EUROGLYCOFORUM - Science Meeting 4667

Final Report

Carbohydrates are one of the most diverse and important classes of biomolecules in nature. Oligosaccharides found on the surface of cells as part of glycoproteins and glycolipids play key roles in the control of a myriad of normal and pathological processes in living organisms. In order to understand glycan diversity and function, it is essential to have access to structurally defined oligosaccharides in sufficient purity and quantity. However, this is still a very challenging task. In recent years, advancements in carbohydrate synthesis, analysis and glycoarray technology have facilitated the development of chemical approaches to "glycomics" that provide a better understanding of the biological processes involving complex carbohydrates.

The main aim of the workshop was to foster a strong network of young carbohydrate chemists and glycobiologists who will form the next generation of European leaders in glycoscience.

The workshop was held in Berlin (Germany) on the 17th-20th of March 2013. The location of the meeting was the Seminaris Hotel Avendi situated at Griebnitzsee in Potsdam, which offered a tranquil atmosphere that allowed all participants to come together in a relaxing environment during the duration of the workshop and encourage scientific discussion, networking and establishing new collaborations. The workshop was held jointly with the 7th Glycan Forum and on the 20th of March we had a joint session whereby 4 young speakers (3 of which were bursary awardees) and 1 of the invited senior academics presented their work at the Glycan Forum venue in Berlin.

The meeting brought together 26 young European investigators (junior academics, post-doctoral fellows and 1 PhD student) and internationally recognized leaders in the field of glycoscience (see list of speakers below). A number of travel bursaries were provided to nominated young speakers to ensure the participation of all countries.
The participants came from the following countries: Germany, Italy, France, Czech Republic, United Kingdom, Ireland, The Netherlands, Austria and USA.

During the workshop, the latest developments in oligosaccharide synthesis and its application to glycobiology, structural glycobiology, drug discovery and future challenges for the field were discussed.

Each attendee gave an oral seminar of 15 min (with additional 5 min for discussion) presenting their latest research results. Invited lectures gave of 45 min presentations (with additional 5 min for discussion). The sessions were arranged in blocks allowing time for breaks in between to encourage interactions between speakers and participants.

The format of the workshop meant that there were many discussions not only during breaks but also after each speaker presentation, often surpassing the 5 minutes allocated for questioning. From the meeting several potential collaborations were established and there were talks to hold a subsequent Young Carbohydrate Chemists Workshop in 2015.

Organizers:

Dr. M. Carmen Galan, Dr. Bruce Turnbull and Dr. Daniel Varon Silva

European Young Investigators Workshop-
"Deciphering the Glycome –from Synthesis to applications"

Registration List

Name	Institution	Country	E-mail address
Invited Speakers			
Prof. Peter H. Seeberger	MPIKG	Germany	peter.seeberger@mpikg.mpg.de
Prof. Alexei Demchenko	University of Missouri	U.S.A.	demchenkoa@umsl.edu
Prof. Anne Imberty	CERMAV-CNRS	France	imberty@cermav.cnrs.fr
Prof. Serge Perez	CERMAV-CNRS	France	serge.perez@cermav.cnrs.fr
Prof. Ben Davis	National University of Ireland	Ireland	trinidad.velascotorrijos@nuim.ie
Prof. Gijs van der Marel	Oxford University	U.K.	bgdpa@chem.ox.ac.uk
Dr. Daniel Varon Silva	MPIKG	Germany	daniel.varon@mpikg.mpg.de
Dr. Carmen M Galan	University of Bristol	U.K.	m.c.galan@bris.ac.uk
Dr. Bruce Turnbull	University of Leeds	U.K.	w.b.turnbull@leeds.ac.uk
Dr. Daniel Kolarich	MPIKG	Germany	daniel.kolarich@mpikg.mpg.de
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Travel Bursary Awardee			
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Dr. Emiliano Bedini	Università degli Studi di Napoli "Federico II"	Italy	ebedini@unina.it
Dr. Christian Stanetty	University of Natural Resources and Life Sciences, Vienna	Austria	christian.stanetty@boku.ac.at
Dr. Aliaksei Pukin	Utrecht University	The Netherlands	A.Pukin@uu.nl
Mr. Roberto Cighetti	Università di Milano-Bicocca	Italy	roberto.cighetti@unimib.it
Dr. Lenka Malinovská	Masaryk University	Czech Republic	malinovska@mail.muni.cz

European

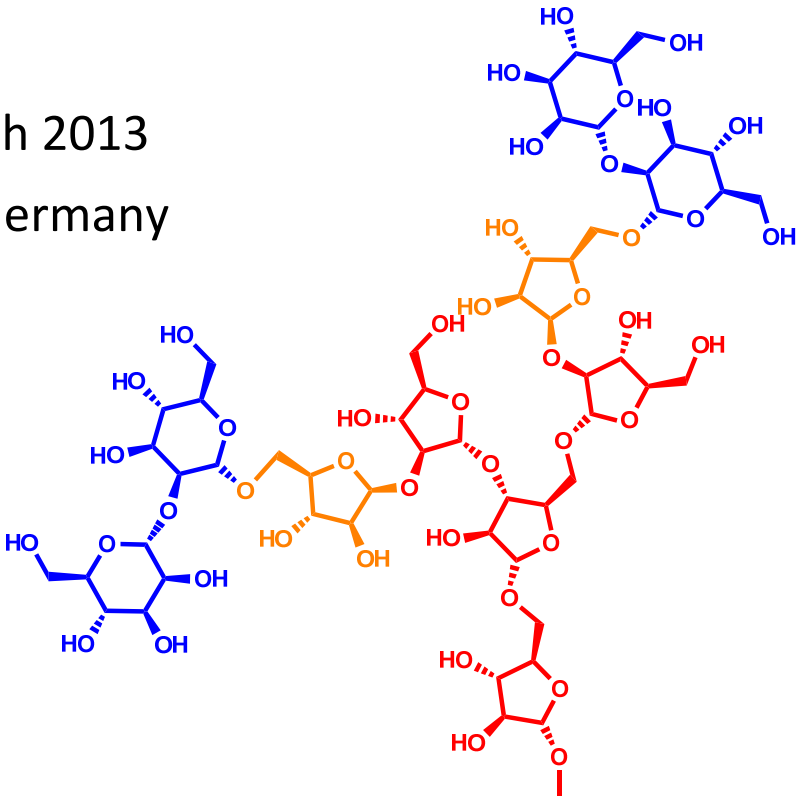
Young Investigators

Workshop

Deciphering the Glycome: From Synthesis to Applications

17th – 20th March 2013

Potsdam/Berlin, Germany



EUROPEAN SCIENCE FOUNDATION

Programme for the European Young Investigators Workshop

"Deciphering the Glycome – from synthesis to applications"

