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2009 Claude S. Hudson Award in Carbohydrate Chemistry

Carbohydrates: A Frontier in Medicinal Chemistry

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Introduction

In the past, the importance of carbohydrates has been overshadowed by oligonucleotides and proteins, two other major classes of biopolymers. Recent advances in glycochemistry and glycobiology have helped renew strong interest in the study of oligosaccharides. It is now commonly acknowledged that oligosaccharides play fundamental roles in the development, recognition, growth, function, and survival of living cells and organisms.[1]

Various natural products including antibiotics and anticancer agents also contain carbohydrates and oligosaccharides. While oligonucleotides and oligopeptides can be assembled chemically from monomeric building blocks using standardized protocols, the synthesis of oligosaccharides still relies on strategies custom-tailored for each target compound. To date, only laboratories with carbohydrate chemistry expertise can synthesize complex oligosaccharides and project duration is still not measured in hours or days (as for the automated synthesis of oligopeptides[2,3] and oligonucleotides[4]) but rather in months or years. Access to oligosaccharides by isolation from natural sources is equally tedious and provides only small quantities of material that often lacks the required degree of purity for detailed biophysical or biochemical studies. This Award Address focuses on the application of synthetic carbohydrates to medical research. Recent progress in the synthesis and development of carbohydrate-based vaccines against infectious diseases and cancer will be discussed. Besides traditional solution phase synthesis of vaccine candidates and immunological relevant carbohydrates, applications of automated synthesis will also be addressed.

Synthesis of Oligosaccharides

To obtain pure and well-defined oligosaccharides, one must typically resort to chemical synthesis. During the past decades, the field of glycochemistry has seen vast improvements, as new techniques have been established. Two main challenges have to be faced in oligosaccharide synthesis: the functional groups (in particular, hydroxyl and amino groups) display similar reactivity and require differentiation for the construction of linear or branched structures. Additionally, the anomeric stereochemistry of the linkage between two carbohydrates must be controlled. Traditionally, oligosaccharide synthesis is performed by the assembly of selectively protected carbohydrate building blocks via a sequence of glycosylation reactions.[5−13]

A carbohydrate building block **1** (Scheme 1) equipped with a suitable anomeric leaving group, the glycosyl donor, generates an electrophilic carbocation intermediate upon activation. This intermediate reacts with nucleophile **2**, forming an *O*-glycosidic linkage. A strategically placed free hydroxyl group of another carbohydrate building block, the so-called glycosyl acceptor, typically serves as the nucleophile. The growing carbohydrate chain can now be extended in two ways: either via the reducing or the nonreducing end. In the former case, the growing carbohydrate serves as an electrophilic glycosylating agent, whereas in the latter case the growing carbohydrate chain serves as nucleophile. Extension via the nonreducing end is preferred, since no cumbersome conversion of an anomeric protecting group to a leaving group is required.

A variety of anomeric leaving groups and the corresponding activators have been developed for oligosaccharide synthesis (Scheme 1). A "good" leaving group is qualified by (i) high thermal stability in the absence of the respective activating agent, (ii) high yield in glycosylations, and (iii) high stereoselectivity. Nature achieves this regio- and stereoselectivity by the use of enzymes, the glycosyl transferases that couple an activated sugar (in the form of a nucleotide sugar) to the free hydroxyl group of another carbohydrate. Enzymes have been applied successfully to the synthesis of oligosaccharides and glycoproteins.

Although a variety of anomeric leaving groups are known, few of them are broadly applied. Glycosyl trichloroacetimidates **4** (Scheme 1) introduced by Schmidt can be activated using catalytic amounts of Lewis acid such as $BF_3 \cdot OEt_2$, AgOTf, or TMSOTf.[14,15] Anomeric phosphates **5**, upon activation with a Lewis acid such as TMSOTf, display high reactivity.[16,17] Thioglycosides[18] **6** are often used and can be activated with reagents such as *N*-iodosuccinimide (NIS) and triflic acid[19] or methylating agents such as MeOTf or dimethyl(methylthio)sulfonium triflate (DMTST). Among the anomeric halides, the chlorides are now rarely used but

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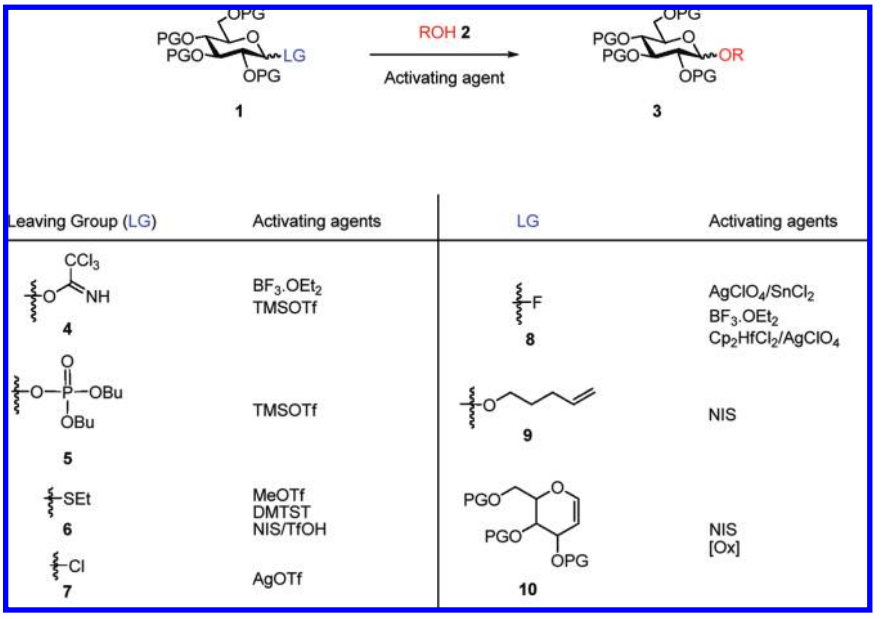
Scheme 1. Glycosylation Reaction Involving Glycosylating Agent **1** and Nucleophile **2** (ROH)[a]



[a] Various anomeric leaving groups (LG) and their corresponding activation methods are shown. PG = protecting group.

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can be activated with AgOTf. Anomeric fluorides **8**,[20] on the other hand, are stable and useful glycosylating agents. Strong Lewis acids are required for their activation such as $AgClO_4$, $BF_3 \cdot OEt_2$ or $Cp_2HfCl_2/AgClO_4$. *N*-Pentenyl glycosylating agents **9**[21] introduced by Fraser-Reid can be activated with NIS. Glycals **10**[22] bear no direct leaving group on the carbohydrate, and glycosylation is promoted by activation with electrophiles.

During the synthesis of complex and branched oligosaccharides, various hydroxyl groups have to be protected such that they can be revealed selectively.

The protecting group (PG) in the C2 position of a glycosylating agent plays a pivotal role in the stereochemical outcome of a glycosylation reaction. Neighboring group participation of an ester group at C2 will favor 1,2-trans glycosidic linkages, whereas nonparticipating groups at C2, such as ethers, will favor the formation of α-glycosidic linkages because of the anomeric effect. Many glycosidic linkages can be accessed by virtue of optimized conditions such as the nature of the anomeric leaving group, the set of protecting groups, and the solvent system. Yet some glycosidic linkages remain difficult to install. This is in particular the case in the synthesis of branched or 1,2-cis linked structures such as the challenging *β*-mannosides.

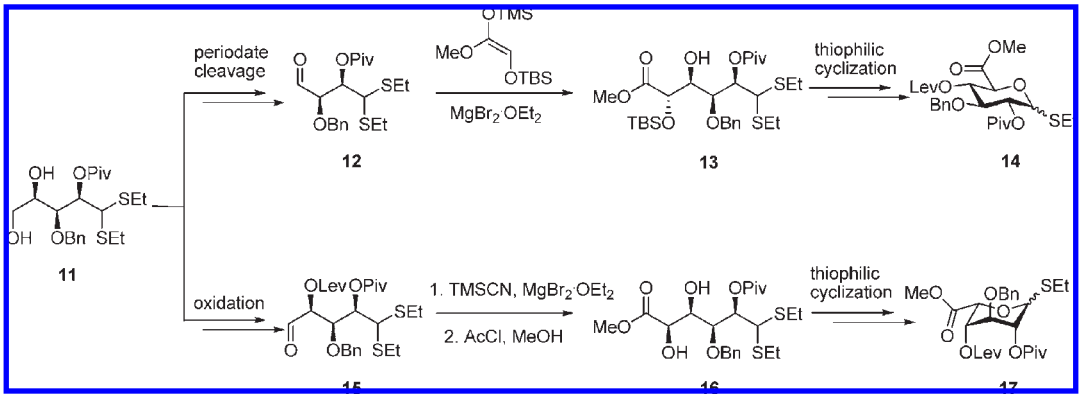
Various chemical methods exist to obtain differentially protected building blocks. In the following section, two relatively novel concepts to prepare these building blocks will be introduced: de novo synthesis and one-pot protection.

