

Drugs and Pharmaceuticals

Current R & D Highlights

(Carbohydrate-based Drugs)

Contents

Introduction		News & Views	27
• Carbohydrate-Based Drugs—An Introduction	1	R&D Highlights	32
Features		R& D Technology	41
• Carbohydrates as Potent Therapeutic Agents for Parasitic Infections	4	New Leads	44
• Design and Development of Carbohydrate Based Therapeutics	12	Natural Products	48
• Carbohydrate-Based Targets and Vehicles for Cancer and Infectious Diseases Vaccines	19	Biotechnology	59
		Patents	63



Carbohydrate-Based Drugs—An Introduction

Carbohydrates are involved in a variety of fundamental biological processes (cellular differentiation, embryonic development, fertilization) and pathological situations (bacterial and viral infections, inflammatory diseases, cancer). They therefore have a large pharmaceutical and diagnostic potential.

Carbohydrates make an ideal therapeutic platform because they are known to be involved in diverse biological processes. Increased understanding of how carbohydrate biology changes between the normal and the disease states has fueled interest in these molecules as excellent starting points for developing a multitude of new drugs and therapeutic approaches.

Carbohydrates are ubiquitous and represent the most abundant class of molecules in nature. All cell surfaces are coated with complex carbohydrates where they act as recognition molecules for other cells, functional molecules, and pathogens.

Complex carbohydrates are chains of monosaccharides, often called glycans, and are often found attached to proteins (to form glycoproteins) and lipids (glycolipids, glycosphingolipids, etc.). Glycoproteins are usually on the cell surface, where they are recognized by bacteria, viruses, and other proteins, such as lectins, in order to facilitate various crucial functions. It is also known that glycans are involved in a variety of biological processes including protein folding and signalling events. Unlike DNA and proteins, however, monosaccharides may be linked to one or more other monosaccharides, such that they form a branched tree structure. In order to

formulate a standardized notation for glycans, the Consortium for Functional Glycomics (CFG) proposed a standard symbolic representation for those monosaccharides that are found most in nature,

A relatively untapped source of new chemical entities of drugs comes from the area of functional carbohydrates. All cell surfaces are coated with complex carbohydrates which extend much further out from the cell than the protein layer. These intricate molecules act as recognition molecules, not just for other cells but also for pathogens that must identify and bind specific cell types for successful infection. On cell surfaces, they form layers known as glycocalyx ranging from 10 to 100 nm in thickness and thus extend much further out from the cell surface than proteins. These *O*- and *N*-glycans (according to the type of linkage to the non-carbohydrate core) are present in many different molecular forms including glycoproteins, proteoglycans, glycolipids, and glycosphosphatidylinositol-linked proteins. Their broad diversity originates from their assembly from monosaccharide building blocks, which can be linked with others at various positions on their pyranose/furanose rings. In addition, branched structures are formed because each ring can establish several linkages. Finally, the density of structural information is further increased by the possibility of α - and β -isomers at the anomeric center. These chemical characteristics bestow carbohydrates with optimal properties desired for recognition molecules. Proteins called “lectins” have evolved three-dimensional domains [carbohydrate-recognition domains (CRD)]

Introduction

that can bind specific carbohydrate structures and thus decode this information.

Cell surface carbohydrates involved in specific cell-to-cell recognition exhibit biological phenomena like cell adhesion, cell activation, inflammatory migration, neurite outgrowth, and cancer metastasis (Ernst and Magnani, 2009).

Pathogens including viruses, bacteria, and even parasites infect their host cells by recognizing and binding to specific carbohydrates on their surfaces. Perhaps the most popular examples are the influenza viruses, which are designated by the viral coat proteins hemagglutinin (H) and neuraminidase (N). Both of these viral proteins in fact bind specific carbohydrate sequences on the host cell surface. The current pandemic “swine flu” is designated H1N1. Soluble proteins, such as lectins, toxins, and antibodies, also bind cell surface carbohydrates and exhibit a fundamental role in many diseases. Antibodies that recognize carbohydrates and promote diseases are identified as therapeutic targets in autoimmune diseases such as certain forms of peripheral neuritis, Guillain-Barre syndrome, and paraproteinemias (Kaida et al., 2009). Other carbohydrate binding antibodies can prevent infectious diseases.

The identification of functional carbohydrate epitopes and their corresponding specific carbohydrate-binding proteins as new targets for drug development is emerging at a rapid rate due to concerted worldwide efforts (US: Consortium for Functional Glycomics; Europe: EurocarbDB; and Japan: Human Disease Glycomics/Proteome Initiative) to organize and support technologies for elucidating the human glycome.

While carbohydrates act as excellent recognition molecules on cell surfaces, many of their intrinsic properties make them poor choices for small molecule drugs. In many cases the binding affinities of monovalent

carbohydrates are in the milli- to micromolar range and therefore relatively weak and usually not adequate to compete with multivalent native interactions. The general hydrophilic nature of carbohydrates and their high density of hydroxyl groups (hydrogen bond donors and acceptors) works against the lipophilicity required for passive absorption through the intestine. Once in the circulation, native carbohydrates are susceptible to renal excretion. Finally, although much progress has been made in both enzymatic and chemical synthesis of glycans, the large-scale synthesis of native carbohydrate structures remains cumbersome and expensive.

Glycometics Compounds

All of these drawbacks have been addressed by rationally designing drug-like compounds of low molecular weight based on the structure of functional carbohydrate. These molecules, called glycomimetics, can reach far higher affinities than their native carbohydrate counterparts by optimization of their entropic and enthalpic binding contributions and by their pre-organization in the bioactive, i.e., bound conformation. By reducing their hydrophilicity, oral bioavailability can be achieved and by avoiding potential metabolic “soft spots” their plasma half-life can be improved.

The classic examples of glycomimetic drugs are the viral neuraminidase inhibitors Relenza and Tamiflu, which are used for the treatment of Influenza A. Both of these drugs mimic the transition state of the hydrolysis of the terminal *N*-acetylneuraminic acid by neuraminidase. In addition, Tamiflu is a prodrug, i.e., it incorporates an additional ethyl ester which increases its hydrophobicity, leading to passive transport and oral bioavailability. Ubiquitous esterases hydrolyze the prodrug into its active principle.

Several other glycomimetic drugs have been designed to inhibit the α -glycosidases in the brush border of the small intestine. Glustat,

Introduction

Zavesca, Glyset, and Glucobay mimic the transition state of the hydrolytic reaction and are used for the treatment of diabetes. Finally, several synthetic heparins (e.g., Arixtra) are approved. While these are not strictly glycomimetics, they participate in carbohydrate-protein interactions and are widely used.

Bioinformatics in Glycomics Research

Carbohydrates are considered the third class of information-encoding biological macromolecules. “Glycomics,” the scientific attempt to characterize and study carbohydrates, is a rapidly emerging branch of science, for which informatics is just beginning. Glycomics requires sophisticated algorithmic approaches. Several algorithms and models have been developed for glycobiology research in the past several years.

Glycans are the most abundant and structurally diverse biopolymers formed in nature. Bound to proteins, as glycoproteins, they are known to affect the functions of proteins. In terms of bioinformatics in glycobiology, there are several paths of research that are currently in progress. Several major glyco-related projects such as

Consortium for Functional Glycomics KEGG Glycan, GLYCOSCIENCES.de are maturing and provide well-structured glyco-related data that are awaiting data mining and analysis. With the exciting new developments in carbohydrate arrays and automated MS annotation, the analysis of the glycome has reached a new level of sophistication, which requires broader informatics support.

Along with the development of these glycan databases bioinformatic methods for analyzing glycan structures have also been developed. These can be classified into the following six categories: glycosylation analysis, glycomics, glycan biomarker prediction, glycan structure analysis, glyco-gene expression analysis, and glycan structure mining.

In research incremental increases in the capability of research tools have made the localization and functional characterization of glycoconjugates possible. Most importantly, carbohydrate chemists and glycobiologists have forged links that have allowed carbohydrate-based therapeutics to enter the mainstream drug discovery process.



Carbohydrates as Potent Therapeutic Agents for Parasitic Infections

M. K. Suthar, S. V. Singh, P. K. Doharey and J. K. Saxena
Division of Biochemistry, Central Drug Research Institute, Lucknow

Introduction

Among major classes of biomolecules, carbohydrates perhaps are the least exploited. It has been known that carbohydrates can serve as structural components of natural products, as energy sources, or, as key elements in various molecular recognition processes, including bacterial and viral infections, cell adhesion in inflammation and metastasis, differentiation, development, regulation and many other intercellular communication and signal transduction events.

All cells are coated with complex carbohydrates called glycans, which form a layer known as the glycocalyx, ranging from 10 to 100 nm in thickness. Glycans are present in many different molecular forms, including glycoproteins, proteoglycans, glycolipids and glycoposphatidyl inositol linked proteins. Their broad diversity originates from linking of monosaccharides to each other at various positions on their pyranose or furanose rings. Each ring can establish several linkages, giving rise to branched structures. The structural complexity of glycans is further increased by the possibility of α and β isomers at the anomeric centre. The precise mechanism of many carbohydrate mediated recognition processes are, however, not well understood. The pace of development of carbohydrate-based pharmaceuticals has been slower than that of the other classes of biomolecules.

Several reasons may be attributed to this slow pace of development e.g. due to unsolved technical problems, no PCR equivalent replication system available for the amplification of minute amounts of carbohydrates to facilitate structure analysis and synthesis, and absence of the solid-phase synthesis system for oligosaccharides to facilitate the study of their functions.

In drug development, glycans conventionally play role in stabilizing and modulating the protein subunit of glycoproteins. Many therapeutic proteins, including antibodies, growth factors and cytokines, are derived from naturally glycosylated proteins, the effect of glycans on serum half-life, immunogenicity and activity of biotechnologically derived proteins have attracted growing attention. Currently, glyco-engineering entails either addition or subtraction of chemicals to the media which influence the cellular synthesis of polysaccharides, addition of glycosyl-transferases to polysaccharide chains *in vitro*, or genetic manipulation of the cells which produce the glycosylated proteins. *De novo* synthesis of complex glycans is a difficult task which is further complicated by the fact that the structure of most potentially therapeutic glycans is unknown. The best studied examples of successful glycan-based drugs are the low molecular weight heparins (LMWHs).

Feature

The anticoagulant activity of heparin is due to its inhibition of the blood coagulation Factors Xa and IIa via binding of a specific heparin pentasaccharide sequence to the serine protease inhibitor antithrombin III (ATIII). LMWHs are therefore important antithrombotic drugs which are derived from unfractionated heparin by a multitude of chemical and enzymatic depolymerization and modification reactions. There are three main LMWHs currently in clinical use: enoxaparin sodium (Lovenox), dalteparin sodium (Fragmin), and tinzaparin sodium (Innohep). In addition to their anticoagulant activity, heparin-derived oligosaccharides exhibit anti-inflammatory, anti Alzheimer's as well as anti cancer activities. Despite potential in all of these the specific heparin ligand structure has still not been identified, but once this has been achieved, promising applications of these glycan-derived drugs are expected.

Concept of glycomics and glycan microarray:

Glycome is the entire complements of sugars, whether free or present in more complex molecules, of an organism. The comprehensive study of glycome is called glycomics which aims to understand the information contained in glycan structures derived from organisms, tissues or cells. Enzymes which tailor glycan determinants and proteins specifically interacting with these glycan make specific 'glyco-code', which serves a versatile communication tool for biosignaling, cell-specific targeting, and host-defense pathways. Interactions of glycans with proteins can be studied in several ways, but it can now be studied using microarrays of immobilised glycan structures. Such arrays consist of many spots, each one formed by the deposition of an individual glycan. The advantages of the microarray concept can be seen in two ways, first as miniaturisation, reduction of the volume of reagents, the reaction volume, and development of automated procedures, and second concerns

the efficacy of recognition between the immobilized carbohydrate and the glycan binding proteins.

Complex glycan structures of human and animal parasites:

Recent years have witnessed the advent of new and improved methods for glycan analysis. These methods, which include nuclear magnetic resonance spectroscopy and mass spectrometry, have led to an explosion in information about the structures of parasite-derived glycans. Protozoan parasites make unique and highly complex glycan structures, for example, African trypanosomes generate GPI-lipid-anchored VSG containing species specific modifications of the anchor moiety and unique biosynthetic steps in GPI biosynthesis that differ from those in mammals. Although the VSG variations are genetically controlled by the parasite and thwart development of vaccines against the VSG itself, there are promising opportunities for development of new therapeutics based on inhibition of enzymatic steps in GPI biosynthesis unique to trypanosomes. Virtually all protozoans, including *Plasmodium* parasites, synthesize GPI anchors which may be a novel target for vaccine production. *Trypanosoma cruzi* also synthesize O-glycans (Ser/Thr-linked) on GPI-anchored mucins with an unusual structure where they are linked by O-GlcNAc and are dominated by galactose-containing structures, in contrast to mammalian O-glycans that are often linked by O-GalNAc and contain considerable levels of GlcNAc and Gal residues in addition to other sugar residues. *Leishmania* synthesizes surface lipophosphoglycan (LPG) with unique phosphodiester of mannose in the backbone and furanosylgalactoside modifications that may be contained within ether-based phospholipids, GPI-anchored glycoproteins, mucin-type membrane and secreted glycoproteins. Different *Leishmania* species, such as *L. donovani*, *L. major*, and *L. mexicana*, differ in the way they modify the

Feature

LPG backbone and the number of repeating units.

Parasitic helminths generate an amazing array of carbohydrate structures which include both N- and O-glycans of surface and secreted glycoproteins, glycolipids, and other polysaccharide-based components. It is consistently observed that worms generate N- and O-glycans with core structures similar to those found in mammalian glycoproteins, but the worms introduce modifications that are unique to parasites. *Haemonchus contortus*, *Trichinella spiralis*, and *Schistosoma* species, synthesize N-glycans with a 3-linked core fucose residues, a modification apparently limited to invertebrates and plants. Schistosomes also synthesize many other antigenic glycans, including Lewis x (Lex) antigen, poly(Lex), lacdiNAc (LDN), fucosylated LDN sequences and glucuronic acid-containing circulating cathodic antigen (CCA). Except for CAA, most of these antigens have been found N-glycans of parasite glycoproteins, but some also occur in O-glycans. Interestingly, Lex, LDN, and LDNF are expressed by all developmental stages of schistosomes, including newly transformed schistosomula, although the surface expression and exposure are different. Using IgM monoclonal antibodies specific to the LDN, LDNF, and Lex antigens, it is apparent that 3-h-old schistosomula express these antigens over their entire surface. This explains the ability of monoclonal antibodies to the LDN antigen to effectively kill the 3-h-old schistosomula in a complement-dependent fashion *in vitro*. The LDN and LDNF structures are common to many parasitic helminths, including *Fasciola hepatica*, *Dirofilaria immitis*, and *H. contortus*, whereas Lex structures are highly restricted, and so far have been found only in schistosomes and the bovine parasitic nematode *Dictyocaulus viviparus*. No parasitic helminths appear to synthesize sialic acid-containing glycans, suggesting a lack of the genes required for

synthesis and mobilization of sialic acid. Consistent with this observation, the genome of the free-living nematode *Caenorhabditis elegans* lacks genes involved in sialic acid and CMP-sialic acid synthesis and utilization.

Glycans as diagnostic reagents for parasitic infections:

Current diagnostics for parasitic infections largely rely on microscopic examination of blood, tissues, body fluids, and stool or urine samples for the physical presence of some parasite life cycle stage. This mode of diagnosis has many drawbacks. Serodiagnosis, which could circumvent many of these problems, has not found much application for several reasons. The most serious problems affecting the development of serodiagnostics for parasitic infections are related to the identification and the production of antigenic targets of high specificity. Attempts to diagnose helminthic infections by serology have been hampered primarily by antigenic cross-reactivity among different helminths. Interestingly, many of the cross-reactive antigens have now been shown to be glycans. For several helminths, these cross-reactive epitopes appear to be LDN and LDNF glycans that occur commonly on outer branch structures of helminth-derived N-glycans. Different helminths may also synthesize unique antigenic glycans that can be utilized as targets for specific differential diagnosis of helminthic infections. The detection of antibodies to parasite glycans requires development of specific glycan-based reagents. Antibodies to glycans could also be used to capture circulating antigens derived from the parasites. Helminths are known to secrete glycoproteins into sera. For example, both circulating cathodic antigen (CCA) and the circulating anodic antigen (CAA) derived from *S. mansoni* are present in sera of infected individuals and their levels as detected immunologically generally correlate with infection status. Thus, the development of a better understanding of glycan antigen

Feature

structures and monoclonal antibodies to these antigens should have broad use in the future for the development of diagnostic procedures for various parasitic infections. The problem of potential cross-reactivity of different glycan antigens could be addressed by using a large panel of conjugates with different glycan structures and the pattern of recognition of such a panel might be valuable in identifying the specific parasitic infection.

Carbohydrates in Vaccine development:

Most of the vaccine discovery effort thus far has focused on generating peptide-, protein- or DNA-based vaccines, but these efforts have had very limited success. The ineffectiveness of these strategies is probably due to a combination of factors. These include our relatively poor understanding of the relevant glycan and protein antigens; the recent appreciation that glycans are often the dominant antigens; the complexity of presentation and availability of target parasite antigens for interactions with immune responses generated against them; the lack of understanding of the molecular interactions between host immune cells and parasites; lack of understanding of parasite interactions with their intermediate hosts; alterations in expression levels of antigens during parasite development in infected host; and the fact that many protozoan parasites reside intracellularly in host tissues and are not readily available to immune attack. Thus, there is a clear need to better define the structures and immunogenicity of surface macromolecules synthesized by parasites and their availability for immune interactions.

There is strong evidence that carbohydrate, rather than protein; antigens dominate the immune responses to many helminthic parasites. Complex carbohydrates are also important to interactions of protozoan parasites with their hosts. Furthermore, antigen processing cells (APCs), such as dendritic cells and macrophages, appear to specifically

recognize many parasite-derived glycans, partly through Toll receptors and C-type lectins, which can lead to enhanced or attenuated responses to parasitic infection. Yet, studies on the use of carbohydrates as immunogens and as potential vaccine candidates have been very limited. This limitation arises from the technical difficulties in obtaining large quantities of parasites that do not readily grow in vitro, the historical difficulty in defining structures of unusual parasite-derived glycan antigens, and the difficulty in synthesizing specific glycan antigens in sufficient quantities for testing and evaluation as vaccine components. But perhaps more importantly, there has been a general underestimation of the efficacy of glycan-based vaccines to combat infections. Virtually all vaccines provide protective immunity through the induction of antibodies.

However, the immune responses to pure protein or pure oligo- or polysaccharide antigens are different. Most antibody synthesis and isotype switching is thought to require cognate interactions between antigen-specific B cells and MHC class II-restricted CD4⁺ T cells (T cell-dependent antigens), but B cell activation and Ig switching can also occur without T cell help (T cell-independent antigens). These latter responses are often associated with imbalanced immunoglobulin isotype responses. Protein antigens in humans, which are T cell-dependent antigens, generate strong IgG1 and IgG3 responses, which are associated with good protective immunity. By contrast, free, unconjugated oligo- or polysaccharides are T cell-independent antigens and are either poorly immunogenic, generate relatively weak IgM responses, or upon appropriate stimulation may induce T cell-independent IgG2 responses. But the conjugation of oligo- or polysaccharides to carrier protein (glycan conjugates) enhances processing, presentation, and recognition of glycan antigens by APCs and T/B lymphocytes, leading to T cell-dependent and

Feature

relatively strong IgG1 responses in humans. These findings have been exploited to develop glycan-based protein conjugate vaccines for several encapsulated microbes, including *H. influenzae* type b, which provide effective immunity against certain types of bacterial infections. Thus, the earlier limitations of glycans as vaccine antigens have been overcome by chemical coupling of glycan antigens to carrier proteins. Conjugates with other glycans are the basis of vaccines to *Streptococcus pneumoniae* (types 4, 6B, 9V, 14, 18C, 19F, and 23F) and *Neisseria meningitidis* (serogroup C). Many other microbial conjugate vaccines are currently in development toward *Salmonella typhi*, *Pseudomonas*, *Klebsiella*, group B *Streptococcus*, and *E. coli*. Thus, glycans as components of vaccines are no longer limited by immunologic considerations of the relatively weak immunogenicity of free glycans.

Glycoconjugates are desirable vaccine targets because:

- i. For most parasites, and certainly for parasitic helminths, major glycan antigens occur on many different glycoprotein species, thus enhancing the display and multiplicity of antigens targeted by antibodies.
- ii. Moreover, these glycoconjugates occur on the outer surfaces of the parasites, thus making them the preferred targets by their availability for direct interactions with immune effectors.
- iii. The average density of glycan antigens on a cell is likely to be much greater than the density of a specific protein antigen, since a single glycoprotein may contain multiple glycan epitopes. Thus, it is possible that anti-glycan antibodies will provide a higher level of bound antibodies and promote complement fixation or opsonization more quantitatively than antibodies to a specific protein.

iv. Antibodies to glycan antigens in experimentally infected animals can fix complement and cause parasite lysis.

v. Finally, it is now possible to synthesize complex glycans by combinations of chemical and enzymatic approaches and to tailor them for specific conjugation to carrier protein. Such semi-synthetic glycoconjugates are highly antigenic and are well recognized by antibodies in sera of infected animals.

Antigenic glycans of parasites *Entamoeba histolytica*, *Plasmodium falciparum*, *P. berghei*, *Leishmania donovani*, *L. Mexicana*, *Trypanosoma cruzi*, *Schistosoma mansoni*, *Fasciola hepatica*, *Onchocerca volvulus*, *Setaria digitata*, *Litomosoides sigmodonti* are well characterized.

Conjugate vaccines against parasites:

The available structural information about parasite glycans and the evidence that glycoconjugate-enriched antigens can provide protective immunity naturally raises hope for the development of conjugate vaccines. There are various types of vaccines to consider for parasites. The complex life cycles of parasites and the pathology they cause provide avenues for the design of different vaccine strategies to limit their propagation, transmission, and pathology.

Malaria conjugate vaccines

Malaria parasites synthesize GPI anchors, which probably constitute over 90% of the total glycoconjugates made by the parasite. Importantly, these parasite GPIs appear to be dominant malarial toxins responsible for many of the severe pathological consequences of the disease. The malarial GPI can activate macrophages and vascular endothelial cells through activation of several signaling pathways, resulting in production of mediators such as nitrous oxide (NO), tumor necrosis factor α (TNF- α), and intercellular adhesion molecule-1 (ICAM-1). Thus, the malarial

Feature

parasite GPI has been targeted for evaluation as a new vaccine candidate. A synthetic version of the malarial GPI, with multiple mannose residues, glucosamine, and 6-myoinositol- 1, 2-cyclic phosphate was chemically synthesized. Immunization of C57BL/6J mice with the synthetic GPI-conjugate in Freund's adjuvant resulted in significant protection after challenge with the rodent malaria parasite *Plasmodium berghei* ANKA, thus suggesting common structures of GPI between all *Plasmodium* species, including those that infect rodents.

Trypanosomiasis

T. cruzi does not synthesize sialic acid, but expresses a trans-sialidase that catalyzes the transfer of sialic acid from host glycoconjugates to the parasite surface. This trans-sialidase may be a virulence factor required for successful infections. Immunization of mice with a plasmid DNA containing a gene encoding the catalytic domain of the trans-sialidase generated antibody and T cell-mediated immune responses. These antibodies recognized the native enzyme and inhibited its activity in vitro. The immunized animals displayed reduced parasitaemia and mortality upon challenge with bloodstream trypomastigotes.

Leishmaniasis

The LPG derived from *Leishmania* is not highly immunogenic in natural infections and in fact it functions in part to prevent complement-mediated lysis by blocking insertion of the C5b-9 membrane attack complex into the promastigote membrane. However, mice vaccinated with preparations of LPG derived from the parasite develop protective immunity.

Schistosomiasis

Among helminths, the immune responses to glycans have been most intensively studied in schistosomes. In humans, primates, and rodents, carbohydrate antigens contained in

schistosome glycoconjugates generate all isotypes of immunoglobulin; IgM, IgG, and IgA and all subclasses of IgG, including IgG1. In regard to specific glycan antigens, *S. mansoni*-infected patients, with either intestinal or hepatosplenic disease, generate mainly IgM, but also IgG and IgA, to LDN, LDNF, and Lex.

Carbohydrates as anti-adhesion drugs:

To colonize a host and cause disease, many bacterial pathogens have evolved means for attachment or adhesion to the host cells and tissues. Adhesion is required so that the organisms are not swept away and provides the pathogens with better access to sources of nutrition, facilitates the delivery of toxic agents into the host tissues and eventually the penetration bacteria into the tissues. The most common means of adhesion, expressed by numerous bacteria, are surface lectins that combine with complementary carbohydrates present on the host cell surfaces. They serve as virulence factors of the organisms and are among the determinants of their organ and tissue tropism. Blocking or inhibition of these lectins by suitable carbohydrates or their analogs for the prevention and treatment of microbial diseases is the aim of anti-adhesion therapy of such diseases. Saccharides are ideal for this purpose as they are unlikely to be toxic or immunogenic, in particular since many of those that inhibit bacterial adhesion are normal constituents of cell surfaces or body fluids, such as human milk. Moreover, since anti-adhesive agents do not act by killing or arresting the growth of the pathogens, it is very likely those strains resistant to such agents will emerge at a markedly lower rate than those that are resistant to antibiotics, and that their spread in the environment will be minimal.

