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Solid-Phase Synthesis of Oligosaccharides and Glycoconjugates by the Glycal Assembly Method: A Five Year Retrospective

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Introduction

Nucleic acids, proteins, and polysaccharides are the three major classes of biopolymers found in eukaryotes. Although the first two systems are principally linear assemblies, polysaccharides are structurally more complex. This structural and stereochemical diversity results in a rich content of "information" in relatively small molecules. Nature further amplifies the structural versatility of polysaccharides by their covalent attachment (i.e., "conjugation") to a diverse array of other biomolecules such as isoprenoids, fatty acids, neutral lipids, peptides, or proteins.[1]

Oligosaccharides and glycoconjugates sharply influence many critical biological functions.[2] They mediate a variety of events, including inflammation, immunological response, metastasis, and fertilization.[3] Cell surface carbohydrates provide biological markers for various tumors and function as sites for other invasive programs including those of pathogenic infection.[4]

The increasing recognition of the roles of oligosaccharides and glycoconjugates in fundamental life-sustaining processes has served to accentuate the need for access to usable quantities of these materials. Glycoconjugates are difficult to isolate in homogeneous form from living cells because they exist as microheterogeneous mixtures. The purification of these compounds, even when possible, is at best tedious and is generally achieved in marginal yields. Given the travails associated with isolation from natural sources, a major opportunity for chemical synthesis presents itself.[5]

The invention of solid-phase peptide synthesis by Merrifield 35 years ago dramatically altered modalities for the synthesis of biopolymers.[6] The preparation of structurally defined oligopeptides[7] and oligonucleotides[8] has benefited greatly from the feasibility of conducting their assembly on various polymer supports. The advantages of solid matrix-based synthesis in terms of allowing for an excess of reagents to be used and in the facilitation of product purification are now well appreciated. It is obvious that the level of complexity associated with the synthesis of an oligosaccharide on a polymer support dwarfs that associated with the other two classes of repeating biooligomers. First, the need to differentiate similar functionalities (hydroxyl or amino) in oligosaccharide construction is much more challenging than is the situation with oligopeptides or oligonucleotides. Furthermore, in these latter two cases, there is no stereoselection associated with construction of the repeating amide or phosphate bonds. By contrast, each glycosidic bond to be fashioned in a growing oligosaccharide ensemble constitutes a new locus of stereogenicity.

Remarkably, a great deal of progress had been achieved in assembling relatively complex carbohydrate ensembles on a solid support. Advances along these lines have involved the need for considerable simplification and refinement of protecting group strategies and the development of glycosylation methodology that is workably stereoselective and amenable to being conducted with one component anchored to an insoluble matrix.[9]

Much of the effort has been directed at the synthesis of glycopeptides on solid support. The groups of Kunz,

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Samuel J. Danishefsky was born March 10, 1936, and raised in New Jersey. His parents were Jacob and Anna Danishefsky. Under the tutelage of his father, he was exposed at an early age to the elements of logical thought and critical analysis, through the study of the Talmud. His formal training included a high school degree at the Talmudical Academy in Manhattan and a B.S. degree at Yeshiva University (1956). In keeping with the example of an older brother (Isadore), he took an interest in chemistry in college. A life-long fascination with organic chemistry followed from absorption with two introductory treatments of the subject - one by Raymond Brewster and the other by Louis and Mary Fieser. This exposure led him to pursue graduate studies at Harvard University where he received a Ph.D. in 1962 under the direction of Professor Peter Yates. From 1961 - 1963 he was an NIH sponsored Postdoctoral Fellow at Columbia University under the mentorship of Gilbert Stork. His first independent academic position, which started in 1963, was at the University of Pittsburgh, where he became Professor in 1971 and University Professor in 1979. In 1980 he moved to Yale University and served as chairman of the department from 1981 - 1987. He was named Eugene Higgins Professor in 1984 and Sterling Professor in 1990. In 1993 he returned to New York as Professor of Chemistry at Columbia University and as Kettering Professor and Head of the Laboratory for Bioorganic Chemistry at the Memorial Sloan-Kettering Cancer Center. His research interests have been in the areas of synthetic strategy, synthetic methodology, cytotoxic natural products and, most recently, fully synthetic carbohydrate-based tumor antigens. His honors include membership in the Connecticut Academy of Science, the American Association for the Advancement of Arts and Sciences, the American Academy of Arts and Sciences and the National Academy of Sciences. He has received the Guenther Award, the Aldrich Award, a Cope Scholar Award, and the Hudson Award from the American Chemical Society. In 1995 he received the Tischler Medal from Harvard University and in 1996 he shared the Wolf Prize in Chemistry with Gilbert Stork. In 1997 he received the Tetrahedron Prize for Creative work in Organic Chemistry. He will receive the 1998 ACS Cope Award.

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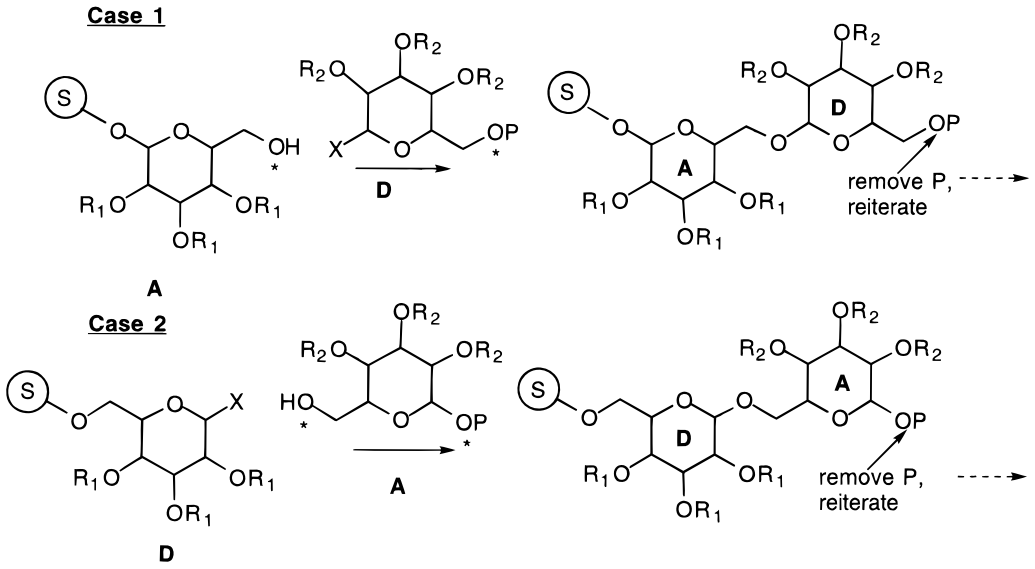
Scheme 1. Glycosyl Acceptor (Case 1) and Donor (Case 2) Bound to the Solid Support[a]



[a] Key: S, solid support; P, unique protecting group; X, activating group; *, uniquely differentiated hydroxyl group.

