Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries.
Edited by Peter H. Seeberger

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PREFACE

Glycobiology has provided many compelling results that place oligosaccharides and glycoconjugates in the center of a host of signal transduction processes at the molecular and cellular levels. It was found that oligosaccharides in the form of glycoconjugates mediate a variety of events, including inflammation, immunological response, metastasis, and fertilization. Cell surface carbohydrates act as biological markers of various tumors and as binding sites for other substances, including pathogens.

A major impediment to the rapidly growing field of molecular glycobiology is the lack of pure, structurally defined complex carbohydrates and glycoconjugates. Although these molecules are often found only in low concentrations in nature, the identification and isolation of complex carbohydrates from natural sources is greatly complicated by their microheterogeneity. Detailed biophysical and biochemical studies of carbohydrates require sufficient quantities of defined oligosaccharides. The procurement of synthetic material presents a formidable challenge to the synthetic chemist. While the need for chemically defined oligosaccharides has steadily increased, the synthesis of these complex molecules remains time consuming and is carried out by a few specialized laboratories.

Many innovative methods in carbohydrate chemistry have been developed and are covered in several very recent (at the time of writing) books on this subject. Although the synthesis of oligopeptides and oligonucleotides has benefited greatly from the feasibility of conducting their assembly on polymer supports, solid support oligosaccharide synthesis has, after some reports in the 1970s, been deemed too difficult for a long time. More recent developments in solution-phase carbohydrate synthesis methodology, combined with a more general appreciation of the advantages of solid support synthesis, have led to renewed interest in this field. The advent of combinatorial chemistry has energized investigations into methods applicable to the generation of diverse libraries of oligosaccharides and glycoconjugates.

This book covers all of the most recent (at the time of writing) developments in the field of solid support oligosaccharide synthesis. Included are chapters discussing different synthetic strategies, glycosylation protocols, the use of solid supports versus soluble polymeric supports and "on-resin" analytical methods. Special topics such as the formation of β-glycosidic linkages on solid support are also discussed.

Combinatorial chemistry has provided new ways for the pharmaceutical industry and for academic researchers to address specific problems in a time- and resource-efficient manner. Given the involvement of specific oligosaccharide structures in signal transduction processes, combinatorial carbohydrate libraries are

expected to provide a wealth of information and to lead to a detailed understanding of the structures involved in these processes. Because of the complexity of the task, only few approaches have been reported. Different approaches are summarized in the last part of this volume and keep the reader abreast of the latest developments in the field.

Finally, solid-phase glycopeptide synthesis is highlighted in two chapters describing exciting new developments in this area.

This book covers the state-of-the-art developments in the field until the beginning of the year 2000. At this time it becomes clear that the tremendous progress has set the stage for the conception of an automated oligosaccharide synthesizer. The ingenuity and hard work of many synthetic chemists will eventually lead to a situation already familiar within the peptide and oligonucleotide arenas; defined oligosaccharides and glycoconjugates will become readily available for biochemical and biophysical studies.

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1 Solid-Phase Carbohydrate Synthesis: The Early Work

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1.1 INTRODUCTION

The first steps toward solid-phase oligosaccharide synthesis date back to the early 1970s.[1] Intriguing features associated with the solid-phase paradigm that prompted researchers to explore oligosaccharide synthesis on solid support included maximized yields by use of excess reagents, ease of purification, and synthesis speed. By 1970 solid-phase peptide synthesis,[2] the concept of which had just been extended to the synthesis of depsipeptides,[3] had already been automated.[4] Given the immense impact of automated solid-phase oligopeptide[5] and later oligonucleotide synthesis[6] on the development of the biochemistry and biology of these molecules, the enthusiasm for developing related methodology for the synthesis of oligosaccharides is quite understandable.

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. While oligopeptides and oligonucleotides consist of merely linear chains, oligosaccharides, bearing up to four sites of potential elongation, are often branched, requiring flexible protecting group strategies for the effective differentiation of an array of similar functionality (hydroxyls and amines). The formation of a new stereogenic center in every glycosylation step further complicates oligosaccharide synthesis. Additionally, traditional acid-sensitive linker systems used for peptide synthesis are often incompatible with the Brönsted or Lewis acidic glycosylation conditions. Thus, a series of problems have to be considered in the planning process: (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.

Because of the lack of availability of several of these required methodologies, the initial attempts described below were ultimately not continued. However, they explored most of the fundamental issues that provide the basis for solid-phase oligosaccharide synthesis practiced today. In this chapter we focus on pioneering work carried out in the 1970s.

1.2 SOLID-PHASE STRATEGIES

Fréchet and Schuerch were the first to report on the synthesis of di- and trisaccharides on a solid support in 1971.[7] Glucosyl bromide **2** was attached to allyl alcohol functionalized Merrifield resin **1** by simple alcoholysis, preparing the first resin-bound monomer **3**. The reaction was carried out in benzene or tetrachloromethane with excess donor over 2–4 days in the presence of 2,6-dimethylpyridine, providing **3** in yields up to 96%, as determined by weight gain of the resin.[8] These conditions, resulting in a rather slow reaction, were chosen to minimize side reactions often associated with activation by metal ions. The use of *p*-nitrobenzoate as temporary protecting group at C6 aimed at achieving high α-selectivity in the coupling reactions, as was established by solution studies.[9] After removal of the *p*-nitrobenzoyl group, the coupling was reiterated twice yielding resin-bound trisaccharide **5** in near-quantitative stepwise yield. The yield was determined by weight gain of the resin and on the basis of the free hydroxyls of the latest attached sugar monomer. Cleavage from the resin was accomplished by ozonolysis followed by reduction of the ozonide with dimethyl sulfide in varying yields between 51% and 91% to furnish 2-hydroxyethyl glycoside **6**. As no suitable analytical method was available at the time, a high degree of α-glycosidic linkages in the product was assumed on comparison of the optical rotation of model compounds obtained by solution syntheses (Scheme 1.1A). Attempts to achieve β-selectivity in the solid-phase glycosylation by changing the electronic properties of the C6-protecting group[9] were not successful.[8] While long reaction times and the failure to selectively synthesize β-linked glycosides severely limited the generality of this approach, α-linked 1,6-oligomers were prepared reasonably well.

Zehavi and coworkers introduced the original concept of a photolabile linkage[10] of the first carbohydrate monomer to the polymeric phase (Scheme 1.1B).[11] Applying essentially the same coupling conditions as Fréchet, disaccharide **8** was obtained in approximately 90% yield per coupling step. Unfortunately, photolytic release of the disaccharide from the resin did not proceed as well on a preparative scale as in previous solution-phase model studies,[12] and debenzylated reducing isomaltose **9** was obtained in only 12.5%, based on resin-bound monomer. High α-selectivity in these glycosylation reactions was demonstrated by digestion experiments using α- and β-glycosidases.

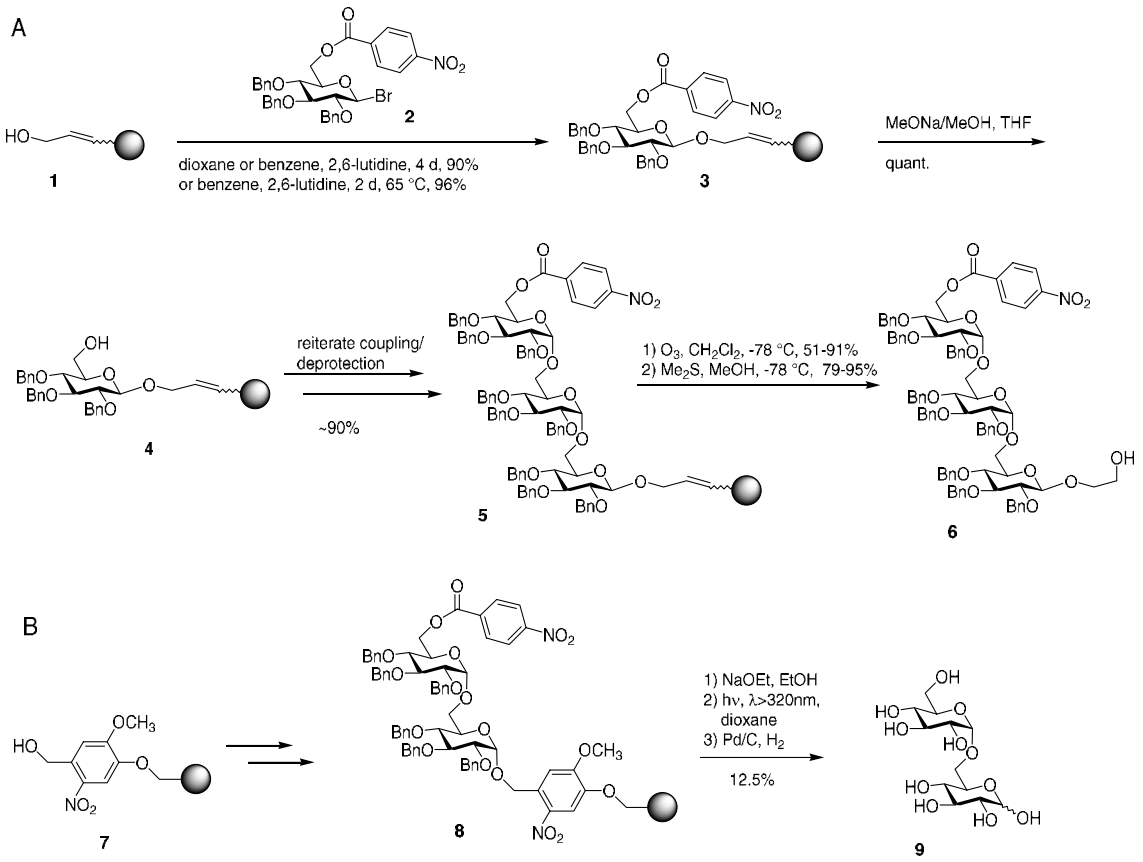
Anderson and coworkers introduced a thioglycosidic linkage[13] to the solid support in 1976 to afford the free reducing oligosaccharide after release from the support (Scheme 1.2A).[14] Using a set of protecting groups similar to those mentioned above,

resin-bound monomer **12** was obtained either by coupling of a stoichiometric amount of thiosugar **11** to chloromethylated polystyrene, or by glycosylation of the corresponding thiol functionalized resin with an excess of glucosyl donor in comparable yields (about 80%). The free C6 hydroxyl group was glycosylated with excess glucosyl donor **13** under repeated alcoholysis conditions to furnish support-bound disaccharide **14** in 75% yield. Refluxing disaccharide **14** in benzene in the presence of methyl iodide and benzyl alcohol as acceptor, furnished free disaccharide **15** as the major component in a mixture of products. Gas-liquid chromatography (GLC) analysis of the disaccharide fractions revealed an α/β-diastereomeric ratio of 11.5–19:1, confirming the findings by Schuerch and Zehavi on similar systems.[9]

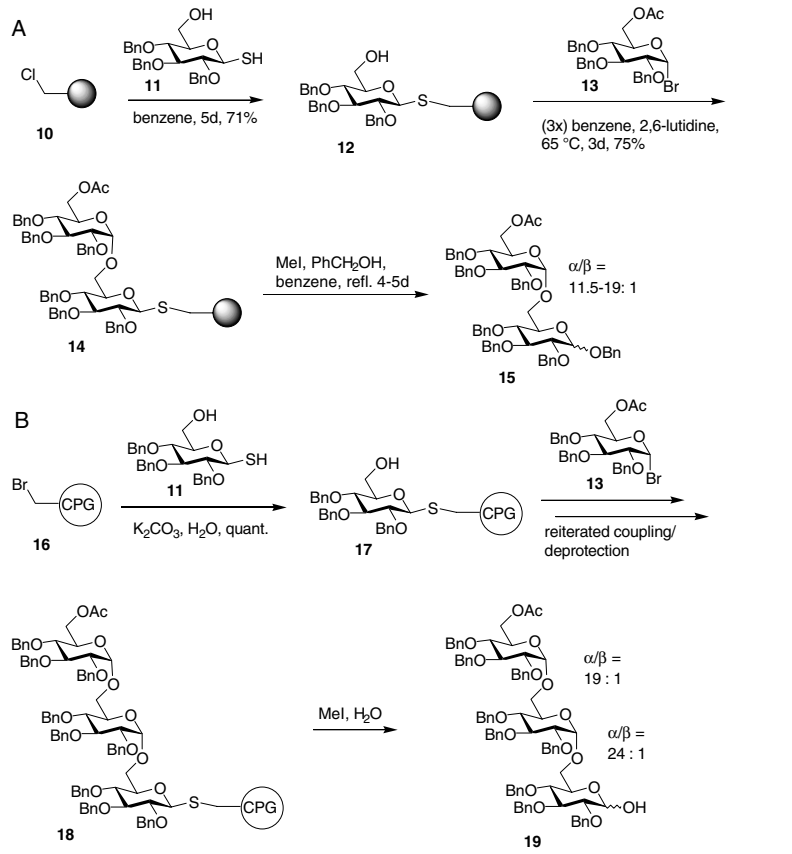
In addition to differently functionalized polystyrene (Merrifield's resin), controlled-pore glass, as a nonswelling inorganic polymeric support was already evaluated for solid-phase oligosaccharide synthesis in its pioneering days. Schuerch reported the attempted glycosylation of a zirconia-coated glass surface carrying unsaturated alcohol acceptor sites, but only poor glycosylation yields (<20%) could be achieved.[15] A second attempt by Anderson et al. was based on porous glass beads (pore size 2500 Å) functionalized with bromobenzyl attachment sites.[14] The first thiosugar monomer **11** was quantitatively coupled to the support and subsequently glycosylated up to the trisaccharide employing excess donor **13** in repeated alcoholysis reactions. However, coupling yields were low even after prolonged reaction times. HPLC analysis of the cleaved trisaccharide **19** showed essentially the same α/β ratios as for reactions carried out on polystyrene support (Scheme 1.2B).

In addition to these selective α-(1→6) glucosylations, several β-selective glycosylation reactions have been studied on the solid support making use of participating ester groups. Gagnaire and coworkers described two approaches to solid-phase oligosaccharide synthesis linking the first carbohydrate monomer to the polymeric support via an ester bond. Glucosamine acceptor **21** was immobilized by esterification with acid chloride functionalized "popcorn" polystyrene at the C6-hydroxyl. Benzoylation of the remaining free C4-hydroxyl and selective removal of the benzoyl propionate protecting group furnished acceptor **22**. Repeated glycosylation with excess glucosamine chloride donor **23** employing Helferich conditions furnished β-linked disaccharide **24** in 85% yield.[17] This was the first time that a sterically more hindered secondary acceptor was glycosylated on a polymer matrix. Cleavage with sodium methoxide in methanol/dioxane and subsequent reacetylation rendered free disaccharide **25** in 51%, yield based on polymer-bound monomer **22** (Scheme 1.3). A β-(1→6)-linked glucosamine dimer had previously been prepared on a "popcorn" polystyrene by the same group in a similar fashion.[18] A major drawback of this approach on "popcorn" polystyrene was considerable loss of material at several stages during the syntheses due to partial solubilization of the matrix.

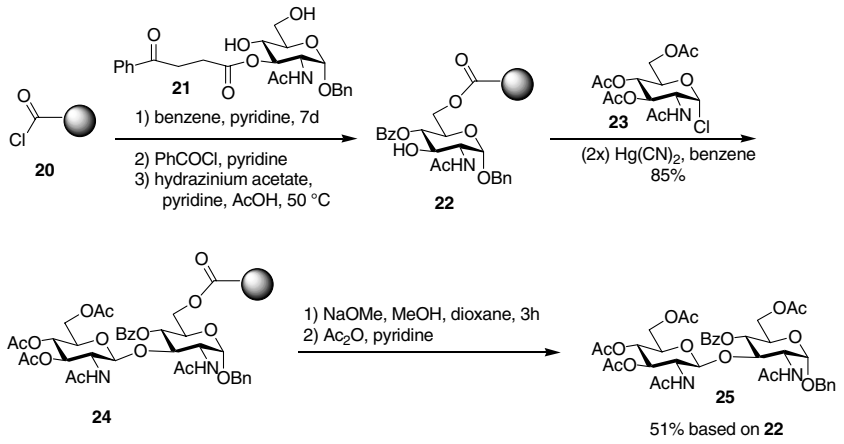
Gentiotetraose, an all β-(1→6)-linked tetramer of glucose was selectively prepared by Gagnaire and coworkers (Scheme 1.4).[19] 6-*O*-Trichloroacetylated glucosyl bromide **27** was attached to succinoylated 2% crosslinked polystyrene. After selective



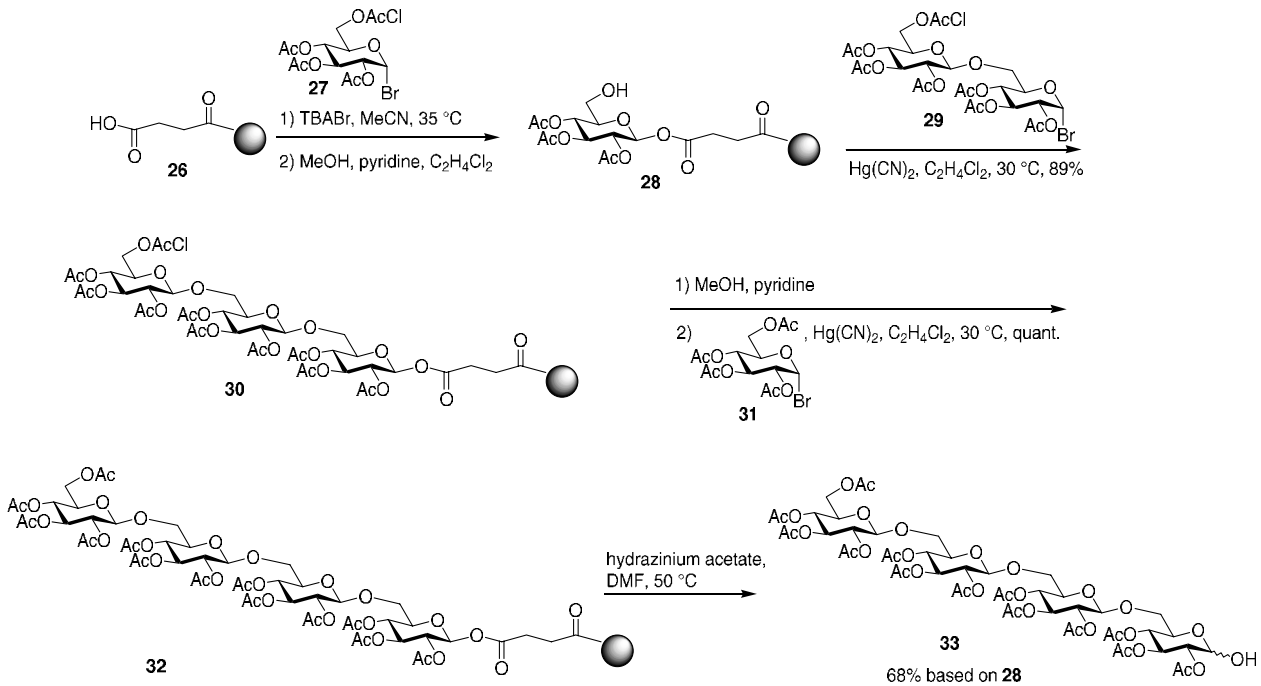
Scheme 1.1 Early solid-phase approaches to α-(1$\rightarrow$6)-linked oligosaccharides.



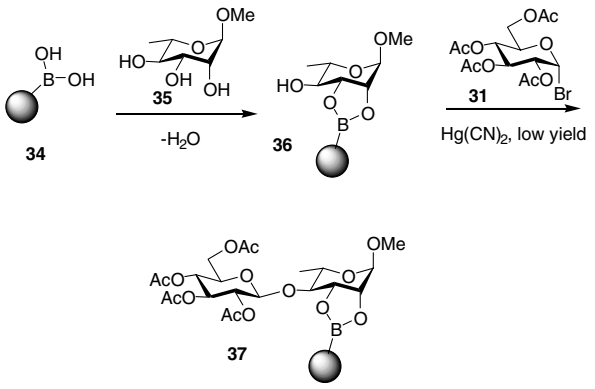
Scheme 1.2 Solid-phase oligosaccharide synthesis employing thioglycosidic linkages to different solid supports.



Scheme 1.3 Solid-phase synthesis of β-(1→3)-linked glucosamine dimers employing ester linkages to the support.



Scheme 1.4 Solid-phase synthesis of β-(1→6)-linked gentiotetraose employing ester groups for permanent protection, for temporary protection, and as linkage to the support.



Scheme 1.5 Disaccharide synthesis on a solid support using a cyclic boronic acid ester linker.

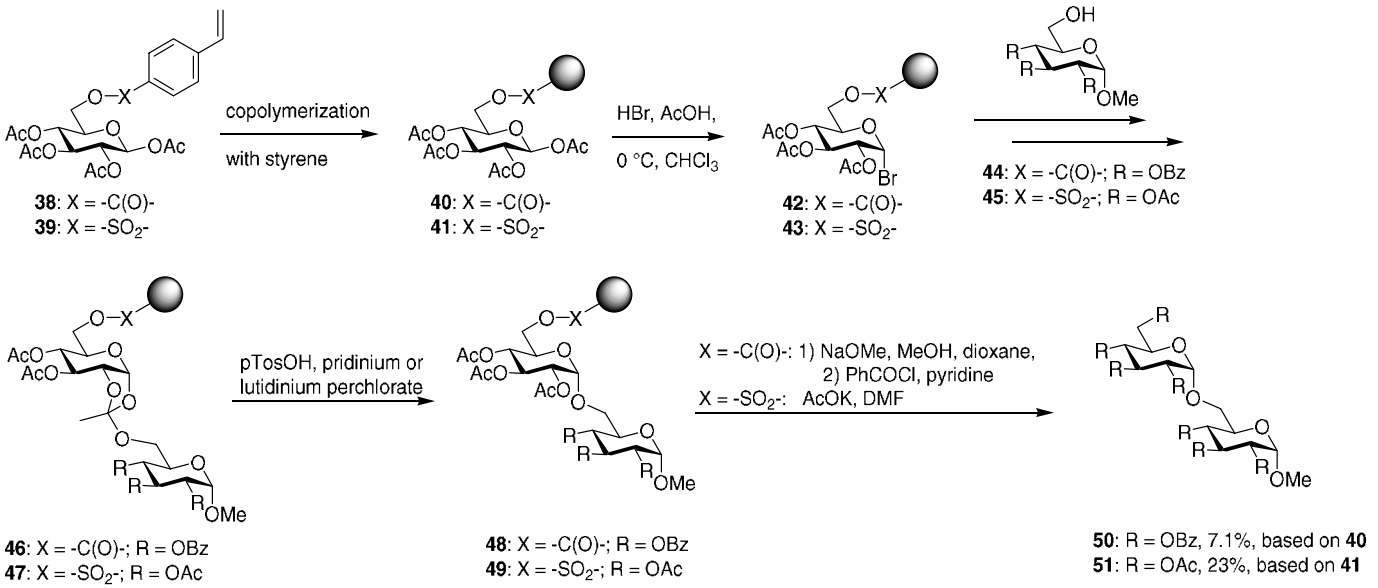
removal of the trichloroacetyl group with ammonia in dioxane subsequent glycosylation with disaccharide donor **29**, deprotection and glycosylation with glucosyl bromide **31** employing Helferich conditions afforded resin-bound gentiotetraose **32**. Cleavage from the support with hydrazinium acetate[20] furnished the crude tetramer **33** in almost 70% yield containing traces of di- and trisaccharides.

Changing the 2-*O*-acetyl group in **31** to a nonparticipating benzyl group resulted in preferential α-glycosylation yielding 76% of disaccharide (α/β ratio 4.4:1).[21] The same support and donor were employed in the diastereospecific synthesis of β-(1→3)-linked glucose dimer laminarabiose in good yield on 2% crosslinked polystyrene.[22] It should be noted that the stereochemical outcome of these solid-phase syntheses was virtually identical to that in solution phase. An interesting feature of these syntheses is the exclusive use of ester groups for both permanent and temporary protection of hydroxyl groups and attachment to the support. The esters were differentiated by their lability to treatment with base.

Fréchet proposed a resin-bound cyclic boronic acid ester as an unconventional mode of attaching the first sugar monomer to the solid phase (Scheme 1.5).[23] This linkage was very selectively formed with *cis*-1,2 and *cis*-1,3-diols under mild azeotropic conditions, leaving one hydroxyl for further chain elongation. Simple hydrolysis of the cyclic esters afforded the free sugars. Unfortunately, couplings using monosaccharide **31** as glycosyl acceptor proceeded sluggishly and in poor yields,[1b] thus rendering this approach unattractive.

1.3 OLIGOSACCHARIDE SYNTHESIS ON SOLUBLE POLYMERS

All the described solid-phase glycosylation protocols required long reaction times to proceed in reasonable yields because of the slower reaction kinetics on support than in solution. Furthermore, since no analytical means were available to monitor the progress of the reaction on the bead, development of optimal reaction conditions was difficult. The approach described by Guthrie and coworkers in the early 1970s for



Scheme 1.6 The first approaches to oligosaccharide synthesis employing soluble polymer supports.

polymer-supported oligosaccharide synthesis was unique in many respects. The polymer support was created by copolymerization of styrene with a sugar monomer suitably functionalized with a polymerizable *O*-protecting group.[24] This linear polymer allowed for glycosylation reactions in homogeneous solution, thus avoiding some of the principal shortcomings of any solid-phase approach; on the other hand, the support could be readily precipitated for purification to take advantage of the solid-phase paradigm. This was the only approach utilizing a glycosyl donor that was generated on the support and was reacted with excess acceptor in solution. Carbohydrate monomers **38** and **39** were copolymerized with styrene to yield soluble polystyrene polymers **40** and **41**, containing approximately 0.15 and 0.06 mol% of sugar monomer, respectively. Disaccharide formation was effected via glycosyl bromide **43** and orthoester **47** following the Kochetkov orthoester glycosylation method (Scheme 1.6).[25] Treatment of the resin with potassium acetate in refluxing DMF (dimethylformamide) yielded gentiobiose octaacetate **51** in 42% yield, based on the support-bound monomer **41**.[26] When a benzoyl linkage to the support **40** was used instead of the arylsulfonyl linkage present in **41**, cleavage by methanolysis furnished 23% of free disaccharide, based on available support-bound disaccharide **48**.

This approach had several drawbacks. Most side reactions in glycosylation reactions occur within the glycosyl donor moiety, thus terminating the growth of the corresponding chain under this donor-bound paradigm. Moreover, repeated glycosylations could not be used to improve coupling yields. In addition, the strongly acidic conditions used for glycosyl bromide formation could affect acid-labile glycosidic bonds in the oligosaccharide chain. Another disadvantage of the soluble-polymer-supported synthesis was a substantial loss of material during the precipitation and filtration steps following each reaction on the support.

1.4 THE PERIOD OF STAGNANCY (1976–1991)

The pioneering work in solid-phase oligosaccharide chemistry provided the foundation for the rapid progress that several groups have made in the area as described in the following chapters. These early approaches explored some of the important fundamental issues, including different strategies (donor- vs. acceptor-bound synthesis), various solid supports (soluble and insoluble), and linker systems. Unfortunately, at that time "this approach [was] not competitive with the more classical solution chemistry methods, due mainly to the lack of suitable glycosidation reactions" (Fréchet),[1b] that would meet the demands and conditions of solid-phase synthesis. The absence of suitable on-bead analytical tools for effective reaction monitoring made reaction development particularly difficult. Soluble polymers circumvented in theory some problems associated with solid supported synthesis such as reaction kinetics and reaction monitoring. The considerable loss of material during the workup steps also compromised the advantages of the solid-phase paradigm, since it was less effective and more laborious than syntheses on solid support. Eventually, the field came to a complete standstill for a 20-year period, and with only one exception mentioned below, no further progress was reported. Major

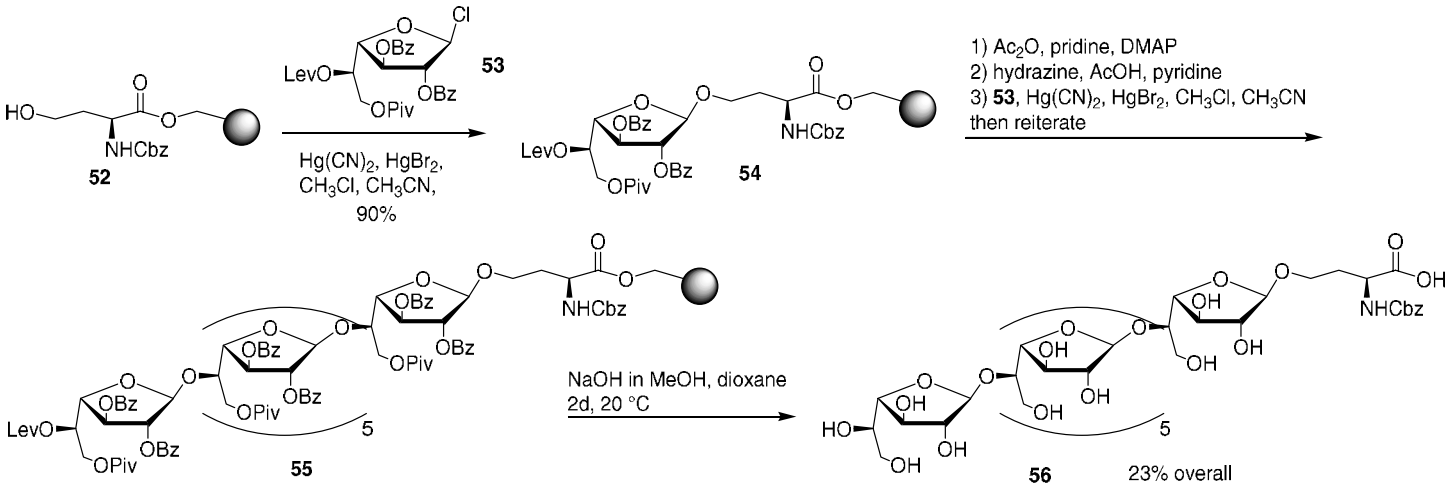
advances in solution-phase oligosaccharide synthesis with regard to donor reactivity, glycosylation selectivity, protecting group diversity, and analytical techniques were necessary before solid-phase oligosaccharide synthesis (SOS) set the stage for the developments described in the following chapters.

During the 1980s and early 1990s some solid-phase[27] and soluble-polymer-supported[28] syntheses of bacterial capsular oligosaccharides were reported by chemical formation of phosphodiester bonds. Van Boom et al. reported the only notable advance based on chemical glycosylations on solid support, expanding the initial attempts to the solid-phase synthesis of a large antigenic oligosaccharide that exhibited properties of a synthetic vaccine (Scheme 1.7).[29] Linear β-(1→5)-linked galactofuranosyl homopolymers of varying length, found to be immunologically active in *Aspergillus* and *Penicillium* species,[30] were chosen as targets for a repetitive oligosaccharide synthesis on Merrifield resin. These synthetic structures were the basis for studies correlating oligosaccharide length and immunogenicity. Merrifield's resin was functionalized with L-homoserine, resulting in a 0.5 mmol/g loading of acceptor resin **52**. Coupling with a twofold excess of galactofuranosyl chloride **53** employing Helferich conditions furnished resin-bound monosaccharide **54** stereoselectively in 90% yield as determined after cleavage from the resin. In order to facilitate the purification of the final products, acetylation of any free hydroxyl groups was chosen as a capping step after each glycosylation reaction. Chain elongation was achieved by selective removal of the C5-levulinoyl protecting group in resin-bound monomer **54** with hydrazine, pyridine, and acetic acid and subsequent β-stereospecific glycosylation with donor **53**. The deprotection, glycosylation, and capping steps were reiterated up to the heptamer stage. Base hydrolysis released heptamer **56** in 23% overall yield, corresponding to 89% average yield over 13 coupling and deprotection steps. Complete deprotection afforded the oligomers up to the heptamer in bioconjugatable form. These semisynthetic constructs were used for biological tests in rabbits demonstrating an increase of immunogenicity with increasing oligosaccharide chain length.

While chemical polymer-supported oligosaccharide synthesis with the abovementioned exception came to a halt during the 1980s, interest in enzymatic methods for oligosaccharide and glycopeptide[31] synthesis on both insoluble[32] and soluble[33] polymeric supports continued since the first disclosure by Zehavi in 1983. The intriguing features of this approach include α/β-specificity and regioselectivity, which reduces the need for elaborate protecting group manipulations in many cases. These methods have been reviewed elsewhere[34] and exceed the scope of this chapter.

High selectivity and substrate specificity of glycosyl transferases make them valuable catalysts for special linkages in polymer-supported synthesis. There is, however, still a rather limited set of enzymes available to date, and the need to synthesize a variety of natural and non-natural oligosaccharides prevails. Particularly with regard to combinatorial approaches, chemical solid-phase oligosaccharide synthesis promises to meet the demands most effectively.

With the development of novel, powerful, and selective glycosylating agents,[35] exemplified by the introduction of glycosyl trichloroacetimidates[36] to



Scheme 1.7 Solid-phase synthesis of an immunogenic heptasaccharide by van Boom et al.[29]

polymer-supported oligosaccharide synthesis by Krepinsky in 1991,[37] interest in chemical solid-phase oligosacharide synthesis was revived. The following chapters give an overview of the achievements in the field, based on significant advances in glycosylation methodology, polymer supports, linker systems, "on bead" analytical tools, and protecting group development. Today, solid-phase oligosaccharide synthesis is competitive not only with classical solution-phase methods but also with many of the major problems solved, as automation has come within reach. These achievements promise significant impact on the glycosciences.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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2 The Glycal Assembly Method on Solid Supports: Synthesis of Oligosaccharides and Glycoconjugates

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2.1 INTRODUCTION

The three major classes of biopolymers found in eukaryotic systems are nucleic acids, proteins, and polysaccharides. The latter class is the most complex with respect to structural and stereochemical diversity. Polysaccharides indeed possess a massive "information" content. Furthermore, polysaccharides are commonly found in nature covalently attached (conjugated) to other biomolecules such as proteins, isoprenoids, fatty acids, and lipids.[1]

Polysaccharides are involved in a number of significant biological functions, beyond merely acting as structural elements and serving as sources of energy.[2] For example, they play key roles in such processes as pathogen binding, inflammation, metastasis, and fertilization.[3] To study such processes, there has been an increasing need to gain access to usable quantities of these materials in pure form.

Oligosaccharides and glycoconjugates in living cells often exist as closely related mixtures. Their isolation from natural sources in homogeneous form is therefore very difficult, involving tedious purification and difficult characterization. This sequence of steps tends to result in low yields. This difficult situation presents chemical synthesis with a major opportunity to positively affect progress in the biochemical understanding of the processes described above.[4]

The application of solid-phase synthesis and automation has revolutionized much of the chemical and biochemical research related to peptides and nucleic acids.[5] Thus, it is likely that successful methods to synthesize oligosaccharides and glycoconjugates

on solid supports could bring with them a similar impact in carbohydrate related research.

The development of methods for the synthesis of oligosaccharides on a polymer support requires the simultaneous solution to a myriad of problems.[6] The high "information" content of these structures means that their synthesis involves a level of complexity that dwarfs the one associated with peptides and oligonucleotides. One must tackle the usual considerations concerning the nature of the support material, selection of a suitable linker, and monitoring of reaction progress, possibly by "on resin" techniques. Choices must also be made as to whether the carbohydrate attachment should occur via its "reducing" or "nonreducing" end. In a way this decision is not unlike that involved in undertaking to grow a peptide chain via its carboxy or amino terminus. However, unlike the solid-phase synthesis of the other classes of biopolymers, in oligosaccharide synthesis there is a more challenging requirement for the construction to differentiate among numerous and similar functionalities (hydroxyl or amino). An efficient and highly flexible protecting group strategy must be adopted. Finally, and perhaps most importantly, each glycosidic bond to be fashioned constitutes a new locus of stereogenicity. Therefore, high-yielding and stereospecific coupling reactions are necessary and must be amenable to being conducted with one component anchored to an insoluble matrix.

As this book will attest, remarkable progress has been achieved in the assemblage of oligosaccharides and glycoconjugates on solid support. In this chapter we report on our laboratory's advances, which have led to the efficient assembly of relatively complex and biologically relevant structures, including the Lewis^b blood group and globo-H polysaccharides. The synthesis of these compounds is also described.

2.2 WHY GLYCAL ASSEMBLY? STRATEGIC CONSIDERATIONS

As was alluded to above, two possible approaches immediately present themselves for the synthesis of oligosaccharides on solid support. These involve the decision as to the mode of attachment of the first carbohydrate to the matrix.

In one scenario, the first carbohydrate is anchored via its "reducing" end (see Scheme 2.1, case 1). Here the support-bound carbohydrate will function as an acceptor in the coupling step 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 requires that the donor (**D**) of the previous step be furnished with a uniquely removable blocking group at the site of the next proposed elongation. Clearly, under the glycosylation conditions, the solution-based donor **D** cannot possess simultaneously a free hydroxyl and the intact glycosyl donating function. This need to expose a unique hydroxyl group in the polymer-bound construct will likely necessitate awkward functional group adjustments in the preparation of **D**.

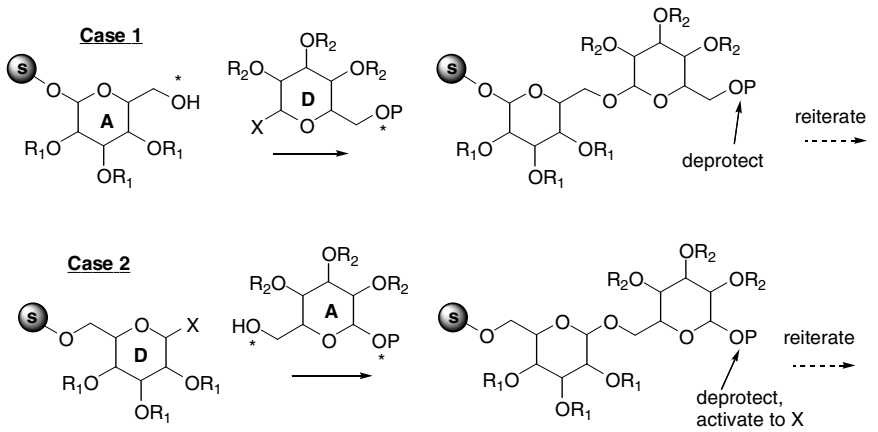
Connection of the glycosyl acceptor to the solid support (case 1) allows for the use of excess donor **D** (usually more fragile than **A**), a feature that can be used to advantage to drive reactions to completion. Nevertheless, this strategy requires a capping step to prevent the formation of deletion sequences, which would complicate

final purification. Moreover, and perhaps most importantly for the broader context of glycoconjugate synthesis, the completed oligosaccharide construct would likely require retrieval from the solid matrix before conjugation to the peptide or lipid, unless this portion were already present as part of the linker to the solid support.

In the other scenario, which we have found more novel, for reasons that will be explained shortly, the oligomer undergoing elongation is mounted to the solid support somewhere in the nonreducing region. In this case, the reducing end (i.e., glycosyl donor portion) of the molecule is available for coupling to a solution-based acceptor **A** (Scheme 2.1, case 2).

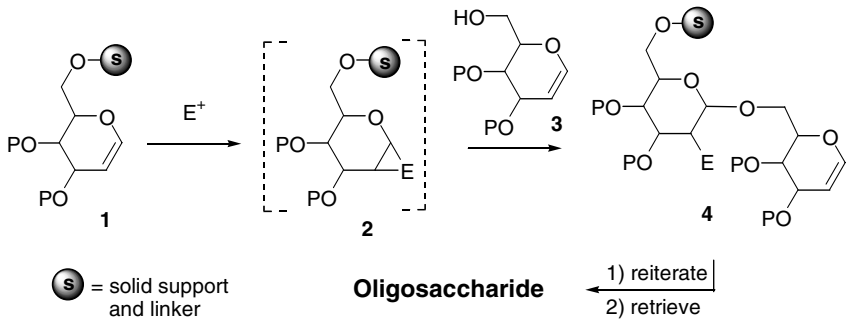
The use of **A** has two requirements. The first is that the precise acceptor site on **A** be properly identified. The second is that the reducing end of **A** be functionalized so that new donor capacity can be installed at the anomeric carbon of the elongated construct, in anticipation of the next coupling event. Not unlike the possible situation of case 1, a serious question of functional group compatibility must be anticipated during glycosylation in case 2. During the coupling step, acceptor **A** cannot possess a fully equipped, next-stage anomeric donor function at the same time as it carries a free hydroxyl group.

The reason for favoring the second scenario described above has to do with our development of the "glycal assembly" method, which appeared to offer several advantages for the solid-phase synthesis of oligosaccharides via this strategy (case 2, Scheme 2.1). Scheme 2.2, with the expression **1**→**2**→**4**, captures the essence of the method and reveals the potential attractiveness of this approach. In this scheme, the nature of E+ and of the oniumlike species **2** has been left unspecified, as well as any indication of stereochemistry. Nevertheless, it is apparent that the glycal terminus of **1** could be converted to a donor function as represented in a general way with **2**. For



Key: S, solid support and linker; P, unique protecting group; X, activating group; * indicates a uniquely differentiated hydroxyl group

Scheme 2.1 Strategies for building an oligosaccharide chain: glycosyl acceptor linked via its reducing end (case 1) and donor linked via a nonreducing end (case 2).



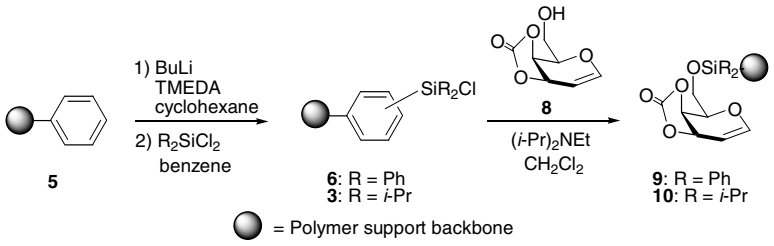
Scheme 2.2 General strategy for the synthesis of oligosaccharides on a solid support using the glycal assembly method.

example, compound **2** could be an isolable entity such as a 1,2-anhydrosugar,[7] in which case E^+ corresponds to an epoxidizing agent. Moreover, through employment of a glycal as the solution based acceptor, the scheme benefits from relative simplicity in the identification of strategic hydroxyls for glycosylation. It is in fact a lot more straightforward to distinguish the hydroxyls of a pyranosidal glycal than those of a pyranose.

Compound **2** acts as a support bound glycosyl donor to yield **4** when treated with acceptor glycal **3**, along with any necessary agents to promote the glycosylation (Scheme 2.2). The process can be repeated to assemble the desired oligosaccharide. This is followed by retrieval from the support and purification by chromatographic methods. *Two major factors influence "reiterability" and therefore the success of this approach: (1) the polymer-bound glycal double bond must be effectively converted to a donor function and (2) the coupling conditions must be tolerant of the glycal double bond present in the acceptor, which, for example, is labile in acidic environments.*The use of 3,3-dimethyldioxirane (DMDO) as the epoxidizing agent effectively converts **1** to a 1,2-anhydrosugar. This is the simplest of the possible cases and the most ideal situation. It is particularly successful when applied to an appropriately protected galactose series. As will be outlined in the following sections, some situations have demanded the elaboration of the glycal double bond to other donor functions, such as anomeric sulfides.

2.3 LINKER DESIGN

Following adoption of the glycal paradigm for the solid-phase assembly of oligosaccharides, the next strategic consideration involved the choice of a solid support and implementation of a method of linkage. Our first preference for the insoluble support was polystyrene 1% divinylbenzene copolymer, which is commonly used for the solid-supported synthesis of peptides. This polymer has high loading capacity, is compatible with a wide range of reaction conditions, and is cheap.



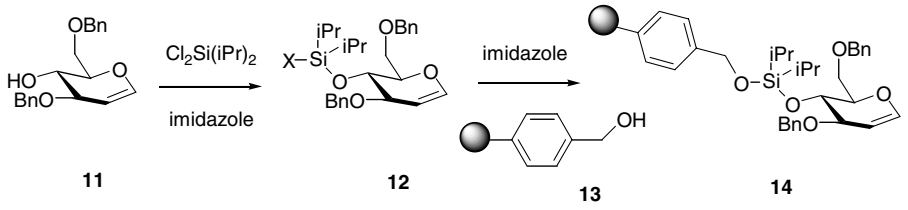
Scheme 2.3 Glycal attachment to a polystyrene resin via a silyl linker.

Launching of the program required attachment of the first glycal monomer, through its nonreducing end, to a polystyrene-based matrix. A covalent attachment through a silicon–oxygen bond would mimic one of the most common protecting groups used for alcohols. Using chemistry developed by Chan and coworkers,[8] lithiation of polystyrene **5** at aromatic sites, followed by silylation with a dihalosilane, such as diphenyldichlorosilane, resulted in polymer-bound silyl halide **6** (Scheme 2.3). This silicon–chlorine linkage now became the attachment site for glycal **8** to create polymer bound glycal **9**. Unreacted sites were capped by reaction with methanol. The extent of glycal loading was on average 0.3 mmol of saccharide per gram of polymer.

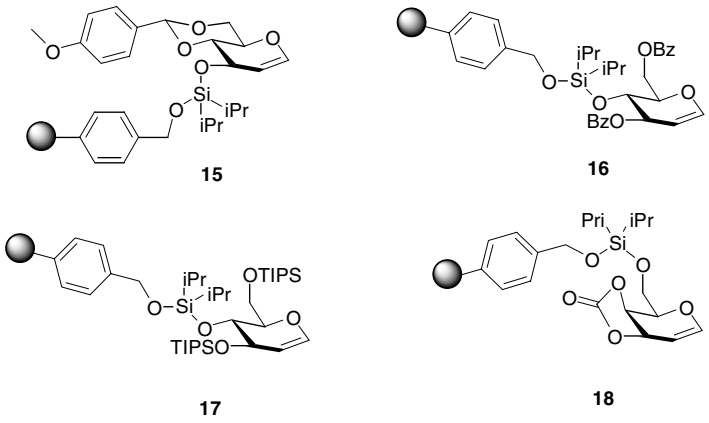
The success of this approach depended on the ability to load the monomer through the silylation reaction and, most of all, on the robustness of the silyl ether linkage during the coupling events. A significant improvement in stability was subsequently achieved through the use of a diisopropyl linker (bound glycal **10**) in place of the diphenyl arrangement (Scheme 2.3).

This loading approach is successful for the relatively unhindered 6-hydroxyl, but proved to be inefficient for more sterically encumbered alcohols. A modification of the abovementioned method has been developed to enable attachments through these more hindered sites.[9] This process is accomplished with inexpensive materials, and the linker is compatible with a variety of reaction conditions. Moreover, it allows for facile recycling of the polymer support for further use once the final carbohydrate assemblage is cleaved from the resin.

The success of this modification, exemplified by the use of 3,6-dibenzyl-glucal (Scheme 2.4), relies on the enhanced reactivity of a dialkyldihalosilane relative to its



Scheme 2.4 Polymer support silylene linking method for hindered hydroxyl-bearing glycals.



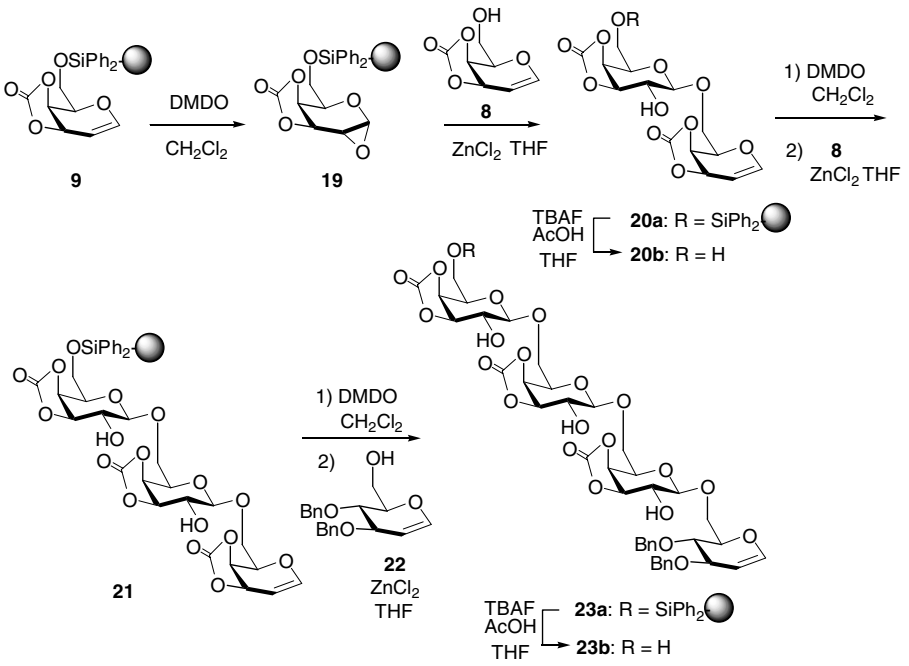
Scheme 2.5 Glycals loaded via silylene linkage.

mono-halogenated counterpart. This differential in reactivity allows for a single linkage of more sterically demanding systems in the first stage, while discouraging 2-fold silylation (see **11**→**12**). A more reactive nucleophile, such as a primary alcohol, even if polymer bound, can now join at the less active remaining silylating site. The commercially available hydroxymethyl-modified Merrifield[10] or Wang[11] resins perform well in the second-stage silylation, which effectively constitutes the loading step to furnish **14**. The unreacted sites on **13** are capped by reversing the order of additions (imidazole and diisopropydichlorosilane are added to the polymer followed by methanol). Scheme 2.5 shows a variety of other glycals, which were successfully loaded via this method. Construct **17** was fashioned from extremely hindered hydroxyl groups. Typical loading capacities were in the range of 0.12–0.19 mmol/g.

We are now poised to proceed with glycal assembly on solid support, and the synthesis of oligosaccharides will be described in the following sections. As will be evident, the synthesized structures became increasingly complex as our confidence increased in the applicability of the glycal assembly method to solid-phase synthesis. This confidence goes hand in hand with our ability to manipulate glycal double bonds, in the context of the solid matrix, into becoming other appropriate donors. The synthesis of glycopeptides will be described in Section 2.10.

2.4 SOLID SUPPORT GLYCAL ASSEMBLY VIA 1,2-ANHYDROSUGAR DONORS

Reduction to practice was first realized in the context of the synthesis of a linear tetrasaccharide, outlined in Scheme 2.6.[12] Polymer-bound galactal **9** was converted to the 1,2-anhydrosugar **19** by epoxidation with DMDO.[13] Polymer-bound **19** acted as a glycosyl donor when reacted with a solution of **8** in the presence of zinc chloride, resulting in disaccharide **20a**. The glycosylation procedure was reiterated using galactal **8** as acceptor, to afford **21**. One further reiteration using glucal **22** yielded

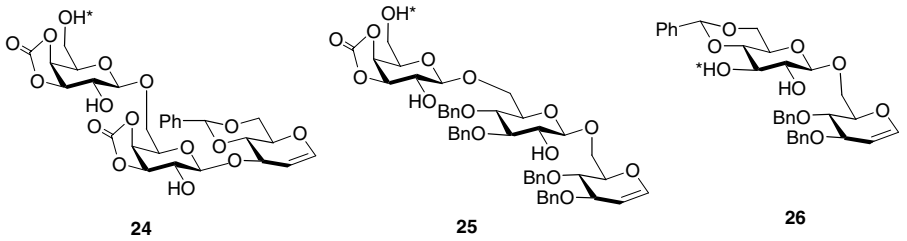


Scheme 2.6 Solid-phase synthesis of a tetrasaccharide using the glycal assembly method.

tetrasaccharide **23a**. The cleavage of the product from the polymeric support could be performed at any stage of the sequence as a means of checking the progress of each new coupling. This was done by stirring polymer bound saccharide in a 1:1 mixture of 0.1 M acetic acid in THF and 0.1 M TBAF in THF for 3–4 h, followed by filtration and rinsing with THF. The saccharides were purified by column chromatography on silica gel using methanol/ether eluent mixtures. Cleavage after the final coupling gave linear tetrasaccharide **23b** in 32% overall yield from **9**. This first success translated to an average yield of 70% per coupling cycle (consisting of epoxidation and glycosylation), assuming quantitative retrieval during fluoride-mediated cleavage.