Attachment sites for bacterial pathogens on animal tissues:

Most bacterial lectins are surface-bound, commonly in the form of submicroscopic multi-subunit proteins designated as fimbriae

Feature

or pili, the expression of which is dependent on growth conditions. An individual bacterium may co-express more than one lectin. Carbohydrates present on host tissue provide means of attachments and specificity (Table 1). Enterobacterial surface lectins the most prevalent and best characterized bacterial surface lectins with respect to structure, biosynthesis and function are products of enterobacterial strains, namely the mannose specific type 1 fimbriae of *E. coli*, and the galabiose-specific P fimbriae of uropathogenic *E. coli* (UPEC). More recently the N-

acetylglucosamine-specific F17 fimbriae of *E. coli* have also been studied in considerable detail. Attachment of a pathogen to a tissue does not of itself initiate disease. It must be coupled to specific responses that lead to infection. Following adherence, the target cells are activated, with resultant production of cytokines that engender acute inflammation and other symptoms of disease, while in the bacteria the interaction leads to up-regulation of signal transduction systems that allow responses to the changing environment.

Table 1. Carbohydrates of host tissues acting as attachment sites for parasites:

Parasite	Carbohydrate of host
<i>E.coli</i> type 1	Man α 3Man α 6Man
<i>E.coli</i> K99	NeuAc(α 2-3)Gal β 4Glc
<i>H. influenza</i>	[NeuAc(α 2-3)] _{0,1} Gal β 4GlcNAc β 3Gal β 4GlcNAc
<i>H. pylori</i>	NeuAc(α 2-3)Gal β 4GlcNAc Fuc α 2Gal β 3(Fuc α 4)Gal
<i>K. pneumonia</i>	Man
<i>N. meningitidis</i>	[NeuAc(α 2-3)] _{0,1} Gal β 4GlcNAc β 3Gal β 4GlcNAc
<i>P. aeruginosa</i>	Gal β 3Glc(NAc) β 3Gal β 4Glc

Anti-adhesion therapy:

Co-administration of methyl α -mannoside with type 1 fimbriated *E. coli* into the urinary bladder of the mice reduced the rate of urinary tract infection by two thirds, while methyl α -glucoside, which is not inhibitory to the fimbriae, was without effect. Methyl α -mannoside is also effective against *K. pneumoniae* type I and Sia3LacNAc is strong anti-adhesive agent against *H. pylori*. The protective effect of anti-adhesive sugars against different pathogenic bacteria support the concept that antiadhesive carbohydrates

might be useful in the fight against bacterial infections. Human milk contains different oligosaccharides, some in high concentrations (mg to few hundreds mg/liter), several of which are inhibitors of the surface lectins of various bacteria, Of particular interest in this respect are the fucosylated oligosaccharides (e.g., Fuc α 2Gal β 4GlcNAc) that are effective inhibitors of the adhesion of the enteropathogen *Campylobacter jejuni* to human cells. Infants breast-fed on milk containing high levels of these oligosaccharides suffer from a considerably lower incidence of diarrhea than those fed on

Feature

milk with low levels of such oligosaccharides. Two major mucus-associated glycoproteins that carry oligomannosides, namely IgA and Tamm–Horsfall glycoprotein interact with the type 1 fimbrial lectin in a mannose specific fashion. Significantly, as mentioned earlier, Tamm–Horsfall knockout mice were very recently shown to be considerably more susceptible to bladder colonization by type 1 fimbriated *E. coli* than normal mice, whereas they were equally susceptible to P fimbriated *E. coli*. These results provide the first in vivo evidence indicating that under physiological conditions, Tamm–Horsfall glycoprotein can serve as an effective soluble receptor for type 1 fimbriated *E. coli*, inhibiting them from adhering to the uroplakin Ia receptors present on the urothelial surface.

Future perspectives and conclusions:

At molecular level, recognition of carbohydrates and related structures in biological systems represents a new frontier of research. Glycans have potential therapeutic benefit as drug themselves or as drug targets. Understanding of the molecular details of protein-glycan interaction will provide the key for therapeutic targeting of this molecular encounter. Breakthroughs in this field may lead to the development of effective vaccines and enhanced serodiagnostics to control parasitic infections. There is possibility in developments of glycomimetics (carbohydrate analogs) that are more potent inhibitors of bacterial adhesion agents than the available saccharides.

References

1. Astronomo RD., Burton DR.2010. Carbohydrate vaccines: developing sweet solutions to sticky situations. *Nat Rev.* 9, 308-324.
2. Ernst B., Magnani JL.2009. From carbohydrate leads to glycomimetic drugs. *Nat Rev.* 8, 661-677.
3. Gesslbauer B., Kungl AJ.2006. Glycomic approaches toward drug development: Therapeutically exploring the glycosaminoglycanome. *Curr Opin Mol Ther.* 8(6), 521-528.
4. Nyame AK., Kwarar ZS., Cummings RD.2004. Antigenic glycans in parasitic infections: implications for vaccines and diagnostics. *Arch Biochem Biophys.* 426, 182–200.
5. Sears P., Wong C.1996. Intervention of carbohydrate recognition by proteins and nucleic acids. *Proc Natl Acad Sci USA.* 93, 12086-12093.
6. Sharon N.2006. Carbohydrates as future anti-adhesion drugs for infectious diseases. *Biochim Biophys Acta.* 1760, 527–537.

Views expressed in the journal are those of the authors and the Editorial Board/Publisher takes no responsibility for the same. We are a secondary abstracting service and the veracity of information is of the source quoted and not our primary responsibility.

Editor



Design and Development of Carbohydrate Based Therapeutics

Surendra Singh Bisht and Rama P. Tripathi

Medicinal and Process Chemistry Division, Central Drug Research Institute, Lucknow, India

A large number of carbohydrates and carbohydrate derivatives are used as therapeutics. Understanding the bioactive conformation and carbohydrates-protein interaction, allow the rational design of small drug like molecules with increased affinity, stability, and bioavailability.

Introduction

Recent advances in glycobiology, glycochemistry and glycomedicine have given a strong interest in the study of carbohydrates. Among their role in metabolism and structural building blocks, carbohydrates are constituent of every cell surface.^{1,2} The recognition of carbohydrates in the form of glycoconjugates,³ the significance of carbohydrates in cell-cell recognitions,⁴ cell-external agent interactions,⁵ inflammation,⁶ immune response,⁷ metastasis⁸ and fertilization⁹ is widely accepted.

On the other hand the molecular diversity introduced in the carbohydrate structures is extremely high, it is due to the occurrence of the two possible anomers and to the presence of commonly four or five attachment points per sugar unit with different stereochemistry, affording highly diverse or complex linear or branched structures.¹⁰ Due to their relevant biological role and molecular diversity, carbohydrates are promising candidates for drug design and disease treatment.

Different approaches in carbohydrate based therapeutics design

We can design carbohydrate-based

therapeutics following different approaches¹¹ which are-

- **The 'native glycan approach':** This approach is used for the development of vaccines against viruses and bacteria. For example anticancer therapeutic vaccines and antibacterial vaccines were developed by this approach.
- **The 'glycomimetic approach':** In this approach the new class of small drug molecules were developed by understanding the carbohydrate-protein interactions. This approach should guarantee a higher *in vivo* stability and better pharmacokinetic properties.
- **The 'carbohydrate scaffolds approach':** In this approach the scaffolding structures for the presentation of binding functional groups in a specific orientation can be generated from carbohydrates. The advantage of this approach is high functional and structural diversity from carbohydrates. For example peptidomimetics and inositol analogs have been synthesized according to this approach.
- **The 'glyco-fused therapeutic approach':** This approach involves the synthesis of the drug structure onto a sugar moiety. The advantages of this approach are the solubility issue (which is the main problem of organic based drugs) or modulate the pharmacokinetic properties.

Feature

Carbohydrate and carbohydrate based therapeutics

○ Carbohydrate based antibacterial vaccines

Natural polysaccharides conjugated to carrier proteins are made as vaccines against *Neisseria meningitidis*, *Streptococcus*

pneumoniae, *Haemophilus influenzae* type b (Figure 1, Hib)^{12,13} and other synthetic oligosaccharide epitopes offer opportunities for vaccination against HIV, *Plasmodium falciparum*, *Vibrio cholerae*, *Cryptococcus neoformans*, *S. pneumoniae*, *Shiga toxin*, *Bacillus anthracis* and *Candida albicans*.¹⁴⁻¹⁷

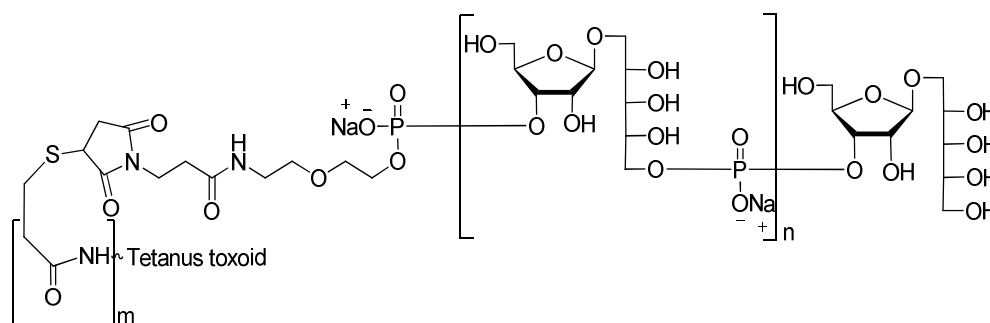


Figure 1. Synthetic conjugate polysaccharide vaccine against *Haemophilus influenzae* type b

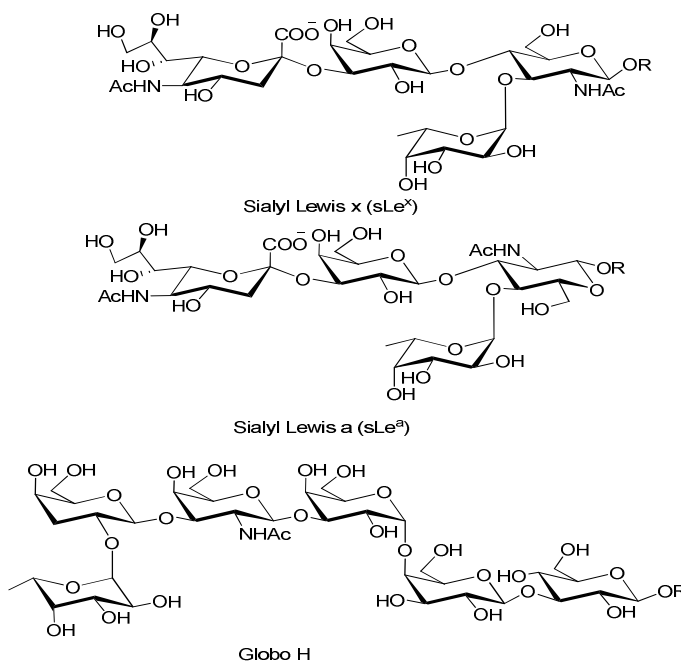


Figure 2. Chemical structures of some TACAs

○ Carbohydrate-based anticancer vaccine

Synthetic oligosaccharides have used for the development of therapeutic vaccines for cancer. Cancer cells often display alterations in their glycan repertoire if compared to normal cells, resulting in the accumulation of new

structures such as sialyl Lewis x (sLe^x), sialyl Lewis a, Globo-H (sLe^a), etc.; on the cell surface (Figure 2)^{18,19}, usually referred to as tumor-associated carbohydrate antigens (TACAs).

A three-component vaccine composed of a

Feature

TLR2 agonist, a promiscuous peptide T-helper epitope and a tumor-associated glycopeptides²⁰ gave promising anticancer activity in mice.

○ *Monosaccharide mimics*

The monosaccharide mimics as drugs are shown in figure 3, prominent examples are the inhibition of α -glycosidases for the treatment

of diabetes by voglibose,²¹ miglitol²², the prevention of influenza virus infection by zanamivir²³ and oseltamivir.²⁴ Promising results have also been recently obtained in the treatment of cystic fibrosis with miglustat.²⁵ topiramate is an anticonvulsant drug.²⁶

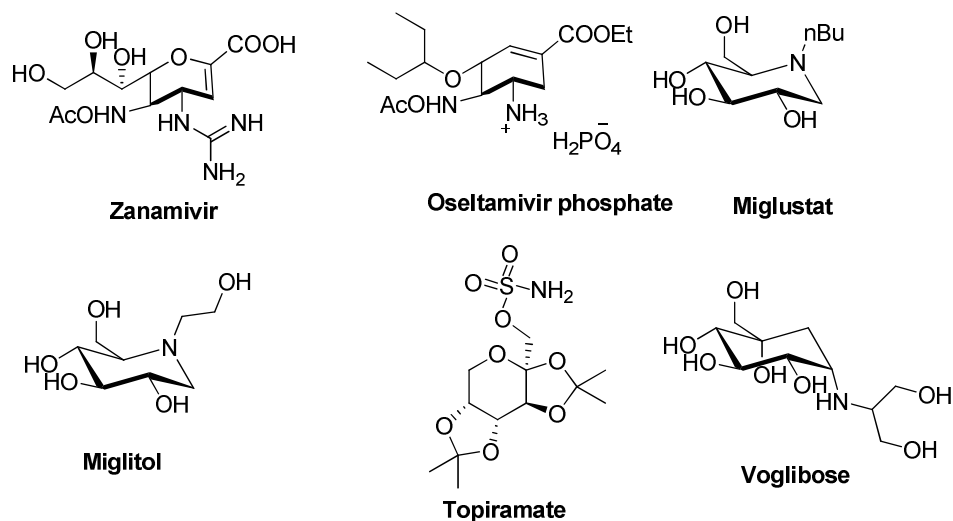


Figure 3. Monosaccharide mimics as drugs

○ *Oligosaccharide mimics*

Validamycin A^{27,28} (Figure 4), the major active ingredient of the fermentation culture of *Streptomyces hygrosopicus* subspecies *limoneus* is an agricultural antibiotic with good

control on diseases of fungi. Acarbose²⁹ (Figure 4) is another bioactive oligosaccharide of natural origin and it is used to treatment type 2 diabetes mellitus.

Dear Readers

We are happy to receive your encouraging response to our journal. We shall, however, appreciate receiving your critical comments and suggestions for further improvements. We are always looking forward to your specific contributions on various aspects of the journal. The contributions shall be duly acknowledged.

Editor

Feature

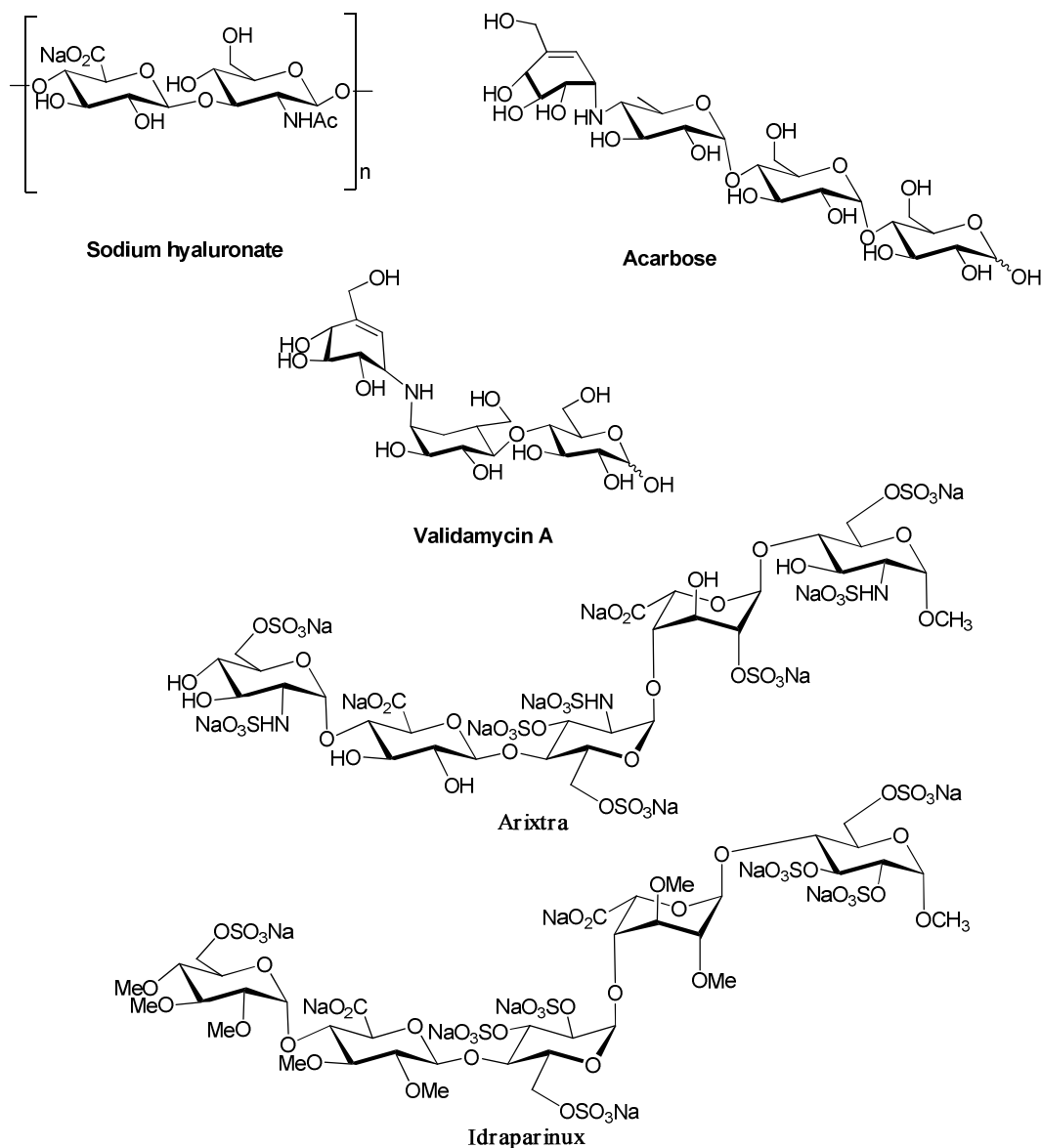


Figure 4. Oligosaccharide mimics

- ***Glyco-fused therapeutics***

Sodium hyaluronate³⁰ (Figure 4) surgical aid in cataract extraction, corneal transplant, retinal attachment surgery; relief of mild to moderate pain due to osteoarthritis of the knee. It also used for treatment of dry eyes. Indraparinix (Figure 4) is another synthetic heparin develop for anticoagulation therapy (Figure 4).³¹

The synthesis of GABA_A receptor ligands, where the bioactive moiety pyrrolobenzodiazepines^{32,33} have been engineered on a sugar scaffold was an example of this approach (Figure 5). In this case, the sugar moiety plays a crucial role in improving the drug solubility and confers conformational constraints to the drug, which is a key issue in the design of GABA_A receptor ligands.

Feature

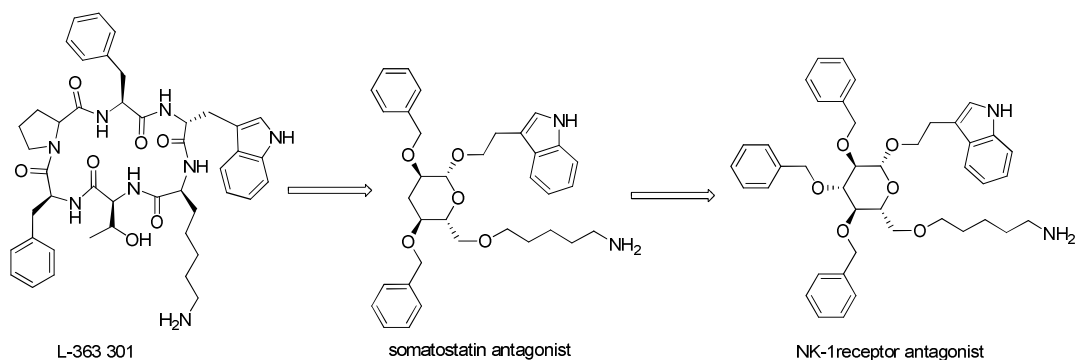


Figure 5. Somatostatin mimetic based on a glucose scaffold

○ ***Aminoglycoside as antibacterial drugs***

Streptomycin³⁴⁻³⁶ was the first aminoglycoside antibiotic identified; it inhibits protein synthesis by binding tightly to the

conserved site of 16S rRNA in the 30S ribosomal subunit. Kanamycin and amikacin³⁴⁻³⁶ are an aminoglycoside antibiotic having the same mode of action as streptomycin.

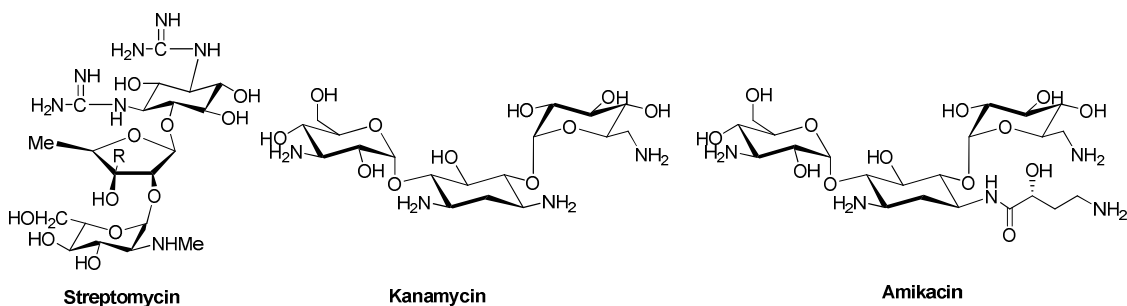


Figure 6. Aminoglycosides as antibacterial

○ ***Naturally occurring biologically active sugar amino acids***

Sugar amino acids (SAAs) were found in

nature as construction elements. Interestingly two different 3-amino-3-deoxy uronic acids, derivatives of 3-amino-3-deoxy- *O* -

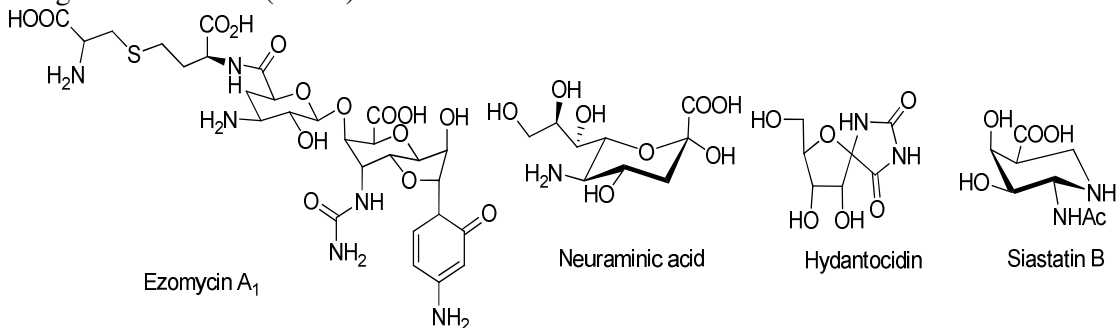


Figure 7. Several naturally occurring sugar amino acids

gulopyranuronic acid and 3-amino-3,4-dideoxy-*O*-xylohexopyranuronic acid, were found in natural antibiotic ezomycin A (Figure 7).³⁷ The *N*- and *O*-acyl derivatives of

dideoxy-*O*-xylohexopyranuronic acid, were found in natural antibiotic ezomycin A, which is located peripherally on

Feature

glycoproteins.

The naturally occurring furanoid SAA (+)-hydantocidin exhibits herbicidal activity, which represents a spiro hydanthion derivative.³⁸ Siastatin B inhibitor for both α -glucuronidase and *N*-acetylneuraminidase was isolated from a *Streptomyces* culture.³⁹

Conclusion

Several trials made in the field of carbohydrates for the generation of therapeutics, which carried few drugs to the

clinic. The success stories of developing vaccine candidates using glycoproteins, development of drug molecule from monosaccharide, and oligosaccharide mimics mark the beginning of an era where carbohydrates will be used more as therapeutic, diagnostic and nutritional agents. Drugs will be created from carbohydrates by rational design or combinatorial approaches and engineering of the active drug on glycidic structures.