De Novo Synthesis of Carbohydrate Building Blocks

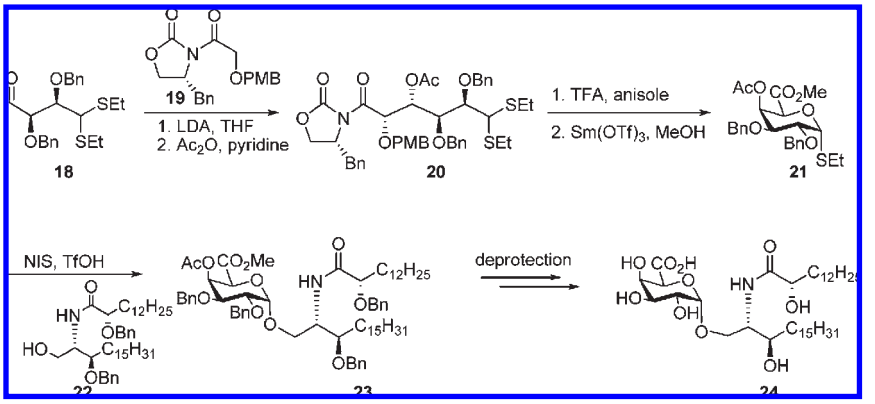
Chemical assembly of oligosaccharides and glycoconjugates requires large quantities of fully functionalized monosaccharide building blocks. Traditionally, these differentially protected and functionalized monosaccharides have been accessed from naturally occurring sugars as starting materials in a series of protection−deprotection maneuvers in order to establish a desired protecting group pattern. One fundamental difficulty of this process is the differentiation of up to five chemically similar hydroxyl groups in hexoses via temporary protecting groups as well as the selective masking of the anomeric hydroxyl. Consequently, protocols for the preparation of carbohydrate building blocks can rely on up to 20 synthetic steps, making this process both time-consuming and expensive.[23] Dissection of carbohydrate building blocks into shorter fragments reduces the number of hydroxyl groups, which have to be differentiated, makes the synthesis more convergent, and hence reduces the number of required synthetic steps. Additionally, the number of carbohydrates available as suitable starting materials is rather restricted. Syntheses of rare sugars from commonly available monosaccharide precursors are more tedious, since the selective removal of hydroxyl groups, the incorporation of new functional groups or a switch from the D- to the L-series may be required. All of these drawbacks can be overcome by the stereocontrolled synthesis of carbohydrates from smaller substrates by C−C bond forming reactions, commonly referred to as "de novo synthesis" of sugars.

A plethora of de novo routes to monosaccharides has been reported in the past 30 years.[24−26] However, none of these syntheses delivered suitably protected carbohydrate building blocks, required for the use in oligosaccharide synthesis. Thus, the large scale preparation of carbohydrate building blocks remains a key bottleneck on the way to synthetic oligosaccharides. De novo synthesis of scarcely available and expensive monosaccharides is particularly attractive.[27−31] Uronic acids, such as D-glucuronic acid (GlcA) and L-iduronic acid (IdoA), are structural elements of various glycosaminoglycans and play an important role in cell−cell communication, inflammatory response, and wound healing. Both uronic acid building blocks can be synthesized, de novo, diverging from a D-xylose-derived common advanced intermediate **11** (Scheme 2).[27] C4-Aldehyde **12** is elongated by a chelate-controlled Mukaiyama-type aldol reaction to afford the acyclic glucuronic acid **13**. For the synthesis of the iduronic acid **17**, in contrast, a chelate-controlled cyanation/Pinner reaction sequence with the C5-aldehyde **15** is chosen. In this way, both uronic acids were obtained in a highly diastereoselective manner. After a few

Scheme 2. Convergent de Novo Synthesis of Glucuronic **14** and Iduronic **17** Acids from Common Advanced Intermediate **11**



Scheme 3. Total Synthesis of Glycosphingolipid **24** from *Sphingomonas yanoikuyae*



protecting group manipulations, both thioacetals were cyclized to thioglycosides **14** and **17** using *N*-iodosuccinimide as a thiophilic reagent. Efforts to use these building blocks in automated synthesis of different classes of glycosaminoglycans are ongoing.

D-Galacturonic acid (GalA), another uronic acid, constitutes a structural motif of many naturally occurring bacterial glycolipids. Glycosphingolipid **24** from *Sphingomonas yanoikuyae* activates natural killer T (NKT) cells and induces a strong immune response after binding to CD1d (Scheme 3). We recently reported a concise and efficient total synthesis of glycosphingolipid **24** based on the de novo [4 + 2] assembly of the GalA building block utilizing an Evans aldol reaction between C4-aldehyde **18** and oxazolidinone **19** as a key step.[29] Thioacetal **20** was cyclized to thioglycoside **21** after treatment with TFA/anisole, and the auxiliary was efficiently removed with $Sm(OTf)_3$ in methanol. The difficult glycosidic bond between GalA thioglycoside **21** and ceramide **22** was constructed in 85% yield and 4.2:1 α:*β*-selectivity after extensive optimization studies. Thus, the de novo approach helped to reduce the number of synthetic steps in the GalA construction to only four steps, starting from an aldehyde readily derived from L-arabinose.

One-Pot Protection of Carbohydrates

Another approach for the efficient preparation of carbohydrate building blocks has been reported by Hung et al.[32] Monosaccharides were decorated with various orthogonal protecting group patterns in a TMSOTf-catalyzed one-pot procedure. The reaction conditions were optimized for D-glucopyranosides but should be applicable to D-mannosides, D-galactosides, and 2-azido-2-deoxy-D-glucosides.

Starting from per-*O*-silylated α-glucosides and *β*-thioglucosides **25**, fully protected monosaccharides as well as C2-, C3-, C4-, and C6-alcohols were obtained selectively (Scheme 4). The reaction sequence begins with TMSOTf-catalyzed arylidenation of **25** with an arylaldehyde, yielding *O*4,*O*6-arylidene **27**, followed by TMSOTf-catalyzed regioselective triethylsilane-mediated reductive O3-etherification of the acetal with a second equivalent of an arylaldehyde to furnish building block **29**.

The silyl ether at C2 can be cleaved with TBAF[a] to the corresponding alcohol **30** or it can be acylated with acid anhydride and a catalytic amount of TMSOTf to yield ester **31**. At this stage it is possible to open the arylidene regioselectively with $BH_3 \cdot THF$ and catalytic amounts of TMSOTf to yield the C6 alcohol **33** in the same vessel. Alternatively, the arylidene can be opened to liberate the C4 hydroxyl (**32**) using hydrochloric acid and sodium cyanoborohydride. This procedure allows for facile large scale synthesis of various orthogonally protected monosaccharides.

Bioinformatic studies analyzing the diversity of mammalian oligosaccharide linkages of isolated *N*-linked and *O*-linked glycans and glycosphingolipids revealed that nature uses only few connectivities.[33] The glycospace, the body of different structures that can, in principle, be constructed,

[a] Abbreviations: DMAP, 4-dimethylaminopyridine; Lev, levulinyl; Piv, pivaloyl; TBAF, tetrabutylammonium fluoride; TBS, *tert*-butyldimethylsilyl; TBDPS, *tert*-butyldiphenylsilyl; Tf, trifluoromethanesulfonic; TIPS, triisopropylsilyl; TMS, trimethylsilyl; UDP, uridine diphosphate.

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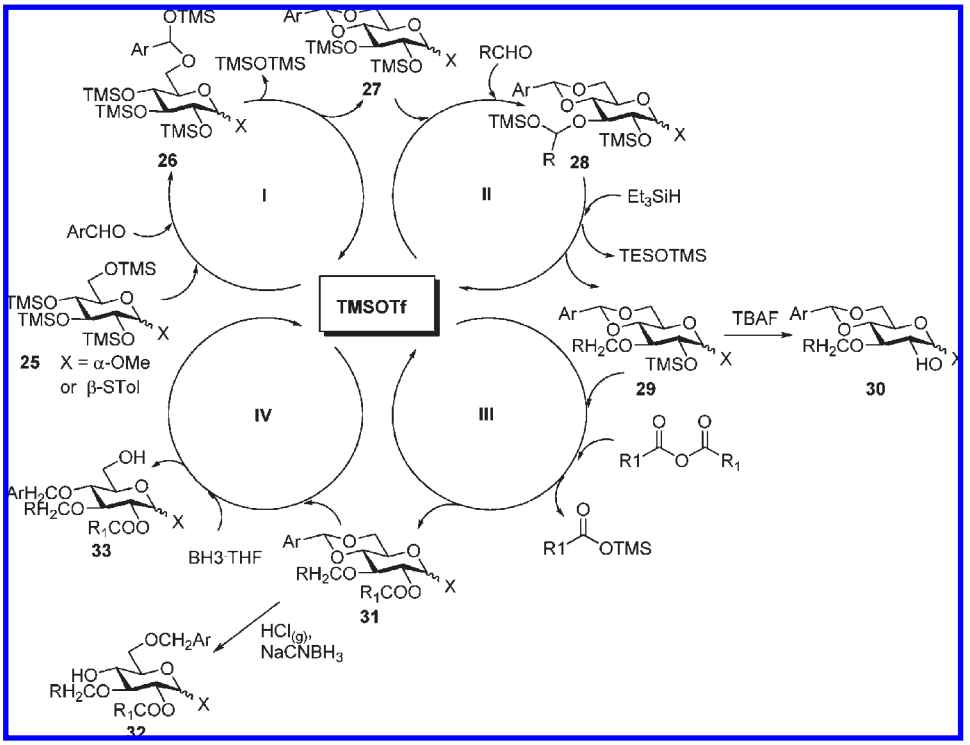
Scheme 4. Catalytic One-Pot Protection of Per-Silylated Monosaccharides **25**



is less complex than anticipated considering the theoretically possible connections. Database analysis showed that in principle 36 building blocks would suffice to assemble 75% of known mammalian oligosaccharides. Bearing in mind that more than 100 building blocks are now commercially available for solid phase peptide synthesis, commercial manufacture of bulk quantities of differentially protected carbohydrates can be envisaged. With the de novo synthesis or Hung's one-pot procedure, the synthetic route to various differentially protected building blocks can be kept short and efficient. Bacterial carbohydrates display more diversity in both the type of the carbohydrate and the connectivity between them. Nevertheless, many immunogenic bacterial carbohydrates can be constructed with the same set of 36 building blocks. Efficient methods for the assembly of oligosaccharides from the corresponding building blocks are needed. Ideally, these methods would be automated such that large libraries of oligosaccharides can be synthesized quickly.