Meldal, Paulsen, Wong, Bock, and Waldmann, among others, have provided massive advances in this field. We may refer the interested reader to recent reviews for more detailed descriptions.[10]

The development of protocols for the solid-support synthesis of oligosaccharides and glycopeptides requires solutions to several problems. Considerable thought must be given to the nature of the support material. The availability of methods for attachment of the carbohydrate from either the "reducing" or "non-reducing" end would be advantageous. Also, selection of a linker that is stable during the synthesis, but can be easily cleaved when appropriate, is critical. A protecting group strategy that allows for high flexibility is desirable. Most important is the matter of stereospecific and high-yielding coupling reactions. Finally, monitoring of chemical transformations by "on resin" methods is obviously beneficial. Tremendous progress on the synthesis of glycoconjugates, particularly glycopeptides, by chemical and enzymatic methods has been achieved through the work of many groups.[10a−f] This work has been reviewed recently[10g] and we will focus in this account on the research conducted in our laboratory over the last five years.

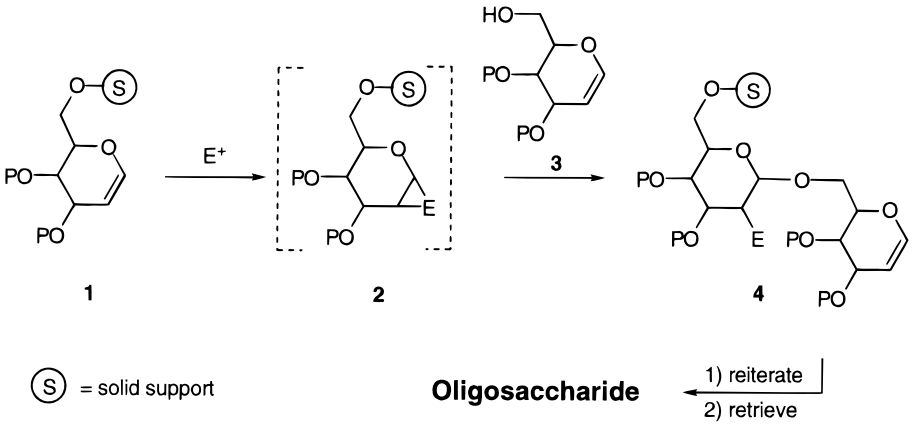
We report here on the progress of the advances in the development of solid-support synthesis methodology in our laboratory that has led to the efficient assembly of complex, biologically interesting oligosaccharides and glycoconjugates. This progress has allowed us to assemble a number of biologically relevant structures including the Lewis[b] blood group pentasaccharide and the globo H hexasaccharide. The synthesis of these compounds will be placed in the context of our general methodological advances.

Fundamental Considerations

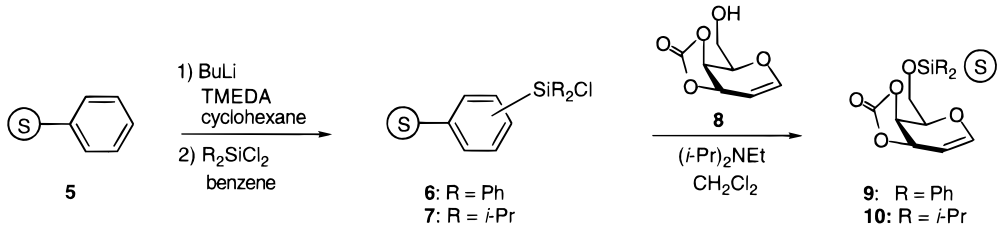
In considering how matrix-supported synthesis can be applied to oligosaccharide and broader glycoconjugate problems, two broad strategies present themselves. In one variation, the first carbohydrate is anchored to the support via its "reducing" end (see Scheme 1, Case 1). The solid support-bound carbohydrate will function as an acceptor in the coupling event to a solution-based donor **D**. As the next cycle is contemplated, a unique acceptor hydroxyl must be exposed in the now elongated, resin-bound carbohydrate construct. This strategy virtually demands that in Case 1, the donor (**D**) employed in the previous glycosidation step would have been furnished with a uniquely removable blocking group at the site of the next proposed elongation. Coexistence of a free hydroxyl in the solution-based donor **D**, *which already bears the intact glycosyl donating function*, is unlikely under the glycosylating conditions. The need to definitively expose the unique hydroxyl group in the context of the polymer support will more than likely necessitate multistep functional group adjustments in synthesizing **D**. The attractiveness of the method is correspondingly compromised.

Alternatively, the oligomer undergoing elongation may be mounted to the support somewhere in a "non-reducing" region, with the reducing and glycosyl donating moiety available for coupling with solution-based acceptor **A** (Case 2). The use of **A**, of course, demands that the precise acceptor site be properly identified. Furthermore, (and, as was the situation in Case 1) in anticipation of the next coupling event, the reducing end of acceptor **A** (Case 2 acceptor) is so functionalized that a new donor capability can be installed at the anomeric carbon of the elongated construct. Not unlike the situation that would prevail in Case 1, a serious question of compatibility under the conditions of glycosylation in Case 2 must be anticipated if one attempts to enter acceptor **A** with a fully equipped, next-phase anomeric donor function already in place.

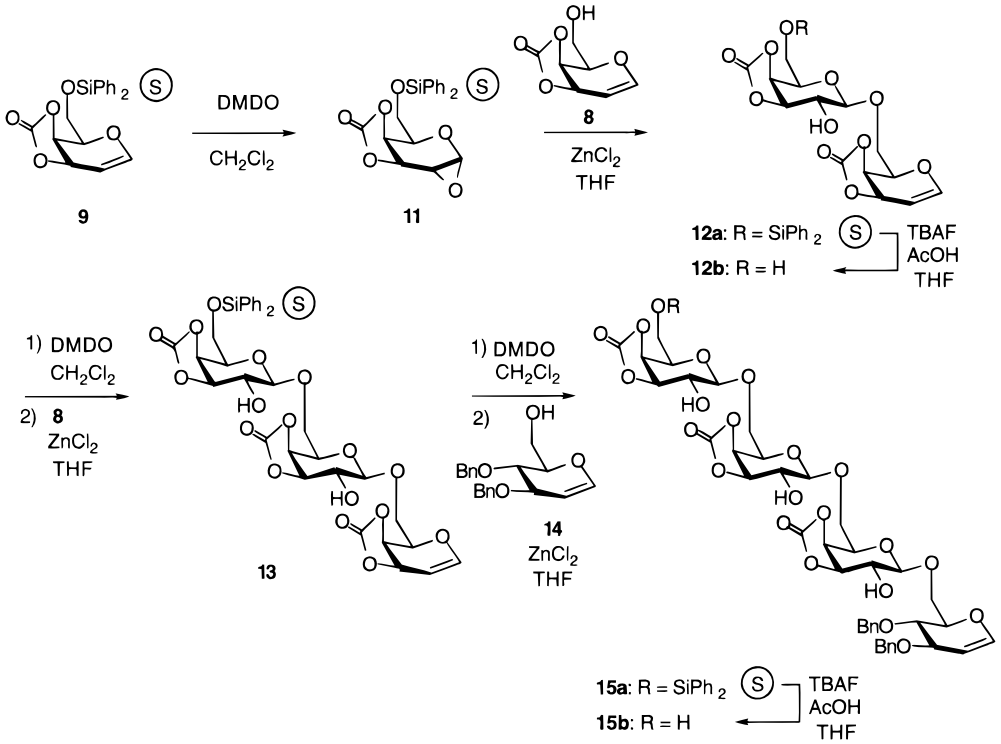
Scheme 2. General Strategy for the Synthesis of Oligosaccharides on a Solid Support Using the Glycal Assembly Method



