Book of Abstracts

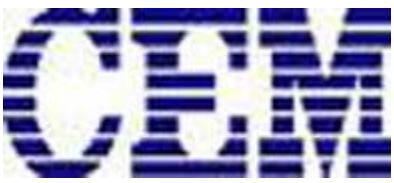
European Young Investigator Workshop

Carbohydrate Chemistry:
From Synthesis to Applications

Lyon – France

April 11–15, 2011

The organizers would like to thank the following companies and organizations for their financial or technical support. Without them it would not have been possible to carry out such a meeting.



Welcome

Dear Participants,

We cordially welcome you to this European Young Investigator Workshop on carbohydrate chemistry here in Lyon. The idea to arrange such a workshop was born two years ago during the last Gordon Research Conference on carbohydrates. However, this is not the first meeting of this type. In March 2007, Paul V. Murphy organized a CERC-3 meeting on carbohydrate chemistry in Dublin, Ireland. Although CERC does not exist any more, the aim of this forum is still the same as four years ago.

With this meeting we would like to provide an opportunity for young scientists to meet each other in a small group in order to better interact and to provide an overview about the different research topics in glycochemistry. In addition, we hope that the scientists participating in this workshop will be united by their strong and common interest to initiate collaborations across the borders. In recent years, carbohydrate chemistry has celebrated some kind of renaissance and many young investigators in this field started their independent research only a few years ago. Therefore, it is time to get to know each other and maybe to think about the next scientific challenges facing us in glycosciences.

We are grateful to our numerous sponsors and local institutions for helping us in organizing this scientific conference.

We hope you will enjoy your time in Lyon and profit from this conference by sharing ideas, having inspiring discussions and making new colleagues and friends.

Best wishes,

Sébastien Vidal
Co-chairman

Daniel B. Werz
Co-chairman

Scientific and Social Program

	Monday 11 April	Tuesday 12 April	Wednesday 13 April	Thursday 14 April	Friday 15 April
9.00-9.50		**IL-2 Unverzagt**	**IL-5 Mulard**	**IL-8 Jiménez-Barbero**	**IL-9 Driguez**
9.50-10.10		Lecourt	Hackenberger	Nitz	Fridman
10.10-10.30		Linclau	Chambert	García-González	Westerlind
10.30-11.00		**Coffee break**	**Coffee break**	**Coffee break**	**Coffee break**
11.00-11.20		Kandasamy	Stubbs	Reichardt	Ardá
11.20-11.40		Pokorná	Lopin-Bon	Chevolot	Marcelo
11.40-12.00	**Arrival and Registration**	**Elicityl**	**New J. Chem.**	Lahmann	Morales
12.00-14.00		**Lunch**	**Lunch**	**Lunch**	**Lunch**
14.00-14.50		**IL-3 Nishimura**	**IL-6 Wessel**		
14.50-15.10		Turks	Legentil		
15.10-15.30		Velasco-Torrijos	Biskup		
15.30-15.50		Cumpstey	Gouin		
15.50-16.10		**Thermo Fisher Scientific**	**Grace Discovery**	**Social**	
16.10-16.30	**16h00-16h50 IL-1 Crich**	**Coffee break**	**Coffee break**		
16.30-16.50		Blériot	Descroix		
16.50-17.10	Turnbull	Compain	Renaudet		**Departure**
17.10-17.30	Codée	Gallienne	Vincent	**Event**	
17.30-17.50	**Coffee break**	**CEM**	Krylov		
17.50-18.10	Boonyarattanakalin	**Free time**	**Free time**		
18.10-18.30	Norsikian				
18.30-18.50	Galan	**18h30-19h20 IL-4 Murphy**	**18h30-19h20 IL-7 Wong**		
18.50-19.10	Varón Silva				
19.30-21.00	**Dinner**	**Dinner**	**Dinner**	**Gala Dinner**	

IL = Invited lecture

Invited Lectures

Methodology Development and Physical Organic Chemistry; A Powerful Combination for the Advancement of Glycochemistry

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The lecture will consist of a personal overview of the current challenges faced by organic chemists working in the area of glycochemistry and glycoscience,[1] and a presentation of recent work in our laboratories directed at their solution.

(1) (a) Bohé, L.; Crich, D. *Trends. Glycosci. Glycotech.* **2010**, *22*, 1-15. (b) Bohé, L.; Crich, D. *Comptes Rendus* **2011**, *14*, 3-16. (c) Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153. (d) Aubry, S.; Sasaki, K.; Sharma, I.; Crich, D. *Topics in Current Chemistry* **2011**, DOI: 10.1007/128_2010_102. (e) Guinchard, X.; Picard, S.; Crich, D. In *Modern Tools for the Synthesis of Complex Bioactive Molecules*; Arseniyadis, S., Cossy, J., Eds.; Wiley: Hoboken, 2011, p in press.

Synthesis of Glycoproteins

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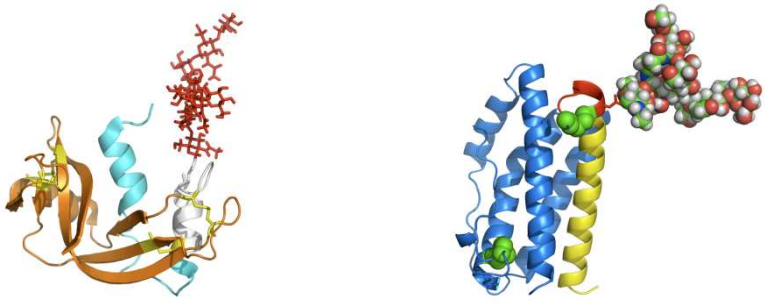
The use of recombinant therapeutic glycoproteins is one of the fast growing applications in human therapy.[1] Typical for these secretory and cell surface proteins is the attachment of oligosaccharides to asparagine residues (N-glycosylation). Despite many efforts in this field the function of N-glycosyation is poorly understood, which is mainly caused by the lack of pure glycoproteins. Since purification of natural glycoproteins is quite tedious due to heterogeneity in the sugar part, the total synthesis of homogeneous glycoproteins has become an attractive target.[2] Native chemical ligation has enabled the synthesis of entire proteins including those carrying posttranslational modifications.[3] We have chosen this approach using bovine ribonuclease and human interleukin 6 as model glycoproteins. Ribonuclease (RNase) is an established model system for protein synthesis and refolding. RNase C is containing a single complex type N-glycan at Asn 34. Ligations were planned in a sequential manner [4] by using a combination of recombinant and chemically synthesized protein segments.[5] In order to expand this approach to the synthesis of libraries of glycoforms several new challenges need to be met. This includes the synthesis of the desired N-glycans and the convenient coupling of these oligosaccharides to the peptide chains.



Scheme 1. Renderings of synthetic glycoproteins RNase C (left) and interleukin 6 (right)

[1] S. Dubel, *Appl. Microbiol. Biotechnol.*, **2007**, *74*, 723. [2] D. P. Gamblin, E. M. Scanlan, B. G. Davis, *Chem. Rev.*, **2009**, *109*, 131. [3] P. E. Dawson, T. W. Muir, I. Clark-Lewis, S. B. Kent, *Science*, **1994**, *266*, 776. [4] D. Bang and S. B. Kent, *Angew. Chem. Int. Ed.*, **2004**, *43*, 2534. [5] a) C. Piontek, P. Ring, O. Harjes, C. Heinlein, S. Mezzato, N. Lombana, C. Pöhner, Markus Püttner, D. Varón Silva, A. Martin, F. X. Schmid, C. Unverzagt, Angew. *Chem. Int. Ed.*, **2009**, *48*, 1936; b) C. Piontek, D. Varón Silva, C. Heinlein, C. Pöhner, S. Mezzato, P. Ring, A. Martin, F. X. Schmid, C. Unverzagt, *Angew. Chem. Int. Ed.*, **2009**, *48*, 1941.

Identification of Disease Specific Glycopeptide Epitopes

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Recently we demonstrated that robust compound library of synthetic MUC1 glycopeptides allowed for the first time rapid and precise identification of the specific epitope recognized by anti-KL-6 monoclonal antibody, a probe for detecting human serum biomarker of interstitial pneumonia [1]. We revealed that an essential epitope recognized by anti-KL-6 MAb is Pro-Asp-Thr-Arg-Pro-Ala-Pro in which Thr is modified by Neu5Acα2,3Galβ1,3GalNAcα. Anti-KL-6 MAb could not differentiate this core 1 structure from core 2-based glycopeptides involving this epitope and showed a similar binding affinity toward these compounds, indicating that branching at *O*-6 position of GalNAc does not influence the interaction of anti-KL-6 MAb with serum MUC1s involving an essential epitope. This is the reason why anti-KL-6 MAb often reacts with tumor-derived MUC1s as well as a biomarker of interstitial pneumonia, namely KL-6 originally discovered as a circulating pulmonary adenocarcinoma-associated antigen. Novel monoclonal antibodies obtained by this epitope reacted specifically with core 1-based structures and did not recognize MUC1s bearing core 2 type *O*-glycans. In the present lecture, key technologies that can accelerate a comprehensive approach toward rapid and precise determination of such glycopeptide epitopes targeting novel diagnostic/therapeutic antibodies [2]-[7].

[1] Ohyabu, N.; Hinou, H.; Matsushita, T.; Izumi, R.; Shimizu, H.; Kawamoto, K.; Numata, Y.; Togame, H.; Takemoto, H.; Kondo, H.; Nishimura, S. –I. *J. Am. Chem. Soc*. **2009**, *131*, 17102-17109. [2] Matsushita, T.; Nagashima, I.; Fumoto, M.; Ohta, T.; Yamada, K.; Shimizu, H.; Hinou, H.; Naruchi, K.; Ito, T.; Kondo, H.; Nishimura, S. –I. *J. Am. Chem. Soc*. **2010**, *132*, 16651-16656. [3] Naruchi, K.; Nishimura, S. –I. *Angew. Chem. Int. Ed*. **2011**, *50*, 1328-1331. [4] Matsushita, T. et al. *Biochemistry* **2009**, *48*, 11117-11133. [5] Miura, Y.; Kato, K.; Takegawa, Y.; Kurogochi, M.; Furukawa, J. –i.; Shinohara, Y.; Nagahori, N.; Amano, M.; Hinou, H.; Nishimura, S. –I. *Anal. Chem*. **2010**, *82*, 10021-10029. [6] Kurogochi, M.; Matsushita, T.; Amano, M.; Furukawa, J. –i.; Shinohara, Y.; Aoshima, M.; Nishimura, S. –I. *Mol. Cell. Proteomics* **2010**, *9*, 2354-2368. [7] Hashimoto, R.; Fujitani, N.; Takegawa, Y.; Kurogochi, M.; Matsushita, T.; Naruchi, K.; Ohyabu, N.; Hinou, H.; Gao, X-D.; Manri, N.; Satake, H.; Kaneko, A., Sakamioto, T.; Nishimura S-I. *Chem. Eur. J*., **2011**, *17*, 2393-2404.

From Glycosidation-Anomerisation to Glycomimetics

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The 1,2-trans glycoside is normally isolated in some reactions with 2-acyl containing donors using $TiCl_4$ or $SnCl_4$ due to acyl group participation, yet on other occasions the α-product[1] or a mixture of products is obtained. The formation of α-products can be explained when glycosidation and anomerisation occur in tandem (glycosidation-anomerisation).[2] A greater understanding of the factors that influence $TiCl_4$ and/or $SnCl_4$ induced anomerisation would be helpful in predicting when anomerisation will be productive for generating both α-*O*- and α-*S*-glycosides. We have used anomerisation as a key step in syntheses of both 1,2-cis *O*- and *S*-glycolipids that are components/structural analogues of *Sphingomonous* cell wall antigens.[3] We will present in detail factors these synthesis and factors that alter the rates and the stereoselectivity of $SnCl_4$ and $TiCl_4$ promoted anomerisations of acylated glycosides. Rates of anomerisation are faster for glucuronic acid or galacturonic acid derivatives and stereoelectronic effects contribute. Anomerisation of *S*-glycosides are consistently faster than corresponding *O*-glycosides. Anomeric ratios (stereoselectivity) depend on saccharide residue, catalyst, catalyst concentration, temperature, protecting group and electron withdrawing power of the aglycon. Very high ratios of the α-anomer can be achieved even for *S*-glycosides, where the anomeric effect is not as strong as for *O*-glycosides. The data will be useful in predicting when glycosidation reactions catalysed by $TiCl_4$ or $SnCl_4$ might give products that contain high proportions of the 1,2-*cis* glycoside, even in the presence of 2-acyl protecting groups.[4] The synthesis of glycophanes using this methodology will be presented. This will include the development of novel applications of the glycophanes such as new inhibitors of carbohydrate-protein interactions and echinomycin mimetics.[5]

References

1. (a) Poláková, M.; Pitt, N.; Tosin, M.; Murphy, P. V. *Angew. Chem. Int. Ed.* **2004**, *43*, 2518-21. (b) Tosin M.; Murphy, P. V. *Org. Lett.,* **2002**, *4*, 3675-78.

2. O'Brien, C.; Polakova, M.; Pitt, N.; Tosin, M.; Murphy, P. V. *Chem. Eur. J.* **2007**, *13*, 902-909.

3 Pilgrim, W.; Murphy, P.V. *Org. Lett.,* **2009**, *11*, 939-942

4. Pilgrim, W.; Murphy, P.V. *J. Org. Chem.* **2010,** *75*, 6747–6755

5. (a) André, S.; Torrijos, T. V.; Leyden, R.; Gouin, S.; Tosin, M.; Murphy, P. V.; Gabius, H. J. *Org. Biomol. Chem.* **2009**, *7*, 4715-4725. (b) Leyden, R.; Velasco-Torrijos, T.; André, S.; Gouin, S. G.; Gabius, H.-J.; Murphy, P. V. *J. Org. Chem.* **2009**, 74, 9010-9026 and unpublished results.

Towards a synthetic carbohydrate-based vaccine against endemic shigellosis: dream or reality?

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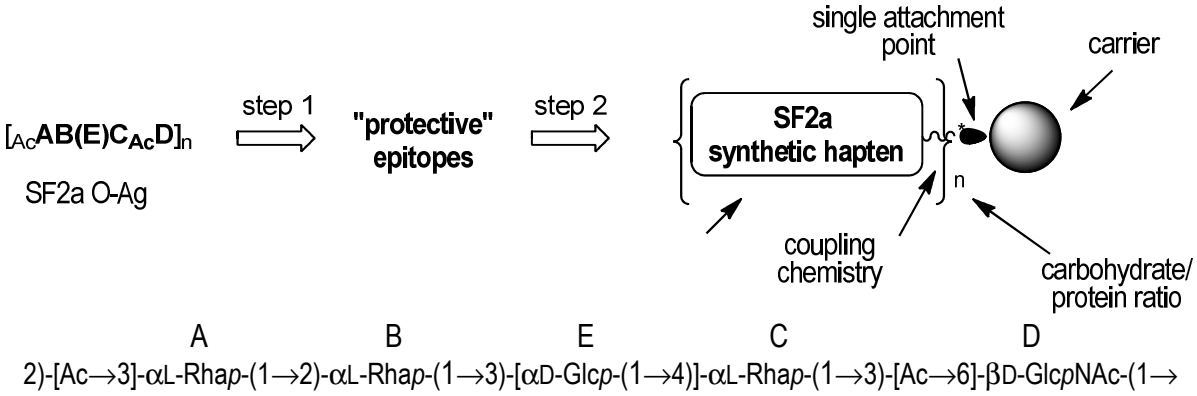
Shigellosis, or bacillary dysentery, caused by the non capsulated Gram negative bacteria *Shigella*, is a burden worldwide. *Shigella flexneri* is the major responsible for the endemic form of the disease in developing countries.[1] Serotype diversity and geographical distribution strongly support the need for a multivalent vaccine. Protection against re-infection is mainly achieved by antibodies specific for the O-antigen (O-Ag) moiety of the lipopolysaccharide (LPS), a major bacterial surface antigen and virulence factor. In recent years, vaccine candidates encompassing synthetic oligosaccharides mimicking the protective determinants carried by the O-Ag have been considered as a possible alternative to detoxified LPS-protein conjugates. In this context, the multidisciplinary strategy (Scheme 1) in progress in the laboratory will be illustrated with emphasis put on *S. flexneri* 2a (SF2a).[2,3]



Scheme 1. General strategy towards a synthetic carbohydrate-based SF2a vaccine candidate and structure of the repeating unit of the SF2a O-Ag[4]

The repeating units of most *S. flexneri* O-Ags comprise a common linear tetrasaccharide backbone (ABCD). Diversity and serotype-specificity rely on branched α-D-Glucosyl (E) and O-Acetyl (Ac) "decorations". The possible impact of such decorations on vaccine development will be discussed in the context of multivalency. The influence of the recently disclosed SF2a O-Ag acetylation pattern[4] will be detailed based on available antigenicity data involving three synthetic mono- and/or di-*O*-acetylated decasaccharides, the synthesis of which will be described.

