Maintenance of cell surface glycan density by lectin-carbohydrate interactions: A cellular homeostatic mechanism

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We have recently introduced the concept of lectins as density dependent glycan binding proteins in innate immunity (1). More recently, studies have shown that mutations in the pathway for the formation of specific branch chains in N- and O-linked glycoproteins in mouse cells results in global compensation of glycan epitope density in the remaining chains of the mutant's N- and O-linked glycans, respectively (2, 3). High-resolution mass spectrometry demonstrate that certain glycan epitopes such as LacNAc and Lewisx moieties on the surface of cells from different organs are compensated in the mutant N- and O-linked glycans by carbohydrate chain extensions possessing the missing epitopes. Similar glycan epitope compensation in gangliosides that interact with MAG in mice missing GM3 has also been reported (4). These studies suggest that glycan epitope density on the surface of mouse cells from different organs and the brain may be regulated by lectin binding, cross-linking and signaling. This suggests a model in which lectin-carbohydrate interactions in the glycocalyx of metazoans are involved in cellular homeostasis.


(2) Takamatsu et al. (2010) “Physiological and glycomic characterization of N-acetylglucosaminyltransferase-IVa and –IVb double deficient mice” Glycobiology 20, 485-497.


Synthetic glycosyltransferase acceptor substrates and substrate analogs: A lifetime achievement by Khushi Matta for the detailed characterization of novel enzymes

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Glycosyltransferases found ubiquitously in all cells are involved in the biosynthesis of sugar linkages. Sugar-transfer reactions are often abnormal in diseases such as cancer. These enzymes are usually highly specific for both the sugar donor and the sugar acceptor substrates. Khushi Matta’s group has a long history of accomplishments in this field. They have synthesized an extremely large variety of glycosyltransferase acceptor analogs which have been used by us and by biochemists for the discovery of new enzymes and for the characterization of detailed substrate specificities of glycosyltransferases. Our knowledge of glycosyltransferases and biosynthetic pathways would not be as advanced without the systematic, targeted and insightful chemical synthesis by K. Matta. Many other chemists have followed in his footsteps. Matta has therefore made critical contributions to the discovery of new enzymes and pathways, to an understanding of enzymatic catalysis, the role of glycosylation, as well as the mechanisms of disease. In addition, the knowledge gained led to the design of specific inhibitors that have the potential to re-engineer glycosylation.

CARB 3

Chemistry builds the platform for defining “Glycan Signatures” on cancer cells

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To meet the challenge of identifying “cancer specific glycan structures”, we initiated a study termed “predictive strategy”. This involves the synthesis of unique carbohydrate acceptors that can distinguish between closely related glycosyltransferase activities. Analysis of glycosyltransferase activity in cancer cell lines, tumor specimen and sera then allows the prediction of associated glycan structures. In one aspect, I will present an overview of our chemical synthesis efforts with focus on enzymes involved in O- and N-glycan biosynthesis. Next, I will discuss a unique enzyme activity that we recently discovered called “reverse” or “exchange” sialylation. This property of the mammalian sialyltransferase, ST3Gal-II, allows reversible synthesis of CMP-NeuAc from donors containing the NeuAca2,3Galβ1,3GalNAc- units in the presence of 5’-CMP, and it also allows radiolabelling of cancer associated mucins. The utility of this enzymatic property for the synthesis of novel carbohydrates and identification of cancer specific signatures will be discussed.

CARB 4

Efficient imidazolium cation tagged solution-phase assembly of a nonasaccharide

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Soluble imidazolium cations (IC) have shown potential as supports for catalyst or reagent immobilization and their utility in solution phase oligosaccharide synthesis. The solubility of IC tagged organic species can be turned on and off by variation of anions to make them phase separate from less polar organic solvents such as chloroform/dichloromethane and aqueous media. Thus, IC tagged carbohydrate species containing non-coordinating anion such as hexafluorophosphate can be purified from the reaction mixture after conventional workup and concentration by simple washing with solvents such as diethylether. This methodology provides; a) homogeneous reaction conditions, b) eliminates the excessive use of column chromatography for the purification of products after each glycosylation step, and c) convenient characterization of IC tagged glycosides by conventional spectroscopic techniques. Herein, we report a convenient IC-tagged assembly of the target branched nonasaccharide from Corynebacterium glutamicum using an efficient catch and release purification on Silica gel.

**CARB 5**

**New methods and strategies for oligosaccharide synthesis**

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Described is a research program on the development of new catalytic glycosylation methods and novel protective group chemistry that enables rapid assembly of homogenous glycoconjugates. Case study on the syntheses of biologically relevant structures will be provided.

**CARB 6**

**Chemo-enzymatic preparation of N-glycan arrays and their applications**

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Glycan arrays have emerged as versatile platforms in Functional Glycomics for the high-throughput screening of binding specificities for lectins and antibodies or to determine the substrate requirements of carbohydrate processing enzymes.

A remaining major bottleneck for a more extended use of glycan arrays is the supply with sufficiently pure and well-characterized ligands. Focusing on N-glycans our laboratory has combined the synthesis of N-glycan core structures with their enzymatic modification on the chip using recombinant glycosyltransferases to generate a library of structures with systematic variations in number of antennae, terminal sugars and core modifications.\(^1\)\(^-\)\(^3\) A surface-based MALDI-tof MS method has been developed to evaluate the enzymatic on-chip glycosylation\(^4\) and as a powerful tool to analyze biomass degrading enzymes in environmental samples.\(^5\)

The printed and modified glycan arrays have been employed in the screening of substrate specificities of carbohydrate processing enzymes\(^6\) and to determine lectin and antibody specificity employing fluorescent, MALDI-Tof\(^4\) and autoradiography as complementary readout techniques.


(5) submitted 2011


**CARB 7**

**Chemoenzymatic synthesis of fluorous-tagged heparin oligosaccharides**

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Heparin, a highly sulfated polysaccharide, has been used as an anticoagulant for more than 75 years. While commercial heparin is currently prepared by porcine tissue extraction, there is no efficient method to synthesize heparin from a non-animal source.
The varying sulfo group patterns and locations within the heparin chain make it an extremely difficult target to synthesize chemically. Here, we show our efforts to synthesize heparin oligosaccharides using a chemoenzymatic approach. First, an acceptor disaccharide with a fluorous protecting group was synthesized in less than 15 steps. The disaccharide was then extended to an oligosaccharide with the desired number of sugar residues using glycosyltransferases. Finally, heparin and heparan sulfate biosynthetic enzymes, including O-sulfotransferases and N-sulfotransferase, and C5-epimerase, were used to introduce sulfo groups and to epimerize glucuronic acid to iduronic acid, both of which are crucial to heparin's anticoagulant activity.

**CARB 8**

*Synthesis of alkyl fluorous thiols and their use in automated oligosaccharide synthesis*

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Thioglycosides are one of the most stable and versatile glycosyl donors for the construction of simple or complex oligosaccharides. However, their activation often requires conditions such as extremely cold temperatures that make their use in automated syntheses less attractive. Herein we report the syntheses of two new fluorous thiol tags and compare their utility as glycosyl donors and acceptors in both manual and automated syntheses of oligosaccharides based on the fluorous solid-phase extraction of intermediates.

**CARB 9**

*Synthesis of polyfluorinated carbohydrates*

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The synthesis of polyfluorinated carbohydrates is an area of current interest. This class of compounds are probes to investigate the 'polar hydrophobicity' concept, first proposed by DiMagno, in which binding energy to protein targets would be derived from hydrophobic desolvation and multipolar interactions.

We have described short syntheses of a number of tetrafluorinated monosaccharides, and this presentation will disclose the synthesis of a number of novel sugar targets.
Methodology development and physical organic chemistry: A powerful combination for the advancement of glycochemistry

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Ongoing work from the author's laboratory on the elucidation of glycosylation mechanisms will be presented.

CARB 11

New synthetic approach to carbohydrate-based drug discovery

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This lecture will present some new development of glycosylation methodology, especially for the synthesis of polysialic acid and identification of cancer markers, and development of therapeutics.

CARB 12

Development of pre-activation based one-pot carbohydrate synthesis methodologies

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Oligosaccharide synthesis is traditionally tedious and time-consuming. During the past two decades, many innovative methods have been developed to facilitate this process. Preferential activation of a highly reactive armed donor in the presence of a less reactive disarmed acceptor is the foundation of the popular reactivity based one-pot synthesis strategy. However, to prepare building blocks with desired anomeric reactivities, extensive protective group manipulations must be carried out, thus lowering overall synthetic efficiency. To solve this problem, a new pre-activation based one-pot glycosylation approach is developed for the efficient assembly of oligosaccharides independent of anomeric reactivities. This is achieved by pre-activating the glycosyl donor, followed by sequential addition of building blocks in the same reaction flask. Synthesis of several complex oligosaccharides by this method will be discussed. Moreover, the results from mechanistic studies will be presented. The pre-activation based one-pot method represents an important advance towards streamlining oligosaccharide synthesis, which can potentially evolve into a general glycosylation approach complementing the current automated solid-phase technology.

CARB 13
Various nano-guises of the TF antigen for anticancer therapy

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The Thomsen Friedenreich antigen is a tumor associated carbohydrate antigen (TACA) that is implicated in the processes of tumor invasion, metastasis and immune recognition. It is an important disaccharide used in many previous anticancer immunotherapies and vaccine preparations. We have synthesized TF antigen in various guises and used them to coat nanoparticles. These particles were evaluated as antiadhesive agents to prevent tumor metastasis and as vaccine platforms to promote an immune response to TF-bearing glycopeptides. Recent progress on these fronts will be discussed.

CARB 14

Lipopolysaccharide transport and assembly in Escherichia coli

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The outer membrane of Gram-negative bacteria contains an outer leaflet composed of lipopolysaccharide (LPS) that is transported to this location by a pathway that is essential for viability. It has been suggested that inhibitors of this pathway could be useful antibiotics. In Escherichia coli, eight essential proteins have been identified to function in the proper assembly of LPS following its biosynthesis. This assembly process involves release of LPS from the inner membrane, transport across the periplasm, and insertion into the outer leaflet of the outer membrane. I will talk about the mechanism of LPS transport and assembly and the development of tools that could lead to the discovery of inhibitors of this process.

CARB 15

Personalizing cancer treatment with the aid of glycan array technology

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Cancer cells undergo dramatic changes in carbohydrate expression during the onset and progression of the disease. Aberrantly expressed glycans on cancer cells can serve as important targets for immune surveillance and/or for immune responses induced by cancer vaccines. However, immune responses to glycans have been largely understudied due to difficulties in obtaining structurally-defined carbohydrates and measuring carbohydrate-protein interactions. Our group uses chemical synthesis to obtain a diverse collection of carbohydrates, which are then printed onto glass microscope slides to produce glycan microarrays. These arrays provide a high-

...
throughput tool to profile the repertoire of anti-glycan antibodies in human serum. We have used this technology to evaluate immune responses induced by a poxvirus-based prostate cancer vaccine (PROSTVAC-VF) that is currently in Phase III clinical trials. We have profiled over 140 patients from two Phase II trials and have identified serum antibodies with statistically significant correlations with overall survival. These antibodies are promising new biomarkers for predicting which patients will respond favorably to PROSTVAC-VF. In addition, our results have implications for improving vaccine design.

CARB 16

Building well-defined glycoproteins

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The expression of complex carbohydrates in human cells is critical in the development and physiology of living systems. Unlike proteins and nucleic acids, glycoprotein biosynthesis is not under genetic control, resulting in heterogeneous mixtures, the so-called glycoforms. Each component of these glycoforms may have different biological properties. Access to pure samples from natural sources is very challenging, despite some exceptions. Chemical site-selective glycosylation has emerged a powerful instrument to access and study single glycoforms. A number of methodologies based on a tag-and-modify strategy that enable construction of defined glycoproteins will be discussed. We first explored cysteine in the selective formation of disulfides. The susceptibility of disulfides to reduction prompted the development of different methods for the formation of more stable thioethers. Finally, a site-selective Traceless Staudinger strategy for glycoproteins synthesis consisting of incorporation of an azide containing amino acid followed by modification with a sugar phosphine was developed.

CARB 17

Synthesis and characterization of sialyl glycopolymers for site-specific protein conjugation

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Protein-based biotherapeutics often suffer poor bioavailability and biodistribution times due to enhanced clearance within the body. Glycoengineering aimed at adding carbohydrates to proteins to increase in vivo activity and prolong the duration of action has become a promising approach for biotherapeutic enhancement. Particularly, sialylation has shown enhanced protein pharmacokinetic properties such as improving protein stability with reduced immunogenicity and extended plasma half-lives. Thus, O-
cyanate chain-end functionalized and glycine chain-end functionalized glycopolymers presenting multiple copies of sialic acid units were synthesized via cyanoxyl-mediated free-radical polymerization in one-pot fashion. The resultant glycopolymers were characterized by $^1$H NMR spectroscopy. Non-site specific protein conjugation via isourea bond formation with O-cyanate chain end functionalized glycopolymer and site-specific protein conjugation via sortase-mediated ligation with glycine chain-end functionalized glycopolymer were investigated and characterized by SDS-PAGE. The synthetic strategy presented in this work exhibits oriented multivalent carbohydrate modification of protein in straightforward, aqueous mild conditions.

CARB 18

APTS labeled oligosaccharides: Probes for the evaluation of plant cell wall biosynthetic enzymes

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Fluorescently labelled saccharides, representing structures found in pectic glycan arabinogalactan and rhamnogalacturonan II, were synthesised by chemical glycosylation of O-6 of diacetone-D-galactose followed by deprotection and reductive amination with amino-substituted fluorophore APTS. This convenient method installs a common aminogalactitol-based tether in order to preserve the integrity of the reducing end of specific carbohydrates of interest. APTS-labelled glycans prepared in this manner were purified by carbohydrate gel electrophoresis and subjected to capillary electrophoresis analysis, as a basis for the subsequent development of high sensitivity assays for glycosyltransferases involved in plant cell-wall biosynthesis.

CARB 19

WITHDRAWN

CARB 20

Study of protecting group effect on the stereoselectivity of GlcN$_3$ donors

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GlcN$_3$ donors are commonly used for the synthesis of alpha-GlcNH$_2$ linkages. However, the stereoselectivity often significantly changes in different reactions. Protecting groups play an important role in controlling the reaction result. A series of GlcN$_3$ donors with different combination of protecting groups were prepared and tested in glycosylation reactions. The results indicated that both the number and the position of the ester
groups can affect the stereoselectivity. The optimum results can be achieved when the right combination was applied.