Scheme 3. Glycal Attachment to a Polystyrene Resin



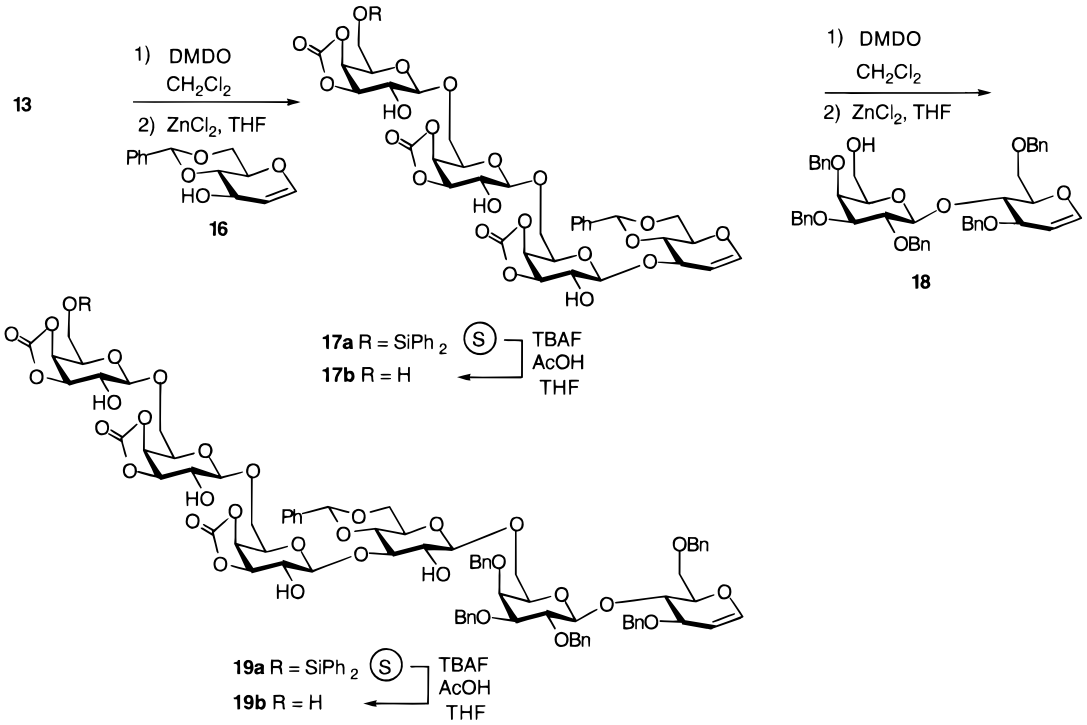
Scheme 4. Solid-Phase Synthesis of a Tetrasaccharide Using the Glycal Assembly Method



It was in dealing with these problems that we perceived of a large advantage of the glycal assembly method. The simplest statement of glycal assembly is captured in the expression **1** → **2** → **4**. Of course, here we leave the nature of E^+ or the onium-like species **2** unspecified. We also, for the moment, confine ourselves to β-glycosidic linkages. These uncertainties notwithstanding, the potential attractiveness of the method for solid-state-supported construction can hardly be missed. We would operate in the paradigm of Case 2. The terminal glycal functions as a readily constituted donor function **2**. Through employment of a glycal as the solution-based acceptor, the scheme benefits from relative simplicity in the identification of strategic hydroxyls for glycosylation.

Compound **2** could be an isolable entity such as a 1,2-anhydrosugar,[11] in which case E^+ corresponds to an epoxidizing agent, or could be a transient species, as would result upon activation of the glycal with an iodo-

Scheme 5. Solid-Phase Synthesis of a Hexasaccharide

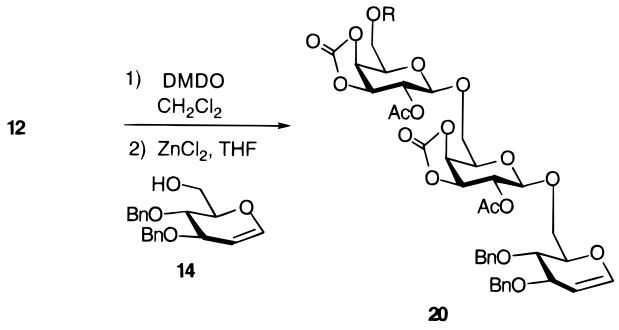


nium source.[12] Compound **2** acts as a support-bound glycosyl donor. When treated with acceptor glycal **4** and any necessary agents to promote the glycosylation, glycoside **4** is obtained. The process can be reiterated to assemble the desired oligosaccharide. The assembly phase is followed by retrieval from the support and purification by chromatographic methods.

To inaugurate the program under the glycal paradigm, we selected polystyrene 1% divinylbenzene copolymer, which is commonly used in solid-support peptide synthesis because of its high loading capacity, compatibility with a wide range of reaction conditions, and low price. We then took advantage of the chemistry of Chan and coworkers.[13] The Canadian group had shown that lithiation of polystyrene can be conducted at aromatic sites and that silylation would be possible with a dihalosilane such as diphenyldichlorosilane, resulting in polymer-bound silyl halide **6**. This silicon−chlorine linkage would be used as an attachment site for a differentiated glycal to create polymer-bound glycal **9**.

This method was first applied in the synthesis of a linear tetrasaccharide outlined in Scheme 4.[14] Polymer-bound galactal **9** was converted to the 1,2-anhydrosugar **11** by epoxidation with 3,3-dimethyldioxirane.[15] Polymer-bound **11** acted as a glycosyl donor. Treatment of the epoxide generated with a solution of **8** in the presence of zinc chloride resulted in the formation of disaccharide **12a**. The glycosylation procedure was reiterated twice, first using acceptor galactal **8** and then acceptor **14** to yield tetrasaccharide **15a**. Fluoridolysis with tetrabutylammonium fluoride (TBAF) cleaved the product from the polymeric support to give tetrasaccharide **15b** in 32% overall yield from **9** (Scheme 4).