One-Pot Glycosylations

One-pot strategies provide short routes not only to carbohydrate building blocks but also to glycosylation sequences. In the OptiMer strategy building blocks are added sequentially to the reaction mixture.[34] The order of addition depends on the reactivity of the building blocks and is performed in decreasing order of reactivity. To this end, the relative reactivity value (RRV) of carbohydrate building blocks was quantified with respect to the type of carbohydrate, the protecting group pattern, and the anomeric leaving group.[35]

Aided by a computer program, fed with the reactivity profile of more than 100 different thioglycosides, the optimal set of building blocks can be predicted for the synthesis of an oligosaccharide consisting of up to six monomers. The reactions are then performed in solution with the oligosaccharide chain being extended from the nonreducing to the reducing end. *p*-Methylphenylthioglycosides are used in the OptiMer approach for practical reasons such as the ease of activation with thiophilic *N*-iodosuccinimide (NIS) and dimethyl(methylthio)sulfonium triflate (DMTST). Shorter oligosaccharides can conveniently be synthesized with this method, as exemplified by the synthesis of the Globo-H hexasaccharide.[36] A difficulty inherent to this method is the large number of glycosyl donors needed. Considering that one structure might contain more than one identical linkage, several differentially protected building blocks have to be prepared for each linkage differing in relative reactivity.

Automated Oligosaccharide Synthesis

Solution phase synthesis of complex carbohydrates is intrinsically slow. Each deprotection and coupling step is typically followed by time-consuming workup and purification procedures. Inspired by the success of solid phase peptide and oligonucleotide syntheses, Fréchet and Schuerch in the early 1970s carried out the first studies concerning the solid phase synthesis of oligosaccharides.[37,38] In 2001 the first automated synthesizer for solid phase oligosaccharide assembly was introduced.[39] The fundamental principle of solid phase synthesis lies in the covalent linkage of one reaction partner, in this case the growing chain of oligosaccharides, to an insoluble solid support such as a resin. Reagents can be added to a suspension of the solid support that will react with, or modify, the reactant attached to the support. An excess of reagents ensures that reactions are driven to completion, and all unreacted starting material or other products remaining in solution can be removed by simple filtration and washing. The desired product is cleaved from the solid support once the synthesis sequence is terminated. A cleavable linker is placed between the reactant and the solid support and only a single purification step is needed once the final product is detached

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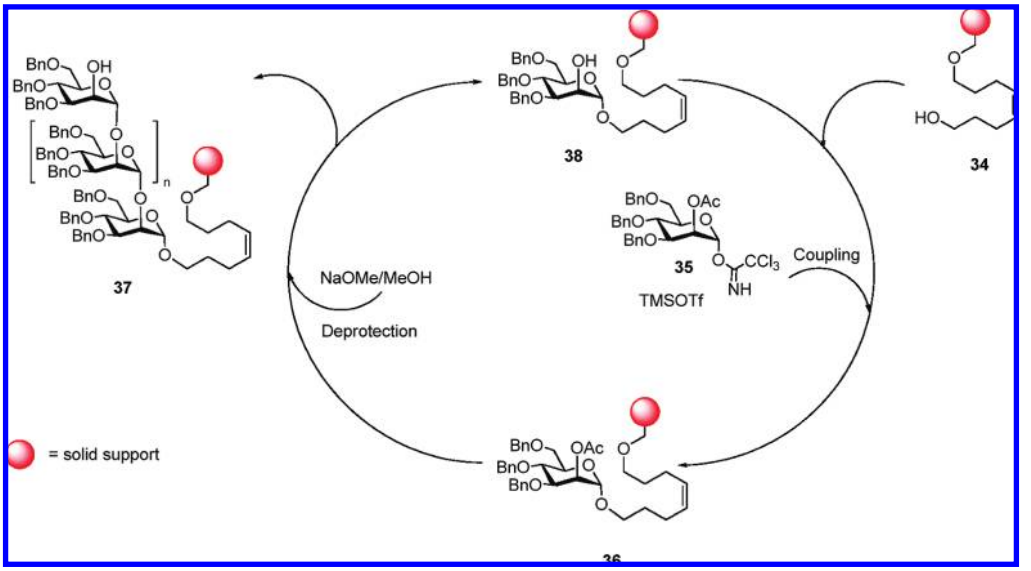
Scheme 5. Automated Coupling Cycle Showing Coupling, Deprotection, and Final Detachment from the Solid Support Employed for the Synthesis of an Oligo-α-mannoside Bearing a Pentenyl Linker on the Reducing End



from the resin. The principle of automated solid phase synthesis is illustrated by the synthesis of a poly-α-mannoside (**37**, Scheme 5). The "acceptor-bound" strategy was used whereby the reactive glycosylating agent is delivered in solution to the nucleophilic hydroxyl group that is exposed on solid support: the hydroxyl group of octenediol functionalized polystyrene resin **34** served as nucleophile ready for coupling with glycosylating agent **35**. The anomeric trichloroacetimidate leaving group was activated by catalytic amounts of TMSOTf. The C2 acetate controls the anomeric configuration via neighboring group participation such that exclusively 1,2-trans glycosidic linkages were obtained. Following the coupling reaction, the C2 ester group was hydrolyzed with sodium methoxide in methanol to generate a nucleophile for the subsequent glycosylation with building block **35**. The coupling/deprotection cycle was repeated until the desired chain length was obtained. Finally, oligosaccharide **37** was cleaved from the solid support. In the case of the octenediol linker, cross-metathesis with ethene using Grubbs' catalyst removes the oligosaccharide from the solid support. Finally, HPLC was used before global deprotection as the only purification step to yield the unprotected sugar. Heptamannoside **37** ($n = 5$) was synthesized in 20 h with an overall yield of 42%. In comparison, the manual synthesis of the same heptamannoside on solid support took 14 days to yield 9% product overall.[40] This observation highlights the advantage of automated solid phase synthesis compared to the manual approach. With the introduction of the fluorescent 9-fluorenmethoxycarbonyl (Fmoc) group for temporary hydroxyl group protection, monitoring of protecting-group removal by UV/vis spectroscopy is possible. Analysis of Fmoc deprotection by mildly basic amines such as piperidine thus provides a qualitative assay for the efficiency of each glycosylation and deprotection cycle during automated assembly. This strategy has been applied by our group to the syntheses of the Lewis blood group oligosaccharides Lewis X (Le^x), Lewis Y (Le^y), and the Le^y−Le^x nonasaccharide.[41]

Recent advances in automated synthesis now allow glycosylations in the synthesizer to be performed at temperatures as low as −40 °C. Challenging 1,2-cis linkages including α-galactosidic[42] or β-mannosidic linkages can be installed by automated synthesis.[43]

Application of Synthetic Carbohydrates as Vaccine Candidates

With efficient synthetic tools in hand, sufficient amounts of pure and well-defined carbohydrates can be obtained for the construction of vaccine candidates. The rationale behind the strategy of carbohydrate-based vaccines is that specific polysaccharides located on the surface of bacteria or viruses allow for selective recognition of those cells by the immune system of an organism. Immunization with polysaccharide antigens, in principle, enables protection against intruders bearing these sugar moieties on their surface.[44,45] Antigens, in general, can be classified as T cell dependent (TD) or T cell independent (TI). While TD antigens require immune stimulation from $CD4^+$ T cells to generate an antibody response, TI antigens can elicit an immune response independent of T cells. TI antigens can be further divided into two classes, TI-1 and TI-2. TI-1 antigens such as bacterial lipopolysaccharides are often B cell mitogens and induce polyclonal activation of B cells. In contrast, polysaccharides belong to the group of TI-2 antigens that can only be recognized by mature B cells by cross-linking of surface-exposed immunoglobulin receptors.[44] Frequently, these carbohydrates consist of highly repetitive structures such as capsular polysaccharides from bacteria. Vaccines based on carbohydrates have a long history; a milestone was set as early as 1930 when Francis and Tillett described induction of an antibody response in patients by pneumococcus-specific polysaccharides that was later shown to be protective.[46,47] Numerous attempts have been made since to use capsular polysaccharides from bacteria as vaccines. However, as polysaccharides are TI-2 antigens, vaccines based on pure carbohydrates are limited. Antibody responses against TI-2 antigens lack T cell help. Without T cell help, no immunological memory is generated and neither isotype switching from IgM to other Ig subclasses nor B cell affinity maturation occurs. Children below the age of 2 have immature B cells, are unable to produce anti-polysaccharide antibodies, and generally respond poorly to TI-2 antigens. In many cases, bacterial polysaccharides are poor immunogens in humans because of structural homology to human glycolipids and glycoproteins. One way to tackle this problem is to conjugate the

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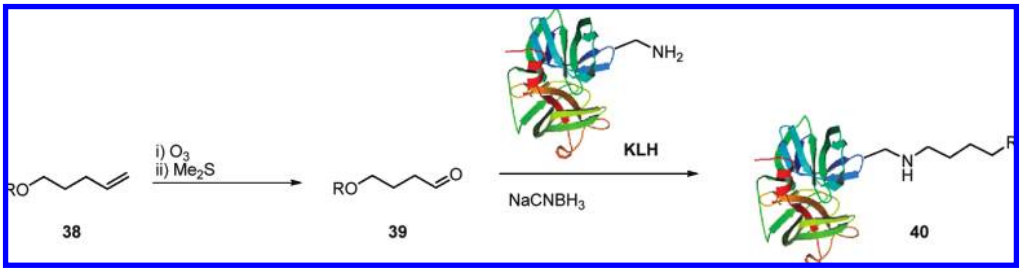
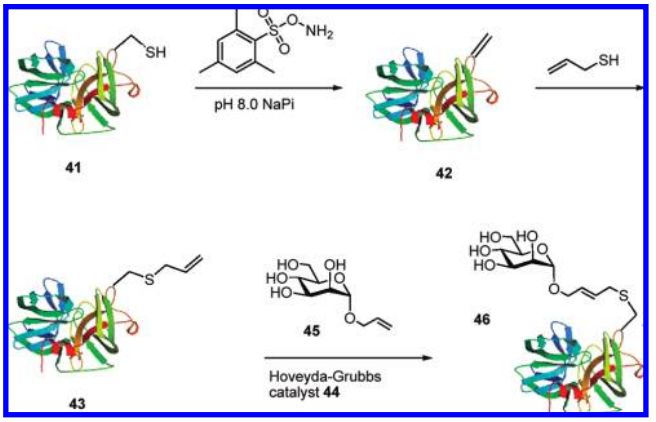
Scheme 6. Coupling Strategy for the Covalent Attachment of a Pentenyl-Linker Equipped Carbohydrate **38** (with R Being the Carbohydrate Moiety) to a Lysine Residue of a Protein (Here KLH) via Reductive Amination



