**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.












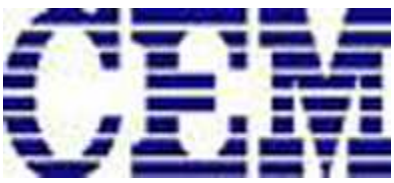



# Welcome

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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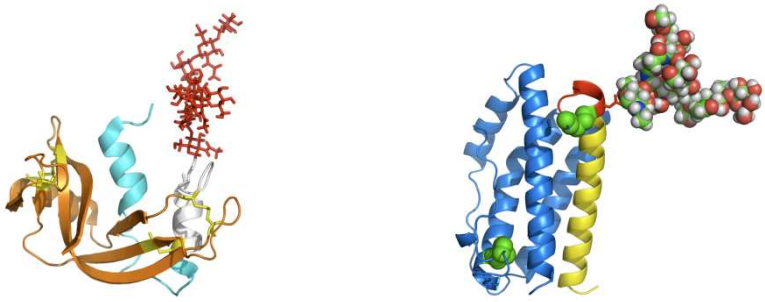
The use of recombinant therapeutic glycoproteins is one of the fast growing applications in human therapy.[1] Typical for these secretory and cell surface proteins is the attachment of oligosaccharides to asparagine residues (N-glycosylation). Despite many efforts in this field the function of N-glycosyation 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 Neu5Ac$\alpha$2,3Gal$\beta$1,3GalNAc$\alpha$. 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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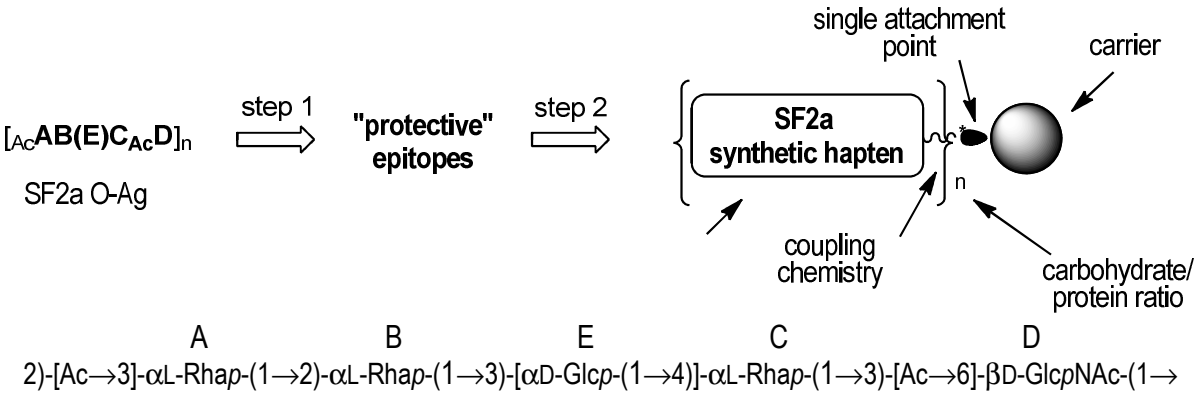
Shigellosis, or bacillary dysentery, caused by the non capsulated Gram negative bacteria *Shigella*, is a burden worldwide. *Shigella flexneri* is the major responsible for the endemic form of the disease in developing countries.[1] Serotype diversity and geographical distribution strongly support the 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-$\pi$ 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.

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