Scheme 6. Solid-Phase Synthesis of a Trisaccharide

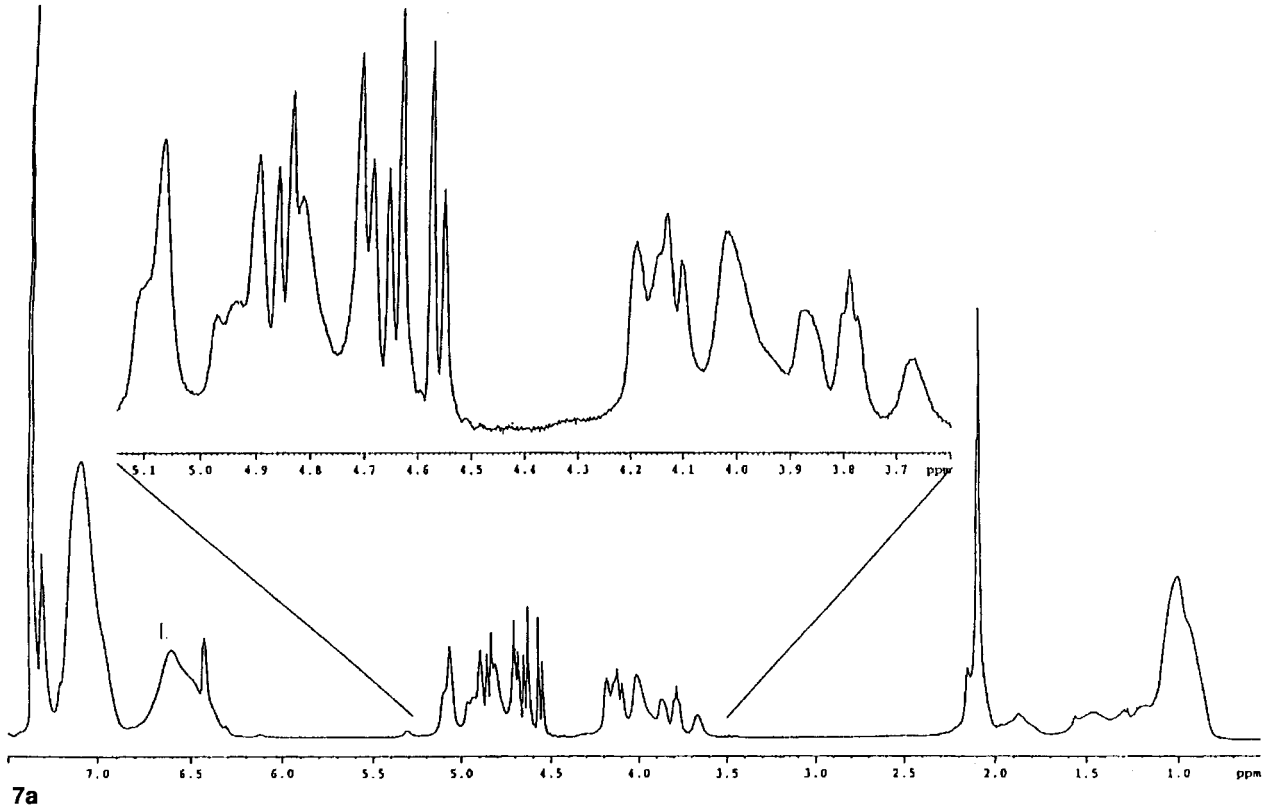


The versatility of this approach was demonstrated in the synthesis of a variety of oligosaccharides. The synthesis of hexasaccharide **19b** included the use of secondary alcohol glycosyl acceptors as well as disaccharide acceptors (Scheme 5).

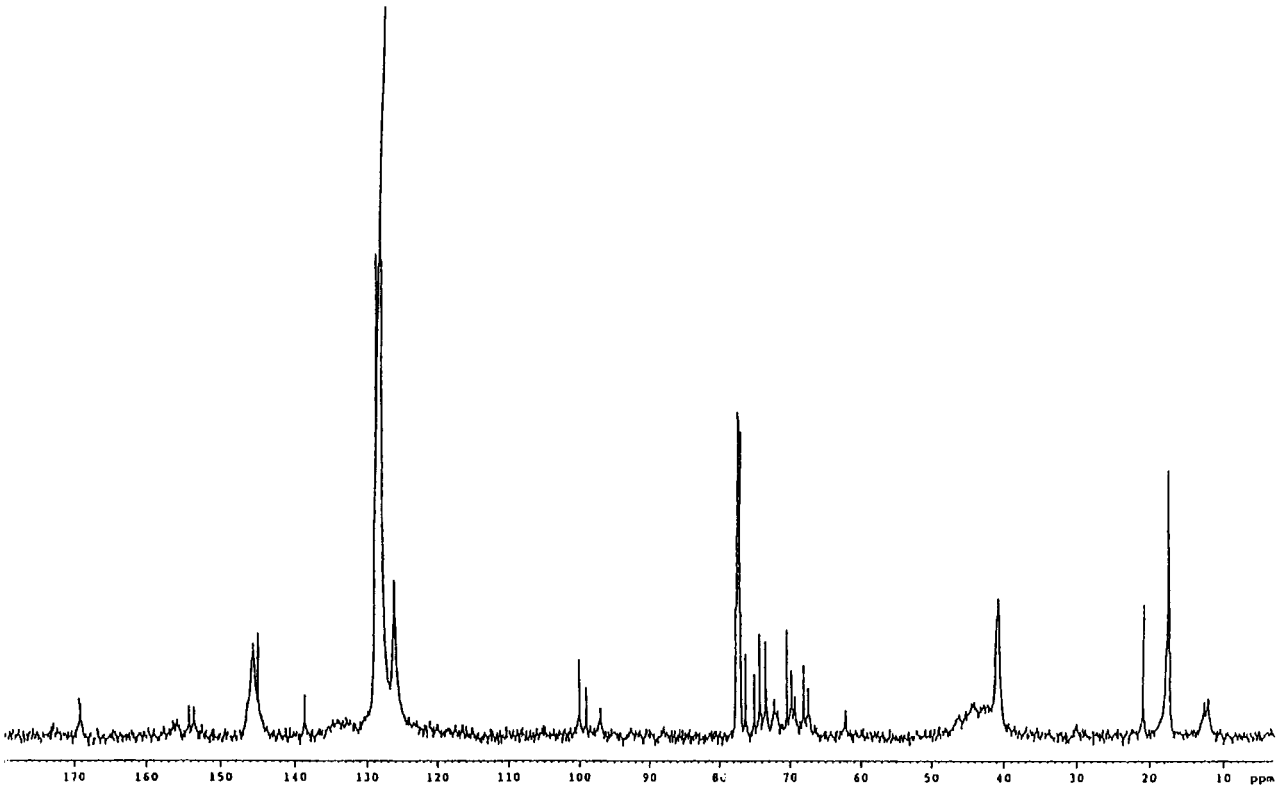
Upon opening of a 1,2-anhydrosugar during glycosylation, a C2 hydroxyl group is exposed. The latter may, in turn, serve as a glycosyl acceptor to form branched oligosaccharides as will be demonstrated on the example of the synthesis of a H-type blood group determinant (vide infra).[16]

Development of reliable solid-phase methodology for the synthesis of oligosaccharides and glycopeptides had been greatly hampered by the lack of convincing "on-resin" analytical techniques. As already outlined, it was customary to cleave the products or intermediates of multistep syntheses from the resin to allow for the use of classical spectroscopic means [e.g.; solution-state nuclear magnetic resonance (NMR) and mass spectrometry]. The cleavage method for analysis is time-consuming and wasteful in the context of multistep syntheses.

Scheme 7. (a) ^{1}H HR-MAS NMR Spectrum of 20 and (b) ^{13}C NMR HR-MAS Spectrum of 20