Scheme 7. Conjugation of Carbohydrate **45** to a Protein via Cross-Methathesis



polysaccharide to a carrier protein. A number of commercially available vaccines against *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), and *Salmonella typhi* are based on conjugates.[48]

Conjugation of Carbohydrates to Carrier Proteins

Bacterial polysaccharides are typically conjugated to carrier proteins to enhance their otherwise poor immunogenicity in humans. A plethora of conjugation methods have been established to attach sugars to proteins.[49−51] Most modifications rely on the reaction of a lysine residue with an activated ester or an isocyanate.[52] Reaction of the primary amine functionality of lysine with an aldehyde in the presence of a reducing agent such as sodium cyanoborohydride yields the corresponding secondary amine. Conjugation via reductive amination was used to attach a synthetic tetrasaccharide, *n*-pentenylglycoside **38**, which resembles oligosaccharides found on the spores of *B. anthracis* to keyhole-limpet-hemocyanine (KLH) carrier protein (Scheme 6). Ozonolysis of the alkene yielded the corresponding aldehyde **39** that, upon addition of KLH, formed an imine with the primary amine group of the lysine residues located on the outside of the protein. Reductive amination led to the desired glycoconjugate **40**.

Glutamate and aspartate residues can be activated by carbodiimides to yield activated esters that will react with nucleophiles to form stable adducts. In addition, the thiol group of cysteine can be used for conjugation of proteins to molecules equipped with an electrophilic group such as maleimide.[52]

Recently, Davis et al. described the site-selective surface modification of proteins using olefin metathesis.[53,54] Cysteine residue **41** (Scheme 7) on the protein surface was converted into dehydroalanine (Dha) **42** using *O*-mesitylenesulfonylhydroxylamine (MSH).[54] The resulting α,β-unsaturated amide was then treated with a thiol to yield a thioether. Allyl ether **43**, formed upon treatment of **42** with allylmercaptan, proved to be particularly suitable for further aqueous cross-metathesis, with allyl equipped mannose **45**, in the presence of Hoveyda−Grubbs catalyst **44** to obtain a functionalized protein **46**.[53] It has also been shown that treatment of Michael acceptor **42** with a glycan bearing an anomeric thiol group yielded the corresponding thioether glycoprotein.[54]

This method enables the site-selective conjugation of oligosaccharides to carrier proteins to yield well-defined glycoconjugates. Oligosaccharides equipped with a linker bearing a terminal olefin can conveniently be manufactured with the automated oligosaccharide synthesizer.

The following section will discuss the synthesis of specific glycoconjugates and their application as vaccine candidates for bacterial, viral, and parasitic infections as well as cancer.

Haemophilus influenzae Type B

The commercially available vaccine against Hib, used to eradicate childhood meningitis, highlights the potential of carbohydrate vaccines. Prompted by the success of vaccines containing purified Hib capsular polysaccharides, fully synthetic fragments of the native polysaccharides were conjugated to a carrier protein.[55,56] The antigenicity of the synthetic conjugate was comparable to the native polysaccharide linked to the same carrier protein. Key step in the large scale synthesis of the Hib polysaccharide was a one-step polycondensation reaction using H-phosphonates (Scheme 8).[57] Ribosylribitols **47** and **48** were derived from D-glucose and peracetylated β-D-ribofuranose, respectively. Phosphate-containing terminal residue **47** and H-phosphonate **48** were oligomerized in high yield using pivaloyl chloride (PivCl) as a polycondensation reagent. Oxidation with iodine was followed by deprotection of the benzyl ethers and reduction of the azide by hydrogenation. Synthetic oligomers **49** consisted on average of eight repeating units and were reproducibly obtained in high yield after purification by size exclusion chromatography. Treatment of amine **49** with 3-maleimidopropionic acid *N*-hydroxysuccinimide ester gave **50**, and the vaccine was finally obtained by conjugation to tetanus toxoid. This vaccine (Quimi-Hib) was licensed in Cuba in 2003 and has been routinely used for prophylactic immunization of infants and children in this country.[58]

Streptococcus pneumoniae

The first pneumococcal conjugate vaccine (Prevnar) was licensed in the U.S. in 2000 by Wyeth Vaccines and consists of purified polysaccharides of seven *Streptococcus pneumoniae*

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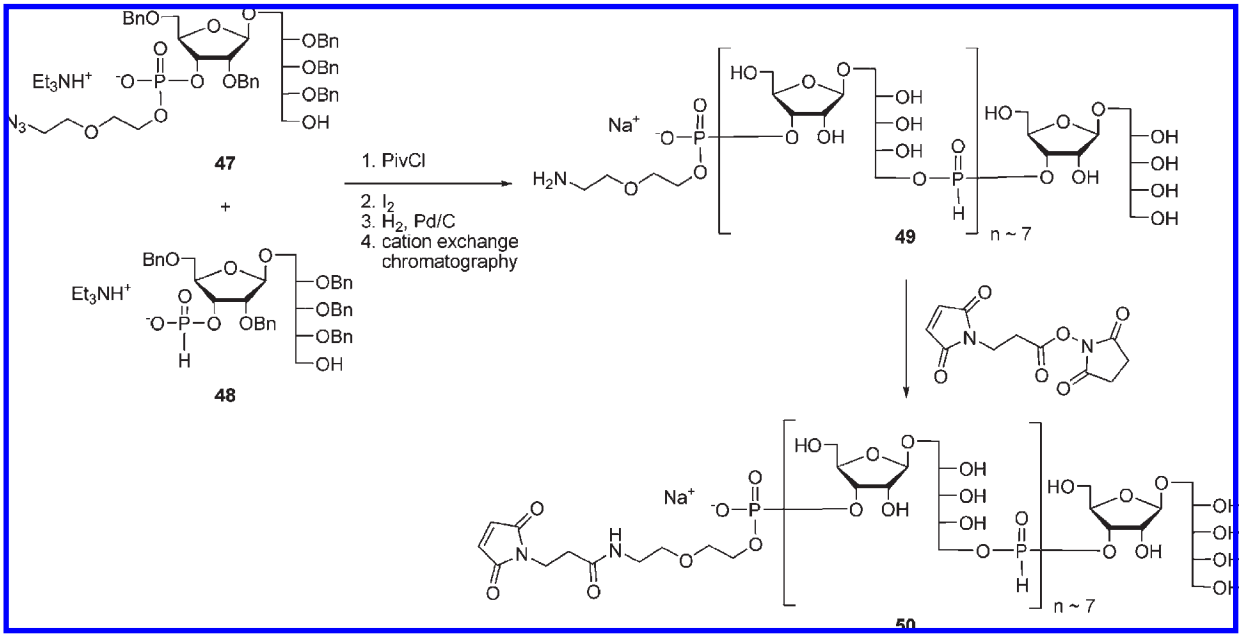
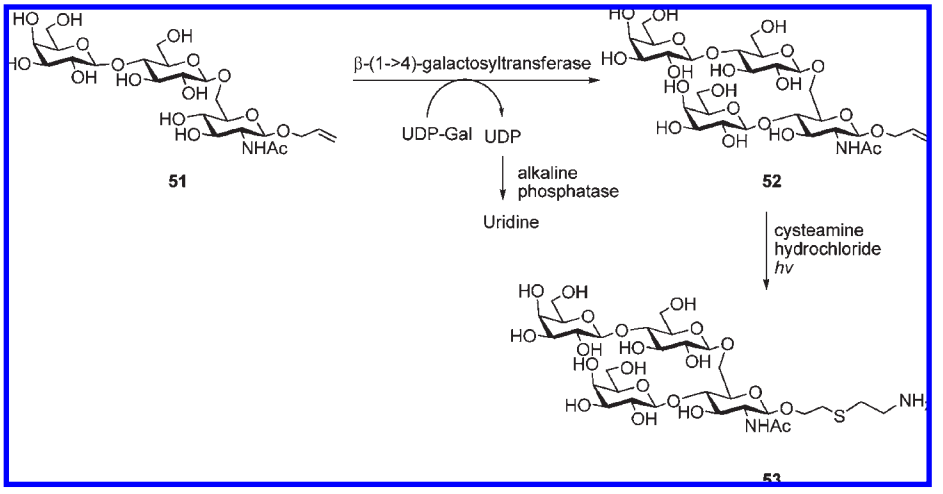
Scheme 8. Synthesis of Hib Capsular Repeating Unit **50** Used for Conjugation to Human Serum Albumin or Tetanus Toxoid



Scheme 9. Enzymatic Key Step in the Synthesis of the Branched Tetrasaccharide Repeating Unit (**53**) of *S. pneumoniae* Type 14



serotypes conjugated to a nontoxic variant of diphteria toxin (CRM_{197}).[59] Synthetic approaches have resulted in promising vaccine candidates. For instance, di-, tri-, and tetrasaccharide-CRM_{197} conjugates were synthesized to protect against *S. pneumoniae* type 3 infection, and the induction of polysaccharide-specific antibodies was shown in a mouse model.[60] For *S. pneumoniae* 6B a study regarding the immunogenicity and protective capacity of di-, tri-, and tetrasaccharides showed that these oligosaccharide structures were capable of inducing fully protective antibodies in rabbits and mice.[61] Synthetic and chemoenzymatic approaches to other *S. pneumoniae* serotypes have yielded encouraging results, indicating that synthesis might become a serious alternative to purification of polysaccharides.[62,63] The branched tetrasaccharide repeating unit of *S. pneumoniae* type 14 was prepared chemoenzymatically (Scheme 9). Trisaccharide allyl glycoside **51** was prepared via solution phase synthesis by coupling of a peracetylated lactosyl building block and a suitably protected galactosamine. The key step during the synthesis of tetrasaccharide **53** was the enzymatic glycosylation of acceptor **51** with UDP-galactose catalyzed by β-(1→4)-galactosyltransferase. Alkaline phosphatase was added to prevent feedback inhibition by released UDP. Tetrasaccharide **52** was obtained in almost quantitative yield. Allylglycoside **52** was then converted into the amine linker equipped glycoside **53** by reaction with cysteamine under UV irradiation. The amine was used for the subsequent coupling of the tetrasaccharide to carrier proteins. Acceptor **51** was tolerated by the enzyme, and in particular, the anomeric allyl group did not pose any problems to the enzymatic reaction except for a decrease in reaction rate compared to the acceptor with a free anomeric hydroxyl group.