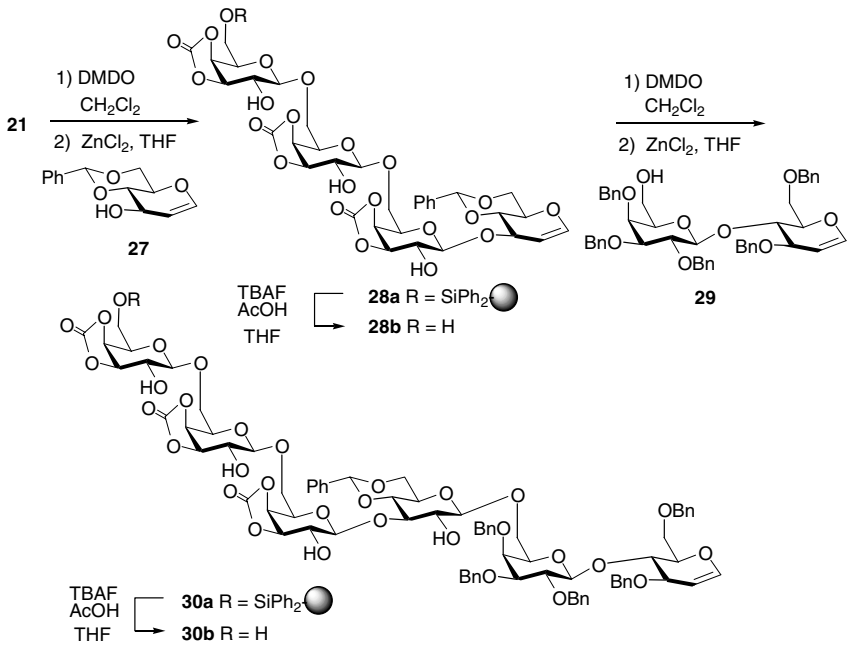
The method results in little or no "interior deletion" products. The final oligosaccharide is purified from highly polar byproducts with very straightforward chromatography. Most likely, any 1,2-anhydrosugar that has failed to couple to the acceptor is hydrolyzed during the intervening rinsing procedure. This hydrolysis has the dual benefit that it results in a permanently terminated sequence, which is also highly polar and therefore easily separable from the desired final product.

The versatility of this approach was demonstrated in the synthesis of a variety of oligosaccharides (Scheme 2.7). The synthesis of hexasaccharide **30** (Scheme 2.8) exemplified the use of a glucal acceptor with a secondary alcohol (**27**) as well as the use of a disaccharide acceptor such as **29**. These typical, varied systems can be included in the protocol without complications.



Scheme 2.7 Oligosaccharides assembled on solid phase via glycal assembly. The asterisk denotes the achorage site of the first glycal to the solid support via $SiPh_2$ linker.

The method can also be applied to the synthesis of branched oligosaccharides by capitalizing on the fact that the opening of a 1,2-anhydrosugar during glycosylation results in a newly exposed C2-hydroxyl group. This uniquely exposed hydroxyl function can serve in turn as a glycosyl acceptor and will be a key factor in the synthesis of blood group determinants.



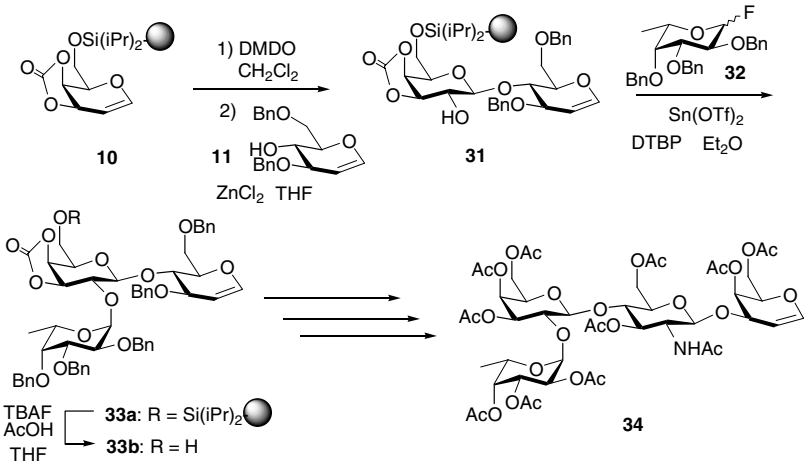
Scheme 2.8 Solid-phase synthesis of a linear hexasaccharide.

High-resolution magic-angle spinning NMR (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 "on resin" spectra of high quality.[14] This capability was first exemplified with the monitoring of the formation of crude product from the multistep synthesis of a trisaccharide similar to **21** (Scheme 2.6). This technique is less time-consuming and less wasteful of material than the cleavage method in the analysis phase. Since their introduction, on-resin NMR techniques have greatly facilitated the development of novel synthetic schemes of oligosaccharides and glycopeptides on solid support. We refer the reader to Chapter 8 in this book, which is dedicated to this topic.

2.5 SOLID-PHASE SYNTHESIS OF THE BLOOD GROUP H DETERMINANT

The synthesis of carbohydrate domains having blood group determining specificities[15] was the first striking demonstration of the power of glycal assembly on solid support. These branched structures are naturally expressed as glycoproteins or glycolipids and play key roles in cell adhesion and other binding phenomena.[16] Furthermore, glycoconjugates related to 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.[17]

The H-type 2 determinant (Scheme 2.9) is found largely on the surface of erythrocytes and the epidermis of type O persons, at the termini of membrane associated glycoproteins.[18] Persons of blood types A and B also possess this determinant, which is further glycosylated at its galactose nonreducing terminus with a galactosamine (type A) or galactose moiety (type B). The solid phase assembly of



Scheme 2.9 Solid-phase synthesis of an H-type 2 blood group determinant.

the H-type 2 tetrasaccharide **34** is depicted in Scheme 2.9.[19] Galactal **10**, bound to polymer via the diisopropysilyl linker, was treated with a solution of DMDO, followed by glucal acceptor **11** and zinc chloride, thereby providing disaccharide **31**. With its unique C2-hydroxyl newly uncovered, compound **31** now acted as a polymer-bound acceptor vis-à-vis the solution-based fucosyl donor **32**. Polymer-bound trisaccharide **33a** was thus obtained, through the agency of tin triflate. The addition of the nonnucleophilic base di-*tert*-butylpyridine (DTBP) was necessary in this instance to protect the glycal double bond. Treatment of **33a** with TBAF provided trisaccharide glycal **33b** in 50% overall yield from **10**.

When this synthesis was undertaken, no solid support methodology existed to construct glycosidic linkages bearing C2-acylamino functions. We therefore had to take recourse to solution-phase chemistry in preparing the H type 2 blood group determinant tetrasaccharide glycal **34**. The trisaccharide glycal was cleaved from the solid support and extended via iodosulfonamidation in solution. Protecting group manipulations eventually led to **34** (Scheme 2.9), whose glycal functionality provided a handle for further functionalization at the reducing end. As will be shown later, a protocol for the azaglycosylation on the solid phase has been developed, so that the abovementioned 1-sugar homologation can now be made directly on solid support.

2.6 SOLID SUPPORT GLYCAL ASSEMBLY VIA THIOETHYL GLYCOSYL DONORS

The use of glycals on the solid support has led to the efficient construction of β-galactosyl linkages, even with hindered acceptors such as C4 hydroxyls flanked by protecting groups at C3 and C6. We have taken advantage of the stability of the 1,2-anhydrogalactose donor to very mild Lewis acids such as anhydrous zinc chloride. This stability may be a result of the constraints imposed on the molecule by the carbonate protecting group bridging the C3 and C4 hydroxyls (Scheme 2.10, compound **35**).

The analogous β-glucosidic linkages cannot be prepared as efficiently as the galactosidic linkages with the methodology developed thus far. This may be because no similarly constrained glucosyl epoxy donor is available. The glucosyl system **36** (Scheme 2.10) is highly reactive in the presence of zinc chloride and degradation of the donor is competitive with glycosylation, especially with hindered acceptors.

35 **36**

Scheme 2.10 Galactosyl and glucosyl 1,2-anhydrosugar donors.

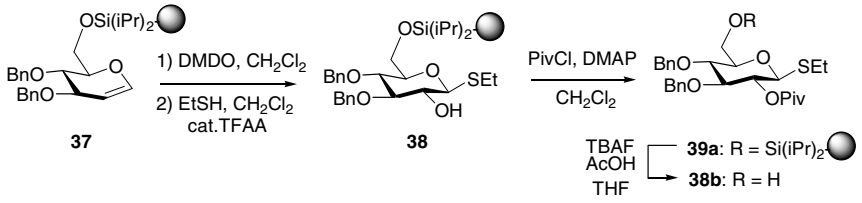
An approach has therefore been introduced to overcome this problem, which involves the conversion of glycals into thioethyl glycosyl donors.[20] On activation with thiophilic reagents, thioethyl donors bearing a participatory protecting group at C2, such as a pivaloyl group, become very powerful β-glycosylating agents.[21] Pivaloyl groups are chosen because they have been shown to prevent orthoester formation during the coupling step.[22]

Polymer-supported glucal **37** was converted to the protected thioethyl glucosyl donor **39** as outlined in Scheme 2.11. Compound **37** was first epoxidized by the action of DMDO. The resulting 1,2-anhydrosugar was opened by a mixture of ethanethiol and dichloromethane (1:1) in the presence of a trace of trifluoroacetic acid. Polymer-bound **38** was thus obtained in 91% yield. This was a substantial improvement over the 78% yield obtained by the same protocol in solution. Protection by reaction with pivaloyl chloride occurred in quantitative yield to furnish **39a**.

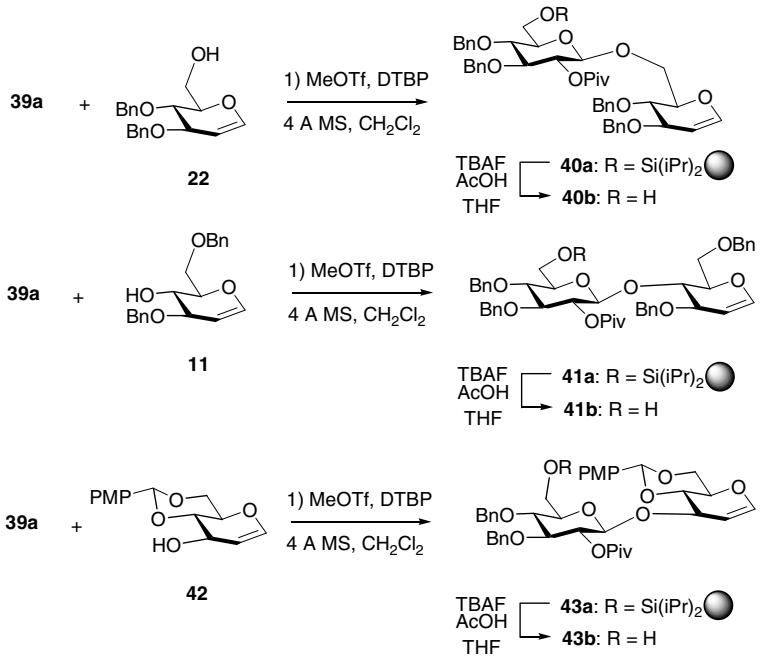
This polymer bound thioglucoside **39a** reacted with solution-based glycal acceptors as outlined in Scheme 2.12. Activation was performed by using methyl triflate, in the presence of one equivalent of the nonnucleophilic base di-*tert*-butylpyridine (DTBP). This base was added to prevent decomposition of the glycal bond resident in the acceptors. The formation of β-glucosyl (1→4) and β-glucosyl (1→3)-linked disaccharides **41a** and **43a** was almost free of contaminating side products and provided the disaccharides in good yields (Scheme 2.12). Only the formation of the β-glucosyl (1→6)-linked disaccharide **40a** was accompanied by formation of detectable side products.[23]

The solid-phase synthesis of systems with branching from C2 is also accessible through this methodology, as demonstrated by the synthesis of **46b** outlined in Scheme 2.13. The C2-pivaloyl protecting group of the β-glycosyl (1→4)-linked disaccharide **41a** was removed by treatment with DIBAL. The exposed C2 hydroxyl group could now function as the polymer-bound glycosyl acceptor. Formation of the synthetically challenging β-(1→2) glycosidic linkage was accomplished in 59% yield when glucosyl donor **45** was used (Scheme 2.13).[23]

The synthesis of a tetrasaccharide containing exclusively β-(1→4) glucosidic linkages (Scheme 2.14) further demonstrated the efficiency of this solid-phase methodology. Transformation of polymer-bound disaccharide glycal **41a** into the C2-pivaloyl thioglycosyl donor **47** was followed by coupling to provide the trisaccharide **48a** in 45% overall yield based on **37** as determined after cleavage from the solid support to furnish **48b**. The procedure was reiterated to convert **48a** to a

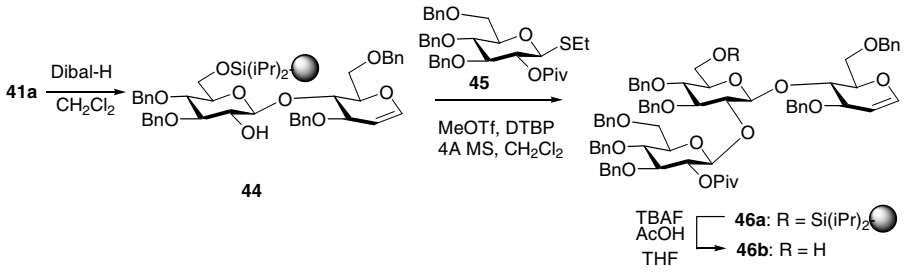


Scheme 2.11 Synthesis of polymer-bound thioethyl glucosyl donor.

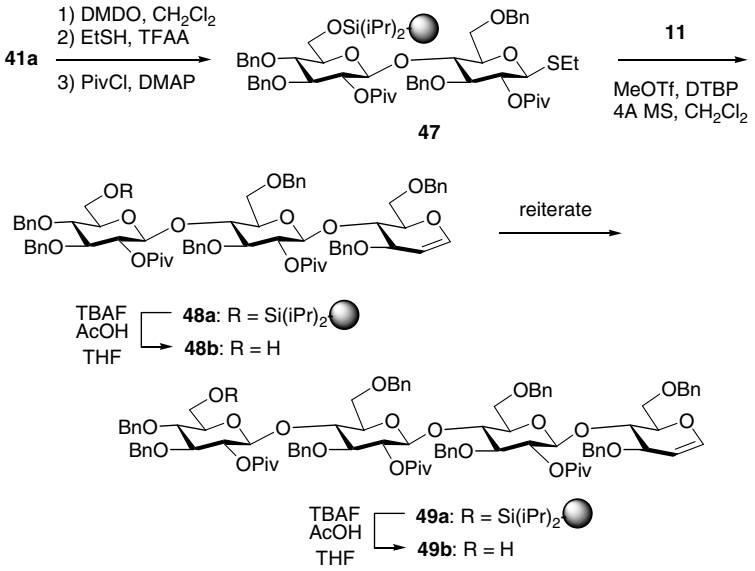


Scheme 2.12 Synthesis of disaccharides using a polymer-bound thioethyl glucosyl donor.

trisaccharyl thioethyl donor and to couple again to glycal acceptor **11**. Cleavage from the solid support at the conclusion of the sequence furnished the desired tetrasaccharide **49b** in 20% yield over 12 steps from **37** as determined after cleavage from the support by fluoridolysis. This overall result corresponds to an average yield of 84% per step (Scheme 2.14).[23]



Scheme 2.13 Synthesis of a branched trisaccharide via thioethyl donors.

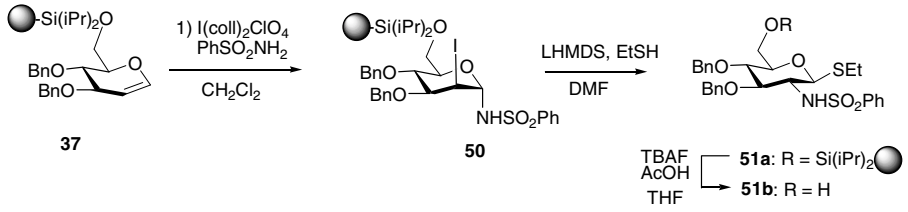


Scheme 2.14 Synthesis of a β-(1→4)-linked tetrasaccharide.

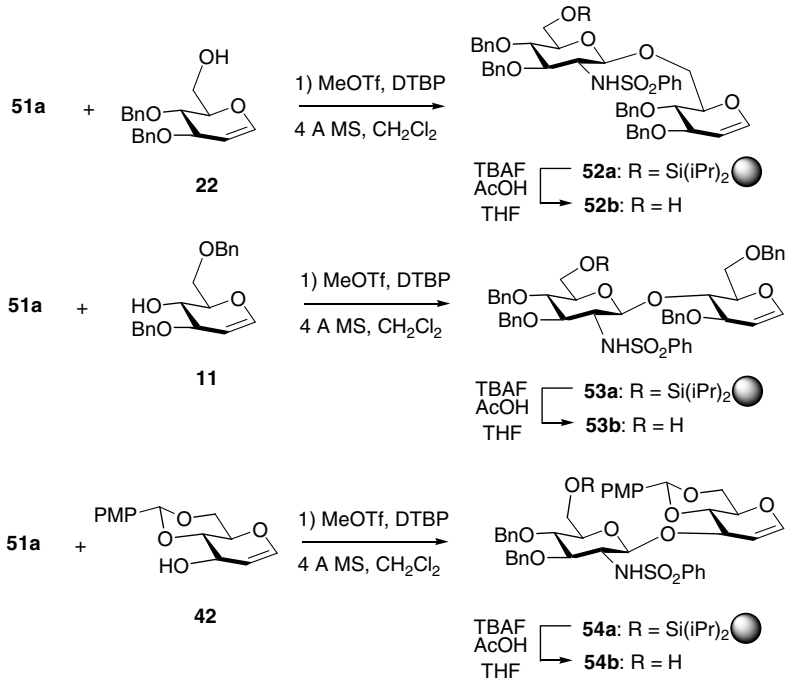
2.7 SOLID SUPPORT ASSEMBLY VIA THIOETHYL 2-AMIDOGLYCOSYL DONORS

As was pointed out above in the synthesis of the H type 2 antigen, our previous approach toward the blood group determinants on solid support was hampered by a serious shortcoming in our methodological arsenal. We had to take recourse to solution-phase methodology for construction of the *N*-acetylaminoglucosidic linkages that are prevalent in these biologically important molecules.[19] Fortunately, this obstacle has recently been overcome.

It was known from solution-based studies that an iodonium electrophile adds to the glycal linkage, in the presence of a sulfonamide, in a transdiaxial fashion.[24] This results in formation of a 1-α-sulfonamido-2-β-iodo product. Furthermore, displacement of iodine can be induced by nucleophilic attack of a thiolate nucleophile



Scheme 2.15 Synthesis of polymer-bound thioethyl 2-amidoglucosyl donor.



Scheme 2.16 Synthesis of disaccharides using a polymer-bound thioethyl 2-aminoglucosyl donor.

at the anomeric position. A concomitant suprafacial movement of the sulfonamide from C1 to C2 results in the formation of thioethyl 2-amidoglysosyl donors. Fortunately, extension of this capability to the solid phase proved to be feasible (Scheme 2.15). Polymer supported glucal **37** was treated with iodonium *sym*-collidine perchlorate to form iodosulfonamide **50** as an intermediate.[25] Transdiaxial displacement through the agency of ethanethiolate yielded 65% of the protected thioethylglucosyl donor **51**.

The polymer bound thioethyl 2-amido glucosyl **51a** is a competent 2-amidoglucosyl donor toward glycal acceptors when activated with methyl triflate in the standard manner.[21] The formation of β-2-aminoglucosyl (1→4; **53**) and β-2-aminoglucosyl (1→3; **54**)-linked disaccharides proceeded in over 70% yield (Scheme 2.16). The β-2-aminoglucosyl (1→6)-linked disaccharide **52** was formed in lower yields.

2.8 SOLID-PHASE SYNTHESIS OF THE LEWIS^b BLOOD GROUP DETERMINANT

The Lewis^b blood group determinant (Le^b, Fig. 2.1) is another antigen of particular interest. This determinant has been identified as a mediator for the binding of

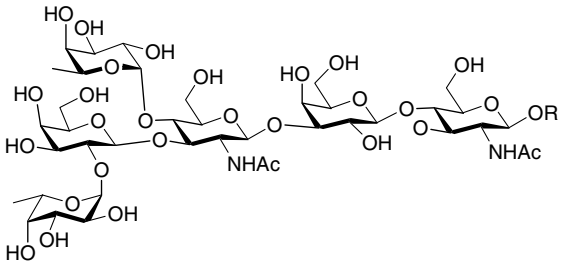
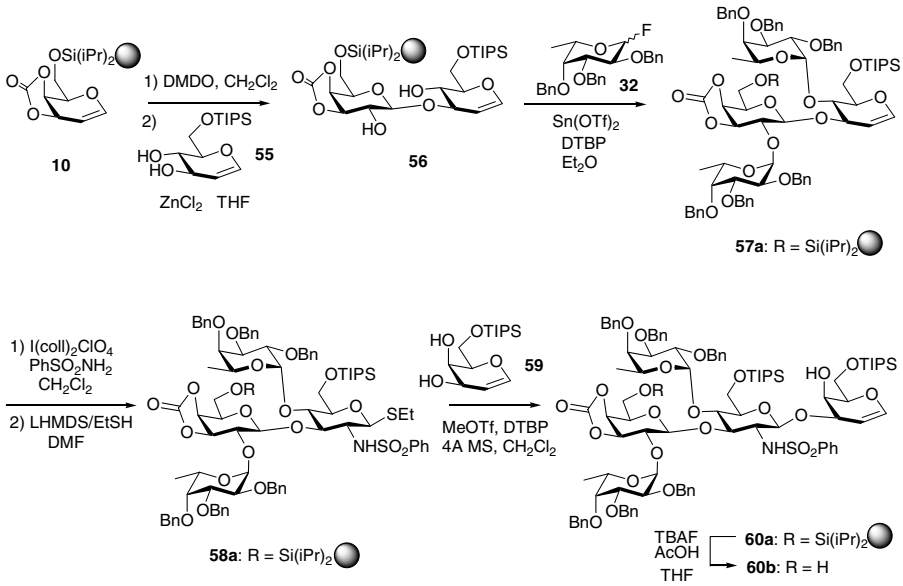


Figure 2.1 Structure of Lewis^b hexasaccharide.

Helicobacter pylori to human gastric epithelium.[26] Clinical studies have identified *H. pylori* as a causative agent in gastric and duodenal ulcers.[27] Considerable evidence exists to suggest that carbohydrate-based treatments could be an effective means to combat infection.[28] Since bacterial attachment is a prerequisite to infection,[29] soluble Le^b oligosaccharides may serve as therapeutic alternatives to broad-spectrum antibiotics.

The first problem to be addressed in the solid-phase assembly of Le^b involved the synthesis of the core tetrasaccharide (Scheme 2.17).[25] Polymer-bound galactal **10** was epoxidized with DMDO. The resultant epoxide reacted with a solution of glucal **55** to give polymer-bound disaccharide diol **56**. This reaction proceeded in a highly regioselective fashion, wherein glycosylation occurred only at the allylic C3 position



Scheme 2.17 Solid-phase synthesis of a Le^b (Lewis^b) blood group determinant.

of **55**. Bisfucosylation of **56** using donor **32** provided polymer-bound tetrasaccharide glycal **57**. Recourse to the aminoglycosylation protocol allowed conversion of the branched tetrasaccharide glycal **57** into the thioethyl donor **58**. The latter was coupled to galactal acceptor **59** to yield 71% of the desired pentasaccharide **60a** (Scheme 2.17). Retrieval of the pentasaccharide was accomplished using TBAF to afford **60b** in 20% overall yield from **10**.[25]

A hexasaccharide of the Le^b system had previously been obtained using a combination of solid-phase (up to the tetrasaccharide **57** stage) and solution-phase chemistry.[19] This compound was conjugated with human serum albumin by the method of Bernstein[30] to provide a neoglycoprotein whose biological properties are currently (at the time of writing) being investigated.

2.9 SOLID-PHASE SYNTHESIS OF THE HEXASACCHARIDE GLOBO-H ANTIGEN: PROGRESS AND LIMITATIONS

The globo-H carbohydrate antigen (Fig. 2.2) was first identified chemically from breast tumor extracts by Hakomori et al.[31] It was immunocharacterized by Colnaghi et al. (mAb MBr1)[32] and more recently by Lloyd et al. (mAb VK-9).[33] Globo-H was identified on a number of human cancers (including those of the prostate and the breast) and in a restricted number on normal epithelial tissues.[34] Its overexpression on cancerous tissues makes it an attractive target for active immunotherapy with vaccines. We have synthesized a globo-H hexasaccharide and shorter isomers using solution-based glycal assembly methods.[35] Vaccines containing the fully synthetic hexasaccharide conjugated to the carrier protein keyhole limpet hemocyanin have been found to elicit globo-H-specific responses in mice.[36] These vaccines have been tested in human clinical trials, with excellent serological markers.[37]

We have approached the synthesis of the globo-H hexasaccharide on solid support following closely the strategy that was adopted in solution. The synthesis, outlined in Schemes 2.18 and 2.19, required the coupling of a polymer-bound trisaccharide glycal to a very complex trisaccharide glycal acceptor.[38] The 1,2-anhydrosugar derived from polymer-bound galactal **10** reacted with galactal **59** under standard conditions to furnish disaccharide **61**. The solution-phase synthesis required delivery of carefully

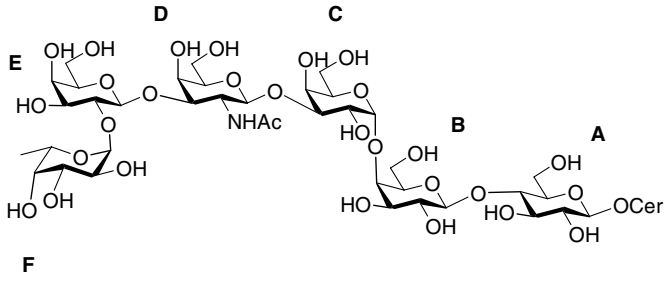
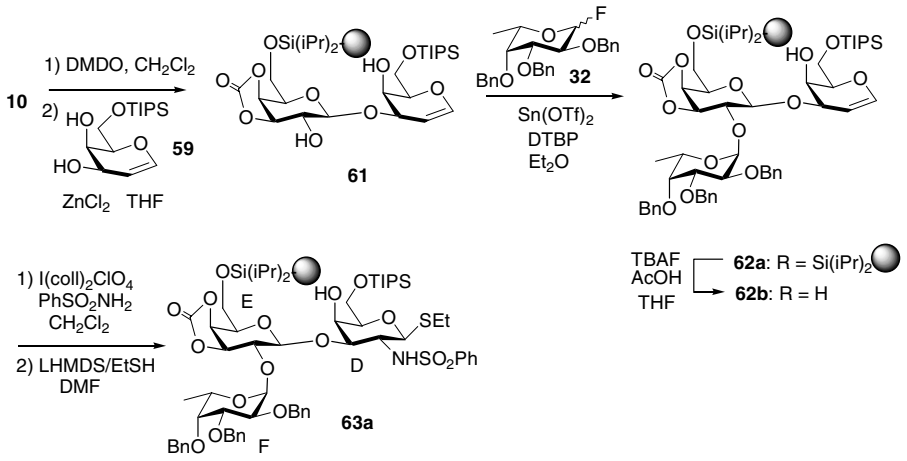
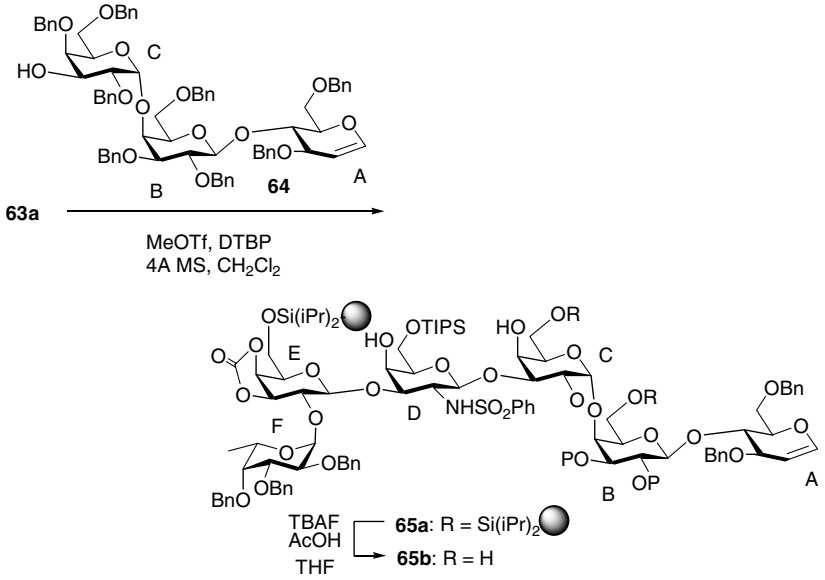


Figure 2.2 Structure of globo-H antigen.



Scheme 2.18 Solid-phase synthesis of the globo-H trisaccharide antigen.

measured amounts of fucosyl donor **32** in order to effect monofucosylation at the C2 hydroxyl of galactose E. In the solution-phase situation, excess **32** led to fucosylation at the C4 hydroxyl of galactose D. To our delight, polymer-bound disaccharide **61** was converted almost exclusively to the trisaccharide **62** even when large excesses of **32** were employed. The glycal double bond was then elaborated into the thioethyl



Scheme 2.19 Solid-phase synthesis of the globo-H hexasaccharide antigen.

2-amidoglycosyl donor **63**, ready for coupling to the trisaccharide glycal **64**, which was prepared by assembly in solution phase.[35] Unfortunately, the yield of compound **63** was very low. Examination of the products obtained after cleavage from the solid support indicated the substantial presence of an unusual sulfonamidation product. We believe this material to be the β-iodo-β-sulfonamido species, which formed during prolonged reaction times from the desired β-iodo-α-sulfonamide, via isomerization at the anomeric center. This relative stereochemistry prevented the desired suprafacial movement of the sulfonamide from C1 to C2 during attack by the thiolate anion. This isomerization can be kept in check during the solution-phase work with short reaction times. This containment is unfortunately not possible in the polymer-supported case and is a reminder that translation of methodology from solution phase to polymer support is never straightforward when dealing with systems of such complexity. A search for a solution to the isomerization dilemma is in progress. Nevertheless, coupling of compound **63** to the solution-based trisaccharide **64** was achieved (Scheme 2.19) and afforded the desired hexasaccharide glycal **65**.

2.10 SOLID-PHASE SYNTHESIS OF *N*-LINKED GLYCOPEPTIDES

Glycoproteins are subdivided into two major groups: *N*-linked and *O*-linked families; the former is the most abundant in nature.[39] The biosynthesis of the glycoproteins results from cotranslational glycosylation usually occurring in the endoplasmic reticulum. The naturally occurring *N*-asparagine glycopeptides are β-*N*-linked to a chitobiose segment of a high mannose antennary structure (Fig. 2.3). This pentasaccharide core carbohydrate is the medium to which virtually all *N*-asparagine glycoproteins are attached. The specific role of *N*-linked proteins is not well understood, however their necessity has been demonstrated.[40] It is possible that the glycosylation has a conformational effect on the protein, which influences the rate or even energetics of protein folding.[41] Several groups have achieved significant advances in the field of glycopeptide synthesis.[6,42,43]

Our approach to the synthesis of *N*-linked glycopeptides on the solid support aimed at the implementation of a highly convergent synthetic strategy.[44] It was envisioned that a terminal glycal of a polymer-bound oligosaccharide would be subjected to iodosulfonamidation. Treatment of the intermediate with azide resulted in formation of a β-anomeric azide with the usual suprafacial movement of the α-sulfonamide from C1 to C2. Reduction of the azide and coupling of the resulting anomerically pure β-amino functionality would provide a protected glycopeptide.

Our first synthesis of a polymer-bound anomeric amine involved a trisaccharide model system (Scheme 2.20).[45] Disaccharide **66** was extended in standard fashion to trisaccharide **67** and fully protected to give **68**. This latter compound was treated with anthracenesulfonamide and $I(sym\text{-coll})_2ClO_4$ to form the intermediate **69**. Reaction of the iodosulfonamide **69** with tetra-*n*-butylammonium azide followed by acetylation provided the anomeric azide **70**.

The anthracenesulfonamide linkage was cleaved under mild, solid support compatible conditions such as thiophenol or 1,3-propanedithiol and Hünig's base.

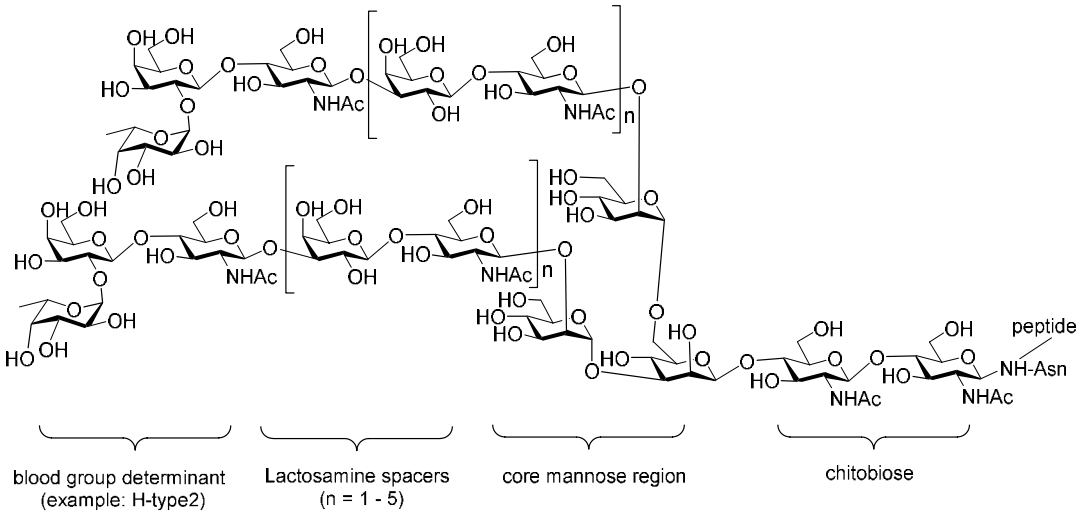
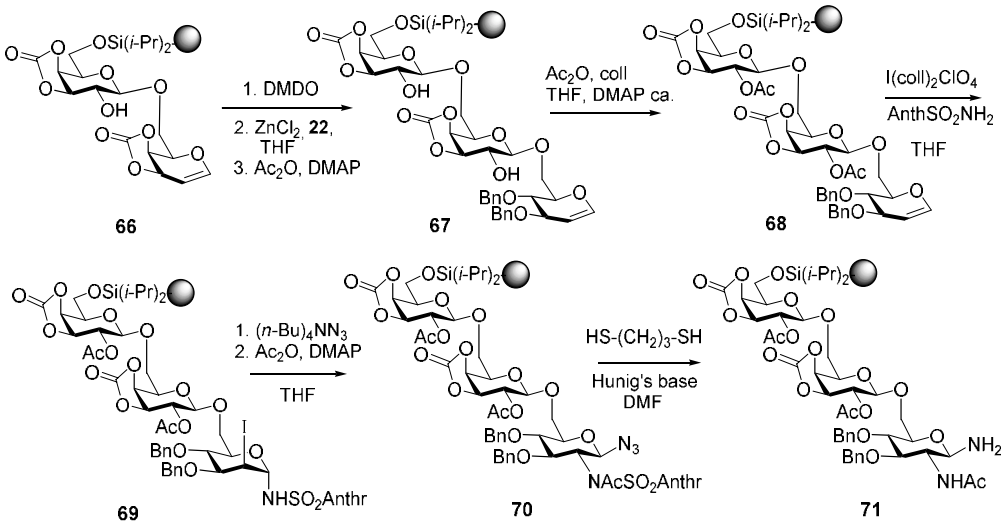
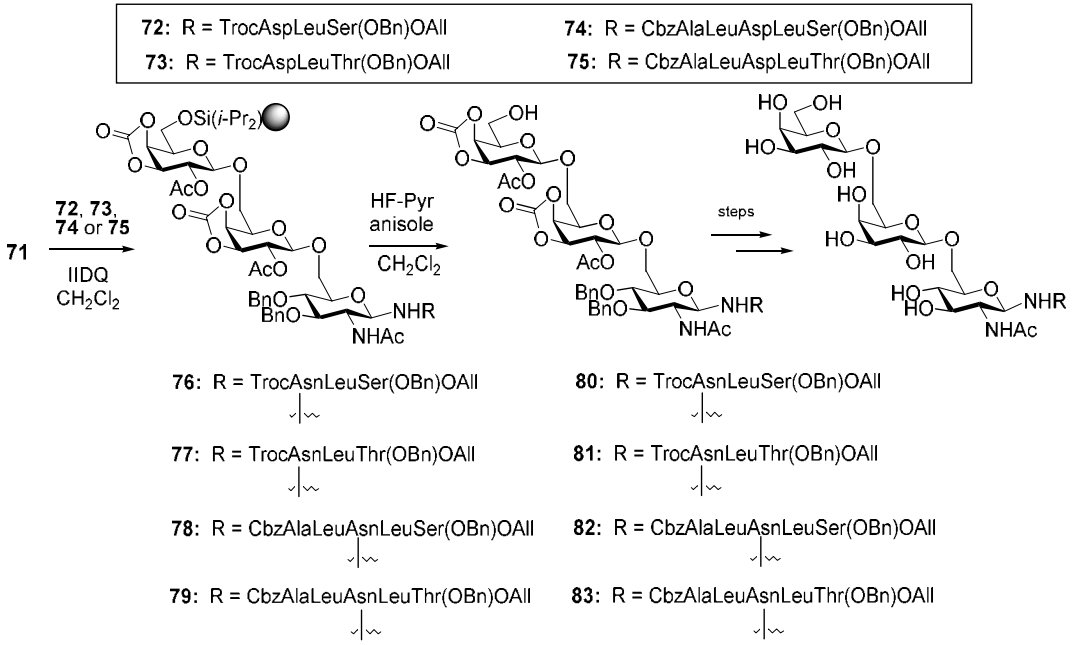


Figure 2.3 Complex-type biantennary structure.



Scheme 2.20 Solid-phase anomeric amine synthesis.

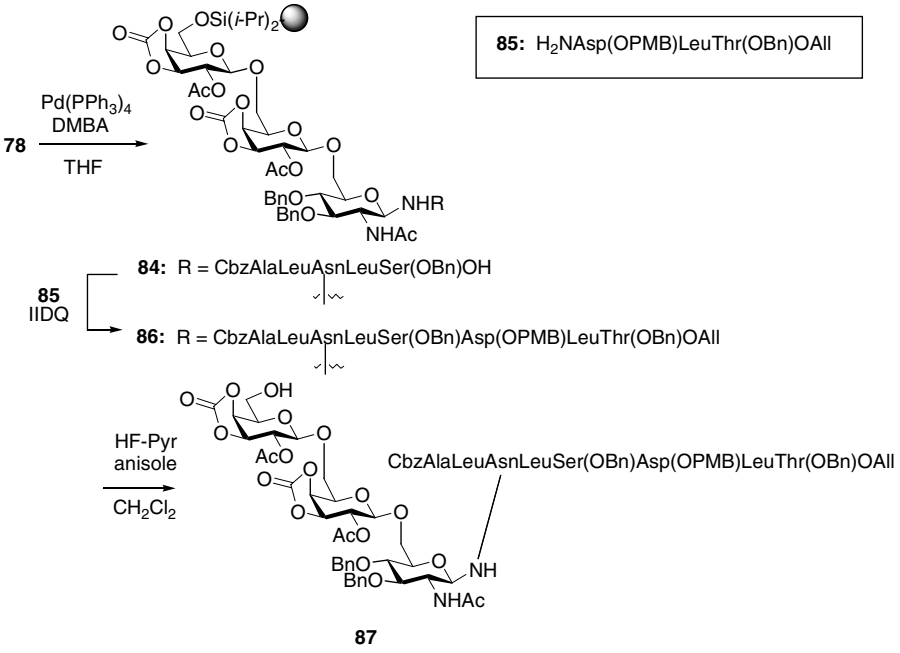


Scheme 2.21 Synthesis of *N*-linked glycopeptides on a solid support.

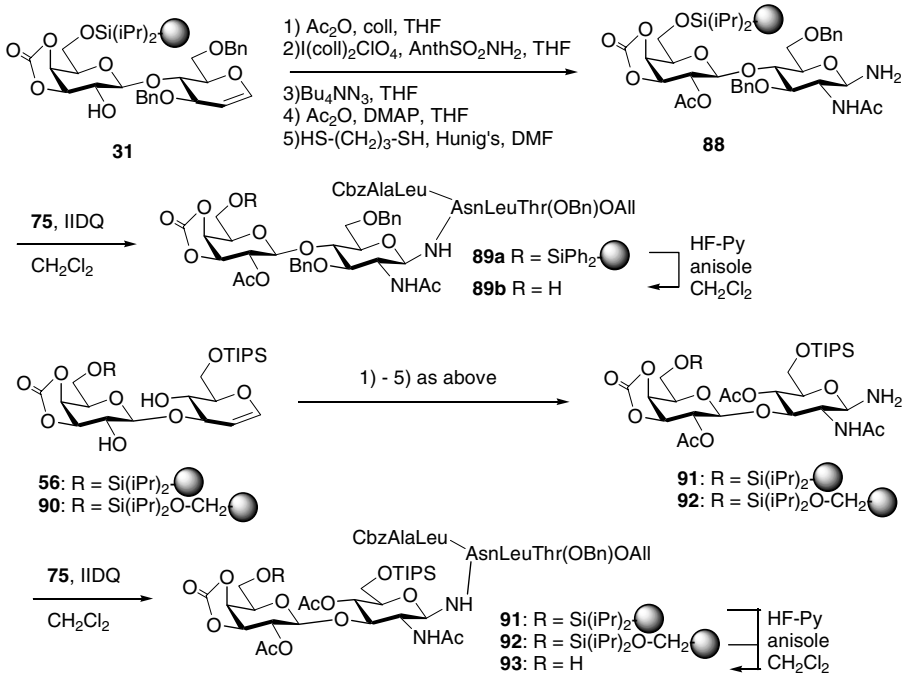
Treatment of **70** with 1,3-propanedithiol and *i*-Pr_2NEt effected both the cleavage of the sulfonamide and the reduction of the azide to afford **71**.

The coupling of the resulting amine **71** with either tripeptides **72** or **73** or pentapeptides **74** or **75** is illustrated in Scheme 2.1. The oligopeptides were synthesized separately by solution-phase techniques. The protected glycopeptides **76**–**79** are formed respectively, in the presence of IIDQ. Removal from the solid support with HF·pyridine provided the glycopeptides **80**–**83**, in 20–35% overall yields, based on the initial loading of polymer-bound galactal carbonate. These yields correspond to approximately 90% yield per step. Reverse-phase silica column chromatography was sufficient to obtain these compounds in pure form. The remaining protecting groups were cleaved under standard conditions to provide the completely deblocked glycopeptides.

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 functionality for linking to a carrier molecule to generate other glycoconjugates. Scheme 2.22 depicts the strategy for elongation of the peptide portion of the glycopeptide while still bound to the polymer support. Solid-phase-bound trisaccharide pentapeptide **78** was deprotected to give the acid **84**. Solid support-bound **84** was then coupled to tripeptide **85** with a free *N* terminus to



Scheme 2.22 Synthesis of *N*-linked glycopeptides on a solid support.



Scheme 2.23 Solid-phase synthesis of glycopeptides.

give glycopeptide **86**. Retrieval from the solid support afforded trisaccharide–octapeptide **87** in 18% overall yield from **10**.

The methodology described above has proved to be effective for other glycosylation sequences. Scheme 2.23 shows conversion of a variety of disaccharides to their respective glycopeptides. For example, lactal derivative **31** was transformed into a lactosamine-linked glycopeptide where C6 of the glucosamine residue is protected as its benzyl ether, in 24% overall yield. Disaccharides **56** and **90**, with a β-(1→3) glycosylation pattern, differ in their mode of attachment to the solid support. In compound **56** the linkage was established via a silyl ether, while in **90** it was fashioned via a silylene linkage. They can both be elaborated to disaccharide–pentapeptide **93** in 20% and 44% yields, respectively, based on the initial loading of polymer-bound galactal carbonate.

2.11 CONCLUSIONS

In this chapter we have described the progress that our laboratory has made in the solid support synthesis of oligosaccharides and glycopeptides using glycal assembly. 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 and bring complex structures within reach. The flexibility and capabilities of our synthetic approach were demonstrated on some select structures of biological interest.

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

A number of challenges still remain before a flexible, high-yielding, and absolutely selective strategy for the synthesis of oligosaccharides on the solid support becomes available. Once these problems are solved, the construction of an automated oligosaccharide synthesizer will become feasible.

The rapid access to complex glycoconjugates will open the door to detailed studies concerning the structure and function of this class of biooligomers.

ACKNOWLEDGMENTS

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3 The Sulfoxide Glycosylation Method and its Application to Solid-Phase Oligosaccharide Synthesis and the Generation of Combinatorial Libraries

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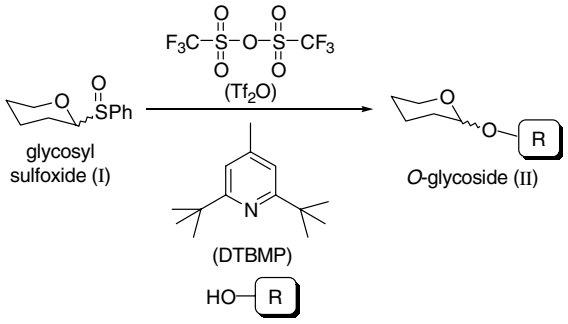
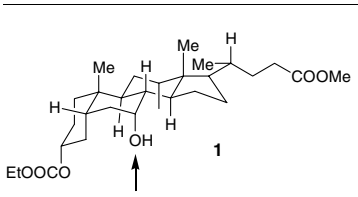
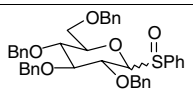
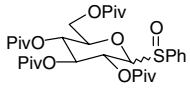
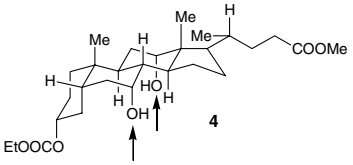
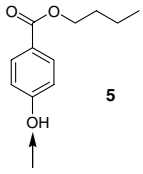
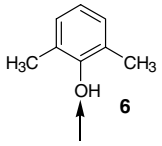
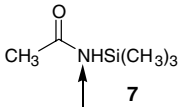
3.1 INTRODUCTION

The sulfoxide method was introduced by Kahne and coworkers,[1] and was heralded as "a new method for rapid glycosylation of unreactive substrates in high yield under mild conditions." The reaction involves the sulfoxide donor [sulfoxide (I)], an activating agent (usually triflic anhydride), a hindered, nonnucleophilic base (2,6-di-*tert*-butyl-4-methylpyridine, DTBMP) and a nucleophilic acceptor (most often an alcohol) (Scheme 3.1). The glycosylation of sterically hindered steroidal alcohols, phenols and the *N*-glycosylation of an acetamide was reported (Table 3.1).



Scheme 3.1 The sulfoxide glycosylation method.

TABLE 3.1 Glycosylation of Unreactive Substrates

Glycosyl Acceptor	Glycosyl Donor	Reaction Solvent	Yield and Ratio of Anomers
 **1**	 Sulfoxide **2**	toluene CH_2Cl_2 CH_3CN	86%; 27:1 (α:β) 80%; 1:3 (α:β) 50%; 1:8 (α:β)
	 Sulfoxide **3**	CH_2Cl_2	83%; all β
 **4**	Sulfoxide **2**	toluene	58%; α, α
 **5**	Sulfoxide **2**	toluene	78%; 9:1 (α:β)
	Sulfoxide **3**	CH_2Cl_2	60%; all β
 **6**	Sulfoxide **2**	toluene	70%; 2:1 (α:β)
	Sulfoxide **3**	CH_2Cl_2	80%; all β
 **7**	Sulfoxide **2**	toluene	66%; 5:1 (α:β)

Since 1989, the sulfoxide method has been investigated extensively by the laboratories of Kahne and Crich and employed by many others.

Figure 3.1 depicts some oligosaccharides that were synthesized using the sulfoxide glycosylation method. Chemical yields and stereoselectivity, for glycoside formation, are highlighted. Crich and Dai reported the synthesis of a trisaccharide **8**, a component of the *Hyriopsis schlegelli* glycosphingolipid; one of the linkages was successfully established using the sulfoxide method.[2] Yan and Kahne synthesized trisaccharide **9** en route to Lewis^x.[3] Zhang and coworkers applied the sulfoxide glycosylation method to the assembly of compound **10**, the nephritogenoside trisaccharide unit.[4] Berkowitz et al. considered the sulfoxide method in the assembly of artificial disaccharides such as **11**.[5] Kim et al. constructed the three glycosidic linkages in the calicheamicin oligosaccharide **12**.[6]

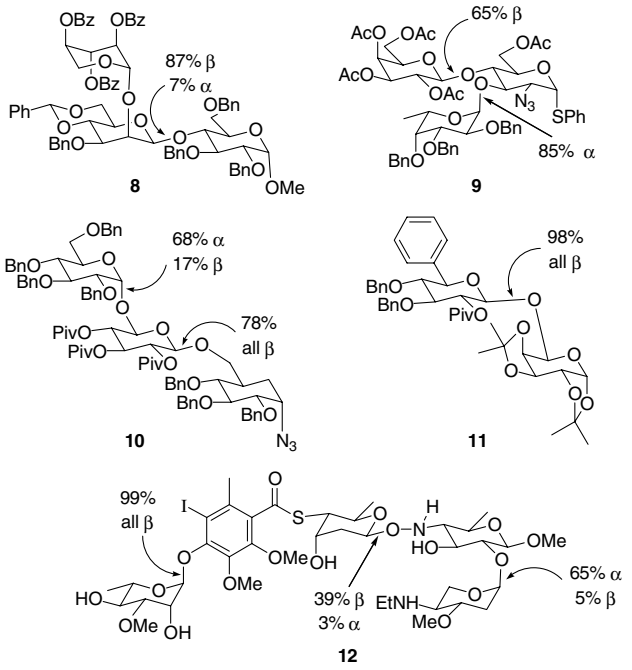
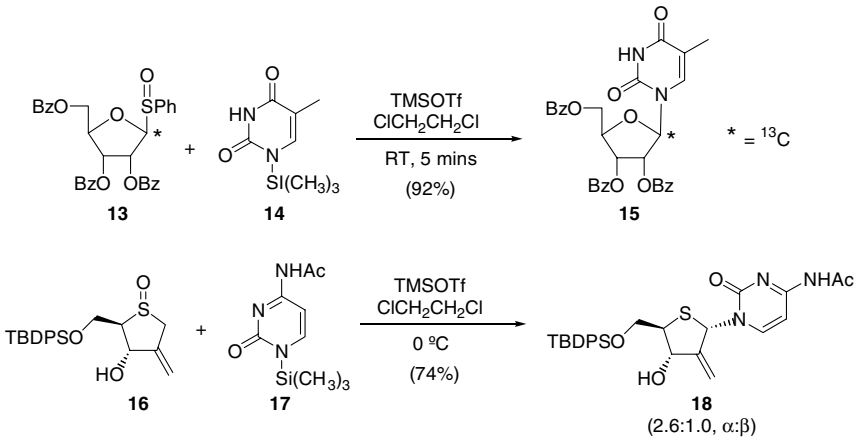
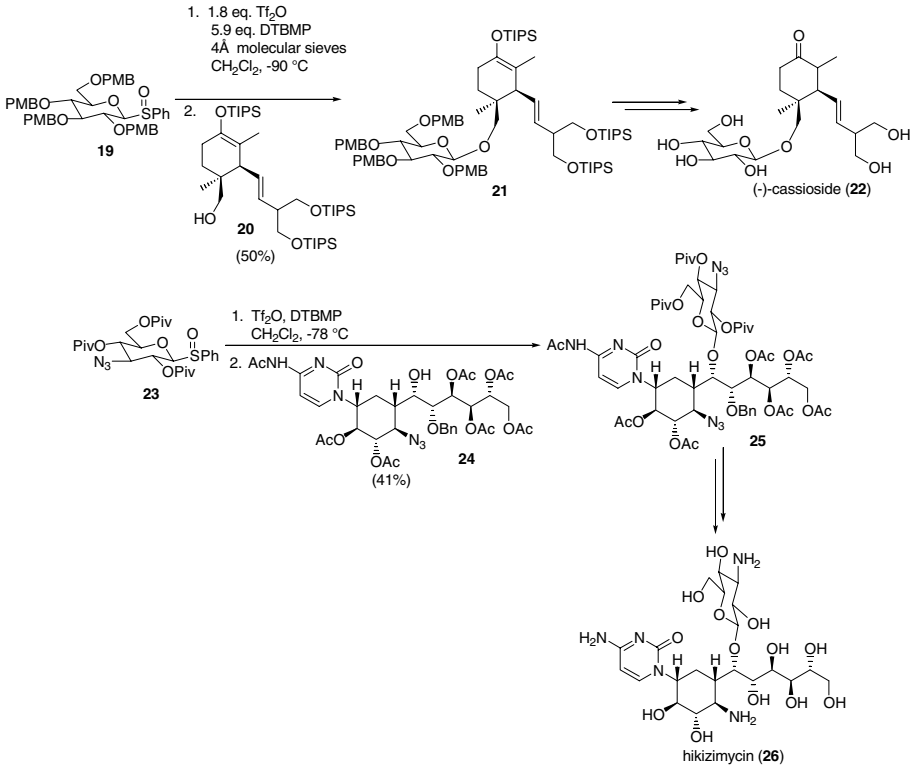


Figure 3.1 Oligosaccharides synthesized using the sulfoxide glycosylation method.



