**CARB 1**

**Novel strategies for vaccine design: Learning from the immune system**

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Capsular polysaccharides are found on the outermost surfaces of many pathogenic bacteria. Each bacterial pathogen possesses a polysaccharide with unique structure that is distinctively recognized by our immune cells. Because these polysaccharides are located on the surface of pathogens, they are easily accessible by the immune cells and therefore are essential vaccine candidates. To induce polysaccharide specific professional immune response (e.g., T cell mediated B cell response), these polysaccharides are conjugated with carrier proteins and the conjugation products are called glycoconjugate vaccines. In a series of experiments we have revised the model that describes how protein-polysaccharide conjugate vaccines interact with the adaptive arm of the immune system. Moreover, based on our mechanistic findings, we designed and synthesized a model vaccine that is substantially more immunogenic than a currently available vaccine.

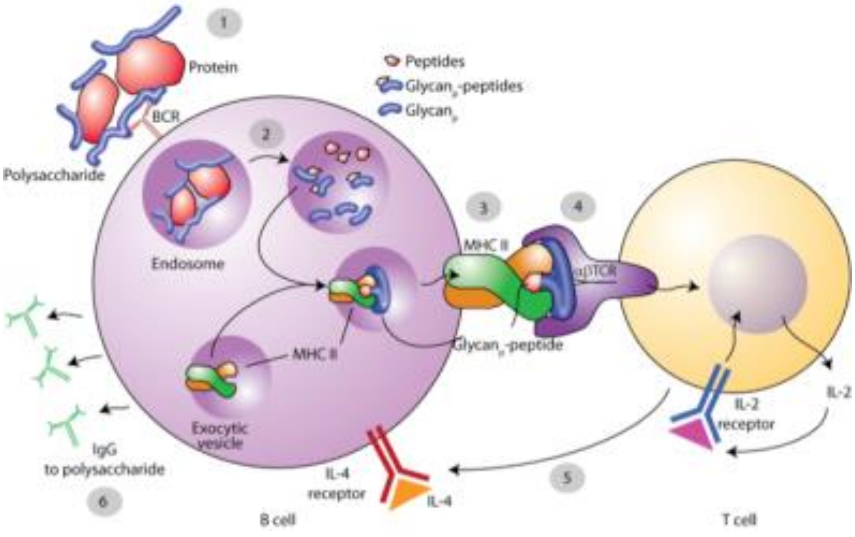


Fig. 1 Mechanism of T cell- mediated immune activation by a model glycoconjugate vaccine. A schematic depiction of the steps in order: uptake (1) and processing (2) of glycoconjugate vaccine and presentation of carbohydrate T cell epitope by MHCII (3), T cell recognition of the carbohydrate epitope (4), stimulation of T cell and B cell by secretions of interleukins 2 and 4 (IL-2 and IL-4) by the T cell (5) and carbohydrate-specific IgG secretion by the B cell as a result of antibody class switch (6).

**CARB 2**

## Combining computational carbohydrate grafting with glycan array data to define the 3D epitopes of carbohydrate binding antibodies

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Glycan array screening is a high-throughput method for identifying potential ligands for glycan-binding proteins. Such screening data provides specificity information, and helps identify the minimal glycan fragment that is responsible for recognition. However, such screening provides no structural insight into the origin of the observed specificity among glycans that contain this minimal binding determinant in their sequence. When applicable, crystallographic and NMR methods can provide 3D structures for these complexes, however these methods are neither rapid nor high-throughput.

Here we demonstrate that given a 3D structure for the minimal glycan determinant aligned in the binding site of the receptor protein, the potential for any glycan, containing this motif, to bind may be predicted by Computational Carbohydrate Grafting (CCG). In CCG, the protein-minimal motif complex is screened against a virtual library of 3D structures of glycans. CCG can be successfully employed to identify previously undiscovered putative binding partners and to explain cross-reactivity. In addition, a CCG analysis can be used to identify false negative data points arising from chemical linker effects in the experimental glycan array.

We illustrate the ability of this high-throughput approach to predict the glycan specificities of a range of carbohydrate binding proteins, including antibodies, and lectins.

Acknowledgments: We thank the National Institutes for Health (P41RR005351, R01GM094919 (EUREKA)) as well as the Science Foundation of Ireland (08/IN.1/B2070) and the European Research Development Fund for support.

**CARB 3**

## Conjugation of oligosaccharids and glycopeptides to protein carriers and phage as micropbial and viral vaccine candidates

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Glycoconjugates that constitute *Brucella* A and M epitopes have been synthesized as discrete antigens and as an antigen expressing both epitopes. The prospects for these antigens as either a universal or specific reagents to detect and diagnose brucellosis

will be illustrated. In addition to the simple conjugation methods employed to directly conjugate oligosaccharides to carrier proteins such as tetanus toxoid and a non-protein carrier such as copovidone used in ELISA, the challenges for synthesis of *Candida albicans* glycopeptide conjugates will be described.Two thiol analogues of gp120 glycans, their chemical conjugation to a filamentous phage carrier, and the LC-UV-MS methodology developed to characterize the resulting glycoconjugates will also be reported.

**CARB 4**

**Characterizing protein glycoconjugates by SEC-UV-MALS-dRI**

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Glycoconjugation is an important approach to enhancing protein stability that requires careful characterization in order to understand the end results of site-specific behavior. In this case study, we utilize SEC combined with multi-angle light scattering (MALS), UV absorption, and differential refractive index (dRI) detection, to determine the conjugation profile, analyze the impact on aggregation and guide the glycoconjugation process.

**CARB 5**

**Analytical control strategies for polysaccharide conjugate vaccines**

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The development of bacterial capsular polysaccharide-protein conjugates represents a major advancement in prophylaxis against bacterial infections caused by *Haemophilus influenzae* type b, *Neisseria meningitidis* serogroups A, C, Y, W135 and thirteen *Streptococcus pneumoniae* serotypes.

These vaccines are complex multicomponent biological products comprised of several polysaccharide conjugates and excipients. Since polysaccharide conjugates and their multicomponent formulations are not considered to be well defined biologicals, it is reasonably expected that, if consistently manufactured, these will have comparable potencies. To ensure equivalence between vaccine lots used in clinical trials and, later, commercially, a very comprehensive, complex analytical control strategy to monitor important characteristics needs to be developed. This talk will outline and discuss critical quality attributes for polysaccharide conjugate vaccines, focusing on the analytical control strategies and methods for the release and characterization of the intermediates, polysaccharide conjugate components and the final multicomponent vaccine formulations. Strategies to monitor stability during storage will be discussed as well.

## CARB 6

### Innovative approaches for carbohydrate-based conjugate vaccines

***Francesco Berti**, francesco.berti@novartis.com. Research, Novartis Vaccines, Siena, Italy*

In the last 30 years glycoconjugate vaccines have been shown to be the safest and most effective vaccines developed. They are a potent tool for prevention of life-threatening bacterial infectious diseases like meningitis and pneumonia.

Various conjugation procedures and carrier proteins have been used to prepare conjugate vaccines tested in pre-clinical and clinical trials. Many variables impact their immunogenicity and a tight control through physicochemical tests is important to ensure manufacturing and product consistency. Conjugate vaccines can be adequately controlled by physicochemical tests and the prediction of human immunogenicity based on animal models might not be reliable.

Several approaches for glycoconjugates preparation and the quality control as well as those variables which might affect their product profile are discussed. The preparations of fully synthetic or enzymatically-produced carbohydrate antigens are described as potential future applications for development of glycoconjugate vaccines.

Complex physico-chemical technologies (e.g. NMR Spectroscopy and Mass Spectrometry), which are currently applied for characterization and potentially understand the relationship between the polysaccharide structure and its immunological efficiency, are also described.

## CARB 7

### Glycoconjugate vaccines: Development challenges

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The phenomenal success of Pneumococcal and Meningococcal glycoconjugate vaccines has laid a strong foundation for the development of the next generation of candidates in both the bacterial and immunotherapeutic conjugate vaccine areas. There is a greater emphasis towards the development of sustainable models that address scale-up, control and consistency. The presence of multiple reaction sites and the carrier proteins in conjunction with the need to preserve potentially critical immunogenic epitopes in the polysaccharides presents special challenges for the conjugation chemistry development. The development challenges for complex multi-valent and multi component vaccines are much greater. The presentation will discuss examples of using integrated approaches, using Design of Experiments (DOE), Statistical Process Control,

process-analytical tools and scale-down models, that could be applied towards a seamless development strategy for multi-valent and multi component vaccines.

**CARB 8**

**Glycoconjugate vaccines produced in bacterial cells through enzymatic conjugation**

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Glycoconjugate vaccines are a safe, cheap and efficient way to improve human health. GlycoVaxyn AG has developed a proprietary technology that enables the manufacture of glycocconjugate vaccines in *Escherichia coli*. These bioconjugates are composed of a antigenic polysaccharide coupled to a specific asparagine residue in an engineered protein carrier. The process is based on an enzymatic conjugation in living bacterial cells under very mild conditions. The conjugation of an antigenic polysaccharide to a carrier protein of choice occurs via an *N*-glycosidic linkage, resulting in a robust and reproducible process and yielding a highly defined product. The novel conjugation method has been used to develop various conjugate vaccines.

The development of different glycoconjugate vaccines produced in bacterial cells will be sumarized. Progress in the production and analysis of these bioconjugates is presented.

**CARB 9**

**Alternate technology for preparation of glycoconjugate vaccines**

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Glycoconjugate vaccines are very important to public health as evident by the decrease in incidence of targeted diseases and the increase in the number of such vaccines in development. While these are very effective tools for controlling and preventing infectious diseases caused by encapsulated bacteria, there are difficulties in production, control and regulation of glycoconjugate vaccine manufacture. These vaccines are often prepared by chemical conjugation of a saccharide antigen to carrier protein with only modest coupling efficiency. Glycoconjugate vaccines are prepared by random coupling methods, which results in a product whose active ingredient is not well defined and is

not easily characterized in detail. We have developed alternate methodologies for preparation of glycoconjugate vaccines to address some of these problems. The efficiency of conjugation by reductive amination was improved by introducing hydrazide groups on the carrier protein. Hydrazide based reduction amination was used to prepare a vaccine against Group A meningococcal meningitis. This vaccine, MenAfriVac, has dramatically reduced disease in the meningitis belt of Africa. The hydrazide based reductive amination method was also applied to preparation of glycoconjugates of pneumococcal polysaccharides. Recombinant carrier proteins with known crystal structures are being employed to reduce the ambiguities in vaccine structure. A glycoconjugate vaccine against cholera was prepared based on the O-specific polysaccharide of LPS and the non-toxic Hc fragment of tetanus toxin. Recombinant Hc fragment is easily purified and has a definable structure. We used enzymes of pathways for bacterial polysaccharide biosynthesis to chemoenzymatically prepare a Group C meningococcal polysaccharide glycoconjugate. We used mass spectrometry to map the location of conjugation sites on the carrier protein.

*This work was partially supported by a CRADA between FDA and PATH, Seattle, WA.*

*"My comments are an informal communication and represent my own best judgment. These comments do not bind or obligate FDA."*

**CARB 11**

**Rearrangement of a carbohydrate backbone discovered en route to higher carbon sugars**

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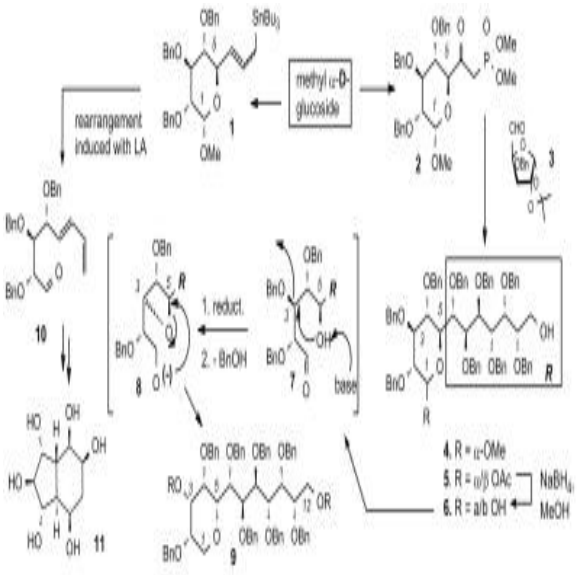
We have elaborated a convenient method for the preparation of higher carbon sugars (HCS) from simple hexoses;[i] examples are shown in Figure 1. Methyl a-**D** -glucoside is converted into allyltin derivative **1** which, upon treatment with a Lewis acid, undergoes a rearrangement to dienoaldehyde **10** used further for the synthesis of highly oxygenated bicyclic derivatives (e.g. **11** ). Alternatively it is converted into phosphonate **2** which reacts with aldehyde **3** to afford (after further transformations of the primary HCS enone) C12-alcohol **4** , acetolysis of which gave **5** . Reduction of hemiacetal function with sodium borohydride induced an unusual rearrangement leading to anhydrosugar **9**.




The plausible mechanism of its formation postulates removal of the acetate from the C1-position to **6** which is in equilibrium with the open chain structure **7** . Next step consists of the elimination of benzyl alcohol with formation of oxetane **8** , which rearranges to **9** .[ii]Examples of other rearrangements in the field of higher carbon sugars will also be discussed.[i]

Acknowledgments: The support from Grant: POIG.01.01.02-14-102/09 (part-financed by the European Union within the European Regional Development Fund) is acknowledged.

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**CARB 12**

**Levoglucosenone derivatives with amino and thiol groups via allylic rearrangements**

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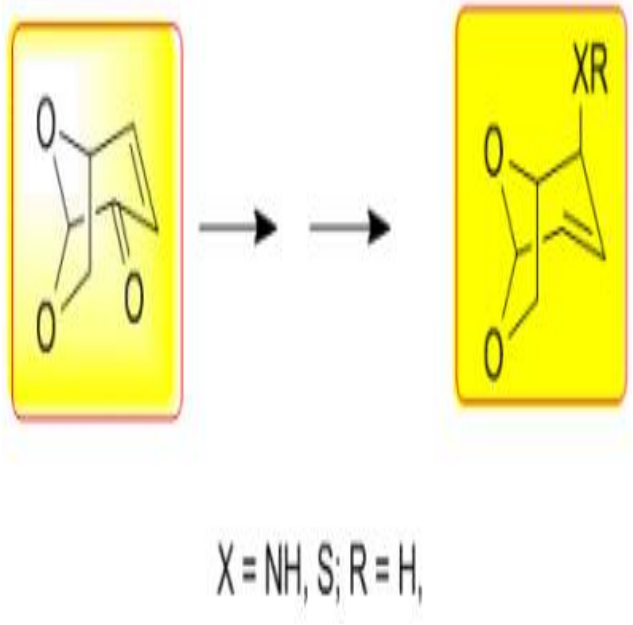
Levoglucosenone is easily available from cellulose. It is a convenient, chiral synthon functionalized with an α,β-unsaturated ketone group. The allylic alcohol deriving from the LAH reduction of levoglucosenone was transformed into derivatives containing C=S and C=NR functionalities. These derivatives were rearranged to form corresponding allylic products. The formation of the carbonyl group is the rearrangement driving force. The products can serve as chiral synthons containing amino and thiol groups at a strategic C-4 position. All reactions proceeded with a very high stereoselectivity.




**CARB 13**

**3,6-Anhydro-D-glycals: The study of their unusual structures and chemical reactivity**

***Cecilia H Marzabadi****, cecilia.marzabadi@shu.edu, Vikram Basava, Sergiu M Gorun. Department of Chemistry and Biochemistry, Seton Hall University, South Orange, New Jersey 07450, United States*

The unusual, 3,6-anhydro sugar of D-glucal **1** has been synthesized and its structure characterized spectroscopically and by x-ray diffraction. The anhydroglycal has been subjected to a series of known glycal addition reactions and the steroselectivity of these reactions studied. The results of other studies aimed at the formation of related anhydroglycals using a similar methodology will be discussed.

[figure 1]

**CARB 14**

**Endo- and exo-cyclic modifications of monosaccharides through expansions and rearrangements**

*Somak Paul, Krishna Gopal Maiti, Narayanaswami Vijayaganesh Ganesh, Arunima Mukherjee, Gour Chand Daskhan, Supriya Dey,* ***Narayanaswamy Jayaraman****, jayaraman@orgchem.iisc.ernet.in. Department of Organic Chemistry, Indian Institute of Science, Bangalore, Karnataka, India*

Monosaccharide modifications provide a rich avenue to implement organic transformations relevant to sugar chemistry. Activation of a monosaccharide monomer through unsaturations and other activation-specific moieties is an optimal approach in the course to implement chosen organic transformations. Further, the reactivities of each carbon differ subtle, and such differences become pertinent to understand how reactions on a sugar scaffold can vary greatly. An example *par excellence* is the glycosidic bond stabilities on, for example, *gluco*- and *galacto*-sugars, where the later series exhibit higher reactivities to hydrolyze the glycosidic bond. On the other hand, hard-soft acid base gradations are immensely applicable to understand the reactivity of each carbon of a sugar, as for example, the hard acidic anomeric center *vs* the soft acidic *C*-3 carbon in a sugar and respective reactivities towards a hard or soft nucleophile. Yet another immense area of monosaccharide modifications is the *endo*--cyclic ring expansion, such that cyclic six-membered sugar is transformed to a cyclic seven-membered sugar. Such transformations typically involve multiple synthetic sequence, with the facility that the intermediates allow a diversity orientation to the synthesis. Thus, in addition to an often focused synthesis of seven-membered sugars, the intermediates of the synthesis lead to secure other modified sugars, for example, modifications at *C*-2, *C*-3 *etc*... Thermal rearrangements are elegantly suitable to a monosaccharide, by which C-C bond formations could be implemented in a rather facile manner. Such rearrangements and ring expansions can be extended to higher oligosaccharides too, that in the class of cyclic oligosaccharides. Thus, synthesis of ring-expanded cyclic oligosaccharides is a case study, where a naturally-occurring cyclic oligosaccharide would have a congener, an un-natural, ring-expanded cyclic oligosaccharide. Many examples that constitute several years of effort in monosaccharide modifications through expansions, shifts and rearrangements will be discussed in the presentation.

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## CARB 15

### Scope and mechanism of nickel-catalyzed transformations of glycosyl trichloroacetimidates to glycosyl trichloroacetamides and subsequent conversion to alpha-urea-glycosides

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The development and mechanistic investigation of a highly stereoselective methodology for preparing alpha-linked-urea neo-glycoconjugates and pseudo-oligosaccharides is described. This two-step procedure begins with the stereoselective nickel-catalyzed transformations of glycosyl trichloroacetimidates to the corresponding alpha-trichloroacetamides. The alpha-selective nature of the conversion is controlled with a cationic nickel(II) catalyst. Mechanistic studies have identified the coordination of the nickel catalyst with the equatorial *C2*-ether functionality of the alpha-glycosyl trichloroacetimidate to be paramount for achieving an alpha-selective transformation. A cross-over experiment has indicated that the reaction does not proceed in an exclusively-intramolecular fashion. The second step in this sequence is the direct conversion of alpha-glycosyl trichloroacetamide products into the corresponding alpha-urea glycosides by reacting them with a wide variety of amine nucleophiles in presence of cesium carbonate. Only alpha-urea-product formation is observed, as the reaction proceeds with complete retention of stereochemical integrity at the anomeric center

## CARB 16

### Synthetic carbohydrate vaccines: From empirical development to rational design

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### Abstract

Vaccines based on conserved carbohydrate structures on the pathogen cell surface can prevent infections. These complex carbohydrates are often made of unique glycan motifs that are distinct from mammalian cell surface glycans.[1]. Such differences form the basis for developing effective vaccination strategies. Complexities involved in the

cultivation of pathogen, subsequent purification and characterization of cell surface glycans (CSG) make developing vaccines a challenging task.[1]Low immunogenicity and lack of structural understanding on carbohydrate immune recognition add to this complexity. Chemical synthesis helps to access these complex structures with utmost purity and definition. Currently, synthetic carbohydrate antigens are developed empirically based on the repeating units, without considering details of antigen-antibody interactions. Structural information on glycan-antibody interaction is rapidly emerging.

This talk will describe the refinement of carbohydrate antigen structures by employing a combination of *in vitro* methods such as glycan arrays, surface plasmon resonance and STD-NMR. Combination of these methods revealed key features of glycan structure relevant to antigenicity and immunogenicity. Based on the results of *in vitro* analyses, neoglycoconjugates were designed and tested in animal models. Synthetic oligosaccharides based on CSGs of *Yersinia pestis* [2]; *Clostridium difficile* [3], *Streptococcus pneumoniae* and leishmania parasites were explored. Our results demonstrate that the design of synthetic carbohydrate antigens can be rationalized based on *in vitro*mapping of carbohydrate-antibody interactions. This strategy will greatly accelerate the vaccine development process.

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**CARB 17**

## De novo synthesis, conjugation, and immnunogenicity of the Burkholderia pseudomallei manno-heptose capsule

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Melioidosis is an infectious disease caused by *Burkholderia pseudomallei* and is associated with high morbidity and mortality rates in endemic areas. It is difficult to treat with antibiotics and there is no licensed vaccine. Capsular polysaccharides have historically made effective vaccine candidates and many capsule-based vaccines are currently licensed, including those against *Neisseria meningitidis*, *Streptococcus*

*pneumoniae* and *Haemophilus influenzae* infection. *B. pseudomallei* expresses a capsular polysaccharide (CPS) consisting of a homopolymer of unbranched 1-3 linked 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptopyranose. This polysaccharide is a major virulence determinant and is thought to be present in all examples of this species, offering the opportunity to develop a univalent vaccine protective against melioidosis.

Access to CPS antigen is currently through a traditional bioprocessing route. This requires expensive containment facilities and yields a product with a naturally heterogeneous chain length containing some impurities, adding a considerable complicating factor to the manufacture of pharmaceutical grade vaccine. We have taken an entirely synthetic approach to access CPS antigen of defined chain length and purity well suited for development. The synthetic antigen was designed to incorporate all of the defining structural characteristics of natural CPS in addition to an amine-based linker to allow efficient conjugation to protein carriers. The choice of a hexasaccharide antigen provided a size likely to be immunologically relevant whilst retaining favorable handling characteristics.

Antigen was synthesized using a modular assembly approach and coupled to the non-toxic Hc domain of tetanus toxin to promote recruitment of T-cell help for antigen display. Mice immunized with the glycoconjugate developed IgM and IgG responses capable of recognizing natural CPS, demonstrating the immunogenicity of the synthetic CPS and providing a basis for future efficacy studies. This work may lead to an effective licensed vaccine which will allow the warfighter to continue to operate independently of detection technologies in a potential BW environment.

**CARB 18**

**"To click or not to click": The design of a carbohydrate based anticancer vaccine for effective and long term antitumor immunity**

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The development of an effective vaccine construct targeting tumor associated carbohydrate antigens (TACAs) is an appealing approach towards tumor immuno-therapy. To enhance the immune responses to TACAs and overcome their low immunogenicity, a typical approach is to covalently attach the antigens to an immunogenic carrier. While much emphasis has been put on the carrier moiety and antigen structure in vaccine design, the impact of the linker on immune responses to the TACAs has not been studied much. Among many bio-conjugation methods explored,

the copper mediated alkyne azide cycloaddition click reaction is becoming increasingly popular for vaccine construction. In this talk, we will discuss our results in comparing vaccine constructs containing the triazole linker and a flexible alkyl amide linker. We demonstrate that the linker has a significant effect on anti-TACA humoral responses. The knowledge gained can provide valuable guidance towards the development of effective TACA based anti-cancer vaccines.

**CARB 19**

**Development of chemical synthetic antitumor vaccines**

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The treatment of cancer is a considerable challenge in the world. And cancer immunotherapy is considered as a safer and more effective way to treat cancer. And vaccine plays an important role in immunotherapy. Compared with the traditional vaccine, chemical synthetic vaccine has confirmable structure andhomogeneous component. And in general, it contains the indispensable antigen structure and immunostimulating assistant structure. However, for the development of high-efficiencytumor vaccines, there are two problems need to be solved. The first one is to searchforan effective and specific B-cell epitope. The second one is to construct a rationalvaccine system to elicit strong immune response.

In our research, MUC1 glycopeptides,which are over-expressed and aberrantly glycosylated in tumor cells,were chosen as the target antigens for cancer vaccines.And to overcome the weak immunogenicity of MUC1 glycopeptides,Bovine Serum Albumin (BSA) wasintroduced to improve the immunogenicity of MUC1 glycopeptides[1]. However, the carrier protein always has complex structure andcan induce side effects, soshort peptide T-cell epitopesfrom carrier protein was adopted[2]. A kind of immunostimulants,Toll-like receptor 2 ligand, wasconstructed in the three-component vaccine to enhance immune response[3].To improve the immune response,cluster effect was introduced in the vaccines [4]. Besides, a self-assembly adjuvant-free vaccine containing Q11 peptide and B-cell epitopes was developed[5]. These vaccines we developed can efficiently trigger the immune system to elicit high level of antibodies against MUC1 glycopeptides.