[1] K.L. Kotloff *et al.*, *Bull. W. H. O.* **1999**, *77*, 651–666. [2] F. Bélot *et al.*, *Chem. Eur. J.* **2005**, *11*, 1625–1635. [3] A. Phalipon *et al.*, *J. Immunol.* **2009**, *182*, 2241–2247. [4] J. Kubler-Kielb *et al.*, *Carbohydr. Res.* **2007***, 342*, 643–647.

Carbohydrates in Pharmaceutical Research

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Carbohydrates and their derivatives are being employed in pharmaceutical industries in research and in production, be it as small molecules, natural products, or biologicals. Research applications include the use as chiral pool starting materials e.g. to furnish peptide mimetics or chiral scaffolds for the generation of compound libraries. Bioactive carbohydrates are being studied intensely, and some of those or their mimetics[1] have progressed to the market. Specific examples will be discussed.

[1] H. P Wessel, S. D. Lucas, *Oligossacharide mimetics. In Glycoscience: Chemistry and Chemical Biology*; B. Fraser-Reid, K. Tatsuda, J. Thiem, Eds.; Springer Verlag: Heidelberg **2008**, Part 9, 2079-2112; [2] H. P. Wessel, Saccharide-peptide hybrids. In Oligosaccharides in Chemistry and Biology: A Comprehensive Handbook. Synthesis of Oligosaccharides, Glycoconjugates and Glycomimetics, Part II: Synthesis of Oligosaccharide Mimetics; B. Ernst, G. Hart, P. Sinay¨, Eds.; Wiley/VCH: Weinheim, **2000**; Vol. I, 565–586.

Interplay of Chemistry and Biology: Tackling the Problems of Infectious Diseases and Cancers

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Protein glycosylation is the most complex post-translational process; it is predicted that more than 90 percent of human proteins are glycosylated. The significance of glycosylation at the molecular level is however not well understood, and as such the pace for the development of carbohydrate-based drug discovery and diagnosis is relatively slow. It is thus important to develop new tools to study the effect of glycosylation on the structure and function of proteins and other biologically active molecules. This lecture will focus on the development of new methods for the synthesis of homogeneous glycoproteins with well defined glycan structure, glycan arrays for the high-throughput analysis of protein-glycan interaction and design of click-induced fluorescence probes for use to identify new cancer biomarkers for diagnosis and drug discovery. New glycoprotein vaccines and small molecules have been designed and developed to tackle some of the problems associated with cancers and infectious diseases.

The specific interaction of carbohydrates with proteins. A 3D view 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, embryogenesis, 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, the determination of the structural and conformational factors and the physicochemical features which govern the molecular recognition of these molecules is of paramount importance. This presentation is focused on the application of NMR methods to the study of molecular recognition processes between a variety of polypeptides and carbohydrate molecules and analogues as well as sugar-sugar interactions. Special attention will be paid to the conformational and structural details of the interaction process, with particular emphasis in the origin and strength of CH-π interactions. The use of isotope-labeled receptors and ligands (with ^{13}C, ^{15}N, or ^{19}F stable isotopes) highly facilitates the analysis of the interactions between carbohydrates and glycomimetics with the corresponding receptors.

Recent advances in GAGs chemistry: Design and synthesis of new FGF-R agonists

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Heparin (HP) is a complex sulfated Glycosaminoglycan (GAG) involved in various essential biological processes from blood coagulation to cell-cell communication, growth and differentiation. It also plays a critical role in several pathological conditions such as cancer, angiogenesis, some neurodegenerative diseases like Alzheimer's, atherosclerosis and microorganisms infectivity [1]. HP, as well as its structurally related Heparan sulfate (HS), contains highly negative charges coming from sulfate and carboxylate groups that greatly impact its abilities to interact with biological factors, therefore resulting in specific properties. For example, the sulfation pattern of HS has an impact on the complex formation efficiency with Fibroblast Growth Factors (FGFs) and their receptors. The result of this interaction leads to intracellular signal transduction and may improve recovery through angiogenesis and arteriogenesis after heart ischemia as well as in treatment of peripheral nerve injury or peripheral arteries occlusion disease. The communication will focus on the recent advances of our group in finding potent and selective compounds that may promote this process.

[1] For a Review Article, see N. S. Gandhi and R. L. Mancera, *Chem Biol Drug Des*, **2008**, *72*, 455-482.

[2] L. D. Thompson, M. W. Pantoliano, B. A. Springer, *Biochemistry*, **1994**, *33*, 3831-3840.

Oral Presentations

A study of oxathiane glycosyl donors and the basis for their stereoselectivity

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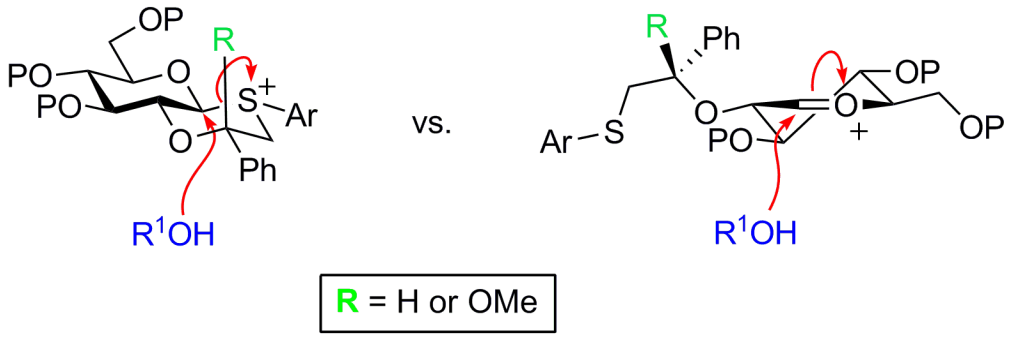
Neighboring group participation has long been used to control the synthesis of 1,2-*trans*-glycosides. More recently there has been a growing interest in the development of similar strategies for the synthesis of 1,2-*cis*-glycosides, in particular the use of auxiliary groups that generate sulfonium ion intermediates.[1-2] However, there has been some debate over the role of sulfonium ion intermediates in these reactions:[3-4] do sulfonium ions actually engage in neighboring group participation, or are they a resting state of the system prior to reaction through an oxacarbenium ion intermediate?

I will describe the reactivities and stereoselectivities of a family of bicyclic thioglycosides in which an oxathiane ring is fused to the sugar to form a transdecalin-like structure (Scheme 1).[2] The importance of a ketal group in the oxathiane ring for maintaining high stereoselectivity has been investigated using a combination of experiment and density functional theory calculations.[5] The experimental data will be discussed in the context of changes in the calculated balance of S_N1 and S_N2 mechanisms, and the two-conformer hypothesis for stereoselective addition to oxacarbenium ions.



Scheme 1. Oxathiane glycosyl donors: S_N2 vs. S_N1 reaction mechanisms

[1] J. H. Kim, H. Yang, J. Park, G. J. Boons, *J. Am. Chem. Soc.* **2005**, *127*, 12090-12097.
[2] M. A. Fascione, S. J. Adshead, S. A. Stalford, C. A. Kilner, A. G. Leach, W. B. Turnbull, *Chem. Commun.* **2009**, 5841-5843.
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Mannuronic Acids: Reactivity and Selectivity

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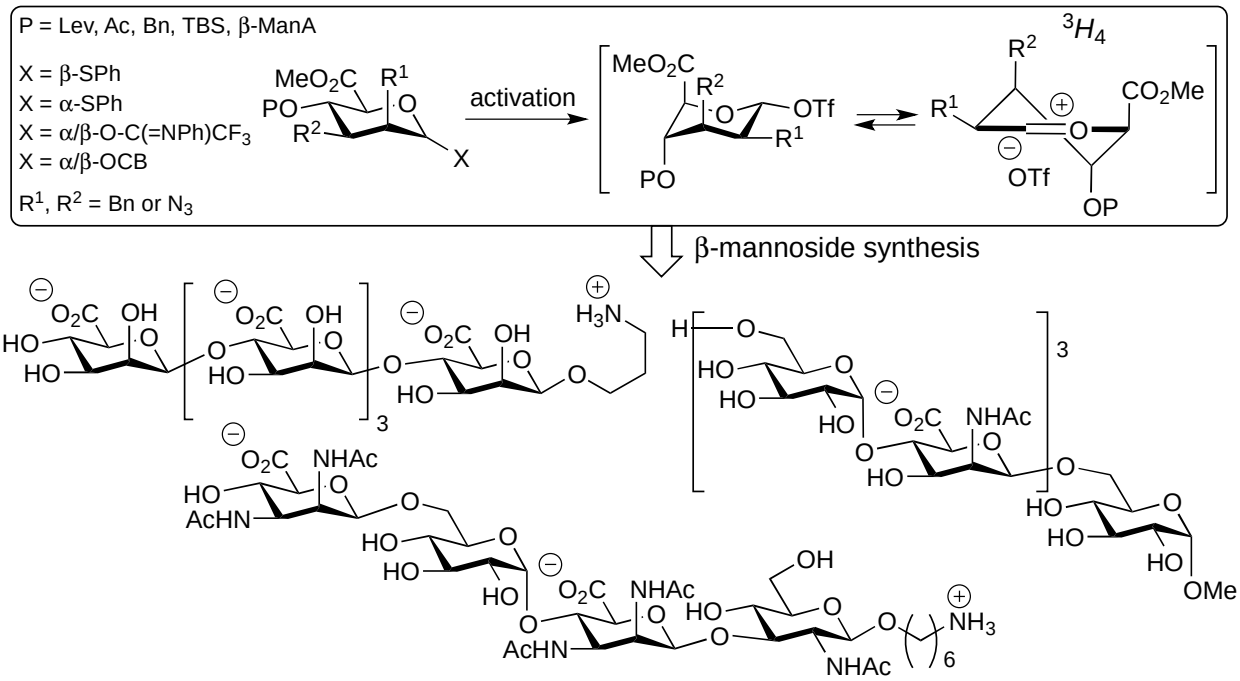
Our continued interest in uronic acid containing oligosaccharides has inspired a study towards the glycosylating properties of mannuronic acids. In these studies we've discovered that mannuronic acid donors generally provide 1,2-*cis* linked products with various nucleophiles, in good to excellent yield with excellent β-stereoselectivity.[1] To explore the reaction mechanism(s) underlying this striking selectivity we've investigated various different uronic acid donors.[2] These studies have revealed a strong stereodirecting effect of the C-5 carboxylate function in the mannose series. Furthermore, surprising equatorial anomeric triflates have been identified as intermediates in glycosylations featuring mannuronate donors.[3] In this presentation the reactivity and selectivity of mannuronic acids will be discussed including their use in the synthesis of various bacterial oligosaccharides.[4]



Scheme 1. Mannuronic acids in the stereoselective synthesis of bacterial oligosaccharides.

[1] J.D.C. Codée, L.J. van den Bos, A. de Jong, J. Dinkelaar, G. Lodder, H.S. Overkleeft, G.A. van der Marel, *J. Org. Chem.* **2009**, *74*, 38-47. [2] J. Dinkelaar, A. de Jong, R. van Meer, M. Somers, G. Lodder, H.S. Overkleeft, J.D.C. Codée, G.A. van der Marel, *J. Org. Chem.* **2009**, *74*, 4982-4991 [3] M.T.C. Walvoort, G. Lodder, J. Mazurek, H.S. Overkleeft, J.D.C Codée, G.A. van der Marel, *J. Am. Chem. Soc.* **2009**, *131*, 12080-12081. [4] M.T.C. Walvoort, G. Lodder, H.S. Overkleeft, J.D.C. Codée, G.A. van der Marel, *J. Org. Chem.* **2010**, *75*, 7990-8002.

Synthesis of Regio- and Stereoregular α(1-6) Mannopyranan from *Mycobacterium tuberculosis* by the Ring-opening Polymerization of Tricyclic Orthoesters of D-mannopyranose

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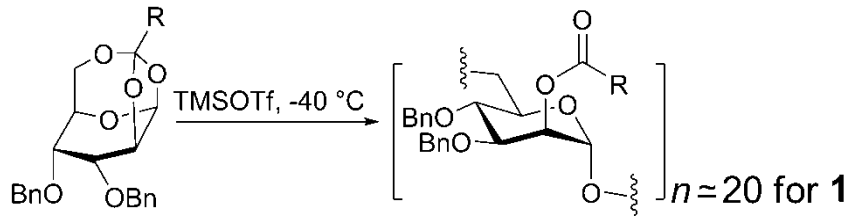
Oligo- and polysaccharides have recently been recognized for their various significant biological activities including eliciting mammalian immune response. Polysaccharides are found as major components of the intricate cell wall structure of bacteria and contribute to bacterial virulence, causing severe diseases in humans, such as tuberculosis and leprosy. Chemically defined mimics of envelope components of *Mycobacterium tuberculosis* (*Mtb*) serve as important tools for biological studies of the bacterial interactions with mammalian hosts. For rapid synthesis of mannopyranan, tricyclic orthoester building blocks of mannose provide suitable structure for ring-opening oligo- and polymerizations. The building blocks 3,4-*O*-benzyl-β-D-mannopyranose 1,2,6-orthobenzoate (**1**) and 3,4-O-benzyl-β-D-mannopyranose 1,2,6-orthopivalate (**2**) were synthesized and utilized as monomers in ring opening oligomerizations. The building blocks **1** and **2** (Scheme 1) were efficiently prepared in six high yielding chemical reactions. The whole synthesis requires only two purification steps. The transformation conditions were adjusted to fit the high humidity climate for versatility in possible industrial scale-up.



1: R = C_6H_5
2: R = $C(CH_3)_3$

Scheme 1. Polymerization of 1,2,6-Tricyclic Orthoesters of D-mannopyranose

Polymerizations of monomer **1** when TMSOTf was used as a catalyst resulted in α(1→6) mannopyranan regio- and stereoselective polymannose with high yields. Preliminary results suggest that the size of α(1-6) mannopyranan polymer products can be controlled by varying the monomer concentrations. Chemically defined structures of LM will facilitate biological studies of bacterial interaction with host immune systems, leading to effective treatments and prevention of tuberculosis.

[1] C. Yongyat, S. Ruchirawat, S. Boonyarattanakalin, *Bioorganic & Medicinal Chemistry* **2010**, *18*, 3726-3734.

Synthesis of New Potential Antiviral Constructs Against Influenza

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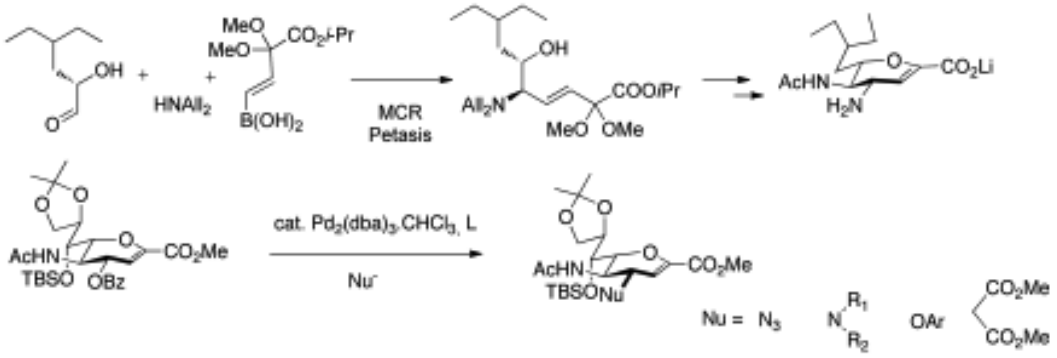
Influenza of type A causes a severe viral infection of the respiratory system. Although vaccination is the primary strategy for the prevention of influenza infections during epidemics, vaccine production by current methods cannot be carried out at the pace required to stop the progress of a new strain of the influenza virus.[1] Also, recent alarming reports on the emergence of drug resistance make the development of new anti-influenza molecules a priority. Therefore, effective antiviral agents are required to prepare for a pandemic. In this general context, our intention is the development of new constructs specifically directed against neuraminidase (N), a crucial glycoprotein for the viral infection and important target for drug development. Using the multi-component Petasis reaction,[2] a recent variation of the Mannich reaction with a vinylboronic acid as the nucleophile, we recently were able to produce, in a short synthetic sequence and with a high stereocontrol, the Relenza-type compound carrying at the 6 position a hydrophobic substituent analogous to the Tamiflu one (Scheme 1).[3] We also developed a highly effective regio- and stereoselective palladium-catalyzed allylic substitution of 2,3-unsaturated derivatives of N-acetyl neuraminic acid (Neu5Ac2en), mostly relying on a suitable protecting group pattern of the starting material. This methodology was applied to the construction of C-C, C-N and C-O bonds and led with high stereoselectivity to the major formation of the C-4 regioisomers.[4]



Scheme 1

[1](a) E. De Clercq *Nat. Rev. Drug Discov.* **2006**, *5*, 1015-1025. (b) M. von Itzstein *Nat. Rev. Drug Discov.* **2007**, *6*, 967-974. [2] (a) N. A. Petasis, I. Akritopoulou, *Tetrahedron Lett.* **1993**, *34*, 583-586. (b) N. A. Petasis, *In Multicomponent Reactions*; Wiley-VCH, Ed.; J. Zhu,;H. Bienaymé: **2005**, pp 1999-1223. [3] J.-F. Soulé, A. Mathieu, S. Norsikian, J.-M. Beau, Org. Lett. 2010, 12, 5322-5325.[4] (a)C. W. Chang, S. Norsikian, J. M. Beau, Chem. Eur. J. 2009, 15, 5195-5199.