Scheme 3.2 Synthesis of nucleoside analogs.

Sulfoxides have also been used in the synthesis of nucleoside analogs (Scheme 3.2). Chanteloup and Beau reported the synthesis of ribofuranosyl sulfoxide **13** and its use in the glycosylation of a series of silylated pyrimidine and purine bases.[7] Although **16** is not an anomeric sulfoxide, its reaction with cytosine derivative **17** is conceptually related.[8]

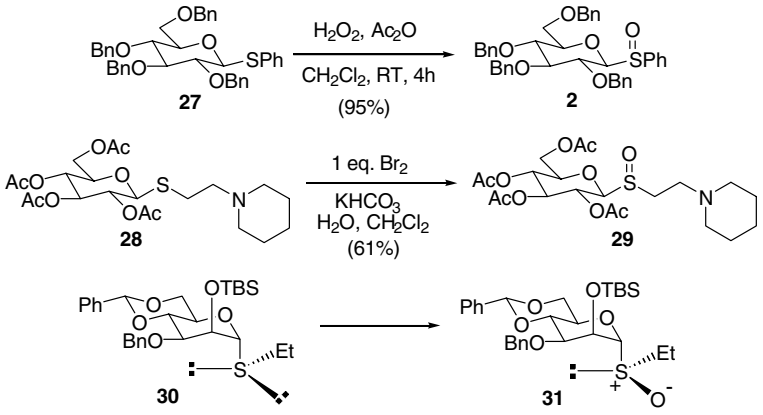


Scheme 3.3 Synthesis of glycoside natural products.

The sulfoxide method has come to the rescue in the syntheses of some complex glycoside natural products, where many other glycosylation methods failed. Two examples are shown in Scheme 3.3. Boeckman and Liu reported an enantioselective synthesis of cassioside (**22**).[9] While the focus of the synthesis was the assembly of the aglycone **20**, utilizing a highly diastereoselctive cycloaddition of a trienol silyl ether, the glycosylation turned out to be a challenge in its own right. They initially performed this glycosylation using the perbenzylated glucose sulfoxide **2** (see Table 3.1); but found that the hydrogenolytic removal of the benzyl protecting groups was not compatible with other functionality in the molecule. Similar problems were reported in the synthesis of ciclamycin 0.[10] The solution, in both cases, was found in the *p*-methoxybenzyl ether protecting group, which can be removed oxidatively.[11] Ikemoto and Schreiber encountered a challenging glycosylation in their synthesis of hikizimycin (**26**); sterically hindered alcohol **24** was ultimately glycosylated using sulfoxide **23**.[12]

3.2 SYNTHESIS OF SULFOXIDE DONORS

The preparation of thioglycosides was thoroughly reviewed by Garegg.[13] These versatile glycosides can be used directly in glycosylation reactions, or can be converted to a number of other glycosyl donors, including sulfoxides.



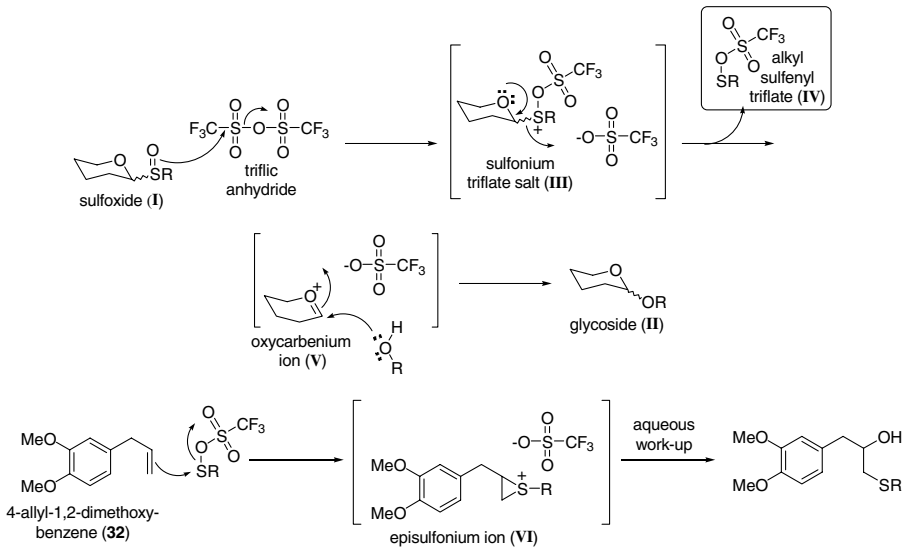
Scheme 3.4 Synthesis of sulfoxide donors.

Thioglycosides are most commonly oxidized to a mixture of diastereoisomeric sulfoxides using *m*-CPBA.[3] Care must be taken to monitor and minimize overoxidation to the sulfone; low temperatures are usually required. Kakarla et al. have used $H_2O_2/Ac_2O/SiO_2$ in dichloromethane to produce sulfoxide **2** in excellent yield on very large scale.[14] Juodvirsis et al. prepared mixtures of sulfoxides (e.g., **29**) and sulfones using $Br_2/KHCO_3$ (Scheme 3.4).[15] Interestingly, Crich and coworkers employed a number of reagents (*m*-CPBA, monoperoxyphthalate [MMPP], sodium periodate and Oxone®) to oxidize α-mannopyranosyl thioglycosides (e.g., **30**), and observed stereoselective formation of the *R*-sulfoxides.[16] This was rationalized on the basis of the exo-anomeric effect, which favors the conformation in which the *pro-R* lone pair on sulfur is more exposed to attack by oxidizing agents.

3.3 MECHANISM OF THE SULFOXIDE GLYCOSYLATION

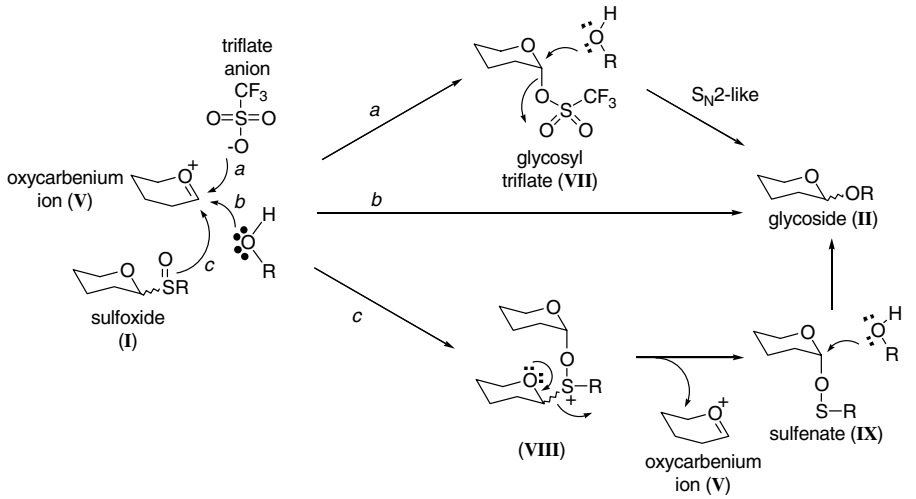
A simplistic view of the mechanism is depicted in Scheme 3.5. The sulfoxide (I) is activated by triflic anhydride to generate a sulfonium salt (III). It was observed early on that the outcome of the glycosylation was not influenced by the configuration at the two variable stereogenic centers in the sulfoxide donor (i.e., anomeric carbon and sulfur).[1] This was taken as evidence for the intermediacy of the oxycarbenium ion (V), which arises from rapid elimination of an alkylsulfenyl triflate(IV) from III. Reaction of V with a nucleophile then gives rise to a mixture of α- and β-glycosides (II).

The generation of phenylsulfenyl triflate(IV) (R = Ph) can be a problem if there are thioglycosides in the starting materials or product, as these functional groups can be activated as glycosyl donors by phenylsulfenyl triflate. This problem can be dealt with in two ways: inclusion of a scavenger for phenylsulfenyl triflate (e.g., 4-allyl-1,2-dimethoxybenzene, **32**) or by ensuring that the thioglycosides are sterically hindered and/or electronically deactivated.[10]



Scheme 3.5 Simplistic mechanism for the sulfoxide glycosylation.

In attempts to improve the yields of problematic glycosylation reactions, it has become clear that the mechanism is much more complex than Scheme 3.5 suggests. In essence, the oxycarbenium ion(V) is susceptible to attack by nucleophiles other than the glycosyl acceptor (Scheme 3.6). Two intermediates for which there is experimental evidence are glycosyl triflates(VII)[17] and glycosyl sulfenates(IX).[18]



Scheme 3.6 Triflate and sulfenate intermediates in the sulfoxide glycosylation.

Crich and Sun propose that path *b* predominates when the glycosyl acceptor is present during activation of the sulfoxide. However, they have evidence to suggest that when the sulfoxide is activated, prior to addition of the acceptor, the glycosyl triflate forms within minutes.[17] This has important consequences in terms of the stereochemistry of the glycosidic linkage.

Gildersleeve and coworkers have suggested that the formation of a sulfenate(IX) can be competitive with glycoside formation; this has been confirmed by characterization of reaction products generated at low temperatures (–78°C).[18] The sulfenate itself becomes a glycosylating agent at higher temperatures (–20–20°C). This is consistent with earlier reports that the "activated sulfoxide" appears to be extremely reactive at –78°C (oxycarbenium ion, V) and yet stable at room temperature (sulfenate, IX).[1]

Clearly, the relative proportions of the different intermediates depends on the structure and conformation of the glycosyl donors, the nucleophilicity of the glycosyl acceptor and the order of addition of reagents. To optimize a glycosylation reaction, a knowledge of these potential intermediates and how they affect the outcome of a reaction, is essential.

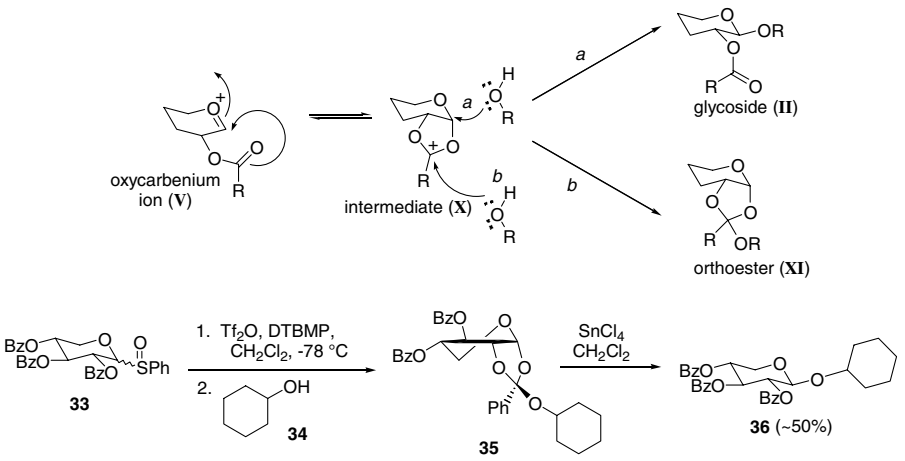
While triflic anhydride activation of sulfoxides is the norm, other activation procedures have been employed. In the synthesis of the ciclamycin 0 trisaccharide, catalytic triflic acid (TfOH) was employed.[19] Trimethylsilyl triflate (TMSOTf), in conjunction with triethyl phosphite (a scavenger for phenylsulfenic acid), has also been used.[20]

3.4 STEREOSELECTIVITY

In the absence of neighboring-group participation, the anomeric effect prevails giving rise to an axial glycoside as the major product. An incoming nucleophile approaches from the axial direction for stereoelectronic reasons. Solvent influences the stereoselectivity of the glycosylation, with arene solvents giving the best selectivity for axial glycosides.

Interestingly, when a PMB ether is present at C2, β-glycosides seem to predominate (see Scheme 3.3 and Ref. 11). Yan and Kahne attribute this to extreme steric requirements of the glycosyl acceptors in the examples reported.[11] Boeckman suggests that the β-selectivity is due to the high reactivity of the reactive intermediate, and perhaps participation by the PMB ether oxygen.[9]

When there is a substituent at C2 capable of engaging in neighboring group participation, a 1,2-*trans* glycoside is formed almost exclusively (Scheme 3.7). Dichloromethane is the preferred solvent for these glycosylations. When there is an ester, or amide, at C2, the formation of intermediate X is possible. If the nucleophile attacks X at the anomeric carbon, it does so from the face not obstructed by the acetoxonium ring, to give the desired glycoside with excellent stereoselectivity (path *a*). However, the nucleophile can also follow path *b*, to generate an orthoester (XI). This side reaction can prevail in sulfoxide glycosylations when an acetate protecting group is employed. Crich and Dai reported that the orthoester **35** was the major

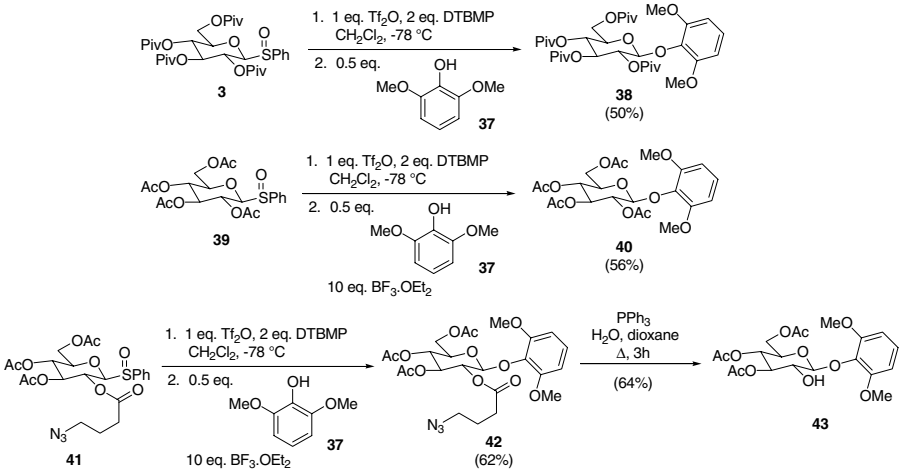


Scheme 3.7 Orthoester formation.

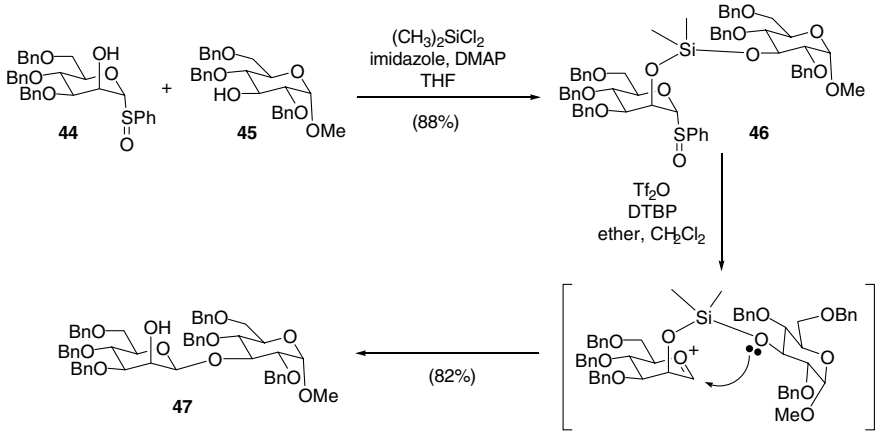
product isolated from the attempted glycosylation of cyclohexanol **34** with perbenzoylated xylosyl sulfoxide **33**.[21] The desired β-glycoside **36** could be isolated in moderate yield, by Lewis acid–catalyzed rearrangement of the orthoester. The pivaloate ester, $(CH_3)_3COOR$, has been widely used, in conjunction with the sulfoxide glycosylation method, because the bulk of the *tert*-butyl group, discourages orthoester formation. Unfortunately, pivaloate esters can be difficult to install and remove, and while their steric bulk reduces orthoester formation, it also impedes attack by a nucleophile in glycoside formation.

Thompson et al. have recently overcome this limitation in 1,2-*trans*-glycoside formation. An acetate at C2 ***is*** effective for stereoselective glycoside formation, when used in conjunction with $BF_3{\cdot}OEt_2$ and DTBMP.[22] The base is believed to increase the nucleophilicity of the glycosyl acceptor, while Lewis acids are known to catalyze the rearrangement of orthoesters to the corresponding β-glycosides. As shown in Scheme 3.8, these reaction conditions actually gave a better yield than glycosylation with the corresponding perpivaloyl sulfoxide (3) under standard conditions. An azidobutyryl ester at C2 also gave good results; this group is useful because it can be selectively removed in the presence of acetates elsewhere in an oligosaccharide. The model studies in Scheme 3.8 provided valuable information that was applied to the glycosylation of the vancomycin aglycone.

The formation of β-mannosides has long been a challenge in synthetic carbohydrate chemistry, because both the anomeric effect and an ester group at C2 conspire to favor an α-glycoside (1,2-*trans*). Stork and coworkers have used a "temporary silicon connection" in conjunction with the sulfoxide glycosylation method, to achieve this. The glycosyl donor (e.g., **44**) is covalently linked to the glycosyl acceptor (e.g., **45**) via a silicon tether, rendering the glycosylation reaction intramolecular (Scheme 3.9).[23]

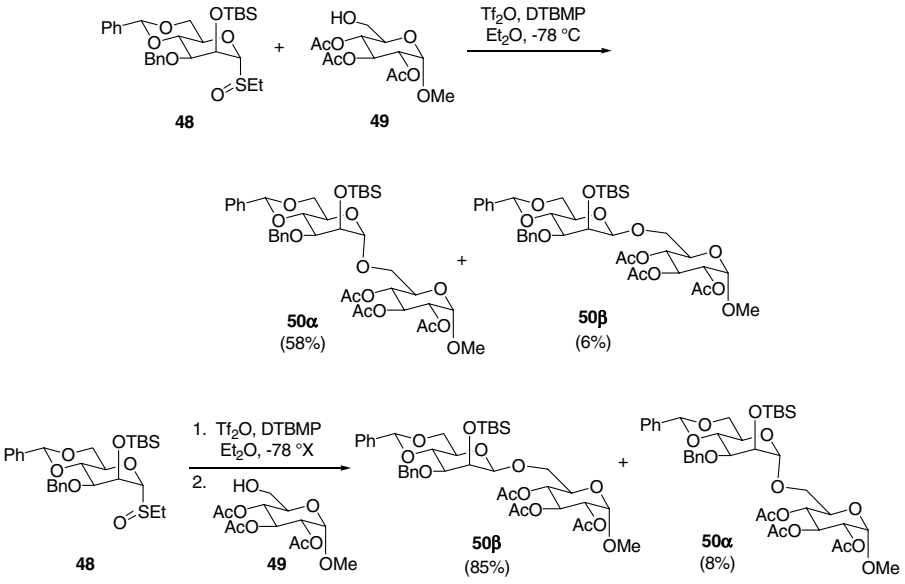


Scheme 3.8 Optimization of participating groups at C2.



Scheme 3.9 The temporary silicon connection.

Crich and Sun have reported an efficient protocol for the stereoselective synthesis of β-mannopyranosides,[24] utilizing sulfoxides as glycosyl donors. Interestingly, a reversal of anomeric stereoselectivity is observed, simply by changing the order of mixing reactants and reagents (Scheme 3.10). When the glycosyl acceptor **49** is present during activation of sulfoxide **48**, the α-anomer **50**α predominates (this implies path *b*, Scheme 3.6). When sulfoxide **48** is activated prior to addition of nucleophile **49**, the anomeric triflate forms (path *a*, Scheme 3.6), and the β-glycoside **50**β results from an S_N2-like attack.



Scheme 3.10 Reversal of anomeric stereoselectivity in β-mannopyranoside synthesis.

3.5 SOLID-PHASE OLIGOSACCHARIDE SYNTHESIS

The mild conditions employed for the activation of sulfoxides, and the high reactivity of the glycosyl donor, suggested that the method might translate well to the solid phase. Moreover, the nonpolar nature of the sulfoxide, triflic anhydride, and DTBMP (solution-based reagents) should enable them to partition effectively into a non-polar, polystyrene-based resin.

Initial solid phase synthesis[25] was carried out on Merrifield's resin (1% crosslinked chloromethylated styrene/divinylbenzene copolymer, 200–400 mesh) because of its track record in solid-phase peptide synthesis.[26] Unfortunately, the Merrifield resin has limitations as a carbohydrate carrier to study interactions between the carbohydrates and relevant binding proteins. The hydrophobic nature of the resin leads to nonspecific, irreversible protein adsorption.[27] Later work utilized Rapp's TentaGel, an amphiphilic, polyethylene glycol resin.[28]

The major requirement of a linker group (i.e., the functionality that joins the first monomer to the polymer) is that it be stable to the various sets of reaction conditions employed in the synthesis. It must then be possible to cleave the linkage to the resin in high yield, without affecting the integrity of the molecule. The basis of the linker used by Kahne and coworkers has been the *p*-hydroxythiophenyl glycoside (Fig. 3.2).[25,28] The use of a substituted thiophenyl glycoside was a natural extension of the methodology, and the phenolate anion in the para position was used to displace benzylic chloride functional groups on the surface of the resin. The thiophilic nature of mercury(II) salts has been used to good effect in cleavage of oligosaccharides from

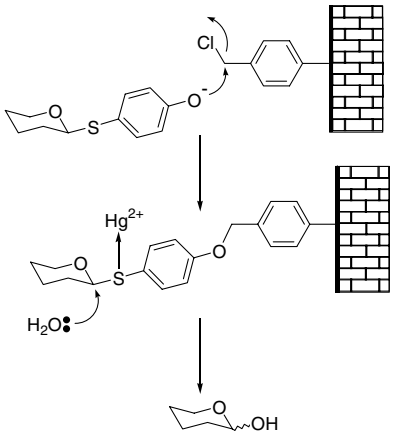


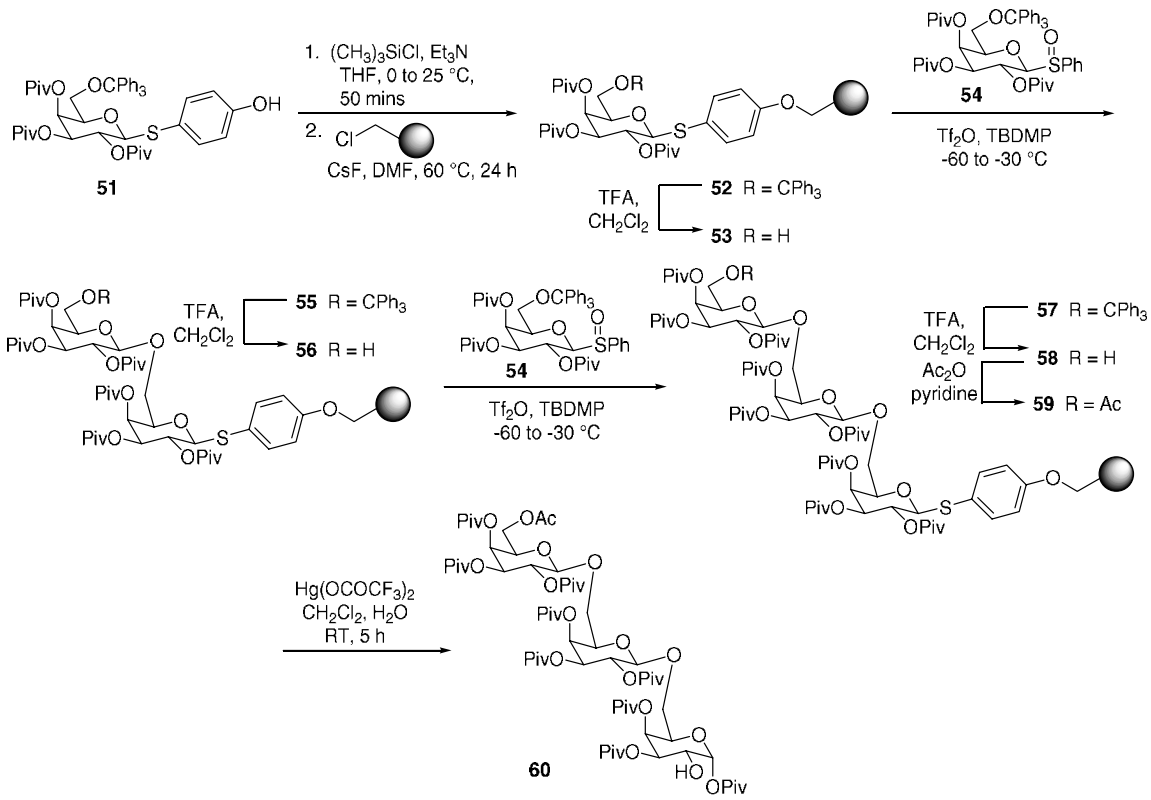
Figure 3.2 The *p*-hydroxythiophenol linker.

the resin; a limitation of this cleavage procedure is that a mixture of α- and β-lactols is invariably produced. More recent work involved a slightly longer linker group and an amide linkage to the resin.[28]

The first test of the sulfoxide glycosylation method in a solid-phase environment was the iterative assembly of the β-(1→6)-linked galactose trimer,[29] illustrated in Scheme 3.11. It provided a sensible test case for the glycosylation methodology because differential protection and deprotection of the primary hydroxyl was straightforward; and glycosylation of primary alcohols is easier than that of more sterically hindered secondary alcohols. Trisaccharide **57** was assembled on the resin, using an excess of sulfoxide **54** and a repetition of the glycosylation reaction, to drive the glycosylation to completion.[25] The trityl group in **57** was converted to an acetate, to give **59**, and then the trisaccharide cleaved from the resin. Interestingly, this cleavage was accompanied by migration of the pivaloyl ester from C2 to C1, suggesting the intermediacy of X (Scheme 3.7).

The Lewis blood group antigens have attracted a great deal of attention, because of their biological significance, and also because they present a considerable synthetic challenge. The presence of both α- and β-glycosidic linkages and the branched nature of the oligosaccharides, makes them a good test case for any glycosylation method and protecting group strategy.

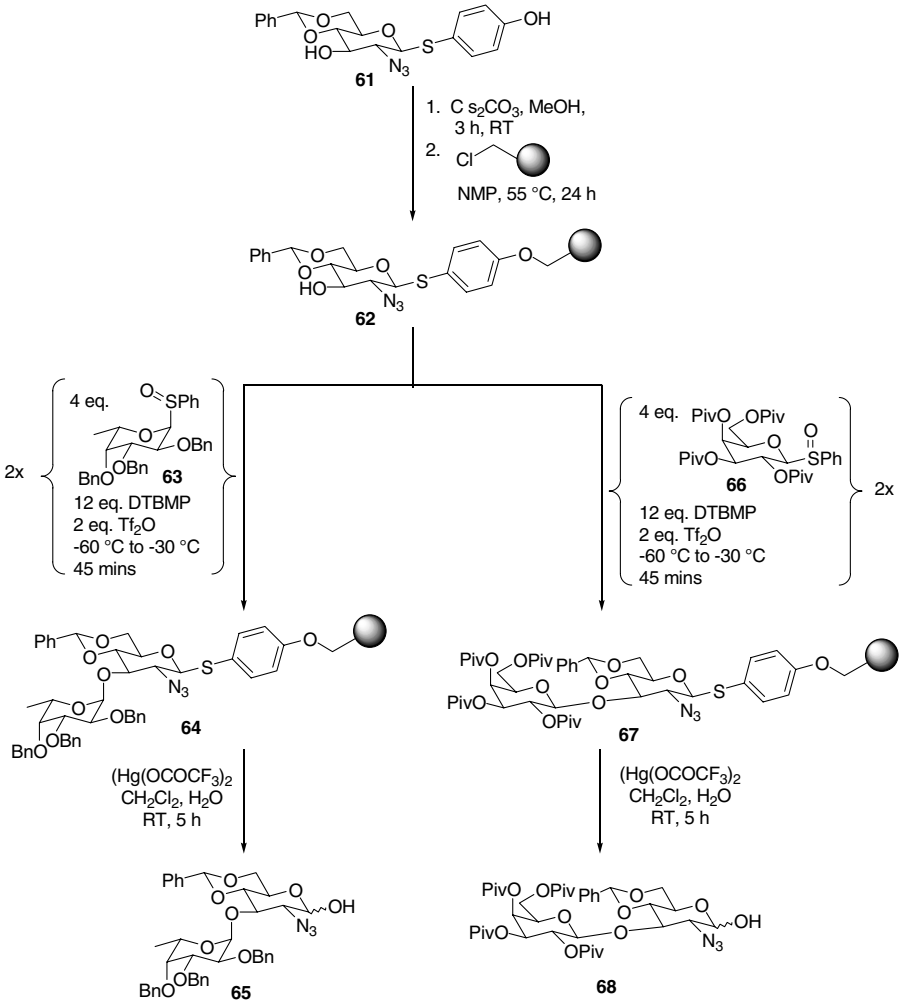
Yan and Kahne reported the solution synthesis of Lewis^a, Lewis^b, and Lewis^x using the sulfoxide method exclusively.[3] This work was carried out for two reasons: to demonstrate the potential generality of the sulfoxide method and to serve as a model study prior to solid-phase synthesis. The pivotal, central monosaccharide, which serves as a basis for this family of oligosaccharides, is *N*-acetylglucosamine. 2-Amino-2-deoxysugars are ubiquitous in glycoconjugates (e.g., glycoproteins and glycolipids) and therapeutic glycosides (e.g., antitumor agents and antibiotics), and so it was important to develop methodology to permit incorporation of these important building blocks. While an *N*-acetylglucosamine derivative has been used in



Scheme 3.11 Iterative assembly of β-(1→6)-linked galactose trimer.

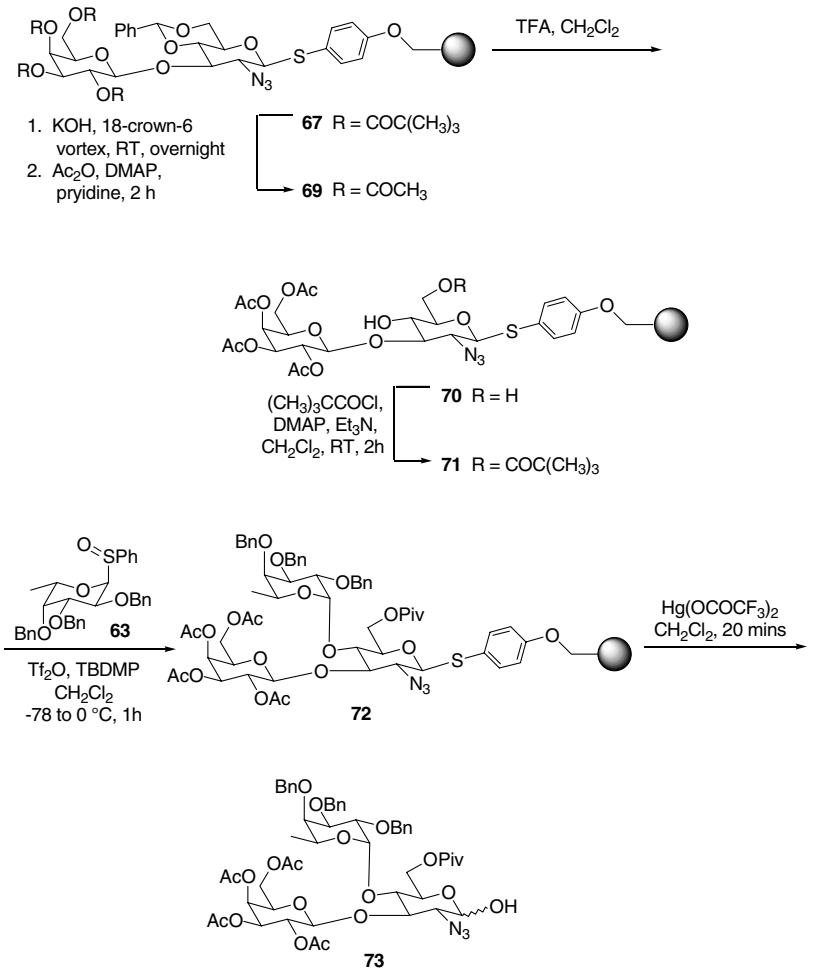
solid-phase oligosaccharide synthesis,[30] a masked form of the acetamido group is preferred. A number of masked amino groups at C2 have been investigated, in conjunction with the sulfoxide method, including phthalimido,[31,32] tetrachlorophthalimido,[32] azido,[25,31] trifluoroacetamido,[32] and carbamate protected amines (Fmoc[32] and Alloc).[32]

In 1994, Yan et al. reported stereoselective glycosylations of resin-bound glycosyl acceptor **62** (Scheme 3.12).[25] An α-(1→3)-glycosidic linkage, like that found in Lewis^x, was formed using perbenzylated fucosyl sulfoxide **63**. A β-(1→3)-glycosidic linkage, like that found in Lewis^a, was formed using perpivaloyl galactosyl sulfoxide **66**.



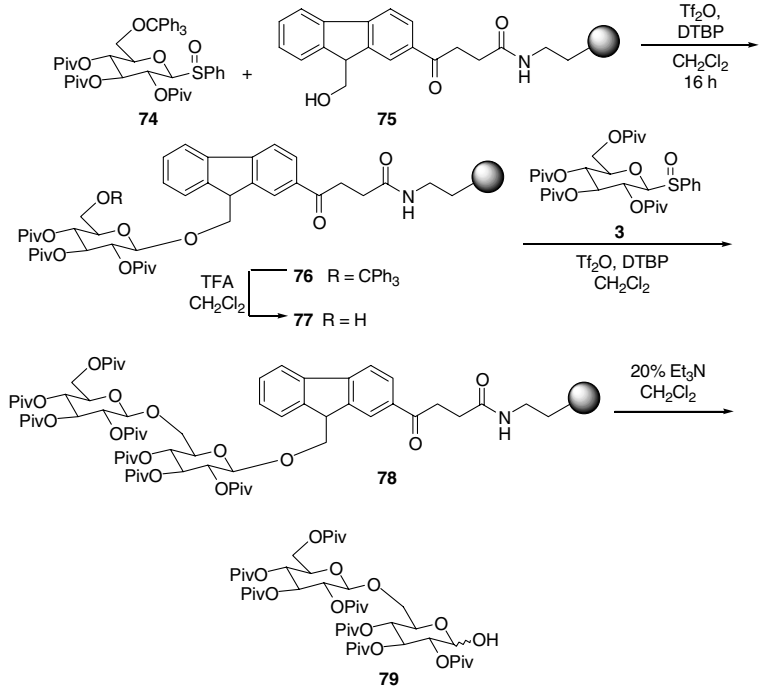
Scheme 3.12 Stereoselective glycosylation of resin-bound glycosyl acceptor.

Resin-bound disaccharide **67** has been converted to Lewis[a] derivative **73** (Scheme 3.13).[31] It proved necessary to replace the pivaloyl protecting groups in the galactose residue with acetates, to reduce steric hinderance. The benzylidene protecting group on the *N*-acetylglucosamine residue was then cleaved, and the primary alcohol selectively protected as a pivaloate ester **71**. Formation of the α-glycoside using perbenzylated fucopyranose sulfoxide **63** then afforded **72**, which was detached from the resin to give **73**.

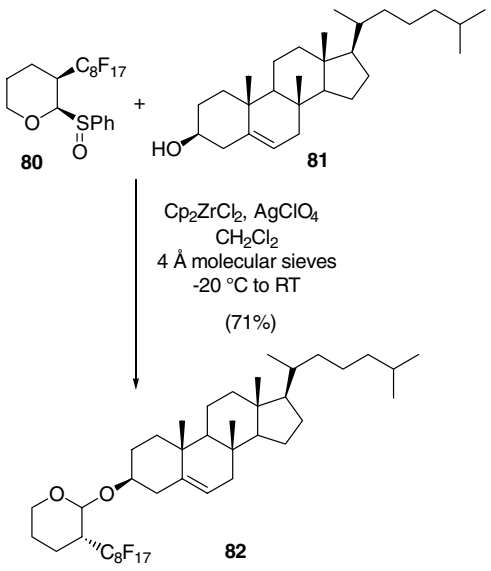


Scheme 3.13 Synthesis of a Lewis[a] trisaccharide.

It has long been recognized that polymeric supports for oligosaccharide synthesis do not need to be insoluble.[33] Wang et al. applied the sulfoxide glycosylation method to the synthesis of disaccharide **79** (Scheme 3.14). The key feature of this work was the base-labile linker. Sulfoxide **74** was used to glycosylate polyethylene glycol



Scheme 3.14 Disaccharide synthesis using the sulfoxide glycosylation method.



Scheme 3.15 Formation of fluorous pyranosides.

(PEG) derivatized with a fluorenylmethanol group **75**. Deprotection and glycosylation was similar to that described in Scheme 3.11. Treatment with mild base then effected β-elimination to release **79** from the polymer.

An alternative to polymer-supported synthesis, which is gaining momentum, is fluorous synthesis. This concept exploits the ability of a highly fluorinated compound to partition predominantly into the fluorocarbon phase in a liquid–liquid extraction between an organic solvent and a fluorinated solvent. Wipf and Reeves have recently reported the development of a fluorous tetrahydropyran (THP^F) protecting group/label that utilizes a modification of the sulfoxide glycosylation method for introduction of the THP^F group.[34] The standard reagents (Tf_2O, DTBMP) gave low yields of pyranosides, and elimination to generate the dihydropyran was a significant problem. Alternative reaction conditions (Scheme 3.15) gave good yields of the desired products (e.g., **82**) which were purified, taking advantage of fluorous media; the THP^F can be recycled.

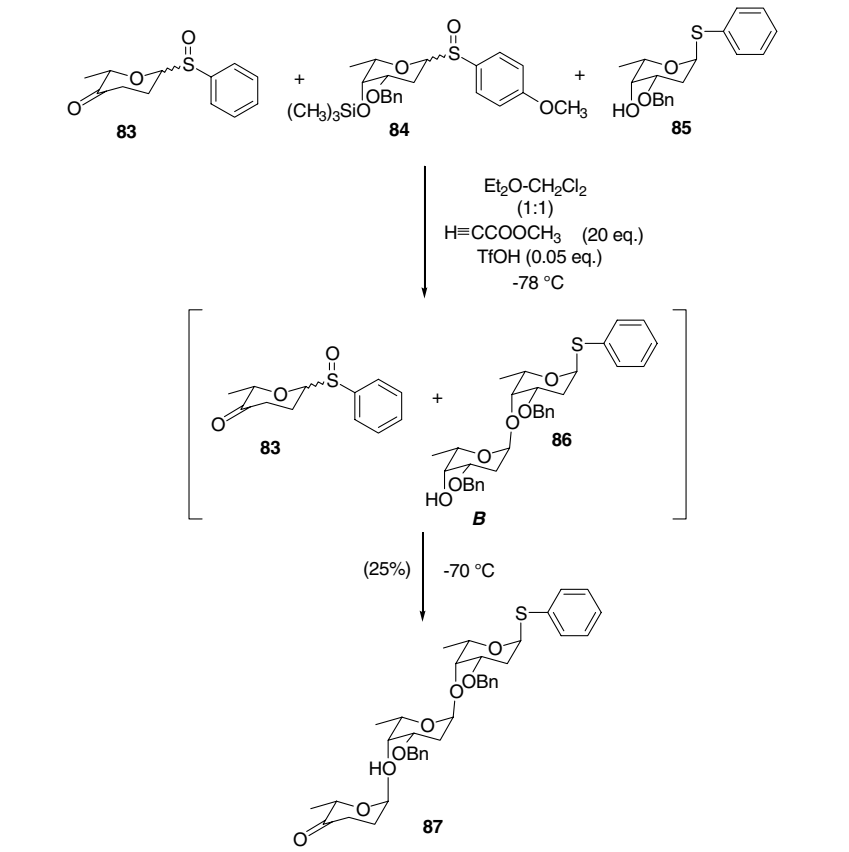
3.6 LIBRARIES OF OLIGOSACCHARIDES

Combinatorial approaches to the synthesis of libraries of peptides, nucleotides, and many classes of small organic molecules are well documented.[35] However, the field of oligosaccharide combinatorial chemistry is still in its infancy.[36] The slow development is due to the inherent complexities in the synthesis of oligosaccharides.

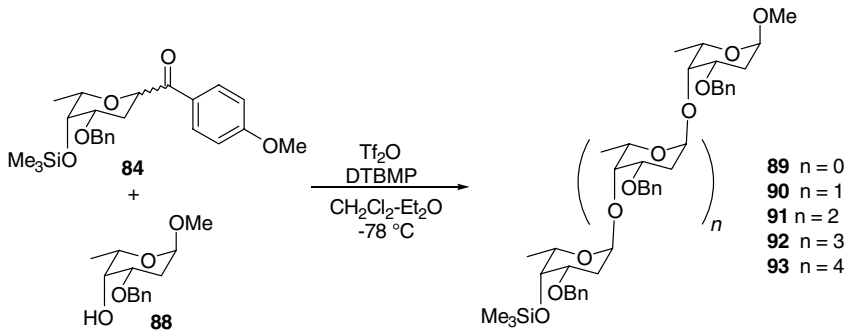
In 1993, Raghavan and Kahne reported the remarkable one-step synthesis of the ciclamycin 0 trisaccharide **97** (Scheme 3.16).[19] In related studies, Raghavan investigated the controlled polymerization of 2,6-dideoxysugars (Scheme 3.17).[37] When a 2:1 ratio of **84**:**88** was employed, the disaccharide **89** ($n = 0$) was obtained in 45% yield, along with 20% yield of the trisaccharide **90** ($n = 1$). On increasing the **84**:**88** ratio to 5:1, a statistical mixture of the di-, tri-, tetra-, penta-, and hexasaccharides was obtained. These five compounds were separable by chromatography. In essence, this was the first example of a carbohydrate library produced using the sulfoxide glycosylation method.

The advantages of a solid-phase approach to the synthesis of libraries are well recognized. The use of excess solution-based reagents, and repetitive couplings, can be used to drive reactions to completion. Pools of resin can be tagged or spatially resolved, so that the reaction history of any bead is accessible. Moreover, it has been suggested that there are situations where on-bead assays may be advantageous. In a sense, the presentation of oligosaccharides on a bead of resin, mimics the manner in which they might be displayed on the surface of a cell.[28,36f]

In 1996, Liang and coworkers described the parallel synthesis and screening of a library of di- and trisaccharides that were screened for binding to *Bauhinia purpurea* lectin (peanut agglutin).[28] This protein binds to carbohydrates on the surface of erythrocytes, causing them to agglutinate; it thus provides a good model system for carbohydrate binding proteins that recognize cell surface carbohydrates. An established ligand for the protein is Gal-β-(1→3)-GalNac (see **113**, Fig. 3.4).



Scheme 3.16 One-step synthesis of ciclamycin *O*-trisaccharide.



Scheme 3.17 Controlled polymerization of 2,6-dideoxy sugars.

The library was synthesized on TentaGel resin. Six monomers (Fig. 3.3) were attached to the resin via an amide linkage (Scheme 3.18), and each batch of resin was encoded with a chemical tag according to the methodology of Still.[38] The mixture of resin-bound monomer was then split into 12 batches and each batch subjected to glycosylation, via the sulfoxide method, with a different glycosyl donor (Fig. 3.3), and tagged accordingly. All the batches of resin were combined and treated with trimethylphosphine; this reduced the azides to primary amines. The resin was then

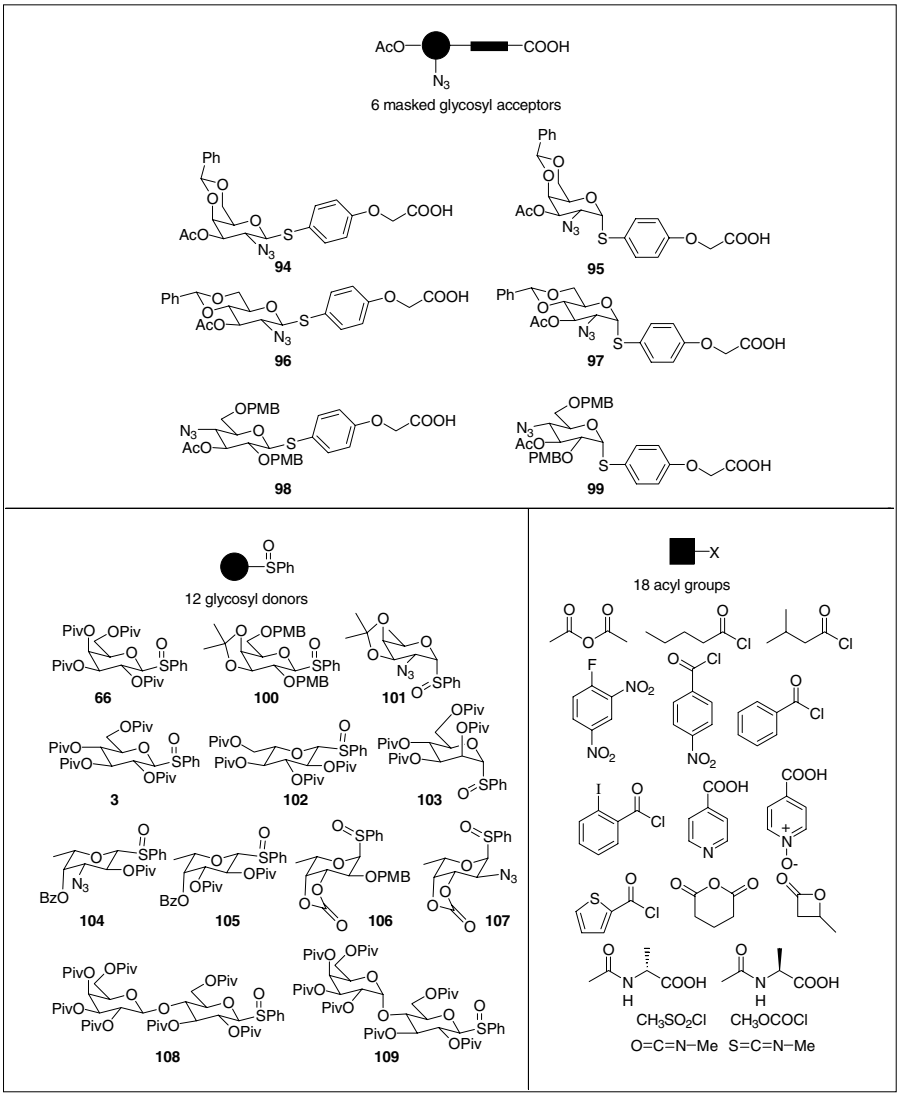
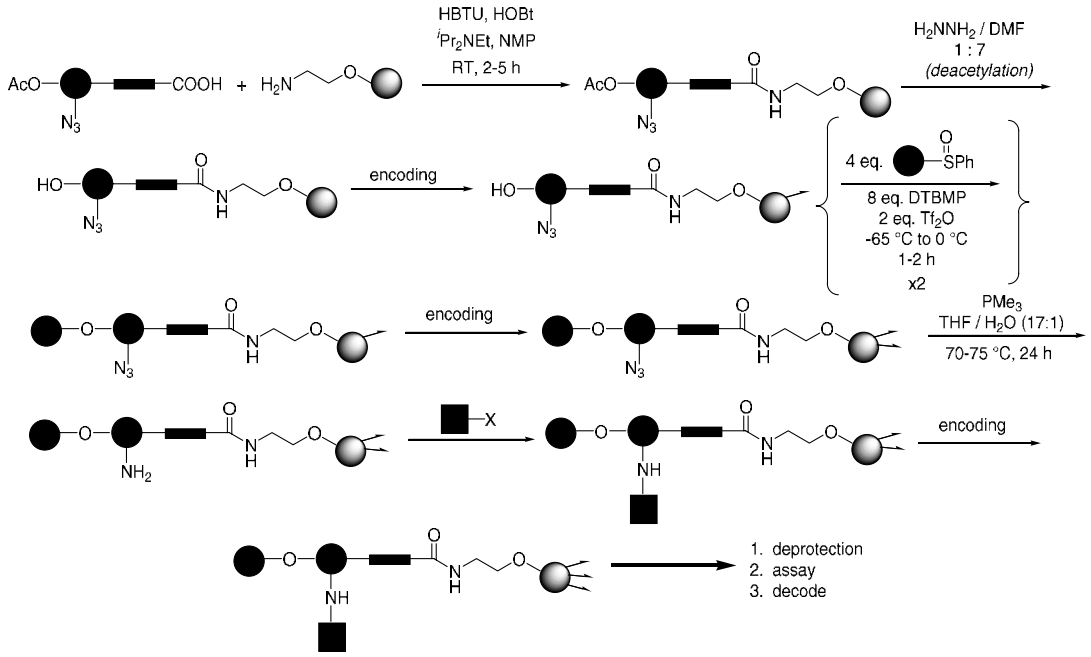
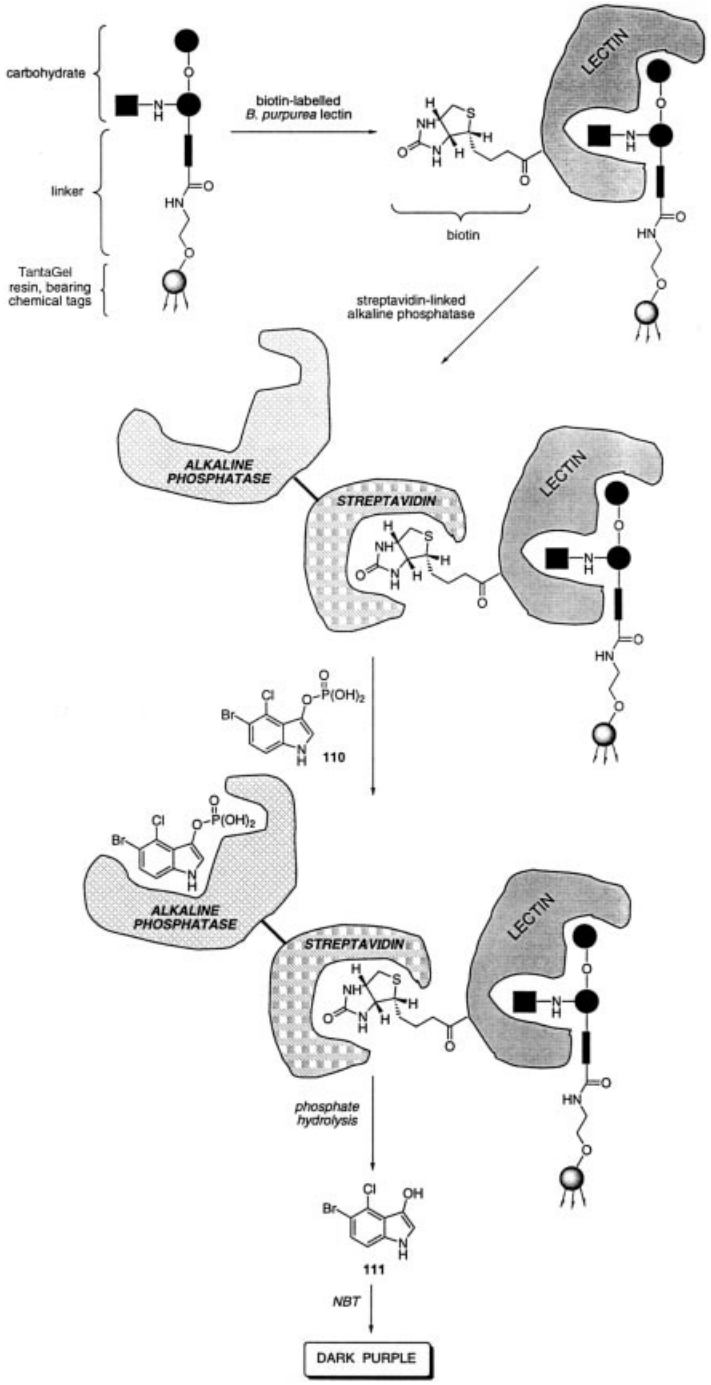


Figure 3.3 Building blocks for the library.



Scheme 3.18 Generic representation of library synthesis.

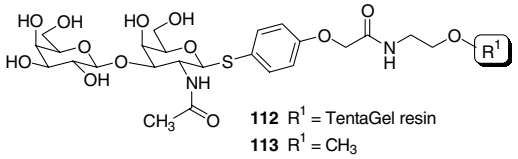


Scheme 3.19 Oligosaccharide binding to *Bauhinia purpurea* lectin leading to a colorimetric response.

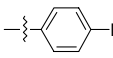
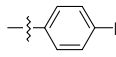
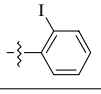
split into 18 batches which were each treated with an acylating agent (Fig. 3.3) and encoded with a chemical tag. Finally, all the beads were mixed together again and deprotected (with trifluoroacetic acid to remove benzylidenes and PMB ethers, followed by lithium hydroxide to hydrolyze ester and carbonate protecting groups). There are theoretically $(6 \times 12 \times 18) = 1296$ compounds in the library. It was estimated that in 10 mg of derivatized resin, there were about 9000 beads and about six copies of each library member.