17th – 20th March 2013 – Berlin, Germany

Sunday		
1530	Introduction	
1540	**OP1 Daniel Varon Silva**	Glycosylphosphatidylinositol Anchors: A Complex and Functional Bridge Between Proteins and Membranes
1600	**OP2 Carmen Galan**	Novels Tools for Glycobiology: From Synthesis to Applications
1620	break	
1645	**IL1 Gijs van der Marel**	Synthesis and evaluation of immune-modulating agents
1730	End	
1800	Welcome reception	

Monday		
900	**IL2 Peter Seeberger**	Automated Oligosaccharide Synthesis
945	**OP3 Jeroen Codee**	Glycuronic acids: reactivity, selectivity, automated synthesis and applications
1005	**OP4 Christian Stanetty**	Synthesis of Phosphorylated Hepto-Oligosaccharides from Bacterial LPS
1025	break	
1100	**OP5 Daniele Lo Re**	Carbohydrate based synthesis of enantiomerically pure glycomimetics alkaloids
1120	**OP6 Jenifer Hendel**	Towards the Inhibition of Pathogen- Host interactions: The Rational Design and Synthesis of Sialyl Galactose Glycomimetics
1140	**OP7 Tom Wennekes**	Mechanism-Based Covalent Influenza Neuraminidase Inhibitors & Getting a Grip on Microbial Sialic Acid Glycobiology
1200	lunch	
1330	**IL3 Anne Imberty**	The sweet tooth of bacterial and fungal pathogens: from structural glycobiology to antiadhesive strategies
1415	**OP8 Lenka Malinovská**	Lectins from Burkholderia cenocepacia – what do they really like?
1435	**OP9 Chakkumkal Anish**	Pathogen Detection Based on Antibodies Against Cell Surface Glycans
1455	break	
1530	**OP10 Véronique Blanchard**	Serum glycome profiling - a biomarker for diagnosis of ovarian cancer
1550	**OP11 Daniel Kolarich**	Deciphering the glycocode: The glycoprotein alphabet can be decoded using Glycoproteomics
1610	**IL4 Serge Perez**	Neutron and Synchrotron Radiations for Glycosciences
1700	End	

Tuesday		
900	**IL5 Alexei Demchenko**	From Chemical Glycosylation to Expeditious Oligosaccharide Synthesis
945	**OP12 Trinidad Velasco-Torrijos**	Facile Approach towards Synthetic Glycolipids: Potential as Soft Materials and Antimicrobial Agents
1005	**OP13 Bruce Turnbull**	Understanding and exploiting bacterial toxins
1025	break	
1100	**OP14 Hui Cai**	Fully Synthetic Multiple-Valent Glycopeptide-Lipopeptide Anti –Tumor Vaccines: Novel Cluster Effect
1120	**OP15 Michele Fiore**	Synthesis of homo- and hetero-glyco-clusters, Prophylactic Vaccine Against Cancer
1140	**OP16 Aliaksei Pukin**	Multivalent Carbohydrates In Action: Strong Inhibition of Sugar-Binding Proteins
1200	End	
1400	Excursion	

Wednesday - joint with Glycan Forum		
0900	Herbert Jäckle	Welcome from MPI
0915	Sabine Flitsch	Welcome from Euroglycoscience Forum
0930	Chi-Huey Wong	Chemical Approach to Carbohydrate-mediated Biological Recognition
1020	X. Liu	Glycosciences: From Synthetic Methods to Glycobiology
1100	break	
1130	Biao Yu	Assembly of Naturally Occurring Glycosides, General Tactics and New Glycosylation Methods
1210	Koiichi Fukase	Synthesis of regulatory molecules and probes for innate immunity and glyco-imaging
1250	Lunch	
1400	**Bruce Turnbull**	Introduction
1405	**IL6 Ben Davis**	Sugars and Proteins
1450	**OP17 Richard Daniellou**	GLYCOPEPS: towards the enzymatic synthesis of thioglycoconjugates
1510	**OP18 Emiliano Bedini**	Semi-synthesis of Chondroitin Sulfate Polysaccharides
1530	break	
1600	**OP19 Roberto Cighetti**	New sugar-based small molecules to investigate Lipopolysaccharide recognition
1620	**OP20 Ulrika Westerlind**	Towards Multivalent Mucin Glycopeptides
1640	Daniel Kolarich	Introducing three important neighbouring areas of glycoscience
1645	Bernd Stahl	Human milk oligosaccharides: the unrivalled model for functional dietetic glycans
1705	Bruno M. Moerschbacher	NanoBioEngineering of Partially Acetylated Chitosans to be Used as Novel Functional Biopolymers
1725	Thomas Rosenau	Towards a better understanding of cellulose swelling, dissolution and regeneration at the molecular level
1745	End	

Invited Lecture 1

Synthesis and evaluation of immune-modulating agents

Gijs van der Marel

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Abstract

Two classes of immune-modulating agents are discussed. First, synthetic teichoic acids, phosphodiester-containing biopolymers, that occur in the cell wall of Gram-positive bacteria, are evaluated as well-defined synthetic antigens. Solution, solid phase and different fluorous tag synthetic approaches towards teichoic acids fragments are discussed.

The second class comprises conjugates, in which ligands for a mammalian pathogen recognizing receptor of the innate immune system - a molecular adjuvant - are covalently linked to an antigenic peptide. The synthesis of conjugates containing one (a TLR or a NOD2 ligand) or two ligands as well as their potential to function as an anticancer vaccine are presented.

Invited Lecture 2
Automated Oligosaccharide Synthesis

Peter H. Seeberger

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While peptides and oligonucleotides are now readily accessible using automated solid phase synthesis, access to complex carbohydrates has been very difficult and time consuming. Described is the development of a fully integrated platform based on automated oligosacharide sythesis[1,2,3] and carbohydrate arrays to address biological problems. Particular emphasis will be placed on the latest verison of the automated synthesis platform that is currently being made available commercially for laboratories around the world.