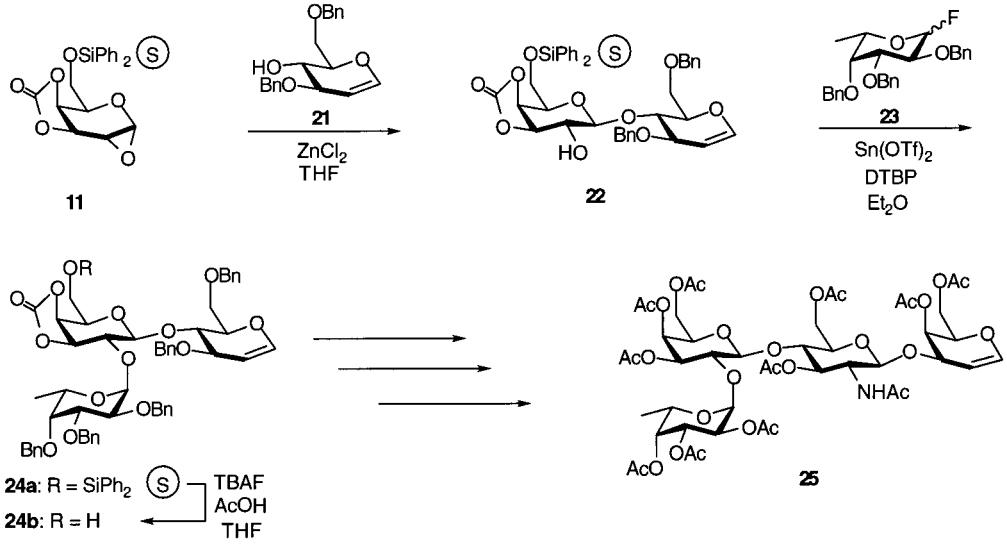
7a



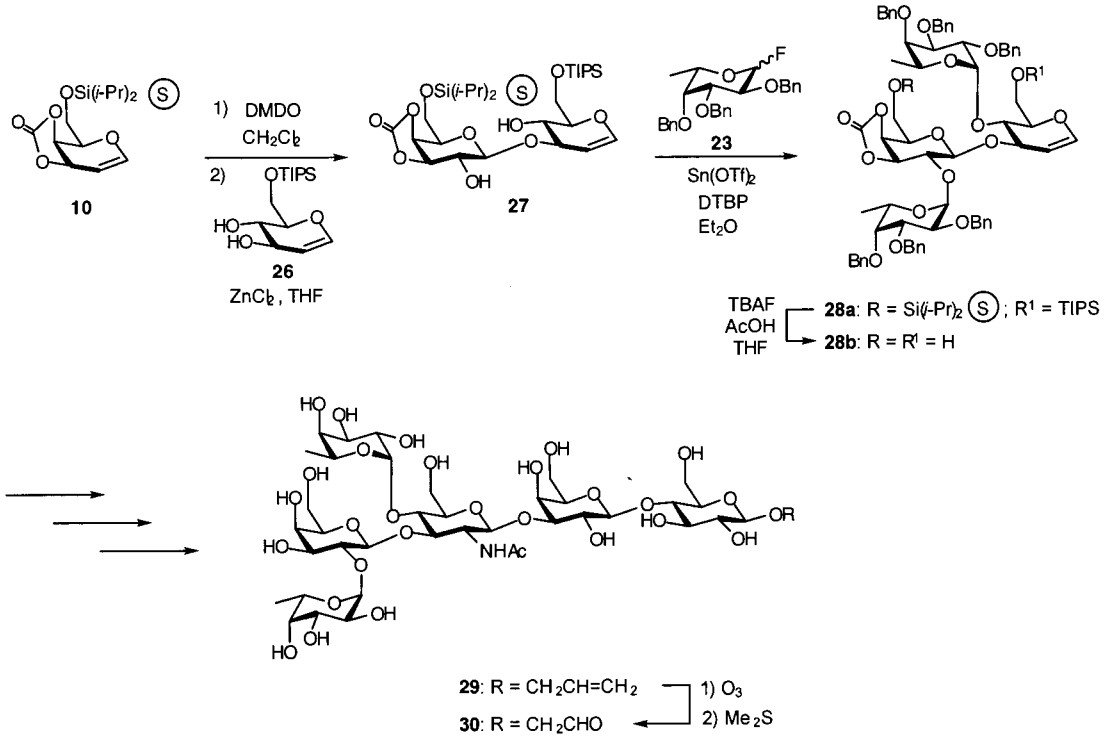
7b

It was with this in mind that we turned to "on-line" NMR analysis as a method of assaying our progress. Indeed, the development of high-resolution magic-angle spinning (HR-MAS) NMR experiments proved to be an ideal way of monitoring the solid-support synthesis by obtaining ^{1}H NMR, ^{13}C NMR, and $^{1}H-^{13}C$ NMR spectra of high quality. Since its introduction, this technique has greatly facilitated the development of novel synthetic

Scheme 8. Solid-Phase Synthesis of a H-Type Blood Group Determinant



Scheme 9. Solid-Phase Synthesis of a Tetrasaccharide Precursor to the Le^b Blood Group Determinant



schemes of oligosaccharides and glycopeptides on a solid support.[14]

The effectiveness of our approach with respect to solid-support oligosaccharide synthesis was conveniently documented by on-resin analysis. The crude solid-phase bound product of the multistep synthesis of trisaccharide **20** is illustrative of the power of the method (Scheme 6). The ^{1}H NMR (Scheme 7) of this material showed that only one product was obtained.

Solid-Phase Synthesis of Blood Group Determinants

The methodology just outlined found striking applications in the synthesis of carbohydrate domains with blood-group-determining specificities.[18] These structures, which are carried naturally in the form of glycoproteins or glycolipids, were found to play key roles in cell adhesion and other binding phenomena.[19,20] Furthermore, glycoconjugates related to these blood group substances have been recognized as markers for the onset of various tumors. These tumor-associated antigens are currently being studied in vaccines for cancer immunotherapy.[21,22]

Initial efforts targeted the assembly of a H-type 2 tetrasaccharide (Scheme 8).[23] Polymer-bound 1,2-anhydrosugar **11**, when treated with a solution of glucal acceptor **21**, provided disaccharide **22**. Compound **22** was fucosylated using a solution of fucosyl donor **23**[24] to furnish trisaccharide **24**. Treatment of **24a** with TBAF

provided trisaccharide glycal **24b** in 50% overall yield from **9**.

Due to the lack of solid-support methodology at that time to fashion glycosidic linkages bearing C_2-acylamino functions, we had to take recourse to solution-phase chemistry in preparing the H-type 2 blood group determinant glycal. The glycal provides a handle for ready functionalization at the reducing end.

The Lewis^b blood group antigen (Le^b) was also of particular interest in this regard because it has been identified as a mediator for the binding of *Helicobacter pylori* to human gastric epithelium.[25] Clinical studies have identified *H. pylori* as a causative agent in gastric and duodenal ulcers[26] and antimicrobial treatments are an effective means to combat infection.[27] Because bacterial attachment is a prerequisite for infection,[28] analogues of the Le^b oligosaccharide may serve as therapeutic alternatives to broad spectrum antibiotics.

Our first approach to Le^b addressed the problem of the core tetrasaccharide, which was assembled on the polymer support as depicted in Scheme 9.[27] Polymer-bound galactal **10** was epoxidized with dimethyldioxirane and then reacted with a solution of glucal derivative **26** to give polymer-bound disaccharide diol **27**. This reaction proceeded in a highly regioselective fashion wherein glycosylation occurred at the allylic position at C3 of **26**. Bisfucosylation of **27** using donor **23** provided polymer-bound tetrasaccharide glycal **28a**. Treatment of **28a** with TBAF gave **28b**, which was obtained in a 40% overall yield from **10**.

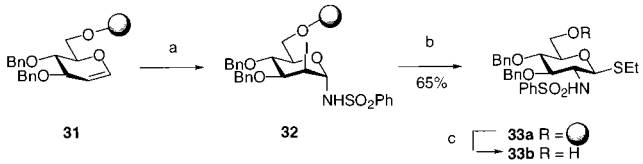
Glycal **28b** was further converted into a hexasaccharide of the Le^b system using solution chemistry. Compound **30** was conjugated with human serum albumin by the action of sodium cyanoborohydride to provide the desired neoglycoprotein.[29] The biological properties of this glycoconjugate are currently being investigated.

