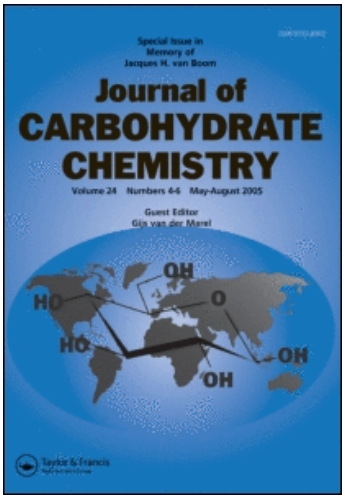

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SOLID PHASE OLIGOSACCHARIDE SYNTHESIS

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SOLID PHASE OLIGOSACCHARIDE SYNTHESIS*

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INTRODUCTION

Polysaccharides are the structurally most complex of the major classes of biopolymers. While nucleic acids and proteins are linear assemblies, polysaccharides are structurally and stereochemically more diverse. Additional complexity is added to the polysaccharide structure by the formation of glycoconjugates such as glycolipids and glycoproteins.[1] Oligosaccharides in the form of glycoconjugates mediate a variety of events, including inflammation, immunological response, metastasis, and fertilization.[2,3] Cell surface carbohydrates act as biological markers of various tumors and as binding sites for other substances, including pathogens.[4]

The increased understanding of the important roles oligosaccharides and glycoconjugates play in fundamental life-sustaining processes has stimulated a need for access to usable quantities of these materials. Glycoconjugates commonly exist as microheterogeneous mixtures and are difficult to isolate in homogeneous form, therefore yielding only small amounts of the desired material. The problems associated with isolation from natural sources give rise to opportunities for chemical synthesis.[5]

The developments in synthetic carbohydrate chemistry have centered on finding solutions to two important challenges: the need to differentiate similar functionality (hydroxyl or amino) contained on each monosaccharide and the need to induce the selective formation of a variety of glycosidic linkages. Unlike linkages found in the other two classes of repeating biopolymers, each glycosidic bond to be fashioned in a growing oligosaccharide ensemble constitutes a new locus of stereogenicity. In re-

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sponse to these two key challenges, a wide variety of protecting group strategies[6] and increasingly powerful and selective glycosylating agents have been developed.[7] Still, the synthesis of complex oligosaccharides and glycoconjugates remains a difficult and time-consuming task.

The introduction of solid support synthesis methods for preparation of structurally defined oligopeptides[8] and oligonucleotides[9] has led to tremendous improvements in terms of synthesis speed and efficiency. The advantages of solid phase synthesis, such as maximized yields by use of excess reagents, ease of purification, and synthesis automation, are now well appreciated. The biopolymers obtained by these rapid synthetic methods significantly impacted the fields of peptide and nucleic acid biochemistry. Glycobiology would benefit in many ways from straightforward synthetic methods, which could be used by nonexperts to prepare oligosaccharides.

Since the level of complexity associated with the synthesis of oligosaccharides on a polymer support is much greater than that associated with the other two classes of repeating biooligomers, this task seemed for a long time too difficult to tackle. The development of protocols for the solid support synthesis of oligosaccharides and glycopeptides requires the scientists in the field to find solutions to several problems: 1) selection of an overall synthetic strategy and development of methods for attachment of the carbohydrate to the polymeric support through the ''reducing'' or the ''nonreducing'' end, 2) choice of a solid support material, 3) selection of a linker (''support-bound protecting group'') that is stable during the synthesis but can be easily cleaved when desired, 4) a highly flexible protecting group strategy, 5) stereospecific and high-yielding coupling reactions, and 6) ''on-resin'' methods to monitor chemical transformations.

Pioneering efforts in the area of solid support oligosaccharide synthesis were undertaken in the early 1970s by several research groups, utilizing the limited set of glycosylating agents available at the time. While some fundamental issues were explored, the lack of glycosylation reactions compatible with polymeric supports impeded further progress. This early work has been reviewed[10] and is not covered in this chapter.

Since these initial attempts, a great deal of progress has been achieved in assembling relatively complex carbohydrate ensembles through simplification and refinement of protecting group strategies and the development of new and powerful glycosylation methodology. This progress is a result of the efforts that have focused on solid support oligosaccharide synthesis since the early 1990s. The different approaches to solid support oligosaccharide synthesis by chemical rather than enzymatic methods are summarized in this chapter.

Much effort has been focused on the use of soluble, polyethylene glycol (PEG) based polymeric supports for the synthesis of oligosaccharides. Reaction development using these soluble polymers has proven quite facile, since the reactions are run under homogeneous conditions and conventional analytical methods may be employed.[11] A number of glycosylation strategies have been explored by Krepinsky and coworkers[12] and van Boom et al.[13] An orthogonal glycosylation strategy in conjunction with soluble polymeric supports has been developed.[14] In 1996 a very innovative ''gatekeeper'' approach[15] to the synthesis of oligosaccharides containing β-mannosidic linkages was introduced. This work, which used a PEG polymer,[16] was reviewed in 1998[17] and is not covered in this chapter.

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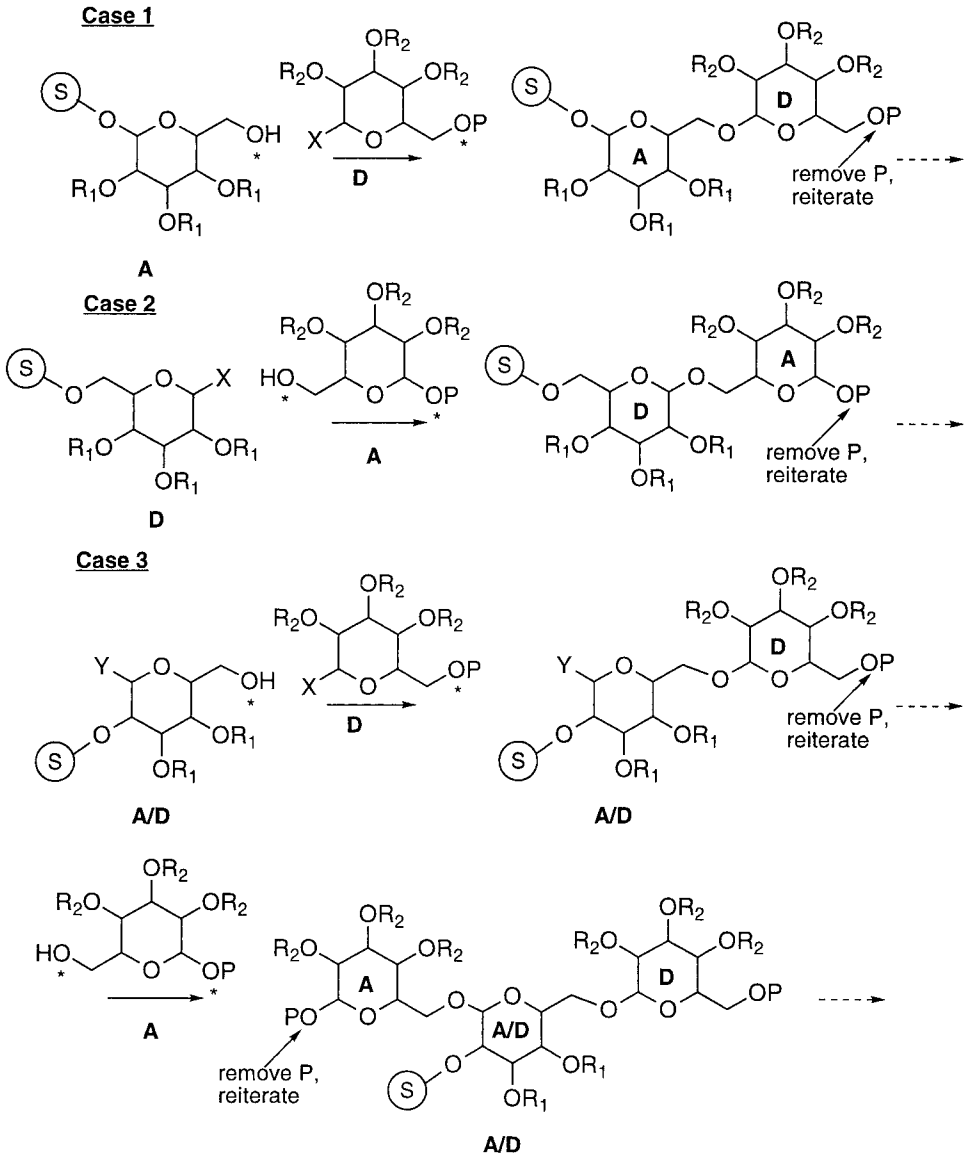
SYNTHETIC STRATEGIES

Three major strategies have been explored for the solid support synthesis of oligosaccharides and glycoconjugates. In one variation, the first carbohydrate is anchored to the support via its ''reducing'' end (see Scheme 1, Case 1). The carbohydrate bound to the solid support functions as an acceptor in the coupling event to a solution-



Scheme 1. Glycosyl acceptor (Case 1) and donor (Case 2) bound to the solid support, and bidirectional synthesis (Case 3): S, solid support; P, unique protecting group; X, activating group; asterisk, uniquely differentiated hydroxyl group.

based donor (**D**). In the cycle, a unique acceptor hydroxyl must be exposed in the now elongated, resin-bound carbohydrate construct. In Case 1, this strategy virtually demands for the donor (**D**) employed in the preceding glycosidation step a uniquely removable blocking group at the site of the next proposed elongation. The need to expose the unique hydroxyl group in the context of the polymer support will necessitate multiple functional group manipulations in synthesizing **D**.

Alternatively, the carbohydrate that is to undergo elongation may be mounted to the support somewhere in a ''nonreducing'' region, thereby making the reducing end available as a glycosyl donor for coupling with solution based acceptor **A** (Case 2). The use of **A**, of course, demands that the precise acceptor site be properly identified. In anticipation of the next coupling event, the reducing end of acceptor **A** is so functionalized that a new donor capability can be installed at the anomeric carbon of the elongated construct. This approach necessitates in the acceptor an anomeric group that does not serve as a glycosyl donor itself but rather may be converted in a straightforward manner into a glycosyl-donating moiety.

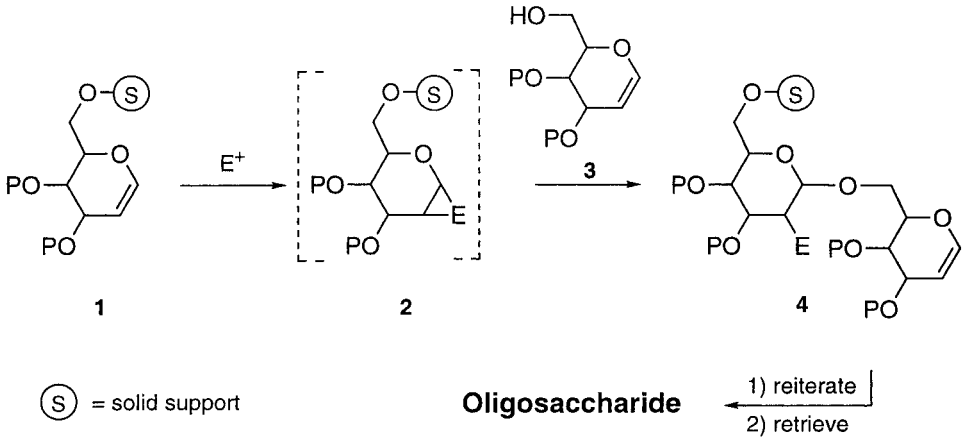
A hybrid of both strategies is the bidirectional synthesis approach (Case 3), which is based on the orthogonal glycosylation concept. The first monosaccharide (**A/D**) may serve as glycosyl donor as well as glycosyl acceptor and is attached to the support matrix through a position other than the anomeric center. This glycoside contains an anomeric leaving group (as for Case 2), and in addition exhibits an uniquely distinguished acceptor site (analogous to Case 1). During the initial phase of the synthesis the support-bound sugar functions as a glycosyl acceptor **A** without compromising the chemical integrity of the anomeric moiety. In this fashion, oligosaccharide synthesis following the Case 1 paradigm may be carried out. If an orthogonal set of glycosylating conditions is used, the support-bound sugar may be activated to serve as a glycosyl donor in chain elongation to follow a Case 1 synthetic scheme.

