007) by the Portuguese artist Rodrigo Oliveira (Figure 1). This art work was selected, and since 2015 is owned by the Museum of Art, Architecture and Technology (MAAT) It was exhibited from 22 March to 21 August 2017 in the Utopia/Distopia exhibition. It is made up entirely of sugar cubes. This art work had been kept disassembled for about ten years. The remains of the art work together with some original cubes still in the original packaging, were kept in a corrugated cardboard box. Some of the cubes had become yellow, while those in the original packaging remained white. Since it was not allowed to collect samples from the art work, these cubes were given by the artist himself Rodrigo Oliveira.

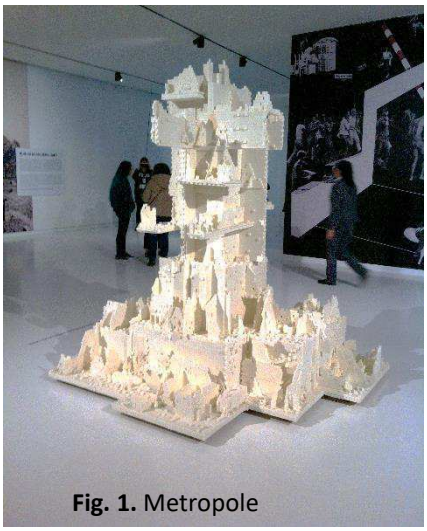

Fig. 1. Metropole

To preserve this art work, it is important to understand the reason of yellowing of the sugar cubes.

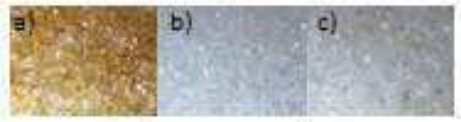

Figure 2. a) yellow cube; b)white cube; c) yellow cube after washing with methanol

Yellow sugar cubes were washed with methanol and the surface, before and after washing, were observed using the naked eye observation and the binocular magnifying glass and compared with the surface of the cubes that remained white (Figure 2). All cubes presented a similar image with clear crystal surfaces. Melting point of samples of yellow and white sugar were determined using a melting point apparatus with microscope. FTIR and ^{1}H NMR of the residue of methanol washings was obtained.

Although this work did not yet allow to fully characterize the main causes of yellowing of sugar cubes, it gives a good indication that the phenomena occurred only at the surface of the crystals that constitute sugar cubes and probably results from an external contamination instead of a degradation of sucrose.

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POSTERS | BS – Biomolecule Structure

TAILSPIKE PROTEINS AS HIGH-AFFINITY SENSORS FOR PATHOGENS

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The unrestrained use of antibiotics against bacterial infection has accelerated the occurrence of resistances in many pathogens. In order to reduce antibiotics use, sensitive and robust pathogen detection is necessary. Bacteriophage tailspike proteins (TSP) are thermostable and detergent resistant proteins with binding sites for bacterial cell surface polysaccharides [1]. They therefore offer a promising design platform for bacterial cell surface polysaccharide sensors.

In this work we have analyzed complexes of *Shigella flexneri* (*S. flexneri*) O-polysaccharides with the tailspike protein of *Shigella* bacteriophage Sf6 (Sf6TSP) [2]. The pathogen *S. flexneri* causes dysentery in infants and is an important diagnostic target. The conformational space populated by the polyrhamnose backbone of the *S. flexneri* O-polysaccharide in complex with Sf6TSP could be well described with 2D ^{1}H,^{1}H-trNOESY NMR experiments and the results are in good agreement with the conformations obtained from molecular dynamics (MD) simulations. To analyze the impact of amino acid exchanges in the glycan binding site of Sf6TSP, MD simulations were used to search for increased O-polysaccharide binding affinities. All predicted mutants with affinity increase were then further analyzed with surface plasmon resonance. One of the high-affinity mutants obtained was used to construct a TSP-based *Shigella* sensor. The resulting fluorescent probe was suitable for the detection of low amounts of bacteria in solution, offering a starting point to faster and more sensitive *Shigella* diagnostics.

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STRUCTURAL STUDIES AND BIOSYNTHETIC ASPECTS OF THE O-ANTIGEN POLYSACCHARIDE FROM SHIGATOXIN-PRODUCING E. COLI O179

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The O-antigen polysaccharide (PS) is the outermost part of the lipopolysaccharide (LPS) of gram-negative bacteria, and one of the most important surface antigens used for classification of strains in different serotypes. According to the serological properties of their O-antigen polysaccharides (O-PS), the strains of *E. coli* are presently differentiated into more than 180 serogroups; information about the structure, NMR chemical shifts, cross-reactivity, and glycosyltransferases (GTs) involved in the biosynthesis of their O-antigens PS can be found in ECODAB (*E. coli* O-antigen database).[1] The structure of the O-specific chain of most of the aforementioned *E. coli* serogroups have been reported so far in the literature, but a few O-PS (such as that of the *E. coli* O179) has not yet been characterized.

In this study, we investigate the structure of the O-specific chain of the *O*-deacylated lipopolysaccharide (LPS-OH) of *E. coli* O179 using NMR data, the program CASPER and analysis of biosynthetic information available in the ECODAB. The PS consists of branched pentasaccharide repeating units composed of mannose, glucose, glucuronic acid, and *N*-acetylglucosamine. The chemical structure is consistent with the genetic information available in the literature, since four genes encoding for GTs could be identified in the O-antigen gene cluster (GeneBank accession number AB812076.1).

The O-antigen PS of *E. coli* O179 share structural similarities with that of *E. coli* O6, *S. boydii* type 16, and the capsular polysaccharide of *E. coli* K43,[2-4] explaining the serological cross-reactivities observed with strains belonging to these O- and K-antigen groups.[5,6] The aforementioned antigens have an identical trisaccharide structural element at the reducing end of their biological repeating units, and the O-antigen gene clusters encode some proteins with high sequence similarities. For instance, the proteins with GeneBank accession numbers BAQ01964.1 and BAQ01963.1 (encoded in the O-antigen gene cluster of *E. coli* O179), share similarities with WfaV (GeneBank accession number ABD19781.1, 60% identity, *E.* value = 5×10^{-173}), and WfaV (GeneBank accession number ABD19780.1, 40% identity, *E.* value = 1×10^{-89}) of *Shigella boydii* type 16, respectively. Consequently, the functions of those proteins could tentatively be assigned to GTs involved in the biosynthesis of the structural elements β-D-Man*p*-(1→3)-D-Glc*p*NAc and β-D-Man*p*-(1→4)-D-Man*p*, respectively.

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EXCLUSIVE RECOGNITION OF CORE FUCOSYLATION BY A NOVEL LECTIN FAMILY

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Core (a1,6) fucosylation consists in the addition of an α1,6 linked-fucose to the innermost Nacetyl glucosamine (GlcNAc) of the N-glycan core of mammalian glycoproteins. It influences the intrinsic properties and bioactivities of glycoproteins and has reported links to cancers.
Core fucosylation profiling in glycoproteins is important in numerous biomedical applications but most lectins actually used are not strictly specific for this fucosylation. Recently, a lectin PhosL discriminating selectively and strictly core fucosylation was extracted from mushroom[1]. PhosL represents therefore a promising molecular tool for glycosylation analysis[2]. We manage to produce it in a recombinant form in order to have better availability, quality management and reproductibility of the lectin. It presents the same characteristics as the native protein. In the absence of structural data, we determined the crystal structures of the 40 amino acids protein in apo or complexed form with three bisected core fucosylated Nglycans.
We unraveled an interlaced trimer that forms a novel protein fold that we termed b-prism III and a novel binding site architecture. Our work provides the structural basis and a protein consensus motif for the exclusive recognition of a1,6-fucosides defining a new lectin family.

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VACUUM-ULTRAVIOLET CIRCULAR-DICHROISM STUDY OF OLIGOSACCHARIDE CONFORMATIONS USING A SYNCHROTRON-RADIATION SPECTROPHOTOMETER

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Vacuum-ultraviolet circular-dichroism (VUVCD) spectroscopy using a synchrotron radiation (SR) is a powerful tool for analyzing saccharide structures (especially unsubstituted saccharides) in aqueous solution because they contain high-energy chromophores such as hydroxy groups and acetal bonds whose n–□* electronic transitions are only detectable in the vacuum-ultraviolet (VUV) region below 190 nm [1, 2]. However, the relationships between the structures and VUVCD of saccharides remain controversial due to the interpretation of the observed CD spectra in the VUV region not being assigned explicitly. We have recently measured the VUVCD spectra of various methyl aldopyranosides in aqueous solution and theoretically revealed the pairwise relationships between configurations (gauche and trans rotamers of hydroxymethyl group at C-5, and □- and □-anomers of hydroxyl group at C-1) and VUVCD of monosaccharides [3]. In the present study, we measured the VUVCD spectra of the unsubstituted malto-, laminari-, isomalto-, and cello-oligosaccharide series with the aim of clarifying the contributions of the different types of constituent disaccharides and increases in chain length to the structures and CD spectra of oligosaccharides.

Each oligosaccharide series with various degree of polymerization [DP] exhibited characteristic VUVCD spectra in aqueous solution [4]. Disaccharides exhibited markedly different CD spectra depending on the types of glycosidic linkages, and the CD spectra of each oligosaccharide series (with the exception of the isomalto-oligosaccharide series) varied with the chain length below 190 nm while retaining the spectral shape of the constituent disaccharide (Figure 1). These results indicate that the basic structures of oligosaccharides were greatly affected by the configurations of their constituent disaccharides, which had unique torsion angles restricted by the intramolecular hydrogen bonds between glucose units. Based on comparisons between the experimental and theoretical data, we suggest that the chain-length dependence of CD above 180 nm reflects the backbone structure of oligosaccharides (e.g., helical structures), while those below 180 nm are influenced by other factors associated with higher-energy chromophores such as the hydroxyl groups. Investigating the structural characteristics of oligosaccharides is not only inherently interesting but also an important step toward understanding the structure and dynamics of polysaccharides. The further accumulation of VUVCD data and their theoretical assignments is important for understanding the more detailed structures of oligosaccharides and their biological functions, which opens new fields in the structural biology of glycoconjugates.

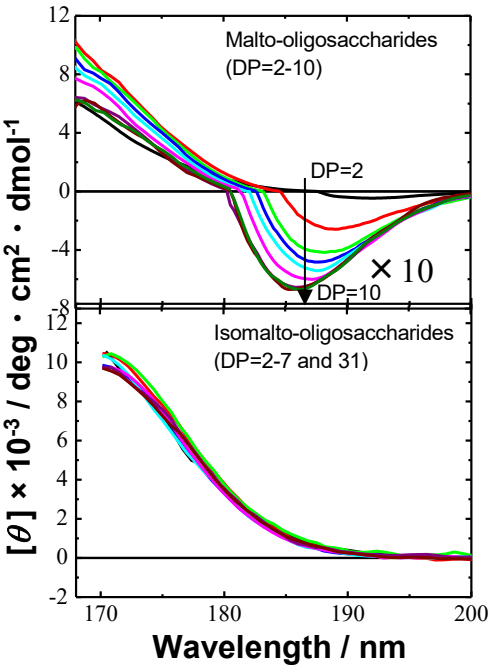

Fig. 1. VUVCD spectra of malto- and isomalto-oligosaccharides in aqueous solution at 25°C.

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THE 3D STRUCTURE OF RHAMNOGALACTURONAN II SLOWLY UNRAVELS

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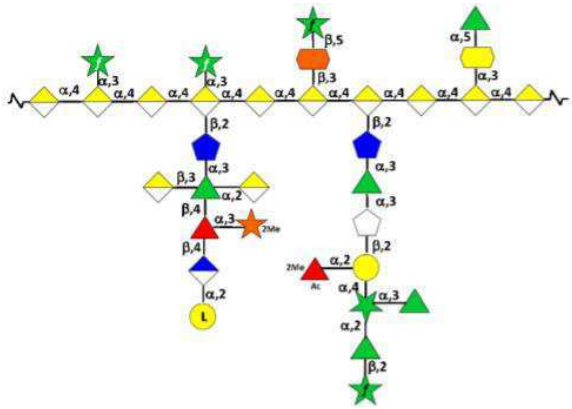
Walls of growing plants are extremely complex and sophisticated composite materials incorporating a dynamic assembly of polysaccharides, proteins, and phenolics. Among the polysaccharides, the pectins encompass a group of acidic heteropolysaccharides, with distinct structural and functional domains. Pectins offer a repertoire of structural complexity; they can nevertheless be described in terms of a canonical structure which form the distinct specific domains. These domains are named after their major monosaccharide constituents. Whereas most of these domains are notable for their structural heterogeneity, another one, so-called Rhamnogalacturonan-II (RG-II), exhibits a remarkable conservation throughout the plant kingdom, which may indicate its major role in the structure and growth of higher plants. RG-II is one of the most complex plant polysaccharide on earth. As a monomer, it contains 13 different sugars and 21 distinct glycosidic linkages organized in a backbone and several branches of different lengths. Nevertheless it is in its dimeric association that it takes its full biological and physicochemical relevance.

The progress of the elucidations of the different structural levels of RG-II have paralleled the development of enabling methodologies and technologies over a 40 year period of time. Starting from the pioneer description of the structural constituents (1), followed by a high-resolution NMR structural elucidation (2), the anatomic dissection of RG-II by but bacterium from *B. thetalotaomicon* and subsequent X-ray resolution of some degraded fragment (3), the picture of the structural composition of RG-II emerges. The integration of all these pieces of information within a specialized macromolecular builder integrating knowledge-based energetic constraints provides a reliable three-dimensional description of the RG-II monomeric unit.



The results of several studies had led to the suggestion that an interaction between borate and pectin is important for wall structure and for plant growth and development. In this interaction, borate would act as a cross-linking of two RG-II monomers forming the bio-active structure of RG-II. Some stereochemical reasoning; somehow confronted by several observations have suggested that in planta, the dimeric formation could be mediated by a borate diester linkage between D-Apif in each monomer.

The availability of the 3-dimensional structure of one RG-II monomer provides a way to establish the molecular basis of the born-diester linkage. We conducted a detailed investigation of the structural features of the Boron-Carbohydrate via DFT computational method. Upon translating these results to the case of RG-II, we came to the conclusion that D-Apif should not be the only one monosaccharide involved in the bridging and that more complex interactions have to be invoked.

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STRUCTURE OF LIPOOLIGOSACCHARIDES FROM HALOPHILIC BACTERIA

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Extremophiles are organism that can live in harsh environment. They can produce unique metabolites as they adapt to their particular conditions and are hence an interesting source of biologically active compounds [1]. Directly exposed to their environment, Lipopolysaccharide (LPS) and Lipooligosaccharide (LOS) are the main component of the outer membrane of Gram-negative bacteria. LPSs are composed of three main parts: a polysaccharide named the O-antigen, a core oligosaccharide and the Lipid A that is anchored in the outer membrane. These endotoxins are known to interact with mammal's innate immunity, being agonist or potential antagonist of the Toll-Like Receptor (TLR4) and Myeloid Differentiation factor 2 (MD-2). Depending on their structures, endotoxins can be potent modulators of TLR4/MD2, leading to potential therapies [2]. In the case of extremophiles, LPS can possess unusual structure and immunological activities [3]. New structural features can then be found in halophiles, organisms that live in high salinity conditions.

In this context, endotoxins from different halophiles were characterized. *Spiribacter salinus* is a gamma-proteobacterium isolated from intermediate-salinity pound in Spain [4]. Its Lipid A was characterized using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) and MS^2 techniques [5]. The structure of *Halopeptonella vilamensis* LOS was also investigated. It is a gamma-proteobacterium isolated in a saline lagoon in Argentina [6]; its Lipid A was analyzed by MALDI-MS and MS^2 and its oligosaccharide content was studied with NMR spectrometry.

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COMPUTATIONAL ANALYSIS OF INTERACTION OF ENDOSTATIN WITH GLYCOSAMINOGLYCANS

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Glycosaminoglycans (GAGs) play a key role in a variety of biological processes in the extracellular matrix (ECM) via interactions with their protein targets. Due to their high flexibility and electrostatics-driven nature of their interactions, GAG-containing systems are very challenging for *in silico* approaches. In this study, we characterized the interactions of endostatin, a naturally occuring proteolytic C-terminal fragment of collagen XVIII known to be an anti-angiogenic and GAGs of different type, length and sulfation pattern. We applied molecular docking and molecular dynamic simulations to obtain the stable conformations of protein-GAG complexes. MM-GBSA approach was used to calculate the binding free energies and to characterize the amino acid residues of endostatin which have the most favourable contribution to GAG binding. We also investigated the influence of endostatin-Zn^{2+} binding on a conformational change in endostatin-heparin (HE) complexes using computational approaches. We detected particular Asp residues in endostatin that unfavourably contributed to the binding to all analyzed HE and heparan sulfate (HS) ligands. Several mutations on Asp residues were analyzed using *in silico*. We are expressing recombinant simple, double and triple endostatin mutants to perform binding assays, calculate the affinity of the mutants for HE and HS and validate the *in silico* findings.

RELATIONSHIPS BETWEEN THE O-POLYSACCHARIDES OF *ESCHERICHIA COLI* AND OTHER ENTERIC BACTERIA

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The O-specific polysaccharide chain of the lipopolysaccharide called the O-antigen is an important component of the outer membrane of the cell wall of gram-negative bacteria. Being exposed to the bacterial cell surface, the O-antigen is subject to intense selection by the host immune system and bacteriophages giving rise to diverse O-antigen forms and providing the basis for typing of bacteria. The O-antigen forms of many bacteria are unique, whereas some are structurally and genetically related to others. Multiple genetic mechanisms of diversification of the O-antigen forms, such as lateral gene transfer and mutations, have been reported.

Escherichia coli is the predominant facultative anaerobe of the colonic flora, and some specific serotypes are associated with enteritis, hemorrhagic colitis, and hemolytic uremic syndrome. *Shigella* spp. are human pathogens that cause diarrhea and bacillary dysentery (shigellosis). The bacteria *Salmonella enterica* are responsible for a food-borne infection (salmonellosis), and specific serotypes cause typhoid fever and paratyphoid fever. Structural and genetic relationships between the O-polysaccharides of some *E. coli* serotypes and *Shigella* spp. on the one hand[1, 2] and between those of some other *E. coli* serotypes and *S. enterica* on the other hand[3] are well known. Multiple *E. coli* serotypes also are related to a number of other members of Enterobacteriaceae, including the most close species *Escherichia alberti*[4] and such taxonomically remote enteric bacteria as *Proteus* spp. and *Yersinia* spp. Other examples are *Hafnia alvei*, *Klebsiella pneumoniae*, *Enterobacter aerogenes, Citrobacter* spp., and *Cronobacter* spp.

In this work, structures and genetics of biosynthesis of related O-polysaccharides of various enteric bacteria are summarized. The molecular evolutionary relationships between the O-antigens of these bacteria and the origins of their diversity are discussed. These data help understanding the role of gene polymorphism in pathogenicity.

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ENZYMATIC TOOLS TO GLYCOENGINEER ANTIBODIES

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Streptococcus pyogenes EndoS is a bacterial endoglycosidase that specifically hydrolyzes the □-1,4-di-*N*-acetylchitobiose core glycan linked to residue Asn297 of human immunoglobulin G (IgG) antibodies (1). This renders antibodies incapable of eliciting host effector functions through either complement or Fc □□receptors, providing the bacteria with a survival advantage. On account of this antibody-specific modifying activity, EndoS is being developed as a promising injectable therapeutic for autoimmune diseases that rely on autoantibodies (2). Additionally, EndoS is a key enzyme used in the chemoenzymatic synthesis of homogenously glycosylated antibodies with tailored Fc γ receptor-mediated effector functions (3). Despite the tremendous utility of this enzyme, the molecular basis of EndoS specificity for, and processing of, IgG antibodies has remained poorly understood.

We showed that EndoS is composed of five distinct protein domains, including glycosidase, leucine-rich repeat, hybrid Ig, carbohydrate binding module, and three-helix bundle domains, arranged in a distinctive V-shaped conformation. Our data suggest that the substrate enters the concave interior of the enzyme structure, is held in place by the carbohydrate binding module, and that concerted conformational changes in both enzyme and substrate are required for subsequent antibody deglycosylation. The EndoS structure presented here provides a framework from which novel endoglycosidases could be engineered for additional clinical and biotechnological applications.

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COMPARATIVE STUDY OF THREE NOVEL β-N-ACETYLHEXOSAMINIDASES FROM PHYLOGENETICALLY DIVERSE SOURCES

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β-N-acetylhexosaminidases (EC 3.2.1.52) are glycoside hydrolases (GH) that catalyze the cleavage of terminal β-D-GluNAc and β-D-GalNAc residues in N-acetyl-β-D-hexosaminides. B-Nacetylhexosaminidases have recently gained relevance due to their roles in human physiology, acquired and hereditary diseases (e.g. Tay-Sachs disease[1]) and as biomarkers (e.g. type 1 diabetes, alcoholism) as well as for their untapped potential for the enzymatic synthesis of carbohydrates and glycomimetics.[2] Bacterial and fungal β-N-acetylhexosaminidases have been involved in myriad functions including cell wall recycling, nutrient assimilation, biofilm spreading, hyphal expansion and branch initiation. Owing to their substrate promiscuity, β-N-acetylhexosaminidases can be repurposed to catalyze the transfer of varied substrate functionalities.[2] We have selected three DNA sequences encoding β-N-acetylhexosaminidases from a bacterium and a fungus from the CAZy[3] GH20 family and an archaeon from the GH3 family to evaluate their glycolytic potential as versatile biotechnological tools. We cloned several constructs of the chosen β-N-acetylhexosaminidase genes, expressed and purified them as catalytically active recombinant proteins. Next, their glycan hydrolytic properties were characterized using standardized spectrophotometric assays both as free enzymes and as covalently immobilized biocatalysts on functionalized resin supports. We have also characterized their thermal stability and pH activity profiles. To shed light on their structure-function relationship, we have also conducted the preliminary biophysical (e.g. sedimentation-velocity analytical ultracentrifugation) and structural characterization by X-ray diffraction of these enzymes. Here, we present the comparative analysis of the hydrolytic activity, stability and structural properties of a set of three novel β-Nacetylhexosaminidases and discuss the potential of these versatile enzymes for various biocatalysis applications.

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NOVEL BACTERIAL AND FUNGAL α-GLUCURONIDASES FOR THE DEVELOPMENT OF MULTIENZYMATIC PROCESSES

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α-Glucuronidases are glycoside hydrolases (GH) from the CAZy[1] GH67 and GH115 families (E.C. 3.2.1.139 and E.C. 3.2.1.131) that are able to catalyze the enzymatic release of 4-methyl-D-glucuronic acid units from xylooligosaccharides (α-glucuronidases) and from polymeric glucuronoxylan. These enzymes hold promise for the improvement of the efficient exploitation of plant hemicellulosic biomaterial, which is notoriously resilient to enzymatic hydrolysis. We have mined the DNA sequences encoding three novel α-glucuronidases from a bacterium and from two different fungal species. We cloned, expressed and purified these three α-glucuronidases and performed a thorough characterization of their capacity to hydrolyze aldotriouronic units using a commercially available kit. Of the tested enzymes, the most stable and active α-glucuronidase came from *Streptomyces* spp. This enzyme was selected for a more thorough biochemical, biophysical and structural characterization. In particular, we analyzed the glycohydrolytic properties of the free *versus* the resin immobilized enzyme preparation.[2] We have also characterized its thermal stability and pH activity profile. In addition, we have also conducted a preliminary biophysical and structural characterization by X-ray diffraction of this enzyme. Since GH67 α-glucuronidases require the preprocessing of the lignocellulosic substrates by xylanases that trim the polymeric xylan into smaller xylooligosaccharides,[3] we have studied the potential synergy between *Streptomyces* spp. α-glucuronidase and the bifunctional endo-β-xylanase (Xyl2) from *Fusarium oxysporum*,[4] which we have recently characterized in the laboratory, in the enzymatic degradation of complex xylan. The chosen experimental approach involved a first step whereby the colorimetric substrate Remazol Brilliant Blue (RBB)-xylan was degraded into smaller xylooligosaccharides, which were subsequently subjected to the glycohydrolytic activity of the α- glucuronidase. Using this strategy, we have demonstrated that the multienzymatic preparation of a bifunctional endo-β-xylanase and an α-glucuronidase is a superior biocatalyst for the processing of highly branched glucuronoxylan.

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CHEMICAL STRUCTURE OF THE *E. COLI* O6:K15 CAPSULE POLYSACCHARIDE

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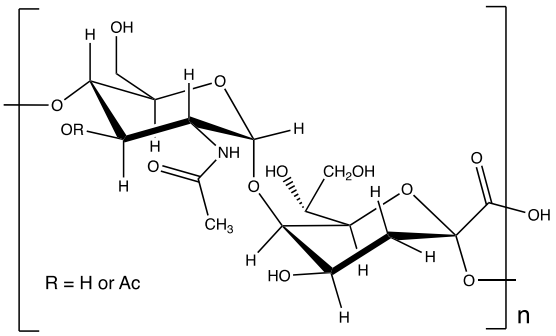
Capsular polysaccharides are important virulence factors in pathogenic bacteria.[1] Characterizing their components and production pathways will improve our ability to design preventative therapies, in the form of vaccines, or drugs interfering with development or reproduction of targeted pathogens. Among many strains affecting human health, the *Escherichia coli* O6:K15 serotype has been identified as both, an enterotoxigenic[2] and uropathogenic[3] bacteria. Despite its relevance as a disease causing bacteria, this strain remains poorly characterized.

We present a preliminary report on the genes involved in the production of the capsular polysaccharide and the structure description of the resulting product, based on chemical and NMR analysis. The polysaccharide was prepared and isolated from a culture of *E. coli* F8361-41:O6:K15:H16. Chemical analysis revealed that the K15 polysaccharide is comprised of a disaccharide repeat unit of N-acetylglucosamine and Kdo (3-deoxy-d-*manno*-oct-2-ulosonic acid). N-acetylglucosamine was determined to be 4-substituted by methylation analysis. The Kdo unit was shown to be 5-substituted by periodate oxidation, β-elimination, and methylation analysis of the methyl ketoside methyl ester. Finally, complete ^{1}H and ^{13}C NMR assignments of the K15 polysaccharide were accomplished using (^{1}H,^{13}C)HSQC, (^{1}H,^{13}C)HMBC, (^{1}H,^{13}C)HSQC-TOCSY, and (^{1}H,^{13}C)HSQC-NOESY experiments for both O-acetylated and non-acetylated forms of the polysaccharide. Analyses of the spectra indicate that N-acetylglucosamine is O-acetylated at C3. The substitution pattern determined by methylation was confirmed by NMR experiments and the anomeric configuration of the glycans revealed by the chemical shifts of the anomeric CH in N-acetylglucosamine. Chemical shifts for the equatorial and axial H3's, together with the ^{13}C chemical shift of C3 in Kdo indicate an α-configuration. Thus, the K15 polysaccharide structure consists of a repeat unit of:

→4) -α-D-Glc*p*NAc-(1→5) -α-Kdo-(2→



In future work we plan to elucidate the specific roles of the different enzymes in in the production of the capsule, and in particular the mechanism for which Kdo is transferred in the α-configuration.

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STRUCTURAL MODIFICATION OF *STREPTOCOCCUS* CAPSULAR POLYSACCHARIDES BY MUTAGENESIS

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The capsular polysaccharide (CPS) represents a key virulence factor for most encapsulated streptococci. *Streptococcus suis* and Group B *Streptococcus* (GBS) are both well-encapsulated pathogens of clinical importance in veterinary and/or human medicine and responsible for invasive systemic diseases. CPS differences are the basis for serological differentiation of the species into serotypes.

S. suis serotypes 2 and 1/2, which possess identical gene content in their *cps* loci, express CPSs that differ only by substitution of galactose (Gal) by *N*-acetylgalactosamine (GalNAc) in the CPS side chain. The same sugar substitution differentiates the CPS of serotypes 14 and 1, whose *cps* loci are also identical in gene content. Using mutagenesis and CPS structural analysis, it was found that a single amino acid polymorphism in the glycosyltransferase CpsK defines the enzyme substrate predilection for Gal or GalNAc and therefore determines CPS composition, structure, and strain serotype.[1]

S. suis and GBS are the only Gram-positive bacteria which express a sialylated CPS at their surface. An important difference between these two sialylated CPSs is the linkage between the side-chain galactose and sialic acid, being α-2,6 for *S. suis* but α-2,3 for GBS. It is still unclear how sialic acid may affect CPS production and, consequently, the pathogenesis of the disease caused by these two bacterial pathogens. The role of sialic acid and the putative effect of sialic acid linkage modification in CPS synthesis were investigated using inter-species allelic exchange mutagenesis. It was shown that sialic acid (and its α-2,6 linkage) is crucial for *S. suis* CPS synthesis, whereas for GBS, CPS synthesis may occur in presence of an α-2,6 sialyltransferase or in absence of sialic acid moiety. To evaluate the effect of the CPS composition/structure on sialyltransferase activity, two distinct capsular serotypes within each bacterial species were compared (*S. suis* serotypes 2 and 14 and GBS serotypes III and V). In spite of common CPS structural characteristics and similarities in the *cps* loci, sialic acid exerts differential control of CPS expression by *S. suis* and GBS.[2]

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MOLECULAR CHARACTERIZATION OF GLYCOSADASES IN GH 97, SUBFAMILY c

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Glycoside hydrolase family 97 (GH97) is one of the most interesting glycosidase families since it contains inverting and retaining glycosidases. GH97 enzymes can be further classified into five subfamilies (GH97a−97e) based on phylogenetical analysis [1]. Among them, GH97a and GH97b have been known to include inverting α-glucoside hydrolases and retaining α-galactosidases, respectively. In this study, we characterize substrate recognition specificity of GH97c enzymes, BT3661 and BT3664, derived from *Bacteroides thetaiotaomicron* [2].

Recombinant BT3661 and BT3664 were produced using *Escherichia coli*. The recombinant enzymes were purified by a Ni^{2+}-affinity column chromatography. Their hydrolysis activities toward *p*-nitrophenyl (*p*NP) glycosides were examined. BT3661 and BT3664 hydrolyzed both *p*NP β-L-arabinopyranoside (*p*NP Ara) and *p*NP α-D-galactopyranoside (*p*NP Gal), but not *p*NP α-D-glucopyranoside or *p*NP α-D-xylopyranoside. Steady state kinetic parameters of *p*NP Ara and *p*NP Gal were determined. The k_{cat}/K_m value of BT3661 for *p*NP Ara (0.41 $s^{-1}mM^{-1}$) was similar to that for *p*NP Gal (0.14 $s^{-1}mM^{-1}$). On the other hand, the k_{cat}/K_m value of BT3664 for *p*NP Gal (1.9 $s^{-1}mM^{-1}$) was 25 times higher than that for *p*NP Ara (0.076 $s^{-1}mM^{-1}$). Thus, BT3661 is identified to be a bifunctional β-L-arabinopyranosidase/α-galactosidase, and BT3664 was confirmed to be an α-galactosidase [3]. The findings demonstrated that BT3661 possesses the novel specificity in GH97.

Hydrolytic activities of BT3661 toward polysaccharide substrates, gum arabic from acacia tree and arabinogalactan from larch wood, which are considered to contain β-L-arabinopyranoside and α-D-galactopyranoside at their non-reducing ends, were investigated. The amount of arabinose or galactose was estimated using HPAEC-PAD and its release rate was calculated. The enzyme catalyzed to release arabinose (2.0 μmol/min/mg protein) and galactose (1.4 μmol/min/mg protein) from gum arabic, and arabinose (41 μmol/min/mg protein) from arabinogalactan. BT3661 is likely to physiologically involve in the degradation of these polysaccharides.

We determined the three-dimensional structure of BT3661 to characterize its novel substrate specificity in GH97 members. The tertiary structure consists of three domains similar to other GH97 enzymes. The structural comparison indicates that the active-site pocket of BT3661 is similar to GH97b retaining α-galactosidase except for residues involved in the stabilization of a C6 hydroxy group of the α-galactosyl moiety. While GH97b α-galactosidase has Glu351 to stabilize the hydroxyl group of C6 through a hydrogen bond, BT3661 possesses Asn338 at the equivalent position. Mutational analysis was conducted to understand the role of Asn338 in substrate recognition. Asn338 was replaced by Glu, Ala and Asp, which are placed at the corresponding position with Asn338 in GH97 retaining glycosidases. The k_{cat}/K_m value of N338E for *p*NP Ara increased but that for *p*NP Gal decreased. The k_{cat}/K_m value of N338A for *p*NP Gal notably increased but that for *p*NP Ara decreased. These results suggested that the length of the side chain of the residue at the Asn338 position is concerned with specificity of BT3661.

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CLEAVAGE OF CAPSULAR POLYSACCHARIDES OF MULTIDRUG-RESISTANT PATHOGEN *ACINETOBACTER BAUMANNII* BY BACTERIOPHAGE AND PROPHAGE-DERIVED DEPOLYMERASES

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Acinetobacter baumannii is gram-negative conditionally pathogenic bacteria, which cause nosocomial infections, such as pneumonia, wound and catheter-related urinary tract infections, peritonitis, meningitis, endocarditis, and bloodstream infections. Treatment of the infections is complicated by the ability of the bacteria to acquire and accumulate various mechanisms of antibiotic resistance, which poses a serious public health problem. An alternative method of combating *A. baumannii* may become phage therapy, which is a highly specific approach with minimum medical contraindications. One of the virulence factors of *A. baumannii* is a capsular polysaccharide (CPS) composed of many oligosaccharide repeats (K units), which forms a thick protective layer around the bacterial cell. Due to polymorphism of the capsule gene locus (K locus, KL) CPS structures are highly diverse (more than 120 KL types have now been identified). A prerequisite of infection and lysis of *A. baumannii* cells by a bacteriophage is specific recognition followed by cleavage of the CPS by phage tail-spike receptor proteins that possess a CPS depolymerizing activity.

The aim of this work was the establishment of the biochemical basis for phage therapy of the infections caused by *A. baumannii*, including elucidation of the mechanisms of cleavage of the CPSs of these bacteria with recombinant bacteriophage depolymerases or depolymerases derived from prophages found in the same or a different *A. baumannii* strain.

The CPSs were isolated by phenol-water extraction from cells of clinical isolates of *A. baumannii* belonging to different KL types and were purified by Sephadex G-50 gel chromatography. Their structures were either identified by comparison with published data or de novo established by sugar analysis along with one-dimensional (^{1}H and ^{13}C) and two-dimensional (^{1}H,^{1}H COSY, ^{1}H,^{1}H TOCSY, ^{1}H,^{1}H ROESY, ^{1}H,^{13}C HSQC, and ^{1}H,^{13}C HMBC) NMR spectroscopy. The CPSs from six strains (NIPH70, NIPH146, NIPH601, LUH5549, B05, AbWRMAC3340) were cleaved with recombinant bacteriophage depolymerases and those from three strains (AYE, AB5256, B8300) with prophage-derived depolymerases. The oligosaccharide products were fractionated by Fractogel TSK HW-40S gel chromatography and studied by negative or positive ion mode high-resolution electrospray ionization mass spectrometry and one- and two-dimensional NMR spectroscopy. It was found that all depolymerases studied possess a glycosidase activity and cleave specifically the CPSs of *A. baumannii* by the hydrolytic mechanism to give a monomer or/and an oligomer (a dimer and in one case a trimer) of the K units.

This work was supported by the Russian Foundation for Basic Research (project No. 17-04-01254).

UNVEILING MULTIPLE BINDING MODES FOR CELLODEXTRIN PHOSPHORYLASE

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Cellulose is a linear homo-polysaccharide consisting of β-(1-4) linked D-glucose residues, it is the most abundant biopolymer in the world and an essential component of the plant cell wall. Nonetheless, cellulose extraction requires energetically expensive and non-environmental friendly procedures due to difficulties in purification. For this reason, the *in-vitro* synthesis of cellulose is nowadays of high interest. The enzymatic synthesis of cellodextrin leads to pure crystalline cellulose with a particular type of allomorphism known as cellulose type II. [1] The synthesis of cellodextrin oligomers from GH94 glycoside hydrolase cellodextrin phosphorylase (CDP, EC 2.4.1.49) involves the elongation of short β-(1→4)-glucans (acceptors) through the addition of glucose units donated from α-D-glucose-1-phosphate (donor).[1] Recently, the first X-ray crystal structure of cellotetraose-bound CDP has been published, allowing a better understanding of the enzyme molecular recognition. In addition, acceptors permissiveness and donor specificity have been investigated to probe the possibility of cellulose derivative synthesis. [2]

On the other side, it is well known that the crystallographic picture lacks the dynamics view of the enzyme-substrate interaction, from which a more clear understanding of acceptors and donors selectivity could be achieved. Indeed, it is widely accepted that protein-ligand crystal structures might not represent the whole picture of molecular recognition events.

In this study, STD NMR experiments for the investigation of the binding epitope of CDP donor (α-D-glucose-1-phosphate) and acceptors (glucose, cellobiose, laminaribiose and cellotriose) in solution were carried out. In addition, ligand orientation inside the binding pocket was studied by average DEEP-STD NMR methodology. Finally, LOGSY-titration experiments were conducted in order to distinguish between buried and solvent-accessible part of the ligand.

The collected data show differences in the protein contacts to the α- and β-anomers at the reducing ring, highlighting contact differences between β-(1→4) and β-(1→3) -glucans. For cellobiose and cellotriose, the magnetization was spread along the whole length of the ligand, suggesting a more complex binding mode. This result was then confirmed with the identification of a plane of symmetry in the ligand-enzyme interaction, and the analysis of the water exposure.

Overall, STD NMR experiments did not only allow us to obtain insights on enzyme-ligand interaction for small molecules impossible to co-crystallize, but also to characterize an additional unproductive binding mode for cellotriose, where the ligand enters the binding pocket with the reducing ring instead of the non-reducing ring.

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PEPTIDOGLYCAN DEACETYLASE *Bs*PDAC ACTING ON CHITIN OLIGOSACCHARIDES. STRUCTURE AND CHARACTERIZATION

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Chitosans are a family of fully or partially deacetylated polymers derived from chitin. They are the only natural polycationic polysaccharides, property that allows them to interact with anionic biomolecules such as proteins, DNA, and membrane phospholipids, eliciting diverse biological functions.
Consequently, chitosans and chitosan oligosaccharides (COS) are bioactive molecules with many current and potentially new applications in several fields. Their physicochemical and biological properties are determined by their specific structures characterized by the degree of polymerization (DP), degree of acetylation (DA) and, pattern of acetylation (PA), which defines the distribution of GlcNH2 units along the oligometic chain. Since chemical deacetylation methods yield products with random PA, there is a growing interest in developing selective enzymatic approaches to afford sequence-defined COS to evaluate their biological functions and develop new applications.
Well-defined partially acetylated chitooligosaccharides (paCOS) can be obtained by enzymatic methods and, in this regard, deacetylases from CAZY's Carbohydrate Esterase Family 4 (CE4 enzymes) are of great interest for their potential to be used as biocatalysts. The CE4 family comprises enzymes with deacetylase activity on different biopolymers such as chitin, peptidoglycan, acetyl xylan and poly-β1,6-*N*-acetylglucosamine. Although each enzyme subclass preferentially acts on their natural substrates, some enzymes show a broader specificity deacetylating a wider range of substrates.
In this context, our interest is focused on chitin de-*N*-deacetylases (CDAs) and related CE4 enzymes active on chitin oligomers. The deacetylation patterns exhibited by these enzymes are diverse, some being specific for one position of the oligomeric substrates and others following multiple attack or processive mechanisms leading to partially or fully deacetylated products. A major challenge is to decipher the determinants of substrate specificity aiming at engineering the deacetylation pattern for the production of tailored and sequence-defined paCOS. Based on the first 3D-structure of a CDA in complex with substrates [1], we proposed the "subsite capping model" to rationalize the deacetylation pattern exhibited by different CDAs [1,2].
We here present a bioinformatics, biochemical, and structural study of the enzyme PdaC from *Bacillus subtilis* (*Bs*PdaC), a peptidoglycan deacetylase with preliminary reported activity on COS [3]. We cloned and expressed the full-length and its isolated catalytic domain, and solved the crystal structure of the CE4 catalytic domain [4]. The enzyme deacetylates COS with DP ≥3 following a multiple attack mechanism in which all but the reducing-end GlcNAc residues are deacetylated. Docking of substrates revealed the protein-ligand interactions that define the initial deacetylation events experimentally observed, which are guiding engineering strategies to modify the substrate specificity.

Acknowledgements.

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POLYSACCHARIDES OF THE RED SEAWEED *GRATELOUPIA TURUTURU*

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Grateloupia turuturu is a red seaweed native from the Asian Pacific Ocean shores that belongs to the order of Halymeniales. It is an invasive species with a high rate of reproduction and growth; thus, it is common to find it in different parts of the world, including the Argentine seashore. A previous report from 1975 indicates that, as it is usual in the Rhodophyta, this seaweed biosynthesizes large amounts of sulfated galactans[1].

The polysaccharides produced by this seaweed were extracted from samples collected from the coast of Miramar (Province of Buenos Aires, Argentina). We worked on two different stages of the plant: cystocarpic (F_1) and juvenile (F_2). They were cleaned and ground to withstand a sequential extractive process with water at different temperatures: 20 h at room temperature (F_nA), 8 h at 90 °C (F_nA90 I) and a second step of 8 h at 90 °C (F_nA90 II). The first extraction with hot water was the most effective: F_1A90 I and F_2A90 I were obtained with yields around 50% and 30% respectively, whereas the other fractions were obtained with yields lower than 20% (F_1A and F_2A 9%, F_1A90 II 19%, and F_2A90 II 17%). The analysis of the composition of the extracts showed that they were constituted mainly by carbohydrates (50-60%), with a decreasing tendency in the proportion of sulfate as the extraction sequence progressed (for F_nA, 27%; for F_nA90 I, 25%, decreasing to 16% for F_nA90 II). In addition, the extracts obtained contained proteins and uronic acids, in proportions close to 7% each.

As expected, galactose was the major neutral sugar in all products (83-90%). Small amounts of xylose, glucose, 3-*O*-methylgalactose and traces of rhamnose and 3,6-anhydrogalactose were also found. The absolute configuration of the galactose units present in the polysaccharides was determined by reductive amination with chiral amines and subsequent analysis by gas chromatography[2]. A D / L ratio greater than one was determined in all cases (close to 2.5 in F_nA and F_nA90 II, and reaching 3.5 in F_nA90 I). This result could indicate the presence of carrageenans and agarans as separate entities, or the presence of the so-called D / L hybrids, whose existence was never proved yet. GPC analysis showed that all fractions carried high molecular weights, and also large dispersions. The average molecular weights of these fractions was close to 2,000 KDa.

The F_2A extract was fractionated by column chromatography using a mixture of DEAE Sephadex A-50 / Sephadex G-100 (ratio 1: 3)[3]. Elution was effected with NaCl solutions of increasing concentrations (0.1 to 4M). Two main fractions were obtained (P_1 and P_2), by elution with NaCl concentrations 1M and 1.5M respectively, and similar yields (26%). The composition analysis of P_1 and P_2 showed about 57% of carbohydrates, 21.4% of sulfate for P_1 and 24.5% for P_2, proportions of uronic acids close to 8% for both, and less than 4% proteins. The uronic acid present was identified as D-glucuronic acid. The sugar components of both fractions showed no appreciable differences with respect to the F_2A extract, disregarding enantiomeric issues. However, the D / L ratio of the galactose units present in each of the fractions varied with respect to the initial extract, yielding values of 1.68 for F_2A-P_1 and 3.09 for F_2A-P_2. Further studies in progress will throw light on the fine structure of the system of galactans from *Grateloupia turuturu.*

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FUCOIDANS FROM THE BROWN SEAWEED *SCYTOSIPHON LOMENTARIA*

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Brown seaweeds biosynthesize a variety of polysaccharides, namely alginates, laminarans and fucoidans[1]. The fucoidans contain large amounts of L-fucose and sulfate, together with minor amounts of other sugars like xylose, galactose, mannose and uronic acids[2,3]. For the fucan moiety, most examples show 3-linked α-L-fucopyranosyl units, but some products carry also 4-linked and 2-linked units, and fucofuranosyl non-reducing terminals[4]. In recent years the fucoidans have received major attention given their important and promising biological activities[5] (anticoagulant, antitumoral, antiinflamatory and antiviral) and biomedical applications[1]. In this work we present the chemical analysis and the antiherpetic activity of fucoidans from *Scytosiphon lomentaria.* This brown seaweed was collected in summer at the shores near Comodoro Rivadavia (Chubut Province, Argentina). In order to obtain such polysaccharides, the milled alga was extracted with 80% ethanol at room temperature and then at 70 ºC (each for 24 h), followed by HCl pH 2 at room temperature, 7 h. The products extracted with HCl were precipitated with cetrimide and subsequently fractionated by redissolution with solutions of increasing concentrations of NaCl. The extract and the fractions were chemically characterized. In addition, on some fractions, structural elucidation studies were carried out. The extract showed important percentages of sulfate, and fucose and galactose as major sugars. Glucose was predominant in the fraction that remained soluble after the addition of the cationic detergent, indicating that some laminaran is present. The other fractions showed an increasing proportion of sulfate as the ionic strength raised up to 3 M, whereas the uronic acids showed the opposite tendency. The fraction redissolved with 0.5 M of NaCl showed a heterogeneous composition with important percentages of xylose and mannose. The fraction redissolved in 2 M NaCl proved to be mainly composed of fucose, mannose, xylose and galactose, while that of 3 M NaCl evidenced to be a practically pure galactofucan. In this last fraction, fucose residues were found linked in positions 2-, 3- and 4-, with terminal fucopyranose units sulfated in O-3 and O-4; whereas that obtained with 2 M NaCl, showed 3-linked fucose units, sulfated and branched in O-2, O-4 or in both positions. The soluble fraction and that redissolved with 0.5 M NaCl were inactive against HSV-1, whereas the original extract and galactofucan fraction showed the highest activity against this virus with IC_{50} ranging from 0.76 ± 0.06 to 1.34 ± 0.13, without evidence of cytotoxicity (CC_{50}> 1000 µg / ml).

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SCREENING METHODOLOGY FOR EVALUATION OF CHITIN DEACETYLASE LIBRARIES: APPLICATION TO *VIBRIO CHOLERAE* DEACETYLASE MUTANT LIBRARIES FOR ENGINEERED SPECIFICTY

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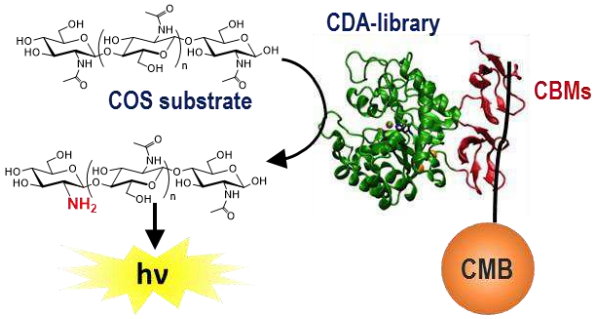
Directed evolution has proven to be a powerful and reliable strategy to modify and improve enzymes with regard to their catalytic efficiency, stability or substrate specificity [1]. The key to a successful directed evolution approach is the selection or screening technique to be applied. In order to evolve a certain property, diversity in the protein sequence is created and the screening effort depends on the library size, the larger the more distant are the new targets from the original properties. Even though the general trend was to increase the library size, sequential rounds of "focused" randomization and screening of smaller libraries has proven to be an interesting option in order to optimize the hit prospecting efforts (Iterative Saturation Mutagenesis and focused random mutagenesis strategies) [2].

In our group, we are aiming at the development of a biotechnological platform for the production of chitosan oligosaccharides with defined patterns of acetylation (paCOS), combining protein and metabolic engineering approaches. In order to achieve our goals, directed evolution of relevant enzymes involved in the chitin transformation routes (deacetylation, hydrolysis, synthesis…) is seen as an engineering tool that could accelerate the discovery of new materials and applications.

We solved the first 3D structure of a chitin deacetylase from *Vibrio cholerae* (*Vc*CDA) in complex with different substrates helping us to get structural insights into substrate specificity [3,4]. In addition to rational structure-guided engineering towards novel specificities, we are also applying directed evolution approaches, for which efficient high throughput screening (HTS) assays are required.

Here, we report the design and development of a HTS assay in microplates format using a medium scale robotic platform for the screening of directed evolution libraries of CDA and other CE4 enzymes active on COS and its proof-of-concept application to the engineering of substrate specificity of *Vc*CDA. The assay is based on fusion of the target CE4 catalytic domain to be evolved to a chitin binding module (CBM), capture of the expressed proteins from cell-free extracts with chitin-coated magnetic beads, and evaluation of the deacetylase activity of the immobilized enzyme variants on COS substrates by monitoring product formation with a coupled assay leading to a fluorescence readout [5].



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ATOMIC FORCE MICROSCOPY INVESTIGATION OF THE BIOFILM POLYSACCHARIDE FROM *BURKHOLDERIA MULTIVORANS* C1576

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Biofilms (BF) are the most common mode of bacterial life. To setup biofilms bacteria adhere to a surface, or to other cells, and biosynthesize a macromolecular matrix which surrounds the cells, entrapping useful molecules. The major BF matrix components are exopolysaccharides (Epols) which constitute an aqueous gel-like structure. In diseases, BF-associated infections are particularly relevant because they are very difficult to eradicate. Therefore, the mechanisms involved in matrix stability and the role of matrix components in BF functions are important. We investigated the structure of Epols extracted from biofilms of different species of the *Burkholderia cepacia* Complex, a group of opportunistic pathogens causing serious lung infections. The one produced by *B. multivorans* strain C1576 (EpolC1576) is constituted by Rha sequences bearing also methyl substituents (Figure 1)[1]. These features suggested a less polar nature of the Epol backbone with respect to other polysaccharides, as proved by its ability to bind aromatic fluorescent probes[2]. In addition, molecular modeling showed that the EpolC1576 forms a flexible random coil conformation in aqueous solution, with transient hydrophobic domains that may accommodate nonpolar guest molecules[2].

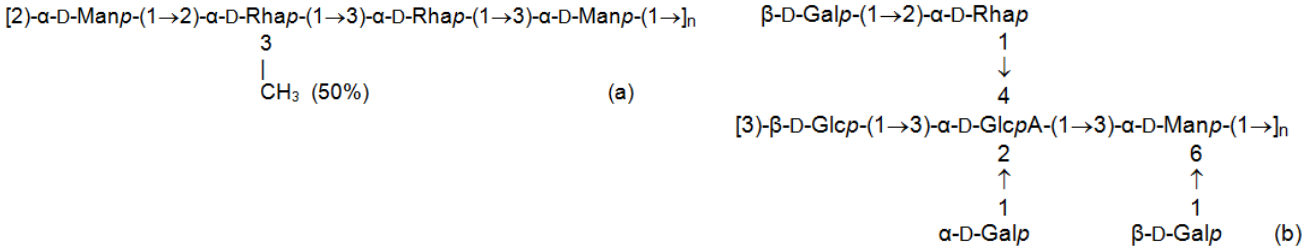

Fig. 1. repeating units of EpolC1576 (a) and cepacian (b)

To investigate the role of this Epol in biofilms, AFM experiments were performed by spray-drying polymer solutions on mica surfaces. The obtained images revealed a spherical shape of the whole polysaccharide confirming the flexible nature of the backbone. In addition, at high Epol concentrations AFM showed large aggregates with packing typical of spherical objects. The aggregation on one side confirmed the hydrophobic nature of the Epol and, on the other hand, suggested its role in biofilm matrix formation.

For comparison, AFM experiments on the polysaccharide cepacian (Figure 1) which is produced by the same strain but in non-biofilm conditions will be discussed.

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NMR AND MOLECULAR DYNAMICS STRUCTURAL STUDIES OF GLYCOSAMINOGLYCANS AND THEIR INTERACTION WITH MIDKINE AND PLEIOTROPHIN

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Midkine (MK) and Pleiotrophin (PTN) are members of a family of Glycosaminoglycan-binding growth factors which play an important role in early central nervous system development, as well as in pathologies as inflammation and cancer. Recent studies have demonstrated that both cytokines strongly bind to some glycosaminoglicans (GAGs) as heparin and chondroitin sulfate chains (CS), giving rise complexes that can modulate important biological processes [1] (**Figure 1**). For this reason, have been carried out studies using fluorescence polarization which has allowed us to characterize protein-ligand binding of glycomimetics of several GAGs in solution [2].

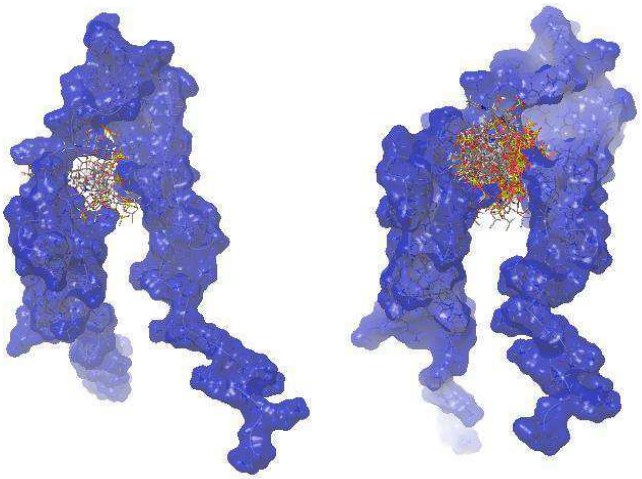

Fig. 1. Three-dimensional models of the complex between CS tetrasaccharides and midkine obtained by docking (Glyde).

On the other hand, in order to characterize the molecular bases of this interaction we have analyzed it using NMR transient techniques (NOESY and STD) complemented with molecular dynamics calculations. [3] Here we report the results obtained from the combined approaches and preliminary conclusions.

Acknowledgements:

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O-ANTIGEN POLYSACCHARIDE CONFORMATIONS OF *SHIGELLA FLEXNERI* SEROGROUP 2

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Shigella is a family of bacteria that are among the main diarrheal disease-causing pathogens worldwide. These diarrheal diseases have a high morbidity and claim more than 800 000 annual fatalities - predominantly young children in poorly developed regions[1]. *Shigella flexneri* is the dominant cause of shigellosis infection - causing up to 60% of shigellosis cases. Shigella strains are serotyped based on the O-antigen lipopolysaccharide (LPS) structure which is strain specific and the target of vaccine development. Except for serogroup 6, the *S. flexneri* serotypes share a common tetrasaccharide repeating unit backbone: →2)-α-L-Rha*p*^{III}-(1→2)-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→3)-β-D-Glc*p*NAc-(1→. Serotypes differ mainly by site-selective glucosylation and/or O-acetylation. The similarity of *S. flexneri* serotypes suggests that a large degree of cross-protection may be possible. It has been suggested that broad vaccine protection is possible against 15 serotypes of *S. flexneri* by making use of shared group antigens in a vaccine that includes just four serotypes[2].

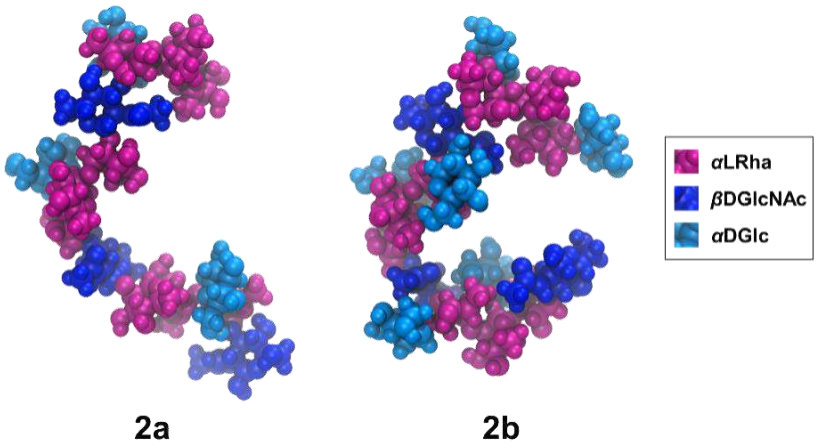

Fig. 1. Representative 3RU conformations of the dominant clusters for *S. flexneri* serotypes 2a and 2b.

S. flexneri serotype 2a is most prevalent worldwide and has Glc at O4 of Rha^{I}. Serotype 2b has the same structure as 2a but with an additional Glc at O-3 of Rha^{III}. Glucosylation has been shown to have a significant impact on the conformation of the O-antigen LPS by changing the linear backbone to adopt a more compact structure which was also shown to increase virulence[3]. A previous study of the conformational preferences of *S. flexneri* O-antigens reported the individual linkage conformations from relatively short molecular dynamics simulations supported by NMR NOE analysis[4]. This work expands the investigation of *S. flexneri* LPSs by analyzing the oligosaccharide conformations of three repeating units (3RU) of *S. flexneri* serotypes 2a and 2b. Initial results show that both serotype 2a and 2b adopt slightly curved and twisted conformations (Figure 1). The simulations are run for a relatively long time period (300 ns) in aqueous solution and the dynamic conformational changes are then investigated by clustering analysis.

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CONFORMATIONAL PHASE SPACE ANALYSIS OF LINKAGE-ISOMERS α-MANNOSE DISACCHARIDES BY HAMILTONIAN REPLICA EXCHANGE MD SIMULATIONS AND NMR EXPERIMENT

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Mannose-containing oligosaccharides show the highest variability in terms of the types of inter-glycosidic linkage isomers between the individual sugar residues. This differentiation, together with the possibility for the mannose to be present in both the epimeric forms at the anomeric center, leads unequivocally to a complex net of conformational topologies which are associated with distinct biological roles. A range of mannose-containing biomolecules that cover these structural motifs are widely found in nature. Foremost amongst these structures are the N-glycans associated with immunity and infectious disease, particularly the mammalian high mannose-type N-linked glycan which plays an important role in the folding of glycoproteins in the endoplasmic reticulum[1]. Further mannose-containing forms can be found in the cell wall polysaccharides of *Saccharomyces cerevisiae*[2] and *Mycobacterium smegmatis*[3] or in glycosylphosphatidylinositol anchored glycoproteins.

In this context, the main objective of the project is to build up a coherent understanding of the dynamics regulating these structures, exploring their conformational phase space by making use of robust computational and spectroscopic approaches. A series of α-linked mannose-disaccharides units comprising all the regioisomeric forms are considered and elucidated as reference building units of these biomolecules.

A dual approach to this end shall be considered:

- A number of NMR methods (e.g. J-HMBC,[4] 1DLR,[5] 1D-HMBC[6]) relying on spin-spin coupling constant measurements are employed on both 13C-enriched and unlabeled samples, with the aim of primarily define Karplus relationships between interglycosidic torsion angles (φ, ϕ) and the corresponding 3*J*CH.
  Further conformational dependence relationships, based on finite perturbation theory (FTP) formulation in semiempirical INDO methods,[7] of anomeric 1*J*CH on the dihedral angle, ϕ, are considered as validation.
  Complementary conformational sensitive parameters obtained by measurements of nuclear Overhauser (NOE) and transverse rotating-frame overhauser (T-ROE) effects are utilized to obtain effective proton-proton distances across the glycosidic linkages.
- Atomistic details of the conformational sampling are obtained with the CHARMM36 additive (AFF) and Drude polarizable force fields (PFF) for carbohydrates.[8] Hamiltonian Replica-Exchange with concurrent solute scaling and biasing potentials (HREST2-BP) are used for enhanced conformational sampling to obtain an understanding of the full range of conformations sampled by the mannose disaccharides. The biasing potentials used to enhance sampling are based on 2-dimensional grid-based correction maps (bpCMAP) along the torsional dihedrals φ/ϕ of each glycosidic linkage.

A comprehensive structural description of these structures is meant to be achieved along with the evaluation of the AFF/PFF and the HREST2-BP method as tools for the investigation of the conformational properties underlying these biologically relevant molecules.

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HIGHLY BRANCHED XYLOGALACTOFURANAN ISOLATED FROM BIOMASS OF HETEROTROPHIC MUTANT MICROALGA *CHLORELLA VULGARIS* G11

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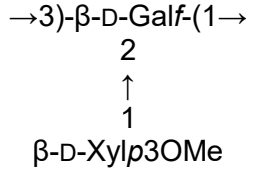
Cultivating microalgae, both marine and freshwater species, have fast growing interest for many researches. Microalgal biomass is a rich source of various nutritive, energy-rich and bioactive compounds that make it useful as biofuel or food supplement [1-3]. Among these compounds, specific cell wall polysaccharides may have immunostimulatory and antitumor effects[3,4]. There are several mutant strains of green microalga Chlorella with high level of lutein, a yellow carotenoid pigment widely used in food, pharmacy and cosmetics[5, 6]. These mutants are also interesting as potent producers of unusual polysaccharides.

The present study is focused on isolation, purification and characterization of polysaccharides produced by heterotrophic mutant form of microalga *Chlorella vulgaris* G11. Macromolecules (proteins and polysaccharides) were isolated from discolored and defatted biomass by subsequent extractions with water at various temperatures (20°, 60° and 100°C) yielding crude fractions F1a-c. The proteins were removed from these fractions by the treatment with proteolytic enzymes and precipitation with Cu^{2+}. Purified polysaccharides were then separated by preparative gel chromatography using Biogel p-100 (Bio-rad, USA) as stationary phase. Purity and composition of isolated polysaccharides were monitored by organic elemental analysis, GC/FID/MS for monosaccharide composition and linkage analyses, GPC for molecular mass, FTIR, FT Raman and correlation NMR spectroscopy.

The preparative chromatography of purified fraction F1c (boiling water extract) yielded three mayor fractions PS-1 (2500 KDa), PS-2 (136 kDa) and PS-3 (25.8 kDa). The last one demonstrated significant difference in spectra and composition in comparison with the previous two. Xylose and galactose were found to be the mayor units of PS-3, while PS-1 and PS-2 were rich of rhamnose and contained xylose and galactose in lesser amounts. Methylation analysis and correlation NMR confirmed that PS-3 consisted of terminal *O*-3-methoxy-β-xylopyranose and 1,3-bound β-galactofuranose units at nearly equimolar ratio. The terminal non-methylated β-xylopyranose and α-rhamnopyranose were found as minor units. This polysaccharide was defined to be branched xylogalactofuranan having xylose units attached at *O*-2 of the (1-3)-β-galactofuranan backbone:



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MOLECULAR INSIGHTS INTO THE INTERACTION BETWEEN DC-SIGN AND THE αGAL EPITOPE THROUGH NMR

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DC-SIGN is a well-studied C-type lectin that plays important roles in the immune regulation.[1] Its promiscuity in terms of glycan binding specificity is very remarkable and has been highlighted in different glycan array studies.[2] [3] Even though it is usually described as a Mannose and Fucose binding lectin, it can also bind terminal αGal, but not αGalNAc nor βGal, with weaker affinity. Herein, we have used NMR and molecular modeling strategies in order to gain insights into the molecular recognition between the Carbohydrate Recognition Domain of DC-SIGN and the aGal epitope. Even though much weaker in affinity with respect to the interaction with Fucose and Mannose, this interaction could contribute to its high promiscuity to oligosaccharides and to explain observed differences in selectivities between Gal and GalNAc containing glycans such as A/B Blood group antigens.[4]

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POLYSACCHARIDES PROFILE OF *PORPHYRIDIUM CRUENTUM* MICROALGAE

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Microalgae are photosynthetic microorganisms considered an important and promising source of high added-value compounds, namely fatty acids and other lipids, essential amino acids and polysaccharides. *Porphyridium cruentum* is a red saline microalga that have raised particular interest due to its ability to excrete into the medium high levels of sulfated polysaccharides (sEPS) [1], recognized as compounds exhibiting a wide range of biological activities, promising application in several economic sectors [2]. The production of those polysaccharides depends on the growth medium conditions, having influence in the amount, as well as on polysaccharides structures [3, 4], and ultimately on biological activity (structure-activity relationship). Thus, the present work consists on the production of the microalgae *P. cruentum* in three different salinities to study their influence in the production yields and structure of extracellular polysaccharides.