Bioinformatics studies have revealed that a relatively small number of building blocks is required to synthesize a large portion of the occupied glycospace.[4,5] Automated oligosaccharide synthesis relies on access to usable quantities of monosaccharide building blocks. In order to shorten synthetic routes, we have designed *de novo* methods using purely chemical[6] as well as enzymatic means. [7]

The synthesis of glycosaminoglycans, a highly complex class of carbohydrates that includes heparin, is even more complex as it requires the sulfation at particular positions. For that purpose a new strategy and a new instrument had to be designed that now yields glycosaminoglycan oligosaccharides in days rather than months.[8,9]

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Invited Lecture 3

The sweet tooth of bacterial and fungal pathogens: from structural glycobiology to antiadhesive strategies.

Anne Imberty

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Lectins are carbohydrate-binding proteins that interact with specific sugars or glycoconjugates and mediate several biological activities. Bacterial lectins are involved in host recognition, biofilm formation, tissue adhesion and virulence. *Pseudomonas aeruginosa* and *Burkholderia sp* are opportunistic pathogens responsible for lung infections that are life-threatening for cystic fibrosis patients and immuno-compromised individuals. The fungal pathogen *Aspergillus fumigatus* is also responsible for air-borne nosocomial lung infections. These pathogens produce a variety of lectins that display high affinity for fucosylated oligosaccharides that are present on human tissues. We used combined titration microcalorimetry, x-ray crystallography and molecular modeling approaches to decipher the thermodynamical and structural basis for high affinity binding of pathogen lectins to host carbohydrates [1,2].

The design and synthesis of high affinity lectin ligands have been performed in collaboration with chemists. The complete characterization of carbohydrate specificity, affinity and atomic details of interaction between bacterial lectins and their ligands allowed for the design and synthesis of high affinity glycomimetics and glycodendrimers that can act as antiadhesive compounds.

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Invited Lecture 4

Neutron and Synchrotron Radiations for Glycosciences

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Centre de Recherches sur les Macromolécules Végétales, CNRS, Grenoble, France.

To fully address emerging challenges, scientists must investigate Real materials, in Real conditions and in Real Time. The intricacy of such challenges is far beyond the conventional scientific disciplines (Biology, Chemistry, Physics, Material Science, Geosciences, and imaging) and requires the use of unique large scale facilities that allow scientists to investigate materials at the atomic scale, thus enabling them to perform discoveries that translate into innovations and technology breakthrough. Synchrotron and Neutron sources are more and more used to these ends also facing an increased demand for industrial applications.

Compared to conventional laboratory X-rays the spatial resolution and in-depth information achievable can be increased by several orders of magnitude by using synchrotron radiation and neutrons, in there complementarity. The application of neutron radiation is well adapted to the field of organic and bio-macromolecular structures using the Hydrogen-Deuterium effect. It is also well suited to characterize molecular vibrations throughout low-energy spectroscopy, and to the characterization of magnetic structures and excitations. Neutron radiation can also be applied to bulk structures submitted to strains and excitations. Synchrotron radiation is increasingly applied to macromolecular crystallography and in particular to the elucidation of protein and virus crystal structures. It also offers to investigate fast chemical reactions, and to carry on high-energy spectroscopy (measurements of electron energy levels) and imaging. This is also well suited to surface studies (e.g. defects, corrosion,…). The use of neutron and synchrotron radiation has been the domain of physicists and other natural scientists for many years, the predominant experimental techniques used are diffraction, small-angle scattering and tomography.

Following a general presentation of the neutron and synchrotron radiations infrastructures in Europe, and their major contributions to the advancement of sciences and technology, the lecture will concentrate on how structural glycosciences at large can benefit from such unique facilities. The critical role in training the next generation of scientists in Synchrotron and neutron facilities will be also emphasized.

HERCULES. Higher European Research Course for Users of Large Experimental Systems. http://hercules-school.eu

Invited Lecture 5

From Chemical Glycosylation to Expeditious Oligosaccharide Synthesis

Alexei V. Demchenko, PhD

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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, foremost of which can be found in the area of development of therapeutic agents and diagnostic platforms. Although carbohydrates are so desirable for biological and medical community, chemically these molecules are very challenging targets because of the need for chemical functionalization, protecting and leaving group manipulations, controlling anomeric stereoselectivity, etc. Advances in chemistry and biochemistry have certainly simplified the synthesis of carbohydrates. However, the development of practical and general methods for chemical glycosylation[1] and expeditious oligosaccharide synthesis[2] remain demanding areas of research.

At the core of this presentation is the development of new methods, strategies, and technologies for chemical glycosylation and expeditious oligosaccharide assembly.[3-7] These innovative tools for oligosaccharide synthesis will be discussed in light of recent results.[8-10] The effectiveness of methods developed will be illustrated by the synthesis of medicinally relevant oligosaccharides and conjugates thereof. This work was generously supported by awards from the NIGMS, NSF, Pfizer, and Mizutani Foundation for Glycoscience.

References

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Invited Lecture 6

Sugars and Proteins

Ben Davis

University of Oxford

Our research comes under the broad heading of the chemistry of Carbohydrates and Proteins. The reactions and manipulation of sugars and proteins have fascinated organic chemists for over a century and this work is culminating today in a host of new drugs for treating diseases. It is becoming increasingly clear that oligosaccharides (carbohydrates in small clusters) and alterations in proteins (modifications) are examples of chemically complex biological markers that can act in important recognition processes such as microbial infection, cancer metastasis and cellular adhesion in inflammation, in addition to many intracellular communication events. Their remarkable structural diversity means that they can often mediate highly specific and therefore complex processes.

Our work comes under the broad headings of the Chemistry, Chemical Biology and Biotechnology of Carbohydrates and Proteins. Interests encompass organic synthesis and methodology, inhibitor design, biocatalysis, enzyme mechanism, protein engineering, drug delivery, molecular modelling, molecular biology, imaging and translation into *in vivo* systems. The application of an understanding of such systems on a fundamental level leads to the design, synthesis and modification of potential therapeutic and biotechnologically applicable systems.

Glycosylphosphatidylinositol Anchors: A Complex and Functional Bridge Between Proteins and Membranes

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Glycosylphosphatidylinositol (GPI) are complex glycolipids that are present in free form or attached to proteins and other biomolecules anchored at the outer leaflet of eukaryotic cell membranes.[1] Besides their anchoring role, these glycolipids are involved in modulation of the host immune system during parasitic infections and in formation of lipid rafts that are implicated in many regulatory functions such as cell signaling.