Mycobacterium tuberculosis

Tuberculosis is caused predominantly by infection with *Mycobacterium tuberculosis* and is a major cause of mortality worldwide.[64] Despite the development of new treatments, this disease remains a major global health concern. Annually, tuberculosis causes more than 7 million new cases and

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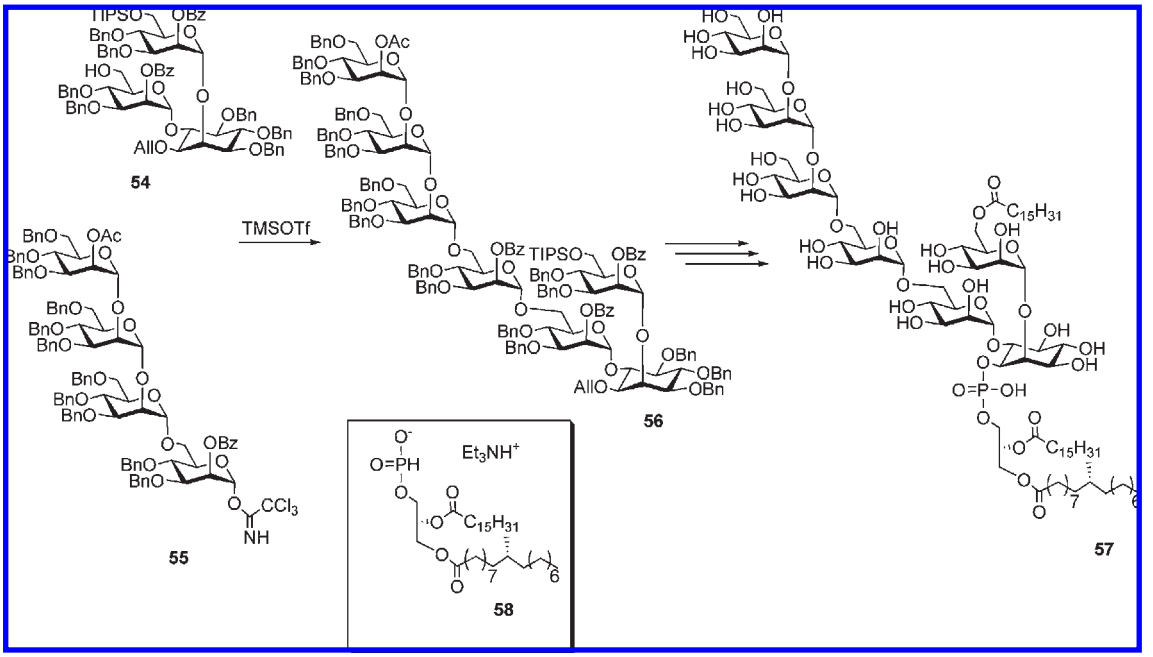
Scheme 10. Synthesis of *M. tuberculosis* PIM **57** via [4 + 3] Coupling



2 million deaths.[65] The exploration of novel drug targets and vaccines against *M. tuberculosis* is essential. In contrast to other bacterial pathogens, *M. tuberculosis* is not characterized by a unique capsular polysaccharide that could serve as a vaccine target. Instead, the mycobacterial cell wall consists of complex glycolipids such as lipomannan, lipoarabinomannan (LAM), and mannan-capped lipoarabinomannan.

Several enzymes participate in the biosynthesis of mycobacterial cell wall components and are particularly attractive targets for antibiotic action. One strategy for the identification of novel anti-TB agents is the elucidation of pathways involved in the biosynthesis of the *M. tuberculosis* cell wall. The synthesis of a docosanasaccharide motif of the arabinogalactan polysaccharide has been performed in the Lowary lab.[66] This key motif as well as related synthetic oligosaccharide fragments may serve as tools to investigate the biosynthesis of arabinan-containing polysaccharides.

Another strategy is the expression of enzymes involved in the mycobacterial cell wall biosynthesis and the development of high-throughput assays for the identification of inhibitors. Recombinant expression and purification of glfT, an enzyme that catalyzes the formation of β-(1→5) and β-(1→6) linkages in the biosynthesis of the mycoyl-arabinogalactan-peptidoglycan complex, have been performed.[67] Well-defined intermediates of the cell wall biosynthesis of *M. tuberculosis* have been accessed such as oligosaccharide fragments of LAM.[68] The Seeberger group reported the total synthesis of phosphatidylinositol mannosides (PIMs), a key class of antigenic glycolipids found on the cell wall of *M. tuberculosis*.[69] On the basis of a [4 + 3] glycosylation strategy, phosphatidylinositol dimannoside (PIM_2) and hexamannoside (PIM_6) were synthesized. Together with synthetic arabinomannan oligosaccharides,[70] these molecules aid the search for carbohydrate antigens for vaccinations against tuberculosis.

For the total synthesis of PIM_6 a challenging glycoslyation strategy was envisaged (Scheme 10). Dimannoinositol **54** was glycosylated with tetramannose trichloroacetimidate **55** and catalytic amounts of TMSOTf to yield glycosylinositol **56**. The complex glycosylation between **54** and **55** is only high yielding when the reactivities of glycosylating agent and nucleophile are properly matched. Subsequent protecting group manipulations and selective palmitoylation followed by phosphorylation with H-phosphonate **58**, derived from the Roche ester, gave fully protected PIM_6. Late stage introduction of the lipids into the glycan backbone maximized synthetic convergence and allowed access to PIM motifs with different lipids. Global debenzylation furnished native PIM_6 **57**.

Shigellosis

Shigellosis (bacillary dysentery) is a food-borne illness caused by infection with bacteria of the genus *Shigella*. Serotypes belong to four groups: group A (*Shigella dysenteriae*), group B (*Shigella flexneri*), group C (*Shigella boydii*), and group D (*Shigella sonnei*).[71] Subunit vaccines based on use of the major protective antigen, i.e., LPS, oligosaccharides have been synthesized that mimic the *O*-specific polysaccharide (*O*-SP) portion of *Shigella* LPS (Figure 1).

These conjugates are recognized by anti-*O*-SP protective monoclonal antibodies.[72] More recently, a synthetic oligosaccharide-based vaccine candidate against *S. flexneri* 2a infection has been developed.[73] By mapping of the carbohydrate epitopes recognized by protective IgG antibodies, the contribution of each monosaccharide was investigated to lead to more immunogenic carbohydrates.[74] Furthermore, the identification of appropriate core oligosaccharides responsible for eliciting a protective antibody response, and the variation of this lead structure, might contribute to higher immunogenicity as well as cross-protection against diverse *Shigella* serotypes.

Anthrax

Anthrax is a severe mammalian disease caused by the spore-forming bacterium *B. anthracis*.[75] Although treatment with antibiotics is possible at an early stage of the disease, the gastrointestinal and inhalation form of anthrax is often

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resistant to treatment because of the rapid progress of the disease. Spores of *B. anthracis* have been used as biowarfare agents to terrorize civilian populations, rendering an efficient detection system of spores necessary. *B. anthracis* bears unique oligosaccharides on the surface of the spore for interaction with the host. A convergent total synthesis of a *B. anthracis* tetrasaccharide antigen equipped with a linker on the reducing end of **59** (Figure 2) was established.[76] The nonreducing terminal sugar, anthrose **60**, is not even found in closely related species.[77]

A convergent [2 + 2] approach was chosen for the synthesis, and a terminal pentenyl group was used as the point of attachment for conjugation to a carrier protein in vaccine development (Scheme 11). Synthesis of the terminal anthrose building block **70** started from commercially available D-fucose where the configuration of C4 was inverted by triflation of the free hydroxyl group, followed by a S_N2-type displacement with sodium azide. Building blocks **61**, **62**, and **63** were derived from L-rhamnose. Glycosylating agents **61**, **64**, and **66** were equipped with participating ester groups at C2 to ensure the 1,2-trans relationship in all couplings. To complete the total synthesis, the two disaccharide units **65** and **66** were coupled to afford tetrasaccharide **67**. Sodium in liquid ammonia removed all permanent protecting groups and transformed the azide moiety into an amine. The formation of the amide with 3-hydroxy-3-methylbutanoic acid led to desired pentenyl equipped tetrasaccharide **59**.

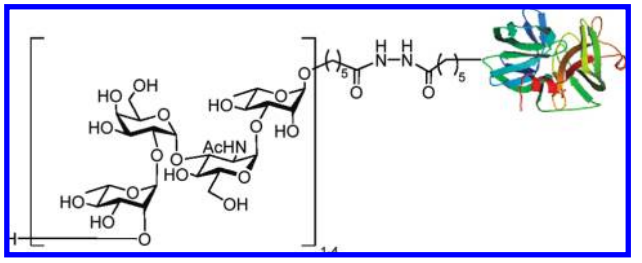


Figure 1. Conjugates of the synthetic tetrasaccharide repeat unit of the *O*-SP of *S. dysenteriae* type 1 to human serum albumin.[72]

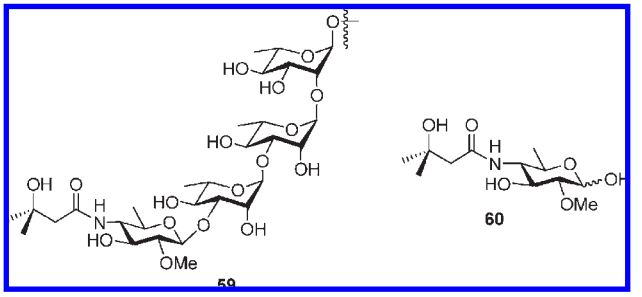


Figure 2. Structure of the terminal tetrasaccharide **59** of the major surface glycoprotein of *B. anthracis* and the structure of anthrose **60**.