P. cruentum was grown by Necton, S.A., Portugal, at 18, 32, and 50 g/L NaCl media in photobiorreactors at 20-21ºC with air bubbling and solar light, performing a photoperiod of 12/12 hours of light/dark. To obtain the exopolysaccharides, the growth medium was centrifuged (4500 rpm, 10 min) to remove the biomass, boiled during 30 min to stabilize the medium, and centrifuged again. The supernatant was dialyzed (12-14 kDa) in order to obtain the polymeric material, observing that the medium with 18 and 32 g/L of salinity yielded more polymeric material (115.5 and 149.8 mg per liter of growth medium, respectively), compared with the higher salinity (50 g/L), which only produces 78 mg/L. The polymeric material was mostly constituted by polysaccharides (65-68%), 4.1-5.3% of sulfate esters, and 2.1-3.6% of protein. The characterization of each sample revealed that all samples presented a similar polymeric material composition. The sEPS were mostly composed by xylose, followed by galactose, uronic acids, glucose, and minor amounts of mannose. An ethanol precipitation at 83% was performed to purify the sulfated EPS, allowing enrich up to 80% of the total mass in polysaccharides in the precipitate. The glycosidic linkage analysis of the precipitate and supernatant of the three salinity samples revealed highly branched polysaccharides due to the presence of high content of terminally-linked Xyl (20%) and 2,3,4-Gal (15%). An analysis of methylation before (native) and after desulfation were performed allowing to determine that the sulfate esters were mainly at C-6 of Glc and, in minor extent, at C-3 of Glc and C-6 of Gal, for all the samples. The main linear linkages are 3- and 4-Xyl, and 3-Glc. There was no great difference between the polysaccharides glycosidic-linkage composition of supernatants and precipitates or between the different salinities.

From the above, it is proposed that the environmental salinity (32 g/L) can be used to produce sEPS of *Porphyridium cruentum* that could be used in several fields, namely for biomedical purposes.

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DIFFERENTIAL EPITOPE MAPPING- (DEEP) STD NMR TO REVEAL THE NATURE OF PROTEIN-CARBOHYDRATE CONTACTS

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Saturation Transfer Difference (STD) NMR spectroscopy is extensively used to obtain epitope maps of ligands binding to protein receptors under fast exchange conditions [1], [2]. STD NMR reveals structural details of biomolecular recognition processes, which are fundamental to direct lead optimisation efforts in drug discovery, and is an excellent technique for structural studies of protein-carbohydrate interactions.

Standard procedures seek uniform saturation of the receptor to identify regions of the carbohydrate contacting the protein binding pocket. However, in this way, the experiment does not provide information about the “nature” of the amino acids surrounding the ligand in the bound state.

Here we report a novel protocol (*DiffErential EPitope Mapping*-STD NMR or DEEP-STD NMR) to identify the type of protein residues contacting the carbohydrate [3]. We demonstrate that the approach constitutes a novel versatile method to *orthogonally* explore the nature (aliphatic, aromatic, polar or hydrophobic) of the amino acid residues lining the surface of the binding pocket and their orientation relative to the ligand.

As a proof of principle, we selected two relevant protein-ligand interactions from different areas of interest: i) the interaction of 3-nitrophenyl-α-galactoside (3NPG) with subunit B of Cholera Toxin (CTB) [4] and ii) the interaction of 2,7-anhydro-Neu5Ac with the intramolecular *trans*-sialidasefrom *Ruminococcus gnavus* belonging to glycoside hydrolase GH33 family [5], an important contributor of gut microbiota adaptation to the host [6]. For both systems, high resolution X-Ray structures are available, allowing us to validate the protocol. The approach is solid, versatile and promises to expand the use of STD-NMR beyond its current limitations. We envision that DEEP STD NMR will become a valuable tool in the field of structural glycobiology.

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HYDROXYPROPYL β CYCLODEXTRIN (HPBCD) SUBSTITUTION PATTERN: QUANTIFICATION OF SUBSTITUTED HYDROXYL MOITIES AND COMPARISON

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Cyclodextrins (CD) are cyclic oligosaccharides made of α-D-glucopyranose units linked by α 1→4 glycosidic bond. Naturally released by bacterial degradation of starch, they have attracted lots of attention for their solubilization properties related to their unique structure: a hydrophobic cavity surrounded by an outer hydrophilic layer. β-CDs consist of 7 α-D-glucopyranose units and can form inclusion complexes with a wide range of chemical molecules. Therefore, they have been extensively studied as technological solutions for active pharmaceutical ingredient (API) solubilization and bioavailability improvement, catalytic applications, biopolymers or new drug delivery systems. However, their poor water solubility might hinder several applications. Substituting hydroxyl moieties of β-CD with hydroxypropyl groups is one of the solutions to increase their water solubility and decrease their toxicological potential [1]. HPBCD are thus widely used as excipients for pharmaceutical applications but also as API for neurodegenerative diseases related to cholesterol imbalance [2]. As a consequence, legal authorities are asking for better analytical characterization of HPBCD products and their relative impurities. HPBCD are currently characterized by the degree of substitution (DS) which only reflects a mean substitution average. DS can be measured by various analytical techniques such as nuclear magnetic resonance (NMR) or mass spectrometry (MS) [3].

Improved characterization of HPBCD substitution pattern can be achieved by 2D NMR but gives poor quantitative information. Here, a chemical method was optimized to better characterize different batches of HPBCD. HPBCD products were permethylated, hydrolysed and acetylated [4]. The objective was first to characterize all types of substituted anhydroglucose units in experimental batches of HPBCD prepared in Roquette Frères Laboratory and then to compare them to competitive HPBCD products. Each type of hydroxyl substitution was quantified by GC-MS. Generated molecules were characterized by GC-MS and further quantified by GC-FID. A total of 6 samples, including the 2 experimental laboratory batches of HPBCD, have been characterized. Statistical analysis of the results revealed significant differences in the localization of the hydroxyl groups for products having similar DS. This study highlights the preferential substitution site per product and this could bring insight to better explain the various application properties of HPBCD products having a similar DS.

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PEA-DERIVED MALTODEXTRINS AS SOLUBILIZATION ENHANCERS

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Objectives: Nutraceutical ingredients market has increased in food industry, although technological issues remain unsolved. They need to be bioavailable in body fluids. Enhancing their solubility can be achieved by forming inclusion complexes using cyclodextrins or amylose. Maltodextrins are a complex mixture of low and high molecular weight molecules released from starch hydrolysis. They have been largely investigated for their technological properties as bulking macromolecules replacements [1]. Maltodextrins have been recently studied as potential complexing agents for active ingredients in aqueous solution such as ibuprofen [2]. Here, we investigated pea-derived maltodextrins – pea is naturally rich in amylose – as potential solubilizer.

Methods: 1-naphthol was selected as host-molecule for its low water solubility and physico-chemical features. Solubilization was performed in an aqueous solution 1:1 molar ratio of either maltodextrins or betacyclodextrins, these latter being used as an inclusion complex control. Supernatants were freeze-dried and characterized by vibrational spectroscopy (FTIR & Raman), liquid and solid NMR, XRD and microscopy (SEM & cryo-TEM).

Results: Pea-derived maltodextrins achieved in fully solubilizing 1-naphthol in aqueous solution. 2D NMR NOESY experiments pointed out specific interactions between 1-naphthol and maltodextrins. The involvement of the substituted benzene ring in 1-naphthol solubilization was highlighted by both FTIR and Raman spectroscopy. XRD and solid-state NMR didn't reveal typical amylose-complex fingerprint [3], meaning that different mechanisms enabled 1-Naphthol solubilization. SEM analysis of the betacyclodextrins-naphthol complex evidenced planar crystals. On the other hand, the analysis by SEM showed coiling-like layered microstructure whereas the TEM identified small organized domains.

Conclusion: Although 1-naphtol has low water solubility, we manage to fully solubilize it with maltodextrins. As maltodextrins contain linear amylose degradation products, it was assumed that their solubilization properties could be explained by inclusion complexe formation. However, the results could not support this hypothesis. This model would rather reflect a different structural organization induced by maltodextrins [4]. Interactions between 1-naphthol and maltodextrins were partially identified and mainly attributed to hydrogen bonding. This study could be extrapolated to natural bioactive ingredients solubilization using maltodextrin as low-price biosourced solubilizer.

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POSTERS | MD – Carbohydrates for Medicine and Diagnosis

INVESTIGATING *EXO*-GLYCALS REACTIVITY RECENT PROGRESS AND APPLICATIONS

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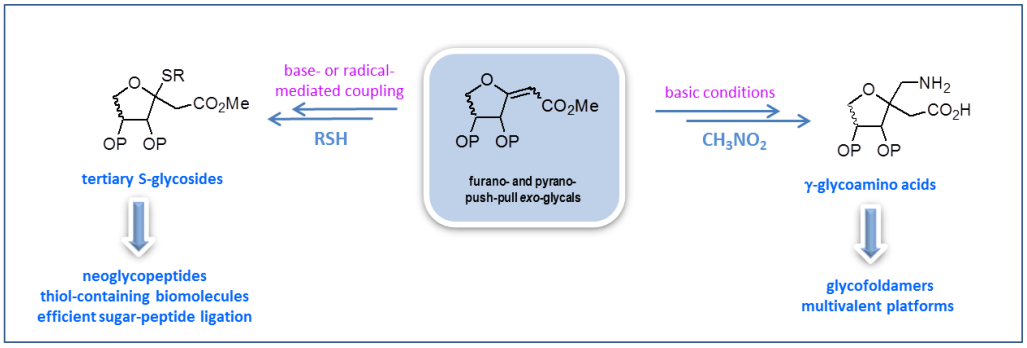
Exo-glycals or *C*-glycosylidene compounds having an exocyclic double bond at the anomeric centre are readily available by Wittig olefination of carbohydrate lactones with stabilized phosphoranes [1]. Investigating the chemistry of this class of unsaturated carbohydrates possessing an interesting push-pull substitution of the double bond, characterized by an electron-withdrawing and an electron-donating group, is a challenging and innovative task [2].

For original access to new compounds of interest in the field of carbohydrates, *exo*-glycals can be valuable substrates in the well-known Michael addition. This prompted us to investigate the formation of a carbon-sulfur bond at the quaternary center by the addition of various thiols derivatives. The hydrothiolation of the trisubstituted double bond was performed with different thiols, thio-sugars, and thiol containing amino acids and peptides under radical and basic conditions. This opens the way to an efficient sugar-peptide ligation method and permits the preparation of tertiary *S*-glycosides, complex neoglycopeptides and thiol-containing biomolecules [3].

Exo-glycals can also be valuable substrates for Michael additions of nitroalkane anion to access new interesting bis *C*,*C*-glycosyl compounds. These can be subsequently converted into the corresponding anomeric γ-amino acids and incorporated in glycopeptides for folding properties studies (glycofoldamers) [4,5] and multivalent platforms synthesis [6].



Both approaches and their scopes and limitations will be presented and their applications will be discussed.

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(AUTOMATED SOLID PHASE) SYNTHESIS OF *S. AUREUS* WALL TEICHOIC ACID FRAGMENTS

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Staphylococcus aureus is a gram-positive bacterium that causes (hospital acquired) infections to the skin, respiration and bloodstream [1]. The peptidoglycan of *S. aureus* is highly functionalized with anionic polymers, called wall teichoic acids (WTAs). These polymers consist of polyribitol phosphates (RboP) and they are modified with D-alanine and/or α- and β-*N*-acetyl glucosamine (GlcNAc) residues. WTAs are involved in cell shape, cell division, biofilm formation, phage infectivity, and pathogenesis as well as resistance to cationic antimicrobial peptides [2].

It has been shown that β-O-GlcNAcylation is required to maintain β-lactam resistance in Methicillin-resistant *S. aureus* (MRSA), and GlcNAc residues have been found to function as antigenic epitopes of *S. aureus* WTAs for serum IgG and as ligands for mannose binding lectins (MBL) [3]. Furthermore, WTA-β-GlcNAc epitopes promote anti-WTA IgG-mediated complement activation leading to opsonophagocytosis [3]. These findings make GlcNAc-WTAs promising candidates for immunological studies, which ultimately may lead to the development of new and improved vaccines against *S. aureus* and MRSA-infections. However, the isolation of WTAs is laborious and results in mixtures of heteregeneous WTA fragments, which makes structure-activity relationship studies difficult or even impossible.

In this study we report the synthesis of well-defined polyribitol phosphate fragments, unsubstituted or decorated with α- and/or β-GlcNAc and D-alanine residues. The fragments have been equipped with a spacer handle for further conjugation purposes (Fig 1). We also describe the first automated solid phase synthesis of a WTA octa- and dodecamer. Initial immunological evaluation of the fragments will be presented.

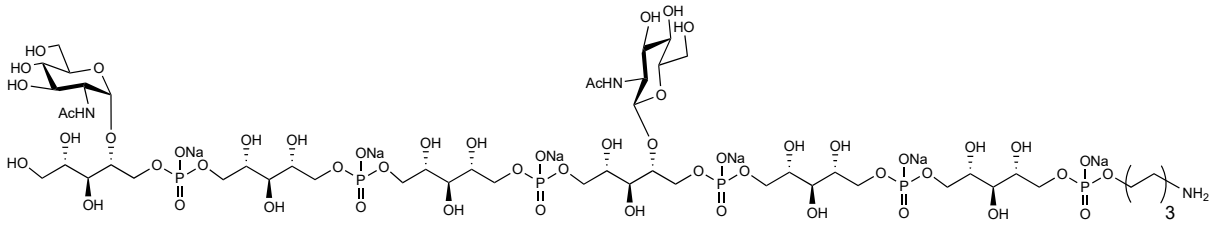

Fig. 1 – One of the generated WTA fragments

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GLYCOSIDASE ACTIVATED GLYCONAPHTHALIMIDE PRODRUGS FOR ANTICANCER APPLICATIONS

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The administration of certain chemotherapeutic drugs is hindered due to their lack of specificity in targeting tumors cells, leading to undesired side effects. Fluorescent probes for the diagnosis of cancers often employ specific interactions with cell-receptors, which are over-expressed in cancerous tissues, helping with the cancer diagnosis and subsequent removal through surgery. Small-molecule glycoconjugates are widely used as both therapeutic agents and biological probes. The glycan unit increases the hydrophilicity and lectin binding properties, which modulates tumour cell targeting and cellular uptake [1].

We have developed a new class of glycosylated naphthalimides, which offer the possibility of releasing therapeutic agents in a controlled manner using naturally occurring enzymes (glycosidases) that are over-expressed in the tumour environment. This offers an attractive approach for the release of therapeutic payloads selectively in tumour sites, circumventing certain side-effects. Glycosylated naphthalimides remain inactive and outside of cells prior to enzymatic release, thus functioning as effective prodrugs (Fig. 1). This methodology has been successfully validated in three different cell lines, evaluating their cell viability and following the process with confocal microscopy [2]. Due to the excellent photophysical properties of these molecules, in addition to the release of therapeutic agents, they have applications as fluorescent probes for cancer imaging and diagnosis, acting as a multifaceted tool in both the treatment and diagnosis of cancer.

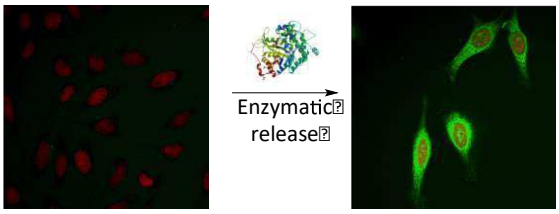

Fig. 1 – Enzymatic release of a fluorescent glycosylated-naphthalimide in HeLa cells. Imaged using confocal microscopy

Current work is focused on the use of this enzyme-dependent release process for the release of cytotoxic drugs. We have developed a prodrug of Amonafide, a topoisomerase II inhibitor that exerts excellent cytotoxicity for different cell lines but failed in clinical trials due to undesired side effects [3], with the aim of evading these adverse responses. This new glycosylated naphthalimide is able to release Amonafide (naphthalimide unit) after enzymatic exposure, inducing cell death.

In conclusion, a family of glycosylated naphthalimides that can be activated by glycosidases has been developed. The activated compounds undergo rapid cell uptake in a range of cancer cell lines. This approach is being used successfully for the release of therapeutic agents through activation by glycosidases.

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EXPLORING THE CA19-9 GLYCOPROTEOME OF BORRMANN TYPE IV ADVANCED GASTRIC CANCER FOR GUIDED THERAPEUTICS

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Histologically Borrmann type IV advanced gastric carcinomas are an heterogeneous group of tumours characterized by rapid cancer cell proliferation and infiltration accompanied by extensive stromal fibrosis, presenting poorly differentiated cells or signet ring cells [1]. It accounts for approximately 10% of all gastric cancers and its prognosis is worse than the other types of gastric cancer, due to aggressive behavior and the lack of effective therapies. These observations highlight the urge for novel targeted therapeutics based on the biological behavior of this disease. The carbohydrate antigen sialyl Lewis a (CA19-9) is the most commun serum marker for the diagnosis of digestive cancers. It is a ligand for vascular cell adhesion molecule E-selectin and facilitates hematogenous metastasis through mediating adhesion of cancer cells to vascular endothelium [2]. Nevertheless, the CA19-9 antigen has never been studied in Borrmann type IV tumours. Moreover, the CA19-9 antigen has been frequently detected in non-malignant cells of different organs hampering its potential for targeted therapeutics. Here we devote to addressing the CA19-9 antigen in the context of Borrmann type IV tumours and providing a comprehensive glycoproteomics approach envisaging novel and more specific targeted therapeutics.

This work aims to provide insights on the clinical significance of CA19-9 in Borrman type IV tumours and cancer associated glycoproteins yielding the CA19-9 for target for guided therapies. The expression and clinical significance of CA19-9 was evaluated in a retrospective design including 21 formalin-fixed, paraffin-embedded Borrmann type IV tumors and 18 lymph node metastases. Targeted glycoproteomics studies were performed in the OCUM-1 cell line overexpressing the CA19-9 antigen by nanoLC-ESI-MS/MS. The validation of relevant CA19-9 expressing glycoproteins was performed by in situ proximity ligation assays (PLA) and western blot in clinical samples. The CA19-9 expression on tumors and lymph nodes metastasis is 90.5% and 83.3%, respectively. Also, the expression of this antigen and the presence of distant metastasis was significantly correlated ($p=0.03$) as well as the correlation with lymphocytic infiltrate ($p=0.04$). Furthermore, the OCUM-1 cell line express 60% of this antigen make them a good cell line model of Borrmann type IV gastric cancer. Glycoproteomics analysis followed by bioinformatics data curation resulted in the identification of 23 CA19-9 expressing glycoproteins reported to yield clinical significance in other forms of gastric cancer. In particular we have identified several key cancer-associated glycoproteins related with cell-adhesion. The expression of clinical relevant glycoproteins were confirmed by western blot and PLA; more importantly it was not detected in apparently histologically normal tissues adjacent to the tumour. Our results show the clinical relevance of CA19-9 in Borrmann type IV gastric cancer. Future studies should devote to addressing the clinical and biological significance of these findings towards novel therapeutic approaches.

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MULTIVALENT CYCLOPEPTIDES-BASED GLYCOCONJUGATES FOR LECTIN INHIBITION

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Synthetic glycoclusters and glycodendrimers have stimulated increasing interests over the past decade. Among the large variety of multivalent scaffolds reported so far, our group is focusing on cyclopeptide-based glycoconjugates for diverse biological applications. In this context, well-defined structures with various size, sugar density and combination have been prepared using either single or orthogonal chemoselective procedures. In this presentation, we will present the synthesis and evaluation of nanomolar lectin ligands [1].

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NEW CLASS OF C-MANNOPYRANOSIDE ANTAGONISTS AGAINST ADHERENT-INVASIVE INFECTIONS

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Antagonists of the uropathogenic *Escherichia coli* type-1 fimbrial adhesin (FimH) are recognized as attractive alternatives for antibiotic therapies and prophylactic strategies against acute and recurrent bacterial infections.

In this study, C-linked α-D-mannopyranosides possessing aromatic aglycones were investigated to fit within the hydrophobic sugar binding pocket of the FimH tyrosine gate (Tyr48-Tyr137). The results were summarized into a set of structure-activity relationships to be used toward designing potent FimH inhibitors. Alkene linkers afforded improved affinity and inhibitory potential, because they could provide favorable binding interactions with hydrophobic side chains located in the middle of the tyrosine gate [1].

Of particular interest was a C-mannoside derivative, prepared by a Heck reaction between a family of aryl iodides and C-allyl α-D-mannopyranoside. One of them, an *ortho*-substituted biphenyl aglycone, showed an affinity enhancement in the nM range. Docking of its high-resolution NMR structure to the FimH adhesion, indicated that it could present its *ortho*-substituted phenyl ring directly in contact with isoleucine-13 (Ile13), located in the clamp loop that undergoes conformational changes under shear force exerted on the bacteria upon binding to its receptor (Figure1) [1]. Molecular dynamic simulations confirmed that a subpopulation of the C-mannoside conformers were able to interact in this secondary binding site of FimH, thus unraveling a new mode of binding, useful in the design of potent inhibitors against the *E. coli* adhesion.

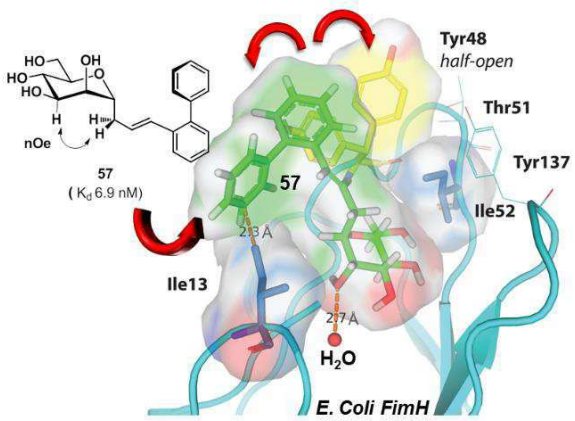

Fig. 1 – NMR-derived solution conformation of C-linked *ortho*-biphenyl *α*-D-mannopyranoside superposed in the FimH active site, (PDB: 4auy)

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SYNTHESIS OF FULLY ISOTOPE-LABELED HEPARAN SULFATE OCTASACCHARIDE

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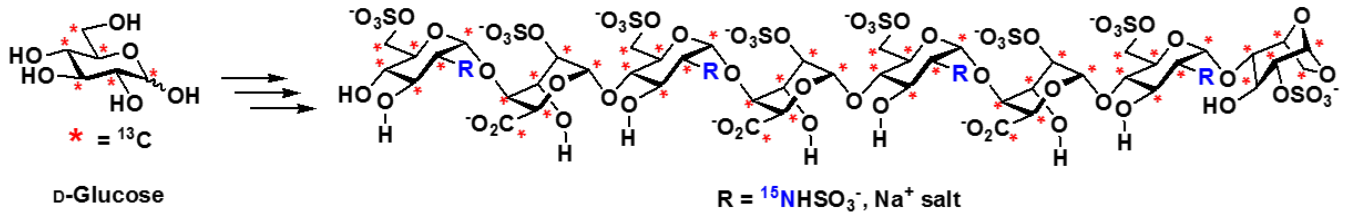
Heparan sulfate, which is a linear polysulfated polysaccharide, ubiquitously distributes on the cell surface. It's a known mediator of several biologically important events including viral and bacterial infection, cell growth, inflammation, wound healing, tumor metastasis, lipid metabolism, and diseases of the nervous system.



Here the first chemical synthesis of a uniformly ^{13}C- and ^{15}N-labeled HS was accomplished. To prepare the desired labeled octasaccharide, the full labeled building blocks GlcN and anhydro-L-idose were synthesized respectively from commercially available ^{13}C-labeled D-glucose. Both building blocks were designed to offer excellent stereoselectivity during glycosylations and permit the final functional group transformations. Convergent [2+2] and [4+4] glycosylation effectively generated the fully protected octasaccharide building blocks, follow by partial deblocking, selective oxidation, *O*-sulfonation, global deprotection, and final *N*-sulfonation delivered the target ^{13}C- and ^{15}N-labeled HS octasaccharide.

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NEW INSIGHTS ON THE STRUCTURE OF HEXAGONALLY FACETED PLATELETS FROM HYDROPHOBICALLY MODIFIED CHITOSAN AND α-CYCLODEXTRIN

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The ability of α-cyclodextrin to interact with linear alkyl chains was exploited in our research group to design non-spherical particles by a self-assembly process, in which a polysaccharide grafted with linear alkyl chains (C16-C18) was mixed to α-cyclodextrin in water [1-5]. These particles, called platelets because they have a flattened morphology, exhibited intrinsic biological activities. Heparin nano-platelets were used as biomimetic strategy against heparan sulfate dependent viruses (herpes simplex type 1 and type 2 viruses (HSV-1 HSV-2), human papilloma virus (HPV-16) and respiratory syncytial virus (RSV) [5]. The concept was applied to other glycosaminoglycans (*i.e.* chondroitin sulfate). The nano-platelets showed exalted anti-HSV-2 activity in comparison with native chondroitin sulfate [4]. More recently, we demonstrated the ability of chitosan micro-platelets to have synergistic activity when mixed with amphotericin B deoxycholate against *Candida albicans* [1] and *Candida glabrata* [2].

In this work, we varied the composition of lipids grafted on chitosan (oleic acid, palmitic acid or stearic acid) and characterized their size and morphology by transmission electron microscopy (TEM), cryogenic TEM, confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and atomic force microscopy (AFM). Whatever the alkyl chain grafted on chitosan, the platelets have a preferentially hexagonal shape with sharp edges, variable sizes, and thickness. The presence of terraces suggests a multi-layered structure intercalating the polysaccharide and lipids/α-cyclodextrin complexes [6]. Data also revealed that mixing of fatty acids with α-cyclodextrin resulted from solid particles with a crystalline structural organization [7] and that this interaction was the driving force for the formation of the platelets.

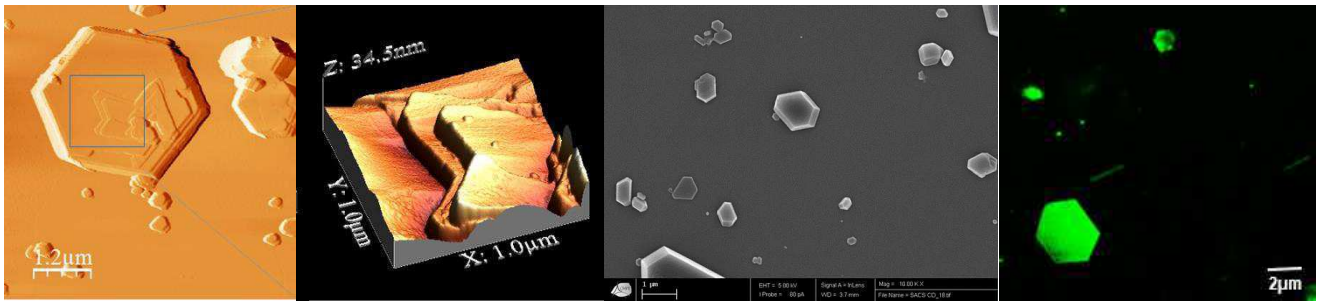

Fig. 1 – AFM, SEM and CLSM images of chitosan platelets composed of stearoyl chitosan/α-CD.

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DEVELOPMENT OF A NOVEL DRUG DELIVERY SYSTEM COMPRISING CARBOHYDRATES FOR DERMAL AND NAIL DELIVERY

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Predominantly, the majority of fungal infections (dermal and nail) are caused by dermatophytes, such as Trichophyton rubrum known as one of the most prominent. Among fungal infections, nail infections or onychomycosis exhibit the most difficulties and limitations in their treatment. Onychomycosis affects around 5-10% of the population in the world. Onychomycosis is a common infection of the nail caused by dermatophyte affecting mostly toenails in adults being associated with limited treatment options [1]. In this study novel dosage forms were prepared and evaluated for their suitability in treatment of onychomycosis. Films were prepared comprising polymeric excipients such as chitosan, (hydroxypropyl)methyl cellulose, hydroxyethyl-cellulose, carboxymethylcellulose according to solvent evaporation method. Developed formulations as shown in Table 1 were evaluated in terms of physical appearance, stability and adhesiveness.

Table 1: Composition of formulations containing polymers.

Ingredients	Chitosan [g]	Ethanol [mL]	HCl [mL]	HEC [g]	HPMC [g]	NaCMC [g]	PPG [g]	PVP [g]	TEC [mL]	Water [mL]
F1	-	20	-	1.0	0.2	0.4	0.23	-	-	-
F2	-	20	-	-	-	0.4	0.23	1.0	-	-
F3	0.5	20	10	-	-	-	0.23	0.4	-	-
F4	-	20	-	1.0	0.2	-	-	-	0.5	-
F5	-	20	-	1.0	-	0.4	0.23	-	-	10

HCl: Hydrogen chloride; HEC: Hydroxyethyl cellulose; HPMC: Hydroxypropyl methylcellulose; NaCMC: Carboxymethyl cellulose sodium salt; PPG: Polypropylene glycol; PVP: Polyvinylpyrrolidone; TEC: Triethyl citrate

Furthermore skin and nail irritation studies were conducted. Five potential formulations (F1-F5) were designed while F1 and F4 exhibited the most promising results in terms of stability with 26 min and 40.67 min, respectively, and suitability in nail application. F1 as the most favorable dosage form revealed with 2.9438 kg/m/s in terms of adhesive force the most adhesive properties in contrast to the other preparations. All formulations were found to be non-skin irritating and safe to use. Taken together, these findings suggest novel designed films containing polymeric excipients as a fruitful platform for the treatment in onychomycosis.

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CONCISE SYNTHESIS OF 2,7-ANHYDROSIALIC ACID DERIVATIVES AND ITS APPLICATION

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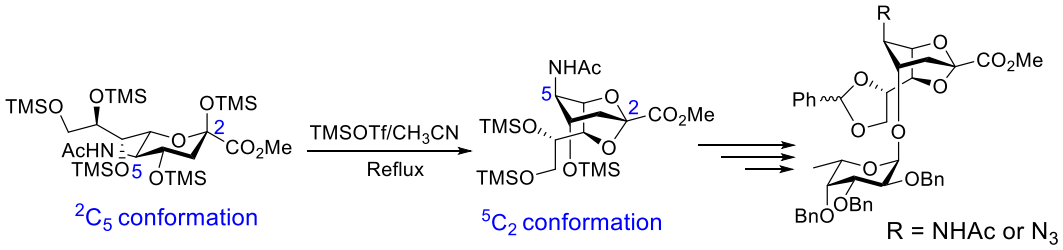
In *N*-acetylneuraminic acid, apart from O9 and O8, a possible glycosylation site is the O4 position. For example, gangliosides HLG-2 and HPG-7 are considered to be potential lead compounds for carbohydrate-based drug development to treat neural disorders. However, the construction of their *α*(1→4) fucosyl sialic acid and *α*(2→4) linkages between sialic acids is difficult because of the regioselectivity problem. Herein, *N*-acetyl-2,7-anhydroneuraminic acid was synthesized in three steps from Neu5Ac methyl ester through per-*O*-trimethylsilylation, heating-assisted intramolecular anomeric protection (iMAP) and desilylation. The iMAP simultaneously circumvents both the 2- and 7-OH protection. Upon protecting the 8- and 9-OH groups as a benzylidene acetal, only 4-OH is free for glycosylation. These 2,7-anhydro-8,9-*O*-benzylidenesialic acid derivatives were examined as acceptor for an *α*-selective fucosylation to construct the glycosidic linkage of fucosyl *α*(1→4)2,7-anhydroneuraminic acid. The corresponding disaccharides were obtained in high yields with excellent *α*-stereoselectivity from the NIS/TfOH-promoted glycosylation reactions. This protocol can be applied to synthesize important gangliosides upon successful cleavage of the 2,7-anhydro backbone of the disaccharides.



$^{2}C_{5}$ conformation

$^{5}C_{2}$ conformation

Reference:

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SYNTHESIS OF THIOMALTOOLIGOSACCHARIDES BY A THIO-CLICK APPROACH

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Over the last few years, the radical addition of thiols to alkenes (also called thiol-ene coupling or thio-click reaction) has emerged as a valuable tool for the synthesis of carbohydrate derivatives [1-6]. We have shown that photoinitiated hydrothiolation of 2-acetoxy-D-glucal with various sugar thiols in the presence of 2,2-dimethoxy-2-phenylacetophenone (DPAP) gave 1,2-*cis*-α-*S*-disaccharides with complete regio- and stereoselectivity in good to excellent yields [2]. To extend the applicability of these fully selective addition reactions, we have studied the feasibility of this approach for the synthesis of α-*S*-linked oligosaccharides.

Herein we describe the preparation of biorelevant thiomaltooligomers [7] by using thiol-ene coupling reactions. The hydrothiolation reaction of 2-acetoxy-D-glucal **1** with thiol **2** resulted in the corresponding α-*S*-linked disaccharide **3**. Deprotection of the thiol at position 4 gave the disaccharide thiol **4**, which was then reacted with glucal **1** affording the "thiomaltotrioside" **6**. The 2-acetoxy-D-glucal derivative **5** was obtained from **3** by a two-step procedure. The thiol-ene coupling reaction of compounds **4** and **5** led to the formation of the tetrasaccharide **8**. The free radical addition of thiol **7** to 2-acetoxy-D-glucal **5** resulted in the corresponding α-*S*-linked pentasaccharide **9** (Fig. 1). All hydrothiolation reactions took place with full regio- and stereoselectivity.

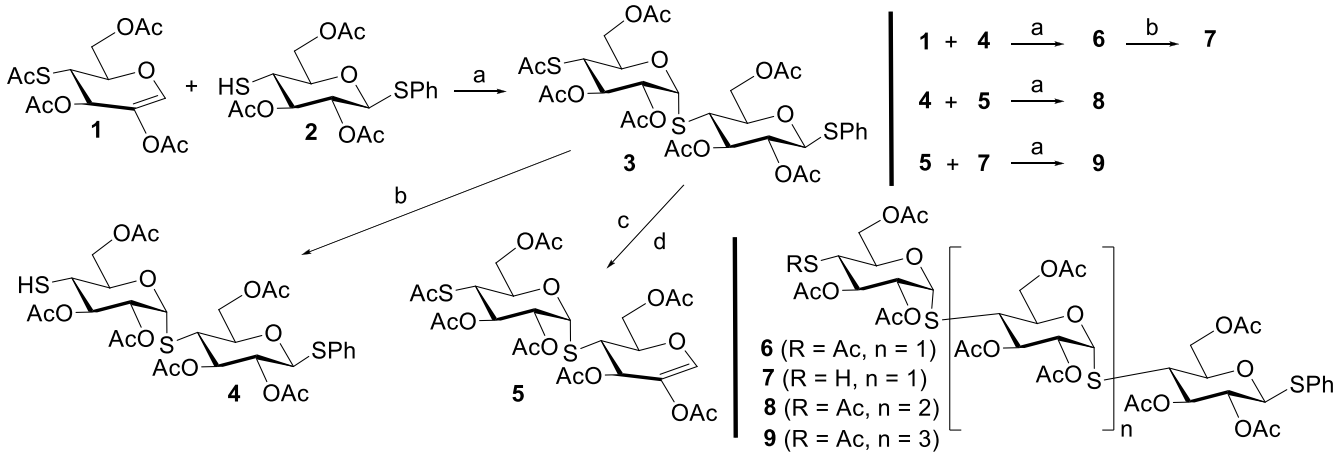

Fig. 1 – Synthesis of thiomaltooligomers up to pentasaccharide. Reagents and conditions: (a) thiol (1.3 equiv.), DPAP (3 x 0.1 equiv.), 3 x 15 min irradiation, toluene, -80 °C; (b) CH_3ONa, CH_3OH; (c) Br_2, CH_2Cl_2; (d) DBU, CH_2Cl_2.

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C-GLUCOSYL DERIVATIVES OF SOME IMIDAZO-FUSED HETEROCYCLES: SYNTHESES AND GLYCOGEN PHOSPHORYLASE INHIBITION

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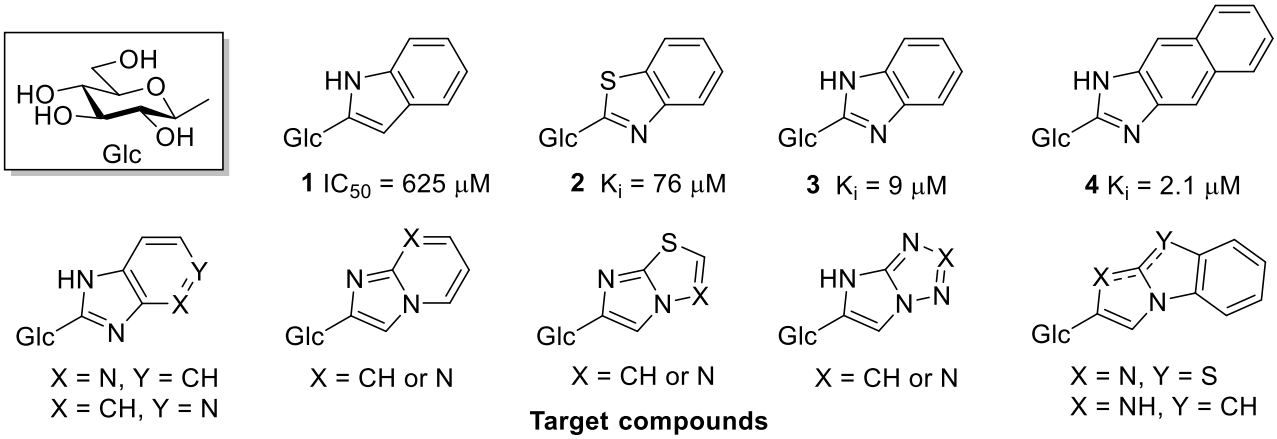
The inhibition of glycogen phosphorylase (GP) can be considered as a powerful concept aimed at finding new therapies against type 2 diabetes [1], ischemic lesions [2] and cancer [3]. A large set of small molecules have been shown to inhibit this enzyme [1] including different derivatives of D-glucose which bind to the catalytic site of GP [4].

Within glucose based inhibitors of GP *C*-glucopyranosyl azoles exhibiting even submicromolar inhibitory effects represent a very promising subclass [5]. This group comprises some condensed heterocyclic derivatives (e.g. **1-4**) [6-8], and among them benzimidazole [6] **3** and naphtho[2,3-*d*]imidazole [7] **4** are the most active ones. The comparable pairs of this small library indicate that the number (**1** *vs* **3**) and the nature (**2** *vs* **3**) of the heteroatoms as well as the size of the heteroaromatic ring (**3** *vs* **4**) have a strong bearing on the inhibitory efficiency.



Target compounds

In order to get a deeper insight into the structure-activity relationships of such type of condensed molecules synthesis and study of further analogs were envisaged. The present work has been focused on imidazole derivatives fused by a set of six- and five-membered hetarenes.

In the presentation details for the syntheses of the new compounds as well as results of their enzyme kinetic assays will be summarized.

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INDIUM-MEDIATED ALLYLATION OF DISACCHARIDES

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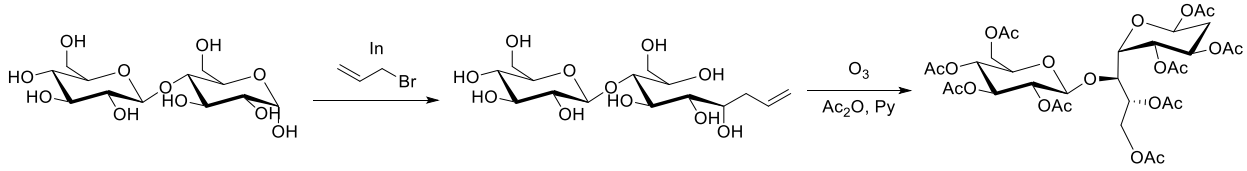
The demand for efficient synthetic approaches towards natural as well as unnatural complex carbohydrate structures is still continuously increasing and an important research aim in Glycoscience because the isolation of complex natural glycans is not efficient to obtain quantitative amounts and unnatural derivatives are often interesting variants for biomedical as well as medicinal applications [1,2]. In this context, the development of diastereoselective and efficient synthetic routes to elongated carbohydrates and oligosaccharides has been in our focus to contribute to this field. Therefore, we became interested in indium-mediated allylations for the elongation of carbohydrate moieties [3,4]. With the use of this reaction followed by ozonolysis, various elongated disaccharides could be produced in a short, diastereoselective and efficient synthesis. Here we present how effective this synthetic reaction sequence can be utilized to disaccharides as starting materials in order to obtain complex di- and oligosaccharides. Results and experimental details will be presented.



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THE SYNTHESIS OF HEXAVALENT GLYCODENDRIMERS AS ANTI-VIRAL AGENTS

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Glycodendrimers are highly branched polymers that have diverse functional groups. The many branches and types of terminal functional groups create the multivalent effect. The multivalent effect refers to the simultaneous interactions of multiple binding sites on one molecule to multiple receptor sites on another. This effect makes glycodendrimers useful in the inhibition of viruses. Fusion can be inhibited between the virus and the targeted cell, and thus infection of the host cell can be prevented. Our research is focused on the synthesis of multivalent glycodendrimers as viral entry inhibitors.

In the present study, a four-step pathway was used to synthesize four hexavalent glycodendrimers, each terminated with a unique sugar linker. The first part of this study was a two-step process in which the sugar linkers were synthesized. The linker [1] was reacted with a sugar in a microwave (CEM MARS 5) for 30 minutes under aqueous conditions with aniline. The sugars used were gentiobiose, melibiose, cellobiose, and *N*-acetylglucosamine, giving yields for the protected linkers as follows: 67.0%, 57.7%, 60.0%, and 59.5%, respectively. The second step of the sugar linker synthesis involved deprotecting the linkers giving final yields as follows: 68.2%, 67.4%, 90.5%, and 73.2%, respectively. After the sugar linkers were completed, they were reacted with a hexavalent dendrimer core and TBTU overnight under basic conditions, with yields ranging from 55%-85%. Once these glycodendrimers are purified, they will be sulfated and subjected to an ELISA assay to test for target protein binding. If the ELISA assay is positive, the glycodendrimers will be evaluated in a live cell assay to test for viral inhibition of infectivity.

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NANOSCALE MAPPING OF MULTIPLE LECTIN SITES ON CELL SURFACES DETECTED BY CARBOHYDRATE-FUNCTIONALIZED AFM TIPS

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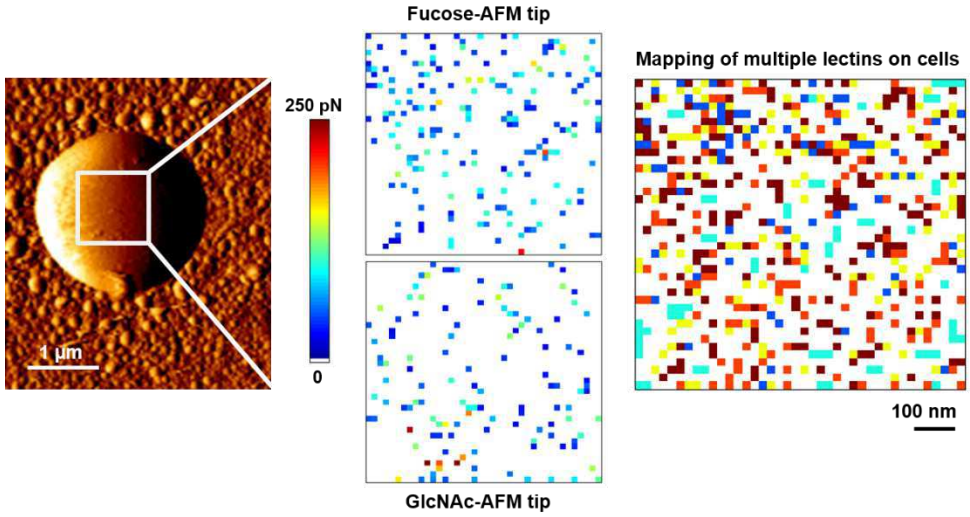
Molecular recognition events driven by protein-carbohydrate interactions play fundamental roles in various physiological and pathological processes in living organisms, including cohesion inside tissues, innate immune response, cancer cell metastasis and infections. Unlike widely investigated carbohydrates, a detailed knowledge of both the spatial organization of specific lectins and their identification on cell surfaces remains an essential prerequisite for the understanding of pathogen adhesion to host tissues and subsequent infection prevention. In the present work, we report an elegant and selective means to reveal the localization with nanometre scale resolution of multiple carbohydrate-binding sites and their identification directly on the surface of fungal pathogen *Aspergillus fumigatus* [1]. Indeed, single-molecule force spectroscopy experiments were conducted with different carbohydrate-functionalized atomic force microscopy (AFM) tips to perform several nanoscale recognition maps, corresponding each to a unique specific receptor-ligand interaction. These recognition maps were then superimposed to build a spatial mapping of lectin site distribution on the pathogen surface. The nanoscale reconstructed mapping reveals a high coverage ratio of various carbohydrate-binding sites, allowing them to be spatially localized, identified and quantified [1]. In a subsequent step, the identified binding sites were blocked with the appropriate carbohydrate, attesting the possibility to control lectin-mediated host-pathogen interactions.



These reconstructed maps offer an efficient means to track the spatially-resolved localization, identification and quantification of multiple lectin sites directly on the surface of any pathogen or cell. This versatile and non-destructive approach represents an important new tool in nanobiology, capable of addressing various biologically and medically unresolved questions by tracking biological functions driven by lectins [1,2]. These insights clearly open exciting avenues in nanomedicine to prevent and treat many infectious diseases by controlling host-pathogen interactions with the development of vaccines or inhibitory drugs that preferentially target the identified carbohydrate-binding sites.

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A LIQUID STABLE BIOLOGICAL ACTIVE SEMI-SYNTHETIC GLYCOCONJUGATE VACCINE AGAINST *HAEMOPHILUS INFLUENZAE* TYPE b

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Haemophilus influenzae type b (Hib) is a causative organism of childhood pneumoniae and meningitis [1]. Commercial licensed glycoconjugate vaccines against Hib save the lives of millions of children in both developing and developed countries [2]. The major issue with Hib glycoconjugate vaccines is lack of stability of Hib polysaccharides in the presence of water and adjuvants [3]. Phosphodiester bonds present in the polyribosylribitol phosphate of the Hib capsular polysaccharide undergo significant cleavage or migration in the presence of an adjuvant like aluminum hydroxide. To overcome this problem, we rationally designed and synthesized oligosaccharide analogs of the Hib capsular polysaccharide. These novel oligosaccharides were stable at temperatures up to 70 ºC when tested in water and formulations containing adjuvants. Hib analogs were conjugated to the carrier protein CRM197 and tested for their immune response in rabbits. HiberiX® served as a positive control. The synthetic Hib oligosaccharide analog in liquid formulation with-/without aluminum hydroxide showed antibody titers comparable to the commercial vaccine HiberiX® over three administration study. A booster dose with the same vaccine batch administered after a 12-week resting period elicited antibody titers comparable to the commercial vaccine HiberiX® indicating prolonged stability of the Hib analog containing glycoconjugate vaccine.

Biological activity of the induced Abs was analyzed in a serum bactericidal assay and shown to be comparable to those induced by the commercial vaccine. Overall an innovative approach using organic syntheses was applied to solve the long existing hydrostability problem of the commercial Hib vaccine. The structural changes on a molecular level imparted stability plus retained immunogenicity, cross reactivity and functionality on induced Abs. Taken together, using structure driven rational design of glycan molecules we introduced a new class of glycoconjugate vaccines exhibiting optimized characteristics.

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HIGH THROUGHPUT SCREENING APPROACH FOR THE SEARCH OF HUMAN FUCOSYLTRANSFERASE VI (Fut6) INHIBITORS

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Carbohydrate structures (or glycans) that are present on cell-surfaces constitute a very important mode of intercellular communication. Fucosylation is one of the most important types of glycan modifications, and fucosylated glycans are involved in a multitude of cellular interactions and signal regulation in normal biological processes. Increased formation of sialyl Lewis^X (a fucosylated cell-surface glycan) has been observed in several types of cancers, e.g. colorectal, prostate, and pancreatic cancers, and has been associated with the abnormal upregulation in the expression of specific fucosyltransferase enzymes (FUTs) [1]. Therefore, the role that upregulated FUTs play towards the progression of cancers to metastatic stages, makes them important targets for potential anticancer drugs. The search for small molecule inhibitors of FUTs would be useful in drug development.

The search for small molecule inhibitors of enzyme targets in drug development depends upon high-throughput screens and activity assays that are facile and sensitive, something that is lacking for the enzymes of the type we wish to target. In this project, to screen for human fucosyltransferase VI (Fut6) inhibitors we implemented a strategy that we have developed towards assaying the activity of carbohydrate-active enzymes (glycosidases and glycosyltransferases) in forming specific glycan structures [2,3]. This involves utilizing synthetic, fluorogenically labeled oligosaccharides, and specific glycosidase enzymes whose activity results in a fluorescence signal when the specific labeled glycan structures that they recognize are hydrolyzed. We have tested a small library of synthetic compounds, which are structural mimics of a previously reported human fucosyltransferase inhibitor [4], in a facile high-throughput manner, and determined their inhibitory constant around micromolar range.

We will continue to screen compound libraries from McMaster Centre for Microbial Chemical Biology (CMCB) High Throughput Screening Lab. Lead compounds identified will serve as further starting points for generating derivatives through a general combinatorial chemistry approach. Further modification will be evaluated to incorporate functional groups amenable to *in situ* click chemistry. In addition to the assay for Fut6 inhibition, we will also develop our approach towards assaying several other human FUT enzymes and glycosyltransferases that have been implicated in cancer.

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CuAAC AND *IN SITU* SCREENING FOR THE RAPID DISCOVERY OF DIVALENT GLYCOSIDASE INHIBITORS

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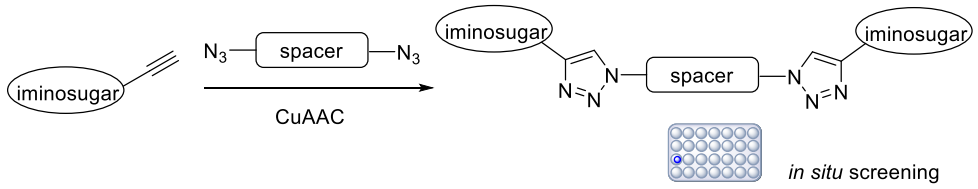
Multivalent glycosidase inhibitors with extremely enhanced activity with respect to the monovalent parent, on a valence-corrected basis, have been successfully developed only for α-mannosidases [1] and, to a lesser extent, for hexosaminidases [2]. To achieve multivalency, the design of the adequate multimeric iminosugar by anchoring the monomeric inhitope to a functionalized platform through appropriate spacers is crucial. Concerning the search for divalent inhibitors, short and flexible spacers may favor the divalent effect through the so-called “recapture” mechanism. On the other hand, for the simultaneous interaction of both inhitopes with the enzyme, the nature and length of the spacer is critical to promote the right orientation of the binding motifs. Additionally, adventitious interactions of the spacer itself with the enzyme should not be discarded. As no structural information is available for many glycosidases, it is difficult to anticipate which spacer is the optimum. Thus, the development of a combinatorial methodology to explore the structure-activity relationships between different dimeric inhibitors and a particular glycosidase, could allow us to select the best spacer to maximize the divalent effect in glycosidase inhibition.

In this communication we present an efficient tool for the rapid discovery of divalent glycosidase inhibitors harnessing pyrrolidine iminosugars into dimeric structures via CuAAC click reaction. We have prepared different libraries of dimeric iminosugars that could be *in situ* screened against mammalian α-fucosidases and β-galactosidases. This method has allowed the rapid identification of a potent dimeric inhibitor of α-fucosidases (IC_{50} = 0.50 nM). Besides, a modest divalent effect was observed in the inhibition of β-galactosidases.



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CHARACTERIZATION OF THE SULFATED GLYCAN-BINDING SPECIFICITIES OF HUMAN LACTOFERRIN AND ITS DERIVATIVE

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Lactoferrin (Lf) has a wide range of potential therapeutic applications such as anticancer, anti-inflammatory, and neuroprotective effects. Lf is a multifunctional lectin contained in most mammalian exocrine secretions, including breast milk and tears. This lectin is a glycoprotein belonging to the transferrin family, and is composed of two globular domains, the N-lobe and C-lobe. Thus far, the N-terminal region within Lf has been predicted as a binding site of sulfated glycosaminoglycan (sGAG).

Recently, it was reported that chondroitin sulfate (CS) in the tumoral microenvironment affected the proliferative and adhesion abilities of lung cancer cells, and contributed to the enhancement of metastatic potential. However, the contribution of sGAGs in the regulation of lung cancer cell proliferation by Lf N-lobe is unknown. In this study, we examined the effect of sGAGs on the proliferation inhibitory activity of Lf N-lobe in three human lung cancer cell lines (poorly-differentiated non-small cell lung adenocarcinoma PC-14, moderately-differentiated non-small cell lung adenocarcinoma PC-3, and differentiated non-small cell lung adenocarcinoma PC-9). The results indicated that : i) the proliferation inhibition of the three lung cancer cell lines by Lf N-lobe differed depending on their degree of differentiation, ii) the level of activity of Lf N-lobe against PC-3 cells was controlled by the presence of CS, iii) cellular uptake of Lf N-lobe into PC-3 cells was inhibited by the presence of CS, iv) Lf N-lobe had high affinity for a specific CS subtype, and v) 11 amino acid residues within Lf N-lobe had high affinity for a specific CS subtype.

These results suggested that the CS moieties were important for regulation of lung cancer cell proliferation by Lf N-lobe.

OLIGOGALACTOSIDES AS POTENTIAL GALECTIN INHIBITORS

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Galectins are a structurally related family of proteins that binds to β-galactoside motifs through a highly conserved carbohydrate recognition domain (CRD) [1]. They are involved in a pleiotropic number of fundamental biological processes. Galectins are key contributors to the homeostasis but also take part in many pathologies such as cancer progression, inflammatory and immune diseases, diabetes…Human galectin-3 is one of the prominent members of this lectin family, identified as a potential therapeutic target. All galectins recognize type I and type II lactosamines (β-Gal*p*-(1-3/4)-β-D-Glc*p*N). Noticeably, galectin-3 shows a marked preference over other galectins but galectin-9 for binding to poly-lactosamines [2] (Table 1 and Figure 1).

Table 1 – Affinity of various lactose/lactosamine derivative for galectin-3

Compounds	Lactose	LN2	LN3	LN5
K_d[a] (μM)	26	1.3	0.35	0.19

[a] Determined by frontal chromatography affinity (data from reference 2)

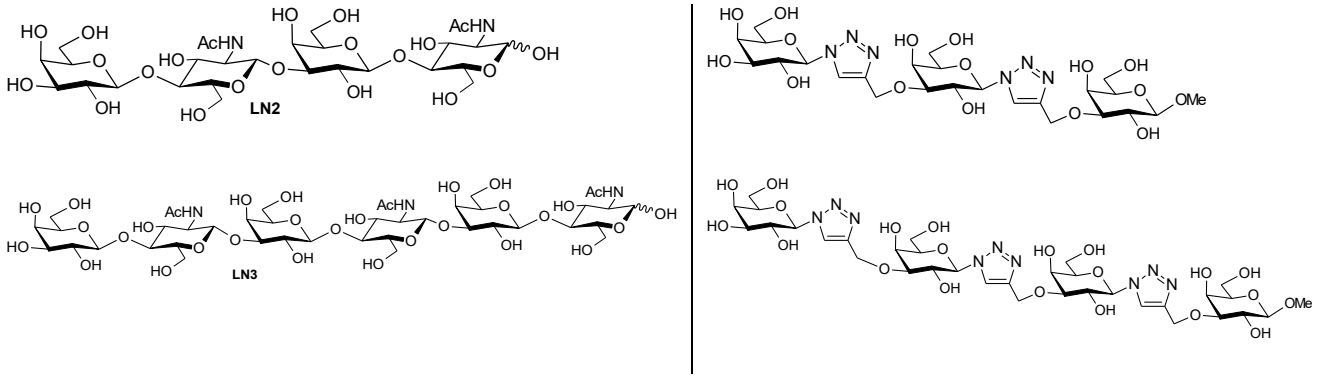

Fig. 1 – Structure of representative natural oligo-lactosamine motifs (left panel) and corresponding oligo-galactoside mimics (right panel)

We hypothesized that (oligo)-galactosides obtained *via* iterative copper catalyzed azide-alkyne Huisgen cycloaddition could act as lactosamine mimics towards the galectin-3. In these derivatives, the glucosamine units are replaced by a triazole ring. We will present the synthesis of these original multivalent inhibitors and discuss their binding properties on the basis of fluorescence polarization measurements as well as crystallographic stuctures.

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CHITOSAN-COATED POLY(ISOBUTYLCYANOACRYLATE) NANOPARTICLES HAVE INTRINSIC TRICHOMONACIDAL AND ANTI-LEISHMANIAL ACTIVITIES

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Topical parasitic infections such as trichomoniasis [1] and cutaneous leishmaniasis still represent a major health problem. Systemic administration of drugs is the first intention for the prevention and the treatment of these topical infections. However, this administration route leads to (i) drug toxicity (ii) drug resistance and (iii) low drug efficacy or even inactivity, due to the low dose available on mucosal and cutaneous tissues. In this context, drug-free bioadhesive nanoparticles (NPs) composed of poly(isobutylcyanoacrylate) coated with chitosan (M_w 20,000 g/mol, denoted PIBCA/Chito20) exhibited *in vitro* intrinsic anti-*T. vaginalis* and anti-*Leishmania major* activities [2]. The present work correlated NP anti-parasitic activity to their internalization. Flow-cytometry revealed that *T. vaginalis* were found to uptake PIBCA/Chito20. Electron microscopy techniques SEM and TEM were simultaneously conducted to monitor morphological changes induced by PIBCA/Chito20 NPs on the external and internal structures of parasites. SEM and TEM observations showed that PIBCA/Chito20 NPs underwent a drastic morphological transformation with the appearance of abnormal morphologies (Fig. 1). Numerous pits were seen on the membrane as early as 10 min after incubation of NPs with the parasite (Arrows on Fig. 1.B). Gradual increase in contact time increased NP endocytosis (Arrows on Fig. 1.A) and induced a proportional damage to *T. vaginalis* membrane.

NP activity was then evaluated *in vivo* against *L. major* after either intralesional or topical applications to mice. q-PCR, histological and immunohistochemical examinations of skin lesions showed a significant decrease of the inflammatory granuloma and a reduction of the parasitic load, in comparison with amphotericin B-deoxycholate, used as a control. These results provide a strong evidence that drug-free PIBCA/Chito20 NPs could be considered as a strategy to cure topical trichomoniasis and leishmaniasis.

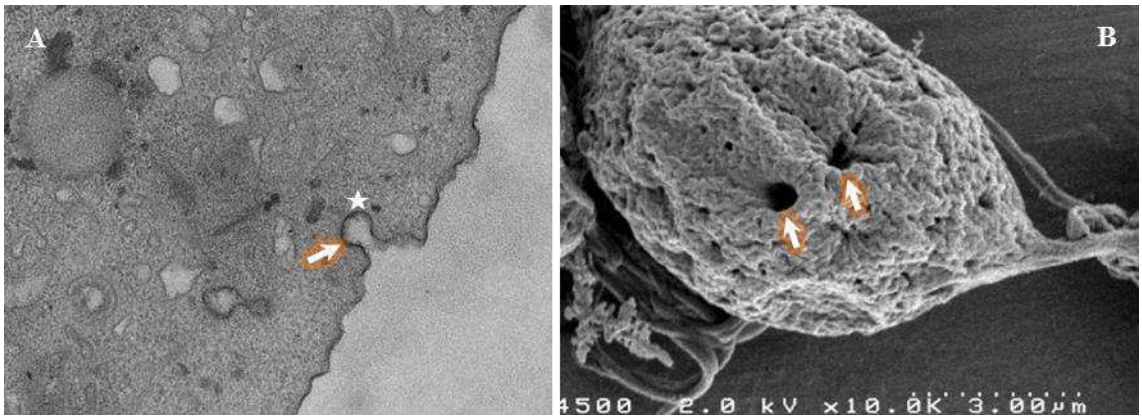

Fig. 1 – TEM (A x 15,000) and SEM (B) images of *T. vaginalis* after different incubation times with PIBCA/Chito20 NPs. 10-min (A) and 3 h-incubation (B). Numerous pits (arrows on A and B) and crater-like depressions (arrows on B) appeared at several points of the parasite surface. The beginning of the process of ingestion of nanoparticles is indicated by a star (★) in A.

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DETOXIFICATION OF AMINOGLYCOSIDE ANTIBIOTICS THROUGH CHEMICAL MODIFICATIONS TO ENHANCE BINDING TO BACTERIAL RIBOSOMES

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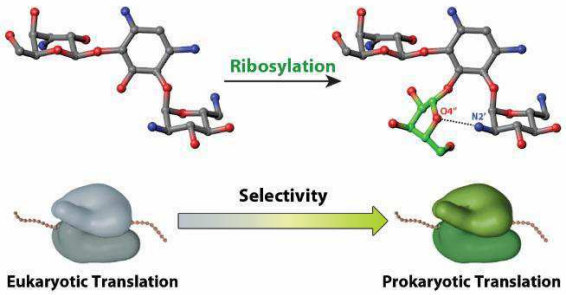
Aminoglycosides (AGs) are an important family of clinically useful antibiotics that perturb the fidelity of bacterial protein synthesis by binding to the decoding A-site region of the bacterial ribosome. Despite emergence of resistant bacterial pathogens, these antibiotics remain a central component of the treatment of severe infections. Unfortunately, the use of AGs to treat systemic infections is very limited due to their nephrotoxicity and ototoxicity. *In vitro* translation experiments have shown that several AGs inhibit mitochondrial translation and cytosolic eukaryotic translation, suggesting that toxicity is due to perturbation of the fidelity of both mitochondrial and eukaryotic translation.

Westhof and co-workers have reported crystal structures of complexes between an oligoribonucleotide representing the prokaryotic decoding A-site and different 4,5-disubstituted 2-deoxystreptamine AGs. In these structures the ribofuranose sugar ring interacts with the target A-site nucleotides through a set of hydrogen bonds. Notably, the 2' amine in these AG structures is within range to form a hydrogen bond with the ribofuranose sugar ring oxygen. This bond likely assists in positioning of the ribofuranose ring in the A-site. Based on this crystallographic data we rationalized that attachment of a *β-O*-linked ribofuranose ring to the C-5 position of 4,6-disubstituted 2-deoxystreptamines with a 2'-equatorial amine or to the C-5 position of 4-monosubstituted 2-deoxystreptamine AG scaffolds would enhance occupancy of the prokaryotic A-site rRNA binding domain [1].

In this study we synthesized and investigated seven pairs of natural and synthetic aminoglycosides and their corresponding ribosylated derivatives, synthesized by attaching a *β-O*-linked ribofuranose to the 5-OH of the deoxystreptamine ring of the parent pseudo-oligosaccharide antibiotic. All the ribosylated derivatives had higher selectivity for inhibition of prokaryotic relative to eukaryotic *in vitro* translation. The ribosylated derivatives of the pseudo-disaccharide aminoglycoside apramycin and its demethylated analogue maintained a potent antimicrobial activity.