References

1. Van Teeffelen, J. W.; Brands, J.; Stroes, E. S.; Vink, H. *Trends Cardiovasc. Med.* **2007**, *17*, 101-105.
2. Nieuwdorp, M.; Max, N.; Marijn C. M.; Hans L. M.; Can I.; Lysette N. B.; John J. P. K.; Erik S. G. S.; Hans V. *J. Appl. Physiol.* **2008**, *104*, 845-852.
3. a) Wang, P.; Bertozzi, C. Eds., *Glycochemistry: Principles, Synthesis, and Applications* (Marcel Dekker, New York, 2001);
b) Bisht, S.S.; Fatima, S.; Tamrakar, A.K.; Rahuja, N.; Jaiswal, N.; Srivastava, A.K.; Tripathi, R.P. *Bioor Med Chem Lett*, 2009, *19*, 2699-2703; (c) Pandey, V.P.; Bisht, S.S.; Mishra, M.; Kumar, A.; Siddiqui, M.I.; Verma, A.; Mittal, M.; Sane, S.A.; Gupta, S.; Tripathi, R.P. *Eur J Med Chem*, 2010, *45*, 2381-2388.
4. Okajima, T.; Irvine, K. D. *Cell*, **2002**, *111*, 893-904.
5. Lis, H.; Sharon, N. *Chem. Rev.*, **1998**, *98*, 637-674.
6. Varki, A. 1993, *3*, 97-130.
7. Lowe, J. B. *Cell*, **2001**, *104*, 809-812.
8. Fuster, M. M.; Esko, J. D. *Nat. Rev. Cancer*, **2005**, *5*, 526-542.
9. Bleil, J. D.; Wassarman, P. M. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 5563-5567.
10. Werz, D.B.; Ranzinger, R.; Herget, S.; et al. *ACS Chem. Biol.*, **2007**, *2*, 685-691.
11. Sibylle, A. W.; Gruner, E. L.; Elisabeth, L.; Horst, K. *Chem. Rev.*, **2002**, *102* (2), pp 491-514
12. Verez-Bencomo, V.; Fernandez-Santana, V.; Hardy, E, et al. *Science*, **2004**, *305*, 522-525.
13. Fernandez-Santana, V.; Cardoso, F.; Rodriguez, A.; et al. *Infect. Immun.*, **2004**, *72*, 7115-7123.
14. Wang, L.X. *Curr. Opin. Drug Discov. Devel.*, **2006**, *9*, 194-206.
15. Taylor, R.K.; Kirna, T.J.; Bosea, N. et al. *Biodiversity*, **2004**, *1*, 1036-57.
16. Stallforth, P.; Lepenies, B.; Adibekian, A.; Seeberger, P.H. *J. Med. Chem.*, **2009**, *52*, 5561-5577.
17. Pozsgay, V. *Curr. Top. Med. Chem.*, **2008**, *8*, 126-40.
18. Hakomori, S.I. *Cancer Cells*, **1991**, *3*, 461-70.
19. Dube, D.H.; Bertozzi, C.R. *Nat. Rev. Drug Discov.*, **2005**, *4*, 477-488.
20. Ingale, S.; Wolfert, M.A. Gaekwad J. *Nat. Chem. Biol.*, **2007**, *3*, 663-667.
21. Chen, X.; Zheng, Y.; Shen, Y. *Curr. Med. Chem.*, **2006**, *13*, 109-116.
22. Campbell, L. K.; Baker, D. E.; Campbell, R. K. *Ann. Pharmacother.*, **2000**, *34*, 1291.
23. Itzstein, M.V.; Wu, W. Y.; Kok, G. B.; Pegg, M. S.; Dyason, J. C.; Jin, B.; Phan, T. V.; Smythe, M. L.; White, H. F.; Oliver, S. W.; Colman, P.M.; Varghese, J. N.; Ryan, D.M.; Woods, J. M.; Bethell,

Feature

- R. C.; Hotham, V. J.; Cameron, J.M.; Penn C. R. *Nature*, **1993**, *363*, 418-423.
24. Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D. B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc.* **1997**, *119*, 681-690.
25. Norez, C.; Antigny, F.; Noel, S. et al. *Am. J. Respir. Cell Mol. Biol.*, **2009**, *41*, 217-225.
26. Maryanoff, B.; Nortey, S.; Gardocki, J.; Shank, R.; Dodgson. *Journal of Medicinal chemistry*, **1987**, *30*, 880-887.
27. Meister, R.T. 1994. Farm Chemicals Handbook '94. Meister Publishing Company. Willoughby, OH.
28. Thomson, W. T. 1982. Agricultural Chemicals Book IV Fungicides. Thomson Publications. Fresno, CA.
29. Truscheit, E.; Frommer, W.; Junge, B.; Müller, L.; Schmidt, D.D.; Wingender, W. *Angew. Chem. Int. Ed. Engl.*, **1981**, *20*, 744-761.
30. Li, X.; Shah, A.; Franklin, P.; Merolli, R.; Bradley, J.; Busconi, B. *J. Orthopaedic Surg. Res.*, **2008**, *3*, 43.
31. Petitou, M.; van Boeckel, C.A. *Angew. Chem. Int. Ed.*, **2004**, *43*, 3118-3133.
32. Araujo, A.C.; Nicotra, F.; Costa, B.; Giagnoni, G.; Cipolla, L. *Carbohydr. Res.*, **2008**, *343*, 1840-1848.
33. Araujo, A.C.; Nicotra, F.; Airolidi, C.; Costa, B.; Giagnoni, G.; Fumagalli, P.; Cipolla, L. *Eur. J. Org. Chem.*, **2008**, 635-639.
34. Chan, E. et al. Tuberculosis, 2nd edition. Philadelphia, PA: Lippincott Williams & Wilkins, 2003, pp. 773-789.
35. Di, P.G, Bonora S. *J. Antimicrob. Chemother.*, **2004**, *54*, 593-602.
36. Ho, Y. et al. *J. Antimicrob. Chemother.*, **1997**, *40*, 27-32.
37. Knapp, S.; Jaramillo, C.; Freeman, B. *J. Org. Chem.* **1994**, *59*, 4800.
38. Nakajima, M.; Itoi, K.; Takamatsu, Y.; Kinoshita, T.; Okazaki, T.; Kawakubo, K.; Shindo, M.; Honma, T.; Tohjigamori, M.; Haneishi, T. *J. Antibiot.* **1991**, *44*, 293.
39. Umezawa, H.; Aoyagi, T.; Komiyama, T.; Morishima, H.; Hamada, M.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 963.

Drugs & Pharmaceuticals Industry Highlights

The monthly periodical focuses on National as well as Global information on Drugs and Pharmaceuticals Industry.

Coverage

- Indian Pharma Industry News
- Indian Regulatory News
- Global Pharma News
- Product News
- Healthcare News
- R&D News
- Biotechnology
- Natural Products
- Patents
- In Parliament

Please send your contributions, enquiries and subscription to:

Scientist-in-Charge

S & T Knowledge Resource Centre
Central Drug Research Institute
PO Box 173, Lucknow-226 001, India
Website: www.cdriindia.org



Carbohydrate-Based Targets and Vehicles for Cancer and Infectious Diseases Vaccines

The last decade has seen carbohydrates used not only as targets for effective vaccines against bacteria, but also developed as adjuvants and vaccine carriers for protein antigens for immunotherapy. This chapter focuses on carbohydrate targeting in bacterial and parasitic models for vaccine development, and in the current leading edge technologies for inducing T cells specific to tumor antigens for cancer therapy. This is made possible by the fact that the cells of the immune system, and specifically antigen presenting cells, express carbohydrate receptors which provide both a danger signal to the cell, and deliver protein attached to the carbohydrate for effective processing and presentation to T cells. Examples of such carbohydrate receptors include the mannose and scavenger receptors. The mechanisms by which they lead to effective antigen processing and stimulation of the immune system are also considered.

Bacterial Carbohydrates as Danger Signals

The immune system has evolved a set of receptors on phagocytic and antigen presenting cells (APCs) to recognize specifically and rapidly bacterial carbohydrates, especially in the form of liposaccharides. The best characterized are bacterial lipopolysaccharides (LPS) found abundantly on all Gram negative bacteria such as *Escherichia coli*. Interaction of LPS with receptors, such as CD14, on dendritic cells (DCs), monocytes and macrophages promotes phagocytosis of the bacteria, and provides an activation or danger signal to the cell. LPS-stimulated DCs express potent costimulatory molecules for T cell activation, such as CD40, CD80 and CD86,

which makes them uniquely potent at priming both CD8 and CD4 T cell responses. LPS-activated monocytes and macrophages do not only become more effective at destroying the bacteria, but also secrete large amounts of proinflammatory molecules such as tumor necrosis factor alpha (TNF α), which cause general reactions to infection, such as fever.¹ Although these responses help to eliminate the bacteria, excessive proinflammatory reactions can be deleterious to the host. Indeed, toxic shock induced by LPS injection is lethal in many species. The diverse biological activities of LPS may be further dissociated into chemical moieties with the lipid A portion being the main determinant of toxicity.

Although unmodified LPS may therefore not be a safe adjuvant, cell-wall skeleton (CWS) fractions of mycobacterial cells (present in Freund's complete adjuvant, the widely used adjuvant for experimental animals) have also been found to contain carbohydrate-lipid or protein conjugates with immunostimulatory activity. It has been suggested that DCs and macrophages express both Toll-like receptors, TLR-2 and TLR-4, and a receptor for mycobacterial CWS whose signaling pathways promote an activation state of the immune system. Synthetic adjuvants such as muramyl dipeptide (MDP) derivatives and trehalose-dimycolates (TDM) have been developed to activate similar signaling pathways. Mycobacterial lipoarabinomannan (LAM), mannosylated LAM (ManLAM) and LAM lacking the terminal mannosyl units (AraLAM) induce distinct responses in human polymorphonuclear (PMNs) and mononuclear

Feature

phagocytes. Thus, AraLAM and ManLAM affect mononuclear, but not PMN, phagocyte functions, but both forms are chemotactic for monocytes and monocyte-derived macrophages (MDMs).

Bacterial Carbohydrates as Human Vaccines

Bacterial carbohydrates, particularly those of the bacterial capsules of *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis* can be the targets of protective antibodies. These antibodies may have the dual function of opsonizing bacteria for phagocytosis and targeting them for destruction by complement. In addition, their toxin neutralizing effect on the soluble LPS released during bacterial destruction may limit the extent of potentially harmful pro-inflammatory reactions. Purified capsules can often elicit antibody responses in adults and children older than 2 years, but these thymus-independent responses only promote the generation of low-affinity antibodies and fail to generate long-term memory. The use of immunogenic carrier proteins containing helper T cell epitopes linked to the polysaccharide creates more powerful immunogens able to induce high affinity antibodies and prime for boosting either with the glycoconjugate or the polysaccharide itself.

Prior to the implementation of vaccination programs, the weighted worldwide incidence of *H. influenzae* type B (Hib) diseases except nonbacteremic pneumonia was 71/100,000 in patients younger than 5 years. Vaccination against Hib using a polysaccharide vaccine was initiated over 25 years ago. However, the initial formulation suffered from poor immunogenicity, particularly in infants. A dramatic improvement in efficacy was observed upon the introduction of four Hib glycoconjugate vaccines in the 1990s. Indeed, since the immunization programs began, the disease has been almost eliminated in countries with high immunization coverage with an estimated 38,000 cases prevented each year.

However, Hib vaccination still has had only limited impact globally. It is hoped that the development of vaccines that are practical for administration in the Third World, probably using glycoconjugates in a reduced number of doses and in combination with other vaccines, may extend the full benefit of vaccination to less privileged countries, where most Hib disease occurs.

The weighted worldwide incidence of meningitis in patients younger than 5 years prior to the implementation of vaccination programs was estimated at 57/100,000. Human trials in the 1970s and 1980s showed that polysaccharide vaccines prevent meningococcal meningitis. Most of these vaccines have focused on group A meningococcus. A study elegantly summarizing the results of eight such trials suggested that the protective effect within the first year was consistent across all trials, with an overall vaccine efficacy of 95% (Exact 95% CI 87%, 99%). Protection extended into the second (in two studies) and third (in one study) years after vaccination, but the results were not statistically significant, with variations in the level and duration of protection, particularly among young children. Recently, a meningococcal serogroup C glycoconjugate vaccine was successfully implemented nationally in the UK. The glycoconjugate approach to increase immunogenicity and priming ability has now been extended with A, C, W 135 and Y, but not B serotypes in registration phases. The potential inclusion of new virulence factors such as outer membrane proteins (OMPs) and lipopolysaccharides (LPS) may further the development of effective meningococcal immunogens, currently focused on providing a vaccine for serogroup B.

S. pneumoniae (pneumococcus) is a major cause of morbidity and mortality worldwide, causing over 1 million of the 4 million annual deaths from acute lower respiratory infections in children under 5 years of age. The currently

Feature

licensed pneumococcal vaccine comprises 23 capsular pneumococcal polysaccharides. Unfortunately, many of these are poorly immunogenic in young children. Similarly to Hib, clinical trials of protein-polysaccharide conjugates have shown promising results in safety and immunogenicity studies. However, the development of a conjugate vaccine against pneumococcal disease is further complicated by the existence a very large number of serotypes. Since nonvaccine serotypes are already present in the community as confirmed in studies of the etiology of acute purulent otitis media, these nonvaccine serotypes may become more common in vaccination areas.¹¹ Indeed, a shift in *S. pneumoniae* serotypes colonizing the nasopharynx in children receiving the vaccine can be observed.¹¹ The identification of protective antigens (proteins or carbohydrates) common to clinically important strains could provide alternative immunogens for inclusion in a generic pneumococcal vaccine. Serotype diversity may be anticipated to be a problem for other capsular bacterial vaccines, for example although a group B streptococcus (GBS) polysaccharide-protein conjugate vaccine given to women of reproductive age was well tolerated and highly immunogenic, new capsular serotypes are now causing an important proportion of clinical infections.

Bacterial Carbohydrates as the Basis of Novel Vaccine Approaches

The use of glycoconjugates of capsular polysaccharides with carrier proteins has proven highly effective in generating serotype-specific protective immunity. The problem of targeting multiple serotypes is a serious one. In addition, there is a need in generating vaccines that are not only effective but affordable and easy to administer in poor countries where the burden of bacterial disease is highest.¹⁴ The identification of conserved antigenic regions in carbohydrates as well as proteins, in order to provide cross-strain protection, is a direct approach to tackle this problem. Two more

unusual generic approaches are exemplified below.

Staphylococcus aureus and *S. epidermidis* are common causes of nosocomial infection and are major pathogens for domesticated animals. *S. aureus* is frequently found in community-acquired infections. Poly-N-succinyl β -1-6 glucosamine (PNSG), a chemical form of the *S. epidermidis* capsular polysaccharide/adhesin (PS/A) is a target for protective antibodies. *S. aureus* cells in infected human sputa and lung also elaborate PNSG and immunization of mice with PNSG protects them against metastatic kidney infections. However, PNSG is not usually expressed by the bacteria in vitro, and indeed the challenge strains can be initially PNSG negative. Antigens like PNSG, present only under specific in vitro and in vivo conditions, offer exciting potentials as new targets for the development of protective bacterial immunity.

N. meningitides is a major cause of meningitis and sepsis. Despite nearly 25 years of work, there is currently no vaccine for meningococcal B strains. The capsular polysaccharide of this organism is conserved and antibodies to it confer protection against disease. The immunogenicity of meningococcal B polysaccharide-based vaccines is poor. Although the use of immunogenic protein carriers is expected to enhance immunogenicity of the polysaccharide, this is not generally found to be case for group B capsular polysaccharides. There is also a concern that a portion of the antibody elicited by the capsule has autoimmune activity towards brain glycolipids and NCAM molecules on specialized cells. To avoid this problem, a panel of murine monoclonal antibodies (Mabs) to capsular polysaccharide epitopes on meningococcal B that are distinct from host polysialic acid was identified. These antibodies confer passive protection in animal models. The Mabs were then used to identify molecular mimetics from phage display peptide libraries. Although the

Feature

resulting mimetic peptides have thus far failed to induce high levels of anti-capsular antibodies, this approach when optimized may be an exciting alternative line of research in vaccine development targeting bacterial polysaccharide structures.

Parasite Carbohydrates As Targets of Protective Immunity

Bacterial carbohydrates have been known for many years to be the target of protective immunity, with effective human vaccines developed on this solid basis. Parasite carbohydrates, in contrast, are still largely understudied as targets of protective immunity or as vaccine components.

Evidence to show that protective antibodies can target carbohydrates is growing for a variety of parasitic diseases. *Trypanosoma cruzi* is the causative pathogen of Chagas disease. Antibodies that lyse trypomastigotes in a complement-mediated reaction are thought to mediate protection against virulent *T. cruzi*. Titers of antibodies to α -galactosyl determinants markedly increase in Chagas's disease. Binding of these antibodies to *T. cruzi* causes complement-mediated lysis of trypomastigotes. Anti-gal antibodies from human serum may similarly inhibit the growth of *Plasmodium falciparum*, the parasite responsible for lethal malaria. Most of the lytic power of the serum anti-Gal induced by Chagas's disease is removed by absorption with Gal α 1, 3Gal β 1, 4GlcNAc. Lytic antibodies are also partly absorbed by *Serratia marcescens* but not by *E. coli*O111. However, crossreactivity between some bacterial polysaccharides and *T. cruzi* may also occur. Indeed, rabbits and human volunteers immunized respectively with purified meningococcal polysaccharide C and the AC-polysaccharide vaccine produced antibodies crossreactive to *T. cruzi* infective forms. Furthermore, these crossreactive antibodies were able to target trypomastigotes for complement-mediated lysis. Nonsialylated

epitopes expressed on infective forms of the parasite are the target of these antibodies, and could be considered as new immunogens for the development of *T. cruzi* vaccines. A radiation-attenuated *Shistosoma mansoni* vaccine in chimpanzees induced specific IgM and IgG to glycans on antigens released by cercariae. These antibodies were crossreactive to soluble antigens from larvae, adult worms, and eggs. Egg deposition was the major antigenic stimulus after challenge. Glycan epitopes recognized included GalNAc β 1-4GlcNAc- (LacdiNAc), fucosylated LacdiNAc, Lewis X (weakly), and those on keyhole limpet hemocyanin. Antibodies to peptide epitopes became prominent only during the chronic phase of infection, as glycan-specific IgM and IgG decreased. It is unclear whether these anti-glycan responses are protective or a "smoke screen" to divert the immune system away from more vulnerable larval peptide epitopes. C57BL/6J and CBA/J mice vaccinated with irradiated cercariae have protective antibodies recognizing carbohydrate epitopes on schistosomal glutathione S-transferase. Lacto-N-fucopentaose III, a carbohydrate structure relevant for cell trafficking, is recognized predominantly. In contrast to the primate studies, there is no binding to its nonfucosylated homologue, lacto-N-neotetraose, or to oligosaccharides present on keyhole limpet hemocyanin. It may be that the fine specificity of the anti-carbohydrate response determines its role in protective immunity.

As well as carbohydrates on parasites, carbohydrates of the host can play a role in parasitic infection by serving as receptors for the attachment and entry of parasites into cells. A clear example is provided by *P. falciparum*'s use of chondroitin sulphate to attach itself to vascular endothelium, particularly of the placenta. Attachment of infected red blood cells allows this parasite to sequester itself and multiply to high density. The inhibition of these carbohydrate-protein

Feature

interactions leads to protective immunity, as demonstrated by the protective effect of anti-pfEMP1 antibodies, which target the parasite lectin or by interfering with this interaction directly using competing carbohydrates in vitro.

Parasite Carbohydrates As Immunomodulators

Galactosyl residues and mannan have been described to be immunostimulators or as carriers to target specific receptors on APCs. These moieties, found on many bacteria, yeasts and parasites, are also involved as classical exogenous “danger” signals that usually activate APCs to promote a potent Th1 response. In contrast, a phosphorylcholine-containing glycoprotein (ES-62) secreted by the filarial nematode, *Acanthocheilonema viteae*, promotes the maturation of DCs with the preferential capacity to induce Th2 responses. Other glycoconjugates with immunomodulatory roles include lipophosphoglycan (LPG), a major surface glycoconjugate of *Leishmania promastigotes*. *Leishmania* parasites use LPG to impair the normal activity of phagocytes, and thus protect the parasites within phagolysosomes. The LPG-mediated escape mechanisms of promastigotes from human phagocyte responses have been shown to include impairment of oxidative burst and chemotactic activity.

Similar to some bacterial toxins, a dominant glycolipid from *P. falciparum* may contribute to the fever response associated with malaria infection. Parasite-derived glycosylphosphatidylinositol (GPI), free or associated with protein, induces TNF α and interleukin-1 production by macrophages and regulates glucose metabolism in adipocytes. Deacylation with specific phospholipases abolishes cytokine induction, as do inhibitors of protein kinase C. When administered to mice in vivo GPI from the parasite induces cytokine release, a transient pyrexia, and hypoglycemia. When administered with

sensitizing agents it can elicit a profound and lethal cachexia. Antibody to the GPI inhibits these toxic activities, suggesting that GPI could be a useful target for inclusion in vaccines against malarial disease.

Targeting the Mannose Receptor for Vaccine Development

The mannose receptor (MR) is primarily present on DCs and macrophages. It recognizes carbohydrates (mannose, fucose, glucose, GlcNAc, maltose) on the cell walls of infectious agents (mainly bacteria and yeast). Upon binding, there is aggregation and receptor mediated endocytosis and phagocytosis. The MR is prototypical member of the multilectin receptor family and provides a link between innate and adaptive immunity.

Human DCs and macrophages bind agalactosyl IgG, found in several autoimmune diseases, which has the terminal galactose residues removed, thus exposing N-acetylglucosamine and providing a binding site for the MR. Antibodies or antigen-antibody complexes are taken into DCs or macrophages and generate Ig-derived peptides that bind MHC class II molecules and activate T cells. The expression and functional state of the MR is governed by various cytokines, immunoglobulin receptors and pathogens. It is downregulated during IFN γ mediated macrophage activation even though the affinity of ligand binding is not affected, however the functional properties are modified. IFN γ treatment of MDMs increases their capacity to kill *Candida albicans* in an MR-dependent manner. The addition of IL-4 acts synergistically with IFN γ to enhance MR-dependent uptake. In addition, IL-4 increases cell surface expression of MR and MR-mediated endocytosis whereas IFN γ decreases these effects. However, both IL-4 and IFN γ either alone or together increased MR-mediated phagocytosis; IL-13 exerted similar effects to IL-4. This demonstrates that phagocytosis of microorganisms could be

Feature

enhanced in the presence of T1- and T2-type cytokines at sites of inflammation. Furthermore, when the MR binds to microorganisms, a variety of intracellular responses are triggered such as cytokine secretion, lysosomal enzyme secretion, and modulation of other cell surface receptors. In addition, glycosylated viral envelope proteins (HIV, HSV) stimulate IFN α production by DCs. This stimulation can be inhibited by sugars specific for the MR implying that the MR is an important receptor for the recognition of enveloped viruses by DCs. Thus, the MR has more functions than just phagocytosis of pathogens.

Targeting the Mannose Receptor for Drug Therapy

The biology of MR has given new insights of its use as a target for delivering drugs to macrophages that have internalized bacteria or other infectious organisms. One study targeted the macrophage MR with a norfloxacin antibiotic, which is active against intracellular bacteria. The antibiotic was conjugated to mannose with a poly(L-lysine citramide imide) carrier which successfully targeted the MR of macrophages infected by intracellular bacteria. Thus, targeting the MR with mannose linked to drugs to kill ingested microorganisms or viruses, is a new and exciting approach for therapeutic drug delivery. In addition, mannosylated poly(L-lysine) (Man-PLL) was synthesized as a carrier molecule and mixed with a plasmid DNA encoding chloramphenicol acetyltransferase (CAT) to form a DNA-Man-PLL complex. The complex bound specifically to the MR in the liver after intravenous injection, indicating that a cell-specific gene delivery system can be developed by regulating the biodistribution of DNA-carrier complex. Furthermore, the MR has been investigated for MR-mediated gene transfer into macrophages using mannosylated cationic liposomes and high transfection activity due to recognition by the MR was demonstrated.

Targeting the Mannose Receptor for Antigen Delivery

The high expression of MR on DCs and macrophages indicates that the MR is a key molecule in antigen recognition. The MR on macrophages and immature DCs is involved in endocytosis and phagocytosis and is an important pathway for antigen uptake and delivery to MHC class II molecules. Recently, we demonstrated that the MR is also involved in antigen uptake and delivery to MHC class I molecules, particularly with mannan modified by oxidation.

The MR is found in endosomes where it transits to the cell surface to bind ligands. It recycles from the cell surface through the endosomal pathway. Dissociation of the bound ligands occurs at the lower pH found in endosomes. Endocytosis by DCs via the MR takes place in small coated vesicles, shortly after internalization the MR and its ligand appear in larger vesicles, followed by colocalization with MHC class II molecules in lysosomes. Mannosylated peptides and proteins are able to stimulate MHC class II restricted peptide specific T cells with 200-10,000 fold higher efficiency than peptides or proteins which have not been manno-sylated. Furthermore, uptake by DCs via the MR results in 100-fold enhanced presentation of soluble antigens to T cells than antigens internalized via fluid phase pinocytosis. Recently, a DC receptor for endocytosis, DEC-205, was found to mediate a 100-fold increase in antigen presentation via the MHC class II pathway to CD4⁺ T cells.

After internalization, the MR transports antigens to MHC class II-containing compartments in immature DCs for antigen processing and presentation to T cells. Human peripheral blood mononuclear cells cultured in GM-CSF and IL-4 for 5 days, develop into DCs which are able to efficiently present antigens to T cells. It has been demonstrated that DCs can endocytose antigens via the MR

Feature

and deliver processed peptides to MHC class II molecules. In addition, the antigen presenting function of the DCs has been shown to be associated with high level expression of the DEC-205 MR in mice.

CD1 proteins have been implicated to have antigen presentation function. Human CD1b can present nonprotein antigens from mycobacteria to T cells, including lipid mycolic acid and LAM. The antigen presentation pathway for LAM has been characterized and the macrophage MR is clearly responsible for uptake. MR is abundant in early endosomes and the MHC class II loading and presentation pathway. LAM is taken into early endosomes via the MR, transported to late endosomes and then loaded onto CD1b molecules for T cell presentation. This study links the MR to presentation of glycolipids via CD1 and indicates that the MR may play a critical role in processing of carbohydrate antigens.

A fusion protein containing the cysteine-rich domain of the murine MR and the Fc portion of human IgG1 was able to bind cells which were MHC class II, sialoadhesin and CD11c positive, and negative for other markers such as F4/80, FDC-M2, CD11b, B220 and CD4. These cells have been found to localize to B cell follicles and initiate humoral immune responses and activation of T cells.

The use of mannan to aid in the induction of T1-mediated immune responses and cytotoxic T cells (CTLs) has also been investigated. Cationic liposomes, containing HIV-1 DNA and coated with mannan, can significantly enhance HIV-specific CTL responses, T1-type cytokine $IFN\gamma$, IgG2a and IgA antibodies and delayed-type hypersensitivity responses. Furthermore, HER2 protein conjugated to either mannan or to polysaccharides having cholesteryl groups was able to induce CD8+ CTLs which rejected HER2+tumors.