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**CARB 20**

**Development of fully synthetic self-adjuvanting cancer vaccines**

***Geert-Jan Boons****, gjboons@ccrc.uga.edu. Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30606, United States*

The expression of tumor associated carbohydrates correlates strongly with poor survival rates of cancer patients. The differential expression of tumor-associated carbohydrates has been exploited for the development of therapeutic cancer vaccines. Several clinical trails have shown that vaccines based on a tumor associated carbohydrate conjugated to a carrier protein elicit mainly inferior IgM antibodies. To address this problem, we have designed, chemically synthesized and immunologically evaluated a number of fully synthetic cancer vaccine candidates that have the potential to reverse immunotolerance. We have identified compounds that can elicit robust humoral and cellular immune responses to tumor associated carbohydrates and are efficacious in reversing tolerance and generate a therapeutic response in a mouse model of mammary cancer.

**CARB 21**

**Microwave-assisted multicomponent cascade couplings with carbohydrates**

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The underlying goal of synthetic chemistry is to generate high-value compounds through the development of methods. As the importance for natural products, pharmaceuticals, diagnostics, agrochemicals and other compounds continues to grow, a surgent in proficiency and efficiency demands new ways to prepare molecules. Domino or cascade reactions represent a long-standing class of transformations that aim to overcome or bypass issues with purification and protecting groups through the generation of complexity in a one-pot sequence of coupling events. One of the most simple, yet effective ways to accomplish diversity generating reactions is through multicomponent couplings which, for the purpose of this talk, will infer to mean three components or more in a single reaction vessel. The concept of domino or cascade couplings can also be extended to substrate equals product and subsequent product

can equal substrate for further complexity generation such as in an intramolecular cyclization event. These green chemistry reactions have been embraced in the mantra of Diversity-Oriented Synthesis (DOS), which aims to capture that each product equals substrate can generate unique structures from a single reaction event.

In this presentation, we will examine how microwaves can influence reacted motifs from cascade coupling events for further derivitization of products that equal substrates and utilize the knowledge gained to explore anomeric alkoxide glycosylation. Furthermore, we will employ multicomponent cascade reactions containing chiral D-sugars for diastereoselective control in the formation of natural product-like compounds of high value.

**CARB 22**

## Domino reactions in glycochemistry: A strategy to build original frameworks with biological profile

***Amélia P. Rauter**, aprauter@fc.ul.pt. Department of Chemistry and Biochemistry, University of Lisbon, Faculty of Sciences, Lisbon, Portugal*

Carbohydrate-based drug discovery has been a growing area of research due to the biological processes in which carbohydrates are involved. However, multistep syntheses are often required using these multifunctional and stereochemically rich molecules as synthons. We have explored domino reactions involving furanulose scaffolds for the synthesis of bicyclic structures embodying a butenolide, which occurs in biologically active natural and synthetic products. An efficient and facile two-step stereoselective route was developed to access pyranose-fused butenolides, based on Wittig reaction followed by acid hydrolysis, which resulted in furanose ring opening, its closure to the pyranose form and intramolecular lactonization, in one single step. This reliable method has proven successful also for the synthesis of bicyclic thiosugar analogues. Interestingly, the D- or L-configuration of the target molecule can be controlled by the hexofuranulose C-5 configuration, opening the way to such complex systems in a facile and non-expensive two-step procedure. This route was applied to furanuloses bearing and amino group at C-5 but the required neutralization to release the amino group for cyclization resulted in transformation of the iminopentopyranose-fused butenolide intermediate into a butenolide with a C-C linked appendage. Exploitation of the 5-azido Wittig precursor gave an easy access to 1,2-dihydropyridin-3-ones in a two-step synthesis, based on hydrogenation, N-protection and acetic acid elimination after treatment with acetic anhydride in pyridine. Enones of this type have been key intermediates in the synthesis of nojirimycins and are usually obtained in low overall yield requiring multistep syntheses.

Our exploratory work has been also devoted to a number of rearrangements to transform simple sugars into biologically active molecules. As example we present the conversion of aldonolactones into oxetane δ-amino acids by a ring contraction rearrangement. Also transformation of penturono-5,1-lactone into glycomimetics

embodying a difluoromethylene replacing the endocyclic oxygen, via Wittig-type difluoromethylenation and TIBAL promoted rearrangement, will be highlighted.

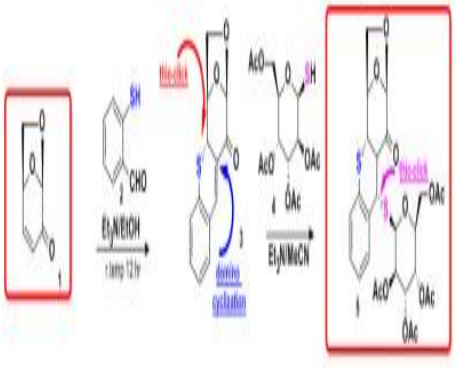
**CARB 23**

**Thio-click and domino approach to carbohydrate heterocycles**

***Zbigniew J. Witczak***, *zbigniew.witczak@wilkes.edu. Department of Pharmaceutical Sciences, Wilkes University, Wilkes-Barre, PA 18766, United States*

Recently thio-click chemistry became a well-accepted part of the click reaction toolbox and is more and more often employed in various thio-conjugations. This strategy requires specifically modified chiral building blocks with sugar framework. One of the ideal synthons fitting into this category is levoglucosenone **1.** However, new protocols allowing for stereoselective functionalization of selected pools of new precursors must be further elaborated. The robustness of this strategy has been demonstrated in a number of new chiral intermediates derived from the enone **1** as depicted below.

A regioselective functionalization of **1** leading to new chiral building blocks such as thio-click/domino cyclization adduct **3** , and its thio-sugar analog **5** , was accomplished in our laboratory. The tentative mechanism of the formation of domino reaction products, with alternative reverse sequence of the reaction will be discussed in detail.



**CARB 24**

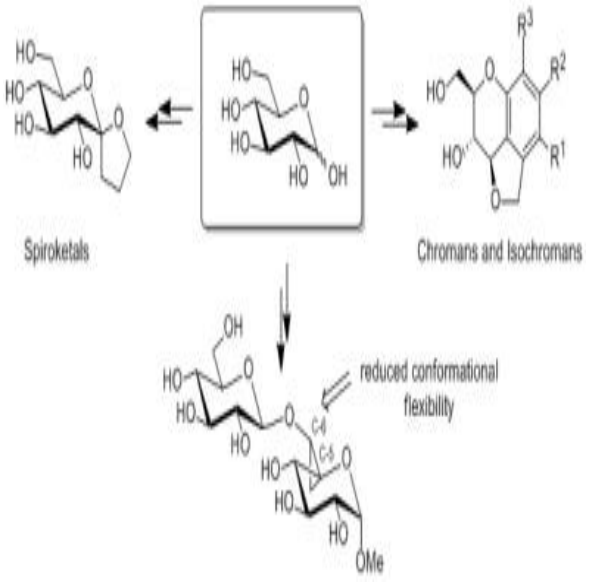
**Adventures with sugars, three-membered rings, and triple bonds**

***Daniel B. Werz***, *d.werz@tu-braunschweig.de. Technische Universitaet Braunschweig, Institut fur Organische Chemie, Braunschweig, Germany*

The development of novel strategies for the synthesis of carbohydrate mimics is a major task in modern bioorganic and medicinal chemistry.[1]Besides traditional approaches especially domino reactions which have the potential to build up a high degree of complexity in only a few steps have come into the focus in the recent past.

The talk will discuss how cyclopropanes as well as carbon-carbon triple bonds, both being systems which are high in energy are utilized to trigger a cascade of consecutive reactions starting from carbohydrates. Carbopalladation reactions are used to synthesize chromans and isochromans starting from bromoglycals.[2] Cyclopropanated sugars showing a highly reactive donor-acceptor cyclopropane[3] are converted via a domino sequence consisting of oxidation and ring-enlargement into spiroketals.[4]

In addition to spiroketal formation the talk will demonstrate in which way a spiroannulated three-membered having only minimal impact on the structural integrity of the carbohydrate, reduces the rotational flexibility of the C6-O bond.[5]Corresponding syntheses for building blocks and their behavior in glycosylation reactions will be discussed.




1) Review on carbohydrate mimics: Koester, D. C.; Holkenbrink, A.; Werz, D. B. *Synthesis* **2010** , 3217-3242. 2) (a) Leibeling, M.; Koester, D. C.; Pawliczek, M.; Schild, S. C.; Werz, D. B. *Nature Chem. Biol.* **2010** , *6*, 199-201. (b) Leibeling, M.; Milde, B.; Kratzert, D.; Stalke, D.; Werz, D. B. *Chem. Eur. J.* **2011** , *17*, 9888-9892. 3) Schneider, T. F.; Kaschel, J.; Werz, D. B. *Angew. Chem. Int. Ed.* **2014** , *53*, in press. 4) Brand, C.; Rauch, G.; Zanoni, M.; Dittrich, B.; Werz, D. B. J. Org. Chem. **2009** , 74, 8779-8786. 5) (a) Brand, C.; Granitzka, M.; Stalke, D.; Werz, D. B. *Chem. Commun.* **2011** , *47*, 10782-10784. (b) Brand, C.; Kettelhoit, K.; Werz, D. B. Org. Lett. **2012** , 14, 5126-5129.

**CARB 25**

**Controlling siRNA properties with nucleobase modifications**

***Peter Beal**, pabeal@ucdavis.edu. Department of Chemistry, University of California, Davis, Davis, CA 95616, United States*

Unmodified short interfering RNAs (siRNAs) are highly potent and effective at directing the selective digestion of specific messenger RNAs inside living cells. However, the native RNA structure has numerous drawbacks as a therapeutic agent. For instance, unmodified RNA is sensitive to nucleases, has poor delivery properties and can stimulate immune responses. While chemical modifications have been described that address these issues, the vast majority of this work has focused on structural changes to the ribose of the component nucleotides. Modification of the nucleobases can have profound effects on the properties of oligonucleotides, yet this has been a comparatively unexplored route to the modulation of siRNA properties. Here we report the effects of newly developed base modifications for siRNAs. Purine derivatives with substituents that project into either the minor groove or the major groove of siRNA are discussed. These modifications are shown to modulate RNAi potency, binding to off-target proteins and Toll-like receptor mediated immune stimulation.

**CARB 26**

**Carbohydrate conjugates for systemic delivery of RNAi therapeutics**

***Muthiah Manoharan****, mmanoharan@alnylam.com. Department of Drug Discovery, Alnylam Pharmaceuticals, Cambridge, MA 02142, United States*

Therapeutic RNA agents that act through the RNA interference (RNAi) pathway are specific and potent inhibitors of gene expression. Chemically synthesized versions of these inhibitors are called short interfering RNAs (siRNAs). These agents may be designed to target disease pathways previously considered “non-druggable”. Recently, systemic delivery of RNAi therapeutics to liver hepatocytes by subcutaneous administration has been achieved at Alnylam Pharmaceuticals using GalNAc-siRNA conjugate delivery platform. In this approach, the siRNAs are conjugated with multivalent *N*-acetylgalactosamine (GalNAc) residues that are recognized by the asialoglycoprotein receptor (ASGPR). ASGPR is a C-type lectin receptor expressed on human hepatocyte cell surfaces at 0.5 to 1 million copies per cell. ASGPR recognizes an exposed terminal galactose to mediate clearance of serum glycoproteins via clathrin-mediated endocytosis. Recognition by the ASGPR requires multi-valency with appropriate spatial distribution and orientation of the GalNAc residues. Binding affinities of these trivalent ligands are in low nM range. GalNAc-siRNA conjugates are active in numerous animal models and hold significant promise for targeting disease-causing genes produced in liver. Alnylam is advancing several GalNAc-siRNA conjugates through pre-clinical and clinical development to address genetically defined diseases with significant unmet medical need. Recent advances with the GalNAc-siRNA conjugate platform will be presented using several disease targets.

**CARB 27**

**Structural basis for target RNA recognition by human Argonaute-2**

***Ian J MacRae****, macrae@scripps.edu. Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037, United States*

Argonaute proteins form the functional core of the RNA-induced silencing complex (RISC) that mediates RNA silencing in eukaryotes. Argonaute proteins are loaded with small RNAs, which are used to guide RISC to complementary mRNA targets. To understand how Argonaute uses the sequence information encoded in guide RNAs, we determined high-resolution crystal structures of human Argonaute2 (Ago2) in complex with a guide siRNA and a variety of target RNAs, bearing different degrees of complementarity to the guide. These structures show that Ago2 binds to the 5' end in the guide RNA and splays out nucleotides 2 to 6, termed the “seed region”, in a helical conformation for base-pairing with mRNA targets. A narrow cavity adjacent to the guide 5' end specifically recognizes adenosine nucleotides in target RNAs. Target binding to the seed region promotes conformational changes in Ago2 that shift guide nucleotides 12 to 16 into a helical conformation, thereby generating a “supplementary seed region”. We suggest that these and additional observed conformational changes associated with target binding are involved in recruitment of further silencing factors to RISC.

**CARB 28**

**Medicinal chemistry strategies for improving the drug-like properties of antisense oligonucleotides**

***Punit P Seth****, pseth@isisph.com. Medicinal Chemistry, Isis Pharmaceuticals, Carlsbad, CA 92010, United States*

Antisense oligonucleotides (ASOs) bind to RNA by Watson-Crick base-pairing and modulate its intermediary metabolism to produce a pharmacological effect. Unmodified oligonucleotides are highly polar, poly-anionic macromolecules which have poor drug-like properties. To address these limitations, medicinal chemists have developed chemical modification and conjugation strategies to improve ASO binding affinity, metabolic stability and pharmacokinetic properties. Recent developments which improve potency and therapeutic efficacy of ASO drugs in animal models will be presented.

**CARB 29**

**Design and synthesis of oligonucleotides as potential therapeutics for the treatment of glioblastoma and ALS**

***Masad J. Damha***[1]*, masad.damha@mcgill.ca, Jovanka J. Bogojeski*[1]*, Adam Katolik*[1]*, Ken Yamada*[1]*, Glen F. Deleavey*[1]*, Nobuhiro Tago*[1]*, Nathaniel Clark*[3]*, Phuong U. Le*[2]*, Kevin Petrecca*[2]*, P.John Hart*[3]*. (1) Department of Chemistry, McGill University, Montreal, Quebec H3A0B8, Canada (2) Department of Neurology and Neurosurgery, Montreal Neurological Institute and Hospital, McGill University, Montreal, Quebec H3A2B4, Canada (3) University of Texas Health Science Center, San Antonio, Texas 78229, United States*

This presentation will describe the synthesis of modified oligonucleotides (antisense/siRNA) for targetting a novel gene product, DRR, which play a key role in promoting tumor invasiveness. In collaboration with Dr. Kevin Petrecca (Montreal Neurological Institute, McGill, Montreal) we were able to halt cancer invasion by targeting the DRR gene product through local administration of specific (antisense) compounds. This second part of this presentation will describe the design and synthesis of nucleic-acid based inhibitors of the RNA Lariat Debranching Enzyme via structure-guided syntheses of RNA branchpoint analogs. These analogs contain sugar/ phosphate modifications but mimic the conformation of the branchpoint and flanking nucleosides in intron RNA lariats. Several previously synthesized RNA-based inhibitors are already in hand, ready for testing for determining the structures of Dbr1/inhibitor complexes using single crystal X-ray diffraction studies carried out by Dr. P. John Hart and co-workers (U Texas HSCSA). The ultimate goal of this endeavor is to develop an effective therapy to treat TDP-43 mediated amyotrophic lateral sclerosis (ALS).

**CARB 30**

**Slow off-rate modified aptamers and their use as diagnostic and therapeutic agents**

***Nebojsa Janjic***[1]*, njanjic@somalogic.com, John C. Rohloff*[1]*, Douglas R. Davies*[2]*, Thale C. Jarvis*[1]*, Amy D. Gelinas*[1]*, Daniel W. Drolet*[1]*, Jeffrey D. Carter*[1]*, Urs A. Ochsner*[1]*, Daniel J. Schneider*[1]*. (1) SomaLogic, Inc., Boulder, CO 80301, United States (2) Emerald Bio, Bainbridge Island, WA 98110, United States*

The identification of nucleic acid ligands by the SELEX procedure has enjoyed considerable success over the past two decades. Nevertheless, chemical diversity of nucleic acids is more limited compared to that available in proteins and this has constrained the scope of targets for which high-quality nucleic acid ligands can be obtained. To bridge this gap in diversity, we have recently been using nucleic acid libraries with various functional groups introduced at the 5 position of deoxyuridine residues through amide linkages. For this purpose, we have assembled a library of modified nucleotides with moieties that mimic amino acid side chains and privileged fragments of small molecule drugs. Because base pairing is not compromised, such modifications are compatible with SELEX and can also be used for fully chemical post-SELEX optimization. A major benefit of using modified libraries of this type is a substantial increase in the number of targets accessible to SELEX including many proteins that have traditionally represented difficult targets. We have termed this new

class of ligands slow off-rate modified aptamers, or SOMAmers, to account for their unique composition and binding properties. We have recently succeeded in obtaining high-resolution crystal structures for several SOMAmer protein complexes which have allowed us to gain an appreciation for the role modified nucleotides play in creating novel intramolecular motifs and shaping the protein-binding surfaces. Compared with conventional aptamers, SOMAmers engage their protein targets with interfaces that are notably more hydrophobic in character, thus expanding the range of epitopes available for binding. Overall, these advances to the SELEX method have allowed us to assemble a collection of over 1,100 SOMAmers to major protein families such as growth factors, cytokines, enzymes, hormones and receptors. This large and growing collection of affinity reagents has substantial potential for use in diagnostics and therapeutics.

**CARB 31**

**New convergent approaches to therapeutically relevant RNA derivatives**

***Dennis Gillingham**, dennis.gillingham@unibas.ch, Na Fei, Daniel Bachmann, Kiril Tishinov. Department of Chemistry, University of Basel, Basel, Basel-Stadt 4056, Switzerland*

While solid-phase synthesis has made the generation of native RNA and DNA polymers trivial, modified derivatives are not so simple. I will present the various strategies we have been developing to create chemically tailored RNA derivatives. These range from new phosphoramidates for solid-phase synthesis to direct chemical modifications on native RNA and DNA polymers. Although the commercial production of therapeutics will almost certainly be achieved through standard solid-phase synthesis, at the screening and optimization stage modular chemical strategies to create libraries of nucleic acid derivatives would be valuable.

**CARB 32**

**Induction of RNAi responses by self-delivery of bioreversible phosphotriester siRNN prodrugs**

***Bryan R Meade**[1,2], Bryan@solsticebio.com, Steven F Dowdy[1]. (1) University of California, San Diego, La Jolla, CA 92093, United States (2) Solstice Biologics, San Diego, CA 92121, United States*

siRNA-induced RNAi responses have great potential to selectively treat human disease, especially cancer with its myriad of genetic mutations. However, due to their 14 kDa size and 40 negative anionic charges, siRNAs have no bioavailability to enter cells and require a delivery technology. We have focused our delivery efforts on synthesizing short interfering RiboNucleic Neutral (siRNN) prodrug molecules that contain bioreversible, phosphotriester groups that are specifically cleaved by cytoplasmic thioesterases to convert the siRNN prodrug into intracellularly active siRNA that induces robust RNAi responses. We have developed and optimized phosphotriester

phosphoramidite synthesis pathways, deprotection and purification conditions that result in high yielding siRNN oligonucleotides. siRNNs have favorable drug-like properties, including high stability in human serum, absence of innate immune stimulation and solubility. Targeted delivery of siRNN prodrugs results in robust RNAi responses *in vivo*. siRNN prodrugs represent a novel approach to induce RNAi responses by delivering one molecule at a time into cells with the smallest possible size of <20 kDa.

**CARB 33**

**Peptide nucleic acid analogs: Designs for selective binding with DNA/RNA and cell permeation**

***Krishna N Ganesh****, kn.ganesh@iiserpune.ac.in. Department of Chemistry, Indian Institute of Science Education and Research, Pune, INDIA, Maharashtra 411008, India*

Peptide nucleic acids (PNA) are a class of non-ionic DNA mimics, in which the sugar-phosphate backbone is replaced by ethylenediamine-glycine backbone. They bind complementary DNA or RNA with high affinity and selectivity. Hence they promised to be potential therapeutic agents. However they have serious drawbacks in terms of their aqueous solubility and cell penetration ability. Further their equal affinity for iso-sequential DNA / RNA decreases their target specificity by half. In order to overcome these drawbacks, we have designed, synthesized and evaluated several PNA analogues, which are conformationally constrained, in backbone. These include pyrrolidine, cyclopentyl and cyclohexyl moieties that are chiral and recently acyclic PNA analogues that possess cationic (amino and guanidine) groups to make them more soluble in water. These have been labeled with fluorescence groups to examine their cell membrane permeability properties. This lecture presents a comparison of the biophysical properties such as their differential complementation with iso-sequential DNA / RNA, selectivity in binding parallel and antiparrallel sequences and their cell permeation abilities.

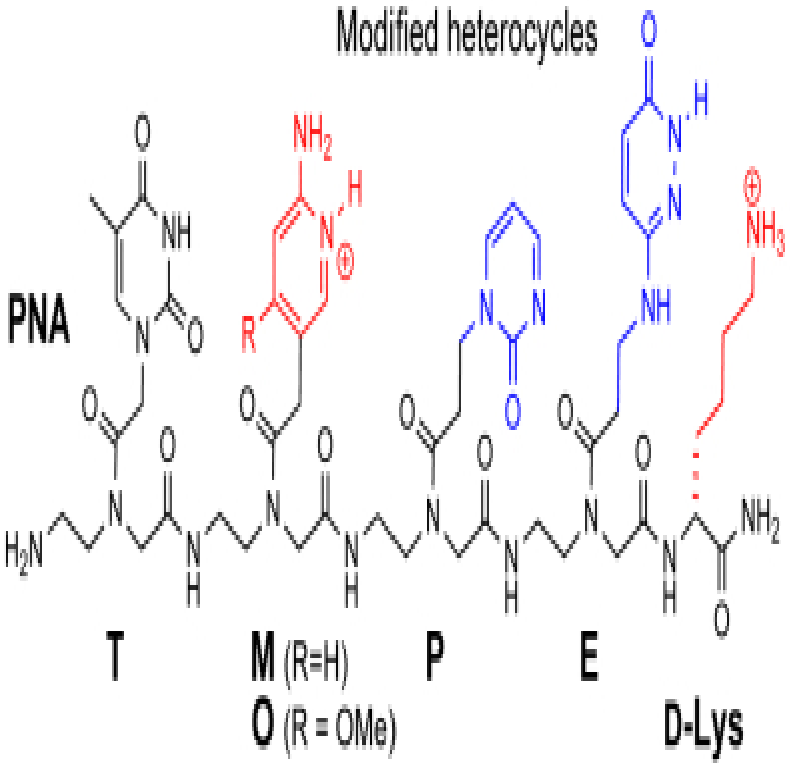
**CARB 34**

**Sequence selective recognition of double-stranded RNA using nucleobase-modified peptide nucleic acids**

*Thomas Zengeya, Ming Li, Pankaj Gupta, Oluwatoyosi Muse,* ***Eriks Rozners****, erozners@binghamton.edu. Department of Chemistry, Binghamton University, Binghamton, NY 13902, United States*

The import role that non-coding double-stranded RNAs play in biology and development of disease makes them attractive targets for molecular recognition. However, designing of small molecules that selectively recognize RNA has been a challenging and involved process because RNA helix presents little opportunity for shape-selective recognition. We discovered (1) that nucleobase-modified peptide nucleic acids (PNA), as short as

six nucleobases, bind with low nanomolar affinity and high sequence selectively to double-stranded RNA via triple helix formation under physiological conditions.




Interestingly, little to no binding is observed to dsDNA of the same sequence, which suggests that the modified PNAs have unique selectivity for dsRNA. We also demonstrated that this binding could be used for highly specific recognition of medicinally relevant double-stranded RNA, such as bacterial A-site and microRNAs. PNAs carrying **M** and Lys modifications were efficiently taken up by HEK-293 cells, while the unmodified PNA showed little uptake (2). This presentation will discuss our most recent results on sequence selective recognition of microRNAs using chemically modified PNA analogues under physiological conditions. The implications for development of novel anticancer approaches will be discussed.