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SULFONE-BASED GALECTIN-3 INHIBITORS TO TARGET A NEW POCKET NEAR THE CANONICAL CRD

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The overall goal of the present project is to synthesize galectin ligands to allow for detailed biophysical characterization of galectin-ligand interactions. A key feature of galectins is binding affinity towards galactoside-containing glycoconjugates [1]. Natural small ligand fragments of the galectins, such as N-acetyl lactosamine (LacNAc) and lactose, show low inhibition potency why detailed biophysical characterization of galectin ligand recognition is imperative to facilitate denovo ligand design.

Here we present the modification at galactose C3 to obtain sulfone-based moieties to target a binding pocket perpendicular to the conserved site in galectin-3. These inhibitors are bound with ~70uM against human galectin-3 (almost similar to previously reported phenyl triazole [2]) and they are well tolerated in the protein pocket. Interestingly, the afffinity is dependent on the oxidative state of the sulfurus; on losing the number of oxygen on sulphur subsequently i.e from sulfone to sulfoxide to sulphide, every time affinity decreases almost by 2 folds. This suggests that the interactions of both sulfone oxygen with galectin-3 are important. This was supported by the mutation study; the sulfone triazoles towards R144s galectin-3 mutant displayed similar affinity as corresponding sulphide triazole.

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GLYCO-NANOPROBES AS DIAGNOSTIC AND AS DRUG DELIVERY SYSTEMS

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Cell surface carbohydrates play key roles in cell recognition mechanisms. Protein-carbohydrate interactions typically exhibit high specificity and weak affinities toward their carbohydrate ligand [1]. This low affinity is compensated in nature by the architecture of the protein, the host presenting the carbohydrate ligands in a multivalent manner or as clusters on the cell or mucosal surface [2]. O-glycosylation is a ubiquitous post-translational modification that is highly dynamic and responsive to cellular stimuli through the action of the cycling enzymes [3]. Expression of specific O-glycans is linked to changes in gene expression in, for example, inflammatory bowel disease, cystic fibrosis and several types of cancer [4]. Understanding these glycosylation patterns at molecular and functional levels will allow mechanisms associated with bacterial-host interactions, bowel disease and other cancers to be defined, which will facilitate the development of new effective therapeutics and diagnostic tools for these conditions.
In this poster, the chemical synthesis of novel multivalent mucin-type O-glycan [5] probes for the screening of O-glycosylation-linked interactions in cancer cells will be discussed.

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DEFINING THE STRUCTURAL ORIGIN OF ANTIBODIES RECOGNITION OF TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS

Ron Amon,[a]# Oliver C. Grant,[b]# Shani Leviatan Ben-Arye,[a] Spandana Makeneni,[b] Anita K. Nivedha,[b] Tal Marshanski,[a] Christoffer Norn, [c] Hai Yu,[d] John N. Glushka,[b] Sarel J. Fleishman,[c] Xi Chen,[d] Robert J. Woods[b]* and Vered Padler-Karavani[a]*

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Anti-carbohydrate monoclonal antibodies (mAbs) hold great promise as cancer therapeutics and diagnostics. However, their specificity can be mixed, and detailed characterization is problematic, because antibody-glycan complexes are challenging to crystallize. Here, we developed a combined computational-experimental approach to define the structural features of anti-carbohydrate antibodies as potent tools for cancer therapeutics. We apply this methodology on an antibody against the tumor-associated carbohydrate antigen sialyl-Tn (STn). Initially we clone this antibody followed by quantitative glycan microarray screening to obtain specificity profile and apparent K_D values against potential antigens. Subsequently, glycan-antigen contact surface was defined by saturation transfer difference NMR, and key amino acid residues in the antibody binding site identified by point-mutagenesis. This elaborated experimental information was used to select optimal 3D-model of the antibody-glycan complex out of 12,000 possibilities by automated docking and molecular dynamics simulation. Specificity was validated by computational carbohydrate grafting of the human glycome. This powerful approach would provide tools for rational design of potent antibodies targeting carbohydrates, succeeding the need for the challenging antibody-glycan crystal structures. This work is the first of its kind describing a new concept in methodology of relevance to biomedical sciences, and is at the interface between multiple fields including computational biology, applied immunology and glycobiology. It combines state-of-the-art technologies, such as glycan microarray, antibody and carbohydrate modelling, simulation and docking, proteomics and antibody engineering.

GLYCOCONJUGATED AMINOPORPHYRAZINES AS POTENTIAL PHOTOSENSITIZERS IN PHOTODYNAMIC THERAPY

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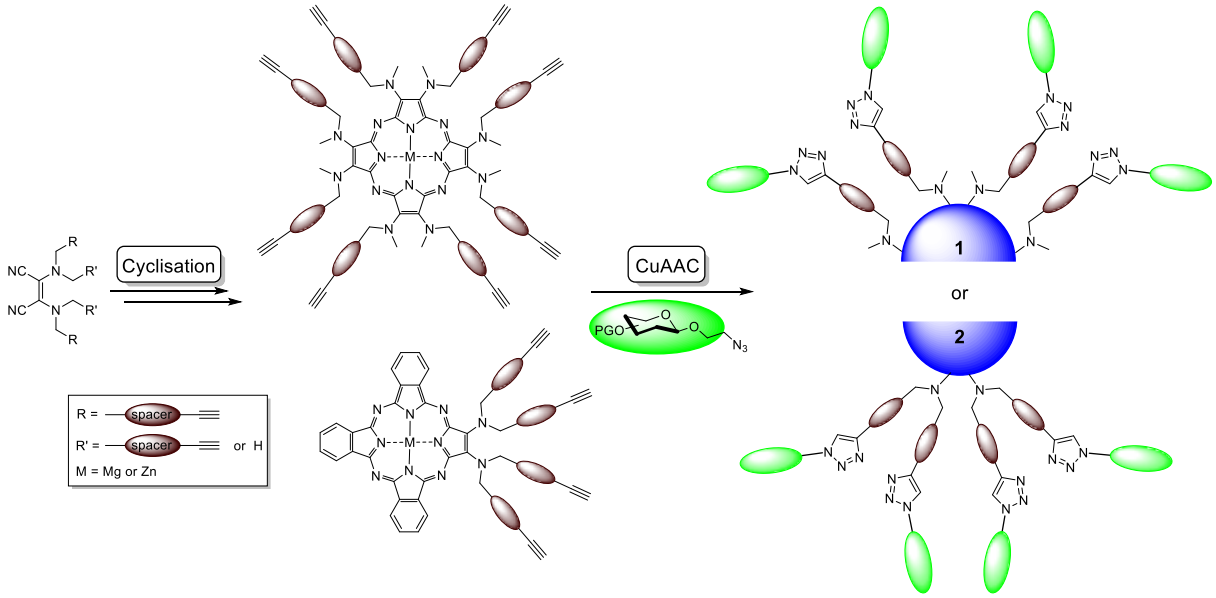
Over the last two decades photodynamic therapy (PDT) became an important pillar in cancer treatment. Though there are several photosensitizers (PS) already available, the research on new PS with improved photophysical and biological properties is mandatory. One major disadvantage of tetrapyrrolic macrocycles like porphyrins, phthalocyanines and porphyrazines (Pz) is their low solubility in physiological fluids [1]. Besides this, an enhanced accumulation in or on the surface of cancer cells is desirable. These issues can be addressed by attaching carbohydrates to the periphery of PS which is why this type of compounds are the subject of intense research [2-4].

Here we present the synthesis of octa-glycoconjugated aminoporphyrazines **1** and AB_3-type aminoporphyrazines **2** showing in UV/Vis spectra a Q-band maximum around 715 nm and 685 nm, respectively, which is well suited for PDT. Our approach to new 3rd generation photosensitizers, based on glycoconjugated porphyrazines, is to synthesize aminoporphyrazines whereby the carbohydrate moieties are attached to the Pz by Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) via 1,2,3-triazoles. This postmodification allows the use of carbohydrates with various protecting groups (PG) and keeps the possibility to exchange the Mg(II)-ion easily with other metal ions.



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PREPARATION AND CHARACTERIZATION OF N-SUCCINYL-CHITIN NANOPARTICLES AND THEIR APPLICATIONS IN THE OTONEUROLOGICAL PATHOLOGY

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Succinate, a natural endogenous metabolite that is generated during the Krebs cycle, has been used as a substrate for the development of metabolic cytoprotectants for nonspecific treatment of otoneurological disorders arising from hypoxic and ischemic pathogenesis. When administered exogenously under anaerobic conditions, succinate reduces the concentrations of lactate, pyruvate, and citrate, while supporting necessary ATP production. Its antihypoxic and cytoprotective effects in cases of unbalanced consumption of endogenous metabolic substrates have been exploited successfully in several pharmacological drugs based on succinate solutions (e.g., Reamberin, Cytoflavin, Remaxole, Mexidol, etc.). The main disadvantage of these drugs is their short cycling time in blood and low accumulation in target organs. One way to increase the circulation of an active substance in the blood is to bind it with polymeric nanoparticles. Targeted drug delivery, in this case, is provided by the internalization of the nanoparticles in areas of microvasculature damage, which have high permeability and retention. One of the most promising materials for the development of this type of therapeutic drug carrier is the biodegradable and biocompatible polymer chitin. The aim of the present work was to prepare and characterize N-succinyl-chitin (SCH) nanoparticles and to evaluate their pharmacological action following intravenous administration in an animal model of acute hearing pathology.

SCH containing about 40 mol% succinyl groups was obtained by N-acylation of partially deacetylated chitin nanofibers with succinic anhydride. Treatment of an aqueous dispersion of SCH with ultrasound gave a stable aqueous dispersion of oval nanoparticles with hydrodynamic diameters ranging from 200 to 300 nm. The fractal dimension changed from d_f=1.2 for the chitin nanowhiskers to d_f=1.5-1.7 for the SCH nanoparticles. The cytoprotective activity of the SCH nanoparticles was evaluated in vivo in an acute hearing pathology model (220–250 g male Wistar rats, n=90) following prophylactic and therapeutic administrations. Ototropic action was estimated using the amplitude of otoacoustic emissions at the frequency of the distortion product otoacoustic emissions in the range of 4–6.4 kHz before acoustic stimulation, as well as at 1 h, 7 h, and 7 days after acoustic stimulation.

The SCH nanoparticles had a positive ototropic effect, independent of the administration regime. Prophylactic administration of SCH nanoparticles gave the following results when compared with meglumine sodium succinate: (i) better penetration (the preparation effects extended more widely throughout the cochlear spiral); (ii) an earlier restoration of cochlear function at the studied frequencies; and (iii) a more prolonged presence in the blood circulation (i.e., a more prolonged cytoprotective action).The therapeutic administration of SCH nanoparticles (after acoustic stimulation) resulted in a positive otoprotective effect that manifested more rapidly when compared with similar experiments using meglumine sodium succinate. Thus, the speed of action of SCH nanoparticles in aqueous dispersion dosage form indicates a prolonged presence in the blood circulation system and an increased biodistribution in the stria vascularis due to the enhanced penetration through the histohematic barrier.

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POTENT METABOLIC SIALYLTRANSFERASE INHIBITORS BASED ON C-5 MODIFIED FLUORINATED SIALIC ACIDS

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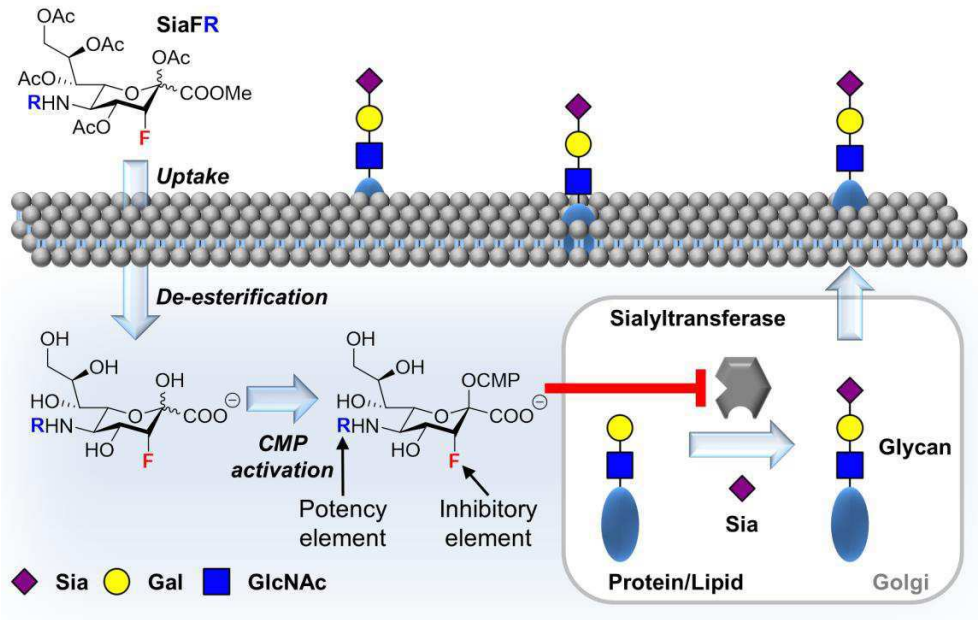
Sialic acids are complex sugars that are abundantly expressed at the termini of mammalian glycans outside the cell. They are involved in numerous biological processes ranging from neuronal development to immune cell trafficking. However, sialic acids are also presented on several bacterial pathogens and aberrant sialylation of cancer cells mediates immune evasion, migration and metastasis [1]. In these cases, inhibition of the sialic acid biosynthesis may hold considerable therapeutic potential. Sialyltransferase (ST) enzymes catalyze the addition of sialic acids to glycoproteins and lipids and are prime targets for inhibition. An ester protected fluorinated metabolic precursor of the donor substrate has been reported as a cell permeable inhibitor of STs [2]. We found that the addition of carbamates to the C-5 position significantly enhanced and prolonged the inhibitory activity in various mouse and human cell lines compared to C-5 amides [3]. These new compounds allow for the inhibition of sialylation at lower concentration, for a longer period of time and in more resistant cell lines. The observed preference of STs for carbamate sialic acids could also be considered in the design of other sialylation inhibitors, as well as metabolic labeling reagents that utilize the same enzymatic machinery. Finally, the specificity, high potency and prolonged inhibition qualifies them as good candidates for further therapeutic development.



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CYCLOPENTANOID D-GALACTOSIDASE INHIBITORS AND POTENTIAL PHARMACOLOGICAL CHAPERONES FOR G_{M1} GANGLIOSIDOSIS

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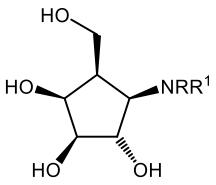
In context with our interest in potential pharmacological chaperones for G_{M1} gangliosidosis related β-D-galactosidase mutants, we have investigated 4-*epi*-isofagomine related compounds [1] as well as, more recently, nitrogen containing cyclopentanoid D-galactose analogs **1** [2]. Based on these initial results, we have tried to improve/modulate compound activities towards higher and more selective chaperoning efficacies. Examples of such very recent attempts will be presented.



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SYNTHESIS AND CHARACTERIZATION OF NEW COPPER(I) COMPLEXES COMPRISING CARBOHYDRATES FOR CANCER THERAPY

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In the field of metallodrugs cisplatin in one of the leading chemotherapeutics with well-established efficacy against a variety of cancers. Despite the clinical success, its use is limited due to severe side effects. Copper complexes have attracted much interest as alternative chemotherapeutic drugs. During the recent years our group has been committed to the discovery of new copper(I)-phosphane derived complexes, most of which revealed higher cytotoxicity than cisplatin against ovarian and breast cancer cell lines [1].

Carbohydrates are an excellent class of tunable ligands for use in medicinal inorganic chemistry, because of their role in recognition and adhesion events. It is proven that modified carbohydrates can interfere with carbohydrate–protein interactions and can inhibit cell–cell recognition and adhesion phenomena, essential processes in cancer growth and progression [2]. Moreover, the coordination of a carbohydrate to a metal potentially leads to complexes having a reduced toxicity and an improved solubility.

Due to the high energy demand of developing tumors, which can only be satisfied by glycolysis, coordinating carbohydrates derivatives to metal complexes may improve the cytotoxic potential of the new compounds by increasing the selectivity toward cancer cells [3].

In this context, the work presented here was intended to reach a new family of copper complexes through linking sugar derivatives to copper centers, thus combining our previous good results of Cu(phosphane)-based complexes with the promising features that carbohydrates can play in bioorganometallic chemistry. The synthesis and characterization of new copper compounds with carbohydrate and phosphane or heteroaromatic ligands will be disclosed herein (Fig. 1).

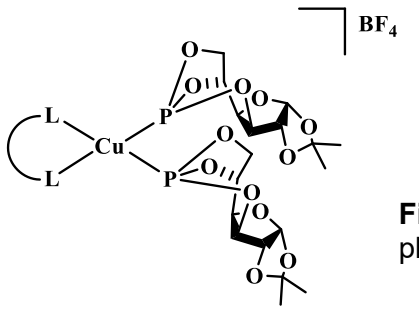

Fig. 1 – General structure of the coordination compounds with LL= phosphane or *N*-heteroaromatic ligands.

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SYNTHESIS OF 4-AZIDO-β-GALACTOSAMINE DERIVATIVES FOR INHIBITOR OF *N*-ACETYLGALACTOSAMINE 4-SULFATE 6-*O*-SULFOTRANSFERASE

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Sulfated carbohydrate chains in glycoproteins and glycolipids play important roles in infection by microorganisms and diseases. Inhibitors of sulfotransferases, responsible for biosynthesis of these carbohydrate chains, could be medical agents against such infections and diseases. *N*-Acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST) is the sulfotransferase responsible for biosynthesis of chondroitin sulfate (CS-E). To investigate the function of CS-E, the development of specific inhibitors of GalNAc4S-6ST is important. We have previously cloned GalNAc4S-6ST, which transfers sulfate from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the C-6 hydroxy group of the GalNAc4S residue of chondroitin sulfate A (CS-A) and forms CS-E containing GlcA-GalNAc4S6S repeating units. We furthermore synthesized phenyl α- or β-2-acetamido-2-deoxy-D-galactopyranosides containing a sulfate group at the C-3, C-4, or C-6 hydroxy groups and examined their inhibitory activity against recombinant GalNAc4S-6ST [1]. We found that phenyl β-GalNAc4S inhibits GalNAc4S-6ST competitively and also serves as an acceptor. The sulfated product derived from phenyl β-GalNAc4S was identical to phenyl β-GalNAc4S6S. These observations indicate that β-GalNAc4S derivatives are possible specific inhibitors of GalNAc4S-6ST. Among inhibitors examined at that time, 3-estratrienyl D-GalNAc4S was the strongest inhibitor; the K_i of 3-estratrienyl D-GalNAc4S for the competitive inhibition was 0.008 mM, which was much lower than that of phenyl D-GalNAc4S, 0.98 mM [2]. We studied that the modification of the GalNAc4S derivatives **A** (Fig. 1, transform a) following the synthetic strategy outlined in (Fig. 1, transform b) which involves the preparation of 4-azido-β-galactosamine derivatives **C**. Amide **B** is sulfate analogue with deceased negative charge and higher cell membrane permeability and one of our purposes is to synthesize cell membrane permeable selective inhibitor for GalNAc4S-6ST.

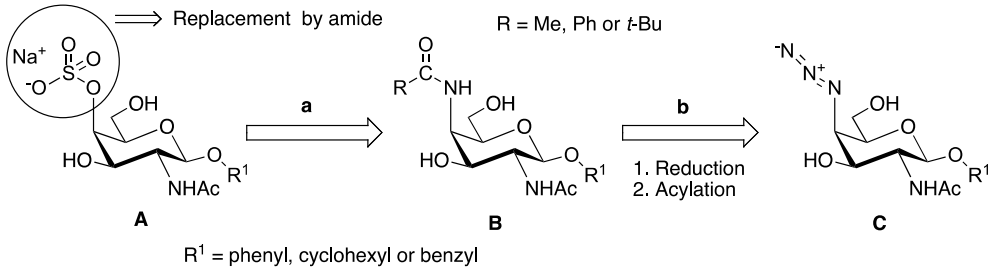

Fig. 1. Design of structure (a) and synthetic strategy (b).

We have found that 4-azido-β-GalNAc derivatives **C** are preferred for inhibitory activity and that a specific functional group at 4-position rather than aglycon would play an important role in the activity.

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NEW MEANS OF TARGETED DRUG DELIVERY OF ANTITUMOR DRUGS FOR HCC THERAPY BASED ON N-ACETYLGALACTOSAMINE DERIVATIVES

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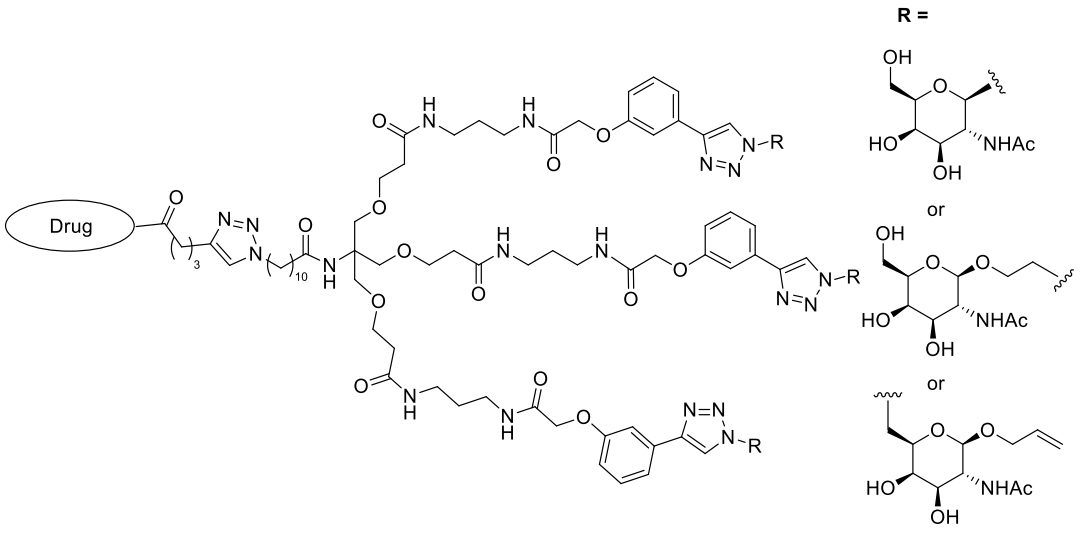
According to the WHO, the hepatocellular carcinoma (HCC, liver cancer) is the 5th most common and the second most deadly disease among human malignant tumors [1]. Modern drugs used for chemotherapy have a number of serious shortcomings, which can be overcome using the targeted drug delivery (TDD) to the tumor cells.

A promising target for a delivery to hepatocytes is the acialoglycoprotein receptor selectively recognizing galactose derivatives [2]. Asialoglycoprotein receptor (ASGP-R) belongs to a wide family of C-type lectins and it is currently regarded as an attractive protein in the field of targeted drug delivery. It is abundantly expressed in hepatocytes and can be found predominantly on the sinusoidal surface especially of HepG2 cells. Therefore, ASGP-R can be used for the TDD of anticancer therapeutics against HCC and molecular diagnostic tools.

We have synthesized trivalent ligands with the structure shown below. These ligands have higher affinity for the receptor than the native ligand *N*-acetylgalactosamine. This effect is based on the peculiarities of the receptor's structure [3] and the cumulative binding effect [4]. Then ligands were conjugated to antineoplastic agents (paclitaxel and gemcitabine) used in clinical practice.



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HERICIUM ERINACEUS ISOLECTINS RECOGNIZE MUCIN-TYPE *O*-GLYCANS AND POLYFUCOSYLATED *N*-GLYCANS AS TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS IN CANCER CELLS

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Cancer-specific glycans are promising biomarkers for the detection of carcinomas [1,2]. Two different lectin groups, HEL1 and HEL2 (*H. erianceus* lectin 1 and 2), from the mushroom fruiting body of *Hericium erinaceus* were identified by using peptide mass fingerprinting based on customized protein sequence databases derived from RNA-Seq data [3]. The HEL2 group included four isoforms designated HEL2a–d. Codon-optimized genes encoding HEL1, HEL2a, and HEL2b were expressed in *Escherichia coli* to produce fully active soluble proteins designated rHEL1, rHEL2a, and rHEL2b. Interestingly, these recombinant lectins showed different molecular weights: approximately 15 kDa for rHEL1 and approximately 100 kDa for rHEL2a and rHEL2b on tricine-PAGE under non-denaturing conditions. rHEL2a and rHEL2b exhibited agglutination activities, but rHEL1 did not show any agglutination activity toward animal erythrocytes. The hemagglutination activity of rHEL2 lectins was strongly inhibited by glycoproteins containing mucin-type O-glycans, with slightly different glycan specificities. Glycan array analysis and isothermal titration calorimetery revealed that rHEL2a and rHEL2b interacted strongly with O-linked glycans harboring the core 1 O-glycan motif, Galβ(1,3)GalNAc with slightly different binding specificities (Fig. 1A). Moreover, the glycan binding specificities of rHEL2a and rHEL2b were comparable to that of peanut agglutinin in their ability to recognize O-glycans attached to leukosialin as tumor-associated carbohydrate antigens on the surface of K562 human leukemia cells [4] (Fig. 1A). On the other hand, rHEL1 interestingly showed the binding specificities highly fucosylated N-glycan ligands on glycan array (Fig. 1B). In addition, rHEL1 detected the polyfucosylated N-glycans in SW116 human colorectal carcinoma cell [5] (Fig. 1B). These results indicate that rHEL1 and rHEL2 isolectins could be used as a powerful tool for analyzing polyfucosylated N-glycans and mucin-type O-glycans expressed on the surface of cancer cells in diagnosis assays.

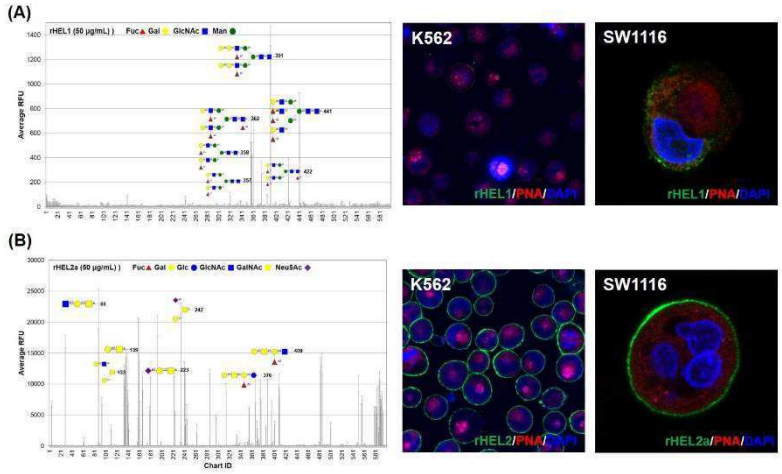

Fig. 1 – Glycan array profiles and cancer specific binding activities of *H. erinaceus* isolectins rHEL2a (A) and rHEL1 (B).

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POLYPLEX FORMATION OF DNA WITH CATIONIC CHITOSAN DERIVATIVES

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Since the 1990s a lot of knowledge has been gained on the development of nonviral gene delivery systems based on polyplexes of DNA with different cationic polymers. Polyplexes have shown their feasibility in gene therapy, but are still far from clinical applications.

In the majority of cases, only colloidal properties of polyplexes (hydrodynamic diameter and ζ-potential) are taken into account as critical for cellular uptake. Dissociation of polyplexes into the cell is also a critical process affecting overall transfection efficiency. It has been reported that thermodynamic parameters associated with the formation of chitosan-DNA polyplexes are correlated with transfection efficiency, and less stable complexes exhibit higher biological response [1] and that free cationic polymer promote transfection process [2]. Thus, we hypothesized that by varying the degree of substitution in cationic chitosan derivatives the balance between DNA binding and DNA release could be found.

A series of samples of *N*-[4-(*N'*,*N'*,*N'*-trimethylammonium)benzyl]-chitosan chloride (TMABC) with different degree of substitution was synthesized as previously reported [3] from chitosan with Mw 3.7×10^4 and the degree of acetylation of 26 %. Salmon sperm double-stranded DNA with an average chain length of 300 bp was used as a model polynucleotide. Binding affinity and stoichiometry of TMABC:DNA polyplexes were studied by fluorescent intercalator displacement assay showing that DNA binding affinity increases with increasing of the degree of substitution of TMABC. Binding constants of TMABC:DNA polyplexes were calculated using Scatchard analysis of the binding curves.

The formation of TMABC:DNA polyplexes was also studied by dynamic light scattering and turbidimetry. Turbidimetric titration curves are represented by a leap in optical density at TMABC:DNA ratio close to 1:1. The hydrodynamic radii of polyplexes reached its maximum at the 1:1 ratio and decreased with increasing of TMABC excess. All methods proved that after certain TMABC:DNA ratio polyplex properties do not change and an excessive amount of TMABC remains unbound. Obtained results are in agreement with our previous results [3] and give answers on the question why excessive amounts of low-substituted TMABC are necessary to enhance transfection efficiency. The degree of substitution ca. 20 % was enough to make TMABC soluble in physiological conditions and promote transfection more efficiently than TMABC with higher degrees of substitution.

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RATIONAL DESIGN OF COVALENT INHIBITORS FOR A BACTERIAL GLYCOSYLTRANSFERASE

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In Gram-negative bacteria such as *Haemophilus* and *Neisseria*, the retaining α-1,4-galactosyltransferase LgtC [1] catalyses the transfer of a galactose moiety from a UDP-Gal donor to lactose acceptor structures in the lipooligosaccharide (LOS) envelope (Fig. 1A). The resulting digalactoside epitope increases serum resistance [2] and is an important virulence factor. Small-molecule inhibitors of LgtC are therefore sought after as chemical tools for glycomicrobiology and potential anti-virulence agents.

We have recently identified, from screening, non-substrate-like inhibitors of LgtC that react covalently with the non-catalytic Cys246 in the acceptor binding site [3]. This discovery inspired us to design a novel type of carbohydrate-based covalent inhibitor for LgtC, which combines an acceptor substrate scaffold with an electrophilic warhead (Fig. 1B). We reasoned that such substrate-based covalent inhibitors may exhibit greater target selectivity and potency than our existing non-substrate-based chemotypes.

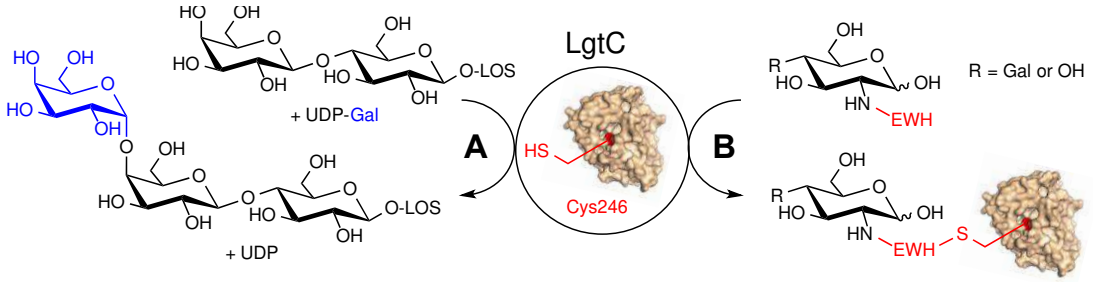

Fig. 1 – (A) the LgtC reaction; (B) our covalent inhibition strategy (EWH: electrophilic warhead).

Herein, we present the first results from this approach, including the design, chemical synthesis and initial biochemical evaluation of our target inhibitors.

A covalent docking protocol [4] was used to identify the optimal position for attachment of the electrophilic warhead on a lactosamine scaffold. Lactosamine derivatives with two different electrophilic warheads, as well as the corresponding glucosamine derivatives, were prepared by multi-step synthesis. All four target molecules were tested against LgtC in a biochemical assay. While the lactosamine derivatives retained some substrate activity, the corresponding glucosamine derivatives exhibited inhibitory activity (IC_{50} 0.15-1mM), and were not turned over as substrates by the enzyme. For selected inhibitors, we have also investigated their mode of action, using detailed enzymological experiments, mass spectrometry, dialysis, and site directed mutagenesis.

Non-catalytic cysteines are a common motif in the active site of many bacterial glycosyltransferases [3]. Covalent targeting of these residues is therefore a broadly applicable strategy for inhibitor development against these enzymes. Our results provide important insights for the application of such an approach.

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DESIGN, SYNTHESIS, AND EVALUATION OF IMPROVED AMINOGLYCOSIDES

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Research in our laboratories into the design, and synthesis of a series of highly active paromomycin derivatives will be presented resulting in a lead compound that displays antibacterial activity equal to or greater than the parent paromomycin for several strains of Gram-negative and Gram-positive bacteria with significant improvement in activity against many strains of antibiotic resistant bacteria. Selected compounds, including the lead, also display enhanced selectivity for bacterial over several ESKAPE pathogens. All aspects of synthesis, biological evaluation, and conformational analysis by NMR spectroscopy will be covered in the presentation.

CHEMOENZYMATIC SYNTHESIS OF PSEUDAMINIC ACID CONTAINING GLYCOSIDES

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The cell-surface bacterial carbohydrate pseudaminic acid (Pse) has been shown to play a role in the virulence of several multidrug resistant pathogens [1]. Whilst the biosynthesis of Pse is well characterized, studies of enzymes which process this sugar have been hindered by a lack of access to Pse and Pse-based glycosides [1,2]. In this study, a chemoenzymatic synthesis was designed, utilizing *Campylobacter jejuni* and *Aeromonas caviae* Pse biosynthetic enzymes to generate nucleotide-activated Pse (CMP-Pse) and derivatives with varying N-linked functionality at C7. A highly promiscuous sialyltransferase was then used with CMP-Pse and a range of glycosyl acceptors to generate a library of di- and trisaccharides containing Pse (figure 1b).

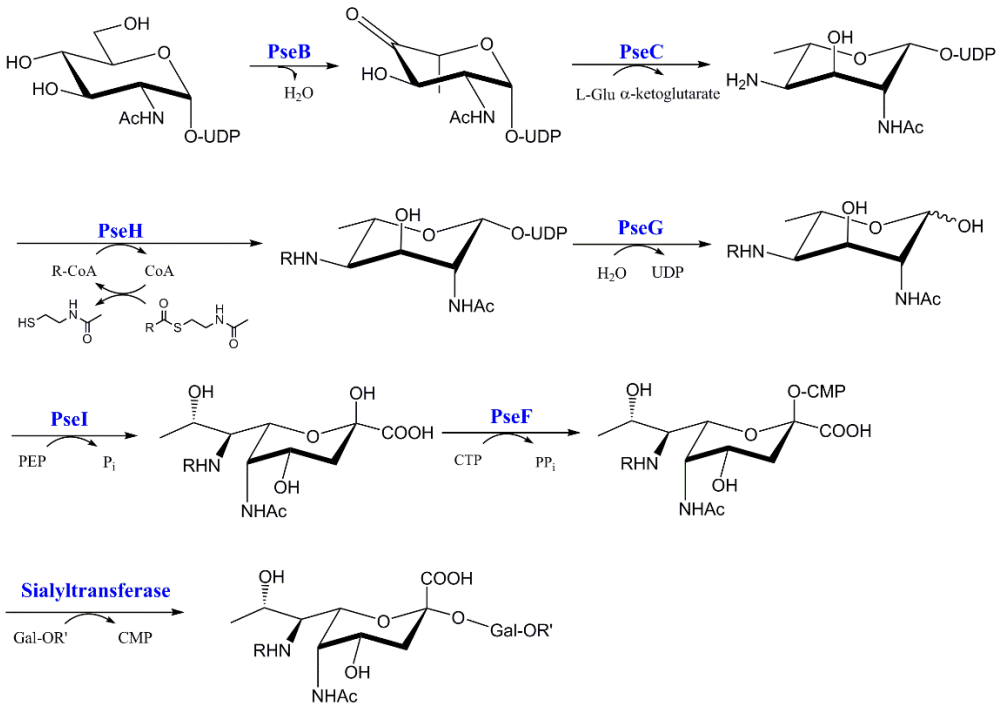

Fig. 1 – Chemoenzymatic synthesis route for the synthesis of Pse containing glycosides

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PDA-BASED NANOMICELLES FOR THE DIAGNOSIS AND TREATMENT OF HEPATIC AND PROSTATE CANCER

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Cancer is one of the chronic diseases with worse prognosis and whose treatment still implies harmful side effects. Among the different approaches developed so far against this threat, nanomedicine is the most promising one for the development of efficient therapeutic and diagnostic (theranostic) agents. Thus, the design and synthesis of nanovectors that allow the targeted delivery of anticancer drugs and imaging agents, is highly needed. Based on these premises, in the present work we report on the design and synthesis of mannose-, galactose- and a dipeptide-coated nanomicelles for their application as smart nanovectors for drug delivery into hepatic and prostate cancers (see Fig. 1). These nanomicelles are easily produced by a simple method through the supramolecular self-organization and photo-polymerization of diacetylenic-based amphiphiles pre-functionalized with the desired specific ligand [1]. The polymerization which takes place without catalyst or initiators afford in pure form micelles unaffected by the dilution.

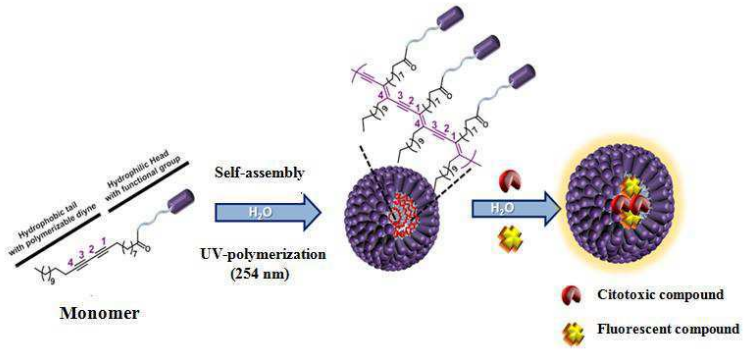

Fig. 2 – Synthesis and drug loading of the smart nanovectors

The loading efficiency, release profile, specific interaction with their cognate receptors as well as the antitumor activities of these nanosystems are some aspects which will be discussed. The interesting antitumor activity of mannose-coated nanomicelles, in accord with the recent discovery of the overexpression of a mannose-6-phosphate receptor in prostate cancer [2], encourages us to design and synthesize micelles functionalized with a robust mannose-6-phosphate analogue. The primary results of this approach will also be discussed.

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INTERACTION AND CONFORMATION STUDIES OF A NOVEL ANTITHROMBIN BINDING OCTASACCHARIDE NOT INCLUDING THE ACTIVE PENTASACCHARIDE SEQUENCE

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Heparan sulphate (HS) is a linear highly sulfated polysaccharide, containing D-glucuronic (GlcA) or L-iduronic acid (IdoA) linked to glucosamine (GlcN), present on all mammal cell surfaces. HS plays a key role in pathophysiology by interacting with different proteins [1]. Heparin, a higher sulfated member of HS family, is a commonly used anticoagulant drug in surgery and in clinics [2]. Heparin-AT binding depends on a specific pentasaccharide sequence containing a 3-O-sulfo group in its central GlcN residue (GlcNS,6S/Ac-GlcA-GlcNS,3,6S-IdoA2S-GlcNS,6S). An important role is also played by the peculiar conformational behavior of the L-iduronic acid. L-iduronic acid (IdoA) as the pyranose ring may assume in solution three equienergetic conformations ($^1C_4$, $^2S_0$, and, less frequently, in $^4C_1$), of which the $^2S_0$ was demonstrated to be the "active" form in the binding to antithrombin (AT) [3]. Recently, a novel chemo-enzymatic synthesis has given access to highly tuneable AT-binding oligosaccharides, opening new opportunities for characterizing heparin-protein interactions [4]. In our previous work, the conformation of two synthetic AGA*IA-containing hexasaccharides obtained by chemoenzymatic synthesis and differing in the sulfation of the IdoA unit was investigated in the free and in the bound state with AT [5]. Here, the conformation of a novel octasaccharide obtained by chemoenzymatic synthesis, not including the classical active pentasaccharide sequence but able to bind and activate AT [6], was studied by Saturation Transferred Difference NMR, transferred-NOEs and Molecular Dynamic simulation. Results confirmed the significant role of the flexibility of IdoA residues and of the contact network between the AT-binding site and residues surrounding the 3-O-sulfated glucosamine. These results would question the common opinion of the high specificity of the binding between heparin and AT.

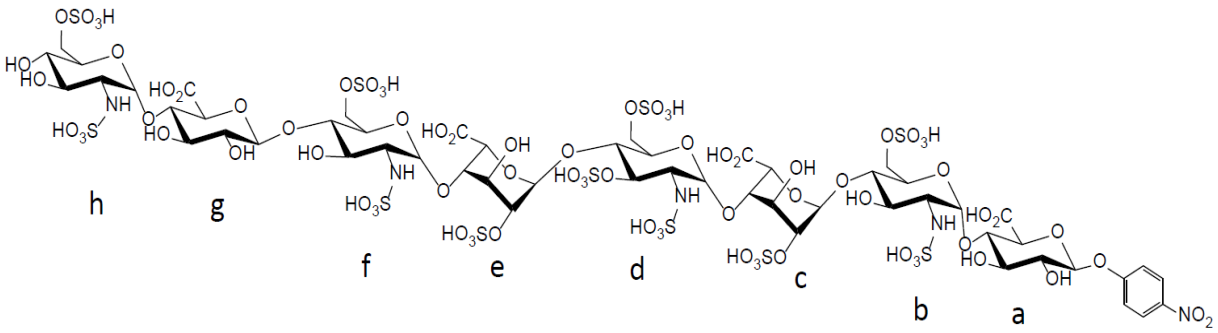

Fig. 1 – Structure of octasaccharide

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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NEW RUTHENIUM COMPLEXES DERIVATIZED WITH GLUCOSE AIMED AT SELECTIVE CANCER THERAPY

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Among the treatment options for cancer, chemotherapy remains of the most relevant modalities. Cisplatin is among the most used broad-spectrum chemotherapeutic drugs and one of the very few coordination compounds in clinical use [1], being also used in combination with other anticancer molecules. However, it displays high toxicity and therefore the search for new and selective metal complexes remains of interest, bearing in mind the potential of inorganic complexes for cancer therapy. Previous published results showed the high effectiveness and selectivity by ruthenium complexes of general structure $[Ru(Cp)P(L\text{-}L)]^+$, with P = Phosphane and L-L = N-O or N-N heteroaromatic bidentate ligands [2].

Based on these findings and with the aim of increasing the selectivity of our previous molecules, we were prompted to synthesize new related derivatives comprising ligands having glucose units. The rational for the inclusion of glucose moieties is based on the knowledge that cancer cells need and have higher consumption and uptake of this sugar due to their overexpression of membrane glucose transporters (GLUT). Hence, it is anticipated that a selective targeting of malignant cells relatively to healthy ones can be achieved with glucose-containing complexes.

In this communication we present the synthesis of new ruthenium (II) organometallic compounds having diglucosyl bipyridine ligands (Fig. 1). Their access firstly involved the preparation of the disubstituted bipyridine ligand, through bis-coupling of a bipyridine dicarboxylic acid with a partially protected glucose derivative by Mitsunobu reaction. Differently substituted glucose derivatives were used to study the scope of the reaction. The resulting ligand was subsequently coordinated to the ruthenium by treatment with $[Ru(Cp)(Ph_3)_2(Cl)]$ and CF_3SO_3Ag. The new molecules were characterized by NMR, FTIR, UV-Vis. Preliminary biological evaluation was assessed in two cancer cell lines.

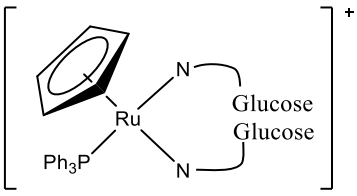

Fig. 3 – General structure of the synthetized ruthenium-diglucosyl bipyridine complexes

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SYNTHESIS OF NEW NUCLEOS(T)IDE ANALOGUES AND EVALUATION OF THEIR CHOLINESTERASE INIBITORY ACTIVITIES

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Nucleosides and nucleotides are essential biomolecules in multiple biological processes, such as the synthesis of nucleic acids, cell cycle or cell signaling. These events are essential in health and also for the progress of diseases, such as cancer and viral infections, in which they are overactivated. Hence, the synthesis of nucleoside and nucleotide analogues may be a useful therapeutic strategy, aiming at interfering or inhibiting these processes [1]. Besides having potential anticancer and antiviral properties, other types of bioactivities for these groups of molecules have been reported, including the ability to inhibit cholinesterases, which are biological targets for Alzheimer's disease therapy. In particular, previous results from our group showed the potent inhibition of acetylcholinesterase by isonucleosides comprising a theobromine or a triazole motif linked to C-5 or C-6 of a monosaccharide unit [2,3].

In this context, in this communication we present the synthesis of various xylofuranosyl isonucleosides comprising various types of nucleobases at C-5 of the sugar moiety. Their access was based on the Mitsunobu coupling between a purine or pyrimidine derivative and a partially protected xylofuranose. Moreover, isosteric functions for a phosphate group, such as a sulfonamide or a phosphoramidate, were installed at the isonucleoside anomeric position, leading to isonucleotides, which constitute a rather unexploited type of nucleotide analogs.

The ability of the new isonucleos(t)ides to inhibit cholinesterases was studied and some compounds showed potent activities with Ki values in the micromolar concentration range. The synthetic work and the results of the bioactivity evaluation will be disclosed and discussed.

Acknowledgements: The authors thank Fundação para a Ciência e Tecnologia (FCT) for financial support through projects UID/MULTI/00612/2013 and IF/01488/2013/CP1159/CT0006.

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SYNTHETIC MARADOLIPIDS AS MINCLE AGONISTS

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Macrophage inducible C-type lectin (Mincle) is expressed on antigen presenting cells and is an important player in innate immunity due to its capacity to recognise pattern-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), which are involved in pathogen recognition and in tissue homeostasis, respectively [1]. There are a number of ligands that can bind and activate Mincle [2], with prominent examples including the mycobacterial glycolipid, trehalose dimycolates (TDM), and the synthetic analogue thereof, trehalose dibehenate (TDB), both of which exhibit promising adjuvant activity [3].

In the present work, we report on the efficient synthesis of *iso*-branched trehalose diesters (maradolipids or *iso*-TDEs) **1a-f** and linear trehalose diesters (TDEs) **2a-c** (Fig.1), and investigate their ability to activate bone marrow derived macrophages (BMDMs) and illicit cytokine and chemokine production [4]. Both classes of TDEs were found to activate macrophages in a Mincle-dependent manner, with longer chain TDEs leading to a robust inflammatory response, as indicated by the production of interleukin (IL)-1β, IL-6, IL-10, IL-12 and macrophage inflammatory protein (MIP)-2 (Fig. 2) [4]. The *iso*-TDEs lead to greater cytokine production and a faster immune response, thus indicating that these trehalose glycolipids may have superior adjuvant activity [4].

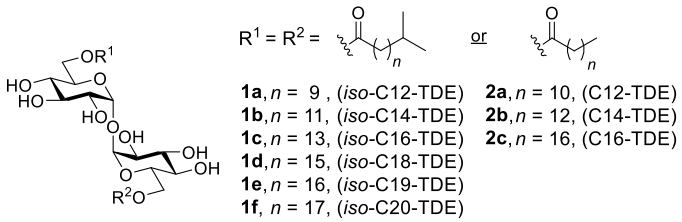

Fig. 4 – Maradolipid and linear TDEs targets

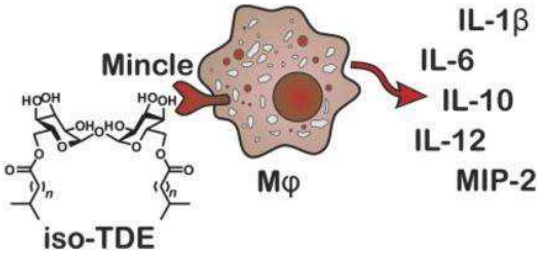

Fig. 2 – Mincle-mediated immune response of macrophages through maradolipid binding

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GLYCOCLUSTERS AS TOOLS FOR INHIBITION OF LECTIN MEDIATED BACTERIAL ADHESION

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Lectins are carbohydrate recognizing proteins involved in intercellular interactions [1]. Amongst various biological processes that lectins play a crucial role in, we focus on the lectin mediated bacterial adhesion to the host tissues. Pathogenic bacteria use lectins to read the glyco-code presented on the host cell surface and adhere to the tissue, which is the initial step of bacterial infection development [1]. Several lectins with different sugar specificity were identified in *Burkholderia cenocepacia* [2] and *Pseudomonas aeruginosa* [3], opportunistic pathogens causing nosocomial infections in a cystic fibrosis patient. Our goal is to find a molecule with a high affinity towards lectin to competitively inhibit the bacterial adhesion to the host tissue and stop the infection at its very beginning.

Glycomimetics with calixarene scaffold core were prepared. These cores were modified with a different number of saccharides positioned on linkers with variable length. According to the specificity of targeted lectin, L-fucose, D-galactose or D-mannose monosaccharide was used. Hemagglutination with purified lectins was used to evaluate lectin activity, as a classical, simple and inexpensive method. As lectins are multivalent proteins, they can bind to cells with suitable saccharides on their surfaces, cross-link the cells and form clusters easily visible in the microscope. Multivalent glycomimetics were used to inhibit lectin hemagglutination activity and the compound potency was compared with monosaccharide inhibitor. The capability of glycomimetics to function on the cellular level was tested in bacterial cross-linking studies.

We were able to discover promising compounds capable to inhibit lectin activity in solution and with proven interaction with the bacterial cell wall. These compounds should be further tested, as they could be potential candidates for antiadhesive therapy development.

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A NOVEL CARBOHYDRATE-BASED PROBE FOR BACTERIAL PROTEOMICS

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Bacteria possess a multitude of carbohydrate-recognising proteins (CaRPs), such as carbohydrate-active enzymes, lectins and porins [1]. Many CaRPs directly underpin bacterial virulence, pathogenicity and intrinsic resistance to antimicrobial agents and bacteriophage [2,3]. Moreover, carbohydrate metabolism is different in low- and high-virulence strains, for example of the Gram-negative pathogen *Klebsiella pneumoniae* [4]. Chemical tools for the proteome-wide analysis of CaRPs are therefore of great interest for bacterial proteomics and metabolomics, and to enable the identification of new targets for antibiotics and diagnostics development.

We have developed a carbohydrate-based probe for the labelling of CaRPs in bacterial lysates (Fig. 1). Our design combines a monosaccharide scaffold as a recognition motif, with an electrophilic warhead for covalent labelling of target proteins, and a reporter group at the anomeric centre for detection.

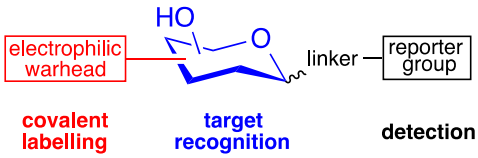

Fig. 5 – Probe design

Herein, we report the application of this probe for the proteome-wide profiling of different strains of *Klebsiella pneumoniae*, the causative agent of pneumonia and one of the most important nosocomial pathogens. Because of its rapid resistance development to latest-generation cephalosporins and carbapenems, *Klebsiella pneumoniae* has recently been classified by the WHO amongst its 12 "Priority Pathogens" [5].

For initial experiments, we used a fluorophore as the reporter. We successfully separated both anomers of the probe and found that, intriguingly, they differ significantly in their fluorescence emission.

Lysates from different clinical isolates of *Klebsiella pneumoniae,* including the carbapenem-resistant strain KP16, were incubated for 30 mins at 30°C with either α- or β-probe, separated by gel electrophoresis, and analysed by Coomassie staining and in-gel fluorescence scanning. Fluorescence detection revealed a distinctive 5-band signature for the carbapenem-resistant strain KP16, including two low-abundance proteins. LC-MS/MS analysis of selected bands showed, that target proteins of the probe include known carbohydrate-binding proteins as well as currently uncharacterised proteins.

Our protocol represents an operationally simple workflow for the profiling of bacterial lysates, which is potentially broadly applicable with many other species and strains also.

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(1-4)-*S*-THIODISACCHARIDES AS POTENTIAL ANTI-GLIOMA DRUGS

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(1-4)-*S*-thiodisaccharides are group of compounds containing the 1-4-thio bridge what is related to their anti-cancer properties. In our previous experimental study we showed that (1-4)-*S*-thiodisaccharides induced oxidative stress and apoptosis in cancer cells in the micromolar range. These compounds were found to kill human breast, ovarian and cervix cancer cells [1,2]. However, the mechanism of their action has not been clearly defined. In this study we are introducing the mechanism of action of two compounds: (1-4)-*S*-thiodisacharide and anhydrosugar with salicylic group connected *via* sulfur bridge denoted respectively as FCP6 and FCP8 [Fig1] on glioma cells. Our *in vitro* glioma model consists of U87 cell line (ATCC) and two novel glioma cell lines derived from patients H6PX and H7PX. To determine the mechanism of (1-4)-*S*-thiodisaccharides activity, we performed a series of tests including cytotoxic, clonogenic and apoptosis assays. We also evaluated the influence of (1-4)-*S*-thiodisaccharides on protein synthesis processes, endoplasmic reticulum stress and the thioredoxin system inhibition. We also checked (1-4)-*S*-thiodisaccharides function within cell as an electrophile that can cause in this form glioma cell death, using dithiothreitol as reducing reagents. Both (1-4)-*S*-thiodisaccharides induced cytotoxicity, have anti-proliferate properties and induced apoptosis of glioma cell. It was found that all the effects to be results of evocation of oxidative stress, endoplasmic reticulum stress, inhibition of protein synthesis, reduced overall cellular thiol level and inhibition of thioredoxin reductase activity. As a part of research, we also performed RT-PCR analysis and observed a significant increase of expression of two key markers of endoplasmic reticulum stress GRP78 and CHOP and FASLG which is pro-apoptotic protein. FCP6 seems to be the most promising anticancer agent due to the effects obtained in low micromolar concentrations (IC_{50}= 65,2 μM on U87 cell line) and deserve further biological investigation.

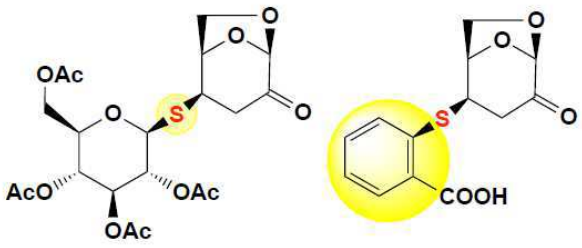

Fig 1 – FCP6 and FCP8

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LOW ESTER AMIDATED CITRUS PECTIN AND FLAXSEED GUM AS SUITABLE CAREERS FOR WOUND HEALING AGENTS

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Construction of novel more effective wound healing dresses is still actual task for medicine. Natural and modified polysaccharides are interesting as potential biodegradable non-toxic carriers for application of wound healing drugs. High and low ester pectins have recently been investigated for its use in various biomedical applications such as drug delivery, skin protection and cell immobilisation [1]. On the other hand, flaxseed gum was found to have antioxidant properties [2] and can be used as a career for silver nanoparticles for antimicrobial and wound-dressing applications [3]. Both pectin and flaxseed gum are interesting because of their moistening, smoothing, air permeability and other effects supporting wound healing. Gradual biodegradation of the polysaccharide matrix leads to a gradual release of the drug from the hydrogel carrier and thus can promote tissue regeneration without additional stress. In addition, these polysaccharides contain free carboxylic groups that can interact with cationic peptides, which were used as appropriate therapeutic agents. Some of these agents are insoluble in water and thus an oppositely charged polysaccharide may uptake them and uniformly distribute them in the bulk gel.

Bioactive peptides like GHK (glycyl-L-histidyl-L-lysine) are able to accelerate wound healing and skin repair by stimulation of both synthesis and breakdown of collagen and glycosaminoglycans, and by stimulation of the replicative activity of fibroblasts and keratinocytes [4]. GHK also attracts immune and endothelial cells to the site of an injury.

In this study several bioactive peptides and their mixtures with several oligosaccharides were tested *in vivo* on female rats as potential wound healing agents. Hydrogels prepared on the basis of deesterified and partially amidated citrus pectin and flaxseed gum were chosen as a promising matrix for this application. Polysaccharides were dissolved in water (1 % m/m); the pH value was justified to 7.4 by 0.2 M NaOH. The agents were suspended in the solution, and in the case of pectin $CaCl_2$ solution was added to induce gel formation. The solution of flaxseed gum was highly viscous, so it was unnecessary to add any compounds supporting gelation. For comparison macadamia nut oil ointment (ambiderman) was also used as a matrix for transport of these preparations. The gels doped with therapeutic agents were characterised by spectroscopic and other analytical methods.

Obtained results demonstrated that some of tested oligopeptides including GHK showed significantly more effective healing than in the case of control animals without the treatment. In comparison with ambiderman, both polysaccharide hydrogels were found to be effective carriers for transport of wound healing agents and thus can be used for construction of wound-dressing materials.

Acknowledgements: Financial support from the Czech Science Foundation (project 17-02448S) is greatly acknowledged.

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UNNATURAL GLYCOPEPTIDES AND GLYCOPROTEINS AND THEIR USE IN ANTIGEN DETECTION AND IMMUNIZATION

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Carbohydrates are essential for the survival and proliferation of many microorganisms, for cancer progression, and they play a major role in immunogenicity. Therefore, these macromolecules are at the center of many vaccines that are currently undergoing clinical trials or are already commercially available, and are used for cancer diagnosis. Detection of glycosylated macromolecules by antibodies is affected by the binding affinity between the antigen and the antibody, and this in turn can be modulated by changes in the 3D conformation of either of the binding partners.

MUC1 is a glycoprotein overexpressed in around 80% of human cancers. However, while in healthy cells, the MUC1 backbone displays complex oligosaccharides, in tumours it's decorated with truncated carbohydrates. Consequently, different tumour-associated carbohydrate antigens become exposed and are involved in triggering immune responses. Over the years, several studies have demonstrated that circulating anti-MUC1 antibodies in serum may be used as a favourable prognosis for patients with different types of cancer because these antibodies can limit tumour outgrowth and dissemination. Consequently, efforts have been devoted towards the rational design of MUC-1 based antigens to be used as diagnostic tools for detection of anti-MUC1 antibodies in human serum. In this work, we designed and synthesized various MUC1 antigens that featured a hydrogen-by-fluorine substitution at that proline residue which displayed enhanced affinity to two anti-MUC1 antibodies. By combining MD simulations and X-ray crystallography, we provide an explanation for the better affinity of our derivatives towards two antibodies, which relies on stronger CH/Π interactions. Finally, we demonstrated then that these novel derivatives are more efficient than natural antigens in detecting low concentrations of circulating anti-MUC1 antibodies in human serum of patients with prostate cancer (adenocarcinoma and benign prostatic hyperplasia).

Improving antibody-antigen binding affinity may also be useful for the generation of more immunogenic agents. We are therefore exploring new synthetic glycoproteins as immunizing agents. By developing site-selective glycosylated proteins, we will also help to elucidate the mechanisms triggered by glycoproteins in the context of immunization.

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ADVANCEMENTS IN THE STRUCTURAL CHARACTERISATION OF AN ADENOCARCINOMA-RELATED MATRIX EPITOPE DEFINED BY THE MONOCLONAL ANTIBODY A10

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The monoclonal antibody A10, raised against murine Ehrlich tumour cells, was previously shown to efficiently inhibit their growth both *in vitro* and *in vivo* [1]. Interestingly, A10 reacted strongly and selectively with numerous human adenocarcinomas [2], suggestive of its potential for exploitation in immunotherapy. To this end, the structural elucidation of its epitope is plainly demanded. In the context of our study, A10-positive extracts of the Ehrlich tumour, namely Ca10, were isolated from culture supernatant by tangential flow filtration. Ca10 proved to be prone to oxidation by sodium periodate in a time- and concentration-dependent manner yet resistant to Pronase, evidencing its carbohydrate nature. The effect of enzymatic as well as chemical degradation (*β*-elimination) of Ca10 was then evaluated by NMR spectroscopy and PAGE. Manifestly, it remained unaffected upon treatment with a mix of exoglycosidases and PNGase F. On the other hand, diffusion-ordered spectroscopy (DOSY) revealed a substantial diffusion coefficient increase upon β-elimination pointing to the presence of *O*-glycosylation, while Ca10 resistance to *O*-glycanase treatment argued against the saccharide association through mucin-like linkage. NMR spectroscopy was selected as the method of choice for the in-solution characterisation of the saccharide chains derived from *β*-elimination. A collection of ^{1}H-^{13}C HSQC, TOCSY, and NOESY experiments aided the resolution of signals overlapping in the ^{1}H dimension, and identified heparan sulfate (HS) as the main carbohydrate component of the Ca10 extract. The sulphation degree and pattern of this HS is currently under study, along with the mapping of the A10-defined epitope.

Acknowledgements: This work was supported by the Spanish Ministry of Economy and Competitiveness (grants RTC-2015-3805-1 and CTQ2015-64597-C2-2-P) and an R&D contract between CIB-CSIC and Inmunotek SL.

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CYCLODEXTRIN-BASED MOLECULAR VECTOR THERANOSTICS

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Nucleic acids need *gene vectors* to go through cells membranes and reach the cytosol (for RNA) or the nucleus (for DNA) without been degraded. Viral vectors have demonstrated high efficacy to accomplish the task and promote transfection. Indeed, the still very limited examples of approved gene therapies are based on purposely-engineered viruses. Yet, some concerns remain regarding biosecurity and high production costs. Alternatively, a range of synthetic vectors with safer toxicity profiles and cheaper manufacturing processes have been developed, but at the expense of a reduced transfection efficiency. Molecularly well-define systems, as contrasted to polymeric vectors, offers higher opportunities for optimization schemes. Our efforts have focused in the development of single-diastereomer *polycationic amphiphilic cyclodextrins* (paCDs) [1] that can undergo nucleic acid-promoted co-assembly in aqueous media to form transfectious nanocomplexes (CDplexes) [2,3]. One step further, here we present a paCD *theranostic* prototype designed to monitor nucleic acid delivery *in vivo* by magnetic resonance imaging (MRI). The strategy consist in the single-branch incorporation of a tricarboxylic cyclen moiety as Gd-chelating element in the paCD structure (Fig. 1).The synthesis as well as preliminary siRNA complexation and MRI visualization data will be presented.

