Glycomimetic Drugs - A New Source of Therapeutic Opportunities

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Abstract: **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. Consequently, they are involved in disease indications as diverse as inflammation, cancer, and infectious disease. In general, native carbohydrates lack the properties necessary for efficacious drugs and historically have not been successful candidates to capitalize on these applications. Understanding the bioactive conformation and molecular interactions of functional carbohydrates, however, allows the rational design of small molecule glycomimetics that exhibit improved drug-like properties such as increased affinity, serum half-life, stability, and bioavailability. Recent advances in analytical techniques (i.e., NMR, x-ray crystallization), molecular modeling, and synthetic organic chemistry allow the design of potent glycomimetic compounds, which opens the door to a new class of therapeutic drugs to target molecular mechanisms that can address many of the current unmet needs in the treatment of disease.** [*Discovery Medicine* 8(43):247-252, December 2009]

Introduction

Exploration for new targets is fundamental for innovation in drug discovery and for the search for improved therapeutic solutions for diseases. 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 glycophosphatidylinositol-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)] that can bind specific carbohydrate structures and thus decode this information.

Description

Examples of the wide variety of carbohydrate-protein interactions that may occur at the cell surface are shown in Figure 1. 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

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(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. The hemagglutinin (H1) in this viral strain binds carbohydrates expressed on human cells terminating in the sugar sequence NeuAcα2-6Galβ1-4R and therefore establishes the first contact with the host cell. After replication, the virus sticks to the surface of the host cell by the same interaction. Now, the neuraminidase comes into play. By hydrolyzing the terminal *N*-acetylneuraminic acid the replicated virus is released and ready for another infection. Currently, two successful drugs for influenza (Tamiflu, Relenza) are mimicking (glycomimetics) the transition state of the enzymatic cleavage of the terminal *N*-acetylneuraminic acid. The future looming pandemic, known as the avain flu (H5N1), is more deadly but currently only efficiently infects birds. The reason for this different species specificity lies entirely on binding host cell surface carbohydrates. The H5 hemagglutinin recognizes only avian cells that express NeuAcα2-3Galβ-R rather than the NeuAcα2-6Galβ-R commonly found on human cells. Thus, just a simple mutation that alters carbohydrate binding specificity of the avian flu's hemaglutinin would cause a global pandemic with a potentially more deadly virus resulting in millions of deaths worldwide.

Soluble proteins, such as **lectins**, **toxins**, and **antibodies**, also bind cell surface carbohydrates and exhibit a fundamental role in many diseases. Lectins may arise from pathogens, such as the virulence factors (PA-IL and PA-IIL) of *Pseudomonas aeruginosa* which aid infection and virulence of the bacteria or they are endogenous in humans and play significant roles in human disease. Over 80 human lectins have been identified but only a small portion thereof has been studied in detail (Table 1). Most of the lectins act at the cell surface, but in certain disease states, some (e.g., selectin) can be released into the bloodstream and cause physiological effects such as the activation of inflammatory cells. Many of the toxins listed as bioterrorism agents