**CARB 21**

**Efficient and alpha only preparation of alpha\(2\rightarrow9\) oligosialic acids for anti-\(N. meningitidis\) vaccine development**

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The development of synthetic polysialic acid vaccines with high immunogenicity and understand the optimal epitope has attracted much attention in recent years. Convergent block synthesis strategy was considered the best method to synthesize oligosialic acid but wasn't realized because the a-selectivity decreased with the increasing of sialyl donor's length in conjugation reaction until we found a new sialylation reagents which combine 5-\(N,4-O\)-carbonyl protections and anomeric dibutyl phosphate as a leaving group.\(^\text{[1]}\) Convergent synthesis strategy was accomplished in this report, a-selectivity retained even though the size of sialyl phosphate donor or oligosialoside acceptor increases.\(^\text{[2]}\)

Dodecasialoside have been synthesized via this convergent strategy, and we believe that this method should be applied to the synthesis of higher oligomers. Our research in using these synthesized polysialic acid with homogeneous structures to construct polysialic acid-protein conjugate as vaccine candidate is underway.


**CARB 22**

**Synthetic study toward pseudopentasaccharide repeating unit of Streptococcus pneumoniae zwitterionic polysaccharide**

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The Pseudopentasaccharide \([\beta\text{-D-Glcp}(1-3)-\alpha\text{-AATp}(1-4)-\alpha\text{-D-GalpNAc}(1-3)-\beta\text{-D-GalpNAc-D-ribitol}]\) is a repeating unit of zwitterionic C-polysaccharide isolated from noncapsulated pneumococcal strain CSR SCS2. Pseudopentasaccharides are linked to each other by phosphodiester linkages between C-5 of D-ribitol residue and C-6 of \(\beta\text{-D-glucopyranosyl}\) residue. The number of phosphocholine residues in the repeating unit varies in different pneumococcal strains. The present work employs a convergent [2+3]
glycosylation strategy to achieve the required pseudopentasaccharide repeating unit. An important feature of this synthetic scheme is the installation of axial amino functionality in challenging AAT moiety by inversion of C4-OH at the pentasaccharide stage. This synthesis employs three orthogonal protecting groups (Nap, Allyl and TBS) that can be selectively removed to install phosphocholines and to oligomerize the repeating unit. Then the synthesized oligosaccharides will be used as tools to understand the biological roles of above mentioned C-polysaccharides.

CARB 23

General strategy for synthesis of GPI anchors

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Glycosylphosphatidylinositol (GPI) anchors are ubiquitous among eukaryotes. Their complex structures, including a conserved pseudopentasaccharide core, suggest biological functions beyond the basic role of anchoring the attached protein to the plasma membrane. Further studies in this direction depend on availability of a diverse set of homogeneous GPIs which are only accessible via chemical synthesis. To address this need we have devised a modular approach to synthesis of GPI anchors that enables access to various branched GPI pseudooligosaccharides starting from a small number of interchangeable building blocks. Five levels of orthogonal protection (exemplified by the fully orthogonally protected central mannose building block bearing TDBPS, 1-methylnaphtyl, benzyl, levulinoyl and allyl protecting groups) are the center point of our synthetic strategy. They enable convergent assembly of the pseudooligosaccharide core and the regioselective installation of the appropriate substituents via phosphate bridges. Syntheses of the selected GPI anchors utilizing this strategy will be discussed.

CARB 24

Critical roles of carbohydrates in producing the sustainable biofuels of the future

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During the last 20 years, the US biofuel industry grew at a faster rate than almost any other manufacturing industry, providing home-grown fuels, jobs, and rural wealth. In 2012, the industry is at a critical stage, faced with diminishing government incentives, higher grain and other feedstock prices, and concerns over “fuel versus feed” issues. The Renewable Fuel Standard (RFS2) addresses some of these issues by placing a cap on the amount of conventional biofuel (corn ethanol) we can use and by mandating increasing use of “advanced” and “cellulosic” biofuels made from non-food feedstocks.
How can we make these advanced and cellulosic biofuels in an environmentally and economically sustainable manner? The key is in efficiently converting the carbohydrates in biofuels feedstocks into both fuels and higher value coproducts. The sustainable biorefineries of the future will use chemical, biochemical, and thermochemical processes to produce “drop-in” fungible fuels and numerous coproducts. Several examples will be given.

CARB 25

Carbohydrate-mediated integrated biorefineries: Optimizing our use of biomass to help address the resource needs of a burgeoning global population

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Products from agricultural, forestry and industrial processes, and their waste streams, and also food processing wastes are significantly underutilized sources for production of food, feed, fuel, chemicals and materials. The state-of-the-art of new knowledge translating to practical applications with emphasis on the carbohydrate reactions with enzymes and microbial catalysts is reviewed. The complex aspects of the emerging bio-industry, emphasize sustainable cellulosic biofuels and co-products, as exemplified in integrated biorefineries, and by the grand challenges, realities, and opportunities presented going forward. Real world, current day practices and academic world, knowledge-building technologies are woven together in this mosaic. The topic is important, timely, complex and controversial. Is it “food or fuel”? “food and fuel”? or “food and feed and chemicals and materials and energy”?

CARB 26

Carbohydrate chemistry underpinning the development of commercially viable processing technologies for the production of industrial sugar feedstocks from sweet sorghum and sugarcane

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New fermentation organisms hold tremendous potential for the production of biobased fuels, chemicals, and materials from sugar feedstocks (Top Value Added Chemicals from Biomass, Vol 1, DOE, 2004). Novozyme's CEO Steen Riisgaard recently said "in a few years sugar will be the new oil" as sugar is a superb feedstock for the production of platform chemicals. While leading projects have been announced in Brazil, several private-sector groups in the U.S. are pursuing development of new domestic industrial sugar feedstocks to supply the anticipated bioprocessing demand. Sugarcane (Saccharum officinarum) and, in particular, sweet sorghum (Sorghum bicolor) have been widely recognized as promising sugar feedstock crops because they are amongst
the plants giving the highest yields of carbohydrates per hectare and easily available. Sweet sorghum’s easy cultivation from seed, low input requirements, and wide geographic suitability across the U.S., plus the current high price of sugar from sugarcane, have put more focus on sweet sorghum as a sugar feedstock. Typically, sweet sorghum has higher fiber and reducing sugar contents and a different impurity profile relative to sugarcane; these properties make extraction and processing more challenging, although there are some relative advantages as well. Three fundamental processing areas have been identified by industry for the large-scale manufacture of liquid biofuels and chemicals from sweet sorghum. These are (1) clarification of the raw juice that will make it suitable for concentration and/or fermentation; (2) stabilization of juice or partially concentrated juice for cost-effective seasonal use; and (3) concentration of the juice into syrup for storage, year-round supply, and efficient transport. The carbohydrate chemistry underpinning the development of commercially viable processing technologies of stable sweet sorghum juice and syrup feedstocks is complex and will be discussed in detail.

CARB 27

Chemical glycomics of Toxoplasma gondii and Plasmodium falciparum infections

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Toxoplasma gondii is the causative agent of toxoplasmosis while Plasmodium falciparum causes the most lethal form of malaria. T. gondii is unique as it can invade virtually any nucleated cell, although the mechanisms are not completely understood. Parasite attachment to the host cell is a prerequisite for reorientation and penetration and likely requires recognition of molecules at the host cell surface. It has been reported that the affinity of tachyzoites, the invasive form of T. gondii, for host cells can be inhibited by a variety of soluble sulfated glycosaminoglycans, such as heparin sulfate. Here, we report that protein components of the parasite rhoptry, dense granule and surface bind glycosaminoglycans.

Plasmodium falciparum merozoites contain complex GPI anchors that have been identified as key toxins in malaria pathogenesis. This lecture will explore the role of GPI anchors during the infectious process and their connection to malaria symptoms such as fever and anemia using synthetic glycans as tools.

CARB 28

HIV-1 Inhibitors from nature: Understanding and optimizing potent cyanobacterial carbohydrate-binding proteins

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Carbohydrate-binding proteins (CBP) can be isolated from a variety of species, including plants, invertebrates, vertebrates and prokaryotes. Some show highly specific recognition for mannose structures present on the HIV-1 glycoprotein gp120 envelope, resulting in remarkable anti-HIV activity.

Cyanovirin-N (CVN) and its homolog microvirin-N (MVN) from cyanobacteria exhibit potent antiviral activity in nanomolar range. Studies on CVN revealed two carbohydrate-binding sites, only one is functional present in MVN. With a much better clinical safety profile for MVN, we propose two strategies to increase MVN's antiviral potency. The first is through installation of a second carbohydrate-binding site and the second by creating an obligate domain-swapped dimer of MVN both through homology modeling and site-directed mutagenesis. Preliminary results indicate that one MVN mutant is properly folded with a second carbohydrate binding-site and another MVN mutant exists predominantly as dimer. In future studies we will evaluate the antiviral and cytotoxic activities of these mutants.

CARB 29

Synthetic HIV-1 glycopeptides for characterization of neutralizing epitopes

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Characterization of the fine epitopes for broadly neutralizing antibodies (bNAb) is an important step for the design of an effective HIV-1 vaccine. Recently, a new class of bNAb, including the somatic variants PG9 and PG16 and the PGT series (PGT121-137 and PGT141-145) antibodies has been isolated from HIV-infected “elite controllers”. These antibodies neutralize primary HIV-1 strains across clades with remarkable breadth and potency. Initial studies on the potential epitopes have revealed an interesting common feature of antigen recognition by these antibodies: they all target quaternary structures at the variable domains (V1/V2 and/or V3 regions) of gp120 and their neutralizing activities are glycan-dependent. These studies implicate that novel variable domain glycopeptides may constitute the epitopes for these bNAb. For further characterization of the fine epitopes, we initiated a project on synthesis of a series of homogeneous glycopeptides of the V1/V2 and V3 domains. These HIV-1 glycopeptides were synthesized using a combined chemical and enzymatic method, with installation of structurally well-defined N-glycans at the conserve glycosylation sites along the polypeptide chain. The synthetic challenge, as well as the use of the synthetic glycopeptides for glyco-pepscan of neutralizing epitopes and for structural studies, will be discussed.

CARB 30
Design of HIV mimotopes by directed evolution of DNA-supported glycoclusters

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2G12 is one of few protective and broadly-neutralizing antibodies which bind to a cluster of high-mannose glycans on the HIV envelope protein gp120. Efforts to raise 2G12-like antibodies against rationally-designed synthetic mimics of this glycocluster have so far met with little success. We describe an alternative approach to design of improved glycocluster antigens, in which DNA-supported clusters of glycans are subjected to evolutionary pressure by selection with 2G12, thereby increasing their gp120-like character and increasing the likelihood that a 2G12-like antibody response can be elicited.

CARB 31

Glycan array for study of protein-ligand interaction and drug discovery

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This lecture will present new development of glycan array and its application to the study of multivalency, heteroligand binding and specificity of protein-ligand interaction, including the analysis of influenza subtypes, specificity of antibodies against HIV and breast cancer sugar epitopes, and development of vaccines against influenza and breast cancer.

CARB 32

Exploring nature’s strategies for making unusual sugars

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Many naturally occurring bioactive secondary metabolites possess unusual sugar moieties that serve as molecular recognition elements critical for biological activities. Altering the sugar substituents of these compounds holds considerable potential for modulating their pharmaceutical properties. Studying the biosynthesis of these sugars is not only important for understanding the chemistry underlying the responsible enzymes, but will also assist us in the production of these and other sugar molecules with improved biological activities. To reach these goals, we have investigated the
biosynthesis of unusual sugars found in a variety of natural systems. We have established several of the biosynthetic pathways of these sugars and characterized the key enzymes involved in their formation. Some recent results of these efforts will be presented.

CARB 33

Combining selective acetylation and glycosyl iodide cyclic ether glycosylation to achieve brief syntheses of functionalized oligosaccharides

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Oligosaccharides are known to be important in cell-to-cell communication processes that initiate many biological processes. Isolating biologically relevant carbohydrates from biological sources is challenging - as is the chemical synthesis, which inevitably requires multiple steps and protecting group manipulations. A methodology of selective acetylation has been developed to shorten the synthesis of complex oligosaccharides. The reaction uses common chemical reagents under microwave assistance to generate partially acetylated building blocks, which otherwise would take many steps to synthesize via conventional methods. Glycosyl iodides exhibit good reactivity and selectivity during the glycosylation reactions. When incorporated with the reaction of introducing cyclic ethers to the anomeric position, functionalized oligosaccharides can be prepared in a relatively short synthetic route and reasonable yields.

CARB 34

Chemoenzymatic methods for synthesizing complex carbohydrates and glycoconjugates

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Carbohydrates and glycoconjugates are biologically and pathologically important molecules. Among different modern synthetic strategies, the application of glycosyltransferases and other related carbohydrate biosynthetic enzymes in producing these complex biomolecules has great advantages. We have developed several efficient one-pot multienzyme systems for synthesizing complex carbohydrates and glycoconjugates including those containing naturally occurring and non-natural carbohydrate modifications. These include one-pot multienzyme sialylation, fucosylation, galactosylation, N-acetylgalactosaminylation, N-acetylgalactosaminylation, and glucuronylation processes. Identification of carbohydrate biosynthetic enzymes or generation of their mutants with good solubility, high expression level in E. coli, high activity and broad substrate specificity allows the activation and transfer of modified or unmodified monosaccharides to take place easily for preparative and large-scale synthesis of complex oligosaccharides, glycolipids, and glycopeptides containing. These
compounds are essential probes for carbohydrate-binding proteins and potential prebiotics and therapeutics.

CARB 35

Predicting RNA structure and stability

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RNA has a large number of vital functions in the cell and sequencing of genomes is providing a huge database of RNA sequences. Experimental results can be combined with thermodynamics to help decode this database into secondary structures that identify targets for therapeutics. NMR can rapidly provide constraints for algorithms that predict secondary structure, including pseudoknots. Secondary structures provide foundations for predicting 3D structures. Force fields that approximate interatomic interactions facilitate prediction of 3D structures. Benchmarks will be presented that test the accuracy of force fields. Applications of the pipeline from genome sequence to RNA structure for influenza RNA will be described.

CARB 36

Carbohydrate-based nanotechnology

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Carbohydrates in form of glycoconjugates decorate the surface of cells and are key to many important biological recognition events. The placement of well-defined, synthetic glycans on surfaces can be used for delivery, molecular imaging and sensing applications. Described is a host of nanostructures equipped with synthetic cell-surface carbohydrates. Nanoparticles,¹ surfaces,² metallo-dendrimers³ and supramolecular assemblies⁴ not only possess interesting structural but also electrochemical and fluorescent properties. These novel structures were applied to diagnostic and imaging applications in vitro and in vivo. Reported are also molecular logic operations using such nanostructures.⁵

In addition, the use of continuous flow reactors to produce large quantities of nanoparticles will be discussed.⁶
Progress toward the rational design of small molecules targeting RNA

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Herein, we present studies aims at developing rational methods to target RNA with small molecules. This approach is centered on the development of a database of RNA motif-ligand partners that are identified through two-dimensional combinatorial screening. The information in this database is then mined against RNA sequence/secondary structures that cause disease. These studies have developed bioactive small molecules targeting the RNA that causes Myotonic Dystrophy.