The on-bead assay was conducted according to Scheme 3.19, which shows the chain of events, which leads to a colorimetric response, when an oligosaccharide binds effectively to the *B. purpurea* lectin. The lectin was covalently linked to biotin, a small molecule with an extremely high affinity for streptavidin. The bead–lectin–biotin conjugates were then exposed to streptavidin, linked to the enzyme alkaline phosphatase. Alkaline phosphatase hydrolyses phosphate esters [e.g., 5-bromo-4-chloro-3-indolyl phosphate (BCIP), **110**]. When the 5-bromo-4-chloro-3-hydroxyindole (**111**) is released, in the presence of nitro blue tetrazolium (NBT), it forms a dark purple, insoluble dye, thus staining beads where there was a favorable binding interaction.

Of the 10 mg portion of beads, only 25 (<0.3%) beads stained dark purple. These were picked out and decoded, by releasing and analyzing the tags; 13 of them contained the same core disaccharide, with two α-linkages, as depicted in Figure 3.4. Of the remaining 12 stained beads, there appeared to be no recurring themes or features, and so these were regarded as "noise."



112 R^1 = TentaGel resin
113 $R^1 = CH_3$

	R^1	R^2
114	TentaGel resin	
115	CH_3	
116	TentaGel resin	
117	CH_3	
118	TentaGel resin	
119	TentaGel resin	

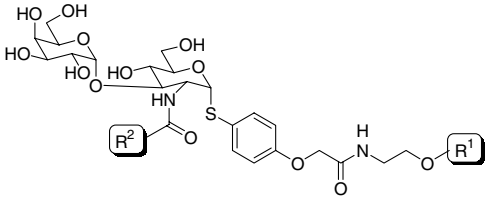
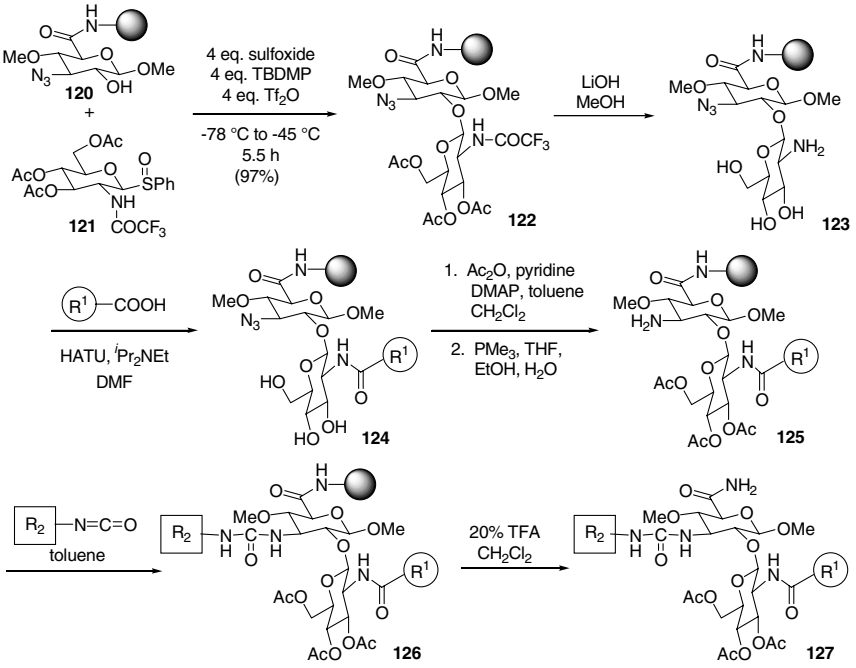
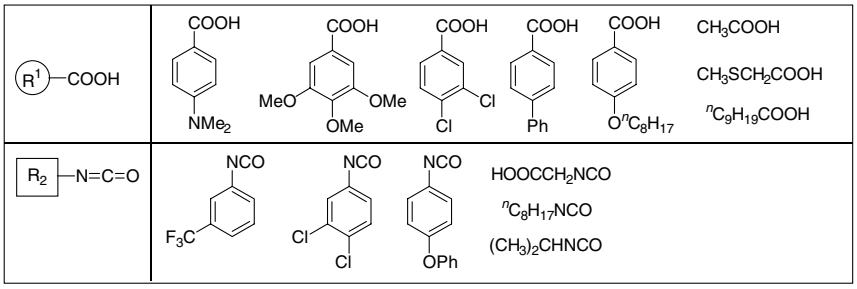


Figure 3.4 Ligands for *Bauhinia purpurea* lectin.

Surprisingly, the known ligand **112** (containing two β-linkages) bound much more weakly than **114**, **116**, **118**, and **119**. This illustrates the power of combinatorial chemistry to identify novel molecules with superior properties, which would be unlikely to be discovered by design. Intrigued by the nonstaining of **112**, compounds **113**, **115**, and **117** were synthesized and their affinities for the lectin were determined in solution. All three compounds, in solution, inhibited the binding of the lectin to **112**, at concentrations in the 20–50-g/mL range. The difference in relative binding affinity in solution versus on the bead was proposed to be a consequence of the polyvalent presentation.





Scheme 3.20 Synthesis of a disaccharide bearing two amines and an amide.

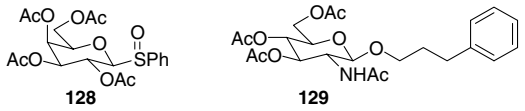


Figure 3.5 Compounds **128** and **129**.

While oligosaccharides themselves are important binding and recognition elements, sugar molecules have also become popular as scaffolds for the display of other functionality.[36e,39] Silva and coworkers recently reported the solid-phase synthesis of a 48-member library of disaccharide derivatives (Scheme 3.20).[32] A glucuronic acid glycosyl acceptor was anchored to a solid support via an amide linkage between the C6 carboxylic acid and amino groups on the surface of the resin to give **120**. Rink amide resin is a relatively cheap, mechanically resistant solid support that is compatible with the sulfoxide glycosylation reaction conditions. 2-Deoxy-2- trifluoracetamido glucosyl sulfoxide **121** was appended in a β-(1→4) fashion. The acetate esters and trifluoroacetamide were hydrolysed and then the amino group acylated (R^1) to give **124**. The free alcohols reacetylated and the C3-azido group on the glucuronic acid residue was then reduced to give **125**, which was reacted with a series of isocyanates (R^2) to introduce alkyl and aryl carbamates at this position. The disaccharide derivatives **127** were cleaved from the resin and characterized by LCMS.

Dzumela and McGarvey have recently described plans for the preparation of an oligosaccharide library using what they describe as the sulfoxide random glycosylation method.[40] They have synthesized **128** and **129** as a prelude to this exercise (Fig. 3.5).

3.7 OUTLOOK

The sulfoxide glycosylation protocol has tremendous potential in the synthesis of oligosaccharides: in solution, in the solid phase, and in library format. One of the current limitations, highlighted in Scheme 3.13, is the difficulty associated with protecting group manipulations. For oligosaccharide synthesis to become a truly iterative process, more sophisticated protecting group strategies need to be developed, wherein each alcohol might be selectively liberated and glycosylated in a graceful fashion. A trisaccharide is the largest oligosaccharide produced on a resin to date, using the sulfoxide method. Although much has been accomplished, many challenges lie ahead, to improve the technology to produce oligosaccharides for the investigation of their behavior in fascinating situations of biological and medical importance.

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4 The Use of *O*-Glycosyl Trichloroacetimidates for the Polymer-Supported Synthesis of Oligosaccharides

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4.1 INTRODUCTION

The chemical synthesis of oligosaccharides has seen years of dynamic progress, based mainly on the development of highly reactive glycosyl donors and advanced protective group strategies during the 1980s.[1] The eminent role of carbohydrate conjugates and polymers in biological systems was a strong stimulus of this development.[2] Despite these achievements, the synthesis of larger and more complex glycoconjugates in solution phase remains a demanding task.[3] Therefore, a well-established method for the preparation of oligosaccharides on a polymer support might be superior to the solution techniques with respect to efficiency, applications in combinatorial synthesis,[4] and future automatization. We report here on the use of *O*-glycosyl trichloroacetimidates, as well established powerful glycosyl donors, in this endeavor. Glycosyl trichloroacetimidates have been investigated on solid and soluble supports in combination with different linker systems in order to arrive at a highly efficient glycosylation methodology.

4.2 POLYSTYRENE-BASED SUPPORTS

4.2.1 Thioglycosides as Linkers

The first application of *O*-glycosyl trichloroacetimidates as glycosyl donors for the glycosylation of an acceptor attached to Merrifield resin was reported by our group in 1996.[1] This approach was based on the use of a thiol functionalized resin (Scheme 4.1).

Scheme 4.1 A thiol functionalized resin.

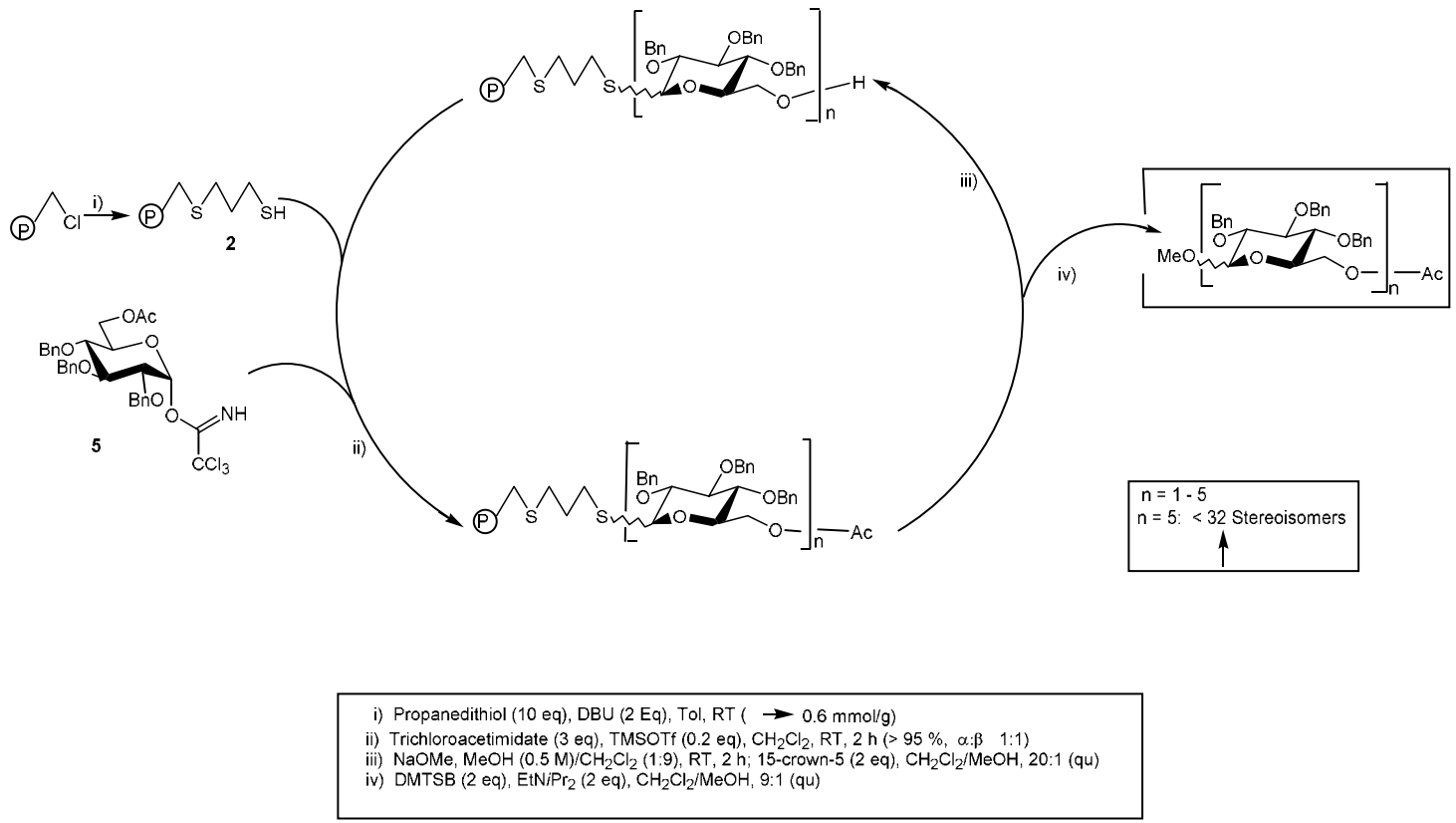
Among the different supports, resins **2** and **3**, simply derived from Merrifield resin by *O*-alkylation using the corresponding protected thiol compounds or *S*-alkylation of symmetric 1,3-propanedithiol for **2**, proved to be the most efficient in terms of reaction yields and chemical stability. These two resins turned out to be very good glycosyl acceptors, thus allowing a very efficient introduction of the first sugar residue. On the basis of this linker strategy, oligosaccharide synthesis was performed, first using **2** as support. Scheme 4.2 depicts the synthesis of glucose (1→6) oligomer using donor **5**.

The anomeric anchoring of the glycosidic chain as a thioether allowed the final liberation of the oligosaccharide part using classical glycosylation conditions for thioether glycosyl donors. Dimethyl(methylthio)sulfonium salts were used as the thiophilic reagents, buffered with Hünig's base in dichloromethane, and methanol as the glycosyl acceptor. Released compounds can thereafter be purified as their 1-*O*-methyl glycosides. This synthesis was completed until the stage of the pentamer. This new linker system allowed the analytical cleavage of small resin samples and thus the monitoring of reactions using MALDI-TOF (matrix-assisted laser desorption ionization–time of flight) mass spectrometry. The initial loading of 0.4–0.6 mmol/g (S elemental analysis of **2**) proved to be too high to allow solid-phase reactions, and most of all glycosylations, to reach completion. The use of resin **3** was found to be more advantageous because of the greater chemical stability of the ether linkage during different reaction conditions. Moreover, lower and thus more practical loading was easily obtained (0.1–0.3 mmol/g, S elemental analysis). By taking advantage of these two improvements, more complex synthetic targets were envisaged. The synthesis of an α-(1→2)-linked pentamannoside **7** was then accomplished[2] (Scheme 4.3) from resin **3** (0.2 mmol/g) using mannosyl donor **6**.

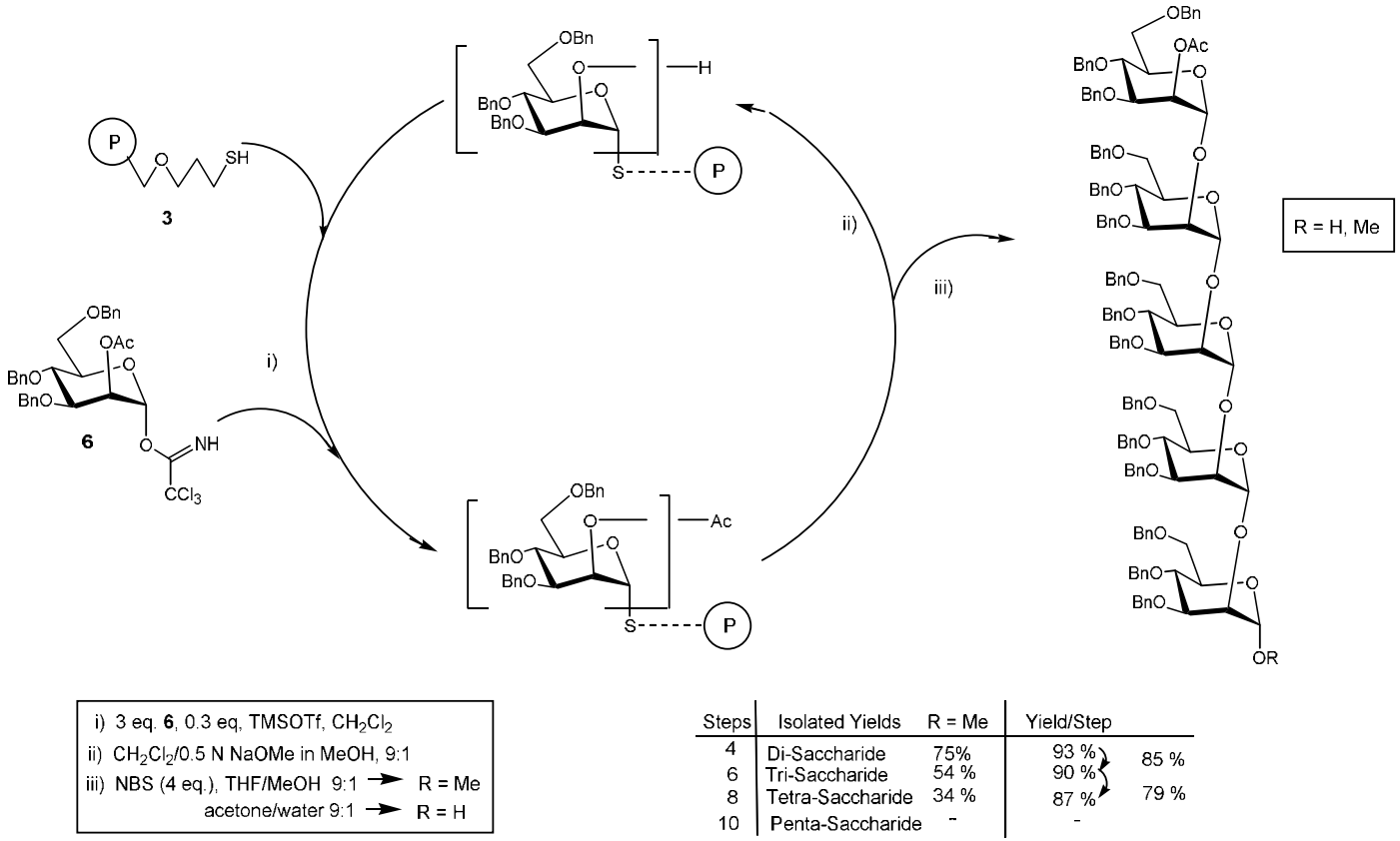
This synthesis demonstrated that the neighboring-group participation effect on the stereoselectivity of glycosylation reactions can be extended to solid-phase processes. In this case, milder and more practical cleavage conditions than previously discussed were established. The use of *N*-bromosuccinimide as the thiophilic reagent in acetone/water or tetrahydrofuran/methanol permitted the release of oligosaccharides in form of lactols or 1-*O*-Me glycosides, respectively. The tetrasaccharide derivative was isolated in 34% yield from thiol resin **3** (80% yield per step).

Using the same linker system, the synthesis of disaccharide **10** was reported (Scheme 4.4). This last work nicely illustrated the application of the use of the anomeric effect for the synthesis of a 1,2-*cis*-glycosidic linkage in the absence of possible neighboring-group effects. The target molecule was prepared using fucosyl donor **9** for the glycosylation of attached acceptor **8**. In solution, this type of glycosylation requires the combined use of diethyl ether as solvent and low temperatures for good stereocontrol. Unfortunately, the Merrifield resin derivative used here does not swell in diethyl ether alone. However, the judicious use of a 1/1 mixture of dichloromethane and 1,4-dioxane at –25°C afforded the target disaccharide in 54% overall yield as pure α-anomer.

With this solid-phase methodology in hand, the synthesis of branched oligosaccharides could be investigated. This led to the preparation of pentasaccharide **15** related to *N*-glycan chains (Scheme 4.5).[3]



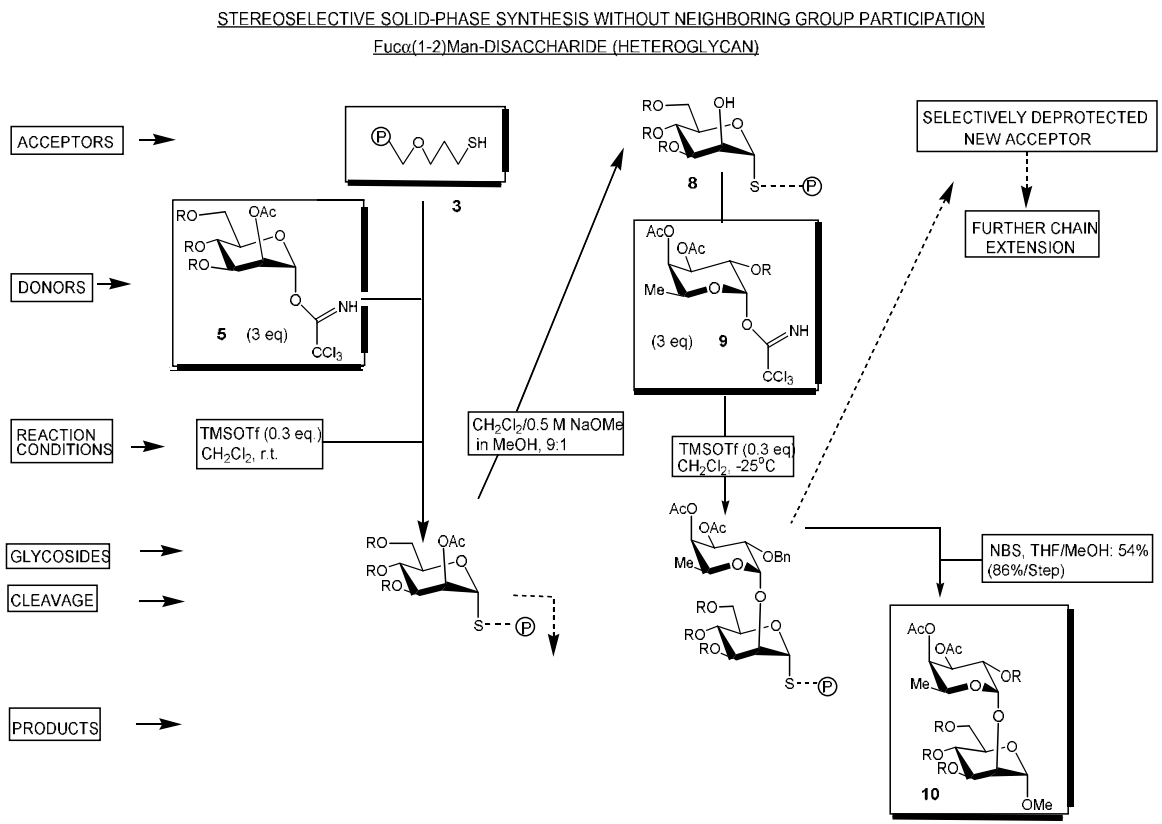
Scheme 4.2 Synthesis of glucose (1→6) oligomer using donor **5**.



i) 3 eq. **6**, 0.3 eq, TMSOTf, CH_2Cl_2
ii) CH_2Cl_2/0.5 N NaOMe in MeOH, 9:1
iii) NBS (4 eq.), THF/MeOH 9:1 → R = Me
acetone/water 9:1 → R = H

Steps	Isolated Yields	R = Me	Yield/Step	
4	Di-Saccharide	75%	93 %	85 %
6	Tri-Saccharide	54 %	90 %	
8	Tetra-Saccharide	34 %	87 %	79 %
10	Penta-Saccharide	-	-	

Scheme 4.3 Synthesis of an α-(1→2)-linked pentamannoside **7** from resin **3**.



Scheme 4.4 Synthesis of disaccharide **10**.

Starting from thiol resin **3**, the first glycosylation using mannosyl donor **11** furnished resin **12**. After deacetylation, acceptor resin **13** was used as the starting material for a glycosylation–deprotection–glycosylation sequence in which two glycosidic bonds were formed simultaneously during each glycosylation step. The target pentasaccharide was obtained in 20% overall yield (82% per step). The strategy used for this work was based on the use of neighboring-group participation to ensure stereoselective 1,2-*trans*-glycosylation using the previously described mannosyl donor **6** and Teoc-protected glucosamine donor **14**. The pentasaccharide **15** is one of the most complex oligosaccharides synthesized on a solid support using a very elaborate and efficient synthetic approach.

4.2.2 Sulfonate Linkers

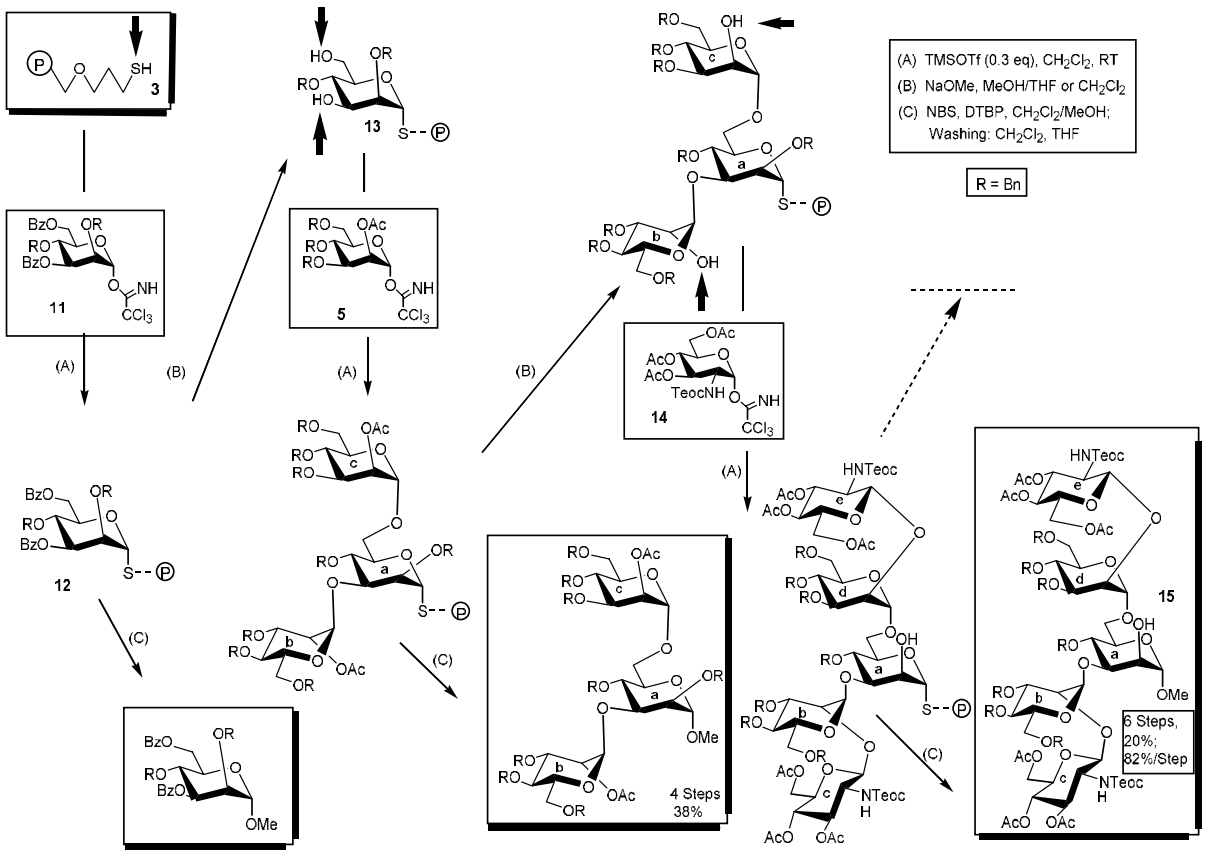
Another sulfur-containing linker was described by Hunt and Roush in 1996 (Scheme 4.6).[4]

The sulfonyl chloride resin **16** was obtained in three steps (70% overall) from Merrifield resin. The loading was determined by gravimetric analysis to be 0.65 mmol/g. This resin allowed for the attachment of the first sugar residue **17** using classical sulfonylation conditions. It should be noticed that in this case the first sugar residue is linked to the solid support via the C6 hydroxyl, thus giving access to 6-deoxy compounds. The authors described the use of acetyl esters and triethylsilyl ethers as temporary protecting groups for the synthesis of trisaccharide **22**. The first glycosylation was accomplished using trichloroacetimidate donor **19** at low temperature. This step was performed two times in similar conditions to ensure a complete reaction. The target compound was released as a 6-deoxy-6-iodo derivative by nucleophilic substitution at the sulfur atom using NaI in 2-butanone at 65°C for 20 h. Subsequent radical reduction led to the corresponding 6-deoxy derivative. Analytical cleavages were performed at each stage of the oligosaccharide chain elongation. Thus disaccharide **21** was recovered, after on-bead acetylation of the corresponding acceptors and cleavage in 87% yield from glycal **18**. The target trisaccharide was prepared in 67% overall yield (nine steps from attached glycal **18**). It is remarkable that a glycosylation using donor **19** was accomplished without anchimeric assistance from a neighboring group. Use of low temperatures ensured excellent stereoselectivity control since the authors reported the isolation of only trace amounts of α-linked disaccharide; this corresponds to an improvement compared to the corresponding glycosylation in solution.

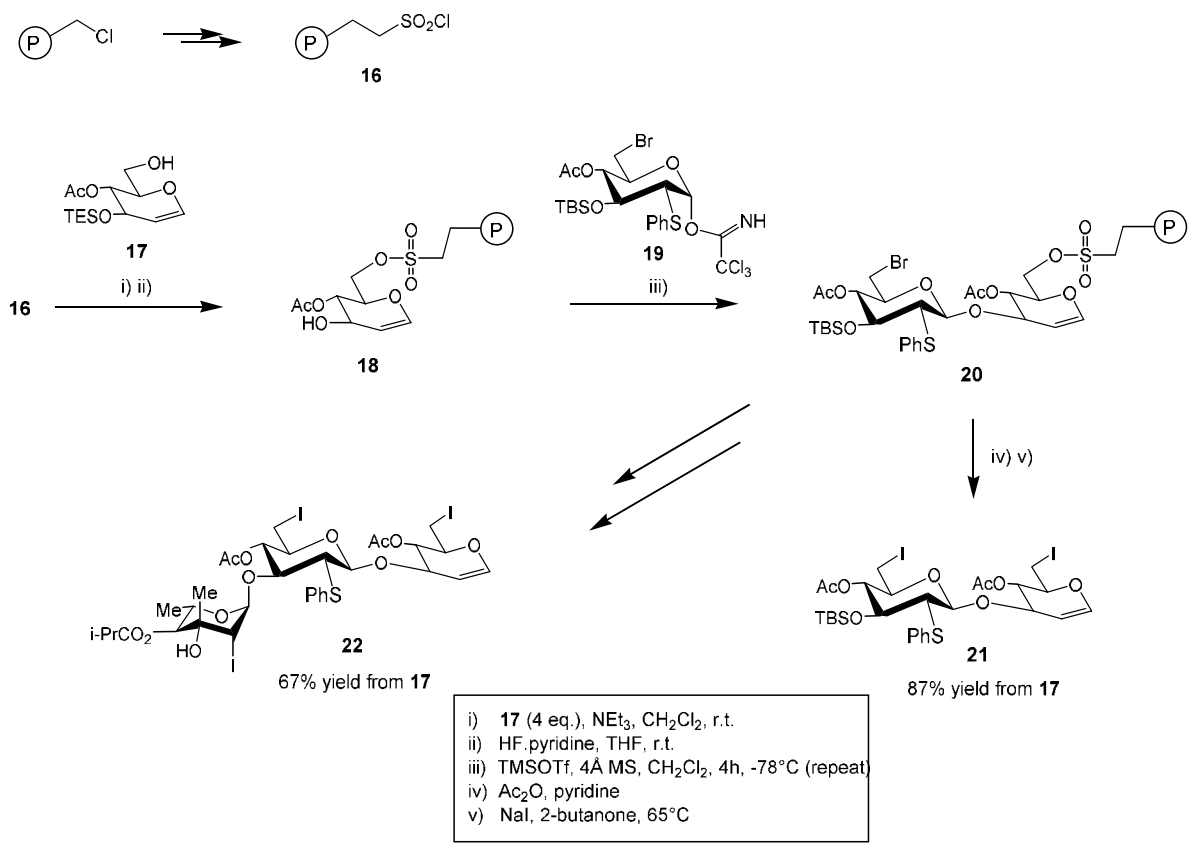
4.2.3 Substituted Benzyl Ether Linkers

A major contribution to the use of glycosyl trichloroacetimidates in the solid-phase synthesis of oligosaccharides came from the group of T. Ogawa (Scheme 4.7).[5]

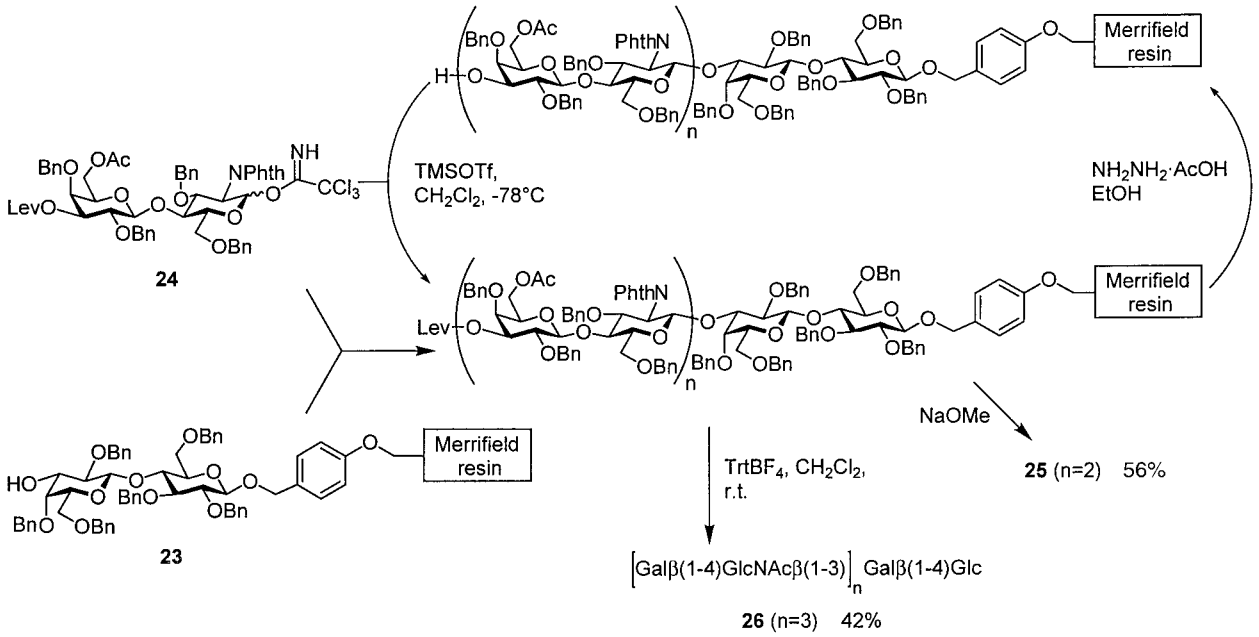
These authors developed the use of a 4-alkyloxybenzyl ether–type linker attached to the Merrifield resin via an ester function that allowed the cleavage of the oligosaccharides under two different conditions. Using the reactivity of the activated



Scheme 4.5 Preparation of pentasaccharide **15**.



Scheme 4.6 A sulfonate linker.

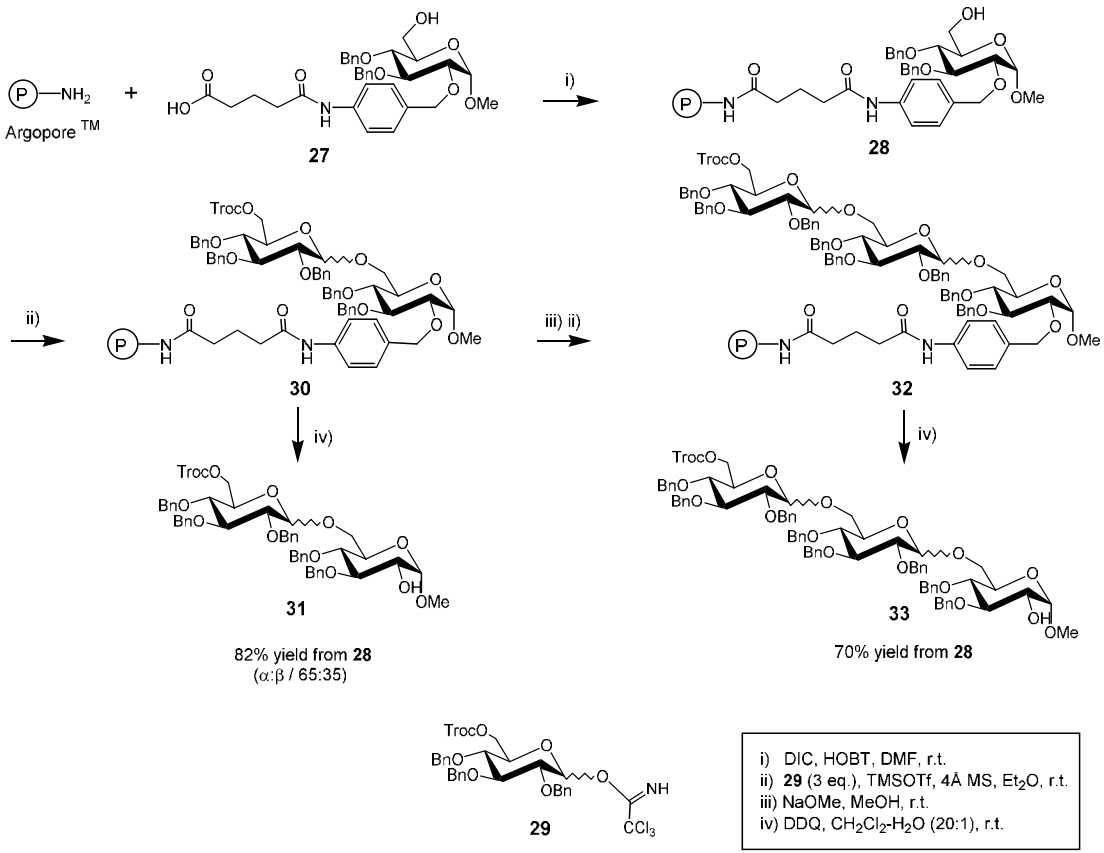


Scheme 4.7 Cleavage of attached oligosaccharides.

benzylic position, they described the cleavage of attached oligosaccharides under mildly acidic conditions ($TrBF_4$ in CH_2Cl_2 at room temperature) as a free hydroxyl derivative. The alternative approach is to use a simple basic transesterification as cleavage reaction (NaOMe in MeOH) in order to release the desired compound as its 4-alkyloxybenzyl ether derivative. To deal with an acid- and base-sensitive linker, these authors had to develop a new protecting group pattern for the synthesis of linear polylactosamine oligosaccharides. The choice of the levulinoyl group as temporary protecting group permitted mild deprotection reactions using hydrazinium acetate, conditions fully compatible with the linker system. Starting from Merrifield resin (0.66 mmol/g) the first lactose unit was introduced using an ester synthesis step to arrive at resin **23**. Extension of the chain was achieved using the lactosamine donor **24**. The choice of phthaloyl as amino protecting group ensured the formation of the pure β linkage. The glycosylation steps (1.5 equiv donor, 0.2 equiv TMSOTf, CH_2Cl_2, −78°C, 2 h) were performed twice at each stage. Using a modified linker system, basic cleavage after the second elongation step furnished methyl ester **25** in 56% overall yield. Extension to the octasaccharide and subsequent acidic cleavage permitted the isolation of octasaccharide **26** in excellent 42% yield. Although mentioned by the authors, no oxidative cleavage using the well-known 4-alkyloxybenzyl reactivity of the linker was reported. There was no mention of analytical reaction monitoring.

A publication by Kusumoto et al.[6] added two more perspectives for the solid-phase synthesis of oligosaccharides using trichloroacetimidate donors on polystyrene-derived supports (Scheme 4.8). First, these authors described the use of a new 4-acylaminobenzyl ether linker that should be more acid-stable than the corresponding 4-alkyloxybenzyl derivative and could be easily cleaved under mild oxidative conditions (DDQ in CH_2Cl_2/H_2O:20/1). To test this concept, the acid **27** was prepared in solution using classical techniques. This compound was subsequently attached to an aminomethyl polystyrene support (ArgoPore) using standard conditions for peptide bond formation and allowing the introduction of the linker and of the first sugar residue in one step.

The second innovation brought by these authors is the use of a highly crosslinked macroporous poly(styrene–divinylbenzene) support. The great advantage of this support is its solvent tolerance notably of diethyl ether and methanol, which could considerably extend the interest of trichloroacetimidate donors for the synthesis of oligosaccharides on this type of support. These contributors also suggest a greater accessibilty from the reagents to the reactive sites on the solid support due to the larger pore size (90 Å for the dry resin). Starting from partially protected resin-attached glucose **28**, they tried to take advantage of the new support in order to investigate the possibility of α-selective glycosylations. Therefore, they used glucosyl donor **29** in diethyl ether at room temperature. By optimizing the support loading in acceptor and the glycosylation conditions, an isolated yield of 82% of (1→6)-linked disaccharide **31** as α/β mixture (α/β: 65/35) was obtained after cleavage. The trichloroacetimidate donor proved to give a good combination of yield and selectivity compared to other donors. Subsequent deprotection of the Teoc group [1 M NaOMe in methanol at room temperature (r.t.)] and glycosylation under the conditions described above gave after cleavage the corresponding trisaccharide **33** in 70% yield from **28**.



Scheme 4.8 Use of trichloracetimidate donors of polystyrene supports in solid-phase oligosaccharide synthesis.

4.2.4 Metathesis-Based Linkers

Most of the advances in the field of solid-phase synthesis of oligosaccharides are directed toward the elaboration of new linker systems allowing a broader stability toward basic and acidic conditions and more efficient cleavage reactions. Those new linker systems should allow for the synthesis of more complex target molecules. The exciting progress in the metathesis reactions, both in solution and on the solid phase, and in the design of efficient and stable metathesis catalysts prompted several groups to investigate metathesis-based linkers for the solid-phase synthesis of oligosaccharides using trichloroacetimidate donors.

Our preliminary results concerning a ring-closing metathesis (RCM)-based linker[7] demonstrated the validity of this approach (Scheme 4.9).

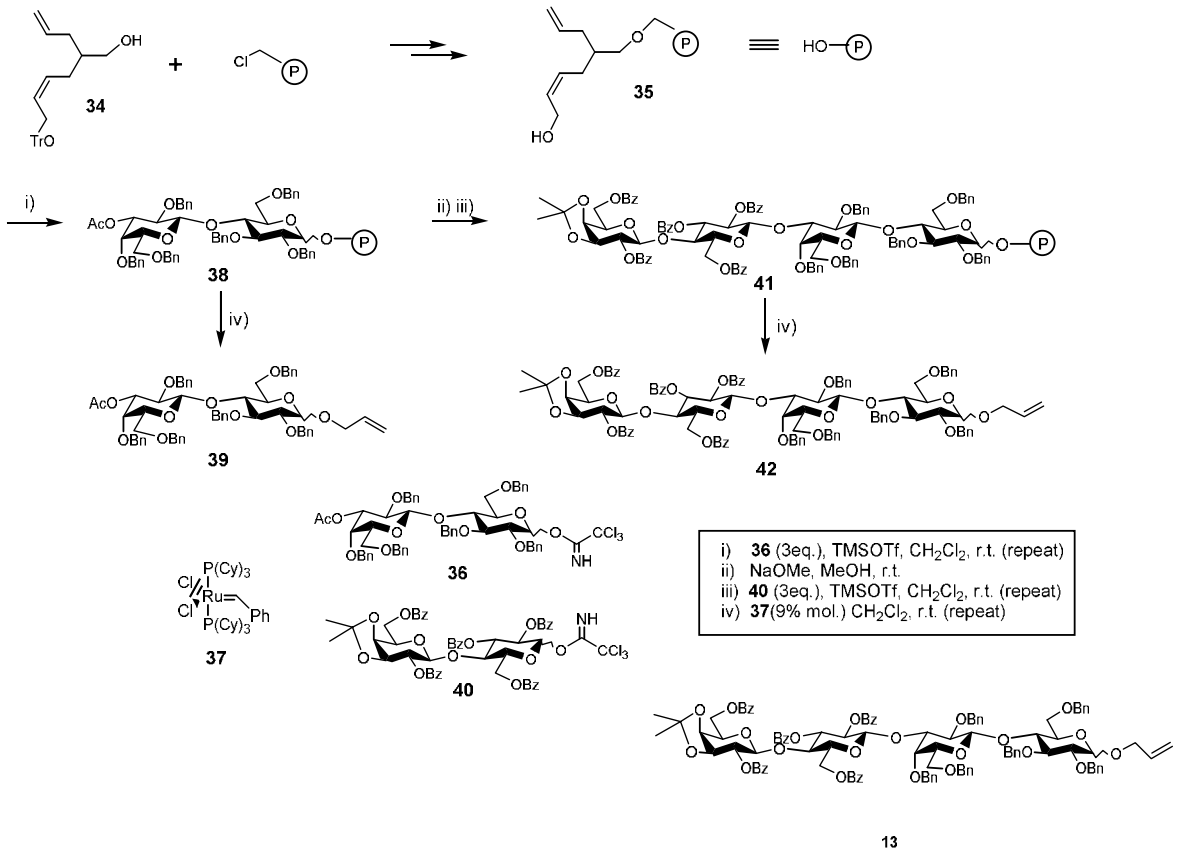
In the course of our ongoing program concerning the solid phase synthesis of human milk oligosaccharides, we prepared linker **35** in two steps from Merrifield resin and alcohol **34**. The loading of resin **35** was calculated to be 0.2 mmol/g by 4-nitrobenzoyl chloride derivatization and elemental analysis. Thereafter, resin **35** was employed in the glycosylation process with lactosyl donor **36**, (3 equiv donor, 0.3 equiv TMSOTf, r.t., 1 h, repeated twice). At this stage we investigated the cleavage using Grubbs catalyst **37**, which was chosen for its optimal combination of reactivity and functional group tolerance.

Two repeated exposures of resin **38** to the catalyst (9% mol) for 18 h in dichloromethane at room temperature afforded the expected allyl lactoside in an encouraging isolated yield of 81% from resin **35** (90% per step). Traces of dimerized compounds resulting from cross-metathesis were detected as the only side products. Extension of the oligosaccharide chain was subsequently performed first by deacetylation (excess NaOMe in 4/1: CH_2Cl_2/MeOH at r.t.) and glycosylation with known lactosyl donor **40** in conditions similar to those mentioned above. Cleavage was performed twice as described above, but with a reduced reaction time of 6 h; in this case tetrasaccharide **42** was isolated in 51% yield from **35** (84% per step). No dimerized products were detected.

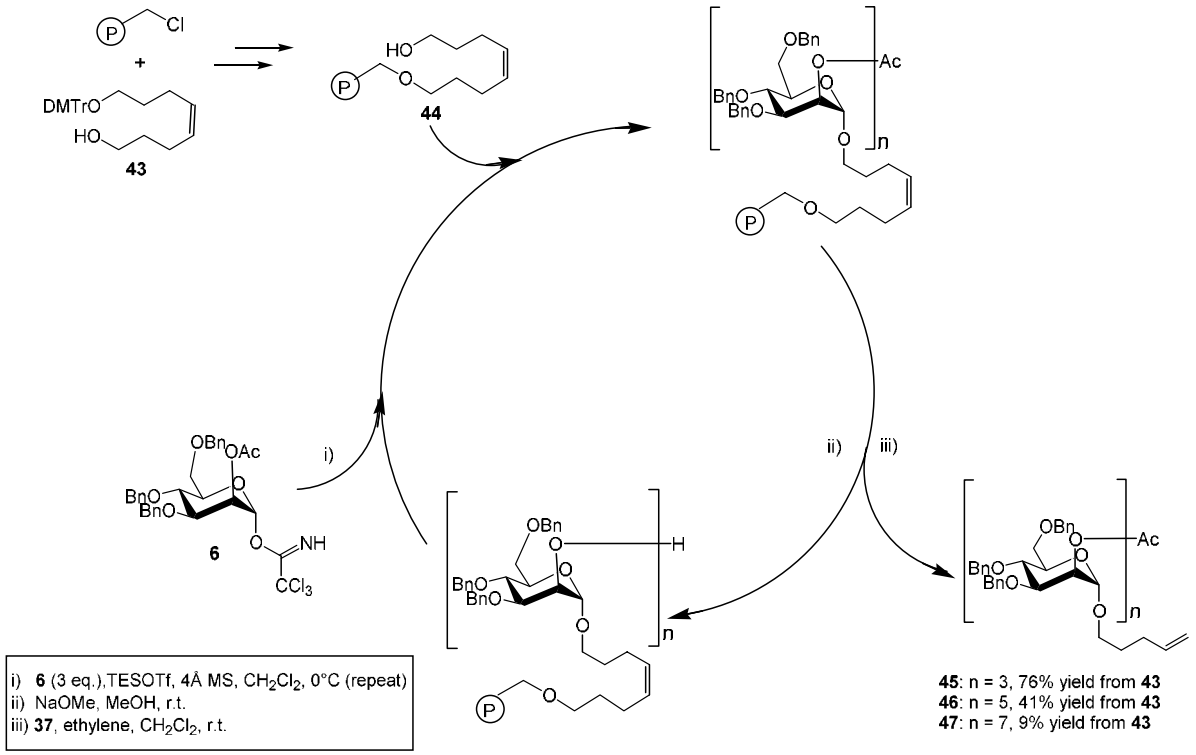
From the analytical point of view, reactions were efficiently monitored using a combination of FTIR (Fourier transform infrared), TLC (thin-layer chromatography), and MALDI-TOF mass spectroscopy analysis of crudes resulting from the cleavage of small resin samples.

A report[8] by the Seeberger group introduced a cross-metathesis-based linker (Scheme 4.10).

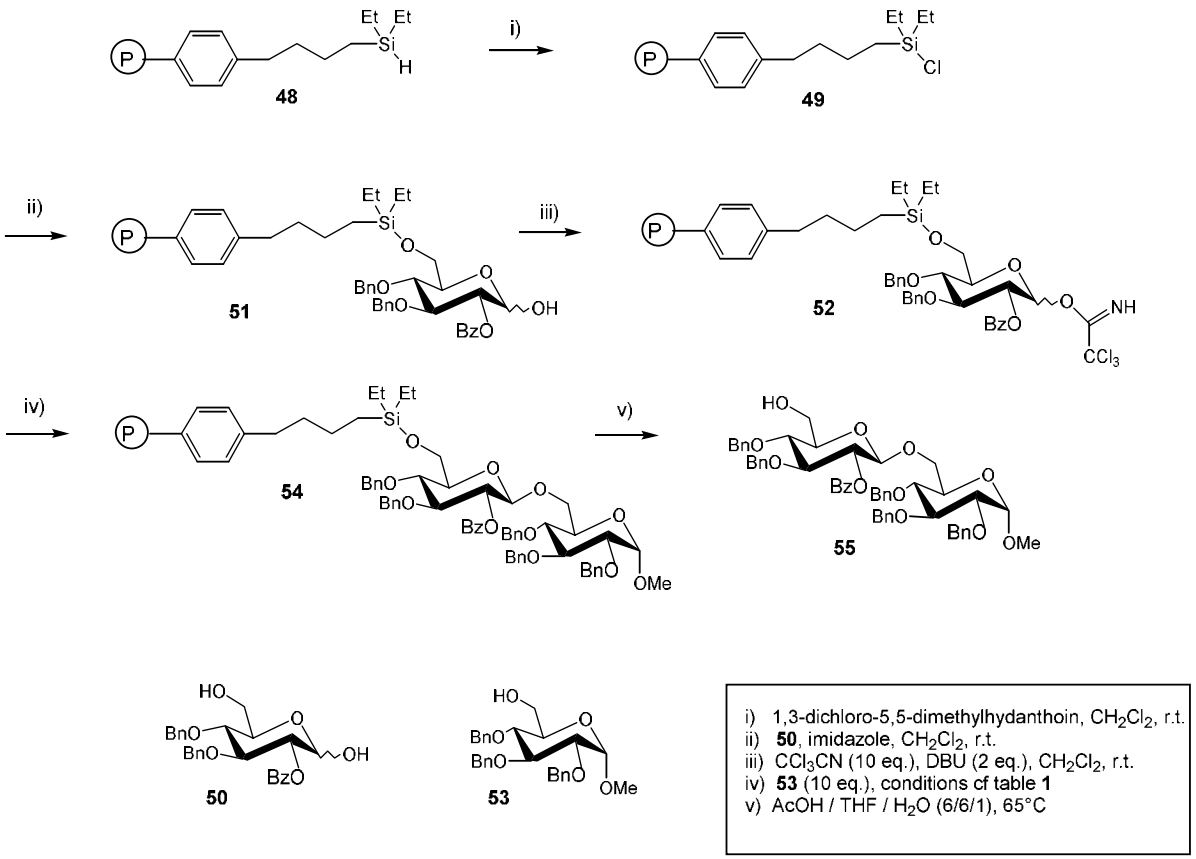
Monoprotected diol **43** was synthesized in solution and linked to the Merrifield resin using simple ether synthesis conditions. A subsequent capping step of unreacted methyl chloride was then performed (NaOMe, MeOH). Deprotection of the monomethoxytrityl group was performed in mild acidic conditions. By using a colorimetric assay, the authors were able to determine the loading of the resin during this deprotection step; loading was calculated to be in the range of 0.45–0.55 mmol/g. With resin **44** as first acceptor, these contributors then performed the synthesis of α-(1$\rightarrow$2)-linked oligomannoside using donor **6** and a two-step deacetylation/glycosylation cycle. Here again, stereoselective formation of the new glycosidic



Scheme 4.9 A ring-closing metathesis-based linker.