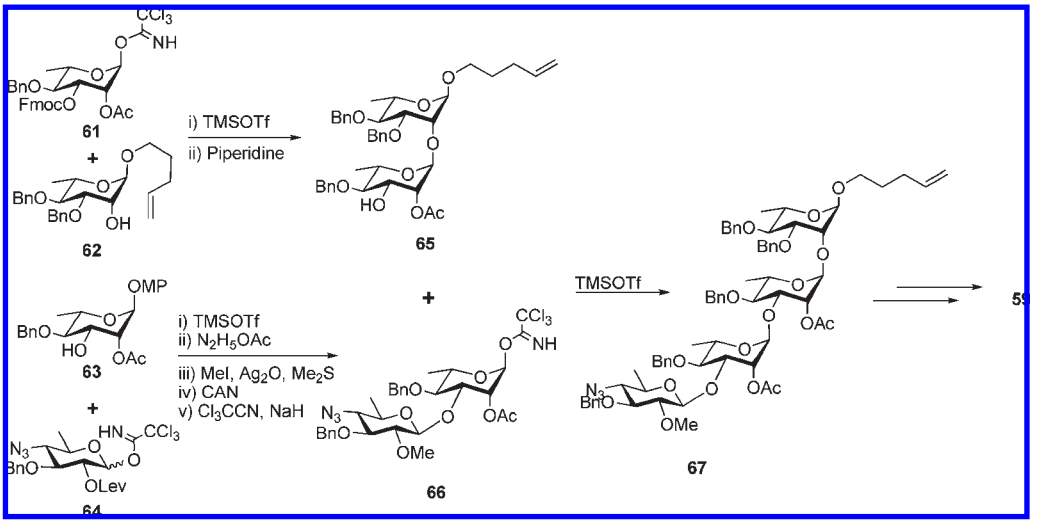
Covalent attachment of carbohydrate antigen **59** to the carrier protein keyhole-limpet-hemocyanine (KLH) by reductive amination (cf. Scheme 6) and immunization of mice with this construct led to substantial antibody responses.[78] Tetrasaccharide-specific monoclonal IgG antibodies bound specifically to native *B. anthracis* endospores and showed no cross-reactivity to endospores of other bacteria, indicating that these antibodies provide a highly sensitive and specific detection system for *B. anthracis* endospores. Total syntheses of oligosaccharides from the major vegetative cell wall polysaccharide of *B. anthracis* have been reported as well.[79,80] Both immunological evaluations and the preparation of derivatives are under investigation.

Synthesis of tetrasaccharide **59** relied on the traditional synthesis from fully functionalized monosaccharides and their connection via glycosylation reactions. A de novo strategy has been reported by O'Doherty and co-workers (Scheme 12)[81] that makes use of palladium-catalyzed glycosylations between a carbohydrate fragment and pyranone unit **69** that is subsequently converted to a sugar via a reduction/dihydroxylation sequence. Asymmetric synthesis from relatively inexpensive acetylfuran **68** furnished the pyranone unit.

Antiparasite Vaccines

Similar to bacteria, many parasites are characterized by unique glycoconjugates on their surface that may be used as targets for eliciting a protective immune response. Pure and defined oligosaccharide structures of parasites are essential for their further evaluation as vaccines. The benefit of synthetic approaches in vaccine development has been shown for malaria, a parasitic disease caused by *Plasmodium*. *Plasmodium falciparum*, the most deadly species of this genus, remains one of the leading causes of morbidity and mortality.[82]

Scheme 11. Synthesis of *B. anthracis* Antigen Tetrasaccharide **59**



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In endemic regions, virtually every individual becomes infected at least once a year and about 2 million patients die from severe malaria annually, most of them children below the age of 5. Clinical manifestations are exclusively linked to the blood stage of infection.[83] Glycosylphosphatidylinositol (GPI) of plasmodial origin has been described as "malaria toxin", since it leads macrophages to secrete proinflammatory cytokines such as TNF-α, IL-1, and nitric oxide, thus playing a central role in the pathogenesis of malaria.[84] A hexasaccharide from plasmodial GPI was synthesized in our laboratory, covalently linked to a carrier protein, and mice were immunized with this antitoxic vaccine candidate (Figure 3).[85,86] Vaccination of mice with the GPI hexasaccharide before infection with *P. berghei* ANKA resulted in protection from pathology such as malarial acidosis, pulmonary edema, cerebral syndrome, and fatality. Immunized mice showed clearly reduced death rates (75% survival) compared to sham-immunized mice (0−9% survival). Parasitemia was unaltered, indicating the specificity of the induced antibody response in blocking an overwhelming proinflammatory immune response, leading to pathology without interfering with parasite replication. The finding that no cross-reactivity with mouse GPI structures was observed renders this GPI glycan a promising vaccine candidate.[86] Clinical studies will show the efficacy of the vaccine to protect people in endemic areas from developing clinical symptoms of malaria. Since small amounts of synthetic material will be sufficient for vaccination purposes, about 4 kg of antigen will be needed each year.

The synthesis of either lipidated GPI structures or GPI glycan analogues from *Plasmodium* not only serves for vaccine development[87,88] but can also be used as tools to elucidate biochemical pathways involved in malaria pathophysiology.

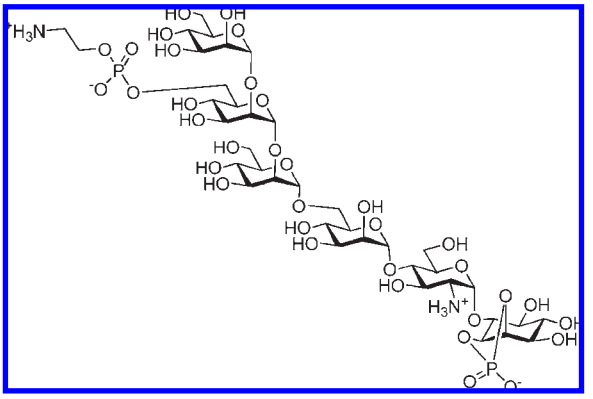


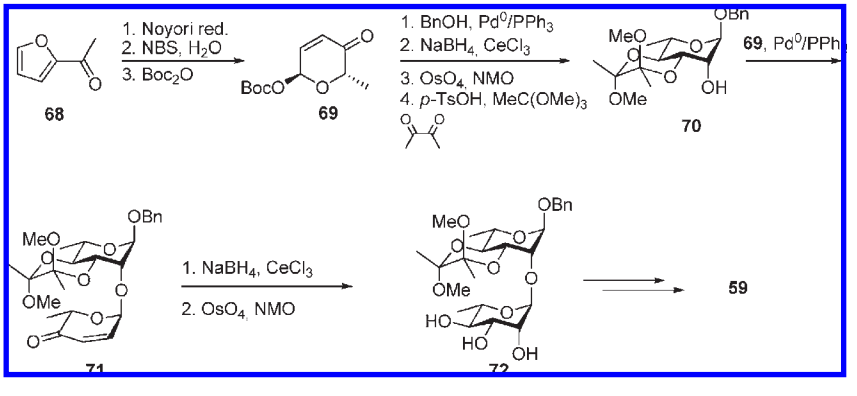
Figure 3. Structure of GPI hexasaccharide from *P. falciparum*.

By use of a synthetic GPI glycan array, anti-GPI antibody levels in infected humans were correlated with protection from severe malaria.[89] Moreover, with the help of synthetic GPI structures, a putative mechanism contributing to malaria anemia was identified.[90] Together with immunological tools, synthetic GPI structures can be used to identify GPI receptors responsible for the induction of a proinflammatory response. Purified GPI from cultured parasites are recognized by TLR2 and to a lesser extent by TLR4.[91] However, TLRs are dispensable for the induction of cerebral malaria as well as parasite control during murine malaria.[92,93] By use of synthetic plasmodial GPI glycan, a new receptor was identified responsible for induction of a pro-inflammatory response in macrophages.[94]

Leishmaniasis is another parasitic disease where the use of synthetic carbohydrate structures may lead to the development of a vaccine. Visceral leishmaniasis, transmitted by phlebotomine sandflies, is a systemic protozoan disease that is endemic in East Africa and the Indian subcontinent.[95] A family of glycoconjugates, the phosphoglycans, play an important role in the persistence of *Leishmania* parasites in infected host macrophages. Lipophosphoglycan (LPG), an abundant promastigote surface glycolipid, has been considered a vaccine target for several years. A unique tetrasaccharide antigen found on the *Leishmania* LPG was prepared using solution- and solid-phase synthetic strategies.[96,97] To enhance the immunogenicity of the vaccine and increase its efficacy, virosomal formulations of the synthetic oligosaccharide were prepared.[98] Conjugation of the cap tetrasaccharide to phospholipid and the influenza virus coat protein hemagglutinin resulted in both IgM and IgG anti-glycan antibodies in immunized mice. The observation that the antibodies elicited upon immunization recognized the natural carbohydrate antigens expressed by *Leishmania* indicates that virosomes might represent a useful delivery system for the application of carbohydrate vaccines.