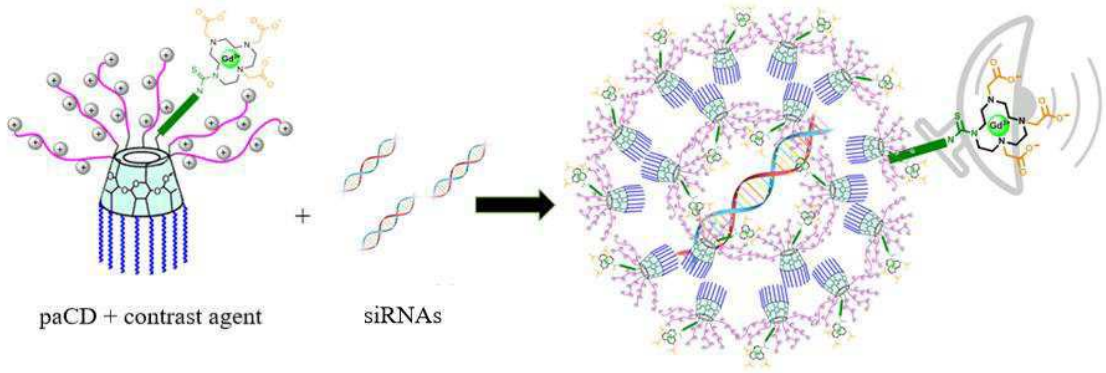

Fig. 1 – Schematic representation of a paCD-based theranostic agent with dual transfection-MRI visualization properties

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MOLECULAR ENGINEERING OF CYCLODEXTRIN-BASED ARTIFICIAL VIRUSES: SELECTIVE CHEMICAL MANIPULATION AT CONTROL OF GENE DELIVERY CAPABILITIES

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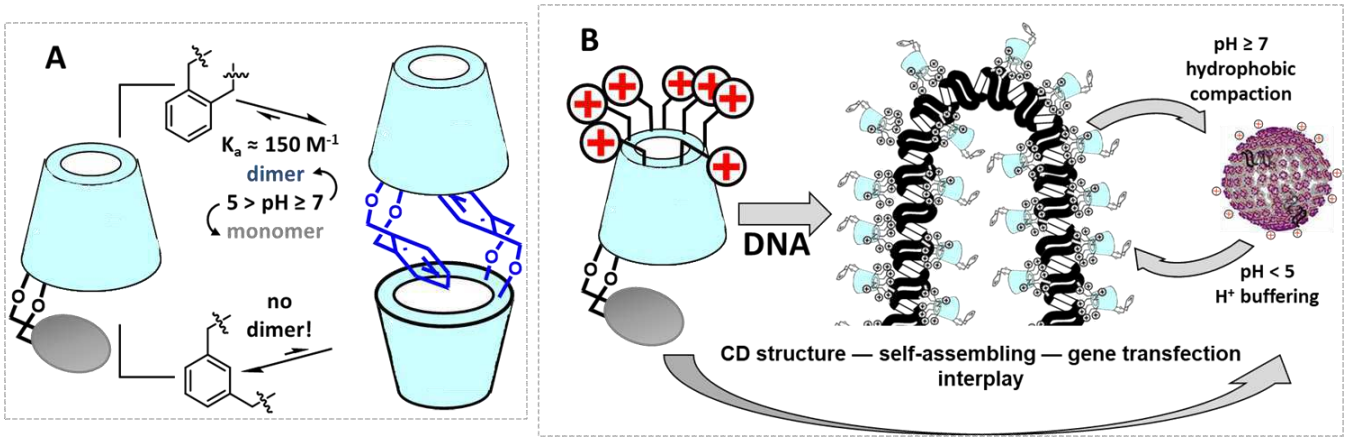
The realization of the gene therapy paradigm has consistently stuck at the difficulties encountered at delivering the gene material at the therapeutic target [1]. Despite the long road walked at viral particle manipulation, safety, technical and economic concerns still persist [2]. Fueled by the advance in nanotechnology, alternatives have arisen from materials with reversible nucleic acid condensing and compacting capabilities (e.g. cationic polymers and lipids) [3] that mediate cell transfection and gene expression into a variety of tissues and living organisms, however the poor understanding and control over the mechanisms for nucleic acid condensation and release are limiting progress to clinics. In this context, molecular scaffolds with hierarchical self-assembling capabilities offer unique prospects [4]. As a proof of concept, we have recently shown how hinge-type aromatic appendages selectively installed in cyclodextrin (CD) scaffolds exquisitely control their dimerization and inclusion properties in a pH-dependent manner (Figure A) [5]. Herein, we have implemented selective chemical functionalization schemes to provide these CD derivatives with cationic nucleic acid-binding domains. A set of CD-based facial amphiphiles featuring different type of aromatic hinges, cationic displays and scaffold sizes has been synthesized in order to explore the interplay between molecular structure, DNA-templated self-assembling process, the (bio)physical properties and stability of the resulting nanometric aggregates, and their ability to promote transgene delivery and expression into different cell lines (Figure B).



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FROM A TEA CUP TOWARDS A NEW DRUG CANDIDATE: EXPLORING THE MULTITARGET MECHANISM OF ACTION OF A PROMISING C-GLUCOSYL ISOFLAVONE WITH THERAPEUTIC EFFECTS AGAINST TYPE 2 DIABETES

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Today, around 375 million people live with type 2 diabetes (T2D) – a chronic illness generally resulting from the consumption of high fat-rich diets, obesity and sedentary lifestyles. T2D is the clinical diagnosis for insulin resistance and associated glucose intolerance, being commonly referred to as the non-insulin-dependent type of diabetes [1]. However, whereas in the prediabetic state patients are hyperinsulinemic due to reduced hepatic insulin clearance and increased pancreatic insulin secretion, in advanced stages of the disease the once exacerbated insulin secretion gives rise to significant reductions in β-cell mass and function [2]. Ultimately, this results in insufficient circulating insulin levels, often requiring the administration exogenous insulin or insulin analogues to control blood glucose levels of T2D patients [3].

Genista tenera is an endemic plant to the island of Madeira, Portugal, and used in folk medicine for the control of diabetes. After the discovery of 8-β-D-glucosylgenistein as the major component of the ethyl acetate extract of this plant, our investigation disclosed the potent antidiabetic activity of this compound, which comprises not only a remarkable antihyperglycemic effect in rats, but also the ability to inhibit the fibrillization of human islet amyloid polypeptide which, in the long run, accumulates in the pancreas and contributes to the above described reductions in β-cell mass and function [4]. These extremely promising observations compelled us to pursue the multitarget pharmacological mechanism of action underpinning the striking antihyperglycemic effects observed. In this communication, we will reveal the latest results of the chronic administration of 8-β-D-glucosylgenistein to diet-induced obese (DIO) C57Bl/6 mice, including a detailed inspection of glucose homeostasis, lipid profile and key pathophysiological traits of T2D in the liver, kidney and pancreas of treated animals. Moreover, the potential implications of our findings in the prevention of Alzheimer's disease in T2D patients will also be discussed.

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POSTERS | GD – Glycosylation and Disease

GLUCOSYL-GALACTOSYL-HYDROXYLATION OF CCN1 REGULATES ITS SECRETION

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CCN1 is a secreted protein and belongs to the CCN family consisted of the matricellular proteins. CCN1 has an N-terminal signal peptide, followed by four functional domains (Fig. 1). Since these functional domains involve in binding to various cell surface receptors, CCN1 plays important roles including cell proliferation, migration and angiogenesis through a variety of signaling pathways. In the previous studies, CCN1 undergoes post-translational modifications (PTMs) such as phosphorylation and *O*-fucosylation, and *O*-fucosylation influences the function of CCN1 [1,2]. Because CCN1 is associated with tumor malignancy [3], PTMs of CCN1 could be a molecular target for preventing carcinogenesis; however, PTMs of CCN1 are poorly understood.

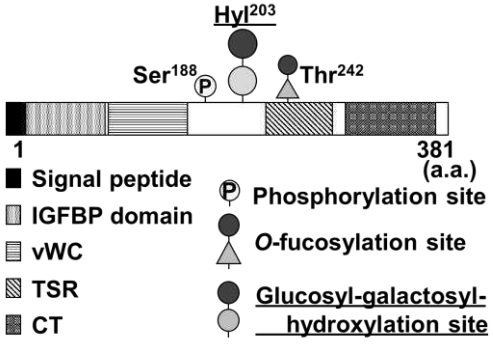

Fig. 1 - Schematic diagram of human CCN1.

In this study, we investigated the novel PTM of CCN1 to deepen the knowledge about the function of CCN1. We purified CCN1 from a CCN1-overexpressing HT1080 cell line, and then we analyzed it by LC-MS in order to explore novel PTMs of CCN1. As a result, we first demonstrated that CCN1 was glucosyl-galactosyl-hydroxylated at Lys203. Glucosyl-galactosyl-hydroxylation is commonly observed at Lys of the collagen family proteins, and glucosyl-galactosyl-hydroxylation of collagen is important for its fibrillogenesis, crosslinking and stability. In addition, collagen-like proteins, such as adiponectin and mannose-binding lectin, are also glucosyl-galactosyl-hydroxylated at Lys of the collagenous domain [4,5]. However, CCN1 does not have the the collagenous domain, so the glucosyl-galactosyl-hydroxylation of CCN1 is novel and unexpectable. We next established a glucosyl-galactosyl-hydroxylation-defective mutant (K203R) CCN1-overexpressing cell line so that we would examine the role of glucosyl-galactosyl-hydroxylation. In consequence, we observed that the secreted level of K203R mutant CCN1 was decreased compared to that of wild-type CCN1. This result suggests that glucosyl-galactosyl-hydroxylation at Lys203 of CCN1 promotes its secretion.

In conclusion, we first demonstrated the glucosyl-galactosyl-hydroxylation at Lys203 of CCN1. Moreover, glucosyl-galactosyl-hydroxylation reglated the secretion of CCN1. These findings in this study provide new insights into PTMs of CCN family and suggest that glucosyl-galactosyl-hydroxylation of CCN1 may be a therapeutic target for diseases associated with CCN1.

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AUXILIARY-MEDIATED SYNTHESIS OF HOMOGENEOUSLY GLYCOSYLATED SOLUBLE FAS LIGAND VARIANTS

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Glycosylation is one of the most abundant and complex posttranslational modifications of proteins. Since its alterations are frequently observed in many pathogenic conditions [1-3], it is crucial to understand how individual glycan structures at specific protein sites affect protein structure and functions in order to elucidate disease promoting mechanisms. On the basis of its frequent appearance [4] and its important role in preventing protein misfolding and aggregation, *N*-glycosylation seems to be especially important to study. Therefore, homogeneously *N*-glycosylated protein variants are urgently required. To obtain such homogeneously *N*-glycosylated protein variants, a chemoenzymatic approach using soluble Fas ligand (sFasL) as a test candidate was chosen. sFasL consists of the extracellular part of Fas ligand, a transmembrane protein of the tumor necrosis factor family which plays an important role in T-cell homeostasis and immune response [5]. The function of the soluble part is still not fully understood, however, the protein seems to take part in cancer proliferation [6]. The protein will be assembled from three segments, each of them synthesized by solid phase peptide synthesis (SPPS), using native chemical ligation (NCL). The C-terminal peptide, containing two native *N*-glycosylation sites will be equipped with a recently introduced ligation auxiliary at the N-terminus [7]. In addition to mediating NCL at glycine, this photocleavable, polyethyleneglycol (PEG)-containing auxiliary can also facilitate peptide purification between cycles of enzymatic carbohydrate chain elongation. After the incorporation of asparagine-*N*-acetyl-glucosamine building blocks by SPPS at the sites of *N*-glycosylation, glycan extension is achieved by using glycosidases/glycosynthases and a homogeneous, activated *N*-glycan core structure isolated from egg yolk [8]. Subsequent elongation with suitable glycosyltransferases increases the number of accessible glycopeptide variants. These glycopeptide variants will be linked to the larger N-terminal segment, which in turn is synthesized from two smaller SPPS fragments by NCL. In this way, several *N*-glycan variants of soluble Fas ligand will be obtained and subjected to further chemical and biological investigation.

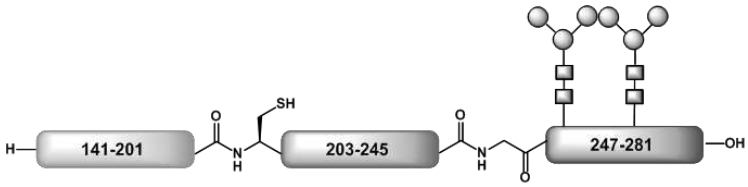

Fig. 1 - Homogeneously *N*-glycosylated sFasL (position 250, 260), assembled in three segments using SPPS and (auxiliary mediated) NCL

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DEVELOPMENT OF BISUBSTRATE COVALENT INHIBITORS OF PROTEIN *O*-GLCNAC TRANSFERASE (OGT)

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Reversible posttranslational modification of certain serine and threonine residues in nucleocytoplasmic proteins by attachment of a single β-*N*-acetylglucosamine (*O*-GlcNAc) is highly conserved in higher eukaryotes and is essential for proper development of the animal embryo [1, 2]. The protein *O*-GlcNAc transferase (OGT; EC 2.4.1.255) uses the donor substrate UDP-GlcNAc to transfer the sugar moiety onto target proteins, while deglycosylation reaction is carried out by *O*-GlcNAc hydrolase (OGA). Substantial substrate promiscuity enables this single pair of enzymes to control the proteome linked to the regulation of a range of cellular processes [3]. Dysregulation of the *O*-GlcNAc cycle is manifested in multiple metabolic pathologies, such as diabetes [4] and cancer [5], as well as neurological diseases such as Alzheimer's and Parkinson's [6].
Elucidation of the precise biological role(s) of protein O-GlcNAcylation is hampered by the lack of functional inhibitors of OGT. The known drug-like small molecules originated from fragment screening are yet to be improved to reach useful level of selectivity and potency [7]. The metabolic OGT inhibitor, i.e. per-acetylated derivative of 5-*thio*-GlcNAc is *a priori* non-selective when considered in the context of several other families of essential UDP-GlcNAc processing glycosyl transferases present in the cell [8].

Recently, we have reported synthesis and evaluation of *S*-linked UDP-peptide conjugates as bisubstrate hOGT inhibitors [9]. The targeted compounds were obtained by tethering the UDP moiety of the donor substrate to a series of peptides derived from several hOGT acceptor proteins through a three-carbon linker (Figure 1).The conjugate VTPVC(S-propyl-UDP)TA appeared to be the most potent synthetic binder of the hOGT (Ki = 1.5 μM) and inhibited the enzyme activity in *HeLa* cell lysates.

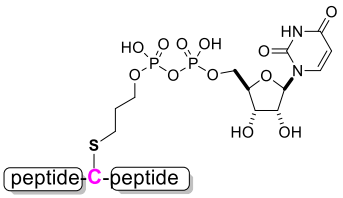

Figure 1

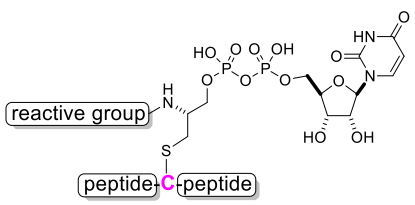

Figure 2

Here we report the inital results on development of covalent hOGT inhibitors using the scaffold of S-linked UDP-peptde conjugates by installation of a series of cysteine reactive probes aiming non-catalytic Cys^{917} onto stereo-defined amino handle in the linker (Figiure 2). Starting with custom phosphorylated lanthionine derived building block a highly efficient modular synthesis featuring assembly of the phosphorylated peptide followed by super-fast uridylation and attachment of the requisite reactive handle was implemented. In the initial tests, some of the new derivatives showed covalent inhibition of hOGT at concentration as low as 10 nM.

Details of the synthetic approach and the results of the prelliminary biochemical/biophysical elucidation of novel compounds will be presented.

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SYNTHESIS AND FUNCTIONAL STUDY OF LIPID-LINKED BACTERIAL PEPTIDOGLYCAN COMPONENTS

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The rise of antibiotic drug resistant bacteria such as VRE (vancomycin resistant enterococcus), MRSA (methicillin-resistant *Staphylococcus aureus*), MDR-TB, and XDR-TB (multidrug resistant and extensively drug-resistant tuberculosis) has stimulated the development of new antibiotics. Bacterial peptidoglycan, a major component in the bacterial cell wall, is composed of long glycan chains cross-linked by short peptides to form a mesh-like structure and is essential for bacterial shape and growth. Among those enzymes involved in bacterial cell-wall (peptidoglycan) biosynthesis, transglycosylase (TGase) and translocase MraY, are essential enzymes and considered as potential antibacterial drug targets. The former catalyzes the transfer of the sugar moiety from the activated Lipid II or polymeric peptidoglycan such as Lipid IV (a glycosyl donor) to Lipid II (a glycosyl acceptor), with concomitant release of an undecaprenyl pyrophosphate moiety. Lipid II is the naturally occurring minimum monomer of liner peptidoglycan polymers. The latter catalyzes the transfer of the monophospho-MurNAc-pentapeptide moiety from Park's nucleotide (UDP-MurNAc-pentapeptide) onto the undecaprenyl phosphate, to give Lipid I with concomitant release of UMP.

In this work, we *systematically modified* both complex and unique enzyme substrates (Lipid II and Park nucleotide) and used them for the comprehensive study to build the structure-activity relationship. Besides, the substrate-based inhibitors and chemical probes were also discovered for new assay development or bacterial imaging application.

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USE OF SUPERACID CONDITIONS TO HIGHLIGHT UNPRECEDENTED TRANSIENT INTERMEDIATES IN GLYCOCHEMISTRY

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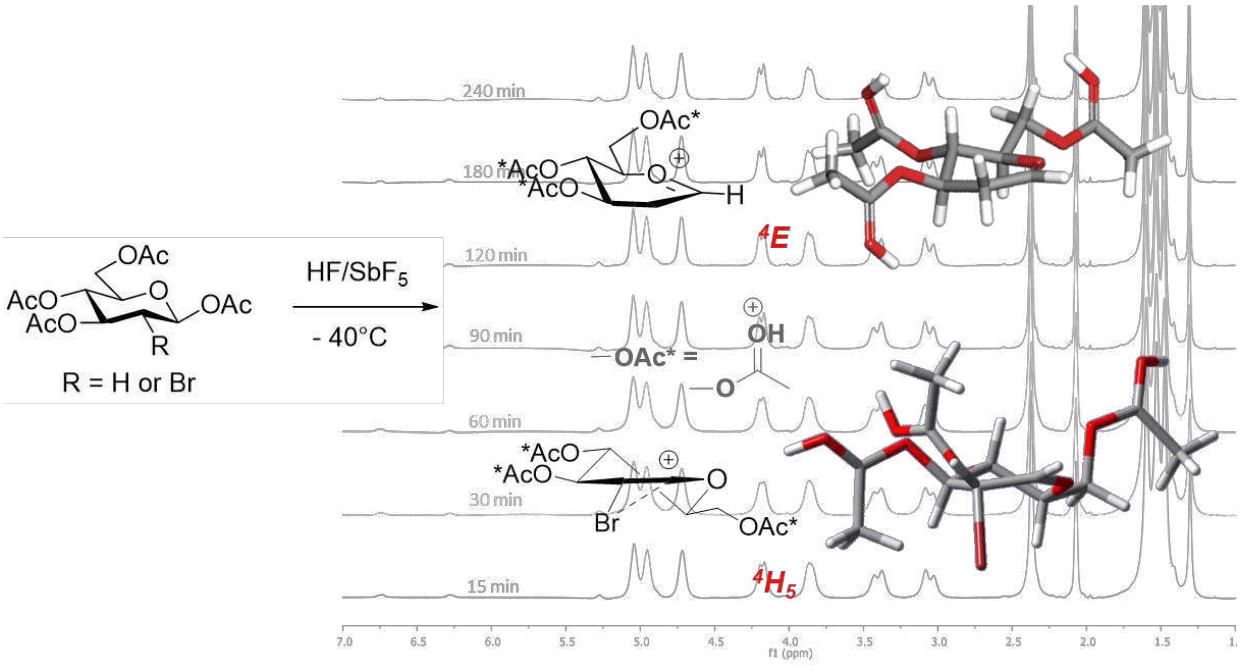
Glycosylation is the central reaction in glycoscience but details of its mechanism are still not totally understood. Thus, oxocarbenium and dioxalenium ions are considered to be key intermediates in the glycosylation reaction, but observation of these intermediates remains a challenging project that could be used to rationalize the stereochemical outcome of glycosylation reactions.

Several groups [1-3] are currently involved in the observation of the oxocarbenium ion using different methods, but the extremely short life of this intermediate makes its experimental observation particularly difficult. Our approach consists of generating these intermediates in superacid media [4]. It allowed their study by low-temperature *in situ* NMR supported by calculations giving access to the preferential conformation of these unprecedented transient intermediates [5]. The most recent results of this promising strategy will be presented.



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SYNTHESIS OF DONOR SUBSTRATES OF 4-AMINO-4-DEOXY-L-ARABINOSE TRANSFERASES

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Glycosylation of bacterial lipopolysaccharide (LPS) by addition of 4-amino-4-deoxy-L-arabinose (Ara4N) to lipid A and Kdo/Ko inner core units was implicated as a major cause of antibiotic resistance due to the masking of anionic charges [1-3]. 4-Amino-4-deoxy-L-arabinose transferases (ArnT) utilize α-Ara4N undecaprenylphosphate as donor substrate, but functional and structural information on the reaction is still scarce. In a first series towards the synthesis of the native substrate and potential inhibitors derived therefrom, both anomeric forms of Ara4N-phosphodiester derivatives containing saturated and unsaturated medium chain-length alkyl groups have been prepared and will be biologically evaluated.

Starting from methyl xyloside **1**, available in multigram amounts [4], the TIPDS-protected hemiacetal **4** was generated allowing the preparation of a separable mixture of the corresponding H-phosphonates **5a** and **5b** [5]. Approaches to enrich the α-H-phosphonate anomer will be presented. Formation of the phosphodiester linkage was achieved in fair yields upon PivCl mediated activation followed by oxidation. Cleavage of the silyl ether and conversion of the 4-azido into the 4-amino group through a Staudinger reduction gave the target compounds **8a, 8b, 9a** and **9b**.

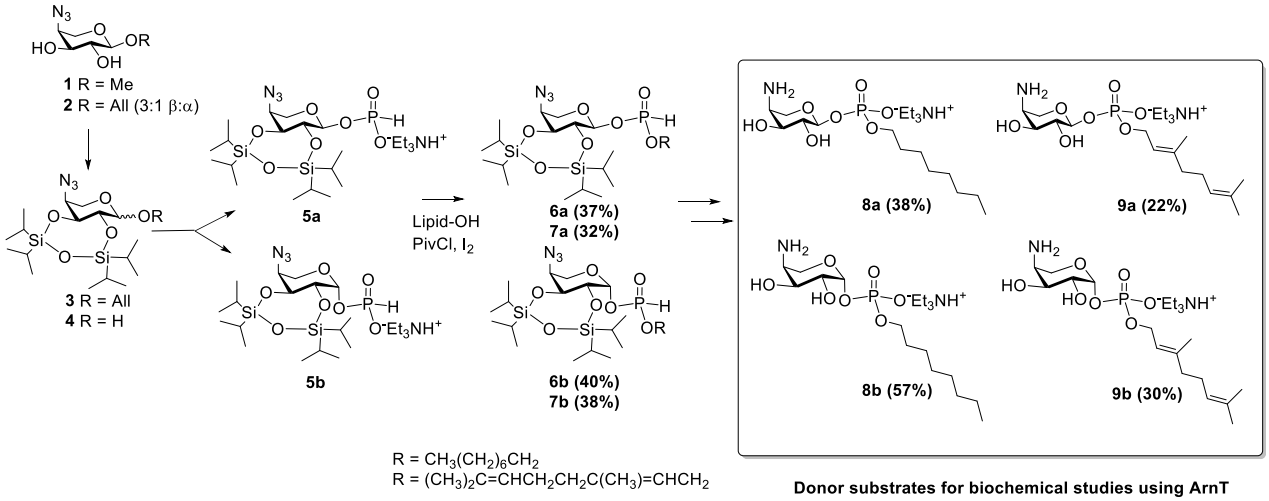

Fig.1 - Synthetic route for compounds **8a, 8b, 9a** and **9b**.

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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL (TRIAZOLYL)METHYL AMIDE-LINKED PSEUDODISSACARIDE NUCLEOSIDES AS POTENTIAL GLYCOSYLTRANSFERASE INHIBITORS

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Glycosylation is the most important post-translational modification occurring in proteins and other biomolecules, being involved in many cell processes such as cell-cell interactions and immune response [1]. Glycosyltransferases are enzymes responsible for the formation of glycans and glycoconjugates. Changes in glycosylation patterns on glycoproteins and other glycoconjugates occur in cancer and is considered a hallmark of this disease [2]. Therefore, inhibition of glycosyltransferases is a promising therapeutic strategy in anticancer therapy [3].

A few mimetics of nucleotide diphosphate sugars, natural substrates of glycosyltransferases, have been synthesised throughout the years [4].

In this communication, the synthesis of four novel nucleotide sugar mimetics containing a (triazolyl)methyl amide linkage as a more enzymatic/hydrolytic stable and neutral bioisostere of the diphosphate moiety is presented. Variations on the nucleoside ring A (i.e. furanose and pyranose motifs) and on the regiochemistry of the N-glycosidic bond (N7 and N9-linked nucleosides) (Fig. 1) [5], were made.

The synthetic methodology for these compounds involved the synthesis of two precursors (an azido glucoside and N-propargyl glucuronamide nucleosides) which were coupled via a “click” azide-alkyne 1,3-dipolar cycloaddition reaction [5]. These compounds were also evaluated for their stability in biological media. Preliminary results on the evaluation of the cytotoxicity of the compounds against MDA-MB-231 breast cancer cells overexpressing glycosyltransferases will also be disclosed.

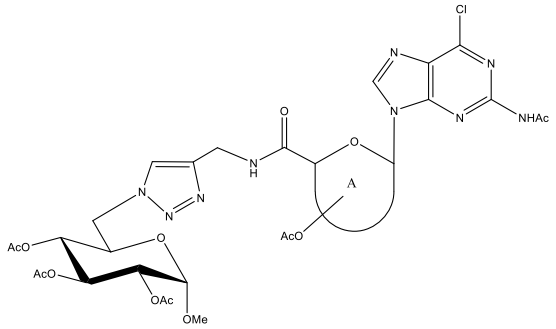

Fig. 1 – General structure of the synthesised compounds

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FUNCTIONAL ANALYSIS OF THE PROMOTER REGION OF C-MANNOSYLTRANSFERASE DPY19L3

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C-mannosylation is first identified in RNase 2 derived from human urine [1]. *C*-mannosylation is a novel sugar modification that adds α-mannose to the first tryptophan residue in the consensus sequence W-X-X-W/C (in which X represents any amino acid) of the protein. It has been reported that the function of *C*-mannosylation affects enzyme activity [2], protein secretion [3], and intracellular signal transduction [3, 4]. In the previous study, DPY19L3 was identified as one of *C*-mannosyltransferase for secreted protein R-spondin1 [5]. However, the regulation mechanism of gene expression of DPY19L3 is still poorly understood. *C*-mannosylation, which is a very specific sugar modification, has been found in various proteins in recent studies, and is steadily strengthening its position as a new sugar modification. Therefore, elucidation of the function of *C*-mannosylation is important for the development of glycobiology.

To identify the promoter of the human DPY19L3 gene, we cloned the fragment with the length of 2065 bp located at position −15 to +2050 bp relative to the transcription start site into the promoter-less pGL3-Basic luciferase reporter, named DPY19L3-2050. The result showed that luciferase assays revealed a significant increase in promoter activity of DPY19L3-2050 compared to pGL3-Basic empty vector in HEK293T cells, indicating a functional promoter is contained in the region of −15~+2050 bp of the human DPY19L3 gene. To further determine the crucial region for regulating human DPY19L3 gene expression, a series of luciferase reporter plasmids containing different fragments were generated, named DPY19L3-1868, DPY19L3-1655, DPY19L3-1500, respectively. These plasmids and DPY19L3-2050 were transfected into HEK293T cells, and promoter activity was measured by luciferase assays. The result showed that DPY19L3-1500 surprisingly decreased promoter activity compared to DPY19L3-1655. Therefore, the region from -1655~-1501 is important for transcriptional activity of the human DPY19L3 gene. Then, using online software TF bind, we found several putative binding sites of transcription factor. Mutations were added to determine the function of these putative binding sites. The result showed that promoter activity revealed a significant decrease in mutation plasmid compared to DPY19L3-2050.

In conclusion, we first determined the crucial region for regulating human DPY19L3 gene expression. Moreover, we narrowed down candidates of transcription factors that regulated gene expression of human DPY19L3. These findings in this study provide new insights into the elucidation of the function of *C*-mannosylation and suggest that *C*-mannosylation may be applied for the treatment of diseases associated with transcription factors regulating gene expression of human DPY19L3.

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IDENTIFICATION AND CHARACTERIZATION OF CHOLESTERYL GLUCOSIDE 6'-ACYLTRANSFERASE IN HELICOBACTER PYLORI FOR ITS ROLE IN PATHOGENESIS

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Helicobacter pylori infects approximately half of the human population and is the main cause of various gastric diseases. This pathogen is auxotrophic for cholesterol, which it converts upon uptake to various cholesteryl α-glucoside derivatives, including cholesteryl 6α-acyl and 6α-phosphatidyl α-glucosides (CAGs and CPGs). Owing to a lack of sensitive analytical methods, it is not known if CAGs and CPGs play distinct physiological roles or how the acyl chain component affects function. Herein we established a metabolite-labeling method for characterizing these derivatives qualitatively and quantitatively with a femtomolar detection limit. By using this labeling method, we provide a series MS/MS database of CGds and reveal overall lipid profiles of CGds in H. pylori. Through these profiling, we found that these bacteria acquire phospholipids from the membrane of epithelial cells for CAG biosynthesis. The resulting increase in longer or/and unsaturated CAG acyl chains helped to promote lipid raft formation and thus delivery of the virulence factor CagA into the host cell, supporting the idea that the host/pathogen interplay enhances bacterial virulence. In addition, we will report the identification and characterization of the enzyme catalyzing the CAG firmation, namely cholesteryl α-glucoside 6'-acyltransferase (CGAT). The enzyme is located in the bacterial outer membrane and secreted extracellularly via outer membrane vesicles. To inhibit CGAT was shown to effectively abolish the bacterial adhesion, indicating the enzyme to be a potential target for therapeutic intervention.

A FLUORESCENT ACTIVITY ASSAY FOR HIGH-THROUGHPUT SCREENING OF *O*-GlcNAc TRANSFERASE INHIBITORS

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The addition of *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) to serine and threonine residues of proteins is a dynamic and ubiquitous post-translational modification in mammalian cells [1]. This modification is mediated by only two enzymes; *O*-GlcNAc transferase (OGT), which catalyzes the addition of the *O*-GlcNAc unit, and *O*-GlcNAcase (OGA), which catalyzes hydrolysis of the carbohydrate unit from the protein sidechain. OGT is emerging as a major regulator of cell and organism physiology, regulating central processes such as transcription [2]. Further, dysregulation of the *O*-GlcNAc modification has been implicated in numerous disorders including Alzheimer's Disease and cancers [3,4]. Unfortunately, there are limited chemical biology tools available to study this modification and its fundamental roles in health and disease. Inhibitors of OGT, in particular, are expected to have great potential both for studying the role OGT in living systems as well as for the treatment of diseases which are linked to excessive OGT activity.

To date, few inhibitors of OGT have been developed, and these generally suffer from various drawbacks that limit their broad utility. To address this need, we have developed a convenient one step fluorescence-based activity assay to directly measure the glycosyltransferase activity of OGT. Notably, this assay circumvents the need for radioactive OGT substrates, suggesting it could be widely used by the community. To enable this assay, we synthesized several fluorescent OGT substrates. We demonstrated that these analogues are processed by the enzyme and permit *in vitro* enzyme kinetic studies. Using these substrates, we then implemented a fully-automated HTS campaign against a small-molecule library encompassing 60,000 compounds. Hits obtained from this screen have been validated and are presently undergoing optimization in a medicinal chemistry program to create suitable tool compounds for OGT that are potent, selective, and cell-permeable. We expect that suitable compounds will be of great benefit for the study of OGT activity in live cells and the role of this enzyme in human health and disease. Finally, we expect that the concepts embodied by this assay should be useful in the creation of new screens for many other enzymes catalyzing group transfer reactions.

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A C-TYPE LECTIN RECEPTOR (CLR)-FC FUSION PROTEIN LIBRARY AS A TOOLBOX TO EXPLORE THE INTRICATE INTERPLAY OF CLR-PATHOGEN INTERACTIONS

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Glycosylation is a highly diverse process that produces an abundant and highly regulated repertoire of complex glycoconjugates [1]. Myeloid C-type lectin receptors (CLRs) in innate immunity are glycan-binding proteins specialized in the recognition of glycoconjugates present on pathogens and host cells [2]. CLRs expressed by antigen presenting cells impact phagocytosis, antigen processing and presentation to T cells [3]. We have generated a comprehensive CLR-Fc fusion protein library containing about 20 immunologically relevant CLRs that represents a valuable screening platform for the identification of novel pathogen/CLR interactions [4].
The impact of CLR multivalency in carbohydrate recognition was evaluated using *N*-glycan microarray in an exemplary study for DC-SIGNR, a CLR that recognizes *N*-linked high-mannose oligosaccharides. Different DC-SIGNR constructs were employed including DC-SIGNR carbohydrate recognition domain (CRD, monomer), DC-SIGNR extracellular domain (ECD, tetramer), and DC-SIGNR-Fc (dimer). The glycan array data revealed a similar glycan recognition pattern for all DC-SIGNR constructs, with enhanced binding intensity for increased CLR valency. *Candida albicans*, a pathogenic fungus with a cell wall composed of mainly mannans, glucans and chitin, was further employed to evaluate pathogen recognition by the different DC-SIGNR constructs. Flow cytometry- and confocal microscopy-based binding assays highlighted DC-SIGNR as a novel pattern recognition receptor for *C. albicans* [5].
To extend the applications of our CLR-Fc fusion protein library, we are currently developing methods to detect interactions with enveloped viruses. In an exemplary study, we focused on Rift Valley Fever virus (RVFV), a phlebovirus transmitted by arthropods to livestock as well as human beings. *N*-linked glycans present in RVFV envelope glycoproteins are crucial for protein folding, viral infectivity, and immune evasion [6]. An affinity chromatography purification process to remove remaining host cell-derived proteins from RVFV preparations was performed to allow for an easy and fast viral screening by ELISA using our CLR-Fc library. To enable a multiplexed screening of virus/CLR interactions, we have also developed a novel flow cytometry-based assay in which the different CLR-Fc fusion proteins are immobilized on protein G-coated beads.

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THE RESTRAINED CONFORMATION OF KIFUNENSINE RESULTS IN ENZYME SPECIFICITY AND HIGH-AFFINITY BINDING

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The potency of an enzyme inhibitor depends on its ability to conformationally bind along the reaction coordinate of the target protein [1]. Using *ab initio* metadynamics (Car-Parrinello method [2]) and X-ray crystallography, the conformational flexibility of kifunensine in complex with GH47 was analysed [3].

Synthesized by the actinobacterium *Kitasatosporia kifunense*, kifunensine was discovered to inhibit class I glycoside hydrolase family 47 (GH47) enzymes [4,5]. These α-1,2-mannosidases are involved in N-glycan processing by cleaving the α-1,2-mannosyl linkages of $Man_9GlcNAc_2$ [6]. GH47 enzymes follow a ${}^3S_1 \rightarrow {}^3H_4 \rightarrow {}^1C_4$ conformational itinerary [7]; whereas two other mannosidase families, GH38 and GH92, which target α-1,2-, α-1,3- and α-1,6-mannosyl linkages, follow an ${}^OS_2 \leftrightarrow B_{2,5} \leftrightarrow {}^1S_5$ pathway [8,9]. The potential therapeutic uses for kifunensine include treatment for sarcoglcanopathies [10] and lysosomal storage disorders [11].

All possible conformations of kifunensine bound to a range of GH47, GH38 and GH92 enzymes were mapped onto a conformational free-energy landscape. The analysis of structures from small molecule crystallography and GH47, showed a low energy region around a ${}^1C_4$ conformation. The restrained "ring-flipped" ${}^1C_4$ conformation was confirmed by an atomic resolution (1 Å) structure of kifunensine bound to *Caulobacter* sp. GH47. To stabilise the conformation, a catalytically essential calcium ion coordinates the O2' and O3' hydroxyls of kifunensine [12]. Nanomolar binding affinity, K_d of 39 nM, reflects the preference for the low energy product ${}^1C_4$ conformation. For GH38 and GH92 complexes, a higher energy region around a ${}^{1,4}B$ conformation was energetically accessible. In comparison, kifunensine has a low binding affinity to GH38 and GH92, K_i of 5mM [13] and 100-200 μM [9] respectively, due to its inability to bind along the reaction coordinate.

This work highlights the application of natural substrates in the design of conformationally restricted glycoside hydrolase inhibitors.

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THE IMPACT OF FLOTILLINS ON ENDOCYTOSIS, TRAFFICKING AND SIGNALLING OF THE *P. AERUGINOSA* LECTIN LECA

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In the past decade, there has been growing evidence for the need to emphasize the role of lectins in bacterial pathogenicity [1]. The opportunistic bacterium *Pseudomonas aeruginosa* is one of the leading causes of nosocomial infections and particularly colonizes immunocompromised patients. One of its lectins, namely LecA, contributes to the virulence of *P. aeruginosa* and was also shown to aid in the transformation of a common extracellular bacterium like *E. coli* to an invasive pathogen [2,3]. LecA preferentially binds to α-galactose residues of the glycosphingolipid receptor globotriaosylceramide (Gb3), which assembles in cholesterol-rich lipid raft domains. In this work, we demonstrate the interaction with another lipid raft marker, the flotillin protein family. Flotillin-1 and -2 oligomerize as heterotetramers at the cytoplasmic leaflet of the plasma membrane. They form specific microdomains that represent active signalling platforms and play vital roles in cargo endocytosis and trafficking within the cell [4]. By CRISPR-Cas9 knockout of flotillin-1 and siRNA mediated knockdown of flotillin-2 in the lung epithelial cell line H1299, we created a flotillin depletion model to study its impact on endocytosis, trafficking and signalling of LecA. We compared wildtype (WT) and flotillin-depleted cells by immunostaining early, late and recycling endosomes as well as lysosomes and identified differences in the kinetics of LecA internalization. Our recent report suggested that LecA induces Src kinase-dependent CrkII activation in H1299 cells [5]. We now found that the phosphorylation of Src^{Y418} and $CrkII^{Y221}$ is shifted to earlier time points in the flotillin depletion model with respect to WT cells, strengthening our microscopy data. In ongoing experiments, we are screening for novel LecA interaction partners by pull-down experiments and proteomic as well as lipidomic analyses. Understanding the complex process of host cell invasion is a crucial step within the overall aim of piecing together the puzzle of a bacterial infection and developing novel therapeutic approaches [6].

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METABOLIC OLIGOSACCHARIDE ENGINEERING AS TOOL FOR THE HEPATIC STAGE OF MALARIA INFECTION

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We use metabolic oligosaccharide engineering to generate galactose derivatives containing terminal alkene reporter groups for labeling reactions with 6-Methyl-tetrazine derivatives. These galactose derivatives are applied during the hepatic stage of a Malaria infection. Malaria is still one of the major global health problems, causing about 500000 deaths each year [1]. In a time where increasing resistance to present treatments is observed, vaccination would be the application of choice.

The asymptomatic liver stage is considered as bottle-neck phase during the infection and represents a favored target for vaccine development. Since the presence of the α-Gal epitope is proven on *Plasmodium* sporozoites and anti-α-Gal specific antibodies (IgM) were shown to be increased in malaria endemic regions, as well as to provide a protective effect against Malaria in in vivo experiments, we are investigating the presence of galactose containing glycans on the surface of human hepatic cells during the liver stage of infection [2,3]. We synthesized three different galactose derivatives bearing terminal alkene groups in C2 or C6 position and varying chain length. Reaction kinetics were evaluated using a high-throughput method in 96-well-plates and the metabolic incorporation was explored in HepG2 cells. The incorporation of all galactose derivatives was successfully proven, and pentenyl-substituted derivatives were shown to result in the best labeling efficiency.

In the next step, sporozoites from *Plasmodium berghei* were used to infect HepG2 cells in the presence of one of the introduced galactose derivatives. We evaluated the level of incorporated artificial galactose derivatives via fluorescence intensity and compared the intensity of non-infected cells vs. cells infected with sporozoites from *Plasmodium berghei*.

We introduce the method of metabolic oligosaccharide engineering as a tool to investigate further on the nature of glycan structures during the liver stage of Malaria infection, in order to gain knowledge about possible candidates for vaccine development.

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ACTIVITY OF GLYCOENGINEERED GLYCOSIDASE WITH MULTIPLE MANNOSE-6-PHOSPHATES IN CELLS

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Lysosomal acid hydrolases are crucial for degradation and recycling of various biomolecules. They are mostly produced in a form of glycoproteins with oligosaccharides containing terminal mannose-6-phosphate (M6P). Genetic deficiencies of lysosomal enzymes cause incomplete digestion of biomolecular substrates and thus the progressive accumulation of non-degraded substrates in the lysosome. This event results in the onset of lysosomal storage disorders (LSDs). In particular, Tay-Sachs and Sandhoff diseases, a class of LSDs, which are caused by deficiency of β-N-acetylhexosaminidase, result in progressive accumulation of GM2-ganglioside in lysosomes. To remove GM2-ganglioside in diseased cells, multiple mannose-6-phosphate (M6P)-appended β-N-acetylhexosaminidases were prepared by click chemistry between the enzyme containing the alkynylated amino acid and azide-appended multiple mannose-6-phosphate (M6P) peptides. The multiple M6P-appended β-N-acetylhexosaminidase was found to be efficiently internalized into cells via M6P receptor-mediated endocytosis and then to reach lysosomes to regain its enzyme activity. Furthermore, the glycoengineered enzyme efficiently cleaved GM2-ganglioside in primary diseased cells, indicating the restoration of its activity in cells. The present strategy is highly attractive to construct multiple M6P-containing enzymes which can be used to study LSDs.

N-GLYCAN OF B CELL MATURATION ANTIGEN IS ASSOCIATED WITH RECEPTOR EXPRESSION AND SURVIVAL BY AFFECTING GAMMA SECRETASE-MEDIATED CLEAVAGE

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The homeostasis of long-live plasma cells in the bone marrow microenvironment is important for long-term effect of vaccines. Several interactions between receptor and ligand regulate the lifespan of plasma cells. Among these receptors, a tumor necrosis receptor family member named B cell maturation antigen (BCMA) plays an essential role for plasma cell survival in bone marrow microenvironment. Malignancies or inflammatory accessory cells also take the advantage of BCMA to maintain their survival to deteriorate the symptoms of diseases. Investigation on the interaction of BCMA and its ligand, a proliferation-inducing ligand (APRIL) and B cell-activating factor (BAFF), by specific antibodies and recombinant proteins are considered as potential therapeutic treatment. Besides, chimeric antigen receptor T cells (CAR-T) aimed to BCMA-expressing malignancy cells showed promising results in clinical trial. Recently, BCMA was reported to be modified by a single *N*-glycan on extracellular domain by aid of sugar alkynyl probes coupled with mass spectrometry. Glycosylation is an important post-translational modification and regulates many aspects of cellular responses, such as ligand binding or receptor expression. In this report, we studied the role of *N*-glycan on BCMA. We found the glycosite-mutated BCMA expressed lower than wild type. The mutant BCMA and glycosidase treatment exhibited decreased surface retention and increased soluble BCMA in culture medium. The mass spectrometry analysis showed the purified soluble BCMA were extracellular domain with almost non-glycosylated. Thus, we thought N-glycan was associated with surface expression of BCMA. It was reported that gamma secretase can shed BCMA from plasma membrane. We added gamma secretase inhibitor (GSI) and significantly elevated the surface BCMA on glycosidase-treated or glycosite-mutated cells and also reduced the soluble BCMA in culture medium. Interestingly, we found that almost GSI-induced increased BCMA was non-glycosylated. We also reduced the mRNA expression of the catalytic subunit of gamma secretase, PSEN1, by shRNA lentivirus and similar phenomenons as GSI did were also observed. The apoptosis assay showed that these increased non-glycosylation can enhance the cellular protection to non-receptor mediated (Dexamethasone) or death receptor-mediated (TRAIL) apoptosis. Together our findings, we suggested that *N*-glycosylation of BCMA is associated with surface receptor expression to fine tune the survival response by affecting gamma secretase-mediated cleavage.

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THE INHIBITION OF *N*-LINKED GLYCOSYLATION BREAKS THE RESISTANACE OF HUMAN OVARIAN CANCER CELLS TO CISPLATIN AND ETOPOSIDE

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Glycosylation is found as the most frequent post-translational modification of proteins, frequently altered in tumour cells [1]. Alterations in cellular glycosylation are among the crucial factors involved in cellular signaling, angiogenesis, development and progression of drug resistance in cancer cells. Changes in the expression or activity of glycosylotransferases and in the conformation of peptide backbones are the most important factors, which lead to an increase in the amount of *O*-GlcNAcylation of intracellular proteins and branched-*N*-glycans. Abundance of *N*-glycans correlates not only with the grade of cancer but also with the sensitivity to anticancer treatment [2].

The aim of the present study was to investigate the effect of two inhibitors of *N*-linked glycosylation – castanospermine (CAS) and tunicamycin (TM) – on the activity of anticancer drugs: cisplatin and etoposide in human ovarian cancer cells. We decided to choose two cell lines: cisplatin-sensitive A2780 and cisplatin-resistant A2780cis cells. Cisplatin is used as a standard drug in the first-line treatment of ovarian cancer. Etoposide is often introduced as a part of the second-line treatment in the relapsed and cis-platin-resistant ovarian cancers according to the van der Burg protocol [3]. We decided to combine these routinely used anticancer drugs with CAS and TM. One of the most important features of CAS is inhibition of glucosidases I and II [4]. TM blocks the initial steps in the formation of *N*-linked oligosaccharides in bacterial and eukariotic organisms [5].

We determined the effect of CAS and TM on the cytotoxic and genotoxic activity of cisplatin and etoposide in human ovarian cancer cells. CAS alone did not decrease the viability of cisplatin-sensitive and cisplatin-resistant cells (up to 25 μg/ml). A similar effect was observed for TM-treated in cisplatin-sensitive cells (up to 25 μg/ml): whereas, TM decreased the viability of cis-platin-resistant cells. Concomitantly, we observed a slight increase in the level of DNA damage after combined treatment with cisplatin+TM and etoposide+TM. TM caused approximately 3-fold sensitization of A2780cis cells to both anticancer drugs. Interestingly, among the cis-platin-sensitive cells we observed only a slight positive effect of TM combined with etoposide, but not with cisplatin. Unlikely TM, CAS did not influence cytotoxicity of cis-platin or etoposide in A2780 and A28780cis cells.

Results obtained in this study suggest that TM, but not CAS, can enhance the sensivity of A2780 and A2780cis cells to etoposide. Moreover, TM can decrease resistance of A2780cis cells to cisplatin. It indicates that the inhibition of *N*-linked glycosylation may be helpful in designing novel schemes of combined treatment in cis-platin-resistant ovarian cancer.

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FROM D-GALACTOSE TO PRIVILEGED HETEROCYCLES

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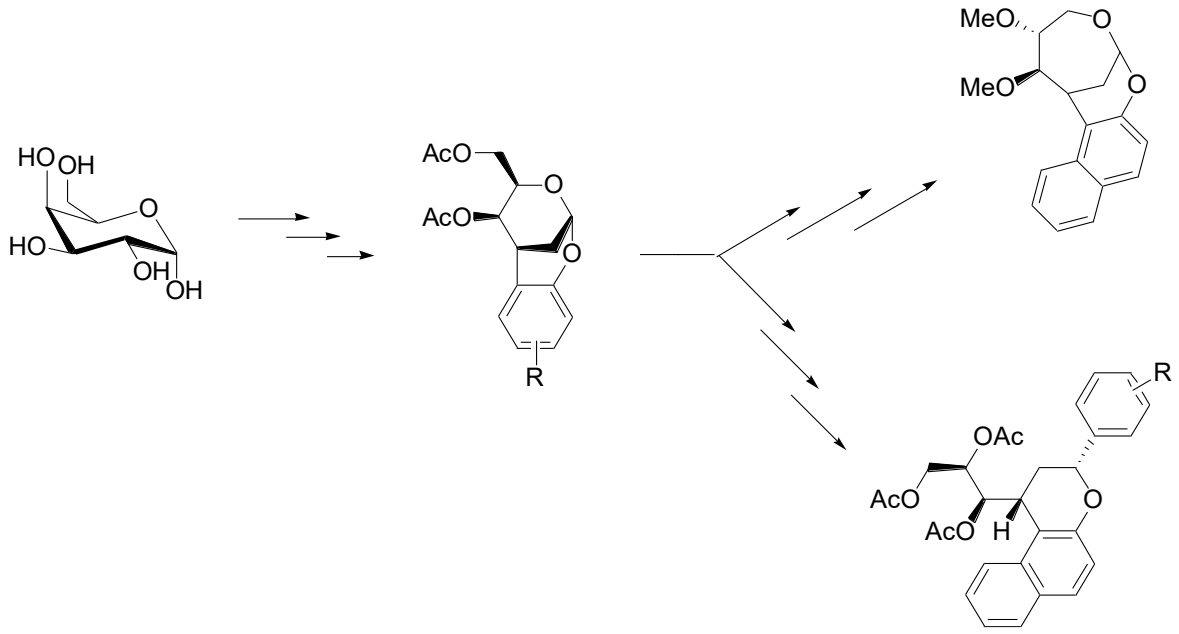
Carbohydrates are the most abundant group of natural products and are involved in a wide range of biological processes. Among biologically active heterocyclic scaffolds, the benzopyran structure frequently appears in many natural products and synthetic bioactive molecules that are of high therapeutic value [1]. Flavonoids and oxepanes are among the privileged structures in medicinal chemistry [2].

The synthesis of these oxygen heterocycles requires an ability to form carbon-oxygen bonds. $Al(OTf)_3$, a versatile Lewis acid, has been demonstrated to be a good catalyst to effect such glycosidations, coupling glycals with a range of aglycons [3]. We seek to discuss the use of $Al(OTf)_3$ for the synthesis of bridged chiral benzopyrans from D-galactose. And further ring-opening of the bridged chiral benzopyrans culminating into privileged heterocycles *viz.* chromans, chromenes, oxepanes and flavonoids.



Scheme 1 - Synthesis of chiral multicyclic compounds

The scope of the reactions will be detailed and mechanistic considerations for the synthesis of these chiral multicyclic compounds will be discussed.

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LPG1X FAMILY FROM LEISHMANIA MAJOR: FOUR GALACTOFURANOSYLTRANSFERASES WITH UNEXPECTED CATALYTIC PROPERTIES

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Galactofuranosyltransferases (Gal*f*Ts) (E.C. 2.4.x.x) are poorly described enzymes from the CAZY family 40. They catalyze the transfer of a rare galactofuranose (Gal*f*) from an activated UDP-galactofuranose onto a suitable acceptor. This constitutes a crucial step for the biosynthesis of glycoconjugates expressed at the surface of various pathogenic species such as *Aspergillus, Salmonella, Mycobacteria tuberculosis* and *Leishmania*. Thus, Gal*f*Ts are considered as potential therapeutic targets against these various diseases [1]. Up to date, only the mycobacterial Gl*f*T2 is fully characterized [2]. In addition, the gal*f*-glycoconjugates such as LPGs (lipophosphoglycans) and GIPLs (Glycosylinositolphospholipids) and some glycoproteins [3,4] play a key role in the survival and infection of *Leishmania* parasites. Therefore, they are classified as virulence factors in the neglected tropical disease leishmaniosis [5]. In this study the four putative Gal*f*Ts of *L. major* have been cloned, overexpressed and their kinetics parameters for several donors have been determined. We showed that they are good to excellent Gal*f*Ts, but unexpectedly, they can also catalyze the transfer from some NDP-pyranoses.

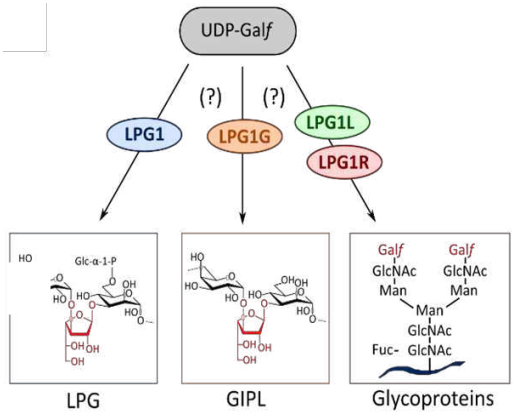

Fig. 1 - Putative biological roles of Leishmania major GalfTs

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CLONING AND EXPRESSION OF AMYLOSUCRASE FROM DEINOCOCCUS WULUMUQIENSIS AND THE APPLICATION OF ITS TRANSGLYCOSYLATION ACTIVITY

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Amylosucrase (ASase; EC 2.4.1.4) is a versatile enzyme that exhibits three activities: hydrolysis, isomerase, and transglycosylation. The transglycosylation activity of ASase uses sucrose as a sole substrate instead of expensive UDP-glucose and has a wide acceptor range, making it a useful enzyme widely be applied in the food industry. In this study, a putative ASase from *Deinococcus wulumuqiensis* (DWAS) was cloned and expressed in *Escherichia coli*. The optimal pH and temperature for the sucrose hydrolysis activity of DWAS were determined to be pH 9.0 and 45°C, respectively. To increase the solubility and stability of isovitexin, DWAS was introduced using sucrose as a donor and isovitexin as an acceptor. As a result, isovitexin glucosides were produced, one major and two minor products were observed by high performance liquid chromatography. Also, the molecular weight was determined using Liquid chromatography–mass spectrometry. As results, the major product was isovitexin monoglucoside and two minor products were isovitexin diglucoside and isovitexin triglucoside.

POSTERS | ID – Inflammation and Disease

DEVELOPMENT AND EVALUATION OF PHARMACOLOGICAL CHAPERONE THERAPEUTICS FOR GAUCHER DISEASE

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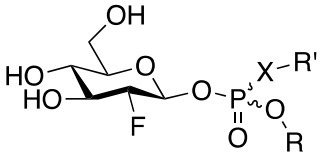
Gaucher disease (GD) is a genetic disorder which is characterized by insufficient amounts of the enzyme glucocerebrosidase (GCase) in the lysosome. Mutations in the genome lead to the formation of GCase with altered folding kinetics, which triggers the degradation of the enzyme, even though mutated GCase has been shown to retain its catalytic function. The concept of pharmacological chaperones is to use small molecules which assist mutant proteins in their folding process and then stabilize that structure for transport. Thus, degradation in the endoplasmic reticulum can be prevented and the concentration of *e.g.* GCase in the lysosome can be increased.



F-Glc-P

X=O, R=R', symmetric phosphate
X=O, R≠R', asymmetric phosphate
X=CH_2, asymmetric phosphonate

2-Fluorosugar phosph(on)ates (**F-Glc-P**) have been discovered as promising chaperone candidates for the treatment of GD.[1] The synthesis of the next generation of 2-fluorosugar phosph(on)ates is presented along with the evaluation of their potency as pharmacological chaperones. Thereby, two transgenic mouse lines expressing human GCase containing mutations[2] allow for the first *in vivo* study for this class of chaperones.

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NMR STUDIES OF THE SPECIFIC INTERACTION OF DC-SIGN WITH DIFFERENT OLIGOSACCHARIDES

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The DC-SIGN (dendritic cell-specific ICAM-3 grabbing non-integrin) protein has represented one of the major interests for the scientific community over the last twenty years, becoming a promising therapeutic target for the treatment of several diseases. Since its direct implication in pathogen infection was evidenced at the beginning of the century, a lot of effort has been made to unveil the structural details at atomic level underlying the specific process of recognition by pathogenic agents. These events involve the participation of carbohydrate-based structures attached to surface glycoproteins of pathogens like viruses (HIV, Ebola) and bacteria (Mycobacterium tuberculosis), amongst others [1]. Meanwhile, other related protein, Langerin, has been reported to act as a natural barrier against these diseases. Therefore, a detailed knowledge about the selectivity of the DC-SIGN lectin towards glycosylated structures is mandatory for the development of selective inhibitors that could avoid the DC-SIGN-mediated infections without blocking Langerin [2].

This protein belongs to the C-type lectin superfamily, which includes all these proteins with the common calcium-dependent carbohydrate recognition domain (CRD). As described for other calcium-dependent proteins, the affinity of DC-SIGN for oligosaccharides is low and usually around the milimolar or submilimolar range. However, it is well known that DC-SIGN forms tetramers by self-association through the neck region, thus acquiring a multivalent configuration which reduces the dissociation constants even near the submicromolar range. Additionally, its ability to bind a very extensive range of sugar motifs, essentially including mannose-containing and fucose-cotaining glycan scaffolds [3], is also challenging for the design of specific inhibitors, and more detailed analyses are needed to clarify the role of the entire glycan structure and its spatial distribution in the affinity.

On account of the increasing interest for this lectin and its potential use, we herein present the ongoing stages of our project, focused on the NMR studies of the key molecular recognition features related to DC-SIGN interaction mechanisms. The specificity towards fucosylated ligands, like histo-blood group antigens A and B, has been investigated in order to accurately describe the minimal structural elements of the fucose moiety that can be recognized and the importance of both the nature and the arrangement of the surrounding pyranose rings. For that purpose, the DC-SIGN CRD has been obtained making use of different isotopic labelling strategies (^{15}N-labelled, ^{15}N and ^{13}C-labelled) to develop a complete NMR methodology for this study.

^{15}N-HSQC-based titrations, alongside saturation transfer difference (STD) and transferred-NOE experiments, have been performed with fucose-containing ligands. Our conclusions have revealed interesting data about the binding event that have led us to propose precursory binding poses. These results will provide the initial milestones for the forthcoming studies with other ligands, including longer and branched ligands.

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THE FORMATION OF POLYSIALIC ACID / LACTOFERRIN COMPLEXES COUNTERACTS THE RELEASE OF NEUTROPHIL EXTRACELLULAR TRAPS

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N-acetylneuraminic acid residues are the building blocks of polysialic acid (polySia) [1]. In mammals this negatively charged polysaccharide is present in several body fluids like ejaculates, milk and blood [2-4]. Interestingly, we observed that lactoferrin interacts with polySia *in vitro* as well as *in vivo*. Lactoferrin is an important antimicrobial component and its best known function is to scavenge and retain iron [5-6]. However, lactoferrin has also a direct impact against the invasion of several pathogens including bacteria, viruses, fungi and parasites. Moreover, lactoferrin acts as an anti-inflammatory component. For instance, the release of neutrophil extracellular traps (NETs) is inhibited by lactoferrin [7]. NETs are generated by neutrophil granulocytes after their activation by e.g. lipopolysaccharides. After the initiation of NETosis neutrophils form a sticky meshwork consisting of a mixture of DNA and histones, which is additionally equipped with several anti-microbial molecules (e.g. lactoferrin and neutrophil elastases). NET-fibers can effectively capture and kill invading microbes. However, the main disadvantage of NETosis is its cytotoxicity against endogenous cells leading inter alia to autoimmune diseases [8]. In order to prevent a harmful NET formation, the release of NET-fibers must be strongly regulated. We observed that complexes of polySia and lactoferrin increase the efficiency of lactoferrin to counteract the release of NET. Since lactoferrin and polySia seems to interact with each other in several body fluids like ejaculates, serum and milk, the described effect might represent a natural modulation-system of NETosis and may represent a promising application during NET triggered diseases like thrombosis, sepsis and infertility.

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USING SYNTHETIC HEPARAN SULFATE TO TREAT ACUTE LIVER INJURY

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Acetaminophen (APAP, also known as paracetamol) is widely used to treat headache, muscle aches, and fever. However, APAP overdose is the leading cause of drug-induced liver injury in US and Europe. Here, we report a non-anticoagulant heparan sulfate octadecasaccharide (HS 18-mer) that protects from APAP induced acute liver injury in a murine model. This protective effect is specific for HS 18-mer; HS 12-mer, HS 6-mer or a heparin-like 18-mer with the anticoagulant activity exhibit no hepatoprotection effect. The HS 18-mer binds to HMGB1 (high mobility group box 1) which is a DNA-binding protein released from the nuclei of necrotic hepatocytes, and attenuates HMGB1-mediated neutrophil infiltration to the injury site. The HS 18-mer likely potentiates the endogenous protective effects of HS chains on shed syndecan-1 given that increased plasma levels of syndecan-1 are present in both APAP overdose mice and patients. Finally, we demonstrate that the HS 18-mer administered six hours after APAP overdose is still protective in the mouse model, demonstrating the potential benefit over the current standard of care for late presenting APAP overdose patients. Discovery of HMGB1-neutralizing heparan sulfate oligosaccharides will offer a practical approach to treat liver diseases.

POSTERS | V – Vaccines

GLYCOSYLATION PATTERNS AND IMMUNE RESPONSES TO 2013 H3N2 INFLUENZA A VIRUS HEMAGGLUTININ

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Hemagglutinin (HA), a major surface antigen of influenza A viruses (IAV), is heavily *N*-glycosylated in recent A(H3N2) isolates. The presence of glycans can alter host antibody responses by shielding or modifying immunodominant antigenic sites on the globular head and/or immunosubdominant sites on the stem region of HA, as well as by interacting with the innate immune response. To better understand the role of site occupancy in the generation of adaptive immune responses, we grew a 2013 A(H3N2) strain in the absence or presence of an inhibitor of *N*-linked glycosylation, NGI-1. This inhibitor reduces *N*-linked glycosylation in mammalian cells by targeting the oligosaccharyltransferase enzyme complex. Mass spectrometry-based analysis of NGI-1-treated virus revealed reduced glycosylation in ten out of twelve HA glycosylation sites, compared to virus grown without the inhibitor. Overall, the HA of NGI-1-treated IAV had approximately 21.3% less glycans than untreated virus. Both the globular head and the stem region of HA molecule were affected by NGI-1 treatment. We immunized naïve mice with two intramuscular injections of 10 µg of HA in concentrated purified NGI-1-treated or untreated IAV. As measured by both ELISA and hemagglutination inhibition assays, there were significant differences in total serum cross-strain and protective antibody responses between lower- and higher-glycosylated HA. Mass spectrometry indicated that residues 45 and 144 were the most impacted by NGI-1-treatment, with a loss of approximately 70% of their glycans, even though they were least (23-26%) occupied by *N*-linked glycosylation in untreated virus. We constructed reverse-genetics viruses without *N*-glycan addition sites at position 45, 144, or 45/144 of 2013 A(H3N2) HA, and used these to immunize naïve mice as described above. Antibody responses generated by glycosylation mutant viruses were compared to those of the wild-type virus (containing glycosylation at both, 45 and 144, sites). The reverse genetics viruses induced significantly higher homologous and cross-protective antibody responses than did the parent wild-type IAV, with the strain lacking glycans at residue 45 inducing the highest response. Together, our data suggest that even low level of site occupancy by *N*-linked glycosylation may play important roles in interactions of the influenza virus with the immune system and diverting efficient adaptive immune responses.

COVALENT TLR4 LIGAND – ANTIGEN CONJUGATES AS VACCINE MODEL

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Irrespective of its target, a vaccine usually contains a mix of antigens and an adjuvant that is required to stimulate the immune system. The exact molecular composition of existing vaccines is largely unknown, complicating the improvement and development of existing and new vaccines. Therefore attention is currently focused on the generation of well-defined molecular structures with a controllable immunological function, including co-stimulation. A promising strategy to obtain these vaccine modalities takes advantage of pattern-recognition receptors, such as Toll-like receptors. Toll-like receptor 4, for example, is able to recognise lipopolysaccharides and their endotoxic component, known as lipid A, which can be found on the cell surface of Gram-negative bacteria. Based on the structure of lipid A, a new class of potent monosaccharide adjuvants has been discovered, the aminoalkyl glucosamine 4-phosphates (AGPs), in which the reducing end glucosamine of lipid A is substituted with a functionalized serine residue [1, 2]. CRX-527 is one of the most potent AGPs and was therefore selected to use as a built-in adjuvant for the generation of novel conjugate vaccine modalities.

Herein, we describe the design and synthesis of conjugate **1** composed of CRX-527 and an ovalbumin derived peptide having the MHC-I epitope SIINFEKL, incorporated to a longer peptide motif (Figure 1). For its generation we improved the syntheses of the potent TLR4 agonist CRX-527 and (R)-3-alkyloxytetradecanoic acids and developed an efficient conjugation and purification strategy. The initial in vitro evaluation of the first AGP-based conjugate **1** will be presented.