This chapter discusses the different approaches to solid phase oligosaccharide synthesis developed to date, grouped by the overall synthetic paradigm they obey.

THE GLYCOSYL DONOR BOUND STRATEGY: THE GLYCAL ASSEMBLY APPROACH

Overview of the Glycal Assembly Approach

While the donor-bound paradigm (Case 2) minimizes the number of protecting group manipulations that have to be carried out on the solid support, it mandates the presence of a latent glycosyl donor moiety in the solution-based glycosyl acceptor. Glycal building blocks fulfill these requirements, since they serve as glycosyl acceptors but may be converted into powerful glycosylating agents. Danishefsky and coworkers successfully explored the application of the glycal assembly method to the solid support synthesis of oligosaccharides and glycoconjugates.[18] The general principle of the glycal assembly method is outlined in Scheme 2. Attachment of a glycal to a solid support through the nonreducing end results in the presence of a terminal glycal function that may be readily converted into donor **2**. Since glycals are employed as the solution-based acceptor, the protecting group scheme used for the identification of hydroxyls predestined for glycosylation remains relatively simple.



Scheme 2. Use of the glycal assembly method in a general strategy for the synthesis of oligosaccharides on a solid support.

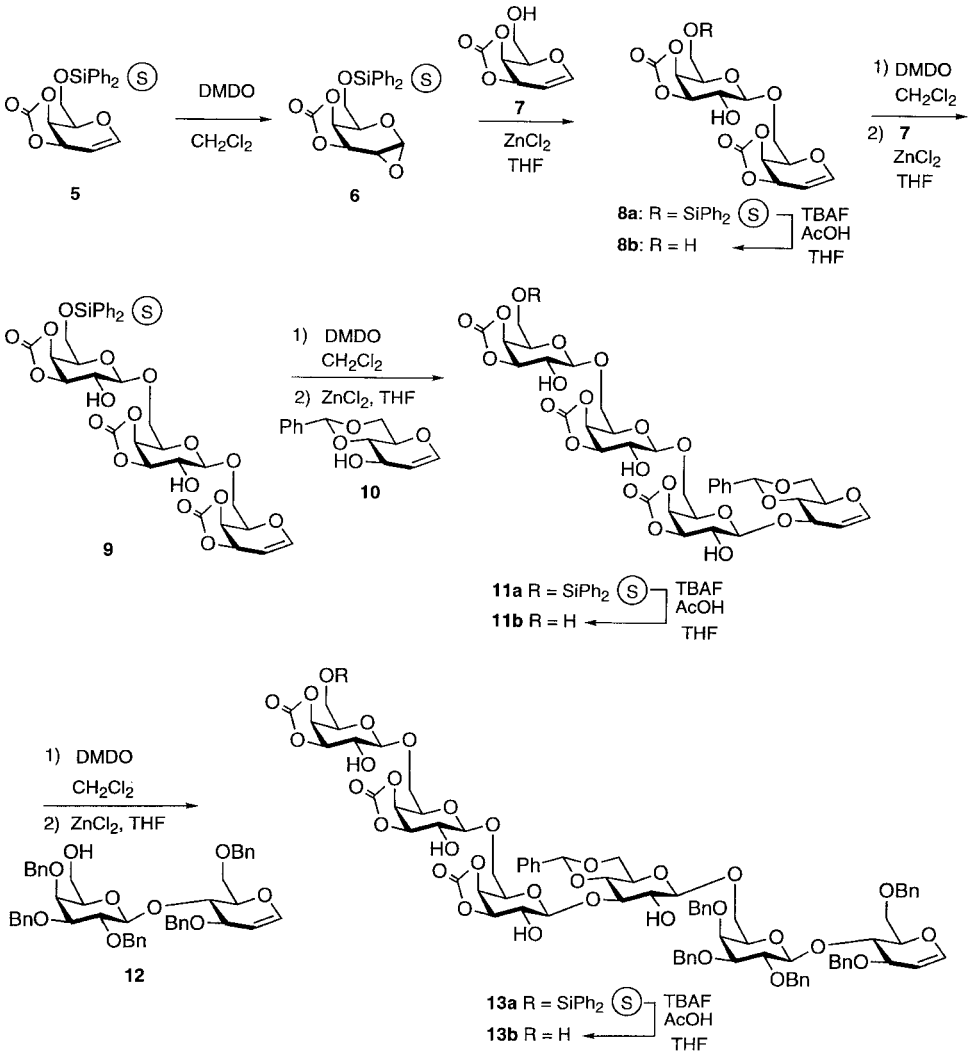
Compound **2** could be a 1,2-anhydro sugar,[19] derived by reaction with an epoxidizing agent, or it could be a transient species, derived by activation with an iodonium source.[20] Support-bound **2** acts as a glycosyl donor when treated with a solution of acceptor glycal **3**, along with any necessary agents to promote the glycosylation, thereby forming **4**. The process can be repeated to assemble the desired oligosaccharide, followed by retrieval from the support and purification by chromatographic methods.

All studies on the solid support synthesis of oligosaccharides by the glycal assembly approach employed a 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. The first glycal was linked to the solid support through a disilane linkage that could be cleaved rapidly and completely by treatment with fluoride.[21]

The validity of the approach was first demonstrated by the synthesis of a linear tetrasaccharide[22] and a hexasaccharide **13**[23] as outlined in Scheme 3. Polymer-bound galactal **5** was converted to the 1,2-anhydro sugar **6** by epoxidation with 3,3-dimethyldioxirane.[24] Polymer-bound **6** acted as a glycosyl donor when reacted with a solution of **7** in the presence of zinc chloride, resulting in the formation of disaccharide **8a**. Upon repetition, this glycosylation procedure accommodated the secondary alcohol glycosyl acceptor **10** as well as disaccharide acceptor **12**. Fluoridolysis with tetrabutylammonium fluoride (TBAF) was used to cleave the desired products from the polymeric support and furnish hexasaccharide **13** in 29% overall yield from **5**.[16]

Solid Phase Synthesis of Blood Group Determinants

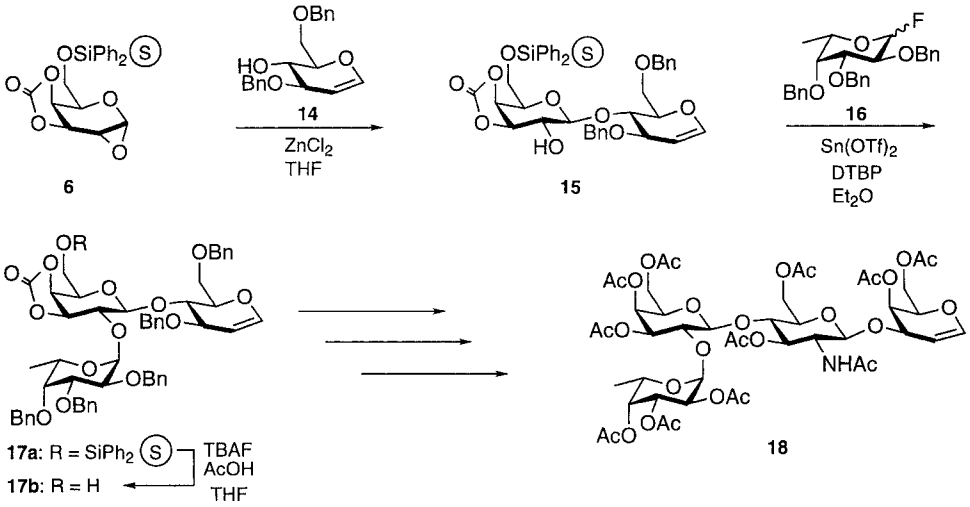
Carbohydate blood group determinants in the form of glycoproteins or glycolipids were found to play key roles in cell adhesion and other binding phenomena.[25,26] Furthermore, glycoconjugates related to these blood group substances have been



Scheme 3. Solid phase synthesis of a hexasaccharide by the glycal assembly method.

recognized as markers for the onset of various tumors. These tumor-associated antigens are currently being studied in vaccines for cancer immunotherapy.[27,28] The glycal assembly approach outlined above found its first applications in the synthesis of a H-type 2 tetrasaccharide (Scheme 4).[29,30]

Treatment of polymer-bound 1,2-anhydro sugar **6** with a solution of glucal acceptor **14** provided disaccharide **15**. Upon opening of a 1,2-anhydro sugar during glycosylation, a C2 hydroxyl group is exposed, which may in turn serve as a glycosyl acceptor to form branched oligosaccharides.[31] Compound **15** was fucosylated using a solution of fucosyl donor **16**[32] to furnish trisaccharide **17**. Treatment of **17a** with TBAF provided trisaccharide glycal **17b** in 50% overall yield from **5**.



Scheme 4. Solid phase synthesis of an H-type blood group determinant by the glycal assembly method.

Because of the lack of solid support methodology to fashion glycosidic linkages bearing C2-acylamino functions, solution phase chemistry had to be employed to access the H-type 2 blood group determinant glycal **18**. Ready functionalization at the reducing end was achieved through the terminal glycal handle.

The Lewis^b blood group antigen (Le^b) is of particular interest because it has been identified as a mediator for the binding of *Helicobacter pylori* to human gastric epithelium.[33] Clinical studies have identified *H. pylori* as a causative agent in gastric and duodenal ulcers,[34] and antimicrobial treatments are an effective means to combat infection.[35] Since bacterial attachment is a prerequisite for infection,[36] analogs of the Le^b oligosaccharide may serve as therapeutic alternatives to broad spectrum antibiotics.

The first approach to Le^b addressed the synthesis of the core tetrasaccharide **22**, which was assembled on the polymer support as depicted in Scheme 5.[37] Polymer-bound galactal **19** was epoxidized with dimethyldioxirane and then reacted with a solution of glucal derivative **20** to give disaccharide diol **21**. This reaction proceeded in highly regioselective fashion wherein glycosylation occurred at the allylic position at C3 of **20**. Bisfucosylation of **21** using donor **16** provided polymer-bound tetrasaccharide glycal **22a**. Treatment of **22a** with TBAF gave **22b**, which was obtained in a 40% overall yield from **19**. Initially, solution chemistry was used to further convert glycal **34b** into a hexasaccharide of the Le^b system, whereupon **34b** was conjugated with human serum albumin to provide the desired neoglycoprotein.