Ionic-Liquid-based Catch and Release Oligosaccharide Synthesis (ICROS). Chemical and Enzymatic Applications.

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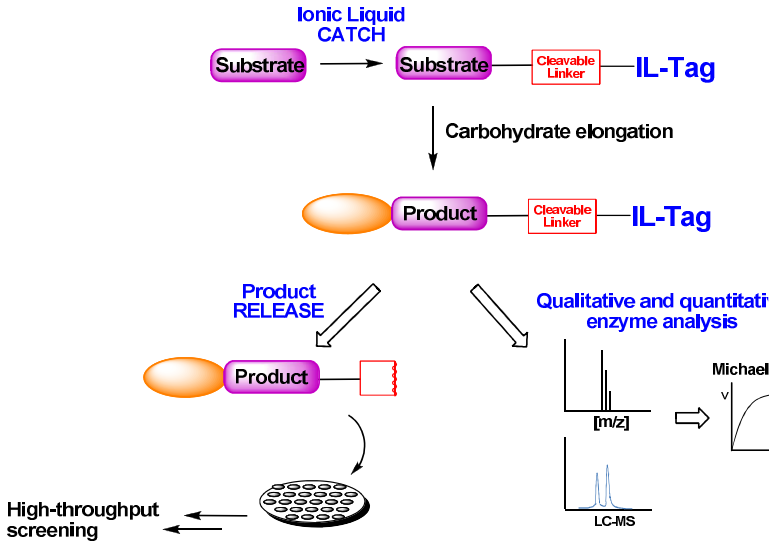
The development of general and efficient methodologies for the preparation of complex oligosaccharides has been the subject of research for many years. Herein, we describe an ionic based "catch-and-release" oligosaccharide strategy for the chemical[1] and enzymatic[2] synthesis and fast purification of oligosaccharides.

We demonstrate that the methodology is compatible with thioglycoside and trichloroacetimidate glycosylation strategies and amenable to protecting group manipulations. Furthermore, we have also shown that the ICROS methodology is compatible with glycosyltransferase enzymatic transformations.



The ionic-liquid-based tags (ITags) developed are designed for easy attachment to substrates and simple product release that is amenable to conjugation to array platforms for further high-throughput biological screening. In addition, the ITags can be used as fast, robust and sensitive tools for reaction monitoring and quantitative kinetic analysis.

[1] A. T. Tran, R. A. Burden, D. Racys and M. C. Galan, submitted, 2010. [2] M. C. Galan, A. T. Tran and C. Bernard, *Chem. Commun.* **2010**, *46*, 8968-8970.

General Strategy for the Synthesis of Branched Lipidated and Phosphorylated GPI-Anchors

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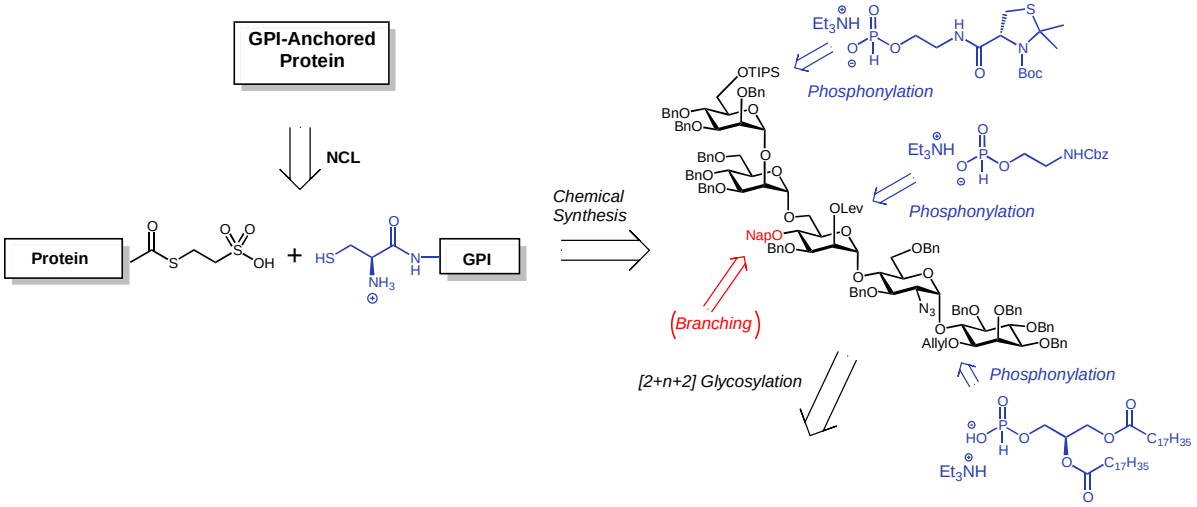
Proteins and glycoproteins can be attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. The structure of this glycolipid is diverse and cell type dependant. Although a conserved core pseudo-pentasaccharide-containing Man3-GlcNH2-inositol structure has been observed, additional modifications such as lipidation, phosphorylation and additional glycosylation can also be found.[1]

Mammalian GPI molecules have been described to contain additional branched carbohydrate structures and phosphorylation. These modifications extend the diversity of the GPI and have been reported as critical moieties for the function and recognition of GPI anchored proteins.[2]



Scheme 1. Retrosynthesis of lipidated and phosphorylated GPI-anchored protein.

In order to understand the role of the branching glycosylations and other modifications in the function of GPIs and GPI anchored proteins, we reported here new strategies for the total synthesis of different branched GPI anchors. The strategies are based on the chemical synthesis of lipidated and phosphorylated GPIs useful for the subsequent ligation to recombinantly expressed proteins (scheme 1).[3] The syntheses of the GPI-glycan moieties were performed using pre-synthesized building blocks by [2+n+2] and [3+n+2] glycosylation strategies. The branching glycosylation has been introduced through the use of an orthogonal protected mannose building block elongated at 4-*O* with a mono-, di- and trisaccharide. Finally, sequential phosphorylations using pre-synthesized *H*-phosphonates and incorporation of a cysteine residue on a phosphate-ethanolamine delivered the desired GPI anchors ready for native chemical ligation to homogenous GPI-anchored proteins.

(1) Ferguson, M. A. J.; Williams, A. F. *Annual Review of Biochemistry* **1988**, *57*, 285.
(2) Debierre-Grockiego, F.; Schwarz, R. T. *Glycobiology* **2010**, *20*, 801.
(3) Becker, C. F. W.; Liu, X. Y.; Olschewski, D.; Castelli, R.; Seidel, R.; Seeberger, P. H. *Angew. Chem. Int. Ed.*. **2008**, *47*, 8215.

Carbene-mediated activation of the anomeric C-H bond: a stereospecific entry towards α- and β-ketopyranosides

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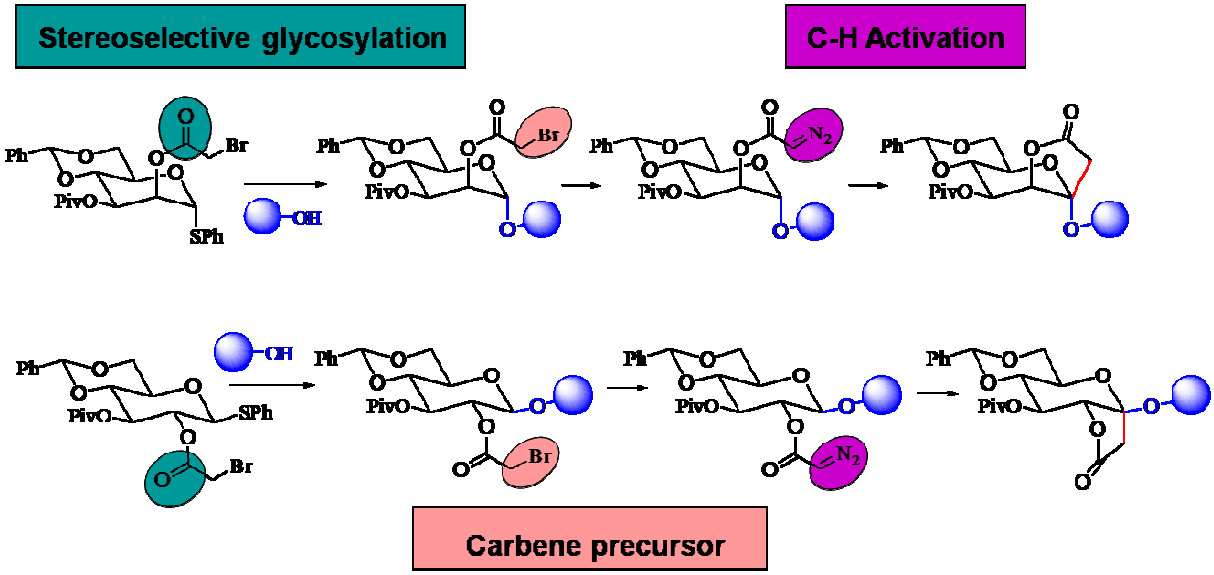
New synthetic tools in carbohydrate chemistry have attracted tremendous interest over the past decades due to the important role of complex oligosaccharides in many fundamental biological processes. In this context, quaternarization of a specific position represents a highly potent entry towards chemical tools for glycobiology. However, such a modification of the sugar backbone requires a multi-step approach relying on: 1) selective protection/deprotection of a given position 2) oxidation of the resulting alcohol 3) addition of an organometallic reagent. Herein we would like to report a new approach towards ketopyranosides relying on the *precise one-step substitution of the anomeric C-H bond to create a new C-C bond*. Thus, a bromoacetate at position 2 first controls the stereoselectivity of a glycosylation step by anchimeric assistance, and, after conversion into a diazoacetate, then promotes activation of the anomeric C-H bond (scheme 1).[1]



Scheme 1. Carbene-mediated activation of the anomeric C-H bond.

This approach relying on late activation of the anomeric C-H bond provides a straightforward entry towards both α- and β-ketopyranosides.

[1] Mélissa Boultadakis-Arapinis, Pascale Lemoine, Serge Turcaud, Laurent Micouin, Thomas Lecourt *J. Am. Chem. Soc.* **2010**, *132*, 15477-15479.

Synthesis, glycosylation, and conformation of tetrafluorinated monosaccharides

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It is now commonly accepted that organofluorines do not engage in strong interactions with hydrogen bond donors. Nevertheless, attractive interactions, electrostatic in origin, are possible with electron poor or positively charged "fluorophilic" regions.[1]

The hydrophilic nature of sugars is not beneficial for achieving good affinities to protein binding sites. Affinity can often be improved by incorporating hydrophobic moieties. Perfluoroalkyl groups are more hydrophobic than alkyl groups.[2]

DiMagno introduced the concept of 'polar hydrophobicity', in which protein-carbohydrate affinity would be increased by polyfluorination, due to a combination of hydrophobic desolvation and fluorophilic interactions.[3] The hexafluorinated pyranose **1** (Figure 1) was shown to cross the erythrocyte membrane (mediated by a glucose transporter) ten times as fast as glucose.

However, sugar stereochemistry is important for binding selectivity, and on this basis we proposed to investigate tetrafluorinated carbohydrates, such as the "F_4-Gal" and "F_4-Glc" **2** and **3**.[4] In addition, the fluorination will significantly modify the hydrogen bond donating/accepting capacities of the adjacent alcohol groups.

The above will be discussed more in detail, and a brief overview of the results obtained so far for the synthesis and glycosylation of this kind of sugar analogues will be given.[4,5] The solid and solution phase $^4C_1$-conformation of "F_4-Gal" derivatives will be discussed (Figure 2).

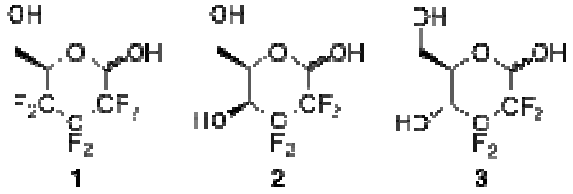

Figure 1. Heavily fluorinated monosaccharides.

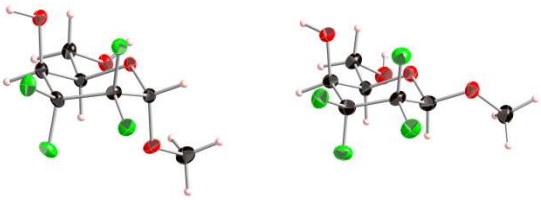

Figure 2. X-ray structures of (*gt*)-methyl α and β-2,3-dideoxy-2,2,3,3-tetrafluorogalactoside.

[1] (*a*) K. Müller, C. Faeh, F. Diederich, *Science*, **2007**, *317*, 1881; (*b*) M. Zürcher, F. Diederich, *J. Org. Chem.,* **2008**, *73*, 4345. [2] Gao, J.; Qiao, S.; Whitesides, G. M. *J. Med. Chem.*, **1995**, *38*, 2292. [3] (*a*) H. W. Kim, P. Rossi, R. K. Schoemaker, S. G. DiMagno, *J. Am. Chem. Soc.*, **1998**, *120*, 9082; (*b*) J. C. Biffinger, H. W. Kim, S. G. DiMagno, *ChemBioChem*., **2004**, *5*, 622. [4] Timofte, R. S.; Linclau, B. *Org. Lett.* **2008**, *10*, 3673. [5] (a) Boydell, A. J.; Vinader, V.; Linclau, B. *Angew. Chem. Int. Ed.* **2004**, *43*, 5677. (b) Linclau, B.; Boydell, A. J.; Timofte, R. S.; Brown, K. J.; Vinader, V.; Weymouth-Wilson, A. C. *Org. Biomol. Chem.* **2009**, *7*, 803.

REPAIRING FAULTY GENES BY SMALL MOLECULES: DEVELOPMENT OF NEW DERIVATIVES OF AMINOGLYCOSIDES FOR TREATMENT OF HUMAN GENETIC DISEASES

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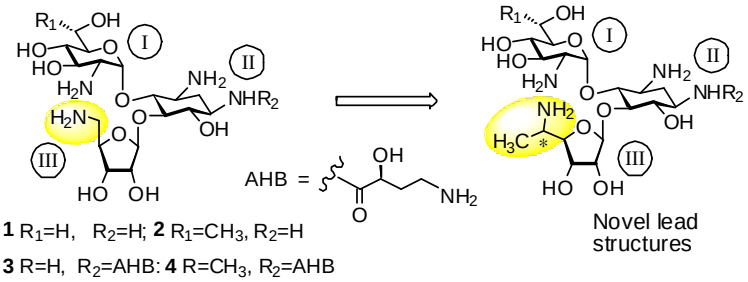
A large number of human genetic disorders including cystic fibrosis, Duchenne muscular dystrophy and several types of cancer are resulted from nonsense mutations, single point alterations in the DNA, where one of the three stop codons (TGA, TAG or TAA) replaces an amino acid-coding codon, leading to a premature termination of the translation and eventually to a nonfunctioning protein. Aminoglycoside antibiotics were the first small molecule drugs that gave promising results as a potential therapy of such genetic disorders. However, high human toxicity (nephrotoxicity and ototoxicity) and reduced readthrough efficiency at safe doses, largely limits their use for suppression therapy. To date, no systematic studies have been performed to optimize aminoglycosides activity for readthrough activity and reduced toxicity.



1 R_1=H, R_2=H; **2** R_1=CH_3, R_2=H

3 R=H, R_2=AHB: **4** R=CH_3, R_2=AHB

Figure1. Novel aminoglycosides as potent readthrough inducers

Towards these ends we recently reported on the development of novel pseudo-trisaccharide derivatives of paromomycin (Figure 1, **1**-**4**) with significantly improved readthrough activity and reduced toxicity in comparison to those of parent paromomycin and gentamicin.[1-3] stop codon mutations was examined in both *in vitro* and *ex vivo* mammalian systems.[1-3] In attempts to further improve the readthrough efficiency we discovered that the introduction of a chiral C5''-methyl group on the ribosamine ring (ring III) of these lead structures (**1**-**4**) significantly improves the suppression activity of the observed derivatives while the toxicity was retained unchanged and similarly low as that of the parent leads.[4] By separately synthesizing each C5''-diasteromers of the ribosamine ring and solving their absolute configuration, we also discovered significant preference of the (*S*)-C5''-methyl group over that of (*R*)-C5''-methyl group in the resulted pseudo-trisaccharides, both in cell-free and cell-based readthrough activity tests. The new developed pharmacophore, (*S*)-C5''-methyl group, was further introduced in combination with other structural elements and created most powerful pseudo-trisaccharide derivatives exhibiting the highest readthrough efficacy and reduced toxicity ever reported. These new biochemical data, mostly unpublished together with very recently communicated data[4] will be presented in terms of design, synthesis, structure-activity-toxicity relationship, and their impact both on fundamental understanding of aminoglycosides-induced toxicity and their potential for the treatment of genetic diseases.