Scheme 4.10 A cross-metathesis-based linker.



Scheme 4.11 Application of the traceless silyl linker strategy.

bonds were ensured by neighboring group participation. However, in order to optimize the glycosylation conditions, parallel syntheses were performed up to the trisaccharide stage. After cleavage, using 20% mol of Grubbs catalyst **37** under an atmosphere of ethylene for 36 h, the 1-*O*-pentenyl trisaccharide **45** was obtained in 76% isolated yield. To this end, glycosylation steps were performed twice using 3 equiv of donor **6** and 0.15 equiv of TESOTf at 0°C for one hour. The use of TMSOTf as activator, under the same conditions but at room temperature led to compound **45** in a slightly lower yield of 71%. Further extension of the chain led to pentasaccharide **46** and heptasaccharide **47,** respectively, in 41% and 9% yields (stepwise yield of 84–95%). Deacetylations were achieved using NaOMe in a mixture of methanol and dichloromethane. Monitoring of the glycosylation steps was performed by gravimetric analysis but also FTIR microspectroscopy and high-resolution NMR, which permitted the on-bead study of the stereoselectivity of the process.

To date, heptasaccharide **47** is the largest oligosaccharide assembled on a solid support using only monosaccharide building blocks. This turns out to be an excellent demonstration of the efficiency of the *O*-glycosyl trichloroacetimidate approach.

4.2.5 *O*-Glycosyl Trichloroacetimidates Attached to the Solid Support via Silyl Linkers

To our knowledge, only one attempt has been made toward the preparation and use of a fixed glucosyl trichloroacetimidate.[9] Takahashi et al. described the synthesis of an attached *O*-glucosyl trichloroacetimidate and extended the use of the well known traceless silyl linker strategy (Scheme 4.11).

Starting from the commercial silyl resin **48** (0.75 mmol/g) and after chlorination, diol **50** was regioselectively fixed through the C6 position via a silylation reaction in the presence of imidazole in dichloromethane. The corresponding trichloroacetimidate derivative was obtained by treatment with 10 equiv of trichloroacetonitrile and 2 equiv of DBU in dichloromethane at r.t.. Preparative cleavage of a resin sample indicated a loading of 30% at this stage. Glycosylation was then studied using glucose acceptor **53** in the presence of different Lewis acids, with or without addition of base (2,6-di-*tert*-butyl pyridine) and at r.t. for 12 h (see also Table 4.1). Acidic cleavage was performed thereafter ($AcOH/THF/H_2O$: 6/6/1, 65°C, 10 h).

The yields reported correspond to the weight of the crude product **55** after cleavage, and purity was determind by HPLC analysis. This new strategy was not validated by

TABLE 4.1 Glycosylation Results with Donors 52 and 53

Lewis Acid	DTBP	Yield (%) of **55**	Purity (%)
TfOH	Yes	95	69
TfOH	No	47	76
$BF_3{\cdot}OEt_2$	Yes	70	70
$BF_3{\cdot}OEt_2$	No	59	50

other results until the time of writing, but it offers another possible way to use trichloroacetimidate donors.

4.3 CONTROLLED-PORE GLASS AS A SOLID SUPPORT

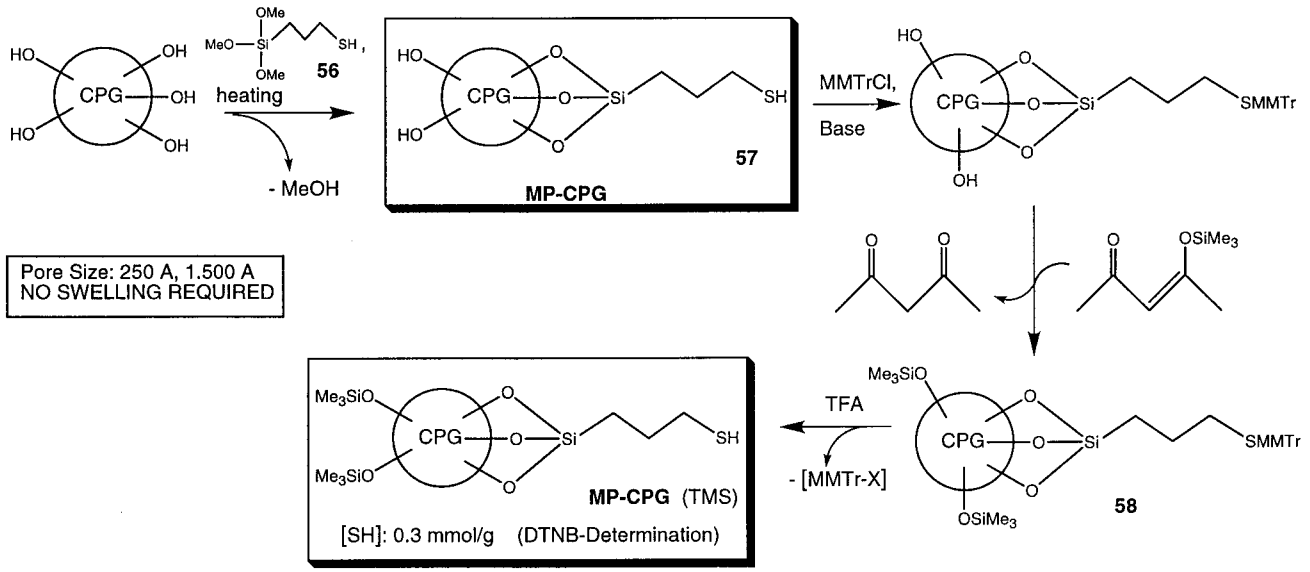
Although popular for the solid-phase synthesis of oligonucleotides, the controlled-pore glass (CPG) type support has not attracted much interest for the solid-phase synthesis of oligosaccharides. However, its compatibility with polar solvents and the absence of limitations due to solvent swelling requirements should be one advantage in this field. The main achievement in the use of CPG support for the solid phase synthesis of oligosaccharides using glycosyl trichloroacetimidates has been reported by our group in 1998 (Scheme 4.12).[15]

This work was based on the adaptation of the thiol linker strategy established on Merrifield resin (Section 4.2.1.). Solution-phase preparation of thiol **56** was followed by its successful attachment on CPG under standard conditions affording mercaptopropyl CPG support **57**. Protection of the thiol function and subsequent necessary capping of the unreacted free silanol groups on the support by silanization yielded fully protected support **58**. Monomethoxytrityl was removed using TFA and this deprotection step permitted the calculation of the free thiol group loading of the support (0.3 mmol/g) by a colorimetric assay. To test the mercaptopropyl CPG support, the synthesis of α-(1→2)-oligomannan was chosen as a standard example (Scheme 4.13).

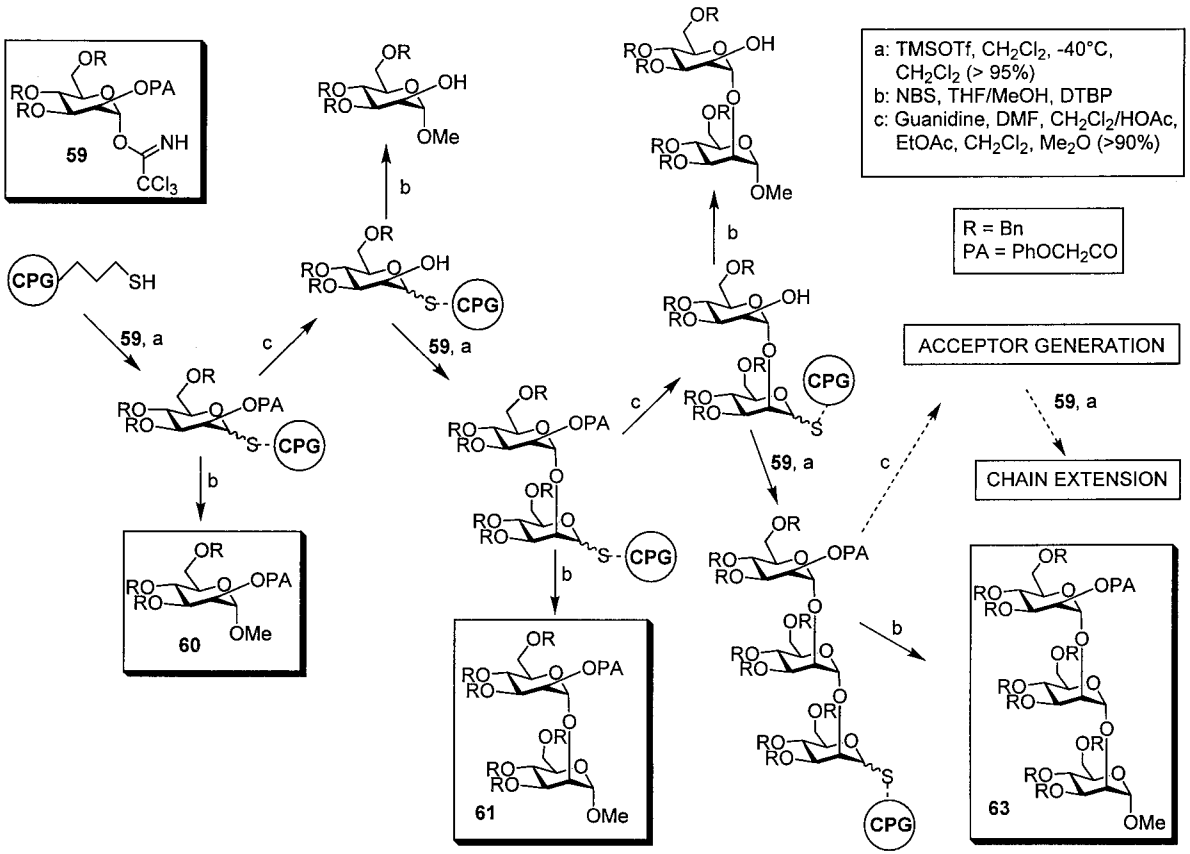
Adaption to the CPG support implicated the preparation of donor **59** possessing a phenoxyacetyl group as temporary protecting group, which is easily cleaved on the solid support under mild conditions (guanidine in DMF). Concerning the glycosylations, conditions used during the Merrifield approach were adapted by using a lower temperature (–40°C). The same cleavage conditions as for the Merrifield support were successfully applied on CPG. Solid-phase synthesis was performed until the trisaccharide stage; cleavages were performed after each glycosylation step, affording monosaccharide **60**, disaccharide **61,** and trisaccharide **62**. MALDI-TOF and TLC analyses were performed from cleavage of small support samples to monitor the reactions.

Iadonisi's group reported the preparation of an amino CPG support (30–35 μmol NH_2/g) of β-(1→6)-linked glucose dimer (Scheme 4.14).[16]

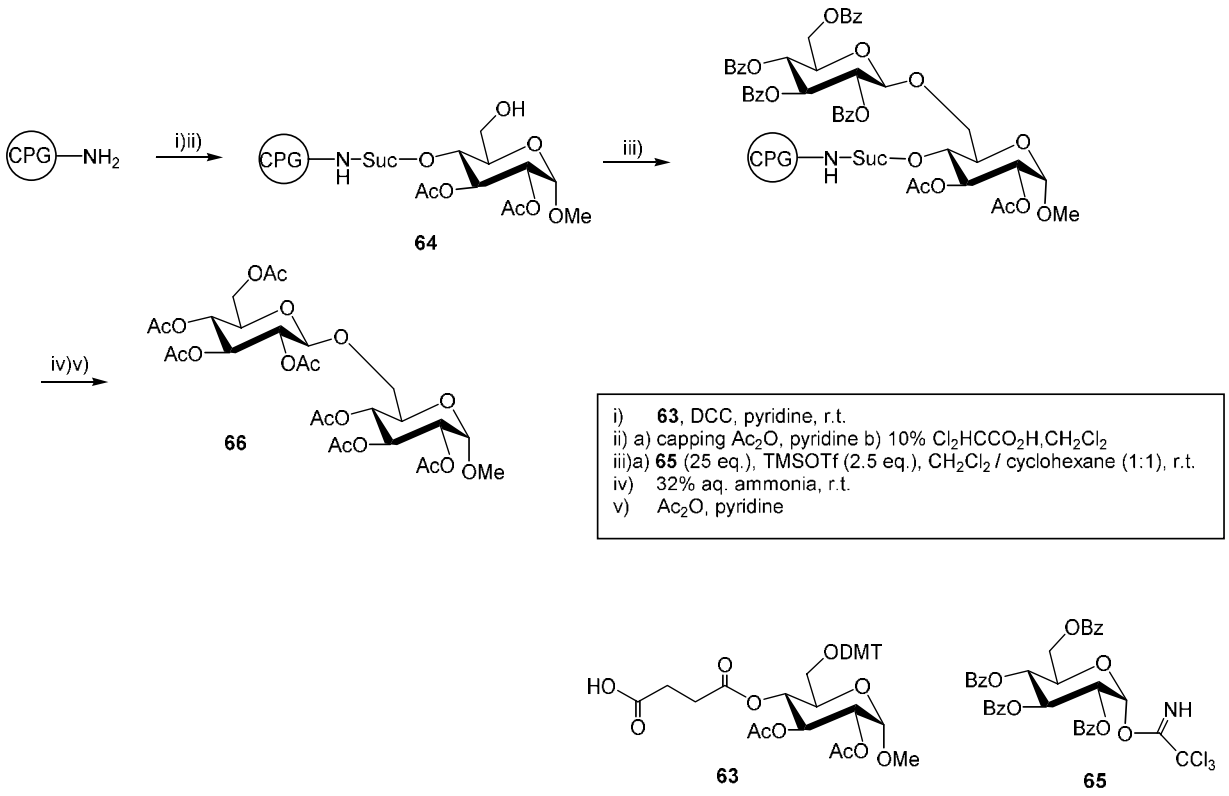
The first glucose unit was attached to the support by a succinate linker via the C3 position. Glycosylation was performed using peracetylated or perbenzoylated *O*-glucosyl trichloroacetimidate (i.e., **65**). Optimal conditions involve 25 equiv of perbenzoylated donor **65** in the presence of 2.5 equiv of TMSOTf as activator in 1/1 dichloromethane/cyclohexane for 3 h. Disaccharide was cleaved using aqueous ammonia and then peracetylated for purification to yield 95% of **66**. Adopting similar conditions, the same group reported the glucosylation of a fully protected oligonucleotide on amino CPG and the one-step deprotection with concomitant cleavage of the condensation product; glycosylation was calculated to have proceeded with 71% yield.[17]



Scheme 4.12 Application of CPG support in solid-phase oligosaccharide synthesis using glycosyl trichloroacetimidates.



Scheme 4.13 Synthesis of α-(1$\rightarrow$2)-oligomannan.



Scheme 4.14 Preparation of an amino CPG support of β-(1→6)-linked glucose dimer.

4.4 SOLUBLE POLYMERS AS SUPPORTS

4.4.1 MPEG as Support

The use of polyethyleneglycol (PEG) derivatives as support for the solid-phase synthesis of oligosaccharides has been reported by Krepinsky et al. as early as 1991.[10] In this report, these authors presented a synthesis of lactosamine from an immobilized glucosamine acceptor and peracetylated galactosyl donor in 70%. To our knowledge, this was the first example of a glycosidic bond formation using a polymer-supported acceptor and a trichloroacetimidate donor in solution. The support employed was polyethyleneglycol monomethyl ether (MPEG; average molecular weight = 5000). The acceptor was attached to the support via a succinate linkage and was cleaved by hydrazinolysis.

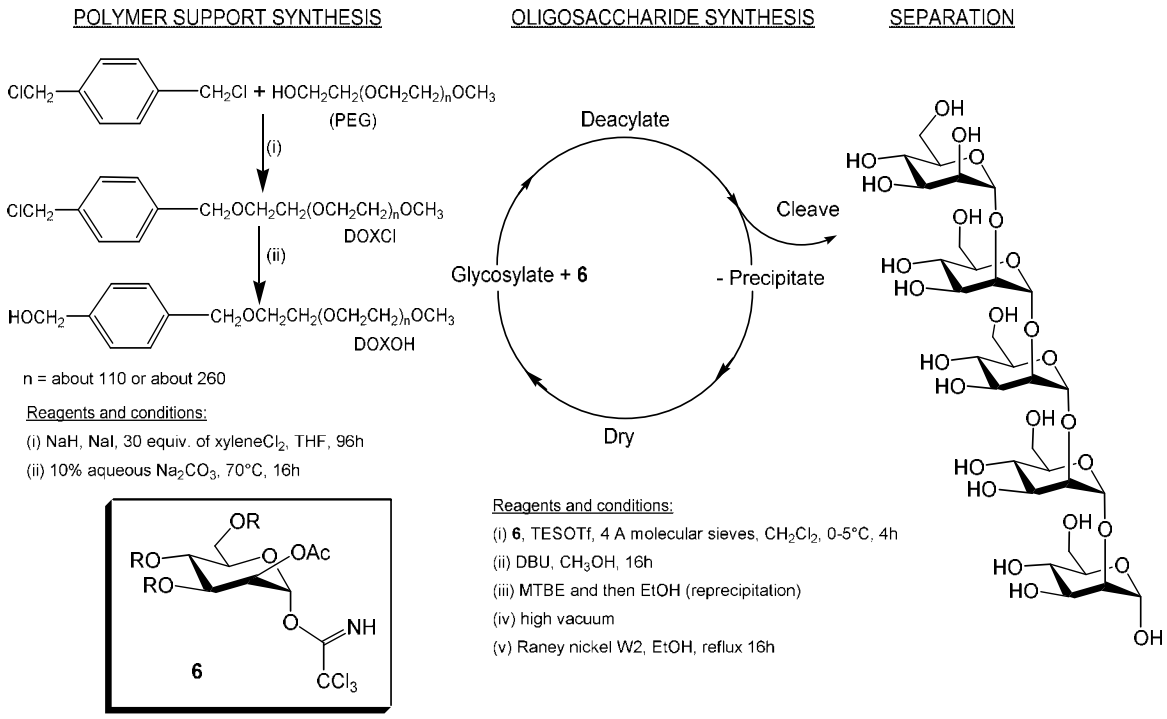
This strategy appears to be very attractive because of the possibility of completely solubilizing the support in most of the common solvents. From a chemical perspective, that property allows one to benefit from all the solvent conditions used in classical solution chemistry. This could prove to be very advantageous, especially to obtain stereoselective glycosylation without neighboring-group assistance. Moreover, isolation and purification of the polymer is easily achieved by precipitation usually in diethyl ether or methyl-*tert*-butyl ether (MTBE) and recrystallisation from ethanol. One major drawback of this type of support is its tendency to solidify at low temperature, thus limiting the variety of temperature conditions.

In a second publication,[11] these authors improved their approach by designing a new linker (Scheme 4.15) synthesized from MPEG. The new support, MPEG-DOX (DOX = α,α′-dioxyxylyl), was prepared as MPEG-DOX-Cl in one ether synthesis step using α,α′-dichloro-*p*-xylene, or as MPEG-DOX-OH by hydrolysis from the latter. Compared with the succinate linker, the authors claimed a greater stability and the possibility of cleaving the target oligosaccharide by controlled hydrogenolysis as a free OH or as a *p*-tolylymethyl derivative. With this new linker strategy in hand, the synthesis of D-mannopentaose from MPEG-DOX-OH using donor **6** was performed and a reiterative sequence presented in Scheme 4.15.

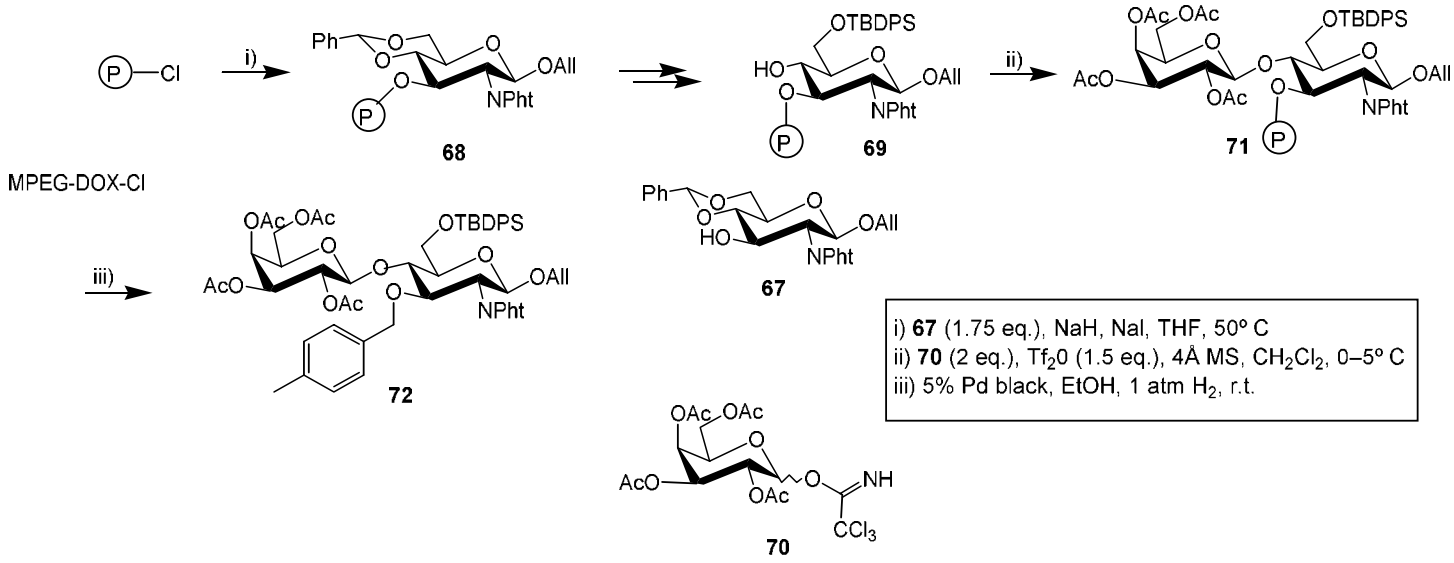
The final compound was isolated after peracetylation; no trace of β-anomer was formed during the process. As in their first report, these authors also investigated the MPEG-DOX linkage of the first sugar residue via hydroxyl groups other than the anomeric one (Scheme 4.16).

Thus, synthesis of lactosamine **72** was reported using the new linker system and an 1-*O*-allyl glucosamine building block **68**. Finally, the controlled hydrogenolysis concept was applied to the synthesis of lactose derivatives (Scheme 4.17).

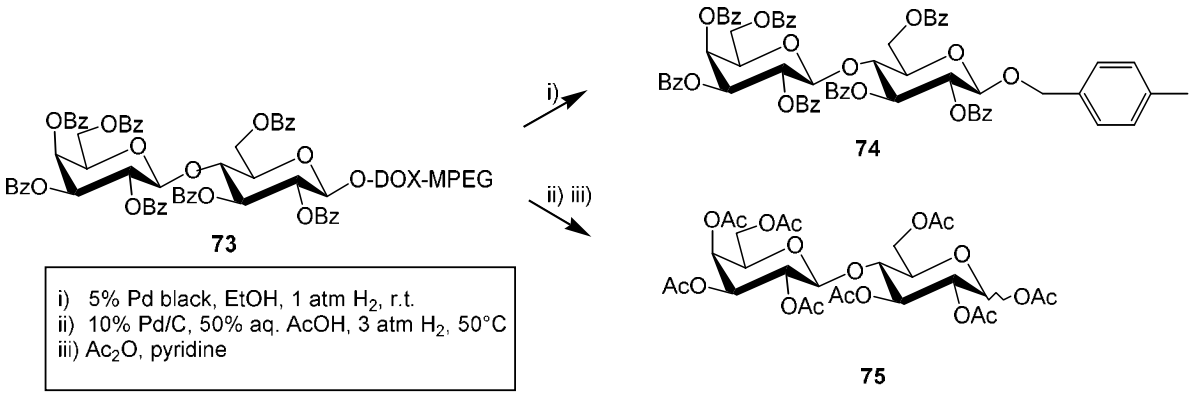
Using 5% Pd black, 1 atm H_2 in ethanol at r.t. for 48 h, *p*-tolylmethyl derivative **74** was selectively obtained. Under stronger conditions (10% Pd/C, 50% aqueous AcOH, 3 atm H_2 at 50°C) MPEG-DOX was completely cleaved to release, after acetylation, the derivative **75**. This selective cleavage was reproduced using different 2-*O*-protected or 2-*O*-glycosylated hexopyranoses.



Scheme 4.15 Synthesis of D-mannopentaose from MPEG-DOX-OH using donor **6**.



Scheme 4.16 MPEG-DOX linkage of the first sugar residue via nonanomeric hydroxyl groups.



Scheme 4.17 Application of the controlled hydrogenolysis concept in lactose derivative synthesis.

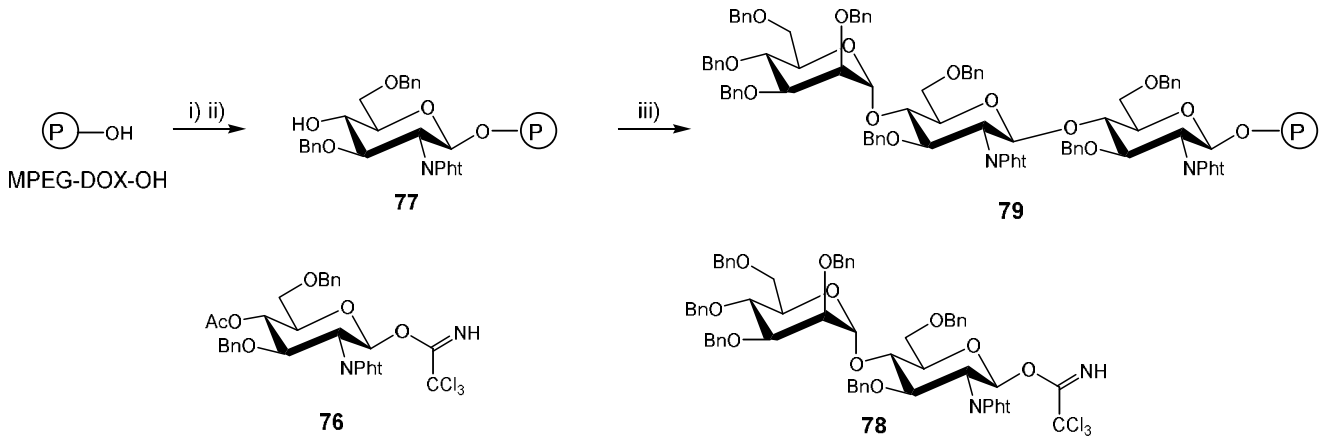
Studying the influence of the activator and using the MPEG-DOX-OH support (Scheme 4.18),[12] the same group reported the synthesis of trisaccharide **79** using donors **76** and **78**; both glycosylations were performed by action of 3 equiv of donor, in the presence of 0.1 equiv of dibutylboron triflate (DBBOTf) as activator and 4-Å molecular sieves at –45°C for 30 min.

Glycosylation of MPEG-DOX-OH with **76** was achieved in 95% yield. After deacetylation using DBU in methanol, glycosylation using disaccharide donor **78** was performed and resin-linked trisaccharide was obtained in 85% yield.

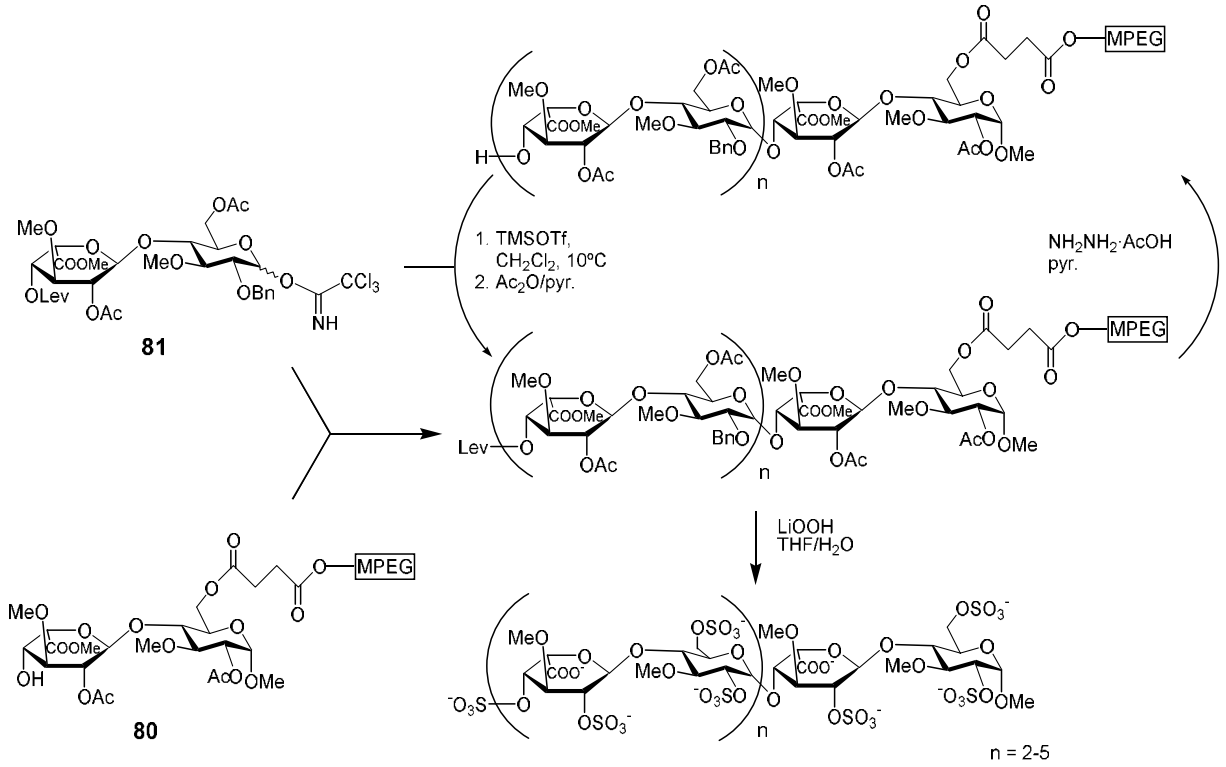
Dreef-Tromp et al. described the synthesis of *O*-methylated heparan sulfate–like oligomers up to the 12 mer on MPEG (average molecular weight = 5000) (Scheme 4.19).[13] In a first stage, synthesis of the fully protected support bound oligosaccharides was achieved by reiterative delevulinoylation–glycosylation–capping elongation cycles. These syntheses started from resin-bound acceptor **80** and were based on the use of donor **81**. The simple, base-sensitive succinate linker that was employed prompted the authors to use the levulinoyl moiety as temporary protecting group. Deprotection was easily performed under mildly basic conditions, which were compatible with the linker and the acetates used for capping, by action of hydrazinium acetate in pyridine. The glycosylations were optimized by performing the reaction at 10°C and by using 2.5 equiv of donor, 0.45 equiv of TMSOTf as activator, and 4 Å molecular sieves in dichloromethane. Couplings were found to work with more than 95% efficiency in this case. Unreacted hydroxyl groups were then capped by acetylation before performing the next elongation cycle. The cycles were completed twice only when the glycosylation efficiency proved to be less than 95%. Monitoring was effected by standard ^{1}H NMR spectroscopy. The fully protected oligosaccharides were released from the support with concomitant removal of the acetyl protecting groups by action of lithium hydroperoxide. Subsequent debenzylation, *O*-sulfation, and purification of the target compounds were performed using standard techniques.

4.4.2 Low-Molecular-Weight MPEG as Support

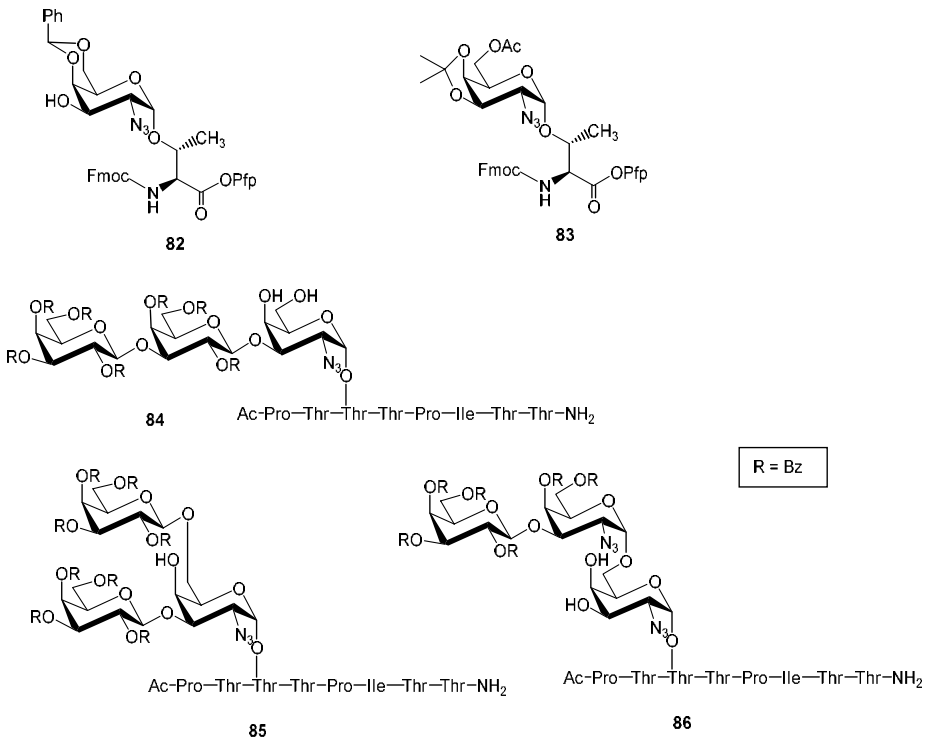
Chan et al. reported the use of a low-molecular-weight poly(ethyleneglycol)-ω-monomethyl ether (MPEG; average molecular weight = 550, n = ~8–20).[14] The advantage of this particular PEG derivative is the possibility of purification by conventional chromatographic methods and to characterize the product by using common NMR techniques. Here also the α-(1→2)-linked tetramannoside was chosen as the target using the abovementioned mannosyl donor **6**. Glycosylation steps were performed using 2.0 equiv of donor in the presence of 0.8 equiv of TMSOTf and 4-Å molecular sieves. The reactions were performed in dichloromethane at room temperature. Deacetylations were achieved using potassium carbonate in wet methanol at r.t. for 4 h. Resin-linked tetrasaccharide was cleaved and debenzylated in one step using standard hydrogenation conditions. The tetrasaccharide was isolated after peracetylation in 10% overall yield.



Scheme 4.18 Glycosylation of MPEG-DOX-OH in trisaccharide synthesis.



Scheme 4.19 Synthesis of *O*-methylated heparan sulfate-like oligomers.



Scheme 4.20 Solid-phase synthesis of various octapeptides.

4.5 OLIGOSACCHARIDE SYNTHESES ON PEPTIDES ATTACHED TO A SOLID SUPPORT

An attempt to synthesize an oligosaccharide chain by the trichloroacetimidate method using a glycosylated peptide as primer has been reported by Paulsen and Bock.[18] The support employed here was a polyethylene glycol dimethylacrylamide copolymer (PEGA) derivatized with the well-known Rink amide as an acid-labile linker. This constitutes a standard system for solid-phase peptide synthesis. These authors performed the solid-phase synthesis of different octapeptides (Scheme 4.20) containing a threonine residue glycosylated with 2-azido-2-deoxy-α-D-galactose unsubstituted in position 3 or 6. To this end, buiding blocks **82** and **83** were employed.

The oligosaccharide chain was subsequently elongated by using different perbenzoylated trichloroacetimidate donors. Glycosylations were performed by using an excess of donor (8–10 mol equiv) in dichloromethane at low temperature (–15 to –30°C) and TMSOTf as catalyst. The reaction time used was 12–24 h. Reaction monitoring was performed by analytical cleavage of small resin samples. Different, linear (**84**) or branched (**85**, **86**) trisaccharide-containing peptides were isolated after TFA-mediated cleavage from the support. Whereas the neighboring-group participation was successfully applied to 2-*O*-benzoylated donors or Teoc-protected 2-amino-2-deoxy donors with encouraging yields, the 2-azido-2-deoxy donors proved to give the expected α linkage, but only in modest yields. In the case of aminosugar donors, it is to be noticed that a change of support from PEGA to polyhipe proved to be favorable. Using this last support, these contributors attempted even a direct glycosylation of a pentapeptide containing a threonine residue with a free hydroxy group. All attempts made with different L-fucosyl and D-xylosyl donors gave no glycosylation product; however, under more forcing conditions, side reactions with ^{t}Bu-protected glutamic acid led to α-glycosidically bound esters.

4.6 CONCLUSIONS AND OUTLOOK

O-Glycosyl trichloroacetimidates exhibited excellent glycosyl donor properties in polymer–supported oligosaccharide syntheses. In particular, linkage to the Merrifield resin as solid support turned out to be successful. As linkers, thioglycosides, 4-alkyloxybenzyl, and 4-acylaminobenzyl glycosides, and, above all, ring closing and cross-metathesis, offered efficient oligosaccharide synthesis on the resin and finally cleavage from the resin. Also, attachment of the first sugar to the resin via anomeric positions as either acceptor or donor was successfully probed.

Even controlled-pore glass (CPG) could be successfully employed as solid support with *O*-glycosyl trichloroacetimidates as glycosyl donors. Thus, limitations of solvents and reaction temperatures in the glycosylation step, as experienced with the Merrifield resin, are restricted to those observed in solution-phase synthesis. Therefore, regio- and stereocontrol of the glycosylation reactions should be available from well-established solution-phase methodologies.

Soluble polyethylene glycol (PEG) as polymer support was also successfully investigated. Precipitation after each reaction step in order to remove excesses of reagents and soluble byproducts seemed to work well. Another topic of great interest is direct glycosylation of solid support connected peptides in order to arrive finally at glycopeptides. *O*-Glycosyl trichloroacetimidates also proved successful in this endeavor.

These results demonstrate that *O*-glycosyl trichloroacetimidate–based oligosaccharide synthesis on solid support may eventually become a valuable alternative to solution-phase synthesis because useful experience is available for the selection of the polymer support and choice of the linker system and the glycosyl donor. Further standardization of the building blocks and the protective group pattern will finally provide the yields and the anomeric control in order to successfully plan automated syntheses of oligosaccharides also in a combinatorial manner.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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5 Synthesis of Oligosaccharides on Solid Support Using Thioglycosides and Pentenyl Glycosides

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5.1 INTRODUCTION

Today, a multitude of methods for formation of glycosidic linkages is available to the synthetic organic chemist. Covered here are solid-phase oligosaccharide syntheses relying on the use of thioglycosides and *n*-pentenyl glycosides as glycosyl donors. They differ from many other glycosyl donors because they are stable under a variety of reaction conditions commonly employed in oligosaccharide synthesis but at the same time can be selectively activated when required. Thus, they serve both as a means of anomeric protection and activation. Activation of thioglycosides and *n*-pentenyl glycosides proceeds on addition of a soft electrophile under mild conditions, which is helpful for the construction of sensitive target molecules. Their chemical stability—especially that of thioglycosides—has been exploited mainly in block syntheses of oligosaccharides in solution where they are activated late in the synthetic sequence. On solid support, the main advantage of the chemical stability of thioglycosides and *n*-pentenyl glycosides is that these glycosidic linkages can be used to anchor the growing oligosaccharide chain on the resin.

5.2 THIOGLYCOSIDES AS GLYCOSYL DONORS

Thioglycosides were the first example of an anomeric derivatization that serves the dual role of protection and activation. They were introduced in glycosylation reactions by Ferrier et al. in 1973, who used mercury(II) salts as activator.[1] After the development of improved methods of their synthesis and activation, thioglycosides together with trichloroacetimidates are now the most commonly used glycosyl

donors.[2–6] Thioglycosides are conveniently obtained by a number of substitution reactions of anomerically activated carbohydrates.[2,4] Examples include thiolysis of glycosyl bromides and chlorides under basic conditions, thiolysis of glycosyl acetates and trichloroacetimidates under Lewis acid catalysis, tin-mediated thioalkylation of glycosyl acetates, and the Lewis acid–catalyzed reaction of glycosyl acetates with trimethylsilyl thioethers. The direct conversion of aldoses with thiols into thioglycosides under conditions normally used with alcohols for Fischer glycosidation is not possible. Under these conditions dithioacetals rather than thioglycosides are obtained.

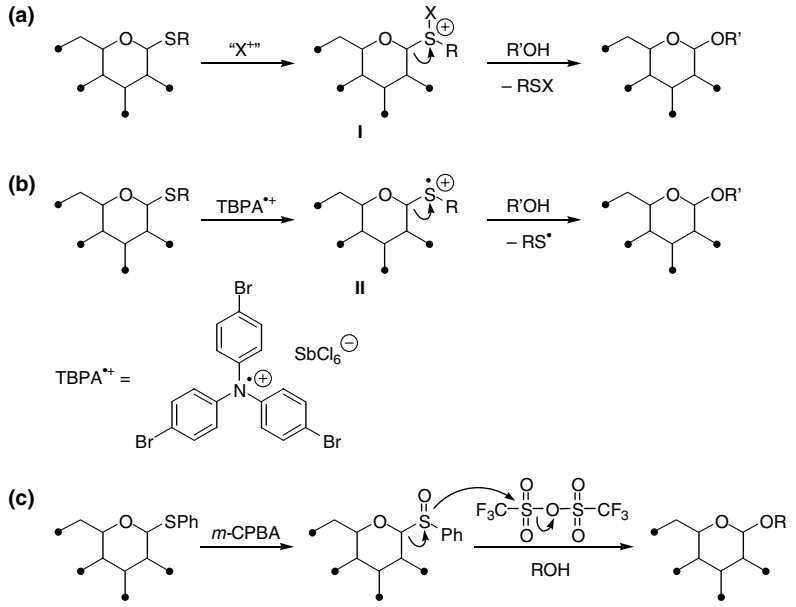
5.2.1 Activation Procedures

A distinct advantage of thioglycosides is the fact that they are stable under a wide range of reaction conditions commonly employed in the synthesis of oligosaccharides whereas at the same time they can be employed for most of the coupling procedures currently in use. Thus, thioglycosides may be introduced at an early stage of a synthesis and later on be converted into a glycosyl donor, making them interesting intermediates in the block synthesis of oligosaccharides.

Thioglycosides readily react with bromine and chlorine to afford glycosyl bromides[7] and glycosyl chlorides,[8] respectively, which can be transformed into *O*-glycosides under promotion by Ag^+, Hg^{2+}, or tetraalkylammonium bromide.[9] Alternatively, the halogenoses may be hydrolyzed to the lactols, which can be further derivatized to give trichloroacetimidates.[10] The Hg^{2+}-promoted conversion of thioglycosides into *O*-glycosides introduced by Ferrier[1] was the first example of the direct use of thioglycosides in *O*-glycosylation reactions. Subsequently, Hanessian et al. demonstrated the use of the 2-pyridylthio group.[11] The application of heavy-metal salts is, however, of limited utility since they suffer from low reactivity and inconvenience. Nicolaou et al.[12] as well as Hanessian et al.[11] used *N*-bromosuccinimide (NBS), but this method also has disadvantages, such as variable reactivity and somewhat poor selectivity.

Recently, a number of highly specific thiophilic activators have been introduced (Scheme 5.1a). Methyl triflate[13] efficiently *S*-alkylates the thioglycoside function to give an anomeric sulfonium triflate. This species (I), or the derived glycosyl triflate, is then substituted by the alcohol. However, methyl triflate is toxic and in the presence of slow-reacting glycosyl donors can give rise to methyl ethers in addition to *O*-glycosides. This can be overcome by the use of the soft electrophile dimethyl(methylthio)sulfonium triflate (DMTST)[14] which methylsulfenylates the thioglycoside and gives fast glycosylations at room temperature or below. A side reaction has been reported in which an acetamidosugar underwent *N*-methylsulfenylation by DMTST.[15] Care must also be taken in the presence of the allyloxycarbonyl protecting group, which is not compatible with DMTST.[16] Other examples of thiophilic electrophiles include phenylselenyl triflate (PhSeOTf),[17,18] *N*-iodosuccinimide-triflic acid (NIS-TfOH),[19,20] and iodonium di-*sym*-collidine perchlorate (IDCP).[21] IDCP is not commercially available, but it is easily prepared by the procedure of Lemieux and Morgan.[22]

Treatment of thioglycosides with *N*-bromosuccinimide (NBS) in the presence of diethylaminosulfur trifluoride (DAST) or HF-pyridine complex furnishes glycosyl fluorides.[23] These can be activated with $AgClO_4/SnCl_2$ in the presence of another



Scheme 5.1 Mechanism of thioglycoside activation (a) by thiophiles "X^+" such as *N*-bromosuccinimide (NBS),[11,12] methyl triflate,[13] dimethyl(methylthio)sulfonium triflate (DMTST),[14] phenylselenyl triflate (PhSeOTf),[17,18] *N*-iodosuccinimide/triflic acid (NIS/TfOH),[19,20] and iodonium di-*sym*-collidine perchlorate (IDCP)[21]; (b) by tris(4-bromophenyl)ammoniumyl hexachloroantimonate ($TBPA^{\bullet+}$)[25]; and (c) via anomeric sulfoxides.[26] The stereochemical outcome of these glycosylations follows the same general trends as with many other glycosyl donor/promoter combinations (*m*-CPBA = *meta*-chloroperbenzoic acid).

thioglycoside, thus it is possible to use thioglycosidic acceptors, a feature that is exploited in the two-stage activation procedure.[23,24]

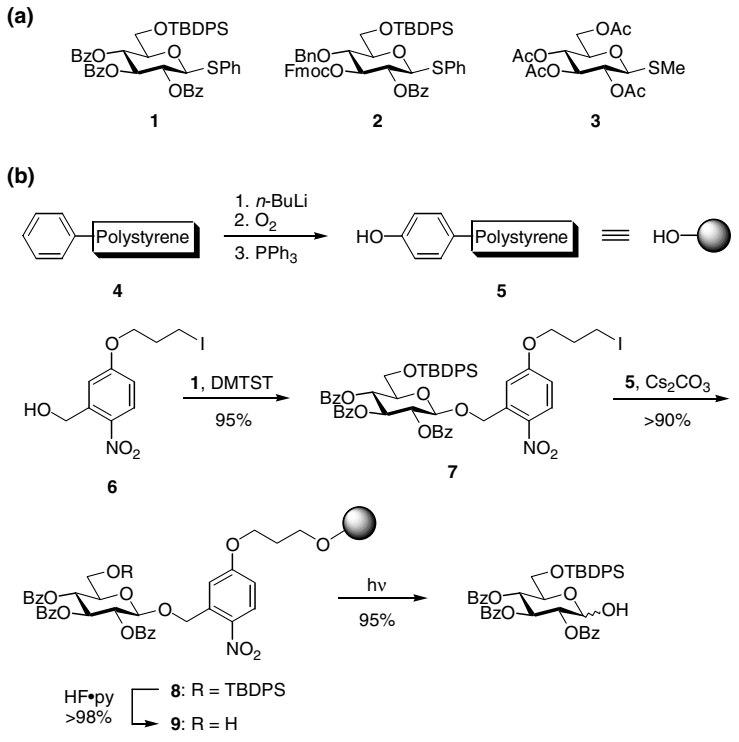
Tris(4-bromophenyl)ammoniumyl hexachloroantimonate ($TBPA^{\bullet+}$) is a rather novel activator for thioglycosides which has been developed by Sinaÿ et al. (Scheme 5.1b).[25] This method involves a single-electron transfer step from sulfur to the radical cation $TBPA^{\bullet+}$, thus generating a glycosyl radical cation II that extruses a thiyl radical on addition of the hydroxylic glycosyl acceptor. Another new method of *O*-glycosylation using thioglycosides has been reported by Kahne et al. (Scheme 5.1c).[26] In this two-step procedure thioglycosides are first oxidized by *meta*-chloroperbenzoic acid (*m*-CPBA) to give anomeric sulfoxides, which are activated by triflic anhydride in a second step. Related to this method is an approach developed by Ley et al. involving oxidation of thioglycosides to the sulfone stage followed by displacement of the sulfonyl group by alcohols in the presence of magnesium bromide etherate and sodium bicarbonate.[27,28]

5.2.2 Applications to Solid-Phase Synthesis

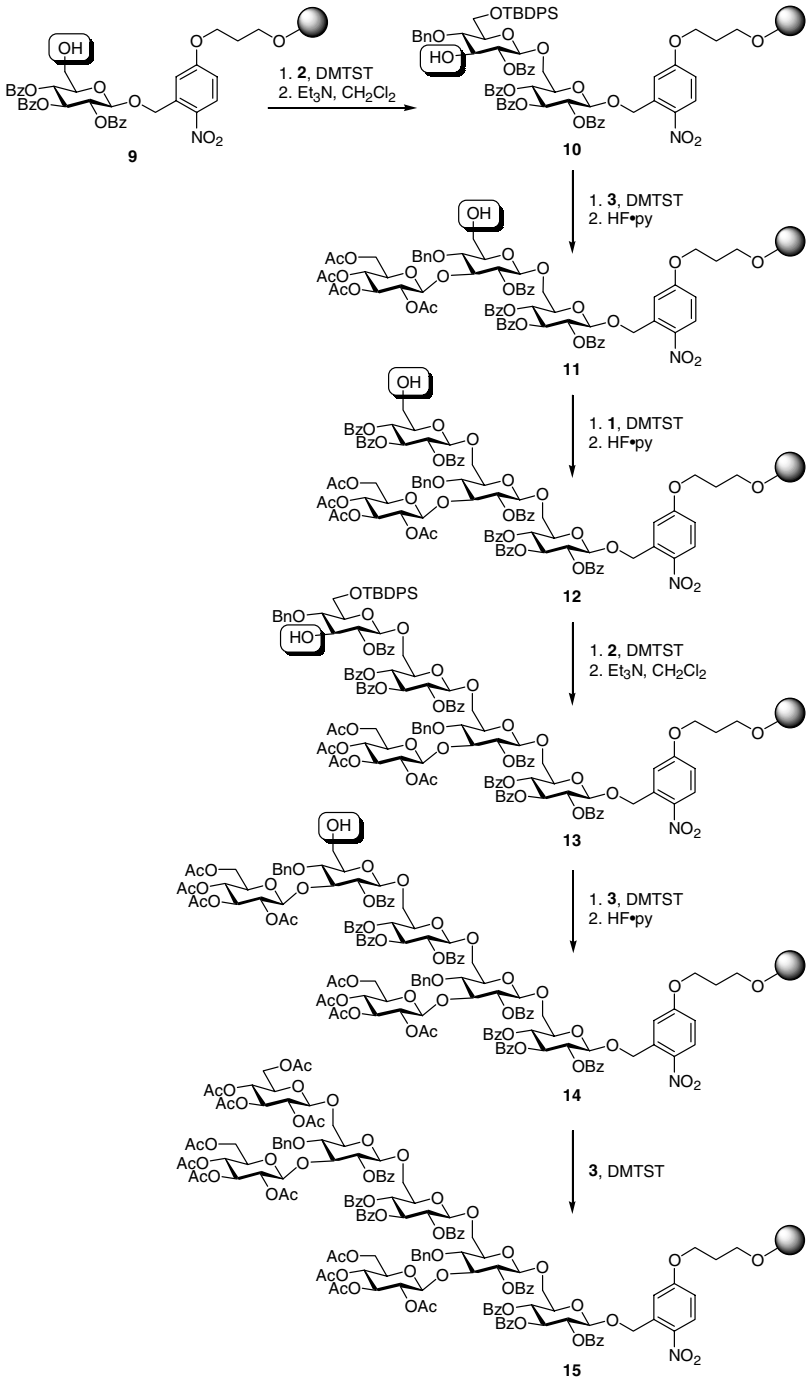
Thioglycosides have been applied in solid-phase oligosaccharide synthesis by several research groups.[29–35] Kahne et al. used them as precursors for the generation of

glycosyl sulfoxides, which are valuable glycosyl donors in solid-phase synthesis because of their high reactivity on activation by triflic anhydride even at low temperatures and their nonpolar character.[36,37] Danishefsky et al. developed a method for the conversion of resin-bound glycals into 2-*O*-pivaloyl thioglycosides.[31] These were subsequently coupled to several hindered glycosyl acceptors to furnish β-(1→2), β-(1→3), β-(1→4), and β-(1→6) linkages. Especially in the case of glucosyl donors, the use of thioglycosides is advantageous over the so far used 1,2-anhydrosugar derivatives. Subsequently, thioglycoside intermediates were also employed by the same group for the transformation of immobilized glycals into β-glycosides of glucosamine.[32] Further examples of solid support syntheses with thioglycoside donors have been reported by the groups of Boons,[33] Kanie and Wong,[34] and Takahashi.[35] Thioglycosides have been also used in glycosylation reactions with soluble polymers[38,39] and for anchoring oligosaccharides on a solid support.[36,37,40–43] Some of this work is described elsewhere in this book in more detail and thus is not covered in this chapter.