These approaches toward the synthesis of blood group determinants on a solid support were hampered by a serious shortcoming in the methodological arsenal of the glycal assembly method. While this strategy permitted rapid and concise access to β-glycosidic linkages, solution phase methodology solution had to be used for construction of *N*-acetylamine glucosidic linkages prevalent in biologically important blood group determinants, gangliosides, and N-linked glycopeptides.[37]

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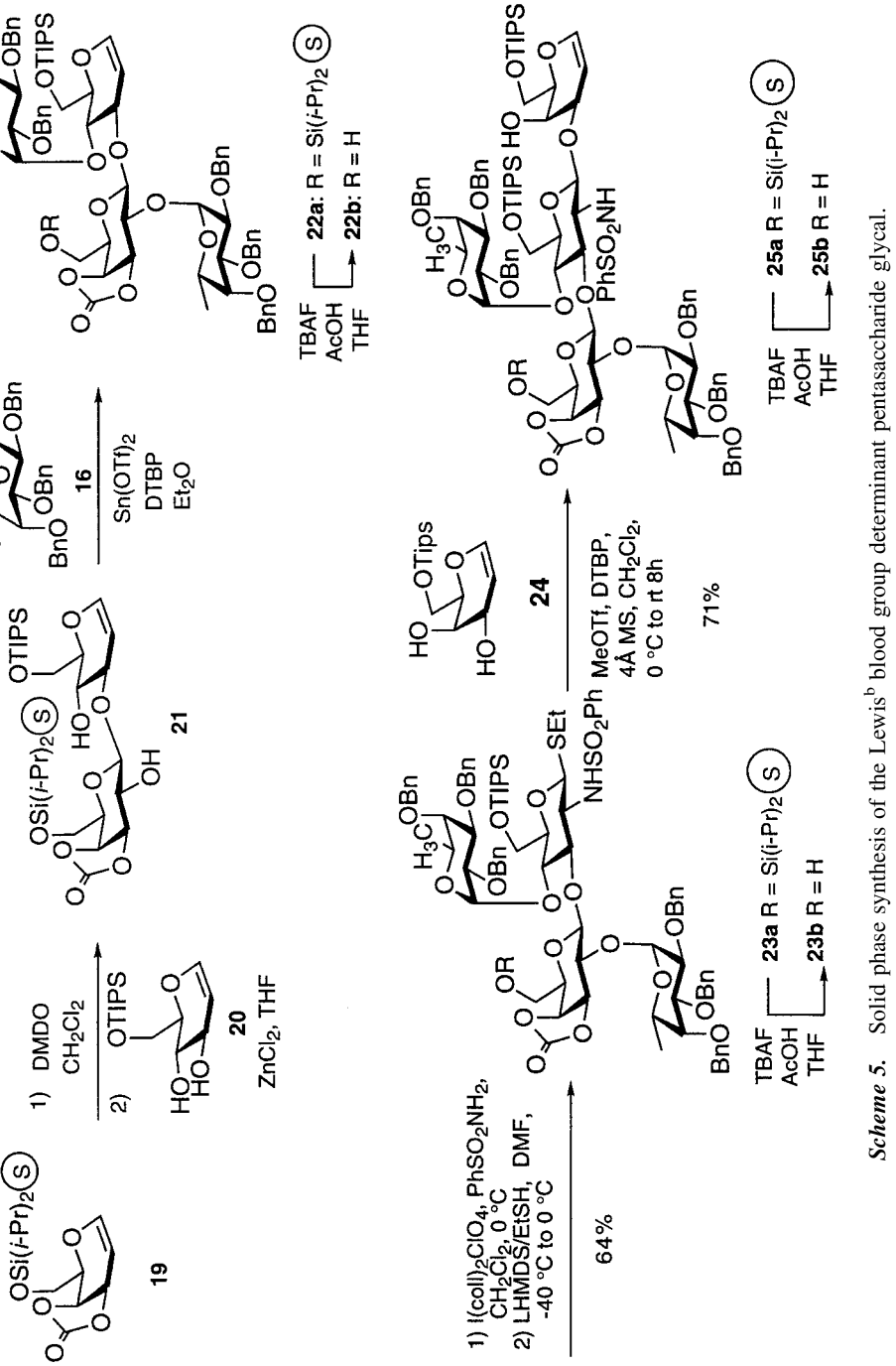


Scheme 5. Solid phase synthesis of the Lewis^b blood group determinant pentasaccharide glycal.

To install the appropriate functionality at C2, solution phase chemistry has relied on a trans-diaxial addition of an iodonium electrophile in the presence of an amine to form an iodosulfonamide. Displacement of iodine proceeds presumably through an aziridine intermediate and may be induced by a thiolate nucleophile to fashion thioethyl 2-amidoglycosyl donors.[38] Successful transfer of this method to the solid support allowed polymer-bound glycals to be converted into thioethyl glycosyl donors. These donors were in turn coupled with a variety of glycosyl acceptors, including glycals.[39]

After an efficient coupling protocol for the synthesis of β-2-amidoglucosidic linkages had been established, the difficulties encountered during the earlier synthesis of the Lewis^b pentasaccharide glycal could be overcome. Branched tetrasaccharide **22a** was converted into the thioethyl donor **23**. Coupling to galactal acceptor **24** yielded 71% of the desired pentasaccharide **25a** (Scheme 5). TBAF was used to effect retrieval of the pentasaccharide, affording **25b** in 20% overall yield from **19**.[32]

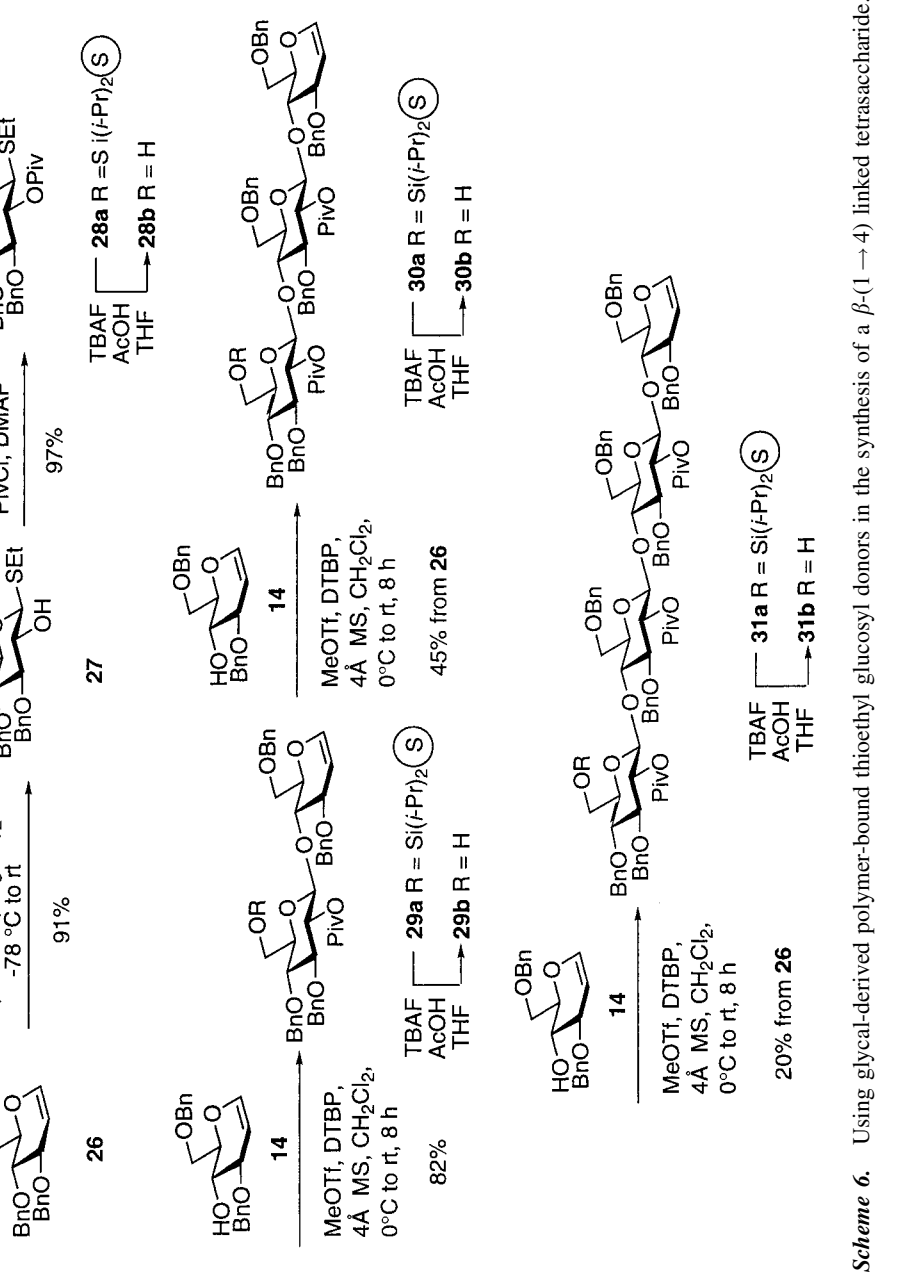
Generation and Use of Thioethyl Donors on the Solid Support

While the use of glycals on the solid support allowed for the construction of β-galactosyl linkages with great efficiency even with hindered glycosyl acceptors, the analogous β-glucosidic linkages could not be prepared reliably. In the galactose series, the anhydride is relatively stable to very mild Lewis acids, particularly anhydrous zinc chloride, because a conformationally constraining cyclic carbonate protecting group is used. 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 and thus are prone to donor deterioration.

To overcome the problems associated with the formation of β-glucosidic linkages, an approach that allowed for the conversion of glycals into thioethyl glycosyl donors was developed in solution phase.[40] These thioethyl glycosyl donors constitute a class of extremely powerful glycosylating agents upon activation with thiophilic reagents.[41] 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 been shown by Kunz and others to prevent the formation of orthoester products during glycosylations.[42]

Conversion of **26** to the protected thioethyl glycosyl donors **27** was achieved through epoxidation with dimethyldioxirane to yield the 1,2-anhydro sugar, followed by opening of this intermediate by ethanethiol in the presence of a trace of acid. Thioethyl glycoside **27** was obtained in 91% yield and transformed into fully protected thioethyl donor **28a** by pivaloylation in near quantitative yield. Support-bound thioglycoside **28a** was activated using methyl triflate as a thiophile, while the non-nucleophilic base di-*tert*-butylpyridine (DTBP) provided stability for the glycal linkage during coupling. In model studies the formation of β-glucosyl $(1\rightarrow4)$, β-glucosyl $(1\rightarrow3)$, and β-glucosyl $(1\rightarrow6)$ linked disaccharides was achieved in good yield and complete selectivity.[43]

After an efficient coupling protocol involving support-bound thioethyl glucosyl donors had been established, this methodology was applied to the synthesis of tetrasaccharide **31** containing exclusively β-$(1\rightarrow4)$ glucosidic linkages. Transformation of disaccharide glycal **29a** into the C2 pivaloyl thioethyl glycosyl donor was followed by



Scheme 6. Using glycal-derived polymer-bound thioethyl glucosyl donors in the synthesis of a β-(1 → 4) linked tetrasaccharide.

coupling to provide trisaccharide **30a** in 45% overall yield from **26**. Conversion of **30a** to the thioethyl glycosyl donor was followed by coupling to glycal acceptor **14** to yield the desired tetrasaccharide **31a**. After cleavage from the support **31b** was obtained from **26** in 20% yield over nine steps, corresponding to 84% average yield per step (Scheme 6).