(1) Nudelman *et al., Bioorg. Med. Chem. Lett*., **2006**, *16*, 6310. (2) I. Nudelman *et al., J. Med. Chem*., **2009**, 52, 2836. (3) Nudelman et al., *Bioorg. Med. Chem*., **2010**, *18*, 3735. (4) Jeyakumar *et al., Med. Chem. Commun*., **2011**, DOI: 10.1039/c0md00195c.

Production of Ca-dependent high-affinity lectins with defined specificity by mutagenesis of PA-IIL lectin

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The bacterium *Pseudomonas aeruginosa* is a human opportunistic pathogen that can infect almost every human tissue in conditions of a lowered immunity barrier. The lectin PA-IIL plays an important role in the bacteria's virulence. It displays unusually high affinity for L-fucose in the micromolar range. [1] This characteristic is correlated with the remarkable presence of two calcium ions in the binding site of the protein. [2] Using genome analysis, several lectins with sequence similarities have been identified in other bacteria. These homologues have similarly strong affinities but different specificities towards monosaccharides despite having only slight structural differences.

In vitro mutagenesis was performed focusing on three single point mutants of PA-IIL in positions 22-23-24. The mutated amino acids belong to the "specificity-binding loop". The structure of all mutants was determined by X-ray crystallography and their thermodynamic characterization was performed using isothermal titration calorimetry. The mutants were designed also by *in silico* methods, and the resulting structures were used for docking experiments. The *in vitro* mutagenesis in combination with computational methods allowed the key importance of amino acid 22 for the specificity of the lectin to be identified. [3] *In silico* approach was used also for a saturation mutagenesis in all positions.

We have further focused on amino acids in the binding loop that can modulate hydrophobic interactions, e.g. Thr98 that participate in the sugar binding through a water bridge and Thr45 that interacts with a methyl group of L-fucose. Some interesting mutants in these positions were prepared using site-directed mutagenesis. In addition to site-directed studies, we also use methods of random mutagenesis for preparation of a large-scale mutant library with altered or improved binding properties.

[1] E.P. Mitchell, C. Sabin, L.Šnajdrová, M. Pokorná, S. Perret, C. Gautier, C. Hofr, N. Gilboa-Garber, J. Koča, M. Wimmerová, A. Imberty, Proteins. **2005,** *58(3),* 735-46. [2] E.P. Mitchell, C. Houles, D. Sudakevitz, M. Wimmerová, C. Goutier, S. Pérez, A.M. Wu, N. Gilboa-Garber, A. Imberty, Nat Struct Biol. **2002,** *10(4),* 918-21. [3] J. Adam, M. Pokrná, C. Sabin, E.P. Mitchell, A. Imberty, M. Wimmerová, BMC Struct Biol. **2007**, *7*, 36-48.

Synthesis of novel carbohydrate-heterocycle conjugates *via* C(3)-modifications of hexoses

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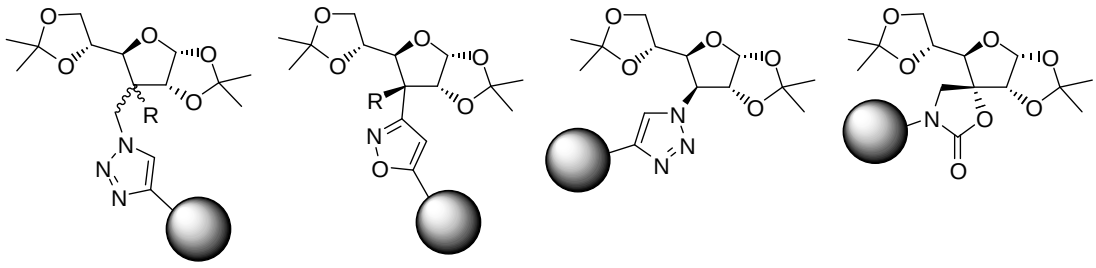
Triazole-carbohydrate conjugates exhibit a broad spectrum of biological properties such as inhibitory effects on the proliferation of leukemia cells [1] and glycosidases [2]. Similarly, oxazolidinones exhibit distinct biological activities and, in particular, sugar-based spiro-oxazolidinones have raised a special interest [3]. On the other hand, isoxazoles have been successfully used to link sugars with other molecules of medicinal interest [4]

We report here synthesis of novel carbohydrate-azole and carbohydrate-spiro-oxazolidinone conjugates (Scheme 1). Our synthetic approach is based on Cu(I)-catalyzed click-dimerization, nitrile oxide 1,3-dipolar cycloaddtion reaction, and oxazolidinone formation at C(3) of glucose unit, respectively. Additionally, disacharides that are connected by an extended linker containing bis-triazole, bis-isoxazole, and bis-spiro-oxazolidinone units are produced in a similar way.
Diacetone-D-glucose, diacetone-D-allose, and diacetone-D-galactose derived azides, as well as diacetone-D-glucose derived nitrometyl derivatives are the key-intermediates in these syntheses. Practical aspects of large scale synthesis of C(3)-modified hexoses and the biological activities of the selected products will be discussed.



Scheme 1. Novel carbohydrate-heterocycle conjugates

[1] K. El Akri, K. Bougrin, J. Balzarini, A. Faraj, R. Benhida, *Biorg. Med. Chem. Lett*. **2007**, *17*, 6656-6659. [2] L. L. Rossi, A. Basu, *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3596-3599. [3] C. Gasch, J. M. Illangua, P. Merino-Montiel, J. Fuentes, *Tetrahedron* **2009**, *65*, 4149-4155 and references therein. [4] K. S. Wankhede, V. V. Vaidya, P. S. Sarang, M. M. Salunkhe, G. K. Trivedi, *Tetrahedron Lett*. **2008**, *49*, 2069-2073.

O-Serine Glycosides and Aspartic Acid Conjugates: Easy Access to Synthetic Glycosphingolipid Analogues

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In recent years, synthetic carbohydrate chemists have devoted considerable efforts towards the synthesis of glycosphingolipid analogues. The discovery of the potent biological activities of compounds such as alpha-galactosylceramide derivative KRN700 has prompted a search for non-natural glycoconjugate mimics of glycosphingolypids.[1] Many examples of synthetic analogues of this family of compounds have been reported, and significant advances in the understanding of their structure-activity relationships have been achieved. However, the search for more specific and potent analogues that can lead to new therapies for cancer and autoimmune diseases remains a goal for scientists working in this field.

Glycopeptide conjugates allow for rapid synthetic access to a wide variety of glycolipid analogues of biological relevance. Using synthetic carbohydrate chemistry, we are preparing glycolipids incorporating *O*- serine glycosylated building blocks, as well as derivatives generated from aspartic acid (shown in Figure 1). The advantages of using this type of approach include (i) rapid introduction of orthogonal functionality in the glycoside building block, (ii) availability of synthetic precursors and (iii) well established synthetic methodologies.

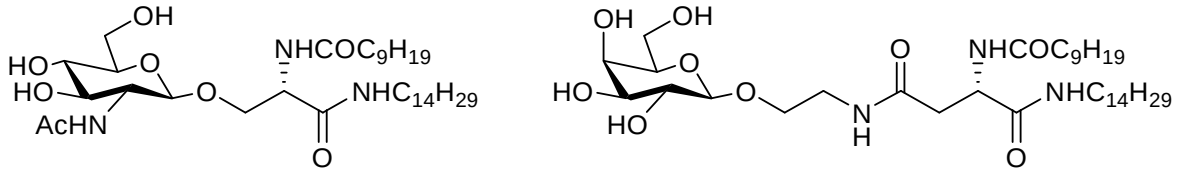

Figure 1. Structure of synthetic glycolipids featuring serine and aspartic acid derivatives.

We have found that the presence of the long acyl chain affects greatly the reactivity of the glycoconjugates and determines the synthetic sequence. The introduction of the aspartic acid moiety confers certain degree of rigidity to the glycoconjugate structure, which in turn will influence the overall conformational preferences of the glycolipids.

[1] A. Schiefner, M. Fujio, D. Wu, C.H. Wong, I. A. Wilson, *J. Mol. Biol.* **2009**, *394*, 71–82.

Synthesis of aminosugars using transition metal catalysis

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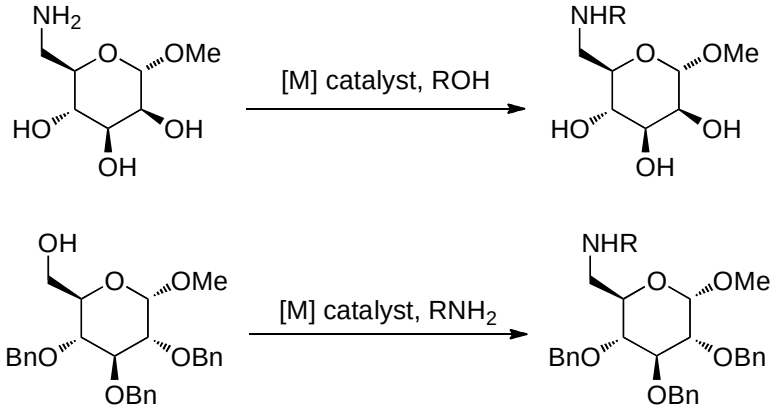
Two significant goals of current carbohydrate chemistry should be the development of new chemistry applicable to the carbohydrate "backbone", and the transformation of carbohydrates into biologically active molecules, for example glycomimetics. Homogeneous transition metal complex catalysed redox coupling between amines and alcohols to form higher order amines is currently in vogue [1], but its use beyond simple substrates has hardly been studied.

Here we present results of our investigation into how this reaction can be used to modify carbohydrates, including: alkylation of carbohydrate amines at different ring positions using alcohols or amines as alkylating agents; alkylation of carbohydrate amines without affecting unprotected carbohydrate alcohols; amination of carbohydrate alcohols.



[1] For a review: Guillena, G.; Ramon, D.J.; Yus, M., *Angew. Chem.* **2007***, 46,* 2358–2364.

Seven-membered iminosugars: ring isomerisation and glycosidase inhibition

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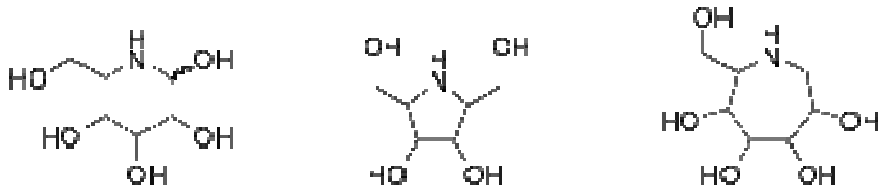
Iminosugars, in which the ring oxygen has been replaced by nitrogen, constitute the most promising class of sugar analogues because their glycosidase and/or glycosyltransferase inhibition profile make them promising therapeutics.[1] As a consequence, some iminosugar derivatives are already on the market to treat diabetes or Gaucher disease while others are currently involved in clinical trials to treat cancer, viral infections or genetic diseases such as cystic fibrosis. While five- and six-membered iminosugars have been largely investigated, the unusual seven-membered analogues have been rather unexplored [2] despite an expected potential related to their conformational flexibility.



Scheme 1. Structure of five-, six- and seven-membered iminosugars

We have launched a program to explore the synthetic access, the biological and the synthetic potential of these polyhydroxylated azepanes.[3] We will present herein recent results regarding these flexible iminosugars.

[1] Iminosugars: From Synthesis to Therapeutic Applications; Compain, P., Martin, O.R., Eds.; Wiley-VCH: Weinheim, 2007.
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[3] H. Li, Y. Blériot, C. Chantereau, J.-M. Mallet, M. Sollogoub, Y. Zhang, E. Rodriguez-Garcia, P. Vogel, J. Jimenez-Barbero, P. Sinaÿ, *Org. Biomol. Chem.* **2004**, *2*, 1492-1499.

Iminosugars: an Opportunity for Discoveries in Chemistry and Biology

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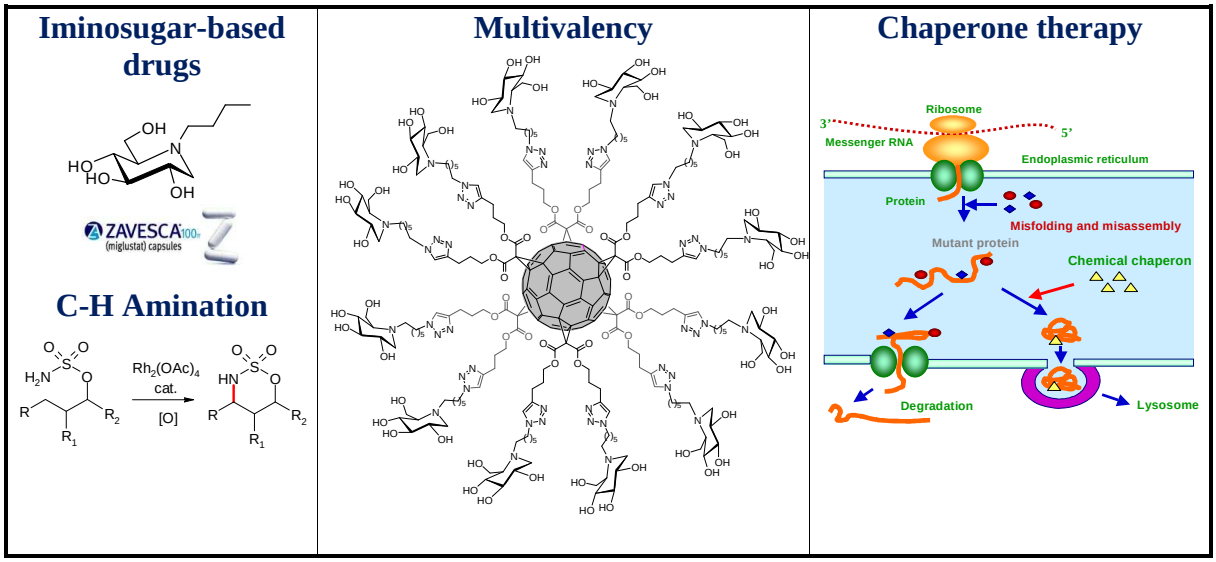
Since the emergence of the first synthetic iminosugars in the mid-1960s, the pace of discoveries in this field has been breathtaking. In addition to glycosidases, the scope of their biological activity as inhibitors has been extended to numerous enzymes.[1] Several iminosugars are now under development as therapeutic agents or already used as drugs to treat a wide range of diseases.[1] In this context, the general objective of our research is to combine innovative synthetic methodologies and original structures to meet fundamental challenges in glycobiology and to discover original iminosugars of therapeutic interest. For example, we have recently developed a new bond construction strategy based on iterative multifunctionalization of unactivated C-H bonds in azacycloalkanes related to iminosugars.[2a]



A dodecavalent iminosugar with a fullerene core was recently synthesized;[2b] this unprecedented neoglycoconjugate was found to display a binding enhancement of up to 3 orders of magnitude over the corresponding monovalent ligand. This is the first evidence of a significant multivalent effect in glycosidase inhibition.[2b] In addition, we have recently disclosed promising iminosugar-based pharmacological chaperones for the treatment of Gaucher disease, a rare genetic disorder.[2c,d]

1. *Iminosugars: from Synthesis to Therapeutic Applications*; Compain, P.; Martin, O. R., Eds.; Wiley-VCH, 2007.

2. (a) Toumieux, S.; Compain, P.; Martin, O. R *J. Org. Chem.* **2008**, *73*, 2155; (b) Compain, P.; Decroocq, C.; Iehl, J.; Holler, M.; Hazelard, D.; Mena Barragán, T.; Ortiz Mellet, C.; Nierengarten, J.-F. *Angew. Chem. Int. Ed.* **2010**, *49*, 5753; (c) Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. *ChemBioChem* **2006,** *7*, 1356; (d) Oulaïdi, F.; Front-Deschamps, S.; Gallienne, E.; Lesellier, E.; Ikeda, K.; Asano, K.; Compain, P.; Martin, O. R. *ChemMedChem* **2011**, *6,* 353.