**CARB 35**

**Butyl nucleic acid (BuNA): A versatile acyclic nucleic acid analog for molecular device**

***Kesavan Venkitasamy****, vkesavan@iitm.ac.in. Department of Biotechnology, Indian Institute of Technology Madras, Chennai, Tamil Nadu 600036, India*

(*S*)-Butyl Nucleic Acid (BuNA)[1], composed of acyclic backbone containing phosphodiester linkage and bearing natural nucleobases was synthesized from (*R*)-aspartic acid. Circular dichroism (CD), UV-melting and non-denatured gel electrophoresis (native PAGE) studies revealed that (*S*)-BuNA incorporation does not alter B-type-helical structure of the duplex. It was further proven that BuNA is capable of make duplexes with their complementary strands and integration of (*S*)-BuNA nucleotides into DNA duplex do not alter the properties of DNA. BuNA was used further for the construction of an A-switch. To demonstrate its use as an A-switch, a stretch of poly adenine nucleotides of (*S*)-BuNA was studied by circular dichroism (CD) and Ultraviolet (UV) spectroscopy under neutral and acidic conditions. Acid-base titration revealed two state transitions at pH 4.8 and highly pH-dependent structural conformation reversibility. Thermal melting ($T_m$) studies suggest that at neutral pH, poly BuNA(A) is a weakly organized single strand, while at low pH it adopts a highly organized and rigid structure. Furthermore, MALDI-TOF-MS data revealed intermolecular interactions which led to the formation of A-motif composed of double helical structure. Since, BuNA does not suffer from depurination under acidic conditions, which allowed us to determine thermodynamic parameters of the A-motif. This was the first report of the construction of an A-switch using artificial nucleic acids.

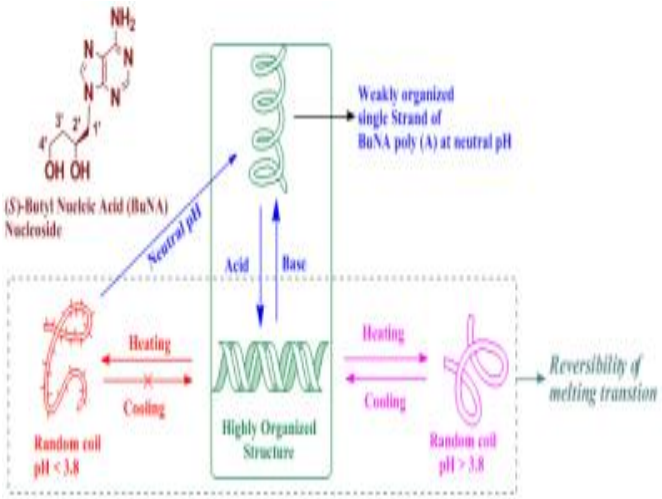



*Fig 1. BuNA derived molecular device*

[1](a) Vipin Kumar, Venkitasamy Kesavan *RSC Advances*, **2013** , 3(42), 19330 (b) Vipin Kumar, Kiran R Gore, P.I. Pradeep Kumar, Venkitasamy Kesavan *Org. Biomolecular. Chem*. **2013** , 11(35)5853

**CARB 36**

**Spherical nucleic acids (SNAs) as potent immuno modulatory agents**

***Sergei M Gryaznov**, sgryaznov@aurasense.com. AuraSense Therapeutics, Skokie, IL 60077, United States*

New macro-molecular constructs, called Spherical Nucleic Acids (SNAs), were designed, synthesized and studied as sequence-specific and versatile TLR-addressed regulators of innate immune system. These multi-valented SNAs consist of two essential components: a mono-dispersed colloidal gold nanoparticle-based core, and nucleic acids, which are covalently attached to the core surface. These nucleic acid pharmacophore exert their activity *via* aptamer-type interactions with TLR receptors, in either agonistic – ImmunoStimulatory (*is-SNAs*), or antagonistic – ImmunoRepressive (*ir-SNAs*) manner. The SNAs exhibit interesting and unexpected activity both *in vitro* and *in vivo,* which marks them as attractive candidates for further therapeutic development. Thus, cellular uptake, stability, and overall TLR9 activating efficacy of the SNAs with natural CpG-motif containing phosphodiester oligonucleotide conjugates were significantly improved *vis-a-vis* un-conjugated oligo counterparts. A detailed overview of the SNA synthesis, structure, specific gene and therapeutically important pathway regulatory activity *in vitro* and *in vivo* will be presented.

**CARB 37**

**Assay for ribosome binding drugs**

***Dev P. Arya**, dev.arya@nubadllc.com. Chemistry, Clemson University, Clemson, SC 29634, United StatesNUBAD LLC, Greenville, SC 29605, United States*

The urgent need for new antibacterial antibiotics has led to synthesis and screening approaches for novel bioactive compounds. Bacterial ribosome is a well established drug target for the development of antibacterial compounds. We have developed high-throughput capable assays for ribosome targeted drug discovery. One such assay examines the compounds ability to bind to a model ribosomal RNA A-site. We have also coupled this assay to other functional orthogonal assays. Such analysis can provide valuable understanding of the relationships between two complementary drug screening methods and could be used as standard analysis to rapidly correlate the affinity of a compound for its target and the effect the compound has on a cell.

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2. King, A.; Watkins, D.; Kumar, S.; Ranjan, N.; Gong, C.; Whitlock, J. and Arya. D.P. “Characterization of Ribosomal Binding and Antibacterial Activities Using Two Orthogonal High Throughput Capable Screens”. *Antimicrobial Agents and Chemotherapy*, **2013** Oct;57(10):4717-26.

**CARB 38**

**Synthesis and biophysical properties of nucleobase-functionalized LNA (locked nucleic acid)**

***Patrick J. Hrdlicka**, hrdlicka@uidaho.edu. Department of Chemistry, University of Idaho, Moscow, ID 83844-2343, United States*

Oligonucleotides that are modified with conformationally restricted nucleotides such as Locked Nucleic Acid (LNA) monomers are used extensively in molecular biology and medicinal chemistry to modulate gene expression at the RNA level. Major efforts have been devoted to design LNA derivatives that induce even higher binding affinity and specificity, greater enzymatic stability and more desirable pharmacokinetic characteristics. Most of this work has focused on modifications of LNA's oxymethylene bridge. Here, we describe a different approach toward modulating the properties of LNA, i.e., through functionalization of LNA nucleobases. More than twenty structurally diverse nucleobase-functionalized LNA monomers have been synthesized and incorporated into oligodeoxyribonucleotides (ONs), which were then characterized with respect to thermal denaturation, enzymatic stability and fluorescence properties. ONs modified with C5-functionalized LNA pyrimidines that are conjugated to small alkynes display significantly increased affinity toward DNA/RNA targets, improved mismatch discrimination and markedly increased protection against 3′-exonucleases relative to conventional LNA. In contrast, ONs modified with monomers that are conjugated to bulky hydrophobic alkynes display significantly lower affinity toward DNA/RNA targets but also much greater resistance against 3′-exonucleases. ONs modified with C5-fluorophore-functionalized LNA uridine monomers enable excellent fluorescent discrimination of targets with single nucleotide polymorphisms (SNPs). This concept is successfully extended to alpha-L-LNA and C8-functionalized LNA purines. We therefore anticipate that functionalization of nucleobase-functionalization will serve as a general and synthetically straightforward approach for modulation of pharmocodynamic and pharmacokinetic properties of oligonucleotides modified with LNA or other conformationally restricted monomers.

**CARB 39**

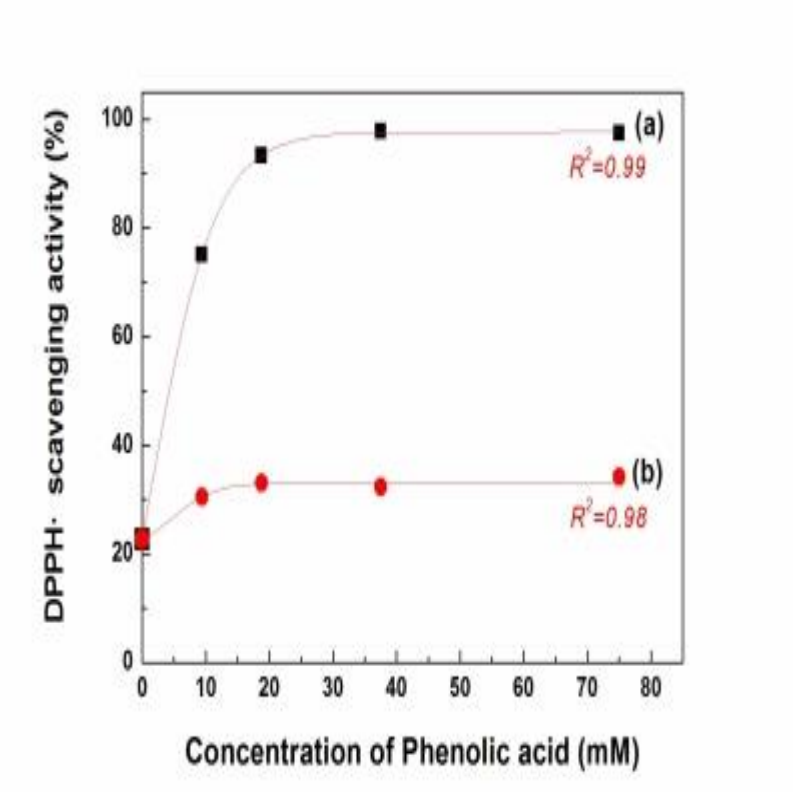
**Preparation and properties of multifunctional cotton fabrics treated by phenolic acids**

***Kyung Hwa Hong**, hkh713@kongju.ac.kr. Department of Fashion Design & Merchandising, Kongju National University, Gongju, Chungnam 314-701, Republic of Korea*

Phenolic acids are found in many plant-based natural antioxidants and are known to offer diverse health-promoting effects such as antimelanogenic, antioxidant, antineoplastic, and bacteriostatic properties. Furthermore, they not only inhibit pathogen growth but also have little toxicity to human beings. Therefore, in this study we treated cotton fabrics with two different phenolic acids, gallic acid (GA) and 4-hydroxybenzoic acid (4-HBA), through a pad-dry cure process, and investigated the properties such as

mechanical properties, antibacterial ability, antioxidant ability, etc. Consequently, the phenolic acid treatment did not significant influence on color, touch, and tensile strength of cotton fabrics. However, it was found that the cotton fabrics treated by both GA and 4-HBA showed high antibacterial ability against S. aureus and K. pneumonia; however, only the GA treated cotton fabrics showed reasonable antioxidant ability.

| | | Reduction % of Bacteria | |
|---|---|---|---|
| | | *S. aureus* | *K. pneumoniae* |
| Pristine cotton | | - | - |
| GA treated cotton | 9.375mM | 96.2 | 94.8 |
| | 18.75mM | 99.8 | 97.5 |
| | 37.5mM | 99.9 | 99.9 |
| | 75mM | 99.9 | 99.9 |
| 4-HBA treated cotton | 9.375mM | 99.4 | 98.1 |
| | 18.75mM | 99.9 | 99.9 |
| | 37.5mM | 99.9 | 99.9 |
| | 75mM | 99.9 | 99.9 |

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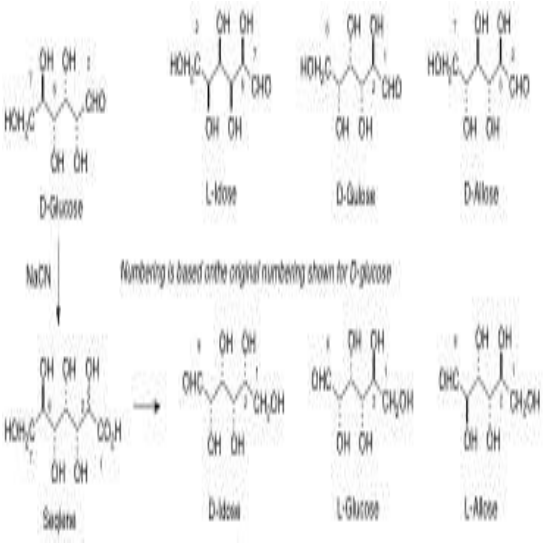
**CARB 40**

## D-glucose to derivatives of L-glucose, D-gulose, D-idose, L-idose, D-allose, L-allose, and other rare sugars

***Zilei Liu***[1]*, zilei.liu@sjc.ox.ac.uk, George W. J. Fleet*[1]*, Fernando Martínez*[1]*, Akihide Yoshihara*[2]*, Ken Izumori*[2]*. (1) Department of Chemistry, University of Oxford, Oxford, United Kingdom (2) Rare Sugar Research Center, Kagawa University, Kagawa, Japan*

In order to provide a viable alternative to the biotechnology of Izumoring, chemical synthesis of rare sugars need to be short and cheap. The reaction of D-glucose with sodium cyanide give Seqlene at prices of $1 to $5 per kilogram for ton amounts depending on the ratio of the isomers. Seqlene or equally cheap materials can provide with minimal protection [usually just isopropylidene] access to derivatives of a number of rare sugars including: L-glucose, D-gulose, L-idose, L-allose, D-idose, D-allose…, some of which are not easy to access by biotechnology.




**CARB 41**

## Fluoro azetidines and difluoro prolines from D-glucose: Inhibition of pancreatic cancer cell growth by a fluoro azetidine

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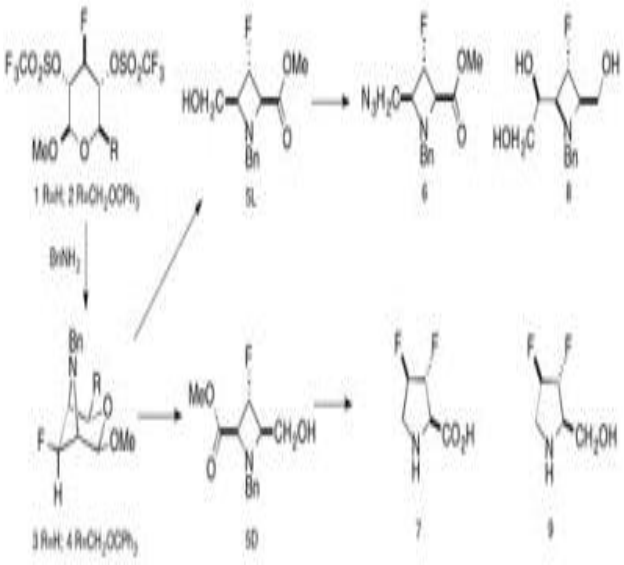
Azetidine 2-carboxylic acid (Aze) is a naturally occurring amino acid and is toxic by mis-incorporation in place of prolines in human. Aze analogues may be useful building blocks for novel peptidomimetics. It is not possible to make peptides with 3-hydroxy Aze

as a component since at pH >8, the ring is fragmented by a retro-aldol reaction. This paper describes the synthesis of 3-fluoro-Aze intermediates to form stable peptide analogues. D-Glucose can be efficiently transformed into the ditriflates **1** and **2** , which with benzylamine give good yields of the bicyclic azetidines **3** and **4** . Elaboration gives either enantiomer of *trans, trans*-fluoro Aze **5D** and **5L** in good yield. The ester **5L** can undergo displacement of the OH group to give the azido Aze **6** for incorporation of δ-amino Aze into peptides. Alternatively, ring expansion during the nucleophilic displacement gives the *trans, trans*-difluoro proline **7** as a novel building block. Meanwhile, novel fluoro iminosugars such as **8** and **9** are also accessible. None of the fluoro iminosugars affect any glycosidase. However one of them is comparable to 5-fluoruracil and Gemcitabine in its effect on human pancreatic cancer cell growth.



**CARB 42**

**Step-economical syntheses of tumor-associated carbohydrate antigens (TACAs) and immunogenic glycolipids using ReSET and glycosyl iodide gycosylation**

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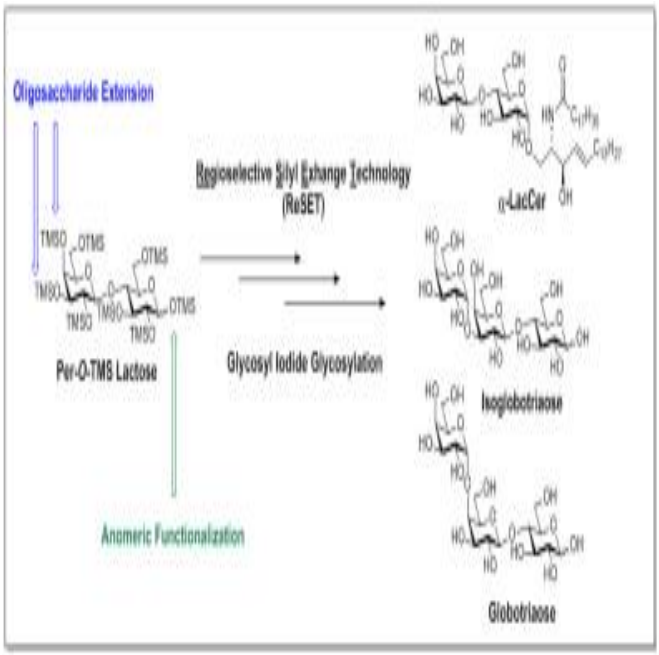
Carbohydrates mediate a wide range of biological interactions, and understanding these processes benefits the development of new therapeutics and chemical biology research tools. Isolating sufficient quantities of carbohydrates and glycolipid from biological samples remains a significant challenge; as well as chemical synthesis which usually require multiple steps and purifications. Two methodologies, Regioselective Silyl Exchange Technology (ReSET) and glycosyl iodide glycosylation, have now been combined to simplify the synthesis of the globo series trisaccharide (globotriaose and isoglobotriaose) and a-lactosylceramide (a-LacCer). These glycoconjugates are tumor-

associated carbohydrate antigens and immunostimulatory glycolipids that hold promise as potential immunotherapeutics. Glycosyl iodide can also be further functionalized by using trimethylene oxide (TMO) to introduce iodopropyl linker at the anomeric carbon as a chemical handle. The terminal iodide can be converted to corresponding azide followed by copper-catalyzed azide-alkyne cycloaddition to afford multivalent glycoconjugates of Gb3 for further investigation as anti-cancer vaccine.




**CARB 43**

**Studies of flammability and thermal degradation for flame retardant cotton fabric with P-N containing derivatives**

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The effectiveness of a phosphoramidate *Tetraethyl piperazine-1,4-diyldiphosphoramidate*(**TEPP** )as a flame retardant (FR) on cotton twill fabrics was compared with that of a previously studied *Diethyl 4-methylpiperazin-1-ylphosphoramidate*(**DEPP** ). **TEPP** was formed in a reaction between two phosphonates and a piperazine then cotton twill fabrics were treated with **TEPP** at different levels of add-on (2 - 19 wt%) and characterized using vertical flammability, limiting oxygen index (LOI), microscale combustion calorimetry (MCC), and thermogravimetric analysis (TGA) methods. The results showed better flame retardancy and thermal behavior for **TEPP** fabrics when compared with **DEPP** fabrics. When the morphological structure of the formed char from the burned areas was examined by Scanning Electron Microscopy

(SEM), the results revealed a fairly insignificant difference in the mode of action between the two types of fabric.

**CARB 44**

**Difference in dimannose binding affinities between P51G-m4-CVN and its mutants at position 41**

***Zhen Li**, zhen-li@uiowa.edu, Sai Ramadugu, Hemant Kashyap, Claudio Margulis. Department of Chemistry, University of Iowa, Iowa City, Iowa 52241, United States*

CVN, a strong HIV inhibitor, binds to oligomannose displaying the Manα1-2Manα motif. (Bewley, C. A. *Structure,* **2001** , 9, 931-940) Glu41 of CVN is a residue that is known to form strong hydrogen bonds with the sugar moiety. In order to better understand the role of Glu41 in binding to these sugars we have carried out a set of computational free energy studies. (Ramadugu, S., Li, Z. *et al*. *Biochemistry,* **2014** , DOI: 10.1021/bi4014159) Specifically, we have utilized the thermodynamic integration and Bennett Acceptance Ratio approaches to estimate the free energy changes that result from the mutation of residue Glu41 of P51G-m4-CVN (Fromme, R. *et al*. *Biochemistry,* **2007** , 46, 9199-9207) into Ala and Gly. The analysis of our results is split into electrostatic and dispersion contributions to the free energy. Interestingly, we find that whereas the Coulombic contribution to the free energy significantly favors Glu41, there are significant cancellations because of dispersion interactions. In more general terms, we learn that it is not enough that structural hydrogen bond interactions are observed between an amino acid and the ligand to infer that said ligand is necessarily key to the overall free energetics of binding.

**CARB 45**

**One pot synthesis of various N-heterocycles using glycal and olefins**

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The use and applications of carbohydrate-based oxazolines cannot be overstated. Carbohydrate-fused oxazolines have been prepared bearing the oxygen at the C-1 position (*O*-glycooxazolines) and these molecules have been used as: *i*) glycosyl donors in the synthesis of oligosaccharides; *ii*) as activated donor substrates in the enzymatic synthesis of glycoconjugates; or *iii*) as protecting groups of the anomeric center.There has been recent interest in the preparation of carbohydrate-based oxazolines due to their recurrence in natural products such as the chitinase inhibitor Allosamidin and the trehalase inhibitor Trehazolin. We are developing a convergent approach for the synthesis of a small library of carbohydrate-fused substituted oxazolines based on the framework of Allosamidin as part of our ongoing research program aimed at the generation of carbohydrate-fused heterocyclic compounds.

Compounds of this kind have the potential to be powerful glycosidase inhibitors. We report a novel one-pot procedure for the direct preparation of *N*-glycooxazolines (N at C-1), *N*-glycoaminooxazolines, and *N*-glycothiazolines via the iodonium mediated addition reactions of heteroaryl amides, thioamides, and substituted ureas to various protected glycals . The preparation of oxazolines, thiazolines, and dihydro dioxazines using electron-rich olefins were also prepared using the same methodology. The effects of the sugar protecting group, the nature of the olefin as well as the substituent on the amide and reaction temperature on the product outcomes were also explored in this study.

**CARB 46**

**Effects of chemical and enzymatic modifications on the inclusion formation between starch and oleic acid**

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Starch-inclusion complexes can function as carriers for delivering and protecting bioactive molecules from oxidation and degradation. However, most starch complexes formed are insoluble because of their ordered structure. The objective of this study was to increase the solubility of the starch complexes by modifying starch chemically via acetylation with two levels (low and high) and enzymatically with isoamylase and beta-amylase to create more linear starch molecules with varying molecular sizes. Both soluble and insoluble complexes were recovered and evaluated. The soluble complexes had a higher degree of acetylation than their insoluble counterparts. A combination of high acetylation and beta-amylase treatment increased the X-ray intensity for the soluble and insoluble complexes. The melting temperature of all complexes decreased with the β-amylase treatment and further decreased with acetylation. These results demonstrate that starch can be modified by acetylation and β-amylase treatment to increase the solubility of starch-oleic acid complex.

**CARB 47**

**Automated solid-phase synthesis of oligoxylans**

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Plant cells are surrounded by a polysaccharide-rich matrix that constitutes the cell wall of all higher plants. One of the main components of plant cell wall polysaccharides is the hemicellulose xylan, the second most abundant polysaccharide in nature. Although the structure of xylans varies between plant species, they all possess a common backbone

consisting of β-1,4-linked xylopyranoses. This backbone structure may then be partially acetylated and substituted with arabinofuranosyl or (4-*O*-methyl) glucuronyl residues.

Insights into the structures and functions of plant wall polysaccharides can be provided by high resolution imaging of cell wall microstructures. Monoclonal antibodies raised against plant polysaccharides are powerful molecular probes to monitor the composition of glycans in the cell wall. The specificities of these antibodies can be characterized using carbohydrate microarrays equipped with poly- and oligosaccharides of plant origin.

We describe here the automated solid-phase synthesis of oligosaccharide fragments of the hemicellulose xylan. Conjugation-ready oligoxylans as long as octasaccharides were printed as microarrays and selective binding of anti-xylan antibodies was detected.

**CARB 48**

**Synthesis and characterization of crystalline cellulose derived from Hibiscus sabdariffa stems, an agricultural waste product**

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Tuskegee University is investigating Hibiscus sabdariffa as a specialty crop for farmers in southern Alabama. It would be desirable to find a value added use for the stems instead of relegating them to agricultural waste. Over the last few years, interest in using biodegradable natural products to reduce the waste generated by discarded electronics has rapidly increased with a special focus on crystalline cellulose-based nanocomposites. Cellulose is an ideal candidate for reinforcement fillers in polymers commonly used in electronics because it is an abundant, natural polymer that possesses great strength and biodegradability. In this study, crystalline cellulose was extracted from the stems of Hibiscus sabdariffa via strong acid hydrolysis with sulfuric, nitric, and hydrochloric acids. A comparison of the surface and structural differences of the polymer caused by the varying acids will be examined with x-ray diffraction analysis and scanning electron microscopy. Mechanical and thermal properties of the extracted cellulose will be investigated allowing additional insight into any characteristic improvements or regressions.

**CARB 49**

**Identification, profiling, and purification of lectins from cancer cells using magnetic glyconanoparticles**

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Research has shown that the interactions between cancer cells and carbohydrates can play crucial roles in the survival, growth, invasion and metastasis of cancer cells making this interaction a target in cancer studies. Similarly, there is enough evidence supporting the fact that lectins (carbohydrate binding proteins) are overexpressed on cancer cells and the interactions between lectins and carbohydrates on the cell surface can contribute in cancer growth and development. However, studies to understand these interactions in finer details have been hampered by a lack of quantitative tools. In this presentation, magnetic iron oxide nanoparticle based technology immobilized with carbohydrates (glyco-nanoparticles) has been developed as a tool to isolated and purify endogenous lectins . Good specificity has been achieved in endogenous lectin isolation from the cancer cells using glyco-nanoparticles raising the possibility of discovering novel lectins endogenous to cancer cells. This preliminary work indicates that carbohydrate functionalized magnetic iron oxide nanoparticles can be a new addition to the cancer research toolbox which could facilitate the further exploration of the role carbohydrate recognition plays in tumor development.