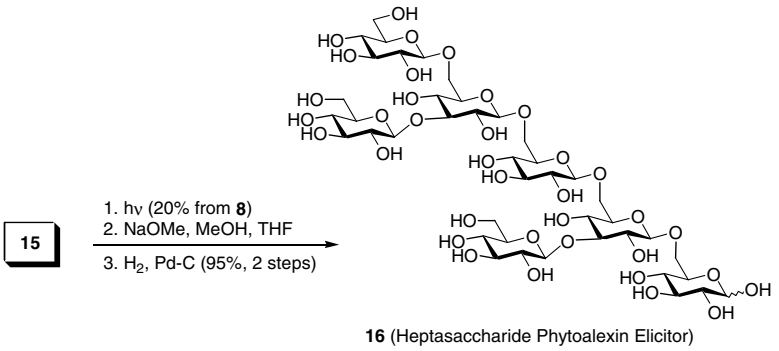
In 1997 Nicolaou et al. published the assembly of a heptasaccharide phytoalexin elicitor (HPE, **16**) on a solid support (Schemes 5.2–5.4).[29] This oligosaccharide



Scheme 5.2 (a) Monosaccharide building blocks **1**–**3** selected for solid phase synthesis of heptasaccharide **16**; (b) attachment of the first carbohydrate onto phenolic polystyrene through a new photocleavable *o*-nitrobenzyl-type linker[29] (Bn = benzyl, Bz = benzoyl, Fmoc = 9-fluoromethyloxycarbonyl, Ph = phenyl, py = pyridine, TBDPS = *t*-butyldiphenylsilyl).



Scheme 5.3 Stepwise assembly of the solid-phase-bound heptasaccharide **15** using thioglycosides as glycosyl donors and DMTST as activator.[29]



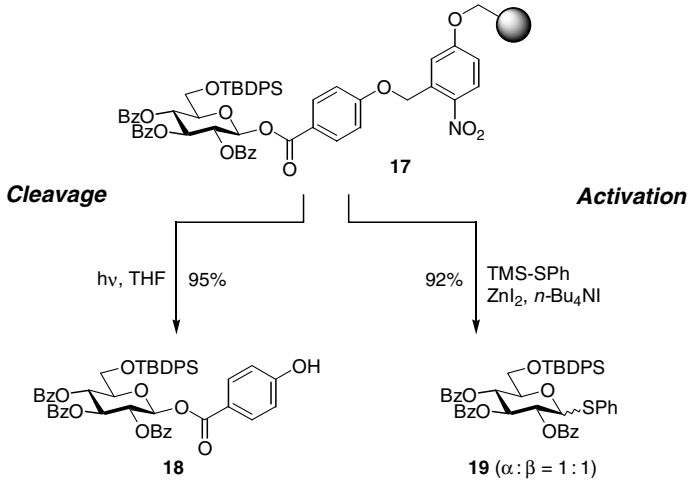
Scheme 5.4 Photolytic cleavage of resin-bound heptasaccharide **15** and deprotection to yield the heptasaccharide phytoalexin elicitor **16**.[29]

consists of seven β-(1→6) and β-(1→3) linked glucose units and has two branch points. Three monosaccharide building blocks were selected for linear chain elongation (**1**), establishing the branch points (**2**), and termination (**3**), respectively (Scheme 5.2). These thioglycosides were activated by DMTST throughout the whole synthesis. Acyl protecting groups at C2 assured stereoselective formation of β-glycosides through neighboring-group participation. For attaching the saccharide to the solid support, a new photolabile linker with improved cleavage properties compared to published linkers[44–46] was constructed. Readily available *o*-nitrobenzyl alcohol **6** was glycosylated with thioglycoside **1** and then coupled to phenolic polystyrene **5**, which had been prepared from polystyrene **4**. Irradiation of **8** liberated the attached monosaccharide in high yield and demonstrated the efficiency of the photocleavage. The assembly of the target oligosaccharide proceeded in a stepwise manner as depicted in Scheme 5.3. HF-pyridine complex was used to remove the silyl protecting groups, whereas the Fmoc group at the 3 position of the branch point building block **2** was selectively cleaved with triethylamine in dichloromethane. The obtained support-bound heptasaccharide **15** was photolytically cleaved from the resin to furnish the protected saccharide with free reducing end in a 20% overall yield from **8** as mixture of α- and β-anomers (Scheme 5.4). The targeted heptasaccharide **16** was finally obtained after deacylation followed by hydrogenolysis.

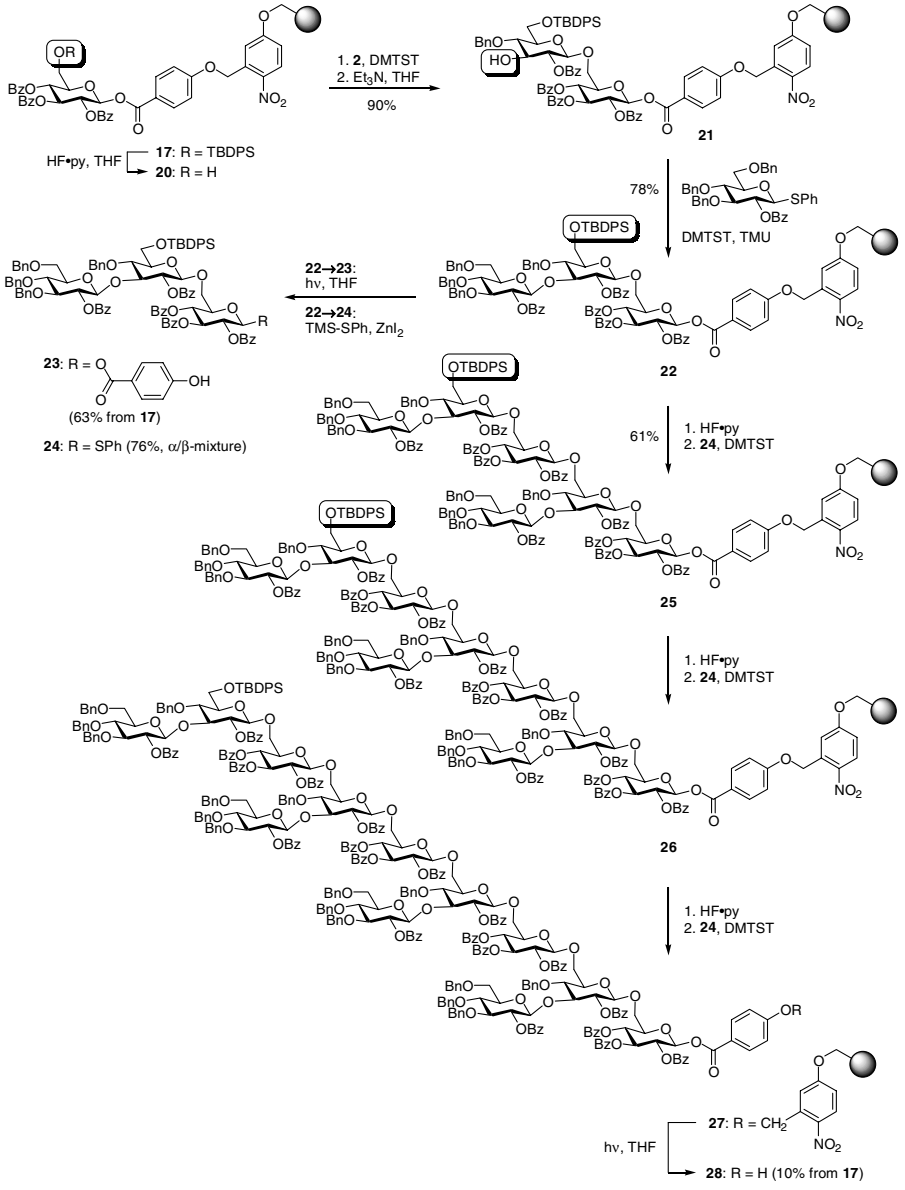
A problem associated with solid-phase synthesis is the characterization of resin-bound reaction products. Although spectroscopic solutions to this problem have been published,[34,47–53] the commonly applied procedure is to cleave the product from a small resin sample and subsequently analyze the product in solution. However, if saccharides with free reducing end are generated in this step, mixtures of anomers are obtained, aggravating the NMR spectroscopic analysis, as was the case for the HPE **16**. Another drawback of the solid support synthesis of **16** is the fact that cleavage products first have to be activated unless they can themselves be coupled to the support-bound growing oligosaccharide chain. With respect to the composition of **16** of repeating trisaccharides, such an approach would be more efficient than the stepwise coupling of monosaccharide building blocks.

With the solid-phase synthesis of a dodecasaccharide, Nicolaou et al. presented a chemical solution to both problems mentioned above.[30] As shown in Scheme 5.5, they incorporated 4-hydroxybenzoic acid as spacer between the photolabile linker and the anomeric position of the first saccharide (**17**). If **17** (or an analogously immobilized larger oligosaccharide) is photolytically cleaved, stereochemically homogeneous β-product **18** (or the analogous oligosaccharide derivative) is obtained, which is ideally suited for analytical investigations. Cleavage from the solid support can also be effected with concomitant activation of the released saccharide under conditions originally described by Hanessian[54] for the cleavage of *O*-glycosides as shown for the conversion **17**→**19**. The thioglycosides thus obtained can readily be used in further (solid-phase) glycosylation reactions.

Scheme 5.6 demonstrates the application of this strategy to the solid-phase block synthesis of the phytoalexin elicitors related dodecasaccharide **28**.[30] Commencing with **17**, two cycles of deprotection and DMTST promoted coupling of a thioglycoside led via **20** and **21** to the resin-bound trisaccharide **22**. Photolysis of a resin sample gave stereochemically pure trisaccharide **23** suitable for NMR spectroscopic analysis. A part of **22** was desilylated with HF-pyridine complex. Another portion of **22** was treated with TMS-SPh, ZnI_2, and *n*-Bu_4NI to give trisaccharide thioglycoside **24**, which was subsequently coupled to deprotected **22** under DMTST activation to furnish resin-bound hexasaccharide **25**. Two more cycles consisting of desilylation and DMTST activated glycosylation with trisaccharide **24** gave, via **26**, solid-phase bound dodecasaccharide **27**. Photolytic cleavage finally yielded the fully protected dodecasaccharide **28** as a single stereoisomer (~10% yield from **17**).



Scheme 5.5 Structure and properties of the photolabile linker **17** used for solid-phase synthesis of dodecasaccharide **28.**[30]



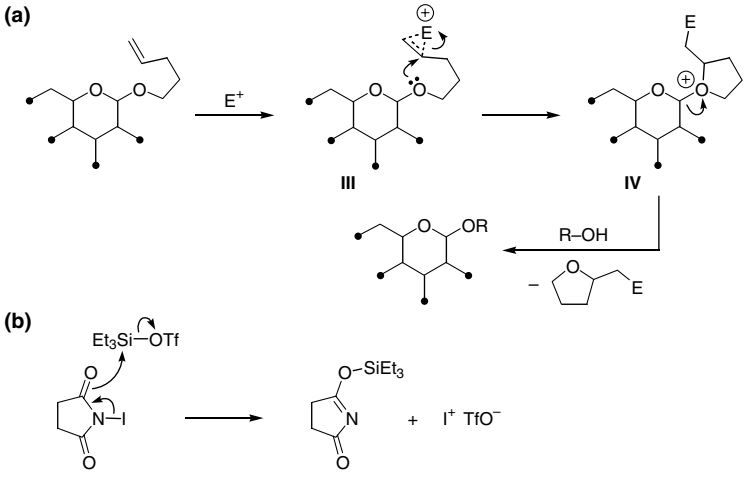
Scheme 5.6 Solid-phase assembly of dodecasaccharide **28**[30] (TMU = tetramethylurea).

5.3 PENTENYL GLYCOSIDES AS GLYCOSYL DONORS

The use of *n*-pentenyl glycosides (NPGs) as glycosyl donors[55,56] was introduced to carbohydrate chemistry in 1988 by Fraser-Reid et al.[57] and was based on the observation of an unexpected side reaction during the synthesis of streptovaricin A. As with the thioglycosides, NPGs can serve both as protection and activation of the anomeric hydroxyl group. Their preparation[55,56] can be carried out by standard procedures for making simple alkyl glycosides. Particularly, it is possible to obtain NPGs from aldoses with 4-pentenyl alcohol and camphorsulfonic acid under slightly modified Fischer glycosidation conditions.[58] However, for some sugars, such as galactose and glucosamine, the Fischer glycosidation gives poor yields. In these instances, NPGs can be prepared from glycosyl bromides or chlorides under Koenigs–Knorr conditions or from glycosyl acetates under Lewis acid catalysis. Alternatively, the synthesis of NPGs via pentenyl 1,2-orthoesters has been described.[59] The advantage of using orthoesters is that several base-promoted protecting group manipulations can be carried out before the acid-induced rearrangement to an NPG occurs, leaving an acyl protecting group at C2. Related to *n*-pentenyl glycosides are glycosyl 4-pentenoates, which have been described by Kunz et al.[60]

5.3.1 Activation Procedures

The activation of NPGs during a glycosylation reaction (Scheme 5.7a) depends on electrophilic addition to the olefin (→III), followed by intramolecular displacement by the anomeric oxygen to form the oxonium species IV. Trapping with a glycosyl

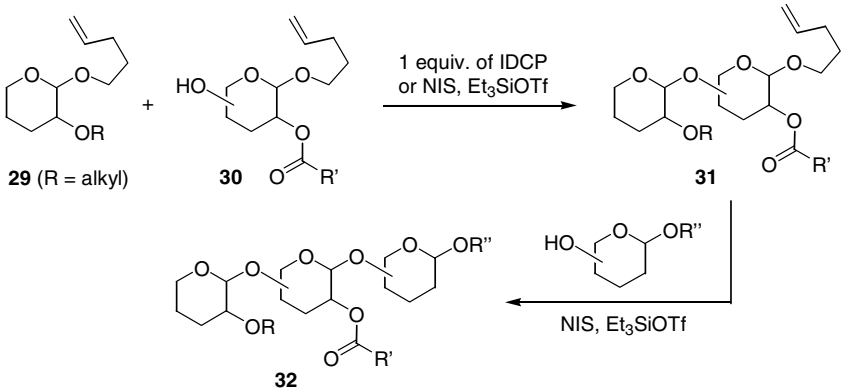


Scheme 5.7 (a) Mechanism of the *n*-pentenyl glycoside–based glycosylation method. Electrophiles commonly employed include iodonium di-*sym*-collidine perchlorate (IDCP) and *N*-iodosuccinimide/triethylsilyl triflate (NIS/Et_3SiOTf); (b) Mechanism for the activation of NIS by Et_3SiOTf.[55]

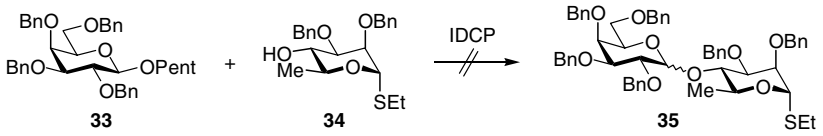
acceptor then leads—in the case of an S_N1-type reaction via a glycosyl cation—to the desired glycoside. *N*-Halosuccinimides like NBS or NIS are widely used sources for halonium ions; when they are used by themselves, the reaction of NPGs is comparatively slow, requiring hours to days to proceed to completion.[56] However, addition of Brønsted or Lewis acids such as triflic acid (TfOH) or triethylsilyl triflate (Et_3SiOTf) significantly accelerates the reaction (Scheme 5.7b). Thus, the promoter of choice for activation of NPGs is NIS/Et_3SiOTf.[61] Under these conditions, glycosylation reactions are often complete within minutes, even when disarmed donors are used.

A promoter of intermediate potency is iodonium di-*sym*-collidine perchlorate (IDCP), which was successfully used for coupling reactive (armed) NPGs.[62] A problem is that some IDCP-promoted reactions tend to stall, leaving substantial amounts of unreacted starting material, an effect probably caused by liberation of collidine during the course of the reaction.

NPGs bearing an alkyl protecting group at C2 react much more readily on electrophilic activation than do NPGs with an 2-*O*-acyl protection. This observation led to the terminology of armed (2-*O*-alkyl) and disarmed (2-*O*-acyl) *n*-pentenyl glycosides; the terms "armed" and "disarmed" were coined by the Fraser-Reid group.[62] It is an example of the well-known principle that acyl-protecting groups depress anomeric reactivity as compared with the less electron-withdrawing ether-protecting groups. However, it was the Fraser-Reid group who demonstrated for the first time that these reactivity differences could be exploited for the chemoselective coupling of two similar glycosyl donors.[63] Thus, activation of a mixture of an armed NPG **29** and a disarmed NPG **30** bearing a free hydroxyl group (Scheme 5.8) affords the cross-coupled disaccharide **31** as the only product. It is worth mentioning that the success of the reaction is independent of the promoter used. Even with NIS/Et_3SiOTf, where coupling occurs within minutes, only the cross-coupled



Scheme 5.8 Armed/disarmed concept for chemoselective coupling of two *n*-pentenyl glycosides (NPGs). A 2-*O*-alkylated (armed) NPG **29** is much more readily activated than is a 2-*O*-acylated (disarmed) NPG **30**.[62]



Scheme 5.9 Attempted cross-coupling of NPG **33** and thioglycoside **34**. Only products arising from self-condensation of **34** have been observed[21] (Pent = *n*-pentenyl).

product is observed. Subsequently, **31** can be used directly for the next coupling event to obtain trisaccharide **32**.

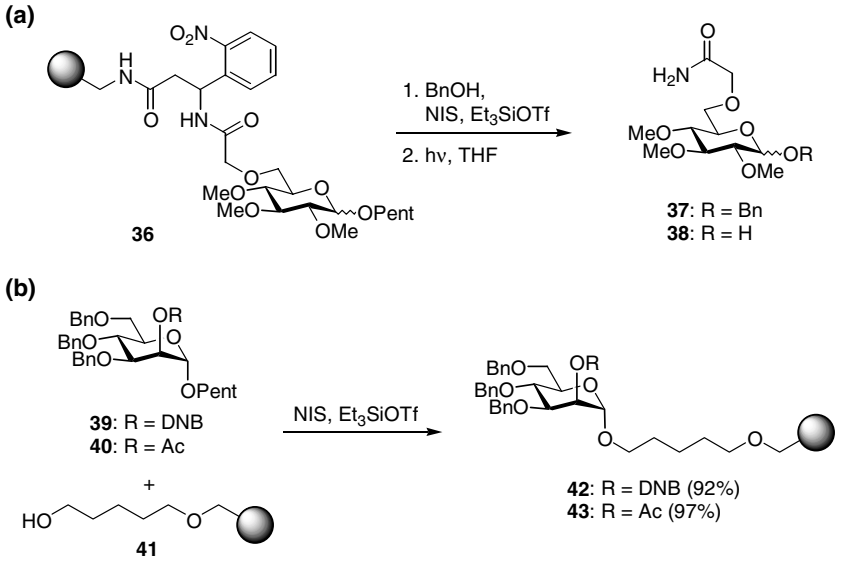
The concept of armed/disarmed glycosyl donors was subsequently extended by other groups to thioglycosides[21] and selenoglycosides.[64] A similar strategy has been used by Friesen and Danishefsky to achieve chemoselectivity in electrophilic addition to glycal double bonds.[65]

Since IDCP and NIS/(Lewis) acid can activate both *n*-pentenyl glycosides and thioglycosides, it is of interest to know which of these two types of glycosyl donors reacts more readily. A first hint can be obtained from an experiment carried out by Veeneman and van Boom (Scheme 5.9).[21] In an attempt to synthesize disaccharide **35**, they added IDCP to a mixture of NPG **33** and thioglycoside **34**, both of which were benzyl-protected. However, analysis of the reaction revealed no formation of the cross-coupled product **35**, but merely products arising from self-condensation of **34**. At least in this case, the halonium ion reacts preferentially with sulfur. However, it cannot be excluded that the observed selectivity is (partially) caused by a long-range inductive effect of the C6 oxygen present in **33** but not in **34**.

5.3.2 Applications to Solid-Phase Synthesis

Almost 10 years after the first publication on NPGs, Fraser-Reid et al. reported their use in the solid phase synthesis of oligosaccharides.[66] Using polystyrene and TentaGel resins, they compared the efficiency of the two possible strategies for polymer-supported oligosaccharide synthesis: the immobilization of either the glycosyl donor or the glycosyl acceptor (Scheme 5.10). Treatment of the glycosyl donor **36**, bound to aminomethyl polystyrene resin via the 3-amino-3-(2-nitrophenyl)propionyl linker,[67] with a large excess of benzyl alcohol in the presence of NIS and Et_3SiOTf, followed by photocleavage of the linker gave some benzyl glycoside **37** beside substantial amounts of hydrolysis product **38** (Scheme 5.10a). Alternatively, pentane-1,5-diol was immobilized onto Merrifield resin to give the polymer-bound glycosyl acceptor **41**. Subsequent glycosylation with the NPGs **39** and **40** furnished the bound glycosides **42** and **43** in excellent yields (Scheme 5.10b).

Thus, the latter approach was clearly preferable and several NPG donors were coupled to **41** under these conditions in order to investigate trends in anomeric selectivity, as monitored by gel-phase ^{13}C NMR spectroscopy (Table 5.1).[66] As expected, neighboring-group active donors **44** and **45** produced solely the



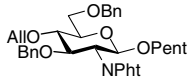
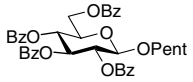
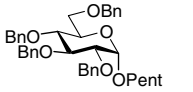
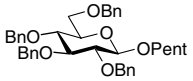
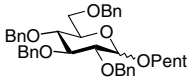
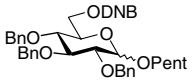
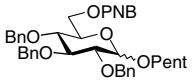
Scheme 5.10 Application of NPGs in solid-phase synthesis; (a) immobilization of the glycosyl donor; (b) immobilization of the glycosyl acceptor.[66]

1,2-*trans* products. Use of NPGs **46** and **47** with nonparticipating C2 substituents shows that in dichloromethane the α/β selectivity is independent of the anomeric configuration of the glycosyl donor but can be influenced by choice of solvent. Donor **48** with the 3,5-dinitrobenzoyl (DNB) protection at C6 gave the highest α:β ratio of 9:1, whereas the mononitro counterpart **49** produced a 4:1 ratio. On the basis of these results, glycosyl donor **48** was enlisted to synthesize in an iterative manner a solid-phase-bound, fully protected derivative of trisaccharide Glcα(1→6)Glcα(1→6)Glc(α-OR) starting from **41**.

The use of benzyl protecting groups in solid-phase synthesis has the disadvantage that these groups cannot be removed by catalytic hydrogenation while the product is still attached to the polymer. Although the reductive removal under Birch conditions represents an alternative solution method, the use of easily cleavable acyl protecting groups would be desirable. However, this approach includes the coupling of disarmed glycosyl donors to deactivated glycosyl acceptors throughout the entire synthesis. Using plastic pins with a polystyrene-crafted surface ("crowns") as insoluble support* in combination with Rich's photolabile linker,[44] Fraser-Reid et al. were able to successfully implement this strategy (Scheme 5.11).[66] Starting with the resin-bound disaccharide **50**, selective dechloroacetylation was achieved by treatment with thiourea, followed by coupling of NPG **51**. The resulting trisaccharide **52** was completely deprotected with ethylenediamine while still resin-bound. For structural

*In contrast to Merrifield resin or TentaGel, this material reacts only on the surface; swelling by the solvent and diffusion of reagents into the material is not required.

TABLE 5.1 Stereoselectivity in Coupling Reaction of 41 to Some *n*-Pentenyl Glycosides[66]

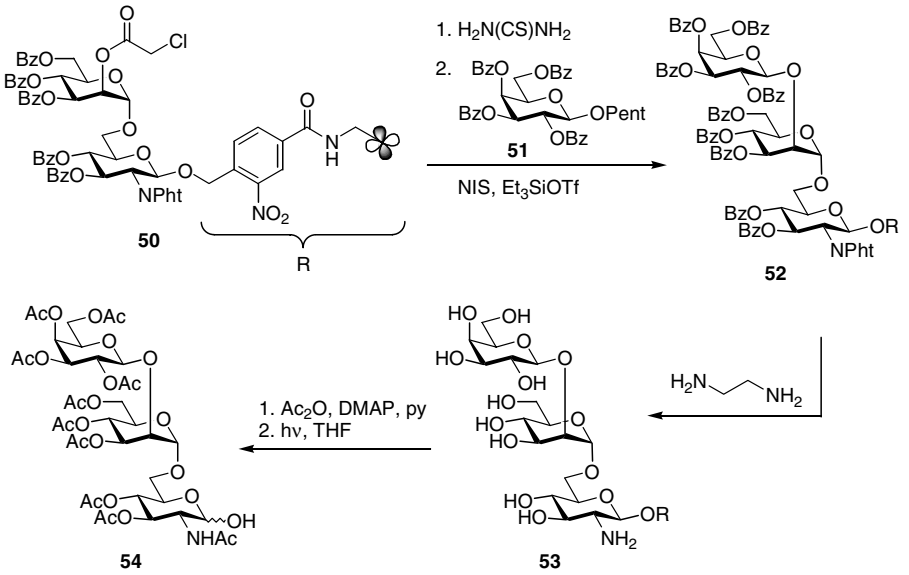
Donor		Solvent	α:β Ratio of Product
	**44**	CH_2Cl_2	Only β
	**45**	CH_2Cl_2	Only β
	**46**	CH_2Cl_2	1:1
	**47**	CH_2Cl_2	1.1:1
	**46/47**	Et_2O:CH_2Cl_2 (11:1)	1.8:1
	**48**	CH_2Cl_2	9:1
	**49**	CH_2Cl_2	4:1

Key: DNB = 3,5-dinitrobenzoyl, Pht = phthaloyl, PNB = 4-nitrobenzoyl.

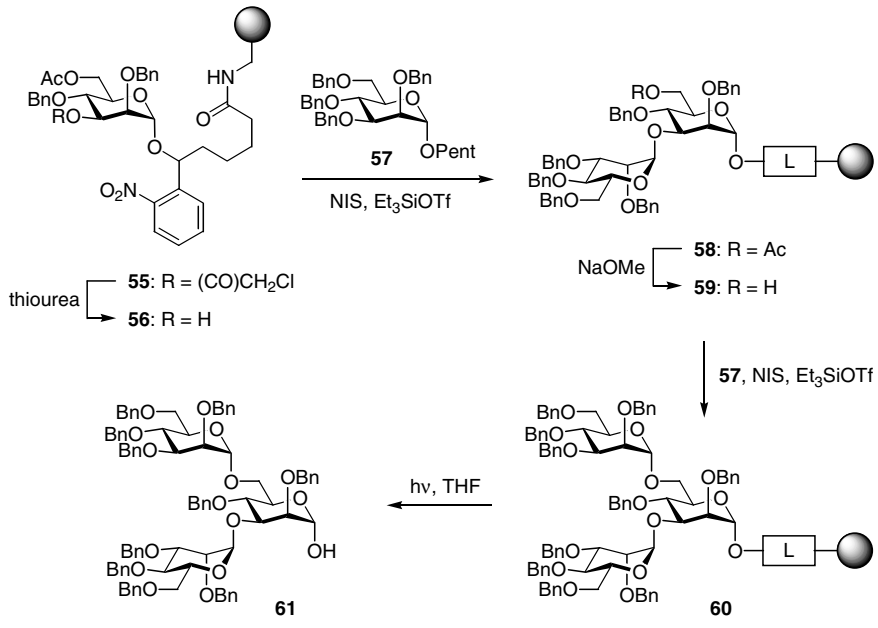
confirmation, **53** was peracetylated and subsequently cleaved from the solid support by irradiation.

Pentenyl glycosides have also been used in the solid-phase synthesis of a branched trimannan employing a new *o*-nitrobenzyl photocleavable linker (Scheme 5.12).[68] This linker was designed to afford improved cleavage kinetics and yields as compared to Rich's linker. Glycosylation of the linker immobilized on an amino functionalized resin furnished **55**, which was selectively dechloroacetylated with thiourea to give **56**. Glycosylation of the hindered C3 hydroxyl group with perbenzylated mannoside **57** yielded disaccharide **58**, which was deacetylated. Further coupling of donor **57** to the primary hydroxyl group of **59** afforded trisaccharide **60**. Irradiation at 365 nm finally gave trimannan **61** in an overall yield of 42% from immobilized linker, representing an average yield of 87% per step.

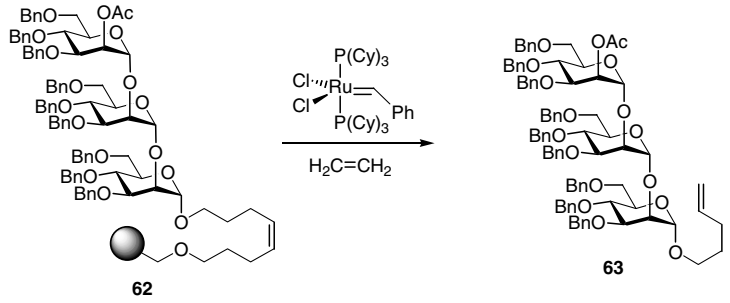
A recent report by Seeberger et al.[69] on a novel octenediol linker is an example of the application of substituted *n*-pentenyl glycosides to anchor oligosaccharides on a solid support (Scheme 5.13). This linker was employed to assemble several oligosaccharides on Merrifield's resin using glycosyl phosphates, thioglycosides, and trichloroacetimidates. Using Schmidt's trichloroacetimidates as glycosyl



Scheme 5.11 Solid-phase synthesis of fully deprotected trisaccharide **53** and subsequent acetylation and photolytic cleavage[66] (DMAP = 4-dimethylaminopyridine).



Scheme 5.12 Solid-phase synthesis of branched trimannan **61.**[68]



Scheme 5.13 Cleavage of an octenediol linker by olefin cross-metathesis to yield NPG **63**.[69]

donors, trisaccharide **62** was synthesized. Cleavage from the resin was accomplished by olefin cross-metathesis by treatment with Grubbs' catalyst[70] under an atmosphere of ethylene to yield fully protected NPG **63** in an overall yield of 76% based on linker functionalized resin (95% per step). As with Nicolaou's linker **17** (Scheme 5.5), a single stereoisomer is obtained on cleavage, facilitating NMR spectroscopic analysis. Moreover, this derivative itself can be used for further coupling in a (solid-phase) block synthesis; or, alternatively, it can be hydrolyzed under halonium activation to give the reducing oligosaccharide. Using an immobilized monosaccharide as model compound, cleavage of the octenediol linker was also attempted under NIS/Et_3SiOTf activation in the presence of benzyl alcohol. However, under these conditions the desired benzyl glycoside was obtained in only low yield, making the metathesis process the method of choice for releasing saccharides from this new linker.

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6 Solid-Phase Oligosaccharide Synthesis Using Glycosyl Phosphates

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6.1 INTRODUCTION

The importance of oligosaccharides in various cell–cell recognition events is now well appreciated.[1,2] In light of the need for the defined oligosaccharide and glycoconjugate structures, major advances concerning the development of versatile and powerful glycosylating agents have been made.[3] Most notably, the development and application of glycosyl trichloroacetimidates,[4] thioethyl glycosides,[5] glycosyl sulfoxides,[6] fluorides,[7] and *n*-pentenyl glycosides[8] has proved very useful in the installation of various glycosidic linkages both in solution and on solid support.[9] However, eventual shortcomings, such as lengthy syntheses, long reaction times, and the use of toxic activating agents, still fuel efforts directed at the development of new glycosylation reactions. Glycosylating agents should be generally applicable and easily accessible and show high yields and selectivity, while ideally using nontoxic, commercially available activators.

In the biosynthesis of oligosaccharides, glycosyl transferases catalyze the glycosylation of an acceptor using glycosyl phosphates best known in the form of nucleotide diphosphate sugars (e.g., UDP-Glc).[10] A number of glycosyl phosphate syntheses have been developed in pursuit of studying enzymatic transformations.[11] Syntheses of glycosyl phosphates by conversion of free reducing sugars,[12] glycosyl trichloroacetimidates,[13] *n*-pentenyl glycosides,[14] glycosyl phosphites,[15,16] vinyl glycosides,[17] H-phosphonates,[18] 1,2-anhydrosugars,[19] and glycosyl nitrates[20] have been reported. While glycosyl phoshites[15] have enjoyed considerable attention in oligosaccharide synthesis,[21] particularly for sialylations,[15,22] the potential of phosphinates,[23,24] phosphinothioates,[25] and

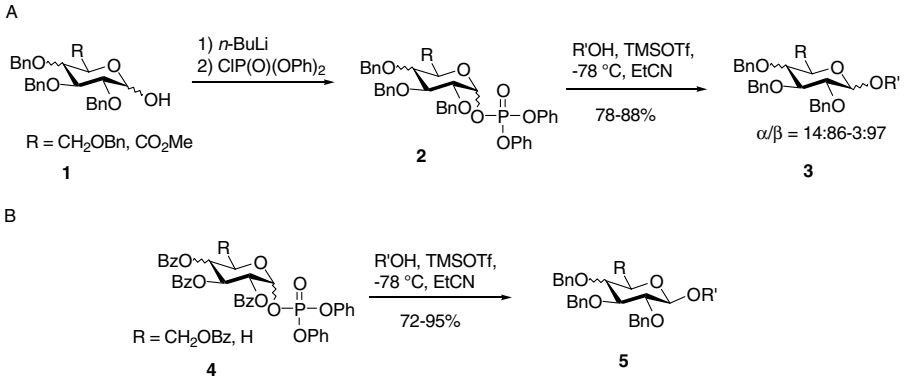
phosphinimidates;[26] or phosphorous(V) compounds as glycosyl donors was not extensively studied until 1999. In this chapter we summarize the most recent developments concerning the synthesis and application of glycosyl phosphates as glycosylating agents.

6.2 GLYCOSYL PHOSPHATE DONORS

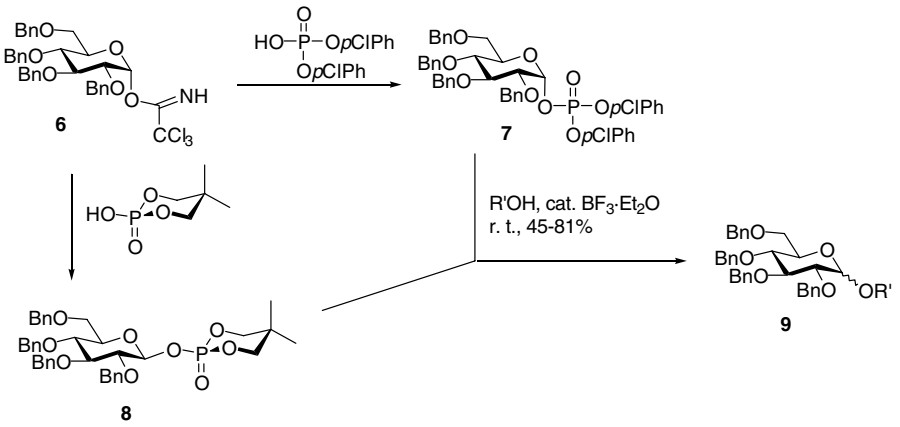
6.2.1 Synthesis and Reactivity

Ikegami and coworkers were the first to address the potential of glycosyl phosphates as glycosyl donors.[23] A series of diphenyl phosphate donors **2** were readily obtained by deprotonating the free reducing sugar with *n*-butyllithium and subsequent treatment with diphenyl phosphorochloridate (Scheme 6.1A). The 2-*O*-benzylated glycosyl donors **2** showed remarkable β-selectivity and high yields in coupling reactions with various glycosyl acceptors. The reactions were carried out in propionitrile in the presence of equimolar amounts of TMSOTf as promoter. Acid-sensitive groups such as epoxides and acetals survived the coupling conditions. Corresponding 2-*O*-benzoyl protected glycosyl diphenyl phosphates **4** exhibited complete β-selectivity with coupling yields up to 95%, including coupling to the hindered C4 position on glucose in over 83% yield.

Although this synthesis preferentially lead to α-phosphates, either anomer could be prepared selectively by direct anomeric phosphorylation of the lactol using diphenyl phosphorochloridate and DMAP choosing thermodynamic or kinetic conditions.[27] Glycosyl α- and β-diphenyl and dialkyl phosphate donors have also been synthesized by nucleophilic displacement of an anomeric trichloroacetimidate. Evaluation of α-diaryl phosphate **7** and cyclic β-phosphate **8** as glycosyl donors under promotion by catalytic amounts of BF_3 etherate in a nonparticipating solvent required ambient temperature to proceed in reasonable yield, although revealing poor stereoselectivities (Scheme 6.2).[28]



Scheme 6.1 Glycosyl phosphates functioning as glycosl donors.

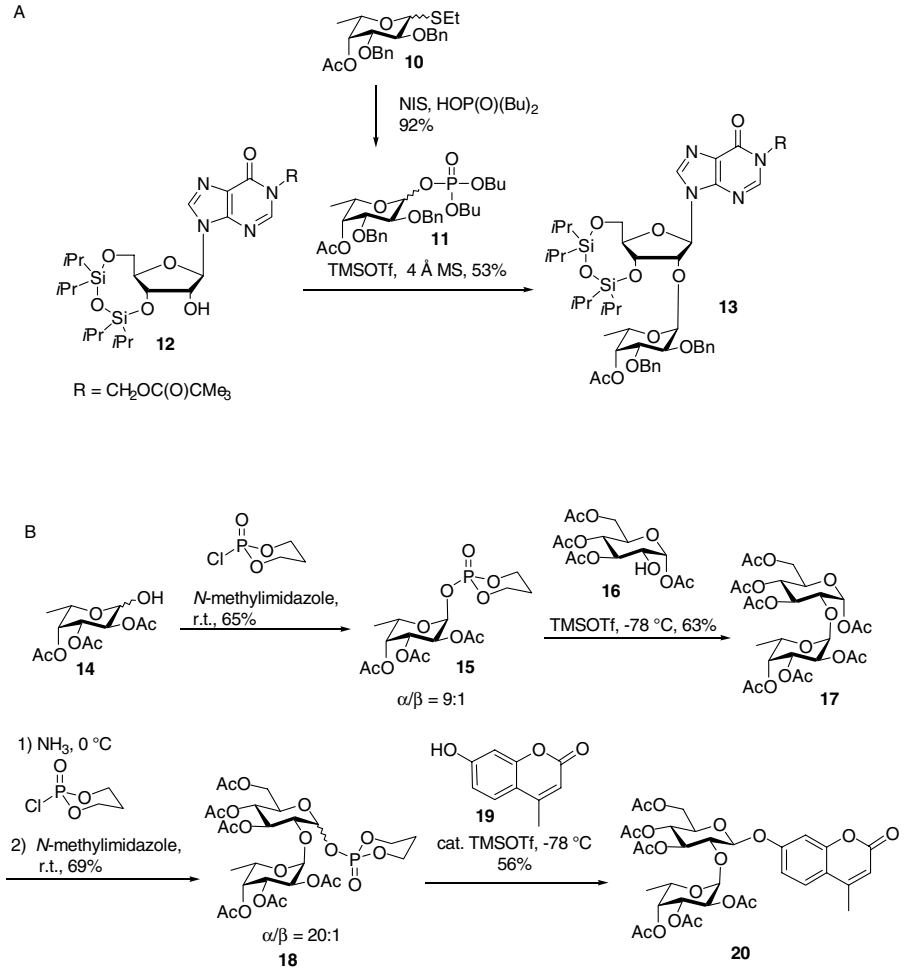


Scheme 6.2 α-Diaryl phosphate and cyclic β-phosphate functioning as glycosyl donors.

When activated by excess or equimolar TMSOTf, glycosyl phosphate donors proved to be very efficient in a few total syntheses of glycosylated compounds. Boger and Honda used a 2-*O*-acetyl mannosyl phosphate for the high-yielding construction of an α-mannosidic linkage in the total synthesis of bleomycin A.[29] α-Fucosidic linkages serve as examples of difficult-to-achieve 1,2-*cis*-glycosidic linkages, which have been installed employing fucosyl phosphate donors in the synthesis of Shimofuridin analog **13** (Scheme 6.3A)[30] and fucosidase substrate **20** (Scheme 6.3B).[31] Interestingly, when fucosylations were carried out in the presence of catalytic amounts of promoter, the coupling proceeded with poor stereoselectivity and in considerably lower yield, indicating either an S_N2-type mechanism or a neighboring-group participation by the 2-*O*-acetyl group.

We have disclosed a straightforward one-pot procedure for the synthesis of glycosyl phosphates from glycals. Diastereoselective epoxidation of suitably protected glycals followed by ring-opening addition of a phosphate diester and in situ protection of the generated free C2-hydroxyl gave access to a wide range of differentiated glucosyl-, and galactosyl phosphates in high yields (Scheme 6.4).[32] Generally, this procedure yielded the more reactive β-phosphates when carried out in noncoordinating solvents such as dichloromethane or toluene. This selectivity, however, could in most cases be effectively reversed by simply switching the solvent to THF as exemplified in the synthesis of **24**. This approach minimizes the number of protecting group manipulations in the preparation of glycosyl phosphates, thus rendering phosphates an attractive alternative to the established donors that often require lengthy syntheses.

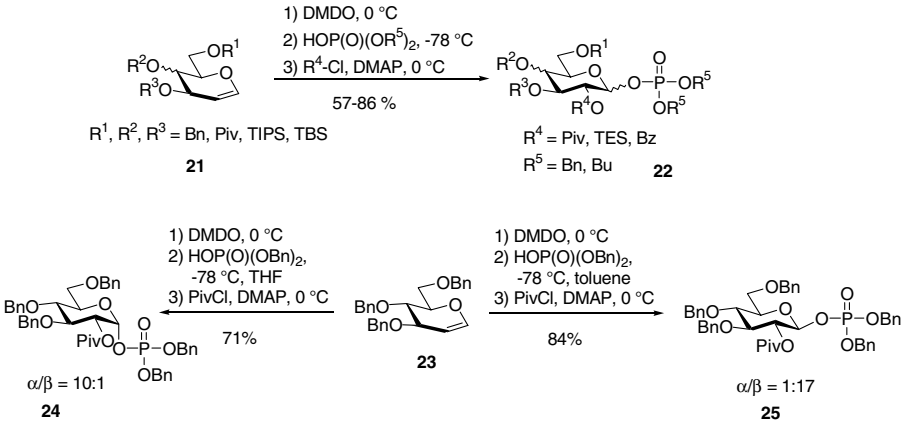
β-Phosphates were smoothly activated by equimolar amounts of TMSOTf at −78°C, while α-phosphates reacted readily at −20°C with various carbohydrate acceptors. Employing these conditions, glycosyl phosphates served as powerful glycosyl donors in the β-selective formation of glycosidic linkages with or without a C2 participating group (Scheme 6.5).



Scheme 6.3 Syntheses of Shimofuridin analog and fucosidase substrate.

The coupling to hindered acceptor sites as in **32** underscores the power of glycosyl phosphate donors in oligosaccharide synthesis. This efficiency was further illustrated in the extremely high-yielding synthesis of the H type II blood group determinant.[33] 2-*O*-benzoyl galactose phosphate **33** was coupled to the hindered C4 position of glucosamine acceptor **32** in 95% yield. Deprotection gave disaccharide acceptor **34**, which was α-fucosylated with complete regio- and stereoselectivity to furnish protected H type II antigen **37** in 93% yield (Scheme 6.6).

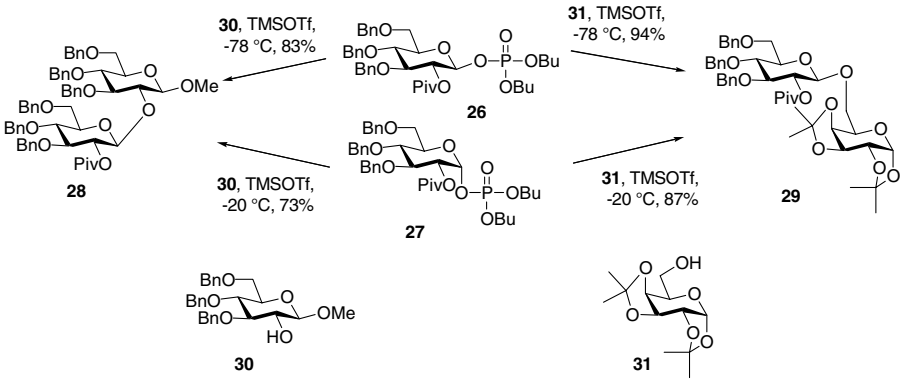
Fucosyl phosphate **36** also very efficiently glycosylated the C4 position of acceptor **32**. The orthogonality of the activating conditions of thioglycosides and glycosyl phosphate donors was exploited in the synthesis of trisaccharide glycal **41** (Scheme 6.7A).[32] Furthermore, the different reactivity of α- and β-glycosyl phosphates was



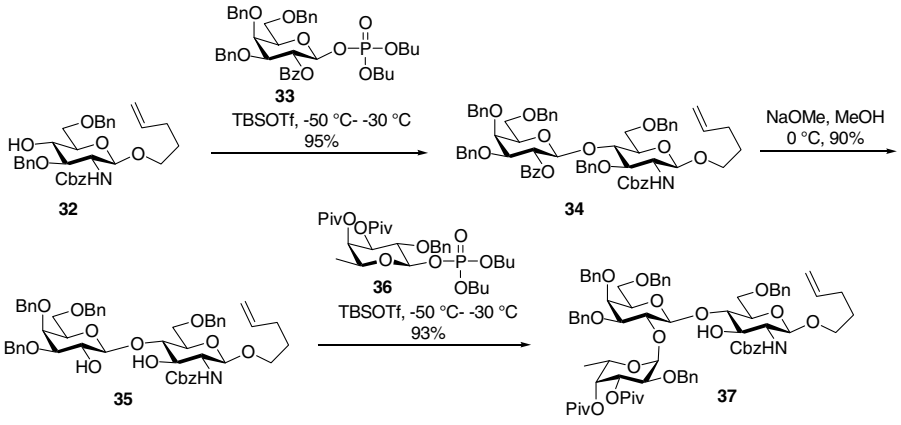
Scheme 6.4 One-pot synthesis of glycosyl phosphates from glycals.

illustrated in the synthesis of trisaccharide **44** without any protecting group manipulations (Scheme 6.7B).[34]

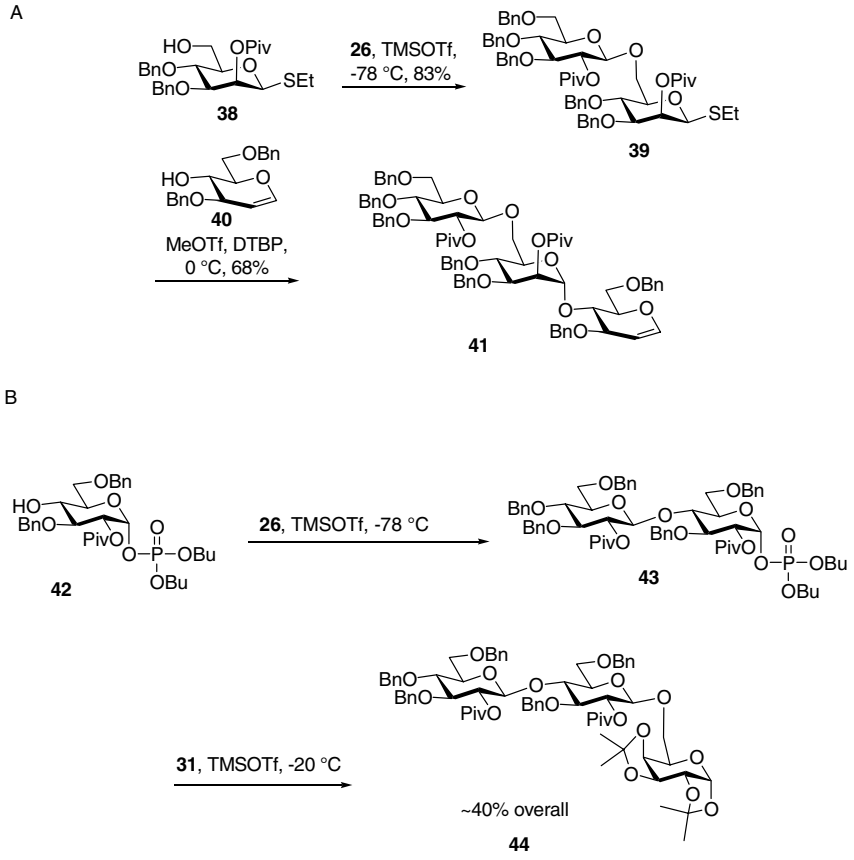
Crich had shown that anomeric α-triflates or close ion pairs are formed on activation of suitably protected glycosyl sulfoxides with excess triflic anhydride, leading to β-selective mannosylation reactions.[35] The observed β-selectivity in reactions involving glucosyl phosphates even in the absence of a participating C2 group and observations by Singh[36] in reactions of a cyclic glucosyl propane-1,3-diyl phosphate with primary acceptors suggest that these reactions may proceed via α-triflate intermediates. Guided by this model, attempts were directed at the selective creation of β-mannosides employing glycosyl phosphates. The formation of these *cis*-glycosidic linkages, present in the core region of *N*-linked glycoproteins,[37] still constitutes a major challenge for the synthetic chemist. α-Mannoside formation is



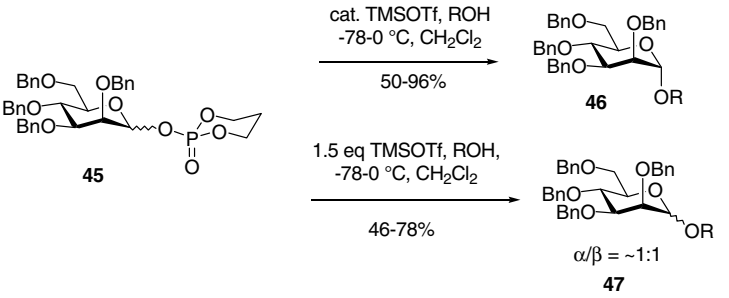
Scheme 6.5 Glycosyl phosphates functioning as glycosyl donors in β-selective formation of glycosidic linkages.



Scheme 6.6 α-Fucosylation of a disaccharide acceptor with complete regio/stereoselctivity.



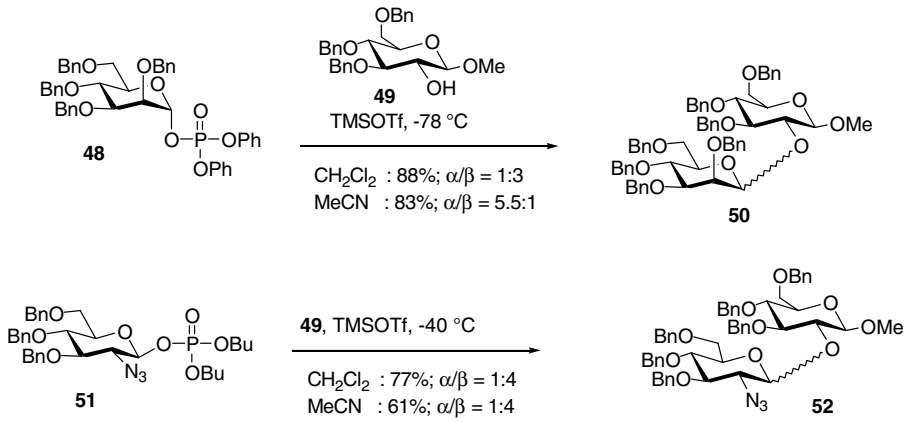
Scheme 6.7 Orthogonal glycosylations to fashion trisaccharides.



Scheme 6.8 Formation of α-mannosides on cyclic mannosyl phosphate activation.

favored principally for steric reasons and the anomeric effect. As expected, only α-mannosides were formed on activation of cyclic mannosyl phosphate **45** with catalytic amounts of TMSOTf in the presence of various glycosyl acceptors (Scheme 6.8).[38] When excess activator was employed, Singh observed considerable increase of β-mannoside formation.