Solid Phase Synthesis of N-Linked Glycopeptides

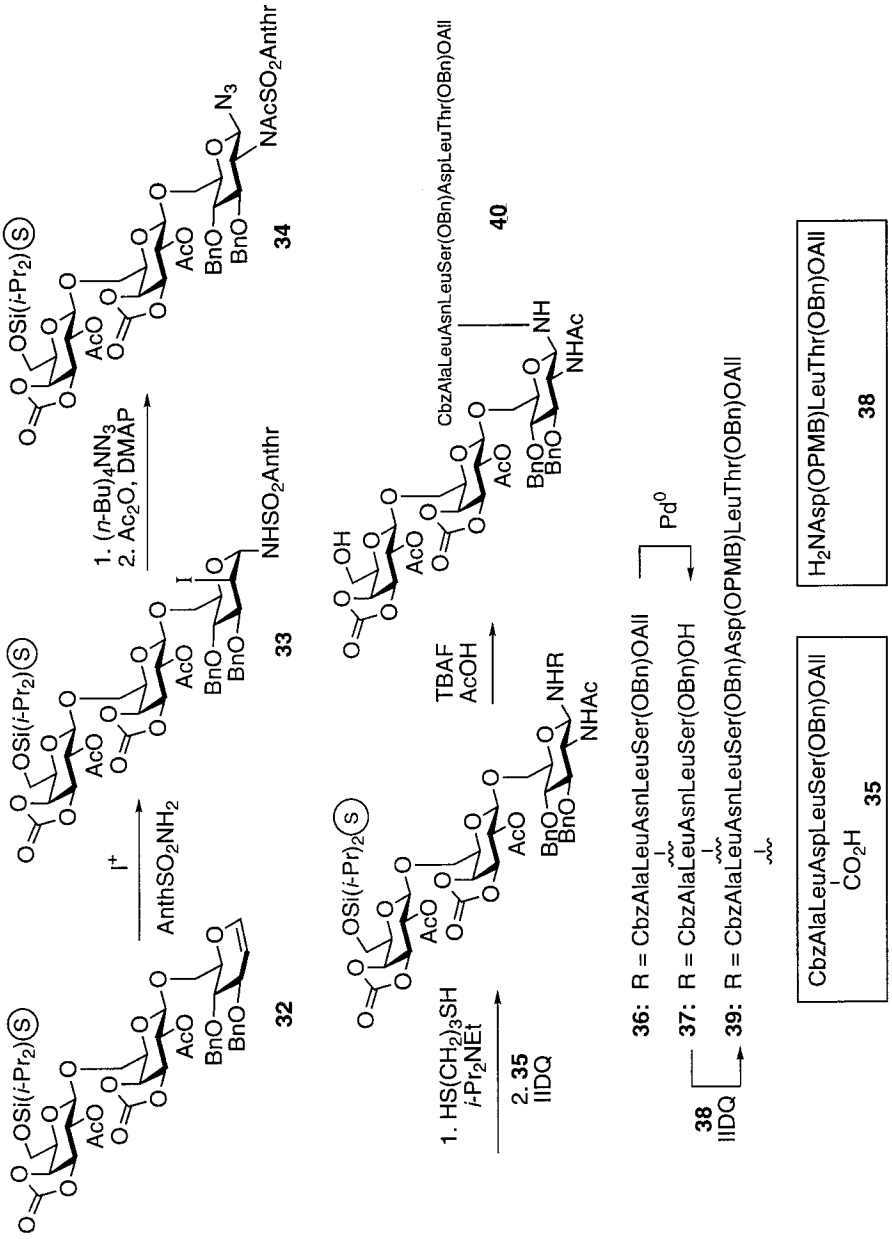
N-linked and O-linked glycoproteins are the two major subgroups of glycoproteins, with the former being the most abundant in nature.[44] The biosynthesis of these glycoproteins results from contranslational glycosylation usually occurring in the endoplasmic reticulum. The sugars of N-linked glycoproteins are usually attached by an oligosaccharyltransferase to an asparagine having the glycosylation sequence Asn-X-Ser/Thr. Advances in glycopeptide synthesis have been achieved by several groups.[45–47]

The synthesis of N-linked glycopeptides[48] on the solid support using a terminal glycal of a synthetic oligosaccharide domain aimed at a highly convergent synthetic strategy.[49] Polymer-supported trisaccharide **32** was reacted with anthracenesulfonamide and I(*sym*-coll)$_2$ClO$_4$ to form intermediate **33** (Scheme 7). Reaction of the iodosulfonamide **33** with tetra-*n*-butylammonium azide, followed by acetylation, provided the anomeric azide **34**. The anthracenesulfonamide linkage can be cleaved under mild conditions such as 1,3-propanedithiol and Hünig's base, which concomitantly effected the reduction of the azide. The resulting amine was coupled with pentapeptide **35** in the presence of IIDQ to afford the protected glycopeptide **36**.

Orthogonal protecting groups on the C- and N-termini of the peptide provided the opportunity to extend the peptide chain while the ensemble was 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. The C-terminus of **36** was deprotected to give the acid **37**, which was coupled to tripeptide **38** with a free N-terminus to give glycopeptide **39**. Retrieval from the solid support afforded trisaccharide-octapeptide **40** in 18% overall yield from **19**.[50]

STRATEGIES USING SUPPORT-BOUND GLYCOSYL ACCEPTORS

The solid support synthesis of oligopeptides and oligonucleotides is routinely carried out by reacting a support-bound structure (acceptor) with an excess of a solution-based reactive species (donor). While the glycal assembly method, which utilizes a support-bound reactive species, has been used very successfully for the synthesis of oligosaccharides and glycopeptides, all other synthetic approaches explored to date have followed an acceptor-bound strategy. The acceptor-bound paradigm allows for the glycosyl donor to be used in excess during elongation of the growing oligosaccharide from the reducing to the nonreducing end. Because the donor is used in excess, side reactions that usually affect the glycosyl donor, resulting in inactive degradation products, do not impact the overall yield. Each hydroxyl group to serve as a glycosyl acceptor needs to be distinguished by a unique protecting group, which may be removed before each coupling cycle. In this section synthetic strategies employing a variety of glycosylating agents are reviewed.

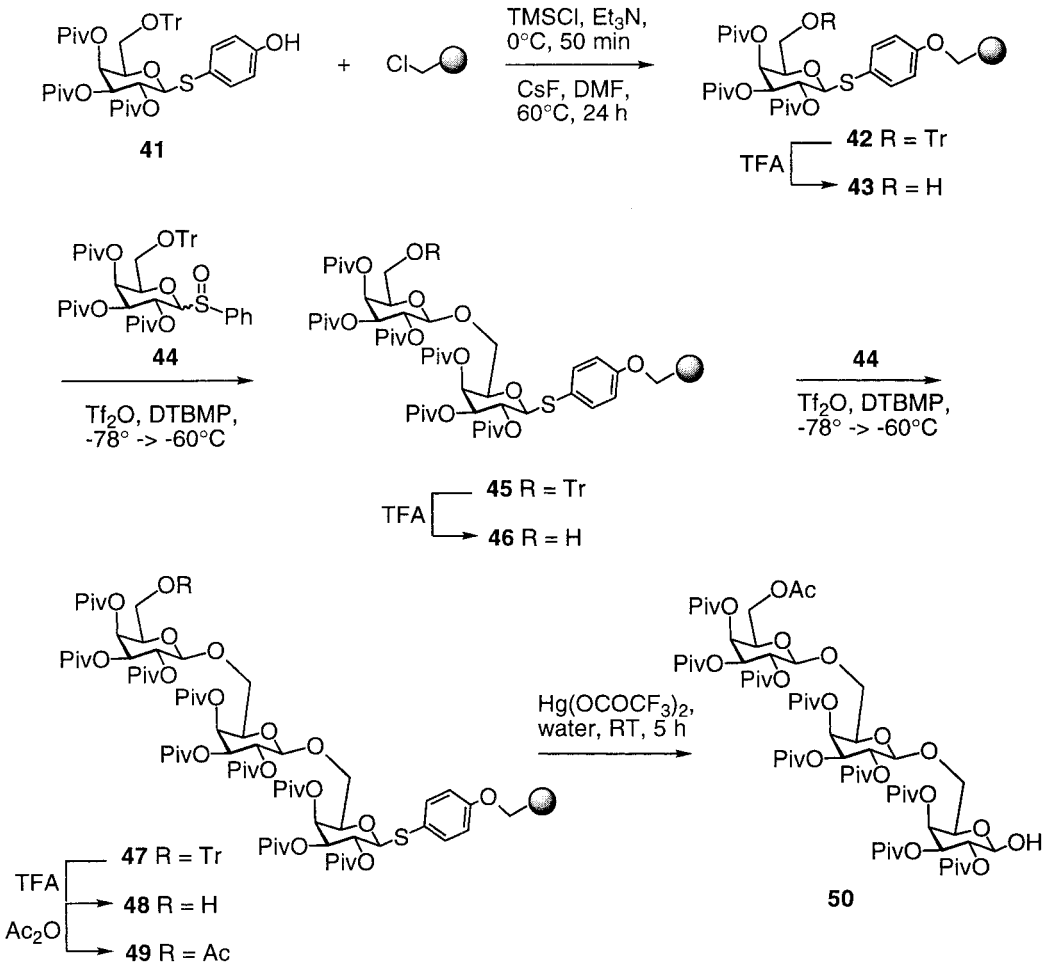


Scheme 7. Synthesis of N-linked glycopeptides on a solid support.

Synthesis of Oligosaccharides Using Glycosyl Sulfoxides

The use of anomeric sulfoxides as glycosylating reagents was reported by Kahne and coworkers in 1989.[51] Upon activation with triflic anhydride at low temperatures, anomeric sulfoxides are transformed into extremely reactive glycosyl donors, which can glycosylate very hindered acceptors. The excellent reactivity of the sulfoxide donors coupled with the selectivity achieved by use of a participating group in the C2 position prompted their use in the synthesis of oligosaccharides on the solid support.[52]

Merrifield's resin (polystyrene cross-linked with 1% divinylbenzene) was chosen as a polymer matrix, and an anomeric hydroxy thiophenyl ether moiety was selected as linker to the solid support (Scheme 8). The thiophenyl linkage is stable to all coupling and deprotection conditions but may be cleaved readily with mercuric trifluoroacetate. The first monosaccharide **42** was selectively deprotected and coupled



Scheme 8. Solid phase synthesis of a trisaccharide by the glycosyl sulfoxide method.

by addition of 4 equiv of galactosyl sulfoxide **44**, in the presence of DTBP as a base, and triflic anhydride as activator. Coupling at $-60°C$ for 30 min, filtration, and washing were followed by deprotection of the acetyl group. One further coupling and cleavage from the polymeric support by reaction with mercuric trifluoride furnished trisaccharide **50** in 52% overall yield. It was estimated that each coupling proceeded in 94–95% yield based on a detachment efficiency of 70–75% (as determined by solution phase studies). The glycosyl sulfoxide strategy successfully accommodated secondary hydroxyl acceptor groups, and α-fucosidic linkages were generated by the use of focusyl sulfoxides.[52]

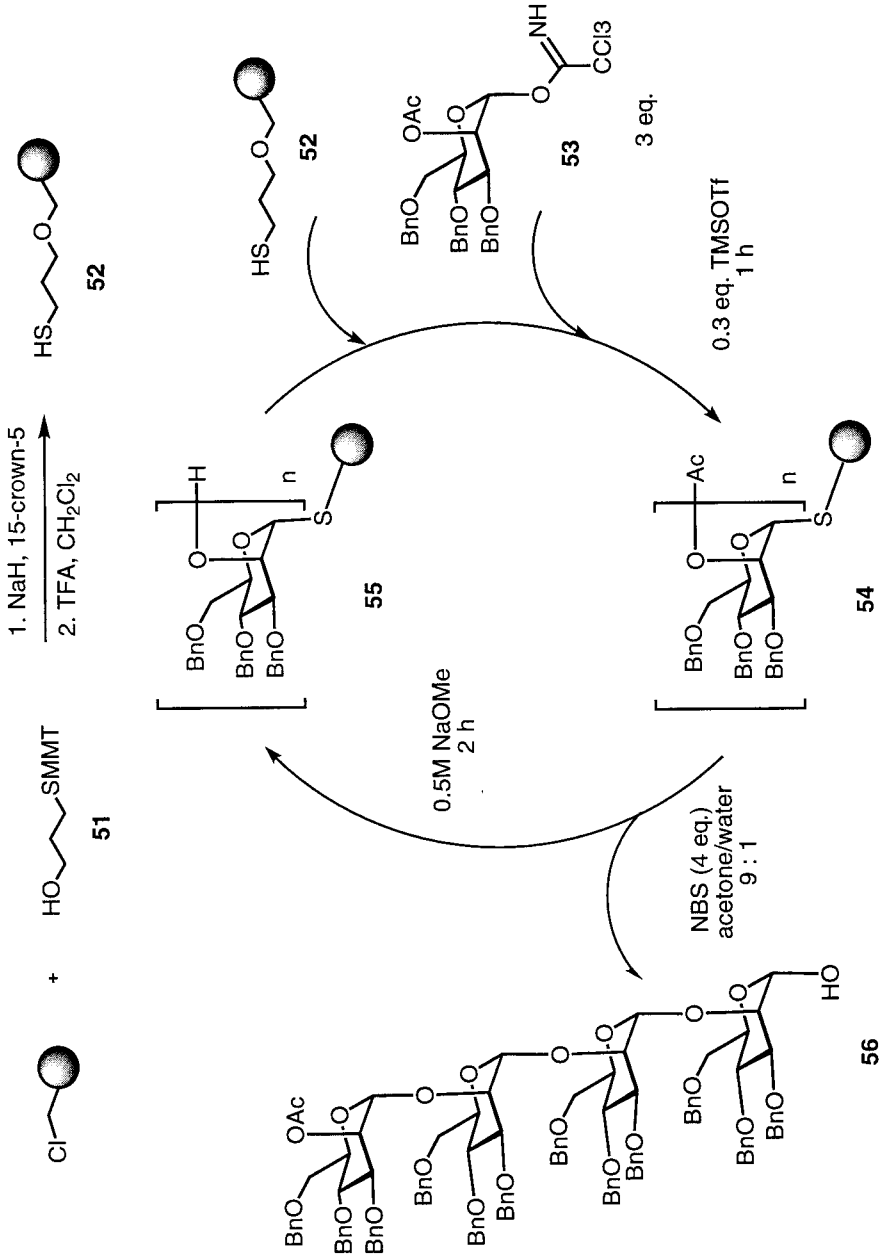
These advances in the solid support synthesis of oligosaccharides using glycosyl sulfoxides were applied to the preparation of a combinatorial library of approximately 1300 disaccharides and trisaccharides.[53] A polystyrene-polyethylene glycol (PS-PEG) copolymer that swells in a wide range of solvents, including water, was employed in the syntheis of these libraries. Screening of the carbohydrate structures against a bacterial lectin from *Bauthinia purpurea* was performed with the carbohydrate structures still attached to the resin beads. Use of a chemical tagging system developed by Still and coworkers[54] allowed for rapid structure determination of the hits in the screening assay. Alternate binding structures with higher affinity than the natural ligand for the lectin of interest were identified in this manner.