Other parasitic diseases for which carbohydrate synthesis might also be of interest with regard to vaccine development are toxoplasmosis and Chagas disease. Infection with *Toxoplasma gondii*, the causative agent of toxoplasmosis, usually leads to minor and self-limiting disease but often causes deformities or fatality in the fetus when a woman contracts the disease during pregnancy.[99] GPI anchors of *T. gondii* elicit an immune response in humans and have been reported to induce TNF-α production in macrophages.[100] Synthesis of GPI structures of *T. gondii* might be helpful to further dissect pathways involved in GPI−receptor interactions as well as the development of a protective vaccine.[87] The pathogenic

Scheme 12. De Novo Synthesis of Tetrasaccharide **59**



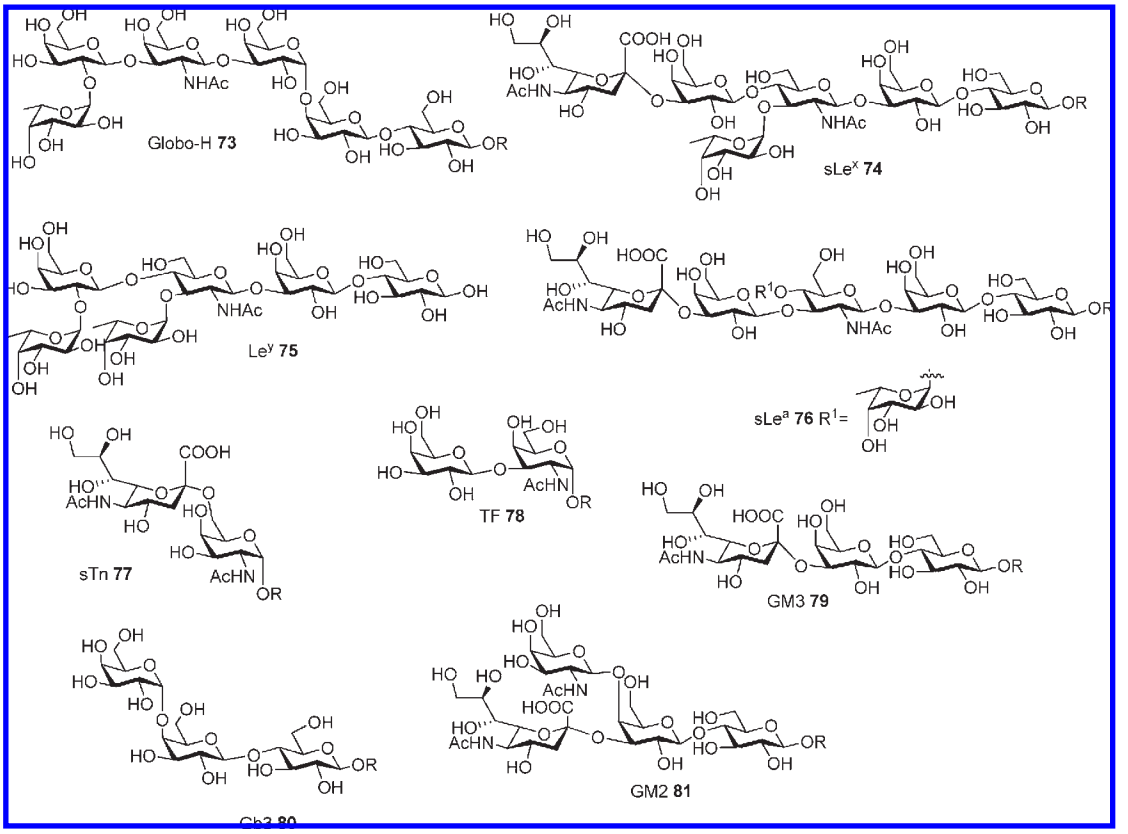


Figure 4. Selection of tumor-associated carbohydrate antigens (TACAs) that are attached to either ceramides or proteins (indicated as R).

protozoan parasite *Trypanosoma cruzi* displays on its surface a family of glycosylinositolphospholipids closely related to GPI anchors involved in the modulation of host T and B cell responses.[101] Similarly, the helminth *Schistosoma mansoni*, the causative agent of schistosomiasis, displays glycans and glycoconjugates on its surface that play a prominent role in the biology of the parasite, particularly in the interaction with host cells.[102] Thus, carbohydrate synthesis will contribute to the identification of receptors and lead to a deeper understanding of how immune responses are modulated during infections with parasitic protozoa or helminths.

HIV

Development of a vaccine against human immunodeficiency virus (HIV) remains an urgent challenge, since HIV is becoming one of the most common chronic infectious diseases worldwide.[103] The creation of an effective vaccine is impeded by the fact that a dense carbohydrate coat on the surface of the virus masks peptide antigens that serve as vaccine targets. The viral coat glycoprotein, gp120, is rich in mannose residues[104] and binds to C-type lectins such as the mannose receptor, langerin, and DC-SIGN.[105] The glycans of gp120 represent attractive targets for therapeutic interventions, i.e., lectins, to prevent viral infection or transmission. Microbicidal lectins have been used for competitive inhibition of HIV entry in cells, and at least one, cyanovirin, has proven efficient in vivo.[106] Cyanovirin, an 11 kDa protein from the cyanobacterium *Nostoc ellipsosporum*, is a lectin with specificity for α-(1→2)-linked mannose residues. We identified structural requirements for the binding of recombinant cyanovirin complexed to synthetic oligosaccharide structures.[107,108] Carbohydrate microarrays were employed as a tool to investigate protein−carbohydrate and protein−glycoprotein interactions in HIV glycobiology. Composed of a series of high-mannose oligosaccharides, these microarrays were used to establish the binding profiles of the gp120 binding proteins DC-SIGN, CD4, 2G12, and cyanovirin, and the carbohydrate structural requirements for these interactions were determined.[109] In addition, a novel HIV-inactivating protein, scytovirin, was identified using this microarray platform. Scytovirin holds potential as an HIV entry inhibitor for both therapeutic and prophylactic anti-HIV applications.[109−111]

For vaccines based on carbohydrates, the identification of neutralizing antibodies that block viral entry into host cells has proven useful. One of these neutralizing antibodies, 2G12, binds exclusively to the carbohydrates of gp120 in the dense cluster of the oligomannose glycans found on the outer domain.[112,113] Multivalent glycopeptide constructs that mimic gp120s binding to 2G12 have been prepared by chemical synthesis.[114−117] Furthermore, structural optimization of synthetic glycopeptides might lead to an improved antigen uptake by APCs and presentation to T cells and thus may constitute a valuable path to an HIV vaccine. Strategies based on glycobiology and glycochemistry, i.e., the combination of carbohydrate microarrays to unravel carbohydrate−protein interactions essential for virus entry and carbohydrate synthesis, may lead to improved prevention and/or treatment modalities for HIV/AIDS.

Anticancer Vaccines

Alterations in the glycosylation status of cancer cells compared to normal cells are common. Transformation to cancer cells is accompanied by the accumulation of new glycan structures such as Globo-H **73**, sialyl-Le^x (sLe^x) **74**, Le^y **75**,

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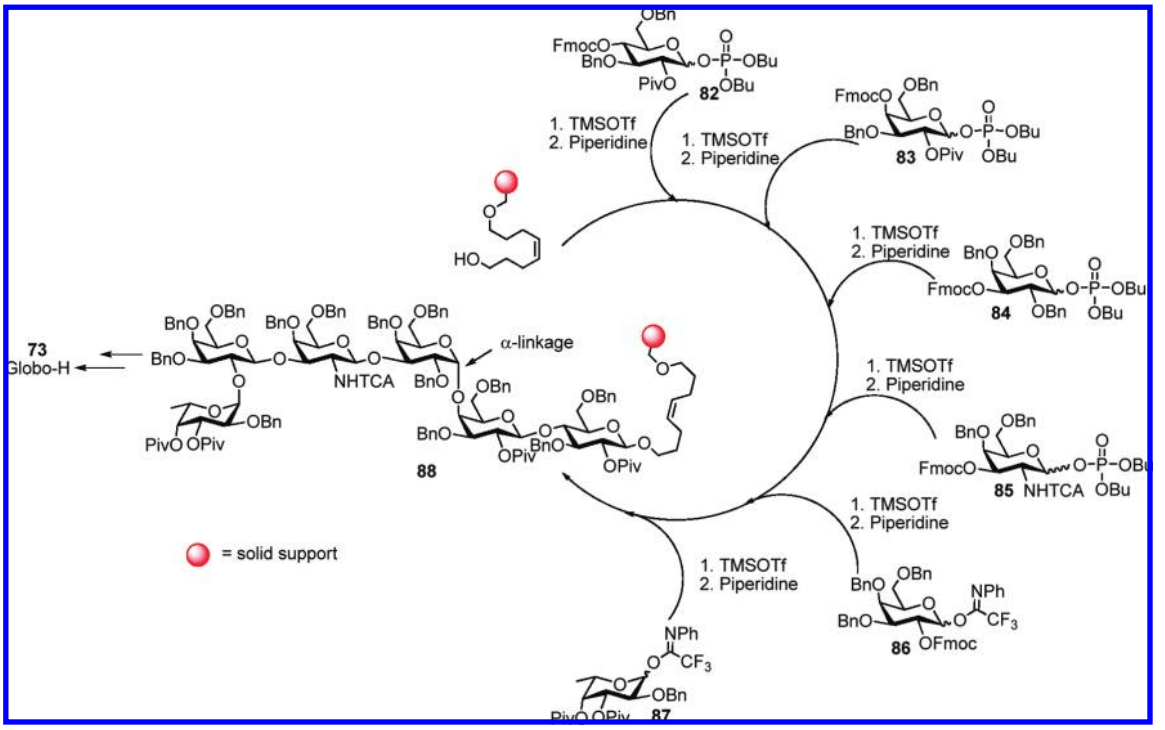
Scheme 13. Automated Synthesis of Globo-H



sLe[a] **76**, sTn **77**, TF **78**, GM3 **79**, Gb3 **80**, GM2 **81** on the cell surface (Figure 4).[118] Expression of these tumor-associated carbohydrate antigens (TACAs) on glycolipids or glycoproteins containing *N*- or *O*-linked glycans often allows adhesion or invasion of cancer cells, thus contributing to the metastasis process. High serum levels of sLe[x] and sLe[a] in pancreatic, prostate, and colorectal cancer as well as high Tn and sTn levels correlate with poor prognosis.