Although all GPIs contain a conserved core pseudopentasaccharide, in nature they occur as heterogeneous mixtures. Investigation of their function and evaluation of the effects these glycolipids exhibit on the attached proteins require well-defined, homogeneous samples of GPIs and GPI-anchored molecules. To address this need, we have developed a general strategy for the total synthesis of lipidated, fully phosphorylated and branched GPI structures.[2] Using this strategy different parasitic GPIs were synthesized, printed on microarrays and evaluated in antibody-binding assays. [3] To evaluate the immunogenicity of these glycolipids, synthetic GPI molecules have been synthesized and conjugated to a carrier protein and used in immunization assays in mice. Additionally, we have also prepared mammalian GPI structures containing a cysteine residue that enables ligation of GPIs to proteins and peptides via protein trans-splicing or by NCL with C-terminal thioester expressed proteins to produce homogeneous GPI-anchored proteins.

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Oral Presentation 2

"Novels Tools for Glycobiology: From Synthesis to Applications"

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Protein- and lipid-bound oligosaccharides play important recognition roles in a diverse range of biological processes such as protein folding, cell-cell communication, bacterial adhesion, viral infection and masking of immunological epitopes.[1] As a consequence, carbohydrates have become important targets in the discovery of novel therapeutics. Despite their importance, the synthesis of these complex molecules still remains a formidable challenge and the development of general and efficient methodologies for the preparation of complex oligosaccharides has been the subject of research for many years.

In our group we are interested in the development of novel methodologies for the expedient synthesis of oligosaccharides and glycoconjugates of biological relevance.[2-4]

1. M. C. Galan*, D. Benito-Alifonso and G. M. Watt. *Org. Biomol. Chem.* (2011), 9 (10), 3598 - 3610**.**

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Oral Presentation 3

Glycuronic acids: reactivity, selectivity, automated synthesis and applications

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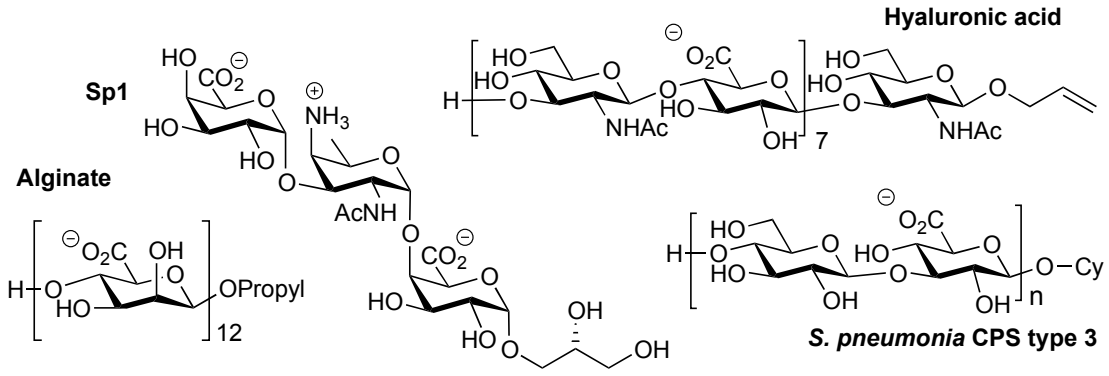
Glycuronic acids are prominent constituents of many naturally occurring oligo- and polysaccharides and they have been subject of many synthetic studies. Because it has long been thought that the C-5 carboxylic acid ester in glycuronic acid building blocks has a detrimental effect on the reactivity of these, the use of these building blocks is often circumvented. This presentation evaluates the influence of the C-5 carboxylic ester function on the reactivity and selectivity of various glycuronic acid building blocks and reports on their use in the (automated solid phase) assembly of a collection of anionic oligosaccharides, such as depicted below.



Scheme 1. Glycuronic acid containing oligosaccharides

Oral Presentation 4

Synthesis of Phosphorylated Hepto-Oligosaccharides from Bacterial LPS

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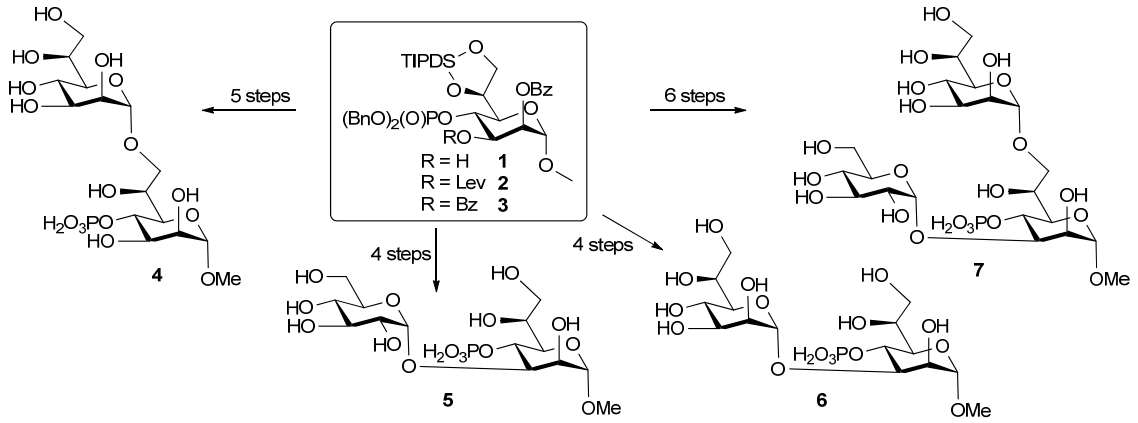
Phosphorylated heptoses of the L-*glycero*-D-*manno* configuration are important constituents of the lipopolysaccharide (LPS) of many Gram-negative bacteria contributing to fundamental binding interactions of LPS with the innate and adaptive immune system. Complementing previous synthetic approaches,[1] we have developed a new synthetic strategy towards Hep-4-phosphate containing inner-core fragments which is based on early phosphate introduction (**1** to **3**) and high yielding 7-*O*-regioselective partial TIPDS cleavage.