The glycosyl sulfoxide method has proven very efficient and flexible in constructing a variety of glycosidic linkages on the solid support. Combinatorial carbohydrate libraries prepared by this strategy hold great potential for the identification of natural and nonnatural lectin ligands. While the synthesis of larger oligosaccharide structures using the glycosyl sulfoxide method still needs to be demonstrated, this strategy holds great potential for many applications.

Synthesis of Oligosaccharides Using Glycosyl Trichloroacetimidates

Among the multitude of glycosylating agents now at the disposal of the synthetic chemist, glycosyl trichloroacetimidates have become the most widely used building blocks. These donors have been used to prepare a large number of very complex oligosaccharides and glycoconjugates in solution phase.[55] High coupling yield, versatility, and excellent selectivity are hallmarks of this approach to oligosaccharide synthesis. Recently the first syntheses of oligosaccharides using trichloroacetimidate donors on the solid support have been reported. Schmidt and coworkers initially used Merrifield's resin and explored a variety of ether and thioether linkers.[56] Attachment of the first residue via a thioether linkage proved most reliable, and cleavage was successfully effected by reaction with a nucleophile. Reaction with water or methanol in the presence of *N*-bromosuccinimide yielded the lactol or the methyl glycoside, respectively.

A two-step coupling cycle (Scheme 9) was used to assemble a series $(1 \rightarrow 2)$ linked mannosides. Removal of the temporary acetyl-protecting group by sodium methoxide in methanol was followed by coupling of the trichloroacetimidate mannosyl donor **53** to the exposed axial C2 hydroxyl group. Repetition of this cycle and cleavage of the thioether linker resulted in assembly of the desired tetrasaccharide **56** in 34% overall yield. Penta- and hexamannosides obtained by using this method were characterized by mass spectrometry, but no yields were reported.[57]



Scheme 9. Synthesis of α $(1 \rightarrow 2)$ mannosides by the trichloroacetimidate strategy.

In addition to 1,2-*trans*-glycosides, disaccharides containing the synthetically more challenging 1,2-*cis*-glycosidic linkage were prepared. Fucosyl trichloroacetimidate was coupled to the C2 position of a support-bound mannosyl acceptor under the agency of trimethylsilyl triflate (TMSOTf) to yield 54% of the desired disaccharide.[57] Promising results were also obtained for the synthesis of a $(1 \rightarrow 2)$ trimannoside, which was prepared on a mercaptoethyl-functionalized controlled pore glass (CPG) solid support. This nonswelling support performed well under the described coupling and deprotection conditions.[58]

More recently, the trichloroacetimidate strategy has been applied to the synthesis of a branched pentasaccharide unit common to most complex *N*-glycan structures (Scheme 10). Reaction of mannosyl donor **57** with thiol-functionalized resin resulted in attachment to the polymer matrix through the reducing end of the sugar **58**. Removal of the benzoyl protecting groups on the C3 and C6 positions was effected by treatment with sodium methoxide to furnish diol **59**. Dimannosylation was accomplished by reaction with trichloroacetimidate mannosyl donor **53** to afford solid support bound trisaccharide **60** in 38% overall yield. Cleavage of the acetyl protecting groups of the axial C2 hydroxyl functionalities revealed support-bound trisaccharide diol **61**. Conversion of **61** to pentasaccharide **63** was achieved by reaction with glucosamine donor **62**. Cleavage of the thioether linker with *N*-bromosuccinimide in the presence of methanol furnished the desired pentasaccharide methyl glycoside in 20% overall yield.

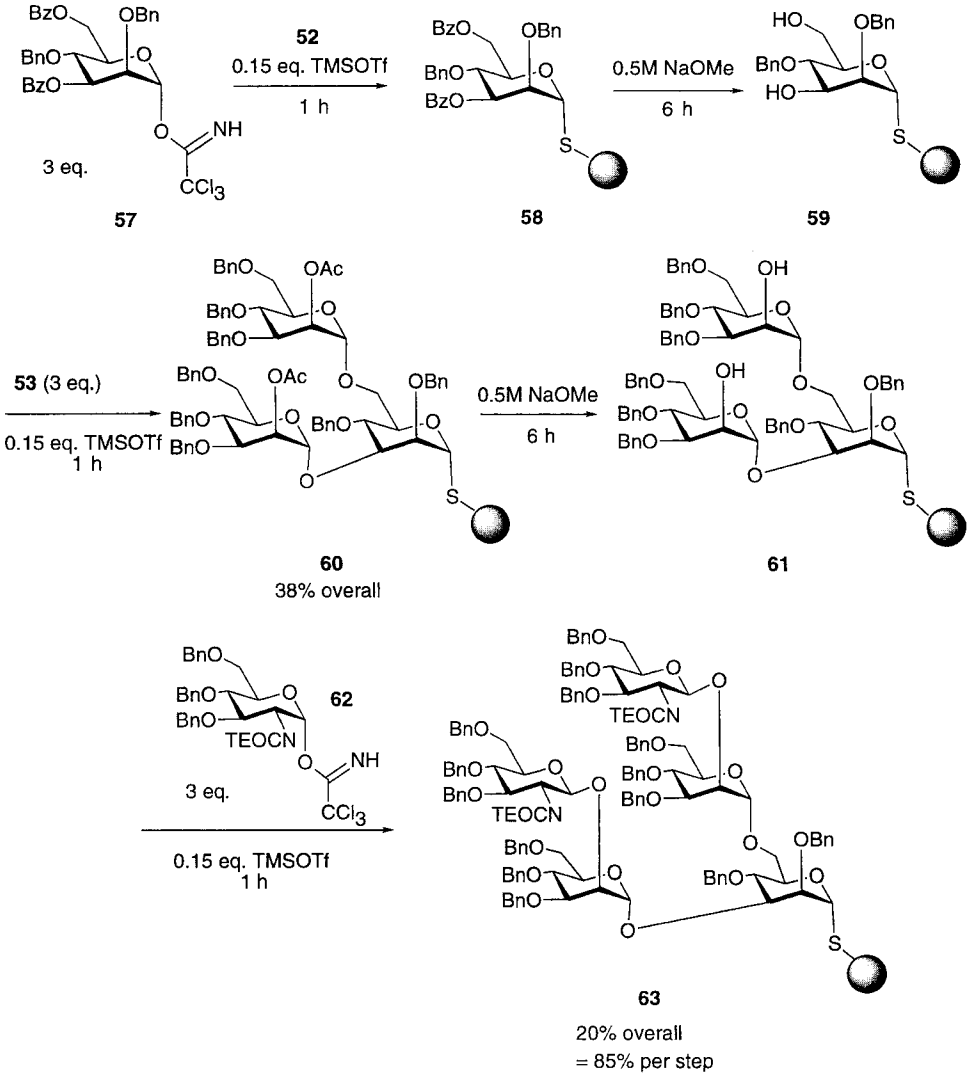
Adinolfi et al.[59] explored the use of different solid supports for the synthesis of disaccharides employing trichloroacetimidate donors. The glycosyl acceptor was connected through the C2, C3, or C6 hydroxyl group to amino-functionalized solid support via a succinimide linker. Polystyrene, PS-PEG copolymer (TentaGel), and CPG supports were tested for their performance with trichloroacetimidate donors. Best coupling yields (95%) were obtained in these studies when the donor was reacted with acceptor bound to CPG or polystyrene support. PEG-containing copolymers performed significantly poorer in these glycosylations.

Hunt and Roush[60] used a solid phase method to prepare 6-deoxy di- and trisaccharides. A sulfonate linker was employed to connect the first monosaccharide, a glycal, via its C6 position to the polymer resin. A galactosyl trichloroacetimidate donor was used in the first coupling reaction, followed by cleavage from the solid support by treatment with NaI to furnish pure disaccharides in 85–91% yield. Reduction with Bu_3SnH (AIBN) provided the desired 6-deoxydisaccharide.

These impressive initial accomplishments using trichloroacetimidate glycosyl donors on the solid support underscore the potential this strategy holds for the synthesis of complex oligosaccharide structures containing a variety of glycosidic linkages. Before the assembly of larger constructs may be contemplated, the overall yield and recovery will have to be improved. Novel linkers that are completely stable throughout the synthesis as well as improved coupling protocols should remedy these shortcomings.