Aminoglycosylation on the Solid Support

Our previous approaches toward the synthesis of blood group determinants on a solid support were hampered by a serious shortcoming in our methodological arsenal. Although the glycal assembly method permitted rapid and concise access to β-glycosidic linkages, we had to take recourse to solution-phase methodology for construction of *N*-acetylaminoglucosidic linkages prevalent in biologically important blood group determinants, gangliosides, and N-linked glycopeptides.[30] Fortunately, we have recently been able to overcome this obstacle by conversion of solid-support-bound glycals into iodosulfonamides.

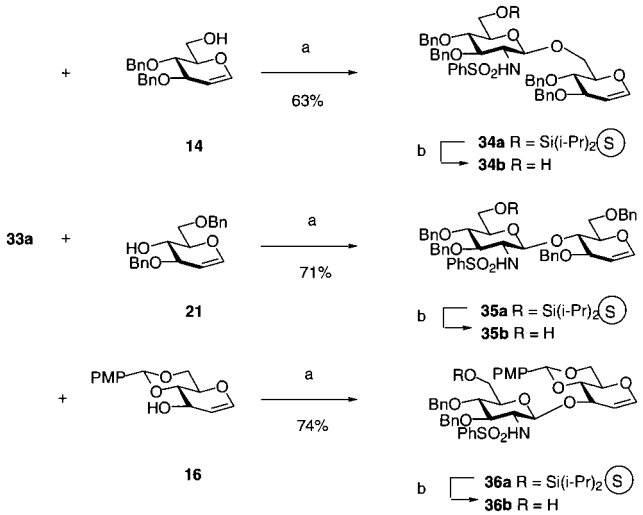
To install appropriate functionality at C2, solution phase chemistry was based on the realization that we must achieve a trans-diaxial addition of an iodonium electrophile to the glycal linkage in the presence of a sulfonamide to form a 1-α-sulfonamido-2-β-iodo product. Displacement of iodine was induced by a thiolate nucleophile to fashion thioethyl 2-amidoglycosyl donors (see Scheme 10).[31] We now attempted to extend this capability to the solid phase. Polymer-supported glucal **31**[32] was treated

Scheme 10. Synthesis of a Polymer-Bound Thioethyl 2-Amido-Glucosyl Donor[a]



[a] (a) $I(coll)_2ClO_4$, $PhSO_2NH_2$, CH_2Cl_2, 0 °C. (b) LHMDS/EtSH, DMF, −40 to 0 °C. (c) TBAF/AcOH, THF, 40 °C, 18 h. Shaded circle = (Si(*i*-Pr)$_2$(polystyrene).

Scheme 11. Synthesis of Disaccharides Using a Polymer-Bound Thioethyl 2-Amino-Glucosyl Donor[a]



[a] (a) MeOTf, DTBP, 4 Å MS, CH_2Cl_2, 0 °C to rt 8 h. (b) TBAF/AcOH, THF, 40 °C, 18 h.

with iodonium sym-collidine perchlorate to form iodosulfonamide **32** as an intermediate. Transdiaxial displacement through the agency of ethanethiolate yielded 65% of the protected thioethyl glycosyl donor **33** (Scheme 10).

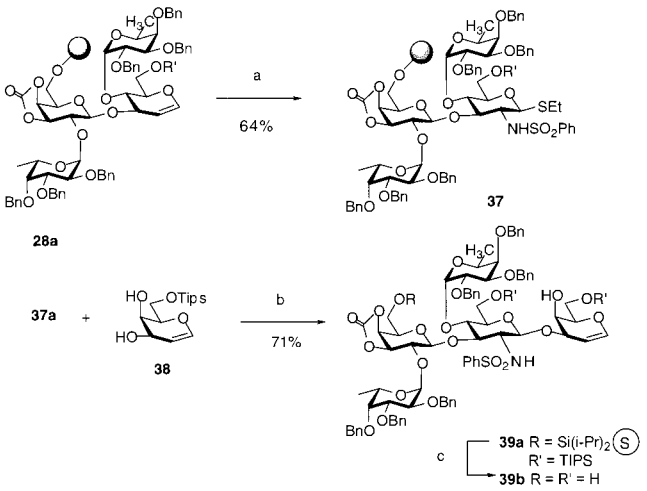
Coupling of donor **33a** by activation with methyl triflate in the presence of one equivalent of the nonnucleophilic base di-tertbutylpyridine (DTBP) proved to be successful in the case of the thioethyl 2-amidoglucosyl donors.[33] The formation of β-2-aminoglucosyl (1→4; **35**)- and β-2-aminoglucosyl (1→3; **36**)-linked disaccharides proceeded in >70% yield (Scheme 11). The β-2-aminoglucosyl (1→6)-linked disaccharide **34** was formed in lower yields.

After an efficient coupling protocol for the synthesis of β-2-amidoglucosidic linkages had been established, this methodology was used to overcome the difficulties during the previous synthesis of the Lewis^b pentasaccharide glycal. Expanding now on these earlier advances, branched tetrasaccharide **28a** was converted into the thioethyl donor **37**. Coupling to galactal acceptor **38** yielded 71% of the desired pentasaccharide **39a** (Scheme 12). Retrieval of the pentasaccharide was accomplished using TBAF to afford **39b** in 20% overall yield from **10**.[34]

Generation and Use of Thioethyl Donors on the Solid Support[35]

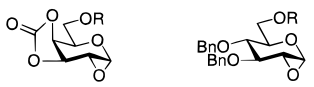
The use of glycals on the solid support allowed for the construction of β-galactosyl linkages with great efficiency,

Scheme 12. Solid Phase Synthesis of the Lewis[b] Blood Group Determinant Pentasaccharide Glycal[a]



[a] (a) (i) $I(coll)_2ClO_4$, $PhSO_2NH_2$, CH_2Cl_2, 0 °C; (ii) LHMDS/EtSH, DMF, −40 to 0 °C. (b) MeOTf, DTBP, 4 Å MS, CH_2Cl_2, 0 °C to rt 8 h. (c) TBAF/AcOH, THF, 40 °C, 18 h.

Scheme 13. Galactosyl- and Glucosyl-1,2-Anhydrosugar Donors

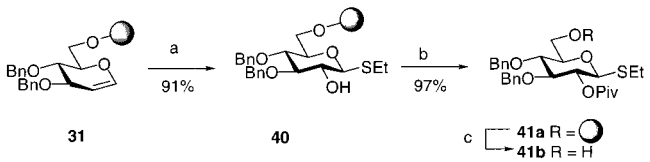


even with hindered glycosyl acceptors, but the analogous β-glucosidic linkages could not be prepared as efficiently. This disparity in capability is in turn related to the glycosyl-donating performances of two donor structure types. In the galactose series, we take advantage of the relative stability of the epoxy donor type to very mild Lewis acids, particularly anhydrous zinc chloride. The stability allows for galactosylation of even hindered acceptors, such as C4 hydroxyls flanked by protecting groups at C3 and C6. No analogous constrained glucosyl epoxy donor is available, and glucosyl systems in the presence of zinc chloride are highly reactive. Unfortunately, with hindered acceptors, degradation of donor is competitive with glycosylation (Scheme 13).