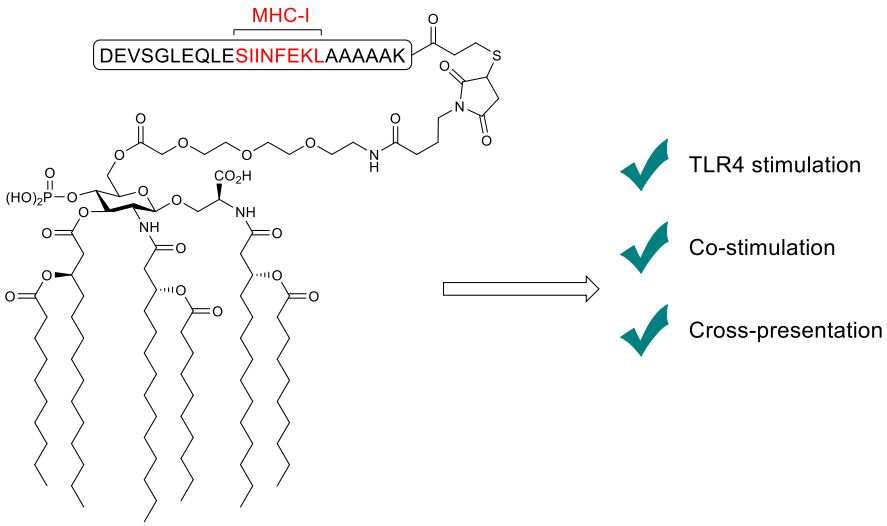

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Fig 1 -. Structure of the AGP-based conjugate **1**.

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CYCLOPEPTIDE-BASED ANTITUMORAL SYNTHETIC VACCINES

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Cancer remains the major cause of mortality worldwide. Despite declining mortality rates due to early diagnosis and multiple treatment modalities, fatal recurrences are common. Among the main treatments, standard chemotherapy still induces unacceptably high toxicity, radiotherapy treatments often kill both normal tissue and tumors while stray cancer cells sometimes escape surgery. Synthetic vaccines offers an attractive alternative to overcome these problems [1]. In this context, we have designed multicomponent cyclopeptide-based constructs (Figure 1) bearing well-defined mixtures of B-cell epitopes, CD4+ and CD8+ T-cell peptide epitopes and palmitic acid as adjuvant [2]. Immunological studies in mice have revealed a spectacular increase of survival [3].

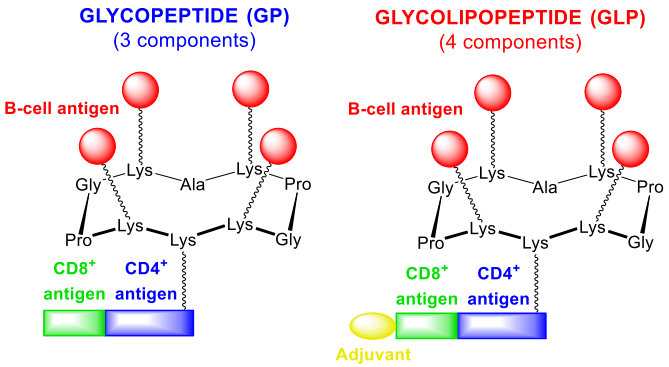

Fig 1.- Example of fully synthetic vaccine prototype.

This approach was more recently extended to the utilization of TACA mimetics and the preparation of multiepitopic constructs to improve efficiency. [4]. Here we present the synthesis of several vaccine candidates and their immunological evaluation.

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SYNTHESIS OF A *NEISSERIA MENINGITIDIS* SEROGROUP Y ANTIGEN FRAGMENT AND ITS 4-FLUORINATED ANALOGUE FOR FULLY SYNTHETIC VACCINE CANDIDATES

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Neisseria meningitidis bacteria are gram-negative diplococci that colonize mainly mucosal surfaces of the upper human respiratory tract. This pathogenic organism is responsible for meningitis and septicemia, which even today raise mortality in children as well as in young adults worldwide. Serotype Y ranks amongst the clinically most relevant strains, due to its highly invasive pathogenesis and widespread abundancy [1,2,3].

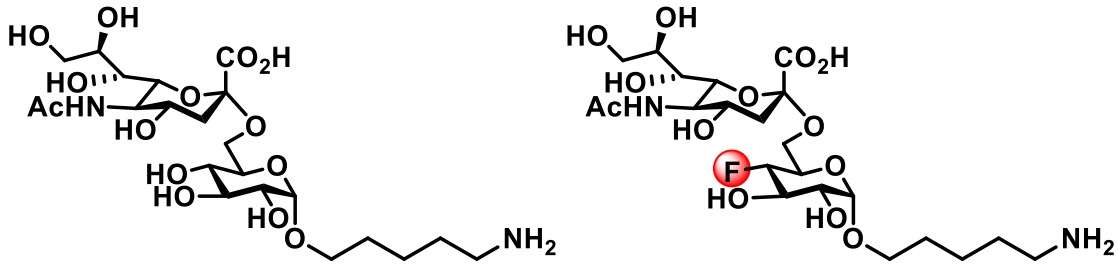

Fig 1 - Natural and fluorinated *Neisseria meningitidis* antigen fragments.

Vaccines based on carbohydrate antigens often lack a satisfying intrinsic immunogenicity and are prone to *in vivo* metabolic degradation. However, fluorination provides an attractive strategy to overcome these drawbacks as fluorinated carbohydrates are presumed to provide enhanced stability regarding enzymatic glycosidic bond cleavage, while maintaining the properties of the natural congener. Furthermore fluorinated mimics usually seem more foreign to the immune system since fluorine atoms are absent from most organisms [4,5,6].
Herein, the synthesis of a disaccharide antigen fragment of *Neisseria meningitidis* serogroup Y and its fluorinated analogue is introduced. Both fragments comprise an aminopentyl handle to allow for construction of fully synthetic vaccine candidates.

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SCALE-UP CONSIDERATIONS AND INVESTIGATION OF THE BIOCONJUGATION OF A SEMI-SYNTHETIC *SHIGELLA FLEXNERI* TYPE 2A CONJUGATE VACCINE UNDER GMP GUIDELINES

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Currently one of the major causes of diarrheal diseases is attributed to Shigellosis, of which *Shigella flexneri* type 2a (Sf2a) is most prevalent strain. In search of an effective conjugate vaccine concept, a synthetic mimic of the O antigen (O-Ag) moiety of the *Shigella* lipopolysaccharides (LPS) was covalently linked to Tetanus toxoid (TT, carrier protein) [1]. Here the grafting of linker/spacer molecule 4-Maleimidobutyric acid *N*-hydroxysuccinimide ester (GMBS) on TT was optimized through a design of experiments (DoE) approach. Using GMB modified TT conjugates were constructed bearing different loadings of Sf2a (5, 9, 17 & 26). Immunological evaluation of these conjugates led to the finding that the Sf2a:TT molar ratio was critical with an optimum in the range of 17 [2].

These initial experiments were performed at lab scale (0.2 mL). Now, before going into full scale production to obtain two GMP vaccine lots for a toxicology study and a phase I clinical trial, several scale-up considerations were investigated. One major factor was the availability of the GMP grade Sf2a, and the intended average molar Sf2a:TT ratio of approximately 17 which led to a production volume of 100 mL. The use of spin filtration as primary purification method, was replaced by tangential flow filtration (TFF) which was scalable and controllable. Also, the removal of all residual chemicals and impurities during the individual production steps were evaluated and compared to specifications (ICH-M7 & ICH-Q3D). Due to the limited amount of Sf2a available, all data related to the conjugation reaction originates from experiments performed using only buffers and chemicals (mock studies).

When assessing the modification reaction using actual TT at 100 mL scale, very similar results were observed for both lab and production scale grafting of GMBS on TT. Additionally, at this scale no distinct differences were observed for induction of aggregates. Now tested for the first time using TFF, DMSO (solvent for GMBS), unreacted GMBS and hydrolyzed GMBS were all successfully removed to below specifications . The mock conjugation reaction, studied using only buffers and chemicals, also performed very well. Here *N*-Hydroxysuccimide, Hydroxylamine-HCl and Cysteamine-HCl were removed to below specifications.

With the exemplary results of the mock studies on impurity removal and TFF performance as reference, two GMP productions were performed at large scale. Both the toxicology and phase-I clinical trial material were released in compliance with all pre-set specifications and guidelines. An average molar Sf2a:TT ratio of approximately 19 was obtained for both batches, which was well within specifications (17±5). Final fill and finish conceived 2 and 10 μg formulations for both productions. which were succesfully subjected to a real time stability study for more than 9 months. The main criterium was the degradation of the conjugate, releasing (free)Sf2a, which did not exceed 10 %.

With the introduction of TFF and elaborate mock studies beforehand we were able to scale-up from the 0.2 mL experimental studies using spin filtration to 100 mL production without any major complications. Both batches complied to all specifications and international guidelines, and were successfully tested in both a toxicology and clincial phase-I study.

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EFFECT OF SACCHARIDE LENGTH ON THE IMMUNOGENICITY OF *NEISSERIA MENINGITIDIS* SEROGROUP X CONJUGATE VACCINE

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Neisseria meningitidis (Nm) is a leading cause of bacterial meningitis and sepsis globally. The six most infectious serogroups (NmA, B, C, W, Y, and X) express negatively charged capsular polysaccharides (CPS). The isolated CPSs conjugated to carrier proteins represent the target antigens for the development of vaccines against Nm strains. An exception is the CPS of NmB, which is identical to polysialic acid of protein-attached glycans expressed in the foetus meaning that immune tolerance exists against this structure and it resulted in a very low antibody response.
A number of mono- and tetravalent conjugate vaccines against Nm have been licensed [1].
Understanding the role and influence of glycoconjugate attributes in the immunogenicity is of major interest for vaccine design. Among the different factors that affect the immunogenicity of conjugated vaccines [2-4], some are related to the carbohydrate moiety, like the length of saccharide chain and the decoration pattern of the sugar (i.e. O-acetylation), while other factors are linked to the carbohydrate-protein interface, such as the conjugation chemistry and the loading in terms of sugar/protein ratio and the site of conjugation.
We have recently shown that conjugates of MenX oligosaccharides with average degree of polymerization (avDP) of 15-20 obtained by sizing of the natural polysaccharides, or enzymatically made oligomers with avDP11-12, are effective in eliciting high levels of bactericidal IgGs [5-7]. Here we have studied the immunogenicity of conjugates with different size, to determine the impact of the saccharide length on the immunogenicity.
For this purpose oligosaccharides of different length (DP 5, DP 10, avDP20 and avDP35) were obtained from CPS hydrolysis and conjugated to CRM_{197} for testing in mouse model. The anti-CPS response induced in mouse was characterized for the specific levels of IgG evoked on the sera and for their functionality by serum bactericidal activity (SBA).

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A SYNTHETICALLY DEFINED GLYCOCONJUGATE VACCINE AGAINST CANCER

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During the past century, carbohydrate-based vaccines have proven successful against multiple diseases. When conjugated, the glycoconjugate vaccines have some advantages over traditional polysaccharide-based vaccines, such as triggering T cell dependent responses and the possibility of being used to immunize children under five years of age. With this in mind, a three-component vaccine composed of designed MUC1 glycopeptide derivatives (antigen), OVA257-264 (to act as an adjuvant) and PEGylated Gold Nanoparticles (to act as carriers) was developed.

Due to its overexpression and shorter glycans in cancer cells, MUC1 is currently being used as a drug target for many cancers. However, MUC1-based therapeutic vaccines face some challenges; MUC1 is low immunogenic, and because it may act as a self-antigen it may lead to immunosuppression. To overcome these issues, a non-natural, designed MUC1 was synthesized and used to obtain the glycoconjugate-based vaccine candidates. These constructs consisted of MUC1-MeCys-FPro, an unnatural MUC1 derivative containing a fluoroproline residue and S-glicosidic linkage, and OVA Cys253-264 conjugated to gold nanoparticles via maleimide-thiol chemistry, through a NHS-PEG2-Maleimide linker.

Once obtained, the vaccine candidates were tested for their ability to trigger the production of antibodies against MUC1 in Balb/c mice. By ELISA, we could observe that the conjugates were able to trigger the production of antibodies specific against MUC1, manly of IgG1 type, which is indicative of a Th2 immune response. Furthermore, we could also see that the anti-sera from the immunized mice were able to bind to the natural MUC1 antigen, which is overexpressed in the human breast cancer cell lines MCF7 and T47D.

To confirm these results, further tests with Human samples from cancer patients will be performed and the more promising vaccine candidate will be further evaluated and tested in breast cancer-challenged mice.

STRUCTURAL ELEMENTS WITHIN LIPOPOLYSACCHARIDES SHARED BY BACTERIA OF THE GENUS *BORDETELLA*. THE QUEST FOR A UNIVERSAL ANTI-*BORDETELLA* VACCINE

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Whooping cough is a highly contagious disease especially dangerous for infants. It is caused by the Gram-negative bacterium *Bordetella pertussis* and to a lesser extent by *B. parapertussis* and *B. holmesii*. Symptoms caused by these bacteria are similar, but the treatment requires different antibiotics. Diagnosis of pertussis is difficult, as *B. pertussis* are slowly growing bacteria. Typically quicker RT-PCR tests do not always distinguish between *B. pertussis* and *B. holmesii* and about 30% of cases are misdiagnosed. What makes the accurate diagnosis of pertussis even more difficult is the fact that *B. pertussis* and *B. holmesii* are often isolated together [1,2].

Lipopolysaccharide is a major surface antigen of Gram-negative bacteria. It has been implicated as a virulence factor in pertussis, but it has never been considered as a possible vaccine antigen due to its toxicity and reactogenicity. The LPS of *B. pertussis* lacks an O-specific polysaccharide chain, contains a distal trisaccharide instead and structurally it constitutes a lipooligosaccharide (LOS) [3]. Since *B. pertussis* LOS is an evolutionary stable component, the oligosaccharide fragments derived from LOS seem suitable for the search of common vaccine antigens among lipopolysaccharides of other *Bordetella* bacteria. The structural features of *B. pertussis*, *B. parapertussis, B. bronchiseptica and B. holmesii* O-antigens were investigated using the NMR structure reporter groups approach. The antisera obtained against neoglycoconjugates of *B. pertussis* LOS-derived oligosaccharides were used to analyze the possible cross-reactivities with LPS of other *Bordetellae*. The presence of such common structural motifs and cross-reactivities between LPS of the genus *Bordetella* would allow for a design of a new vaccine component yielding broader protection and thus more effective in case of mixed infections.

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POSTERS | PM – Glycosciences and Personalized Medicine

NOVEL 3D MODELS TO STUDY THE ROLE OF GLYCOSYLATION IN CANCER DRUG RESISTANCE

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In the scenario of personalized medicine, targeted therapies are currently the focus of cancer drug development. These drugs can block the growth and spread of tumour cells by interfering with key molecules of malignancy. Receptor tyrosine kinases, major targets for treatment of advanced gastric cancer, are transmembrane glycoprotein receptors whose glycan modifications have been shown to modulate the receptor activation [1,2].

In this work, we have addressed the role of aberrant glycosylation, specifically the altered α2,3- and α2,6- terminal sialylation, in gastric cancer malignancy and therapy resistance. For this purpose, we have developed gastric cancer cell models engineered with key sialylation-related enzymes. First, glycomic analyses of the models were performed to fully characterize the sialylation status [3]. To mimic the *in vivo* tumour features, an innovative 3D high-throughput cell culture methodology has been developed for gastric cancer cells [4]. After in-depth characterization of the gastric cancer spheroids, we evaluated the resistance of the glycoengineered cell models by subjecting the spheroids to tyrosine kinase inhibitors that are currently in clinical use and preclinical trials. The phenotypical and functional parameters assessed disclose that cell sialylation leads to different cellular adhesive and invasive features. Furthermore, we demonstrate that by applying 3D cell culture methods, the cell glycocalix undergoes changes compared to the conventional 2D culture systems.

Remarkably, our glycomodels display strikingly different cell cytotoxicity response to several inhibitors of major oncogenic receptors. Furthermore, distinct activation levels of cell receptors are observed by applying targeted therapy drugs, altogether suggesting the altered sialylation occurring in cancer as an important mechanism of drug resistance. Our results demonstrate that cell glycosylation, in addition to being a key feature of tumour progression, plays a critical role in therapy resistance to tyrosine kinase inhibitors in gastric cancer. These findings shed new light on the mechanisms underlying cancer drug resistance and propose aberrant sialylation as new predictive biomarker for patients' treatment response.

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SYNTHESIS OF SQUARY GROUP MODIFIED PHOSPHOGLYCOLIPID ANALOGS AS LIGANDS FOR GPR55

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Lysophosphatidyl glucoside (LPG) is a structurally unique glycolipid that exhibits neural cell guidance activity by acting on a class A G-protein coupled receptor (GPCR) GPR 55 of NGF-responsive dorsal root ganglion axons.[1] As GPR55 plays important roles in development and is implicated in many disease states, molecules that target this protein is of widespread interest.[2] In this study, we developed a strategy to create novel analogs of LPG that has a squaryl diamide group as surrogate of phosphodiester. The compound which has hydroxy groups configured in a same manner as natural LPG exhibited activity to repel Dorsal root ganglia (DRG) sensory neurons. Our study enables facile synthesis of LPG analogs for systematic structure-activity relationship study of potential modulators of GPR55.

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ANALYSIS OF ELECTROSTATIC INTERACTION OF GANGLIOSIDE GM3 WITH TRANSMEMBRANE PEPTIDE OF INSULIN RECEPTOR

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The insulin receptor is a single transmembrane protein that regulates blood glucose level. The reduction of sensitivity to insulin leads to type 2 diabetes. It has been suggested that the overexpression and the accumulation of ganglioside GM3 on cell membrane reduces insulin signal in type 2 diabetes. The interaction of GM3 with the insulin receptor is considered to be due to an electrostatic interaction between a sialic acid of GM3 and the lysine residue located just above the transmembrane domain of the insulin receptor [1] (Fig. 1). However, the detailed analysis has not been elucidated.

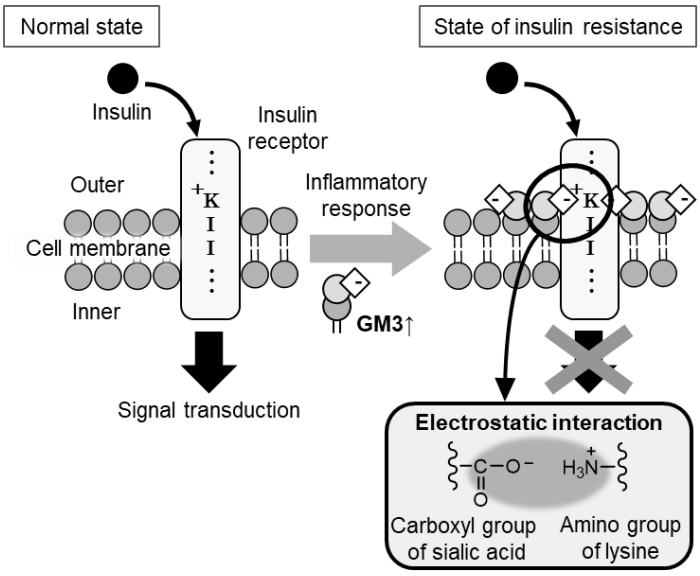

Fig.1 Interaction between Insulin Receptor and GM3

In order to observe this interaction, we constructed a model system by incorporating fluorescently labeled insulin receptor transmembrane peptides into liposomes containing GM3. We successfully visualized the interaction with a confocal laser scanning microscopy.

First, in order to clarify interaction of these molecules in liposomes, we studied the optimal conditions of phase-separating liposomes containing GM3. We found that liposomes consisting of DOPC, GM3 and cholesterol did not undergo phase separation, whereas sphingomyelin (SM) containing liposomes showed phase separation. Here, it is considered that DOPC is localized in Ld (Liquid disordered) phase, and GM3 and SM are localized in Lo (Liquid ordered) phase. This result suggests that the charge repulsion between GM3 may affect the liposome phase separation.

Next, we synthesized the transmembrane peptide of insulin receptor. At the isolation of this peptide by HPLC system, the recovery rate was remarkably low because of its hydrophobicity. We improved this problem by using formic acid as a solvent. In addition, the purity was also improved by using the isopeptide method [2]. The synthesized peptides were incorporated into liposomes and observed by 3D imaging (Fig. 2). As a result, we observed the separation of POPC and peptides, both of which are localized in the Ld phase. We are synthesizing a peptide in which the lysine residue is mutated to non-charged alanine to elucidate the electrostatic interaction of GM3 and insulin receptor.

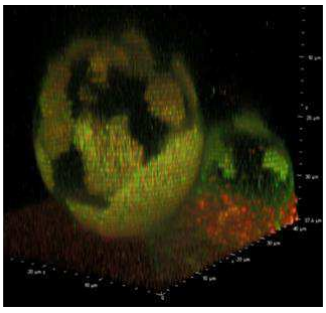

Fig. 2 3D Imaging of Liposome

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LIVE CELL IMAGING ANALYSIS WITH BODIPY LABELED LACTOSYLCERAMIDE

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It is known that the composition and distribution of glycolipids depend on the type and state of cells, and they are involved in various biological phenomena with control of cell function. In general, glycolipid metabolism in cells is carried out in the Golgi apparatus, but the detailed mechanism has yet to be elucidated. In the present study, we investigated the glycolipid metabolism by using live-cell imaging with BODIPY-labeled lactosylceramide (LacCerBODIPY), which is known to undergo glycan transformations [1].

Pulse-chase experiments using LacCerBODIPY were employed to improve spatial-temporal resolution. We succeeded to observe recycling pathway between plasma membrane and organelle of the cells (Fig.1). We then established a live cell imaging system to observe intracellular lipid distribution. The recycling transport of LacCerBODIPY was thus confirmed by the live cell imaging.

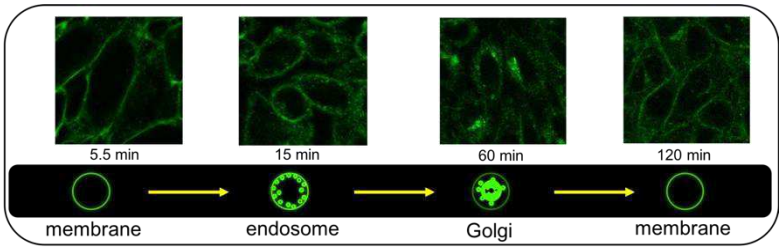

Fig. 1 Intracellular fluorescent labeled glycolipids distribution.

LacCerBODIPY was also used as a probe for monitoring membrane fluidity during the cell differentiation. Diffusion of membrane molecules was analyzed by using the FRAP system in order to elucidate the membrane fluidity. The increase of membrane fluidity was observed immediately after NGF stimulation, whereas the neurite growth of the cell was started from 30 min after the stimulation of NGF. These results suggest that an increase of membrane fluidity by NGF stimulation initiates the neurite growth (Fig.2).

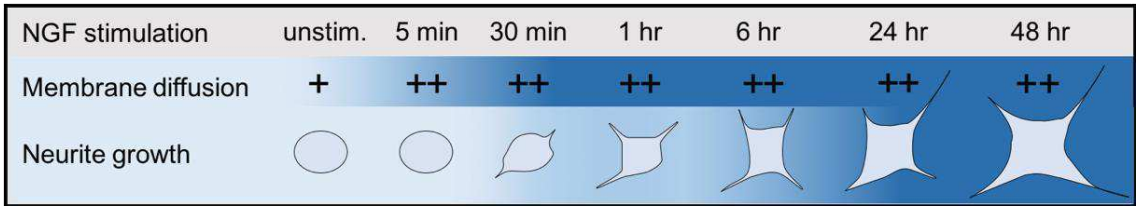

Fig.2 Membrane fluidity and neurite growth with NGF stimulation.

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MEASURING BACTERIAL GLYCOSYL HYDROLASE ACTIVITY WITH A SOLUBLE CAPTURE-PROBE BY MASS SPECTROMETRY

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The detection of bacterial pathogens is of great importance in the diagnosis and management of human infectious diseases, in veterinary medicine and in many other fields such as water and food quality control. The development of rapid, selective and sensitive detection methods is increasingly necessary in this field. The importance of carbohydrates for the bacterial metabolism is reflected by the high number of bacterial genes encoding Carbohydrate active enzymes (CAZymes).[1] One common approach to determine the presence of bacteria is the measurement of enzymatic activities associated to their metabolism, especially measuring glycosyl hydrolases involved in the breakdown of oligosaccharides into monosaccharides.

In the present work, we chose lactose functionalized with an azido ethyl linker ($LacN_3$) as the carbohydrate probe for detecting β-galactosidase activity. β-galactosidase enzyme [EC 3.2.1.23] cleaves lactose into galactose and glucose and forms part of the carbohydrate degrading metabolism of bacteria, plants and animals. Based on our previous report on hydrophobic immobilization of glycans on indium tin oxide (ITO), [2] we have developed an alkyne terminated MALDI-tof active surface for the analysis of enzymatic activity via immobilization through bio-orthogonal “click-chemistry” reaction. After immobilization, the surface could be extensively washed to remove the excess of salts, buffers or media components. Clean and good intensity MALDI-tof spectra were acquired and the enzymatic activity was inferred by the ratio of non reacted probe ($LacN_3$) towards the product ($GlcN_3$). The system was applied to the detection of enzymatic activity in bacterial extracts without any purification/extraction step and also in living cultures of gram negative *E. coli* ATCC 25922 and gram positive *S. aureus* ATCC 29213.

Due to the emergence of antibiotic resistance in many hospital bacterial pathogenic strains, additional information related to the antibiotic susceptibility of bacterial isolates is essential for an appropriate infection treatment. Conventional antimicrobial resistance assays are based in the detection of bacteria growth in the presence of the antibiotics tested, whether in solid agar or liquid media. [3] The detection method described was further employed for the accurate determination of minimal inhibitory concentration (MIC) in cultures of *E. coli* ATCC 25922 in the presence of the model antibiotic ampicillin.

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PREPARATION OF MULTIVALENT GLYCONANOGELS AND INTERACTION STUDIES

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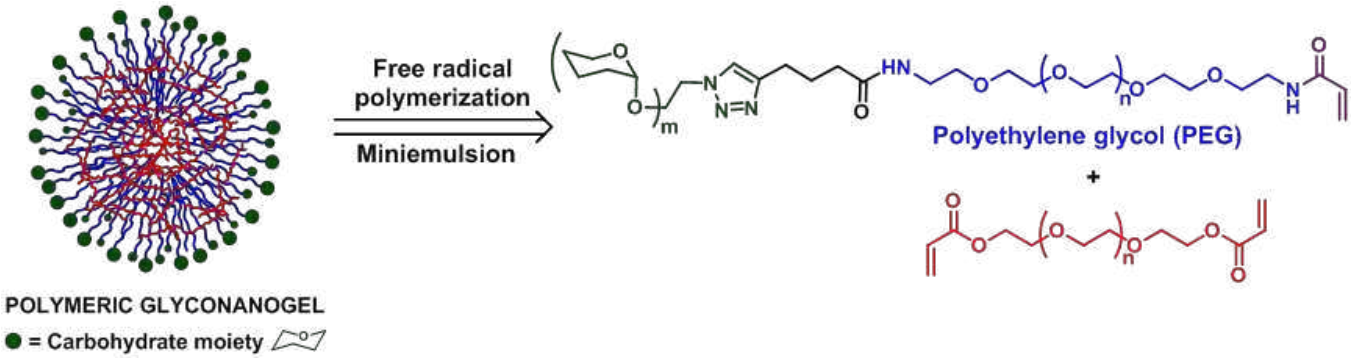
Lectins are proteins able to recognize carbohydrates with high specificity and selectivity, and this carbohydrate-protein interaction is implicated in several important biological events, such as cell growth, cell differentiation, cell adhesion, cell migration, immunomodulation, inflammation, etc.[1] Therefore, lectins can be considered as potential targets for intervening in relevant diseases. However, this interaction is characterized by a low affinity, which is compensated in Nature by a multivalent presentation of carbohydrates.[2]

With the aim of obtaining a multivalent carbohydrate presentation, we have prepared mannose-decorated nanogels from FDA-approved Poly(ethylene glycol) (PEG)[3] by free radical polymerization using inverse miniemulsion. This polymer is functionalized on one end with an alkyne group, in order to be coupled with several carbohydrates by Click Chemistry, and in the other end with an acrylate group to be polymerized.

This type of systems can be decorated with simple carbohydrates moieties or with more complex structures, such as glycodendrons. By increasing the multivalency by coupling of dendrons of three or nine units of mannoses, we are trying to enhance the biological activity of these systems.



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POSTERS | NG – Natural Glycoconjugates

TOTAL SYNTHESIS OF CHOLESTERYL 6'-*O*-PhOSPHATIDYL AND CHOLESTERYL 6'-*O*-LYSOPHOSPHATIDYL α-GLUCOSIDES FROM *HELICOBACTER PYLORI*

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Roughly half of the world population are carriers of *Helicobacter pylori* (*H. pylori*), a Gram-negative bacterium that colonizes the stomach tissue.[1],[2] Chronic infection with *H. pylori* is associated with several clinical conditions such as gastritis, gastric adenocarcinoma, peptic ulcer, etc.[3] A unique feature of the bacteria is the ability to confiscate cholesterol from their host and to convert the confiscated substrate to different glycolipids (Cholesteryl-α-glucoside (CG), cholesteryl-6'-*O*-acyl-α-glucoside (CAG)) and glycophospholipid compounds glycolipids (Cholesteryl-6'-*O*-phosphatidyl-α-glucoside (CPG) and cholesteryl-6'-*O*-lysophosphatidyl-α-glucoside (lyso-CPG)) (**Figure 1**).[4] Preliminary studies have shown that these glycolipids have impact on the transfer of cytotoxin-associated gene A (Cag A) to host cells.[5] As such, the immunological response of the host is modulated that favors the survival of the bacteria in harsh environment. In this oral communication, we like to share with you a general strategy for synthesis of native CAG, CPG, and lyso-CPG compounds. The strategy features the of use of *in situ* transformation of glycosyl adducts in construction of the α-glucosidic bond.[6,7]

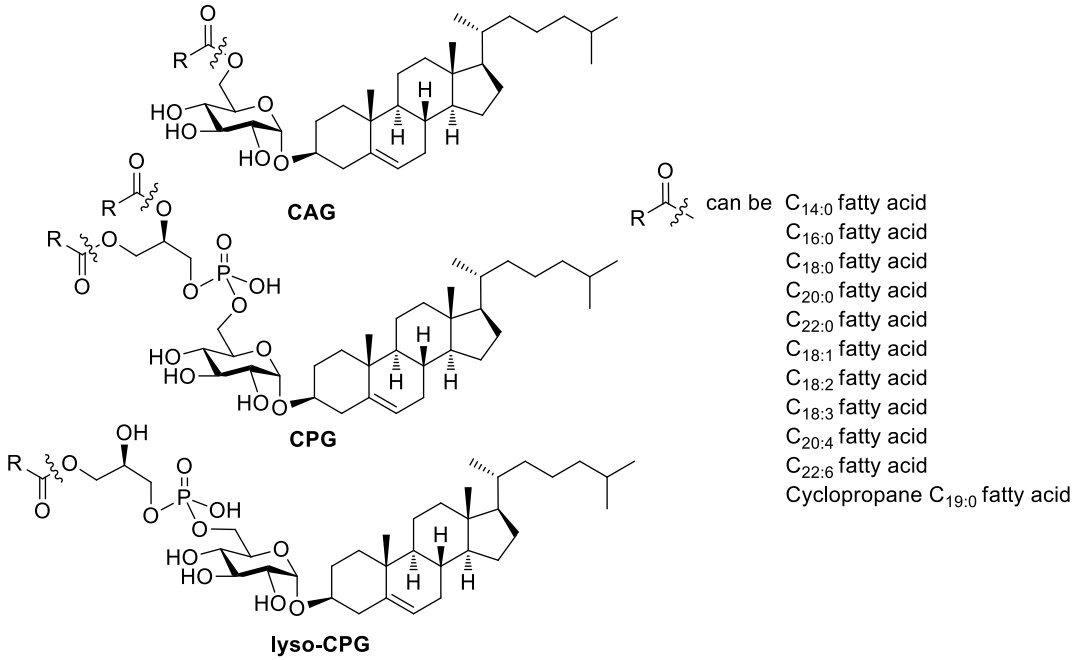

Figure 1. CAG, CPG, and Lyso-CPG compounds with various fatty acid chains

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MODULATION OF MOLECULAR RECOGNITION AND BIOACTIVITIES OF IMMUNOSTIMULATORY GLYCOLIPIDS BY LIPID CHAIN MODIFICATION WITH POLAR FUNCTIONAL GROUP

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Various bacterial membrane glycolipids are recognized by innate immune receptors such as TLRs, and lipid antigen-presenting molecules, CD1a–d. These proteins activate immune system and lead to release of various proinflammatory cytokines. These proinflammatory cytokines activate adaptive immunity to affect other immune cells. The recognition of lipid moieties by the lipid recognition proteins, such as TLR2, TLR4 and CD1a-d, is conducted by extensive hydrophobic pocket. We focused on a few polar residues in the hydrophobic pocket of lipid recognition receptors, because these shielded hydrogen-bond in hydrophobic pocket has been shown to be relatively stable[1,2]. We thus designed the ligands containing polar functional groups in the lipid chain, which were expected to interact with the hydrophilic regions of the binding pocket, in order to improve activity and selective activation of lipid recognition receptors.

We designed and synthesized several glycoconjugates and lipidconjugates including α-GalCer (CD1d ligand), Pam_2CSK_4 (TLR2 ligand), phosphatidylinositol (PI; potential TLR2 or CD1d ligands) that contain polar functional groups in the lipid chain (Figure 1). The binding assay and immunostimulatory activity test using these ligands indicated that the position of polar group in the acyl chain influenced the ligand activities, presumably because the polar functional group interacted with the hydrophilic regions of the binding pocket in TLR2 and CD1d. Moreover, The designed α-GalCer displayed higher binding affinities and cytokine production activities compared to the CD1d ligand containing an unmodified lipid chain. These results suggested that the polar functional groups may specifically interact with polar residues in the lipid binding pocket, and these residues would be a target for the regulation of lipid recognition in these lipid binding proteins[3,4].

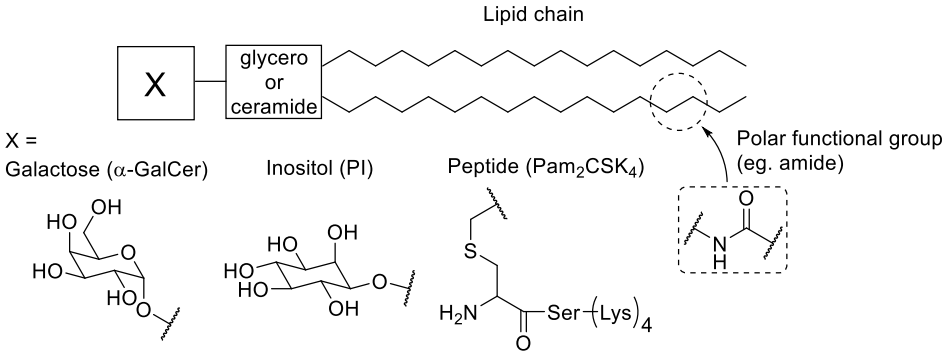

Figure 1.

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SULFOQUINOVOSIDASES CATALYZE HYDROLYSIS OF PLANT SULFOLIPID: COMPARATIVE STRUCTURAL AND KINETIC STUDIES

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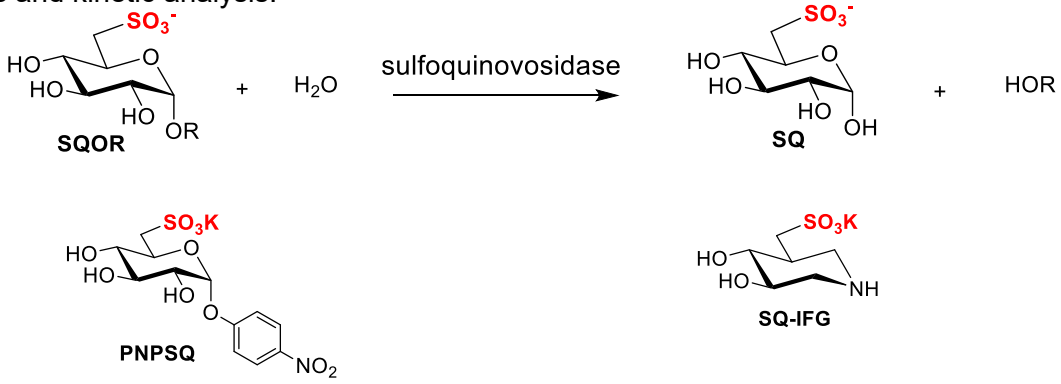
Sulfoquinovose (SQ, 6-deoxy-6-sulfoglucose) is a sulfur-containing sugar that forms the polar head group of the plant sulfolipid, sulfoquinovosyl diacylglycerol (SQDG)[4]. Two sulfoglycolysis pathways have been discovered that facilitate the degradation of SQDG: the sulfoglycolyticEmbden-Meyerhof-Parnas (sulfo-EMP) pathway and the sulfoglycolytic Entner-Doudoroff (sulfo-ED) pathway[1,2]. Entry of SQDG to the sulfur cycle requires hydrolysis of SQDG to form SQ, which is catalyzed by a sulfoquinovosidase, YihQ. Structural analysis of *Esherichia coli* YihQ revealed substrate recognition by a non-contiguous QQRWY motif[3]. On the other hand putative SQases within gene clusters encoding sulfo-EMP and sulfo-ED pathways contain a KERWY motif; it is not known whether these genes encode genuine sulfoquinovosidases.

This presentation focuses on the discovery and characterization of a second sulfoquinovosidase that bears a KERWY motif: *At*SQase from *Agrobacterium tumefaciens*. *At*SQase can hydrolyze the artificial substrate *p*-nitrophenyl sulfoquinovoside (PNPSQ), which enabled its kinetic and structural characterization. Through the synthesis of a series of analogues of PNPSQ it is shown that YihQ and *At*SQase are highly specific for both correct D-*gluco* stereochemistry and the sulfonate group. The first SQase inhibitor SQ-IFG was designed and synthesized. Structural analysis of both enzymes allowed identification of the catalytic and substrate binding residues. Their roles were supported by mutagenesis and kinetic analysis.



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CHARACTERIZATION OF MPIASE, A GLYCOLIPID ESSENTIAL FOR THE MEMBRANE PROTEIN INTEGRATION

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Integration of membrane proteins into biomembranes is a vital event in cells. Recently we identified a novel factor named MPIase (membrane protein integrase) that is essential for the membrane protein integration in the inner membrane of *E. coli*.[1)2)] MPIase catalyzes both translocon-dependent and -independent protein integration (Fig. 1a) .

We purified MPIase from the extract of the inner membrane of *E. coli* by using repeated liquid-liquid partition chromatography. Despite its enzyme-like activity, amino acid analysis indicated that MPIase is non-proteinaceous and comprises unstable aminosugars. The breakthrough for the structure determination was MALDI-TOF-MS measurements. A characteristic series of peaks suggested that MPIase contained repeating trisaccharide units. Extensive NMR analyses and synthetic works revealed the structre of MPIase to be as shown in Fig.1b. In summary, MPIase is a glycolipid whose glycan consists of GlcNAc, uronate of ManNAc (ManNAcA), and *N*-acetyl-4-aminofucose (Fuc4NAc). The number of repeating trisaccharide units is about 10, which means that about 30 *N*-acetyl-aminosugars are included in its glycan. About 30% of GlcNAc is 6-*O*-acetylated. The glycan links with the diacylglycerol moiety through a pyrophosphate linker. The fatty acids vary with the culture lots.

We performed the structure-activity relationship studies and examined physicochemical properties of the membranes in the presence of MPIase. Based on these results, we proposed a working model for the integration facilitated by MPIase (Fig.1c). The glycan part of MPIase captures the substrate protein to prevent aggregation, and then more hydrophobic interactions between membrane lipids and the substate protein would facilitate the procession into the membrane. Moreover, the alteration of the physicochemical properties of lipid membranes would assist the integration activity.

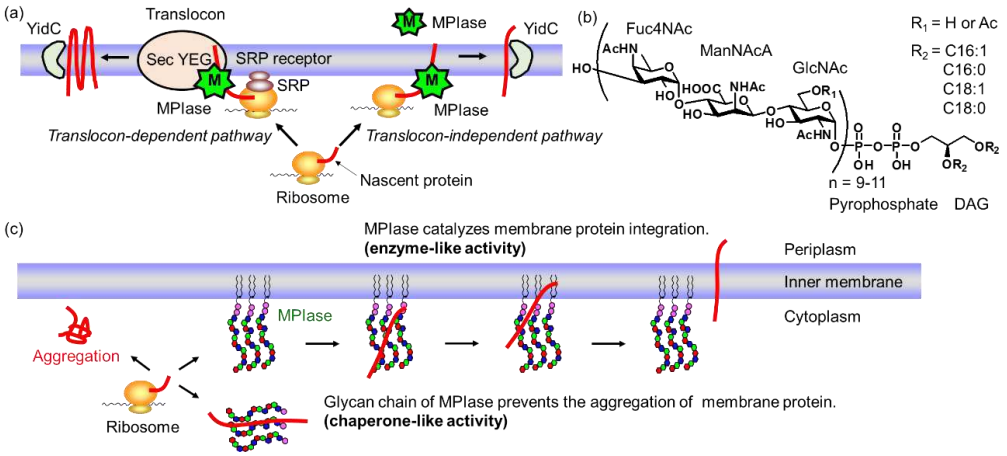

Fig.1 (a) Schematic model for membrane protein integration in *E. coli*. (b) Structure of MPIase (c) Putative mechanism for translocon-independent integration involving MPIase.

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PROGRESS TOWARD A NOVEL SYNTHESIS OF GLYCOSYLATED ASPARAGINE RESIDUES

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Some approaches that provide glycosylated asparagine residues employ β-glycosylamines, intermediates that are prone to mutarotation and are not hydrolytically stable. β-Glycosylamines are most commonly generated by the Kochetkov reaction which is often plagued by long reaction times, poor product yields, and diglycosylamine byproducts. We present here our progress toward generating a set of glycosylated asparagine residues using β-glycosylhydrazides as nucleophiles instead of β-glycosylamines.

POSTERS | G – Glycans

SIMPLE METHOD FOR DE NOVO STRUCTURAL DETERMINATION OF GLUCOSE-, GALACTOSE, MANNOSE-OLIGOSACCHARIDES AND APPLICATIONS ON IN SITU STRUCTURAL DETERMINATION IN LC/MS

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Carbohydrates play crucial roles in many biological processes. To elucidate the chemical and biological properties of carbohydrates, relevant structure–function relationships must be studied. The structural identification is difficult because of the large number of carbohydrate isomers and small amount sample can be extracted from biological system. Mass spectrometry (MS) is a highly sensitive analytical method and is frequently used in the molecular structure identification. However, the structural determination of oligosaccharides through mass spectrometry remains challenging. Most MS methods only determine part of the structures, or oligosaccharides have to be derivatized prior to MS measurement. Here we demonstrated a new MS method[1] which can be used for rapid and completely *de novo* structural determination of underivatized and tagged glucose- galactose- mannose-oligosaccharides. This new method involves sequential low-energy resonance excitation CID experiments of oligosaccharides in a typical ion trap mass spectrometer, a logical procedure to determine the structural decisive fragments for tandem CID sequence based on the dissociation mechanism we discovered recently, and a special prepared database. Two approaches are demonstrated. First, this method was first applied to determine the structures of more than 30 underivatised linear and branched glucose-, galactose-, and mannose-trisaccharides, tetrasaccharides, and pentasaccharides and 25 synthesized tetrasaccharides with tag on reducing sugar. The linkage, anomeric configuration, and branching location can be clearly identified. High-performance liquid chromatography and a mass spectrometer with a built-in logical procedure are established to demonstrate the capability of the in situ CID spectrum measurement and structural determination of oligosaccharides in the chromatogram. Second, this method was applied to determine the structures of more than 20 underivatised linear glucose-, galactose-, and mannose-trisaccharides, tetrasaccharides. The constitute monosaccharide, linkage, and anomeric configuration can be identified. Applications of these two approaches on the structural determination of N-glycans are demonstrated.

References

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INTRAMOLECULAR GLYCOSYLATION OF PEPTIDE-TETHERED GLUCOSE DERIVATIVES

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Intramolecular glycosylation using substrates in which the glycosyl donor and acceptor are linked via a tether connecting the functional groups, except for reactive sites, is also known as molecular clamping [1]. Peptides are promising as tethers for synthesizing oligosaccharides via the molecular clamping method because sequential couplingbetween tethers can be easily achieved using the common solid-phase peptide synthesis method [2, 3]. However, in intramolecular glycosylation using peptides as tethers, the regioselectivity and stereoselectivity are difficult to control because of the conformational flexibility or specific secondary structure of peptides, which depend on the peptide sequence. We have synthesized a disaccharide precursor in which each hydroxyl group at the C6 position of a thioglycoside donor and a glycosyl acceptor are tethered by L-glutamic acid dipeptide derivatives. A glycosylation reaction gave both anomers, and their ratio was changed through variations in the solvent. Moreover, we revealed that the tethered thioglycoside was not activated by N-iodosuccinimide and a catalytic amount of trifluoromethanesulfonic acid in an acetonitrile-containing solvent, indicating that the amide in the tether dipeptide could inhibit the activation of thioglycoside under certain conditions. To investigate the effect of dipeptide tethers on the regioselectivity and stereoselectivity of glycosylation, glycosyl donors (1) and acceptors (2) in which each primary hydroxy group at C6 was condensed with a carboxyl group of the side chain of D/L-glutamic acids or aspartic acids were synthesized in six steps from D-glucose and in eight steps from methyl-α-D-glucoside, respectively. In this presentation, the advantages and limitations of these tethers for regioselective and stereoselective glycosylation are presented and discussed.

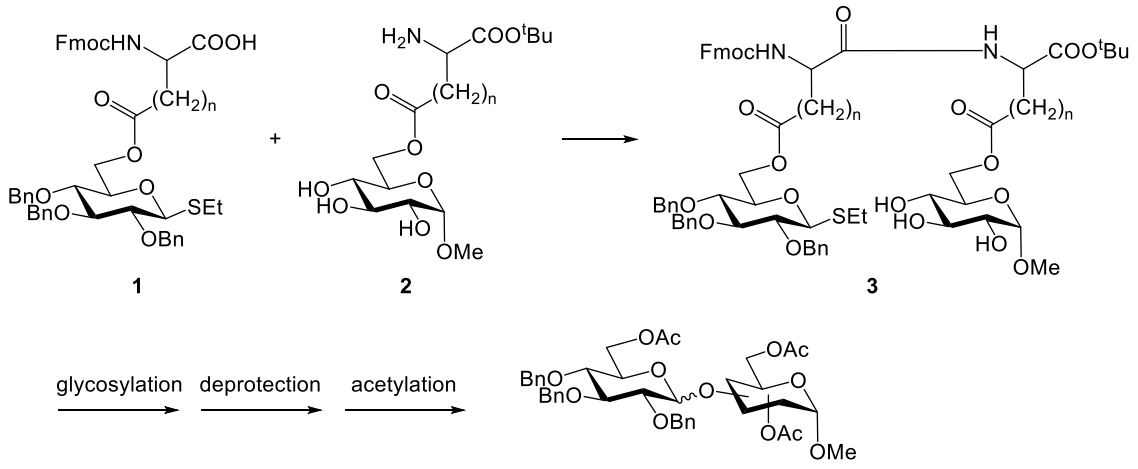

Fig. 1

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ENZYMATIC SYNTHESIS OF α–1,2-FUCOSYL *PARA-LACTO-N*-HEXAOSE AND ITS ISOMERIC DERIVATIVES

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Enzymatic synthesis of *para-lacto-N*-hexaose and its isomeric structures as well as those α1,2-fucosylated variants naturally occurring in human milk oligosaccharide (HMOs) was achieved using a sequential one-pot enzymatic system. Three glycosylation routes comprising bacterial glycosyltransferases and corresponding sugar-nucleotide–generating enzymes were developed to facilitate efficient production of extended type-1 and type-2 *N*-acetyllactosamine (LacNAc) backbones and hybrid chains. Further fucosylation efficiency of two α1,2-fucosyltransferases on both type-1 and type-2 chains of the hexasaccharide was investigated to achieve practical synthesis of the fucosylated glycans. The availability of structurally defined HMOs offers a practical approach for investigating future biological applications [1].

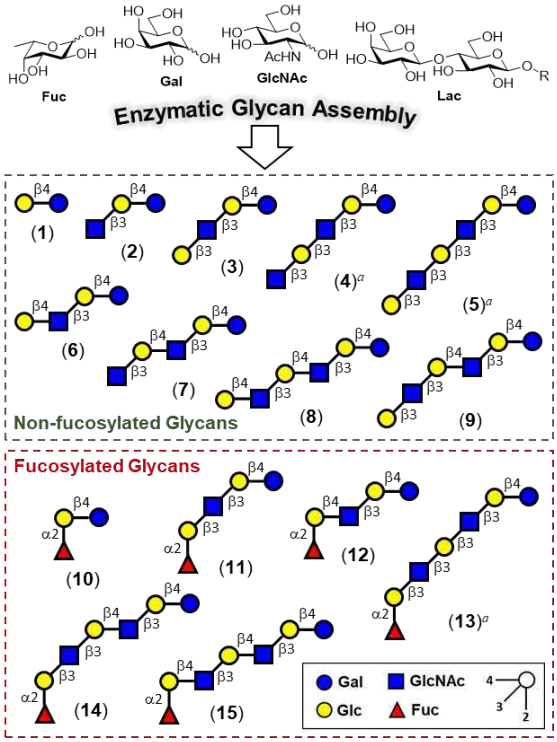

Fig. 1 – Fifteen human milk oligosaccharide (HMO)-type glycans and related isomeric glycans synthesized in this study. [a] Not reported in human milk, but found as the backbones of the human tumor-associated antigen.

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INVOLVMENT OF THE α2,6 SIALYLATION OF GLYCANS IN THE DIFFERENTIATION OF MYOBLASTS

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Myogenesis is a physiological process which depends on the proliferation, the differentiation and the fusion of myoblasts in order to form myotubes and then the future muscular fibers. These mechanisms are regulated by myogenic factors such as the MRFs (Myogenic Regulatory Factors) and two members of the Paired box (Pax) protein family, Pax3 and Pax7. Among them, the balance between Pax7 and MyoD is essential for the cell fate determination and the entry in differentiation. The myoblastic fusion requires the presence of glycans on the cell surface, especially the N-glycans. We established that among a panel of glycogenes expressed in the myoblastic murin cell line C2C12, more than a half are transcriptionnaly deregulated during differentiation [1, 2]. We demonstrated that during the C2C12 cell differentiation, decrease of α2,6 sialylation is associated with a decrease of gene expression coding for ST6Gal1. We have interested in the implication of α2,6 sialylation in the regulation of Pax7 expression. For this purpose, we created C2C12 clones, which expressed shRNA against ST6Gal1. After a phenotypic and molecular characterization of some clones (proliferation time course, index fusion, ST6Gal1 expression) we have analyzed the sialylation of N-glycans present at the cell surface by lectinoblotting and mass spectrometry technics.

We show that the clones ST6Gal1- have a less proliferative capacity due to a low expression of Pax7, and an earlier differentiation. The knockdown of ST6Gal1 brings a decrease of sialylation, especially on the tri and poly-sialylated N-glycans for the benefit of the mono and bi-sialylated forms. The earlier fusion of the clones ST6Gal1- leads to a decrease of the amount of the reserve cells (Pax7+/MyoD-) and to the high fusion index observed in the clone ST6Gal1-. The K.O. of ST6Gal1 inactivates the proliferation and Smad 2/3 pathway whereas it activates the Akt pathway which favors the cell fusion.

We can conclude that the α2,6 sialylation: (1) is required for Pax7 activation and Smad 2/3 pathway activation, and so C2C12 proliferation; (2) loss induces an higher fusion of myoblasts; (3) contributes to the maintain of a population of reserve cells.

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CHEMOENZYMATIC SYNTHESIS AND CHARACTERIZATION OF PARAMAGNETIC MULTIANTENNARY N-GLYCAN COMPLEXES

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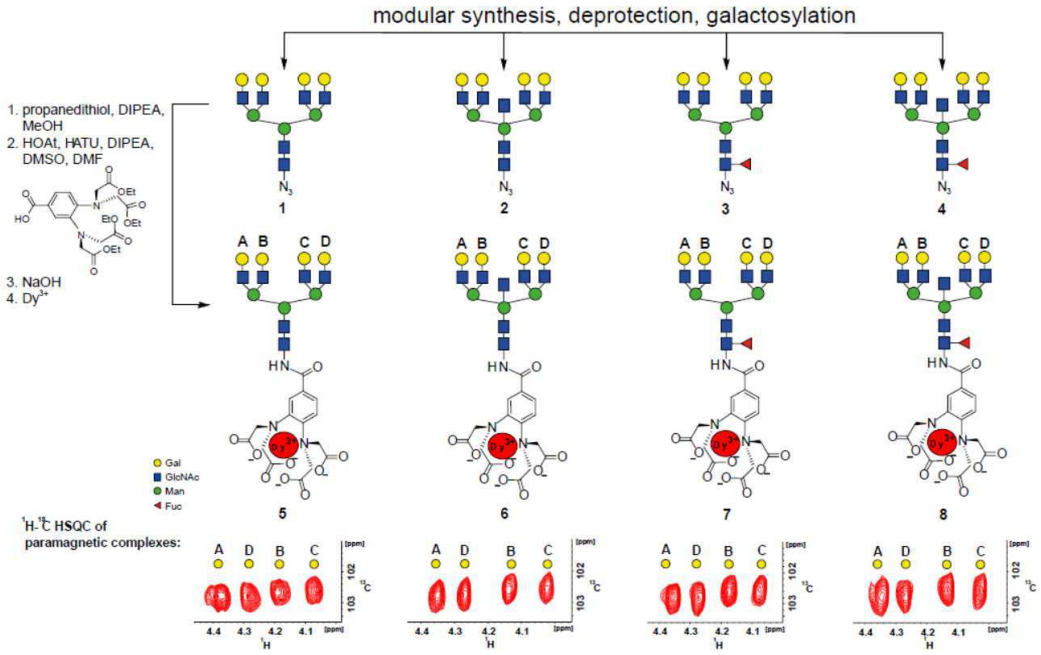
Complex-type N-glycans are ligands of lectins, toxins, enzymes and antibodies. In order to understand carbohydrate-protein interactions, pure and labeled N-glycans are required. The chemoenzymatic synthesis of these compounds [1] is a reliable approach to obtain defined structures in high purity. Recently, by means of paramagnetic NMR spectroscopy the analysis of branch-selective interaction of multiantennary *N*-glycans with lectins was accomplished. [2,3]
We developed a versatile chemical synthesis for complex *N*-glycans, that provides access to multiantennary bisected *N*-glycans in high yields even in a one-pot double glycosylation. [4] A selective deprotection leads to *N*-glycans bearing an anomeric azido group. [5,6] This approach was expanded to tetraantennary *N*-glycans with bisecting and/or fucose residues. The galactosylated tetraantennary *N*-glycans (**1-4**) were conjugated to a lanthanide binding tag. [2] The ^{1}H-^{13}C HSQC NMR spectra of the four paramagnetic complexes (**5-8**) fully resolve the anomeric signals of the terminal galactose residues of each branch (**A-D**). Paramagnetic NMR spectroscopy has revealed branch selective interaction of two lectins (RCA120, DSL) with the tetraantennary *N*-glycan complex **5**. [2,3] Studies of the interactions of the *N*-glycan complexes (**5-8**) with other lectins are ongoing.



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TOWARD CHARACTERIZING THE GLUCOSIDASES IN THE GREEN MICROALGAE, *CHLAMYDOMONAS REINHARDTII*

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Chlamydomonas reinhardtii is a green unicellular eukaryotic model organism for studying biological questions such as the *N*-glycosylation pathway. Moreover, the growing interest of using C. reinhardtii as an emerging cell factory for the industrial production of biopharmaceuticals require overall an in-depth understanding of protein *N*-glycosylation in this organism [1-3]. *N*-glycosylation is an universal post-translational modification which results in the covalent attachment of an oligosaccharide onto asparagine residues belonging to the consensus sequence Asn-X-Ser/Thr/Cys, in which X represents any amino acid except proline. This modification is initiated in the endoplasmic reticulum (ER) with the biosynthesis of a lipid-linked oligosaccharide (LLO) precursor [3,4]. Understanding this modification is fundamental, because it's determine the critical quality attributes that can influence folding, half-life, activity, and immunogenicity of proteins and biopharmaceuticals in particular[2].
Despite a relatively well-known *N*-glycosylation pathway in *C. reinhardtii* [1-3,5], glycoenzymes and specific steps still need to be characterized. Previous results demonstrated that the LLO in *C. reinhardtii* is a linear structure composed of 5 mannose and 3 glucose residues [5]. In this work, characterization of α-glucosidase I and II (*GSI* and *GSII*) insertional mutants from *C. reinhardtii* is performed. In addition, a pharmacological approach using glucosidase plant alkaloid inhibitor (castanospermine) in vivo on wild type strain of *C. reinhardtii* is conducted. The accumulation of glycoproteins with different glycan profiles ($Glc_3Man_5GlcNAc_2$ for GSI mutants and $Glc_2Man_5GlcNAc_2$ for *GSII* mutants) is expected and will be analyzed by complementary approaches: immunodetection, gas chromatography and mass spectrometry.

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A HIGHLY REGULAR FUCAN SULFATE PREPARED FROM FUCOIDAN: STRUCTURE AND BIOLOGICAL ACTIVITY

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Sulfated polysaccharide preparations (fucoidans) of brown seaweeds are known to influence blood coagulation, thrombosis, inflammation, oncogenesis, angiogenesis and some other biological processes. Many fucoidans are mixtures of several different polysaccharides, some of which have very complex branched molecules containing numerous monosaccharide units (Fuc, Gal, Man, GlcA, Xyl). The polysaccharide molecules are often characterized by irregular sulfation pattern. These features strongly hinder the determination of detailed fucoidan structures. As a result, revealing the structure-activity relationships within fucoidans seems to be a challenging task.

In this communication we describe the preparation of a highly regular fucan sulfate **FS** by chemical modification of fucoidan **AJ** isolated from the brown seaweed *Analipus japonicus*. Structure of **AJ** as multiple branched acetylated and sulfated fucan was elucidated previously [1]. To simplify the structure of **AJ** it was desulfated, deacetylated and further subjected to Smith degradation to give a linear (1→3)-α-L-fucan (Figure 1). Exhaustive sulfation of the latter resulted in formation of **FS**. The structure of **FS** was characterized by NMR spectra, MW and electrophoresis.

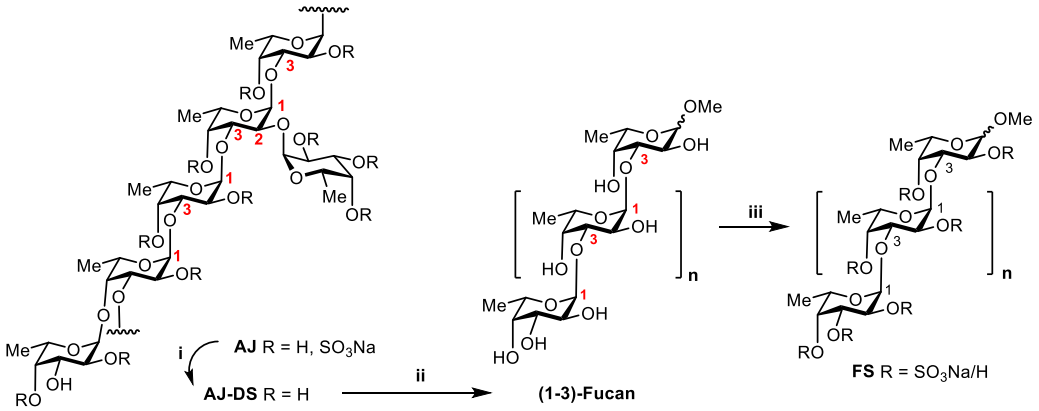

Fig. 1 – Chemical modification of the fucoidan from *Analipus japonicus*. Conditions: (i) a) Dowex 50 (PyH^+), b) DMSO/ MeOH; (ii) Smith degradation; (iii) a) $Py{\cdot}SO_3$, DMF, HSO_3Cl, b) $NaHCO_3$.

The ability of **FS** to influence blood coagulation and platelets aggregation was studied in vitro. It was shown that the effect of **FS** was similar to that of low molecular weight heparin (enoxaparin) in APTT test. Also **FS** significantly potentiate the inhibition of thrombin and factor Xa by antithrombin III. Due to low molecular weight, **FS** itself did not induce platelet aggregation. At the same time, it effectively inhibited platelets aggregation induced by ristocetin.

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PERVAPORATION MULTILAYER MEMBRANES BASED ON POLYELECTROLYTE COMPLEXES OF CHITOSAN AND ANIONIC POLYSACCHARIDES

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Pervaporation has gained wide acceptance as a membrane separation method, and it is recognized as the most adaptable technique for the separation of water-organic mixtures. Several studies have demonstrated the greater effectiveness of hydrophilic pervaporation for membranes prepared from polyelectrolyte complexes (PEC) than from individual polymers [1]. However, the role of PEC in the separation process, and the mechanism of selective transport of polar multiple-charged ions through this type of membrane remain to be established. Most authors maintain that the unique properties of PEC reflect the existence of a specific hydrophilic-hydrophobic balance that can be easily changed by varying the composition of the complexes and the conditions of their preparation. The separation characteristics of multilayer PEC membranes formed by combining layers of polymeric electrolytes depend largely on the strength of the interactions between the macrocounterions. This interaction can change the structure of the film towards increasing order. This factor of interpolymer interaction is believed to prevail over other "structural" factors, such as the degree of crosslinking, the flexibility of the backbone, the presence of aromatic fragments, etc. Thus, obtaining regular non-defect layers of interpolymer complexes with good separating capability is considered possible only when using polyelectrolytes that have a high charge density.
Chitosan, a partially or completely deacetylated derivative of chitin, is a cationic polyelectrolyte with a high charge density, which can form PECs with polyanions, suggesting the possibility of numerous practical applications including pervaporation. The aim of the present work was to prepare multilayer composite membranes based on chitosan and anionic polysaccharides (hyaluronic acid, alginic acid, sulfoethyl-cellulose). These membranes were evaluated regarding their structure, morphological features and their performance as dehydrating pervaporation membranes for the separation of water-alcohol mixtures. Studies of the transport properties of the pervaporation membranes in the separation of water-alcohol mixtures of various compositions show their high selectivity and high permeability [2,3] comparable with the best commercially available pervaporation membranes.