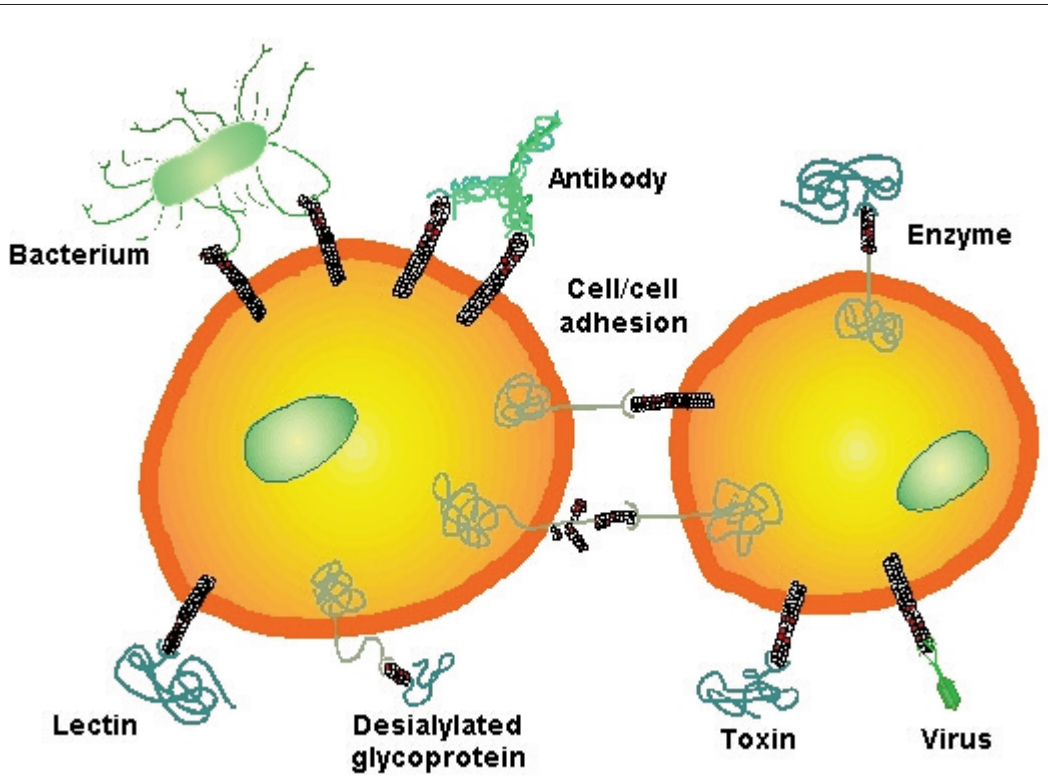

Figure 1. Cell surface carbohydrates are recognition molecules used in specific binding and interactions among cells, pathogens, and molecules.

(i.e., botulism, ricin, cholera) as well as those responsible for mortality in some infectious diseases (i.e., diphtheria, tetanus, *Clostridium difficile*, *Shigella*, *E. coli*) bind to specific cell surface carbohydrates (Smith et al., 2004), a required event for recognition, entry, and death of the target cell. 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. A hopeful example is the antibody 2G12, which has been shown to neutralize HIV infectivity. It binds to a common carbohydrate epitope (Manα1-2Man) on the HIV coat protein gp120 (Scanlan et al., 2002). Viruses use human enzymes to glycosylate their coat with non-immunogenic human carbohydrates in order to camouflage themselves from the human immune system. Antibody 2G12, however, recognizes a subtle change in the packing of the human carbohydrate epitope now located on a viral protein, and thereby identifies the Achilles heel of the virus. Rationally designed glycomimetics would allow it to capitalize on this subtle change and have the potential to produce a glycomimetic conjugate vaccine with broad neutralizing activity towards HIV infection.

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. As these efforts progress, the size of the lectin families (see Table 1) will further increase and in many cases present new therapeutic targets for rationally designed drugs based on the bioactive conformation of the corresponding functional carbohydrate structure. Detailed discussion of some of these targets is available in several recent review articles (Ernst and Magnani, 2009; Brown et al., 2007).

Glycomimetic Compounds Solve the Problems of Carbohydrate Drugs

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.

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.

Rational Design of Glycomimetic Drugs

The first step in the rational design of a glycomimetic drug is to fully understand the molecular base of the interaction between the functional carbohydrate epitope and its protein receptor (lectin). Empirical techniques include nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography of the carbohydrate ligand co-crystallized with its lectin. NMR techniques,

Table 1. Families of Human Carbohydrate-binding Proteins (Lectins)

Intracellular Lectins	Extracellular Lectins
• L-type lectins (β-sandwich) • P-type lectins (unique β-rich) • Calnexin	• C-type lectins(unique α/β) - Selectins - DC-Sign - Asialoglycoprotein receptor - Dectins - Mannose-binding protein - Etc. • I-type lectins (Siglecs, Ig superfamily) • R-type lectins (β-trefoil) • Galectins (β-sandwich)

such as transfer nuclear overhauser effect NMR (NOE NMR), can determine the exact binding conformation the carbohydrate acquires in lectin's binding site (Scheffler et al., 1997), whereas saturation transfer difference NMR (STD NMR) can identify those areas of the ligand being in a direct contact with the lectin (Herfurth et al., 2005; Rinnbauer et al., 2003).

Using this information, glycomimetics can be designed to eliminate or modify positions not involved in binding and optimize those areas crucial for interacting with the protein receptor surface. Knowing the bioactive conformation also provides the information necessary to design glycomimetics in which this conformation is pre-organized already in solution leading to reduced entropy (DS) costs upon binding. Gains in increased affinity by stabilizing the bioactive conformation have been substantial in several studies of glycomimetics (Thoma et al., 2001). Increases in binding affinity can also be accomplished by exploring additional interactions. An example of this strategy was successfully realized with the design of the anti-influenza glycomimetics Tamiflu and Relenza (Kim et al., 1997; von Itzstein et al., 1993).