To overcome this shortcoming, we introduced an approach in our solution-phase approach that allowed for the conversion of glycals into thioethyl glycosyl donors.[36] Thioethyl glycosyl donors bearing a participatory protecting group at C2 constitute a class of extremely powerful β-glycosylating agents upon activation with thiophilic reagents.[10] The glycal-derived donors were equipped with a C2 pivaloyl neighboring group and coupled to glycal acceptors to fashion a variety of glycosidic linkages with high efficiency. Pivaloyl neighboring groups had previously been shown by Kunz and others to prevent the formation of ortho ester products during glycosylations.[37]

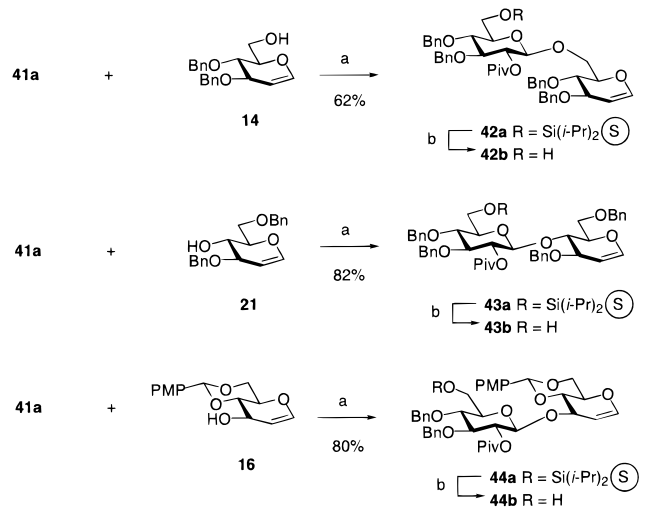
Conversion of **31** to the protected thioethyl glycosyl donor **40** was achieved through epoxidation with dimethyldioxirane to yield the 1,2 anhydrosugar, followed by opening of this intermediate by a mixture of ethanethiol and dichloromethane (1:1) in the presence of a trace of trifluoroacetic acid (Scheme 14). The thioethyl glycosyl donor **40** was obtained in 91% yield. This result constitutes a significant improvement over the 78% yield obtained in solution.

Scheme 14. Synthesis of a Polymer-Bound Thioethyl Glucosyl Donor[a]



[a] (a) (1) DMDO, CH_2Cl_2, 0 °C, 2.5 h; (2) EtSH, $(CF_3CO)_2O$, −78 °C to rt, CH_2Cl_2. (b) PivCl, DMAP, CH_2Cl_2, rt, 4 h. (c) TBAF/AcOH (2:1), THF, 4 °C, 18 h.

Scheme 15. Synthesis of Disaccharides Using a Polymer-Bound Thioethyl Glucosyl Donor[a]

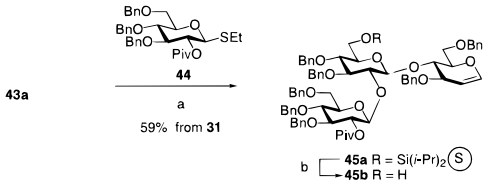


[a] (a) MeOTf, DTBP, 4 Å MS, CH_2Cl_2, 0 °C to rt 8 h. (b) TBAF/AcOH (2:1), THF, 40 °C, 18 h.

The thioethyl glycoside **40** was converted to the pivaloyl-protected thioethyl glycoside **41a** by reaction of pivaloyl chloride in the presence of DMAP in near quantitative yield. The support-bound thioglycosides were activated using methyl triflate as a thiophile, while one equivalent of the nonnucleophilic base di-tertbutylpyridine (DTBP) was added to provide stability for the glycal linkage during the coupling experiments. The formation of β-glucosyl (1→4)- and β-glucosyl (1→3)-linked disaccharides **43a** and **44a** was almost free of contaminating side products and provided the disaccharides in good yields (Scheme 15). Only the formation of the β-glucosyl (1→6)-linked disaccharide **42a** was accompanied by formation of detectable side products.[31]

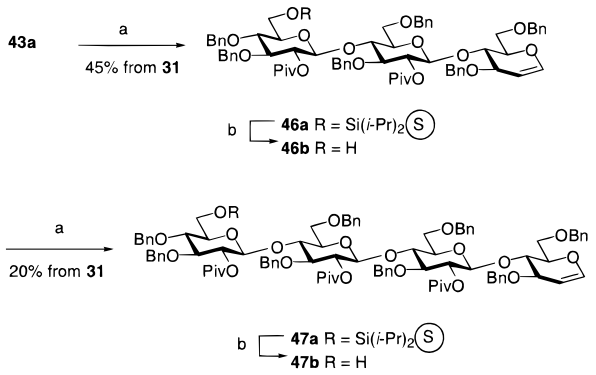
The synthesis of systems with branching from C2 is also accessible through this methodology as demonstrated in the context of the solid-phase synthesis of **45b**. The C2 pivaloyl neighboring group of the β-glucosyl (1→4)-linked disaccharide **43** was removed by treatment with DIBAL. The exposed C2 hydroxyl group could now function as the glycosyl acceptor in the synthesis of the branched trisaccharide **45b**. Formation of the synthetically challenging β-(1→2) glycosidic linkage was accomplished in 59% yield when the glycosyl donor **44** was used (Scheme 16).

Scheme 16. Synthesis of a Branched Trisaccharide[a]



[a] (a) (1) Dibal-H, CH_2Cl_2, −78 °C, 5 h; (2) **44**, MeOTf, DTBP, 4 Å MS, CH_2Cl_2, 0 °C → rt, 8 h. (b) TBAF/AcOH (2:1), THF, 40 °C, 18 h.

Scheme 17. Synthesis of a β-(1→4) Linked Tetrasaccharide[a]



[a] (a) (1) DMDO, CH_2Cl_2, 0 °C; (2) EtSH, $(CF_3CO)_2O$, CH_2Cl_2; (3) PivCl, DMAP, CH_2Cl_2, rt, 4 h; (4) **21**, MeOTFm DTBP, 4 Å MS, CH_2Cl_2, 0 °C to rt 8 h. (b) TBAF/AcOH (2:1), THF, 40 °C, 18 h.

After an efficient coupling protocol involving support-bound thioethyl glucosyl donors for the synthesis of disaccharides had been established, this methodology was applied to the synthesis of a tetrasaccharide containing exclusively β-(1→4) glucosidic linkages. Transformation of disaccharide glycal **43a** into the C2 pivaloyl thioethyl glycosyl donor was followed by coupling to provide the trisaccharide **46a** in 45% overall yield based on **31** as determined after cleavage from the solid support to furnish **46b**. Furthermore, conversion of **46a** to the thioethyl glycosyl donor was followed by coupling to glycal acceptor **21**. The desired tetrasaccharide **47b** was obtained in 20% yield over nine steps from **31** as determined after cleavage from the support by fluoridolysis. The overall yield corresponds to a yield of 84% per step (Scheme 17).