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EndoE AND EndoP: VERSATILE BIOCATALYSTS FOR GLYCOENGINEERING

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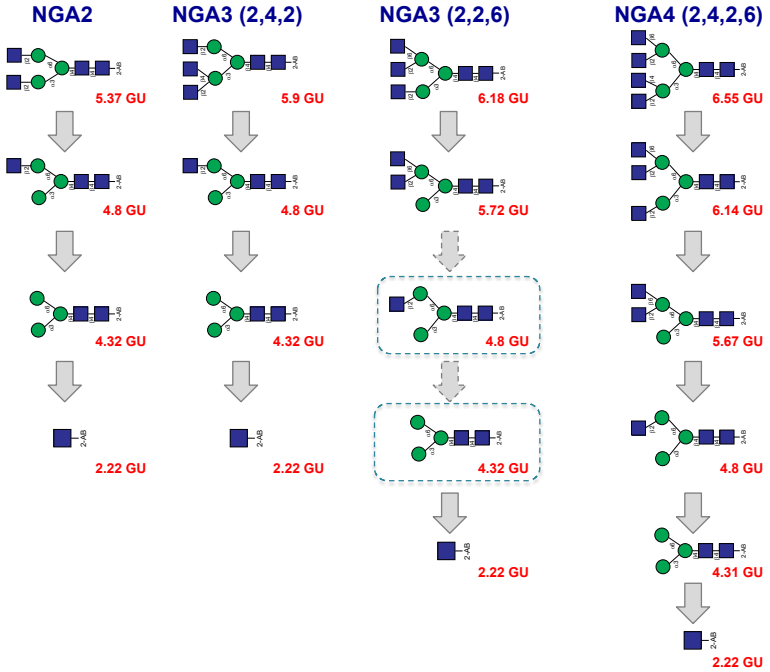
Glycosylation exerts a profound impact on the structures of antibodies and a variety of physiological processes ranging from fertilization, embryogenesis, signal transduction, and disease progression. The platform for production of homogeneous glycoforms of glycoproteins is emerged from glycosylation remodeling using the endoglycosidase-catalyzed deglycosylation and transglycosylation approach [1] Here, two of the *endo*-β-*N*-acetyl-glucosaminidases (ENGases) EndoE (encoded from *Enterococcus faecalis*) and EndoP (encoded from *Prevotella melaninogenica*) were identified from the *CAZymes* database and examined its hydrolysis activities on glycans of the IgGs, high-mannose glycans, C5-amine linked glycans, and 2-AB tagged glycans. EndoP was found to have the glycosidic activity on complex type *N*-glycans, whereas EndoE was found to have the glycosidic activity on both complex type and high-mannose type *N*-glycans [2,3]. The selective and sequential cleavages of EndoE and EndoP were shown on 2-AB linked tri- (NGA3) and tetra- (NGA4) glycans, it derived further examination of transglycosylation using glycan donors with different linkers at the reducing-end to perform homogeneous glycoforms with well-defined glycan structures and optimal effector functions.

EndoE: selective and sequential cleavages on 2-AB labeled glycans



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CONFORMATIONAL ENERGY MAPPING OF XYLOBIOSE WITH QUANTUM MECHANICS

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Xylobiose is the shortest xylan, a hemicellulose that closely interacts with cellulose in woody plants. Those interactions are important in plant growth and utilization. Conformations of β-xylobiose were studied with a method previously used for cellobiose.[1] Vacuum and continuum solvation efforts found a profound difference, i.e., the optimal ϕ torsion angles differed by about 150°. Our vacuum results agreed with other theoretical [2] and vacuum experimental results. [3] The solvated map accommodated the many crystal structures for cellobiose as well as cellulose. The difference in preferred conformations was attributed to dominant inter-residue O-H…O-H hydrogen bonding in the vacuum phase, whereas the condensed-phase structure involved the less attractive O-5' atom in one of its two hydrogen bonds. The stronger hydrogen bonding in the vacuum phase apparently overcomes the intrinsic preference for anti conformation in a tetrahydropyran analog of cellobiose. Thus it was of interest to study the energy surface of xylobiose, which can only make one inter-residue hydrogen bond.

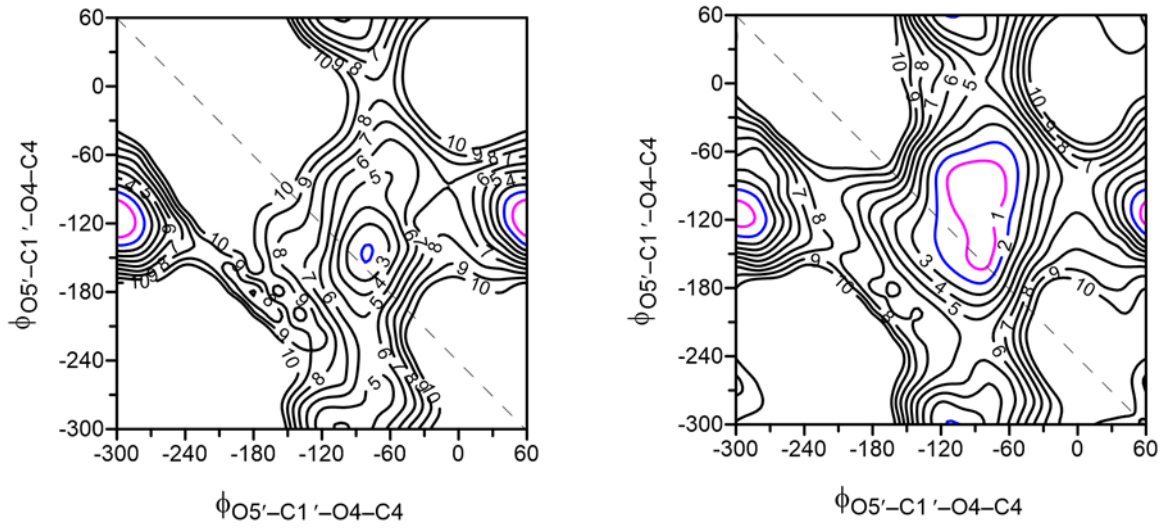

Fig. 1 – B3LYP/6-31+G* (left) and SMD solvation (right) maps. The magenta contours are at 1.0 kcal/mol above the minimum, the blue lines are at 2 kcal/mol. Locations with 2-fold screw-axis symmetry fall on the diagonal dashed lines.

Results. The vacuum map shows, compared to cellobiose, a reduced preference for the edge of the map minimum, whereas the solvation map barely prefers the center. The solution global minimum supports the 3-fold helical shape of xylan dihydrate. [4]

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SYNTHETIC GLYCOLIPIDS WITH CUSTOMISED DISTANCE BETWEEN LIPID AND GLYCO PARTS

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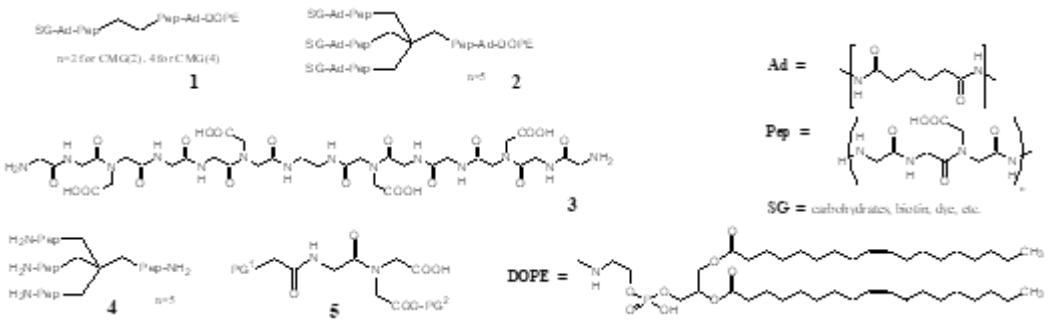
Glycolipids are capable to spontaneous insertion into cell membranes. The application of such molecules is often limited by requirement to use organic solvents for maintain them in a solution-phase suitable for insertion and this can limit their compatibility with live cells. Recently synthetic glycolipid-like constructs, which have a spacer between a synthetically friendly lipid (as a rule, dioleoyl phosphatidylethanolamine, DOPE) and the glycan head have been constructed. These constructs are now known as Function-Spacer-Lipid constructs (FSLs). All FSLs have been designed to disperse as a solution directly in organic solvents free water, saline and biological media. FSLs posses linear 1 or branched 2 structure (Scheme 1, 1-2). Like natural glycolipids, all FSLs found to insert easily into cell membranes. Spacer (S) as part of FSLs is no less important than parts F and L, we usually use CMG 3-4. They: 1) distances the glycan from the cell membrane to the required distance, 2) increases dispersibility in water, 3) can work in stealth mode, because they do not interact with blood components. Changing the amount of tripeptide blocks in CMG we can variate the distance between F and L moieties in an interval up to 20 nm.



Scheme 1

The synthesis of FSLs is a two step procedure and the first step is usually the attachment of lipid tail to the spacer. This reaction proceeds smoothly but not selectively and requires relatively large amount of the spacer while it is often critical to prepare FSLs in at least a gram-scale. The spacer for the synthesis is used in an excess and the reaction mixture contains both as starting material and products of mono addition of the lipid moiety and its multiple addition.

With purpose to obtain the spacer for FSLs in a multigram scale and/or simplification of further separation of the reaction mixture components we synthesized several tripeptides 5 with orthogonal protection groups and used them in design of the spacer CMG(2) 3 (Scheme 1). Varying PG1 and PG2 we found the conditions when the synthesis of CMG(2) from tripeptide 5: 1) can be performed with low time and solvents consumption but hardly scalable; 2) is time consuming but can be easy scaled; 3) leads to the linker 3 with diversified tails. The last molecule, due to orthogonal protection groups, is useful.

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MOLECULAR RECOGNITION OF BLOOD GROUP ANTIGENS BY hGALECTIN-1

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Glycans serve as recognition elements for glycan binding proteins at the surface of most cells and mediate key biological processes such as cell-cell adhesion, migration and other events critical for the development and function of complex multicellular organisms. At the heart of important living processes, protein-glycan interactions are also linked with several diseases, including infection, cancer or autoimmune disorders.

In this context, human galectin-1 (*h*Gal-1), a β-galactoside binding lectin, is involved in a large variety of relevant biological processes such as inflammatory responses, differentiation trafficking, survival of immune cells and establishment and maintenance of T-cell tolerance and homeostasis *in vivo* [1]. Furthermore, *h*Gal-1 overexpression in tumours correlates with a metastatic phenotype [2]. Moreover, there are recent evidences that suggest that deletion of the *h*Gal-1 gene increases survival in mouse models for pancreatic ductal adenocarcinoma (PDA) [3]

Considering its biological relevance and its growing importance as biomedical target, we have investigated the binding of *h*Gal-1 with naturally occurring oligosaccharides containing LacNAc structures. In particular, we have studied the molecular recognition of blood group antigens (LacNAc and its 3'-O-α-Gal derivative, H type II, B type II and A type II) using Nuclear Magnetic Resonance (NMR) techniques. We have combined Saturation Transfer Difference (STD) NMR experiments with ^{15}N-^{1}H Heteronuclear Correlation Spectroscopy experiments to determine the binding features in these systems. In addition, to gain insights into the driving forces for the complex carbohydrate-protein formation, we have employed Isothermal Titration Calorimetry (ITC).

Overall, our results provide a comprehensive analysis from a molecular point of view of the glycan-lectin binding process to guide the design and development of galectin-based therapeutic tools.

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SEMISYNTHESIS OF *N*-,*O*-GLYCOSYLATED BIKUNIN

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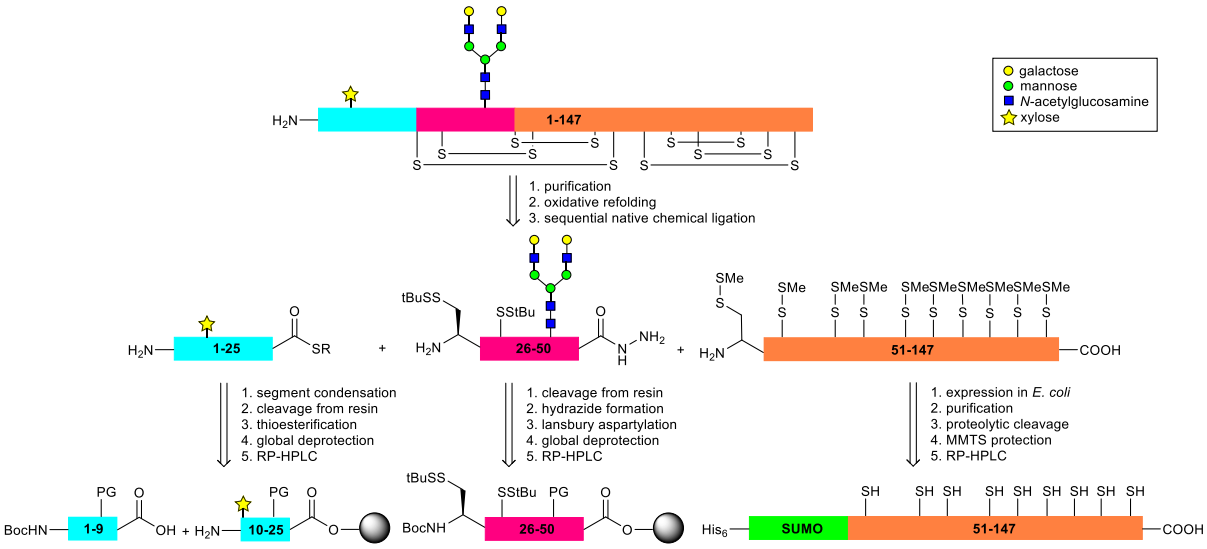
The proteoglycan bikunin is a protease inhibitor with an *N*-glycosylation and an *O*-linked glycosaminoglycan (GAG) found in the blood serum of mammals. Bikunin is related to inflammation, cancer and pancreatitis.[1,2,3] The proteoglycan bikunin is glycosylated at two sites, the quite regular chondroitinsulfate chain is linked to Ser10 and the biantennary *N*-glycan to Asn45.[3,4] In order to examine the correlation between glycosylation and biological activity of glycoproteins the availability of pure glycoforms is essential.[5] The aim of this project was the semisynthesis of *N*-,*O*-glycosylated bikunin via sequential NCL.[6,7,5] The full-length bikunin 1-147 was divided into three fragments. The *O*-glycopeptide 1-25 with a C-terminal thioester was obtained by solid phase glycopeptide synthesis (SPPS) of bikunin 10-25 and segment condensation with bikunin 1-9. The *N*-glycopeptide hydrazide 26-50 was synthesized on the solid phase. Hydrazide formation and convergent sugar coupling via Lansbury aspartylation were carried out in solution.[8] The cysteine peptide bikunin 51-147 was produced by recombinant expression in *E. coli* and enzymatic removal of the His_6-SUMO tag.[9] After sequential ligation the full-length bikunin glycopeptide 1-147 was oxidatively refolded via stepwise dialysis. The semisynthetic bikunin was purified by HPLC and showed inhibitory activity against trypsin.



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CARBOHYDRATE FLEXIBILITY MODULATES THE KINETICS AND THERMODYNAMICS OF PROTEIN BINDING

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Sugar recognition by protein receptors takes place through attractive forces mainly including hydrogen bonding, CH-π interactions and coordination to divalent cations. However, these favorable contacts go together with a reduction of conformational, rotational and translational freedom of the individual binding components, which is translated into an unfavorable entropic contribution to the binding. [1] Carbohydrates are molecules rather flexible and therefore this term is generally significant. As a consequence, protein-carbohydrate interactions are essentially enthalpically driven and display low binding affinities. Not surprisingly, some attempts were made to lower the loss of conformational entropy and get higher-affinity ligands by designing conformationally restricted sugar mimetics. [2]

Histo-blood group antigens A, B and H are tethered oligosaccharides characterized by their conformational rigidity. We have explored in detail the molecular recognition of such glycan epitopes by *h*Galectin-3 using the combination of NMR and ITC experiments with modeling protocols. We observed that the thermodynamics and kinetics of binding are governed by the conformational flexibility of the carbohydrate. In fact, constrained oligosaccharides showed higher affinities of binding. The experimental results indicated that the entropic penalty of binding is favorably reduced when conformationally pre-organized carbohydrates are used as ligands, even reaching positive values. This study highlights the effect of glycan dynamics in the kinetics and thermodynamics of binding and goes beyond the usually provided static picture for protein-ligand complexes. [3]

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A NEW MONOCLONAL ANTIBODY RECOGNIZING THE FULL-LENGTH INTRACELLULAR HUMAN A1,4-GALACTOSYLTRANSFERASE (GB3/CD77 SYNTHASE) AS WELL AS ITS SOLUBLE FORM IN THE BLOOD SERUM

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Human α1,4-galactosyltransferase (Gb3/CD77 synthase), encoded by the *A4GALT* gene, catalyzes the transfer of galactose residue to lactosylceramide and paragloboside, creating globotriaosylceramide (Gb3, CD77, P^k antigen) and P1, which belong to the globo and neolacto glycosphingolipid series, respectively. Both P^k and P1 antigens contain the Gal-α(1,4)-Gal terminal structure and belong to the P1PK histo-blood group system. We found previously that the point mutation c.631C>G in the *A4GALT* gene causes a change of 211 amino acid from glutamine to glutamic acid (p.Q211E) [1]. The variant enzyme shows a broader acceptor specificity and in addition to P^k and P1 it catalyzes the synthesis of rare antigens NOR1 and NOR2, both of which terminate with a Gal-α(1,4)-GalNAc unit [2]. The aim of this study was to a obtain monoclonal antibody against an active recombinant catalytic domain of human α1,4-galactosyltransferase.

Previously, we obtained a soluble, active recombinant α1,4-galactosyltransferase and the mutein with p.Q211E substitution using the baculovirus-insect cell system. The purified enzyme was used to immunize BALB/c mice and several clones of monoclonal antibody-producing hybridoma cells have been obtained. We screened hybridoma culture supernatants in Western blotting and ELISA using recombinant consensus α1,4-galactosyltransferase and the variant enzyme. Specificity of reactive supernatants was further tested in Western blotting on lysates from cells transfected with vectors encoding consensus or variant enzyme, as well as on human serum samples and blood platelets lysates.

The obtained antibody recognizes both the mutein and the consensus α1,4-galactosyltransferase in the soluble form (i.e. without the transmembrane domain), but also the full-length protein overexpressed in CHO-Lec2, Namalwa and 2102Ep cells. Moreover, using this antibody we detected α1,4-galactosyltransferase in human serum samples and blood platelets. To the best of our knowledge, this is the first monoclonal antibody elicited with an active recombinant catalytic domain of human α1,4-galactosyltransferase.

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GENE EXPRESSION ANALYSIS OF GLYCOSYLTRANSFERASES OF SKELETAL MUSCLE CELLS AND ITS EXERCISED CONDITION IN HYPERGLYCEMIC CONDITION

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Diabetes is one of the lifestyle diseases, and which is induced various complications. It has been considered that those complications are coursed by a long-term hyperglycemic condition. However, it has not been revealed about a detailed causal association. Among these complications are cases of motor dysfunction and muscle atrophy, it was suggested that the long-term hyperglycemic condition had an adverse effect on the muscle. Skeletal muscle is important organ in metabolism and exercise, and it is the largest organ in the body. Therefore, we focused on the *N*-glycans of skeletal muscle cells in hyperglycemic condition. Our previous study, we analyzed the *N*-glycan structures of two types conditions of normal blood sugar (Low Glucose Condition Cells; LGCC) and hyperglycemic (High Glucose Condition Cells; HGCC) in the differentiated C2C12 cells, and we confirmed that the amount of the specific *N*-glycan structures were changed, and regardless of sugar condition, the total amount of *N*-glycans was decreased in the exercise condition of C2C12 cells. As a further study, we analyzed the expression level of glycosyltransferase genes associated with these *N*-glycans. C2C12 cells were differentiated into myotubes under conditions of LG or HG and exercised using electrical pulse stimulation (EPS). The gene expression level was analyzed by real-time PCR. As a result, it was observed that the expression level of galactosyltransferase gene was increased in HGCC. In addition, although the total amount of N-glycans were decreased after EPS, there was no significant change in the *N*-glycan related genes. Therefore, we are suggesting that the decreasing of total amount of *N*-glycans by EPS is not gene expression level of glycosyltransferases but impact of the activation of decomposition process.

STRUCTURAL ANALYSIS OF MILK OLIGOSACCHARIDES IN BOVINE COLOSTRUM

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“Milk” is most important nutrient source in early childhood of mammal, and which contains proteins, carbohydrates, lipids, and minerals in well-balanced. Lactose is most abundant component of carbohydrate, but besides this, oligosaccharide and glycopeptide are existed in the milk as well. Recently, it has been revealed in some of their functions, and the information is very important for developing various functional food such as prebiotic food, immunostimulating food, metabotropic food, and understanding the real meaning of milk for mammal. However, there are few reports about the comparative structural analysis of milk oligosaccharides between primiparous colostrum and multiparous colostrum. In addition, the detailed function of glycopeptides and stage-specific composition of milk oligosaccharides also have not been clarified. In our previous research, we confirmed that the specific oligosaccharides contained in both primiparous colostrum and multiparous colostrum, and those concentration were significantly high as compared with those of normal milk.
In this study, we focused on the detailed structure and function of the milk specific oligosaccharides and glycopeptides in the primiparous colostrum and multiparous colostrum. We extracted all oligosaccharides and glycopeptides from bovine colostrum by chloroform-methanol, and separated by gel filtration chromatography. The oligosaccharides and glycopeptides were monitored by phenol-H_2SO_4 and ultraviolet absorption, respectively. The collected oligosaccharides were purified by HPLC, and the structure was estimated by MALDI-TOF MS. The result of HPLC, the oligosaccharide composition of the primiparous colostrum was different from those of the multiparous colostrum. The result of MALDI-TOF MS, it was suggested that the existence of specific oligosaccharides which having two phosphate groups

EXPESSION AND MORHOLOGICAL ANALYSIS OF HYALURONIDASE1 AND 2 IN DIGESTIVE ORGAN OF *XENOPUS LAEVIS*

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Hyaluronidase (HYAL), a hyaluronic acid (HA) degrading enzyme, also plays an initial stage role in degradation of chondroitin sulfate (CS), an HA structural analog. It has been reported that HYAL has five subtypes so far. It has been reported that HYAL1 and HYAL2 use low molecular weight and high molecular weight CS as substrates, respectively. We have elucidated and reported the amount of each subtypes of CS in the gastrointestinal tract of Xenopus, the expression of the enzyme group involved in CS synthesis, namely chondroitin sulfotransferase (CHST), and the cells producing these CHSTs. However, the amount of CS produced in the gastrointestinal tract (GI) of Xenopus did not coincide with the expression level of *Chsts*, and it was necessary to consider this divergence. In the background of this divergence, it is necessary to consider the existence of the degradation mechanism of CS by HYALi and HYAL2. In this study, we tried to clarify the expression levels of *hyal1* and *hyal2* in Xenopus digestive organ, and tried to identify the HYAL-expressing cells.

GI and liver were collected from *Xenopus laevis* (4 male, 70g each). In addition to the esophagus and the stomach, the digestive tract from the duodenum to the anus was trisected (named as upper, middle and lower). Total RNA was extracted from each site to synthesize cDNA. Expression levels of *hyal1*, *hyal2* and *cd44* (HA receptor) were compared and examined by real time PCR. HYAL-expressing cells in GI was detected by *in situ* hybridization (ISH).

Comparing the expression levels of *hyal1* and *hyal2* for each site throughout the GI and liver, *hyal1* and *hyal2* was significantly higher in liver and middle region of intestine, respectively. However, there was no significant difference in the expression level of *cd44* at each site of GI. Expression level of *hyal2* in throughout in GI was extremely high compared to that of *hyal1*. ISH revealed that both *hyal1* and *hyal2* expressed in epithelial cells in any of site of GI.

From the above, the following possibilities are conceivable. 1) High molecular weight CS, which is synthesized or absorbed in GI is degraded by HYAL2. 2) Degraded CS may be further degraded by HYAL1 of epithelial cells or liver after being taken into the body. These results of expression levels of *hyal1* and *hyal2* in Xenopus GI may help to explain the divergence between the expression level of *Chsts* and CS amount

CHEMOENZYMATIC SYNTHESIS OF ASYMMETRIC N-GLYCANS

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Since *N*-glycans have a great influence on the biological function of *N*-glycoproteins, there is a need for libraries containing as many pure *N*-glycans as possible. [1] These should include not only *N*-glycans with symmetrically substituted branches, but also those with an asymmetric pattern.[2] We sought approaches for the synthesis of such asymmetric *N*-glycans by partial enzymatic reactions starting from readily available nonasaccharide azide **1**. [3] Through partial digestion of 1 with β-galactosidase the monogalactosylated compounds **2** and **3** could be obtained in a 3:1-ratio. Furthermore nonasaccharide azide 1 was monosialylated with two different bacterial sialyltransferases providing asymmetric *N*-glycans **4 – 7**. Digestion of the monosialylated *N*-glycans **6** and **7** with β-galactosidase gave *N*-glycans **8** and **9**.

In general the separation of asymmetrically substituted *N*-glycans is challenging. Due to the nearly identical structure the compounds are quite difficult to separate on a preparative scale. So far we have successfully separated eight pairs of asymmetric *N*-glycans by HPLC and identified them by NMR-spectroscopy subsequently.

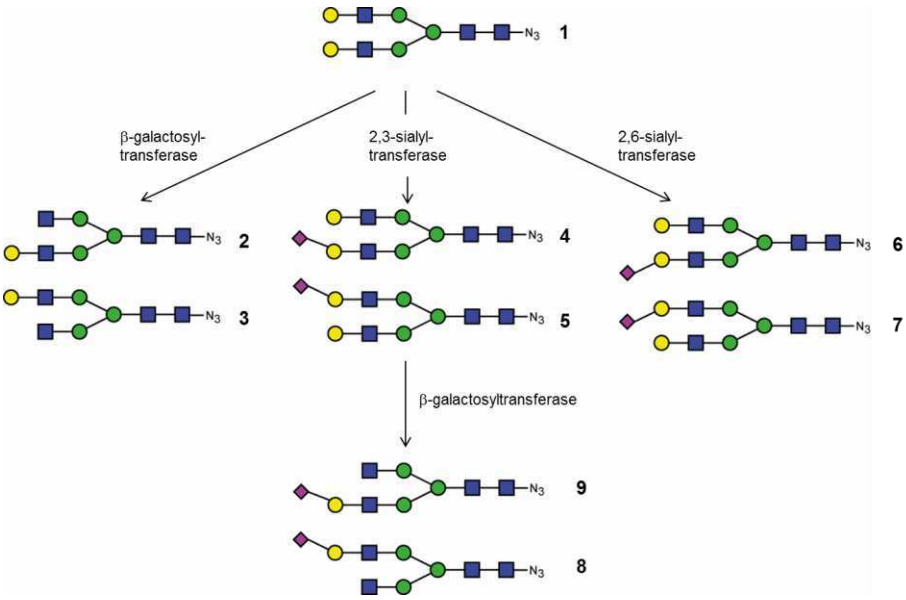

Fig. 1 – Enzymatic synthesis of asymmetric *N*-glycans.

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SECOND GENERATION CHONDROITIN SULFATE (CS) DENDRIMERS AS USEFUL TOOLS TO STUDY CS/PROTEIN INTERACTIONS

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Glycosaminoglycans (GAG's) are a class of sulfated polysaccharides present in connective tissues that are characterized by a heterogenic structure. Within this family of sugars, Chondroitin sulfate plays a significant role in various biological processes, interacting with several proteins including lectins and growth factors.[1] Its heterogeneity demands the development of glycomimetics as tools to study their biological functions. In this sense, the linear synthesis of these polysaccharides is expensive and time-consuming; instead the decoration of a multivalent scaffold with a disaccharide ligand, allows the preparation of useful tools, able to mimic these complex polysaccharides. [2]

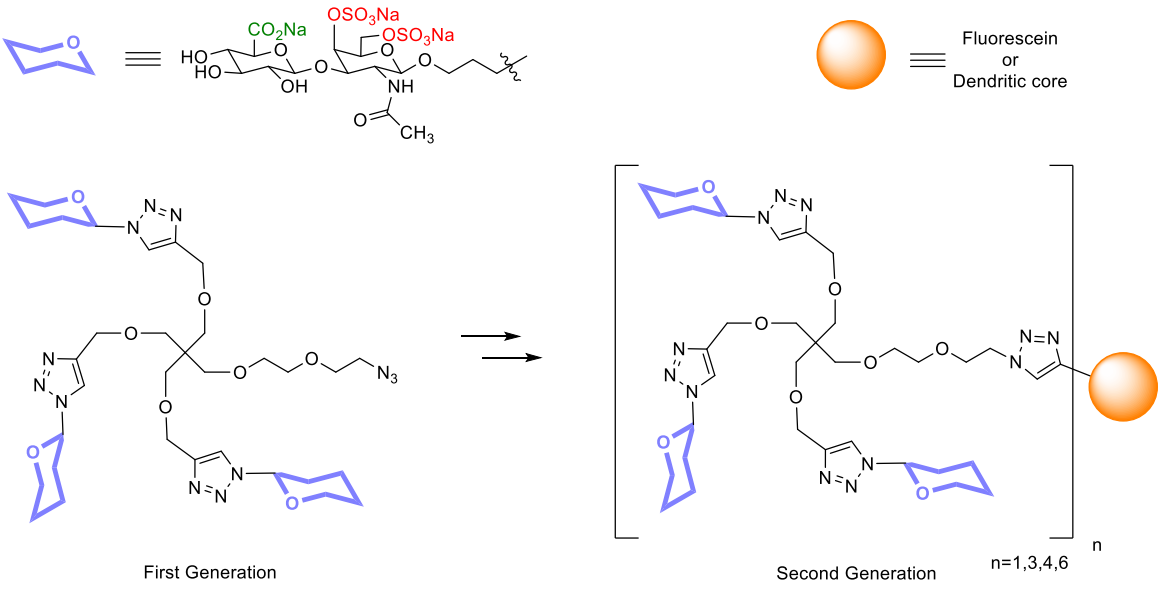

Fig. 1

Herein, we present the synthesis of a novel family of CS Type-E glycodendrimers, generated by means of copper(I)-catalyzed alkyne-azide cycloaddition also called "click chemistry" (**Figure 1**). Glycodendrons orthogonally functionalized in the focal position were first prepared. Using this versatile approach it was then possible to prepare second-generation glycodendrimers [3], with a complete control of the composition, displaying 9, 12 and 18 ligand copies. Moreover, in order to study the interaction of these compounds with lectins by fluorescent polarization, we synthesized a glycodendron functionalized at the focal position with a fluorescent probe.

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THE DEVELOPMENT OF GLYCAN STRUCTURE IS DETERMINED BY INTERPLAY OF GLYCOSYLTRANSFERASES

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The glycosylation is a complex process which is involved in many glycosyltransferases. N-acetylglucosamine (GlcNAc) -terminated biantennary glycan (A2) is a substrate for fucosyltransferase 8 (FUT8), N-acetylglucosaminyltransferase (GnT)-III, GnT-IV, and GnT-V [1], and it is important to investigate the development of glycan structure, which is determined by the competition of these enzymes in cells. We identified the specificity and activity of human FUT8 toward GlcNAc-terminated bi, tri- and tetra-antennary N-glycans in the forms of glycopeptides. We found that the biantennary glycan (A2 type) and tri-antennary glycan [A3(2,4,2) type] terminated with GlcNAc, which is generated by GnT-IV, are good substrates for FUT8, but the A3(2,2,6) type of tri-antennary glycan, generated by GnT-V, and tetra-antennary (A4) glycan are not (Figure) [2]. We also observed that core-fucosylation reduced the activity of GnT-IV to generate A3(2,4,2) from biantennary glycan. Furthermore, we examined the correlation between the types of N-glycans and the expression levels of FUT8, GnT-IV, and GnT-V in cells. It revealed that the expression of GnT-IVa plays a role in directing the branching and core fucosylation of N-glycans in vivo. The high expression of GnT-IVa in MDA-MB-231 cells is correlated to high amount of A3(2,4,2) without core-fucosylation. However, knock-out of GnT-IVa in MDA-MB-231 cells resulted in significant increasing in A2F glycan. This study thus provides insights into the interplay among FUT8, GnT-IV, and GnT-V in N-linked glycosylation during the assembly of glycoproteins.

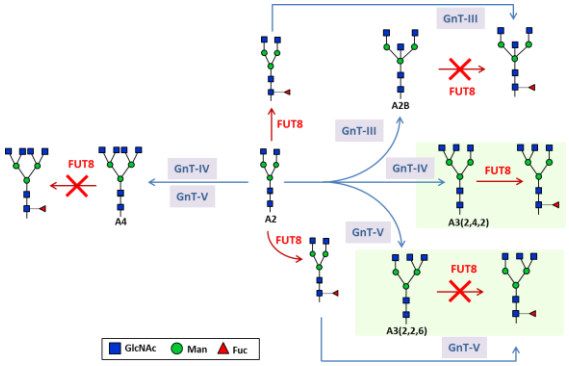

Fig. 1 – The selectivity of substrates of human Fut8.

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CHARACTERISATION OF THE GLYCOME OF CANINE MELANOMA CELLS

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Glycomics is the broad scientific study of glycans focusing on defining their biochemical structures and biological roles in living organisms. Comprehensive mass spectrometric strategies are commonly employed, associating with enzymatic, biochemical and chromatographic techniques, to provide unambiguous information on carbohydrate sequences and glycosidic linkages for the structural definition of glycans from cells and tissues.

Aberrant glycosylation is a hallmark of cancer and it is well established that the structures and expression levels of polylactosamine-containing glycans are altered upon malignant transformation and tumour progression [1]. Such glycans have been implicated in the trafficking of tumour cells and the suppression of innate and adaptive immune systems. We have recently shown that the transformation of human melanocytes to malignant melanoma is associated with the conversion of branched polylactosamines (the I-antigen) to linear polylactosamines (the i-antigen) [2]. The latter are potential ligands for galectins.

Spontaneous cancers in dogs are considered to be excellent models for analogous cancers in humans. However virtually nothing is known about polylactosamine expression in canine melanoma cells. In the current study we have employed MALDI-TOF and GC-MS glycomic methodologies to analyse the *N*-, *O*- and glycolipid glycomes of melanoma cells from a stage III canine melanoma patient. We have found that canine melanoma cells express a similar repertoire of glycans to those on human melanoma cells, except that, as expected, some of the glycans are capped with *N*-glycolylneuraminic acid and alpha linked galactose. Both human and canine melanoma cells express gangliosides GD2 and GD3 that are known binding ligands for Siglec-7, an immune signal-transducing receptor that inhibits natural killer (NK) cells [3]. They both also express elevated levels of linear polylactosamine sequences.

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DISCOVERY OF κ-CARRAGEENAN-LIKE AGAROSE FROM A RED SEAWEED, *Gracilaria coronopifolia*

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Agarose is the major gelling constituent of agar extracted from a family of red seaweeds and used as a gelling agent in the separation, purification, characterization, and analysis of biomacromolecules. The principle of agarose gel has been established at molecular level including water [1-3]. Furthermore, high quality agarose [4] and the agarose substituted with methyl groups at C-2 or C-6 of β-D-galactose residues were identified [5]. We report here on the identification of κ-carrageenan-like agarose from *Gracilaria coronopifolia* collected in Ishigaki Island, Okinawa , Japan.

The yield of polysaccharide from *G. coronopifolia* was 15.4% based on dried seaweeds. Total carbohydrate and sulfate content was 69.5 and 20.3%, respectively. After methanolysis of the polysaccharide in 0.5 M anhydrous HCl-methanol solution at 100°C for 12 h, 3,6-anhydro-methyl-β-D or L-galactoside, 3,6-anhydro-methyl-α-D or L-galactoside, and methyl-β-D-galactoside were identified by thin-layer chromatography. D-Galactose-4-sulfate was identified by IR spectrum. ^{1}H- and ^{13}C-NMR spectra were in agreement with those of standard κ-carrageenan. Specific rotation (0.2% in water) was -1.0°. A very weak and transparent gelation of the polysaccharide occurred at 0.2% concentration with addition of 13.5 mM KCl. We concluded that the polysaccharide isolated from *G. coronopifolia* was a κ-carrageenan-like agarose.

This work is the first to report on the agarose involving sulfate groups at C-4 of β-D-galactose residues.

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SITE MAPPING AND MUCIN-TYPE *O*-GLYCAN PROFILING OF CD43 (LEUKOSIALIN / SIALOPHORIN) BY ELECTRON TRANSFER DISSOCIATION MASS SPECTROMETRY

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Many of the mammalian clusters of differentiation (CD) antigens, which play crucial roles in immunological processes, carry mucin-type *O*-glycans (MTOG)[1]. MTOG imparts rigidity and large hydrodynamic volume to the polypeptide backbone and enables moderate reversible *cis*-interactions with neighbouring cell surface molecules. CD43 (leukosialin / sialophorin) is considered to be a negative regulator of immune activation. CD43 possesses an extended rod-like structure extending to 50 nm and is estimated to carry 80-90 MTOG on its polypeptide backbone[2]. Extracellular domain of CD43 consists of 234 amino acids including 93 (46 Ser and 47 Thr) potential MTOG sites. Studies on the MTOG structure of CD43 have been hampered due to its complexity and a vast number of theoretical proteoforms. Earlier studies by Schmid and co-workers on galactoglycoprotein (Galgp, the secreted form of CD43) had identified 25 occupied Ser/Thr sites using Edman degradation[3].

Herein, we present results of our investigations on comprehensive site mapping and enumeration of diverse glycopeptides from CD43 using the high-energy C-trap dissociation – product dependent – electron transfer dissociation (HCD-PD-ETD) methodology. Soluble CD43-Fc-His recombinant protein was purified from lentivirally transduced Jurkat cells cultured in chemically defined medium and digested using trypsin and Glu-C. The glycopeptides were resolved and analyzed by nano-LC-ESI-MS/MS. The data were analyzed using Proteome Discoverer and Byonic softwares and validated by manual verification. Our analysis revealed that on an average 71 sites were found to be occupied with HexNAc, of which 18 sites were in agreement with the previous report; of the seven sites reported to be occupied in Galgp we found two sites to be not occupied in CD43-Fc-His and five sites were not detectable due to precursor ion mass range limitations. The diversity of site occupancy is exemplified by the analysis of a fragment glycopeptide ^{44}MYTTSITSDPK^{55} from CD43, which has five potential sites. Combinatorially, given the addition of a HexNAc only, there are 32 (2^5) glycoforms possible.

Our data showed mass spectral evidence for the existence of 17 out of 32 glycoforms with the majority of glycopeptides carrying three or higher occupied sites. Additionally, analysis of CD43-Fc-His, from Jurkat cells incubated with the Ac_5GalNTGc, an efficient inhibitor of MTOG [4], provided evidence for metabolic incorporation, reduction in site-occupancy, and poor elaboration. Deciphering the MTOG diversity might provide insights in to functional roles of CD43 glycosylation in immunological processes including autoimmune diseases.

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EXTRACTION, CHARACTERIZATION AND ANTI-CANCER ACTIVITY OF PECTIC OLIGOSACCHARIDES PRODUCED FROM AGRO-WASTE OF ORANGE (*CITRUS RETICULATE*)

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Pectin is a complex polysaccharide present in primary plant cell walls. Pectin is used as gelling agent or stabilizer in jams, jellies and other food products [1]. Pectin is also used in healthcare and pharmaceutical industries [2]. Citrus waste is a potential source for extraction of pectin. In this study, pectin from the agro-waste, the fruit peel of Orange (*Citrus reticulate*) was extracted by Ultrasound Assisted Extraction (UAE) process [3]. UAE treated and untreated fruit peel samples were analysed by FESEM which that the fruit peel powder was more porous as compared to untreated fruit peel powder. This treatment was efficient for the pectin extraction.

The extracted pectin from orange was characterised by FTIR, HPSEC, DSC-TGA, XRD and NMR analyses. FTIR and NMR analyses of extracted pectin showed esterified galacturonic acid residues. TGA showed thermal degradation at 225°C. XRD analysis revealed its semi crystalline nature. HPSEC analysis established the molecular weight as 90 kDa. Pection has a potential role in prevention of carcinogenesis. Anti-cancer activity of orange pectic oligossacharides was analysed. The pectic oligossacharides were produced by a pectate lyase (*Ct*PL1B) from *Clostridium thermocellum* [4]. HPLC analysis showed the action of PL1B on extratced orange pectin produced majorly DP2 oligosaccharide as compared to DP1 and DP3. The viablity of colon cancer (HT29) cells after treatment with 1 mg/ml of orange pectin oligossacharides for 24 h reduced by 18.4% and for 48 h by 38.5%. The microscopic observation of treated HT29 cells revealed the reduced connection between the cells and change in cell morphology from undifferentiated to globular shape.

Pectin is involved in induction of apoptosis and inhibition of galectin-3 receptor [5]. The effect of 1 mg/ml pectin oligossacharides on normal human embryonic kidney cell (HEK293) cells showed no effect on the cell viability even after 48 h. The microscopic observation of treated HEK293 cells showed no change in the cell morphology as compared with the untreated cells.

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MUSHROOM β-GLUCAN MODIFIED GOLD NANORODS FOR PHOTOTHERMAL CANCER THERAPY

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Naturally-occurring β-glucans have been widely regarded as a natural source for functional foods and pharmaceuticals due to their immunomodulatory property and antitumor activity.[1-2] However, physico-chemically stable and the biocompatible β-glucans are rarely explored as a carrier for nanomaterials to overcome the problems of aggregation and nanotoxicity.

Plasmonic gold nanorods (AuNRs) of controllable localized surface plasmon resonance (LSPR) have attracted much attention for the applications in cancer photothermal therapy (PTT) by converting absorbed light into heat during plasmon relaxation process.[3] However, the clinical application of AuNRs have been limited due to the cytotoxicity caused by the residual positively charged cetyltrimethylammonium bromide (CTAB), which is the essential structural template and surface ligand for AuNRs.[4] Therefor, the surface modifications of AuNRs with nature polymeric materials such as mushroom β-glucans is envisioned to be a safe and low-cost strategy to design biocompatible and efficient PTT agents for cancer therapy.

In this project, we have developed a highly stable and biocompatible mushroom β-glucan coated gold nanorods (AuNR-Glu) by integrating *Pleurotus tuber-regium* sclerotial β-glucan (Glu) and plasmonic gold nanorods (AuNRs) possessing photothermal property in the second near-infrared (NIR-II) window for cancer photothermal therapy. Glu coating remarkably enhanced the colloidal stability in various biological media compared to the native AuNRs.[5] Moreover, AuNR-Glu had low cytotoxicity and high photothermal stability which are excellent characteristics as photothermal agent for cancer therapy. *In vitro* experiments showed that AuNR-Glu nanohybrid was effective against MCF-7, SW480 and HT-29 at a low dose of 20 μg/mL under NIR-II at a safe laser power density (0.75 W/cm^2).[5,6] *In vivo* study further showed that tumors were effectively destructed after an intravenous injection of AuNR-Glu followed by a safe NIR-II laser irradiation (1 W/cm^2 for 5 min). Furthermore, no obvious harmful side effect of AuNR-Glu was observed by histological analysis of major organs in treated mice, suggesting a promising application of AuNR-Glu in photothermal cancer therapy.[6] We envisage that such β-glucan-based coating method will provide new opportunities to design biocompatible functional nanomaterials for wider clinical applications.

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LIVE CELL IMAGING BY NANO-WRAPPING SYSTEM

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Live cell imaging technology to observe cellular processes within a cell is possible since the development of fluorescent probes and optical microscopy. However, floating cells, such as immune cells, are non-adherent, and these cells are difficult to visualize their immune response. Therefore, we developed the nano-wrapping technique to hold floating cells using macromolecular ultrathin membranes (nano sheet [1,2]) in the observation field of live-cell imaging. Nano-sheets have thickness of <100 nm to use polylactic acid raw material, and are highly adhesive, flexible, and transparent. At the beginning, we wrapped the suspension of THP-1 monocyte with nano sheet on the cover glass. And then, we dipped this sample in the medium, and observed under the addition of droplet. Consequently, we were able to observe this floating cells without dispersion. Next, we tried to perform live-cell imaging of the calcium response by stimulating the floating cells with histamine. We used YC3.60 transfected CHO-K1 cells and measured the Forster resonance energy transfer (FRET) signaling of the calcium response. In this study, we trypsinized these cells, and wrapped with nano sheet. As a result, we were able to visualize the calcium response of the floating cells with nano-wrapping. We are going to visualize the internalization of an antibodies conjugated glycan using this nano-wrapping technology.

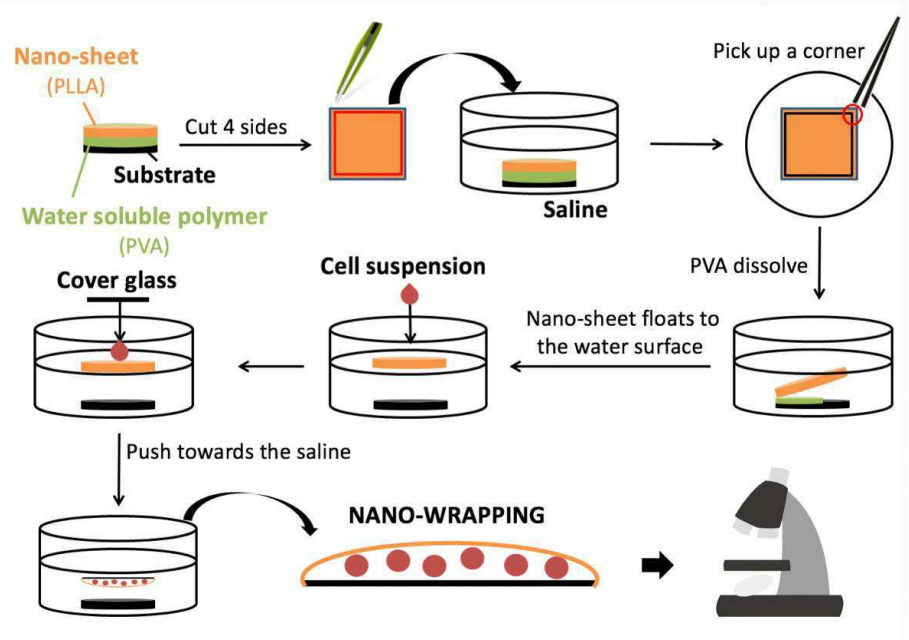

Fig.1. Schematic drawing of nano-wrapping system.

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CELLULASE TREATMENT FOR IMPROVING CHITIN SOLUBILITY IN ALKALI

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The processing of insoluble in many traditional solvents chitin and cellulose is complicated due to their high-ordered structure. During the enzymatic decomposition of these polysaccharides, the enzymes are capable of splitting their crystalline structures. Both catalytic and carbohydrate-binding domains of glycosyl hydrolases may participate in this process [1]. Cellulases can improve the solubility of cellulose in alkali [2]. However, no such studies were carried out with chitin. Except chitinases, chitin undergoes non-specific hydrolysis by different hydrolases, including proteases, lipases, and cellulases. Cellulases are more studied and more commercially available enzymes used in biofuel industry and plant waste utilization. In this work, the effect of different cellulases on chitin crystallinity and solubility in alkali was evaluated.

The treatment of chitin sample (molecular weight 2.7×10^5) was carried out using two commercial cellulases, Cellic (cellulases mixture) and Fiber Care D (endoglucanase), purchased from Novozymes. Enzymes were used in relatively high dosages of 100-200 mg/g to ensure the effect. Enzymatic activities toward soluble carboxymethyl cellulose and chitosan were examined using standard methods of reducing sugars release. Soluble substrates were used to evaluate the endo-enzymes. Crystallinity indices were determined by X-ray diffraction. The solubility of chitin samples in 8% NaOH/4% urea/water solution was compared by weighing the insoluble residue after filtration. The chitin solutions were analyzed using dynamic light scattering.

The dissolution of chitin in the chosen solvent was not complete allowing the evaluation of the enzymatic treatment effect on solubility. The insoluble residue reduced by 10-15 % compared to untreated chitin. The corresponding decrease in crystallinity indices was 3-5 % depending on enzyme type. Despite the smaller monosaccharide release, the treatment with endoglucanase resulted in more significant crystallinity decrease; this action may be related to disruption of hydrogen bonds by carbohydrate-binding domain [3]. Dynamic light scattering of chitin solutions revealed the presence of various aggregates with hydrodynamic radii of approximately 100-150 nm as it was previously reported [4]; the presence of aggregates significantly complicated the estimation of the molecular weight of the dissolved chitin. This study confirmed the possibility of using cellulases in chitin processing; the increased solubility of chitin may be useful in fiber production for biomedical applications.

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GLYCOSYNTHASE TECHNOLOGY ON GH18 CHITINASES: ENGINEERING *Sp*ChiD FOR THE PRODUCTION OF SEQUENCE-DEFINED CHITIN AND CHITOSAN POLYMERS

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Chitosan and chitooligosaccharides (COS), obtained by chitin-modifying enzymes, play remarkable roles in nature. Most of these biological activities are not only related to their degree of polymerization and acetylation, but they also seem dependent on their pattern of acetylation.

Chitinases (EC 3.2.1.14) are glycoside hydrolases (GH) that catalyse the hydrolysis of β□□□ glycosydic bonds of chitin and chitosan polymers. Some chitinases have also transglycosylase activity (TG), allowing them to introduce new glycosidic bonds between donor and acceptor sugar molecules with the consequent generation of oligomeric COS.

We are aiming at the development of a biotechnological platform for the production of sequence-defined low molecular weight chitosans. The project addresses the use of chitinases as a synthetic tool to obtain well-defined oligomers with repeating patterns of acetylation. Such transglycosylating chitinases can be used for the *in vitro* polymerisation of COS, but the hydrolytic activity of these enzymes tends to depolymerise the TG products quickly. Our goal is to develop a glycosynthase using protein engineering to eliminate their hydrolytic activity while concomitantly increasing their transglycosylating activity. Most of the known chitinases belong to family GH18 which act by substrate-assisted catalysis. Since no enzyme nucleophile is present, the strategy used to engineer them as glycosynthases consists in the mutation of assisting residues in the active site and the use of an activated oxazoline donor substrate.

We report here the application of the glycosynthase technology to *Serratia proteamaculans* chitinase D (*Sp*ChiD), a single-domain chitinase from the GH18 family. *Sp*ChiD is an endo-active processive enzyme exhibiting TG activity with COS substrates, depending on both the length and concentration of substrate, and on the enzyme concentration[1,2]. *Sp*ChiD catalytic domain consists of a TIM barrel fold with a highly conserved DXDXE motif, where resides the catalytic triad essential for enzyme activity. Novel *Sp*ChiD mutants have been developed to polymerize activated glycosyl donors to give low molecular weight polymers with defined structures.

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FUELLING THE FUTURE; HYDROLYTIC AND OXIDATIVE ENZYMES FROM *TEREDINIBACTER TURNERAE*

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Industrial lignocellulosic biomass degradation is paving the way towards greener and more sustainable fuels and enzymes are the key. Glycoside hydrolases (GH), boosted with lytic polysaccharide monooxygenases (LPMO) are nature's way of accessing the high value molecules held captive within recalcitrant polysaccharides. This work has looked at a variety of GHs (GH8, GH12, GH5s) and the single LPMO (an AA10) from the Shipworm symbiont *Teredinibacter turnerae*.

A Shipworm (a type of bivalve mollusc) diet consists mostly of woody material, and as such the enzymes produced by *Teredinibacter turnerae* are thought to aid digestion of the lignocellulosic material. Organisms displaying such aptness towards breakdown of plant matter are excellent places to mine for novel hydrolytic or oxidative enzymes, to either improve scientific understanding of enzymatic modes of action on various substrates (an important task in the emerging field of LPMO research) and/or identify new candidates for inclusion in industrial processes.

The enzymes in this study have been analysed for their substrate specificity and activity through a range of biochemical techniques and structures determined using X-ray crystallography.

A NOVEL ULVAN LYASE FAMILY WITH BROAD-SPECTRUM ACTIVITY FROM THE ULVAN UTILISATION LOCI OF *FORMOSA AGARIPHILA* KMM 3901

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The rapid proliferation of members of green macroalgae in eutrophicated waters causes algal blooms. The biomass of these blooms, which is rich in polysaccharides, is underutilised and majorly left to decompose. Ulvan is one of such polysaccharides which constitutes up to 30 % of dry weight in *Ulva* species [1]. Ulvan and its oligosaccharides have potential applications in food and medicine. Ulvan lyases depolymerise ulvan *via* the β-elimination mechanism with the release of oligosaccharides that have unsaturated 4-deoxy-L-threo-hex-4-enopyranosiduronic acid at the non-reducing end. Ulvan lyases belong to the PL24, PL25 or PL28 family in the CAZy database [2-5]. In this study, we identified and biochemically characterised a periplasmic novel broad-spectrum ulvan lyase from *Formosa agariphila* KMM 3901. The lyase was heterologously overexpressed in *Escherichia coli*, and purified using a two-step purification. The purified recombinant enzyme depolymerised ulvan in an endolytic manner with a K_m of 0.77 mg/ml. The enzyme was optimally active at 40 °C and pH 8.

This lyase also degraded heparan sulphate and chondroitin sulphate. Detailed analyses of the end-products of the enzymatic endolytic depolymerisation of ulvan using ^{1}H- and ^{13}C-NMR and LC-MS revealed an unsaturated disaccharide (ΔRha3S) and a tetrasaccharide (ΔRha3S-Xyl-Rha3S) as the principal end-products. This ulvan lyase is a member of the polysaccharide utilisation loci for ulvan.

In contrast to the previously described ulvan lyases, this novel lyase is mostly composed of α-helices that form an $(\alpha/\alpha)_6$ incomplete toroid domain and displays a remarkably broad-spectrum activity. This novel lyase is the first member of a new family, which sequence is not homologous to other ulvan lyases.

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TAILORING FRUCTANS AND FOS BY THE LEVANSUCRASE LEVS FROM *Leuconostoc mesenteroides*

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Fructans are oligo- and polysaccharides composed of fructose units linked by glycosidic bonds. These polysaccharides have properties as sweeteners, probiotic stimulators, emulsifiers and drug nano-carriers. Fructosyltransferases (FTFs) synthesize fructans using sucrose as substrate in reactions in which this disaccharide is both the donor of the fructosyl moiety and the acceptor molecule, leading to products such as fructose polymers or fructooligosaccharides (FOS). These enzymes are classified depending on their linkage specificity as levansucrases (E.C. 2.4.1.10) when the resulting polymer (levan) is linked through β-2,6 linkages or inulosucrases (E.C. 2.4.1.9) when the resulting polymer (inulin) is linked through β-2,1 linkages. Levansucrase LevS from *Leuconostoc mesenteroides* B-512F is a multidomain enzyme able to produce a highly-branched levan polymer. Structural architecture of LevS shows three main domains: The N-terminal domain is implicated in the processivity and regioselectivity of the enzyme; the C-terminal domain containing three-fold repeat sequences and a "transition region" enhances the transferase activity, however this is not essential for the catalysis; meanwhile, the catalytic domain contains the active site responsible of catalysis producing mainly inulin FOS (Fig. 1a)(1). In this study, we apply our discovery concerning the structural and functional relationship of this enzyme in order to generate new biocatalysts to produce tailored-made fructans. Shorter versions of the enzyme deleting N-terminal domain (LevSΔN) and both N- and C-terminal domains as well as the transition region (LevS/CAT) were created (Fig. 1a). Product profile analyses show that LevS synthesizes levan polymer and FOS by a processive mechanism. However, the deletion of the N-terminal domain results in an enzyme catalyzing the synthesis of a low molecular weight (LMW) inulin polymer through a non-processive mechanism. Finally, the enzyme containing only the catalytic domain LevS/CAT synthesizes a LMW polymer of inulin by a non-processive mechanism. (Fig. 1b) With these results we demonstrate the possibility of guide the transfructosylation toward the synthesis of tailor-made fructans and FOS with different type linkage, without compromising the transfructosylation efficiency of LevS.

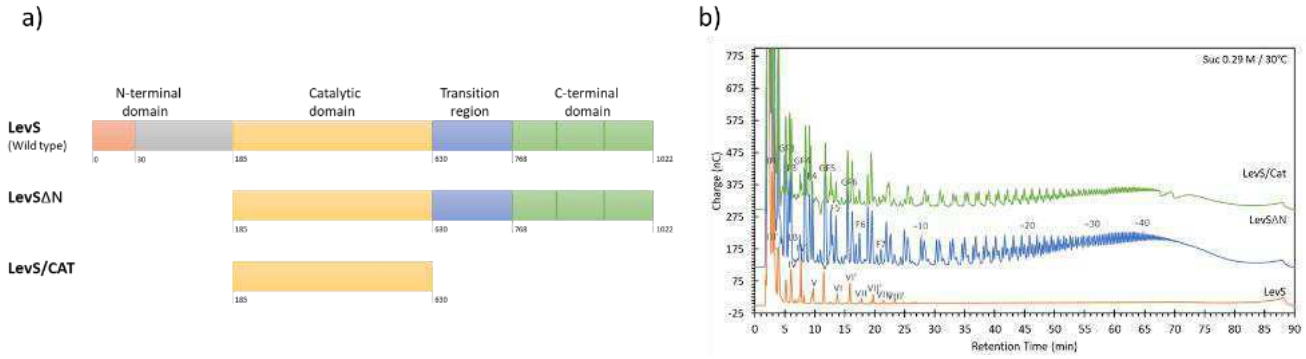

Fig. 1. Product profile of LevS and its truncated versions. a) Schematic representation of LevS and truncated versions, b) FOS profile obtained from sucrose by LevS and its truncated versions.

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CHITOSAN MICROPARTICLES AS ORAL DELIVERY SYSTEM OF RELAXING PEPTIDES

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Bioactive peptides can prevent or control diseases and exert a variety of activities including relaxing function. Microencapsulation of bioactive peptides may constitute a valuable process for preserving their integrity during processing and digestion. Chitosan is a prominent natural biomaterial that is used in delivery systems. In the present study, chitosan microparticles loaded with a relaxing peptide (peptide sequence YLGYLEQLLR) were prepared by ionic gelation, with a mean size of 1.8 μm.

This delivery system was characterized in terms of particle size analysis, Fourier Transform Infrared, peptide encapsulation efficiency and release, mucoadhesion, and bioavailability and cytotoxicity through in vitro assays. The results demonstrated that ca. 86% of the peptide was loaded into the chitosan microparticles. The microparticles showed to be biocompatible and allowed sustained release along 30 min.

This system may be a potential candidate as oral delivery system to carry relaxing peptides or other bioactive peptides.

CHITOSAN MICROPARTICLES ORAL DELIVERY SYSTEM OF RELAXING PEPTIDES

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Bioactive peptides can prevent or control diseases and exert a variety of activities including relaxing function. Microencapsulation of bioactive peptides may constitute a valuable process for preserving their integrity during processing and digestion [1, 2]. Chitosan is a prominent natural biomaterial that is used in delivery systems [3-6]. In the present study, chitosan microparticles loaded with a relaxing peptide were prepared by ionic gelation, with a mean size of 1.813±0.802 µm, zeta potential 43.49±9.09 mV and polydispersion index 0.89.This delivery system was characterized in terms of particle size analysis, Fourier Transform Infrared, peptide encapsulation efficiency, bioavailability and cytotoxicity through *in vitro* assays. The results demonstrated that ca. 86% of the peptide was loaded into the chitosan microparticles. The microparticles showed to be biocompatible. Cell viability was measured by MTT assay and showed an absence of cell toxicity following exposure into formulation contact. Peptide-loaded chitosan microparticles and peptide-loaded chitosan microparticles into guar-gum films showed a faster and distinct permeability than a free peptide. So, peptide-loaded chitosan microparticles and peptide-loaded chitosan microparticles into guar-gum films might be a promising carrier for relaxing peptide buccal administration.

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Evaluation of the potential prebiotic activity of novel polysaccharide rich extracts from the brown seaweed *Ascophyllum nodosum*

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Polysaccharides (PS) form marine sources such as seaweeds have been recognized as a main source of dietary fiber with prebiotic activity [1]. Previous investigations in our group identified and isolated bioactive PS fractions (fucoidans and alginates) extracted under a non-aggressive (no chemical), sustainable and reproducible extraction method, from the brown seaweed *Ascophyllum nodosum*, that had anti-microbial effects and *in vitro* prebiotic potential. In this study, 44 algal extracts were selected to be characterized chemically. Dry weight and ash content was determined by drying samples for 24 h at 65ºC and 2 h at 550ºC, respectively. Total soluble phenols were measured (Folin-Denis method), reducing sugars by the method of Miller et al (1959), total carbohydrates by the DuBois method, total lipids (gravimetrically); free D-mannitol and L-fucose were quantified using Megazyme kits. The *in vitro* prebiotic effects of 18 extracts have been tested vs. *Lactobacillus gallinarum* and *Escherichia coli*. Each algal extract was supplemented as carbon source at 0.3% and 0.1% (w/v) in a sterile 96-well microplate (at 37°C for 24-48 h). The media with no bacteria was used as negative control and glucose and FOS (recognized prebiotics) as positive controls.

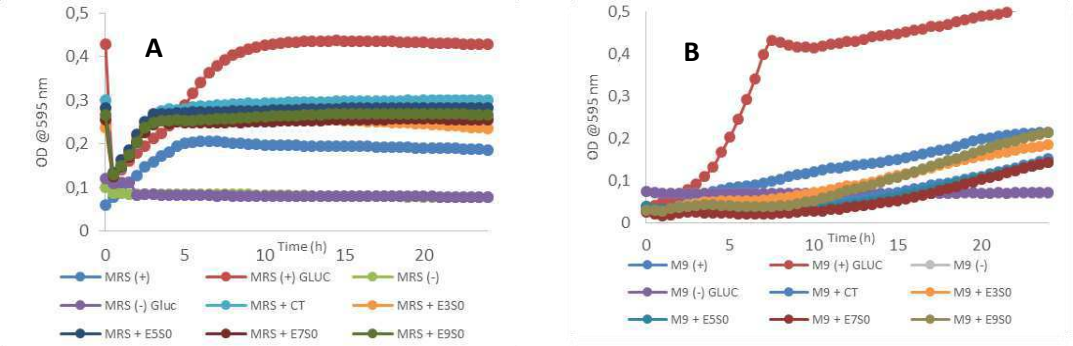

Figure 1: A) Growth of *Lactobacillus gallinarum* for 24 h (STDEV<0.05) using a preparation containing purified alginates and fucoidans (CT) and raw extracts (E3S0, E5S0, E7S0 and E9S0) obtained under different extraction conditions (T (ºC), t(h), E:S) at 0.3% (w/v) per well. **B)** Growth of *Escherichia coli* for 24 h (STDEV<0.05) using a preparation enriched in alginates and fucoidans (CT) and raw extracts (E3S0, E5S0, E7S0 and E9S0) obtained under different extraction conditions (T (ºC), t(h), E:S), at 0.3% (w/v) per well.

Some of the purified fucoidan and alginate fractions (CT) appear to be promising prebiotic candidates as they promoted growth of *Lactobacillus gallinarum*, yet at the same time inhibited *E. coli*, under the conditions tested. Of particular interest was the growth promoting activity of the raw extracts (no purification step); some raw extracts had the best prebiotic potential, which may be of interest when large scale production is targeted. Other bacteria present in the poultry microbiome will be tested and further studies on the structure of these PS are being conducted in order to establish a link between structure and function that explains the mechanism of their antimicrobial and prebiotic bioactivities still remains the main gap for the use of these PS in high value applications [2].

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POSTERS | LBV – Carbohydrates in Lignocellulosic Biomass Valorisation

DEVELOPMENT OF ENDO-GLYCOSIDASE ACTIVITY BASED PROBES

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Cyclophellitol, cyclophellitol aziridine and configurational derivatives thereof are selective and potent, covalent mechanism based inhibitors of exo-acting glycosylhydrolases (Fig A). Activity Based Probes (ABPs) based on these inhibitors have contributed tremendously to our understanding of exo-acting enzyme activity in health and disease.[1,2] The construction of cyclophellitol containing carbohydrate oligomers allows the study of endo-acting glycosidases.

Four classes of cyclophellitol oligomers are being synthesized our laboratory (Fig B). These novel probes are designed to target specific *endo*-glycosidases that monomeric cyclophellitol derivatives have not been able to inhibit. Examples include endo-xylanases and glucanases relevant for biomass valorization, pancreatic amylase important for the digestion of starch in humans and endo mannosidases that act on mannosides present in the fungal cell wall.