Solid Support Synthesis of Oligosaccharides Using Thioglycosides

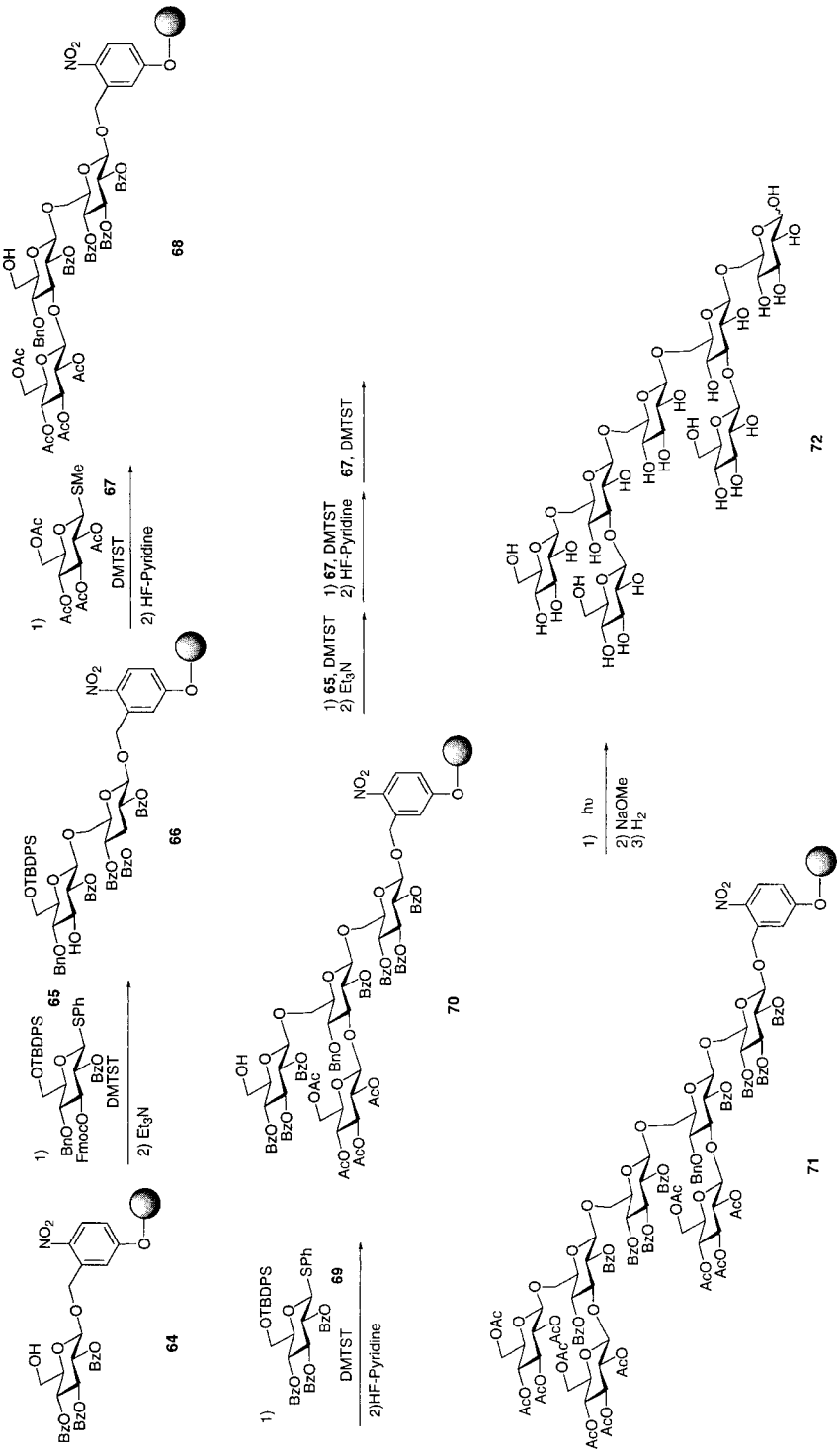
Next to anomeric trichloroacetimidates, thioglycosides are the glycosyl donors most widely used in the synthesis of complex oligosaccharides and glycoconjugats. Thioglycosides may be prepared in high yield and are exceptionally stable, allowing for



Scheme 10. Use of trichloroacetimidate donors in the preparation of a branched pentasaccharide.

prolonged storage even at room temperature. Reaction with thiophiles such as methyl triflate or dimethylthiomethylsulfonium triflate (DMTST) ensures efficient activation of thioglycosides for the formation of glycosidic linkages.[41] In solution phase, thioethyl glycosides have found application in the synthesis of very complex oligosaccharides and in the preparation of a combinatorial carbohydrate library.[61]

Straightforward synthetic access, stability, and high-yielding, selective glycosylation reactions made thioglycoside donors ideal for application to the solid support synthesis of oligosaccharides. Nicolaou and coworkers utilized phenolic polystyrene equipped with a photolabile, readily available *o*-nitrobenzyl linker in the synthesis of heptasaccharide **72** from mycelial walls of *Phytophthora megasperma* (Scheme 11).[62]



Scheme 11. Synthesis of a heptasaccharide employing thioglycoside donors.

This heptasaccharide consists entirely of β $(1 \rightarrow 6)$ or $(1 \rightarrow 3)$ glucosidic linkages and had previously been prepared by using solution phase methods[63] and block synthesis on a soluble PEG polymer.[13] Silyl ethers and fluorenylmethyloxy carbonyl (Fmoc) protective groups were employed to provide temporary protection of the hydroxyl functionalities to be glycosylated in the coupling steps that followed. Removal of the silyl ether protecting group by fluoridolysis to yield **64** was followed by coupling of 4 equiv of the solution-bound thiodonor **65** in the presence of the activator DMTST. The coupling to form **66** proceeded in greater than 95% yield according to material recovered after cleavage from the resin. Consecutive deprotection and coupling steps resulted in assembly of the desired heptasaccharide **71** still attached to the polymeric support. Photolytic cleavage of the linker, treatment with sodium methoxide to remove the ester protecting groups, and hydrogenolysis to cleave the benzyl ethers yielded unprotected heptasaccharide **72**. Alternatively, fully protected heptasaccharide was obtained by acetylation of the anomeric hydroxyl group support in 20% overall yield from **64** after photolytic cleavage from the solid support.[62]

After having established a reliable and efficient reaction sequence for the synthesis of oligosaccharides Nicolaou et al. further improved upon their initial strategy.[64] A 4-benzyloxybenzoic acid spacer was incorporated between the photolabile linker and the anomeric position of the first glycoside to provide access to either unprotected oligosaccharides or oligosaccharide glycosyl donors (Scheme 12). Support-bound acceptor **73** was converted into trisaccharide **77** following the synthetic strategy established earlier (see above). Cleavage from the resin was effected photolytically to furnish the fully protected trisaccharide **80** in 63% yield from **73**. Alternatively, the protected trisaccharide was cleaved from the polymer with concomitant activation by exposure to $PhSSiMe_3/ZnI_2/nBu_4Ni$, furnishing phenylthiotrisaccharide donor **79** (Scheme 12; pp. 628 and 629).

Support-derived trisaccharide building block **79** was used in a block synthesis of dodecasaccharide **82** (Scheme 13; p. 630). Reaction of support-bound glycosyl acceptor **78** and **79** furnished hexassacharide **81**. Removal of a silyl protecting group and coupling with trisaccharide donor **79** was repeated twice, followed by photolytic cleavage of the linker to furnish dodecasaccharide **82** in 10% yield from **73**.[64]

The two examples of solid support oligosaccharide synthesis employing thioglycosides demonstrate the power of this strategy in the preparation of large carbohydrate structures. For the first time, a very large oligosaccharide was constructed via a block synthesis strategy in which every block itself was prepared on a solid support. The synthesis of combinatorial carbohydrate libraries using this strategy should certainly be possible.

Synthesis of Oligosaccharides Using Pentenyl Glycosides

Fraser-Reid and coworkers have established *n*-pentenyl glycosides as a class of powerful and versatile glycosyl donors.[65] Activation by a variety of reagents [e.g., N-iodosuccinimide (NIS), TESOTf] furnished various glycosidic linkages in high yield and excellent selectivity in solution phase synthesis. More recently, the application of *n*-pentenyl glycosides to the synthesis of oligosaccharides on a solid support has been explored.[66]