Synthesis and flocculation property in dye solutions of β-cyclodextrin-acrylic acid-[2-(acyrloyloxy) ethyl] trimethyl ammonium chloride copolymer

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The β-Cyclodextrin (β-CD) is not a perfect flocculant of dyes unless it forms a long chain polymer and is abundantly charged as well. In aqueous solution a cationic copolymer, poly (β-CD-AA-DMC) was synthesized via free radical copolymerization of acrylic acid (AA) esterified β-CD (β-CD-AA), and a cationic monomer [2-(Acryloyloxy)ethyl] trimethyl ammonium chloride (DMC). The copolymer's structure, morphology and thermal stability were demonstrated by FT-IR, 1H-NMR, SEM and TGA analysis. The flocculation properties of the copolymer were evaluated by the decolorization of two reactive dyes'
solutions using a jar test method. The decolorizational efficiency is influenced by both the nature of the anionic dyes and the pH of the initial dye solutions. Electrostatic adsorption as well as bridging of polymer played a dominant role in flocculation of dyes. Moreover, the inorganic salt decreased the efficiency of color removal.

**CARB 39**

**Evaluation of an autoclaved cyclodextrin affinity-based drug delivery system for orthopedic implant infections**

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Orthopedic implants have a 0.5-5.0% bacterial infection rate, often caused by *Staphylococcus aureus*. These infections typically originate during implantation, but can become symptomatic late (up to 24 months after implantation). The formation of a biofilm, which prevents direct access to the infection, makes it difficult to treat these infections. Long-term controlled release of antibiotics from an orthopedic implant could treat initial infections, avoid late-onset infections, and reduce the amount of systemic antibiotics needed. We have achieved near-linear release for over 45 days using cyclodextrin (CD) crosslinked polymers. One barrier that remains is the application and effect of conventional sterilization techniques, such as autoclaving. Mechanical and chemical changes, such as swelling properties, were investigated to evaluate the effect of the autoclave process on the cyclodextrin-crosslinked polymer. Also changes in the loading capacity and release of antibiotics between autoclaved and nonautoclaved polymer were evaluated.

**CARB 40**

**Cyclodextrin-based nanocapsules with tailored antimicrobial star arms prepared by ATRP**

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Cyclodextrin (CD)-based star polymers with arm number from 5 to 10 were prepared via the copolymerization of an antimicrobial macromonomer bearing guanidine short chains and acrylamide (spacer) using atom transfer radical polymerization (ATRP). The structure of the star polymers was confirmed with ¹H-NMR and triple-detector GPC measurements. A corresponding relationship between the charge density of star polymers and minimal inhibitory concentration (MIC) against E.coli was established, which indicated that bacterial inhibition depends on the electrostatic interactions
between antimicrobial star arms and E.coli. The resulting star polymers created a synergetic antimicrobial effect, contributed from the antimicrobial star arms and the disinfectant (butyl paraben) loaded in CD nanocapsules. Apart from lowering MIC values from antimicrobial arms, the remarkable inhibition zone was observed in a ring diffusion test due to the release of disinfectant, thus enhancing the antimicrobial performance of the star polymer/nanocapsules when applied to various substrates, cellulose fibres in particular.

CARB 41

Starch nanocrystals preparation processes and influence of enzymatic pre-treatment

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Starch nanocrystals (SNC) are crystalline platelet resulting from the acid hydrolysis of starch. Promising results as reinforcing filler or barrier elements are achieved with such bio-nanopolysaccharide. However their preparation process is still a limiting factor. Therefore, an enzymatic pre-treatment of starch has been investigated to reduce the acid hydrolysis duration. A screening of three types of enzymes is proposed for a 2h pre-treatment. Porous starches are obtained and observed by SEM. AFM and X-ray diffraction measurements confirmed that the obtained nanoparticles after hydrolysis were SNC. Moreover, kinetic of pre-treated starch was much faster compared to the regular kinetics of hydrolysis for preparing SNC. The extent of hydrolysis normally reached in 24h was obtained after only 6h, and the regular final yield (15% after 5 days) was reached in 36h.

CARB 42

Superabsorbent hydrogel composite made of cellulose nanofibrils and chitosan-g-poly(acrylic acid)

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Superabsorbent hydrogel composites based on cellulose nanofibrils and chitosan-g-poly(acrylic acid) copolymer were developed. The FTIR data confirmed the copolymerization reaction and the formation of the composites. In addition, the XDR pattern indicated that the nanofibrils crystallinity was as high as 90 %. A $2^4-1$ factorial design was employed to evaluate the effect of acrylic acid/chitosan molar ratio, crosslinker, initiator, and filler in the swelling capacity of hydrogel composites. By the
analysis of variance (ANOVA), including F-test and P-values, it was found that the crosslinker and filler correspond to 40% and 30% of the evaluated response, respectively. The addition of nanofibrils improved the swelling capacity from 381 to 486 as well as shortened the time to equilibrium. SEM images showed that adding nanofibrils into the hydrogel matrix increased the average proe dimension. Finally, the swelling behaviors of the composites were responsive to changes in pH and salt concentration.

**CARB 43**

**Study of transfection efficacies of cationic glycopolymers of varying molecular weights, compositions, and architectures**

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The synthesis of well-defined polymers with pre-determined compositions and structures are essential in improving the transfection efficiencies. Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization technique allows successful and facile synthesis of cationic glycopolymers of varying compositions, architectures and molecular weights, in the absence of protecting group chemistry. The linear (block and statistical) and hyperbranched (statistical) cationic glycopolymers of pre-determined molar masses and narrow polydispersities ranging from 3-60 kDa has been synthesized using RAFT polymerization technique. These polymers differ from each other in their architectures (block versus random, linear versus hyperbranched), molecular weights, and monomer ratios (carbohydrate to cationic segment). It is shown that the above-mentioned parameters can largely affect the toxicity, DNA condensation ability and gene delivery efficacy of these polymers in different cell lines. Linear statistical copolymers of high degree of polymerization show superior gene expression than their block analogues. The transfection efficacies of branched copolymers are better than linear copolymers. These statistical copolymers show lower toxicity and higher gene expression in the presence and absence of serum. This is the first example of well-defined synthetic glycopolymers of varying architectures as DNA carriers that works both in the presence and absence of serum proteins.

**CARB 44**

**Sugar based initiators for the cationic ring opening polymerization of 2-ethyl-2-oxazoline**

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Glucose (Glc), galactose (Gal) and fructose based tosylates and triflates were applied as initiators for the living cationic ring opening polymerization (CROP) of 2-ethyl-2-oxazoline (EtOx). In particular, the Glc and Gal triflates induced fast initiation of the CROP as well as a linear increase of the molar mass with conversion. Well-defined Glc as well as Gal a-end functionalized PEtOx was obtained after deprotection. Functionalization of the living oxazolinium chain ends with methacrylate anions resulted in a macromonomer that was applied for the synthesis of a comb polymer being selectively functionalized with Glc at the ends of all side chains via RAFT polymerization. This new general synthetic pathway and the biocompatibility of PEtOx offer the opportunity to benefit from both, the dual responsive properties of the carbohydrate functionalities in drug delivery applications.

CARB 45

**Electro-spinning of alginate and PVA biopolymers to produce cross-linked nanofibrous scaffolds for tissue engineering**

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The objective of this project is to develop electrospun nanofibrous scaffolds for tissue engineering by blending biodegradable polymers such as sodium alginate and polyvinyl alcohol in various ratios and also increase the structural integrity of these fibers by cross-linking them ionically or covalently. The crosslinking is achieved by using glutaraldehyde vapor in various concentrations. The durability experiments showed that nanofibers retained their structural integrity over a long period of time in saline solution. The chemical, physical, and mechanical properties of the biodegradable polymers are investigated.

![Before cross-linking](image1.png) ![After cross-linking](image2.png)

CARB 46

**Preparation and characterization of 3,6-O-Chitosansulphonate**

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A special scope in working with the polysaccharide chitosan was the preparation of 3,6-O-Chitosansulphate to get a structural analogue of the natural blood anticoagulant heparin. Antithrombotic properties similar to that of heparin, could be found for 3,6-O-Chitosansulphate when the concentration of sulphur is high enough. These results were recently reported by our research group (Fasl et al; 2010). Based on the analytical data, substitution patterns for the sulphate groups at the C-6 and/or the C-3 position of the glucosamine unit were suggested, whereas the C-2 position with the amino group should remained unmodified. New results achieved from characterization of 3,6-O-Chitosansulphate in water solution at pH 7 with different titration methods demonstrated the full accessibility of the unmodified primary amino groups but only moderate access to the sulphate groups. Preparation and characterization of 3,6-Chitosansulphate in solution by titration techniques will be shown in detail.

CARB 47

Fabrication of starch film as packaging material: Limitation of applications and approaches to overcome

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Much effort has recently been made to develop environment-friendly biodegradable materials. We made an approach to fabricate polysaccharide (starch) based thin film alternative to conventional packaging material. We prepared starch film by blending technique. Mechanical, thermal and water uptake of the film were investigated. Physical (gamma and UV radiation) and chemical (various monomers) methods were applied to increase the film properties. It was found that both physical and chemical methods had a significant effect on the film properties up to a certain limit. However, applications are limited by factors that include moisture sensitivity. The talk will also give three hypotheses to sort out this problem. Solutions to these issues would open up new markets for the technology, and could allow for these packing materials to be used with high water content products.

CARB 48

On renewable film made from spruce glucomannan
Film materials are wildly used for packaging and coating. Petroleum- and aluminum-based films are now dominating in the fields. To prepare renewable and green film materials, one water-insoluble glucomannan (GM) has been prepared from spruce wood holocellulose and casted to films from different solutions (chloroform, ammonia, DMSO-pyridine) with or without derivatization by e.g. acylation. The GM, obtained in a yield of 6.7% by NaOH/H$_3$BO$_4$ extraction and Fehling reagent precipitation, is composed of galactose, glucose and mannose in a ratio of <0.1:1:3.5. It is large in molecular size ($M_p \sim 27kDa$), almost free from other polysaccharides such as xylan (0.61%) and free from metal ions (<0.2 % ash content). So far biopolymer thin films have been spinning-coated from ammonia solution of un-derived GM. To evaluate the application potentials, the films formed are being systematically characterized for smoothness, mechanical strength, oxygen and water permeability, thermal plastic properties, transparency, and UV absorption.

CARB 49

Design and evaluation of DNA minor groove targeting two-site compounds

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Synthetic compounds that can regulate cell function in a desired fashion are a central goal in chemical biology and offer advantages in development of new drugs. We focused in this work on the design of relatively simple, cell-permeable compounds to selectively target at least 10 base pair DNA sequences. Compounds with one or two linked amidine-phenyl or amidine-benzimidazole-phenyl units to specifically target closely spaced AT sequences with intervening GCs were prepared. Compound interactions with DNA were evaluated with an array of biophysical methods. Issues addressed in this presentation are (i) DNA binding affinity, kinetics, stoichiometry and mode; (ii) binding to single versus two sites; (iii) correlations between heterocycles and linker length with the number of base pairs between the binding sites; (iv) thermodynamics and kinetics of binding. The results presented here are surprising and show that a small set of initial compounds has three different binding modes with DNA.

Supported by NIH

CARB 50
Structural and in vitro mechanistic studies of sugar modified siRNAs to probe molecular recognition and cleavage events relevant to gene silencing functions

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The efficacy of our siRNA mimics has now been well documented. However, important aspects of their structure, enzyme interactions, and mechanisms of action remain to be elucidated. In this regard, I will provide an overview of recent work in my laboratory and those of collaborators concerning [1] Structural NMR studies to determine the solution structure of arabinose and ribose modified duplexes previously observed to have potent gene silencing activity in multiple gene silencing assays; [2] NMR titration experiments and crystallographic/NMR techniques to investigate the binding affinities and binding conformations of chemically modified guide strands within the 5' binding (MID) domain of the Ago2 component of RISC. These studies make use of fluorinated dimer compounds as mimics of the larger and more complex modified siRNA guide strands; and [3] Cleavage assays with purified hAgo2 to determine the fate of the modified siRNA passenger strands upon siRNA uptake by RISC. We believe that insights gained from these studies will directly serve to guide optimization of existing compound designs to improve biocompatibility and potency. This research is funded, in part, by the Canadian Institutes of Health Research.

CARB 51

Direct observation of single molecular event in DNA origami frame

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The DNA origami method developed for the preparation of fully addressable two-dimensional (2-D) structures has been utilized for the selective positioning of the functional molecules and nanoparticles and for the design of various 3-D architectures. We recently developed "DNA frame" using the DNA origami method to examine enzymatic action. In our DNA frame, tensed and relaxed dsDNA can be created by bridging the defined length of dsDNA in the DNA scaffold. The relaxed strand can accommodate the enzymes to bind and bend the target sequences. On the other hand, the tensed strand allows binding of these enzymes, while this strand is a poor substrate for bending, resulting in the lower reaction efficiency. In addition, the DNA frame is valuable for analyzing the motion of the enzyme because of the defined coordinated space. In this presentation direct observation of DNA modifying enzymes will be presented.
Shape recognition of duplex DNA by carbohydrates

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Shape recognition of DNA plays an important role in determining DNA-protein specificity. We have incorporated such shape-specific principles in our design of DNA binding ligands. This presentation will discuss recent progress in our approach to target the major groove of DNA using carbohydrates. Approaches to target different conformations (A and B form) of duplex DNA will be presented. While there have been many efforts to recognize B-DNA in the minor groove, major groove recognition of A or B-DNA by small molecules has eluded us. Our approach opens up complementary methods for targeting DNA sequences. When combined with existing approaches, these are expected to lead to small molecules with higher affinity and selectivity that can directly compete with protein binding in the major groove.

Design, synthesis, and structure regulation of small molecules

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We have obtained a series of small compounds and fully characterized their structures. These compounds can induce the structure change of nucleic acids. Some of them can regulate the structures of nucleic acid from double strand DNA to G-quadruple DNA. Some of them can regulate the structure change of nucleic acid from B-DNA to Z-DNA. Further experiments provide strong evidence to support these results.