Since TACAs are predominantly expressed on cancer tissues, they provide tools not only for diagnostic purposes as tumor markers but also for therapeutic interventions. Yet, many TACAs are of embryonic origin or expressed in normal tissue, at least at low levels. Although TACAs might serve as antigens to elicit an antitumor immune response, they are often not immunogenic enough to induce protection. The aim of an anticancer vaccine development is, therefore, to increase the immunogenicity of TACAs in order to break immune self-tolerance. Various attempts for anticancer immunotherapy have been reported, including the administration of killed tumor cells or plasmid DNA.[119,120] One way of breaking self-tolerance to established tumors lies in the covalent linkage of TACAs to immunogenic carrier proteins such as KLH.[121] Presentation of peptide epitopes by MHC molecules on antigen presenting cells leads to helper T cell recruitment and finally results in antibody- and cell-mediated immune responses against the glycan.[122−124] A sTn-KLH conjugate (Theratope) failed to meet the study goals in a phase III clinical trial but showed modest efficacy.[125] A potent anticancer vaccine based on TACAs, in addition to inducing a substantial B cell response, has to stimulate cytotoxic CD8$^+$ T cells that directly target and destroy tumor cells. Coupling synthetic sTn glycopeptides from the MUC1 antigen to a CD4$^+$ T cell epitope from ovalbumin ($OVA_{323-339}$) demonstrated that CD4$^+$ T cells providing T cell help for B cells to undergo isotype-switching and to produce high-affinity antibodies are also important. Transgenic mice expressing a specific T cell receptor for the $OVA_{323-339}$ epitope were immunized with the construct.[126,127] The high specificity of MUC1-specific antibodies induced by the immunization is an encouraging result for the development of efficient anticancer vaccines. Using TACAs associated with peptides containing CD4$^+$ T cell epitopes increased the levels of antibodies with higher affinity.[128] To increase the carbohydrate density, dendrimeric glycopeptides were synthesized that were improved by introducing promiscuous HLA-restricted T cell epitopes for use in humans.[129,130] Chemical modification of monosaccharides further increases the immunogenicity of carbohydrate-based anticancer vaccines.[131] Modified sialic acid with unnatural *N*-acyl side chains elicited enhanced immune responses when compared to the natural sugars.[132,133] Passive immunization with a monoclonal antibody raised against TACA-KLH conjugates inhibited tumor growth and metastasis in animal models.[134,135] Besides these semisynthetic vaccines composed of synthetic TACAs conjugated to a protein, fully synthetic vaccines involving synthetic TACAs as well as synthetic peptides or lipids have been explored. Tripalmitoyl-*S*-glycerylcysteinylserine (PAM), a strong TLR2 agonist, has been used to increase the efficacy of TACA-based anticancer vaccines.[136] Prominent members of the Globo series of tumor antigens include Globo-H, Gb5, and Gb3. Globo-H was first isolated and identified as an antigen on breast, prostate, and ovarian cancer cells. Synthetic Globo-H carbohydrate−protein constructs have been evaluated in clinical trials as an anticancer vaccine for the treatment of breast and prostate cancers.[137]

Synthetic access to antigens of the Globo series has been accelerated using new methods.[138−141] Automated synthesis delivered Globo-H and Gb3 in about 1 day.[42] Six building blocks were required for the synthesis of the fully protected Globo-H hexasaccharide (**82**−**87**, Scheme 13). A major challenge in any synthesis of Globo-H is the installation of the α-galactosidic linkage, indicated by an arrow in Scheme 13.

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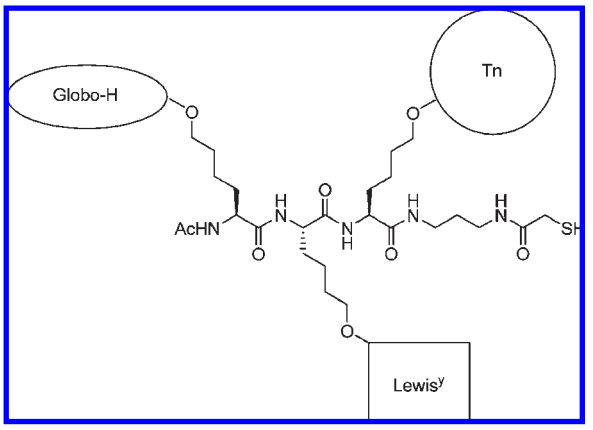

Figure 5. Polyvalent vaccine candidate including Globo-H, Tn, and Le^y.

Good α-selectivity is mandatory for the coupling, since the solid-phase approach allows for purification only after completion of the synthesis. Assembly of hexasaccharide **88** required 25 h before cleavage from the polymer support by olefin cross-metathesis yielded the crude product. Purification and removal of all protecting groups then furnished pentenyl linker equipped Globo-H **73** (R = pentenyl).

To date, no carbohydrate-based anticancer vaccine has succeeded in meeting clinically relevant end points in clinical trials. However, by use of appropriate adjuvants to break tolerance and combine vaccination strategies against TACAs with immunotherapies, a breakthrough may be achieved. Promising results were obtained when a three-component vaccine composed of a TLR2 agonist, a promiscuous peptide T helper epitope, and a tumor-associated glycopeptide was used to immunize mice.[142] Chemical attachment of the TLR2 agonist Pam_3CSK_4 to B and T cell epitopes facilitated uptake by APCs and led to a high local concentration of cytokines. In this approach exceptionally high titers of IgG antibodies recognizing the appropriate antigen on tumor cells were raised. Another strategy to increase the immunogenicity of an anticancer vaccine is targeting several TACAs in a polyvalent approach. Danishefsky et al. have provided a construct that involves a peptide backbone that carries Le^y, Globo-H, and Tn carbohydrate domains connected via a non-natural tetramethylene linker (Figure 5).[143]

Influenza

The strain H5N1, a highly virulent avian influenza virus, has been spreading in East Asia and Europe, illustrating the potential of H5N1 to cause a pandemia. The remarkable similarity between the strain responsible for the Spanish influenza pandemic in 1918 and H5N1 has been detected by gene-sequence analysis and virus reconstruction.[144] Virus entry into host cells is mediated by interaction of the viral protein hemagglutinin (HA) with cell-surface glycans carrying terminal *N*-acetylneuraminic acid (Neu5Ac). While human influenza viruses preferentially recognize the Neu5Acα(2→6)Gal linkage, avian flu viruses bind to the Neu5Acα(2→3)Gal motif (Figure 6).[145]

A possible strategy to prevent virus entry into host cells is binding inhibition using oligosaccharides with high HA affinity. Cyclic peptides containing three sialotrisaccharide units that bind HA inhibited virus entry.[146] More potent inhibitors may be developed by multiple display of oligosaccharides on

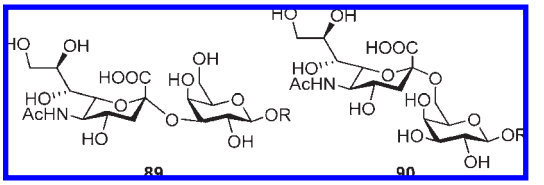

Figure 6. Neu5Acα(2→3)Gal **89** and the Neu5Acα(2→6)Gal **90** motifs.

dendrimers. Poly(amidoamine) dendrimers conjugated with sialic acid were able to inhibit binding of three influenza subtype strains in vitro and prevented infection with the H3N2 subtype in a murine model of influenza pneumonitis.[147]

Conclusions and Future Prospects

The prevention of parasitic, bacterial, and viral infections is an urgent global medical need. Carbohydrate-based vaccines against a host of targets is in principle feasible not only for the diseases mentioned above but also against a number of other pathogens including *Vibrio cholerae*, *Pseudomonas aeruginosa,* or *Borrelia burgdorferi*.[58] Carbohydrate synthesis represents an important tool to access molecules that may be the basis for the development of vaccines against emerging diseases. Alternative delivery systems such as liposomes and dendrimers[148] will have to be tested to enhance the uptake of the glycan structures by APCs and increase antigen presentation to T cells. The use of appropriate adjuvants will be essential to increase the immunogenicity of carbohydrates. The development of new adjuvants in addition to the well-known adjuvants Alum or QS-21 is crucial. The search for immunostimulatory compounds continues.[149] Synthesis of pathogenic glycan structures such as bacterial cell wall components may thus lead to the identification of new adjuvants. Targeting of C-type lectin receptors (CLRs) with synthetic carbohydrate structures induces an additional danger signal to APCs and increases the immune response against model antigens.[150] Carbohydrate−protein interactions have been exploited for receptor-mediated endocytosis in vitro.[151,152] The specificity of these interactions seems to be sufficient for targeted drug delivery in vivo.[153] Carbohydrates will not only be the basis for vaccines but likely also provide new adjuvants and drug delivery systems.

Biographies

Pierre Stallforth obtained his Master of Chemistry from the University of Oxford, U.K., in 2006. He started his Ph.D. studies, funded by the Studienstiftung des deutschen Volkes, under the supervision of Prof. Peter H. Seeberger at ETH Zurich and moved with the group to the Max Planck Institute of Colloids and Interfaces. His research interest lies in the de novo synthesis of bacterial glycolipids and bacterial antigens.

Bernd Lepenies graduated with a degree in Biochemistry/Molecular Biology from the University in Hamburg, Germany, in 2004 and remained there for his Ph.D. at the Bernhard Nocht Institute for Tropical Medicine (2007), supervised by Prof. Bernhard Fleischer. Following his postdoctoral research in the group of Prof. Seeberger at ETH Zurich, he became a group leader at the Max Planck Institute of Colloids and Interfaces sponsored by the German Federal Ministry of Education and Research. His research interests are in chemical biology, targeted drug delivery, and basic immunology with a special focus on infectious diseases.

Alexander Adibekian obtained his M.Sc. in Organic Chemistry/Molecular Biology from the University of Hannover, Germany,

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