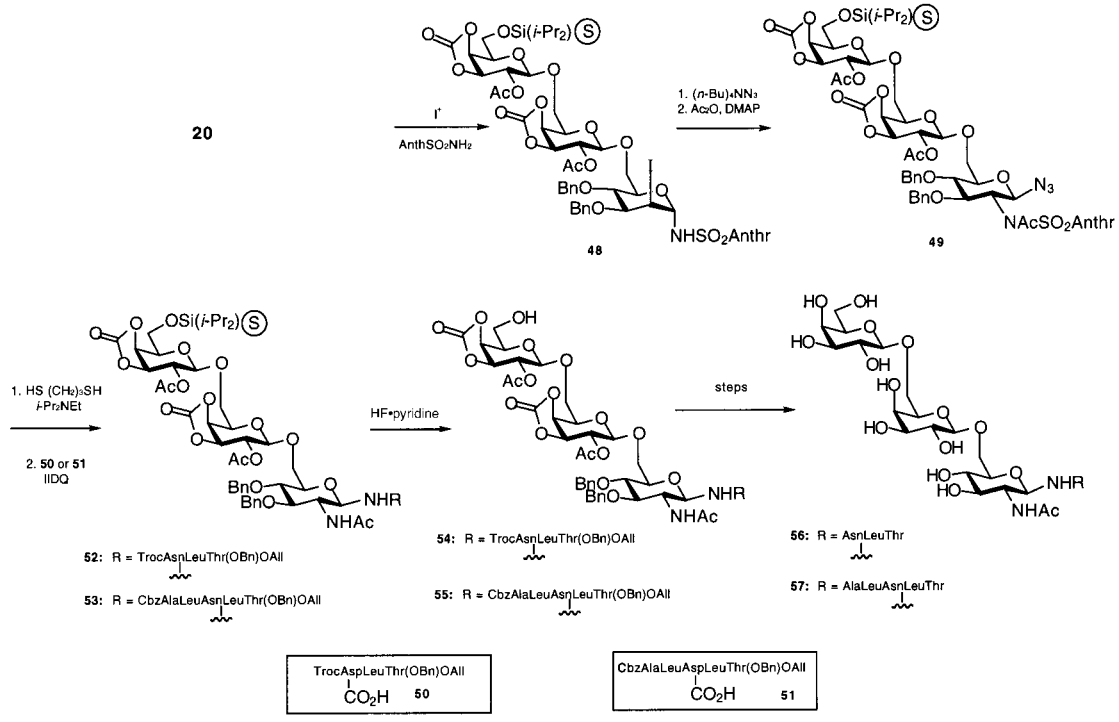
Solid-Phase Synthesis of N-Linked Glycopeptides

Two major subgroups of glycoproteins are known. These are N-linked and O-linked families, with the former being the most abundant in nature.[38] The biosynthesis of the glycoproteins results from cotranslational glycosylation usually occurring in the endoplasmic reticulum. The sugars of N-linked glycoproteins are usually attached by an oligosaccharyl transferase to an asparagine with the glycosylation sequence Asn-X-Ser/Thr. Advances in glycopeptide synthesis have been achieved by several groups.[10,39,40]

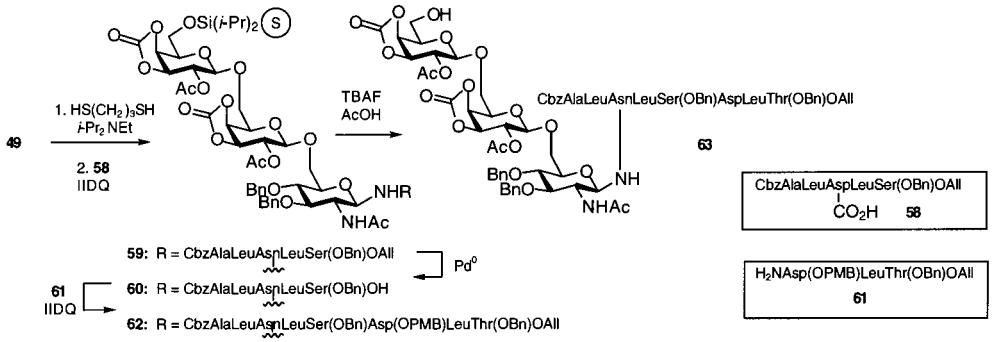
Our approach to the synthesis of N-linked glycopeptides[41] on the solid support aimed at a highly convergent synthetic strategy.[42] It was envisioned that a terminal glycal of a synthetic oligosaccharide domain would be subjected to iodosulfonamidation. Treatment of such an intermediate with azide would result in formation of β-anomeric azide with suprafacial movement of the α-sulfonamide from C-1 to C-2. Reduction of the azide and coupling of the resulting anomerically pure β-amino functionality would provide a protected glycopeptide.

In practice, polymer-supported trisaccharide **20** reacted with anthracenesulfonamide and $I(sym\text{-coll})_2ClO_4$ to form the intermediate **48**. Reaction of the iodosulfonamide **48**

Scheme 18. Synthesis of N-Linked Glycopeptides on a Solid Support



Scheme 19. Synthesis of N-Linked Glycopeptides on a Solid Support



with tetra-*n*-butylammonium azide followed by acetylation provided the anomeric azide **49** (Scheme 18).

The anthracene−sulfonamide linkage can be cleaved under mild, solid-support compatible conditions such as thiophenol or 1,3-propanedithiol and Hünig's base. Thus, treatment of **49** with 1,3-propanedithiol and *i*-Pr_2NEt effected both the reduction of the azide and cleavage of the sulfonamide. The resulting amine was coupled with either tripeptide **50** or pentapeptide **51** in the presence of IIDQ to afford the protected glycopeptides **52** or **53**, respectively. Removal from the solid support with HF•pyridine provided the glycopeptides **54** and **55**, in 30% and 37% overall yields, respectively, corresponding to a yield of ∼90% per step. Reverse-phase silica column chromatography was sufficient to obtain these compounds in pure form. For both **54** and **55**, the remaining protecting groups were cleaved under standard conditions to provide the completely deblocked glycopeptides **56** and **57** in 61% and 48% overall yields from **54** and **55**, respectively.

Orthogonal protecting groups on the C- and N-termini of the peptide provided the opportunity to extend the peptide chain while the ensemble is bound to the solid support. Alternatively, after removal from the support, the liberated peptide terminus may provide a functionality for linking to a carrier molecule to generate other glycoconjugates. Scheme 19 depicts the strategy for elongation of the peptide portion of the glycopeptide while still bound to the polymer support. Solid-phase-bound trisaccharide pentapeptide **59** was assembled as before from **49**, employing pentapeptide **58** in the coupling reaction.

The C-terminus of **59** was deprotected to give the acid, **60**. Solid-support-bound **60** was then coupled to tripeptide **61** with a free N-terminus to give glycopeptide **62**. Retrieval from the solid support afforded trisaccharide−octapeptide **63** in 18% overall yield from **10**.

Conclusions

We have described in this account the progress that our laboratory has made in the solid-support synthesis of oligosaccharides and glycopeptides using glycal building blocks. Protocols for the effective transformation of glycals into powerful glycosyl donors such as 1,2-anhydrosugars and thioethylglycosides have been developed. A variety of glycosidic linkages may now be fashioned in a selective manner, thereby bringing complex structures within reach. The flexibility and capabilities of our synthetic approach were demonstrated on some important structures of biological interest.

Glycal assembly on a solid support eliminates the repetitive purifications usually associated with oligosaccharide synthesis and is a general method as it does not require any specific enzymes or complex starting materials. Both natural and nonnatural sugars may be used in the constructions.

Much progress has been made over the last 5 years since we began exploring the application of the glycal assembly approach to solid-support synthesis. Still, a number of challenges remain before a flexible, high yielding, and absolutely selective strategy for the synthesis of oligosaccharides on the solid support will be available. Once these problems are solved, the construction of an automated oligosaccharide synthesizer will become feasible. The rapid access to complex glycoconjugates will undoubtedly serve to prompt detailed studies concerning the structure and function of this class of biooligomers.

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