Formation of the aminyl radical via one electron attachment to 2'-azido-2'-deoxyuridine and methyl 2-azido-2-deoxy-α-D-lyxofuranoside and subsequent reaction to sugar radical

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We investigated electron attachment reactions with 2'-azido-2'-deoxyuridine (2'-N₃U) and methyl 2-azido-2-deoxy-α-D-lyxofuranoside (2-N₃lyxose) in γ-irradiated aqueous glassy (7.5 M LiCl) systems. The ESR studies showed that the predominant site for the electron attachment is the azide group resulting in the formation of an unstable azide anion radical intermediate (RN₃•-). Loss of N₂ from RN₃•- followed by protonation of
nitrene anion radical (RN•−) gave the neutral aminyl radical (RNH•). Even at 77 K, neither RN3•− nor RN•− intermediates were detected, only the ESR spectra of the neutral aminyl radical was observable. A set of [2H]-labeled 2'-N3U (C2', C3', C4', C5 and C6) and 2-N3lyxose (C5 and OMe-d3) isotopomers and [15N]-azido labeled probes were prepared to characterize radical species produced by bimolecular H-atom abstraction reactions of the neutral aminyl radicals. The aminyl radical formed in 2'-N3U and 2-N3lyxose labeled with [15N], results in a hydrogen abstraction to produce a carbon-centered radical at C5' of nucleoside or C5 of sugar.

**CARB 55**

**Sequence-unrestricted targeting of double stranded DNA (dsDNA): How to "unlock" the dsDNA-targeting potential of invader LNAs**

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Development of molecular tools that enable modulation of gene expression through specific targeting of double stranded DNA (dsDNA), is required to accelerate gene function studies and potentially identify novel classes of therapeutic agents against diseases of genetic origin. Conventional probe technologies such as triplex forming oligonucleotides and peptide nucleic acids experience sequence limitations and low target affinity.

We have recently introduced Invader LNAs (Locked Nucleic Acids) as a radically different approach towards this goal. Invader LNAs are short energetically activated DNA duplexes that are chemically modified with 'energetic hotspots' of intercalator-modified LNA monomers. Both probe strands display extraordinary thermal affinity toward DNA complements, which provides an energetic gradient for specific recognition of mixed sequence dsDNA regions.

In this presentation, I will outline the Invader LNA concept, discuss biophysical characteristics of these probes, disclose results from dsDNA-recognition experiments and introduce simple functional mimics of Invader LNA.

**CARB 56**

**Heterobifunctional ligands to bind and neutralize the *E. coli* shiga like toxin, Stx2**

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Two types of Shiga toxins have been shown to be the major causative agents of Hemolytic Uremic Syndrome caused by the Shiga toxin-producing bacteria *E. coli* O157:H7, Shiga toxin type 1 (Stx1) and Shiga toxin type 2 (Stx2). *E. coli* expressing the latter are reported to be of greatest clinical significance. A crystal structure for Stx1 with bound ligand has facilitated design of STARFISH and PolyBait molecules, which afford protection against the effects of this toxin. However, the crystal structure of Stx2 has only been solved in the absence of bound ligand and neither of the aforementioned ligands affords protection against Stx2. We report a series of studies to correlate the different ligand preferences of Stx2 and an approach for the design and evaluation of heterobifunctional ligands that neutralize Stx2.

**CARB 57**

**Qβ virus-like particle carriers for carbohydrate conjugate vaccines**

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Unique polysaccharides decorating the surface of microbial pathogens are an attractive target for vaccine design. The development of polysaccharide vaccines is hindered by low immunogenicity of carbohydrates. Conjugate vaccines, where glycans are covalently attached to a carrier protein, fully engage the immune system and can generate potent and long-lasting antibody responses. Choice of both protein carrier and adjuvant determines the effectiveness of the carbohydrate vaccine.

We explored the potential of virus-like particles (VLPs) derived from bacteriophage Qβ as carbohydrate vaccine carriers. VLPs are inherently immunogenic and possess the kind of repetitive structure that is optimal for polyvalent display of glycan antigens. We used the copper-catalyzed azide-alkyne cycloaddition reaction to load Qβ VLPs with multiple copies of synthetic oligosaccharides specific for *Streptococcus pneumoniae*, and immunized mice with the resulting conjugates. The role of formulation components and parameters such as glycan loading in the generation of strong and specific anti-carbohydrate immune responses will be discussed.

**CARB 58**

**Sugars and proteins**

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Sugars and post-translational modifications are critical biological markers that modulate the properties of proteins. Our work studies the interplay of proteins, sugars and modifications. This lecture will cover emerging areas in our group in (a) biomolecule construction with an emphasis on new bond-forming processes compatible with biology (Topics (i), (iii)) and (b) use of chemical probes & modulators of carbohydrate & protein processing events (Topic (iii)). (i) Synthetic Biology's development at the start of this century may be compared with Synthetic Organic Chemistry's expansion at the start of the last; after decades of isolation, identification, analysis and functional confirmation the future logical and free-ranging redesign of biomacromolecules offers tantalizing opportunities. New methods are required: despite 80-years-worth of non-specific, chemical modification of proteins, precise methods in protein chemistry remain rare. The development of efficient, complete, chemo- & regio-selective methods, applied in benign aqueous systems to redesign the structure and function of proteins will be presented. (ii) 'Synthetic Biologics' and their applications in areas including drug delivery; probes of in vivo function; non-invasive pre-symptomatic disease diagnosis; targeted high intensity radioprobes; and designed glycoconjugate vaccines. (iii) Chemical Probes and Modulators: Delineation of mechanisms of carbohydrate- and protein-processing systems allows not only an understanding of their fundamental role in Biology and Immunology but also the use of molecules to modulate and manipulate such processes, with a strong associated potential for diagnosis, therapy and intervention.

CARB 59

Development of multivalent entry inhibitors of Norovirus infections from fragment-based screening

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Noroviruses are the major cause of non-bacterial gastroenteritis and no direct treatment or vaccines currently exist. To characterize their natural receptor determinants, we applied nuclear magnetic resonance (NMR) techniques to investigate the recognition of histo-blood group antigens. These terminal carbohydrate structures on glycoproteins or -lipids serve as attachment factors or receptors for the virus to enter the host cells. Following the resulting description of the interaction at atomic resolution we identified a conserved sub-pocket that was then the target of NMR screening. Low molecular weight binders of the receptor recognition site of virus-like particles of the GII.4 Norovirus Ast6139 were identified. On the basis of these NMR experiments a heterobifunctional ligand was then designed and forms the basis for highly potent, multivalent entry inhibitors. The benefits of using entire virus-like particles for the investigation of the receptor specificity and their application towards the rational design of entry inhibitors are discussed.

CARB 60

Synthesis and biological evaluation of cholesteryl glycosides correlated with H. pylori infection

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*Helicobacter pylori* is a gram-negative pathogen infecting more than one half of the world's population. The pathogen is well known as the major cause of various gastric diseases such as chronic gastritis, peptic ulcer and gastric cancer. Although cholesterol alpha-glucoside and its derivatives are present in high percentages of the bacterial membrane, *H. pylori* does not synthesize cholesterol but extracts it from the plasma membranes of human gastric mucosa cells. Cholesterol alpha-glucosyltransferase from *H. pylori* is the enzyme to catalyze the formation of cholesterol alpha-glucoside. The enzyme product, cholesterol alpha-glucoside, was shown to play an important role in preventing the immune evasion because it was found to inhibit the phagocytosis of macrophage and subsequent T cell activation. To better understand these host/pathogen interactions, a program dedicated to the stereoselective synthesis of steroidal glycosides has been initiated. Glycosyl iodides have been used to control the stereoselectivity about the anomeric center to generate cholesteryl derivatives important to these investigations. The products were then employed as standards to characterize these cholesterol derivatives and evaluate their expression levels. Interestingly glucosidase activity was discovered to degrade some of the cholesterol glucosides.
Toward a fundamental understanding of asymmetric phase transfer catalysis through chemoinformatics

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Although asymmetric phase transfer catalysis has been known and practiced for over 25 years, the fundamental features of what constitutes reactive and selective phase transfer catalysts are still unknown. This lecture will describe a multifaceted program designed to learn the "rules" that govern rate and enantioselectivity for simple phase transfer catalyzed alkylation reactions.

The approach involves the creation chiral quaternary ammonium salts by implementation of the tandem [4+2]/[3+2] cycloaddition of nitroalkenes. The rigid, polycyclic amine scaffolds are embellished with a variety of substituents in a convergent region of space using parallel synthesis methods to generate libraries of ammonium ions.

The chiral ammonium salts are evaluated for their catalytic potential by standard kinetic and analytical methods. Next, a Quantitative Structure-Selectivity Profile is developed to explain the roles of the different substituents so that the most important controlling features can be systematically identified and their properties incorporated in designs for more reactive and selective catalysts.

Studies of complex reactions with peptide-based catalysts

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David Gin had an affinity for complex problems and a gift for making great progress in the face of daunting challenges. My lab's research benefited from heartfelt encouragement from Dave over our years together as colleagues that began our independent academic careers in the same year, 1996. My lecture will endeavor to share the results of our studies of catalytic reactions in the settings presented by complex natural products. A particularly focus will be on the general challenge of "site-selectivity," so prominent in complex carbohydrate synthesis. Our endeavors to functionalize unique functional groups within molecules that present arrays of multiple, similar sites, will be discussed.

Diversity and design: New chemical probes for biology and medicine
The identification of new, highly specific ligands for biological targets remains a significant challenge in chemical biology and drug discovery. We are engaged in a two-pronged approach to this problem involving both diversity-oriented synthesis and rational design. In the area of diversity-oriented synthesis, we use structural motifs from natural products as starting points for library design. New synthetic routes are developed to provide flexible, efficient, systematic access to these structures. The resulting libraries access underexploited regions of chemical space and are being screened against a wide range of targets. In the area of rational design, we are using a natural product-based sulfonyladenosine design platform to develop selective inhibitors of various enzymes that catalyze adenylation reactions, including novel antibiotic and anticancer targets. We leverage multidisciplinary collaborations with biologists to evaluate these molecules with the long-term goals of probing key biological processes and exploring new therapeutic opportunities in cancer and infectious diseases.

**CARB 64**

**Stereoselective reactions of oxocarbenium ions: Structure and reactivity of highly reactive intermediates**

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The structures of oxocarbenium ions related to carbohydrate systems are not controlled exclusively by steric effects. Both stereoselectivities and spectroscopic analysis show that electronic effects exert powerful influences on the conformational preferences of these cations. When the oxocarbenium ion is substituted with alkoxy groups, axially substituted conformers are generally favored. This preference appears to be the result of attractive electrostatic effects between the partially negatively charged substituent and the cationic carbon atom. The reactions of these oxocarbenium ions, which generally conform to stereoelectronic models, can be more difficult to analyze than might be expected, particularly with highly reactive nucleophiles like alcohols.

**CARB 65**

**Stereocontrolled glycosidic bond formation: A challenge and an inspiration for organic chemists?**

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Problems inherent in the stereocontrolled synthesis of glycosidic bonds and their surrogates will be discussed. Approaches to the solution of such problems from the author’s laboratory will be presented as will their applications in synthesis.
**CARB 66**

**Advances toward affinity proteomics strategies to target carbohydrate dependant host pathogen interactions**

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Glycoconjugate recognition events between host and pathogen are among the first interactions en route to infection. Identification of the pathogenic factor or factors that recognize host glycoconjugate entities can lead to a better understanding of these events and provide valuable drug targets. Here we present our progress towards the development of affinity proteomics approaches to insolate and identify these pathogenic factors. Development of these methods to suit model pathogenic interactions and those in the surrogate host Caenorhabditis elegans will be discussed. Key chemical strategies and associated mass spectrometry based approaches will be presented.

**CARB 67**

**Glyco-nanoparticles, a tool for cancer cell profiling and molecular imaging**

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Carbohydrates are ubiquitous in nature. Many cellular interactions involve the binding of carbohydrates and glyco-conjugates. In this talk, we present our work in combining biological recognitions of carbohydrates with the properties of magnetic nanoparticles for in vitro and in vivo detections.

Our in vitro studies were focused on profiling of cancer cells. The development of simple and effective techniques to delineate the fine characteristics of cancer cells can have great potential impacts on cancer diagnosis and treatment. We will discuss the results of using a magnetic glyco-nanoparticle (MGNP) based nanosensor system not only to detect and differentiate cancer cells but also to quantitatively profile their carbohydrate binding abilities by magnetic resonance imaging (MRI). Using an array of MGNPs, a range of cells including closely related isogenic tumor cells, cells with different metastatic potential and malignant vs normal cells can be readily distinguished based on their respective “MRI signatures”.

For our in vivo studies, we aimed at developing MGNPs for molecular imaging of vascular inflammation and atherosclerosis. Cardiovascular diseases, often associated with inflammation and atherosclerosis, are the leading cause of death and disability in the world. CD44 is a cell surface receptor expressed on three major cell types present in the atherosclerotic plaques, i.e., vascular endothelial cells, macrophages and smooth
muscle cells. Multiple studies have suggested that CD44 promotes atherosclerosis by mediating inflammatory cell recruitment and vascular cell activation. In order to detect the presence of CD44 in atherosclerotic plaques \textit{in vivo}, we have synthesized magnetic nanoparticles with hyaluronic acid (HA) immobilized on the external surface. The \textit{in vitro} and \textit{in vivo} imaging of atherosclerotic plaques using the HA immobilized magnetic nanoparticles will be presented.

\textbf{CARB 68}

Uncovering new functions for carbohydrates in neurobiology and cancer

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Understanding the remarkable complexity of the brain on a molecular, cellular and systems level is one of the major challenges in science. The principles and tools of chemistry, when combined with biology, can be used to gain new insights into the molecules and interactions involved in cellular communication and memory storage. We will describe the synergistic application of chemistry and biology to explore the structure and function of carbohydrates and their impact in various biological contexts, including neuronal communication, long-term memory and cancer.

\textbf{CARB 69}

Glycomics of disease via lectin microarrays

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This paper will focus on lectin microarray technology and its applications to understanding disease states.

\textbf{CARB 70}

Synthesis of rare sugars by in vitro and in vivo aldolase reactions

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The high stereoselectivity of aldolases in C-C bond construction confers upon them tremendous applications as synthetic biocatalysts. Among the aldolases, dihydroxyacetone phosphate (DHAP)-dependent aldolases are particularly attractive as a set of four possible diastereomers of vicinal diols can be synthesized conferring upon them the potential to be used in the synthesis of rare sugars and other hydroxylated
natural products. Unfortunately, the strict requirement for the donor substrate DHAP, a rather expensive and unstable compound, limits aldolase use in large-scale preparation. Therefore, the capability to generate DHAP from inexpensive sources could ultimately broaden the scope of aldolase reactions making it an attractive challenge.

We employed DHAP-dependent aldolases in the synthesis of several rare sugars via a one-pot four enzyme reaction system in which DHAP is generated from the oxidation of cheap DL-glycerol 3-phosphate. Subsequently, the DHAP generated in situ is coupled with glyceraldehydes by aldolases to give different rare sugars. More significantly, we used engineered bacteria as 'chemists' to carry out the aldolase reactions in vivo. The bacteria were fed with green starting materials (e.g. glucose) and DHAP is generated in vivo via bacterial glycolysis. Desired rare sugars could thus be produced by simple fermentation and isolated from the culture media.