Our laboratory has immunized

cynomolgous monkeys with MUC1 fusion protein conjugated to oxidized mannan. Immunized monkeys generated strong anti-MUC1 antibodies, MUC1-specific CD4⁺ and CD8⁺ T cell proliferative responses and specific CTL precursor cells. In a phase I clinical trial 25 patients with advanced metastatic adenocarcinoma were injected with increasing doses of mannan-MUC1. High titers of IgG1 anti-MUC1 antibodies were produced in 13/25 patients (with antibody titers by ELISA of 1/320-1/20,480). In addition, T cell proliferation was found in 4/15 patients, and CTL responses were seen in 2/10 patients. Recently it was demonstrated, by flow cytometric analysis of peripheral blood mononuclear cells of patients immunized with MUC1-mannan conjugates, that intracellular cytokines IL-2, IL-4, $IFN\gamma$, and TNF α were produced by CD4⁺CD69⁺ and CD8⁺CD69⁺ activated T cells upon MUC1 antigen stimulation. Taken together these studies suggest that patients can successfully be immunized for the generation of both humoral and cellular responses using mannan-antigen conjugates. We are currently performing clinical trials where MR on macrophage/DC is targeted *ex vivo* with mannan-MUC1.

Targeting the Scavenger Receptor for Vaccine Development

The scavenger receptor is primarily present on macrophages and can internalize endotoxins, oxidized low density lipoproteins and other negatively charged proteins. Maleylated ovalbumin has been demonstrated to bind to the scavenger receptor, this enhances its presentation to ovalbumin-specific MHC class I restricted CTL by macrophages and B cells. Maleylated diphtheria toxoid has also been demonstrated to be more immunogenic than nonmaleylated diphtheria toxoid which generated enhanced antibody and T cell proliferative responses. In chickens, immunization with maleylated-bovine serum albumin (BSA) specifically bound to the scavenger receptor and modulated the Th1

Feature

immune response with weak antibodies. In addition, splenocytes expressed high levels of mRNA for IFN γ . Non maleylated BSA induced Th2 immune responses.

Alcohol metabolites malondialdehyde and acetaldehyde when combined form stable adducts (oxidative product). These adducts when conjugated to proteins, such as hen egg lysozyme (HEL), induced a strong antibody response and T cell proliferation. Studies have suggested that the immune responses may be mediated by scavenger receptors that recognize malondialdehyde and acetaldehyde adducted proteins.

Future Prospects

Carbohydrates have been known to be the target of protective immune responses against bacterial diseases for over two decades. Lately, conjugation of carbohydrates to proteins to provide a helper T cell response has dramatically enhanced the efficacy of polysaccharide vaccines aiming to induce bacterial immunity. It is anticipated parasitic diseases may soon follow suit and be the focus

of studies investigating the role of carbohydrates in generating protective immune responses. Recently a number of different novel immunization strategies based on the use of glycoconjugates have been tested in animal models and humans. These have been shown to induce cellular and humoral responses, and may provide a simple, safe and effective approach to the development of vaccines. DCs have emerged as the main stimulating APCs of the immune system. Delivering antigens (e.g., proteins or antigenic peptides) to DCs has proven to be a successful approach for the induction of powerful immune responses, and protection in cancer models. Initial glycoconjugate strategies have focused on targeting the mannose receptor, expressed abundantly on both macrophages and DCs. Recently, scavenger receptors have shown similar promise, and we may anticipate that this field will continue to provide exciting developments.

(Based on the article written by Vasso Apostolopoulos et al., and covered in Landes Bioscience Madame Curie Database)

We request our readers to not only send their reactions to the journals but also contribute to various columns to make this venture a participatory dialogue. Members of Industry are particularly requested to send the latest developments in their companies. Your contributions shall be gratefully acknowledged.

Editors



Carbohydrate Synthesizer Opens Door to New Field of Medicine

German scientists have reported a major advance toward opening the doors of a carbohydrate-based medicine chest for the 21st Century. These carbohydrates may form the basis of revolutionary new vaccines and drugs to battle malaria, HIV, and a plethora of other diseases.

Speaking at the Annual Meeting of the American Chemical Society, Peter H. Seeberger described development of an automated carbohydrate synthesizer, a device that builds these intricate molecules in a few hours - rather than the months or years required with existing technology. "Our automated synthesizer is now the fastest method to make complex carbohydrates," says Seeberger, the principal investigator for the project. "There are currently no competitive methods available. Today, if people working in biology run into a problem related to carbohydrates, they usually drop it because there are no tools available. They can't buy anything from a catalogue. It becomes a royal pain in the neck."

Geneticists trying to synthesize DNA and protein-based molecules experienced a similar pain-in-the neck decades ago, until the invention of automated DNA and protein synthesizers. These devices helped kick start a revolution in genetics and proteomics. The carbohydrate synthesizer may do the same thing for the emerging fields of glycochemistry and glycobiology - named for carbohydrate sugar chains known as "glycans."

Carbohydrates are tough molecules to build because of their complicated, branched

structure. So instead of trying to build carbohydrates from scratch, scientists today use molecules isolated from nature, a painstaking process that could take months. "We make things chemically that people used to isolate," explains Seeberger. "The automated synthesizer puts single sugars, the building blocks of carbohydrates, together like beads on a string."

Carbohydrates play important roles in the immune system, especially in the body's defenses against disease-causing viruses and bacteria. Most of these microbes have unique carbohydrate markers on their surfaces. The immune system recognizes these carbohydrates as foreign material, and creates antibodies that launch an immune response to battle the infection. "Vaccines 'educate' the immune system to recognize a specific molecule on the surface of infectious organisms," explains Seeberger. "The synthesizer allows us to make not one but many carbohydrate structures from a particular organism and test those to see if they protect against the microbe. Synthetic carbohydrates that show promising protective qualities then may become the basis for new vaccines."

In a recent finding, the team discovered a carbohydrate on the surface of the malaria parasite *P. falciparum* that enables the parasite to infect human red blood cells, thus solving a long-standing mystery about how infection happens. Seeberger's group used the carbohydrate synthesizer to develop a malaria vaccine. Clinical trials for the vaccine are scheduled for 2010 in Mozambique and Tanzania. Its unique "anti-disease" mechanism makes it the only vaccine of its kind, he says.

"To my knowledge, ours is the first attempt at an anti-disease vaccine. It doesn't actually kill the malarial parasite; it blocks its toxic action. You create antibodies against the sugar structure, and these antibodies block the carbohydrate toxin from leading to inflammation and anemia, the hallmarks of

News & Views

malarial infection," says Seeberger. He explained that they will pair the carbohydrate vaccine with a traditional, protein-based one to create a "conjugate vaccine," which is best suited to immunize the most vulnerable group of potential malaria victims - children under the age of two.

The malaria vaccine is only one of almost a dozen vaccines from Seeberger's lab headed for clinical trials. And Seeberger believes that carbohydrate-based vaccines could target some of today's most serious infectious diseases, including antibiotic-resistant infections and HIV.

(American Chemical Society 22, March 2009 23:29)

New Method for Producing 'Libraries' of Important Carbohydrate Molecules

Scientists some years back found ways to automate the production of DNA and proteins, making studies of these essential components of life far easier. With complex carbohydrates, it's been a different story. Until now, the construction of so-called "libraries" of carbohydrate molecules for biological study has been slow and tedious. In what may change all that, a team of scientists from the University of Georgia has created a method for the rapid chemical synthesis of complex carbohydrates, and that method could dramatically change the availability of such molecules for research.

"In the past, it has simply taken too long to make these molecules, and it has held back progress in the field," said Geert-Jan Boons, Franklin Professor of Chemistry and director of the research. "Now, we have a new method of synthesis that will make well-defined molecules available for in-depth study."

Other authors of the paper, all from UGA when the work was done, include doctoral students Thomas Boltje and Jin Park and postdoctoral associate Jin-Hwan Kim. The team is part of the Complex Carbohydrate Research Center at UGA, and Boons' appointment in chemistry is part of the

Franklin College of Arts and Sciences. The work was sponsored by the National Institute of General Medicine Sciences of the National Institutes of Health. "The emerging field of glycomics has been severely hampered by a lack of robust, well-defined libraries of carbohydrate molecules, which are greatly needed to decipher the 'carbohydrate codes' used by cells for processes such as cell signaling, embryogenesis and neuronal development," said Pamela Marino, director of the glycobiology portfolio at the NIH's National Institute of General Medical Sciences. "Dr. Boons has established important new methodology for the rapid synthesis of complex oligosaccharides in a manner amenable to automation, moving the field a step closer to achieving automated synthesis of complex sugars."

Glycomics is the study of complete sets of complex carbohydrate structures expressed by specific cells, tissues or organisms. It examines the role of these molecules in areas such as physiology, genetics and disease pathology. The stakes in being able to study and understand the function of oligosaccharides, chains of simple sugars found on the cell surface of all plant and animal cells, are immense. They are involved in such cellular processes as protein folding, the regulation of cell signaling, and fertilization. These complex carbohydrates also are being recognized by pathogens during infection, help control immune cell response and have a role in the development of cancer and autoimmune diseases.

The problem is that building carbohydrate chains for biological study has been difficult at best and slow. Unlike DNA, which can be induced to replicate itself millions of times in a laboratory for study, these compounds must be built a molecule at a time, and, even worse, they can be "linked" in different ways, making chemical bonding problematical at best. The problems associated with how to build carbohydrates in the lab go back more than a

News & Views

century. Although there has been, according to the scientists, "tremendous progress" in chemical or enzymatic approaches used to build the compounds, it is "still very time-consuming, and it not uncommon that the preparation of a single, well-defined derivative can take as long as a year." The new method offers the promise of cutting that time to hours because the procedures allow scientists to eliminate intermediate purification steps and will be amenable to automation.

"One of the ways to understand the problems we've had is that while DNA and proteins are linear molecules in which the nucleoside or amino acid building blocks are linked together one way, carbohydrates are branched, and they can be linked in two different ways," said Boons. "And it's very hard to control the configuration of these linkages in the laboratory. And that is essential if we are to find ways to build these new libraries of molecules for study." The new method in the *Nature Chemistry* paper allows researchers to control the configuration of these linkages and install various branching points, making it much easier to synthesize these carbohydrate molecules without intermediate purifications. To see how well the new method works, the team chose the important carbohydrates glucose and galactose to study, and the results for both showed that the method is sound, rapid and potentially important for the construction of complex carbohydrate molecules to study. Further research will confirm that the method will work on other complex carbohydrates, but all indications now are that it will.

(ScienceDaily May 24, 2010)

Common Carbohydrate Blocks Malaria

Molecules that block the malaria parasite from infecting red blood cells - causing the illness that kills nearly one million people every year - have been found by researchers in Australia and Britain. The team of researchers, which includes immunologists Michelle Boyle,

Jack Richards and James Beeson of the Walter and Eliza Hall Institute in Melbourne, showed that heparin-like molecules interfere with the malaria parasite's ability to attach to, and therefore invade, red blood cells.

The molecule is related to the blood-thinning drug heparin and could provide a new approach for treating the mosquito-borne disease. Although they are not new to science, their malaria-blocking properties were not previously recognised.

Beeson and colleagues investigated carbohydrate molecules that are similar to heparin, suspecting that they had a different approach to attacking malaria. Heparin occurs naturally in the blood, but not at high enough levels to have anti-malarial properties. The researchers found that the heparin-like molecules bind to a protein called MSP1, which is necessary for the parasite to attach to the cell. Apart from being one of the few molecules known to block the parasite's entry into the blood cell, Beeson said that fundamental understanding of how these carbohydrates work removes some of the hurdles in future drug development. "We have identified related compounds that are more potent against malaria than heparin but do not prevent blood clotting," said Beeson. "These could form the basis of new anti-malarial drugs."

So far, the heparin-like molecule's ability to block the malaria parasite has only been shown in culture, and a potential drug for human use is still a long way off, according to Beeson. But given this new way of potentially stopping malaria infections, Beeson envisions a two-pronged approach to malaria treatment in the future: giving patients one drug that stops the parasite's replication in the cell and one that prevents it from entering.

(www.cosmosmagazine.com 4.6.2010)

New Approach for Highly Selective Chemotherapy Delivery

A new approach developed by researchers

at University of California, Irvine, can vastly improve the targeting of chemotherapeutic drugs to specific cells and organs. The findings of the study could pave the way to precisely targeted cancer treatments. For the study, the researchers used liposomes, small spheres (less-than 100 nanometer in diameter) of naturally occurring lipid molecules, as “packages” for the cancer chemotherapeutic agent doxorubicin, and a small peptide molecule to “address” the package to the targeted tissue. Using this technology, they showed that the doxorubicin was directed almost entirely to the targeted site with virtually no uptake by other organs, including lung, kidney and heart.

The new approach is based on the fact that a dense region of sugar-containing molecules called polysaccharides surrounds all tissues and organs, including all tumors. Most importantly, the particular chemical composition of the polysaccharides is different in each tissue and organ of the body. The chemical compositions of the polysaccharides of tumour regions are also different from normal tissue.

The scientists developed a nanocarrier system that can recognize specific types of polysaccharide, and has demonstrated effective, organ-specific delivery of nanocarriers, and their therapeutic contents, based upon this polysaccharide-targeting approach. In their study, the researchers used a peptide derived from a protein found in the microorganism *Plasmodium*, which has an exceptional ability to exclusively target the polysaccharides of liver following entry into the bloodstream.

The drug doxorubicin is a chemotherapeutic agent commonly used as treatment for a variety of cancers. When administered in a chemotherapeutic regimen, doxorubicin distributes widely in the body, including the heart, rather than specifically in tumor regions. The serious heart damage that

results from systemic administration places limits on the dosage that a patient can receive. By encapsulating doxorubicin into a liposome package and including a peptide targeting message on the carrier, the researchers showed that doxorubicin can be effectively delivered to the liver, and away from the heart, with a specificity of greater than 100:1. The study has appeared online in the *International Journal of Pharmaceutics*.

(Health News September 25th, 2009)

Mechanism Behind Carbohydrate Synthesis may Pave Way for New TB Drugs

The mechanism behind how carbohydrates are synthesised from small sugar units has shed new light on a promising way to target new medicines against tuberculosis, revealed a new study. While working with components of the tuberculosis bacterium, researchers from the University of Wisconsin-Madison identified an unusual process by which the pathogen builds an important structural carbohydrate. The mechanism also offers insight into a widespread but poorly understood basic biological function - controlling the length of carbohydrate polymers.

“Carbohydrate polymers are the most abundant organic molecules on the planet, and it’s amazing that we don’t know more about these are made. There’s not much known about how length is controlled in these carbohydrate polymers,” said Laura Kiessling, a professor of chemistry and biochemistry at UW-Madison. Most carbohydrates exist as many sugar molecules linked into long chains, or polymers, but the right number of sugars in the chain is vital for them to work properly. However, Kiessling has said that not much is known about how carbohydrate length is determined.

Unlike some biological chains - such as DNA and proteins - that are built off a template that guides the length of the final

News & Views

product, carbohydrate-synthesizing enzymes work without templates.

The research team focused on an enzyme called GlfT2 that is responsible for building a critical carbohydrate component of the TB bacterial cell wall, and found that a small fatty component at the starting end binds to the enzyme and helps it track the length of the growing polymer. As the enzyme adds more and more sugar units to the opposite end, the chain becomes increasingly unmanageable. Kiessling said that “if the chain gets too long, it gets hard to hold on to both of the ends, so the chain falls off” the synthesizing enzyme, forming a completed carbohydrate polymer.

The researchers believe that the enzymes responsible for building different types of carbohydrates exceed their comfort level at different points, leading to molecules of different prescribed lengths. He said that the report was the first description of this “tethering” mechanism - named for the fatty lipid that tethers the start of the polymer to the enzyme - in carbohydrate synthesis, though it may prove to be common among other organisms as well. The work gives significant insight into developing new therapeutics against TB. The GlfT2 enzyme has two binding sites - one for each end of the growing carbohydrate - that make it an especially appealing candidate.

The new study has appeared in the online Early Edition of the Proceedings of the National Academy of Sciences. (ANI)

(Thaindian News June 23rd, 2009)

Polysaccharides in Black tea to help fight diabetes.

Besides boosting the immune system, black tea can be used to control diabetes, according to a new study. The research has been published in the Journal of Food Science, published by the Institute of Food Technologists. To reach the conclusion, researchers from the Tianjin Key Laboratory in China studied the polysaccharide levels of green, oolong and black teas and whether they could be used to treat diabetes.

Polysaccharides, a type of carbohydrate that includes starch and cellulose, may benefit people with diabetes because they help retard absorption of glucose. The researchers found that of the three teas, the polysaccharides in black tea had the most glucose-inhibiting properties.

The black tea polysaccharides also showed the highest scavenging effect on free radicals, which are involved in the onset of diseases such as cancer and rheumatoid arthritis.

“Many efforts have been made to search for effective glucose inhibitors from natural materials,” says lead researcher Haixia Chen. “There is a potential for exploitation of black tea polysaccharide in managing diabetes,” the expert added..

(Science Daily, August 13, 2009 www.thaindian.com)



Glycomics and disease markers.

An, Hyun Joo *et al.*

Current Opinion in Chemical Biology,
13(5-6), 601 (Dec., 2009)

Glycomics is the comprehensive study of all glycans expressed in biological systems. The biosynthesis of glycan relies on a number of highly competitive processes involving glycosyl transferases. Glycosylation is therefore highly sensitive to the biochemical environment and has been implicated in many diseases including cancer. Recently, interest in profiling the glycome has increased owing to the potential of glycans for disease markers. In this regard, mass spectrometry is emerging as a powerful technique for profiling the glycome. Global glycan profiling of human serum based on mass spectrometry has already led to several potentially promising markers for several types of cancer and diseases.

Alteration of protein glycosylation in liver diseases.

Blomme, Bram *et al.*

Journal of Hepatology, 50(3), 592(Mar., 2009)

Chronic liver diseases are a serious health problem worldwide. The current gold standard to assess structural liver damage is through a liver biopsy which has several disadvantages. A non-invasive, simple and non-expensive test to diagnose liver pathology would be highly desirable. Protein glycosylation has drawn the attention of many researchers in the search for an objective feature to achieve this goal. Glycosylation is a posttranslational modification of many secreted proteins and it has been known for decades that structural changes in the glycan structures of serum proteins are an indication for liver damage.

The aim of this paper is to give an overview of this altered protein glycosylation in different etiologies of liver fibrosis / cirrhosis and hepatocellular carcinoma. Although individual liver diseases have their own specific markers, the same modifications seem to continuously reappear in all liver diseases: hyperfucosylation, increased branching and a bisecting N-acetylglucosamine. Analysis at mRNA and protein level of the corresponding glycosyltransferases confirm their altered status in liver pathology. The last part of this review deals with some recently developed glycomic techniques that could potentially be used in the diagnosis of liver pathology.

Toward multivalent carbohydrate drugs.

Pieters, Roland J.

Drug Discovery Today: Technologies(In Press)

Proteins that bind and/or convert carbohydrate structures represent a vast potential for therapeutic development. The use of multivalency is nature's solution to overcome the limited affinities of carbohydrate ligands and has also proven to be a successful strategy for inhibitor development. Suitable protein targets are those with closely spaced binding sites such as the cholera toxin B-subunit. However, also protein targets of unknown tertiary and quaternary structure, such as the adhesion protein of the pig pathogen *Streptococcus suis*, have experimentally proven to be highly suitable. To enhance the search process for new multivalent ligands for new targets, a glycodendrimer microarray technology is developed which rapidly identifies multivalency effects using little precious protein and ligand material.

Hexosamine analogs: from metabolic glycoengineering to drug discovery.

Wang, Zhiyun

Current Opinion in Chemical Biology,13(5-6), 565(Dec., 2009)

R & D Highlights

Metabolic glycoengineering, a technique pioneered almost two decades ago wherein monosaccharide analogs are utilized to install non-natural sugars into the glycocalyx of mammalian cells, has undergone a recent flurry of advances spurred by efforts to make the methodology more efficient. This article describes the versatility of metabolic glycoengineering, which is a prime example of []chemical glycobiology,' and gives an overview of its capability to endow complex carbohydrates in living cells and animals with interesting (and useful!) functionalities. Then an overview is provided describing how acylated monosaccharides, a class of molecules originally intended to be efficiently-used, membrane-permeable metabolic intermediates, have led to the discovery that a subset of these compounds (e.g. tributanoylated hexosamines) display unanticipated []scaffold-dependent' activities; this finding establishes these molecules as a versatile platform for drug discovery.

Lectinomics: II. A highway to biomedical/clinical diagnostics.

Gemeiner, Peter *et al.*

Biotechnology Advances, **27(1)**, 1, (Feb., 2009)

The review assesses current status and attempts to forecast trends in the development of lectin biorecognition technology. The progressive trend is characterized scientometrically and reflects the current transient situation, when standard low-throughput lectin-based techniques are being replaced by a novel microarray-based techniques offering high-throughput of detection. The technology is still in its infancy (validation phase), but already shows promise as an efficient tool to decipher the enormous complexity of the glycode that influences physiological status of the cell. Further enhancement in robustness and flexibility of lectin microarrays is predicted by using recombinant and artificial lectins that will render production of lectin microarrays cost-

effective and more affordable. Mass spectrometry is expected to play an important role to characterize the binding profile of new lectins. Differences in glycan recognition by lectins and anti-carbohydrate antibodies are given on a molecular basis, and strong and weak points of both biorecognition molecules in diagnosis are briefly discussed.

Pharmacological significance of glycosylation in therapeutic proteins.

Li, Huijuan *et al.*

Current Opinion in Biotechnology, **20(6)**, 678(Dec., 2009)

Glycoproteins represent the major share of marketed and clinical development phase therapeutic proteins. A thorough understanding of the nature and function of the carbohydrate moiety and its impact on pharmacology properties is essential in discovering and developing safe and efficacious glycoprotein biopharmaceuticals. This review summarizes the processes of N-linked and O-linked glycosylation and both established and emerging platforms for expression of recombinant glycoproteins. Recent or illustrative examples of N-linked and/or O-linked glycosylation impacting drug pharmacology properties (including activity, pharmacokinetics, clearance, and immunogenicity) of marketed and developing therapeutic proteins are presented.

Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats.

Pari, Leelavinothan *et al.*

Biomedicine & Pharmacotherapy (In Press)

The purpose of this study was to investigate the effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats. Diosmin was administered to streptozotocin-induced (45 mg/kg b.w) diabetic rats at different doses (25, 50, 100 mg/kg b.w) for 45 days to assess its effect

R & D Highlights

on fasting plasma glucose, insulin, glycosylated hemoglobin, hemoglobin and carbohydrate metabolic enzymes, it was found that plasma glucose was significantly reduced in a dose-dependent manner when compared to the diabetic control. In addition, oral administration of diosmin (100 mg/kg b.w) significantly decreased glycosylated hemoglobin and increased hemoglobin and plasma insulin. The activities of the hepatic key enzymes such as hexokinase and glucose-6-phosphate dehydrogenase were significantly increased whereas, glucose-6-phosphatase and fructose-1,6-bisphosphatase were significantly decreased. Furthermore, protection against body weight loss of diabetic animals was also observed. These results showed that diosmin has potential antihyperglycemic activity in streptozotocin-nicotinamide-induced diabetic rats.

Carbohydrate-based experimental therapeutics for cancer, HIV/AIDS and other diseases.

Oppenheimer, Steven B.

Acta Histochemica, **110(1)**, 6 (Jan., 14, 2008)

This review, primarily for general readers, briefly presents experimental approaches to therapeutics of cancer, HIV/AIDS and various other diseases based on advances in glycobiology and glycochemistry. Experimental cancer and HIV/AIDS vaccines are being developed in attempts to overcome weak immunological responses to carbohydrate-rich surface antigens using carriers, adjuvants and novel carbohydrate antigen constructs. Current carbohydrate-based vaccines are used for typhus, pneumonia, meningitis; vaccines for anthrax, malaria and leishmaniasis are under development. The link between O-linked [beta]-N-acetylglucosamine glycosylation and protein phosphorylation in diseases including diabetes and Alzheimer's disease is also explored. Carbohydrate-associated drugs that are in current use or under development, such as heparan sulfate

binders, lectins, acarbose, aminoglycosides, tamiflu and heparin, and technologies using carbohydrate and lectin microarrays that offer improved diagnostic and drug development possibilities, are described. Advances in carbohydrate synthesis, analysis and manipulation through the emerging fields of glycochemistry and glycobiology are providing new approaches to disease therapeutics.

Recent advances in carbohydrate-based vaccines.

Hecht, Marie-Lyn *et al.*

Current Opinion in Chemical Biology, **13(3)**, 354 (June 2009)

Vaccinations provide an efficient and cost-effective way to combat devastating human diseases. Besides pathogenic protein markers, cell surface carbohydrates from biological sources are widely used as vaccines. Recently, synthetic immunogenic carbohydrate-protein conjugates have been advanced to vaccine candidates. Progress in the chemical synthesis of oligosaccharides and conjugation methods stimulated the development of novel carbohydrate-based vaccine candidates.

Therapeutic potential for GIP receptor agonists and antagonists.