The enhancement of the pharmacokinetic parameters of a rationally designed glycomimetic can be accomplished by a variety of strategies. Incorporation of moieties that enhance binding to serum proteins can increase the serum half-life of a glycomimetic by using serum protein to act as a sink or reservoir within the bloodstream. If the glycomimetic is being eliminated from the bloodstream by an active transport system (i.e., organic anion transport OAT1), the site recognized by the elimination system can either be removed if unimportant, or replaced with a bioisostere, thereby maintaining activity of the glycomimetic but blocking the ability to be recognized by the transport/elimination system.

Furthermore, other drug-like properties such as oral bioavailability may also be designed into glycomimetics. Low molecular weight, adjusted lipophilicity (as measured by LogP/D), and low numbers of hydrogen bond donors and acceptors may allow the glycomimetic to be absorbed passively into the bloodstream (Lipinski, 1997). If necessary, active transport systems can be accessed by adding appropriate side groups recognized by active transport systems (i.e., PEPT1 transporter, bile acid transporter), or a prodrug approach could be used as demonstrated in the glycodrug, Tamiflu.

Examples of Approved Glycomimetic Drugs

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, 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 (Figure 2).

Glycomimetic Drugs Currently in Drug Development

Efforts are underway in both academic and industrial labs to develop glycomimetic drugs for various lectin targets. Perhaps the most heavily studied and targeted by glycomimetic antagonists are the selectins (Barthel et al., 2007), which are a family of adhesion proteins involved in the extravasation of cells from the bloodstream, with a wide range of uses including inflammatory diseases. DC-SIGN is a lectin on the surface of dendritic cells and is used by a variety of pathogens as portals for infection. Examples include HIV, tuberculosis, dengue virus, hepatitis C, ebola, Marburg, and *Schistasoma mansoni*. Glycomimetic antagonists of DC-SIGN (Timpano et al., 2008; Borrok et al., 2007) could have wide-ranging applications in infectious diseases. I-type lectins cover a large family of lectins that bind sialylated carbohydrates and contain immunoglobulin-like domains. Within this family, efforts to develop glycomimetic drugs have focused mainly on Siglec-4 [myelin-associated glycoprotein (MAG)]. Glycomimetic antagonists of MAG (Vyas et al., 2005; Shelke et al., 2007; Gao et al., 2007) promote neurite outgrowth and have potential therapeutic applications in neuronal repair and spinal cord injury. Finally, bacterial lectins are also promising targets for therapeutic interventions. PA-IL and PA-IIL are virulence factors of *Pseudomonas aeruginosa* and glycomimetic inhibitors

of these lectins can be used in combination therapy to improve the efficacy of standard antibiotic treatments. FimH is a lectin responsible for the adhesion and infectivity of uropathogenic *E. coli* on the urinary tract epithelium. Potent glycomimetic antagonists of FimH (Bouckaert et al., 2005; Sperling et al., 2006) prevent adhesion of bacteria and have the potential to be a novel and safe treatment for a common but sometimes intractable infectious disease.

Conclusions

Complex carbohydrates coat all cell surfaces where they are used as recognition molecules for critical functional interactions with other cells, pathogens, and biomolecules. The entire compliment of these structures, known as the glycome, may be one of the least studied and most complicated of the molecular classifications in humans. Academic consortiums worldwide have organized resources and techniques needed to elucidate the human glycome and its functions. Many exciting new targets have been uncovered, yet this represents just the tip of the iceberg of opportunities and illustrates the vast potential of new therapeutic targets that are expected to emerge from this field. The functional carbohydrates identified in these recognition processes themselves do not make good drug candidates. Rather, their bioactive conformations in their receptor sites can be empirically determined by physiochemical methods and used for the rational design of small molecule mimics (glycomimetics) that have higher affinities and more drug-like properties of long serum half life, metabolic stability, low toxicity, and oral bioavailability. These glycomimetic drugs are new chemical entities and provide innovative therapeutic strategies to address current unmet needs among a wide spectrum of disease