In this communication we will address the challenges associated with the design and synthesis of these probes and their biochemical activity.

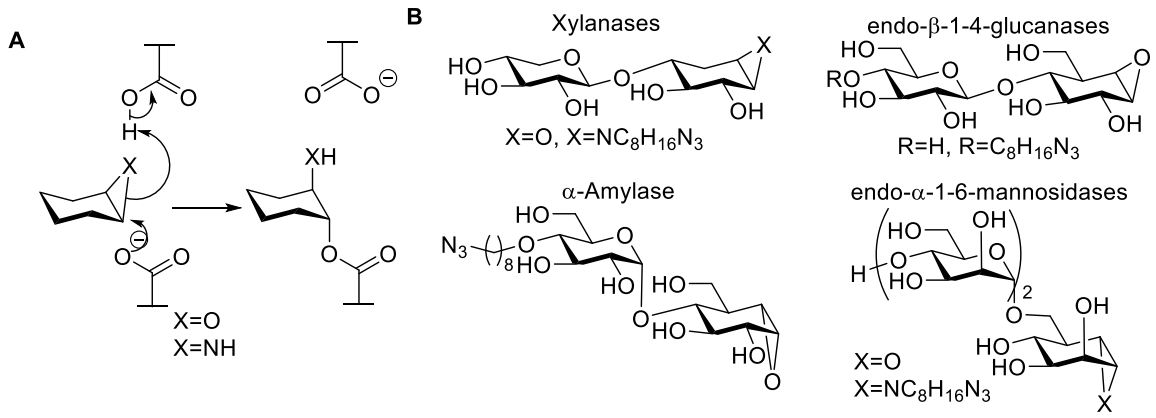

Fig.1 - A: Mechanism of epoxide and aziridine based covalent glycosidase inhibitors. **B**: Novel covalent inhibitors and activity based probes designed to inhibit a variety of endo acting glycosidases.

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ENHANCEMENT OF BIOLOGICAL ACTIVITY OF ARABINOXYLAN FROM WHEAT CHAFF BY ENZYMATIC MODIFICATION

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Nowadays, many carbohydrates from varios sources are used as biologically active molecules in human health or as functional molecules in processing industries. Differences in physiochemical properties of arabinoxylan (AX) diversify its utilization, which can be related to application of various modification procedures. Enzymatic hydrolysis is an efficient environmental-friendly method for modifying the structure and properties of AXs.

Wheat chaff as agro-industrial waste material with more than 30% of carbohydrates in arabinoxylan form could be a source of this heterogeneous sugar. Cereal arabinoxylan structure is composed of xylan chain with L-arabinofuranose as the main substituent sugars while phenolic acids, particularly ferulic, could be bound at position 5 to some of their arabinose residues. [1, 2] Endo-β-1,4-xylanase is a key enzyme in the degradation of xylan while feruloyl esterase is hemicellulose accessory enzymes that does not release sugars directly; however, this enzyme cleaves ester linkages between phenolic acids and sugar residues making carbohydrate molecule more available for other enzymes to bind and degrade it. [3]

The aim of this study was to produce different arabinoxylan fractions using endo-xylanase and feruloyl esterase. Preparation of arabinoxylan from wheat chaff was carried out under mild alkaline conditions (1.5 M NaOH, 1h, 80 ◦C). Action of two enzymes was performed individually and synergistically. Molecular weight of samples was analyzed by GPC-HPLC. ABTS assay was used to evaluate the antioxidative activity of hydrolysates.

Produced hydrolysates resulted in different molecular weight fractions of arabinoxylan with enhanced antioxidant activity. Arabinoxylan modification by both enzymes individual action led to increase in antioxidant capacities while the most prominent enhancement was achieved by their synergistic action. Obtained results could be a solid base for the exploitation of wheat chaff using environmental-friendly technology to produce a variety of valuable arabinoxylans from agro-industrial waste.

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ENGINEERING A NOVEL GH1 β-GLUCOSYNTHASE

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The capacity of glycosyl hydrolases (GHs) for catalyzing transglycosylation reactions makes them great biotechnological tools for the modification of several molecules of interest, through the addition of one or more units of carbohydrates to different compounds. There are many beneficial effects reported on the glycosides obtained by transglycosylation, such as increased solubility, biosafety or stability in comparison to the original compound.

The fungus *Talaromyces amestolkiae* has been recently postulated as a great GHs producer, deserving a special interest because of the secretion of high efficient β-glucosidases (BGL)[1,2]. Initially the study of these biocatalysts was focused on hydrolytic applications but some of them are able to carry out transglycosylation. In the genome of this fungus different BGLs[2] have been reported, mainly belonging to GH3 family, although it contains also a BGL from GH1 family. This enzyme, named BGL-1, has been recently heterologously expressed in *Pichia pastoris*, reaching high activity levels (80 U/mL). The purified enzyme is active against different *p*-nitrophenyl (*p*NP) glycosides, showing an interesting versatility. In addition, its gluco-tolerance is remarkable (40% activity at 3M glucose).

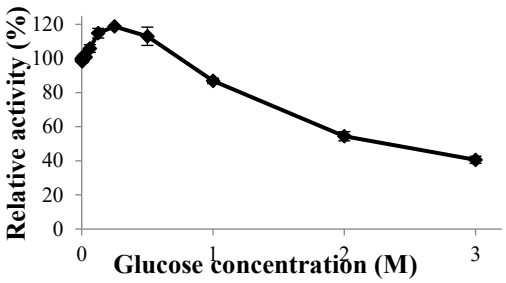
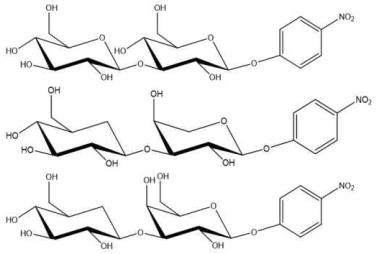


Fig. 1 - Effect of glucose in BGL-1 activity



Glycosides produced by BGL-1 synthase

In order to expand the biotechnological application of BGL-1, it was converted into a glucosynthase, glycosidases in which the catalytic nucleophile residue has been replaced. These mutants lack of hydrolytic activity and catalyze the formation of glycosidic bonds using glycosyl fluoride donors [3]. Preliminary experiments have shown an enhanced ability in the glucosylation of different *p*NP glycosides and several antioxidant plant phenols.

Future studies are expected to deepen in the ability of BGL-1 as an interesting tool for the synthesis of glycosides with biotechnological interest.

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CULTIVATION OF *Moesziomyces aphidis* USING AGRO-INDUSTRIAL RESIDUES FOR THE PRODUCTION OF HEMICELLULASES AND THEIR USE IN BOOSTING ENZYMATIC HYDROLYSIS OF THE SAME LIGNOCELLULOSIC FEEDSTOCK

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Agro-industrial residues are presently underused and attractive renewable resources. Recently, there has been a great interest in its use as low-cost raw materials for the production of value-added compounds as microbial enzymes. Hemicelulases have been gaining increasing attention due to its wide biotechnological applications, especially in the biomass-to-bioethanol context. The aim of this study was the optimization of xylanase production by *M. aphidis* using barley straw (BS) and sweet corn cobs (SCC) previously pretreated through mild alkaline extrusion. Highest xylanase activity was achieved after 10-days cultivation, corresponding to 191 ± 20 and 275 ± 13 IU/mL in BS and SCC, respectively. The presence of salts (from substrates, pretreatment and neutralization) allowed minimal supplementation of the culture media (yeast extract 1 g/L), with negligible influence in the level of xylanase production.

The performance of the hemicellulases crude extract was assessed, in supplementation of Cellic® CTec2, on the hydrolysis of the same substrates. This supplementation proved to increase the yield of hydrolysis of xylan into xylose in BS and SCC, under high solid loadings.

This study was successful in efficiently producing hemicellulases using largely available and inexpensive agro-industrial residues as substrate, contributing to the reduction of enzymatic hydrolysis cost.

Acknowledgements: This work has been co-funded by the European Commission (Horizon 2020 Program under Grant agreement reference no. 654362 (BABET-REAL5 Project).The authors acknowledge the BABET-REAL5 partners CIEMAT and INPT for providing pretreated samples of BS and SCC.

QUANTITATIVE CHARACTERIZATION OF CARBOHYDRATES IN THE LIGNOCELLULOSIC BIOMASS OF THE PERENNIAL PLANTS SIDA AND SILPHIUM.

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Perennial plants Sida and Silphium are grown in tropical and subtropical regions worldwide and presently adopted to grow in Europe[1]. Sida and Silphium plants produce 9 to 20 t biomass per hectare annually,[2] and grow on marginal soils, which cannot be used for efficient agricultural food and feed production. To develop the biorefinery concept for the biomass of these perennial plants and to produce valuable carbohydrates from these materials, the quantitative composition and structure of their lignocellulosic materials is required. The polysaccharide and lignin composition as well as lignin linkages were determined using different analytical techniques such as HPAEC-PAD, GC-MS[3] and 2D HSQC NMR spectroscopy[4] methods. Carbohydrates such as Glucose, Xylose and Arabinose are the most abundant carbohydrates present in Sida and Silphium biomass. Sida and Silphium lignocellulosic biomasses were also pretreated using the Organocat technology,[5] and the Organocat product streams such as pulp, lignin and aqueous phases were analyzed. The Organcat process separated most of the hemicellulose sugars separating them from lignin present in the organic phase. The undissolved pulp contains low amounts of hemicellulose sugars compared to raw biomass.

Lignin composition and lignin linkages were quantified in the raw materials, pulp and extracted lignin fractions using 2D HSQC NMR spectroscopy. Linkage information will be used to further optimize the pretreatment process of perennial biomass in order to separate carbohydrates and lignin efficiently.

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THE ROLE OF CARBOHYDRATES TO ENHANCE BIOGAS PRODUCTION

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Duckweed (*Lemnaceae* sub-family) is a small flowering plant that has a global adaptability across a broad range of climates and can be easily found in quiescent or slowly flowing and polluted water bodies worldwide. This aquatic plant has a longer yearly production period than most of the other plants, and even grows year-round in some warm climate areas. Over the last few decades duckweed has been used in constructed wetlands, replicating natural ecosystems, to treat wastewaters [1]. The benefits of this treatment system comprise low energy input, high rate of nutrient uptake by aquatic plants and high biomass yield. Under stress conditions duckweed can respond by accumulating carbohydrates what can lead to their potential use as feed or as an alternative feedstock for biofuel production, such as biogas [2,3].

The goal of this study was to evaluate the accumulation of carbohydrates by *Lemna minor* in a comparative experiment under different controlled conditions: ideal medium (5 mM KNO_3; 2.5 mM KCl; 2mM KH_2PO_4; 1mM $MgSO_4$.$7H_2O$; 364 µM $CaCl_2.2H_2O$; NaFe EDTA 19 µM), pH 5.8, 16h of photoperiod, photon flux density of 50 µmol/m²s and temperature range between 18ºC-30ºC.

Duckweed growth was evaluated during 21 days, by counting the number of fronds. At the end of the assay, to measure duckweed fresh weight (FW), the surplus water was removed and subsequently the biomass was weighted. The protein content was analysed by the Bradford method, total carbohydrates were analysed using the sulfuric acid digestion, according to [4] and starch determination was carried out using an enzyme mixture of α-amylase and amyloglucosidase to digested starch in plant tissue samples [5].

This first approach shows that temperature and long photoperiod favored the accumulation of carbohydrates in the plant. Results suggested the potential of *Lemna* as feedstock for renewable energy operations.

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A NOVEL STARCH BINDING LACCASE FROM THE WHEAT PATHOGEN *ZYMOSEPTORIA TRITICI* HIGHLIGHTS THE FUNCTIONAL DIVERSITY OF ASCOMYCETE LACCASES

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Laccases are multi-copper oxidases, which are assigned into auxiliary activity family 1 subfamily 3 (AA1_3) in the CAZy database[1]. These enzymes, which catalyze the oxidation of phenolic and nonphenolic substrates coupled to reduction of O_2 to H_2O[2], are increasingly attractive as green oxidation biocatalysts. Basidiomycota laccases are well characterized due to their potential in de-lignification of lignocellulose. By contrast, the insight into the biochemical diversity of Ascomycota counterparts from saprophytes and plant pathogens is scarce.

Here, we report the properties of the laccase *Ztr*Lac1A, from the major wheat pathogen *Zymoseptoria tritici* [3], distinguished from common plant fungal pathogens by an apoplastic infection strategy. Uniquely, we demonstrate that *Ztr*Lac1A is appended to a functional starch-binding module and displays an atypical activity signature disfavoring less polar phenolic redox mediators as compared to biochemically characterized laccases[4][5]. By contrast, the redox potential of the enzyme, determined to 370 mV vs. $Ag|AgCl_{sat}$, is similar to ascomycetes counterparts. The atypical specificity is consistent with distinctive sequence substitutions and insertions in loops flanking the T1 site and the C-terminus of the enzyme compared to characterized laccases.

*Ztr*Lac1A, which is the first modular laccase appended to a starch-specific CBM20 to be described, defines a distinct clade populated predominantly by plant pathogens in the phylogentic tree of AA1_3 enzymes. The possible role of these enzymes in vivo in relation to targeting chloroplatic starch is discussed. These findings expand our toolbox of laccases for green oxidation and highlight the binding functionality of CBM-appended laccases as versatile immobilization tags.

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LGINATE BASED BLOCK POLYSACCHARIDES

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Materials based on polysaccharides represent a new class of materials that potentially could replace or supplement synthetic polymers. When polysaccharides are coupled together terminally rather than laterally, the intrinsic physiochemical properties of the modules are retained. Hence, properties such as sensitivity to pH, ionic strength, temperature, and reaction with divalent cations in the case of alginate, could be utilized to introduce switches to control chain-chain interactions. By exploiting such properties, block copolymers could be used to prepare bottom-up synthesized self-assembling structures [1].

For preparation of terminally conjugated polysaccharides, the first step was modifying the reducing end of a series of biopolymers (alginate, chitosan, chitin and dextran) with bi- and trifunctional hydrazides (NH_2-NH-CO-) and oxyamines (NH_2-O-). We used reductive amination with 2-picoline borane [2], and the reaction was monitored using ion exchange chromatography (HPAEC-PAD). Diblock conjugates of alginate have also been prepared as a proof of principle. Furthermore, alginate oligomers were coupled to an aminooxy-functionalized water-soluble PEG by the same method. The conjugates will be used for the preparation of nanoparticles, using the specific reaction of the alginate with certain divalent cations such as Ca^{2+}, Ba^{2+} and Sr^{2+}.

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WELL-DEFINED OLIGO AND POLYSACCHARIDES AS IDEAL PROBES FOR STRUCTURAL STUDIES

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Polysaccharides are the most abundant organic materials in nature, yet correlations between their three-dimensional structure and macroscopic properties have not been established [1]. Automated glycan assembly (AGA) enables the preparation of well-defined oligo- and polysaccharides resembling natural as well as unnatural structures [2]. A collection of related compounds, modified at specific positions of the chain, is presented. These synthetic glycans are ideal probes for the fundamental study of polysaccharides, shedding light on how the modification patterns affect the polysaccharides properties (i.e. three dimensional shape and aggregation behavior). Molecular modelling simulations and NMR analysis show that different classes of polysaccharides adopt fundamentally different conformations, drastically altered by single-site substitutions [3].

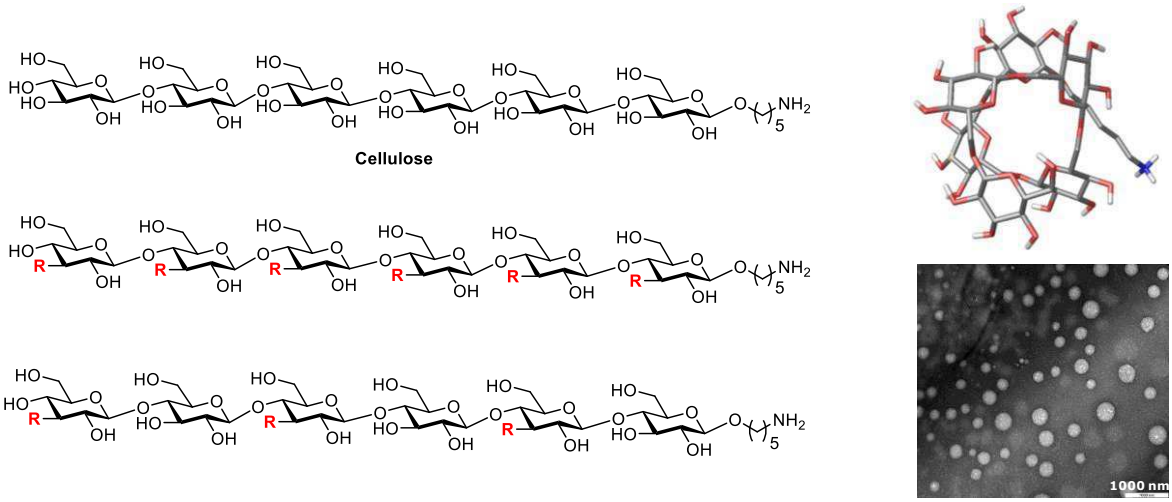

Fig1. Synthetic cellulose oligomers with different modification patterns. Molecular dynamic simulations and oligosaccharide-based nanoparticles (NPs).

The aggregation behavior of these synthetic materials is strongly affected by the single chain conformation and the modification patterns; spherical particles as well as linear fibers are observed. The information gained through this study will guide the development of novel carbohydrate-based biomaterials with tunable properties (e.g. NPs for biomedical applications).

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ANTIBACTERIAL EFFICACY OF CHITOSAN DERIVATIVES AGAINST PLANKTONIC BACTERIA AND BACTERIAL BIOFILMS

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Chitin, the most abundant biopolymer in the marine world, is present naturally in the exoskeletons of crustaceans, as well as in several insects and fungi. Chitosan is obtained mainly through partial deacetylation of chitin and has recently attracted much attention due to its properties such as biocompatibility and biodegradability in the human body. In addition, it has inherited interesting biological activity like antimicrobial, immunological, bioadhesive and wound healing properties [1]. Apart from other areas of applications, chitosan has mostly been studied in medicine as an antibacterial agent. However, the lack in solubility of this polymer in aqueous medium under physiological conditions has somewhat limited its applications. Hence, in recent years, several strategies including chemical modification of chitosan have been attempted to improve the aqueous solubility and enhance its antimicrobial property [2]. In this study, we have performed synthetic modification, specifically at the primary amino group of chitosan using various cationic and lipophilic moieties. We have introduced cationic groups like trimethyl ammoniumyl and guanidinyl groups using different spacer lengths and degree of substitution (DS). These parameters were then utilised to observe the variation in the antibacterial properties of the materials. Additionally, we combined the cationic moiety with different hydrophobic groups either in the form of varying alkyl chain lengths within a quaternary ammoniumyl group or randomly distributed alkyl chains within the polymer backbone [3]. We also introduced a short AMP, anoplin at different DS into the chitosan chain to improve its antimicrobial effect[4]. Investigation of these materials against clinically important Gram positive and Gram negative strains showed that various factors like presence of spacers, DS, hydrophobicity and quaternisation degree significantly affected the antimicrobial property of the biopolymer. We also evaluated the preliminary toxicity of the highly active chitosan derivatives and conjugates against human red blood cells, to ensure that the biocompatibility of the polymer was maintained. The high inhibitory effect as well as the bactericidal effect of the polymer derivatives towards planktonic bacteria motivated us to further determine their efficacy against the highly resistant bacterial biofilms. We observed that the effect of quaternary ammoniumyl chitosan derivatives against *Staphylococcus aureus* biofilms was more variable. The most effective derivatives contained hydrophobic groups, and their efficacy against biofilms depended on the ratio and length of the alkyl chains. Three-dimensional imaging of the biofilms using confocal laser scanning microscope confirmed the accessibility and the antimicrobial effect of the chitosan derivatives in the full depth of the biofilms.

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IMPACT OF NATURE OF SPACER GROUP ON REVERSE MICELLIZATION BEHAVIOUR OF SOME D-GLUCOSE DERIVED NON-IONIC GEMINI SURFACTANTS

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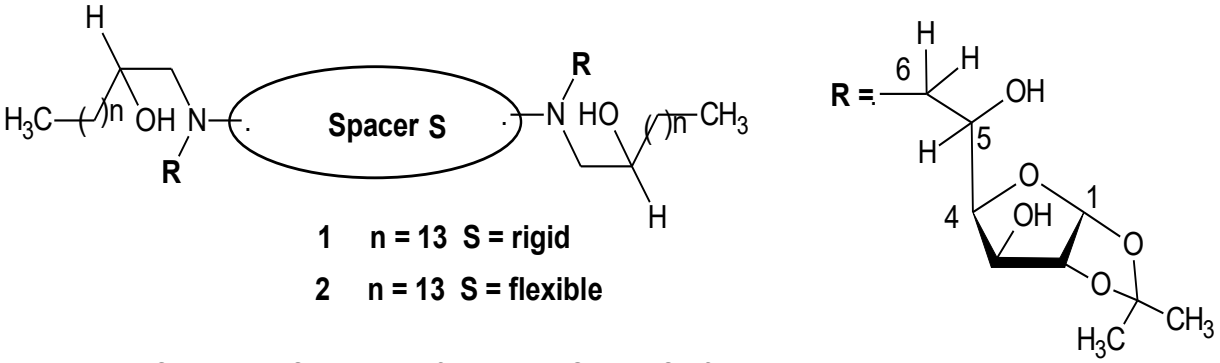
Reverse micelles formed by surfactants can be used for selective solubilisation and reactions which exploit their nanometre size and mimic the membranous biological system *i.e.* non-ionic gemini surfactants are typically employed to encapsulate some aromatic α-amino acids in n-hexane. Carbohydrate based gemini surfactants [1] possess two hydrophilic sugar head groups and two hydrophobic alkyl tails linked by a flexible [2] or rigid [3] spacer, which can be hydrophilic or hydrophobic[4]. Two novel non-ionic Gemini surfactants **1** and **2** with tail lengths of 16 carbons have been synthesized in our laboratory. In first, the spacer is rigid aromatic and in second, the spacer is flexible aliphatic carbon chain. The structure and purity of these surfactants were confirmed using elemental analysis, ESI mass spectrometry and ^{1}H and ^{13}C NMR spectroscopy. These amphiphiles were explored as reverse micellization probes for encapsulation of D- and L- aromatic α-amino acids *viz.* Histidine, Phenylalanine, Tyrosine, and Tryptophan. These amino acid are encapsulated in the sequence Histidine > Phenylalanine > Tyrosine > Tryptophan by both the surfactants. It was found that the non-ionic gemini surfactant with rigid aromatic spacer **(1)** was more efficient to encapsulate these amino acids in *n*-hexane as compared to non-ionic gemini **(2)** with flexible aliphatic spacer.



Scheme 1. Structures of non-ionic Gemini Surfactants

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XYLYLENE CLIPS FOR THE TOPOLOGY-GUIDED CONTROL OF THE SUPRAMOLECULAR PROPERTIES OF CYCLODEXTRINS: TOWARDS ARTIFICIAL VIRUSES

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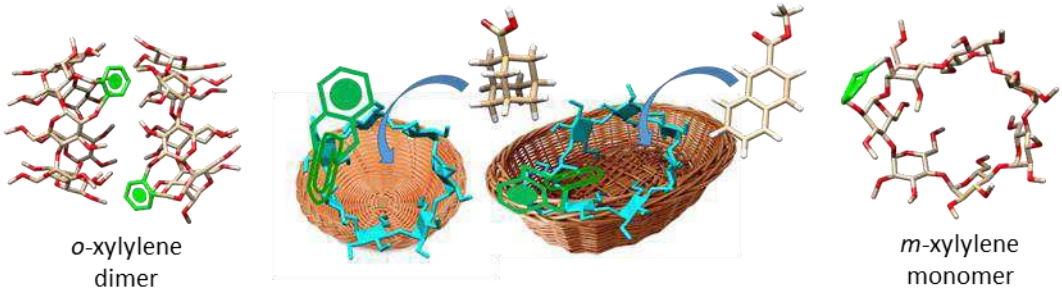
Aromatic appendages attached to macrocyclic platforms, such as cyclodextrins (CDs), can act as functional components promoting non-covalent self-interactions as well as associations with third species, allowing several levels of organization to be implemented. In most reported examples, however, the aromatic component is attached to the cyclooligosaccharide core through a single position and retains substantial mobility, which limits accurate three-dimensional definition. Doubly-linked derivatives with restricted conformational freedom are, in principle, better suited for precise control of receptor topology. Thus, the incorporation of an *o*-xylylene component attached to the secondary O-2(I) and O-3(I) positions at one out of the seven glucopyranoside residues in β-cyclodextrin (βCD), through a hinge-type joint, was found to modulate the inclusion properties and promoting the formation of head-to-head (*HH*) dimers (Fig. 1), which has been exploited in the design of stimuli-responsive gene delivery systems. Particularly appealing is the possibility of installing aromatic "clips" triggering conformational changes important enough to affect the symmetry of the CD platform and achieve a permanent cavity shape reorganization, a yet unattained goal in the field. Here we report that *o*- or *m*-xylylene clips connecting the O-2(I) and O-3(II) positions in consecutive glucopyranosyl subunits of βCD are able to promote distortion of the macrocyclic framework and produce dissymmetrical cavities in a tunable fashion, dramatically impacting the inclusion and self-assembling aptitudes, as determined by NMR, fluorescence spectroscopy, MS, and molecular modeling (see Fig. 1). The ensemble of data supports that the topology of βCD can be molded, from toroidal to ovoid basket-shaped, by using either the *o*- or the *m*-xylylene clip, the shape transition been the consequence of conformational bias at a single glucopyranosyl unit. Notably, this strategy can be exploited to switch *on* or *off* hierarchical modes of polycationic CD co-assembling with nucleic acids.



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THE CRITICAL ROLE OF GLYCONANOMATERIALS SIZE AND SHAPE FOR SELECTIVE BACTERIAL CELLS AGGLUTINATION

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In recent years, infections caused by multidrug-resistant bacterial pathogens have become a huge issue to public healthcare systems highlighting the urgency in finding new ways of fighting this threat [1]. The so-called anti-adherent therapy is a particularly promising new approach to combat bacterial resistance using inhibitors of bacterial adhesion to epithelial cells, one of the first stages of infection [2]. However, development of an effective anti-adhesive therapy is hampered by the structural complexity of carbohydrates, which in turn limits their synthesis. Moreover, adhesin- and in general protein-carbohydrate interactions are characterized by low affinity, and materials that can achieve high affinity interactions, through multivalency, still remain to be developed. The size, shape, number of sugar and their placement are variables that have to be taken into account in order to develop multivalent systems able to inhibit the bacterial adhesion based on sugar-lectin interaction [3]. Based on these premises, in the present work we report different modular approaches for the synthesis of structurally varied glyconanomaterials **I-V** (Fig. 1) and their application in the selective agglutination of uro-pathogenic bacteria.

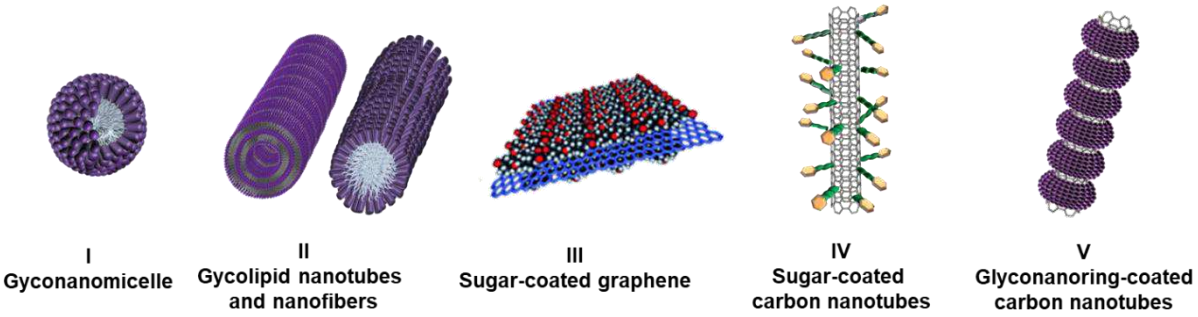

Fig.1 - Sugar-coated nanomaterials used in this study

The *in vivo* studies show that unlike spherical glyconanomicelles **I** [3], 1D sugar-coated nanomaterilas **II, IV, V** [4], and 2D sugar-coated graphene **III** efficiently and selectively bind to *Escherichia coli* type 1 fimbriae, leading to important bacterial cell-agglutination and important inhibition of colony forming units. These findings open the possibility that 1D and 2D sugar-coated nanomaterials could be a novel and effective tool in the control of bacterial pathogenesis.

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POLYVALENT GLYCAN-NANOPARTICLES AS MULTIFUNCTIONAL STRUCTURAL AND MECHANISTIC PROBES FOR VIRAL RECEPTORS, DC-SIGN AND DC-SIGNR

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Multivalent protein-carbohydrate interactions are of critical importance in biology because they initiate the first contact between pathogens and host cells and ultimately lead to infection. Understanding the underlying structural and molecular mechanisms is key to develop specific, potent multivalent inhibitors that can block such interactions and prevent infection [1]. Two proteins that are of particular interest here are the tetrameric lectins, DC-SIGN [2] and DC-SIGNR [3]. They play a key role in facilitating the HIV and Ebola Virus infections by binding to the specific glycans found on the viral surface glycoproteins. Despite extensive research, their flexible, complex and multimeric structure has so far prevented X-ray crystal-lography from being applied to elucidate structural mechanisms. Herein we report the development of two types of glycan-nanoparticles, one is based on a fluorescent quantum dot (QD) and the other is based on a gold nanoparticle (AuNP), for probing multivalent DC-SIGN/R-glycan interactions *via* the fluorescence resonance energy transfer (FRET, Fig. 1A) and fluorescence quenching (Fig. 1B) readout.

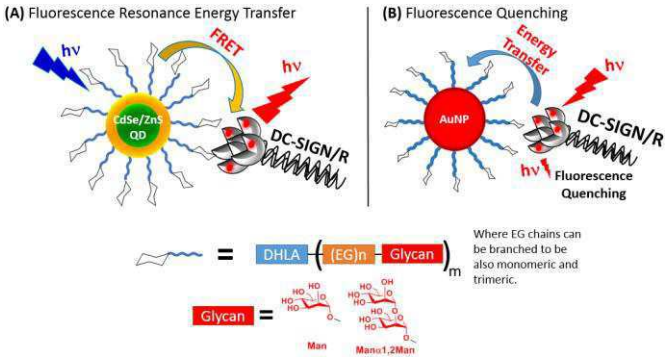

Fig. 1 - Probing polyvalent glycan-nanoaprticle (m = 1, 2, 3) interaction with DC-SIGN/R *via* two readout strategies: (**A**) fluorescence resonance energy transfer (FRET) with glycan-QDs and (**B**) flourscence quenching with glycan-AuNPs.

Using QDs capped with single glycan-PEG functionalized DHLA ligands, we have successfully dissected the different binding modes of DC-SIGN/R by combining FRET readout and TEM imaging, revealing that DC-SIGN binds tetravalently to one QD while DC-SIGNR binds bis-divalently with two different QDs. By quantifying the QD-DC-SIGN/R binding via FRET, we find their binding affinity is significantly weakened upon dilution of the QD surface glycan density with an inert DHLA-zwitterion ligand [4]. We have further synthesized a series of oligomeric glycan-PEG DHLA ligands containing different DHLA: Glycan ratios to further increase the nanoparticle surface glycan density and quantified their binding with DC-SIGN using both FRET (for QD) and fluorescence quenching (for AuNP) readouts. Surprisingly, the glycan-nanoparticle-DC-SIGN binding is weakened considerably with the increasing glycan density and a general affinity trend of m = 3 < m = 2 < m = 1 is obtained for both mannose and dimannose glycan ligands [5]. These results imply that there is an optimal glycan density for specific, high affinity DC-SIGN/R binding.

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NMR SPECTROSCOPIC CHARACTERIZATION OF INCLUSION COMPLEX OF CYCLODEXTRIN-BASED GLYCOCLUSTERS

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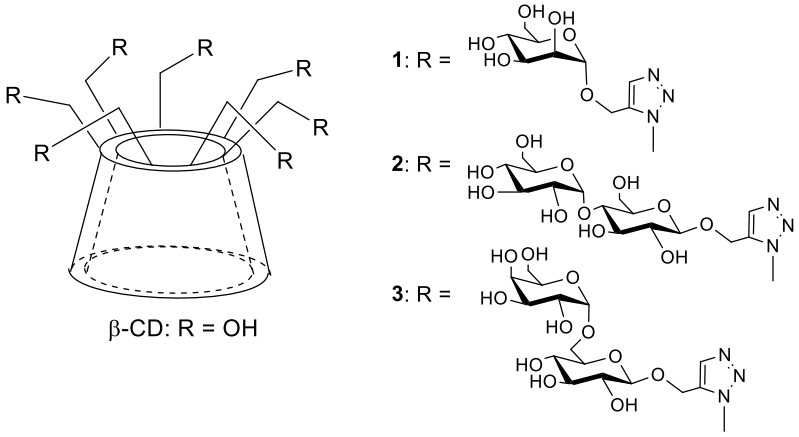
Cyclodextrins (CDs) are the most widely used host molecules and encapsulation of a guest into the CD cavity has a significant effect on the chemical, physical, and biological properties of the guest. Therefore, elucidation of the structure of the inclusion complex has been a challenging task. Recently, a CD-based glyco-cluster (per-D-mannose-grafted-β-CD: **1)** was synthesized by azide-alkyne cycloaddition and encapsulation of anticancer [1], anti-HIV [2], antibiotic [3], and antiphlogistic drugs [4] was investigated. To extend applicability of the glyco-clusters in pharmaceutical field such as construction of drug delivery system (DDS), we are exploring preparation of similar oligosaccharide clusters and characterization of their inclusion complexes. Here, we would like to report preparation of the corresponding maltose- **2** and melibiose-derivative **3** and NMR spectroscopic characterization of their inclusion complexes with ibuprofen sodium salt (IBUNa) as a model guest.

Heptakis(6-azido-6-deoxy)-β-CD was treated with propargyl α-D-mannopyranoside, β-maltoside, and β-melibioside (1.2 eq) in DMF at 80 ºC for 60 h in the presence of catalytic amount of CuBr and PMDETA, and successive purification procedures by precipitation with Et_2O, dialysis with a membrane (MW cutoff 2 kD), and lyophilization gave the corresponding glyco-clusters **1**, **2**, and **3**.

From 600 MHz ^{1}H COSY and TOCSY spectra, almost all proton signals of **1**, **2**, and **3** were assigned and their Inclusion ability of with IBUNa was investigated by NMR spectroscopy. Significant chemical shift change was observed at some protons of both host and guest molecules. Continuous variation (Job's plot) method showed all complexes had 1:1 stoichiometry and their association constants determined by NMR titration were 0.64-2.9 x 10^4 M^{-1}. Furthermore, possible orientations of the guest molecules were deduced by 2D NOESY and ROESY experiments.



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BLOCKWISE STRUCTURED GLUCAN DERIVATIVES

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Aqueous solutions of methyl cellulose (MC) demonstrate thermo-responsive gelation behavior. Hydrophobic-hydrophobic interactions play a key role in gelation, however the mechanism of gelation is not yet fully understood. Earlier models suggested formation of hydrophobic chain networks [1], whereas more recent models argue formation of fibrillar structures [2], caused by coil-helix-like self-assemblies [3].

This work focuses on synthesis and characterization of glucan ethers with a substituent pattern closer to block copolymers having sub-10-nanometer-long blocks of per-methylated glycosyl units (hydrophobic blocks), interrupted by un- or low substituted segments (hydrophilic blocks), and study their structures [4] and structure-property relationship, e.g. thermoreversible gelation, etc.

Instead of copolymerizing co-monomers or successive assembling of short building blocks [5], we use a top-down approach to obtain the blocky copolymer by performing a one-step-one-pot transglycosidation reaction between two different cellulose derivatives with high molecular weights, namely per-methylated cellulose and a fully protected MC. Subsequent deprotection shall provide the desired products with hydrophobic and hydrophilic blocks (Fig. 1).

Introducing a starting material with a mixed substitution pattern of methyl- and protecting groups gives rise to critical synthesis and characterization difficulties which were absent in model transglycosidation reactions between model compounds, per-ethylated cellulose and per-methylated cellulose [6]. Challenges of synthesis, choices of protecting groups (PG), deprotection, and structural characterization of the obtained products by mass spectrometry [7] will be presented.

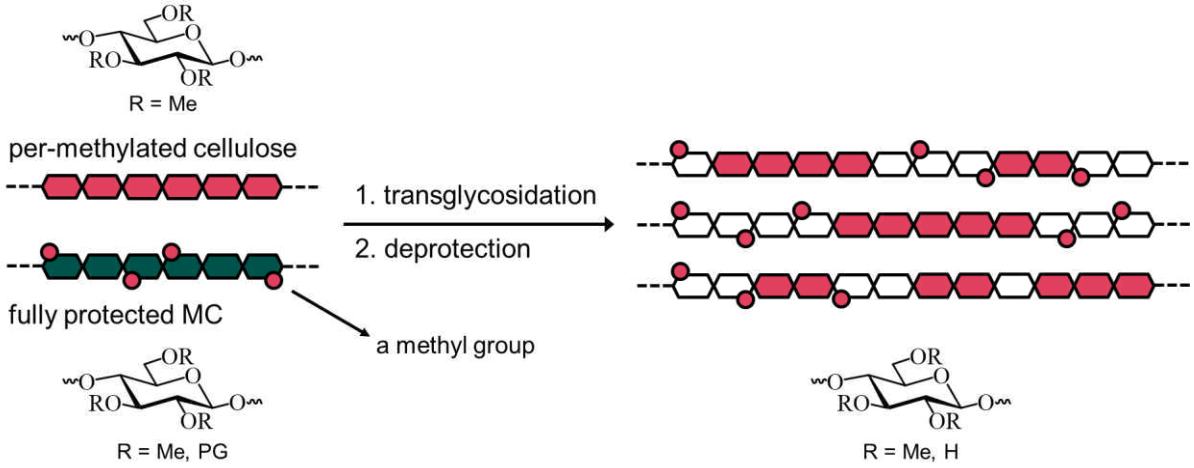

Fig. 1 - Synthesis of blockwise structured glucan ether derivatives by transglycosidation reaction.

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A GH 20 THIOGLYCOLIGASE AS VERSATILE CATALYST FOR GLYCOCONJUGATE SYNTHESIS

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Carbohydrate mimetics, resistant towards enzymatic hydrolysis, have proven useful as inhibitors and therapeutics. Thioglycosides turned out stable glycosides with similar structural and physicochemical properties compared to their O-glycoside counterparts. Chemical synthesis of such compounds is often cumbersome considering the known issues in the control of stereochemistry and numerous protections/deprotection steps. Enzymatic synthesis of thioglycoside linkages in oligosaccharides could overcome these issues and is therefore of immense value. Several thioglycoligases are reported to date, covering both, the formation of α and β glycosidic linkages [1-3].

Herein, we present the first thioglycoligase derived from the mutation of the catalytic acid/base of *Streptomyces plicatus* N-acetyl-β-hexosaminidase (SpHex E314A). SpHex is a retaining GH family 20 *exo*-hexosaminidase cleaving β-1,4 glycosidic linkages between GlcNAcs via substrate assisted catalysis [4]. PNP-GlcNAc and GlcNAc oxazoline were chosen as reactive glycosyl donors and tested against a series of (3,4,6-) deoxythio sugars as glycosyl acceptors. The SpHex variant catalyzed β-thioglycosidic linkage formation with all tested deoxythio sugars of Glu, Gal, Man, GlcNAc, GalNac and ManAc in high yield. Enzyme mediated GlcNAc transfer to various substituted (O-)phenols spanning a broad pKa range gave insights into the donor reactivity. SpHex E314A also catalyzed the reverse reaction hydrolyzing different thioglycosides revealing a 50% decrease in the catalytic efficiency compared to the wildtype enzyme. The remarkable capacity of the SpHex thioglycoligase was successfully expanded to other bioactive acceptors, including cysteine and cysteine bearing oligopeptides, opening a new synthesis path towards GlcNAcylated peptide mimetics.
In summary, we demonstrate that SpHex E314A is a versatile thioglycoligase showing a remarkable thio acceptor promiscuity which renders this variant a potent candidate for generating metabolically stable glycoconjugates.

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CORN STARCH PLASTICIZED WITH ISOSORBIDE AND REINFORCED WITH MCC: A NEW GREEN BIOCOMPOSITE

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The use of plastic materials throughout the last decades has allowed the development of many applications for society but, these materials have a significant pollution problem. Because of this, the tendency for finding more viable and competitive biopolymers, which will be more respectful with the environment than conventional polymers is growing. Among the most promising materials for biopolymers, starch stands out due to its greater availability, lower cost and their origin from natural and renewable sources. Native starch is not a thermoplastic material, being necessary the application of thermal and shear processes, as well as use of some plasticizing agent, to get the conversion to thermoplastic starch (TPS), which can already be processed with the conventional techniques. Water and polyols, especially glycerol, have being the most widely used in bibliography for starches [1]. Recently, the isosorbide has been proposed as a novel and totally green plasticizer (biodegradability, non-toxicity, non-mutagenic and solubility in water).

Despite the advantages of thermoplastic starch, TPS materials have poor mechanical and thermal properties compared to conventional polymers. In order to overcome these shortcomings, several strategies have been developed in recent years, such as addition of particles derived from different biopolymers. Microcrystalline cellulose (MCC) is presented as a possible good candidate because have some inherent good properties like high surface area, strong reinforcing ability, renewability and biodegradability, no health-hazard and low cost [2].

The aim of this work was the development of innovative starch-based biocomposites, completely green and biodegradable, plasticized with the novel isosorbide and reinforced with microcrystalline cellulose. The effect of isosorbide as plasticizer was analyzed and the results were compared with the obtained for materials plasticized with glycerol, which is used as a reference. The effect of the amount of plasticizer and MCC filler was investigated.

The materials used were corn starch; isosorbide and glycerol as plasticizers; and microcrystalline cellulose (MCC) as reinforcement. All samples were prepared by melt processing using an internal mixer and characterized by X-ray diffraction (XRD), thermogravimetric (TGA) and dynamic mechanical analyzers (DMA).

The biocomposites plasticized with isosorbide required lower temperatures but greater shear and increased processing time to get the plasticized material than the necessary for the glycerol plasticized composites. X-ray diffraction revealed low crystallinity in plasticized composites. Native crystallinity of raw starch was lost in TPS and they developed different crystallinity types depending on the plasticizer used. TPS plasticized with isosorbide have shown greater thermal stability and higher values of storage modulus than composites plasticized with glycerol. The MCC addition lead to more crystalline materials with enhanced dynamic mechanical properties.

Acknowledgements: Financial support provided by Xunta de Galicia Government and FEDER (GRC 2014/036) is gratefully acknowledged.

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POLYSACCHARIDE ENGINEERING: LATERAL AND TERMINAL MODIFICATION

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Polysaccharides have been utilized by the humanity for thousands of years, first as parts of natural food and wood-based products. In modern times polysaccharides have been purified and modified in different ways to enhance their functionalities. Today polysaccharides are key ingredients in foods, pharmaceuticals and biomaterials, etc. The various developmental steps in polysaccharide science and technology may conveniently be divides into seven generations: 1) Plant extracts 2) Animal extracts 3) Chemical derivatives of plant and animal extracts 4) Microbially produced glycans 5) Enzymatically polymerized glycans 6) Chemically synthesized glycans and 7) Macromolecular assembly of glycan modules.

The lecture will briefly cover these seven types, but will concentrate on the three last three, focusing by examples (mainly alginates [1-3], chitosans, 1,3-β-glucans[4]) the differences between terminal and lateral modifications. Recently initiated development of new hybrid glycans based on terminal module assembly [5, 6] will be described. Such glycans may contribute to new types of utilization of recalcitrant biomass, and also to supplement or even substitute oil-based polymeric materials.

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ION-INDUCED CONFORMATIONAL CHANGE OF GLYCOCLUSTERS AS A POSSIBLE MECHANISM TO INITIATE CARBOHYDRATE-CARBOHYDRATE INTERACTIONS

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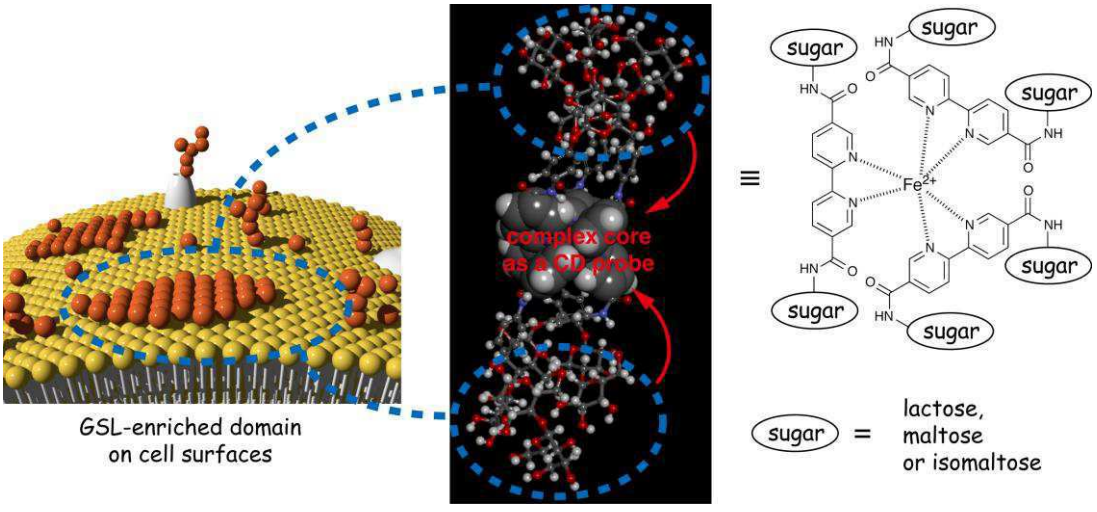
Glycosphingolipids (GSL) in cell membranes laterally aggregate to form GSL-enriched microdomains presenting densely packed carbohydrate clusters (glycoclusters) on cell surfaces. It has been reported that these cell surface glycoclusters play essential roles in cell-cell adhesion via carbohydrate-carbohydrate interactions (CCI), in which GSL on one cell surface recognize GSL on the adjacent cell surface in specific, multivalent and, in some cases, Ca^{2+}-dependent manners. Researchers in this research field believe that Ca^{2+} function as molecular glue to put two carbohydrate units together and the resultant formation of multiple carbohydrate/ion/carbohydrate complexes is a main driving force to mediate the CCI. On the other hand, no attention has been paid for the spatial carbohydrate packings within these glycoclusters. In this work, we developed hexavalent glycoclusters having $[Febpy_3]^{2+}$ cores as circular dichroic (CD) probes. Molecular dynamics (MD) calculation showed that these metalloglycoclusters have unique 3D structures, in which two trivalent glycoclusters locate at northern and southern poles of spherical $[Febpy_3]^{2+}$ cores. Great advantages of our metalloglycoclusters include their $[Febpy_3]^{2+}$ cores as CD probes locating closely to the trivalent glycoclusters. Since the trivalent glycoclusters are energetically coupled with the $[Febpy_3]^{2+}$ cores, ion-induced conformational changes of the former can be readily monitored by the Δ-Λ ratios of the latter. By using such model systems, we investigated carbohydrate packings with/without ions and found that various ions including Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^- , SO_4^{2-} and NO_3^- bind to the glycoclusters and greatly changed their carbohydrate packing not only in carbohydrate- but also ion-dependent manners. In addition, we also found that the sulfates (Na_2SO_4 and K_2SO_4) specifically change rheological properties of aqueous solutions containing the metalloglycocluster having lactoside appendages. These findings suggest an alternative mechanism to initiate CCI; that is, various ions induce conformational change of the glycocluster and these conformational change play important roles to induce CCI.



A CAPPING STEP DURING AUTOMATED GLYCAN ASSEMBLY ENABLES ACCESS TO COMPLEX GLYCANS IN HIGH YIELD

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The products of multi-step automated solid phase syntheses are purified after release from the resin. Capping of unreacted nucleophiles is common place in automated oligonucleotide synthesis to minimized accumulation of deletion sequences. To date, capping was not used routinely during automated glycan assembly (AGA) since previous capping protocols suffered from long reaction times and conditions incompatible with some protective groups. Here, a method using methanesulfonic acid and acetic anhydride for the fast and quantitative capping of hydroxyl groups that failed to be glycosylated is reported. Commonly used protective groups in AGA are stable under these capping conditions. The introduction of a capping step into the coupling cycle drastically improved overall yields by decreasing side-products and simplifying purification while reducing building blocks consumption. To illustrate the method, biologically important tetrasaccharide Lc4, as well as a 50-mer polymannoside were prepared.

PHOTOSWITCHABLE & FLUORESCENT MULTICHROMOPHORIC SACCHARIDES FOR OPTICAL APPLICATIONS

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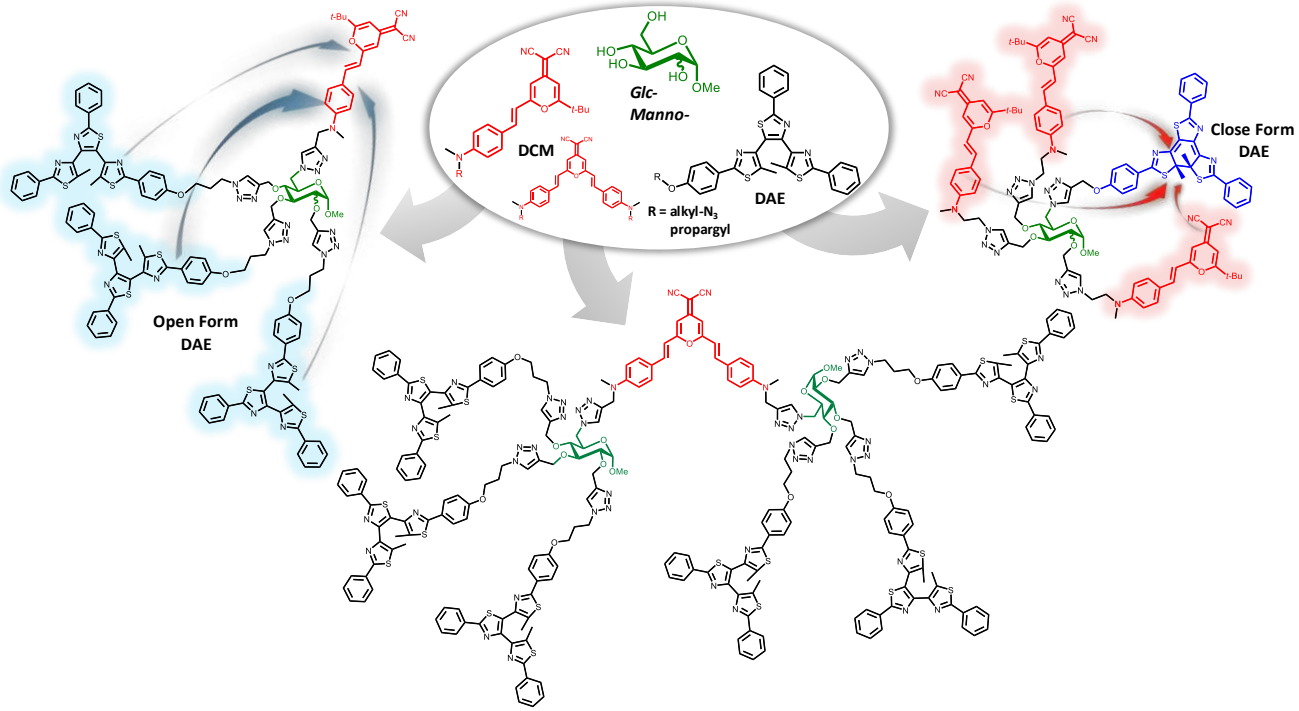
The development of novel optical devices, such as light-driven optical memories or biocompatible nanophotoswitches for super-resolution imaging, attracts considerable interest in the growing field of photoresponsive nanotechnologies [1].

As a continuing program on the development of fluorescent photoswitchable biomolecules for optical applications, we have designed and synthesized several multichromophoric architectures using the Cu(I)-catalyzed azide-alkyne cycloaddition reaction on saccharides derivatives [2,3]. Saccharides are polyfunctional molecules with well-defined stereogenic centers in one molecular unit, and constitute platforms of interest on which multiple chromophoric units can be attached. Furthermore, such platforms offer an important degree of freedom, such as the multivalent effect and the chirality, to tune the photochemical and photophysical properties. To take advantage of the intramolecular energy transfer possibilities for fluorescence photoswitching in the same molecular architecture, we have combined photochromism and fluorescence properties of diarylethene (DAE) and dicyanomethylene (DCM) units. To optimize the system performances (contrast and brightness), the ratio of the linked chromophores (DCM/DAE) and the monosaccharidic platforms (glc- and manno-) were explored.

Synthesis of these photoswitchable multichromophoric saccharides as well as their photochemical and photophysical properties, steady-state absorption & fluorescence under light irradiation, will be presented.



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FUNCTIONAL POLYSACCHARIDE-BASED MATERIALS FOR FOOD AND BIOMEDICAL APPLICATIONS

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Polysaccharides are widely distributed in nature and are easily available, which render them interest due to their functional and sustainable characteristics for food and biomedical applications.

The polysaccharides have been exploited to develop edible and biodegradable films to extend shelf-life and improve quality of food while reducing packaging waste. For example, chitosan has the advantage to be antioxidant and antimicrobial agent. However, to compete with synthetic polymers it needs to meet the requirements of cost-effective materials ensuring the mechanical and gas barrier characteristics for food packaging, which can be achieved by chemical modification. The covalent bonding of a crosslinker, as genipin, enhances the resistance to the typical acidity of food while assigning mechanical properties [2]. Moreover, the grafting to chitosan of some functional molecules can still improve its antioxidant and hydrophobicity character [3]. Also, the addition of nanofillers improves the mechanical and barrier properties and can attribute electrical conductivity and magnetic properties to chitosan derived materials for active and intelligent packaging. Electrical conductivity is a required property for the processing of food at low temperature using electric fields or for sensors application that detect contaminants in foods or monitor changes in packaging conditions or integrity [4].

The majority of polysaccharides from marine organisms are still unexplored, although their great interest for biomedical fields, such as drug delivery and tissue engineering, due to their biocompatibility, biodegradability, and ability to form hydrogels/films. Owing to their structural complexity, there is a strong need for basic research able to identify the structural characteristics that can be responsible to the reported activities, allowing the exploitation of brand new functional materials composed/derived from polysaccharides [1]. Polysaccharides from marine organisms, namely algae and microalgae, may be remarkable for the biomedical field due to the presence of sulfate groups, which do not occur in polysaccharides from other origins. Sulfation seems to be essential to confer antioxidant, immunostimulatory, anti-inflammatory, and anti-viral properties to the polysaccharides. These polysaccharides can be used for coating magnetite particles, allowing to have a biocomposite for hyperthermia treatment in cancer. In addition, this material has the potential to be used for a controlled release of molecules with diagnostic capability, a theranostics approach.

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SYNTHESIS OF PHOTOSWITCHABLE CARBOHADRATE-BASED MACROCYCLES VIA INTRAMOLECULAR GLYCOSYLATION

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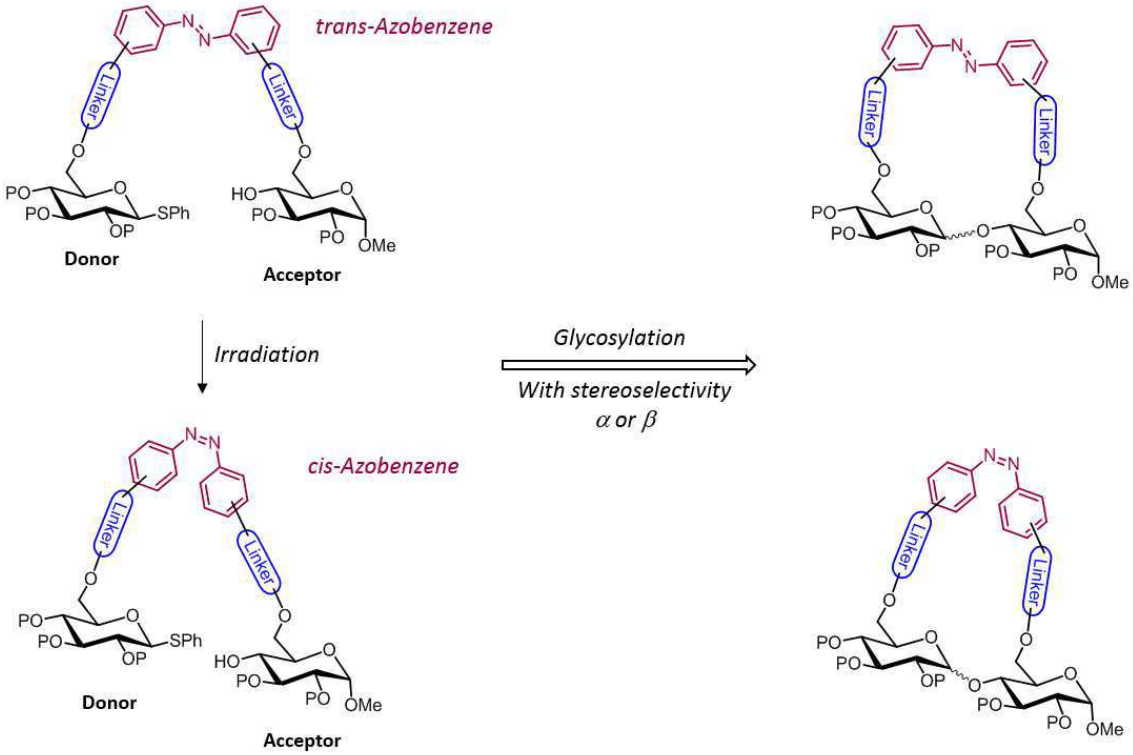
Macrocycles are a unique class of molecules with unique structural, physicochemical and biological properties, leading to their applications in nanotechnology, biology and drug delivery [1,2]. Configurational and conformational diversities induced by imbedding carbohydrate moiety into macrocycles have sharply broadened their applications as biomolecule mimics and biomaterials [3].Azobenzenes have been extensively used for designing smart photo-responsive molecules because of their remarkable photoreversibility and photostability [4]. Herein, we have designed and synthesized a series of photoswitchable carbohydrate-based macrocycles via intramolecular glycosylation wherein the glycosyl donors and acceptors were tethered by azobenzene. The stereoselectivity outcome of the glycosylation from *trans*- or *cis*-substrate was investigated by altering the species of azobenzene and the flexibility of the linker. Furthermore, photochromic and chiroptical properties of the obtained macrocycles will be presented.



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EVALUATION OF TWO ALDITOLS AS GREEN CORROSION PREVENTION INHIBITORS IN AA2024-T3 ALUMINIUM ALLOY USED IN AERONAUTIC AND AEROSPACE INDUSTRY

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Corrosion is a major problem that affects many metals and alloys. The costs and consequences caused by corrosion are of such importance that justifies a comprehensive study in relation to its causes, mechanisms of identification, detection and development of methods of prevention and protection.

The former conventional pre-treatment of AA2024-T3 aluminium alloy involves formulations containing chromium (VI) but REACH (Registration, Evolution, Authorization and Restriction of CHemicals) restricts the use of hexavalent chromium in EU, due to the negative impact of these compounds in environment and human health. In the last few years there has been an increased in interest in developing new corrosion protection alternatives that could be efficient and more environmentally and human-friendly, to replace the chromium (VI) based treatments.

The objective of this study is to evaluate the anticorrosion character imparted by two alditols, (sorbitol and xylitol), and compare their performance with tartaric acid based bath, which is used (e.g. AIRBUS) in the anodising the aluminium alloy used in aeronautical and aerospace industry, the AA2024-T3 alloy. The corrosion resistance of the treated samples was monitored by potentiodynamic polarization assays and Electrochemical Impedance Spectroscopy analysis.

The conversion coatings formed on the aluminium alloy revealed that the use of these carbohydrates provide a good corrosion protection, making this pre-treatment a promising alternative for chromium (VI) based chemical conversion coatings.

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ALGINATE BASED BLOCK POLYSACCHARIDES

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Materials based on polysaccharides represent a new class of materials that potentially could replace or supplement synthetic polymers. When polysaccharides are coupled together terminally rather than laterally, the intrinsic physiochemical properties of the modules are retained. Hence, properties such as sensitivity to pH, ionic strength, temperature, and reaction with divalent cations in the case of alginate, could be utilized to introduce switches to control chain-chain interactions. By exploiting such properties, block copolymers could be used to prepare bottom-up synthesized self-assembling structures [1].

For preparation of terminally conjugated polysaccharides, the first step was modifying the reducing end of a series of biopolymers (alginate, chitosan, chitin and dextran) with bi- and trifunctional hydrazides (NH_2-NH-CO-) and oxyamines (NH_2-O-). We used reductive amination with 2-picoline borane [2], and the reaction was monitored using ion exchange chromatography (HPAEC-PAD). Diblock conjugates of alginate have also been prepared as a proof of principle. Furthermore, alginate oligomers were coupled to an aminooxy-functionalized water-soluble PEG by the same method. The conjugates will be used for the preparation of nanoparticles, using the specific reaction of the alginate with certain divalent cations such as Ca^{2+}, Ba^{2+} and Sr^{2+}.

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POSTERS | CN – Carbohydrates and Nutrition

PREPARATION AND CHARACTERIZATION OF THE AMIDATED ALGINATE AND ITS EFFECT ON THE HYPOCHOLESTEROLEMIC AND HYPOLIPIDEMIC ACTIVITY IN RATS

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Alginate is a copolymer of β-D-mannuronate and α-L-guluronate, which are present in the cell wall of brown algae[1]. Alginic acid is a suitable material for modification to prepare new derivatives because of presence of its carboxyl groups. The high content of carboxyl groups over the entire length of its chain renders it an easily modifiable material with a possibility of achieving a high degree of substitution in the prepared derivatives. The salt of alginic acid (sodium alginate) is readily commercially available and is widely used in many branches of chemistry. Alginic acid was thus selected as the substrate for amidation. The aim of this study was to prepare highly substituted polysaccharide derivative with long alkyl (C-18) chain. The amidation used two-steps: methyl esterification followed by amino-de-alkoxylation with *n*-octadecylamine. The purity and substitution degree was monitored by vibration spectroscopic methods (FTIR and FT Raman) and organic elemental analysis[2]. Molecular weight was analysed by spectroscopic method after reaction with 2,3-dinitrosalicylic acid. These analytical methods confirmed the preparation of *N*-octadecylamide of alginic acid. High level of serum cholesterol is considered a risk factor of cardiovascular diseases. For treatment of cholesterolemia are used different medicaments (statins, cholestyramine), then is possible to mention psyllium and indian plantain[3]. An alternative to bile acid sequestrants are hydrophobically modified polysaccharides for sorption of cholesterol and other neutral sterols. The hypocholesterolemic and hypolipidemic activities of alginate and its hydrophobic derivative, which is prepared by a reaction with *n*-octadecylamine, were compared in rats fed diets containing cholesterol and palm fat at 10 and 50 g/kg, respectively. Amidated alginate, at 20 g/kg, significantly decreased serum cholesterol, serum triacylglycerols, hepatic cholesterol and total hepatic lipids. Alginate at 20 g/kg significantly reduced hepatic cholesterol, but did not influence serum cholesterol, triacylglycerols and total hepatic lipids. Amidated alginate significantly increased the faecal concentrations of neutral sterols, but decreased faecal concentration of bile acids. In conclusion, amidated alginate is an effective hypocholesterolemic agent that is more efficient than its parent polysaccharide. Alginate had not influence on serum lipids (cholesterol and triglycerides). Alginate only decreased cholesterol concentration in the liver tissue. In contrast of this amidated alginate significantly reduced serum cholesterol and triglycerides, cholesterol and total lipids in the liver tissue[4]. In the second experiment with amidated alginate, we compared the effects of different doses. Amidated alginate was in the feed in amount of 0, 10, 20 and 40 g/kg. Amidated alginate at the lowest concentration (10 g/kg) significantly reduced the cholesterolemia to the level of the negative control. It significantly reduced hepatic cholesterol, serum triglycerides, but only with the highest concentration (40 g/kg). The results show that amidated alginate increased concentration of total lipids in the excrements proportionately dosing at the highest dose (40 g/kg). It can be concluded that hydrophobic amidated alginate is an effective hypocholesterolemic and lipid-lowering agent, more effective than hydrophilic alginate. The hypocholesterolemic action of amidated alginate may be attributed to the increased faecal excretion of neutral sterols (cholesterol and coprostanol).