Glycosylation reactions with glucosyl and heptosyl imidate donors completed the short synthetic routes towards the di- and trisaccharides **4** to **7** upon deprotection. These ligands are presently being used to evaluate the binding contribution of heptosyl phosphates when complexed to antibodies and lectins.[2,3]

(Financial support: FWF P 22909)

References

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Oral Presentation 5

Carbohydrate based synthesis of enantiomerically pure glycomimetics alkaloids

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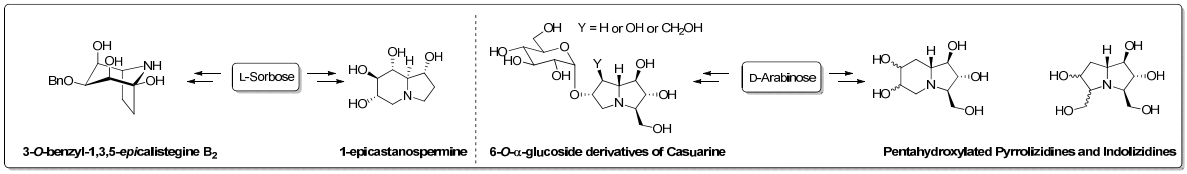
Polyhydroxylated alkaloids, also known as iminosugars or azasugars, are a well-known group of natural compounds. They are normally isolated from water-soluble fractions of medicinal plants,[1] and microbial cultures.[2] Because of the resemblance to carbohydrates, they have been employed as tools in glycobiology to study recognition processes, particularly those concerning the reactions catalyzed by glycosidases and glycosyltranferases.[3] The ubiquity of these enzymes in living organisms, the tasks they play in vital processes like cell function and recognition, and their role in the etiology of diseases like cancer, HIV, and diabetes[4] have raised the need for new and active compounds against them.



Scheme 1. Carbohydrate synthesis of iminosugars

Here we report the synthesis of several unnatural iminosugars using L-sorbose and D-arabinose as chiral starting material.

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Oral presentation 6

Towards the Inhibition of Pathogen- Host interactions: The Rational Design and Synthesis of Sialyl Galactose Glycomimetics

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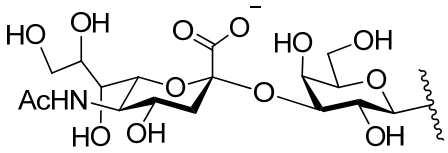
Numerous pathogens (including avian influenza, *H. pylori*, and *S. pneumonia*) employ cell-surface glycans terminating in sialylated galactose (Scheme 1) as their adhesion partner.[1] Consequently, a glycomimetic anti-adhesive therapeutic based on this structure could potentially prevent infection.



Scheme 1. Neu5Ac-α-(2-3)-Gal

One of the initiatives in our research group is to employ sialylated galactose (Scheme **1**) as a chemical scaffold in the rational design of high-affinity inhibitors of hemagglutinin-mediated influenza A virus adhesion. We have developed a 1st generation of glycomimetic targets using advanced computational methods and the progress of the synthesis will be presented. Initially, the anti-adhesives developed in this project will be screened for influenza inhibition. However as mentioned above, several pathogens employ a similar adhesion mechanism as influenza A, and as a result there is potential for the therapeutics we are developing to be effective against a broad range of pathogens.

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Mechanism-Based Covalent Influenza Neuraminidase Inhibitors

& Getting a Grip on Microbial Sialic Acid Glycobiology

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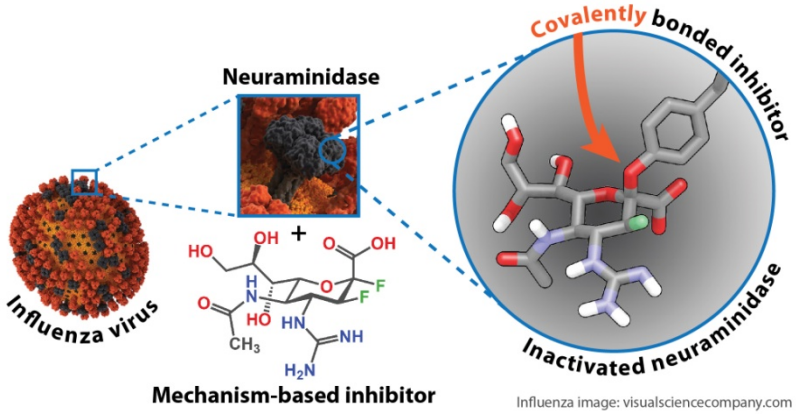
Recent results[1] from an ongoing investigation at UBC into a new class of specific, mechanism-based anti-influenza drugs are reported. These compounds function via the formation of a stabilized covalent intermediate in the influenza neuraminidase enzyme, a mode of action that is confirmed via structural and mechanistic studies. Furthermore it is shown that they function in cell-based assays and animal models, with efficacies comparable to zanamivir and broad spectrum activity against drug-resistant strains in vitro.



Scheme 1. Mode of action for mechanism-based neuraminidase inhibitors

Finally, a brief overview is given of recently started studies at Wageningen University into the interaction of GM1 ganglioside mimics with Cholera toxin[2,3] and the biological role of Pseudaminic & Legionaminic acid in human gut microbes.[4]

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Oral Presentation 8

Lectins from *Burkholderia cenocepacia* – what do they really like?

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Lectins are specific carbohydrate-binding proteins important in both symbiotic and parasitic interactions between microorganisms and hosts. These proteins can mediate adhesion to host cells and promote development of infections. *B. cenocepacia* is an opportunistic human pathogen causing lung infections in immunocompromised individuals. This bacterium produces several soluble lectins located in cytoplasm. However, significant portion of lectins is exported and attached to the bacterial surface by unknown mechanisms. Despite of being closely related, *Burkholderia* lectins differ in binding affinity and thermodynamic properties. They are probably evolutionary optimized for various ligands and could execute different functions in pathogenesis. Lectins vary also in fine binding specificity but all of them recognize D-mannose and they can also bind heptose (an important part of lipopolysaccharides from *Burkholderia*) and its derivatives. Recently, we have also observed their interaction with exopolysaccharides. Thus, LPSs and EPSs from *B. cenocepacia* could be suitable targets for lectins and anchor them directly on the bacterial surface.