Irwin, Nigel *et al.*

Best Practice & Research Clinical Endocrinology & Metabolism, **23(4)**, 499 (August 2009)

Glucose-dependent insulinotropic polypeptide (GIP or gastric inhibitory polypeptide) is a 42-amino-acid hormone, secreted from the enteroendocrine K cells, which has insulin-releasing and extrapancreatic glucoregulatory actions. However, the unfavourable pharmacokinetic profile and the weak biological effects of native GIP limit its effectiveness for the treatment of type 2 diabetes. To overcome this, longer-acting GIP agonists exhibiting enzymatic stability and enhanced bioactivity have been generated and successfully tested in

R & D Highlights

animal models of diabetes. Thus, GIP receptor agonists offer one of the newest classes of potential antidiabetic drug. GIP is also known to play a role in lipid metabolism and fat deposition. Accordingly, both genetic and chemical ablation of GIP signalling in mice with obesity-diabetes can protect against, or even reverse many of the obesity-associated metabolic disturbances. Strong parallels exist with the beneficial metabolic effects of Roux-en-Y gastric bypass in obese, insulin-resistant humans that surgically ablates GIP-secreting K cells. The purpose of this article is to highlight the therapeutic potential of GIP-based therapeutics in the treatment of type 2 diabetes and obesity.

Disease-associated carbohydrate-recognising proteins and structure-based inhibitor design.

von Itzstein, Mark *et al.*

Current Opinion in Structural Biology, **18(5)**, 558 (Oct., 2009)

The role of carbohydrate-related pathways in a wide range of clinically significant diseases has provided great impetus for researchers to characterise key proteins as targets for drug discovery. Carbohydrate-recognising proteins essential in the lifecycles of high health impact pathogens and diseases such as diabetes, cancer, autoimmunity, inflammation and in-born errors of metabolism continue to stimulate much interest in both structure elucidation and structure-based drug design. For example, advances in structure-based inhibitor design against the mycobacterial enzyme UDP-galactopyranose mutase offer new hope in next generation anti-tuberculosis chemotherapeutics. The appearance of H5N1 avian influenza virus has re-stimulated much research on influenza virus haemagglutinin and sialidase. These latest developments on influenza virus sialidase have provided new opportunity for the development of Group 1-specific anti-influenza drugs. The role of siglecs and galectins in a range of disease processes such as inflammation,

apoptosis and cancer progression has also inspired significant structure-based inhibitor design research.

Applications of synthetic carbohydrates to chemical biology.

Lepenies, Bernd *et al.*

Current Opinion in Chemical Biology (In Press)

Access to synthetic carbohydrates is an urgent need for the development of carbohydrate-based drugs, vaccines, adjuvants as well as novel drug delivery systems. Besides traditional synthesis in solution, synthetic carbohydrates have been generated by chemoenzymatic methods as well as automated solid-phase synthesis. Synthetic oligosaccharides have proven to be useful for identifying ligands of carbohydrate-binding proteins such as C-type lectins and siglecs using glycan arrays. Furthermore, glyconanoparticles and glycodendrimers have been used for specific targeting of lectins of the immune system such as selectins, DC-SIGN, and CD22. This review focuses on how diverse carbohydrate structures can be synthetically derived and highlights the benefit of synthetic carbohydrates for glycobiology.

Carbohydrate-based anti-adhesive inhibition of *Vibrio cholerae* toxin binding to GM1-OS immobilized into artificial planar lipid membranes.

Sinclair, Haydn R. *et al.*

Carbohydrate Research, **344(15)**, 1968 (Oct., 12, 2009)

We have studied 'food grade' sialyloligosaccharides (SOS) as anti-adhesive drugs or receptor analogues, since the terminal sialic acid residue has already been shown to contribute significantly to the adhesion and pathogenesis of the *Vibrio cholerae* toxin (Ctx). GM1-oligosaccharide (GM1-OS) was immobilized into a supporting POPC lipid bilayer onto a surface plasmon resonance (SPR) chip, and the interaction between uninhibited Ctx and GM1-OS-POPC was measured. SOS inhibited 94.7% of the Ctx

R & D Highlights

binding to GM1-OS-POPC at 10 mg/mL. The SOS EC₅₀ value of 5.521 mg/mL is high compared with 0.2811 [μ]g/mL (182.5 [ρ]M or 1.825×10^{-10} M) for GM1-OS. The commercially available sialyloligosaccharide (SOS) mixture SunSial E® is impure, containing one monosialylated and two disialylated oligosaccharides in the ratio 9.6%, 6.5% and 17.5%, respectively, and 66.4% protein. However, these inexpensive food-grade molecules are derived from egg yolk and could be used to fortify conventional food additives, by way of emulsifiers, sweeteners and/or preservatives. The work further supports our hypothesis that SOS could be a promising natural anti-adhesive glycomimetic against Ctx and prevent subsequent onset of disease.

Disease-associated carbohydrate-recognising proteins and structure-based inhibitor design.

von Itzstein, Mark *et al.*

Current Opinion in Structural Biology, **18(5)**, 558 (Oct., 2008)

The role of carbohydrate-related pathways in a wide range of clinically significant diseases has provided great impetus for researchers to characterise key proteins as targets for drug discovery. Carbohydrate-recognising proteins essential in the lifecycles of high health impact pathogens and diseases such as diabetes, cancer, autoimmunity, inflammation and in-born errors of metabolism continue to stimulate much interest in both structure elucidation and structure-based drug design. For example, advances in structure-based inhibitor design against the mycobacterial enzyme UDP-galactopyranose mutase offer new hope in next generation anti-tuberculosis chemotherapeutics. The appearance of H5N1 avian influenza virus has re-stimulated much research on influenza virus haemagglutinin and sialidase. These latest developments on influenza virus sialidase have provided new opportunity for the development of Group 1-specific anti-influenza drugs. The

role of siglecs and galectins in a range of disease processes such as inflammation, apoptosis and cancer progression has also inspired significant structure-based inhibitor design research.

Dietary protein-carbohydrate ratio: Exogenous modulator of immune response with age.

Pal, Sudipta *et al.*

Immunobiology, **213(7)**, 557 (Aug. 29, 2008)

Manipulation of dietary variables is one of the most described events to retard the aging process and maintain immune function. The present study deals with the effect of variable dietary protein-carbohydrate ratios (without caloric restriction) on the alteration of immune response of male albino rats at the level of lymphocyte viability, proliferation, cytotoxicity, DNA fragmentation of blood, spleen and thymus and corticosterone levels in plasma and adrenal gland in relation to aging and duration of dietary exposure. Young (3 months) and aged rats (18 months) maintained with control diet [protein (20%)-carbohydrate (68%)] showed age-induced decrease in immune response with an increase in plasma corticosterone level. Consumption of low protein (8%)-high carbohydrate (80%) (LP-HC) diet for short-term period (15 consecutive days) decreased immune response of young rats with little immunopotentiality of aged rats but prolongation of consumption (for 60 consecutive days) of the LP-HC diet potentiated these immunopotentiality effects. High protein (50%)-low carbohydrate (38%) (HP-LC) diet under short-term exposure contrarily showed little immunopotentiality in young with an immunosuppression in aged rats. Prolongation of exposure (for 60 consecutive days) to the HP-LC diet produced similar but more amplified effects in young rats; whereas, in aged rats a pronounced decrease in peripheral immune response with an activation in thymus-dependent immune response was observed under similar

R & D Highlights

conditions. These results thus suggest that diets with variable dietary protein-carbohydrate ratios act as an exogenous modulator of immune response with age and LP-HC diet may be beneficial to slow down/reduce the impairment of immune response in aged individuals.

A novel C-type lectin (Cflec-3) from *Chlamys farreri* with three carbohydrate-recognition domains.

Zhang, Huan *et al.*

Fish & Shellfish Immunology, **26(5)**, 707(May 2009)

C-type lectins are a superfamily of carbohydrate-recognition proteins which play crucial roles in the innate immunity. In this study, the gene of a C-type lectin with multiple carbohydrate-recognition domains (CRDs) from scallop *Chlamys farreri* (designated as Cflec-3) was cloned by rapid amplification of cDNA ends (RACE) approach based on expression sequence tag (EST) analysis. The full-length cDNA of Cflec-3 was of 2256 bp. The open reading frame encoded a polypeptide of 516 amino acids, including a signal sequence and three CRDs. The deduced amino acid sequence of Cflec-3 showed high similarity to members of C-type lectin superfamily. By fluorescent quantitative real-time PCR, the Cflec-3 mRNA was mainly detected in hepatopancreas, adductor, mantle, and marginally in gill, gonad and hemocytes of healthy scallops. After scallops were challenged by *Listonella anguillarum*, the mRNA level of Cflec-3 in hemocytes was up-regulated and was significantly higher than that of blank at 8 h and 12 h post-challenge. The function of Cflec-3 was investigated by recombination and expression of the cDNA fragment encoding its mature peptide in *Escherichia coli* BL21 (DE3)-pLysS. The recombined Cflec-3 (rCflec-3) agglutinated Gram-negative bacteria *Pseudomonas stutzeri*. The agglutinating activity was calcium-dependent and could be inhibited by d-mannose. These results collectively suggested

that Cflec-3 was involved in the immune response against microbe infection and contributed to nonself-recognition and clearance of bacterial pathogens in scallop.

A glycobiology review: Carbohydrates, lectins and implications in cancer therapeutics.

Ghazarian, Haike *et al.*

Acta Histochemica(In Press)

This review is intended for general readers who would like a basic foundation in carbohydrate structure and function, lectin biology, and the implications of glycobiology in human health and disease, particularly in cancer therapeutics. These topics are among the hundreds included in the field of glycobiology and are treated here because they form the cornerstone of glycobiology or the focus of many advances in this rapidly expanding field.

Sweet preferences of MGL: carbohydrate specificity and function.

van Vliet, Sandra J. *et al.*

Trends in Immunology, **29(2)**, 83 (Feb., 2008)

C-type lectins play important roles in both innate and adaptive immune responses. In contrast to the mannose- or fucose-specific C-type lectins DC-SIGN and mannose receptor, the galactose-type lectins, of which only macrophage galactose-type lectin (MGL) is found within the immune system, are less well known. MGL is selectively expressed by immature dendritic cells and macrophages with elevated levels on tolerogenic or alternatively activated subsets. Human MGL has an exclusive specificity for rare terminal GalNAc structures, which are revealed on the tumor-associated mucin MUC1 and CD45 on effector T cells. These findings implicate MGL in the homeostatic control of adaptive immunity. We discuss here the functional similarities and differences between MGL orthologs and compare MGL to its closest homolog, the liver-specific asialoglycoprotein receptor (ASGP-R).

R & D Highlights

Certain dietary carbohydrates promote *Listeria* infection in a guinea pig model, while others prevent it.

Ebersbach, Tine *et al.*

International Journal of Food Microbiology(In Press)

It has been proposed that dietary non-digestible carbohydrates can improve host resistance to intestinal infections by stimulating health-promoting bacteria in the gut. However, evidence from *in vivo* infection studies is scarce, particularly for gram-positive infections. We studied the effect of five non-digestible carbohydrates on the resistance of guinea pigs to *Listeria monocytogenes* infections. Animals were fed a diet supplemented with 10% xylooligosaccharides (XOS), galactooligosaccharides (GOS), inulin, apple pectin or polydextrose for three weeks before oral infection with a mixture of three different fluorescently labeled *L. monocytogenes* strains. Colonisation of *L. monocytogenes* in the intestine was determined by quantification of *L. monocytogenes* in faecal, ileal and caecal samples while translocation was determined by quantification of *L. monocytogenes* in mesenteric lymph nodes, spleen and liver. XOS and GOS significantly ($P < 0.05$) improved the resistance of guinea pigs to *L. monocytogenes*, while inulin and apple pectin decreased the resistance ($P < 0.05$). No significant effect on resistance to *L. monocytogenes* was seen after feeding with polydextrose. No difference in caecal weight or pH between the dietary groups was measured, except for a higher caecal weight and a lower caecal pH of animals fed with XOS, and a lower caecal pH for animals fed with polydextrose. In conclusion, this study shows for the first time that different non-digestible carbohydrates can have entirely different effects on the intestinal colonisation and translocation of a pathogenic bacterium.

Anti-carbohydrate antibodies of normal sera: Findings, surprises and challenges.

Huflejt, Margaret E. *et al.*

Molecular Immunology, **46(15)**, 3037(Sept.,2009)

Researchers have used microchip format glycan array to characterize the individual carbohydrate recognition patterns by antibodies (Ab) in sera of 106 healthy donors. The glycan library included blood group antigens and other most frequent terminal oligosaccharides and their cores of mammalian N- and O-linked glycoproteins and glycolipids, tumor-associated carbohydrate antigens, and common components of bacterial/pathogenic polysaccharides and lipopolysaccharides, totally 205 glycans. The serum Ab interacted with at least 50 normal human glyco-motifs. Apart from expected blood group-, xeno-(heterophil) and infection-related binding activities, we observed a number of new and unexpected features. The surprising, relatively high antibody binding was found to the blood group P1 and Pk trisaccharides and H(type 2) trisaccharide. Novel and very high binding activities have been observed towards Gal[beta]1-3GlcNAc (LeC) related glycans, especially 3'-O-Su-LeC, and towards 4'-O-sulfated lactosamine. Relatively high and uniform Ab binding to GalNAc[alpha]1-3Gal disaccharide demonstrated absence of correlation with fucosylated blood group A GalNAc[alpha]1-3(Fuc[alpha]1-2)Gal antigen- similarly to well known relationship between Gal[alpha]1-3Gal and true, fucosylated blood group B Gal[alpha]1-3(Fuc[alpha]1-2)Gal antigen. The binding intensity to Gal[alpha]1-3Gal[beta]1-4GlcNAc xenoantigen was shown to be rather modest. Absence or very low Ab binding was found against oligosialic acid, sialooligosaccharides except SiaTn, type 2 backbone glycans such as Ley, and biantennary N-chain as well as its truncated forms, i.e. without terminal Sia, SiaGal, and SiaGalGlcNAc motifs. We have also found that Ab are capable of recognizing the short inner core typical for glycolipids (-Gal[beta]1-4Glc) and glycoproteins (-GalNAc[alpha]) as a fragment of bigger glycans.

R & D Highlights

Dusting the sugar fingerprint: C-type lectin signaling in adaptive immunity.

den Dunnen, Jeroen *et al.*

Immunology Letters, **128(1)**, 12 (Jan., 18, 2010)

Pathogen recognition by dendritic cells (DCs) is central to the induction of adaptive immunity. Pattern recognition receptors (PRRs) on DCs interact with pathogens, leading to signaling events that dictate adaptive immune responses. It is becoming clear that C-type lectins are important PRRs that recognize carbohydrate structures. Most pathogens express carbohydrate structures on their surface and these function as a so-called sugar fingerprint that are recognized by specific C-type lectins. Triggering of C-type lectin induces signaling cascades that initiate or modulate specific cytokine responses and therefore tailor T cell polarization to the pathogens. Here we will discuss our current understanding of the innate signaling pathways induced by C-type lectins DC-SIGN and dectin-1 in humans and how these pathways shape adaptive immunity.

Health benefit application of functional oligosaccharides.

Qiang, Xu *et al.*

Carbohydrate Polymers, **77(3)**, 435 (July 11, 2009)

There is no doubt that the functional oligosaccharides have positive effects on human health, both in the prevention and in treatment of chronic diseases. Therefore, there is great interest in health benefits of the functional oligosaccharides. The functional oligosaccharides of various origins (viruses, bacteria, plants and fungi) have been used extensively both as pharmacological supplements, food ingredients, in processed food to aid weight control, to regulation of glucose control for diabetic patients and reducing serum lipid levels in hyperlipidemics and other some acute and chronic diseases. Keeping in view, the pharmacological importance of the functional oligosaccharides

and its derivatives, this article discusses the potential of the functional oligosaccharides to modulate the gut flora, to affect different gastrointestinal activities and lipid metabolism, to enhance immunity, and to reduce diabetes, obesity and cardiovascular risk for further exploitation of health benefits of the functional oligosaccharides.

Carbohydrate-based experimental therapeutics for cancer, HIV/AIDS and other diseases.

Oppenheimer, Steven B. *et al.*

Acta Histochemica, **110(1)**, 6 (Jan., 14, 2008)

This review, primarily for general readers, briefly presents experimental approaches to therapeutics of cancer, HIV/AIDS and various other diseases based on advances in glycobiology and glycochemistry. Experimental cancer and HIV/AIDS vaccines are being developed in attempts to overcome weak immunological responses to carbohydrate-rich surface antigens using carriers, adjuvants and novel carbohydrate antigen constructs. Current carbohydrate-based vaccines are used for typhus, pneumonia, meningitis; vaccines for anthrax, malaria and leishmaniasis are under development. The link between O-linked [beta]-N-acetylglucosamine glycosylation and protein phosphorylation in diseases including diabetes and Alzheimer's disease is also explored. Carbohydrate-associated drugs that are in current use or under development, such as heparan sulfate binders, lectins, acarbose, aminoglycosides, tamiflu and heparin, and technologies using carbohydrate and lectin microarrays that offer improved diagnostic and drug development possibilities, are described. Advances in carbohydrate synthesis, analysis and manipulation through the emerging fields of glycochemistry and glycobiology are providing new approaches to disease therapeutics.

Glycosidases: a key to tailored carbohydrates.

R & D Highlights

Bojarová, Pavla *et al.*
Trends in Biotechnology, **27(4)**, 199(Apr., 2009)

In recent years, carbohydrate-processing enzymes have become the enzymes of choice in many applications thanks to their stereoselectivity and efficiency. This review presents recent developments in glycosidase-catalyzed synthesis via two complementary approaches: the use of wild-type enzymes with engineered substrates, and mutant glycosidases. Genetic engineering has recently produced glucuronyl synthases, an inverting xylosynthase and the first mutant endo-[beta]-N-acetylglucosaminidase. A thorough selection of enzyme strains and aptly modified substrates have resulted in rare glycostructures, such as N-acetyl-[beta]-galactosaminuronates, [beta]1,4-linked mannosides and [alpha]1,4-linked galactosides. The efficient selection of mutant enzymes is facilitated by high-throughput screening assays involving the co-expression of coupled enzymes or chemical complementation. Selective glycosidase inhibitors and highly specific glycosidases are finding attractive applications in biomedicine, biology and proteomics.

Anaphylaxis syndromes related to a new

mammalian cross-reactive carbohydrate determinant.

Commins, Scott P. *et al.*
Journal of Allergy and Clinical Immunology, **124(4)**, 652(Oct., 2009)

Anaphylaxis is a severe allergic reaction that can rapidly progress and occasionally be fatal. In instances in which the triggering allergen is not obvious, establishing the cause of anaphylaxis is pivotal to long-term management. Assigning cause is limited, however, by the number of known exposures associated with anaphylaxis. Therefore identification of novel causative agents can provide an important step forward in facilitating new, allergen-specific approaches to management. In contrast to the view that carbohydrate-directed IgE has minimal, if any, clinical significance, recent data suggest that IgE antibodies to carbohydrate epitopes can be an important factor in anaphylaxis that might otherwise appear to be idiopathic. Here we review the evidence relating to carbohydrates in food allergy and anaphylaxis and discuss the implications of a new mammalian cross-reactive carbohydrate determinant.

Annual Subscription Rates/Periodical

Effective from January 2007 (Vol. 30)

Country	India	Bhutan, Bangladesh	*APPU Member Nepal & Pakistan	Rest of the World Countries
Category of subscriber	(in Rs.)	Surface Mail (in US\$)		Air Mail (in US\$)
A. Corporate Sector	1000	50	65	75
B. Educational & R&D Institutions	600	30	50	60
C. Students & Professionals	400	20	40	50

We offer a discount of 15% to those who wish to subscribe both our periodicals

- Asia and Pacific Postal Union: Australia, China, Korea, Indonesia, Japan, Laos, Malaysia, New Zealand, Philippines, Papua New Guinea, Singapore, Maldives, Peru, Thailand and Sri Lanka



Synthesis and antimicrobial evaluation of carbohydrate and polyhydroxylated non-carbohydrate fatty acid ester and ether derivatives.

Smith, Aoife *et al.*

Carbohydrate Research, **343** (15), 2557(Oct., 13, 2008)

A series of fatty acid ester and ether derivatives have been chemically synthesised based on carbohydrate and non-carbohydrate polyhydroxylated scaffolds. The synthesised compounds, along with their corresponding fatty acid monoglyceride antimicrobials, were evaluated for antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. Of the derivatives synthesised, several of the carbohydrate-based compounds have antimicrobial efficacy comparable with commercially available antimicrobials. The results suggest that the nature of the carbohydrate core plays a role in the efficacy of carbohydrate fatty acid derivatives as antimicrobials.

Cerium(IV)-mediated C-C bond formations in carbohydrate chemistry.

Elamparuthi, Elangovan *et al.*

Tetrahedron, **64**(52), 11925 (Dec., 22, 2008)

We provide a comprehensive study on the addition of radicals to unsaturated carbohydrates in the presence of cerium(IV) ammonium nitrate (CAN). The method is applicable to hexoses, pentoses, and disaccharides, tolerates different protecting groups, and is characterized by mild reaction conditions. Best substrates are malonates and glycals, which afford 2-C-branched carbohydrates in high yields and stereoselectivities. For the first time, the anomeric radicals were trapped with nucleophiles after oxidation and thus the 1-

and 2-position of glucose were functionalized in one step. Nitro compounds are suitable CH acidic radical precursors as well, offering an easy access to C-analogs of glycosamines in moderate to good yields. Finally, selective reductions demonstrate the synthetic potential of cerium(IV)-mediated radical reactions in carbohydrate chemistry.

Synthesis of oxa-aza spirobicycles by intramolecular hydrogen atom transfer promoted by N-radicals in carbohydrate systems.

Martín, Angeles *et al.*

Tetrahedron, **65**(31), 6147 (Aug., 1, 2009)

The nitrogen-centred radical generated by reaction of an N-phosphoramidate or N-cyanamide, attached to a tri- or tetramethylene tether extended from the C-1 of a carbohydrate, with (diacetoxyiodo)benzene (DIB) and iodine can undergo a regio- and stereoselective intramolecular hydrogen atom transfer (HAT) reaction to furnish four different oxa-azaspirobicyclic systems: 1-oxa-6-azaspiro[4.4]nonane, 1-oxa-6-aza spiro[4.5]decane, 6-oxa-1-azaspiro[4.5]decane and 1-oxa-7-azaspiro[5.5]undecane. A tandem 1, 5- or 1,6-HAT-oxidation-nucleophilic cyclisation mechanism is proposed.

InCa-SiteFinder: A method for structure-based prediction of inositol and carbohydrate binding sites on proteins.

Kulharia, Mahesh *et al.*

Journal of Molecular Graphics and Modelling, **28**(3), 297 (Oct., 2009)

Carbohydrate binding sites are considered important for cellular recognition and adhesion and are important targets for drug design. In this paper we present a new method called InCa-SiteFinder for predicting non-covalent inositol and carbohydrate binding sites on the surface of protein structures. It uses the van der Waals energy of a protein-probe interaction and amino acid propensities to locate and predict carbohydrate binding sites. The protein surface is searched for continuous volume envelopes that correspond to a

favorable protein-probe interaction. These volumes are subsequently analyzed to demarcate regions of high cumulative propensity for binding a carbohydrate moiety based on calculated amino acid propensity scores.

InCa-SiteFinder1 was tested on an independent test set of 80 protein-ligand complexes. It efficiently identifies carbohydrate binding sites with high specificity and sensitivity. It was also tested on a second test set of 80 protein-ligand complexes containing 40 known carbohydrate binders (having 40 carbohydrate binding sites) and 40 known drug-like compound binders (having 58 known drug-like compound binding sites) for the prediction of the location of the carbohydrate binding sites and to distinguish these from the drug-like compound binding sites. At 73% sensitivity the method showed 98% specificity. Almost all of the carbohydrate and drug-like compound binding sites were correctly identified with an overall error rate of 12%.

Carbohydrate scaffolds in chemical genetic studies.

Nicotra, Francesco *et al.*

Journal of Biotechnology, **144(3)**, 234 (Nov., 2009)

Small molecules altering protein functions as inhibitors, agonists or antagonists, find application in systems biology enabling an analysis of the in vivo consequences of these alterations. In this context carbohydrates are ideal tools, not only because they are involved in a variety of recognition phenomena of biological relevance, but also because they are ideal scaffolds to generate libraries of bioactive compounds. Examples of design, synthesis and biological assays of different carbohydrate based inhibitors or protein ligands are reported. Exploiting NMR methods, the binding between a small molecules (inhibitor or ligand) and a protein can be detected, the affinity measured, and the interaction topology defined. This set of

information is useful not only to clarify the mechanism of protein-ligand interaction, but also to improve the design of new inhibitors/ligands. The multifunctionality and the conformational rigidity of carbohydrates make this class of compounds the ideal scaffolds to generate libraries exploiting the combinatorial approach. An example of solid phase combinatorial synthesis of a library of 37 compounds is reported.

Sialic acids: carbohydrate moieties that influence the biological and physical properties of biopharmaceutical proteins and living cells.

Byrne, Barry *et al.*

Drug Discovery Today, **12(7-8)**, 319 (Apr., 2007)

Sialic acids are structurally diverse molecules that have important roles in the physiological reactions and characteristics of prokaryotes and eukaryotes. These include the ability to mask epitopes on underlying glycan chains and to repulse negatively charged moieties. Here, we describe the metabolism and immunological relevance of sialic acids and outline how their properties have been exploited by the pharmaceutical industry to enhance the therapeutic properties of proteins such as asparaginase and darbepoetin [alpha].

Synthesis and biodegradation of nanogels as delivery carriers for carbohydrate drugs.

Oh JK, *et al.*

Biomacromolecules. **8(11)**:3326-31(2007 Nov;).