Synthesis of Novel Iminoglycolipids as Pharmacological Chaperones of Mutant Lysosomal Enzymes

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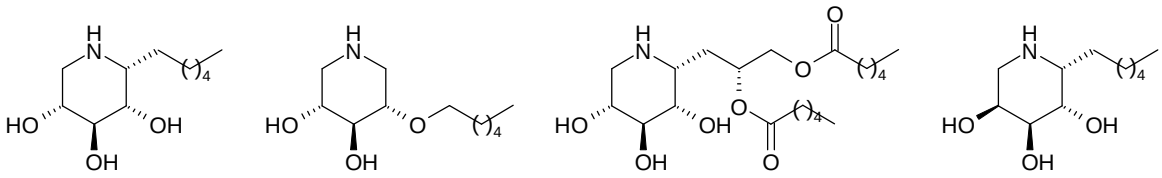
Lysosomal Storage Disorders (LSD) are rare inherited diseases caused by a mutation in one of the glycosidases involved in the catabolism of macromolecules, such as glycosphingolipids, glycoproteins, glycogen and mucopolysaccharides. The resulting dysfunction leads to the accumulation of these macromolecules in organs, which induces serious, often fatal symptoms. An innovative and very promising approach to the treatment of LSD is the use of pharmacological chaperones:[1] such compounds, which are usually potent inhibitors of the involved glycosidases, help stabilize the mutant protein and save it from degradation; this results in an increased enzymatic activity in the lysosome and a concomitant decrease of the symptoms. Our investigations have shown that α-1-*C*-alkyl and *O*-alkyl derivatives of iminoxylitols such as **1** and **2** are potent inhibitors of β-glucocerebrosidase (GCase) and act as effective pharmacological chaperones of the N370S mutant form of GCase from Gaucher disease patients.[2,3]

Similar synthetic methodologies have been recently applied for the synthesis of glucosylceramide mimics such as **3**, and in *galacto*-like series to yield compounds such as **4** as potential inhibitors of β-galactocerebrosidase involved in Krabbe disease or lysosomal β-galactosidase involved in G_{M1}-gangliosidosis. These results pave the way to new therapeutic agents for LSD.



1 **2** **3** **4**

Scheme 1. Novel iminoglycolipids as pharmacological chaperones

The synthesis of these various iminoglycolipids and preliminary biological results on GCase will be described in this communication.

[1] J.-Q. Fan, *Biol. Chem.* **2008**, *389*, 1-11. [2] P. Compain, O. R. Martin, C. Boucheron, G. Godin, L. Yu, K. Ikeda, N. Asano, *ChemBioChem* **2006**, *7*, 1356-1359. [3] F. Oulaïdi, S. Front-Deschamps, E. Gallienne, E. Lesellier, K. Ikeda, N. Asano, P. Compain, O. R. Martin, *ChemMedChem* **2011**, *6*, 353-361.

Enhanced incorporation of unnatural sialic acid precursors into glycans of GNE deficient cell lines

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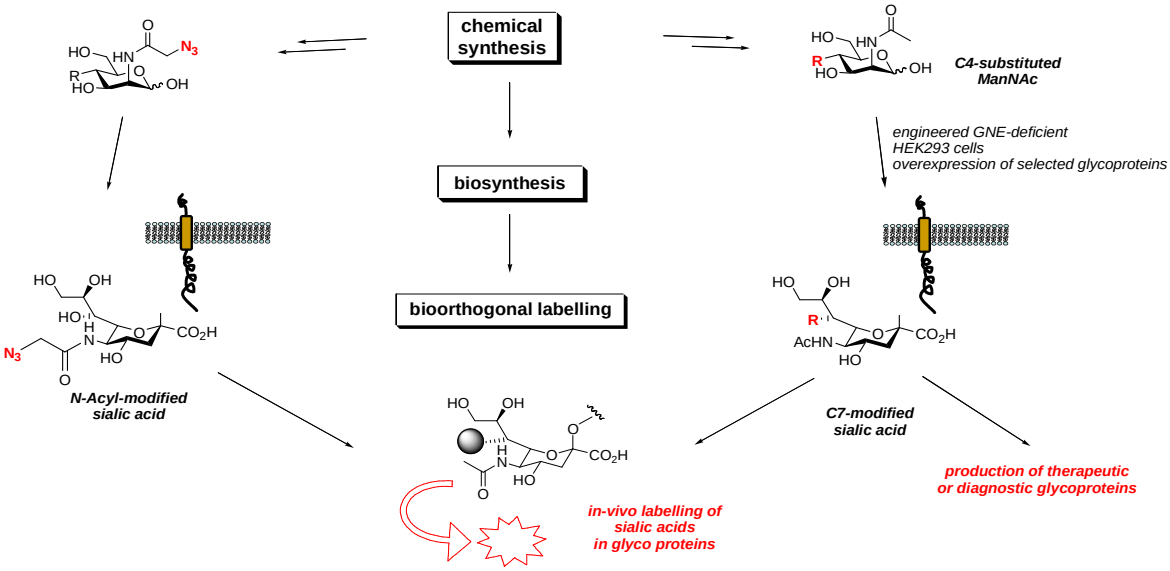
Proteins and peptide hormones of body liquids like blood and tear fluid are essential components of the immune system which support the recognition of and the defence against harmful pathogens. The proper function and the stability of these molecules is dependent on terminally sialylated *N*- and *O*-linked glycans; consequently, alteration of the sialic acid content of these glycoproteins significantly changes their bioactivity. Introduction of additional glycans or the substitution of natural sugars by chemically engineered analogues can improve the quality of therapeutically administered antibodies and growth factors. We employed the concept of biochemical engineering[1, 2] and genetically engineered cell lines which lack components of sugar synthesis pathways to improve the rather low incorporation rate of the involved enzymes. Key regulator of sialic acid biosynthesis is the bifunctional enzyme UDP-GlcNAc 2-epimerase/ManNAc kinase (GNE) converting UDP-GlcNAc into ManNAc. We successfully established GNE deficient cell lines showing a strongly increased incorporation of ManNAc analogues into glycans of cell surface proteins. We also synthesized 4-Azido-MaNAc[3] which was metabolised into 7-Azido-sialic acid and incorporated into glycoproteins by our cells, which was confirmed by western blot and quantified by fluorescence-based HPLC analysis.



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[3] R. Thomson, M. von Itzstein, *Carbohydrate Research* **1995**, *274*, 29.

Nonlinear optical membrane imaging using carbohydrate-based probes

Redon Sébastien,[1,2] Massin Julien, [1,3] Cottet Davy, [4] Wage Hartmut, [4] Douady Julien, [4] Pouvreau Sandrine, [1,5] Andraud Chantal, [1,3] Queneau Yves, [1,2] Girard-Egrot Agnès, [1,6] Bretonnière Yann, [1,3] Chambert Stéphane[1,2]

[1] Université Lyon F-69622, Lyon, France, [2] COB, ICBMS, UMR 5246, INSA-Lyon, France, [3] Laboratoire de Chimie de l'ENS Lyon, CNRS, ENS Lyon, UCBL, UMR 5182, France, [4] LSP, UMR 5588 CNRS-Université Grenoble 1, 38402 Saint Martin d'Hères, France, [5] Physiologie Intégrative, Cellulaire et Moléculaire, UMR CNRS 5123, Université Lyon 1, France, [6] GEMBAS, ICBMS, UMR 5246, Université Lyon 1, France.

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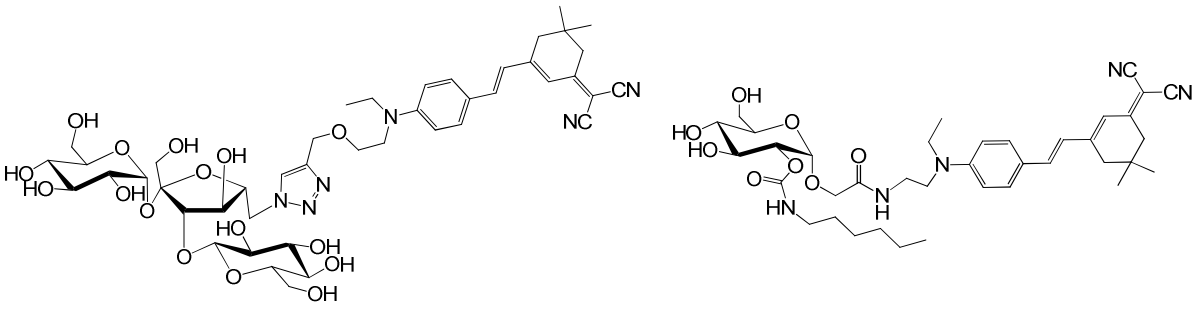
In this interdisciplinary project, by mimicking the overall structure of natural glycolipids we wish to beneficiate of their well known membrane specificity in order to produce new tools dedicated to two promising emerging nonlinear membrane imaging experiments (TPM: two-photon excited fluorescence microscopy and SHIM: second-harmonic imaging microscopy).[1,2] By taking advantage of straightforward methods for modifying and then grafting highly diverse carbohydrate backbones[3,4] onto especially designed push-pull chromophores, a first series of probes was obtained.[5] They were the first neutral synthetic dyes to show a long living SHG signal in cell culture and thus validated our initial postulate by showing the improved properties attained with the use of a carbohydrate hydrophilic moiety. In order to better fulfil the standard requirements of membrane imaging dyes, two new series of probes were designed. Their synthesis as well as their enhanced behaviour will also be discussed (optical characterization, membrane insertion evaluation, biological and imaging properties).



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Deciphering the structure of the trisaccaharide found on *Neisseria meningitidis* pilin

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Protein glycosylation is an abundant polypeptide chain modification in nature. Glycans can be covalently attached to the amide nitrogen of Asn residues (*N*-glycosylation), to the hydroxyl oxygen of, typically, Ser or Thr residues (*O*-glycosylation), and, in rare cases, to the indole C2 carbon of Trp through a C–C linkage. Protein glycosylation has been demonstrated to be essential in eukaryotes for a multitude of cellular functions, but it is only recently that glycoproteins have been discovered and their structures uncovered in prokaryotes. It is now evident that there are clear roles for glycoproteins in bacterial virulence and communication. As many of the carbohydrate moieties found in bacterial glycosylation are not found in humans, the biochemical pathways that synthesise such compounds are therefore targets for therapeutic intervention.

One type of glycosylation of interest is the *O*-glycosylation found on the Pili of the Gram-negative bacterium *Neisseria meningitidis*. Pili which is assembled from monomers of the structural subunit pilin, have been shown to be involved in a variety of cellular processes including cell adhesion and virulence.[1] The glycan moiety comprises a trisaccharide with the structure, Gal-β1,4-Gal-α1,3-DATDH[2] where DATDH represents the structure of an unusual monosaccharide termed 2,4-diacetamido-2,4,6-trideoxyhexose. A similar structure, Gal-β1,4-Gal-α1,3-GATDH has also been found.[3] To date, despite some preliminary investigation into the general structure of the unusual monosaccharide, the true identities of DATDH/GATDH have remained elusive.

Here we describe the synthesis of a set of trisaccharides representing the possible structures of the pilin glycosylation motif. We then use these molecules to demonstrate which of these structures represents potentially the true structure of the trisaccharide. This knowledge coupled with deciphering the function of the proteins involved in the biosynthetic pathway that synthesise DATDH/GATDH will finally allow elucidation of this important type of *O*-glycosylation.

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From Polymer to Size-Defined Oligomers: An efficient and Stereocontrolled Construction of Biotinylated Chondroitin Oligosaccharides

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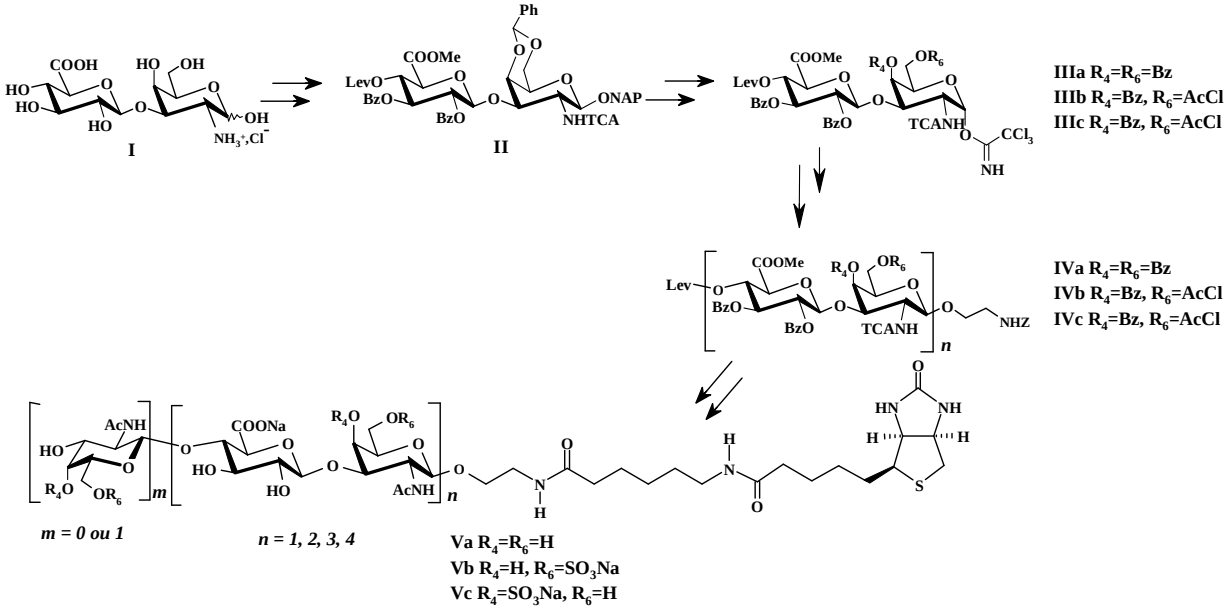
Chondroitin Sulfate (CS) belongs to the family of complex, polyanionic, linear polymer called glycosaminoglycanes (GAGs). CS polymer is one of the major components of cartilage matrix and a defect in the biosynthesis of chondroitin sulfate has been observed in several diseases such as osteoarthritis. Within a program devoted to the study of the biosynthesis of CS chain, a collection of biotinylated oligosaccharide (sulfated (in position 4 or 6) or not) derivatives has been prepared in order to study the substrate specificity of chondroitin synthase, the glycosyltransferase responsible for the polymerization of the chondroitin chain.

Controlled acid hydrolysis of heterogeneous polymeric chondroitin sulfate of bovine origin afforded a basic disaccharide **I** [1] that was used as starting material for the preparation of tailor-made building block **II**. From this key intermediate, several imidates compounds (**III**) has been synthesised and allowed the stereocontrolled construction of a collection of size-defined biotinylated chondroitin oligosaccharides sulfated or not (**V**).[2,3]



Scheme 1. Synthesis of biotinylated chondroitin oligosaccharides

[1] Lopin, C.; Jacquinet, J.-C. *Angew. Chem. Int. Ed.* **2006**, *45*, 2574-2578.
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[3] Jacquinet, J.-C.; Vibert, A.; Lopin-Bon, C. *Chem. Eur. J.* **2009**, *15*, 9579-9595.

Towards new glycoclusters : Synthesis of furanose-supported erythritols by [1,3]-dipolar cycloaddition

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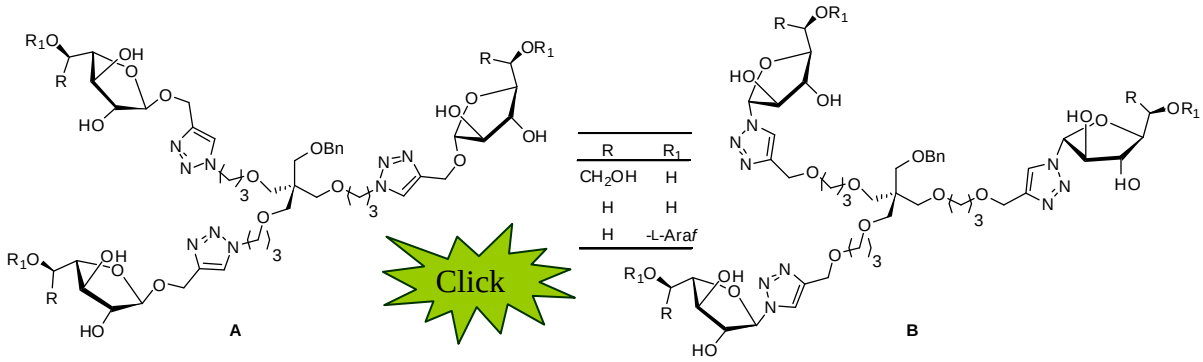
Hexofuranoconjugates are natural carbohydrate-based molecules which are not present in mammalian cells but biosynthesized and metabolized by some microorganisms that could be highly pathogenic (*Mycobacterium* and *Leishmania* sp., etc).[1] Moreover it is common knowledge that natural oligosaccharides including oligofuranosides can positively modulate the mammalian immune system.[2] Owing to their natural distribution, synthetic furanose-containing targets are thus of great interest in order to increase understanding of their biology and to design new pharmacophores.