**CARB 50**

**Novel fluorogenic probes enable fluorescence entrapment in water-in-oil-in-water droplets for ultrahigh-throughput screening of enzymatic activities**

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High-throughput screening is a key technique in the discovery and engineering of enzymes. *In vitro* compartmentalization-based fluorescence-activated cell sorting (IVC-FACS) has recently emerged as a powerful tool for ultrahigh throughput screening of biocatalysts. For IVC-FACS single enzyme expressing cells and fluorogenic substrates are compartmentalized into water-in-oil-in-water (w/o/w) droplets (micro-reactors), which are analyzed and sorted by flow cytometry after a certain period of enzymatic reaction. A limiting factor in such studies is the tendency of the colored dye product to diffuse from the site of cleavage and escape from the w/o/w droplets, reducing resolution and utility.

To address this problem we propose an entrapment strategy, in which a neutral oil-permeable masked-fluorophore is converted into a charged, thereby trapped, fluorophore in the presence of the expressed enzyme of interest. We have shown by FACS enrichment that our novel probes for esterases and glycosidases exhibit superior fluorescence retention in w/o/w emulsions, providing much lower background signal and

better dynamic range compared to traditional fluorescein and coumarin based imaging agents.

Currently we are applying this strategy to the directed laboratory evolution of glycoside hydrolases and engineered variants thereof termed glycosynthases. These evolved glycosynthases will allow the efficient transfer of artificial carbohydrates with bioorthogonal functionalities.

**CARB 51**

**Aspergillus fumigatus produces two arabinofuranosidases belonging to glycosyl hydrolase family 62, one of them possessing a carbohydrate binding module: Heterologous expression and characterization of both enzymes**

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Arabinofuranosidases are important enzymes in the liberation of arabinose from xylan and pectin. In the genome of *Aspergillus fumigatus*, two genes encoding putative GH family 62 arabinofuranosidases (*abf1* and 2) have been identified. The sequence of *abf2* is 1191 bp in length including an intron and a carbohydrate binding module (CBM), while *abf1* with 999 bp shows no introns and CBMs. When the catalytic modules of the respective proteins are compared, they show a 79% identity. The estimated molecular masses of the proteins are 36428 and 42950 for ABF1 and 2, respectively. Using *Pichia pastoris* as host, both proteins have been expressed heterologously and purified from the culture supernatants. ABF1 has a molecular mass of about 30 KDa and ABF2 of 43 KDa as estimated from SDS gels. Both enzymes are active using p-nitrophenyl arabinofuranoside (pNPAra) as substrate, and show a pH optimum of 4-5 (ABF1) and 5 (ABF2). Optimal temperatures are 37ºC and 42 ºC, respectively for ABF 1 and 2. Km for ABF1 is 94.2 mM and for ABF2 3.9 mM. ABF2 is considerably more stable: at 45ºC and 20 min incubation it retains 80% of its activity, compared to 20% for ABF1. Both enzymes liberate arabinose from arabinoxylan and show little activity towards arabinan or debranched arabinan in agreement with other fungal family 62 ABFs. Although both enzymes show similarities in substrate specificity, the presence of a CBM in ABF2 increases its affinity for pNPAra and its heat stabiliby.Grant support: FONDECYT 1130180 and UNAB DI-61-12/R

**CARB 52**

**Towards the total synthesis of *Escherichia coli* O-antigen O111 minimum repeat unit**

***Dina Lloyd****, dina.lloyd@tufts.edu, Clay S Bennett. Department of Chemistry, Tufts University, Medford, Massachusetts 02155, United States*

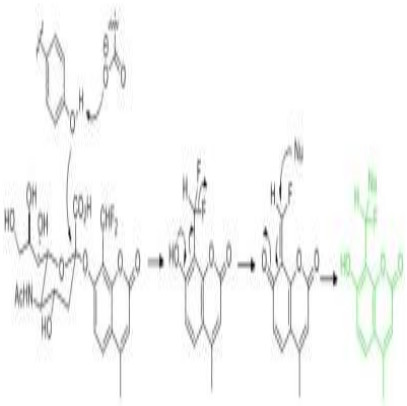
Within the recent years there has been an increase in the number of outbreaks of the hemorrhagic colitis causing bacterium *E. coli* O111 in food sources. Coupled with increasing antibiotic resistance, there is a growing need for new therapeutics to deal with such outbreaks. The *E. coli* O111 O-antigen holds potential as a target for vaccine development. The minimum repeat unit of the O111 O-antigen is a pentasaccharide minimum repeating unit that encompasses four major sugar derivatives, colitose, glucose, galactose, and glucosamine. Progress towards the synthesis of this molecule will be described. Highlights include a highly efficient synthesis of the colitose fragment and the application of our reagent controlled α selective glycosylation for 1,2-*cis*-glycoside synthesis to assemble the fragments.

**CARB 53**

**Development of fluorogenic neuraminidase probes**

***Zhizeng Gao**, zhizeng@chem.ubc.ca, Steve Withers. Department of Chemistry, University of British Columbia, Vancouver, British Columbia V6T 1Z4, Canada*

Neuraminidases (NA) play crucial roles in influenza infections by hydrolyzing the glycosidic linkage between the host cell and the virus, thus releasing the virus to infect new cells. The availability of fluorogenic probes to visualize the activity of neuraminidase in situ would help us understand the infection process. Currently available fluorogenic sialosides are of little use since the fluorophore released rapidly diffuses from the site of cleavage. Here we have developed a sialoside substrate bearing a quinone methide-generating leaving group that is not innately fluorescent but which, upon cleavage, releases a highly chemically reactive and fluorescent aglycone (a quinone methide) that tags the cell or the local environment, minimizing diffusion of the fluorophore once released. Such immobilisation is extremely important for imaging and histological analyses, since all other fluorophores in use are quite highly mobile. This class of probes is 'catalytic' thus generates multiple fluorophores per neuraminidase molecule, thereby giving strong signals. This reagent class will be ideal for use as imaging agents, in histological analyses and possibly in FACS sorting of infected cells.



**CARB 54**

## Proteomic identification of elongation factor 2 (Eft2p) as a candidate biomarker to characterize protein mis-glycosylation in *Saccharomyces cerevisiae*

***Manivannan Yegambaram**, mani1280@yahoo.com, Bhagyashree Manivannan, Rolf U. Halden. Center for Environmental Security, The Biodesign Institute, Arizona State University, Tempe, AZ 85287, United States*

Congenital disorders of glycosylation type Ic (*CDG-Ic*) patients classically present mis-glycosylation of proteins, a diagnostically exploited defect routinely screened for using isoelectric focusing of serum transferrin. To find new biomarkers for identification of this disorder, we screened for mis-glycosylated proteins as a result of lack of α-1,3-glucosyltransferase using *S. cerevisiae* as a model system Using two-dimensional gel electrophoresis (2-DE), enriched glycoproteins were separated by isoelectric point and protein mass. The glycoprotein spots were excised, trypsin digested, and identified utilizing matrix-assisted laser desorption/ionization tandem time of flight (MALDI-TOF/TOF) mass spectrometry. Compared to the wildtype laboratory strain, mis-glycosidation was found in 17% of the total amount of cytoplasmic elongation factor 2 protein (Eft2p) expressed in a *Δalg6* yeast strain. The ability to detect mis-glycosylated Eft2p demonstrated in this study, suggests that similar adverse protein modifications potentially could be identified in humans with CDG type I disorders because the yeast and human elongation factor 2 protein (EF2p) sequences share 66% identity and 85% homology. Future identification of similar mis-glycosylation patterns of EF2p in CDG-I patients could serve as an alternative biomedical diagnostic test for early identification of CDG type I disorders.

**CARB 55**

## Series of partially O-acetylated N-acetyl neuraminic acid (Neu5Ac) using regioselective silyl exchange technology (ReSET)

***Simon S Park**, sipark@ucdavis.edu, Jacquelyn Gervay-Hague. Chemistry, University of California, Davis, Davis, CA 95616, United States*

In mammalian cells, glycans containing sialic acid play a significant role in many biologically relevant processes such as influenza virus infection. In order to adapt to environmental changes, sialic acid adopts variations at the hydroxyl groups, the most common form being the *O*-acetyl groups. Isolation of partially *O*-acetylated sialic acid is challenging due to harsh conditions used in extraction methods; thus, syntheses of these analogues are important. Six partially *O*-acetylated *N*-acetyl neuraminic acid (Neu5Ac) analogues have been made in an efficient manner, four of which are series of naturally occurring 4-*O*-acetylated containing Neu5Ac. The chemical understandings of these compounds can provide further knowledge in biological functions and structural dynamics of sialic acids including its role in immunogenic properties of mammalian cells.

**CARB 56**

## Purification of synthetic oligodeoxynucleotides via catching by polymerization

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Recently, large quantities of pure synthetic oligodeoxynucleotides (ODNs) are needed for preclinical research, patient use, and clinical trials. These ODNs are synthesized on an automated synthesizer. Typically, the synthesis of ODNs generates impurities including failure sequences, which are difficult to remove. The reason is that they have the same properties as the full length ODNs. Currently, ODNs purification technologies can remove those impurities, such as reverse phase high-performance liquid chromatography (RP HPLC), anion exchange HPLC, polyacrylamide gel electrophoresis (PAGE). However, all these methods are inconvenient or costly to scale up. To solve the problem, two non-chromatographic methods of ODNs purification by polymerization are developed. In the first method, during automated synthesis, the full-length ODNs are tagged with methacrylamide group via a cleavable linker while the failure sequences are not. The full-length ODNs are incorporated into a polymer through a copolymerization process, and failure sequences and other impurities are removed by washing. The full-length ODNs are obtained by cleaving from the polymer. In the second method, the failure sequences are capped by a methacrylated phosphoramidite followed by radical acrylamide polymerization and water extraction to retrieve the full-length ODNs. The purity of ODNs has been confirmed by RP HPLC. The identity of ODNs has been established by comparing with authentic ODNs and Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) analysis. These methods do not require any expensive equipment and materials. Therefore, they are useful for large-scale drug ODN purification.

**CARB 57**

## Thio-β-D-glucosides: Synthesis and evaluation as glycosidase inhibitors and activators

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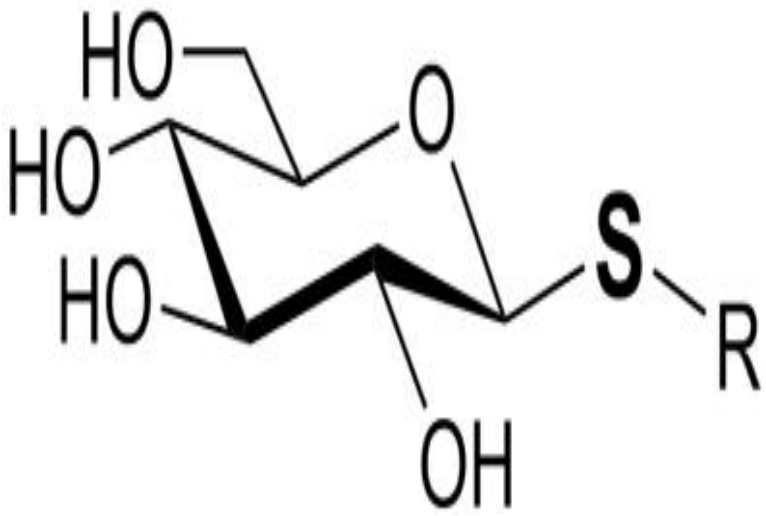
Novel structurally simple 1-thio-β-D-glucopyranosides were synthesized and tested as potential inhibitors towards several fungal glycosidases from *Aspergillus oryzae* and *Penicillium canescens*. Significant selective inhibition was observed for α- and β-glucosidases, while the same compounds produced a weak to moderate activation of α- and β-galactosidases.




**CARB 58**

**Fully Synthetic Multivalent Glycopeptide-Based Antitumor Vaccines**

***Pu-Guang Chen****, woshicpg@126.com, Hui Cai, Yan-Mei Li. Department of Chemistry, Tsinghua University, Beijing, Beijing 100084, China*

Cancer is one of the most dangerous diseases which kill millions of people around the world annually. With the fast developing of immunology, the immunotherapy of cancer became the most promising way against tumor. But choosing a sufficient tumor-specific antigen is the critical prerequisite for developing an effectively antitumor vaccine. MUC1 protein is a member of mucin family which is over-expressed and aberrantly glycosylated in most epithelial tumor cells. So MUC1 tandem repeat sequences peptide combined with tumor-associated carbohydrates antigens (TACAs) seems to be an attractive antitumor target.

However, because of the low immunogenicity of MUC1 peptide, new strategies should be considered to design more effective vaccines to elicit sufficient immune responses. Pam3CSK4 is the agonist of TLR2 which can effectively activate the innate immune system andconsequently activate the adaptive immune system.

It is reported that the multivalent antigen vaccine could elicit stronger immune response than single antigen by cluster effect. So we conjugated the multivalent MUC1 glycopeptideantigen to TLR2 ligand lipopeptideby click chemistry.[1]This vaccine consisting of four copies of MUC1-TACA antigen produced a significant cluster effect and elicited strong immune response without any extra adjuvant. The antibodies induced by the vaccine in micewere prevailing IgG2a which meant the vaccine elicited mainly Th1 cell mediating cellular immune response against tumor cell.

**References:**

[1]. HuiCai, Zhan-Yi Sun, Mei-Sha Chen, Yu-Fen Zhao, Horst Kunz and Yan-Mei Li, Synthetic Multivalent Glycopeptide-Lipopeptide Antitumor Vaccines: Impact of the Cluster Effect on the Killing of Tumor Cells, *Angew. Chem. Int. Ed.* 2014, **53** , 1 – 6.

**CARB 59**

**Self-assembling MUC1 glycopeptide vaccine for cancer therapy**

***Lei Shi**, slbuct@sina.com, Zhi-Hua Huang, Yan-Mei Li. Department of Chemistry, Tsinghua University, Beijing, Beijing 100084, China*

MUC1 is a member of the mucin protein family, which is over-expressed in tumor cells and surrounds all surfaces of the tumor cells, as a significant symbol of tumor. The mucin MUC1 is an attractive target for the development of an immunotherapy against cancer. Its extracellular domain contains numerous tandem repeats of the sequence, which includes five potential glycosylation sites.

MUC1 is weak immunogenic, since it is a self-antigen. Therefore, how to increase the immunogenicity is significant. To overcome the poor immunogenicity of MUC1, many strategies are employed today including combination with carrier proteins, peptide templates, nanoparticles and other adjuvants.

For a novel design of chemical tumor vaccine, we constructed several totally synthetic vaccine candidates with self-assembly domain Q11 and B-cell epitopes[1]. After self-assembling in neutral condition, the vaccine candidates formed fibers and elicited immune response without adjuvant. The fibrillar structure could display multivalent B-cell epitopes under mild condition. The PDTRP sequence with Tn antigen modification on Thr residue could induce an effective immune response. The high titer of the antibody in response to the adjuvant-free vaccine and the recognition of the antibody to human breast tumor cells proved that the vaccines have good immune effect. A T-cell independent pathway and the activation of cytotoxic T cells were detected after immunization. Therefore, the totally synthetic, self-assembling, adjuvant-free MUC1 glycopeptide vaccine was a promising approach for anticancer therapy.

References

[1] Huang, Z. H.; Shi, L.; Ma, J. W.; Sun, Z. Y.; Cai, H.; Chen, Y. X.; Zhao, Y. F.; Li, Y. M. J. Am. Chem. Soc.2012, 134, 8730-8733.

**CARB 60**

**Green synthesis, characterization, and evaluation of sugar coated gold nanoparticles for catalytic applications**

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Gold nanoparticles (GNPs) have gained an immense interest due to their wide applications in the fields of biomedical and pharmaceutical, which is due to their unique physico-chemical properties when they are reduced to their nanoscale size range. Here, we present a novel single step bio-friendly process for synthesis of fructose (monosaccharide), sucrose (disaccharide) and raffinose (trisaccharide) capped GNPs, wherein sugar is directly capped onto gold without the use of any secondary capping/stabilizing agent. Our study is mainly focused on the effect of various lengths of sugars in formation and catalytic reduction activity of sugar capped GNPs. Characterization of synthesized GNPs was done using various analytical techniques such as transmission electron microscopy (TEM), SEM-EDS, FTIR, UV-Vis spectroscopy. p-nitrophenol assay was used to evaluate the catalytic reduction activity of various sugar capped GNPs at different temperature using UV-Vis spectrometer. Using the spectroscopic data, rate constant for three sugar capped GNPs was determined followed by its activation energy and exponential factor using different equations. From the kinetic data, the catalytic reduction activity for three sugars was in the descending order of fructose, sucrose and raffinose GNPs respectively. This difference in the catalytic activity is believed to be due to the size of ligand on gold surface which greatly influences the surface/volume ratio.

**CARB 61**

## Synthesis of the glycoside moiety of solaradixine, a glycoalkaloid found in Solanum laciniatum, using “super-armed” donors and block synthesis strategies

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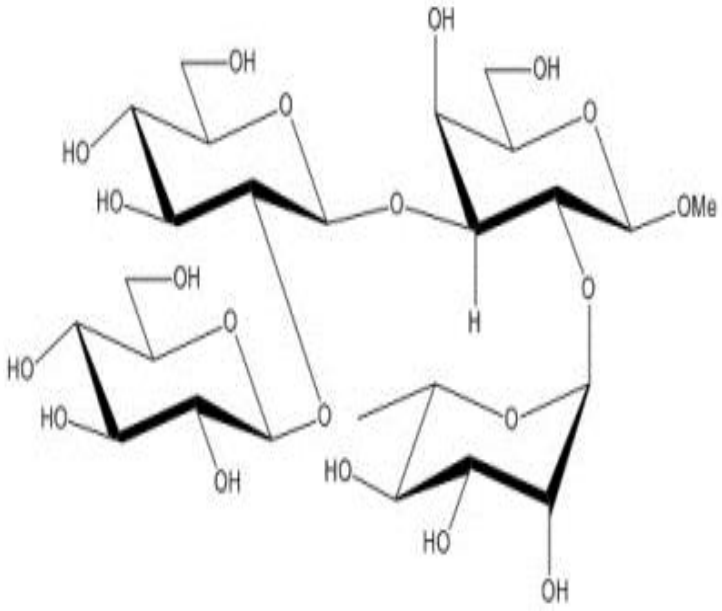
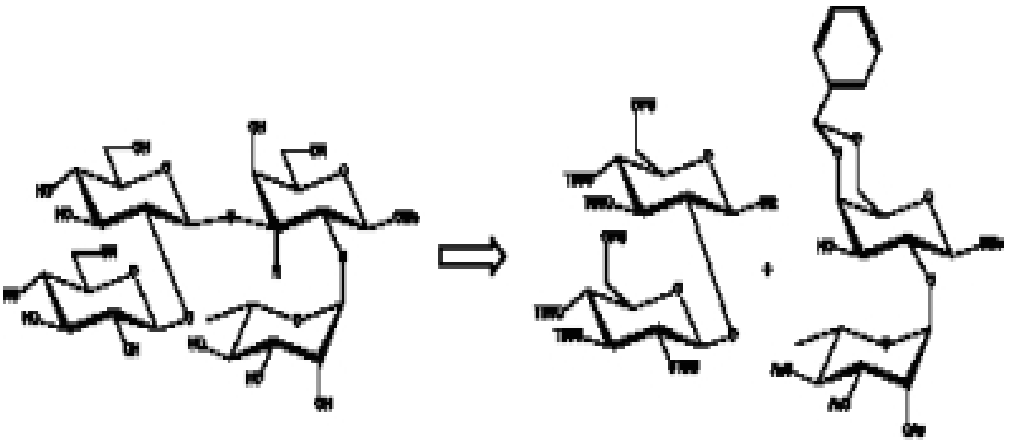
Steroidal glycoalkaloids are glycosylated alkaloids found in a large number of the *Solanum* species. Pharmacological effects of steroidal glycoalkaloids on human cells are dependent on the chemical structures. Of all the modern structural methods for saponins related compounds, NMR spectroscopy yields the most complete picture of the structure and the behavior in solution. $^{1}$H and $^{13}$C NMR chemical shift data are used by the computer program CASPER to predict chemical shifts of oligo- and polysaccharides. The enhancement of the quality of the predictions can efficiently be achieved by focusing on carbohydrate structures of biochemical significance.[1] The main glycoalkaloid from roots of *S. laciniatum* was found to be O(3)-{α-L-Rha*p*-(1→2)-[β-D-Glc*p*-(1→2)-β-D-Glc*p*-(1→]3)-β-D-Gal*p*}-solasodine.[2]




The synthesis of the tetrasaccharide in this sequence was the target of this project. A so called “super-armed” version of a disaccharide donor was synthesized as the first block.[3] Meanwhile, another glycosylation procedure would provide the disaccharide acceptor needed to obtain the targeted product. These strategies allowed a facile and rapid synthesis of the target.

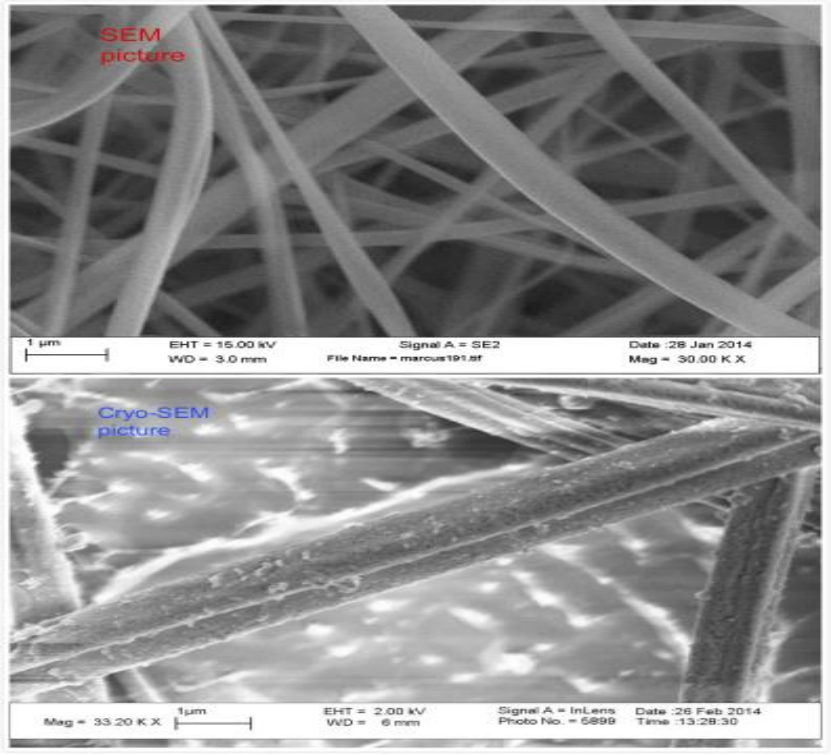


**CARB 62**

**Release profile and anti-inflammatory study of electrospun quercetin-loaded cellulose acetate fiber mat**

***Zhenyu Lin**, marcus.lzy@gmail.com, Qingrong Huang. Department of Food Science, Rutgers University, new brunswick, new jersey 08901, United States*

Quercetin has attracted researchers' attention in recent years due to its anti-oxidative and anti-inflammatory activities. It has a strong potential against skin inflammation problems like acne, skin itching and rash. However, due to the poor skin absorption ability of quercetin, it is valuable to develop a method to enhance its skin absorption efficiency. Electrospun fiber mat, which offers large surface contact area and high affinity to the skin, is possible to address the problem. In this research, quercetin of different loadings and cellulose acetate (CA) were co-dissolved in acetic acid, and then electrospun into fine fiber mats. Fiber diameter could be tuned from 150nm to micron scale, which was determined by Scanning Electron Microscope (SEM).




X-ray diffraction (XRD) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) analyses were performed to examine quercetin crystalline status and molecular interactions within the quercetin-CA fiber mats. Total release profile of quercetin from the fiber mat was assayed by direct immersion in buffered medium while the transdermal release profile was performed by Franz-cell diffusion kit loaded with human skin in 37 °C buffered system. Cryo-SEM was utilized to examine the porous surface morphology of CA fiber mats after immersed in buffered dissolution medium to demonstrate the release mechanism of quercetin. The anti-inflammation properties of quercetin fiber mat were further evaluated by the reduction of mouse ear edema induced by 12-O-tetradecanoylphorbol-13-acetate (TPA).