Biodegradable nanogels loaded with rhodamine B isothiocyanate-dextran (RITC-Dx) as a model for water-soluble biomacromolecular drugs were prepared using atom-transfer radical polymerization (ATRP) in a cyclohexane inverse miniemulsion in the presence of a disulfide-functionalized dimethacrylate cross linker. UV-vis spectroscopy was used to characterize the extent of incorporation of RITC-Dx into the nanogels. The loading efficiency of RITC-Dx into the nanogels exceeded 80%. These

R & D Technology

nanogels were degraded into polymeric sols in a reducing environment to release the encapsulated carbohydrate drugs. The released carbohydrate biomolecules specifically interacted with concanavalin A in water, suggesting that the biodegradable nanogels could be used as carriers to deliver carbohydrate drugs that can be released upon degradation to bind to pathogens based on lectins.

Quantitative glycomics from fluidic glycan microarrays.

X.-Y. Zhu *et al.*

J. Am. Chem. Soc., 2009, **131 (38)**, pp 13646–13650

A hallmark of cell-surface processes involving glycans is their multivalent interaction with glycan binding proteins (GBPs). Such a multivalent interaction depends critically on the mobility and density of signaling molecules on the membrane surface. While glycan microarrays have been used in exploring multivalent interactions, the lack of mobility and the difficulty in controlling surface density both limit their quantitative applications. Here we apply a fluidic glycan microarray, with glycan density

varying for orders of magnitude, to profile cell surface interaction using a model system, the adhesion of *Escherichia coli* to mannose. We show the quantitative determination of monovalent and multivalent adhesion channels; the latter can be inhibited by nanoparticles presenting a high density of mannosyl groups. These results reveal a new *E. coli* adhesion mechanism: the switching in the FimH adhesion protein avidity from monovalent to multivalent as the density of mobile mannosyl groups increases; such avidity switching enhances binding affinity and triggers multiple fimbriae anchoring. Affinity enhancement toward FimH has only been observed before for oligo-mannose due to the turn on of secondary interactions outside the mannose binding pocket. We suggest that the new mechanism revealed by the fluidic microarray is of general significance to cell surface interactions: the dynamic clustering of simple sugar groups (homogeneous or heterogeneous) on the fluidic membrane surface may simulate the functions of complex glycan molecules.



Current R&D Highlights



Synthesis and antitubercular evaluation of new fluoroquinolone derivatives coupled with carbohydrates.

Saraiva, Maurício F *et al.*

Carbohydrate Research, **345(6)**, 761(Apr., 19, 2010)

Authors have describe in this work the synthesis of nine new fluoroquinolone derivatives based on modifications at the C-7 position of the known fluoroquinolones cipro-, gati-, and moxifloxacin, as well as their antitubercular evaluation. The synthesis of these new analogues was improved using microwave irradiation, providing several advantages such as better yields and shorter reaction times, in comparison with classical reaction conditions. Derivatives 4, 5, and 7 exhibited promising antitubercular activities.

Synthesis, HIV-RT inhibitory activity and SAR of 1-benzyl-1H-1,2,3-triazole derivatives of carbohydrates.

da Silva, Fernando de C.

European Journal of Medicinal Chemistry, **44(1)**, 373(Jan., 2009)

This paper describes the synthesis of several 1-benzyl-1H-1, 2, 3-triazoles attached to different carbohydrate templates and their in vitro inhibitory profile against HIV-1 reverse transcriptase. In addition a theoretical comparison of the most active compounds with other classical antivirals was also performed. Our results showed 2a, 2d and 2g as the most active compounds that inhibited the HIV-1 reverse transcriptase catalytic activity with cytotoxicity lower than AZT and SI higher than DDC and DDI. The overall theoretical analysis of the molecular descriptors of 2a, 2d and 2g revealed that their HOMO energy is similar to other antivirals in use (AZT, DDC, DDI and 3TC) and together with the volume may contribute for the biological profile as they may allow new interactions with the

target. In fact the 1, 2, 3-triazole compounds presented more lipophilicity and higher molecular volume and weight than the antivirals studied, which suggested that these features might not only contribute for new interactions with the HIV-RT but also influence the specificity and consequently the low cytotoxicity profile of these compounds. Thus these data point them as promising leading compounds for generating new anti-HIV-RT compounds.

Development of carbohydrate-derived inhibitors of acid sphingomyelinase.

Roth, Anke G. *et al.*

Bioorganic & Medicinal Chemistry, **18(2)**, 939 (Jan., 15, 2010)

The acid sphingomyelinase is an emerging drug target, especially for inflammatory lung diseases. Presently, there are no directly-acting potent inhibitors available for cell-based studies. The potent inhibitor phosphatidylinositol-3,5-bisphosphate (PtdIns3,5P2) is not only unsuited for cell culture studies, but also does not provide hints for further structural improvements. In the SAR study described here, we replaced the inositolphosphate moiety by a carbohydrate derivative and the phosphatidic acid residue by an alkylsulfone ester. The resulting compound is more active than its parent compound and offers new means for further structural modification.

Click Reaction Synthesis of Carbohydrate Derivatives from Ristocetin Aglycon with Antibacterial and Antiviral Activity.

Pintér, Gábor *et al.*

Bioorganic & Medicinal Chemistry Letters (In Press)

New sugar derivatives of ristocetin were prepared by copper-catalyzed 1,3-dipolar cycloaddition reaction using azido-ristocetin aglycon and various propargyl glycosides. Some of them were found to be active against Gram-positive bacteria and showed favorable antiviral activity against the H1N1 subtype of influenza A virus.

New Leads

Novel pyridazine derivatives: Synthesis and antimicrobial activity evaluation.

Kandile, N.G. *et al.*

European Journal of Medicinal Chemistry, **44(5)**, 1989 (May 2009)

A general method for the preparation of new hydrazones is reported. The 1-[4-(2-methoxybenzyl)-6-aryl pyridazin-3(2H)-ylidene] hydrazines or their tautomeric structures (1a-d) were condensed with different aldehydes, dialdehydes, ketones, [alpha]-dicarbonyl compounds and simple carbohydrates to afford the hydrazones and dihydrazones (2a-d), (3a-d), (4a-d), (5a-d), (6d), (7c), (8a-d), (9a-d), (10a-d), (11a-d), (12a,c,d), (13a-d), (14a-d), (15a-d), (16a-d) and (17a-d). The structures of all synthesized compounds were confirmed from microanalytical and spectral data. Some of the products were screened for their antimicrobial activity against *Staphylococcus aureus* and *Streptococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The hydrazone derivative 15d (1-[4-(2-methoxybenzyl)-6-methylphenyl pyridazin-3(2H)-ylidene]-2-(2-carboxydiphenyl methyl) hydrazine) showed the highest biological activity.

Synthesis, HIV-RT inhibitory activity and SAR of 1-benzyl-1H-1, 2, 3-triazole derivatives of carbohydrates.

da Silva, Fernando de C. *et al.*

European Journal of Medicinal Chemistry, **44(1)**, 373 (Jan., 2009)

This paper describes the synthesis of several 1-benzyl-1H-1, 2, 3-triazoles attached to different carbohydrate templates and their in vitro inhibitory profile against HIV-1 reverse transcriptase. In addition a theoretical comparison of the most active compounds with other classical antivirals was also performed. Our results showed 2a, 2d and 2g as the most active compounds that inhibited the HIV-1 reverse transcriptase catalytic activity with cytotoxicity lower than AZT and SI higher than DDC and DDI. The overall theoretical analysis of the molecular descriptors of 2a, 2d

and 2g revealed that their HOMO energy is similar to other antivirals in use (AZT, DDC, DDI and 3TC) and together with the volume may contribute for the biological profile as they may allow new interactions with the target. In fact the 1, 2, 3-triazole compounds presented more lipophilicity and higher molecular volume and weight than the antivirals studied, which suggested that these features might not only contribute for new interactions with the HIV-RT but also influence the specificity and consequently the low cytotoxicity profile of these compounds. Thus these data point them as promising leading compounds for generating new anti-HIV-RT compounds.

Synthesis and antitubercular activity of novel Schiff bases derived from d-mannitol.

Ferreira, Marcelle de L. *et al.*

Carbohydrate Research, **344(15)**, 2042 (Oct., 12, 2009)

Six Schiff base derivatives of d-mannitol, 1,6-dideoxy-1,6-bis-[(E)-arylmethylidene]amino)-d-mannitol (6: aryl=XC₆H₄: X=o-, m- and p- Cl or NO₂), have been synthesized and evaluated for their in vitro antibacterial activity against *Mycobacterium tuberculosis* H37Rv using the Alamar Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in [μ]g/mL. All three nitro derivatives exhibit significant activities: activities of (6d: X=o-NO₂), (6e: X=m-NO₂) and (6f: X=p-NO₂) are 12.5, 25.0 and 25.0[μ]g/mL, respectively. When compared with first line drugs, such as ethambutol, they can be considered as a good starting point to develop new lead compounds for the treatment of multidrug-resistant tuberculosis. Characterization of the new compounds 6 is generally achieved spectroscopically. The structure of compound 3 has been confirmed by X-ray crystallography.

Synthesis and biological evaluation of glycal-derived novel tetrahydrofuran 1,2,3-triazoles by "click" chemistry.

New Leads

Reddy, L. Vijaya Raghava *et al.*
Carbohydrate Research(In Press)

Thirteen new 1, 2, 3-triazoles (5a-e, 15a-d, 17a-b, 19 and 21) were synthesized by "click" reaction of sugar derived azides with commercially available acetylenes. The synthesized triazoles when tested in vitro for their biological activity, compound 5b displayed both antibacterial and antifungal activity at MIC value 12.5 [μ]g/mL, while compounds 15b and 19 showed antibacterial activity at MIC value 25 [μ]g/mL.

Carbohydrate-based anti-adhesive inhibition of *Vibrio cholerae* toxin binding to GM1-OS immobilized into artificial planar lipid membranes.

Sinclair, Haydn R *et al.*

Carbohydrate Research, **344(15)**, 1986 (Oct., 12, 2009)

We have studied [^]food grade' sialyloligosaccharides (SOS) as anti-adhesive drugs or receptor analogues, since the terminal sialic acid residue has already been shown to contribute significantly to the adhesion and pathogenesis of the *Vibrio cholerae* toxin (Ctx). GM1-oligosaccharide (GM1-OS) was immobilized into a supporting POPC lipid bilayer onto a surface plasmon resonance (SPR) chip, and the interaction between uninhibited Ctx and GM1-OS-POPC was measured. SOS inhibited 94.7% of the Ctx binding to GM1-OS-POPC at 10 mg/mL. The SOS EC50 value of 5.521 mg/mL is high compared with 0.2811 [μ]g/mL (182.5 [ρ]M or 1.825×10^{-10} M) for GM1-OS. The commercially available sialyloligosaccharide (SOS) mixture SunSial E® is impure, containing one monosialylated and two disialylated oligosaccharides in the ratio 9.6%, 6.5% and 17.5%, respectively, and 66.4% protein. However, these inexpensive food-grade molecules are derived from egg yolk and could be used to fortify conventional food additives, by way of emulsifiers, sweeteners and/or preservatives. The work further supports our hypothesis that SOS could

be a promising natural anti-adhesive glycomimetic against Ctx and prevent subsequent onset of disease.

Synthesis, HIV-RT inhibitory activity and SAR of 1-benzyl-1H-1,2,3-triazole derivatives of carbohydrates.

da Silva, Fernando de C. *et al.*

European Journal of Medicinal Chemistry,**44(1)**, 373 (Jan., 2009)

This paper describes the synthesis of several 1-benzyl-1H-1, 2, 3-triazoles attached to different carbohydrate templates and their in vitro inhibitory profile against HIV-1 reverse transcriptase. In addition a theoretical comparison of the most active compounds with other classical antivirals was also performed. Our results showed 2a, 2d and 2g as the most active compounds that inhibited the HIV-1 reverse transcriptase catalytic activity with cytotoxicity lower than AZT and SI higher than DDC and DDI. The overall theoretical analysis of the molecular descriptors of 2a, 2d and 2g revealed that their HOMO energy is similar to other antivirals in use (AZT, DDC, DDI and 3TC) and together with the volume may contribute for the biological profile as they may allow new interactions with the target. In fact the 1,2,3-triazole compounds presented more lipophilicity and higher molecular volume and weight than the antivirals studied, which suggested that these features might not only contribute for new interactions with the HIV-RT but also influence the specificity and consequently the low cytotoxicity profile of these compounds. Thus these data point them as promising leading compounds for generating new anti-HIV-RT compounds.

Alteration of the carbohydrate for deoxyguanosine analogs markedly changes DNA replication fidelity, cell cycle progression and cytotoxicity.

O'Konek, Jessica J. *et al.*

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, **684(1-2)**, 1(Feb., 3, 2010)

New Leads

Nucleoside analogs are efficacious cancer chemotherapeutics due to their incorporation into tumor cell DNA. However, they exhibit vastly different antitumor efficacies, suggesting that incorporation produces divergent effects on DNA replication. Here we have evaluated the consequences of incorporation on DNA replication and its fidelity for three structurally related deoxyguanosine analogs: ganciclovir (GCV), currently in clinical trials in a suicide gene therapy approach for cancer, D-carbocyclic 2'-deoxyguanosine (CdG) and penciclovir (PCV). GCV and CdG elicited similar cytotoxicity at low concentrations, whereas PCV was 10-100-fold less cytotoxic in human tumor cells. DNA replication fidelity was evaluated using a supF plasmid-based mutation assay. Only GCV induced a dose-dependent increase in mutation frequency, predominantly GC-->TA transversions, which contributed to cytotoxicity and implicated the ether oxygen in mutagenicity. Activation of mismatch repair with hydroxyurea decreased mutations but failed to repair the GC-->TA transversions. GCV slowed S-phase progression and CdG also induced a G2/M block, but both drugs allowed completion of one cell cycle after drug

treatment followed by cell death in the second cell cycle. In contrast, PCV induced a lengthy early S-phase block due to profound suppression of DNA synthesis, with cell death in the first cell cycle after drug treatment. These data suggest that GCV and CdG elicit superior cytotoxicity due to their effects in template DNA, whereas strong inhibition of nascent strand synthesis by PCV may protect against cytotoxicity. Nucleoside analogs based on the carbohydrate structures of GCV and CdG is a promising area for antitumor drug development.

A vinyl sulfone-modified carbohydrate mediated new route to aminosugars and branched-chain sugars.

Pathak, Tanmaya *et al.*

Carbohydrate Research, 343(12), 1980 (Aug., 11, 2008)

This minireview describes syntheses of various vinyl sulfone-modified carbohydrates and their reactions with nitrogen and carbon nucleophiles for accessing a wide range of aminosugars and branched-chain sugars.

Views expressed in the periodical are those of the authors and the Editorial Board/Publisher takes no responsibility for the same. We are a secondary abstracting service and the veracity of information is of the source quoted and not our primary responsibility.

Editors



Development and characterization of eudragit RS 100 loaded microsponges and its colonic delivery using natural polysaccharides.

Jain V, Singh R.

Acta Pol Pharm. 2010 Jul-Aug;**67(4)**:407-15.

In the present work, paracetamol loaded eudragit based microsponges were prepared using quasi-emulsion solvent diffusion method. The compatibility of the drug with various formulation components was established. Process parameters were analyzed in order to optimize the formulation. Shape and surface morphology of the microsponges were examined using scanning electron microscopy. The formulations were subjected to in vitro release studies and the results were evaluated kinetically and statically. The in vitro release data showed a bi-phasic pattern with an initial burst effect. In the first hour drug release from microsponges was found to be between 17-30%. The cumulative percent release at the end of 8th hour was noted to be between 54-83%. The release kinetics showed that the data followed Higuchi model and the main mechanism of drug release was diffusion. The colon specific tablets were prepared by compressing the microsponges followed by coating with pectin:hydroxypropylmethylcellulose (HPMC) mixture. In vitro release studies exhibited that compression coated colon specific tablet formulations started releasing the drug at 6th hour corresponding to the arrival time at proximal colon. The study presents a new approach for colon specific drug delivery.

Three new secoiridoid glycoside dimers from *Swertia mileensis*.

Geng CA *et al.*

J Asian Nat Prod Res. 2010

Jun;**12(6)**:542-8.

Three new secoiridoid glycoside dimers named swerilactosides A-C (1-3) were isolated from *Swertia mileensis*. Their structures were elucidated based on extensive spectral analyses (1D and 2D NMR, MS, and IR spectroscopic means).

Identification of sakurasosaponin as a cytotoxic principle from *Jacquinia flammea*.

Sánchez-Medina A *et al.*

Nat Prod Commun. 2010 Mar;**5(3)**:365-8

The crude ethanolic extract of leaves, stem-bark and roots of *J. flammea* were tested for their cytotoxic effect against two mammalian cell lines (HeLa and RAW 264.7) and four bacterial species (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*). When tested at the concentration of 100 microg/mL, the root extract showed the highest cytotoxic activity against mammalian cells followed by the stem-bark extract while the leaves extract did not show significant activity. No antibacterial activity was detected for all extracts when tested up to 500 microg/disc in the disc diffusion assay. The cytotoxic root extract was subjected to fractionation using solvents of ascending polarity: petroleum ether, chloroform, ethylacetate, butanol and water. The water fraction which showed cytotoxic activity was further subjected to routine bioassay-guided fraction to lead to the isolation of sakurasosaponin as the active principle. The recorded IC50 value for sakurasosaponin was 11.3 +/- 1.52 and 3.8 +/- 0.25 microM (n=3) against HeLa and RAW 264.7 respectively. The identification of sakurasosaponin was based on analysis of spectroscopic data.

Mass spectral fragmentation analysis of triterpene saponins from *Ardisia crenata* Sims by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry.

Zhang PC *et al.*

J Asian Nat Prod Res. 2010 Jan;**12(1)**:64-9.

Natural Products

Authors used the electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) technique to study the characteristic mass fragmentation patterns of eight triterpene saponins from *Ardisia crenata* Sims. Eight triterpene saponins were analyzed using parent mass list-triggered data-dependent multiple-stage accurate mass analysis at a resolving power of 100,000 in the external calibration mode. The chemical formula with unsaturation numbers was calculated from accurate m/z values of precursor, and product ions were obtained and used to assign the structures of eight triterpene saponins and two trace unknown compounds. The mass accuracies obtained for all full-scan MS and MS(n) spectra were within 7 ppm (< 5 ppm in most cases) in the ESI negative-ion mode. On FTICR-MS and FTICR-MS/MS, the eight triterpene saponins showed characteristic mass fragmentation patterns that facilitated the identification of their structural types, including the individual monosaccharide types, the monosaccharide numbers, and the sequences of the substituted saccharide groups. We proposed their fragmentation mechanisms. Based on their characteristic mass fragmentation patterns and fragmentation mechanisms, two unknown trace triterpene saponins were identified in the mixture.

Four new trace phenolic glycosides from *Curculigo orchioides*.

Zuo AX *et al.*

J Asian Nat Prod Res. 2010 Jan;12(1):43-50

Four new trace phenolic glycosides named orcinosides D (1), E (2), F (3), and G (4) were isolated from the rhizomes of *Curculigo orchioides* Gaertn. Based on comprehensive spectroscopic analyses including IR, FAB-MS, HR-ESI-MS, 1D- and 2D NMR (HSQC, HMBC), their structures were elucidated as orcinol-1-O-beta-D-xylopyranoside (1), orcinol-1-O-beta-D-apiofuranosyl-(1 --> 2)-beta-D-glucopyranoside (2), orcinol-3-O-beta-

D-apiofuranosyl-1-O-beta-D-glucopyranoside (3), and 1-O-beta-D-glucopyranosyl-4-ethoxyl-3-hydroxymethylphenol (4).

Biomaterials in cell microencapsulation.

Santos E *et al.*

Adv Exp Med Biol. 2010;670:5-21.

The field of cell encapsulation is advancing rapidly. This cell-based technology permits the local and long-term delivery of a desired therapeutic product reducing or even avoiding the need of immunosuppressant drugs. The choice of a suitable material preserving the viability and functionality of enclosed cells becomes fundamental if a therapeutic aim is intended. Alginate, which is by far the most frequently used biomaterial in the field of cell microencapsulation, has been demonstrated to be probably the best polymer for this purpose due to its biocompatibility, easy manipulation, gel forming capacity and in vivo performance.

***Acanthopanax koreanum* fruit waste inhibits lipopolysaccharide-induced production of nitric oxide and prostaglandin E2 in RAW 264.7 macrophages.**

Yang EJ *et al.*

J Biomed Biotechnol. 2010;2010:715739. Epub 2010 Mar 23.

The *Acanthopanax koreanum* fruit is a popular fruit in Jeju Island, but the byproducts of the alcoholic beverage prepared using this fruit are major agricultural wastes. The fermentability of this waste causes many economic and environmental problems. Therefore, we investigated the suitability of using *A. koreanum* fruit waste (AFW) as a source of antiinflammatory agents. AFWs were extracted with 80% EtOH. The ethanolic extract was then successively partitioned with hexane, CH₂Cl₂, EtOAc, BuOH, and water. The results indicate that the CH₂Cl₂ fraction (100 microg/mL) of AFW inhibited the LPS-induced nitric oxide (NO) and prostaglandin E₂ (PGE₂) production in RAW 264.7 cells by 79.6% and 39.7%, respectively. These inhibitory effects of the

Natural Products

CH(2)Cl(2) fraction of AFWs were accompanied by decreases in the expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) proteins and iNOS and COX-2 mRNA in a dose-dependent pattern. The CH(2)Cl(2) fraction of AFWs also prevented degradation of IkappaB-alpha in a dose-dependent manner. Ursolic acid was identified as major compound present in AFW, and CH(2)Cl(2) extracts by high performance liquid chromatography (HPLC). Furthermore using pure ursolic acid as standard and by HPLC, AFW and CH(2)Cl(2) extracts was found to contain 1.58 mg/g and 1.75 mg/g, respectively. Moreover, we tested the potential application of AFW extracts as a cosmetic material by performing human skin primary irritation tests. In these tests, AFW extracts did not induce any adverse reactions. Based on these results, we suggest that AFW extracts be considered possible anti-inflammatory candidates for topical application.

Lactobionic acid in a natural alkylpolyglucoside-based vehicle: assessing safety and efficacy aspects in comparison to glycolic acid.

Tasic-Kostov M *et al.*

J Cosmet Dermatol. 2010 Mar;**9(1)**:3-10.

Lactobionic acid (LA) is a newer cosmeceutical active belonging to the class of alpha-hydroxyacids (AHAs), showing advantages over them. The aim of part I of this study was to compare efficacy and irritation potential of LA vs. glycolic acid (GA) from two types of vehicles - gel and emulsion. In part II, effects of LA-containing emulsions based on a new, natural emulsifier of alkylpolyglucoside (APG) type were evaluated. Skin bioengineering was used on 77 healthy volunteers to assess: color as erythema and melanin (MI) index, transepidermal water loss, electrical capacitance and pH of the skin. In part I of the study, the parameters were measured after occlusion and periodically during 2 weeks of test samples application; in part II parameters were measured periodically

during 4 weeks. LA-containing samples has produced better skin performance when compared with corresponding GA-containing ones, particularly the lack of both skin irritation and skin barrier impairment. When used in vehicles based on a new APG-emulsifier, LA and GA have shown better efficacy, emphasizing the importance of vehicle on the effects of topical actives. LA (6%) in the emulsion based on APGs could be proposed as an alternative to low-molecular AHAs in cosmeceuticals.

Fabrication of size-controlled starch-based nanospheres by nanoprecipitation.

Tan Y *et al.*

ACS Appl Mater Interfaces. 2009 Apr;**1(4)**:956-9.

Nanometric and monodisperse starch acetate nanospheres can be prepared through a simple procedure of nanoprecipitation, by the dropwise addition of water to an acetone solution of starch acetate, without any stabilizing agent. This is the first report of the preparation of starch-based nanospheres by this method. The size of the nanospheres obtained can be easily controlled by a number of simple and efficient modifications, i.e., through regulation of the polymer concentration in acetone, the proportions of the water and organic phases, and the molecular weight and degree of substitution of the starch esters. A number of reasons are suggested to explain the observed transitions in the particle size. Fluorescence spectroscopic studies proved that these types of nanospheres could be potentially used for the encapsulation of hydrophobic drugs.

Pressurized liquid extraction of *Tripterygium wilfordii* polyglycosides and optimization of extraction conditions [Article in Chinese].

Yang L *et al.*

Zhongguo Zhong Yao Za Zhi. 2010 Jan;**35(1)**:44-8.

To investigate the extraction method of polyglycosides from *Tripterygium wilfordii*.

Natural Products

The extraction method of pressurized liquid extraction was employed and chromogenic colorimetric technique were used for quantitative analysis. Based on the single-factor experiment, according to the center combination design this paper used three factors and three levels of response surface methodology for process optimization. The optimized conditions were as follows: ratio of solid to liquid was 1:9.5, at the temperature of 115 degrees C for 80 minutes, the actual extract ratio and purity of polyglycosides obtained were 0.21% and 0.52%, respectively. The method of pressurized liquid extraction has obvious advantages over conventional reflux extracting.

Synthesis and biological activity of raltitrexed-carrier conjugates.

Jagiello M *et al.*

Acta Biochim Pol. 2010;**57(1)**:83-7. Epub 2010 Mar 22.