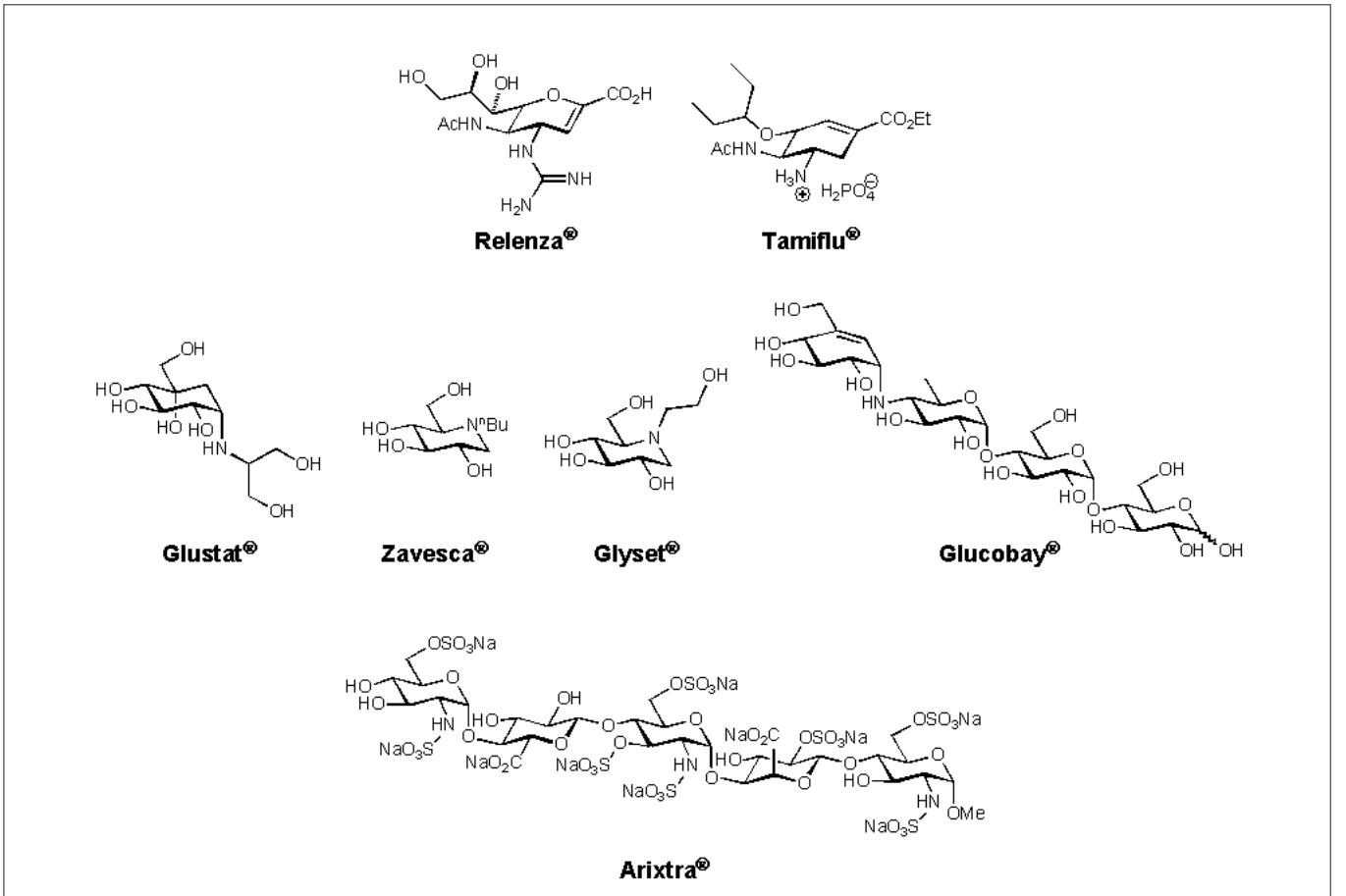

Figure 2. Structures of currently approved drugs. These include glycosidase inhibitors that prevent the digestion of carbohydrates for the prevention of influenza virus infections (Relenza, Tamiflu), the treatment of diabetes (Glustat, Zavesca, Glyset, Glucobay), and sulphated glycosaminoglycans which function as anticoagulants by binding to antithrombin III for the treatment of thrombosis (Arixtra).

applications.

References

Barthel SR, Gavino JD, Descheny L, Dimitroff CJ. Targeting selectins and selectin ligands in inflammation and cancer. *Expert Opin Ther Targets* 11:1473-1491, 2007.

Borrok MJ, Kiessling LL. Non-carbohydrate inhibitors of the lectin DC-SIGN. *J Am Chem Soc* 129: 12780-12785, 2007.