Better β-selectivities were achieved using mannosyl diphenyl phosphate **48** and hindered acceptor **49** under activation with excess TMSOTf to form preferentially the β-mannoside (β:α = 3:1) (Scheme 6.9).[39] The selectivity of the reaction was found to be strongly substrate-dependent. The influence of the acceptor conformation and reactivity on the selectivity of β-mannosylation reactions had also previously been reported by Crich.[40] When the couplings were carried out in a coordinating solvent such as acetonitrile, the selectivity of the mannoside formation was completely reversed (α:β = 5.5:1) (Scheme 6.9). Interestingly, no solvent dependence was found in the coupling of 2-deoxy-2-azido glucosyl phosphate donor **51** with hindered acceptor **49**, but rather high β-selectivity and yields were observed in both solvents.[39]



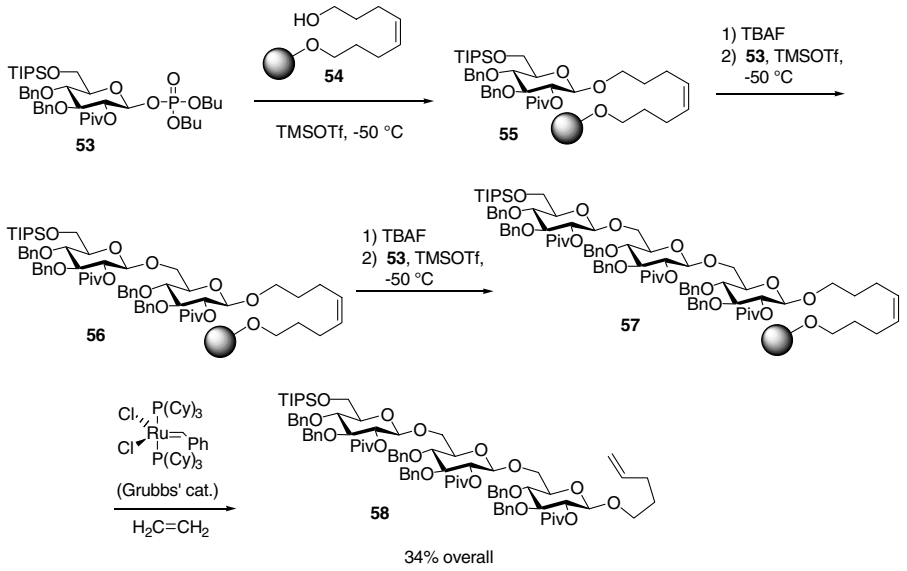
Scheme 6.9 Formation of β-mannosides using glycosyl phosphates.

6.2.2 Special Applications of Glycosyl Phosphates in Oligosaccharide Synthesis

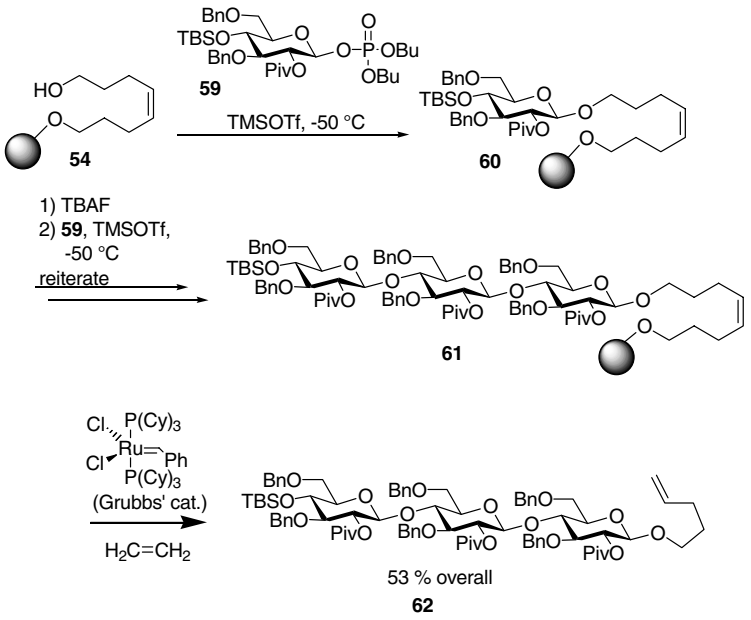
As outlined above, glycal derived glycosyl phosphates have proved to be very easily accessible and effective glycosyl donors. They require only liquid, non-toxic activators, and generally no addition of molecular sieves is necessary. Therefore, glycosyl phosphates lend themselves particularly well to the synthesis on solid support. We also reported on the high-yielding solid-phase syntheses of oligosaccharides employing a novel octenediol linker (Scheme 6.10).[41] β-(1→6)-Linked triglucoside **58** was obtained in 35% overall yield over seven steps using 3 equiv of glycosyl phosphate donor **53** for each coupling step. The glycosylations were carried out at –50°C for one hour followed by removal of the 6-*O*-triisopropylsilyl (TIPS) protecting group with tetrabutylammonium fluoride (TBAF). The trisaccharide was finally released from the support by cross-metathesis using Grubbs' catalyst under an atmosphere of ethylene to yield *n*-pentenyl glycoside **58**.

Using a similar reaction sequence β-(1→4)-glucosidic linkages were very effectively installed on the support using 4-*O*-TBS-protected glucosyl phosphate donor **59**. Thus, repetitive glycosylation and deprotection furnished trisaccharide **62** in 53% overall yield from **54** after cleavage from the resin (Scheme 6.11).

C-Glycosidic linkages are present in various natural products and may inhibit glycosidases due to the structural analogy to their *O*-counterparts.[42] Glycosyl phosphates **63** and **48** served successfully in the α-selective synthesis of aryl

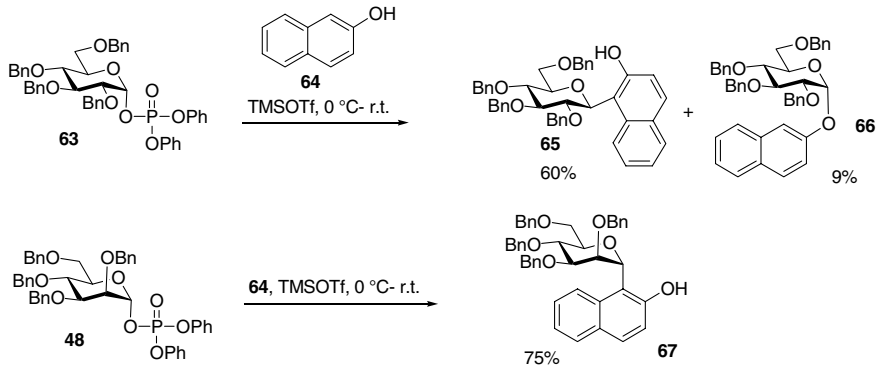


Scheme 6.10 Solid-phase synthesis of oligosacchraides using an octenediol linker.

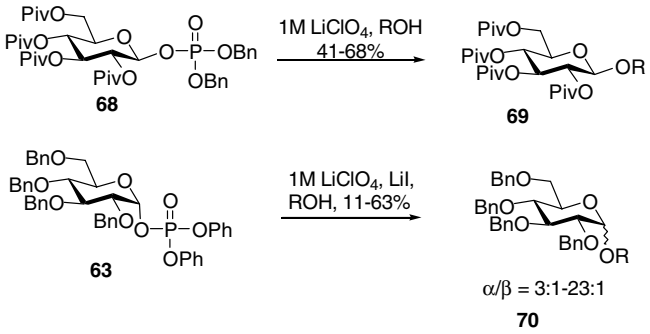


Scheme 6.11 Solid-phase synthesis of a β(1→4) linked triglucoside.

O-glycosides at 0°C, which, on warming to room temperature, rearranged to *C*-aryl glycosides (Scheme 6.12). The preference for the equatorial position of bulky aromatic *C*-aglycons was evident and had been observed before.[42] While couplings employing 2-*O*-acyl protected glycosyl phosphates were unsatisfactory in most cases,



Scheme 6.12 Rearrangement to *C*-aryl glycosides from aryl *O*-glycosides following α-selective synthesis using glycosyl phosphates.



Scheme 6.13 Glycoslation of carbohydrate acceptors with glycosyl α-diphenyl phosphate.

perbenzylated phosphate donors gave high yields also with nonaromatic *C*-glycosyl acceptors.[43]

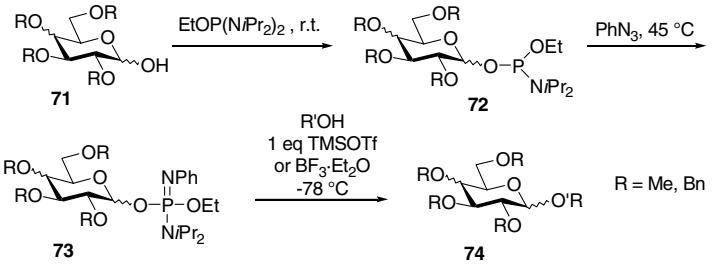
The phosphate leaving group also served in samarium(II)iodide-mediated *C*-glycosidations, as described by Wong.[44] A glycosyl anion equivalent, generated via a two-step sequence, added in high yield to a series of carbonyl electrophiles, thereby stereoselectively creating *C*-glycosidic linkages.

Direct nucleophilic displacements of the phosphate leaving group in inert organic solvents by nucleophiles of the A–B═C–H type, e. g. phenols, have been described by Schmidt.[28] Concentrated solutions of lithium perchlorate in organic solvents enabled similar glycosylations under neutral conditions employing various acceptors, including carbohydrates. While 2-*O*-acylated glycosyl β-phosphates gave exclusively β-glycosides,[45,46] glycosylation reactions employing perbenzylated glycosyl phosphates resulted in varying stereoselectivities depending on the nature of the solvent and the anomeric configuration of the glycosyl donor.[45,47] However, glycosylations of carbohydrate acceptors were unselective. This problem was solved by the use of lithium iodide as an additive for the in situ[48] generation of glycosyl iodide donors.[49] A series of primary and secondary carbohydrate acceptors were glycosylated with glycosyl α-diphenyl phosphate **63** in the presence of lithium iodide resulting in α/β-selectivities of up to 23:1 (Scheme 6.13).

In a similar way glycosyl diphenyl phosphates have been used in the stereoselective preparation of glycosyl azides on S_N2-type displacement of the phosphate group by sodium azide.[27]

6.3 OTHER PHOSPHOROUS(V) GLYCOSYL DONORS

A variety of phosphate analogs have been synthesized and evaluated as glycosyl donors. Glycosyl phosphoramidimidates **73** are readily available from glycosyl phosphoramidites via a Staudinger reaction with phenyl azide (Scheme 6.14). Bearing



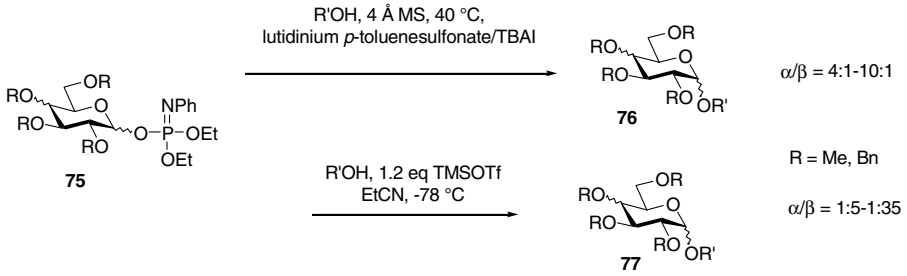
Scheme 6.14 Synthesis of glycosyl phosphoramidimidates from glycosyl phosphoramidites.

a nonparticipating group at C2, glycosyl phosphoramidimidates have been shown to undergo glucosylation and galactosylation reactions with primary acceptor glycosides exhibiting α:β-selectivities of ≤1:20 in some cases, although yields and selectivities generally leave room for improvement.[50] The synthesis of these glycosyl donors proved advantageous since no workup or purification was required for the crude products from the Staudinger reaction.

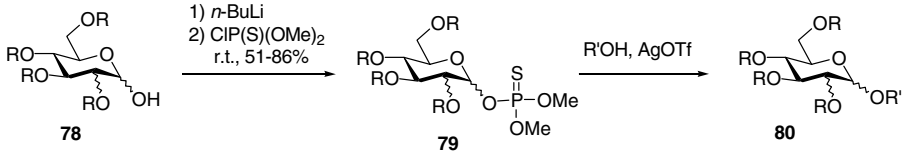
Glycosyl phosphorimidate donors were obtained by the synthetic sequence outlined in Scheme 6.14. These glycosyl donors were coupled with primary acceptor glycosides in moderate to excellent yields, and depending on the reaction conditions (Scheme 6.15) either α- or β-selectivities were observed.[51] β-Selective mannosylations and glycosylation on reaction of secondary hydroxyl containing acceptors did not prove feasible.

The synthesis and application of glycosyl dimethylthiophosphate donors has also been reported.[52] These donors were prepared from the reducing sugars in moderate to good yield and exhibit considerable shelf stability. Glycosylation reactions with a variety of primary and secondary glycosyl acceptors suffered from poor stereoselectivity and required silver triflate as promoter to achieve reasonable coupling yields (Scheme 6.16).

Hashimoto and Ikegami have introduced glycosyl phosphoroamidates as glycosyl donors that combine the advantages of long shelf life with great efficiency in



Scheme 6.15 Coupling of glycosyl phosphorimidate donors with primary acceptor glycosides.

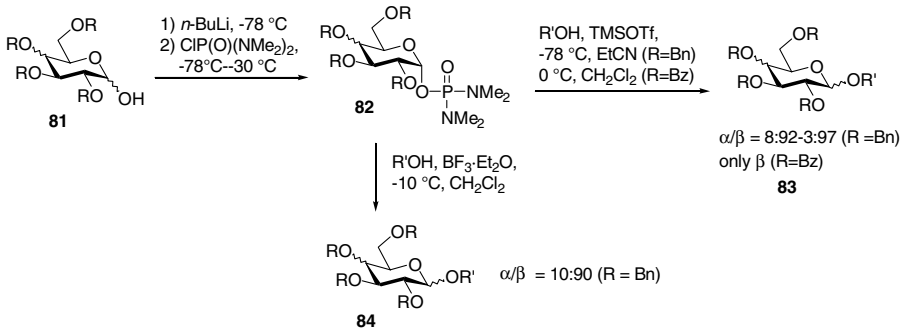


Scheme 6.16 Synthesis and use of glycosyl thiophosphates.

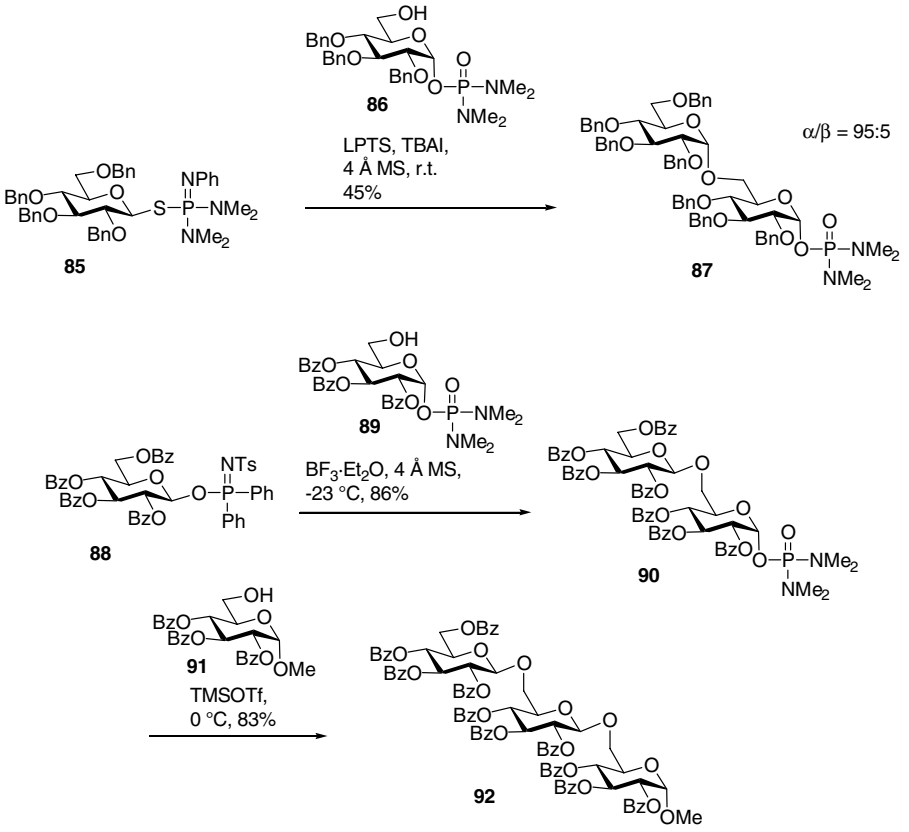
β-glycoside formation on activation at low temperatures (Scheme 6.17).[53] A series of glucosyl and galactosyl phosphoroamidates lacking a C2 a participating group were coupled to primary and secondary acceptors in excellent yield and with α:β-selectivities of ≤3:97 on activation with stoichiometric TMSOTf at −78°C in propionitrile. 2-*O*-Benzoyl-protected phosphoroamidate donors yielded exclusively β-glycosides. Interestingly, this anomeric leaving group was also β-selectively activated by $BF_3{\cdot}Et_2O$ in dichloromethane at −10°C, at conditions compatible with the corresponding glycosyl diphenylphosphinimidate donors. Thus, oligosaccharide syntheses using different kinds of phosphorus-based leaving groups as well as glycosylation of compounds insoluble at very low temperatures in propionitrile, such as steroidal alcohols were enabled.

Exploiting the "armed/disarmed" concept[54] and making use of the added degree of "orthogonal glycosylation"[55] achieved by a fine-tuning of phosphorous-based leaving groups, these researchers demonstrated chemoselective glycosylations in the synthesis of a series of di- and trisaccharides (Scheme 6.18).[56] The phosphoroamidate moiety is stable under highly α-selective glycosylation conditions using glycosyl phosphorodiamidimidothioates,[57] as demonstrated in the synthesis of disaccharide **87**. Perbenzoylated diphenylphosphinimide **89** was also chemoselectively coupled to acceptor phosphoroamidate, furnishing disaccharide phosphoroamidate **90** with high β-selectivity. With the more powerful promoter TMSOTf, **90** was glycosylated again to selectively afford trisaccharide **92** in good yield.

The synthesis of the same trisaccharide core also illustrated the orthogonality between glycosyl phosphite and phosphoroamidate donors as well as between an



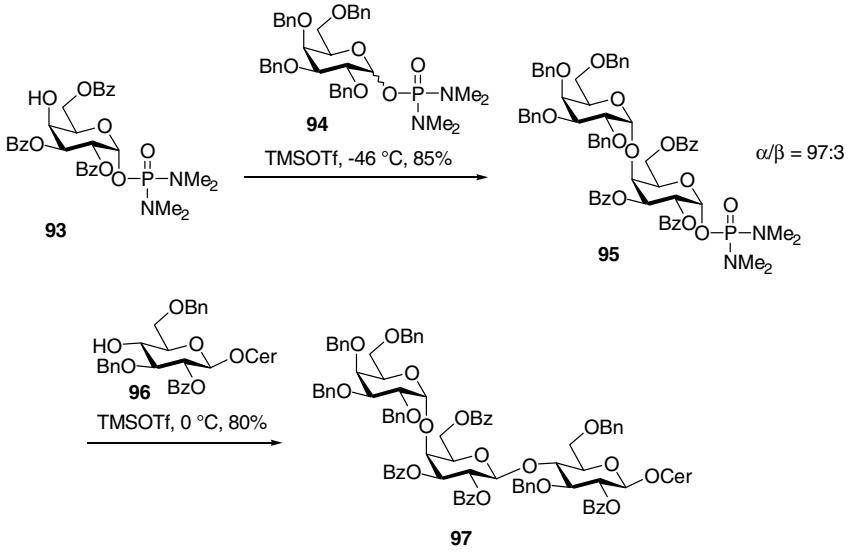
Scheme 6.17 Use of glycosyl phosphoroamidates as glycosyl donors.



Scheme 6.18 Chemoselective glycosylation in synthesis of various di- and trisaccharides.

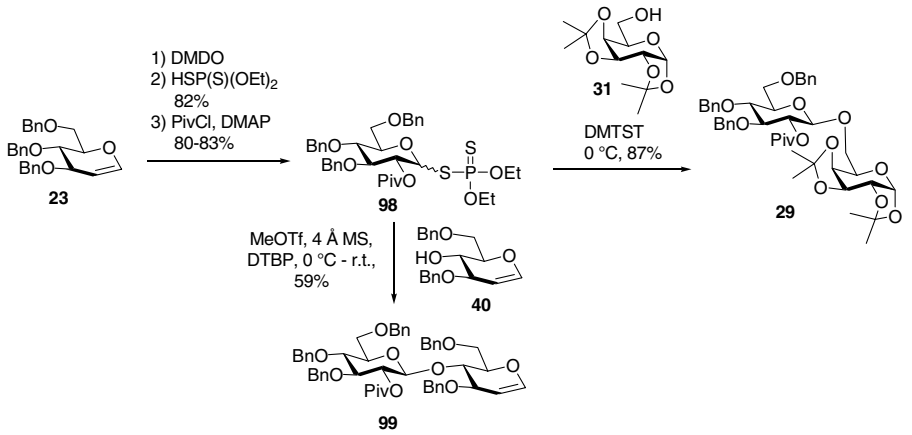
"armed" and a "disarmed" glycosyl phosphoroamidate. The latter concept was further utilized in the convergent synthesis of tumor-associated globotriaosylceramide Gb_3 (Scheme 6.19).[58] Glycosylation of "disarmed" galactosyl phosphoroamidate **93** with "armed" donor **94** proceeded in good yield and excellent α-selectivity. Further coupling to the hindered C4 position of glucosylceramide **96** furnished fully protected Gb_3 in good yield.

Incorporating a bridging sulfur in place of oxygen is expected to alter the reactivity of the phosphorous-based leaving group. 2-Deoxysugar glycosyl phosphorodithioates have been reported as powerful and stereoselective donors in the synthesis of 2-deoxyglycosides of *O*-,[59] *N*-,[60] and *C*-nucleophiles,[61] especially when promoted with iodonium bis(2,4,6-collidine)perchlorate[62] or silver(I) salts.[63] While syntheses of peracetylated glycosyl phosphorodithioates from the anomeric halide[64] or acetate[65] have been described, the efficiency of these compounds in couplings to carboxylic acid acceptors was significantly lower than that of their 2-deoxy counterparts,[66] and no coupling reactions to carbohydrate acceptors have been reported.



Scheme 6.19 Synthesis of globotriaosylceramide.

We have demonstrated that glycosyl phosphorodithioates such as **98**, which can easily be prepared in a high-yielding one-pot procedure from glycals, serve as powerful glycosylating agents.[67] Activation with methyl triflate, or better, DMTST furnished the desired glycosides in excellent yield. Most notably, the activation conditions were mild enough to allow for the efficient coupling to secondary acceptor hydroxyls on acid-sensitive glycals (Scheme 6.20).



Scheme 6.20 Glycoside synthesis using glycal-derived glycosyl dithiophosphates.

6.4 CONCLUSION

Glycosyl phosphates are a class of powerful glycosyl donors that compare favorably to other, more established types of glycosyl donors with respect to their ability to glycosylate hindered glycosyl acceptors in high yield. High anomeric selectivity can be achieved in these glycosylations, including *cis*-(1→2)-glycosidic bonds. Optimal glycosylation conditions require no further additive than stoichiometric amounts of acidic TMSOTf, and high-yielding couplings have been carried out on a solid support. Acid-sensitive compounds can be glycosylated under very mild neutral conditions in concentrated solutions of lithium perchlorate in organic solvents.

Various glycosyl phosphate derivatives offer the potential of fine-tuning the donor reactivity that can be beneficially used to simplify the synthesis of complex carbohydrates. Since glycosyl phosphates and derivatives have become available in a straightforward one-pot synthesis from glycals, thus minimizing protecting group manipulations, they lend themselves as a viable alternative to the established set of glycosyl donors.

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7 Stereoselective β-Mannosylation on Polymer Support

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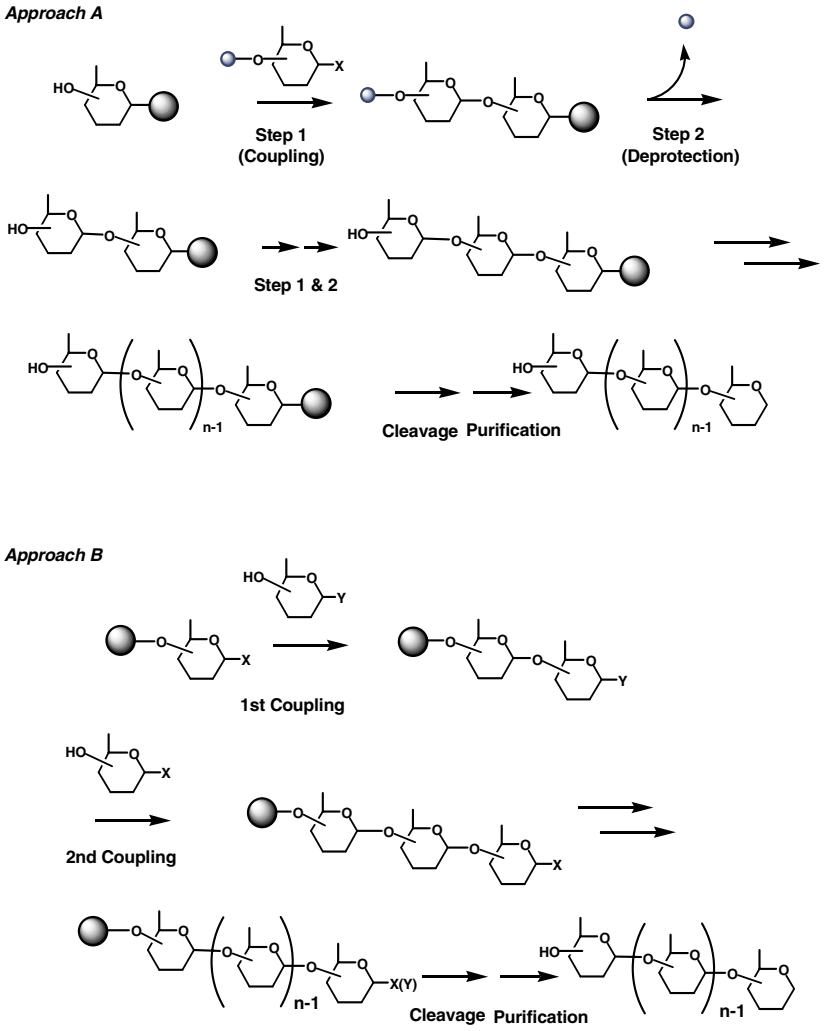
In connection with their significant roles in numerous biological events,[1] chemical synthesis of glycoconjugate-related oligosaccharides has been a subject of intense efforts.[2] Even from a purely chemical point of view, these complex and diverse molecules are challenging and attractive research targets. Since the availability of glycoconjugate-derived glycan chains from natural sources is severely limited, the establishment of methodologies to chemically prepare these molecules is highly desirable. In order to reach this goal, a number of novel reactions effective for *O*-glycoside bond formation have been developed.[3] Consequently, it is now possible to synthesize quite complex oligosaccharides of biological relevance.[4] Relying on chemical synthesis, one can produce homogeneous oligosaccharides in substantial quantities. Additionally, a high degree of flexibility is secured by which one can synthesize oligosaccharides having nonnatural structures. However, preparation of oligosaccharides by purely chemical means requires a series of multistep transformations. Oligosaccharide synthesis consists mainly of repeated glycosylations and protecting group manipulations. Elongation of one sugar residue usually involves two transformations: deprotection to liberate a specific OH group and *O*-glycosylation with an appropriate glycosyl donor. Each sugar residue should be carefully designed so that positionally selective deprotection as well as stereoselective glycoside bond formation can be achieved. Preparation of each "strategically designed" sugar component again requires mulistep operations. After every step, chromatographic purification is usually required to isolate the product in sufficiently pure form that the subsequent transformation can be performed without causing unnecessary complication. This operation is time-consuming and quite often the rate-limiting factor in the whole synthesis. In particular, isolation of the desired product is troublesome, whenever glycosylations involving unreactive acceptors are attempted. Such reactions tend to result in a low yield of the glycosylated product together with a larger amount of unreacted acceptor. In order to obtain more satisfactory conversion, a large excess of glycosyl donor would be required. In either case, the desired product is a relatively minor component of the mixture, and the chromatographic purification process is far from being

straightforward. Obviously, rapid access to various glycan chains is critical in order for chemical oligosaccharide synthesis to become a driving force in glycosciences. Any methodology that would obviate tedious chromatographic separations should be valuable for this purpose.

In contrast to the current situation in carbohydrate chemistry, technologies for chemical synthesis have been remarkably well developed in the areas of other major biological molecules, oligopeptides,[5] and oligonucleotides.[6] Laboratory-scale preparation can be readily conducted even by nonexperts in synthetic organic chemistry, using fully automated synthesizers. Synthetic protocols in these cases have been greatly simplified by adopting solid-phase synthesis as the key technology. In addition to its established popularity as a powerful tool in biological sciences, solid-phase organic synthesis is gaining further attention[7] in connection with library synthesis of small molecules, which are expected to revolutionize drug candidate screening by means of rapid production of a large number of compounds in a combinatorial manner. The advantage of solid-phase synthesis technology in all of these cases stems from the ease of separation of the product. Since phase separation can be simply achieved by filtration of polymer-bound material from the solution followed by washing of the resin, a high degree of conversion can be attained by using a large excess of reagents and/or coupling partners without complicating the isolation process. Multistep transformations can be performed without chromatographic purification at the intermediate stage.

Keeping the structural diversity of glycoconjugate-derived oligosaccharides and their growing demand as biochemical tools in mind, oligosaccharide synthesis on polymer support is currently recognized as the most important topic in the community of synthetic carbohydrate chemistry.[8] In polymer support oligosaccharide synthesis, two approaches can be considered with respect to the direction of chain elongation (Scheme 7.1). In approach A, elongation of glycan chain is performed from reducing end to nonreducing end, while the synthesis starts from polymer-bound nonreducing end sugar residue in approach B. The former approach seems to be more straightforward, since glycosylation steps can be driven to completion and no stereochemical ambiguity exists, and nearly homogeneous product can be obtained at the end. In fact, oligosaccharides with significant complexity containing up to 12 sugar residues have been synthesized by this approach.[9]

On the other hand, approach B is less straightforward in principle, because most of the side reactions in glycosylation reactions arise from the glycosyl donor.[10] It would be obvious that, starting with polymer-supported glycosyl donor, repeated glycosylations result in a rather complex mixture of various products, and the isolation of the correctly assembled oligosaccharide would be quite difficult. However, Danishefsky and coworkers demonstrated that this approach is quite feasible for the rapid production of moderately complex oilgosaccharides. As part of their work on the ingenious use 1,2-anhydrosugars as glycosyl donors in facile oligosaccharide synthesis, they demonstrated that the difficulty inherent to this approach can be reduced to an acceptable level. Polymer-supported glycal is transformed to an 1,2-anhydro(epoxide) derivative that is activated by Lewis acid.[10] Any side products



Scheme 7.1 Strategies for solid-phase oligosaccharide synthesis.

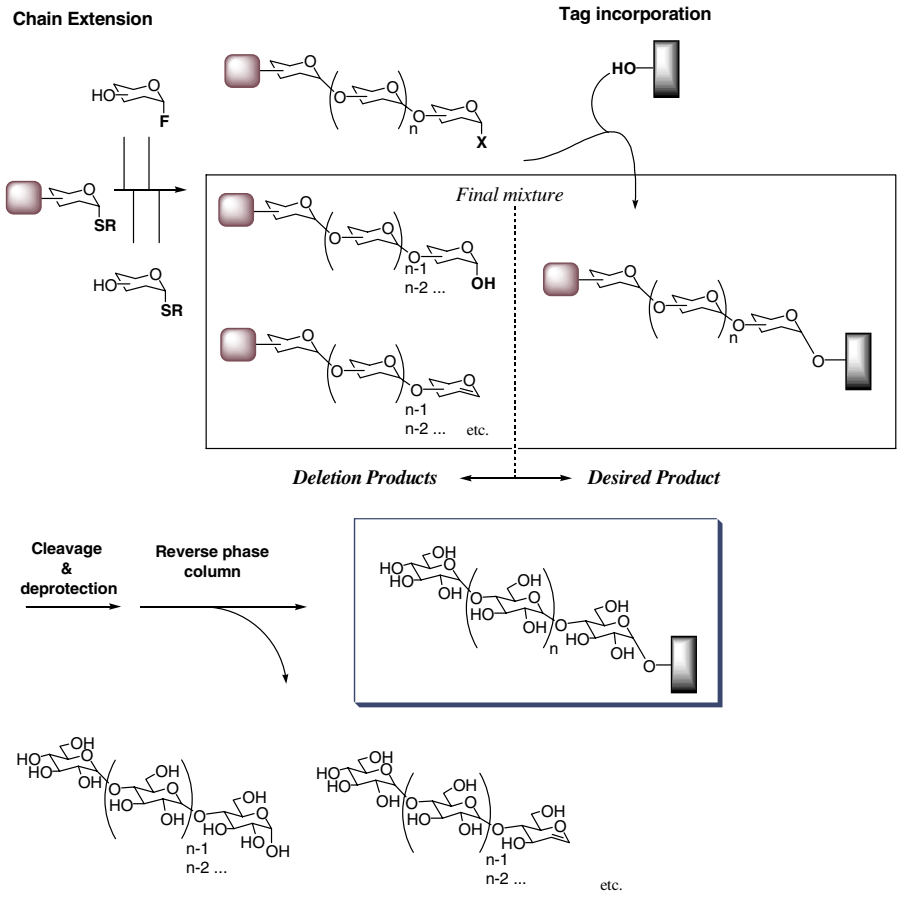
arise from failure of glycosylation cannot be activated, and thus the formation of internal deletion product(s) is eliminated.

Polymer-supported "orthogonal glycosylation" was developed as an alternative tactic applicable to this approach.[11] Chain elongation was performed using thioglycosides (X = SR) and glycosyl fluorides (Y = F) as a set of "orthogonal" donors.[12] Polymer-supported thioglycosides can be activated without affecting anomeric fluorides and vice versa. In this manner, the preparation of a given oligosaccharide by a minimum number of operations is possible. Elongation of *n* sugar residues can be performed with $n + 2$ operations (including cleavage and final purification), while $2n + 2$ operations are required in approach A. In order to facilitate

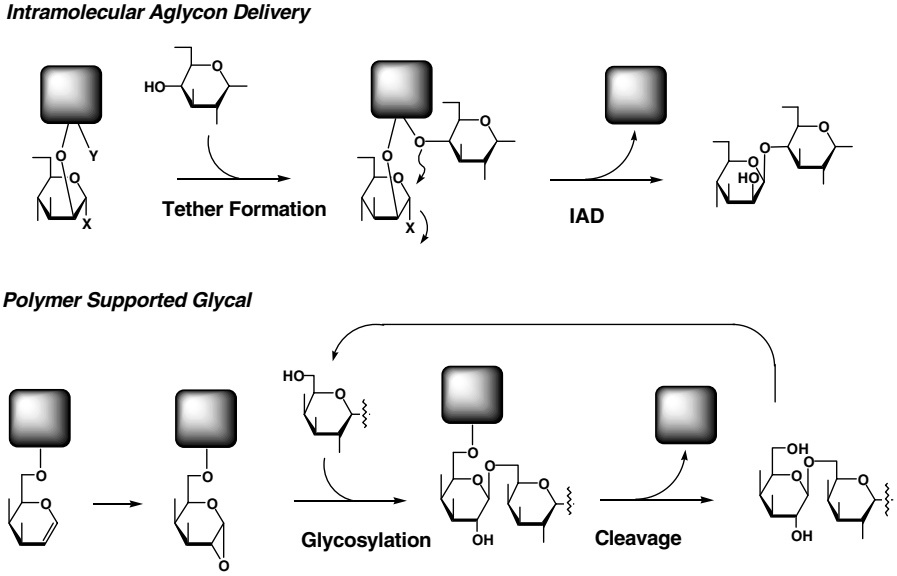
the isolation process, the assembly of oligosaccharides can be followed by an incorporation of a tag at the reducing end. The target oligosaccharide can be distinguished from all byproducts, because only the successfully assembled oligosaccharide is able to incorporate the tag (Scheme 7.2).

In terms of phase separation, both of these approaches rely on the same principle. The growing oligomers remain bound to the solid phase throughout chain elongation, while excess reagents and sugar components (glycosyl donor and acceptor, in approaches A and B, respectively) are washed out into the liquid phase. Both approaches have been successfully adopted in the synthesis of complex oligosaccharides, using either insoluble (e.g., Merrifield resin) or soluble (e.g., polyethylene glycol) polymer supports.[13]

A drastically distinct use of a polymer support in oligosaccharide synthesis was devised in connection with *p*-methoxybenzyl (PMB)-assisted "intramolecular



Scheme 7.2 Orthogonal glycosylation strategy.

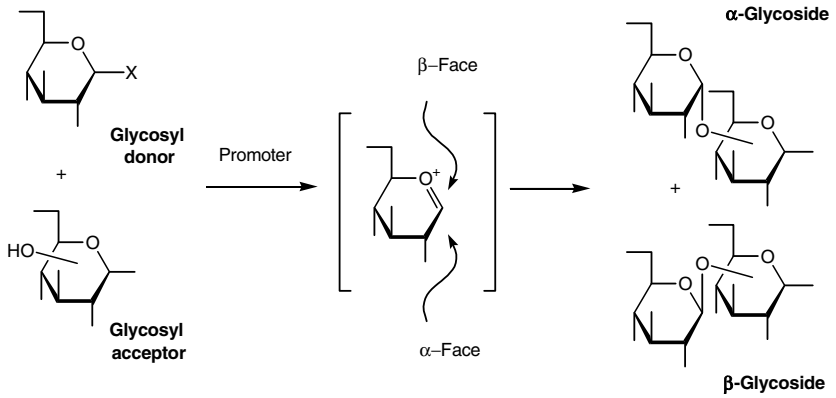


Scheme 7.3 Polymer-supported glycosyl donor.

aglycon delivery" (IAD),[14] which has been demonstrated to be a powerful method for achieving synthetically challenging β-mannoside formation. A salient feature of this system is the ability of the polymer support to serve as a "gatekeeper" that specifically releases the successfully assembled product, while the unreacted substrates and byproduct(s) are retained on the polymer (Scheme 7.3).[15] On the other hand, Danishefsky reported a variation of the solid-phase glycosylation. In this case, the resin-bound 1,2-anhydrosugars are used as donors and, after each glycosylation, product is cleaved from the resin to liberate a hydroxyl group.

7.1 *p*-METHOXYBENZYL-ASSISTED INTRAMOLECULAR AGLYCON DELIVERY: HIGHLY EFFICIENT β-MANNOSYLATION

A number of novel glycosylation reactions have been developed and successfully applied to the synthesis of a wide range of glycolipid, glycoprotein-related glycan chains, glycopeptides, glycosaminoglycans, and oligosaccharide containing natural products. As depicted in Scheme 7.4, *O*-glycosylation is initiated by the activation of glycosyl donor to form a cationic species. This step usually requires a certain promoter that abstracts the leaving group (X). Nucleophilic attack by the alcohol (glycosyl acceptor or aglycon) gives *O*-glycosylated product. A majority of reactions utilize glycosyl donors [X = F,[16] $OC(NH)CCl_3$,[17] SR,[18] S(O)Ph,[19] $OP(OR)_2$,[20] $O(CH_2)_3CH{=}CH_2$[21]] that are relatively stable but can be activated quickly, under mild reaction conditions, to highly reactive species once exposed to specific promoters. Some donors are stable enough to withstand reaction conditions for protecting group



Scheme 7.4 Reaction pathway of *O*-glycosylation reaction.

manipulation, and the preparation of strategically protected glycosyl donors is possible with ease. Alternatively, they can be readily prepared from easily accessible and more robust precursors such as 1-*O*-acetylated or 1-*O*-unprotected derivatives. Taking advantage of these features, block condensation of oligosaccharide fragments has become a feasible strategy for the synthesis of complex oligosaccharide.[22]

Even though the use of these efficient methodologies have been attempted successfully in critical aspects of glycan chain synthesis, stereochemical control, which is the most fundamental problem in chemical glycosylation, has not yet (at the time of writing) been solved in a general sense. Although literature data on the stereochemical outcome of *O*-glycosylation reactions is rapidly accumulating, it is still difficult to interpret them systematically. Quite often, extensive experimentation is required to achieve the formation of a given glycosidic linkage in a satisfactory manner and screening of various types of glycosyl donors, promoters, and reaction conditions (e.g., solvent, temperature) is a common practice.

Among various types of naturally occurring *O*-glycosides with biological interest, the β-glycoside of D-mannose (β-mannoside) has been the most challenging from a synthetic point of view.[23] The difficulty arises from its unique stereochemical features. 1,2-*trans*-Glycosides, such as β-gluco, β-galacto, and α-manno glycosides, can be formed with rigorous stereochemical control by using neighboring-group participation from a C2 ester substituent. In contrast, the formation of a 1,2-*cis*-glycoside, including β-mannosides, requires the presence of a nonparticipating group at C2 and is, to some extent, accompanied by stereochemical uncertainty. This situation is most serious in the case of β-mannosides, because formation of the anomeric C–O linkage with equatorial configuration is disfavored for stereoelectronic reasons (i.e., the anomeric effect[24]). The biological significance of β-mannosides is obvious in light of its prevalence in the core structure common to all types of asparagine (Asn)-linked glycoprotein glycans (Fig. 7.1). In order to pursue systematic synthetic studies on this important class of biological molecules, a general solution to the problems associated with β-mannosylation is required. In particular, the synthesis of so-called complex type oligosaccharides has been a considerable challenge.

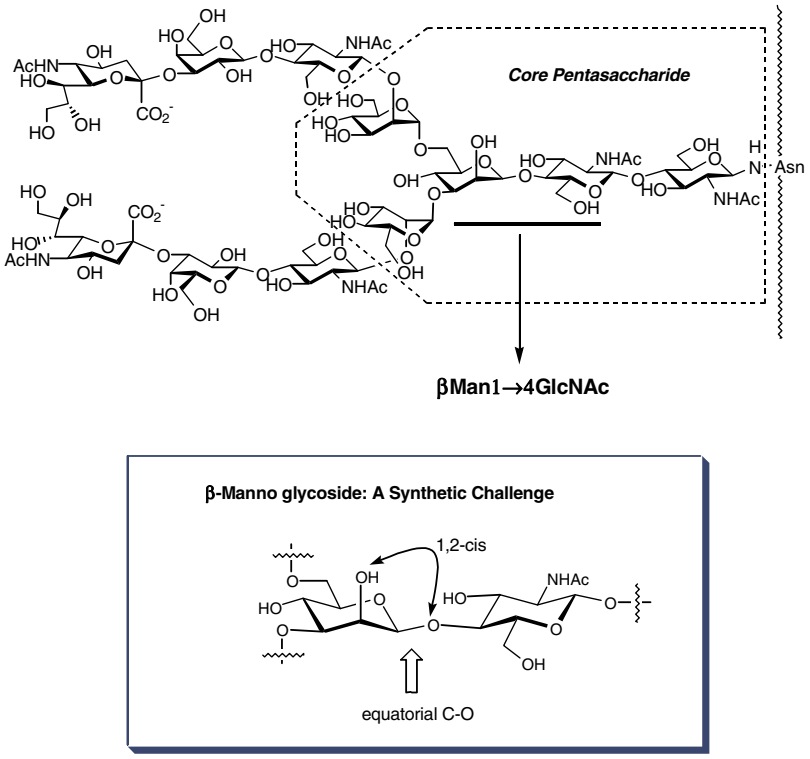
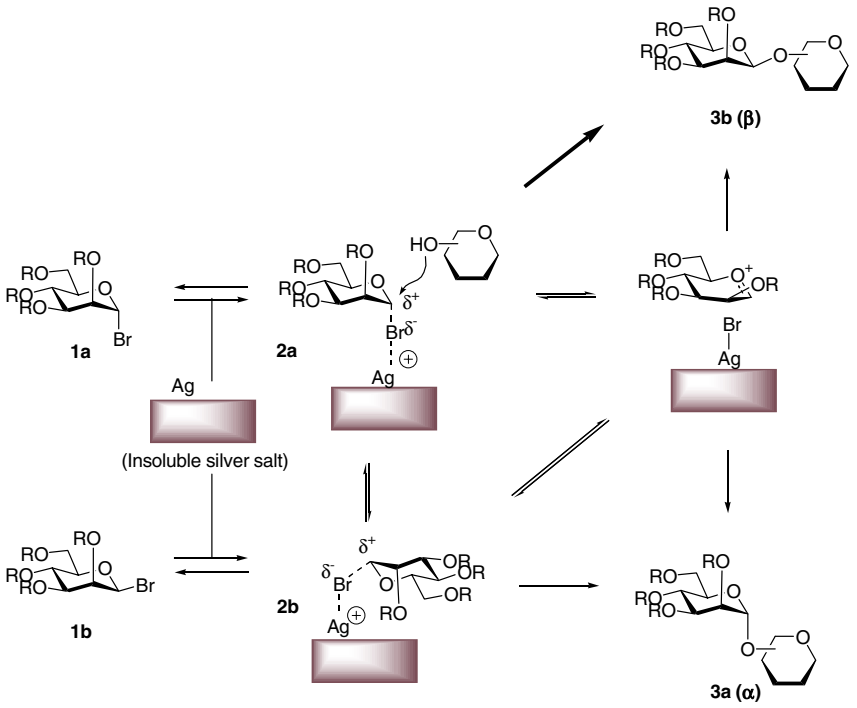


Figure 7.1 Typical structure of Asn-linked glycan chain.

As in the cases of the majority of *O*-glycosylation reactions, the most straightforward tactic to construct β-mannosides would seem to be the direct glycosylation approach. Since the formation of β-mannoside is disfavored under ordinary reaction conditions, a special promoter in combination with a donor equipped with a reactive leaving group is required to realize the goal. Conventionally, this type of reaction has been performed using "insoluble" silver salts as an activator of mannosyl bromide **1**. The unique selectivity observed with this type of reagents has been ascribed to the heterogeneous nature of the salt. Ion pair **2a** generated by abstracting the leaving group is absorbed on a solid surface, and stereochemical scrambling to more reactive **2b** is minimized. Since glycosyl bromide exists almost exclusively as an α-anomer, nucleophilic attack by the hydroxy group proceeds mainly with stereochemical inversion of the anomeric carbon to afford β-linked product **3b** (Scheme 7.5).

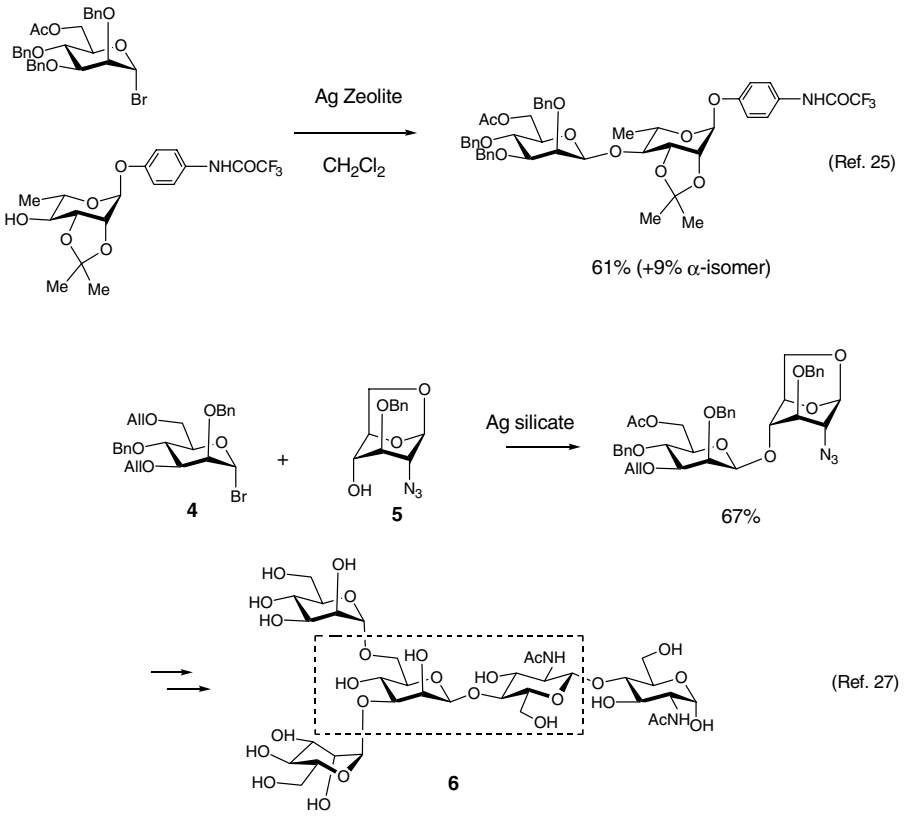
For this purpose, Ag zeolite and Ag, developed by Garegg[25] and Paulsen,[26] respectively, have been widely employed (Scheme 7.6). Reactions using these activators have found practical use in synthetic studies on glycoprotein glycans and glycolipids. For instance, Paulsen reported the chemical synthesis of the core pentasaccharide structure **6** common to all types of Asn-linked glycans, using the Ag



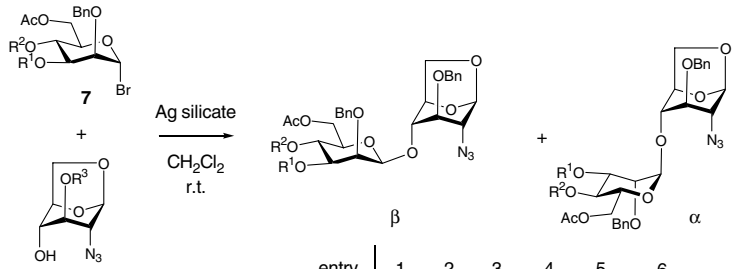
Scheme 7.5 Insoluble Ag salt-promoted β-mannosylation.

silicate-mediated union of **4** and **5** as the key transformation.[27] More recently, van Boeckel reported the use of silver silica–alumina,[28] which was revealed to have some favorable features in terms of reactivity and enhanced cation capacity. His group also systematically investigated the dependence of β-selectivity on the protecting group of mannosyl donor **7** (Scheme 7.7). Very interestingly, an electron-withdrawing acyl group introduced at position 4 enhances the β-selectivity, while it has a negative effect when attached to other positions. Insoluble silver salt has also been applied to the β-selective synthesis of gluco, galacto, 2-deoxy-2-azido, and 2-deoxy glycosides.[29] Although the insoluble silver salt method has found substantial applications in oligosaccharide synthesis, the degree of stereoselectivity is variable and often proceeds in a stereorandom manner. When the reactivity of acceptor is low, the stereochemical scrambling of ion pair (**2a**↔**2b**) on solid surface becomes competitive to S_N2-type reaction (**2a**→**3b**).

Intriguing approaches have been developed using mannosyl fluorides,[30,31] phosphites,[32] and thioglycosides (Scheme 7.8).[33] However, their generalities, particularly in terms of applicability to complex and biologically relevant systems, are yet to be clarified. Quite probably, the most successful donor for direct β-mannosylation is the sulfoxide developed by Crich and coworkers (Scheme 7.9).[34–37] Glycosyl sulfoxides were originally developed by Kahne and have proved



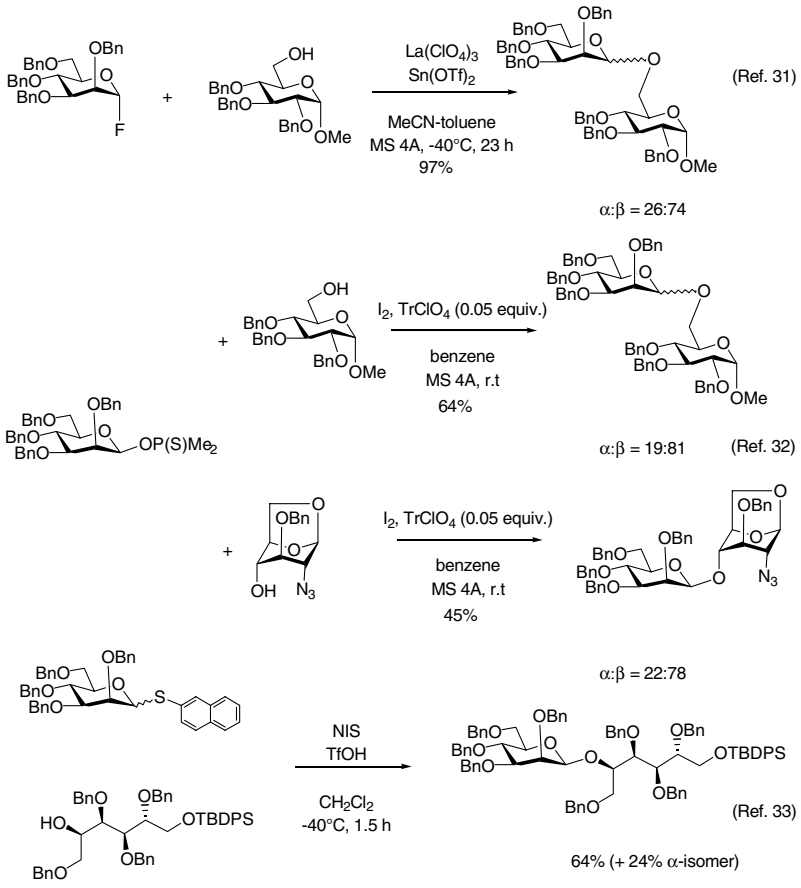
Scheme 7.6 Reactions employing Ag zeolite and Ag activators.



entry	1	2	3	4	5	6
R^1	Ac	Bn	TCA	Bn	Ac	Bn
R^2	Bn	Bn	Bn	Ac	TCA	All
R^3	Bn	Bn	Bn	Bn	Bn	Ac
β:α	1:1	6:1	1:1.2	9:1	>10:1	5.1:1

Scheme 7.7 A reaction demonstrating dependence of β-selectivity on the protecting group of mannosyl donor.