CARB 71

One-step synthesis of glucosamine-grafted maleic anhydride copolymers and their biodegradation

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Glycopolymers are polymers that have carbohydrate pendant groups incorporated into their backbone. They may play an important role in a wide range of biomolecular events such as cellular recognition, adhesion, cell growth regulation, cancer cell metastasis, and inflammation. Carbohydrates fixed within a glycopolymer structure are more efficient than free carbohydrates due to multivalent effect of clustered saccharide. In this work, glucosamine has been grafted to copolymers containing maleic acid blocks by amide linkage. The structure of the products was confirmed by FTIR and $^1$H-NMR and the molecular weight was determined by GPC. Elemental analysis as well as free amine detection by 2,4,6-trinitrobenzenesulfonic acid were used to determine the amount of glucosamine grafted onto the copolymers. A biodegradation method following the ISO standard procedure was developed and used to quantify the decomposition by microorganisms.

CARB 72

Highly efficient one-pot multienzyme system for the synthesis of UDP-GLC(GAL)(MAN) and derivatives

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Glycosyltransferases are key enzymes for creating diverse arrays of carbohydrate structures that mediate many biological processes. Many glycosyltransferases that are responsible for the synthesis of carbohydrate-containing structures require uridinediphospho-sugar donors. In attempts to explore the promiscuity of various glycosyltransferases toward their UDP-sugar donors, a highly efficient one-pot multienzyme system containing a uridylyltransferase, an inorganic pyrophosphatase, and/or a kinase is developed and applied to quickly attain structurally defined UDP-glucose, UDP-galactose, UDP-mannose, and their derivatives from a free monosaccharide or commercially available inexpensive monosaccharide-1-phosphate. These sugar nucleotides have been used as donor substrates of several glycosyltransferases for the synthesis of complex carbohydrates.

Structure and physicochemical properties of Arundo donax hemicelluloses

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Arundo donax hemicellulosic subfractions were obtained by extraction with dimethyl sulfoxide (DMSO), saturated Ba(OH)₂, and 1.0 M aqueous NaOH, and fractionation with gradient 50% and 80% saturation ammonium sulphate precipitation. The detailed structural characteristics of hemicellulosic subfractions were determined by HPAEC, GPC, FT-IR, and 1D (¹H and ¹³C) and 2D (HSQC) NMR. The results revealed that Arundo donax hemicelluloses had considerable heterogeneity, varying in sugar composition, molecular weight, and structural features. The hemicellulosic subfractions precipitated with the lower saturation level of ammonium sulphate (50%) were lesser substituted hemicelluloses as compared with those subfractions obtained with a higher salt concentration (80%). The hemicellulosic subfractions exhibiting higher molecular weights could be precipitated at the lower concentration of saturated ammonium sulphate. FT-IR and NMR analysis indicated that the highly acetylated hemicelluloses were precipitated at a relatively lower concentration of saturated ammonium sulphate (50%), and thus the gradient ammonium sulphate technique can discriminate acetyl and non-acetyl hemicelluloses. The DMSO-soluble hemicellulosic subfraction H⁰₅₀ precipitated by 50% saturated ammonium sulphate mainly consists of low substituted O-acetyl arabinogalactan-(1→4)-β-D-xylan, in which 4-O-Me-α-D-GlcpA-(1→2) units was linked at position O-2, Araf residues at O-3, and O-acetyl groups at O-2 or O-3. The DMSO-soluble hemicellulosic subfraction H⁰₈₀ precipitated by 80% saturated ammonium sulphate is mainly composed of highly substituted arabinogalactan-(1→4)-O-methylglucuronoxylan and β-D-glucans.
Production of macro-algae (*Capsosiphon pulvescense*) based oligomer using enzymatic digestion

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The oligomer production from macro-algae has been studied using enzymatic digestion. The digested solution was separated using hollow fiber membrane according to its molecular weight. We used three molecular cut-off membranes, 3 KDa, 10 KDa, and 30 KDa. The molecular weight of produced oligomer was identified with GPC (Gel Permeation Chromatography) analysis. The produced oligomer which has molecular weight 10 KDa has been characterized with FTIR, LC/MS, NMR and identified as glucan based oligomers. The main composition of oligomer was carbohydrates such as glucose, fucose, galactose and glucuronic acid. The inhibitory effect of oligomer on ACE (angiotensin converting enzyme) was presented *in vitro* and 5 mg of oligomer inhibits the 88% of ACE activity.

HPLC diagram of oligomer compositions

**CARB 75**

Fluorescence-based binding assay of hydrophobic DNA to the lipid bilayer membrane

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We here report fluorescence-based binding assay of hydrophobic DNA to the lipid bilayer membrane. Hydrophobic DNA consists of a hydrophobic region corresponding to the thickness of the lipid bilayer membrane and hydrophilic regions at both ends. The synthesis of the hydrophobic DNA was achieved by ultra-mild phosphoramidite method. In order to investigate interaction between the hydrophobic DNA and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) liposome, we prepared hydrophobic DNA
containing a dielectric-sensitive probe. Fluorescence of the hydrophobic DNA was enhanced and blue-shifted by the presence of POPC liposome. These results indicate that the hydrophobic DNA resided in the hydrophobic region of the liposome.

CARB 76

Synthesis of 2'-C-α-aminomethyl-2'-deoxynucleosides

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An efficient synthetic approach to all of four 2'-C-α-aminomethyl-2'-deoxynucleosides (15; U, C, A, G) from methyl 3,5-di-O-benzyl-2-keto-a-D-ribofuranoside (1) is developed. The synthesis is achieved efficiently via the convergent approach involving glycosylation of persilylated nucleobases with 2-α-phthalimidomethyl ribofuranoside 3. Optimal conditions for subsequent debenzylation depended on the nucleobase. For guanosine derivatives, debenzylation by Pd or Pd(OH)₂ catalyzed hydrogenation gives product in better yield. For uridine, cytidine and adenosine derivatives, boron trichloride induced benzyl ether cleavage gives products in higher yield.

CARB 77

Synthesis of the phosphoramidite derivative of 2'-O-(o-nitrobenzyl)-3'-thioguanosine

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Oligonucleotides containing a 3'-S-phosphorothiolate linkage provide valuable analogues for exploring the mechanisms of ribozyme-catalyzed reactions. Since 3'-S-modified RNAs are very liable to hydrolysis, the synthesis and storage remain challenging. The introduction of 2'-O-photoliable group could be helpful to increase the
stability of these 3'-S-modified RNAs during storage and handling. To construct oligonucleotides containing a 2'-O-(o-nitrobenzyl)-3'-S-guanosine nucleotide via solid-phase synthesis, an efficient approach for the synthesis of 2'-O-(o-nitrobenzyl)-3'-thioguanosine phosphoramidite starting from guanosine in nine steps with 10.2% overall yield is developed.

CARB 78

**Antiproliferative effect of aminoethylated chitooligosaccharide on AGS human gastric cancer cells**

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In this study, aminoethyl-chitooligosaccharide (AE-COS) from COS (below 1 kDa molecular weight) was synthesized and the effect on the proliferation of AGS human gastric adenocarcinoma cells was evaluated and compared. Synthesis of AE-COS from COS was characterized by FT-IR spectroscopy. Treatment with aminoethyl-chitooligosaccharide inhibited cell proliferation in a dose dependent manner. Furthermore, comparing analysis of apoptotic-related gene expression suggests that AE-COS has apoptotic activity by regulating Bcl-2 families responsive genes. Therefore, the present results suggest that aminoethylated COS has inhibitory effect on the proliferation of AGS human gastric adenocarcinoma cells and has promising potential as valuable chemotreventive agents.

CARB 79

**Linked oligonucleotides as tool for targeting single stranded DNA**

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Targeting of single stranded DNA is interesting in various contexts e.g., targeting of single nucleotide polymorphisms. Our hypothesis is that by combining the binding properties of triplex forming oligonucleotides (TFO) and anti-sense oligonucleotides (AON). We will be able to obtain site specific cleavage or chemical modulation of single stranded DNA. Here we present a Clamp system for targeting single stranded DNA with a linked TFO and AON part. The TFO part becomes preorganized for Hoogsteen type base pairing upon hybridization of the AON part. As linker we use different aromatic systems with intercalating properties. With these clamps we are able to obtain an increase in thermal stability of the formed triple helical complex of at least +10 °C, as compared to a reference system having six canonical thymidine nucleotides as linker unit. By incorporating LNA monomers into these linked oligonucleotides we are able to further increase the thermal stability.

CARB 80

Chemoenzymatic probes for detecting and imaging fucoseα(1-2)Galactose glycan biomarkers

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A major focus in the field of glycomics has been the development of new strategies for the rapid detection and quantification of glycans and glycoconjugates. Here we report a robust chemoenzymatic strategy for the rapid, sensitive detection of glycans containing fucoseα(1-2)galactose (Fucα(1-2)Gal), a motif implicated in cognitive processes and cancer pathogenesis. Our approach exploits the bacterial homologue of human blood
group transferase A (BgtA) to chemoselectively install non-natural azido- or ketone-containing sugars onto Fucα(1-2)Gal structures. We demonstrate that this approach can be used to label specific Fucα(1-2)Gal glycoproteins from cell lysates and to detect cell surface Fucα(1-2)Gal glycans. Furthermore, we show that the strategy can be used to discriminate LnCAP prostate cancer cells from normal primary prostate epithelial (PrEC) cells. This chemoenzymatic approach offers a new potential strategy for biomarker detection and expands the glycomics technologies available for investigations into the biologically important Fucα(1-2)Gal motif.

CARB 81

Investigating the inhibitory function of Siglec-G on B cells using antigenic liposomes decorated with carbohydrate ligands

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Lectins of the sialic-acid-binding siglec family play an important role in the regulation of immune cell function. Among siglecs, CD22 and Siglec-G have been proposed to play a role in dampening antigen-induced B cell activation. Recent studies have provided evidence for this inhibitory function through the use of polymers displaying both CD22 ligands and antigen. Here, we present the development of a synthetic carbohydrate-based ligand for Siglec-G. The utility of this ligand was investigated by attaching it to liposomes that present both antigen and the Siglec-G ligand. Stimulation of antigen-specific B cells with the liposomes led to attenuated B cell receptor signalling compared to liposomes bearing the antigen alone. Using Siglec-G KO B cells, the inhibitory effect was completely abolished. Therefore, we have developed a specific ligand that can be used in immunological experiments to modify B cell function. (NIH AI050143 and DAAD-fellowship to FP and HFSP-fellowship to MSM)

CARB 82

Orthogonal glycosyl building blocks to synthesis the glycan libraries of saponin

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Saponins, glycosylated secondary metabolites, are important for cell growth, development, and virus, bacterial defensive system in plants. So far, thousands of homogeneous saponins have been isolated and characterized. These saponins display a tremendous structural diversity and a wide spectrum of biological activities. Many of them contain sugar units, which are often required for biological function in vivo. Due to the limited availability of homogenous saponins, we synthesized and created the saponin-libraries from two types of major saponins, including triterpine, and steroids types, demonstrated by the lead structures of oleanane and diocin. Glucose and
glucosamine were selected as core structures to be orthogonal protected, followed by
the following steps to give saponins glycan libraries: (1) glycosylation with sapogenin;
(2) selective deprotection; (3) glycosylation with a library of glycosyl donors; (4) global
deprotection.

CARB 83

Synthesis and characterization of novel “Birosh” amphiphiles as an alternative to
bicells

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The importance of brain-derived G protein-coupled receptors (GPCRs) in mental
function and health can hardly be overstated. However, our knowledge of the molecular
biophysical basis for how GPCRs bind to their cognate agonists and antagonists to elicit
function is limited by the lack of high resolution 3-D structural information for these
proteins. Overall the specific aim of this work is to develop a novel class of detergents
(“birosh” detergents) that will self-assemble with more traditional detergents in aqueous
solution to form a new class of bicelles that confer native stability and structure to
solubilized Diacyl Glycerol Kinase (DAGK) and other GPCRs. Herein, we describe the
syntheses of the first generation birosh amphiphiles and their respective evaluation in
the proposed DAGK test system. The synthetic methodology utilized employees a long
chain alkyl diol dimer (C18 – C24) obtained via a Grubbs metathesis reaction. The diols
were glycosylated with perbenzoylated maltose under standard β-selective conditions.
Finally, debenzoylation and hydrogenation resulted in the desired bis-maltoside based
surfactants. Biological evaluation with the DAGK system revealed that the long term
stability was enhanced while the native activity was lowered.

CARB 84

Knock-out analysis of candidate methyltransferase genes that might synthesize
3-O-methyl-L-rhamnosyl residues in arabinogalactan proteins of Physcomitrella
patens
The project goal is identification of methyltransferase genes synthesizing \(O\)-methylated sugars in plant cell walls. The model is *Physcomitrella patens*, a moss that produces arabinogalactan proteins (AGPs) containing 15\% 3-\(O\)-methyl-L-rhamnosyl (3-\(O\)-Me-Rha) residues. Present in all plants, AGPs consist of about 10\% polypeptide and 90\% galactan carrying arabinosyl, glucuronosyl, rhamnosyl, and other substituents. *Physcomitrella* AGPs are distinguished by the 3-\(O\)-Me-Rha residues, which have not been found in any angiosperms. The 3-\(O\)-Me-Rha and a high frequency of genetic homologous recombination enable an investigation of methyltransferase genes in *Physcomitrella*. Using a microbial methyltransferase sequence as a guide, candidate genes have been selected from the *Physcomitrella* genome for targeted knock-out. Screening the knock-out lines by 3-\(O\)-Me-Rha/Rha ratio in AGPs has revealed at least one line with 80\% reduced methylation. This line shows altered growth and development of rhizoids. Supported by National Research Initiative grant 2008-35318-04599 from USDA NIFA.

**CARB 85**

**Recent investigation of the synthesis and application of 4,5-oxazolidinone-protected phosphate-based sialic acid donor**

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For the chemical synthesis of natural \(\alpha\)-sialosides, 4,5-oxazolidinone-protected sialic acid donors have been demonstrated to be a very efficient tool, as exemplified by our recent \(\alpha\)-stereoselective synthesis of \(\alpha(2\rightarrow9)\)-linked oligosialic acid (up to dodecamer). Here, we described our recent investigation of the synthesis and application of 4,5-oxazolidinone-protected phosphate-based sialyl donor. In our previous report, we found that the \(\alpha\)-anomeric phosphate is more reactive than its \(\beta\)-counterpart. However, it is inevitably to obtain an anomeric mixture of phosphate compounds after synthesis. To reduce the extra effort for their separation, we reported here that the 4,5-oxazolidinone protected \(\alpha\)-phosphate could be synthesized stereoselectively from the corresponding \(\alpha\)-thiosialoside. We also examined the effect of different leaving group toward using 4,5-oxazolidinone protected sialyl donors in the glycosylation reaction. For applications, the sialyl phosphate donor was employed to prepare \(C\)-glycoside analogue of sialoside. In addition, programmable one-pot synthesis of PSGL-1 hexasaccharide was accomplished.