Bouckaert J, Berglund J, Schembri M, De Genst E, Cools L, Wuhrer M, Hung CS, Pinkner J, Slattegard R, Zavialov A, et al., Receptor binding studies disclose a novel class of high-affinity inhibitors of the *Escherichia coli* FimH adhesion. *Mol Microbiol* 55:441-455, 2005.

Brown JR, Crawford BE, Esko JD. Glycan antagonists and inhibitors: a fount for drug discovery. *Crit Rev Biochem Mol Biol* 42:481-515, 2007.

Ernst B, Magnani JL. From carbohydrate leads to glycomimetic drugs. *Nat Rev Drug Disc* 8:661-677, 2009.

Gao G, Smiesko M, Schwardt O, Gathje H, Kelm S, Vedani A, Ernst B. Mimetics of the tri- and tetrasaccharide epitope of GQ1alpha as myelin-associated glycoprotein (MAG) ligands. *Bioorg Med Chem* 15:7459-7469, 2007.

Herfurth L, Ernst B, Wagner B, Ricklin D, Strasser DS, Magnani JL, Benie AJ, Peters T. Comparative epitope mapping with saturation transfer difference NMR of sialyl Lewis(a) compounds and derivatives bound to a monoclonal antibody. *J Med Chem* 48:6879-6886, 2005.

Kaida K, Ariga T, Yu RK. Antiganglioside antibodies and their pathophysiological effects on Guillain-Barre syndrome and related disorders - a review. *Glycobiology* 19:676-692, 2009.

Kim CU, Lew W, Williams MA, Liu H, Zhang L, Swaminathan S, Bischofberger N, Chen MS, Mendel DB, Tai CY, et al. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J Am Chem Soc* 119:681-690, 1997.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and developmental settings. *Adv Drug Dev Rev* 23:3-25, 1997.

Rinnbauer M, Ernst B, Wagner B, Magnani J, Benie AJ, Peters T. Epitope mapping of sialyl Lewis(x) bound to E-selectin using saturation transfer difference NMR experiments. *Glycobiology* 13:435-443, 2003.

Scanlan CN, Pantophlet R, Wormald MR, Ollman ES, Stanfield R, Wilson IA, Katinger H, Dwek RA, Rudd PM, Burton DR. The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of α1-2-mannose residues on the outer face of gp120. *J Virol* 76:7306-7321, 2002.

Scheffler K, Brisson JR, Weisemann R, Magnani JL, Wong WT, Ernst B, Peters T. Application of homonuclear 3D NMR experiments and 1D analogs to study the conformation of sialyl Lewis(x) bound to E-selectin. *J Biomol NMR* 9:423-436, 1997.

Shelke SV, Gao GP, Mesch S, Gathie H, Kelm S, Schwardt O, Ernst B. Synthesis of sialic acid derivatives as ligands for the myelin-associated glycoprotein (MAG). *Bioorg Med Chem* 15:4951-4965, 2007.

Smith DC, Lord JM, Roberts LM, Johannes L. Glycosphingolipids as toxin receptors. *Semin Cell Dev Biol* 15:397-408, 2004.

Sperling O, Fuchs A, Lindhorst TK. Evaluation of the carbohydrate recognition domain of the bacterial adhesin FimH: design, synthesis and binding properties of mannoside ligands. *Org Biomol Chem* 4:3913-3922, 2006.

Thoma G, Magnani JL, Patton JT, Ernst B, Jahnke W. Preorganization of the bioactive conformation of sialyl Lewis(x) analogues correlates with their affinity to E-selectin. *Angew Chem Int Ed Engl* 40:1941-1945, 2001.

Timpano G, Tabarani G, Anderluh M, Invernizzi D, Vasile F, Potenza D, Nieto PM, Rojo J, Fieschi F, Bernardi A. Synthesis of novel DC-SIGN ligands with an alpha-fucosylamide anchor. *Chembiochem* 9:1921-1930, 2008.

von Itzstein M, Wu WY, Kok GB, Pegg MS, Dyason JC, Jin B, Van Phan T, Smythe ML, White HF, Oliver SW, et al. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* 363:418-423, 1993.

Vyas AA, Blixt O, Paulson JC, Schnaar RL. Potent glycan inhibitors of myelin-associated glycoprotein enhance axon outgrowth in vitro. *J Biol Chem* 280:16305-16310, 2005.