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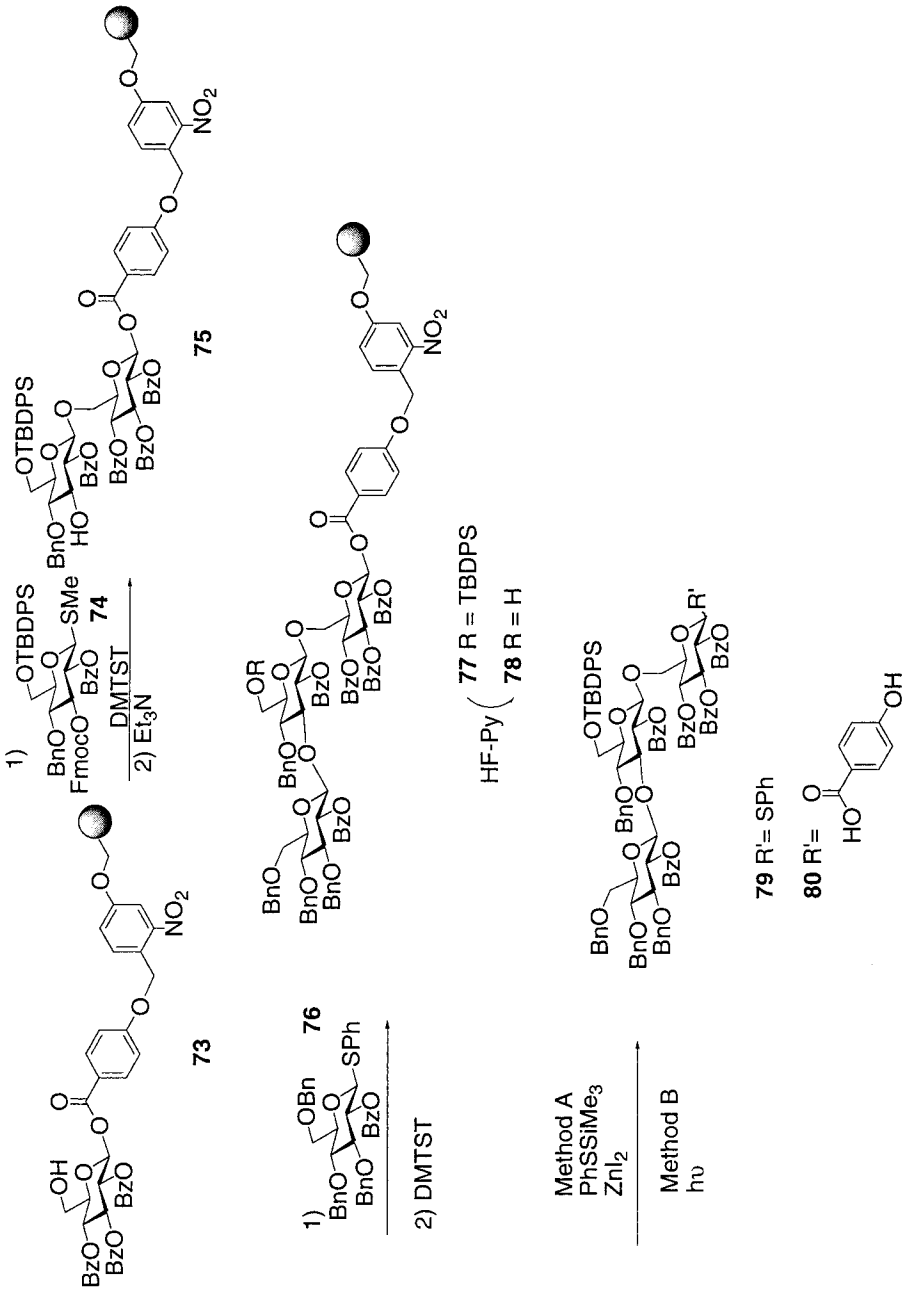
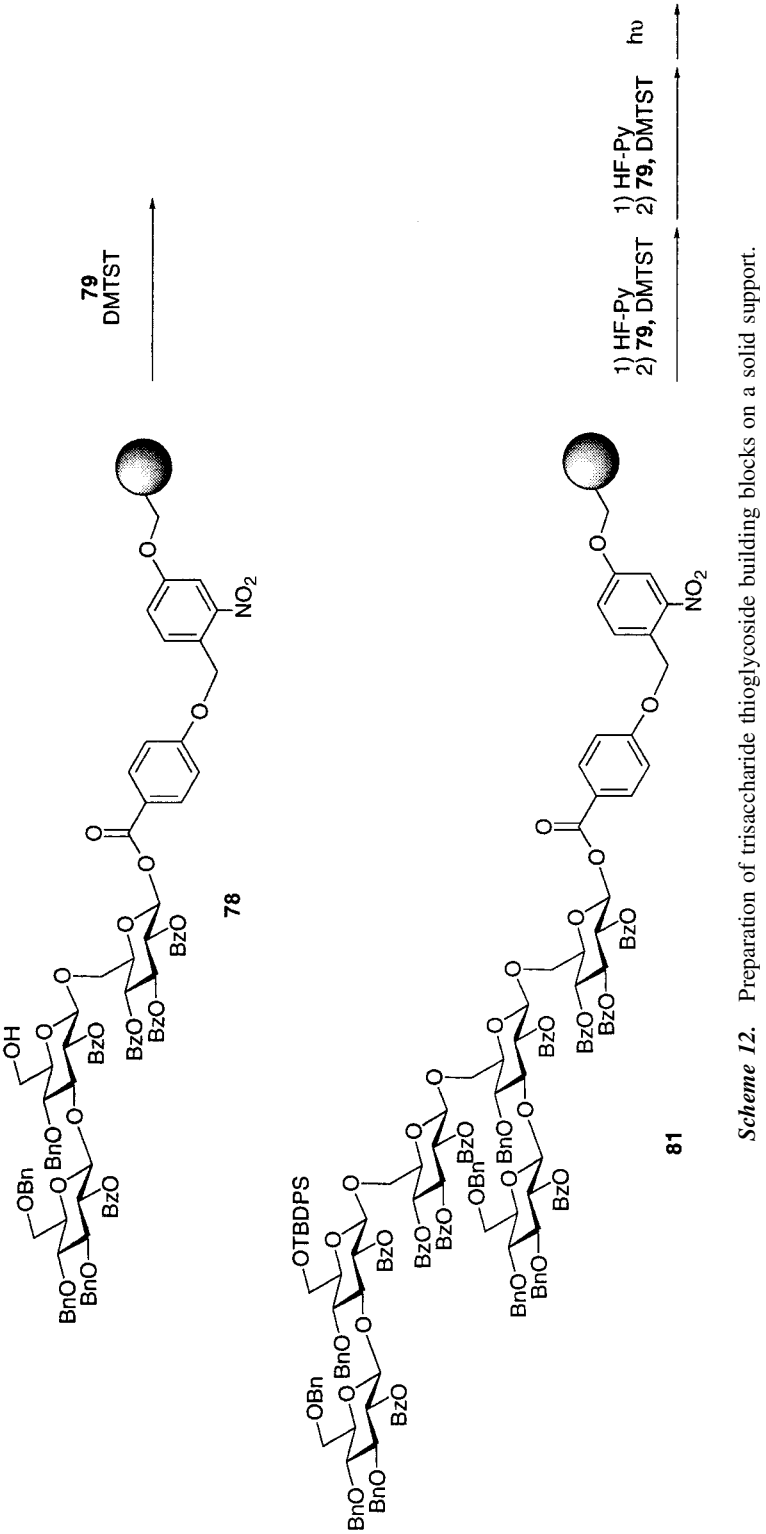
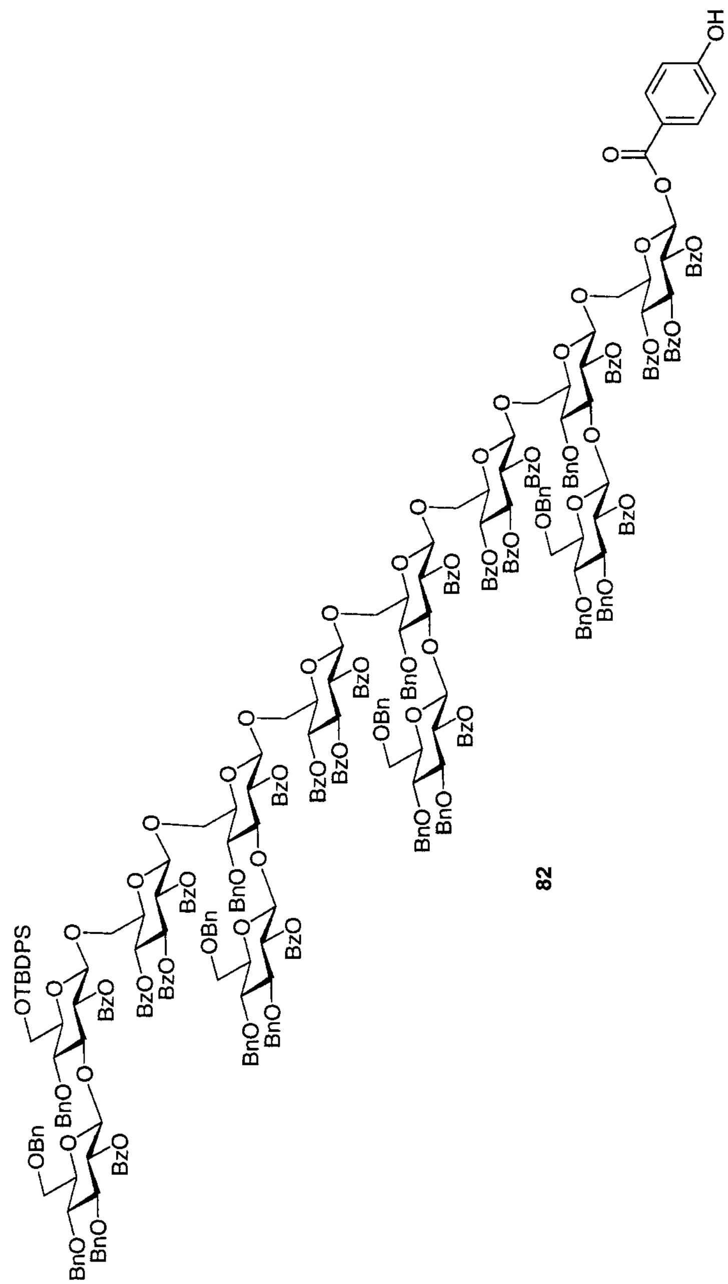




Scheme 12. Preparation of trisaccharide thioglycoside building blocks on a solid support.



82

Scheme 13. Assembly of a dodecasaccharide by the building block strategy on the solid phase.

Both donor- and acceptor-bound synthetic strategies were investigated initially. Attachment of a pentenyl glycoside through the C6 hydroxyl group to the solid support was achieved via photolabile 3-amino-3-(2-nitrophenyl)propionyl linker. Glycosylation using NIS and TESOTf as activating agents did not result in clean formation of the desired disaccharide product but also yielded lactol by donor degradation. An acceptor-bound synthetic strategy was more successful. Model studies with a glucose acceptor that was anchored to the solid support via its anomeric position showed that α- and β-glucosidic and C2 amino glucosidic linkages could be furnished in average coupling yields of greater than 90%. Reaction development was aided by ^{13}C gel phase NMR spectroscopy to analyze the formed products.[69]

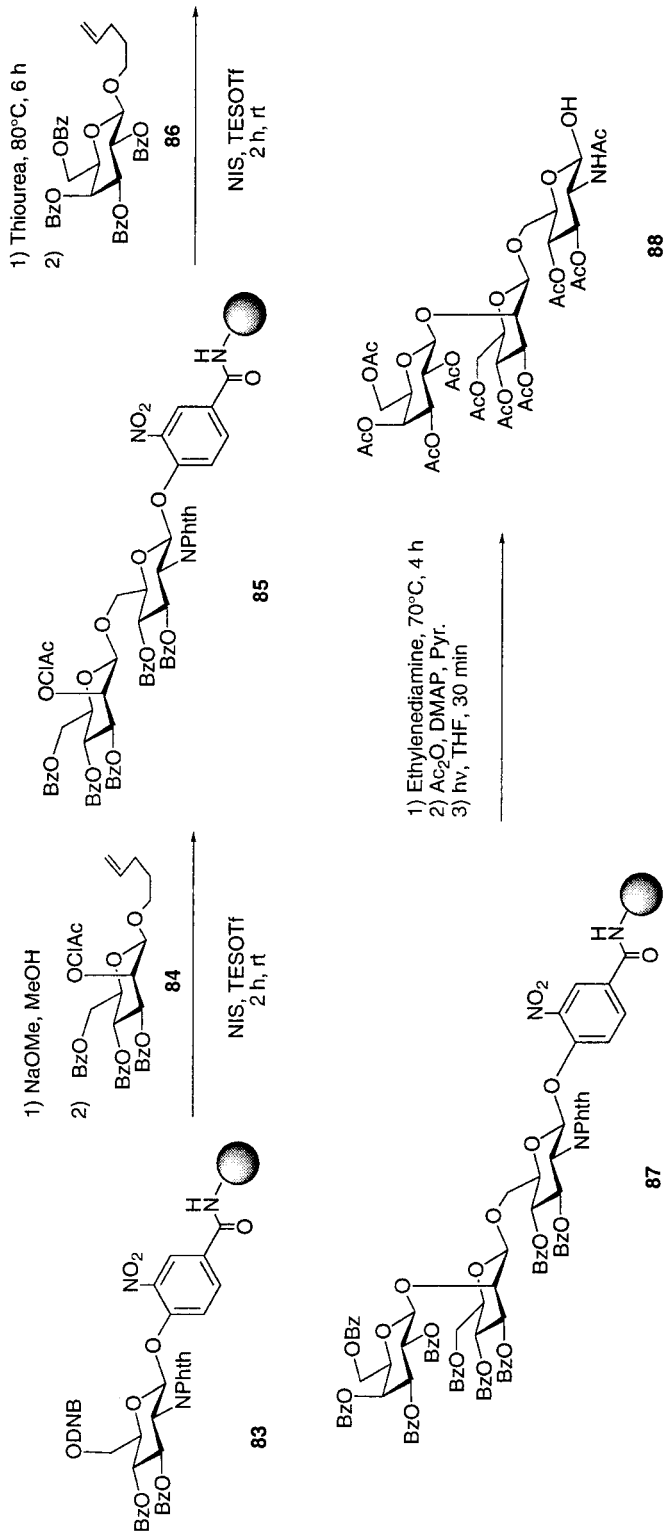
After these initial studies, the synthesis of a support-bound, fully deprotected trisaccharide was achieved (Scheme 14). Chiron's polystyrene-grafted ''crowns'' were chosen as solid support material because they are more amendable to parallel synthesis than traditional polymer supports. A photolabile *o*-nitrobenzyl linker was selected to attach the first amino glucoside **83** through its anomeric position to the solid support. Removal of the C6 dinitrobenzyl protecting group was followed by coupling with pentenyl mannoside **84** and resulted in formation of the desired disaccharide **85**. Removal of the C2 chloroacetyl protecting group and coupling of pentenyl galactose donor **86** furnished trisaccharide **87**. Global deprotection followed by peracetylation and photolytic cleavage from the solid support provided trisaccharide **88**, but no yield was reported.[66]

Bidirectional Glycosylation Strategy for the Solid Phase Synthesis of Oligosaccharides