**CARB 86**

**Resolving anomers by mass spectrometry**
Anomeric configuration of hemiacetal has been regarded as less important in structural determination of oligosaccharide, because hemiacetals isomerize to equilibrium in aqueous solvent. However, previous report indicated that hemiacetals hold the anomeric configurations of their precursor glycosides under collision-induced dissociation conditions in mass spectrometry. Consequently, structural investigation of hemiacetals in gas phase is considered to be valuable.

This study has addressed a possibility of discriminating anomers of sodiated hemiacetals obtained from corresponding glycosides, which proved to be possible. Although computational analysis indicates that ground-state energies between anomers of sodiated hemiacetals are different, confirmation of which has not been feasible. Our results show a possibility of predicting the anomeric configuration of a glycosidic linkage by computation.

CARB 87

Process development and optimization of neopentyl glycol (NG) class amphiphiles

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The Neopentyl Glycol (NG) class amphiphiles are revolutionary new surfactants which have already shown great utility in membrane protein studies. NG class amphiphiles are a more effective surfactant for extracting, solubilizing and/or stabilizing proteins. They are beneficial in the crystallization process due to some unique properties conferred by a revolutionary new architecture. The amphiphilic molecule consists of a central quaternary carbon with two hydrophilic heads and two lipophilic tails, generating subtle constraints on overall conformational flexibility that allows the molecule to pack densely when forming a micelle. This dense packing increases thermal stability of the surfactant/protein complex and most importantly, produces exceptionally low critical micelle concentrations (CMC) and extreme water solubility.

The NG amphiphiles are substitute products for three of today's most popular detergents: lauryl maltoside, octyl glucoside and decyl maltoside. There are remarkable differences in CMC between the new NG class and their counterparts, where approximately 17-fold less of the NG class surfactant achieves the same critical micelle
concentration as the equivalent maltoside or glucoside. These low CMC values reduce the often detrimental effects of excess solubilizing agent on crystallization. Additionally, NG class detergents also demonstrate a superior ability to solubilize expressed proteins without interfering with the protein expression mechanics of cell free protein expression systems.

Herein, we describe the process development work for our commercialization effort from Affymetrix. We have now optimized these syntheses from gram to kilogram scale.

CARB 88

Exploration of O-GlcNAcylated protein interactions using a metabolically incorporated photo-crosslinking sugar

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The O-GlcNAc modification is a dynamic post-translational modification found on hundreds of mammalian proteins. Despite the ubiquity of the O-GlcNAc modification, its roles in regulating the function and interactions of modified proteins remain poorly defined. Recently, we developed a metabolic labeling method to selectively incorporate the diazirine photocrosslinker onto O-GlcNAc-modified proteins in a cellular setting. Subsequent UV irradiation of the cells results in the formation of covalent crosslinks between O-GlcNAc-modified proteins and their neighbors. We have validated our method by examining the interactions of phenylalanine-glycine (FG) repeat nucleoporins, a set of O-GlcNAc-modified proteins that line the nuclear pore. We found that two of these FG-repeat nucleoporins, NUP153 and NUP358, crosslink to nuclear transport factors. We report our current efforts in extending this study to another FG-repeat nucleoparin, NUP98 to determine the O-GlcNAc-dependent interactions in which it engages.

CARB 89

First chemical synthesis of the conjugation ready hexasaccharide antigen of Vibrio cholerae O:139
The title hexasaccharide was synthesized in protected form from thioglycosides and glycosyl bromides as glycosyl donors by stepwise and blockwise approach. The synthesis involved coupling of a HO-3-free, linker-equipped quinovosamine glycosyl acceptor with acetobromogalactose, to give an intermediate disaccharide. The latter was transformed into a glycosyl acceptor with only HO-4 free, which was coupled with an appropriately protected disaccharide glycosyl donor, to give the intermediate tetrasaccharide. Multistep protective group manipulation, which included phosphorylation and conversion of the D-galactose to D-galacturonic acid benzyl ester residue. This resulted in a phosphorylated tetrasaccharide having two correctly positioned hydroxyl groups ready for glycosylation with a glycosyl donor derived from L-colitose. The α-L-colitosylation was effected by halide assisted glycosylation with 2,4-di-O-benzyl-β-L-colitosyl bromide, to yield hexasaccharide in protected form. The synthesis was designed to permit global, one-step deprotection (H₂, Pd/C). It allowed transformation of fifteen functionalities in one operation, namely, removal of ten benzyl protecting groups, removal of a trichloroethyl phosphate protecting group, conversion of two N-trichloroacetyl to N-acetyl groups, a CH₂-Br to a CH₃ group and conversion of an azido to an amino group, to afford the target compound in a form amenable for conjugation.

CARB 90

Self-generating synthesis for the design of Galectin-1 inhibitors

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Galectins, a family of animal lectins, are involved in numerous types of cancer.¹ They exhibit a characteristic carbohydrate-recognition domain and interact selectively with glycoconjugates of cell surfaces. These interactions introduce biomolecular processes as immune reactions, cell growth and tumor progression.²⁻⁴ An improved understanding
of the underlying mechanisms may provide further opportunities for developing therapies based on the immunoregulatory properties of this protein family.

Galectin-1, a prominent member of this protein family, is overexpressed in malignant tissues and binds β-galactosides. The binding constants of such natural carbohydrates presented on cell surfaces range in the micromolar and demonstrate the need for the identification and development of highly affine and selective ligands.

We present a self-generating approach for the synthesis of promising binding partners of Galectin-1.

Such studies combine docking experiments, synthesis and activity assays on living cells.

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CARB 91

Trypanosoma cruzi-derived sugar epitopes: Synthesis and Immunology

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Trypanosoma cruzi is the causative agent of Chagas disease, affecting 16-18 million people annually, with no effective vaccine available. This protozoan parasite expresses cell surface oligosaccharides with terminal alpha-galactosyl and rhamnosyl residues, whose chemical structures are being investigated through syntheses and immunological studies. This led to the discovery of a new orthogonal protecting group manipulation method, and employed a regioselective glycosylation method to synthesize a trisaccharide with potential immunogenicity. Our saccharides were equipped with a thiol functionality and conjugated to maleimide-derivatized KLH (Keyhole Limpet Hemocyanin) carrier protein, then screened in an immunoassay for Chagasic antibody recognition. The best recognized saccharides were used to immunize alpha-1,3-Gal-T-KO mice, which showed prolonged survival following a lethal dose of T. Cruzi compared to control groups. This led to synthesis of a glycolipopeptide composed of a B-cell epitope (alpha-galactosides), a T-cell epitope, and a lipid residue needed for an efficient uptake by antigen presenting cells.
Quantifying the interaction of TAT peptide and cell-surface glycosaminoglycans

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Cell-penetrating compounds (CPCs) are commonly used to enhance the internalization of drugs, but their mechanism at the cell surface is poorly understood. We hypothesize that cell membrane glycosaminoglycans (GAGs) play an integral role in the recognition and subsequent internalization of CPCs and their attached cargo. Here, the interaction of one such CPC, TAT peptide, with four GAGs (heparin, heparan sulfate, dermatan sulfate and chondroitin sulfate A) was studied through microcalorimetry to determine binding constants, enthalpies and stoichiometries. Significant differences in affinity were seen, likely reflecting charge density and hydroxyl stereochemistry variations in GAG structure. These results lead to understanding the role these GAGs play in the cellular internalization of biomaterials, which will contribute to the design of systems with enhanced uptake ability and have implications in targeted delivery to a variety of cell types with different expression levels of these GAG receptors.

Synthesis of pectic oligosaccharides

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The plant cell wall is the major contributor of the world’s biomass. Some of the main components are complex polysaccharides such as pectin. An improved understanding of these complex molecules is crucial to areas such as biofuels, biomedicine and nutrition. A series of linear β-(1→3)-linked, linear β-(1→4)-linked and β-(1→6)-branched galactans have been prepared via a simple convergent strategy. Immobilization on microarrays has allowed high throughput antibody screening.
Ultrasonic detection of carbohydrates using preparative CE and qPCR

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Carbohydrates play crucial roles in a wide range of biological processes and are often present at low levels making their analysis challenging. We have developed an ultrasensitive detection method for carbohydrates using a combination of preparative capillary electrophoresis (CE) and quantitative polymerase chain reaction (qPCR) detection. Preparative CE allows very sensitive separation and collection of chondroitin sulfate disaccharides, prepared from Chinese hamster ovary (CHO) cells, and utilizes a 72 cm x 75 µm silica capillary run with 35 mM sodium borate buffer at pH 9, performed at +30 kV. The key advantage to preparative CE is that the fraction collection can be performed from tiny sample volumes. After collection of peaks, the eluents were conjugated to amine-DNA by carbodiimide coupling reaction. Disaccharides coupled with NH₂-DNA were then biotinylated. Finally, the reaction products are purified using streptavidin magnetic beads, washed extensively with buffer to remove unreacted DNA prior to qPCR analysis.

CARB 95

Cross-metathesis synthesis of 2-(5-aminopentyl)-mannoside for use in surface plasmon resonance microarrays

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Progress in the field of glycomics has long been challenged by the limited availability of technology capable of investigating the complex interactions of carbohydrates. Using surface plasmon resonance (SPR), it is possible to detect carbohydrate-surface protein interactions through the change in refractive index upon binding. Using 2-(5-aminopentyl)-mannoside anchored to acrylamide in microarray format, we hope to investigate the biological activity of a novel C-glycoside. We report a synthetic approach in the preparation of C-glycosides using ruthenium-based olefin cross metathesis.
CARB 96

Triterpene saponins and its synthetic analogs as vaccine adjuvants for humoral and cellular immune activities

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Quillaja saponaria extract is rich in aldehyde-containing triterpene saponins and have shown to possess immune stimulating activities. One of the most active saponin QS-21 is being used as adjuvant in several vaccine preparations for clinical trial and shown to be highly potent. However their commercial use has been limited, due to low stability in solution and high toxicity. Several synthetic analogs have been developed such as GPI-0100 and semisynthetic QS21 analogs. GPI-0100 is prepared by replacing hydrolytically unstable ester side chain with lipophilic dodecylamide linked by hydrolytically stable amide bonds on the glucuronic acid residue. Herein, the progress on semi-synthetic saponin analogs, their design, synthesis, purification, characterization, and structure/activity relationships will be discussed. These saponin analogs have less toxicity, greater stability, and excellent immune stimulating activity. Immunological results showed these adjuvants generate a Th1-type (or mixed Th1/Th2-type) immune response and cell-mediated immunity (CMI), including antigen-specific T cell proliferation and generation of cytotoxic T lymphocytes (CTLs).

This presentation is dedicated to late Prof. David Gin

CARB 97
Design and synthesis of *Quillaja* saponin vaccine adjuvants

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QS-21, an immune-stimulatory saponin natural product, has been employed in numerous cancer and infectious disease vaccine clinical trials. Despite this fact, structure-activity relationship studies of this adjuvant have been limited. On this basis, we report here the chemical synthesis and immunological evaluation of simplified QS-21 analogs involving truncation and replacement of the carbohydrate moieties within the tetrasaccharide quadrant of the QS-adjuvant. These studies allow, for the first time, systematic investigation of the structural requirement and specific identity of the sugar portions for adjuvant activity. In this way, the discovery of adjuvant-active QS-analogs with simpler carbohydrate structures greatly expedites the synthesis of such compounds in the design of novel and simplified vaccine adjuvants.

**CARB 98**

Synthesis of new QS-21 congeners: Improving on nature

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Finding innovative solutions to the complex chemical challenges in synthesizing the active components of QS-21, one of the world's leading adjuvant candidates, has advanced adjuvant science. By applying synthetic innovations to the process for saponin manufacture, David's Triterpene Saponin Synthesis Technology (TriSST) was used to synthesize novel rationally designed saponin derivatives with improved tolerability and enhanced adjuvant efficacy. After successfully synthesizing the active compounds in QS-21, David set out to improve upon nature by redesigning the QS-21 molecule. Using serologic responses against our KLH conjugated cancer vaccines as read-out, he addressed 3 of the natural molecule's shortcomings; instability, toxicity and complexity. The first generation of David's TriSST adjuvants stabilized the hydrolytically labile acyl chain, obviating the burdensome formulation and storage protocols required for natural QS-21. The subsequent generations of these synthetic adjuvants have investigated structure-activity relationships, focusing on rational design of simplified saponin molecules. The diversity of TriSST adjuvants also provides the opportunity to evaluate a variety of antigens against a library of novel adjuvants, providing novel adjuvant-antigen combinations for optimal immunopotentiation and tolerability. David's TriSST is the only means by which this unique saponin adjuvant library can be accessed, providing a dependable, consistent, high purity, and GMP-scalable source of
adjuvant-active QS-21 congeners. Our currently favored TriSST adjuvant construct is synthesized in 22 steps (down from the previously reported 64 steps), is shelf stable and has a more favorable adjuvant potency/toxicity ratio than synthetic or extracted QS-21.

CARB 99

Molecular recognition of the CUG triplet repeats in RNA, the causitive agent of myotonic dystrophy

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Myotonic dystrophy type 1 (DM1) is a triplet repeat disorder wherein aberrant expansion of CTG repeats occurs within the DMPK gene. The disease is believed to occur by an RNA gain of function mechanism wherein expanded CUG RNA recruits MBNL, leading to misregulation of alternative splicing of certain RNAs. This talk will focus on the development of small molecules that selectively complex CTG or CUG repeats with high affinity and, in the case of CUG, inhibit MBNL binding. Strategies for amplifying affinity and selectivity will be discussed as will attempts to determine the structure of the RNA/DNA small molecule complexes.

CARB 100

RNA modification by radical SAM methyl synthases RlmN and Cfr

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RlmN and Cfr are evolutionary related enzymes that install two methyl groups into a single adenosine residue in ribosomal RNA. While RlmN is common in the bacterial kingdom and is thought to contribute to translational fidelity of the ribosome, Cfr has been isolated from pathogenic strains. Together, introduced methylations confer resistance to all antibiotics that target the peptidyl transferase center of the ribosome, and represent a significant concern for the treatment of bacterial infections. A unique chemical challenge of RlmN and Cfr is that their substrates are electrophilic, amidine carbons of the adenosine in RNA. We have established that these enzymes have evolved a novel methyl synthase reactivity to allow for methylation. Mechanistic aspects and functional consequences of these methylation reactions will be presented.