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ANTI-INFLAMMATORY EFFECT OF POLYSACCHARIDES FROM *CYNANCHUM WILFORDII* ON DEXTRAN SODIUM SULFATE-INDUCED ACUTE COLITIS IN MICE AND LPS-INDUCED MACROPHAGES

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In recent years, polysaccharides of plant origin have been proven as important bioactive components[1]. We recently reported on the immune-enhancing effects of a high-molecular-weight polysaccharide fraction of *Cynanchum wilfordii* (HMFO) in macrophages and immunosuppressed mice[2]. Cynanchum wilfordii Hemsley is arguably the most-well known species in Asia and it has been reported to alleviate rheumatoid arthritis, vascular diseases and ischemia-related diseases [3,4].

In this study, we further investigated HMFO for anti-inflammatory effects and its potential molecular mechanisms in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages and in a mouse model of dextran sulfate sodium (DSS)-induced colitis[3]. In RAW 264.7 macrophages, HMFO inhibited several cytokines and enzymes involved in the inflammatory process, such as prostaglandin E_2 (PGE_2), nitric oxide (NO), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) via the attenuation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs). In the mouse model of DSS-induced colitis, HMFO ameliorated the pathological characteristics of colitis, such as the shortening of colon length, and significantly reduced the serum level of pro-inflammatory cytokines. Histological analysis indicated that HMFO improved the signs of DSS-induced colon tissue damage, such as abnormal crypts, crypt loss, and inflammatory cell infiltration. In addition, HMFO inhibited the expressions of iNOS and COX-2 proteins and reduced the levels of phosphorylated NF-κB p65 in the colon tissue of DSS-induced colitic mice.

These results indicated that HMFO attenuated inflammation both *in vitro* and *in vivo* primarily by inhibiting the activation of NF-κB, which supported its potential therapeutic role for the treatment of colitis.

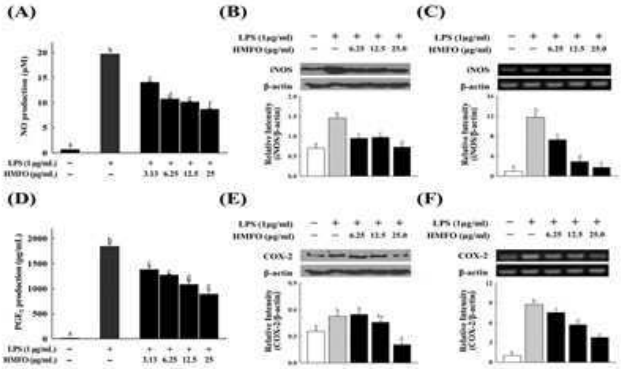

Fig. HMFO inhibited LPS-induced inflammatory mediators by suppressing iNOS and COX-2 activation in RAW 264.7 macrophages

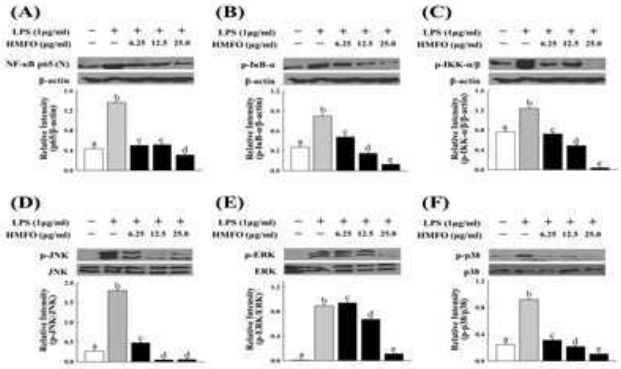

Fig. Effects of HMFO on the NF-κB and MAPK signaling pathways in LPS-induced RAW 264.7 macrophages.

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APPLE POMACE ARABINAN – UNCOVERING ITS APPARENT HYDROPHOBICITY

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Apple pomace is a by-product from apple processing industries, constituted by the pulp, skins, seeds and stalks from the fruit [1]. Currently, apple pomace is valued as a source of pectin, well known by their jellifying properties [2]. Nevertheless, among this apple pomace pectic fraction, resides an arabinose rich-fraction that can be obtained by solid-phase extraction using C18 cartridges (SPE-C18, Supelco-Discovery, 20g), alongside with polyphenols. To determine the structural features responsible for this apparent hydrophobicity, the arabinose rich fraction was dialyzed (12 kDa cut-off membrane, Medicell) against water to remove the polyphenols.

Sugar analysis by GC-FID, after derivatization to their respective alditol acetates, revealed that the retentate was composed of 60% (m/m) of carbohydrates, mainly arabinose (60 mol %). Glycosidic linkage analysis of its partially methylated alditol acetates by GC-MS, demonstrated the occurrence of (1→5)-Ara*f*, (1→3,5)-Ara*f* and (1→2,5)-Ara*f* linkages, which are characteristic features of arabinans. Total polyphenolic content by the Folin-Ciocalteu also showed the occurrence of non-diffusible polyphenols that accounted 6% (m/m) of polyphenols of the retentate. In order to separate polyphenols and carbohydrate pools, this fraction was subjected to ethanol precipitation (80%, v:v). Interestingly, carbohydrates accounted for more than 60% (m/m) in both the precipitate and the supernatant resulting from the ethanol precipitation. Moreover, these fractions had a yellowish colour, indicative of strongly associated phenolic structures to the carbohydrate skeleton. This was confirmed by alkaline fusion followed by HPLC-DAD analysis of these fractions. Hence, the gathered results support the relevance of polyphenols in conferring a hydrophobic character to arabinans.

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GLYCOSYL FLAVONOIDS FROM *SALVIA SCLAREOIDES*: CONTRIBUTION TO THE DEVELOPMENT OF A NEW FUNCTIONAL FOOD

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Salvia sclareoides is a plant of spontaneous Portuguese vegetation that has relevant properties, as part of its cholinergic action and in the prevention of the formation of amyloid plaques. Previous studies suggested [1,2] that this species of *Salvia* can be used as a functional food and also in the development of a food supplement in the context of the prevention and treatment of Alzheimer's disease. Sweet cookies were developed with different concentrations (2 % and 5 %) of *S. sclareoides*, previously ground for particle size reduction (2.00 mm). The nutritional composition was determined, namely: moisture content, ash, total protein, total fat, dietary fibre and salt [3]. The available carbohydrates and the energy value were obtained by calculation. Extracts were prepared from the dried aerial parts of the plant. Different extraction methodologies were used and the phenolic profile of each extract was determined by high performance liquid chromatography with diode array detection. The antioxidant and anticholinesterase activities of these extracts were also evaluated. The ethanol extract of *S. sclareoides*, with 2.00 mm grain size, presented the highest concentration of phenolic compounds (143.74 mg GAE/g dry extract) and antioxidant activity (68.45 %), while the aqueous extract showed the best anticholinesterase activity (30.86 %). The glycosylated flavonoids rutin and (7-*O*-glucosyl)luteolin are clearly the major constituents present in all extracts, along with epicatechin, ellagic acid and (7-*O*-glucosyl)naringenin.

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POSTERS | GMP – Gut Microbiota and Prebiotics

CHARACTERIZATION OF TWO NOVEL SIALYL *N*-ACETYLLACTOSAMINYL NUCLEOTIDES SEPARATED FROM OVINE COLOSTRUM

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The milk/colostrum of some mammalian species is known to contain sugar nucleotides including uridine diphosphate (UDP) oligosaccharides in addition to lactose and milk oligosaccharides, but the detailed structures of these UDP oligosaccharides have not so far been clarified.

In this study we isolated two UDP-sialyl *N*-acetyllactosamines from ovine colostrum and characterized from using ^{1}H-NMR and MALDI-TOFMS spectroscopies. Their structures were found to be Neu5Gc(α2-3)Gal(β1-4)GlcNAcα1-UDP and Neu5Gc(α2-6)Gal(β1-4)GlcNAcα1-UDP.

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PROTEIN ENGINEERING OF LACTO-N-BIOSE FROM *B. BIFIDUM* FOR HMOs PRODUCTION

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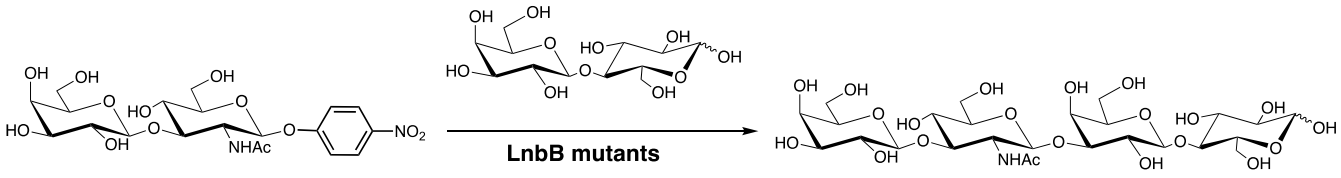
Human milk is considered the gold standard for infant nutrition; it is a complex mixture that besides of providing complete nutrition to the infant, it delivers essential biomolecules. Human milk oligosaccharides (HMO) are a group of glycans that provide essential biological functions such as immune modulators, prebiotics, and nutrients for neonatal brain development [1].

Bifidobacterium bifidum present in the gut's infant produces lacto-N-biosidase (LnbB), which is involved in the catabolism of HMOs. LnbB belongs to the GH20 family N-acetylhexosaminidases that catalyze the hydrolysis of GlcNAc residues located at the non-reducing end of oligosaccharides and glycoconjugates via a retaining substrate-assisted catalytic mechanism [2]. LnbB catalyzes the hydrolysis of the tetrasaccharide lacto-N-tetraose to lacto-N-biose and lactose [3]. Our structural-functional analysis has revealed the multi-domain organization of the enzyme and the important residues for its hydrolytic activity [4].

With the aim of producing a transglycosylating enzyme, we here report the engineering of LnbB following a semi-rational approach [5] targeting conserved residues located in the negative subsites of the substrate binding site. Selected mutants are characterized with regard to their hydrolytic activity on LNB-pNP (lacto-N-biose pNP) and their transglycosylation activity using LNB-pNP and lactose to produce the core 1 HMO lacto-N-tetraose.



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A MECHANISM BASED APPROACH FOR SCREENING METAGENOMIC LIBRARIES FOR UNSATURATED GLUCURONYL HYDROLASES

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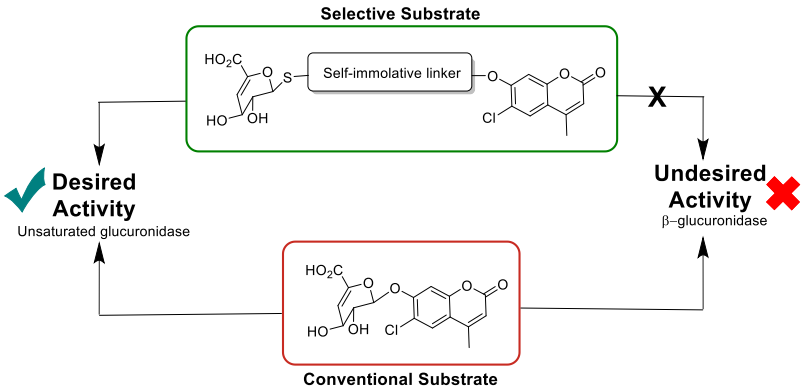
Unsaturated glucuronidases are among the enzymes that a number of pathogens employ to break down glycosaminoglycans, the main structural polysaccharides of our bodies[1]. In order to find out more about these under-studied enzymes and the bacteria that produce them and their relative abundance in the microbial populations of our environment, we set out to screen metagenomic libraries made from environmental samples such as the human gut microbiome for unsaturated glucuronidase activity.

In functional metagenomics, proteins from various uncultured organisms are reproduced in labs, by means of transferring the DNA extracted from environmental samples into host bacterial cell lines[2]. This method offers an exciting novel way of enzyme discovery as these expressed proteins can be readily screened for novel enzymatic activity. However, screening for some of these enzymes is not straightforward, as background activity from the host cells and/or other similar unwanted enzymes from metagenomics genes, can mask the desired activity. Unsaturated glucuronidases are one of these masked enzymatic activities.

In this study, a novel strategy has been developed for screening metagenomic libraries for unsaturated glucuronidases. This was achieved based on the differences between the mechanisms of unsaturated glucuronidases[3] and β-glucuronidases, the main source of background activity. These differences make β -glucuronidases inefficient in hydrolyzing thioglycoside substrates, while unsaturated glucuronidases cleave them rapidly. Two fluorogenic thioglycoside substrates with two self-immolative thiol linkers were designed and synthesized. A small metagenomic library was then successfully screened with these new substrates and the usefulness of the selective substrates were established. We believe that the same strategy is going to be useful when screening metagenomic libraries for some of the other examples of masked activities.



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IN VITRO PECTIN FERMENTATION AND ISOLATION OF PECTIN-DEGRADING GUT MICROORGANISMS FROM KOREANS

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Pectin is a complex polysaccharide present in the cell wall of plants. As it is non-digestible by human digestive enzymes, intestinal microbial enzymes are necessary to degrade and utilize pectin.

In order to isolate pectin-degrading gut microorganisms and investigate related pectin degrading enzymes, we have performed *in vitro* fermentation with three feces of healthy adult on medium containing pectin. As a result of incubation, three feces showed a similar tendency. The level of reducing sugar gradually increased from the early stage of incubation and decreased as culture continued. The pectic polysaccharides decreased during 18 hours incubation.

D-Galacturonic acid, a constituent of main chain of pectin, was produced from 6 to 12 hours of culture, followed by complete consumption. Also, the amount of short chain fatty acid were analyzed during incubation of 3 feces, acetate, propionate and butyrate were produced. Acetate decreased from the middle stage of culture, propionate and butyrate increased to the final stage of culture. However, butyrate was not produced in Donor 2 and 3.

Gut bacteria having pectin degrading ability were isolated purely. The isolated strains were *Enterococcus* species (*E. mundtii, E. faecalis, E. lactis*, and *E. faecium*), *Weissella cibaria*, and *Clostridium perfringens*. Among the isolated strains, *E. mundtii* exhibited the highest pectin degradation rate as 33%.

CHARACTERISATION OF EXOPOLYSACCHARIDES PRODUCED BY *Lactobacillus fermentum* LF2 AND THEIR POTENTIAL APPLICATIONS AS IMMUNE MODULATORS.

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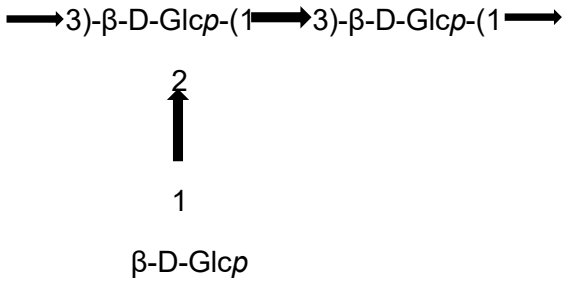
Lactic acid bacteria (LAB) are valuable for commercial food fermentations and have also been recognised as health-promoting micro-organisms. LAB is known to synthesise exopolysaccharides (EPS) which are potential biopolymers due to their unique characteristics, biological activity and safety. *Lactobacillus fermentum* LF2, a strain isolated as a non-starter culture in Cremoso cheese, produces a high crude EPS level (~ 2 g/L) in optimised conditions (SDM broth, pH 6.0, 30 ^{0}C, 72 h). After purification and fractionation of the crude EPS, two polysaccharides were separated: a high molecular weight and a medium molecular weight EPS. The high molecular weight EPS was of high purity, whereas the medium molecular weight was quite complex and its structure is still under study. Using chemical analytical techniques; high performance anion exchange chromatography, gas chromatography-mass spectrometry, size exclusion chromatography and nuclear magnetic resonance spectroscopy, the high molecular weight EPS has been shown to possess a trisaccharide repeating unit having the following structure:



The high molecular weight EPS produced by *Lactobacillus fermentum* LF2 is the same as that which is produced by two other strains of bacteria including: *Pediococcus damnosus 2.6* [1] and *Oenococcus oeni* I4 [2].

Provisional results on the biological activity of the high molecular weight EPS are interesting as we have seen significant TNF-alpha suppression in the presence of the EPS, this will be discussed in the poster.

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EFFECT OF RAW STARCHES AT GUT MICROBIOTA IN MICE

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Gut microbiota are one of the important factors for human health and was influenced by diet. Previously, we demonstrated that raw potato starch-based feeds controlled body weight and insulin resistance in mice. To investigate the association between phenotypic changes and impact on the gut microbiota, corn, wheat, rice or potato starch, substituted based on the nutritional composition of AIN93G, were provided *ad libitum* 6 week-aged C57BL/6 male mice for 16 weeks. Metagenome of microbiota in the mice's feces and cecum were analyzed by 16s rRNA gene sequencing and QIIME pipeline with Greengenes database. The ratio of Firmicutes to Bacteroidetes was decreased by feeding potato starch based-diet and taxonomy assignment indicated that abundances of *Akkermansia, Rikenellaceae* and *Sutterella* were increased at both feces and cecum of mice fed potato starch based-diet.

According to the literatures, it reported that *Akkermansia* and *Sutterella* are associated with improvement of energy metabolism and especially, *Akkermansia* known to be correlated with the abundance of *Rikenellaceae* have been demonstrated to associate with an increase in the large intestine weight and insulin sensitivity. Therefore, it supposes that change of gut microbiota affected according to kind of raw starch and especially, change by potato starch based-diet has associations with beneficial effects on body weight and insulin resistance.

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SYNTHESIS OF PRODRGUS DERIVED FROM AI-2 AND BETA-GLYCOSIDES TO MANIPULATE THE LEVELS OF AI-2 IN THE GUT MICROBIOTA

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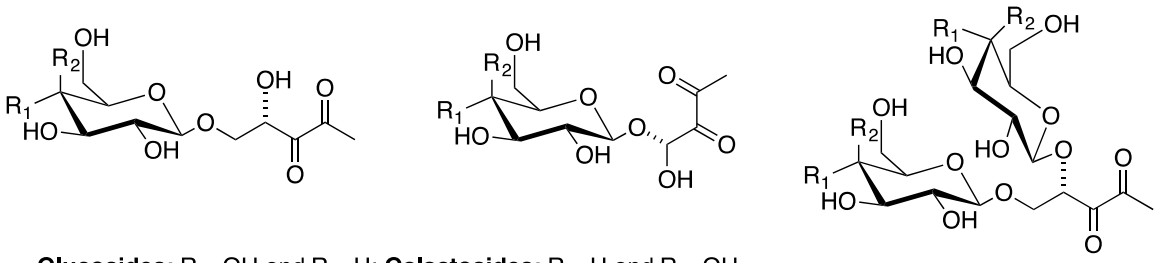
Many species of bacteria control gene expression on a community-wide scale by producing, secreting, detecting and responding to extracellular signaling molecules that accumulate in the environment in proportion to cell density. This process is called quorum-sensing and enables these organisms to coordinate their behaviours which are more beneficial when cells are working in unison.[1] Autoinducer-2 (AI-2) is one of the extracellular signal molecules and is unique as it is produced and detected by many phylogenetically distinct bacteria.[2,3]

In past years we have developed the synthesis of (*S*)-4,5-dihydroxypentane-2,3-dione (DPD), which is AI-2 precursor and analogues in optically pure form.[4,5] Based on previous results, we hypothesise that AI-2 plays an important role in controlling colonization and homeostasis of gut microbiota contributing to the protective properties of these poly-species against pathogens.[1] Here we present a synthetic strategy to obtain new beta-glycoside analogues of AI-2 (Fig.1) that will function as prodrugs to deliver intact AI-2 into the mammalian gut, taking advantage from the enzymes that are only produced in the gut, the beta-glycosidases. The anomeric selectivity of the glycosylation reaction between suitable DPD precursors and several different thioglycosides has been studied and optimised to obtain a good selectivity for the beta anomer.



Glucosides: R_1=OH and R_2=H; **Galactosides:** R_1=H and R_2=OH.

Fig. 1 – Final structure of the desired prodrugs

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SWEET CROSSTALK: GETTING A MOLECULAR-LEVEL GRIP ON GLYCOCODES AT THE HUMAN MUCOSA–MICROBIOTA INTERFACE

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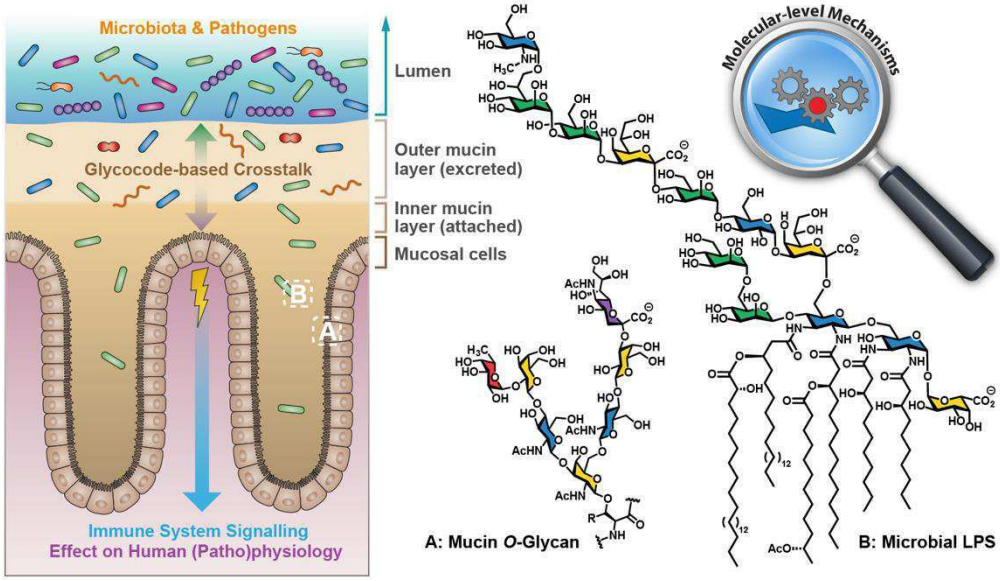
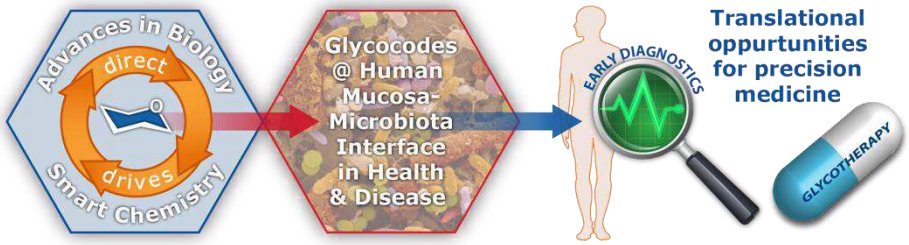
Research of the past decade has uncovered the great diversity and abundance of the human microbiome and how these bacteria directly influence our physiology in health and disease. They protect against invading microbes, aid in degrading non-digestible dietary components, produce key nutrients and vitamins, and prime our immune system. The time has arrived for the mechanistic and functional analysis of mucosal microbiota to unravel the interactions with their host at the molecular-level. Glycans dominate the microbiota-host interface and two classes of glycans in particular are ideally positioned to modulate these interactions between us, our microbiota and amongst the various classes of microbes in it. These key "glycocodes" are the mucin *O*-glycans and the microbial cell envelope glycans. Mapping these glycans, glycan protein-interactions, carbohydrate-active enzymes and their dynamics for individual microbiota members and their co-cultures will therefore be crucial to understand how these complex bacterial communities influences human physiology in health and disease. A better understanding of these glycocodes would also greatly aid the development of early diagnostics and glycotherapeutics for personalized microbiome health. Acquiring a molecular understanding of glycan composition, activities and interacting proteins/ enzymes in their native setting is best achieved by a chemistry guided approach as the non-template driven assembly of glycans prevents the use of conventional molecular biology methods.[1] However, progress is currently hampered by a lack of validated chemical approaches that enable such studies on live microbiota members and well-defined *O*-glycans and microbial glycans in their native environment or advanced model systems.



Overview mucosa-microbiota interface and example of A: mucosal & B: microbial glycocode

The strategy of the Sweet Crosstalk research programme focuses on optimal synergy between chemistry and biology. Smart chemistry and molecular tools drives the research to get a molecular-level grip on the role of these glycocodes at the human mucosa–microbiota interface and their interacting proteins, and advances in biology directs the research.



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GLYCONMRSEARCH - A SEARCH ALGORITHM FOR CHEMICAL SHIFT CORRELATIONS OF UNKNOWN OLIGOSACCHARIDES

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Elucidating the chemical structure of an unknown oligosaccharide is still a challenge. Chemical shifts are a rich source of information about the topology and configuration of oligosaccharides. However, their potential is not fully exploited for oligosaccharides yet. We hypothesize that the chemical shifts of each monosaccharide are unique for each saccharide type with a certain linkage pattern, so that correlated data measured by NMR spectroscopy can be used to identify the chemical nature of an oligosaccharide.

An efficient search algorithm was developed, GlycoNMRSearch, that matches either a subset or the entire set of chemical shifts of an unidentified monosaccharide spin system to all spin systems in an NMR database. The tool is available as a web application at http://glyconmrsearch.santos.pwr.edu.pl.

The new search algorithm is a significant step forward if compared with previous alternatives. It is very precise and false positive results are absent. A similarity score helps to evaluate if a query spin system is identical or similar to existing oligosaccharide structures, making the search for connected chemical shift correlations within all electronically available NMR data of oligosaccharides an efficient way of identifying unknown carbohydrate structures. By adding more data and improving data bank, the chances are high that GlycoNMRSearch will become indispensable for the field of glycomics.

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NMR SIMULATION AND STRUCTURE ELUCIDATION OF SACCHRIDES

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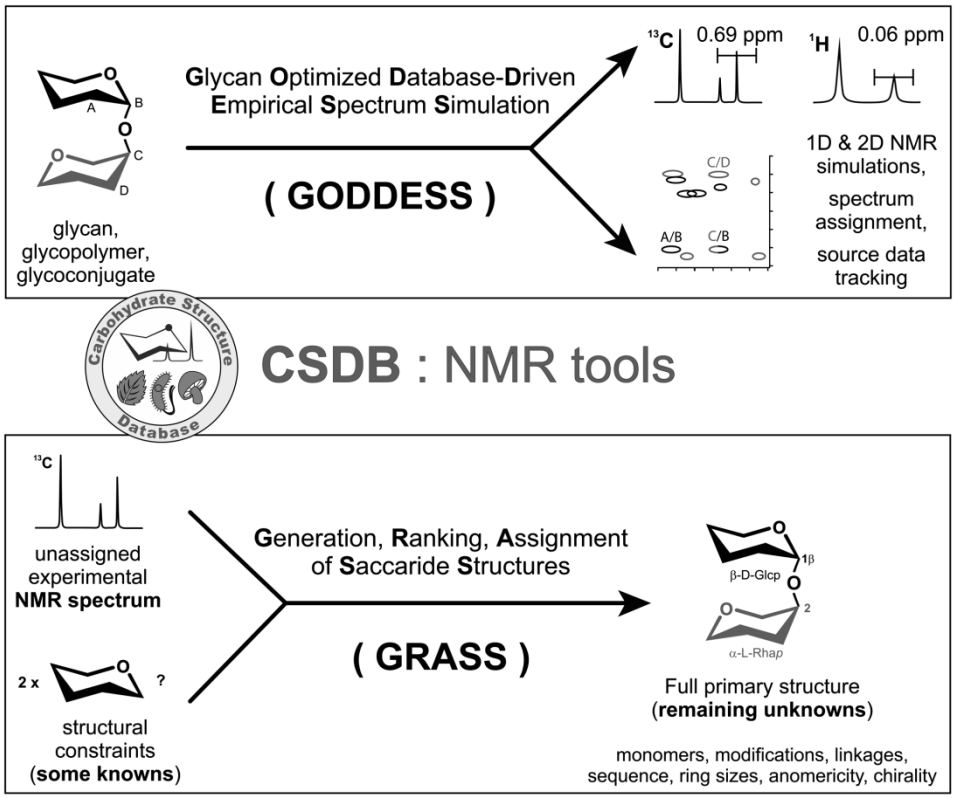
GODDESS (**G**lycan-**O**ptimized **D**atabase-**D**riven **E**mpirical **S**pectra **S**imulation) is a new method to simulate NMR observables of bioglycans, including those containing amino acids, lipids, alditols, phosphates and other non-carbohydrate constituents. This approach was tuned for prediction of ^{13}C and ^{1}H chemical shifts and exhibited the average accuracy of 0.69 ppm per ^{13}C resonance and 0.06 ppm per ^{1}H resonance on a random pool of structures synthesized by microorganisms. The method was proved to be the most accurate method for NMR simulation of carbohydrates [1]. The simulation is based on sequential generalization of the chemical surrounding of the atom under prediction and on heuristic averaging of matching data from the database containing several thousand NMR spectra. The generalization pathways are iteratively mutated to minimize the total structural perturbation with regard to the specific features of carbohydrate molecules.



GRASS (**G**eneration, **R**anking and **A**ssignment of **S**accharide **S**tructures) is a method for automated elucidation of structures of glycans and derivatives which uses unassigned ^{13}C NMR spectra and information from other methods. It is based on the iteration of structures according to the given constraints, and ranking of structural hypotheses according to the similarity between the simulated and experimental spectra. Top 500 matches obtained by the rough empirical prediction are further refined by accurate statistical method (GODDESS). According to the validation results, it excels other methods in the diversity of supported structural features, accuracy and performance [2].

Both GODDESS and GRASS rely upon all the NMR data accumulated in the Carbohydrate Structure Database (CSDB), into which they are integrated. They allow prediction of structures and manual analysis of predictions, including 1D and 2D NMR simulation (COSY, TOCSY, HSQC, HMBC and other) [3], spectrum assignment, estimation of trustworthiness and molecular geometry calculations. Free web services at CSDB platform are available: http://csdb.glycoscience.ru/godess.html and http://csdb.glycoscience.ru/grass.html

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CSDB_GT: GLYCOSYLTRANSFERASE DATABASE AT THE PLATFORM OF CARBOHYDRATE STRUCTURE DATABASE

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Carbohydrate-active enzymes (CAZy) assemble and process glycans in all living organisms. Reliable information on biochemical activities of CAZy is demanded in studies on structural characteristics of bioglycans, whereas application of CAZy in biotechnology facilitates the synthesis of valuable glycan products. We present CSDB_GT, a curated database on glycosyltransferases (GTs) that participate in biosynthesis of natural glycans [1]. CSDB_GT is a dedicated database at the platform of the Carbohydrate Structure Database (CSDB) project [2]. Currently, it provides the coverage on GTs with confirmed functions from the known model organisms *Arabidopsis thaliana* and *Escherichia coli*. CSDB_GT includes protein and gene identifiers (names, IDs and cross-links to other databases), activity data (linkage, donor and substrate), trust level (*in vivo*, *in vitro, in silico*), full glycan structure (if known), taxonomic data, and literature references. The data are manually retrieved by expert glycobiologists from original publications. The database supports search by IDs (UniProt, GenBank, etc.), enzyme name, activity and organism. CSDB_GT is freely available as a web-service at http://csdb.glycoscience.ru/gt.html. This work was funded by the Russian Foundation for Basic Research (grant 18-04-00094).

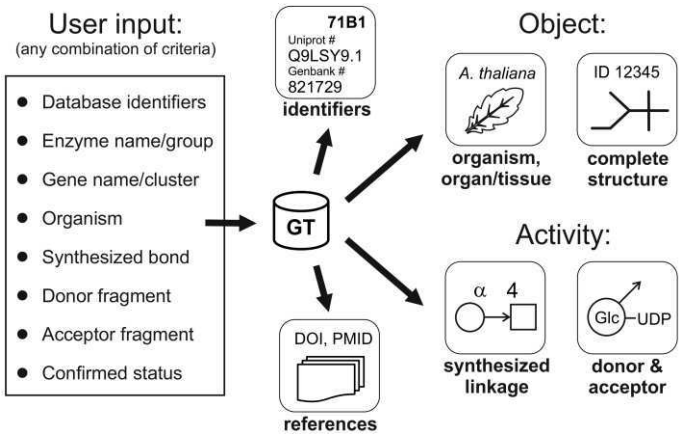

Fig. 1. GT_CSDB user input and output.

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EVOLUTION OF CHITIN DEACETYLASES. THE "SUBSITE CAPPING MODEL".

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Chitin deacetylases (CDAs) catalyze the hydrolysis of the acetamido group in GlcNAc residues of chitin, chitosan, and chitooligosaccharides (COSs). The particular distribution of GlcNAc and $GlcNH_2$ moieties along the oligomeric chain of COSs dictates their role in cell signaling and other biological activities. A major challenge is to understand how CDAs specifically define the acetylation patterns of natural COSs. This is paving the way to engineer novel CDAs to produce non-natural COSs with unique acetylation patterns [1]. A structural analysis of this family of enzymes lead us to the proposal of a model to rationalize the acetylation pattern exhibited by different CDAs: the "subsite capping model" [2]. According to this model, the acetylation pattern exhibited by different CDAs is governed by critical loops that shape and differentially block accessible subsites in the binding cleft of these enzymes [3,4]. We will present here the bioinformatics screening of genomic databases in the search for novel CDAs compatible with our model, together with an analysis of their primary sequence composition. An ensemble of several thousands of putative chitin deacetylases is classified in different phylogenetic trees. The sequence profiles of the loops defining the "subsite capping model" are compared between groups of CDAs exhibiting different deacetylation patterns. The sequence and structure heterogeneity of loops in CDAs is compiled here in the form of hidden-markov models complementing the "subsite capping model" as a rational tool to understand how CDAs define the acetylation pattern.

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INTEGRATION OF OMICS DATA THROUGH GLYCOSMOS

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The Life Science Database Integration Program led by the Japan Science and Technology (JST) and National Bioscience Database Center (NBDC) has been sponsoring the integration of life science databases for over ten years. As a part of this program, most recently, the international glycan structure repository GlyTouCan was developed to fill the gap of the lack of a repository for glycans [1], and it now allows scientists to register glycans and obtain accession numbers for use in publications [2]. These accession numbers also allow glycan databases, as GlyTouCan Partners, to easily link with each other, and GlyTouCan provides these links from each glycan entry page.

The remaining gap to be filled was that between the glycosciences and other omics fields, especially genomics and proteomics, which are closely related to glycans. Considering that glycan function is very closely related to the genes that synthesize them as well as the proteins that they decorate, it was important to integrate these datasets more closely. Thus, new funds were recently granted to develop a Glycoscience Portal, which we call GlyCosmos. Now in the second year, GlyCosmos provides a web interface to not only learn about glycans, but also to learn about their related genes, proteins and lipids. Since the PDBj [3] and jPOSTrepo[4] are also funded by this program, collaborations are now in place to link with protein structures and proteomics data, respectively. GlyCosmos will also provide access to not only GlyTouCan, but also a new Glycoconjugate Structure Repository, called GlyComb, and a new Lipid Structure Repository. GlyCosmos will also include a pathway database for understanding glycan function, and a Total Glycome Database which is a database of glycomes of various types of cells and cell lines. Integration is also being planned with the U.S. GlyGen project, GlyConnect as well as UniCarbKB. Thus in the near future, users should be able to get a grasp of glycan structures and their functions just by accessing GlyCosmos Portal.

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PROGRESSION TOWARDS THE AUTOMATED ASSIGNMENT OF CARBOHYDRATE SPECTRA: CLIP-COSY/RELAY AND HSQC-CLIP-COSY/RELAY NMR EXPERIMENTS

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Nuclear Magnetic Resonance (NMR) spectroscopy is currently the most viable method for the analysis of oligosaccharide mixtures; it is a non-destructive method that can distinguish carbohydrate types, determine the position of glycosidic linkages, and structural analysis (within reason) can be performed without the need for physical separation. Nevertheless, the NMR spectra of oligosaccharide complex mixtures are complex, where manual analysis is monotonous, time consuming, and error prone. Whilst software for the automated assignment of protein backbones [1,2] and small molecules [3,4] is readily available, no such software exists for carbohydrates. If the spectral analysis of carbohydrates could be semi-automated, this would save precious time, minimise human error, and accelerate research.

We have used recently published CLIP-COSY [5] and CLIP-COSY/RELAY (single and double) 2D homonuclear ^{1}H experiments, in combination with HSQC, and HSQC CLIP-COSY/RELAY heteronuclear ^{1}H-^{13}C experiments [6] to begin the automated assignment process. The relevant 1D ^{1}H traces were extracted semi-automatically from each of the CLIP-COSY homonuclear spectra, and the peaks were assigned based on the order they appeared. Subsequently, using the 2D HSQC-CLIP-COSY spectra in accordance with the ranked ^{1}H chemical shifts, we were able to extract the corresponding ^{13}Cchemical shifts and confirm the ^{1}H assignments.

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CONFORMATIONAL DEFECTS OF CARBOHYDRATES IN CRYSTALLOGRAPHIC STRUCTURES

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The growing availability of 3D protein-carbohydrate complex structures promotes the study at atomic level of theirs interactions, which are the basis of numerous biological processes. PDB contains a number of carbohydrate defects, estimated at 30% [1]. This is mainly due to the lack of carbohydrate torsion control during crystallographic structure refinement. Many softwares exist to control torsion, but the difficulty of controlling the constraints leads to its pure and simple deactivation, whatever the resolution.

Here we report an example of the most abundant carbohydrate in the PDB: β-D-GlcNAc. A conformational anomaly is regularly observed at the ω angle, defining the bond between carbon sp^2 and nitrogen sp^2 into the acetamido group. The ω angle should correspond to the typical planar character of N-acetyl group, stabilized by mesomeric effect. However, the angle, usually trans and more rarely cis, happens to have a standard deviation of 21° and 33°, respectively. As a comparison, the ω angle in peptide bonds has a significantly smaller standard deviation: approximately 5°. Indeed, the deformation of the torsion angle is frequently observed in the case of the near perfect $^4C_1$ chair. In most cases, the deformation of the angle is not consistent with puckering. This is probably due to the lack of understanding of crystallographic tools controlling carbohydrate puckering observed in most PDB crystallographic structures.

Community efforts to re-refine structures such as PDB_REDO [2] significantly improve crystallographic models. PDB_REDO optimizes carbohydrates based on geometry libraries. The ω angle anomaly tends to disappear for discretely optimized models, while it disappears completely for fully optimized models. A new correlation study between the deformation of the ω angle and the puckering are performed on the re-refined models. The results show that the deformation of the ω angle is more consistent with puckering. It seems to result from a better balance of torsional restraints within β-D-GlcNAc. In conclusion, PDB_REDO appears to be a reliable database for glycoprotein or protein-carbohydrate complex studies, despite the presence of other types of carbohydrate defects. In addition, the use of carbohydrate validation tools such as pdb-care [3], Privateer [4], should be generalized [5].

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CYCLOMOLDER SOFTWARE: BUILDING CYCLODEXTRIN DERIVATIVES MODELS AND EVALUATING THEIR HOST:GUEST INTERACTIONS

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Background: Many cyclodextrin (CD) derivatives are complex mixtures, since the number of possibilities for positional isomers is very large. The number of possible positions for a substituent group is given by the number of possible positions at glucose unit (three hydroxyl groups: OH (2), OH (3) and OH (6)), multiplied by the number of glucose unit. The number of possible configurations is obtained from the combinatorial calculation divided by the number of glucose units. In the case of β-CD, the maximum number of position isomers (50,388) is achieved by replacing half of the hydroxyl groups [1]. During the synthesis process, homologous derivatives, with low or high molar substitution (MS) ratio, are also formed in addition to the product. The synthesis of uniform CD derivatives is more expensive and complicated, and it also requires regio selective reagents. Therefore, it is easier to find CD derivatives as statistical mixtures with a particular MS ratio than uniform CD derivatives. These issues make it even more difficult to define a suitable in silico model [1]. **Objectives:** The main goal of this work is to develop a software, able to build theoretical models of cyclodextrin derivatives and evaluate their host:guest interactions in an automated and precise fashion, taking into account aspects of the derivative synthesis and making use of a friendly graphical user interface (GUI). **Methods:** The software was written in C++ code, with the QT framework to create a native application with GUI. It also uses others libraries: Avogadro [2], OpenBabel [3] and Qwt 6.1.2 (http://qwt.sourceforge.net). It was possible to allow real-time visualization for the user, and also assist the construction of CDs substituent groups by using Avogadro library. OpenBabel was used to build configurations of CD derivatives, optimize structures and perform conformation searches. Qwt library contains a framework for 2D plots, which enables a graphical analysis of the results obtained for the inclusion complexes. Docking calculations are performed using Autodock Vina software [4], allowing docking of flexible guests to CDs and their derivatives. The starting three-dimensional structures for α, β and γ-CDs came from well-known data at the literature [5]. **Results:** The CycloMolder software consists of two modules: CycloGen and CycloDock. The first module builds theoretical models with more than one chemical structure to represent a cyclodextrin derivative. These structures are divided into configurations and conformations. The configurations can be homologous structures, with different MS ratio, or just positional isomers. Conformers are generated from the built configurations. The second module performs the docking calculations between the host and guest molecules, and displays the final results of the modeled inclusion complexes, including graphs showing the distribution energy and intermolecular interactions present in the host:guest complex. **Conclusions:** The CycloMolder seems to reach the proposed goal of automating and facilitating the construction of models of CD derivatives, taking some important variables into account (including experimental information from synthetic process), like homologous generation and regioselectivity. The work of Xavier-Junior (2017) was published using CycloMolder software [6], where it is possible to perceive the importance of using CD derivatives models characterized by an ensemble of structures, in order to consider the statistical feature of the problem. Thus, we expect that this software could significantly contribute to the studies of host:guest inclusion complexes involving cyclodextrins and their derivatives.

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CARBOHYDRATE-AROMATIC INTERACTIONS IN HALOGENATED SUGAR MIMETICS: THE ROLE OF C-Br···π BONDS

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Protein-carbohydrate interactions play an important role in many biological processes [1], the understanding of the underlying mechanisms being crucial for carbohydrate-based drug design. Besides the ubiquitous hydrogen bond, noncovalent interactions involving aromatic rings are particularly important in sugar binding [2]. These interactions, normally referred to as C-H···π bonds, can involve two or three C-H groups from pyranoses and the aromatic side-chains of aminoacids (i.e. Phe, Tyr, Trp). One of the strategies used to improve properties such as carbohydrate-protein binding affinity is to design carbohydrate mimetics [3]. In this scope, fluorination is quite common, however, the use of heavier halogens (Cl, Br, I) is not usually employed. In the latter case, the existence of halogen bonds, which are noncovalent interactions of the type R-X···B (X = Cl, Br, I; B = Lewis base) [4], is possible. Besides being extremely relevant in the context of biological systems [5], C-X···π halogen bonds share some common features with the C-H···π bonds, namely their directionality. In this work, we employed DFT calculations to compare the energetics and geometries of carbohydrate-aromatic interactions in model systems. In particular, we used a variety of β-D-fucose···phenol, β-D-fucose···indole, and β-D-fucose···benzene dimers possessing C-H···π bonds [6] and compared them with equivalent brominated fucose derivatives (see Fig.1) featuring C-Br···π halogen bonds. With these studies, we provide the first insights on how this specific type of noncovalent interaction can be explored in protein-carbohydrate systems.

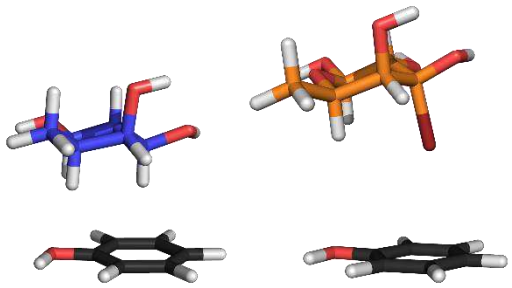

Fig. 1. Example of the optimized structure of a β-D-fucose···phenol dimer interacting via a C-H···π hydrogen bond (left), and a brominated analogue featuring a C-Br···π halogen bond (right).

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INTERNATIONAL CARBOHYDRATE SYMPOSIUM

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P-BS11 NOVEL BACTERIAL AND FUNGAL α-GLUCURONIDASES FOR THE DEVELOPMENT OF MULTIENZYMATIC PROCESSES
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P-BS25 HIGHLY BRANCHED XYLOGALACTOFURANAN ISOLATED FROM BIOMASS OF HETEROTROPHIC MUTANT MICROALGA *CHLORELLA VULGARIS* G11
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P-BS27 POLYSACCHARIDES PROFILE OF *PORPHYRIDIUM CRUENTUM* MICROALGAE
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Juliette Caron, Emmanuel Maes, Yann Guérardel, Jean-Michel Roturier, Pierre Heijboer

P-BS30 PEA-DERIVED MALTODEXTRINS AS SOLUBILIZATION ENHANCERS
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P-MD1 INVESTIGATING *EXO*-GLYCALS REACTIVITY - RECENT PROGRESS AND APPLICATIONS
Nadia Pellegrini-Moïse, Sandrine Lamandé-Langle, Julen Ariztia and Mylène Richard

P-MD2 (AUTOMATED SOLID PHASE) SYNTHESIS OF *S. AUREUS* WALL TEICHOIC ACID FRAGMENTS
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E. Fernandes, D. Ferreira, R. Azevedo, S. Cotton, L. Lima, A. Peixoto, L. P. Afonso, A. Tavares, A. Silva, B. Sarmento, L. L. Santos and J. A. Ferreira

P-MD5 MULTIVALENT CYCLOPEPTIDES-BASED GLYCOCONJUGATES FOR LECTIN INHIBITION
N. Berthet, C. Pifferi, D. Goyard, A. Imberty and O. Renaudet

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Leila Mousavifar and René Roy*

P-MD7 SYNTHESIS OF FULLY ISOTOPE-LABELED HEPARAN SULFATE OCTASACCHARIDE
Xin-Ren Huang, Teng-Yi Huang and Shang-Cheng Hung

P-MD8 New insights on the structure of hexagonally faceted platelets from hydrophobically modified chitosan and α-cyclodextrin
Zeeshan Ahmed, Gilles Ponchel and **Kawthar Bouchemal***

P-MD9 DEVELOPMENT OF A NOVEL DRUG DELIVERY SYSTEM COMPRISING CARBOHYDRATES FOR DERMAL AND NAIL DELIVERY
Flavia Laffleur

P-MD10 CONCISE SYNTHESIS OF 2,7-ANHYDROSIALIC ACID DERIVATIVES AND ITS APPLICATION
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P-MD12 *C*-GLUCOSYL DERIVATIVES OF SOME IMIDAZO-FUSED HETEROCYCLES: SYNTHESES AND GLYCOGEN PHOSPHORYLASE INHIBITION
Eszter Szennyes, Éva Bokor, Tibor Docsa, Ádám Sipos and László Somsák*

P-MD13 INDIUM-MEDIATED ALLYLATION OF DISACCHARIDES
C. Denner and W. Schmid

P-MD14 THE SYNTHESIS OF HEXAVALENT GLYCODENDRIMERS AS ANTI-VIRAL AGENTS
Lauren O. Wells and Katherine McReynolds

P-MD15 NANOSCALE MAPPING OF MULTIPLE LECTIN SITES ON CELL SURFACES DETECTED BY CARBOHYDRATE-FUNCTIONALIZED AFM TIPS
Stéphane Cuenot, Audrey Bouvrée and Jean-Philippe Bouchara

P-MD16 A LIQUID STABLE BIOLOGICAL ACTIVE SEMI-SYNTHETIC GLYCOCONJUGATE VACCINE AGAINST *HAEMOPHILUS INFLUENZAE* TYPE b
Madhu Emmadi, Marilda P. Lisboa, Bopanna Monnanda, Sharavathi G. Parameswarappa, Sylvia Oestreich, Olaia Nieto-Garcia, Arne Von Bonin* and Claney L. Pereira*

P-MD17 HIGH THROUGHPUT SCREENING APPROACH FOR THE SEARCH OF HUMAN FUCOSYLTRANSFERASE VI (Fut6) INHIBITORS
Xiaohua Zhang, Pat Forgione and David Kwan*

P-MD18 CuAAC AND *IN SITU* SCREENING FOR THE RAPID DISCOVERY OF DIVALENT GLYCOSIDASE INHIBITORS
Antonio J. Moreno-Vargas, Ana T. Carmona, Sebastián Carrión-Jiménez, Valeria Pingitore, Elena Moreno-Clavijo and Inmaculada Robina

P-MD19 CHARACTERIZATION OF THE SULFATED GLYCAN-BINDING SPECIFICITIES OF HUMAN LACTOFERRIN AND ITS DERIVATIVE
Masao Nakamura*, Takumi Matsuzaki, Akira Shiga and Atsushi Sato

P-MD20 OLIGOGALACTOSIDES AS POTENTIAL GALECTIN INHIBITORS
Christophe Dussouy, Chandan Kishor, Annie Lambert, Clément Lamoureux, Helen Blanchard and **Cyrille Grandjean***

P-MD21 CHITOSAN-COATED POLY(ISOBUTYLCYANOACRYLATE) NANOPARTICLES HAVE INTRINSIC TRICHOMONACIDAL AND ANTI-LEISHMANIAL ACTIVITIES
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P-MD22 DETOXIFICATION OF AMINOGLYCOSIDE ANTIBIOTICS THROUGH CHEMICAL MODIFICATIONS TO ENHANCE BINDING TO BACTERIAL RIBOSOMES
Sivan Louzoun Zada and Micha Fridman*

P-MD23 SULFONE-BASED GALECTIN-3 INHIBITORS TO TARGET A NEW POCKET NEAR THE CANONICAL CRD
Mukul Mahanti, Kumar Bhaskar Pal, Rohit Kumar, Anders Sudin, Hakon Leffler, Derek Logan and Ulf J. Nilsson*

P-MD24 GLYCO-NANOPROBES AS DIAGNOSTIC AND AS DRUG DELIVERY SYSTEMS
Venkateswararao Boddu and M. Carmen Galan*

P-MD25 DEFINING THE STRUCTURAL ORIGIN OF ANTIBODIES RECOGNITION OF TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS
Ron Amon, Oliver C. Grant, Shani Leviatan Ben-Arye, Spandana Makeneni, Anita K. Nivedha, Tal Marshanski, Christoffer Norn, Hai Yu, John N. Glushka, Sarel J. Fleishman, Xi Chen, Robert J. Woods* and **Vered Padler-Karavani***

P-MD26 GLYCOCONJUGATED AMINOPORPHYRAZINES AS POTENTIAL PHOTOSENSITIZERS IN PHOTODYNAMIC THERAPY
Thomas Klein and Thomas Ziegler*

P-MD27 PREPARATION AND CHARACTERIZATION OF N-SUCCINYL-CHITIN NANOPARTICLES AND THEIR APPLICATIONS IN THE OTONEUROLOGICAL PATHOLOGY
Yury A. Skorik, Valentina A. Petrova, Aleksey A. Panevin, Sergei G. Zhuravskii and Ekaterina R. Gasilova

P-MD28 POTENT METABOLIC SIALYLTRANSFERASE INHIBITORS BASED ON C-5 MODIFIED FLUORINATED SIALIC ACIDS
Johan F. A. Pijnenborg, Torben Heise, Christian Büll, Esther D. Kers-Rebel, Natasja Balneger, Hidde Elferink, Gosse J. Adema* and Thomas J. Boltje*

P-MD29 CYCLOPENTANOID D-GALACTOSIDASE INHIBITORS AND POTENTIAL PHARMACOLOGICAL CHAPERONES FOR G_{M1} GANGLIOSIDOSIS
A. E. Stütz, P. Weber, B. M. Pabst, E. Paschke, W. Windischhofer, M. Tschernutter, T. M. Wrodnigg and A. Wolfsgruber

P-MD30 SYNTHESIS AND CHARACTERIZATION OF NEW COPPER(I) COMPLEXES COMPRISING CARBOHYDRATES FOR CANCER THERAPY
Tânia S. Morais, Catarina Bravo, João Franco Machado, Maria José Villa de Brito, Nuno Xavier and Maria Helena Garcia

P-MD31 SYNTHESIS OF 4-AZIDO-B-GALACTOSAMINE DERIVATIVES FOR INHIBITOR OF *N*-ACETYLGALACTOSAMINE 4-SULFATE 6-*O*-SULFOTRANSFERASE
Hirofumi Nakano*, Seanghai Hor, Takumi Kodama, Kazuya I. P. J. Hidari, Nobuo Sugiura, Hideto Watanabe and Osami Habuchi

P-MD32 NEW MEANS OF TARGETED DRUG DELIVERY OF ANTITUMOR DRUGS FOR HCC THERAPY BASED ON N-ACETYLGALACTOSAMINE DERIVATIVES
R. A. Petrov, S. Yu. Maklakova, E. Yu. Yamansarov, E. K. Beloglazkina and A. G. Majouga

P-MD33 *HERICIUM ERINACEUS* ISOLECTINS RECOGNIZE MUCIN-TYPE *O*-GLYCANS AND POLYFUCOSYLATED *N*-GLYCANS AS TUMOR-ASSOCIATED CARBOHYDRATE ANTIGENS IN CANCER CELLS
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P-MD34 POLYPLEX FORMATION OF DNA WITH CATIONIC CHITOSAN DERIVATIVES
S. V. Raik and Y. A. Skorik

P-MD35 RATIONAL DESIGN OF COVALENT INHIBITORS FOR A BACTERIAL GLYCOSYLTRANSFERASE
Camille Metier, Yong Xu and Gerd K. Wagner

P-MD36 DESIGN, SYNTHESIS, AND EVALUATION OF IMPROVED AMINOGLYCOSIDES
Michael Pirrone, Takahiko Matsushita, Girish C. Sati, Takayuki Kato, Sven N. Hobbie, Thomas Schepfer, Jochen Schacht, Andrea Vasella, Erik C. Böttger, and David Crich

P-MD37 CHEMOENZYMATIC SYNTHESIS OF PSEUDAMINIC ACID CONTAINING GLYCOSIDES
Emily Flack, Harriet Chidwick, Matthew Best, Kun Huang, Tessa Keenan, Sabine Flitsch, Gavin Thomas and Martin Fascione

P-MD38 PDA-BASED NANOMICELLES FOR THE DIAGNOSIS AND TREATMENT OF HEPATIC AND PROSTATE CANCER
Christian Rosales Barrios, Teresa Mena Barragán, Emilio Guillén Mancina, María

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P-MD39 INTERACTION AND CONFORMATION STUDIES OF A NOVEL ANTITHROMBIN BINDING OCTASACCHARIDE NOT INCLUDING THE ACTIVE PENTASACCHARIDE SEQUENCE
Eduardo Stancanelli, Stefano Elli, Po-Hung Hsieh, Jian Liu and Marco Guerrini

P-MD40 SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF NEW RUTHENIUM COMPLEXES DERIVATIZED WITH GLUCOSE AIMED AT SELECTIVE CANCER THERAPY
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P-MD41 SYNTHESIS OF NEW NUCLEOS(T)IDE ANALOGUES AND EVALUATION OF THEIR CHOLINESTERASE INIBITORY ACTIVITIES
Eduardo C. de Sousa, Margarida P. Pereira, Arben Beriša, Rita G. Pereira, Anne Loesche, René Csuk and Nuno M. Xavier

P-MD42 SYNTHETIC MARADOLIPIDS AS MINCLE AGONISTS
Ayesha Khan, Kristel Kodar, Mattie S. M. Timmer and Bridget L. Stocker

P-MD43 GLYCOCLUSTERS AS TOOLS FOR INHIBITION OF LECTIN MEDIATED BACTERIAL ADHESION
Eva Fujdiarová, Lenka Malinovská, Martina Kašáková, Jitka Moravcová and Michaela Wimmerová

P-MD44 A NOVEL CARBOHYDRATE-BASED PROBE FOR BACTERIAL PROTEOMICS
Gerd K. Wagner, Jiaming Peng, Camille Metier, Yong Xu, Steven Lynham and J. Mark Sutton

P-MD45 (1-4)-*S*-THIODISACCHARIDES AS POTENTIAL ANTI-GLIOMA DRUGS
Joanna Sarnik, Monika Toma, Anna Macieja, Tomasz Śliwiński, Zbigniew J. Witczak and Tomasz Popławski

P-MD46 LOW ESTER AMIDATED CITRUS PECTIN AND FLAXSEED GUM AS SUITABLE CAREERS FOR WOUND HEALING AGENTS
Alla Sinica, Pavla Poučková, Marie Zedinová and Vladimír Kral

P-MD47 UNNATURAL GLYCOPEPTIDES AND GLYCOPROTEINS AND THEIR USE IN ANTIGEN DETECTION AND IMMUNIZATION
I. S. Albuquerque, V. J. Somovilla, I. A. Bermejo, N. Martínez-Sáez, J. M. Peregrina, F. Corzana and G. J. L. Bernardes

P-MD48 ADVANCEMENTS IN THE STRUCTURAL CHARACTERISATION OF AN ADENOCARCINOMA-RELATED MATRIX EPITOPE DEFINED BY THE MONOCLONAL ANTIBODY A10
Ioanna Kalograiaki, Marcos Viñuela, José I. Tudela, Ana I. Manzano, José L. Subiza and F. Javier Cañada

P-MD49 CYCLODEXTRIN-BASED MOLECULAR VECTOR THERANOSTICS
M. González-Cuesta, L. Gallego Yerga, V. Ceña*, J. M. García Fernández* and C. Ortiz Mellet

P-MD50 MOLECULAR ENGINEERING OF CYCLODEXTRIN-BASED ARTIFICIAL VIRUSES: SELECTIVE CHEMICAL MANIPULATION AT CONTROL OF GENE DELIVERY

CAPABILITIES
Tania Neva, Juan M. Benito, T. Carmona, Conchita Tros de Ilarduya, Carmen Ortiz Mellet, Francisco Mendicuti and José M. García Fernández

P-MD51 FROM A TEA CUP TOWARDS A NEW DRUG CANDIDATE: EXPLORING THE MULTITARGET MECHANISM OF ACTION OF A PROMISING C-GLUCOSYL ISOFLAVONE WITH THERAPEUTIC EFFECTS AGAINST TYPE 2 DIABETES
A. M. Matos, M. T. Blázquez-Sánchez, I. Lima, I. Mollet, P. Macedo and A. P. Rauter

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P-GD1 GLUCOSYL-GALACTOSYL-HYDROXYLATION OF CCN1 REGULATES ITS SECRETION
Yudai Ishizawa, Yuki Niwa, Takehiro Suzuki, Naoshi Dohmae, Siro Simizu

P-GD2 AUXILIARY-MEDIATED SYNTHESIS OF HOMOGENEOUSLY GLYCOSYLATED SOLUBLE FAS LIGAND VARIANTS
Alanca Schmid, Claudia Bello, Christian Becker

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Vladimir S. Borodkin, Karim Rafie, Martin Hagan, Daan M. F. van Aalten

P-GD4 SYNTHESIS AND FUNCTIONAL STUDY OF LIPID-LINKED BACTERIAL PEPTIDOGLYCAN COMPONENTS
Kuo-Ting Chen, Chia-Ming Hu, Cheng-Kun Lin, Wei-Chieh Cheng

P-GD5 USE OF SUPERACID CONDITIONS TO HIGHLIGHT UNPRECEDENTED TRANSIENT INTERMEDIATES IN GLYCOCHEMISTRY
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P-GD6 SYNTHESIS OF DONOR SUBSTRATES OF 4-AMINO-4-DEOXY-L-ARABINOSE TRANSFERASES
Charlotte Olagnon, Lukas Kerner, Miguel A. Valvano, Paul Kosma

P-GD7 SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL (TRIAZOLYL)METHYL AMIDE-LINKED PSEUDODISSACARIDE NUCLEOSIDES AS POTENTIAL GLYCOSYLTRANSFERASE INHIBITORS
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P-GD8 FUNCTIONAL ANALYSIS OF THE PROMOTER REGION OF C-MANNOSYLTRANSFERASE DPY19L3
Satoshi Yoshimoto, Siro Simizu

P-GD9 IDENTIFICATION AND CHARACTERIZATION OF CHOLESTERYL GLUCOSIDE 6'-ACYLTRANSFERASE IN HELICOBACTER PYLORI FOR ITS ROLE IN PATHOGENESIS
Hau-Ming Jan, Yi-Chi Chen, Tsai-Chen Yang, Danny Yao, **Chun-Hung Hans Lin**

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C. Sofia Proença, Jorge Correia and Maria Eduarda M. Araújo

P-GM19 ALGINATE BASED BLOCK POLYSACCHARIDES
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Tomáš Taubner, Milan Marounek, Andriy Synytsya, Zdeněk Volek, Eva Skřivanová and Dagmar Dušková

P-CN2 ANTI-INFLAMMATORY EFFECT OF POLYSACCHARIDES FROM CYNANCHUM WILFORDII ON DEXTRAN SODIUM SULFATE-INDUCED ACUTE COLITIS IN MICE AND LPS-INDUCED MACROPHAGES
Mi Jang, Young-Ran Song, Tae-Gyu Lim, Young Kyoung Rhee, Chang-Won Cho and **Hee-Do Hong**

P-CN3 APPLE POMACE ARABINAN – UNCOVERING ITS APPARENT HYDROPHOBICITY
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P-CN4 GLYCOSYL FLAVONOIDS FROM SALVIA SCLAREOIDES: CONTRIBUTION TO THE DEVELOPMENT OF A NEW FUNCTIONAL FOOD
Luisa Abraços, Alice Martins, Nuno Neng, José M.F. Nogueira, Maria Luísa Serralheiro, Ana Paula Batista, Anabela Raymundo, Isabel Sousa Tânia Gonçalves Albuquerque, Helena S. Costa, Amélia P. Rauter

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Tadasu Urashima, Masashi Sasaki, Kenji Fukuda, and Sadaki Asakuma

P-GMP2 PROTEIN ENGINEERING OF LACTO-N-BIOSE FROM B. BIFIDUM FOR HMOs PRODUCTION
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P-GMP3 A MECHANISM BASED APPROACH FOR SCREENING METAGENOMIC LIBRARIES FOR UNSATURATED GLUCURONYL HYDROLASES
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P-GMP4 IN VITRO PECTIN FERMENTATION AND ISOLATION OF PECTIN-DEGRADING GUT MICROORGANISMS FROM KOREANS
Ga-Young Kim, Dong-Hyun Jung, So-Jung Bang, Dong-Ho Seo, Cheon-Seok Park

P-GMP5 CHARACTERISATION OF EXOPOLYSACCHARIDES PRODUCED BY *Lactobacillus fermentum* LF2 AND THEIR POTENTIAL APPLICATIONS AS IMMUNE MODULATORS.
Andrew Laws, Sohaib Sadiq, Ana Vitlic, **Hafiz Ahmed**

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