Poly- and oligosaccharides bind rather weakly to their receptors. With the aim of increasing this affinity, L-arabino- and D-galactofuranosyl residues were grafted on U.V. active pentaerythritol supports by [1,3]-cyloaddition to form new glycoclusters bearing furanosyl heads (Scheme 1). It was expected that the new created entities exhibit higher bioactivities due to the well-known cluster effect.[3]



Scheme 1. Targeted glycofuranoclusters

For this purpose, 1-propargyl furanosides and furanosyl azides were synthesized and clicked to azidopropan- or propargyloxypropanerythritol, respectively. Further extension of this methodology was performed for the multi-presentation of alpha-(1,5)-arabinobioside which was obtained according to a chemo-enzymatic approach. It allowed the easy and flexible access to two new classes of glycodendrimers with potential antiparasitic and/or immunostimulating properties.

[1] P. Peltier, R. Euzen, R. Daniellou, C. Nugier-Chauvin, V. Ferrières, *Carbohydr. Res.* **2008**, *343*, 1897-1923. [2] I. Chlubnová, B. Sylla, C. Nugier-Chauvin, R. Daniellou, L. Legentil, B. Kralova, V. Ferrières, *Nat. Prod. Rep.* **2011**, In press. DOI:10.1039/C1031NP00005E. [3] J. Lundquist, E. Toone *Chem. Rev.* **2002**, *101*, 555-578.

Synthesis of Oligoethyleneglycol-linked Carbohydrate Epitopes as Artificial Ligands for the Carbohydrate-binding Protein Malectin

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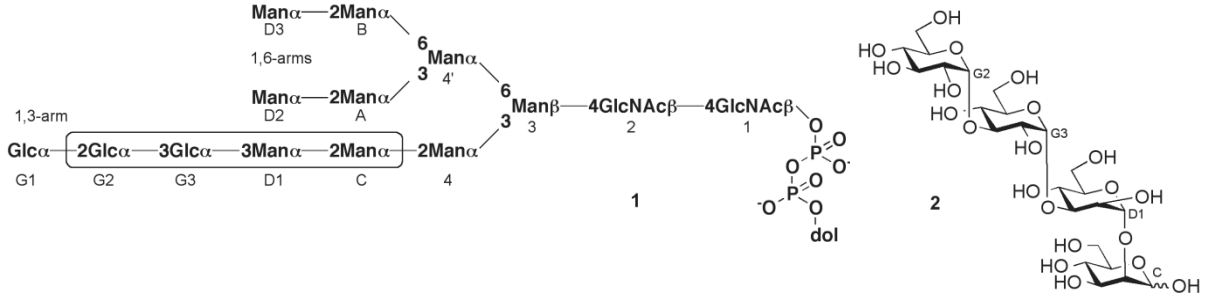
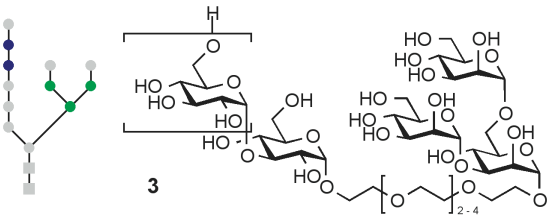
The carbohydrate-binding protein *malectin*, an ER-resident protein, is currently being discussed as a possible player in *N*-glycoprotein quality control in higher eukarya.[1] Its binding affinity to disaccharides and to defined fragments of the $Glc_3Man_9GlcNAc_2$-*N*-glycan precursor **1**, e.g. fragments as the $Glc_2Man_7GlcNAc_2$-*N*-glycan, has been demonstrated.[2,3] As the exact binding epitopes of such carbohydrate structures remained unknown, we commenced a synthetic program aimed at the preparation of defined *N*-glycan precursor fragments as possible *malectin* ligands. Recently, we reported on the synthesis of a Glc_2Man_2-fragment **2** (highlighted region in **1**) and its binding properties with regard to *malectin*.[4]



Based on these results we envisioned a second generation of potential *malectin* ligands, incorporating not only carbohydrate residues from the *N*-glycan precursor's 1,3-arm (highlighted in blue), but from the 1,6-arms (highlighted in green) as well.



Here, we present the synthesis of compounds presenting two distinct, oligoethyleneglycol-linked carbohydrate epitopes (**3**); as well as our results regarding their binding properties with respect to *malectin* (determined from NMR-experiments).

[1] M. Aebi, R. Bernasconi, S. Clerc, M. Molinari, *Trends Biochem. Sci.* **2010**, *35*, 74-82. [2] T. Schallus, C. Jaeckh, K. Feher, A. S. Palma, Y. Liu, J. C. Simpson, M. Mackeen, G. Stier, T. J. Gibson, T. Feizi, T. Pieler, C. Muhle-Goll, *Mol. Cell. Biochem.* **2008**, *19*, 3404-3414. [3] T. Schallus, K. Feher, U. Sternberg, V. Rybin, C. Muhle-Goll, *Glycobiology* **2010**, *20*, 1010-1020. [4] L. N. Müller, C. Muhle-Goll, M. B. Biskup, *Org. Biomol. Chem.* **2010**, *8*, 3294-3299.

Clustering of *Escherichia coli* type-1 fimbrial adhesins using multimeric heptyl α-D-mannoside probes with a carbohydrate core

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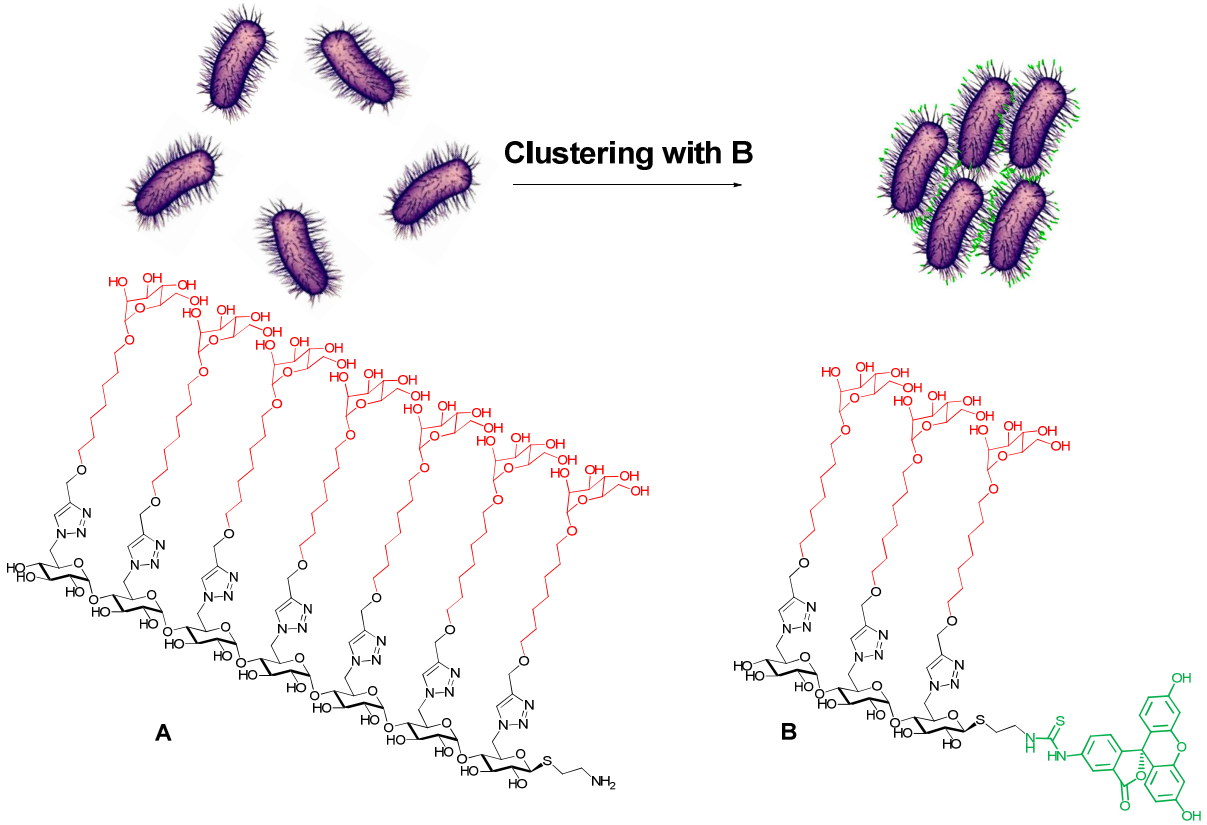
The development of multi-resistant bacterial strains to antibiotic treatments is a serious health problem. Preventing the pathogen adhesion is an appealing alternative, as the bacteria is not killed, and then less prone to develop resistances. Previous results have shown that inhibition of type 1 fimbriated *E. Coli* can be efficiently achieved with synthetic monovalent mannosides bearing hydrophobic aglycons in the anomeric position.

We will present here a protocol for the synthesis of multivalent heptyl mannosides (HM) based on a carbohydrate scaffold. In such regular system, the modulation of valency does not introduce new functional groups, affect the hydrophilicity or the spatial presentation of HM epitopes, as a critical fragment n is repeated. This is of importance to strictly assess how the valency of the ligand influences lectin binding. Activity of the multimers toward the FimH adhesins and uropathogenic bacterial strains will be discussed.



Scheme 1. Structure of a synthetic heptavalent (HM) ligand **A** and of a trivalent probe **B** forming clusters of bacteria in solution.

Synthesis and evaluation of novel polysulfurated glycoasterisks

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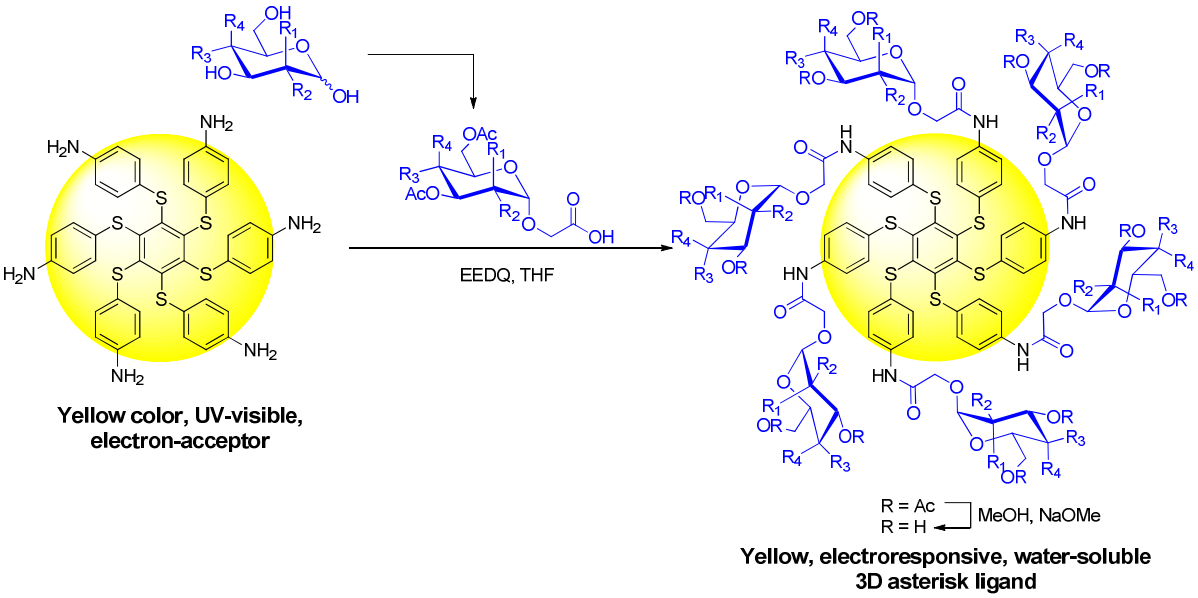
Protein-carbohydrate interactions mediate a wide range of biochemical processes. Amongst them, bacterial infection often proceeds through carbohydrate-binding lectins expressed on bacterial surfaces involved in biofilm formations. Even if the individual associations result from weak assemblies, the assembly of these multiple carbohydrate-protein interactions, typically more than additive, confers to the system the required specificity and avidity for their biological functions.

In order to study this « glycocluster effects », a number of scaffold systems presenting multivalent carbohydrate ligands have been prepared. Dendrimers, polymers, peptides, calixarenes, to name a few, have been used as core molecules for the synthesis of multivalent glycoconjugates.

We have developed a new class of sulfurated, semi-rigid, radial and low-valent glycosylated asterisk ligands. They are well-defined and should potentially present a dual function as a ligand and as a probe thanks to interesting optoelectronic properties.



Scheme 1. Preparation of 1st generation glycoasterisks. [1]

Preliminary results with ConA provided a near nanomolar inhibition concentration, which is unusual in this field. [1] The variation of several parameters such as carbohydrate units, arm's length, cores and possible branching, are currently under investigation to further modulate the ligand affinity of those glycoasterisks. These 2nd generation polysulfurated glycoasterisks will further be evaluated for testing ligand-lectin interactions, multivalency effects, thermodynamic/kinetic parameters, structural biology and the modulation of some biological activities on bacteria, such as in *Pseudomonas aeruginosa*. [2]

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[2] ANR PCV GlycoAsterix, 2008-2011.

Access to bioactive glycoclusters through chemoselective ligations

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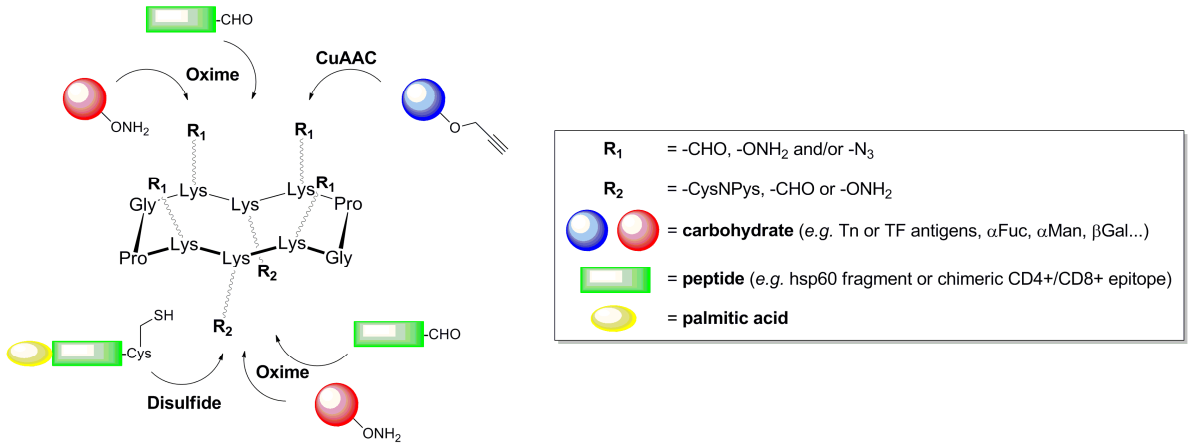
Synthetic glycoclusters have stimulated increasing interests over the last decade [1]. Among the large variety of multivalent glycosylated structures reported so far, we recently showed that cyclopeptides represent attractive scaffolds for diverse biological applications. Cyclopeptide-based glycoclusters with various composition and sugar density can be assembled in a controlled manner using chemoselective procedures (*e.g.* oxime ligation, Huisgen 1,3-dipolar cycloaddition CuAAC) [2-4].



Scheme 1. General synthetic approach for the construction of cyclopeptide-based glycoclusters.

Here we present the synthesis of several compounds, with a special focus on antitumoral synthetic vaccines and inhibitiors for a fucose-specific lectin from *Pseudomonas aeruginosa* (PA-IIL).

[1] Y. M. Chabre, R. Roy, *Adv. Carbohydr. Chem. Biochem.*, **2010**, *63*, 165-393. [2] O. Renaudet, K. Křenek, I. Bossu, P. Dumy, A. Kádek, D. Adámek, O. Vaněk, D. Kavan, R. Gažák, M. Šulc, K. Bezouška, V. Křen. *J. Am. Chem. Soc.*, **2010**, *132*, 6800-6808. [3] O. Renaudet, G. Dasgupta, I. Bettahi, A. Shi, A. B. Nesburn, P. Dumy, L. BenMohamed. *PLoS ONE*, **2010**, *5*, e11216. [4] I. Bossu, M. Šulc, K. Křenek, E. Dufour, J. Garcia, N. Berthet, P. Dumy, V. Křen, O. Renaudet. *Org. Biomol. Chem.*, **2011**, in press (doi: 10.1039/c0ob00772b).