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Oral Presentation 9

Pathogen Detection Based on Antibodies Against Cell Surface Glycans

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Many invading pathogens express unique carbohydrate structures on their cell surface. These complex carbohydrates are often made of unique glycan motifs which are distinct from mammalian cell surface glycans [1]. These distinct differences can be the basis for pathogen detection and vaccination strategies. Complexities involved in cultivation of pathogen, subsequent purification and characterization of cell surface glycans (CSG) make developing antibodies based on these structures a challenging task[2]. Chemical synthesis helps to access these complex structures with utmost purity and definition. The current work describes evaluation of synthetic oligosaccharides based on CSGs of *Yersinia pestis*, *Clostridium difficile* and leishmania parasite as potential candidates for pathogen detection. Glycan array screening of sera from infected individuals were used to define the key epitope and motifs required for immunogenicity. Neoglycoconjugates of synthetic CSGs were prepared and evaluated in animal models to establish their immunogenicity. Robust antibody responses against synthetic CSG were observed in immunised group. High throughput analysis of sera by glycan array demonstrated high affinity binding of antibodies and their class switching in immunized animals. Monoclonal antibodies binding CSGs were developed and subsequently used for specific detection of respective pathogen. The results of current study demonstrate that unique differences in cell surface glycans can be exploited to develop pathogen detection and prevention strategies.

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Oral Presentation 10

Serum glycome profiling - a biomarker for diagnosis of ovarian cancer

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During the development of cancer, changes in cellular glycosylation are observed, indicating that alterations of the glycome occur also in extracellular fluids as well as in serum and could therefore serve as tumor biomarkers. In the case of Epithelial Ovarian Cancer (EOC), common tumor markers such as CA125 often fail to detect the disease at its early stages. Better biomarkers are therefore needed. The aim of the present research work is to identify new potential glycan biomarkers in EOC-patients.

N-glycans were released from serum glycoproteins by PNGase F digestion, purified in a solid phase extraction, permethylated und subsequently analyzed by MALDI-TOF mass spectrometry. 87 samples from preoperative EOC patients and 33 samples from age-matched healthy women were enrolled in this study. Statistical analyses were carried out using the SPSS 18.0 software. A GLYCOV value was calculated from the structures that were over- and underexpressed. We were able to identify statistical N-glycome differences between primary ovarian cancer and control sera, giving better results than those of the established tumor marker CA-125, and for this reason could potentially be used as a biomarker.

Oral Presentation 11

Deciphering the glycocode: The glycoprotein alphabet can be decoded using Glycoproteomics

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Glycoconjugates are well known to be crucial key players of intercellular communication [1]. Comprehensive knowledge on the key molecules and their particular glycan structures present on the cell's surface is a key requisite for deciphering glycan mediated intercellular communication signals. Glycoproteomics provides this information by identifying, characterising and cataloguing glycoproteins present on cell surfaces or biological fluids [2, 3, 4]. Combination of different technologies such as nano-scale liquid chromatography coupled to state of the art mass spectrometers assures high sensitivity and versatility to yield comprehensive data sets such as protein identity, global glycosylation signatures or glycoprotein specific glycosylation from minimal quantities of biological probes. Using these technologies, the primary structure of two important human immunoglobulins, secretory IgA and IgM has been comprehensively investigated [5, 6]. This type of comprehensive information provides the basis for further targeted investigations of the functional role of protein glycosylation on individual glycoproteins.

Such studies are considerably facilitated once synthetically produced and thus well-defined components are made available. Combining glycoproteomics findings with well-established solid phase (glyco)peptide synthesis provides unique opportunities for numerous aspects in glycobiology, such as but not limited to quantitative glycoproteomics and functional glycobiology.

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Oral Presentation 12

"Facile Approach towards Synthetic Glycolipids: Potential as Soft Materials and Antimicrobial Agents"

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The appropriate balance in lipophilic/hydrophilic character is a critical parameter to modulate the interactions of drugs and bioactive compounds. Naturally occurring glycolipids still remain a synthetic challenge for carbohydrate chemists, despite their important roles in many biological processes. In this regard, we have developed a facile approach for the synthesis of glycolipid mimetics, which allow us to modify easily the type, presentation and number of hydrocarbon chains in the glycoconjugates. We thereof are examining their ability to act as anti-adhesion agents for antimicrobial therapy against *Burkholderia cepacia* complex (*Bcc*) pathogens. Some of these glycoconjugates also can behave as supramolecular "low molecular weight organogelators" (LMWOs), which are able to self- assemble in fibrillar networks and induce the gelation of different solvents The synthetic glycolipids have also been incorporated into soft materials such as "Giant Unilamellar Vesicles" (GUVs) (Figure 1).

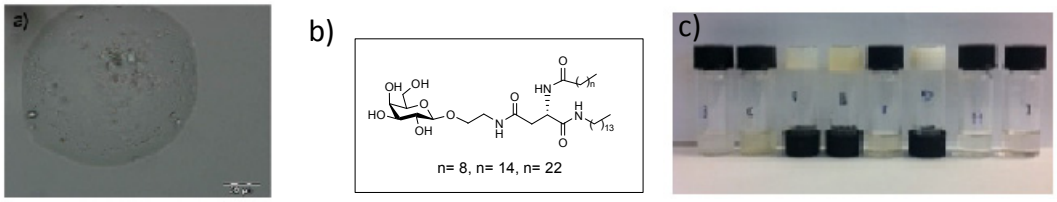

Figure 1: Glycolipid mimetics: a) Image of GUV incorporating synthetic glycolipids; b) general structure of aspartic adid based synthetic glycolipids; gelation ability of different glycolipids in ethanol.

Understanding, inhibiting and exploiting bacterial toxins

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The surface of every living cell is covered in complex carbohydrates. Binding interactions between these sugars and proteins are essential for many biological processes including infection by viruses and bacteria. In this lecture I will describe how we are using a combination of synthetic chemistry and biophysical methods to study a family of carbohydrate-binding proteins that are the toxins responsible for cholera and other diarrhoeal diseases. The bacterial toxins enter cells lining the intestine by first sticking to sugars at the cell surface; therefore, mimics of the carbohydrate ligands have potential as anti-diarrhoeal drugs. Synthetic oligosaccharides have allowed us to study these binding interactions in detail and to understand why people with certain blood groups are affected more severely by cholera. I will also explain how we can modify the toxins for future applications in drug delivery and synthetic biology.