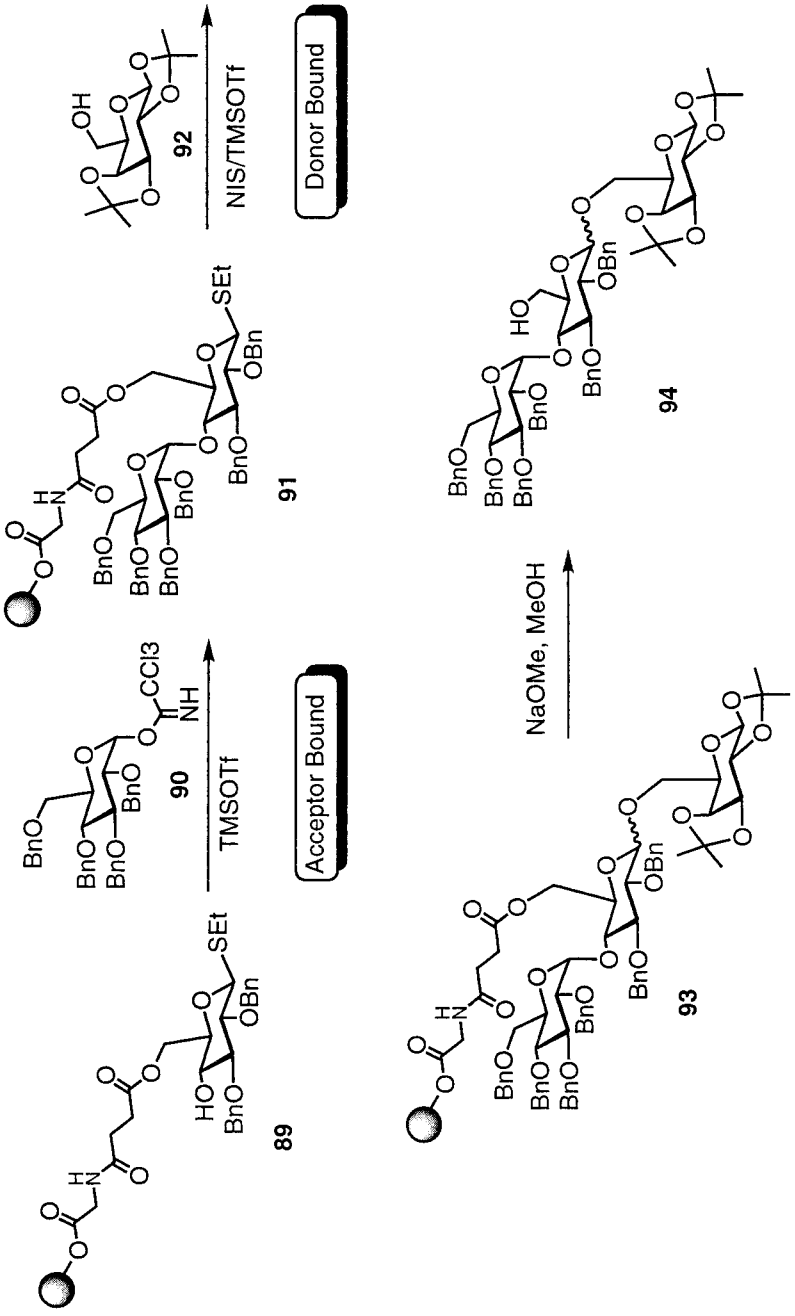
The preceding sections discussed the strengths and weaknesses of the donor- and acceptor-bound synthetic strategies developed to date for the synthesis of complex oligosaccharides. Boons et al. recently described an approach that combines both strategies in a bidirectional solid phase oligosaccharide synthesis.[67]

The use of orthogonal glycosylating agents during the synthesis is an absolute prerequisite for the success of this bidirectional strategy. Trichloroacetimidate glycosides may be activated and function as glycosyl donors in coupling reactions with glycosyl acceptors containing an anomeric thioglycoside. The C6 hydroxyl group of thioethyl glycoside **89** (Scheme 15) was connected to TentaGel resin via a succinate linker. First, the exposed C4 hydroxyl functionality of **89** served as glycosyl acceptor in the reaction with solution-based trichloracetimidate donor **90** in the presence of TMSOTf as promoter. Without the need for further protecting group manipulations, support-bound disaccharide thioglycoside **91** was activated with NIS/TMSOTf [68] to serve as a glycosyl donor in the reaction with solution-based glycosyl acceptor **92**. This strategy served to generate a combinatorial library of trisaccharides while minimizing the necessary protecting group manipulations.

This bidirectional approach stands to combine the advantages of the acceptor-bound approach, such as use of excess donor during the synthesis, with the opportunity to fashion glycoconjugates through the anomeric position formerly possible only in a donor-bound paradigm. Other combinations of orthogonal glycosylating agents and the



Scheme 14. Application of *n*-pentenyl glycosides to solid phase synthesis: preparation of a trisaccharide.



Scheme 15. Bidirectional strategy for the synthesis of oligosaccharide libraries.

applicability of this strategy to the synthesis of larger oligosaccharide structures will have to be addressed in future studies.

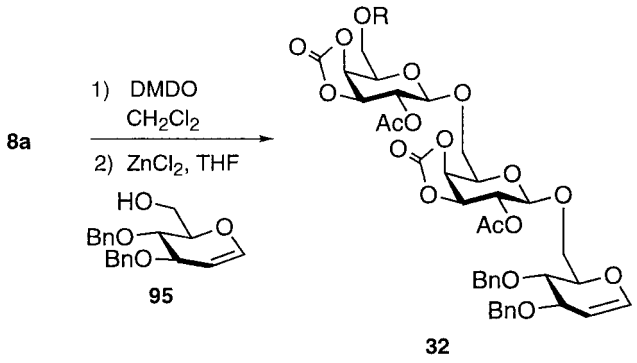
''ON-RESIN'' ANALYTICAL METHODS

Development of a reliable solid phase methodology for the synthesis of oligosaccharides and glycopeptides has been greatly hampered by the lack of convincing ''on-resin'' analytical techniques. In most of the syntheses outlined above, it was necessary 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 NMR and mass spectrometry). The cleavage method analysis is time-consuming and wasteful in the context of multistep syntheses. Polystyrene-PEG copolymers may be analyzed by gel phase NMR spectroscopy but are quite expensive and allow only for relatively low loading.[69] Two NMR-based approaches for the on-resin analysis of polystyrene resins have been developed to aid reaction development by monitoring of the solid support matrix.

High-Resolution Magic Angle Spinning NMR Spectroscopy

The effectiveness of the glycal assembly approach to solid support oligosaccharide synthesis was impressively documented by on-resin analysis. The ''crude product'' of the multistep synthesis of trisaccharide **32** (Scheme 16) was monitored by high-resolution magic angle spinning NMR (HR-MAS).[70]

HR-MAS 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. The ^{1}H NMR (Scheme 17) of the crude product of this synthesis showed that only one product was obtained. Spin-echo techniques were used to suppress NMR peaks derived from the polymeric matrix. Since its introduction, this technique has greatly facilitated the development of novel synthetic schemes of oligosaccharides and glycopeptides on a solid support.



Scheme 16. Solid phase synthesis of a trisaccharide by the glycal assembly method.

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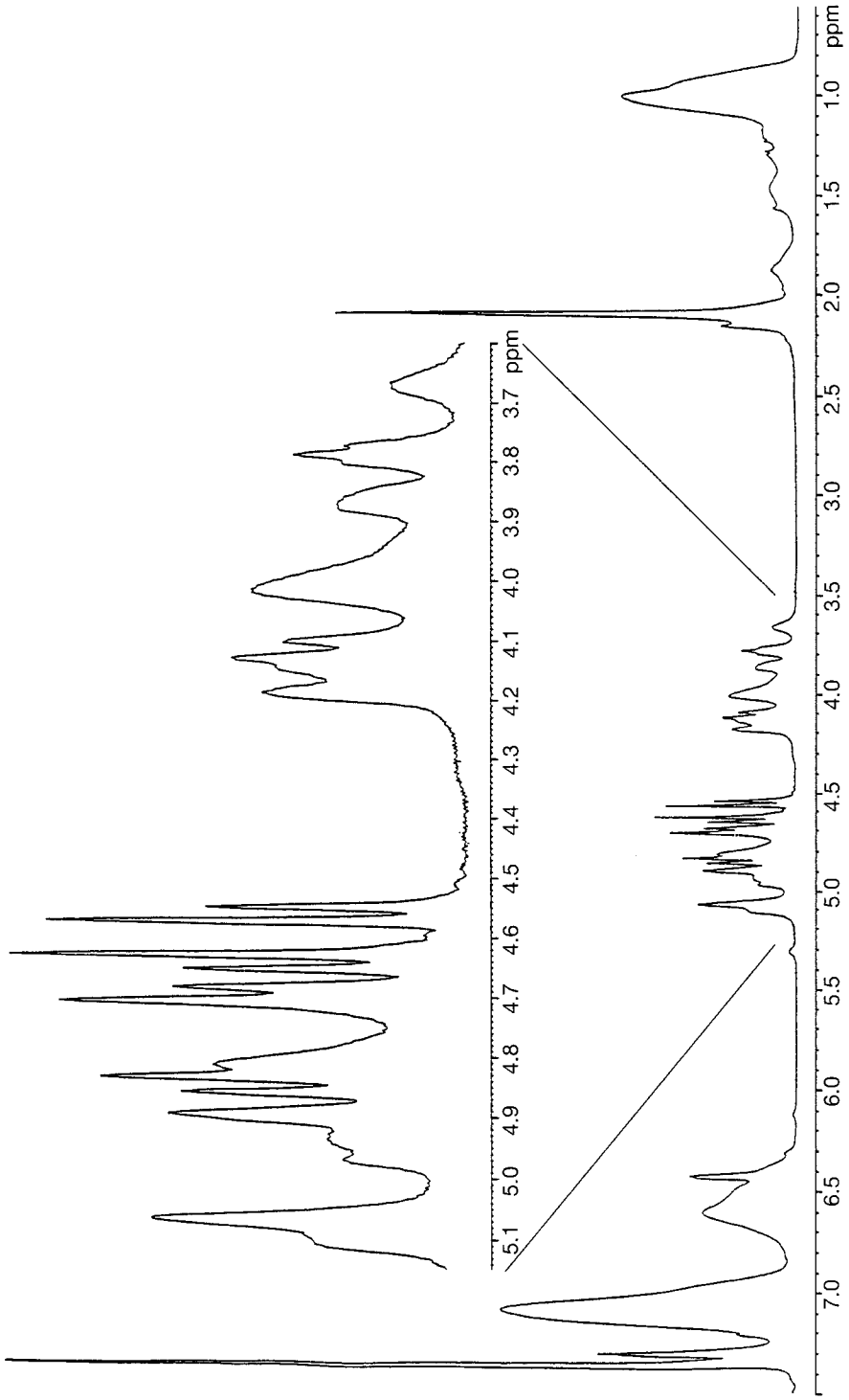

Scheme 17. Spectrum of **32** obtained by high-resolution magic angle spinning ^{1}H NMR spectroscopy.

Gated-Decoupling–^{13}C NMR Spectroscopy After ^{13}C Enrichment

Wong et al. monitored the solid support synthesis of the sialyl Lewis^x tetrasaccharide using ^{13}C-enriched linker and protecting groups.[71] The ^{13}C-labeled linker was attached to the polymer support and functioned as an internal integration standard for the ^{13}C-enriched protecting groups of the growing oligosaccharide. After each coupling reaction, the NMR signal of the protecting group at the growing end of the molecule was compared with the signal of the internal standard to quantify the coupling. This method does not require special NMR hardware such as an HR-MAS probe but can be carried out in standard 5 mm NMR tubes. On the other hand, this method yields no detailed structural information regarding the anomeric purity of the compound prepared. The chemical integrity of the glycosidic linkages formed must be determined after cleavage of the target molecule from the solid support.

CONCLUSIONS AND OUTLOOK

Over the last 5 years a number of very promising strategies for the solid support synthesis of oligosaccharides and glycoconjugates have been developed. Several of the glycosylating reagents successfully used in solution have yielded promising results under the soild phase paradigm. While a number of different solid support matrices and linkers have been explored, much work in this area remains to be done. The rapid developments in combinatorial chemistry on the solid support have created novel support matrices, which will find application to solid phase oligosaccharide synthesis. Innovative linkers will be required to allow for the use of highly flexible protecting group strategies in the synthesis of branched oligosaccharides.

Given the progress in the development of methods for the on-resin analysis of oligosaccharides, reaction development and optimization will be drastically accelerated. Novel glycosylating agents and orthogonal glycosylating schemes will further impact solid support synthesis. Alternative glycosylation methods such as the use of glycosyltransferase-catalyzed reactions on the solid support have also made great strides. For the synthesis of diverse structures, including natural and nonnatural glycoconjugates, chemical synthesis will be of paramount importance.

While much progress has been made, many challenges remain before a flexible, high-yielding and absolutely selective strategy for the synthesis of oligosaccharides on the solid support becomes available. Once these problems have been solved, the construction of an automated oligosaccharide synthesizer may become feasible. Detailed studies concerning the structure and function of oligosaccharides and glycoconjugates will be possible once rapid access to complex glycoconjugates is a reality.

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