## CARB 63

### Synthesis of *Neisseria meningitidis* capsular oligosaccharides for vaccine development

***Chung-Yi Wu**, cyiwu@gate.sinica.edu.tw, Chia-Hung Wang. The Genomics Research center, Academia Sinica, Taipei, Taiwan Republic of China*

We will present the synthesis of the important but problematic *Neisseria meningitidis* Serogroup C[(1)] and W135[(2)] capsular oligosaccharides from disaccharides (with 2 sugars) to dodecasaccharide (with 12 sugars), and further attached them to carrier protein CRM197 to create the *Neisseria meningitidis candidates*, they were tested in a mouse animal model. The results showed that the vaccines successfully induced antibodies to neutralize the capsular oligosaccharides. Our results showed that induced IgG antibody titers were much higher than IgM antibody titers. Furthermore, analysis of the distribution of IgG subclasses showed that the antigen predominantly elicited IgG1 antibodies. Also, IgG3, a typical anti-carbohydrate antibody, was found at high levels in the serum. Consequently, the oligosaccharide–protein conjugate is a TD antigen, which processes antibody isotype switching from IgM to IgG1. For W135, antibodies induced by DT-2 recognized only disaccharides, but could not cross react with tetrasaccharides or longer. In contrast, antibodies induced by DT-4, DT-6, DT-8, and DT-10 all recognized tetra- to decasaccharides but not disaccharides. Patterns on the glycan microarray were the same for both adjuvants, alum and C34. Therefore, we concluded that antibodies induced by DT-4–DT-10 were similar, but different from DT-2. The vaccines then were further examined by bactericidal assay to demonstrate the bactericidal activity of various lengths of sugars, and the results showed that the length as short as tetrasaccharide (with 4 sugars) could sufficiently induce bactericidal activity. The effectiveness of this synthesized vaccine can revolutionize the requirement of high biosafety level for current *Neisseria meningitides* vaccine production, in which the necessary polysaccharides are acquired from pathogenic bacteria and often exist as mixtures of many components. On the other hand, this study provides a new approach to obtain the necessary polysaccharides by synthesis to create a molecular vaccine that is homogeneous, more consistent, and better quality controlled.

## CARB 64

### Enzymatic synthesis of tumor-associated antigen Globo-H starting from bulk substrates

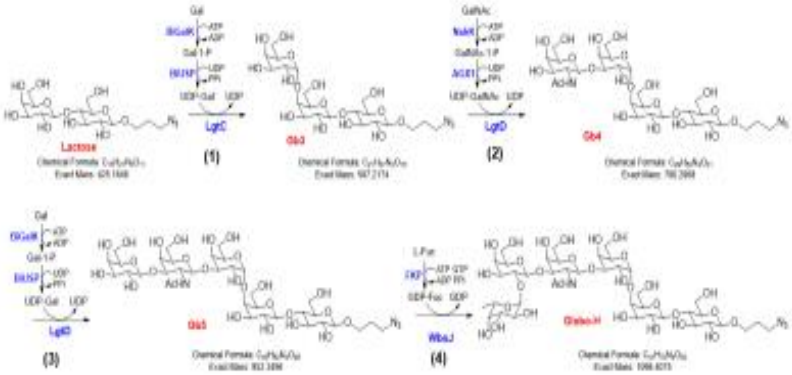
***Kristina Shelley Garner**, kgarner7@student.gsu.edu, Lei Li, Peng George Wang. Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, United States*

The development of a method for the enzymatic synthesis of an important tumor associated carbohydrate antigen Globo-H hexasaccaride. The method uses overexpressed glycosyltransferases coupled with enzymes for the generation of sugar

nucleotides. Our goal is to establish a method of purification of the oligosaccharide: Globo-H in a multi-gram enzymatic synthesis by using inexpensive bulk substrates.

Carbohydrate antigens such as Globo-H are among the best characterized tumor antigens overexpressed on the surface of malignant cells. The hexasaccharide of Globo-H, is a member of a family of antigenic carbohydrates that are highly expressed on various types of cancers. It is expressed on the cancer cell surface as a glycolipid and possibly as a glycoprotein. Globo-H was first discovered in 1983 from a cultured teratocarcinoma cell line. The biological function of Globo-H is not clear but expression is associated with tumor aggressiveness.

As a distinct tumor-associated antigen marker, this epitope has been emerging as an important sequence for the development of anticancer vaccines. This study intends to enzymatically synthesize Globo-H in large scale starting with inexpensive materials.



Initial results showed the synthesis of Gb3, Gb4 and Gb5 from the starting material, Lactose-N3. The next steps would be to synthesize Globo-H. One-pot multi-enzyme synthesis will also be tested, develop methods for facile enzymatic synthesis and quick separation of Globo-H, and link Globo-H to gold nanoparticle or carrier proteins for anti-tumor purpose.

**CARB 65**

**Smart microarray platforms for understanding biochemical interactions**

***Caroline I Biggs****, caroline.biggs@warwick.ac.uk. Department of Chemistry, The University of Warwick, Coventry, West Midlands CV4 7AL, United Kingdom*

Carbohydrate arrays are important research tools in studying infection, probing the mechanisms of bacterial, viral and toxin adhesion and in the development of new

treatments for bacterial infection.[1] Due to the global decrease in new antibiotic discovery and the increase in antibiotic resistance, there is an urgent need for novel new technologies to detect and probe bacterial infection. At the start of the infection process, for example by cholera toxin, HIV or *E. coli*, pathogens adhere onto the host cells, commonly through protein-carbohydrate interactions. Probing these interactions can detect bacteria and provide structural information on their adhesion proteins and carbohydrate specificities [2] and can be achieved efficiently by presenting the carbohydrates in a microarray format.

Current microarrays involve the immobilisation of carbohydrates onto glass slides, followed by the addition of fluorescent-labelled proteins to assess binding.[3] It would be preferable to interrogate whole bacteria, but this increases non-specific binding to the surfaces, reducing resolution and giving rise to false positive diagnoses. We have developed new and versatile methodologies to functionalise many different substrates with carbohydrates, glycopolymers and switchable non-fouling polymers using 'click' type reactions. The use of switchable (thermoresponsive) polymers allows us to have a hydrophilic surface for resisting non-specific interactions, but also create high density arrays,[4,5] which have been characterised by Ellipsometry, Quartz Crystal Microbalance with Dissipation, Drop Shape Analysis and X-ray Photoelectron Spectroscopy. The arrays are as simple as self-assembling on gold, but with the cost saving of working on glass and silicon, and utilise covalently bound carbohydrates.

[**1** ]Park, S. *et al., Chemical Society Reviews* **2013,** *42* (10), 4310-4326. [**2** ]Pieters, R.J., *Medicinal Research Reviews* **2007,** *27* (6), 796-816. [**3** ]Hirabayashi, J. *et al., Chemical Society Reviews* **2013,** *42* (10), 4443-4458. [**4** ]Jones, M.W. *et al., Polymer Chemistry* **2011,** *2* (3), 572-574. [**5** ]Gibson, M.I. *et al.,Chemical Society Reviews* **2013,** *42*(17), 7204-7213.

**CARB 66**

**Binding models for non-substrate-like inhibitors of UDP-galactopyranose mutase**

***Yun Shi**[1], shiyuns@sfu.ca, Valerie Winton[2], Sankar Mohan[1], Laura L. Kiessling[2], B. Mario Pinto[1]. (1) Department of Chemistry, Simon Fraser University, Burnaby, British Columbia V5A1S6, Canada (2) Department of Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706-1322, United States*

UDP-galactopyranose mutase (UGM), an enzyme that catalyzes the interconversion between UDP-galactopyranose and UDP-galactofuranose, is a potential therapeutic target against *Mycobacterium tuberculosis*. Despite numerous efforts at the development of UGM inhibitors, a rigorous structural model for the bound geometry of a non-substratelike inhibitor is not available. Thus, we investigate herein the binding models of two non-substrate-like UGM inhibitors. The location and mode of inhibitor binding are elucidated by an approach that combines atomistic molecular dynamics simulations and saturation transfer difference (STD) NMR spectroscopy. Validation of the computed model is provided by comparisons between theoretical STD effects

calculated from the model and experimental STD values. The binding models thus generated form the basis for future inhibitor optimization.

**CARB 67**

**In(III) triflate-mediated solvent-free synthesis and activation of thioglycosides by ball milling and structural analysis of long chain alkyl thioglycosides by TEM and quantum chemical methods**

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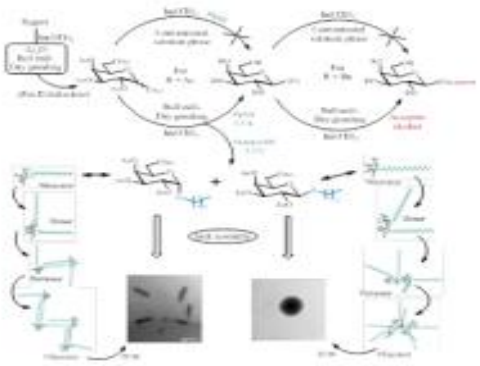
Conventional solution-phase synthesis of thioglycosides from glycosyl acetates and thiols in the presence of In(III) triflate as reported for benzyl thioglucoside failed when applied to the synthesis of phenolic and alkyl thioglycosides, But, it was achieved in high efficiency and diastereospecificity with ease by solvent-free grinding in a ball mill. The acetates in turn were also obtained by the homogenization of free sugars with stoichiometric amounts of acetic anhydride and catalytic $In(OTf)_3$ in the mill as neat products. Per-*O*-benzylated thioglycosides on grinding with an acceptor sugar in the presence of $In(OTf)_3$ yield the corresponding *O*-glycosides efficiently. The latter in the case of a difficult secondary alcohol was nearly exclusive (>98%) in 1,2-*cis*-selectivity. In contrast, the conventional methods for this purpose require use of a co-reagent such as NIS along with the Lewis acid to help generate the electrophilic species that actually is responsible for the activation of the thioglycoside donor *in situ*. The distinctly different self-assembling features of the peracetylated octadecyl 1-thio-α- and β-galactopyranosides observed by TEM could be rationalized by molecular modelling.



**CARB 68**

## Synthesis of 2,3-O-dibenzylribo and xylosides, and their equilibriation studies; Application to oligosaccharide synthesis

***Reignier M. Jeffrey***[1]*, reignierj@gmail.com, Gurdial Singh*[1]*, Patrice G. Plaza*[1]*, Nadia Singh*[1]*, Johnathan Goodman*[2]*, Alessia Bacchi*[3]*, Francesco Punzo*[4]*. (1) Department of Chemistry, The University of the West Indies, St. Augustine, Trinidad and Tobago (2) Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom (3) Department of Chemistry, Università degli Studi di Parma, Parco Area delle Scienze 17/A, Parma 43124, Italy (4) Department of Medical Sciences, Chemistry Section, Università di Catania, Viale Andrea Doria, Catania 95125, Italy*

The preparation of 2,3-di-*O*-benzyl ribose and 2,3-di-*O*-benzyl xylose will be described. These monomeric units contain unprotected hydroxyl groups at the 4 and 5 positions as well as an unprotected anomeric center, therefore they are subject to mutarotation. Equilibria studies, with the use of nuclear magnetic resonance, were performed to determine the relative abundance of the conformers, in solvents of varying polarity, as well as the change in the ratios of the conformers with time. In all cases, the pyranoses dominated. In DMSO the major isomer had a β-pyranose structure. In less polar solvents, the proportion of the furanoses increased

In the case of 2,3-di-*O*-benzyl ribose, the crystal structure was elucidated via x-ray crystallography. This revealed that the molecule adopts a β- pyranose chair conformation comprised of two molecules in the unit cell.

An iterative approach to the synthesis of oligofuranosides will be discussed that employs a biomimetic activation of the anomeric center. This synthetic approach employs the use of a limited protection protocol where acceptors of 2,3 di-*O*-benzyl ribose and 2,3-di-*O*-benzyl xylose are used to form oligomers of the respective sugars which are 1,5- linked and proceed with high β- selectivity.

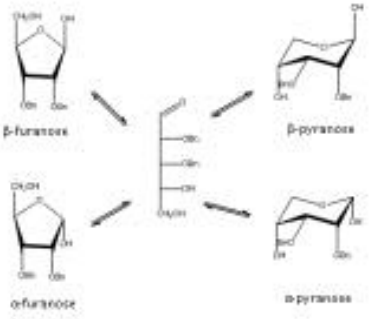



**Figure 1**: Equilibria of 2,3-di-O-benzyl ribose

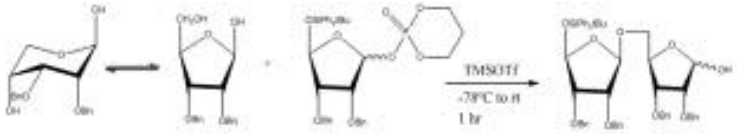



**Figure 2**: Synthetic scheme for the synthesis of a disaccharide of ribose

**CARB 69**

## How cellulose elongates: A QM/MM study of the molecular mechanism of bacteria CESA

***Hui Yang***[1,2]*, hyang2016@gmail.com, James Kubicki*[1,2]*. (1) Department of Geosciences, Pennsylvania State University, University Park, PA 16802, United States (2) Center for Lignocellulose Structure and Formation, Pennsylvania State University, University Park, PA 16802, United States*

Cellulose is the most abundant renewable hydrocarbon source in the world. In bacteria, cellulose is produced via a protein complex, in which BcsA is the catalytically active subunit responsible for the formation of cellulose catalyzing the transfer of the glucose unit from UDP-α-D-Glucose to the cellulose. The catalytic mechanism of BcsA was investigated by using hybrid quantum mechanics and molecular mechanics (QM/MM) approach. The Michaelis complex theoretical model was built up based on the X-ray crystal structure (PDB ID: 4HG6) containing BcsA and BcsB subunits together with one uridine diphosphate molecule and a translocating glucan. A $S_N$-2-type transition structure corresponding to the nucleophilic attack of the non-reducing end $O_4$ on the anomeric carbon $C_1$, the breaking of the glycosidic bond $C_1$-$O_1$, and the transfer of proton from the non-reducing end $O_4$ to the catalytic base Asp343 has been identified via the QM/MM simulation. The proton was found finally transferred to UDP from catalytic base via $O_2$ of the newly added glucose unit, facilitating UDP leaving. Another similar $S_N$-2-type transition structure for adding a second glucose unit to cellulose has also been identified. This study provided detailed insight about how the cellulose is

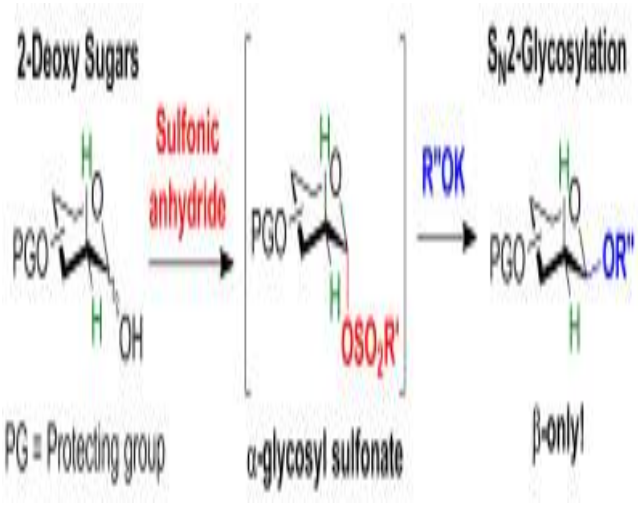
extended by one and not by two glucose molecules at a time. It also provides a theoretical model explaining the inversion of glucose units in each cellobiose-like pair of residues.

**CARB 70**

**Reagent-controlled $S_N2$-glycosylation for the direct synthesis of β-linked 2-deoxy-sugars**

***John P Issa**, john.issa@tufts.edu, Clay S Bennett. Department of Chemistry, Tufts University, Medford, Massachusetts 02155, United States*

Efficient and stereoselective glycosylation remains one of challenging endeavors in organic synthesis. This holds especially true in cases such as β-linked deoxy-sugars, where the outcome of the glycosylation cannot be controlled using the stereochemical information intrinsic to the glycosyl donor. We show that sulfonic anhydrides activate deoxy-sugar hemiacetals as electrophiles which react stereoselectively with nucleophilic acceptors to produce β-anomers exclusively.




NMR studies support this finding, indicating that the hemiacetal is quantitatively converted into an α-glycosyl tosylate, presumably the reactive species in the reaction. This approach demonstrates the viability of a more general reagent-controlled activation protocol which is centered around matching sulfonate reactivity with donor reactivity to effect highly stereoselective glycosylation through an $S_N2$-like reaction manifold.

**CARB 71**

## Synthesis of carbohydrate-drug conjugates: Potential NSAID pro-drugs

***Rachel A Jones****, rajones@ufl.edu. Department of Chemistry, University of Florida, Gainesville, Florida 32611, United States*

Peracetylated carbohydrates have been used previously as glycoconjugates to aid membrane permeability of small drug-like molecules. In addition, there has been much work carried out to modify and improve current drugs *via* conjugation to amino acids and peptides.

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of compound used for the management of pain, in particular as a treatment for osteo- and rheumatoid arthritis. The chronic use of NSAIDs is associated, however, with adverse side effects, many related to the gastrointestinal tract. There is also an increased risk of coronary heart disease and heart failure.

Glucosamine supplements are available to the public as an alternative treatment for the symptoms of osteoarthritis and there are no adverse effects associated with this supplement. We envisaged therefore, that glucosamine could be modified by reaction with an activated NSAID *via* an amino acid linker to develop a series of novel glucosamine-NSAID conjugates.

We describe herein synthetic studies to access glucosamine-NSAID conjugates and subsequent applications of this chemistry in the synthesis of carbohydrate-antibiotic conjugates.

**CARB 72**

## Installation of multiple 1,2-cis glycosidic bonds by automated synthesis

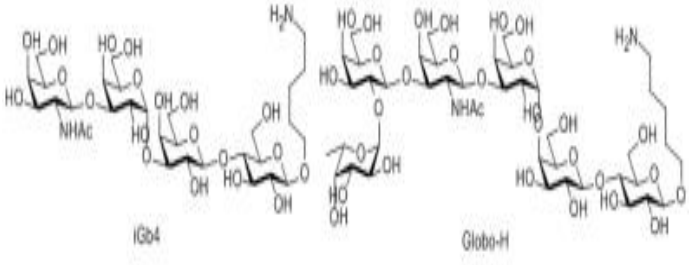
***Heung Sik Hahm***[1,2]*, heungsik.hahm@mpikg.mpg.de, Peter H. Seeberger*[1,2]*. (1) Department of Biomolecular Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany (2) Department of Biology, Chemistry, and Pharmacy, Free University, Berlin, Germany*

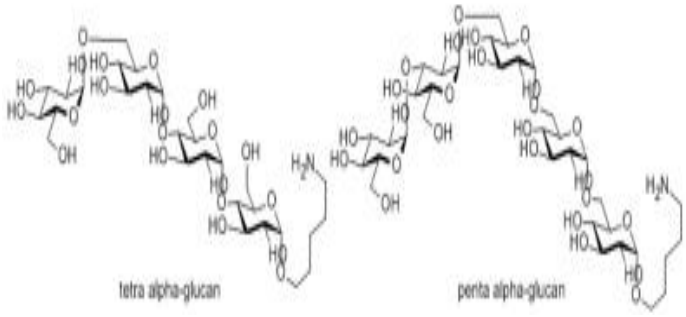
Most efforts toward the improvement of automated synthesis of oligosaccharides focused on how to perform iterative processes most efficiently in automated fashion. The synthesis of oligosaccharides with 1,2-*trans*- glycosidic linkages has been routinely carried out using C2 participating protecting groups. The generation of 1,2-*cis*-glycosidic linkages is still the greatest challenge for automated oligosaccharide synthesis.

Herein, we present efficient 1,2-*cis* glycosidic bond formations with various galactose and glucose building blocks that utilize remote participating group effects in automated

solid-phase synthesis. 4-*O*-Acetyl of galactose, and 6-*O*-acetyl of glucose are most effective to induce α-glycosidic bond formation. 3-*O*-acetyl works as a secondary effector. The optimized building blocks were employed in the synthesis α-Gal containing oligosaccharides such as pentasaccharide α-Gal epitope, *-iso*-Globo-series, and Globo-*series*. Furthermore, tetra, and penta α-glucans containing multiple 1,2-*cis* glycosidic linkages were synthesized using the automated synthesizer. These results encouraged us to synthesize glycan structures of high complexity containing α-glucosidic as well as α-galactosidic linkages.

The most immunogenic carbohydrate antigens out of seven frame-shifted epitopes from two *Streptococcus pneumonia* antigens were identified on a microarray. We built up the efficient fundament using automated synthetic system, and the optimized building blocks to map the most antigenic carbohydrate epitope *en route* to a synthetic carbohydrate vaccine.




**CARB 73**

**O-2 Chiral non-participating groups on mannose donors to test for oxacarbenium ions**

***Dennis M Whitfield****, dennis.whitfield@nrc-cnrc.gc.ca. National Research Council, Ottawa, Ontario K1A 0R6, Canada*

Previous studies (*J. Org. Chem*. **2012** , *77*, 3724) showed that the racemic 1-methyl 1'-cyclopropylmethyl, MCPM, protecting group at O-2 of deactivated glucopyranosyl

donors showed preferential formation of one stereoisomer in glycosylation reactions, especially for β-glycosides. Due to the previously determined *syn* bimodal conformational preference about C-2--O-2 in glucopyranosyl oxacarbenium ions (*Carbohydr. Res*. **2007** , *342*, 2793) this result was interpreted to indicate the presence of oxacarbenium ions in these particular glycosylation reactions. Since mannopyranosyl oxacarbenium ions exhibit the same *syn* bimodal conformational preference about C-2--O-2 it was decided to prepare 4,6-benzylidene protected mannopyranosyl donors with MCPM at O-2 and directly test if the formation of β-mannosides under preactivation glycosylation conditions exhibited any suggestions of oxacarbenium ion formation. Subsequently, similar donors were used to explore the feasability of synthesizing Man(β1,2)Man oligomers as oligosaccharide donors for immunological studies.

**CARB 74**

**Synthesis of disaccharides using glucosamine derivatives as an acceptor**

***Avynash J Bhagaloo****, avynashb@hotmail.com, Gurdial Singh. Chemistry, University of the West Indies, St. Augustine, Trinidad and Tobago*

The dengue virus is an important disease in the tropical and sub-tropical area of the world[1] and results in the mortality of 3 million human's pa annum[2]. To date efforts to obtain an effective vaccine against this disease state have been unfruitful.

Consequently we are engaged in the synthesis of analogue hexasaccharides (Fig. 1) of the naturally occurring epitope located at the ASN153 glycosylation site of the Dengue E glycoprotein[3] (Fig 2). To date we have been successful in the preparation of a mannose trisaccharide donor[4] (Fig 3). We have subsequently investigated use of this donor in glycosylation reactions[5]. Our current focus is on the synthesis of a glucosamine-fucose disaccharide which would serve as a precursor for the synthesis of the hexasaccharide using a convergent synthetic strategy. Herein we report on the synthesis of disaccharides using glucosamine derivatives as an acceptor.

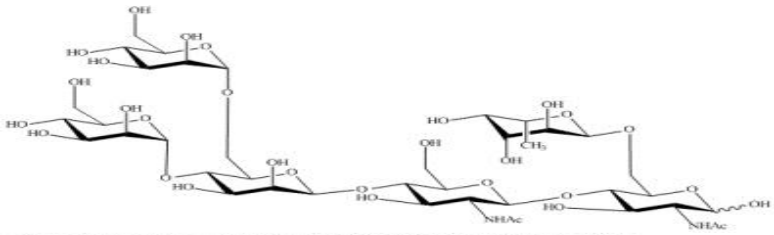


Fig. 1 - Analogue of hexasaccharide located at ASN-153 of the Dengue E glycoprotein

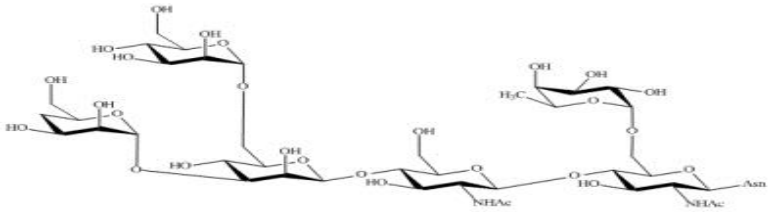


Fig. 2 - Hexasaccharide located at ASN-153 of the Dengue E glycoprotein

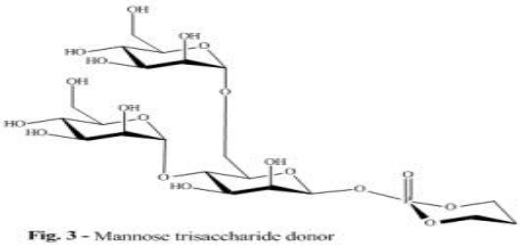


Fig. 3 - Mannose trisaccharide donor


1. Monath, T. P., *Proc. Nati. Acad. Sci. USA* **1994,** *91*, 2395-2400.

2. WHO Dengue and Severe Dengue. http://www.who.int/mediacentre/factsheets/fs117/en/ (accessed 22nd January 2013).

3. Guirakhoo, F.; Hunt, A. R.; Lewis, J. G.; Roehrig, J. T., *Virology* **1993,** *194*, 219-223.

4. Jalsa, N. K.; Singh, G., *Tetrahedron: Asymmetry* **2009,** *20*(6-8), 867-874.

5. Bhagaloo, A. J.; Singh, G. In *Division of Carbohydrate Chemistry*, 246th American Chemical Society National Meeting and Exposition, Indianapolis, IN, September 8-12, 2013; Indianapolis, IN, 2013; pp TECH-71.