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Oral Presentation 14

Fully Synthetic Multiple-Valent Glycopeptide-Lipopeptide Anti –Tumor Vaccines: Novel Cluster Effect

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Using tumor-associated antigen to develop tumor vaccine is the promising method to cure cancer in the future. To this end, the membrane-bound mucin MUC1 glycoprotein is an attractive target. The extracellular domain of this integral membrane protein consists of several tandem repeats 20 amino acids of the sequence HGVTSAPDTRPAPGSTAPPA, including five potential *O*-glycosylation sites. On tumor cells, truncated mucins is extensively over-expressed and generally modified with sialylation. Both the saccharide and the peptide structures contribute to the tumor-associated epitope. However the tumor-selective MUC1 glycopeptides are only moderately immunogenic and additional stimulation is necessary. Therefore, the design of vaccine is crucial and challenging to induce tolerance-breaking immune responses.

We have designed, chemically synthesized and immunologically evaluated a series of fully synthetic multiple-valent vaccine with the mucin MUC1 glycopeptide bearing STn antigen. This glycopeptide was combined with TLR2 ligand lipopeptide Pam_3CSK_4 to construct mo-, di- and tetravalent conjugates by click chemistry. These vaccines were administered in Balb/c mice for immune response evaluation. We have found that the immune responses were altered with different valent conjugates. The antisera induced by the tetravalent glycopeptide-lipopeptide conjugate exhibited most strong binding to tumor cells. This strong binding resulted in a promising therapeutic response of killing ability toward tumor cell by activation of complement dependent cytotoxicity (CDC) complex. This novel cluster effects are derived from the alteration of the induced antibody isotype by the multiple antigen peptide (MAP) structures.

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Oral Presentation 15

Synthesis of homo- and hetero-glyco-clusters, Prophylactic Vaccine Against Cancer

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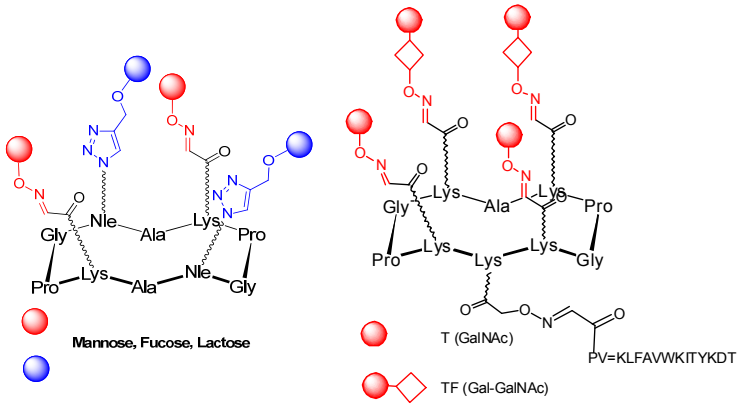
Cancer cells surface are characterized by the presence of diverse truncated structures named Tumor Associated Antigens. The combination of these structures in a single molecule represents an attractive strategy to obtain multiantigenic vaccine candidates with improved immunological properties [1]. To this aim, we developed recently molecular systems that combine two different carbohydrate motifs by using a "one pot" chemoselective ligations [2]. In another study, we prepared a mixture of heterotopic platforms bearing the Tn and TF carbohydrate antigens and an immunostimulating poliovirus peptide using a randomized oxime-based assembly [3]. In this presentation we will describe how these synthetic procedures will be exploited to access to a second generation of glycocyclopeptide-based vaccines against cancers.



Scheme 1.

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Oral Presentation 16

Multivalent Carbohydrates In Action: Strong Inhibition of Sugar-Binding Proteins

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A successful strategy in the development of high-affinity inhibitors of protein-carbohydrate interactions includes a combination of employing the optimal saccharide ligand with the optimal presentation of the inhibitor. This presentation is often multivalent considering that multivalency is an important factor in the recognition of carbohydrates. For the purpose of inhibition of cholera toxin and antibodies relevant in certain neuropathies, chemo-enzymatic syntheses of analogues of human gangliosides GM3, GM2, GM1, GD1a and GalNAc-GD1a were performed. In addition, divalent, tetravalent and octavalent GM2 and GM1 gangliosides were prepared. The potential of these ganglioside analogues to detect toxins and antibodies was shown in a variety of diagnostic/inhibitory tests. These include highly potent multivalent cholera toxin inhibition,[1] detection of cholera and the related heat labile enterotoxin of *E. coli,* detection of IgG and IgM antibodies in serum samples from neuropathy patients.[2]

The ultimate optimization of the spacer in multivalent inhibitors suggests the use of rigid spacers, which will allow for construction of well-matched systems[3] and thus for the design of multivalent ligands with great specificity. Syntheses of rigid glucose based spacers for divalent inhibitors are currently being performed.

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GLYCOPEPS: towards the enzymatic synthesis of thioglycoconjugates

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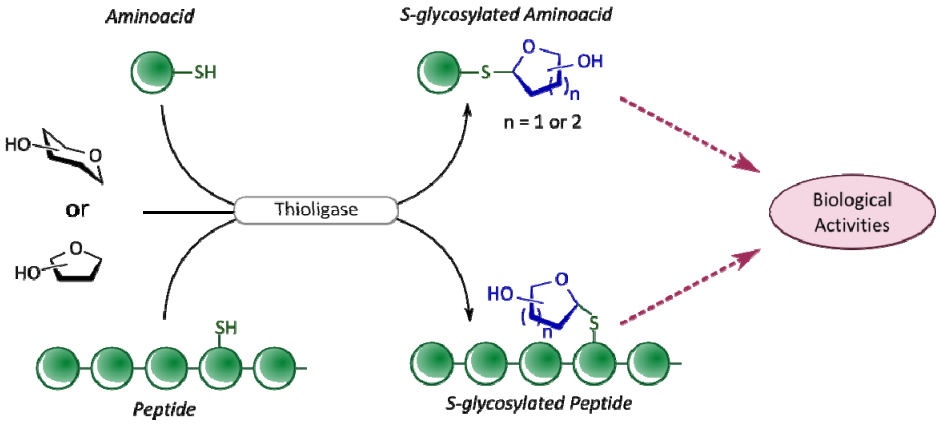
Glycosylation represents the most complex post-translational modification of proteins, and is involved in a number of biological processes.[1] Unfortunately glycoproteins are typically expressed as mixtures of heterogeneous glycoforms that possess the same peptidic backbone but differ in both the nature, the number and site of glycosylation.[2] Consequently, access to well-defined and controlled glycoproteins in order to probe the effects of the sugar motifs on such relevant biological processes is of outmost importance. GLYCOPEPS aims at developing new efficient enzymatic means of production of pure ***S***-glycoforms of amino-acids, peptides and proteins and finely understanding the role of the glycosylation in proteins.