Synthesis of antiadhesive molecules and inhibitors of the LPS Biosynthetic pathway – Towards Antivirulence Molecules

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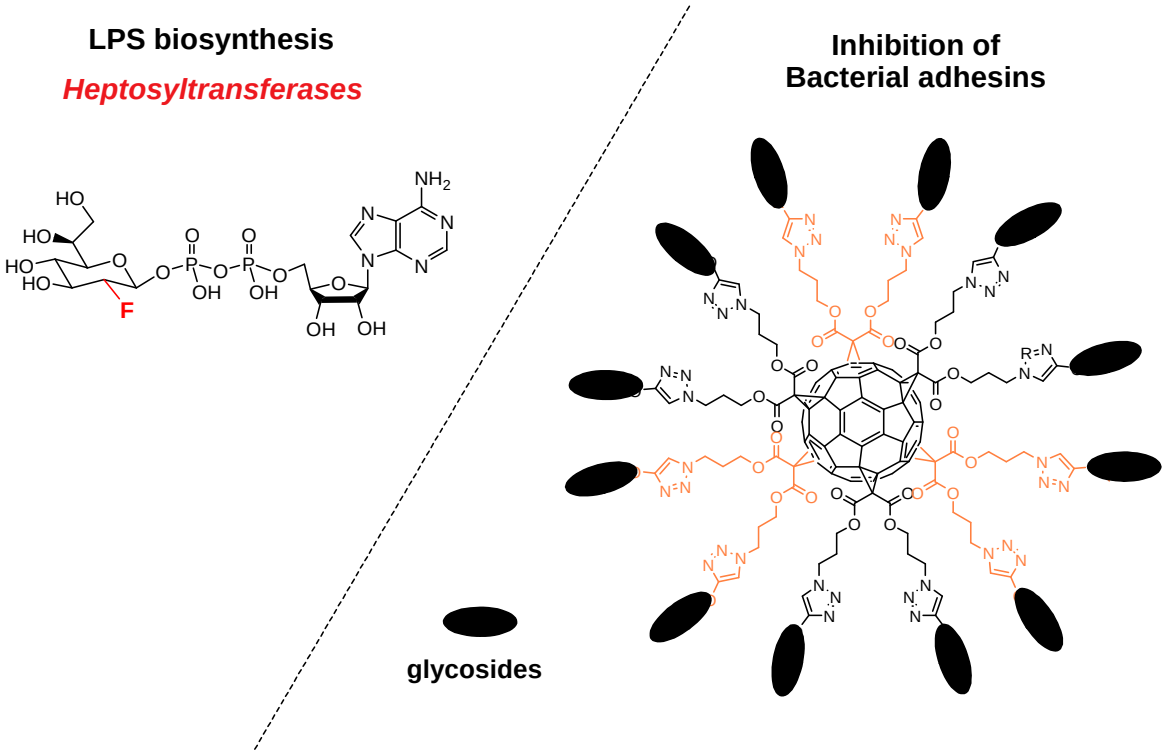
Our laboratory is interested in the mechanistic and inhibition studies of essential enzymes involved in the bacterial cell wall biosynthesis of important human pathogens.[1] Within this context, we have developed novel synthetic methodologies for the preparation of 2-fluoro-glycosides with high anomeric selectivity.[2]



Antivirulence has recently emerged as an alternative antibacterial chemotherapeutic strategy applied to the discovery of new bactericides or bacteriostatic molecules. With this concept in mind, we have recently reported on two complementary approaches that may lead to novel antibacterial agents: 1) the inhibition of LPS heptosyltransferases as a novel approach to inhibit the bacterial cell wall resistance to innate immune response[2] 2) the inhibition of the adhesion of uropathogenic bacteria by multivalent glycosylated fullerenes.[3,4]

[1] A. Caravano, H. Dohi, P. Sinaÿ, S. P. Vincent, *Chem. Eur. J.* **2006**, *12*, 3114–3123 [2] A H. Dohi, R. Périon, M. Durka, M. Bosco, Y. Roué, F. Moreau, S. Grizot, A. Ducruix, S. Escaich, S. P. Vincent *Chem. Eur. J.* **2008**, *14*, 9530-39. [3] M. Durka, K. Buffet, J. Iehl, M Holler, J.-F. Nierengarten, J. Taganna, J. Bouckaert, S.P. Vincent *Chem. Commun.* **2011**, 47, 1321-3 [4] J.-F. Nierengarten, J. Iehl, V. Oerthel, M Holler, B.M. Illescas, A. Muñoz, N. Martín, J. Rojo, M. Sánchez-Navarro, S. Cecioni, S. Vidal, K. Buffet, M. Durka, S.P. Vincent *Chem. Commun.* **2010**, *46*, 3860-3862.

Acid-Promoted Per-O-Sulfation of Large Oligosaccharides Related to Fucoidan Fragments

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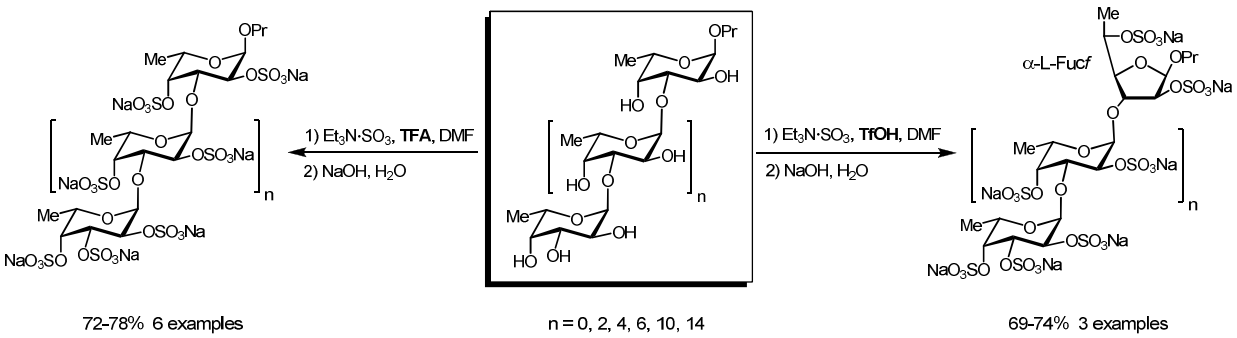
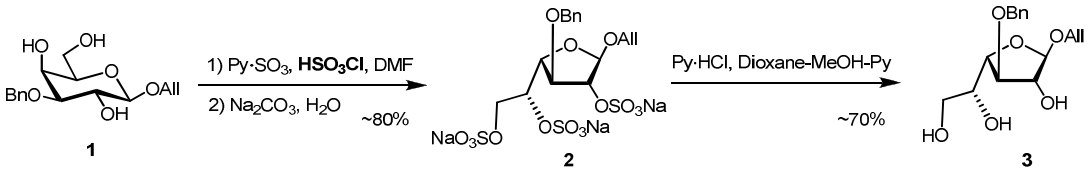
Sulfated polysaccharides fucoidans from brown seaweeds exhibit a wide range of biological activities including anticoagulant, antiangiogenic and antimicrobial as well as the ability to inhibit P- and L-selectin mediated inflammation. In this communication we report on the synthesis of per-O-sulfated oligosaccharides related to the structure of natural fucoidans. Linear oligofucoside chains (up to 16 monosaccharide units) were assembled according to block-wise schemes, with the use of recently developed by us acid-promoted sulfation protocol (Scheme 1) [1].



Scheme 1. Acid-promoted sulfation of linear oligofucosides

We also report on the unusual rearrangement of fucopyranoside residue into fucofuranoside one, which was observed during sulfation (Scheme 1). These conditions can be also applied for transformation of galactopyranoside derivative **1** into galactofuranoside one **2** which appear to be a convenient method for further synthesis of protected galactofuranoside building block **3** (Scheme 2) useful for the assembling of oligosaccharide chains.



Scheme 2. Transformation of galactopyranose into galactofuranose *via* acid-promoted sulfation

Acknowledgments: This work was supported by the Russian Foundation for Basic Research (Grants 10-03-00980-a) and Grant of President of Russian Federation (MK-5544.2010.3).

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The polysaccharide intercellular adhesin and bacterial biofilm formation

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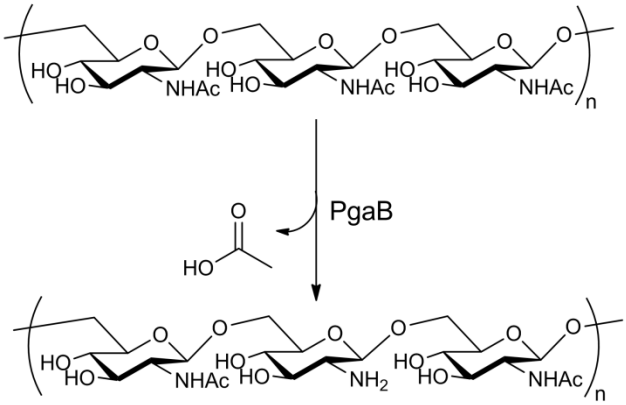
Biofilms are sessile communities of bacteria that adhere to biotic or abiotic surfaces. The formation of bacterial biofilms requires an extracellular matrix to facilitate adherence of bacteria to the surface they colonize.[1] A wide variety of medically important pathogenic biofilm-forming bacterial strains, including S. epidermidis, S. aureus, E. coli, B. pertussis, and Y. pestis generate the same β(1-6)-N-acetylglucosamine (PNAG) homopolymer as a key biofilm matrix exopolysaccharide. In these bacterial strains PNAG undergoes partial enzymatic de-N-acetylation, which is essential for polysaccharide export and surface attachment. In vivo studies have implied that the enzyme responsible for carrying out de-N-acetylation in E. coli is PgaB, an enzyme which has sequence homologues in gram-negative species that form PNAG-dependent biofilms.



Scheme 1. Poly-N-acetylglucosamine de-N-acetylation by PgaB.

This seminar will discuss the chemical synthesis, properties and functionalization of PNAG as well as the in vitro characterization of the de-N-acetylase PgaB and our efforts towards developing inhibitors of this class of enzymes.

[1] D. Davies, *Nat. Rev. Drug Discovery*, **2003**, *2*, 114-122. [2] T. Romeo *et al. J. Bact***. 2008**, *190*, 3670-3690

Production of Natural Product-Based Aerogels from Carbohydrate Precursors Using Supercritical Fluid Technology

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Natural polymer-based materials are regarded as renewable and suitable feedstocks for environmentally sustainable processes. Among them, the use of natural carbohydrates and derivatives are especially attractive because of their non-toxicity, stability, availability and renewability. The broad portfolio of natural polysaccharides with different functional groups (e.g., carboxylic –pectin–, sulfonic –carrageenan–, hydroxyl –agar– and amino –chitosan– groups) and behaviours (anionic –alginate–, cationic –chitosan–, non-ionic –starch–) is a promising starting point for the development of tailor-made materials with controlled properties. Moreover, the usual biodegradability and biocompatibility of these natural polymers, coupled with their capability to be chemically modified, confers them ideal properties for life science applications (e.g., drug delivery systems, tissue scaffolds, biotechnology, pesticides) among other niche markets (e.g., catalysts, environmental remediation). Polysaccharides can undergo gelation by means of different external stimulii (temperature –thermotropic gel–, counterions –ionotropic gel–, covalent cross-linkers –covalently-bound gel–). In this work, the ability of polysaccharides to form gels was exploited to get dry light-weight nanoporous materials (aerogels; ρ=0.05-0.5 g/cm^3) with high surface area (S_a=100-700 m^2/g) using supercritical fluid-assisted drying technology. The inherent null surface tension of supercritical fluids avoids the pore collapse phenomenon in the gel structure during solvent elimination, leading to the outstanding properties of aerogels [1]. Materials engineering and step-by-step process optimization allowed the development of processing approaches to get aerogels from polysaccharide precursors with different gellation mechanisms (ionotropic, thermotropic) and with different morphologies (cylindrical monoliths, beads and microspheres) [2]. The thus obtained natural products were specifically assessed for its use as a chemical compound carrier. Loading of a model compound (ketoprofen) by supercritical CO_2-assisted impregnation was attempted with excellent results (15-20 wt.% drug loaded using alginate and starch aerogel microspheres as matrices).

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Microarray-based Glycomics: Ligand Synthesis and Chip Applications

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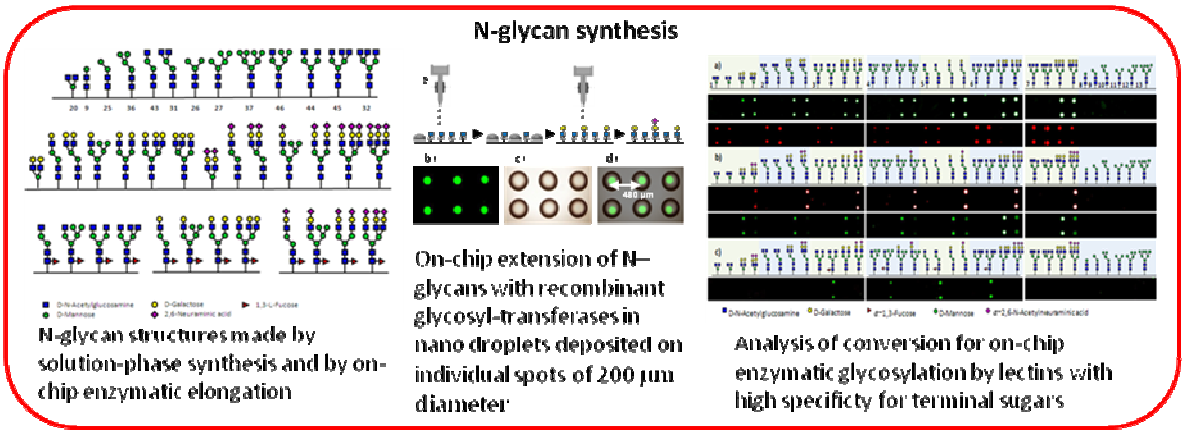
Glycan arrays have recently emerged as versatile platforms in Functional Glycomics for the high-throughput screening of binding specificities for lectin and antibodies or the substrate requirements of carbohydrate processing enzymes. A remaining major bottleneck for a more extended use of glycan arrays is the supply with sufficiently pure and well-characterized ligands. Focusing on individual glycan classes our laboratory is applying several approaches for the efficient synthesis of structurally diverse collections of carbohydrate structures as ligands in glycan arrays.

Combining the modular synthesis of core structures and their on-chip enzymatic extension in nano-droplets we have prepared N-glycan arrays with systematic variations in branching, terminal sugars and core modifications.[1,2] For the preparation of heparan sulfate oligosaccharides with variations in sulfation pattern, sequence and length we have recently started to explore parallel solid-phase synthesis as a promising methodology for accelerating glycan library synthesis.[3]

We are currently started to applying our N-glycan arrays in a variety of relevant glycomics applications, like lectin binding assays and activity screening of glycosyltransferase and hydrolases employing different readout and immobilisation techniques.[4]



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(4) Sanchez-Ruiz, A.; Serna, S.; Ruiz, N.; Martin-Lomas, M.; Reichardt, N.-C. *Angew. Chem. Int. Ed.* **2011**, n/a.

DNA Glycomimetics and DNA Directed Immobilization based Glycoarray

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Glycoarrays are powerful tools for the understanding of protein/carbohydrate interactions and should find applications for the diagnosis of diseases involving these interactions. They are two key issues for obtaining a high performance device. The first one relates to the difficulty in obtaining a large variety of carbohydrates probes and the second one to their immobilisation on the solid support. Herein, we demonstrate that DNA-bearing multivalent glycoconjugates can be synthesized on a phosphorylated scaffold. The synthesis is based on a combination of oligonucleotide phosphoramidite or H phosphonate chemistries on solid support and microwave assisted click chemistry[1-8].

These glycomimetics are immobilised by DNA-directed immobilisation (DDI) onto a DNA chip. The performance of the resulting device is compared to direct covalent immobilisation. The IC_{50} values are measured for different glycomimetics towards their lectins. Furthermore, DDI permits to perform first the interaction between these glycomimetics and the lectin and then to address the resulting complex thanks to the specificity of DNA hybridization.

Next, affinities of different glycomimetics were tested towards *Ricinus communis agglutinin* 120 and *Pseudomonas aeruginosa* 1 Lectin.[7, 9-11]

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One-Point Heparin Conjugation –
Ligate it, Click it and Show it

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Heparin is a highly sulfated, hence highly charged, complex heteropolysaccharide. Polymeric material for medicinal purpose is often coated with heparin to prevent blood clotting. Heparin coating procedures are based both on covalent and non-covalent (by *e.g.* ion interaction) attachment to surfaces. For most of the coating methodologies it is anticipated that each polysaccharide chain forms randomly several bonds to the polymeric material, thus forcing the heparin molecules to accommodate along the polymer surface. Material properties might be improved if one-point coupling between the polymeric surface and the heparin chains could be achieved. The challenge is to produce such a defined and reliable single point attachment and to provide evidence that it has been made.

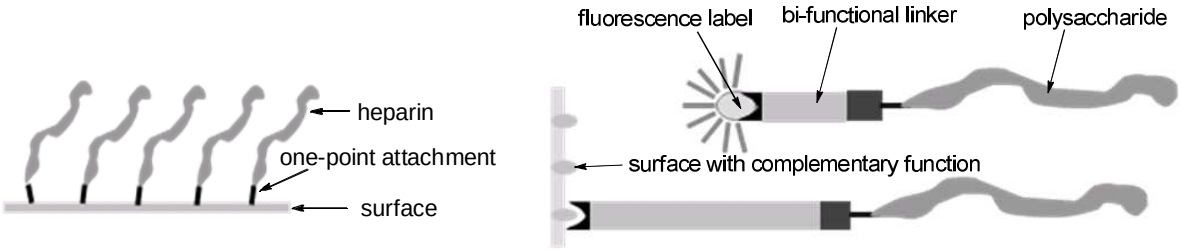

Figure 1. left: idealised surface; right: schematic representation of synthesised conjugates.