Drugs used in chemotherapy give undesirable side effects, e.g., cardiotoxicity, leucopenia, hair loss and others. Covalent binding of a drug with a carrier may change its biodistribution, elimination and/or rate of transformation in the organism. The aim of this work was to synthesize conjugates of anticancer drug - raltitrexed (RTX) with lysozyme, bovine serum albumin (BSA), and dextran T40 and to investigate their cytotoxicity and influence on the cell cycle in comparison with the free drug. Before conjugation RTX was transformed into anhydride by treatment with dicyclohexylcarbodiimide in dimethyl-formamide. Activated RTX was added into aqueous solution of carriers at different pH (from 8.5 to 10.5) for 3 to 15 min. The reaction was stopped by reducing the pH to 7.0. Maximum yield of the reaction was obtained at pH 10 for BSA as well as for dextran. The highest level of substitution was obtained after 5 min of the reaction. In in vitro experiments on three cell lines: SW707, LoVo and A549, all conjugates tested had up to a few hundred times higher

IC(50) than the free drug. Interestingly, it was noticed that the conjugates based on dextran and albumin were more cytotoxic than the free drug in the highest concentrations tested (1000 and 10000 ng/ml). The influence of RTX and the conjugates on SW707 cell cycle was studied. RTX blocked the cell cycle mostly in the G(0)-G(1) and S phase and increased the percentage of apoptotic cells. Cells in the G(2)-M phase were not observed. The conjugates blocked the cell cycle in the S phase and decreased the percentage of cells in the G(0)-G(1) phase.

Glycosidated phospholipids: uncoupling of signalling pathways at the plasma membrane.

Danker K, *et al.*

Br J Pharmacol. 2010 May;**160(1)**:36-47. Epub 2010 Mar 19.

Cell expansion and metastasis are considered hallmarks of tumour progression. Therefore, efforts have been made to develop novel anti-cancer drugs that inhibit both the proliferation and the motility of tumour cells. Synthetic alkylphospholipids, compounds with aliphatic side chains that are ether linked to a glycerol backbone, are structurally derived from platelet-activating factor and represent a new class of drugs with anti-proliferative properties in tumour cells. These compounds do not interfere with the DNA or mitotic spindle apparatus of the cell. Instead, they are incorporated into cell membranes, where they accumulate and interfere with lipid metabolism and lipid-dependent signaling pathways. Recently, it has been shown that the most commonly studied alkylphospholipids inhibit proliferation by inducing apoptosis in malignant cells while leaving normal cells unaffected. This review focuses on a novel group of synthetic alkylphospholipids, the glycosidated phospholipids, which contain carbohydrates or carbohydrate-related molecules at the sn-2 position of the glycerol backbone. Members of this subfamily also exhibit anti-proliferative

Natural Products

capacity and modulate the cell adhesion, differentiation, and migration of tumour cells. Among this group, Ino-C2-PAF shows the highest efficacy and low cytotoxicity. Apart from its anti-proliferative effect, Ino-C2-PAF strongly reduces cell motility via its inhibitory effect on the phosphorylation of the cytosolic tyrosine kinases FAK and Src. Signalling pathways under the control of the FAK/Src complex are normally required for both migration and proliferation and play a prominent role in tumour progression. We intend to highlight the potential of glycosidated phospholipids, especially Ino-C2-PAF, as a promising new group of drugs for the treatment of hyperproliferative and migration-based skin diseases.

Novel synthesis strategies for natural polymer and composite biomaterials as potential scaffolds for tissue engineering.

Ko HF *et al.*

Philos Transact A Math Phys Eng Sci. 2010 Apr 28;**368(1917)**:1981-97.

Recent developments in tissue engineering approaches frequently revolve around the use of three-dimensional scaffolds to function as the template for cellular activities to repair, rebuild and regenerate damaged or lost tissues. While there are several biomaterials to select as three-dimensional scaffolds, it is generally agreed that a biomaterial to be used in tissue engineering needs to possess certain material characteristics such as biocompatibility, suitable surface chemistry, interconnected porosity, desired mechanical properties and biodegradability. The use of naturally derived polymers as three-dimensional scaffolds has been gaining widespread attention owing to their favourable attributes of biocompatibility, low cost and ease of processing. This paper discusses the synthesis of various polysaccharide-based, naturally derived polymers, and the potential of using these biomaterials to serve as tissue engineering three-dimensional scaffolds is also evaluated. In this study, naturally derived polymers,

specifically cellulose, chitosan, alginate and agarose, and their composites, are examined. Single-component scaffolds of plain cellulose, plain chitosan and plain alginate as well as composite scaffolds of cellulose-alginate, cellulose-agarose, cellulose-chitosan, chitosan-alginate and chitosan-agarose are synthesized, and their suitability as tissue engineering scaffolds is assessed. It is shown that naturally derived polymers in the form of hydrogels can be synthesized, and the lyophilization technique is used to synthesize various composites comprising these natural polymers. The composite scaffolds appear to be sponge-like after lyophilization. Scanning electron microscopy is used to demonstrate the formation of an interconnected porous network within the polymeric scaffold following lyophilization. It is also established that HeLa cells attach and proliferate well on scaffolds of cellulose, chitosan or alginate. The synthesis protocols reported in this study can therefore be used to manufacture naturally derived polymer-based scaffolds as potential biomaterials for various tissue engineering applications.

Analysis of 1-deoxy-D-xylulose 5-phosphate synthase activity in Grey poplar leaves using isotope ratio mass spectrometry.

Ghirardo A *et al.*

Phytochemistry. 2010 Jun;**71(8-9)**:918-22. Epub 2010 Mar 18.

Deoxy-xylulose phosphate synthase (DXS) catalyzes the first step of the methylerythritol phosphate (MEP) pathway and it might regulate the metabolic flux in plastidic isoprenoid biosynthesis. We developed a sensitive assay suitable for plant extracts that is based on the decarboxylation of labeled pyruvate (1-(13C)-PYR and detection of (13)CO(2) by isotope ratio mass spectrometry. We tested our method investigating the DXS activity in poplar leaves. Apparent DXS activity showed Michaelis constants of 111 and 158 microM for glyceraldehydes phosphate and pyruvate,

Natural Products

respectively; pH and temperature optima were found at pH 8.6 and 45 degrees C. DXS activity was inhibited when the competitive inhibitor beta-fluoropyruvate was added to the reaction mixture. DXS activity strongly depended on leaf development with higher activity in young leaves and correlated fairly well with leaf isoprene emission potential. In mature poplar leaves, isoprene emission is the main metabolic sink of plastidic isoprenoid intermediates. Consequently, we found lower DXS activity in non-isoprene-emitting lines of poplar than in emitting plants as indicator of a lower demand of metabolic flux within the MEP pathway.

Identification of nevadensin as an important herb-based constituent inhibiting estragole bioactivation and physiology-based biokinetic modeling of its possible in vivo effect.

Alhusainy W *et al.*

Toxicol Appl Pharmacol. 2010 Jun 1;245(2):179-90. Epub 2010 Mar 11.

Estragole is a natural constituent of several herbs and spices including sweet basil. In rodent bioassays, estragole induces hepatomas, an effect ascribed to estragole bioactivation to 1'-sulfoxyestragole resulting in DNA adduct formation. The present paper identifies nevadensin as a basil constituent able to inhibit DNA adduct formation in rat hepatocytes exposed to the proximate carcinogen 1'-hydroxyestragole and nevadensin. This inhibition occurs at the level of sulfotransferase (SULT)-mediated bioactivation of 1'-hydroxyestragole. The K_i for SULT inhibition by nevadensin was 4 nM in male rat and human liver fractions. Furthermore, nevadensin up to 20 microm did not inhibit 1'-hydroxyestragole detoxification by glucuronidation and oxidation. The inhibition of SULT by nevadensin was incorporated into the recently developed physiologically based biokinetic (PBBK) rat and human models for estragole bioactivation and detoxification. The results predict that co-

administration of estragole at a level inducing hepatic tumors in vivo (50mg/kg bw) with nevadensin at a molar ratio of 0.06, representing the ratio of their occurrence in basil, results in almost 100% inhibition of the ultimate carcinogen 1'-sulfoxyestragole when assuming 100% uptake of nevadensin. Assuming 1% uptake, inhibition would still amount to more than 83%. Altogether these data point at a nevadensin-mediated inhibition of the formation of the ultimate carcinogenic metabolite of estragole, without reducing the capacity to detoxify 1'-hydroxyestragole via glucuronidation or oxidation. These data also point at a potential reduction of the cancer risk when estragole exposure occurs within a food matrix containing SULT inhibitors compared to what is observed upon exposure to pure estragole.

From conventional towards new - natural surfactants in drug delivery systems design: current status and perspectives.

Savić S *et al.*

Expert Opin Drug Deliv. 2010 Mar;7(3):353-69.

Surfactants play an important role in the development of both conventional and advanced (colloidal) drug delivery systems. There are several commercial surfactants, but a proportionally small group of them is approved as pharmaceutical excipients, recognized in various pharmacopoeias and therefore widely accepted by the pharmaceutical industry. The review covers some of the main categories of natural, sugar-based surfactants (alkyl polyglucosides and sugar esters) as prospective pharmaceutical excipients. It provides analysis of the physicochemical characteristics of sugar-based surfactants and their possible roles in the design of conventional or advanced drug delivery systems for different routes of administration. Summary and analysis of recent data on functionality, applied concentrations and formulation improvements produced by alkyl polyglucosides and sugar esters in different conventional and advanced

Natural Products

delivery systems could be of interest to researchers dealing with drug formulation. Recent FDA certification of an alkyl polyglucoside surfactant for topical formulation presents a significant step in the process of recognition of this relatively new group of surfactants. This could trigger further research into the potential benefits of naturally derived materials in both conventional and new drug delivery systems.

Two new compounds from the dried tender stems of *Cinnamomum cassia*.

Liu C *et al.*

J Asian Nat Prod Res. 2009 Sep;11(9):845-9.

Two new compounds, cinnamic aldehyde cyclic d-galactitol 3'R,4'S-acetal (1) and cinnamomumolide (2), along with six known compounds, syringaresinol (3), lyoniresinol (4), 5,7,3'-trimethoxyl(-)-epicatechin (5), 5,7-dimethoxyl-3',4'-di-O-methylene(+/-)-epicatechin (6), 2-methoxyl-4-hydroxy cinnamyl aldehyde (7), and glucosyringic acid (8), have been isolated from the dried tender stems of *Cinnamomum cassia*. Their structures were elucidated based on spectroscopic data. Compound 2 was elucidated as 8-methoxyl-9-hydroxy-3',4'-methylenedioxy-3S,4R-diphenyl butyrolactone, named cinnamomumolide, which exhibited activity in protecting against the injury caused by hydrogen peroxide oxidation on human umbilical vein endothelial cells, with an EC(50) value of 10.7 microM. Compounds 3-8 were isolated from the title plant for the first time.

Constituents from the seeds of *Brucea javanica* with inhibitory activity of Tobacco mosaic virus.

Chen QJ *et al.*

J Asian Nat Prod Res. 2009 Jun;11(6):539-47.

Three new constituents were obtained along with 10 known compounds from the seeds of *Brucea javanica*. The structures of these compounds were determined based on spectral and chemical evidence. These new

compounds included a monoterpenoid glycoside and two sesquiterpenes. Bioactivity screening of these constituents showed that compounds 1, 3, 8, 9, and 13 with obvious activities in inhibiting multiplication of the Tobacco mosaic virus.

Homoisoflavonoids from the fibrous roots of *Polygonatum odoratum* with glucose uptake-stimulatory activity in 3T3-L1 adipocytes.

Zhang H *et al.*

J Nat Prod. 2010 Apr 23;73(4):548-52

The EtOAc-soluble fraction of a 90% MeOH extract of the fibrous roots of *Polygonatum odoratum* was found to potentiate insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes. Bioassay-guided fractionation yielded nine homoisoflavonoids (1-9), four of which were new (1-4), together with an isoflavone glycoside (10) and a flavanone glycoside (11). The structures of new compounds were elucidated on the basis of extensive 1D and 2D NMR spectroscopy, and the absolute configurations were deduced by CD spectra. All 11 compounds showed effects of sensitizing adipocytes for insulin in a cell-based glucose uptake assay using 3T3-L1 adipocytes. The results indicate that homoisoflavonoids may be potential insulin sensitizers.

Flavanone and diphenylpropane glycosides and glycosidic acyl esters from *Viscum articulatum*.

Kuo YJ *et al.*

J Nat Prod. 2010 Feb 26;73(2):109-14.

Seven new compounds including three flavanone glycosides, visartisides A-C (1-3), three glycoside acyl esters, visartisides D-F (4-6), and one diphenylpropane glycoside, (4'-hydroxy-2',3',6',3''-tetramethoxy-1,3-diphenylpropane)-4''-O-beta-d-glucopyranoside (7), along with four known flavanone glycosides (8-11) were isolated from the leaves and stems of *Viscum articulatum*. The structure elucidation of 1-7 was based on spectroscopic

Natural Products

data analysis. Biological evaluation showed that 1, 2, and 10 exhibited antioxidant activity using a DPPH method and that compounds 1, 3, and 11 were active in a lipopolysaccharide-induced nitric oxide assay.

Tabletting technology of a dry extract from *Solidago virgaurea* L. with the use of silicified microcrystalline cellulose (Prosolv) and other selected auxiliary substances [Article in Polish].

Marczyński Z.

Polim Med. 2009;**39(4)**:51-60.

Direct tabletting is simpler and more cost-effective from the point of view of good manufacturing practice (GMP) than wet granulation or dry compacting. Moreover, the use of dry plant extracts in the process of direct tabletting, omitting granulation, decreases the possibility of biological activity loss of active substances. Thus, pharmaceutical industry uses this particular process more and more frequently. Only few therapeutic substances form under compression tablets meeting current requirements. Very often addition of auxiliary substances appears to be indispensable. The aim of this study was to obtain uncoated tablets by the method of direct tabletting with the use of selected auxiliary substances. Dry extract from *Solidago virgaurea* L. was the study material. Shrimp chitosan, silicified microcrystalline cellulose (Prosolv), polyvinylpyrrolidone, calcium carbonate and sodium stearyl fumarate were used as auxiliary substances. Eleven tablet batches were manufactured in a reciprocating instrumented tabletting machine (Ewreka). The produced tablets were subjected to morphological tests comprising the tablet size, determination of batching accuracy (determination of mass uniformity of individual tablets), test of mechanical resistance (crushing strength), determination of disintegration time. The statistical hardness of the manufactured tablets was also estimated. Pharmaceutical availability tests were performed of the biologically active substances

released from tablets to the acceptor fluid. The study was based on general and detailed regulations of Polish Pharmacopoeia VII (PP VII). The obtained results allow to conclude that the applied auxiliary substances appeared to be useful in adequate proportions in manufacturing tablets containing dry extract from *Solidago virgaurea* L. The properties of the obtained batches of tablets were in majority consistent with the current requirements. The applied method provides technological reproducibility and high durability of the drug. These tablets as compared to available herbal mixtures and aqueous extracts can be a more comfortable form of a drug.

New potential antitumor compounds from the plant *Aristolochia manshuriensis* as inhibitors of the CDK2 enzyme.

Hegde VR *et al.*

Bioorg Med Chem Lett. 2010 Feb 15;**20(4)**:1344-6. Epub 2010 Jan 11.

The 70% aqueous methanolic extract of the Chinese plant *Aristolochia manshuriensis* was found to contain two novel substituted phenanthrene compounds, SCH 546909 (1), and another phenanthrene glycoside (2). The structures of 1 and 2 were established based on NMR studies. They were identified as inhibitors of the CDK2 enzyme. Compound 1 was found to be a potent inhibitor of the CDK2 enzyme with an IC₅₀ of 140 nM, whereas compound 2 was found to be less active with an IC₅₀ of >10 microM. Copyright 2010 Elsevier Ltd. All rights reserved.

A lectin isolated from bananas is a potent inhibitor of HIV replication.

Swanson MD *et al.*

J Biol Chem. 2010 Mar 19;**285(12)**:8646-55. Epub 2010 Jan 15.

BanLec is a jacalin-related lectin isolated from the fruit of bananas, *Musa acuminata*. This lectin binds to high mannose carbohydrate structures, including those found on viruses containing glycosylated envelope proteins such as human immunodeficiency

Natural Products

virus type-1 (HIV-1). Therefore, we hypothesized that BanLec might inhibit HIV-1 through binding of the glycosylated HIV-1 envelope protein, gp120. We determined that BanLec inhibits primary and laboratory-adapted HIV-1 isolates of different tropisms and subtypes. BanLec possesses potent anti-HIV activity, with IC(50) values in the low nanomolar to picomolar range. The mechanism for BanLec-mediated antiviral activity was investigated by determining if this lectin can directly bind the HIV-1 envelope protein and block entry of the virus into the cell. An enzyme-linked immunosorbent assay confirmed direct binding of BanLec to gp120 and indicated that BanLec can recognize the high mannose structures that are recognized by the monoclonal antibody 2G12. Furthermore, BanLec is able to block HIV-1 cellular entry as indicated by temperature-sensitive viral entry studies and by the decreased levels of the strong-stop product of early reverse transcription seen in the presence of BanLec. Thus, our data indicate that BanLec inhibits HIV-1 infection by binding to the glycosylated viral envelope and blocking cellular entry. The relative anti-HIV activity of BanLec compared favorably to other anti-HIV lectins, such as snowdrop lectin and Griffithsin, and to T-20 and maraviroc, two anti-HIV drugs currently in clinical use. Based on these results, BanLec is a potential component for an anti-viral microbicide that could be used to prevent the sexual transmission of HIV-1.

A sensitive and specific HPGPC-FD method for the study of pharmacokinetics and tissue distribution of Radix Ophiopogonis polysaccharide in rats.

Lin X *et al.*
Biomed Chromatogr. 2010
Aug;24(8):820-5.

Interest in antimyocardial ischemic activity of a graminan-type fructan with a weight average molecular weight of 4.8 kDa extracted from Radix Ophiopogonis (ROP) has necessitated the study of its pharmacokinetics

and tissue distribution. For that, a simple HPGPC-FD method was developed for the sensitive and specific determination of FITC-ROP (fluorescein-isothiocyanate-labeled ROP) in plasma and rat tissues (heart, liver, spleen, lung, kidney, brain and stomach). The analyte was separated on a Shodex Sugar KS-802 high-performance gel column with 0.1 M phosphate buffer (pH 7.0) as mobile phase at a flow rate of 0.5 mL/min, and fluorescence detection at $\lambda(\text{ex})$ 495 nm and $\lambda(\text{em})$ 515 nm. The calibration curve for FITC-ROP was linear over the range 0.25-20.0 or 50.0 microg/mL in all studied biosamples with correlation coefficients > 0.995. The inter-day and intra-day precisions of analysis were not more than 10%, and assay accuracy ranged from 93 to 105% for plasma and from 89 to 108% for tissue homogenates. This method has been confirmed here to be suitable for the study of pharmacokinetics and tissue distribution of ROP and the achieved results are highly instructive for the further pharmaceutical development of ROP, suggesting the promising application of the method to the increasingly important carbohydrate-based drugs.

Reversed-phase screening strategies for liquid chromatography on polysaccharide-derived chiral stationary phases.

Zhang T *et al.*
J Chromatogr A. 2010 Feb
12;1217(7):1048-55. Epub 2009 Nov 18.

Immobilised polysaccharide-based chiral stationary phases (CSPs) are chromatographic materials that combine the remarkable enantioselective performance of the polysaccharide derivatives in addition to solvent versatility for enantiomeric resolution. Their behaviour under normal phase conditions and polar organic mode has been quite extensively discussed in several scientific communications. This article will focus on an approach to developing efficient chiral analytical methods with these immobilised CSPs by applying a limited number of mobile

Natural Products

phases under reversed-phase conditions. The manuscript will review the development of screening strategies by liquid chromatography for the separation of enantiomers in combination with applications compatible with LC-MS. The rational combination of this technique and the different supports will allow the identification of enantiomeric resolutions in reasonable time frames and with high success rates.

Glycoproteomics of paclitaxel resistance in human epithelial ovarian cancer cell lines: towards the identification of putative biomarkers.

Di Michele M *et al.*

J Proteomics. 2010 Mar 10;**73(5)**:879-98. Epub 2009 Dec 3.

Glycosylation, one of the most common post translational modifications (PTMs) of proteins, is often associated with carcinogenesis and tumor malignancy. Ovarian cancer is the sixth cause of cancer-related death in Western countries. Currently, it is treated by debulking surgery followed by chemotherapy based on paclitaxel, alone or in combination with other drugs. However, chemoresistance represents a major obstacle to positive clinical outcome. We used two approaches, Multiplexed Proteomics (MP) technology and Multilectin Affinity Chromatography (MAC) to characterize the glycoproteome of the human ovarian cancer cell line A2780 and its paclitaxel resistant counterpart A2780TC1. Furthermore proteins were separated by traditional 2DE or DIGE and identified by MS (MALDI TOF or LC MS/MS). Seventy glycoproteins were successfully identified in ovarian cancer cells and 10 were found to be differentially expressed between sensitive and resistant cell lines. We focused on four glycoproteins (tumor rejection antigen (gp96) 1, triose phosphate isomerase, palmitoyl-protein thioesterase 1 precursor and ER-associated DNAJ) which were remarkably upregulated in A2780TC1 compared to A2780 cell line and

which may represent biomarkers for paclitaxel resistance in ovarian cancer.

Formulation and evaluation of natural gum-based sustained release matrix tablets of flurbiprofen using response surface methodology.

Shah SN *et al.*

Drug Dev Ind Pharm. 2009 Dec;**35(12)**:1470-8.

This research work was done to design oral sustained release matrix tablets of water-insoluble drug, flurbiprofen, using natural gums as the matrix polymers and to evaluate the drug release characteristics using response surface methodology. The central composite design for two factors at five levels each was employed to systematically optimize drug release profile. Matrix tablets were prepared by direct compression technique. Xanthan and acacia gums were taken as the independent variables. Fourier transform infrared spectroscopy studies were also performed to find out the stability of drug during the direct compression and to check the interactions between polymers and drug. Percent drug release in 2 hours and percent drug release in 8 hours were taken as response variables (Y1 and Y2, respectively). Both the polymers were found to have significant effect on the drug release. Polynomial mathematical models, generated for the response variables using multiple linear regression analysis, were found to be statistically significant ($P < 0.05$). Contour plots were drawn to depict the relationship between response variables and independent variables. The formulated matrix tablets followed zero-order kinetics with negligible drug release in 0.1 N HCl at pH 1.2, which was the objective of this study to produce a formulation avoiding the gastric effects of flurbiprofen.

A new triterpene hexaglycoside from the bark of *Kalopanax septemlobus* (Thunb.) Koidz.

Wang LS *et al.*

Molecules. 2009 Nov 9;**14(11)**:4497-504.

Natural Products

The new triterpene glycoside 3-O-beta-D-xylopyranosyl-(1-->4)-beta-D-xylopyranosyl-(1-->3)-alpha-L-rhamnopyranosyl-(1-->2)-alpha-L-arabinopyranosylhederagenin 28-O-beta-D-gluco-pyranosyl-(1-->6)-beta-D-glucopyranoside, named septemoside A (1), and the known 3-O-alpha-L-rhamnopyranosyl-(1-->2)-O-alpha-L-arabinopyranoside-28-O-beta-D-gluco pyranosyl-(1-->6)-O-beta-D-glucopyranosyl ester of hederagenin (2), were isolated from the bark of *Kalopanax septemlobus*. The structure elucidation of the compounds was based on spectroscopic evidence, including HRESIMS, 1D and 2D-NMR analysis.

Inhibitory effects of guava (*Psidium guajava* L.) leaf extracts and its active compounds on the glycation process of protein.

Wu, Ju-Wen

Food Chemistry, **13(1)**,78 (Mar., 1, 2009)

Hyperglycaemia causes increased protein glycation and the formation of early glycation products and advanced glycation end products (AGEs) which are major factors responsible for the complications of diabetes. This study investigated the ability of guava leaf and

compounds to inhibit glycation process in an albumin/glucose model system and compared the potency of these extracts with Polyphenon 60 which is a commercial polyphenol product extracted from green tea and with the standard antiglycation agent, aminoguanidine. The results showed that the inhibitory effects of guava leaf extracts on the formation of [alpha]-dicarbonyl compounds were over 95% at 50 [mu]g/ml. Phenolic compounds present, namely gallic acid, catechin and quercetin exhibited over 80% inhibitory effects, but ferulic acid showed no activity. The guava leaf extracts also showed strong inhibitory effects on the production of Amadori products and AGEs from albumin in the presence of glucose. The phenolic compounds also showed strong inhibitory effects on the glycation of albumin, especially quercetin exhibited over 95% inhibitory effects at 100 [mu]g/ml. According to the results obtained, guava leaf extracts are potent antiglycation agents, which can be of great value in the preventive glycation-associated complications in diabetes.

Subscription Form

The Scientist-in-Charge
S & T Knowledge Resource Centre
Central Drug Research Institute
Post Box No. 173, Chattar Manzil Palace
Lucknow-226 001, India.

Dear Sir/Madam

I/My organization want to be the annual subscriber of:

Drugs and Pharmaceuticals - Industry Highlights- /- Current R&D Highlights

I/We am/are remitting the subscription amount of Rs./\$ _____ by Demand Draft No. _____ dated _____ towards subscription of the journal(s) as (1) Student/Professional, (2) Educational/R&D Institution, (3) Corporate Sector subscriber.

Name : _____
Address : _____
: _____

Signature with Seal

Please send your payment by Demand Draft only in favour of
The Director, Central Drug Research Institute, Lucknow, payable at Lucknow



BTGlycan arrays: biological and medical applications.