**CARB 75**

## Computational mapping approaches for understanding glycosaminoglycan-protein recognition


***Mark Agostino***[1,2,3], *Mark.Agostino@curtin.edu.au, Neha Gandhi*[1], *Ricardo Mancera*[1]. *(1) School of Biomedical Sciences, Curtin University, Perth, WA 6845, Australia (2) Life Sciences Department, Barcelona Supercomputing Centre, Barcelona,*

*Barcelona 08034, Spain (3) Centre for Immunology, Burnet Institute, Melbourne, VIC 3004, Australia*

Carbohydrate-protein interactions mediate a wide range of biological processes, but are challenging to study at the molecular structural level. The ability of experimental methods to obtain high quality structural information is affected by the high flexibility of carbohydrates, as well as the potential for carbohydrates to exhibit multiple binding modes with their targets. While computational methods are a valuable alternative to experimental methods to obtain structural knowledge, they are affected by the same issues. Additionally, methods such as automated molecular docking, the key computational technique for identifying structures of ligand binding to proteins, are affected by the ability of carbohydrates to form many interactions with their protein targets; often, incorrect solutions can appear to give a better fit than the correct solutions.

To address the challenges faced in the structural determination of carbohydrate-protein complexes, computational mapping approaches have been developed. These methods utilize the interactions between an ensemble of carbohydrate binding modes obtained from docking and the target protein and have proven useful in characterizing a variety of carbohydrate-protein recognition scenarios.[1,2]These methods have now been extended to study glycosaminoglycan-protein (GAG) recognition, an area of emerging importance for the development of anticancer and antiflammatory agents. The site mapping method is demonstrated to provide improved performance for predicting the key protein residues involved in GAG recognition over the top ranked pose from molecular docking. Furthermore, incorporating both site and epitope mapping techniques facilitated virtual screening of GAG disaccharides against acidic fibroblast growth factor, specifically identifying both the known binding ligands and their correct binding modes. This study suggests the potential broad utility of the mapping techniques in virtual glycan screening.

[1]Agostino et al., *Glycobiology*, **2010** , 20, 724-735

[2]Agostino et al., *J Mol Graph Model*, **2013** , 40, 80-90.

**CARB 76**

**Regioselective synthesis of aminated cellulose derivatives for drug delivery**

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Cationic polysaccharides have been a major area of focus due to the potential applications in drug and gene delivery especially through encapsulation of nucleic acids and certain proteins through electrostatic interactions. They have also been sought as paracellular permeability enhancers (PPEs) in an effort to increase permeation across

the epithelial membrane especially for hydrophilic macromolecules of therapeutic significance. These PPEs temporarily alter the integrity of tight junctions and allow for more effective transport - a characteristic that has been identified in the amine containing polysaccharide chitosan. Chitosan has been a template for the behavior of cationic polysaccharides and is most heavily targeted however its limited solubility at neutral pH and inability to target specific areas of the intestine like the ileum or jejunum greatly limit its efficiency in comparison to the best synthetic vehicles available. These drawbacks and others have prompted efforts to functionalize other polysaccharides including cellulose through the addition of permanent positive charges along the polymer backbone. This study explores the synthetic conditions needed to produce ammonium cellulose derivatives regioselectively at C-6 with high degree of substitution (DS) as well as use analytical techniques to discern the potential for these derivatives in oral drug delivery.

**CARB 77**

**Multiple roles of N-linked glycosylation in the voltage-gated potassium channel Kv1.2**

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Potassium channels serve a variety of important physiological functions. In the nervous system, voltage-gated potassium (Kv) channels are important to control action potential waveform. Several Kv channels are glycosylated, though the function of native glycosylation has not been determined fully. We are studying the role of glycosylation in Kv1.2 channels using primary hippocampal neuronal cultures. The glycan structures of Kv1.2 channels were analyzed by glycosidase digestion, confirming differential glycosylation in neuronal cultures compared to more commonly used cell culture lines. The importance of glycosylation for cell surface expression of Kv1.2 was also analyzed, indicating a significance of complex-type glycosylation for proper localization and function in neurons.

**CARB 78**

**Synthesis of trycyclic fused azepinone core of divergolide C/hygrcocin B and the assembly of carbohydrate derived ansachain via Fries rearrangement**

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Although a plethora of natural lactams have been reported in the literature to date, only a few lactams with enamide type of unsaturation are known and divergolide C and hygrocin B are important biologically potent molecules that contain such fused azepinone tryclic core. However, there is no unified methodology for synthesis of such

lactams. Enroute to the total syntheis of divergoldie C, and hygrocins B, we have developed novel methodology for synthesis of the fused azepinones via RCM methdology and the ansa chains derived from D-Glucose and D-arabinose (to settle the sterochemistry of the c8- centers) have been appended via Fries rearrangemen. The details will be presented.

**CARB 79**

**Glycopolymer platform for presentation of tumor associated carbohydrate antigens**

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Tumor associated carbohydrate antigens (TACAs) are overexpressed on tumor cells, which renders them attractive targets for anti-cancer vaccines. To overcome the poor immunogenecity of TACAs, we designed and synthesized a polymer platform via controlled polymerization for antigen presentation by taking advantage of the polymeric backbone to incorporate different immunoactive components on the same chain in order to elicit robust T cell dependent immune response. Immunology studies were carried out to evaluate the efficacy of the glycopolymer as a potential cancer vaccine.

**CARB 80**

**Synthetic glycopeptides mimicking both T. cruzi and tumor mucins show potential for vaccine development**

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Mucins are highly *O*-glycosylated glycoproteins that are rich in serine and threonine repeating units, whose external oligosaccharides are linked to the protein via sugar units of α-*N*-acetyl-glucosamine (GlcNAc), in *T*. *cruzi*, or α-*N*-acetylgalactosamine (GalNAc), in mammals. In *T. cruzi*, these structures helps the parasite to interact with the infected host, while in vertebrates the functions of mucins range from being protective barriers to providing lubrication for epithelial cells. In tumor-related mucins, however, altered glycosylation is a common feature, being verified the presence of mono- and disaccharide structures known as $T_N$ and TF antigens,respectively. Therefore, this study describes the synthesis of the glycopeptides NHAc[βGal]-$(Thr)_2$-[αGalNAc]-$(Thr)_2$-[αGlcNAc]-$(Thr)_2$Gly-OVA **1** and NHAc[βGal-αGalNAc]-$(Thr)_3$-[αLacNAc]-$(Thr)_3$-Gly-OVA **2** as mimetics of both *T. cruzi* and tumor mucins glycoproteins. These glycopeptides were obtained by solid phase synthesis, which involved the previous preparation of the protected glycosyl amino acids αGlcNAc-ThrOH **3** , αGalNAc-ThrOH **4** ,βGal-ThrOH **5** ,αLacNAc-ThrOH **6** and βGal-αGalNAc-ThrOH **7**

by glycosylation reactions. Mice immunizations with glycopeptides **1** and **2** -OVA induced high antibodies titers (1/16000), as verified by ELISA tests, whereas flow cytometry assays showed the capacity of the obtained anti-glycopeptides **1** and **2** antibodies to recognize both *T. cruzi* and MCF-7 tumor cells. In addition, glycopeptides **1** and **2** antisera were also able to inhibit *T. cruzi* fibroblasts cells invasion (70%) and to induce an antibody-mediated cellular cytotoxicity (ADCC) against MCF-7 cells, with 50% reduction of cell viability. Taken together, these data point out glycopeptides **1** and **2** as potential dual synthetic vaccines against *T. cruzi* and breast tumor.

**CARB 81**

## Aggregates of multifunctional saccharide containing small molecules stimulate cell proliferation: Target identification and mechanism exploration

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It is going to report the progress on the identification of the molecular targets, with which the aggregates of the saccharide containing small molecules (i.e. the conjugates of glycoside, amino acid, and necleobase) interact to promote cell proliferation. Exhibiting important biological functions, the aggregates of small molecules formed by self-assembly, are able to serve as functional molecular entities in cellular environment. While all the living cells are covered by complex array of glycans in forms of glycoproteins, proteoglycans, etc., carbohydrates are the most diverse and one of the most important classes of biomolecules in nature, which makes it an attractive candidate as bio-mimicking motif. However, the aggregates of saccharide containing small molecules have received little attention for years. We recently developed a simple way for generating saccharide containing small molecules, which can self-assembly in water to give aggregates in the form of nanofibers or nanoparticles. Unexpectedly, this kind of aggregates was able to act as a growth factor to promote cell proliferation. Further study suggested indispensable function of the saccharide, as removing it from the hybrid put the aggregates out of action. Protein binding and profiling assays were conducted in order to identify the unknown molecular targets, and our study showed that the proliferation-promoting effect may result from its interaction with pyruvate kinase isozyme 2(PKM2).

**CARB 82**

## Conjugation of carbohydrates to biological probes utilizing chemoselective bi-functional linkers

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Glycans form the carbohydrate component of glycoproteins and glycolipids, which are involved in numerous biological processes and as such, understanding the role of these glycoconjugates in both health and disease could resolve in better drug development. Determination of the biological activity of carbohydrates requires the chemical conjugation of carbohydrates to probes, such as fluorophores, dendrimers, and proteins. However, competing reactivity of, for example, hydroxyl functionalities can complicate the stereo- and chemoselective conjugation and thus the judicious use of chemoselective moieties is required. In the past decade, alkoxyamines have been developed for chemoselective ligation of natural carbohydrates to a variety of molecules including other carbohydrates, toxins and microarray slides. In this study we have synthesized a variety of bi-functional alkoxyamines suitable for chemical conjugation to carbohydrates as well as other biological probes such as biotin, dendrons and peptides using chemoselective ligation techniques such as peptide-coupling, click-chemistry and thiol-maleimide ligation.

Key in the construction of these glycoconjugates is the synthesis of novel bi-functional linkers and their ligation to natural carbohydrates. Use of a second chemoselective reactive group, allows us to selectively conjugate the carbohydrate to other biological probes. Using this synthetic approach we can synthesize a wide variety of glycoconjugates for biological applications in order to determine the role of carbohydrates in both health and disease.

**CARB 83**

**Maltodextrins image bacterial infections and drug resistance by positron emission tomography**

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Bacterial infections are a central cause of mortality in the world and affect all areas of medicine ranging from cardiology to oncology. Bacterial infections remain a major health problem despite the availability of effective antibiotics, because their diagnosis is challenging and because they are frequently treated with ineffective antibiotics, due to the widespread rise of bacterial drug resistance. In this report, we present a new PET contrast agent, composed of F-18 conjugated to maltodextrins ($MD^{18}F$), which can for the first time image bacteria in vivo with the specificity and sensitivity needed to detect early stage infections and measure drug resistance in vivo in real time. We show here that $MD^{18}F$ can detect as few as $10^5$ *E.coli* colony forming units (CFUs) in rats, which is 3-4 orders magnitude higher in sensitivity than FDG, the current clinically used bacterial infection contrast agent. In addition, we demonstrate that $MD^{18}F$ can distinguish bacterial infections from inflammation, and has specificity for bacterial infections, giving it the potential to identify infections clinically without a biopsy. We also demonstrate that $MD^{18}F$ can monitor treatment efficacy in vivo and can identify beta lactam resistance in *E.coli* in real time, thus providing physicians with a powerful tool for guiding antibiotic selection. Finally, we demonstrate that $MD^{18}F$ can image implant infections in rats. We

anticipate numerous clinical applications of MD$^{18}$F given the widespread use of PET and the pervasiveness of infections in medicine.

**CARB 84**

**Design, synthesis, and characterizations of dendritic glycoclusters**

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Glycolipids which are able to form self-assembled supramolecular structures can result in useful soft biomaterials. Our group has been working on the synthesis and characterizations of various monosaccharide derivatives, and discovered several novel classes of sugar based low molecular weight gelators (LMWGs). These compounds form unique classes of soft materials such as organogels or hydrogels that may be useful in biomedical research and as advanced functional materials. Since the supramolecular gel networks are held together solely by weak intermolecular forces, they typically are not as stable as polymer gels. Covalently attaching amphiphilic glycolipids to form branched derivatives may enhance the stability of these assemblies. Several branched molecules with sugar moiety at the periphery have been designed and synthesized; these compounds have accurate molecular weight and can form interesting molecular assemblies. In this presentation, our recent progress in the design, synthesis and study of self-assembling properties of various glycoclusters will be discussed.

**CARB 85**

**Ligand controlled C-H carboxylation with $CO_2$ catalyzed by (NHC)Cu/Au complexes**

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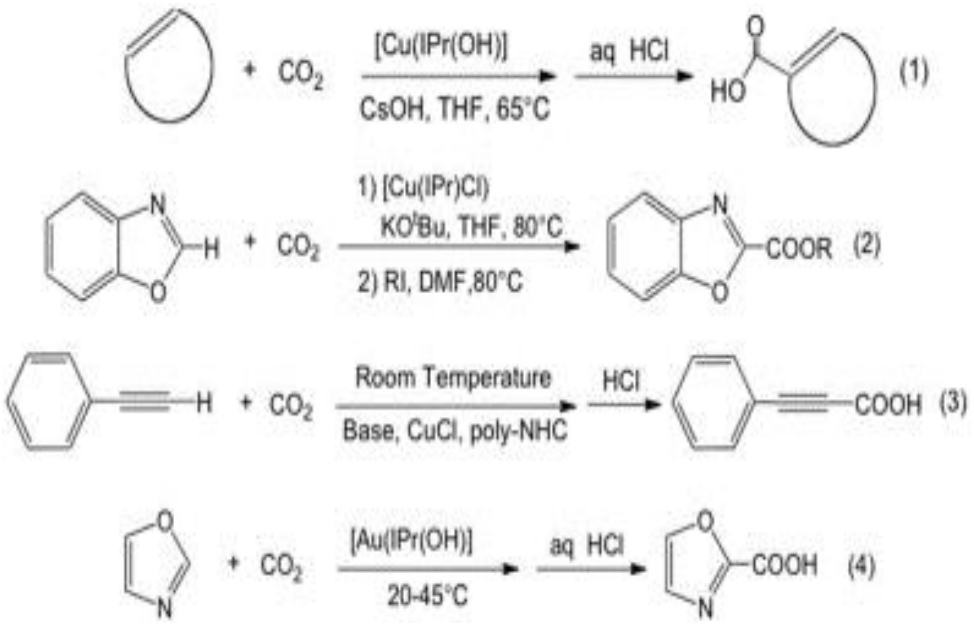
Carboxylic acids are useful compounds in medicinal and organic synthesis. To achieve carboxylic acids from static $CO_2$ by direct C-H bond activation and carboxylation is attractive because carbon dioxide is a renewable and environmentally friendly source of carbon. However, high-cost, low catalytic performances, harsh reaction conditions, and limited substrate scope are remained as obstacles in carboxylation with $CO_2$. It is important to develop new reactions and new protocols for $CO_2$ transformations at mild conditions and in low-cost ways. Herein, a copper-catalyzed transformation of $CO_2$ to carboxylic acids via C(ethynyl)-H bond activation of terminal alkynes with or without base additives is reported in PNAS recently (eq. 1, 2,)[1,2]. Various propiolic acids were synthesized in good to excellent yields under ambient temperature (eq. 3)[3]. It makes progress on the basis of gold catalyzed C-H bond carboxylation with $CO_2$[4,5](eq. 4).




We are interested in the mechanism of the copper and gold catalyzed C(sp, $sp^2$, $sp^3$)-H carboxylation from $CO_2$ by theoretical methods and to understand the selectivity of the reactions. The effect of Poly-NHC as a new ligand will also be examined. The study may lead to the examination of $FeCl_3$catalyzed carboxylation and further to enzymatic carboxylation of C-H bond in small biological molecule.

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**CARB 86**

**Electrochemical properties of chitosan/carbon nanotube/graphene composites used for detections of food additives**

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Composite films of chitosan (CS), carbon nanotube (CNT), graphene oxide (GO), and reduced graphene oxide (rGO) that were cast on glassy carbon electrode (GCE) were investigated on their electrochemical properties and detections of four food additives (sodium nitrite, sodium sulfite, hydroquinone, and catechol) by cyclic voltammetry. As compared to the rGO/CNT/CS/GCE, the GO/CNT/CS/GCE, although exhibiting relatively low current intensity, exhibited selective detection ability for the four food additives. Using sulfonated chitosan (sCS) to substitute for CS, the GO/CNT/sCS/GCE exhibited much enhanced electrocatalytic properties for the four food additives. The linear detection ranges were 1.25-631 μM for sodium nitrite, hydroquinone and catechol, and 2.5-1262 μM for sodium sulfite. The detection sensitivities were 0.0408 ($NaNO_2$), 0.0095 ($Na_2SO_3$), 0.0443 (HQ), and 0.0522 (CC) μA $cm^{-2}$ $\mu M^{-1}$. The detection limits were 0.06 μM ($NaNO_2$), 0.082 μM ($Na_2SO_3$), 0.026 μM (HQ), and 0.026 μM (CC).

**CARB 87**

## Relationship of fluorescence and distribution of CMC/Eu nanocomposites in different reaction time with microwave assistance

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A benign method was used to synthesize CMC/Eu nanocomposites in this study. CMC/Eu nanocomposites were synthesized successfully by $Eu^{3+}$ and CMC (70℃, pH7.0) under microwave treatment with different time. The SEM images showed that CMC/Eu composites have good distribution, and the practical size was 100nm~150nm in nano-scale. The FTIR results indicated the reaction between carboxyl groups in CMC and $Eu^{3+}$. TEM measurement show that the CMC/Eu nanoparticles were polymorphism. The UV-vis spectrum showed absorption band of these nanocomposites were at 210nm in short reaction time) and at 250nm in long reaction time, respectively. The effects of reaction times on the fluorescence spectra of CMC/Eu nanocomposites were complicated (see Figure), but the fluorescence quantum yield (FQY) of the nanocomposites synthesized in 90s was the strongest.

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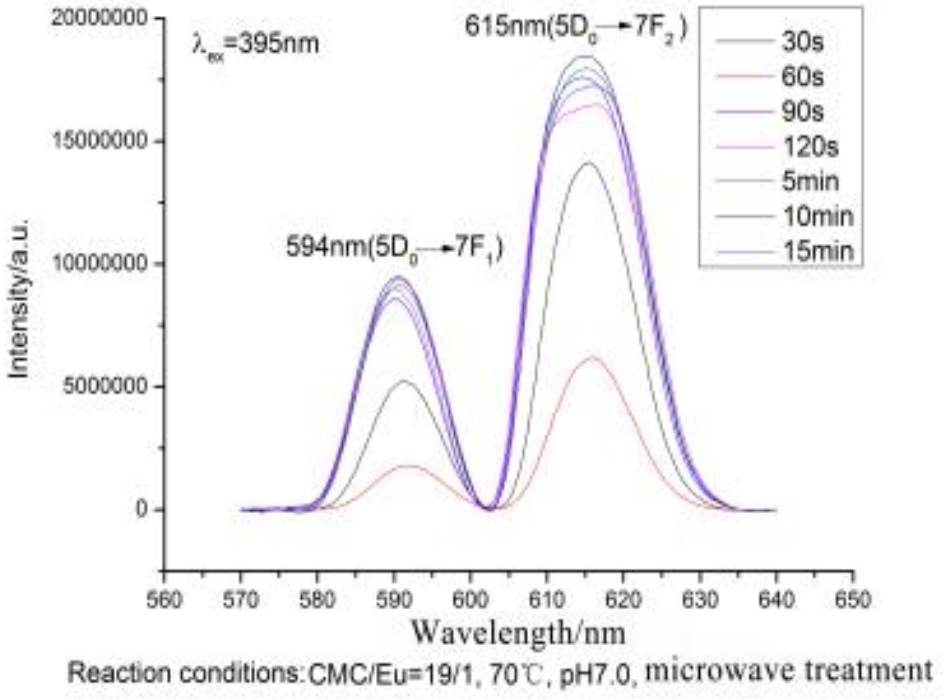


Figure the fluorescence spectra of CMC/Eu nanocomposities synthesized in different time

**CARB 88**

**Galactose-functionalized diblock copolymer nano-objects via polymerization-induced self-assembly**

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A range of novel galactosylated diblock copolymer nano-objects is prepared directly in concentrated aqueous solution via reversible addition−fragmentation chain transfer (RAFT) polymerization using polymerization-induced self-assembly (PISA). First, a galactose-based methacrylic monomer is synthesized without recourse to protecting group chemistry, followed by its RAFT solution polymerization to produce a macro-CTA. The degree of surface functionality of the nano-objects can be tuned by utilizing binary mixtures of this sugar-based macro-CTA combined with a poly(glycerol monomethacrylate)-based macro-CTA. This modular approach also enables the final copolymer morphology to be systematically varied from spheres to worm-like micelles or vesicles and a detailed phase diagram has been constructed. These nano-objects interact *in vitro* with galectins, as judged by a turbidity assay. Interestingly, his assay is

much more sensitive for vesicles than for worms or spheres. The galactosylated vesicles are biocompatible and enable efficient intracellular delivery of an encapsulated molecular cargo.

**CARB 89**

**Targeting cancerous cell lines with glycopolymer micelles**

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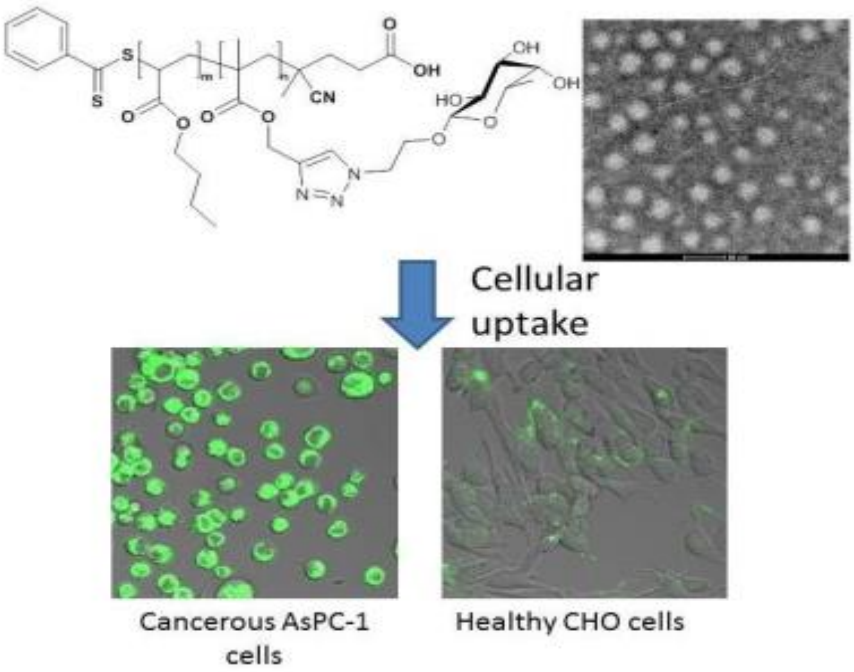
Carbohydrates play a pivotal role in many biological processes. Examples of nature's very successful nanoparticles decorated with glycoproteins are viruses. Viruses are extremely successful in invading their host and escaping detection by the body's defense mechanism. Viruses have developed unique strategies to evade the host immune system and enter a target cell. Viruses come in different, but their most striking features are the well-designed surfaces that facilitate recognition and entry into the host. The powerful nature of viruses and its complex interplay with the host have inspired scientists to use empty viruses as drug delivery carriers. The potential immunogenicity of these natural drug carriers however is a major set-back of these otherwise highly intelligent drug delivery systems. It is therefore tempting to aim at generating synthetic viruses, which have similar properties without the disadvantages. Self-assembled block copolymer can simulate some of the properties of viruses. Polymeric micelles have sizes typically below 100 nm while cylindrical micelles can take on the shape of the famous worm-like Ebola virus that has rather high aspect ratios.

Here, we prepared micelles based on a library of sugars such as mannose, glucose, fructose and fucose. RAFT polymerization was employed to generate various block copolymer. The glycopolymer structures were prepared either directly by the use of glycomonomers or they were clicked onto a reactive backbone, which offers the advantage to create mixtures of sugars onto one block copolymer. These micelles were then tested towards their ability to enter various cell lines. Drugs such as platinum drugs and oligonucleotides were added to evaluate if there is a direct correlation between selective cellular uptake and toxicity of the carrier.