Since the reducing-end anomeric centre is the only unique reactive site in the whole polysaccharide chain, this site was selected for the one-point conjugation. A combination of conjugation techniques, i.e. oxime ligation and click chemistry, together with different functionalised linker and a fluorescence label, provided the desired conjugates. [1]

Targeting Anthracycline-resistant Tumor Cells with Aloe-emodin Glycosides

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Anthracyclines such as doxorubicin (DOX, Figure 1) are antineoplastic agents commonly used in the treatment of hematopoietic and solid tumors. One of the major limitations on the clinical use of anthracylines results from the emergence of tumor cells with resistance to these chemothrapeutic agents.

In search for novel directions to develop anthracyclines with activity against resistant tumors, our attention was drawn to the unique properties of a family of natural anthranoids that includes danthron, rhein, and aloe-emodin (AE, Figure 1) which have antiproliferative activity against several tumor cell lines. Interestingly, although its activity was modest, AE demonstrated similar levels of cytotoxicity against several tumor cell lines and their corresponding anthracycline-resistant lines. Based on these observations, we reasoned that it should be possible to design AE analogs with potent antitumor activity against anthracycline-resistant tumor cell lines.

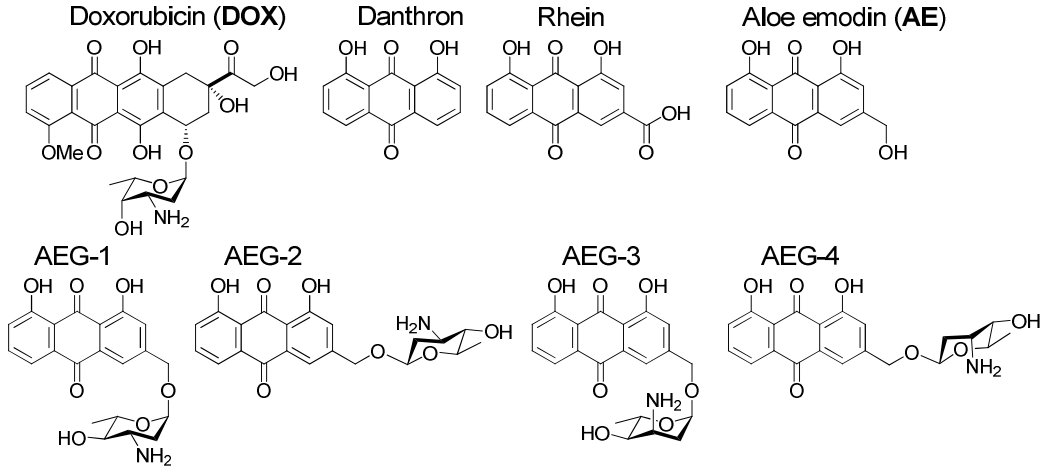

Figure1. Anthracyclines, Anthrnoids and Synthetic AEGs

Hence, we designed and synthesized a small collection of AE glycosides (AEGs **1**-**4**, Figure 1) by attaching a 2,3,6-trideoxy-3-amino-L-sugar to AE anthranoid core in order to improve the DNA minor groove binding properties and as such, the antitumor activity of these analogs. Some AEGs exhibited improved cytotoxic activity of more than two orders of magnitude relative to that of DOX in several tumor cell lines with different levels of anthracycline resistance. Confocal microscopy confirmed that the synthetic AEGs permeated anthracycline-resistant tumor cells while DOX accumulated in these cells membranes. The results of this study demonstrate that AEGs may be used as a promising scaffold for the development of antineoplastic agents that will overcome the widespread problem of anthracycline resistance in tumors in which resistance is conferred by efflux pumps.

Synthesis of Mucin Glycopeptides – Potential Tools to Study Protein Glycosylation in Airway Disease

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We synthesize various glycopeptides with the common aim to generate molecular based tools to study structure and function of protein glycosylation in healthy and disease states. This includes synthesis of glycopeptides to function as standards in quantitative glycoproteomics, generation of antibodies for selective identification or enrichment of specific protein glycosylation, antibody binding epitope identification and microbe ligand mapping. Our current work to synthesize mucin tandem repeat glycopeptides with the aim to generate specific antibodies and to study microbe binding will here be described.

The Mucin glycoproteins have heavily glycosylated tandem repeat peptide regions rich in PTS residues and are the major constituent of the mucus layer. This layer is a part of the innate immune system, and function as a protective barrier against invading pathogens in epithelial tissues [1]. Changes in the expression level and glycan structures of different mucins affect the physical (flow and viscosity) properties of the mucus. In case of chronic airway diseases (asthma, cystic fibrosis (CF) and chronic obstructive pulmonary disease (COPD)), an overproduction of mucin occurs, together with changes in the proportion between the different mucins and their glycan structures [2]. These structural changes are inflammatory-infective responses and contribute to defect flow properties of the mucus, which further induce a chronical inflammatory status and an environment where pathogens can grow and trives instead of being cleared from the system. In order to generate molecular tools to study changes in glycosylation during infection and inflammation in the airways and also to study the role of mucin glycosylation in mechanisms of infection, mucin tandem repeat glycopeptides will be prepared.

Mucin glycopeptide synthesis will perform employing a chemoenzymatic approach. Glycosylated amino acid building blocks with different core and extended core *O*-glycan structures are synthetically prepared and further incorporated into the synthesis of various mucin tandem repeat glycopeptides. In addition to variation of position and density of glycosylation on the mucin tandem repeats further diversity is generated through terminal glycosylation/modification with fucose, sialic acid or sulphate residues employing glycosyl/sulphotransferase enzymes. The large repertoire of glycopeptides generated aims to represent glycosylation on naturally occurring tandem repeats of mucin glycoproteins. These peptides will be immobilized on microarray slides to study binding epitopes including dynamic changes in binding preferences during severity of disease of pathogens known to cause infection in the airways. Immobilization of the synthetic mucin glycopeptides to immune carriers and generation of specific monoclonal antibodies to be used as analytical tools to study changes in glycan structure caused by inflammatory-infective responses will further perform. The mucin glycopeptides synthesized are also of interest as molecular tools to study other mucin rich regions prone to inflammation/infection.

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[2] Thornton *et al.*, *Annu. Rev. Phys.* **2008**, *70*, 459-486.

Structural studies on synthetic mannose selective receptors by NMR and modeling

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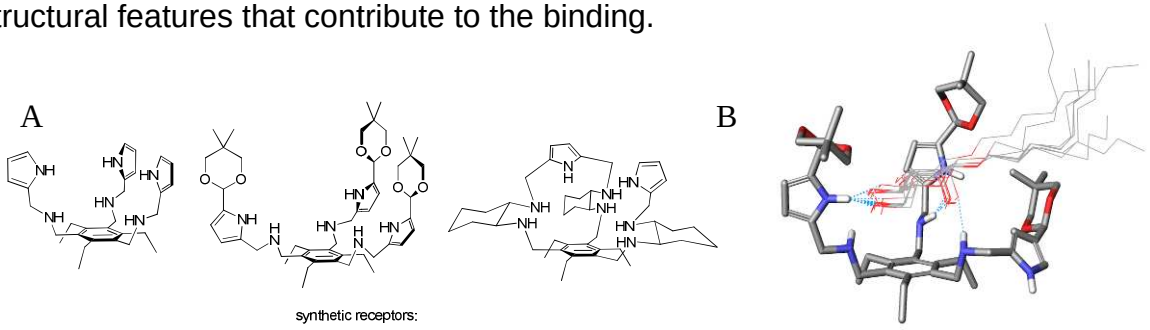
Molecular recognition of carbohydrates by natural receptors (lectins and antibodies) is one of the most important events in living organisms. Unraveling the structural features that govern these processes is a topic of major interest. In recent years, a number of new synthetic receptors for carbohydrates have been reported in an attempt to mimic natural recognition, and shed light on the study of these interactions. Because of its biological relevance, mannose is a challenging and most relevant recognition target for synthetic receptors.

Recently,[1] we have reported on new tripodal benzene receptors decorated with amine and pyrrol groups that have demonstrated to be able to selectively bind b-mannose among the most biologically relevant monosaccharides. A combination of NMR spectroscopy and modeling has been applied to the study of the complexes between these tripodal receptors and octyl-b-D-mannoside, revealing the key structural features that contribute to the binding.



Scheme 1. A: Some of the receptor's structures that recognise mannose, studied in this work. B. The structure of the complex between a synthetic receptor and octyl-b-mannose deduced from NMR and modeling.

[1] (a) A. Ardá, C. Venturi, C. Nativi., O. Francesconi, F.J. Cañada, J. Jiménez-Barbero, S. Roelens, *Eur. J. Org. Chem*., **2010**, 64-71. (b) A. Ardá, C. Venturi, C. Nativi, O. Francesconi, G. Gabrielli, F. J. Cañada, J. Jiménez-Barbero, S. Roelens, *Chem. Eur. J.,* **2010**, *16*, 414-418. (c) A. Ardá, F J. Cañada, C. Nativi, O. Francesconi, G. Gabrielli, A. Ienco, A. J. Jiménez-Barbero, S. Roelens, *Chem. Eur. J.* accepted.

Molecular Recognition and Conformation Analysis of *O*- and *N*-Linked Glycopeptides

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Glycoproteins are a major class of glycoconjugates that plays an important role on molecular recognition.[1] Hence, study of the structure and dynamics of this interacting glycoconjugates are crucial to achieve a better knowledge of the living systems. In addition, NMR has become a major tool to disclose the conformational behaviour and the interaction properties of carbohydrate-protein interactions.[2] In this context, we are interested to investigate the molecular recognition events of either *O*- and *N*-linked glycopeptides in both free and bound state to protein receptors. For that purpose new synthetic glycopeptides containing either unnatural aminoacids (e.g. methyl serine) or uncommon glycosidic bonds (α-*N*-linked glycopeptides) were prepared.

In this communication the implications on the molecular recognition of short tumor-associated glycopeptides **1** and **2** studied by STD-NMR and molecular modelling will be reported.[3]

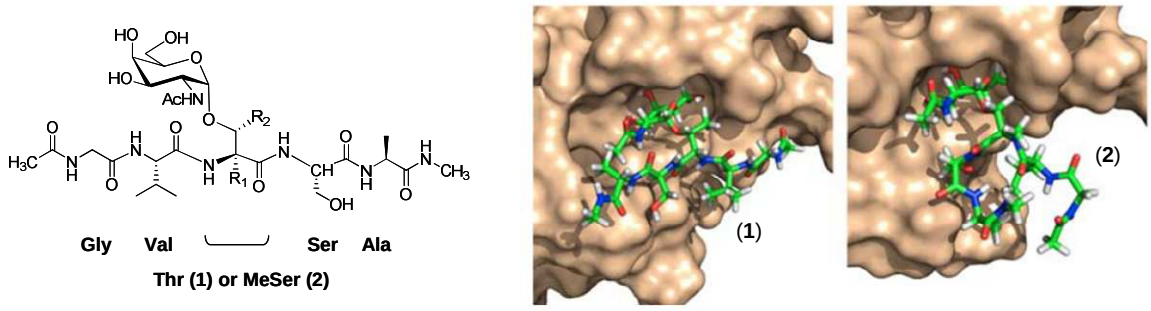

Figure 1. Left: Mucin-like glycopeptides; MeSer = (*S*)-α-methylserine. Right: Structures of glycopeptides **1** (left) and **2** (right) in the bound state.

Afterwards conformation analysis and interaction studies of uncommon α-*N*-linked glycopeptides using STD-NMR and TR-NOESY techniques will be also presented.

[1] A. Varki, R. Cummings, J. Esko, H. Freeze, G. Hart, J. Marth, Essentials of Glycobiology. New York: Cold SpringHarbor Laboratory Press; **1999**. [2] H. Kogelberg, D. Solís, J. Jiménez-Barbero, *Curr. Opin. Struct. Biol.* **2003**, *13*, 646-653. [3] F. Corzana, J. H. Busto, F. Marcelo, M. G. Luis, J. L. Asensio, S. Martín-Santamaría, J. Jiménez-Barbero, A. Avenoza, J. M. Peregrina, *Chem. Eur. J.* **2011**, 15, 3863-3874.

Carbohydrate oligonucleotide Conjugates: cellular uptake depending on glucose presentation and energetic probes to study molecular interactions

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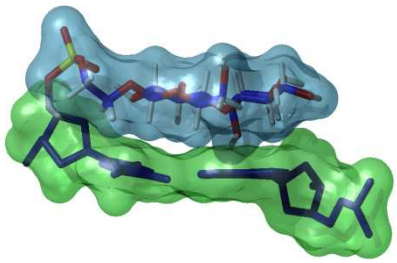
A major drawback for the use of new oligonucleotide-based drugs such as the siRNAs is the poor cellular uptake. Carbohydrate-oligonucleotide conjugates (COCs) are being explored as potential vectors to facilitate the internalization of these promising drugs.[1] We have prepared fluorescently labelled glucose DNA conjugates with different carbohydrate presentation. Results on the cellular uptake of COC antisense sequences in HeLa and U87 cell lines will be described.[2]

Carbohydrate-protein and carbohydrate-DNA/RNA interactions are fundamental for a good number of biological processes.[3] Carbohydrate-aromatic stacking is a common binding motif and we have used COCs as a potent tool to investigate this interaction. Results on the measurement of monosaccharide-benzene stacking interactions will be discussed.[4] In addition, carbohydrate-DNA stacking interactions are present in the recognition of 16S rRNA A-site by aminoglycosides. We have prepared COCs with mono- and disaccharides attached to the 5'-end of short DNA sequences and studied their stacking to the DNA bases using UV, NMR and molecular dynamics calculations.[5]



Scheme 1. Detail of a stacking interaction in a cellobiose-DNA conjugate.

[1] H. Lonnberg, *Bioconjugate Chem* **2009**, *20*, 1065; [2] B. Ugarte-Uribe, S. Pérez-Rentero, R. Lucas, A. Aviñó, J.J. Reina, I. Alkorta, R. Eritja, J.C. Morales, *Bioconjugate Chem* **2010**, 21, 1280; [3] H.J. Gabius, H.C. Siebert, S. Andre, J. Jimenez-Barbero, H. Rudiger, *Chembiochem* **2004**, *5*, 740; [4] J.C. Morales, J.J. Reina, I. Díaz, A. Aviñó, P. M. Nieto, R. Eritja, *Chem. Eur. J.* **2008**, *14*, 7828 [5] R. Lucas, I. Gómez-Pinto, A. Aviñó, J.J. Reina, R. Eritja, C. González, J.C. Morales, *J Am Chem Soc* **2011**, *133*, 1909.

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Scientific and Social Program

	Monday 11 April	Tuesday 12 April	Wednesday 13 April	Thursday 14 April	Friday 15 April
9.00-9.50		**IL-2 Unverzagt**	**IL-5 Mulard**	**IL-8 Jiménez-Barbero**	**IL-9 Driguez**
9.50-10.10		Lecourt	Hackenberger	Nitz	Fridman
10.10-10.30		Linclau	Chambert	García-González	Westerlind
10.30-11.00		**Coffee break**	**Coffee break**	**Coffee break**	**Coffee break**
11.00-11.20		Kandasamy	Stubbs	Reichardt	Ardá
11.20-11.40		Pokorná	Lopin-Bon	Chevolot	Marcelo
11.40-12.00	**Arrival and Registration**	**Elicityl**	**New J. Chem.**	Lahmann	Morales
12.00-14.00		**Lunch**	**Lunch**	**Lunch**	**Lunch**
14.00-14.50		**IL-3 Nishimura**	**IL-6 Wessel**		
14.50-15.10		Turks	Legentil		
15.10-15.30		Velasco-Torrijos	Biskup		
15.30-15.50		Cumpstey	Gouin		
15.50-16.10		**Thermo Fisher Scientific**	**Grace Discovery**	**Social**	
16.10-16.30	**16h00-16h50 IL-1 Crich**	**Coffee break**	**Coffee break**		
16.30-16.50		Blériot	Descroix		
16.50-17.10	Turnbull	Compain	Renaudet		**Departure**
17.10-17.30	Codée	Gallienne	Vincent	**Event**	
17.30-17.50	**Coffee break**	**CEM**	Krylov		
17.50-18.10	Boonyarattanakalin	**Free time**	**Free time**		
18.10-18.30	Norsikian				
18.30-18.50	Galan	**18h30-19h20 IL-4 Murphy**	**18h30-19h20 IL-7 Wong**		
18.50-19.10	Varón Silva				
19.30-21.00	**Dinner**	**Dinner**	**Dinner**	**Gala Dinner**	

IL = Invited lecture