Liang, Pi-Hui *et al.*

Current Opinion in Chemical Biology, 12(1), 86 (Feb., 2009)

Carbohydrates and their conjugates are involved in various biological events, including viral and bacterial infection, the immune response, differentiation and development, and the progression of tumor cell metastasis. Glycan arrays are a new technology that has enabled the high-sensitivity and rapid analysis carbohydrate-protein interaction and contribute to significant advances in glycomics. Glycan arrays use a minute amount of materials and can be used for high-throughput profiling and quantitative analysis and provide information for the development of carbohydrate-based vaccines and new drug discovery.

Increasing carbohydrate diversity via amine oxidation: aminosugar, hydroxyaminosugar, nitrososugar, and nitrosugar biosynthesis in bacteria.

Timmons, Shannon C *et al.*

Current Opinion in Chemical Biology, 12(3), 297 (June 2009)

Bacterial secondary metabolites often contain carbohydrate attachments that play a significant role in conferring biological activity. A small proportion of these bioactive sugars are derived from aminosugar oxidation to ultimately provide hydroxyaminosugars, nitrososugars, and nitrosugars. Recent advances in the elucidation of hydroxyaminosugar-, nitrososugar-, and nitrosugar-containing natural product gene clusters have enabled the proposal of biosynthetic pathways, the *in vitro*

characterization of aminosugar oxidases, and the structure determination of key enzymes. This article focuses upon the key enzymatic transformations in aminosugar, hydroxyaminosugar, nitrososugar, and nitrosugar biosynthesis, as well as the unique chemical reactivity of alkoxyaminosugars, with a particular focus upon developments within the past two years.

Enzymatic Synthesis of Complex Carbohydrates.

Lew Mander *et al.*

DOI: 10.1016/B978-008045382-8.00660-

2

Complex saccharides are highly diverse in structure and biological functions. Accordingly, they are essential for many fields of research. The development of efficient synthetic methodologies for their preparation has thus been in high demand. The chemical synthesis of complex oligo- and polysaccharides, however, is often limited to the milligram scale and is difficult to scale up. Enzymatic synthesis, in contrast, offers an alternative that overcomes these limitations due to the high catalytic activity, lack of undesirable side reactions, mild reaction conditions, and high regio- and stereoselectivity of enzymes. This chapter will outline the development and applications of the following three types of enzymes used in the enzymatic biosynthesis of complex carbohydrates: glycosidases, glycosynthases, and glycosyltransferases.

Carbohydrate engineered cells for regenerative medicine.

Du, Jian *et al.*

Advanced Drug Delivery Reviews (In Press)

Carbohydrates are integral components of the stem cell niche on several levels; proteoglycans are a major constituent of the extracellular matrix (ECM) surrounding a cell, glycosaminoglycans (GAGs) help link cells to the ECM and the neighboring cells, and small but informationally-rich

oligosaccharides provide a "sugar code" that identifies each cell and provides it with unique functions. This article samples roles that glycans play in development and then describes how metabolic glycoengineering -- a technique where monosaccharide analogs are introduced into the metabolic pathways of a cell and are biosynthetically incorporated into the glycocalyx -- is overcoming many of the long-standing barriers to manipulating carbohydrates in living cells and tissues and is becoming an intriguing new tool for tissue engineering and regenerative medicine.

Mucin-type O-glycosylation and its potential use in drug and vaccine development.

Tarp, Mads Agervig *et al.*

Biochimica et Biophysica Acta (BBA) - General Subjects, **1780(3)**, 546 (Mar., 2008)

Mucin-type O-glycans are found on mucins as well as many other glycoproteins. The initiation step in synthesis is catalyzed by a large family of polypeptide GalNAc-transferases attaching the first carbohydrate residue, GalNAc, to selected serine and threonine residues in proteins. During the last decade an increasing number of GalNAc-transferase isoforms have been cloned and their substrate-specificities partly characterized. These differences in substrate specificities have been exploited for in vitro site-directed O-glycosylation. In GlycoPEGylation(TM), polyethylene glycol (PEG) is transferred to recombinant therapeutics to specific acceptor sites directed by GalNAc-transferases. GalNAc-transferases have also been used to control density of glycosylation in the development of glycopeptide-based cancer vaccines. The membrane-associated mucin-1 (MUC1) has long been considered a target for immunotherapeutic and immunodiagnostic measures, since it is highly overexpressed and aberrantly O-glycosylated in most adenocarcinomas, including breast, ovarian, and pancreatic cancers. By using vaccines mimicking the glycosylation pattern of cancer-

cells, it is possible to overcome tolerance in transgenic animals expressing the human MUC1 protein as a self-antigen providing important clues for an improved MUC1 vaccine design. The present review will highlight some of the potential applications of site-directed O-glycosylation.

Carbohydrate moieties as vaccine candidates: Meeting summary.

Lucas, A.H. *et al.*

Vaccine, **28(4)**, 1121 (Jan., 22, 2010)

In September 2007, a meeting entitled '[^]Carbohydrate Moieties as Vaccine Candidates' was held at the National Institutes of Health (Bethesda, MD). This meeting brought together scientists from a number of disciplines to address issues concerning carbohydrate moieties as targets for vaccines for a variety of pathogens and tumors. In addition, the meeting participants addressed fundamental topics of glycoimmunology including the recognition of glycotopes by B and T lymphocytes, the ontogeny of anti-carbohydrate immune responses, peptide mimicry, carbohydrate antigen processing pathways and adjuvants. One session reported progress in the development of new tools such as computational algorithms, glycan arrays and oligosaccharide synthesis and their application to carbohydrate vaccine research. The session titles were: (1) immune response to bacterial carbohydrate antigens; (2) immune response to glycolipids; (3) immune response to carbohydrate antigens on other microbes and on tumors; (4) novel vaccine approaches; (5) novel tools in carbohydrate vaccine research; (6) bench to bedside: carbohydrate moieties as vaccine immunopotentiators.

Recent advances in carbohydrate-based vaccines.

Hecht, Marie-Lyn *et al.*

Current Opinion in Chemical Biology, **13(3)**, 354 (June 2009)

Vaccinations provide an efficient and cost-effective way to combat devastating human diseases. Besides pathogenic protein

markers, cell surface carbohydrates from biological sources are widely used as vaccines. Recently, synthetic immunogenic carbohydrate-protein conjugates have been advanced to vaccine candidates. Progress in the chemical synthesis of oligosaccharides and conjugation methods stimulated the development of novel carbohydrate-based vaccine candidates.

Enzymatic Synthesis of Complex Carbohydrates.

Lew Mander *et al.*

doi: DOI: 10.1016/B978-008045382-8.00660-2

Complex saccharides are highly diverse in structure and biological functions. Accordingly, they are essential for many fields of research. The development of efficient synthetic methodologies for their preparation has thus been in high demand. The chemical synthesis of complex oligo- and polysaccharides, however, is often limited to the milligram scale and is difficult to scale up. Enzymatic synthesis, in contrast, offers an alternative that overcomes these limitations due to the high catalytic activity, lack of undesirable side reactions, mild reaction conditions, and high regio- and stereoselectivity of enzymes. This chapter will outline the development and applications of the following three types of enzymes used in the enzymatic biosynthesis of complex carbohydrates: glycosidases, glycosynthases, and glycosyltransferases.

Recent advances in carbohydrate-based vaccines.

Hecht, Marie-Lyn *et al.*

Current Opinion in Chemical Biology, **13(3)**, 354 (June 2009)

Vaccinations provide an efficient and cost-effective way to combat devastating human diseases. Besides pathogenic protein markers, cell surface carbohydrates from biological sources are widely used as vaccines. Recently, synthetic immunogenic carbohydrate-protein conjugates have been

advanced to vaccine candidates. Progress in the chemical synthesis of oligosaccharides and conjugation methods stimulated the development of novel carbohydrate-based vaccine candidates.

Researchers trick bacteria into generating own vaccine.

Prokerala.com February 25th, 2009

Scientists have tricked bacteria into growing mutant sugar molecules on their cell surfaces that could be used against them as the key component in potent vaccines. Any resulting vaccines, if proven safe, could be developed more quickly, easily and cheaply than many currently available vaccines used to prevent bacterial illnesses. "We are showing for the first time that you don't have to use complicated chemical reactions to make the alteration to the polysaccharide," said Peng George Wang, professor of biochemistry and chemistry at Ohio State University and study co-author. "All we need to do is ferment the bacteria, and then the polysaccharides that grow on the surface of the cell already incorporate the modification." Most vaccines against bacteria are created with polysaccharides, or long strings of sugars found on the surface of bacterial cells. The most common way to develop these vaccines is to remove sugars from the cell surface and link them to proteins to give them more power to kill bacteria, said an Ohio release, written by Emily Caldwell. Polysaccharides alone don't generate a strong enough antibody response needed to kill bacteria. But this new technique would provide an easy approach to make a small alteration to the sugar structure and produce the polysaccharide by simple fermentation. The research is scheduled to appear in the online early edition of the Proceedings of the National Academy of Sciences.

Generating heparan sulfate saccharide libraries for glycomics applications.

Andrew K Powell *et al.*

Nature Protocols **5**, 821 - 833 (2010)

Natural and semi-synthetic heparan sulfate (HS) saccharide libraries are a valuable resource for investigating HS structure–function relationships, enabling high-throughput glycomics studies. Owing to the difficulty of chemical or *in vitro* enzymatic synthesis of HS saccharides, the structural diversity displayed in saccharides from tissue or cell sources cannot be readily accessed. In contrast, saccharide libraries can be generated by partial digestion of tissue-derived HS polysaccharide chains and chromatographic fractionation of the resulting saccharide mixtures. Fractionation is initially on the basis of hydrodynamic volume, using size exclusion chromatography. Further fractionation, on the basis of charge using strong anion exchange, can subsequently be applied. Desalting and sample concentration follows each fractionation step. Chromatographic fractions are generated that contain purified, or partially purified, saccharides. Here we describe a comprehensive protocol for generation of structurally diverse natural saccharide libraries from HS variants that is fast (~3 weeks) and reproducible.

Glycomics data mining.

V Srinivasa Rao *et al.*

J Comput Sci Syst Biol **2**: 262-265.
doi:10.4172/jcsb.1000040

The amount of glycomics data being generated is rapidly increasing as a result of improvements in analytical and computational methods. Correlation and analysis of this large, distributed data set requires an extensible and flexible representational standard that is also ‘understood’ by a wide range of software applications. An XML-based data representation standard that faithfully captures essential structural details of a glycan moiety along with additional information (such as data provenance) to aid the interpretation and usage of glycan data, will facilitate the exchange of glycomics data across the scientific community. We reviewed importance of data warehouse, showing a method of applying data mining techniques using XML, and some of the data issues, analysis problems, and results.

Gift Subscription

We offer Gift Subscription for one year to the contributors for our Periodicals

(i). **Current R&D Highlights** and (ii) **Industry Highlights**

You may contribute detailed Feature articles (upto 2000 words) or your views (upto 1000 words) on any of the topics concerning Drugs and Pharmaceuticals. All those, whose articles or views are accepted for publication will be offered the Gift Subscription for one of the above periodicals.

Kindly send your article(s) entered in a CD or mail to us alongwith a print out as per the Instructions to Authors given on the Back-inside cover.

Scientist-in-Charge

S & T Knowledge Resource Centre
Central Drug Research Institute
PO Box 173, Lucknow-226 001, India
Website: www.cdriindia.org

Patents



Carbohydrate encapsulated nano-particles.

Lin; Chun-Cheng *et al.*

Academia Sinica (Taipei, TW)

US Patent 7,695,738 April 13, 2010 Appl. No.:10/782,076 February 19, 2004

The present invention provides carbohydrate encapsulated nanoparticles. In particular, the present invention provides metallic nanoparticles (e.g. gold nanoparticles) that are encapsulated in biologically important carbohydrate molecules, such as sugars, sugar derivatives, P-blood group antigens and analogues thereof. The present invention also provides methods of employing these carbohydrate encapsulated nanoparticles in diagnostic and therapeutic applications.

Methods and producing low molecular weight heparin.

Sasisekharan, *et al.*

Massachusetts Institute of Technology (Cambridge, MA)

US Patent 7,687,479 March 30, 2010 Appl. No.: 11/518,534 September 8, 2006

The invention relates, in part, to methods and products related to producing low molecular weight heparin.

Analysis of sulfated polysaccharides.

Venkataraman; Ganesh *et al.*

Momenta Pharmaceuticals, Inc. (Cambridge, MA)

US Patent 7,575,886 August 18, 2009 Appl. No.:10/386,402 March 11, 2003

The invention relates to methods and products associated with analyzing and monitoring heterogeneous populations of sulfated polysaccharides. In particular therapeutic heparin products including low molecular weight heparin products and methods of analyzing and monitoring these products are described.

Methods and products related to evaluating the quality of a polysaccharide.

Sasisekharan; Ram *et al.*

Massachusetts Institute of Technology (Cambridge, MA)

US Patent 7,399,604 July 15, 2008 Appl. No.:11/183,323 July 15, 2005

The invention relates to methods and products for characterizing and using polysaccharides. Low molecular weight heparin products and methods of use are described. Methods for characterizing purity and activity of polysaccharide preparations including glycosaminoglycans such as heparin are also described.

Combinatorial complex carbohydrate libraries and methods for the manufacture and uses thereof.

Dukler; Avinoam *et al.*

Glycominds Ltd. (Lod, IL)

US Patent 6,994,966 February 7, 2006 Appl. No.:09/860,559 May 21, 2001

The present invention provides methods of utilizing a complex carbohydrate library which includes a plurality of complex carbohydrate structures each being attached to a specific site of a solid support such as a platform for screening and isolating complex carbohydrate structures capable of specifically and uniquely binding with an entity such as a polypeptide.

Carbohydrate based toll-like receptor (tlr) antagonists.

Upadhyay, Shakti *et al.*

Reliance Life Sciences Pvt. Ltd. (Navi Mumbai, IN)

US Patent Application 20090215710 08/27/2009 Application Number: 12/236358 09/23/2008

The invention provides carbohydrate based compounds, methods of preparation, and compositions useful for modulating signaling through Toll-like receptors. The methods involve contacting a TLR-expressing cell with a carbohydrate based compound of the invention having a core structure comprising of one or more sugar moieties.

Patents

The carbohydrate based compounds are useful for inhibiting immune stimulation involving TLR ligands, especially TLR4 and TLR2. The compounds also are suitable for inhibition of inflammatory conditions resulting from infections. The compounds have use in the treatment of inflammation, autoimmunity, allergy, asthma, graft rejection, graft versus host disease, infection, sepsis, cancer, and immunodeficiency.

Carbohydrate-based synthetic vaccines for HIV.

Wang, Lai-xi *et al.*

University of Maryland Biotechnology Institute (Baltimore, MD, US)

US Patent 7556806, 07/07/2009

Application Number: 10/531124, 10/14/2003

The present invention relates to Trichinella diagnostic reagents that include a β -tyvelose-containing composition and use of such reagents to detect Trichinella, and particularly Trichinella spiralis infections. The present invention also includes diagnostic kits based on such reagents and therapeutic agents based on the the knowledge that β -tyvelose is produced by Trichinella spiralis parasites.

Carbohydrate-based anti-inflammatory agents.

Brandley, Brian K *et al.*

Glycomed, Incorporated (Alameda, CA)

US Patent 5470842, 11/28/1995

Application Number: 07/844102, 03/02/1992

Ligands in the form of N-acetyllactosamines which bind to endothelial leukocyte adhesion molecule-1 (ELAM-1) are disclosed. The ligand compounds can be formulated into pharmaceutical compositions and/or assay compositions used to alleviate inflammation and assay for the presence of (qualitative) and amount of (quantitative) ELAM-1 and thereby determine the presence, location and degree of inflammation.

Carbohydrate derived protein resistant biomaterial.

Guan, Zhibin *et al.*

The Regents of the University of California (Oakland, CA, US)

US Patent 7354747, 04/08/2008,
Application Number: 10/842711, 05/10/2004

Carbohydrate-derived side-chain polyethers that may be synthesized by condensation polymerization of monomers derived from natural occurring carbohydrates. These compounds are protein resistant, biodegradable and may be functionalized at location other than the chain ends. Various devices, apparatus and articles of manufacture may be formed, at least in part, of the compounds of the present invention to achieve desirable protein resistance, biodegradability and/or functionalization.

Lectin derived carbohydrate binding-peptide.

Heerze, Louis D *et al.*

Alberta Research Council (Edmonton, CA)

US Patent 5453272, 09/26/1995,
Application Number: 07/995503, 12/21/1992

The invention is directed to a lectin derived carbohydrate binding-peptide which inhibits cell-mediated immune responses and has the amino acid sequence SPYGRC. The peptide binds terminally linked α -sialic acid (2 \rightarrow 3) β Gal- and α -sialic (2 \rightarrow 6) β Gal-structures and is a acid fragment of the S2 subunit of pertussis toxin produced by Bordetella pertussis.

Carbohydrate conjugates as inhibitors of cell adhesion.

Kretzschmar, Gerhard *et al.*

Hoechst Aktiengesellschaft (DE)

US Patent 5858994, 01/12/1999,
Application Number: 08/509079, 07/31/1995

The invention relates to novel conjugates of tetrasaccharides, preferably of sialyl-Lewis X (SLeX) and sialyl-Lewis A (SLeA), having improved activity as inhibitors of cell adhesion, a process for the preparation of these compounds, and their use as pharmacological active compounds and as diagnostics and pharmaceuticals which contain these

Patents

conjugates.

Inhibition of cell adhesion protein-carbohydrate interactions.

Seed, Brian *et al.*

The General Hospital Corporation
(Boston, MA)

US Patent 6156881, 12/05/2000,
Application Number: 09/229030, 01/12/1999

Disclosed is a method of inhibiting the binding of a cell bearing a cell adhesion protein to a molecule or cell bearing a carbohydrate determinant specific for the cell adhesion molecule. The method involves contacting the cell adhesion protein-bearing cell with an inhibitor molecule bearing the carbohydrate determinant. Also disclosed is a method of inhibiting the binding of the first member of a specific binding pair to the second member of the specific binding pair, involving contacting the first member with an antibody which is specific for the first member and which is covalently bonded to a carbohydrate moiety which interferes with the antibody's ability to fix complement and bind an Fc receptor. The methods of the invention may be used, for example, to reduce inflammation.

Modified proteins comprising a bioactive peptide/protein linked to a sequence of aminoacids containing a human carbohydrate binding module (cbm), and development of a system for the administration of therapeutically active proteins and respective utilizations for biomedical purposes.

Universidade Do Minho

WO 2007/072461, 28.06.2007, A61K
9/70 PCT/IB2006/055023

It has been developed a strategy to simultaneously improve the properties of starch-based biomaterials for biomedical applications and the efficiency of therapeutical proteins. This new strategy which is revealed in this invention consists of the fusion of a carbohydrate binding module (CBM) with bioactive peptides. The human CBM used in this work has starch affinity (SBM). This SBM

was identified as a module of a laforin enzyme, a phosphatase, which has the function to attribute glycogen affinity. As has been demonstrated the SBM does also have starch affinity. This is a low cost polysaccharide available in large quantities and with different properties which has increasingly raised interest for the development of biomedical applications

Vascular delivery systems.

Ben Gurion University of the Negev
Research and Development Authority

WO 2009/133545, 05.11.2009, A61K
47/48 PCT/IL2009/000152

The site-specific expression of selectins on endothelial cells of blood vessels during angiogenesis provides an opportunity to target anti-cancer drugs to the vascular endothelium to extend the range of the therapeutic effect. This invention describes an innovative drug targeting strategy for the selective delivery of the anticancer drugs to endothelial cells by means of polymer-drug conjugates modified with a carbohydrate ligand for the vascular selectins. A model chemotherapeutic drug, doxorubicin, and the E-selectin ligand, sLex, are attached to a biocompatible polymer (HPMA). The selective binding, cellular uptake, intracellular fate, and cell cytotoxicity of the polymer-bound drug are investigated in human endothelial cells.

Polymeric micellar clusters and their uses in formulating drugs.

The School of Pharmacy, University of
London

WO 2008/017839, 14.02.2008, C08B
37/08, PCT/GB2007/003016

Polymeric micellar clusters formed from amphiphilic carbohydrate polymers and their uses in formulating drugs is disclosed, and in particular the finding that amphiphilic carbohydrate polymers are capable of self assembling to form micellar clusters in which the carbohydrate amphiphiles aggregate into hierarchically organised micellar clusters of individual aggregates. The micellar clusters

Patents

may be transformed into stable nanoparticles with drugs, especially hydrophobic drugs that have poor aqueous solubility, and may improve the transfer of hydrophobic drugs across biological barriers.

Modified proteins comprising a bioactive peptide/protein linked to a sequence of aminoacids containing a human carbohydrate binding module (cbm), and development of a system for the administration of therapeutically active proteins and respective utilizations for biomedical purposes.

Universidade Do Minho

WO 2007/072461, 28.06.2007 , A61K 9/70 , PCT/IB2006/055023

It has been developed a strategy to simultaneously improve the properties of starch-based biomaterials for biomedical applications and the efficiency of therapeutical proteins. This new strategy which is revealed in this invention consists of the fusion of a carbohydrate binding module (CBM) with bioactive peptides. The human CBM used in this work has starch affinity (SBM). This SBM was identified as a module of a laforin enzyme, a phosphatase, which has the function to attribute glycogen affinity. As has been demonstrated the SBM does also have starch affinity. This is a low cost polysaccharide available in large quantities and with different properties which has increasingly raised interest for the development of biomedical applications.

Lectin delivery vehicle targeting to destroy tumor growth through specifically binding to carbohydrate motifs on cancer cells.

Badr, Haitham Ali Badr El-Morsi

WO 2010/063294, 10.06.2010, C07K 14/42 , PCT/EG2009/000030

The present invention provides a novel therapeutic lectin distinguish difference in carbohydrate profiles between normal and tumor tissues. The invention also provides for the use of Bid BH3 glycomimetic-induced release of cytochrome c and cell death in cancer cells. Additionally, the invention

provides the use of magnetic drug targeting to treat malignant tumors loco-regionally without systemic toxicity.

Inhibitors of diacylglycerol acyltransferase.

Schering Corporation

WO 2010/059611, 27.05.2010, C07D 213/74 , PCT/US2009/064762 ,

The present invention relates to novel heterocyclic compounds as diacylglycerol acyltransferase ("DGAT") inhibitors, pharmaceutical compositions comprising the heterocyclic compounds and the use of the compounds for treating or preventing a cardiovascular disease, a metabolic disorder, obesity or an obesity-related disorder, diabetes, dyslipidemia, a diabetic complication, impaired glucose tolerance or impaired fasting glucose. An illustrative compound of the invention is shown below: (We bring to your attention that the illustrative compound of the invention is not provided on this electronic version as it is provided in the abstract of the paper copy)

Glycogen synthase kinase-3 beta inhibitors containing 7-hydroxy-benzimidazole-4-yl-methanone derivatives.

Oncotherapy Science Inc.

WO 2010/058512, 27.05.2010 C07D 409/14, PCT/JP2009/004975

GSK-3beta inhibitors comprising 7-Hydroxy-benzimidazole-4-yl-methanone Derivatives are provided. For example, the inhibitors have following general formula (I).

Water insoluble polymer: indigestible water-soluble polysaccharide film coatings for colon targeting.

Roquette Freres

WO 2010/049432, 06.05.2010 , A61K 9/28, PCT/EP2009/064165

The present invention provides a controlled release pharmaceutical dosage form for controlled release of an active ingredient, comprising an active ingredient coated by a polymeric mixture of: - at least a water

Patents

insoluble polymer and - at least an indigestible water-soluble oligosaccharide. The present invention also relates to the use and method for making the same.

Selection of HIV vaccine antigens by use of inpatient sequence variation to identify mutations in the HIV envelope glycoprotein that affect the binding of broadly neutralizing antibodies.

The Regents of The University of California

WO 2010/040136, 08.04.2010, C12Q 1/68, PCT/US2009/059583

Selection of HIV vaccine antigens by use of inpatient sequence variation to identify mutations in the HIV envelope glycoprotein that affect the binding of broadly neutralizing antibodies and polypeptides identified by these methods.

Selective glycosidase inhibitors and uses thereof.

Simon Fraser University

WO 2010/037207, 08.04.2010, C07D 207/12, PCT/CA2009/001302

The application relates to an immoditol compound for selectively inhibiting glycosidases, a prodrug thereof and a pharmaceutical composition comprising the compound or the prodrug. The application also relates to the use of the immoditol compound for treating diseases and disorders related to deficiency or overexpression of O-GlcNAcase, accumulation or deficiency of O-GlcNAc. Such diseases and disorders include neurodegenerative diseases, tauopathy,

cancers, and cardiac disorders

Anionic oligosaccharide conjugates.

Glycan Biosciences Pvt Ltd

WO 2010/037180, 08.04.2010 A61F 11/00, PCT/AU2009/001314,

The invention relates to anionic oligosaccharide conjugates that may be used to mimic the structure and/or activity of the anionic bioactive molecules known as glycosaminoglycans (GAGs).

Cell lines and proteins with variant glycosylation pattern.

Eureka Therapeutics, Inc.

WO 2010/036443, 01.04.2010, C07K 16/18, PCT/US2009/051325,

The present disclosure provides compositions and methods comprising cells producing glycoproteins with variant glycosylation patterns. The methods and compositions may be used in producing antibodies and proteins of therapeutic value.

Carbohydrate-based drug delivery polymers and conjugates thereof.

Nektar Therapeutics

WO 2010/033240, 25.03.2010, C07K 7/08 PCT/US2009/005233,

Provided herein are water-soluble carbohydrate polymers which are monoderivatized at their reducing terminus, such that the carbohydrate polymers can be selectively conjugated at a single location. Also provided are methods of preparation and conjugation of the monoderivatized carbohydrate polymers