**CARB 90**

**Carbohydrate vector systems as potent biomaterials for gene therapy to the liver**

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Hepatocellular carcinoma (HCC) is responsible for more than 80% of liver cancers and is the fourth most common cause of cancer-related deaths. Generally, hepatocellular carcinoma cannot be easily removed surgically (unresectable) and cannot be treated with other therapies such as ablative (e.g. radio-frequency ablation) or trans-arterial chemo-embolization (TACE). Among other treatments, gene therapy which relies on the delivery of genetic materials into cells, offers significant advantage for liver cancer treatment. The major challenges that must be overcome for effective gene therapy of liver cancer include designing and fabrication efficient delivery vectors and the selection of therapeutic genes. With improvement in gene transfer systems and with a better knowledge of the cellular pathways involved in the development and progression of HCC, it should be possible to develop safe strategies that target HCC selectively with little or no toxicity to normal hepatocytes. Therefore, in the last two years, our goal was primarily focused towards the development of a safe, non-toxic gene delivery system without compromising the gene expression. We have carefully studied these glycopolymers and glyconanogels structures (compositions, charges, sizes) to achieve the best gene carrier for Hep G2 transfection. We have so far focused on the structure-property relationship in view of developing materials of *low toxicity* and *high efficiency in gene expression*. By carefully engineering these materials, we have been able to achieve both requirements and those carbohydrate vector systems proved to be quite

remarkable for gene transfer. Besides DNA, we have recently shown that these carbohydrate vectors (glycopolymers and nanogels) can efficiently protect siRNA and efficient knockdown of receptors can be achieved.

**CARB 91**

**Nanoporous gold: Applications in glycan recognition and synthesis**

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Nanoporous gold (NPG) will be discussed as a useful nanomaterial for applications in glycotechnology in a range of fields including assays of glycan recognition, capture and separation of lectins or glycoproteins, and the supported synthesis of glycan structures. NPG is a versatile material formed by direct chemical or electrochemical dealloying of alloys of gold and less noble metals. The material consists of a bicontinuous structure of pores and ligaments typically in the tens of nanometers size range, but readily tunable depending on the preparation conditions or upon subsequent thermal or chemical treatments. It can be prepared in a range of formats, such as thin films, or as macroscopic monoliths. Our labs have shown that NPG can be applied as a support for the synthesis of glycans under either static or flow conditions and that this synthesis can be partially automated using HPLC. NPG monoliths have also been found useful as a support for glycan-modified self-assembled monolayers (SAMs) for the selective capture and elution of lectins, or for the conjugation of lectins to SAMs on its surface for the selective capture and subsequent elution of glycoproteins. NPG electrodes can be used as supports for glycan presenting SAMs and electrochemical methods including square-wave voltammetry used to develop glycoprotein assays, either in kinetic or competitive formats. Thermogravimetric analysis is demonstrated to be highly useful for assessing glycan immobilization and lectin binding within NPG monoliths, and atomic force microscopy useful for imaging lectin binding on exterior or interior surfaces of NPG.

**CARB 92**

**Glycosylated nanobio sensors and a new "chemical tongue" approach to lectin sensing**

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Carbohydrate-protein interactions are inherently weak but also, in the case of monosacharides, very non-specific with most lectins being capable of binding a vast of carbohydrates. In Nature the precise 3-D presentation of branched glycans gives rise to specificity, which allows viral or bacterial docking to host cells. The reproduction of these specific multivalent arrays offers huge promise as the development of new anti-adhesives and pathogen sensors. However the synthesis of native glycans is incredibly challenging and more accessible methods to reproduce their recognition functions are required.

Here we will present recent findings in the design of glycosylated nanoparticles and surfaces designed to detect pathogens and their toxins. In the first approach, gold nanoparticle based sensors that specifically interact with bacteria surfaces to give colorimetric outputs for point of care applications will be described. Secondly, a new 'Chemical Tongue' approach to biosensing will be introduced. Rather than looking for a single carbohydrate-protein binding event, we establish small arrays where the relative binding of lectins to different sugars is evaluated, in a manner similar to the function of taste buds. In combination with a training algorithm, it is possible to distinguish between different lectins including pathogenic agents such as Cholera and Ricin.

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**CARB 93**

**Interaction between boroxole- and glyco-based polymers for drug delivery system**

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It is well known that the architectures (linear, branched, cyclic, and network) of polymeric materials dictate the physicochemical properties even though they have the same chemical compositions. Over the last decade, polymeric materials with excellent control over molecular weight, composition, structure, functionality, and conjugation have been made simpler with the development of unique synthetic such as controlled/living radical polymerizations (CLRPs) and click chemistry. Nanogel that is one of good drug carriers is composed of cross-linked structures by covalent bond, hydrophobic interaction, electrostatic interaction, and coordinate bond, which has been applied in biomaterial field for its high stability, high drug loading, and versatile incorporated materials such as molecules, peptides, proteins, nucleic acids, and inorganic particles. We found that a mixture of boroxole- and glyco-based polymers leads to form gel-construction (bulk and nanoparticles) due to the reversible boroxole-diol interaction. The boroxole group has received significant attention for its wide range of applications in catalysis for stereo-controlled synthesis, diagnosis, and medical treatment for human immunodeficiency virus (HIV), obesity, diabetes, and cancer. On the other hand, the glyco-based polymers of poly(3-gluconamidopropyl methacrylamide) (PGAPMA) and poly(2-lactobionamidoethyl methacrylamide) (PLAEMA) have been polymerized at different structures such as linear, hyper-branched, and conjugated nanoparticles for drug carries. Moreover, these glyco-based polymers were found to recognize a specific cell. In this study, we exploited a formation of gel by simple mixing of well-defined boroxole- and glyco-based polymers that were prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization. The mixed gel and its nanoparticles are expected to apply in carriers for both diagnosis and therapy.

**CARB 94**

## Glycosylated nanomaterials: Detection and neutralization of pathogenic bacteria and toxins

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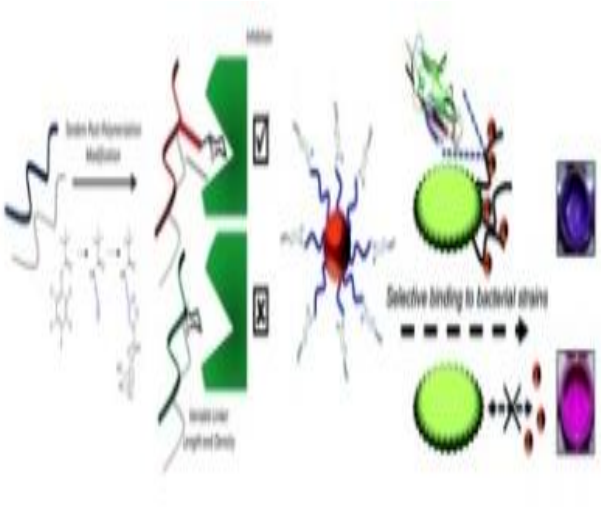
Adhesion to carbohydrates presented on epithelial cell surfaces is often the first step in bacterial infection. The low efficacy of monovalent inhibitors can be circumvented, as in nature, by the presentation of multiple copies of the carbohydrate ligand. A great deal of attention therefore has been directed towards the synthesis of glycopolymers.[1,2]

Although multivalent presentation of carbohydrates on a polymeric scaffold can lead to inhibitors with high affinity for the target lectin or bacterial toxin, they still have low specificity; most sugars will bind many different lectins.[1,2]To overcome this, it is important to exploit structural biology information and tools to explore the binding pocket of different lectins, to guide the synthesis of new glycopolymers/particles.

Here we present two approaches to combatting bacterial infection. 1) Exploitation of a new tandem post-polymerization modification strategy to introduce lectin specificity *via* macromolecular engineering of the carbohydrate density, and glycopolymer chain length.[1] 2) Incorporation of multivalent carbohydrates onto goldnanoparticles to make new colourimetric sensors which can discriminate between pathogenic and non-pathogenic bacteria for point-of-care diagnostic applications.[3]

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**CARB 95**

**Advanced functional magnetic glyconanoparticles for the in vivo treatment and detection of diseases**


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Targeted magnetic nanoparticles have found plethora of applications in various biomedical fields. We exploited the selective interaction between the CD44 receptor and hyaluronan to design advanced functional and targeted magnetic nanoparticles for the *in vivo* treatment and/or detection of diseases characterized by the overexpression of CD44 such as ovarian cancer and atherosclerosis. We relied on Magnetic Resonance Imaging (MRI) and histological examination to confirm the effective accumulation of Doxorubicin-loaded nanoparticles in subcutaneous ovarian tumors. In addition, *in vivo* bioluminescence imaging was used to aid in therapy assessment of the drug-loaded nanoparticles on intraperitoneal ovarian tumors. Appreciating that CD44 plays important roles in the initiation and development of atherosclerotic plaques, hyaluronan coated magnetic nanoparticles were evaluated for its ability to selectively detect and image plaques by MRI in an atherosclerotic rabbit model. The low dose of nanoparticles required to achieve selective and sensitive detection rendered the developed glyconanoparticles advantageous as efficient contrast agents for plaque imaging.

**CARB 96**

## Radiosynthesis of 2-deoxy-2-[18F]-fluororibose, a new positron emission tomography probe

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The development of synthetic methodology for a short radiosynthesis of 2-deoxy-2-[18F]fluororibose (2-FDR) is presented. Radiolabeled analogs of sugars have wide applications as Positron Emission Tomography (PET) tracers for imaging of cell proliferation and heart and brain functions. Several synthetic challenges in the radiosynthesis of 2-FDR arose; for example, the protecting group on the C-3 hydroxyl, trans to a leaving group at C-2, hinders that position from the SN2 attack by fluoride. Also the stabilization of carbocations at C-2 via anchimeric assistance by the group at C-3 caused various skeletal rearrangements and non-stereoselective fluorination. In

order to overcome these hurdles, we installed the completely non-participating *p*-nitrobenzyl (PNBn) protecting group on the hydroxyl group at C-3. Stereoselective fluorination at C-2 under standard fluorination conditions preceded in high yield within 60 min. Reductive deprotection-hydrolysis of the PNBn groups was complete in 30 min. All manipulations were finished in less than two hours. Radiolabeled probe was utilized for the quantitative determination of need for liver transplant due to its functional failure. The details of this investigation will be described at the meeting.

**CARB 97**

**Labeling lipopolysaccharides of living bacteria**

*Jordi Mas Pons[1], Audrey Dumont[3], Annie Malleron[2], Aurélie Baron[1], Grégory Sautejeau[1], Monzer Awwad[2], Antoine Brossay[1], Emilie Fugier[3], Enora Poquet[1], Sam Dukan[3], **Boris Vauzeilles**[1,2], boris.vauzeilles@cnrs.fr. (1) Department of Chemical Biology, Institut de Chimie des Substances Naturelles - CNRS-ICSN, Gif-sur-Yvette, France (2) Laboratoire de Synthèse des Biomolécules, Institut de Chimie Moléculaire et des Matériaux d'Orsay - CNRS and Univ Paris-Sud, Orsay, France (3) Laboratoire de Chimie Bactérienne, Institut de Microbiologie de la Méditerranée, Marseille, France*

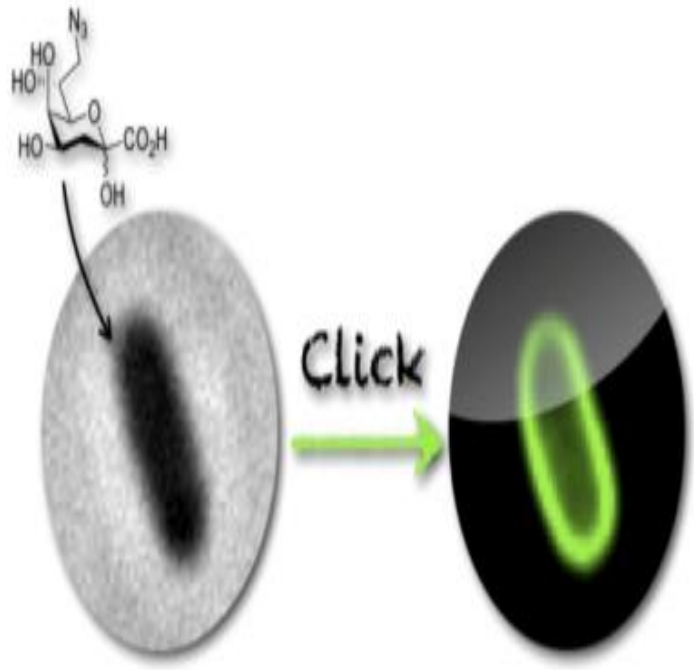
Sudden outbreaks of new epidemics regularly warn us against the severe sanitary and economical impact resistant bacterial infections could have on our industrialized and globalized societies. The rapid identification of viable bacteria is therefore a fundamental challenge.

The outer membrane of Gram-negative bacteria is covered by a dense layer of Lipopolysaccharides (LPS), which are considered to participate into cell integrity, as well as to the level of pathogenicity of a given strain.

We have recently shown that, when they are metabolically active, Gram-negative bacteria can specifically incorporate into their LPS a monosaccharide, which has been modified by the introduction of an *azido* anchor.[1]

This bioorthogonal chemical reporter can then be further exploited in the *click-chemistry* mediated labeling of these bacteria. This overall procedure offers an efficient and rapid strategy to identify living, or metabolically active, bacteria.

This communication will present our latest results in this field, including species-specific labeling of a serious pathogen, *Legionella pneumophila*.[2]

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[2] J. Mas Pons, A. Dumont, G. Sautejeau, E. Fugier, A. Baron, S. Dukan, B. Vauzeilles, Angew. Chem. Int. Ed., 2014, 53, 1275 –1278.

**CARB 98**

**Chemical reporters for mycobacterial cell-wall glycoconjugates**

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Mycobacteria, including the global pathogen *Mycobacterium tuberculosis*, possess a complex cell wall that is characterized by a unique outer membrane—the mycomembrane—which is composed of an array of distinct lipids and glycolipids. Despite the importance of the mycomembrane to mycobacterial physiology and pathogenesis, robust tools for investigating its structure, dynamics, and contributions to disease progression are lacking. One of the major areas of focus in our laboratory is to develop methods for chemically remodeling the mycomembrane that enable delivery of functional cargoes for potential applications in imaging, proteomics, and immunotherapy. Here, the chemoenzymatic synthesis and application of trehalose analogues for metabolic labeling of mycomembrane glycolipids is discussed.

**CARB 99**

## Synthesis and evaluation of glycoside-based inhibitors of Mycobacterium tuberculosis GlgE

*Sandeep Thanna, Sri K. Veleti, Jared J Lindenburger, Donald R Ronning,* ***Steven J Sucheck****, steve.sucheck@utoledo.edu. Chemistry, University of Toledo, Toledo, Ohio 43606, United States*

*M. tuberculosis* (Mtb) GlgE is a (1→4)-α-D-glucan:phosphate-α-D-maltosyltransferase involved in α-glucan biosynthesis and is a genetically validated anti-tuberculosis drug target. Inhibition of GlgE has been suggested to result in a lethal buildup of maltose-1-phosphate (M1P), the substrate the enzyme uses to make alpha-glucan. GlgE exhibits an α-retaining catalytic mechanism during glucan polymn. The X-ray structure of Mtb GlgE was determined as a complex with maltose. In order to identify lead compounds for inhibitor development we synthesized the substrate- and mechanism- based inhibitors, maltosyl-C1-phosphonate and 2,2-dideoxy-2,2-difluoro-α-maltosyl fluoride, respectively. Both target compounds inhibit GlgE in the micromolar range and have been successfully co-crystalized with GlgE. The X-ray structures of the complexes will be used to inform future inhibitor development.

**CARB 100**

## Synthesis of cell-surface carbohydrates for probing infectious diseases

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

**CARB 101**

## Molecular dynamics study on the interaction of O-antigen polysaccharides of the gram-negative bacterium *Shigella flexneri* with the tail-spike-protein of bacteriophage Sf6

***Yu Kang***[1]*, Yu.Kang@mpikg.mpg.de, Stefanie Barbirz*[2]*, Ulrich Gohlke*[3]*,* ***Mark Santer***[1]*, mark.santer@mpikg.mpg.de. (1) Department of Theory and Bio Systems, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany (2) Department of Physical Biochemistry, Universität Potsdam, Potsdam, Germany (3) Department of Macromolecular Structures and Interactions, Max-Delbrück-Center for Molecular Medicine, Berlin, Germany*

We present a computational study on the conformational characteristics of Serotype Y O-Antigen polysaccharide fragments of the Gram-negative bacterium *Shigella flexneri* in solution [1] and bound to the tailspike protein (TSP) of bacteriophage Sf6. The molecular models of the protein-carbohydrate complexes are in good agreement with X-ray structures of TSP co-crystallized with tetrasaccharide fragments on the wild type [2], and octasaccharides with inactive mutants. The bound octasacharide conformer resembles the dominant one in solution. In addition, an investigation has been carried out on how longer O-Antigen fragments could be accommodated at the binding site such as to correctly place the Rha*p* α(1-3) linkage at the active carboxy-residues prior to cleavage. We find that significant excursions from the low-energy equilibrium conformations must occur in order to avoid steric clashes or inconvenient placement, indicating that conformational selection should play a significant role in the interaction of O-Antigen with TSP.

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**CARB 102**

## Glycopeptide conjugates of tumor-associated carbohydrate antigens as anticancer agents

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Tumor-associated carbohydrates were discovered more than 30 years ago and have been used in a variety of studies as haptens for anticancer vaccine design. In addition, some of these small saccharides have been directly implicated in the metastatic process of different solid tumors. We have concentrated on the Thomsen-Friedenreich disaccharide as a basis for the synthesis of nanoparticles containing this antigen in a range of presentations. This talk will highlight our latest in vitro and in vivo results where only specific presentations show interesting activity. The utility of these constructs as imporatnt anti-tumor tools and potential therapeutic agents wiil be discussed.

**CARB 103**

**Unimolecular glycoconjugates for drug release and targeting**

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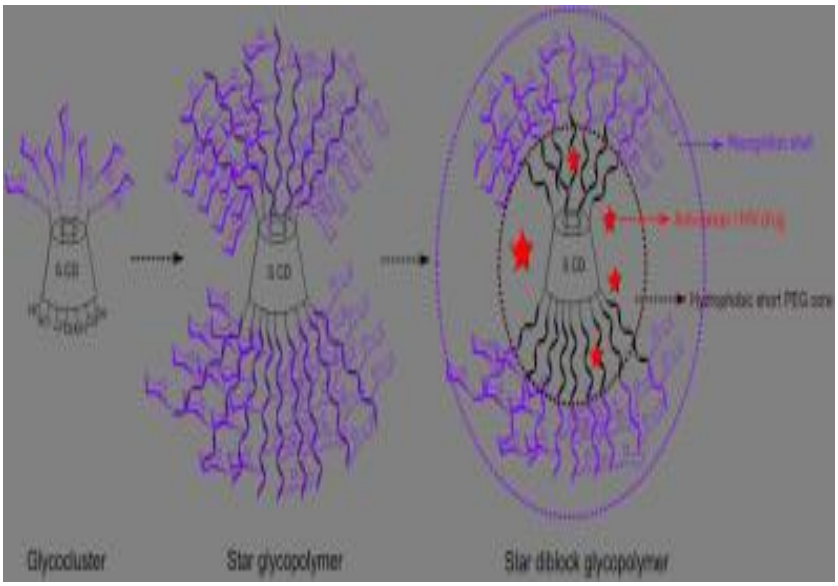
A series of cyclodextrin-based glycoconjugates, including glycoclusters and star glycopolymers, were synthesized via combination of CuAAC Huisgen coupling and copper-mediated living radical polymerization. These glyco conjugates showed high affinity binding to the human transmembrane lectin DC-SIGN and act as inhibitors to prevent the binding of HIV envelope protein gp120 to DC SIGN at nanomolar concentrations. The star block glycopolymers showed high loading capacity of hydrophobic anticancer and anti-HIV drugs, indicating promising applications in HIV therapeutic and smart drug delivery. An encapsulation test of these glycoconjugates *via* UV/Vis and NMR revealed that star diblock glycopolymer bearing a hydrophobic core area showed high loading capacity of hydrophobic anti-cancer and anti-HIV drugs, indicating promising application in HIV-therapeutic and smart drug delivery, potentially utilizing versatile endocytic lectins such as DC-SIGN.




**CARB 104**

**Conjugates and nanoparticles of trehalose glycopolymers stabilize protein therapeutics**

***Heather D. Maynard****, maynard@chem.ucla.edu. Chemistry and Biochemistry, UCLA, Los Angeles, CA 90059, United States*

Proteins are notoriously unstable. This causes protein drugs to be discarded and wasted and is inconvenient to patients. In addition it increases shipping and storage costs for the many scientists who use proteins in their research. This talk will focus on the application of stabilizing polymers that have trehalose in their side chains. The trehalose glycopolymers can be either attached to the protein or simply added to stabilize the biomolecules to extreme stresses such as near boiling temperatures. The polymers significantly outperform the native sugar, trehalose as well as other gold standards in the field like poly(ethylene glycol) (PEG). The polymers can also be made into nanoparticles to encapsulate and release proteins. The synthesis of these polymers and their application to stabilize protein therapeutics will be discussed.

**CARB 105**

**Glycopolymer surfaces for tissue engineering**

*Chrystalleni Hadjicharalambous[1], Maria Chatzinikolaidou[2,1], Ravin Narain[3],* ***Maria Vamvakaki****[2,1], vamvakak@iesl.forth.gr. (1) Materials Science and Technology, University of Crete, Heraklion, Crete 710 03, Greece (2) Institute of Electronic Structure and Laser, Foundation for Research and Technology Hellas, Heraklion, Crete 711 10,*

*Greece (3) Department of Chemical and Materials Engineering, Faculty of Engineering, University of Alberta, Alberta, Edmonton T6G 2G6, Canada*

Surface properties, such as surface chemistry and topography, affect cell–biomaterial interactions potentially guiding cellular behavior and proliferation.[1] Synthetic polymer brushes have been used to tailor the surface properties by altering the grafted polymer molecular characteristics.

In this study, well-defined glycopolymer brushes of high grafting densities based on D-gluconamidoethyl methacrylate (GAMA) and 2-lactobionamidoethyl methacrylate (LAMA), featuring a glucose and lactose functionality, respectively have been synthesized from initiator-modified substrates by direct surface-initiated atom transfer radical polymerization.[2] The polymer film characteristics were investigated by attenuated total reflectance-FTIR spectroscopy, atomic force microscopy, ellipsometry and contact angle measurements. Brushes of different film thicknesses were prepared by varying the polymerization time.

The glycosurfaces were employed in tissue engineering applications. We have investigated the adhesion and viability of MC3T3 pre-osteoblast cells on the PGAMA and PLAMA glycopolymer brushes. Cell attachment, morphology and cytoskeleton organization was observed by scanning electron microscopy and confocal fluorescence microscopy. Cell proliferation was measured using a resazurin-based assay. A marked preference of the MC3T3 cells for the PGAMA substrates was observed, which supported good cell viability and growth. On the other hand, cell proliferation on a 4 nm PGAMA brush was higher (92% of the PS control) than on the 9 nm brush (63%). Moreover, cells cultured on the 4 nm brush displayed enhanced cell spreading compared to cells on thicker brushes, on which cells displayed a spindled and elongated morphology. In summary, our results suggest that the novel glycopolymer brushes possess cell-recognition capabilities which are dependent on the polymer characteristics. Current work focuses on the exploration of the surfaces on pre-osteoblastic cell differentiation to control cell function.

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**CARB 106**

**GLYCOCODE: Precision glycopolymers and their interactions with lectins**

*Qiang Zhang[1], David M Haddleton[1],* ***Remzi Becer***[2]*, r.becer@qmul.ac.uk. (1) Department of Chemistry, University of Warwick, Coventry, United Kingdom (2) School of Engineering and Materials Science, Queen Mary University of London, London, United Kingdom*

Sequence controlled polymers have been attracting more and more attention to deliver the desired properties to the advanced materials by the help of their precisely controlled compositions and architectures. Development of various controlled radical polymerization techniques and “click” reactions provide a sufficient platform to prepare functional polymers.

Understanding the specific multivalent carbohydrate-protein interactions is crucial to determine the structure-property relationships and to design accordingly the next generation of functional glycomaterials. Therefore, we investigate the structure-property relationships between the mammalian lectins and multivalent carbohydrate polymers, which may have applications for anti-adhesion therapy. Moreover, we have investigated the affinity of poly(mannose-methacrylate), helical glycocopolypeptides, gp120, start shaped glycopolymers, and cyclodextrin centered glycopolymers with a selected mannose binding lectin (DC-SIGN) that exists on dendritic cells, using SPR technique. Selected members of a glycopolymer library were used to demonstrate the interactions between DC-SIGN and mannose rich polymers. We extend this study to a broader set of polymers to examine the effect of chain length, end group, architecture, thermoresponsive block, and number of arms in the star shaped polymers on the lectin binding.

**CARB 107**

**Carbohydrate-mediated interactions of glyconanomaterials with bacteria cells**

***Mingdi Yan**, mingdi_yan@uml.edu. Department of Chemistry, University of Massachusetts Lowell, Lowell, MA 01854, United States*

Glyconanomaterials, nanomaterials carrying immobilized glycans, combine the unique properties of nanometer-scale objects with their ability to present multiple copies of glycan ligands. We developed new methods to synthesize glyconanomaterials, which apply to a variety of carbohydrate structures. Analytical methods were developed to determine the binding affinity of glyconanomaterials with lectins, which showed significant affinity enhancement resulting from the multivalent presentation on the nanomaterials. In this talk, selected examples will be presented to demonstrate that cellular interaction of gyconanomaterials is mediated by the type and presentation of carbohydrates on the nanomaterials. We demonstrated that the cellular interactions and uptake of nanomaterials can be enhanced by specific carbohydrate ligands. Recent work using glyconanomaterials as antimicrobial agents will also be presented.