Scheme 1. Strategy of GLYCOPEPS

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Oral Presentation 18

SEMI-SYNTHESIS OF CHONDROITIN SULFATE POLYSACCHARIDES

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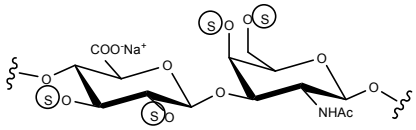
Chondroitin sulfate (CS) is a glycosaminoglycan found in both vertebrates and invertebrates. It is constituted of a 4)-β-GlcA-(1→3)-β-GalNAc-(1→ repeating unit, with a variable sulfation pattern. The most common sulfation patterns are listed in Figure 1.



0: GlcA-GalNAc

A: GlcA-GalNAc(4S)	K: GlcA(3S)-GalNAc(4S)
C: GlcA-GalNAc(6S)	L: GlcA(3S)-GalNAc(6S)
D: GlcA(2S)-GalNAc(6S)	M: GlcA(3S)-GalNAc(4,6S)
E: GlcA-GalNAc(4,6S)	R: GlcA(2,3S)-GalNAc

Figure 1. Typical disaccharide subunits found in natural CSs

CS-A,C is employed for the medium-long term treatment of osteoarthritic patients. It is obtained exclusively by extraction from animal sources, mainly bovine and porcine septa and tracheas, and poultry bones. Apart from ethical problems, this involves several safety risks, as for example transmission of infectious agents. Here we present a new access to CS-A,C and some other rare or unnatural CS polymers. The strategy relies upon the regioselective sulfation of microbial sourced unsulfated chondroitin, through tailored multistep protection/deprotection sequences. [1,2]

References

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New sugar-based small molecules to investigate Lipopolysaccharide recognition

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Toll-Like Receptor 4 (TLR4) is one of the most important receptors of innate immunity, since it is responsible for the sensing of Lipopolysaccharide (LPS) and thus of the presence of bacteria. It works in association with proteins MD-2, LBP and CD14. In our laboratory we develop small molecules based on saccharidic scaffolds that target proteins of the TLR4 pathway and act as antagonists. Their selectivity makes them hit compounds for the treatment of several pathologies related to TLR4 pathway dysfunctions, but they are also good candidates as tools to selectively modify a biochemical route with no need to genetic engineer the cells (the “chemical genetics” approach). In this communication we will show a library of molecules that were developed in our laboratory and their biological characterization to this day.

Visualizing molecular interactions in biological samples or in vivo would also be very useful in order to understand which processes take place during LPS sensing by the immune system. Fluorescence microscopy is an efficient method to visualize molecular interaction both in vivo and in vitro. Fluorescent small molecules that show a good specificity for a protein target could be used as substitutes of antibodies or fluorescent protein fusions. In our laboratory we synthesized several fluorescein-labeled glycolipidic probes and tested them on innate immunity cells. Their interaction with cells was also investigated by confocal microscopy and flow cytometry.

Oral Presentation 20

Towards Multivalent Mucin Glycopeptides

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Mucins are highly glycosylated proteins that populate the cell-surface of epithelial tissues in a membrane bound or secreted form[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[2,3]. By interaction with lectins the cell-surface glycans are responsible for protein cross-linking of membrane-bound receptor complexes resulting in downstream signaling events. The mucin type *O*-glycan ligands are also involved in intercellular binding events, for example through binding to macrophage and T-cell lectin receptors. Through the mucus layer the mucin glycans contribute to the innate immune system by providing a protecting barrier against invading pathogens. With the diversity of glycan structures found in biological systems, understanding of potential roles of the glycans and identification of potential glycan binding proteins interacting with these molecules is a major challenge. The chemical synthesis can provide with well-defined glycan and glycopeptide probes making such systematic glycan binding studies more feasible.

We envisioned developing an efficient synthesis strategy to prepare different multivalent *O*-glycopeptides based on the extended core mucin type glycans making these molecules available for future microarray analysis of protein binding events. Our recent work on the synthesis of complex mucin glycosylated amino acid building blocks and the preparation of a library of multivalent mucin glycopeptides will be described in more detail[4].

References:

1) Rose, M. C.; Voynow, J. A., *Phys. Rev.* **2006,** *86*, 245-278.
2) Carlstedt, I.; Davies, J. R., *Biochem. Soc. Trans.* **1997,** *25*, 214-219.
3) Fukuda, M.; Tsuboi, S., *Biochim. Biophys. Acta, Molecular Basis of Disease* **1999,** *1455*, 205-217.
4) Pett, C.; Schorlemer, M.; Westerlind, U., *Submitted manuscript.*

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Programme for the European Young Investigators Workshop

"Deciphering the Glycome – from synthesis to applications"

17th – 20th March 2013 – Potsdam/Berlin, Germany

Sunday	17 March
1530	Introduction
1540	**OP1 Daniel Varon Silva**
1600	**OP2 Carmen Galan**
1620	break
1645	**IL1 Gijs van der Marel**
1730	End
1800	Welcome reception

Monday	18 March
900	**IL2 Peter Seeberger**
945	**OP3 Jeroen Codee**
1005	**OP4 Christian Stanetty**
1025	break
1100	**OP5 Daniele Lo Re**
1120	**OP6 Jenifer Hendel**
1140	**OP7 Tom Wennekes**
1200	lunch
1330	**IL3 Anne Imberty**
1415	**OP8 Lenka Malinovská**
1435	**OP9 Chakkumkal Anish**
1455	break
1530	**OP10 Véronique Blanchard**
1550	**OP11 Daniel Kolarich**
1610	**IL4 Serge Perez**
1700	End

Tuesday	19 March
900	**IL5 Alexei Demchenko**
945	**OP12 Trinidad Velasco-Torrijos**
1005	**OP13 Bruce Turnbull**
1025	break
1100	**OP14 Hui Cai**
1120	**OP15 Michele Fiore**
1140	**OP16 Aliaksei Pukin**
1200	End
1400	Excursion

Wednesday	20 March
	Joint with Glycan Forum
1400	Introduction
1405	**IL6 Ben Davis**
1450	**OP17 Richard Daniellou**
1510	**OP18 Emiliano Bedini**
1530	break
1600	**OP19 Roberto Cighetti**
1620	**OP20 Ulrika Westerlind**
1640	End

Invited lectures will be 45 minutes including questions

Oral presentations will be 20 minutes including questions