CARB 101

Regulation of gene expression by DNA binding Py-lm polyamides: Targeting transcription factor-DNA interfaces
Many human diseases are caused by dysregulated gene expression. The oversupply or overactivity of one or more transcription factors may be required for the survival, growth, and metastatic behavior of all human cancers. Pyrrole-imidazole polyamides are synthetic molecules programmed to read the DNA double helix by a set of simple chemical principles. These cell permeable small molecules achieve affinities and specificities comparable to DNA-binding proteins. Research efforts are focused on the modulation of gene expression pathways in cell culture by disruption of transcription factor-DNA interfaces.

CARB 102

Chemical modified antisense oligonucleotide and siRNA

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Many modified antisense oligos are being tested in clinical trials with fomivirsen (Vitravene; Isis) as the first oligo drug approved by the U.S. FDA for the treatment of eye cytomegalovirus infection. Currently, such chemical modifications are also being applied to synthetic siRNAs, and some modified siRNAs have been reported to be more potent and stable than their native siRNAs. In the synthesis and incorporation of isonucleosides into antisense oligos, we found that modified antisense oligos not only possess strong nuclease resistance but more interestingly become good substrates of RNase H. An introduction of an amino group into the isonucleoside may increase the thermal stability of the isonucleoside-modified oligonucleotide with its complementary sequence. The chemical synthesis and incorporation of D- and L-isonucleosides into the siRNA duplex and analyze the effect of such a modification at different positions on siRNA structural stability and functional activity will be discussed.

CARB 103

Unnatural base pair systems for DNA-based biotechnology toward diagnostic and therapeutic applications

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Expansion of the genetic alphabet by creating an artificial extra base pair, unnatural base pair, could provide a powerful tool for the site-specific incorporation of new components into nucleic acids and proteins through the genetic information flow in the central dogma. Recently, we have developed a hydrophobic unnatural base pair between 7-(2-thienyl)-imidazo[4,5-b]pyridine (Ds) and 2-nitro-4-propynylpyrrole (Px) and
its variants that exhibit high efficiency and fidelity in PCR as the third base pair. After 40 cycles of exponential amplification PCR (10 cycles PCR repeated 4 times), more than 97% of the Ds–Px pair can be retained in 10-billion-fold amplified DNA fragments. In addition, we found that certain modifications of the unnatural base pair increase the fidelity in PCR without decrease in amplification efficiency. By using the unnatural base pair system, we generated new diagnostic systems for detecting target DNA sequences and new artificial DNA molecules for targeting proteins.

CARB 104

Chemical strategies for delivery of RNAi drugs

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At Alnylam Pharmaceuticals, we have developed and applied multiple chemistry strategies to address the challenge of cellular delivery of drugs that function through RNAi pathways. These include chemical modifications of oligonucleotides, targeted molecular conjugates with appropriate ligands and delivery systems based on liposomal nanoparticles (LNPs). Our progress in these areas will be summarized.

CARB 105

Structure/function studies of chemically modified antisense and siRNA oligonucleotides

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Chemically modified nucleic acids (CNAs) are widely explored as antisense oligonucleotide (AON) or small interfering RNA (siRNA) candidates for therapeutic applications. Research over the last 20 years has led to an improved understanding of how modification affects structure, stability and activity of CNAs, with key insights ranging from conformational changes as a consequence of chemical modification to the modulation of RNA affinity by stereoelectronic effects and hydration. Although crystal structures have only been determined for a subset of the large number of modifications that were synthesized and analyzed in the AON and siRNA contexts to date, they have yielded guiding principles for the design of new CNAs with tailor-made properties, including pairing specificity, nuclease resistance, and cellular uptake. The presentation will cover recent results regarding the structure and activity of selected CNAs.

Supported by the US NIH (R01 GM055237, R01 GM071461).

Reference:

CARB 106

Targeting polymorphic nucleic acids: Modulate their biological functions and utilize these controllable conversions

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Nucleic Acid is polymorphic and exists in diverse conformations. Conversion of different conformation can switch on/off their biological functions. Therefore, targeting polymorphic DNA is important for rational drug design and for developing structural probes of DNA conformation and DNA artificial devices. In this report, we summarize our recent advances on ligand-induced structural transitions, biological effects, and their applications. This work was supported by NSFC, 973 Project, Funds from the Chinese Academy of Sciences and Jilin Province.

References


CARB 107

Shortest strategy for generating phosphonate prodrugs by olefin cross metathesis: Application to acyclonucleoside phosphonates

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Bioavailability is one of the principal hamper for various polar molecules leading to a therapeutic drug. Several, hitherto unknown, unsaturated acyclonucleoside phosphonate prodrugs were synthesized from (acyloxymethyl)-, or
(hexadecyloxypropyl)-allylphosphonate building blocks under olefin cross-metathesis (CM) or Mitsunobu conditions. In case of CM route, a thorough study of ruthenium-based catalyst efficiency was carried out focused on the effects of catalyst scaffold and of supporting ligands to generate the desired phosphonate prodrugs. This presented strategy is appealing for further uses in pharmaceutical and medicinal research.

CARB 108

Chemical and enzymatic synthesis of S-linked glycopeptides

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Post-translational glycosylation introduces enormous structural and functional diversity into glycoproteins. O-linked glycoproteins are important in a variety of these processes. O-linked protein glycosylation can be divided into two general classes – those in which a GlcNAc residue is β-linked to the side chain of Ser/Thr, and those in which a GalNAc residue is α-O-linked to the side chain of Ser/Thr. The latter is characteristic of mucins, O-glycosylated proteins comprising an important class of tumor-associated antigens that have received considerable attention in cancer vaccine therapies, whereas the former is found in the reversible modification of proteins, a process that is important for a variety of cellular processes. Epitope specific antibodies against both classes of O-linked glycoproteins are important tools for the study of their cellular functions, but O-linked glycopeptides often are only weakly immunogenic. In this presentation, orthogonal ligation techniques will be presented for the preparation of α-S-linked analogues that are metabolically more stable than O-linked peptides. In addition, enzymatic approaches will be presented for the preparation of β-S-linked glycopeptides.

References


CARB 109

Catalytic transformations of allylic and glycosyl trihaloacetimidates
C(2)-aminosugars make up one of the most important classes of naturally occurring oligosaccharides and glycoconjugates. Obtaining an adequate supply of these aminosugars from natural sources is challenging. In many cases, high purity C(2)-aminosugars can only be obtained by chemical synthesis. Our group has recently developed an innovative strategy for the synthesis of 1,2-cis-2-aminosugars via nickel catalysis. This method is currently applied to the design and creation of a library of heparin oligosaccharides.

The past decade has seen an explosion in the use of fluorinated molecules for application in medical imaging and pharmaceuticals. In addition, $[^{18}\text{F}]$-fluoride has become the favored isotope for use in PET imaging. Our group has recently developed a rapid allylic fluorination method utilizing trichloroacetimidates in conjunction with an iridium catalyst. We also involve in the development of the rhodium-catalyzed regio- and enantioselective amination of tertiary allylic trichloroacetimidates, forming nitrogen-containing quaternary centers.

### Synthetic Archaea derived glycolipids promote MHC class I activity

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Our carbohydrate synthesis group chooses its targets based on an expectation of activity in immunological assays. The ultimate goal is to create vaccine platforms that can be tuned for any desired immunological outcome. In particular we synthesize glycolipid based delivery systems that can promote predominantly antibody (Th2) or predominantly Cytotoxic T-Lymphocytes -CTL (Th1) responses, or a Th1, Th2 balanced response. Our results show that at least in Ovalbumin mouse model systems specific synthetic glycolipids strongly bias the immune response towards CTL production. Such responses lead to protection in cancer models. The mechanism of immune activation is not known precisely particularly which cell surface receptors are involved but is reproducibly shown to promote long term immune memory. For this purpose we have developed a convenient promoter system for peracythioglycoside donors based on NIS and a BF$_3$-2trifluoroethanol solution that leads to very clean glycosylation reactions which allow for facile purification of otherwise difficult to purify glycolipids. This report focuses on the synthetic complications of handling amphipathic molecules and presents several solutions including phase transfer methodologies and purification procedures.

### Using glycodendrimers for studies of antimicrobial and cancer cell processes
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Glycodendrimers bearing quaternary ammonium endgroups are being used to form multivalent antimicrobial agents. In this presentation, the synthesis of these compounds and their toxicity activity against bacterial cells will be discussed. In addition, the results of resistance assays will be presented. We have found that *E. coli* are unable to develop significant resistance to the dendrimers over a thirty day testing period. Glycodendrimers are also being used to study cancer cellular aggregation processes. Results of cancer cell based assays with glycodendrimers, which reveal that homotypic cancer cellular aggregation can be either enhanced or inhibited by dendrimers in a dendrimer-dependent fashion will be presented.

**CARB 112**

**Enantioselective nucleophilic catalysis**

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Nucleophiles such as 4-(dimethylamino)pyridine (DMAP) and tertiary phosphines catalyze a wide array of useful and interesting reactions. We are pursuing the development of asymmetric processes catalyzed by enantiopure DMAP derivatives and phosphines. In this lecture, I will discuss some of our recent progress.

**CARB 113**

**Efforts toward the expansion of the genetic alphabet**

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Expansion of the genetic alphabet to include a third base pair would be a fundamental accomplishment that would not only have immediate utility for a number of applications, such as site-specific oligonucleotide labeling, but would also lay the foundation for a semi-synthetic organism. We have developed an unnatural base pair, d5SICS -dNaM, that forms based on packing and hydrophobic interactions rather than complementary H-bonding. d5SICS -dNaM, as well as derivatives with linkers attached for site-specific labeling of DNA or RNA, is replicated and transcribed with excellent efficiency and fidelity. Structural studies, as well as several applications, including ongoing selections for unnatural DNAzymes will be discussed. Finally, we are supplementing these efforts with directed evolution to tailor DNA polymerases to more efficiently replicate DNA containing d5SICS -dNaM.

**CARB 114**
Chemical approaches for achieving activation of RNA interference with single stranded oligonucleotides in animals

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Inhibition of gene expression using RNA interference (RNAi) pathway in general uses double stranded RNA (siRNA) and requires cationic lipid formulation for delivery to the desired compartment of the cell and animals. In contrast, RNase H based single stranded antisense oligonucleotides have shown activity in multiple species (including humans) without formulation. Moreover, it has been demonstrated that a single stranded RNA (ssRNA) delivered to cells using cationic lipids has been shown to activate the RNAi pathway. This suggests that a double stranded structure is not necessary to elicit RNAi. This observation made us think that the dsRNA structure could be simplified to a single stranded oligonucleotide that would activate RNAi in cells and in animals.

Our initial mechanistic studies revealed that 5'-phosphate is necessary for ssRNA activity in cell culture. We have done an extensive SAR study of ssRNAs, and coupled this to biochemical studies on the mechanism of activation of the RNA induced silencing complex (RISC). These efforts provided highly potent compounds in cell culture which were shown to function via an argonaute-2 dependent mechanism. These ssRNAs are active in cell culture without cationic lipids, and this activity translated to activity in animals at pharmacologically relevant doses with subcutaneous administration in saline formulations. These studies provide an outline for further optimization of the ssRNA structure for the potential development of human therapeutic agents.

CARB 115

Chemical and structural biology of selenium-nucleic acids (SeNA) for novel drug discovery

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Recently our laboratory has pioneered and developed atom-specific substitution of nucleic acid oxygen with selenium [Se-derivatized nucleic acids (SeNA)], which can be used as atomic probes for structure and function studies of nucleic acids and drug discovery. The atom-specific mutagenesis by replacing nucleotide oxygen with selenium can reveal novel chemistry, structure, function and mechanism of nucleic acids, such as non-coding RNAs. Moreover, Se derivatization leads to a novel paradigm of nucleic acids and allows new drug discovery. Our nucleic acid chemogenetic strategy with
selenium has demonstrated great potentials as a general methodology for structure and function studies of nucleic acids as well as their protein complexes. Furthermore, we found that selenium derivatization can facilitate crystallization and the diffraction quality is high. Excitingly, we have also recently determined the first nucleic acid-protein complex via the nucleic acid Se-derivatization and the MAD phasing; this structure offers opportunity to develop anti-HIV drugs.

CARB 116

Delivery of nucleic acids and proteins into cells by self-assembling Protein-DNA nanostructures

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Delivery of nucleic acids and proteins into cell is an important and challenging problem. With the help of CSAN's (chemically self-assembled nanostructures), nucleic acids can be specifically targeted to selective cells. We have demonstrated that antisense oligonucleotides for eIF4E (eukaryotic Initiation Factor 4E) can be delivered to cells via surface receptors. Upon endocytosis, oligonucleotides (CSAN's) are released from the endosomes into cytoplasm achieving antisense effect. In the future, this idea will be further extended towards delivery of siRNA and miRNA.

CARB 117

Probing the role of carbohydrates in the immune reponse for better vaccine adjuvant design

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Cell types ranging from bacterial pathogens to cancer cells display diverse carbohydrate overcoats that either aid or subvert human and animal immune reponses to these potentially lethal cells. Unfortunately, dissecting the roles of these sugars is complicated by the microheterogeneous mixtures and low relative abundances of particular
structures found in the natural sources. In tribute to the excellent work carried out by the late Prof. Gin in dissecting the role of carbohydrates in the development of potent vaccine adjuvants, this talk will focus on the design and use of synthetic tools, including automated oligosaccharide synthesis and multivalent displays, to probe macrophage and other responses to pathogen-associated carbohydrates and will discuss the implications in designing better vaccine adjuvants.

**CARB 118**

**Synthesis of immunomodulators from mycobacteria**

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Mycobacteria produce a range of immunomodulatory glycolipids that are expressed on the outer surface of their cell wall. Among these are glycopeptidolipids, phenolic glycolipids and lipooligosaccharides. Recent work on the synthesis of representative examples of these species will be presented.

**CARB 119**

**From chemical glycosylation to expeditious oligosaccharide synthesis**

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The involvement of complex carbohydrates in a wide variety of disease-related cellular processes has given this class of natural compounds tremendous diagnostic and therapeutic potential. While scientists have been able to successfully isolate certain classes of natural carbohydrates, the availability of pure natural isolates is still inadequate to address the challenges offered by modern glycoscience. As a consequence, chemical synthesis has become a viable means to obtain both natural complex carbohydrates and unnatural analogues thereof. Unfortunately, chemical synthesis of oligosaccharides of even moderate complexity still remains a considerable challenge, and many more complex structures are not available at all. As such, the development of efficient methods for chemical glycosylation and expeditious oligosaccharide and glycoconjugates synthesis remains a demanding area of research.