**CARB 108**

**In vivo delivery of transcription factors with chemically modified oligonucleotides**

***Niren Murthy**, nmurthy@berkeley.edu. Department of Bioengineering, University of California at Berkeley, Berkeley, CA 94704, United States*

Therapeutics based on transcription factors have the potential to revolutionize medicine, but have had limited medical impact because of delivery problems. In this presentation we demonstrate that a delivery vehicle, termed DARTs (DNA Assembled Recombinant Transcription factors), can for the first time deliver recombinant transcription factors in vivo, and rescue mice from acute liver failure. DARTs are composed of a double stranded oligonucleotide that contain a transcription factor binding sequence, and have hydrophobic $C_{25}$ alkyl chains located at their 3' ends, which are “masked” by acid cleavable galactose residues. DARTs have a unique molecular architecture, which allows them to complex transcription factors, target hepatocytes, disrupt endosomes, and release transcription factors into hepatocytes. We show here that DARTs can deliver the transcription factor Nrf2, to the liver, enhance the transcription of Nrf2 downstream genes, and protect mice from acetaminophen induced liver injury. The DART delivery strategy has tremendous therapeutic potential given the central role of transcription factors in biology and medicine.

**CARB 109**

**Glycopolymer with glycosaminoglycan mimic activity**

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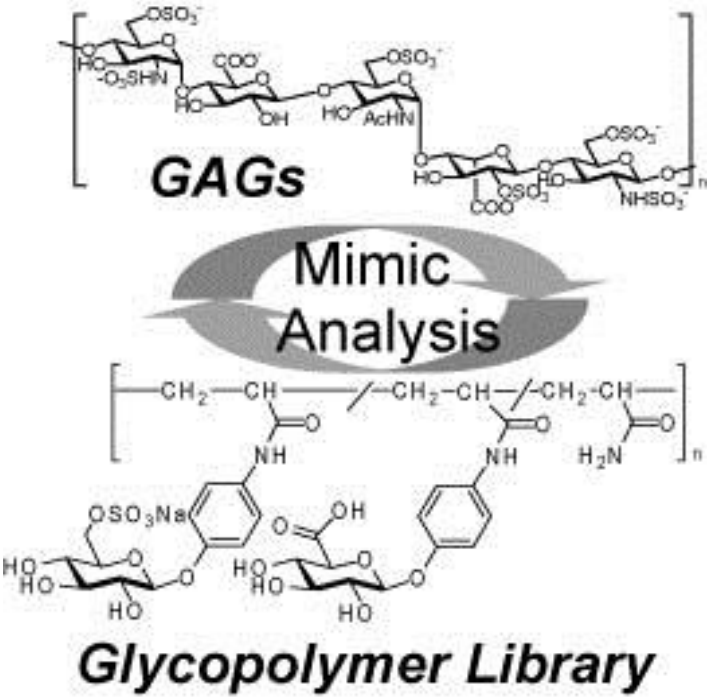
Glycosamonoglycans (GAGs) on the cell surfaces play important roles in biological systems. In spite of the importance of GAGs, it is difficult to clarify the key interaction between GAGs and proteins due to the structural complexity and the high molecular weight. Some researchers have been studying the total synthesis of GAGs, but it is still difficult to obtain the synthetic molecules with GAGs functions. We have synthesized sulfated glycopolymer libralies which mimic GAGs structures




. The acrylamide derivatives with region selective sulfated GlcNAc were synthesized, and glycopolymers with sulfated GlcNAc were polymerized by radical polymerization. The glycopolymer libraries were evaluated by the inhibition of Alzheimer's disease, where the glycopolymers inhibited the aggregation of Amyloid beta peptides and b-secretase activity. The biological activities depended on the sulfated position of GlcNAc, sugar ratio and molecular weight. The glycopolymers including 6-sulfo-GlcNAc showed the strong activities on inhibition of Alzheimer's disease. We will discuss the activities based on the molecular structure and the multivalent effect.

**CARB 110**

**Innovative oral drug delivery vehicles to treat an ancient disease: Tuberculosis**

***Hale C Arca***[1]*, arca@vt.edu, Kevin J. Edgar*[1,2]*. (1) The Macromolecules and Interfaces Institute, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, United States (2) Department of Sustainable Biomaterials, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, United States*

One out of every three people in the world is infected with *Mycobacterium tuberculosis*. Approximately two billion people are carriers of tuberculosis (TB) and have the chance to develop the disease [1]. TB infection is a threat in western countries mostly due to the active TB development in HIV positive patients, but it is a burden in poor countries. Rifampicin is a first line drug used for the TB treatment for more than two decades but still there are challenges in improving its poor bioavailability. Major sources of this problem are instability of rifampicin at the acidic pH of the stomach and its low solubility.

In our work, we proposed to improve rifampicin bioavailability with new cellulose esters used for oral drug delivery systems. Amorphous solid dispersions were prepared with rifampicin and cellulose derivatives (CAP-Adip, CAB-Seb, CA suberate and commercial polymer CMCAB for control purposes). Particle morphology and size were determined by SEM. In addition, XRD studies showed the amorphous nature of the spray dried particles. Afterwards, dissolution studies showed that these novel cellulose derivatives are successful in preventing rifampicin acid degradation (at stomach pH 1.2). Slow release of the antibiotic in the small intestine (pH 6.8) and rifampicin solubility increase were observed. In some of the formulations, 100% drug release was achieved while in other formulations rifampicin was released slowly without the burst effect. These new cellulose esters have strong ability to generate and stabilize supersaturated drug solutions and, great potential for amorphous solid dispersion applications.

Referance

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**CARB 111**

**Photo-switchable glycan ligands on gold nanoparticles**

***Mikkel B. Thygesen***[1]*, mbth@chem.ku.dk, Vijayanand Chandrasekaran*[2]*, Knud J. Jensen*[1]*, Thisbe K. Lindhorst*[2]*. (1) Department of Chemistry, University of Copenhagen, Faculty of Science, Frederiksberg, Denmark (2) Otto Diels Institute of Organic Chemistry, Christiana Albertina University of Kiel, Kiel, Germany*

Glyconanoparticles are emerging probes for unravelling complex carbohydrate-carbohydrate or carbohydrate-protein interactions [1]. Gold glyconanoparticles consist of an inorganic core of $Au^0$surrounded by an organic shell comprising a linker region to which carbohydrate ligands are attached.

The correct presentation of carbohydrate ligands on the surface of nanoparticles reflects a balance between optimal multivalent display of the glycan ligands for their interaction partners and suitable physico-chemical properties of the nanoparticles that confers biocompatibility. To this end, we have designed linkers for self-assembly on gold glyconanoparticles that are flexible yet allow controlled physical repositioning of the glycan ligands within the organic shell. Incorporation of azobenzene units into the linkers facilitates a reversible transition between *E*- and *Z*- configurations by near-UV irradiation that affects a reorganization of the linkers [2]. When assembled on nanoparticle surfaces these linkers may cause steric diversion or contraction of the glycan display, e.g., that can lead to formation or breaking of weak interactions of the ligands.

We report here our initial findings in assembling and evaluating this novel class of glyconanoparticles using azobenzene-linked a-mannosides.

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**CARB 112**

**Chemically synthesized 58-mer LysM protein domain binds lipochitin oligosaccharide**

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Recognition of carbohydrates by proteins is a ubiquitous biochemical process. In legume-rhizobium symbiosis lipochitin oligosaccharides, also referred to as nodulation (nod) factors, function as primary rhizobial signal molecules that trigger root nodule development. Perception of these signal molecules is receptor mediated and nod factor receptor 5 (NFR5) from the model legume *Lotus japonicus* is predicted to contain three LysM domain binding sites. Here we studied the interaction between nod factor and each of the three NFR5 LysM protein domains, which were chemically synthesized. Different LysM domain variants of up to 58 amino acids (AA) designed to optimize solubility were chemically assembled by solid-phase peptide synthesis (SPPS) using microwave heating. Their interaction with nod factors and chitin oligosaccharides was studied by isothermal titration calorimetry (ITC) measurements and by circular dichroism (CD) spectroscopy. The LysM2 showed a change in folding upon nod factor binding providing direct evidence that the LysM domain of NFR5 recognizes lipochitin oligosaccharides. These results clearly show that the *L. japonicus* LysM2 domain binds to the nod factor from *M. loti*, causing a conformational change in the LysM2 domain. The affinity for nod factors compared to chitin oligosaccharides was demonstrated using a newly developed glycan microarray tool. Besides biological implications, our approach shows that carbohydrate binding to a small protein domain can successfully be detected by CD spectroscopy.

**CARB 113**

**Understanding and manipulating protein glycosylation**

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Protein glycosylation is an important post-translational modification. It enhances the functional diversity of proteins and alters their conformation, stability and biological activity. However, because of the complexity of glycoproteins and glycan structures, establishing connections between glycan structures and their functions is difficult. Recently, we demonstrate that chemical synthesis can be employed as a useful tool to determine the effects of protein glycosylation and the molecular mechanisms of these effects. The more precise understanding of protein glycosylation would aid in the development of enzymes and protein-based therapeutics with improved properties.

**CARB 114**

**Carbohydrate-functionalized bivalent polymers**

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Multivalent interactions play important roles in a variety of biological processes.Major roles of multivalent interactions are to enhance weak protein-carbohydrate interactions and change protein receptor proximity. In many cases, a multivalent ligand can bind to a number of protein receptors with enhanced affinity. Extensions of this finding reveal that long polymers result in enhanced multivalent binding (termed 'avidity') compared to short polymers or monomers because of their ability to span a greater number of binding sites. To take advantage of the multivalent binding concept for achieving the highest binding affinity between synthetic oligosaccharides epitopes and proteins of interest, we have designed and synthesized a number of oligosaccharide-derived polymers bivalent polymers. Their ability to bind to ConA, 2G12, and other proteins of interests has been determined using ITC techniques. The synthesis and biological studies of oligosaccharides-derived bivalent polymers will be presented at this conference.

**CARB 115**

**New type of fully synthetic self-adjuvanting glycoconjugate vaccine**

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Most tumors express certain unique or excessive carbohydrates, which are known as tumor-associated carbohydrate antigens (TACAs). TACAs are useful targets for the development of cancer vaccines or immunotherapies. However, TACAs are poorly immunogenic, thus they cannot elicit robust immune responses useful for cancer therapy. To overcome this problem, we have developed a new therapeutic strategy that combines synthetic vaccines made of unnatural sialo-TACA derivatives and metabolic glycoengineering of cancer cell to express unnatural sialo-TACAs. In this regard, we have established a novel strategy of fully synthetic glycoconjugate vaccines with monophosphoryl lipid A (MPLA) as the carrier molecule. These vaccines could induce

robust IgG immune responses without the use of an additional adjuvant, suggesting that they are self-adjuvanting and provoke T cell-dependent immunity that is very useful for cancer immunotherapy. We have further demonstrated that cancer cells can be efficiently and selectively glycoengineered by using a monosaccharide to express the unnatural sialo-TACA analog. Our *in vivo* studies confirmed that immunization using the synthetic vaccine followed by treatment with the unnatural monosaccharide could effectively inhibit tumor growth and metastasis and elongate animal survival.

**CARB 116**

**Applications of intramolecular thiol-ene reactions for the synthesis of thiosugars**

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Thiosugars are carbohydrate analogues where one or more oxygen atoms are substituted with sulfur in both furanoside and pyranoside structures. Due to the unique conformational and electronic properties conferred by the presence of the sulfur atom, these compounds offer fascinating prospects for medicinal chemistry as glycosidase inhibitors and they have been shown to demonstrate potent biological activity as antiviral, antidiabetic and anticancer compounds. We have developed a highly efficient synthetic methodology to access novel thiosugars by employing intramolecular 'thiol-ene' cyclization reactions. Both 5-*exo* and 6-*endo* cyclization pathways have been investigated to access the desired thiosugars. Cyclization reactions occur in high yield with excellent regio- and diastereoselectivity.

**CARB 117**

**Carbohydrate binding module clans**

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At present, 66 families of carbohydrate-binding modules (CBMs) have been isolated by statistically significant differences in the amino acid sequences (primary structures) of their members, and in fact most members of different families show little if any homology. On the other hand, members of the same family have primary and tertiary (three-dimensional) structures that can be computationally aligned, yielding at least some degree of homology, and suggesting that they are descended from common protein ancestors. Almost universally, CBMs are composed of β-strands, with an almost total absence of α-helices. In addition, members of the large majority of CBM families are β-sandwiches, also known as β-jelly rolls. This raises the question of whether members of different families are descended from distant common ancestors, and therefore are members of the same clan, even though their primary structures may no longer retain any similarity. We have attacked this problem by attempting to

computationally superimpose tertiary structure representatives of each of the 51 CBM families that have members with known tertiary structures, using MultiProt. In cases where visual observation has suggested that tertiary structures of different families are very similar, we have determined root mean square deviations (RMSD) and percentages of similarity between adjacent amino acid residues in the two structures. Further criteria beyond low RMSDs and high similarity percentages leading to clan membership are similarities in locations of secondary structure elements, amino acid chain lengths, and bound ligands. These considerations have led us to assign 31 families to seven clans, with two to seven families in each clan. Five of the clans have members with β-sandwiches having six to ten β-strands, while a sixth is composed of structures with β-trefoils, and a seventh has very short chains with only two to four short β-strands.

**CARB 118**

**Rescuing Nod2 in Crohn's disease**

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Microbes are detected by the pathogen-associated molecular patterns (PAMPs) through specific host pattern recognition receptors (PRR). Nucleotide binding oligomerization domain-containing protein 2 (NOD2, CARD15, IBD1) is an intracellular PRR that recognizes fragments of bacterial cell wall or peptidolgycan. Peptidoglycan is an intricate polymer of carbohydrates and peptides. Nod2 is important to human biology, as when it is mutated, it loses the ability to respond properly to bacterial cell wall fragments. In order to determine the mechanisms of misactivation in the Nod2 Crohn's mutants, we developed a cell based system to screen for protein-protein interactors of Nod2. We identified Hsp70 (heat shock protein 70) as a protein interactor of both wild-type and Crohn's mutant Nod2. We describe the regulatory mechanism that Hsp70 plays in Nod2 signaling and how Hsp70 is able to rescue the Nod2 detection of peptidoglycan fragments in Crohn's associated Nod2 variants.

**CARB 119**

**Conformational analysis of nucleosides: A combined PSEUROT/molecular mechanics approach**

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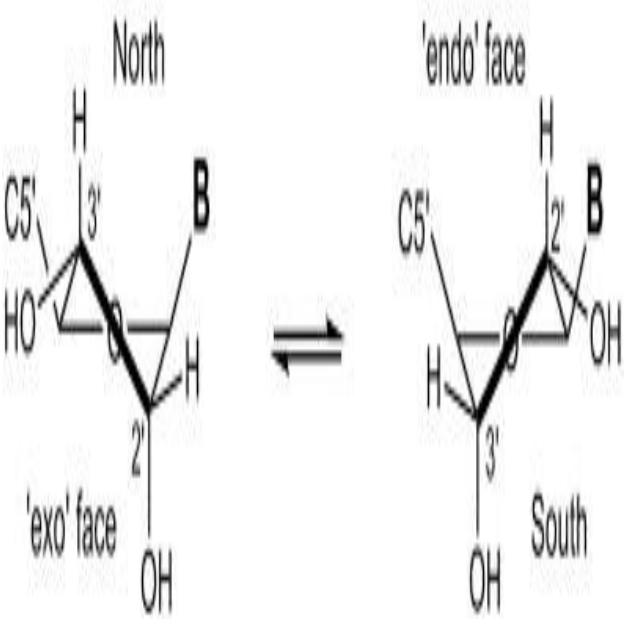
The two-state N/S model of Altona and Sundaralingam is a dominant paradigm in the conformational analysis nucleosides and nucleotides. It is well-demonstrated that most

nucleos(t)ides exist in solution as a mixture of 'north' (C2'-exo/C3'-endo) and 'south' (C2'-endo/C3'-exo) conformers in solution; the precise nature of the N/S conformations and their relative populations is commonly established through the use of the PSEUROT program and $^{1}$H-$^{1}$H coupling constant data ($^{3}J$) obtained from high-resolution $^{1}$H NMR spectra. PSUEROT essentially 'builds' a pair of N/S conformers that best replicate in experimental *J*'s. To do this building properly the user must specify, from a limited selection within PSEUROT, the 'A' and 'B' parameters that correlate the endocyclic torsion angles (which define the N/S conformations) to the exocyclic H-H torsion angles (which form the basis of the calculated $^{3}J$'s). A significant bottleneck to the use of PSEUROT is that the A and B parameters for novel furanose rings not in the PSEUROT database must somehow be derived. Historically, the A and B parameters have been obtained from either from a large body of crystal structures or from high-level quantum mechanical structures. Herein we report that A and B parameters derived from molecular mechanics methods (Amber) provide PSEUROT results virtually indistinguishable from ab initio methods, greatly facilitating the use of PSEUROT.




**CARB 120**

**Quantification of sialic acid based on a robust and affordable quinoxaline derivatization**

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Sialic acids (SAs), a family of 9-carbon containing acidic monosaccharides also known as neuraminic acids are very important carbohydrates terminating cell surface glycoconjugates that are involved in many physiological and pathological processes.

SAs also exist in both free and conjugated forms such as glycoproteins and glycolipids in tissues and fluids such as serum, urine and saliva related to certain disease status. In addition, therapeutic glycoprotein sialylation and the identity of the SAs are very critical to the glycoproteins' efficacy, pharmacokinetics, and potential immunogenicity. Therefore, identification and quantitation of SAs are very important for biomedical research and therapeutic glycoprotein production and applications. Quinoxaline derivatization of SAs has been the most useful approach for sialic acid quantitation of biological samples and glycoproteins by either HPLC or LC-MS/MS. However, stability of SA quinoxaline derivatives and phenyldiame reagents and their cost prevent its practical applications. In this report, we examined SA quinoxaline derivatives formation features with commercially available phenyldiamines and their SAs quantitation competency. We found a stable and inexpensive 4,5-dimethylbenzene-1,2-diamine (DMBA), which selectively reacts with SA to form a stable quinoxaline derivative with high UV and fluorescence properties, proving a convenient and cost-effective HPLC analysis of SAs. DMBA derivatization of both *N*-acetyl-D-neuraminic acid (Neu5Ac) and *N*-glycolyl-D-neuraminic acid (Neu5Gc) and their HPLC quantitation in FBS and glycoprotein fetuin were successfully demonstrated. In addition, DMBA derivatization of SA provides a stable derivative of SA with high MS response, proving a convenient and cost-effective LC-MS/MS analysis of free SAs. The reported DMBA derivatization method provides a robust and affordable approach for quantitation of SAs in both biomatrices and therapeutic glycoproteins.

**CARB 121**

**Using capillary HPAE-PAD to determine carbohydrates in various matrices**

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Monosaccharide and disaccharide determinations are important to various industries from ensuring quality product to researching biological pathways and disease states. Because carbohydrates are poor chromophores, chemical derivitization is needed for absorption. However, derivitization is costly, labor-intensive and may cause changes in molecular configuration. High Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) is a proven sensitive method to directly and selectively determine carbohydrates. In HPAE-PAD, carbohydrates are ionized in strong base and separated by anion-exchange chromatography. The carbohydrates are detected by PAD with a gold working electrode using a four-potential waveform selective and sensitive for carbohydrates. This sensitivity allows carbohydrate analysis down to pmole concentrations or when the samples are limited. This sensitivity is moderated in beverage samples which contain g/L concentrations by minimizing the flow path combined with moderate dilution.

Here we combine the advantages of a reagent-free capillary format IC to determine monosaccharides and disaccharides in various applications, from low concentrations in synthetic urine samples to high concentrations in beverage samples. In a reagent-free

IC system, the hydroxide eluent is electrolytically generated inline to deliver accurate and precise concentrations for isocratic or gradient separations by only adding deionized water. Eluent generation eliminates carbonate contamination and errors from manual preparation. A capillary scale system with µL/min flow rates can run 24/7, always on and always ready for samples. Eluent consumption and waste generation are reduced to 5.2 L/yr and eluent generator cartridges can last up to 18 months.

**CARB 122**

**Aminolysis of maltose**

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In 1963, I. Ježo of the Slovak Academy of Sciences, Bratislava, reported (Chemické Zvewsti 1963, 17, 126–139) that treatment of an aqueous solution of sucrose in the presence ammonium hydroxide and heat (220ºC in a sealed system) yielded numerous sugar-ammonia reaction products including the so-called 2,5- and 2,6-deoxyfructosazines. Ježo also found that the reaction was catalyzed by a small amount of triammonium phosphate; and he correctly concluded that the 2,5- and 2,6-deoxyfructosazines arose from the fructose and glucose formed from the hydrolysis of sucrose under the relatively high temperatures used. More recently, Agyei-Aye *et al.*, showed [Carbohydrate Research 337 (2002) 2273-2277] that the 2,6-deoxyfructosazine could be formed in higher yields under much milder conditions from glucose and diammonium phosphate (DAP) or ammonium salts of weak acids. However, when such reactions carried out commercially (e.g., production of caramel colors), sugar sources such as corn syrup are used. Such syrups are likely to contain maltose (or higher sugars) in addition to glucose, and to date there has been no definitive study on the fate of the maltose. Does it yield 2,6-deoxyfructosazine and similar products from the hydrolysis of maltose and/or does it yield a 2,6-deoxyfructosazine with hexose residues attached to the d-*arabino* and d-*erythro* sidechains? To answer this question, fructose, glucose, maltose, and sucrose were each reacted with DAP in aqueous solutions for 1.5 h at 93ºC in 5 mL reaction vials. The fructose, glucose, and maltose formed dark-brown colored solutions, but the solution of sucrose and DAP was only faintly brown. The reaction mixtures were analyzed according to the procedure described by Agyei-Aye *et al.*, (2002).

**CARB 123**

**Radiolabeled glycoconjugates targeting metastatic melanoma cells**

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Melanoma is one of the seven most common cancers in the US with incidence significantly increased during the last 20 years. It is due to remarkable resistance of melanoma cells to all chemotherapeutic drugs and therapy using the external-beam radiation (EBRT). Systemic therapies targeting melanoma have shown only limited success with tumor response in less than 20% of patients. To increase success of the tumor detection, we have designed a new class of glycoconjugates (RMX-GC) that target metastatic melanoma cells by binding to their upregulated glucose transporters, GLUT-1. RMX-GC are chelator-glucosamine conjugates that can be labeled with positron emitters, 68Ga or 64Cu to image metastatic melanoma metabolism, and Lu177 for radiotherapy. Synthesis of two leads compounds, RMX-GC-08 and RMX-GC-11 was performed by copper-free click chemistry; first by conjugation of 2-acetamido-N-(e-minocaproyl)-2-deoxy-D-glucosylamine to MFCO-N-hydroxysuccinimide or BCN N-hydroxysuccinimide, respectively, followed by coupling of azido-monoamide-DOTA chelator. The resulting products, RMX-GC-08 and RMX-GC-11 labeled with isotopes have shown tumor specific accumulation in MeWo-xenographs models (up to in 4.3%ID/g and tumor-to-normal organ ratio of 2.3). Introduction of PEG or alkyl-amine linker to structure of these conjugates increased their in vivo stability and tumor targeting properties compared to properties of non-linker modified DOTA-glucosamine agents. Our studies have shown that chelator-click-glucosamine agents can be potentially used as metabolic imaging tracers for detection of metastatic melanoma.

**CARB 124**

**Druggability of mammalian C-type lectin receptors**

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Receptor recognition of carbohydrates is an important principle of many aspects of life, such as cellular communication and pathogen recognition. For instance, mammalian lectins are mediators of cell migration, pathogen uptake and processing, and signal integration leading to a defined cellular immune response. Small molecules as modulators for the receptor/glycan interaction by blocking the recognition site of these lectins would be very beneficial for basic research in glycobiology as well as for therapeutic intervention. Unfortunately, X-ray crystallographic analyses of a number of mammalian lectins involved in immune cell regulation have revealed that the carbohydrate recognition sites are rather shallow and featureless. This renders lectin receptors rather unattractive for rational design of small molecule inhibitors. Moreover, previous reports on high-throughput screening campaigns against glycan binding proteins have solidified this opinion, stating that glycan-binding protein are undruggable. These data suggest that the identification of hits and advancing them into lead structures is challenging, if not impossible. Nevertheless, no systematic study has been reported to assess the druggability of a mammalian lectin family. To this end we chose C-type lectins, representing a large mammalian lectin family to re-evaluate the druggability of these receptors. A set of family members was selected and

recombinantly expressed. Biophysical techniques such as NMR and SPR, as well as computational algorithms were applied. We will discuss these results in the light of potential glycomimetic drugs against C-type lectin receptors.