At the core of this presentation is the development of new methods, strategies, and technologies for chemical glycosylation and expeditious oligosaccharide assembly. New innovative tools for oligosaccharide synthesis such as thioimidate glycosylation approach, the temporary deactivation concept, the inverse armed-disarmed strategy, Surface-Tethered Iterative Carbohydrate Synthesis (STICS), thioimidate-only orthogonal and active-latent activation, and O-2/O-5 cooperative effect will be discussed in light of recent results. The effectiveness of the methods developed will be illustrated by the synthesis of pneumococcal oligosaccharides (serotypes 6 and 14) as well as...
Step economy oriented carbohydrate synthesis with allyl glycoside building blocks

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In pursuing step economy-oriented carbohydrate synthesis in solution phase for rapid access to oligosaccharides, we have recently developed a simple glycosylation approach employing only allyl glycosides as building blocks. In a one-pot fashion, an allyl glycoside is isomerized to the corresponding prop-1-enyl glycoside and its subsequent activation with NIS/TfOH in the presence of an allyl glycoside acceptor leads to the formation of a new allyl glycoside. The new protocol could markedly simplify carbohydrate synthesis and improve overall synthetic efficiency. Studies have been carried out to elucidate the mechanism of this new glycosylation reaction. The reaction mechanism proves to be quite complicated. Upon activation of the anomeric prop-1-enyl group isomerized from an allyl group, both the desired new glycoside and addition-reaction byproducts can be produced. TfOH has a dual role in the reaction; it promotes the conversion of the addition-reaction products to the desired new glycoside through multiple competing pathways.

Glycoconjugates reduced eosinophils and basophils response during Bacillus anthracis infection

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*Bacillus anthracis* causes anthrax that lead to toxemia, edema, and death. Glycoconjugates (GCs) on *B. anthracis* Sterne (BA) were studied *in vivo*. C57BL/6 mice were injected with A) PBS; B) BA only, C) GC1 only, D) GC1 only, E) BA+GC1, F) BA+GC3 and sacrificed on day 1 and 5. Blood smears were prepared using Neat stain. Blood cells were counted under microscopy. BA infection demonstrated higher levels of neutrophils and monocytes on day 1. On day 5, production of monocytes and neutrophils were suppressed. BA promoted basophilia and eosinophilia. Both, GC1 (BA+GC1) and GC3 (BA+GC3) treatments decreased eosinophils during 5 days of BA infection. Amount of basophils during GC3 treatment either on day 1 or 5 was very low compared to all studied conditions. GC3 treatment promoted monocytes' production against BA infection. GCs have the ability to inhibit the BA toxins and consequently reduce eosinophils and control the BA infection.
Immunization and vaccination of α1,3-GalT-KO mice with synthetic *Trypanosoma cruzi* carbohydrate epitopes

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Millions of people in Central and South America are infected with the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*), the causative agent of Chagas disease. *T. cruzi* contains cell surface glycoproteins with terminal α-galactosyl residues, known to be highly immunogenic in humans. In some developmental stages of *T. cruzi* other sugars foreign to humans are expressed, e.g. rhamnosides, which may also be immunogenic.

However, the exact structures of the immunogenic carbohydrate epitopes remain unknown. To determine which carbohydrate epitopes can be exploited as potential vaccines α-Gal and α-Rha containing mono-, di-, and trisaccharides were synthesized, conjugated to KLH, and used for immunization of α1,3-GalT-KO mice. Those glycoconjugates capable of eliciting a strong antibody response were used for experimental vaccination. After the immunization mice were challenged with a lethal dose of *T. cruzi*, which resulted in a prolonged survival rate when compared to the unvaccinated control group.

Characterization and inhibition of a novel glucosyltransferase involved in O-antigen synthesis in enterohaemorrhagic *Escherichia coli* serotype O157

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The enterohaemorrhagic O157 strain of *Escherichia coli* displays its O-antigen on the extracellular face of the outer membrane. The O157 antigen is a virulence factor and consists of repeating units with the sequence [-2-D-Rha4NAca1-3-L-Fuca1-4-D-Glcb1-
3-D-GalNAc1-]. In this work, we showed that wbdN found in the O-antigen synthesis gene cluster encodes a Glc-transferase that adds Glc to GalNAc as the second step in O-antigen repeat unit synthesis. The wbdN gene was expressed in E. coli BL21 bacteria; WbdN was purified and characterized using the donor substrate UDP-[14C]Glc and the synthetic acceptor substrate GalNaca-PO3-P3-(CH2)11-O-Phenyl. WbdN has a DxD motif and requires Mn2+ ions for full activity. Mg2+ and Co2+ ions also activate the enzyme. WbdN activity has a broad pH optimum; the enzyme is stable at both 4 °C and -20 °C, and is highly specific for UDP-Glc as the donor substrate. GalNaca derivatives lacking the pyrophosphate group were inactive as substrates. Two imidazolium-lipid compounds strongly inhibited the activity. These inhibitors could be applied to block O-antigen synthesis in specific bacteria. This work was supported by an NSERC Discovery Grant (to I.B.).

CARB 125

O-GlcNAc modification and metabolism in cancer

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The dynamic modification of nuclear and cytoplasmic proteins by the monosaccharide GlcNAc continues to emerge as an important regulator of many biological processes. Herein we describe here the application of chemical reporters to visualize and identify changes in O-GlcNAc modification associated with cancer metabolism and protein regulators of cancer biology.

CARB 126

Thio-click Michael Addition (TCMA) approach to second generation of functionalized (1-5)-S-C-thio-oligosaccharides as new specific inhibitors of Galectin-3

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Galectin-3, a galactose binding protein involved in tumor metastasis, apoptosis, and inflammation differs significantly in its recognition of galactosyl residues within oligosaccharides. Specific inhibitors of Galectin-3 are very valuable tools as probes for protein structure and mechanism and are also attractive for their potential therapeutic applications. One of the potential classes of specific inhibitors of Galectin-3 is new family of S-C-thiodisaccharides.

Our prior synthesis of S-C-thiodisaccharides from unsaturated carbohydrate enones resulted in the generation of compounds with the potential to inhibit cellular proliferation as well as specific inhibitors of galectins. In our studies, we synthesized several new
analogs of new class of (1-5)-S-C-thiodisaccharides\(^2\) by base catalyzed thio-click Michael addition (TCMA) of reactive oligosaccharide 1-thiols bearing galactose moiety to fully protected and functionalized L-arabinose enone.

This presentation will summarize new developments in modification and functionalization of strategically important C-2 position of target molecules and their biological and chemical characterization. The design and discovery of this new generation of specific Galectin-3 inhibitors will be discussed in details.


CARB 127

**Structural identification of an unknown Trisaccharide in bovine milk**

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We have discovered a trisaccharide in bovine milk which contains \(N\)-Acetyl neuraminic acid substituted lactosyl-6' phosphate. We used mass spectrometry, HPLC and a new hydride reduction. With these methods we have identified this trisaccharide. We find the identity of the substitution of phosphate. With new chemistry we are able to discern whether the molecule is phosphorylated or sulfated. We have also discovered a simple method to isolate sialyl lactose-6'-phosphate. We precipitate protein with cold ethanol, centrifuge, remove supernatant and then dry the supernatant.

CARB 128

**Industrial production of the oligosaccharides based on organic synthesis**

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The successful industrialization and the commercialization of carbohydrate science is dependent on the establishment of industrial processes which can produce large quantities of functionalized glyco-conjugates in a stable and reliable manner.

We have developed and achieved industrial scale production of monosaccharides and oligosaccharides as building block raw material for chemical synthesis of functional oligosaccharides. This will make it possible to produce large quantities of functionalized glyco-conjugates.
We will introduce the synthetic saccharide building blocks available in large quantities. Then we will describe the synthesis of functional oligosaccharides and sugar conjugates with various aglycones.

**CARB 129**

**Preparation and characterization of chitosan/natural hydroxyapatite with chondroitin sulfate and amylopectin scaffold for bone tissue engineering**

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Artificial graft materials are important for bone tissue engineering. Novel tricomponent scaffolds of Chitosan/natural Hydroxyapatite with Chondroitin Sulfate (Chitosan/HAp-CS) and Amylopectin (Chitosan/HAp-AP) have been developed for the first time via freeze-drying method and physicochemically characterized as bone graft substitutes. Chemical interactions and dispersion of HAp, CS and AP in Chitosan matrix have been evaluated. The porosity and water uptake/retention ability of the composite scaffolds decreased whereas thermal stability increased as compared to Chitosan scaffold. The pore size of the Chitosan/HAp, Chitosan/HAp-CS and Chitosan/HAp-AP scaffolds varied from 60-180 µm, 60-400 µm and 80-500 µm, respectively. Cell proliferation, alkaline phosphatase activity and production of Type-1 collagen checked *in vitro* using MG-63 cell line was higher in composite scaffolds.

Excellent thermal stability, interconnected porosity, controlled biodegradation and
enhanced cell proliferation observations suggest that the novel Chitosan/HAp-CS and Chitosan/HAp-AP scaffolds are promising biomaterials for bone tissue engineering.

CARB 130

Glyco-macroligand microarrays with controlled orientation and varying carbohydrate density exhibit enhanced sensitivity and selectivity

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Glycan arrays have become powerful high-throughput tool for examining binding interactions of carbohydrates with lectins, antibodies, cells, and viruses. Recently, it has been applied for antibody detection and profiling, vaccine development, biomarker discovery, and drug screening applications. We report here a new type of glycanc array, an oriented and density controlled glycopolymer microarray, fabricated based on end-point immobilization of glycopolymer that was imprinted with boronic acid ligands in different sizes. Briefly, O-cyanate chain-end functionalized lactose-containing glycopolymer was pre-modified by lysozyme-boronic acid, BSA-boronic acid and polyacrylamid-boronic acid ligands and then immobilized onto an amine-functionalized glass slide via isourea bond formation, followed by releasing the boronic acid ligands, respectively. The imprinted glycol-macroligands showed enhanced lectin (Arachis hypogaea) binding compared to non-imprinted one. Furthermore, SPR results confirmed the same trend of density-dependent lectin binding as glycoarray. This glycoarray provides multidimensional glycan arrays with enhanced performance for probing the ligand specificities of glycan-binding molecules.

CARB 131

Hydrogen bonding, the foundations of helicity in a tetramer of α2-8 sialic acid

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Hydrogen bonding patterns define structures in biomolecules. Using a $^{15}$N, $^{13}$C α2,8-linked sialic acid tetramer as a model system at 263K, we detected intra-residue hydrogen bonds between the HN (at C5) and the oxygen atom (at C8) for residues I, II and III of the tetramer. The hydrogen bonds are supported by slower $^1$H/$^2$H exchange rate for the HNs. We generated two models based on the hydrogen bonds, one with two residues per turn ($5_{10}$ helix) and one with four residues per turn ($2_{10}$ helix). We used the models to obtain interatomic distances and report that the $5_{10}$ helical model is in better
agreement with the experimental NOE data. The models are further supported by heteronuclear coupling between H7 and C2 for residues II and III. Thus, hydrogen bonds can be a determinant factor for shaping the oligosaccharide three dimensional structure.

CARB 132

Conformational study of glycal-type neuraminidase inhibitors

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The conformational flexibility of two glycal-type neuraminidase inhibitors has been studied, using several molecular modeling techniques. In agreement with the experimental data available, an intramolecular hydrogen bond, representing a key structural feature that controls the conformer distribution in solution, has been identified. The contribution of each substituent on the overall equilibrium was evaluated using simplified glycal-type compounds. Additionally, four methods for estimating NMR coupling constants from dihedral angles were evaluated and the Haasnoot method was found to be appropriate for this class of sugars. These results should allow a better understanding of the structural parameters governing the physico-chemical properties of glycal-like compounds.

CARB 133

Aldose to ketose conversion using Tin/Ti-Lewis acid models: A computational study

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Mineral acid-catalyzed conversion of naturally abundant glucose (aldose) to platform chemicals such as hydroxy-methyl-furfural (HMF) and levulinic in aqueous solutions suffer a major drawback of poor selectivity, while fructose (ketose) yields HMF or levulinic acid with high selectivity. Recently, it has been reported that large pore Ti-beta zeolite can catalyze the isomerization of aldose to ketose analogous to enzyme Xylose isomerase. In this paper, we report detailed reaction mechanism of isomerization of glyceraldehyde, glucose to dihydroxyacetone and fructose, respectively by Tin/Ti-beta zeolite active site models using the DFT and MP2 levels of theory. Both hydroxylated sites (open site) and non-hydroxylated (close) sites were chosen for this investigation of Ti-beta. Additionally, the influence of the number of hydroxyl groups and alkali metal ions (Na+) on the glucose isomerization process will be discussed. The
concept behind this paper is to identify the active site of Tin/Ti-beta zeolites and suggest a reaction pathway through the simulated results.

This work was supported by the U.S. Department of Energy under Contract DE-AC0206CH11357. This material is based upon work supported as part of the Institute for Atom-efficient Chemical Transformations (IACT), an Energy Frontier Research Center funded by the U.S. Department of Energy, Office of Science, and Office of Basic Energy Sciences.

CARB 134

Understanding the interactions of inositol phosphates with protein kinase B using long time-scale simulations

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Inositol phosphates are an important family of molecules formed from the metabolism of phosphoinositide lipids. The specific arrangement of phosphorylation on their inositol rings is responsible for their distinct interactions and roles within the cell. Molecular dynamics (MD) simulations allow the molecular basis, and associated free energies, of these interactions to be elucidated. In order to carry out these simulations we have generated parameters for a family of inositol phosphates, not available in current force fields.

We have used a total of 3μs of MD simulations to better understand the interactions of protein kinase B (PKB) with inositol phosphates. Whilst it is widely accepted that PKB has a strong preference for 3-phosphorylated phosphoinositide lipids, its poor discrimination for their equivalent inositol phosphate head-groups has puzzled many. However, our atomic-level view provided by MD simulations has rationalised these findings by predicting different binding modes of inositol phosphates compared to their parent lipids.

CARB 135

Protonation of biomass-derived substrates: A computational challenge

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Biofuels have been gaining popularity as an alternative to petroleum based fuels in response to increased regulation of greenhouse gas emissions and decreasing global
production of crude oil. At LANL, we are developing a route from biomass to biofuels that encompasses dehydration of sugars followed by carbon chain extension to furans. For this project, a computational study was performed to help explain the diverse reactivity patterns of these furans during acid catalysis. In particular, we have developed a protocol for calculating pKₐs of the protonated furan substrates based on a thermodynamic cycle. After discussing the challenges encountered while developing this protocol, we protonate each furan substrate at various sites and calculate the resulting pKₐs to determine the preferred site (most basic) of protonation. Finally, we show that the nature of the most basic site of each substrate adequately explains its behavior (ring opening, decomposition, or no reaction) during acid catalysis.

CARB 136

Visualizing structure and dynamics of disaccharide simulations

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We examine the effect of several solvent models on the conformational properties and dynamics of disaccharides such as cellobiose and lactose. Significant variation in timescale for large scale conformational transformations are observed. Molecular dynamics simulation provides enough detail to enable insight through visualization of multidimensional data sets. We present a new way to visualize conformational space for disaccharides with Ramachandran plots.