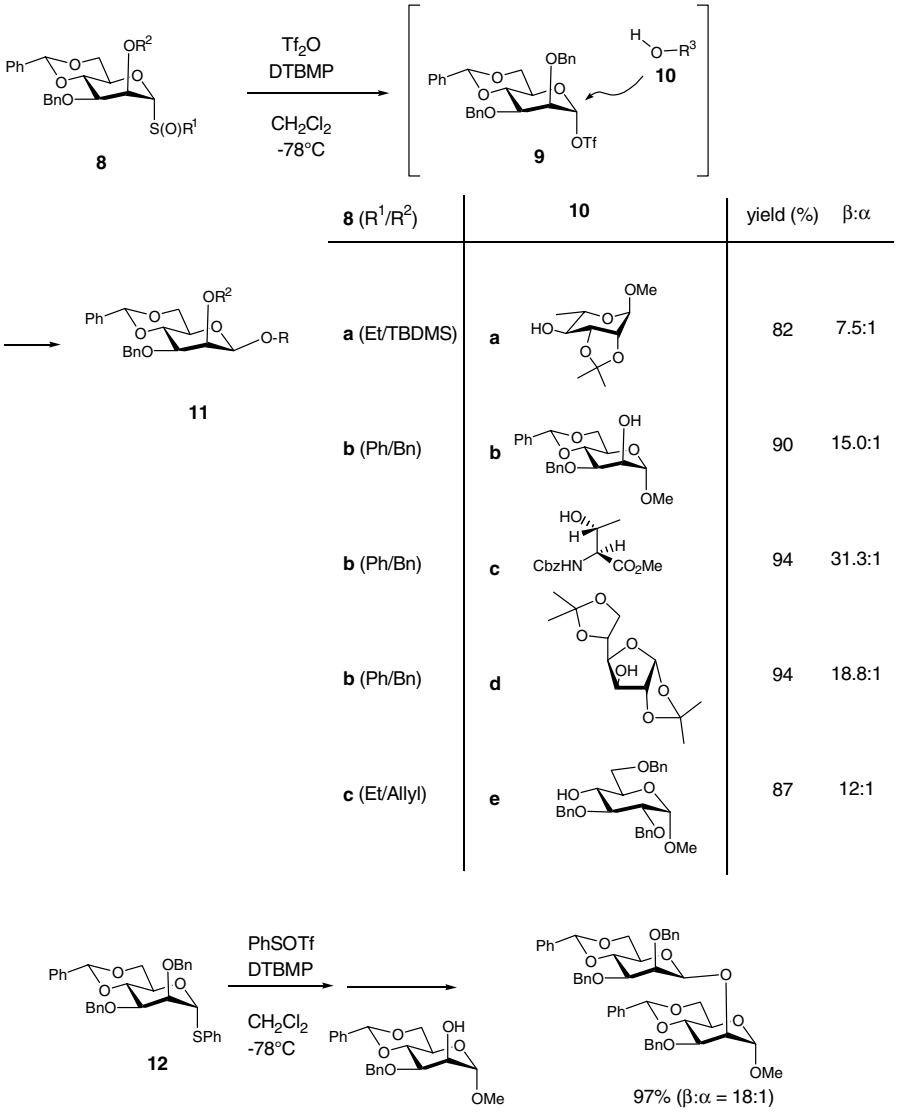
to be exceptionally powerful glycosyl donors that can be quickly activated at low temperature by Tf_2O.[19] Even highly hindered alcohols can be glycosylated successfully. Kahne further applied this method to solid-phase oligosaccharide synthesis and the generation of an oligosaccharide library.[38] After careful optimization of the reaction conditions, Crich discovered that highly selective β-mannosylation using 4,6-*O*-benzylidene-protected sulfoxide **8** can be performed. Thus, an impressively high level of β-selectivity was obtained when aglycon was added after the sulfoxide was preactivated at −78°C with Tf_2O. This selectivity was lucidly explained by in situ formation of α-glycosyl triflate **9**, which was verified by low temperature NMR studies.[39] It is quite reasonably assumed that this highly reactive species reacts with glycosyl acceptor in an S_N2-like manner with inversion of configuration to give β-glycoside as the major product. Although reaction with *N*-acetylglucosamine derived 4-OH proceeded only in a modest yield, glucose derived **10**, which is also known to be a hindered aglycon, was very successfully reacted with **8c** to afford corresponding disaccharide **11** with remarkably high yield and β:α ratio[37]



Scheme 7.8 Some examples of direct β-mannosylation.

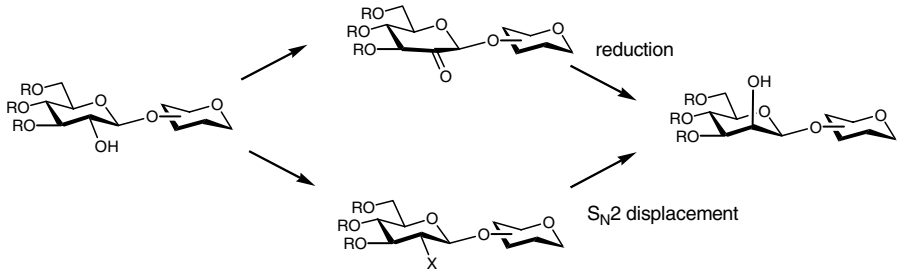
(Scheme 7.9). Equally effective and selective β-mannosylation was achieved with thioglycoside **12** using PhSOTf as a promoter.[36] Intermediacy of the anomeric triflate was again proposed.

In general, the most predictable strategy has been a glycosylation–inversion protocol; β-gluco (1,2-trans) glycosylation followed by the inversion of the C2 stereochemistry by oxidation–reduction[40–50] or by S_N2-type replacement (Scheme



8 (R^1/R^2)	10	yield (%)	β:α
a (Et/TBDMS)	**a**	82	7.5:1
b (Ph/Bn)	**b**	90	15.0:1
b (Ph/Bn)	**c**	94	31.3:1
b (Ph/Bn)	**d**	94	18.8:1
c (Et/Allyl)	**e**	87	12:1

Scheme 7.9 Glycosyl sulfoxide for highly selective β-mannosylation.



Scheme 7.10 Glycosylation–inversion approach to β-mannosides.

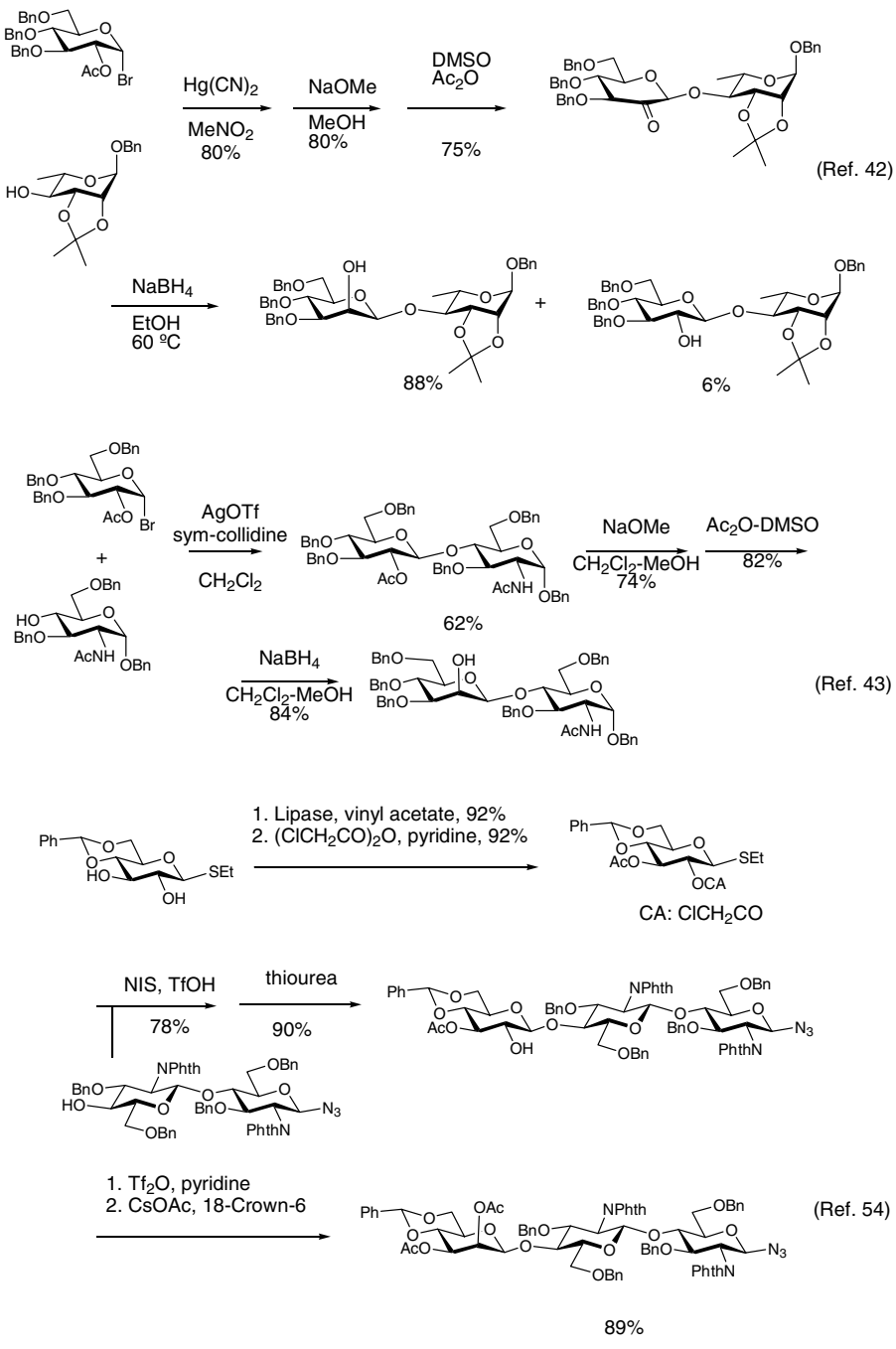
7.10).[51–56] Because β-gluco (1,2-*trans*) glycosides can be made without any stereochemical ambiguity, this approach is quite rational in terms of anomeric stereoselectivity. A number of variations of this approach have been reported and applied to the synthesis of glycoprotein glycans (Scheme 7.11). The most sophisticated among them is the intramolecular S_N2-type reaction developed by Günther and Kunz (Scheme 7.12).[56] Thus, inversion of the C2 position was performed with triflate **13** by intramolecular nucleophilic attack of 3-*O*-carbamate. Unverzagt developed a highly versatile synthetic route to Asn-linked glycan **14** by combined use of this approach with galactosyl transferase and sialyltransferase mediated glycosylation.[57]

Hodosi and Kovác reported the highly efficient β-mannosylation, which makes use of mannose-derived 1,2-*O*-stannylene acetal **15** in combination with an aglycon-derived triflate (Scheme 7.13).[58] A remarkable feature of this method is its extreme simplicity. Even totally unprotected mannose can be used as precursor of **15**. Undoubtedly, this method can be seen as one of the most facile methods for the stereoselective synthesis of β-mannosides.

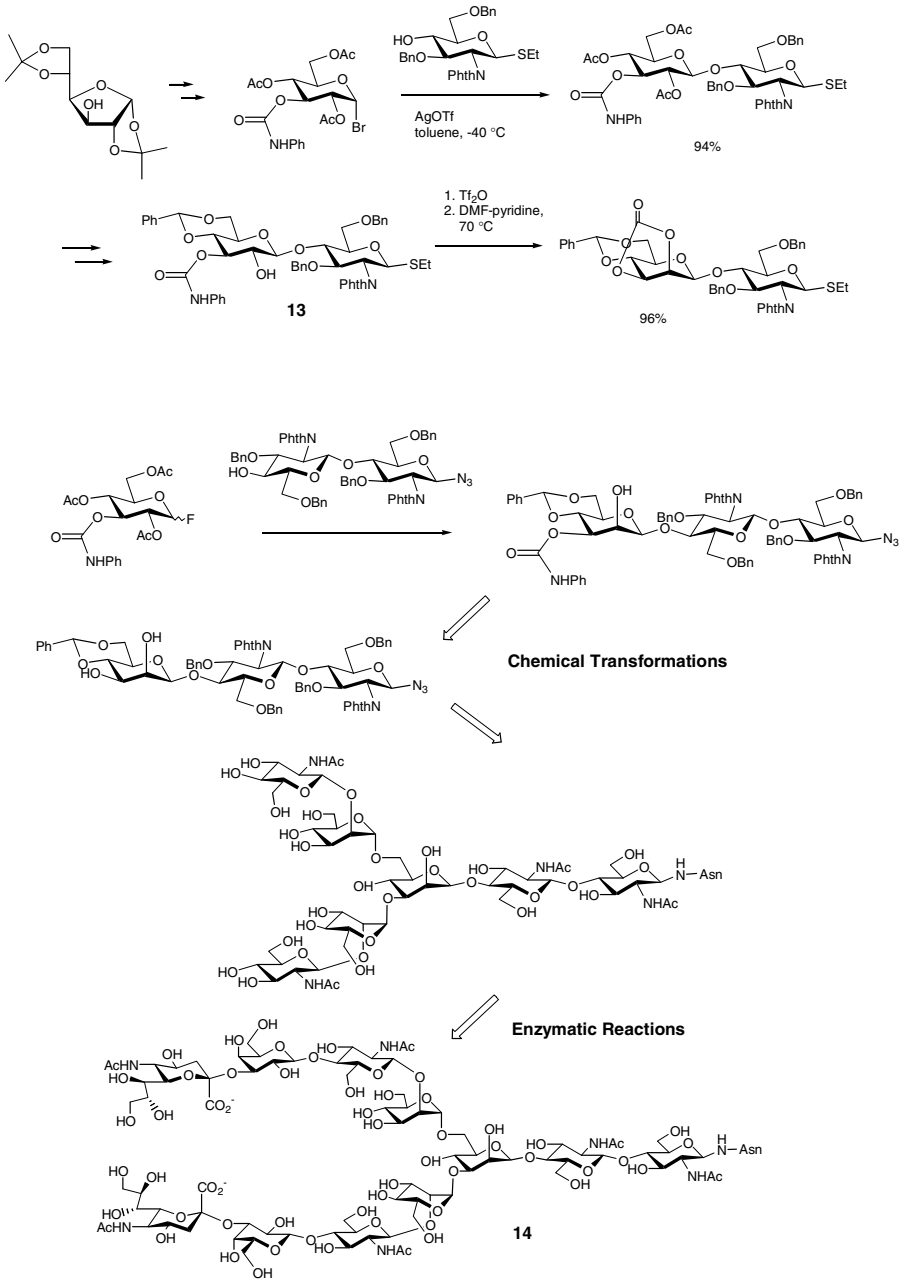
Another interesting approach was developed by Ikegami and coworkers, who used an anomeric orthoester as the key intermediate (Scheme 7.14).[59] Formation of orthoester **16** from lactone was effected by TMSOTf and TMSOMe. Subsequent Lewis acid mediated reduction afforded β-mannoside in high selectivity, presumably because of the stereoelectronically controlled hydride delivery from the α face.

Formation of β-manno glycoside by using enzymatic means (mannosidase and mannosyl transferase) have been investigated with substantial success (Scheme 7.15). For instance, Crout and coworkers developed a highly efficient synthesis of βMan1→4βGlcNAc1→4GlcNAc, a core trisaccharide of Asn-linked glycans, by using β-mannosidase together with *N*-acetylhexosaminidase.[60] This system affords the very facile access to βMan1→4βGlcNAc1→4GlcNAc in a preparatively useful scale. An efficient expression system of recombinant yeast β(1→4) mannosyl transferase was developed by Flitsch and coworkers, who performed a milligram scale β-mannosyltransferase-catalyzed glycosylation.[61]

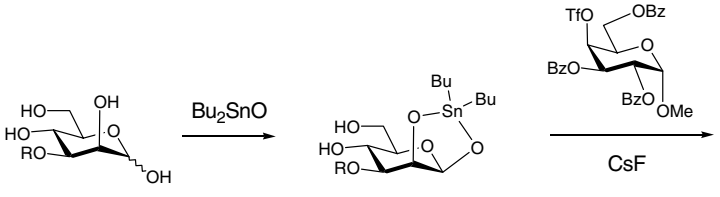
Seeking for general and highly stereoselective β-mannosylation, our attention was drawn to the approach based on the emerging concept of intramolecular aglycon

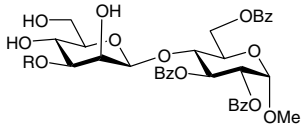


Scheme 7.11 Some examples of the C2 inversion approach.



Scheme 7.12 Intramolecular S_N2 reaction by Kunz.

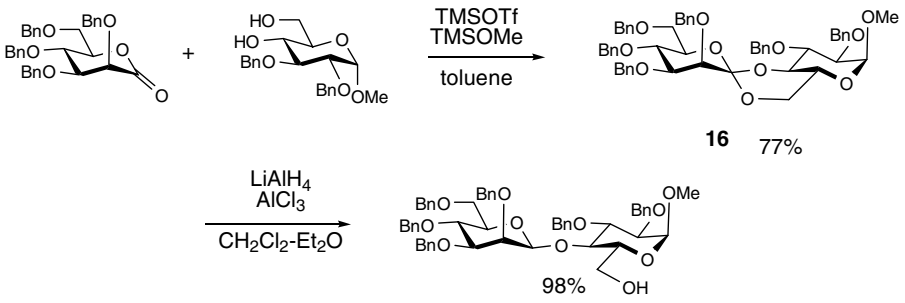




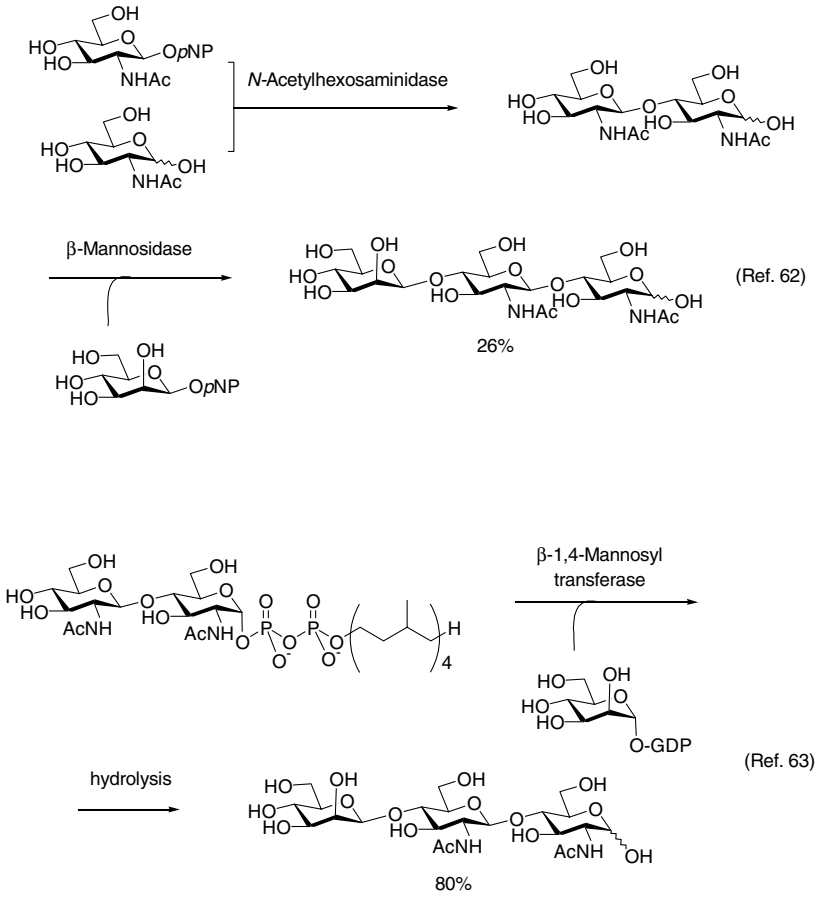
15	R	solvent	yield (%)
a	H	DMSO	59
b	Bn	DMF	67

Scheme 7.13 β-Mannosylation using mannose-derived 1,2-*O*-stannylene acetal in combination with an aglycon-derived triflate.

delivery (IAD), which was first reported by Baressi and Hindsgaul (Scheme 7.16).[62–64] This approach is highly attractive because exclusive formation of the correct stereoisomer can be expected. The aglycon is linked to the axially oriented oxygen at the 2 position, and can approach the oxocarbenium ion only from the β face. Formation of α-isomer is essentially prohibited by the stereochemical constraint. Starting with 2-*O*-acetylated thiomannoside, isopropenyl ether **17** was prepared and converted to the mixed isopropylidene acetal **18**. The latter compound serves as the tethered intermediate for IAD. Subsequent treatment with NIS activates the thioglycosidic linkage to give β-mannoside. Shortly after this disclosure, Stork and coworkers reported the use of silylketal-like intermediate **19** for a similar purpose (Scheme 7.17).[65,66] Although these important achievements convincingly demonstrated the potential of IAD for β-mannosylation, the absolute



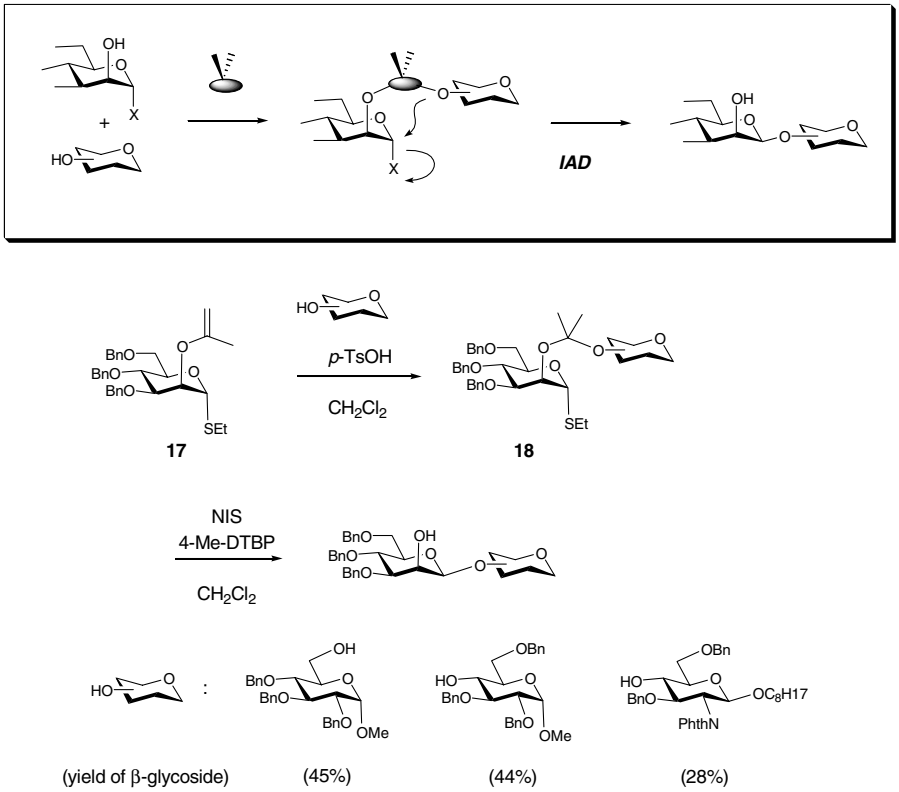
Scheme 7.14 β-Mannoside via anomeric orthoester.



Scheme 7.15 Enzymatic synthesis of βMan1→4βGlcNAc1→4GlcNAc.

yield of β-mannoside was comparable only with that obtained by conventional approaches.

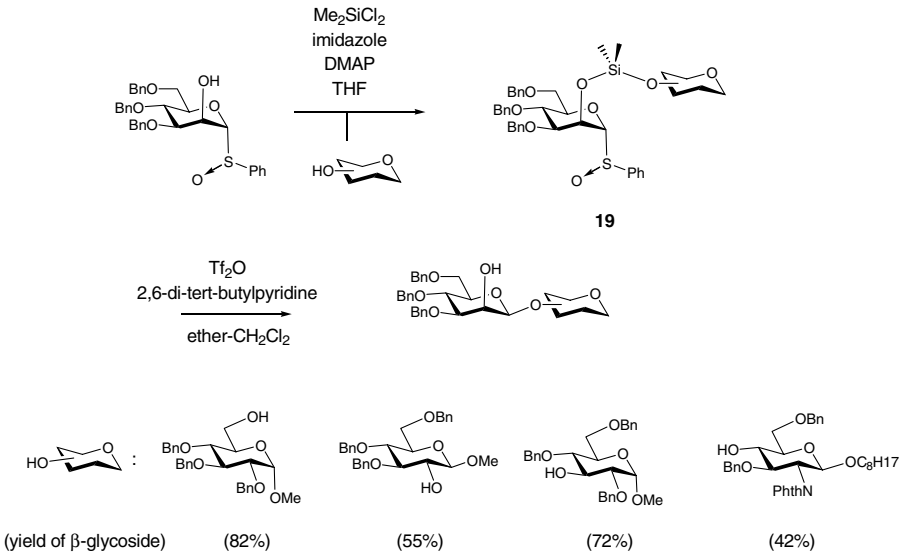
In order to enhance the efficiency and practical utility of IAD, we started our investigation using mannosyl donor **20** equipped with 2-*O*-*p*-methoxybenzyl (PMB or MPM) group (Scheme 7.18).[14] PMB has been widely used as a versatile protecting group that can be removed quickly under specific and mild conditions, by the action of DDQ[67] or CAN.[68] Since the deprotection with DDQ presumably proceeds via hydrolytic quenching of quinonemethide like intermediate **21**, it was expected that, when the reaction is performed in the presence of aglycon with exclusion of moisture, mixed acetal **22** could be formed. Subsequent activation of the anomeric position should trigger IAD to selectively afford β-mannoside. This scenario is highly attractive, because (1) various methods for the introduction of the PMB group have been established and (2) the conditions required for the formation of the mixed acetal



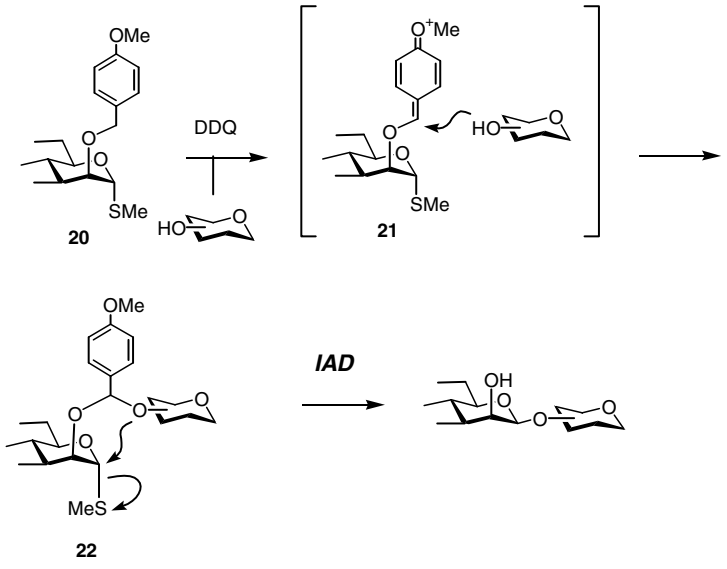
Scheme 7.16 Intramolecular aglycon delivery for β-mannosylation.

are mild and near neutral, and may well be comparable to the presence of various types of protecting groups. More importantly, compared to other IAD systems developed previously, more efficient charge delocalization and therefore clean IAD via a mesomerically stabilized transition state was expected, due to the assistance of an electron donating *p*-methoxy group.

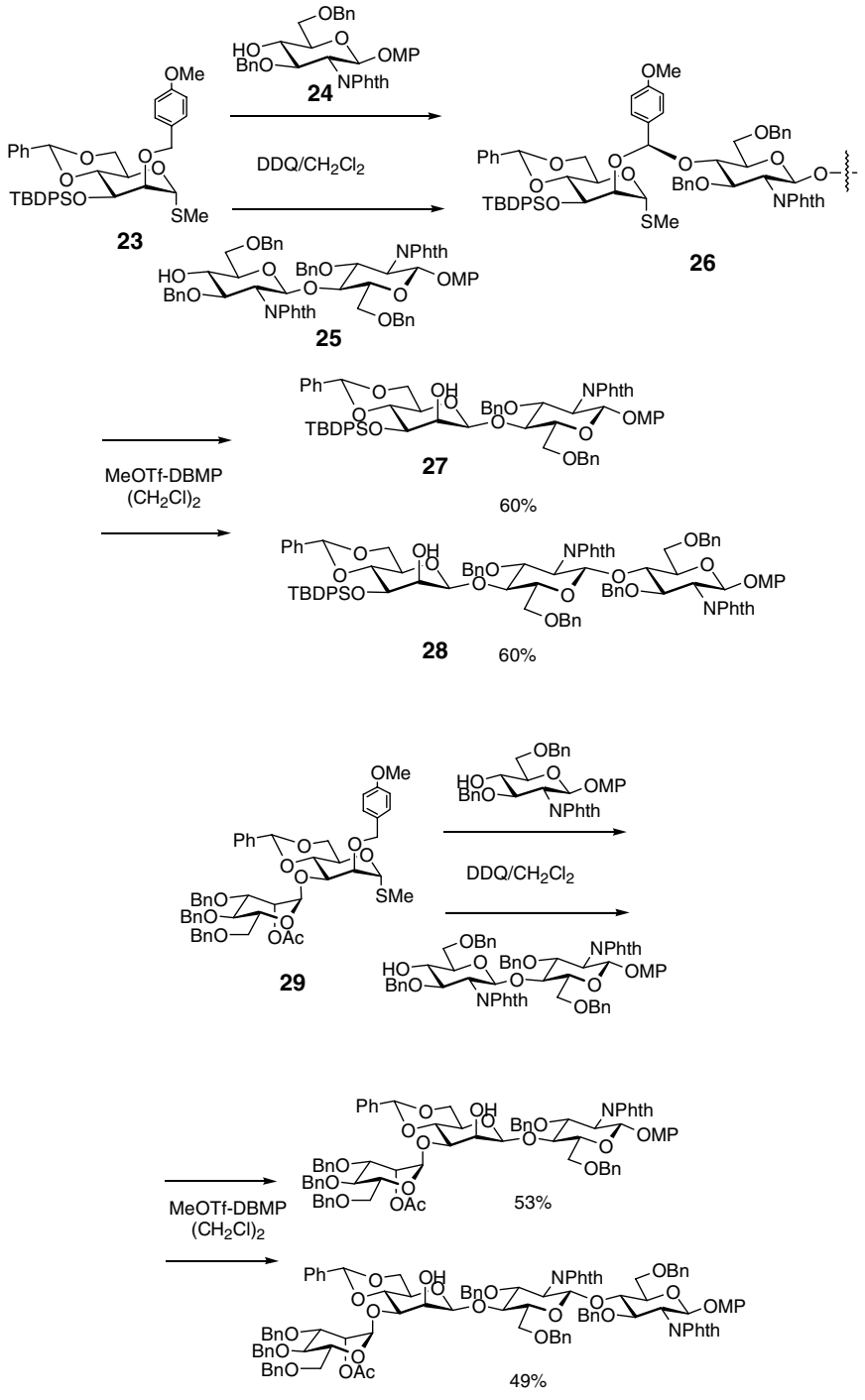
In practice, DDQ-mediated mixed acetal formation proved to be quite efficient and was complete within a few hours at room temperature. As expected, subsequent IAD proceeded stereoselectively to give β-glycoside. Methyl trifluoromethanesulfonate (MeOTf) proved to be the most suitable for this purpose. For instance, thiomannoside **23** afforded 60% yield of β-glycoside **27** and **28**, via mixed acetal **26** (Scheme 7.19).[69] One of the most distinct features of this type of transformation is its applicability to oligosaccharide fragment condensation. For instance, disaccharide donor **29** was reacted with **24** and **25** to give corresponding products with reasonable efficiency (Scheme 7.19). Even a 3+2 coupling using trimannoside donor **30** and disaccharide-derived acceptor **25** was successful. Resultant **31** was transformed to **32**, thereby completing the



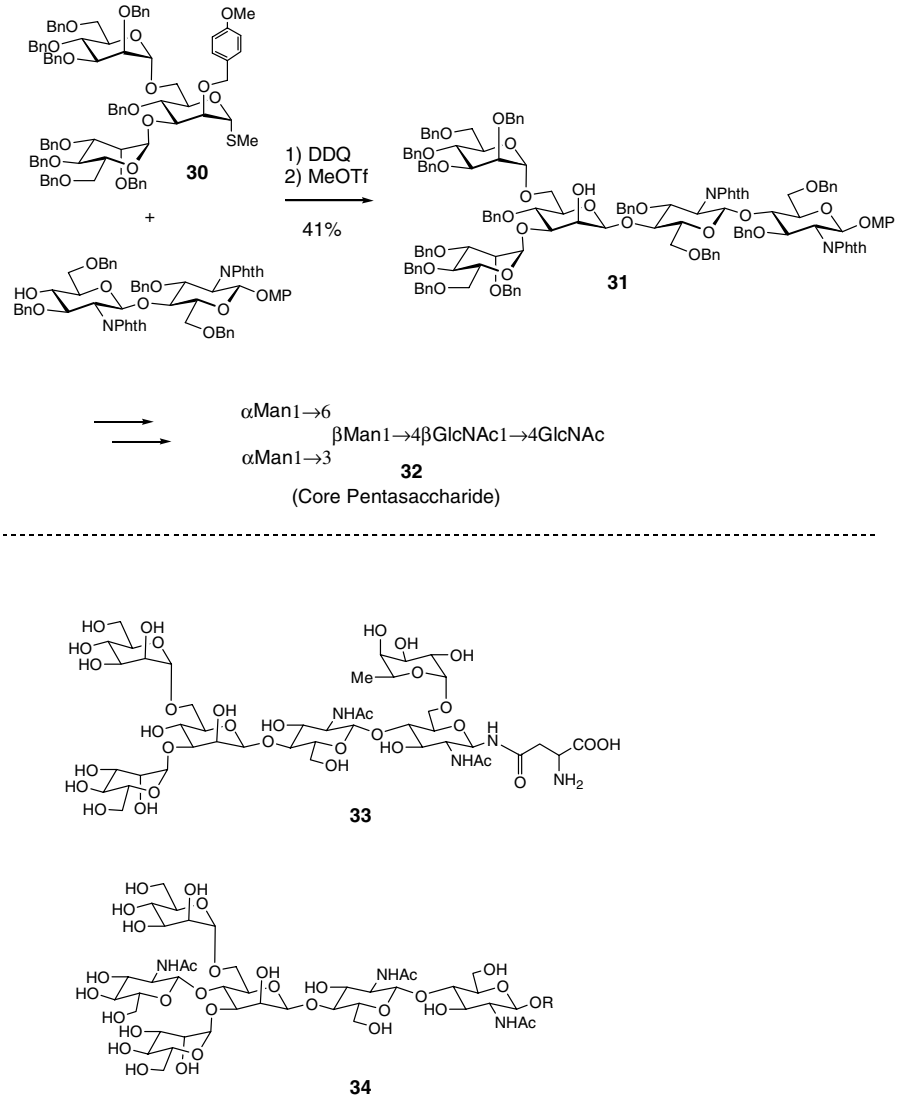
Scheme 7.17 β-Mannoside synthesis using a silylketal-like intermediate.



Scheme 7.18 *p*-Methoxybenzyl-assisted intramolecular aglycon delivery.



Scheme 7.19 Synthesis of core structures of Asn-linked oligosaccharides.



Scheme 7.20 Convergent synthesis of core pentasaccharide.

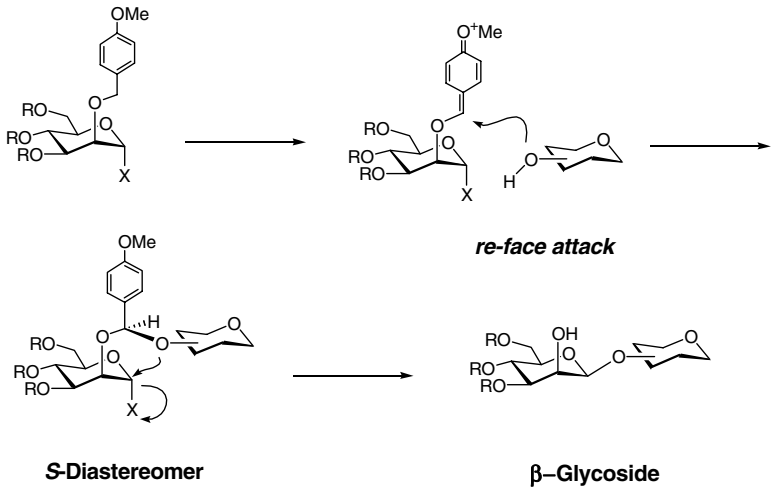
convergent and fully stereocontrolled synthesis of the core pentasaccharide structure of Asn-linked glycans (Scheme 7.20).[70]

Since all hydroxyl groups of the β-linked mannose residue can be distinguished, stereo- and regiocontrolled synthetic routes to essentially all types of Asn-linked glycans can be conceived. This aspect was demonstrated by the synthesis of

fucose-containing hexasaccharide **33**[71] and bisecting GlcNAc carrying structure **34**,[72] and subsequently by completion of the chemical synthesis of the full structure of complex type undecasaccharide.

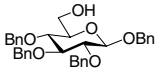
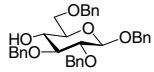
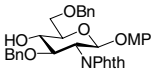
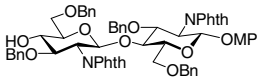
The stereochemical issue of the acetalic carbon was subsequently addressed. Specifically, two diastereomers are possible at the stage of mixed acetal formation. Quite remarkably, the formation of mixed acetal proved to be highly selective to give almost exclusively the *S*-diastereomer. Mixed acetals with various substitution patterns were synthesized and uniformly stereoseletive formation of *S*-isomers was observed in each case (Scheme 7.21).[73]

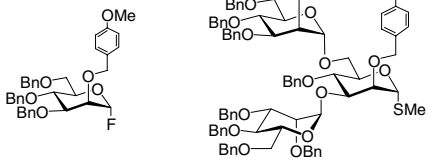
The efficiency of PMB was further enhanced by modifying the protecting group pattern. As summarized in Table 7.1, highly efficient β-mannosylation was achieved with 4,6-*O*-disiloxane-protected **38** or 4,6-*O*-cyclohexylidene-protected **35**. The latter afforded di- and trisaccharide products **36** and **37a** in ~80% yield (Scheme 7.22).[74] Since 4,6-*O*-isopropylidene carrying **39** was less efficient compared to **35**, the rigidity of the pyranose ring seems to be important. Trisaccharide product **37b** obtainable from **35** was successfully used as the key intermediate in the first purely chemical synthesis of prototypical complex-type glycan chain **40**.[75] In this case, trisaccharide product **37b** was transformed to **41** and glycosylated with sialic acid containing trisaccharide donor **42** to give **43**. Subsequent deprotection and regioselective glycosylation at the 6 position afforded undecasaccharide that was transformed into target **40**. The same oligosaccharide structure was synthesized previously by Unverzagt using a chemoenzymatic approach.[57]



Scheme 7.21 Stereochemical assignment of mixed acetal.

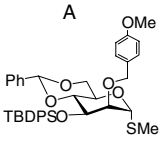
TABLE 7.1 Summary of β-Mannosylation Reactions

Acceptor	Donor	Yield	Promoter[a]	Ref.
	A	74	1	72
	A	52	2	14
	A	40	2	14
	B	60	3	73
	C	53	3	73
	E	83	3	73
	F	60	3	73
	G	75	3	73
	B	53	3	73
	C	49	3	73
	D	41	3	73
	E	85	3	73

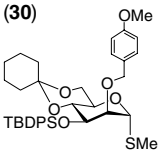


A

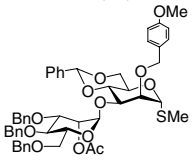
D (**30**)



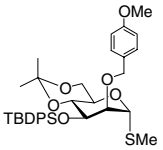
B (**23**)



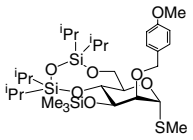
E (**35**)



C (**29**)

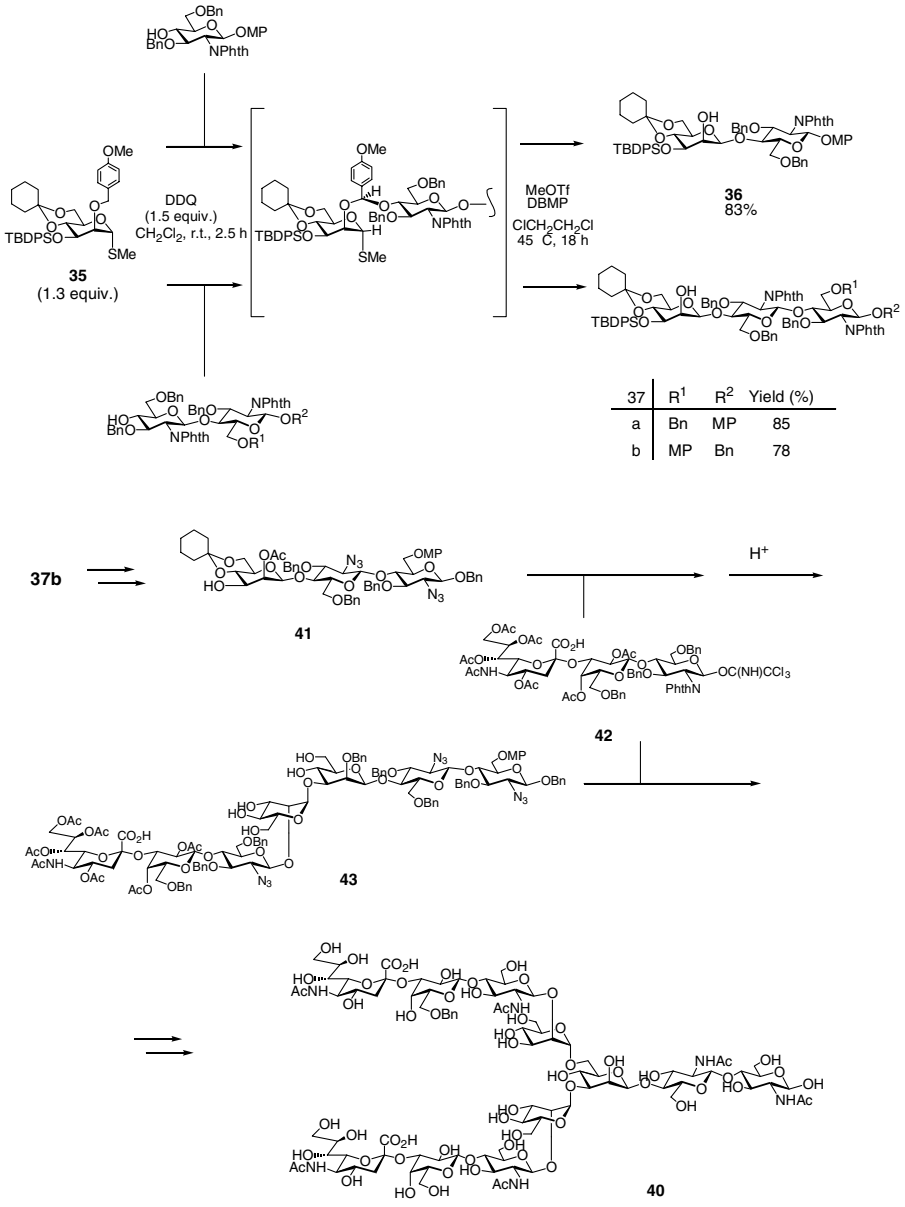


F (**39**)



G (**38**)

[a](1) $AgClO_4$–$SnCl_2$/Et_2O; (2) $AgClO_4$–$SnCl_2$/CH_2Cl_2; (3) MeOTf/$ClCH_2CH_2Cl$.



37	R^1	R^2	Yield (%)
a	Bn	MP	85
b	MP	Bn	78

Scheme 7.22 Cyclohexylidene-protected mannosyl donor.

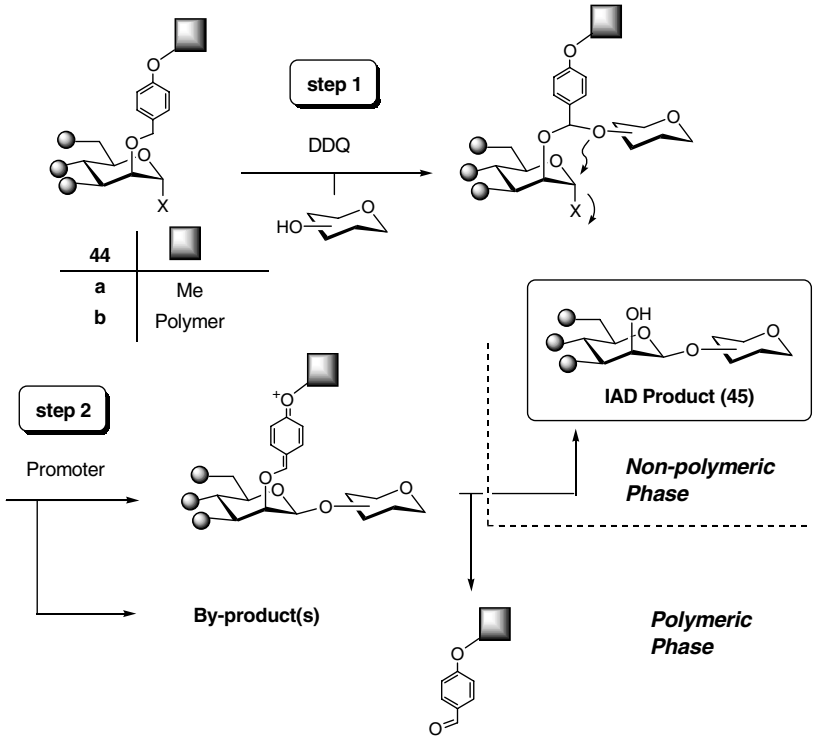
7.2 INTRAMOLECULAR AGLYCON DELIVERY ON POLYMER SUPPORT: GATEKEEPER-CONTROLLED GLYCOSYLATION

The intramolecular aglycon delivery (IAD) approach for β-mannoside synthesis using the PMB group for tether formation, was revealed to be highly efficient. This approach not only guarantees the exclusive formation of the correct stereoisomer, even at the stage of oligosaccharide fragment coupling, but also has enough flexibility to be applied to branched structures of Asn-linked glycan chains.

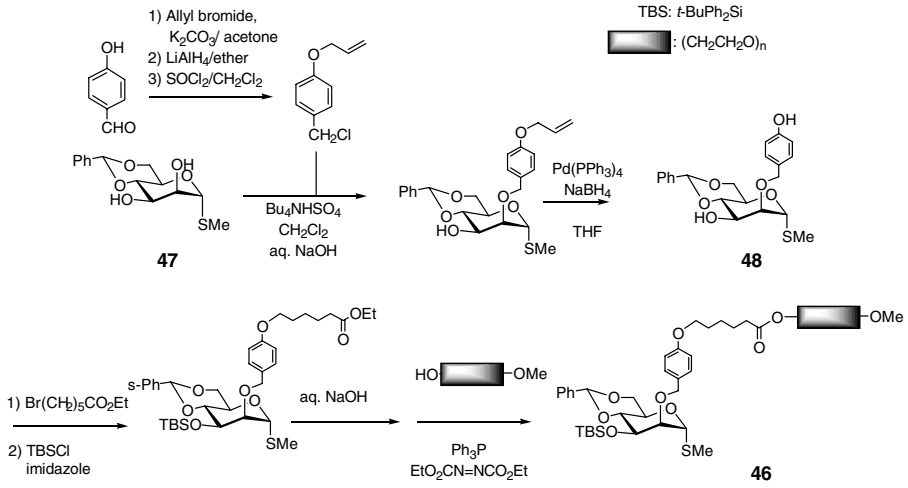
IAD consists of two steps: oxidative formation of the mixed acetal from mannosyl donor **44a** and aglycon (step 1) and initiation of IAD (step 2) by an appropriate promoter. Of particular note was that the IAD was accompanied by the cleavage of PMB and the glycosylated product is obtained as a 2-OH derivative. Therefore, if this transformation is performed starting from the mannosyl donor **44b** that has a polymer linked PMB-like group at C2, the desired β-mannoside **45** is expected to be removed from the nonpolymeric phase (Scheme 7.23). Since unreacted aglycon can be washed away from polymer-bound mixed acetal after step 1 and all major byproducts in step 2 arises from the failure of IAD process should stay bound to the polymer, the nonpolymeric part after two-step transformations should become highly enriched with the desired product. In that sense, the polymer support serves as a "gatekeeper," which specifically releases the correct product. Its function is clearly distinct from those of conventional polymer supports, which bind to the growing oligosaccharide chain throughout the synthesis.[15]

Polymer-supported donor **46** having a PMB-like group at C2 was designed and prepared in an analogous manner as PMB carrying thioglycoside **23**. Polyethylene glycol [PEG, MW (molecular weight) ~5000] was used as the polymer support. As it is endowed with several favorable features, PEG has gained considerable popularity as a "soluble" polymer support in various aspects in organic synthesis. It is well known that PEG derivatives are soluble in a variety of organic and aqueous solvents and most of reactions can be performed under homogeneous conditions.[76] This particular property is very suitable for our purpose, because we noticed that reasonably high substrate concentration (100–200 mM) is required for the optimum mixed acetal formation, and it was assumed that pseudo-high-dilution conditions inherent to solid phase reactions should be avoided. In spite of their solubility, PEG supported materials can be readily precipitated from certain solvents, for instance, *t*-butyl methyl ether (TBME), ethanol, or isopropanol, whenever necessary.[76,77] It was required to incorporate a linker having a *p*-methoxybenzyl-like functional group, in order to effect tether formation by DDQ mediated oxidative conditions. As a precursor to the linker, *p*-allyloxybenzyl chloride was envisaged, which was prepared very conveniently from *p*-hydroxybenzaldehyde by a three-step process.

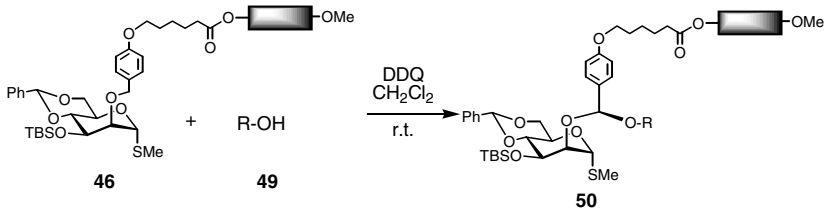
Preparation of the donor **46** was started from 4,6-*O*-benzylidene protected thiomannoside **47** (Scheme 7.24). Alkylation with *p*-allyloxybenzyl chloride under phase transfer conditions[78] was followed by 3-*O*-silylation and Pd(0)-mediated deallylation[79] to give **48**. The phenolic OH group was alkylated with ethyl 6-bromohexanoate and carboxylic acid, liberated by alkaline hydrolysis, was reacted with PEG monomethyl ether (MW ~5000) under Mitsunobu conditions to afford **46**.



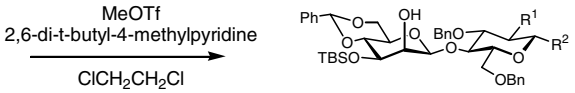
Scheme 7.23 Intramolecular aglycon delivery on polymer support.



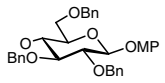
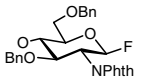
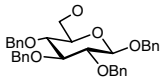
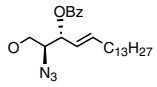
Scheme 7.24 Preparation of polymer-supported mannosyl donor.

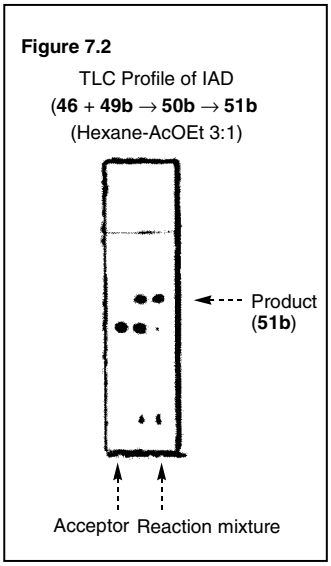


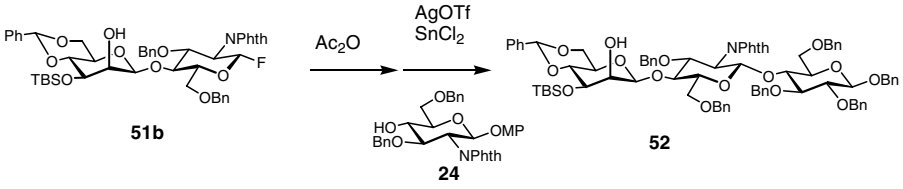
46 49 50



51

49	R-O	yield (%)
a		50
b		37
c		43
d		54





51b 24 52

Scheme 7.25 Polymer-supported β-mannosylation.

β-Mannosylation was first performed by using **49a**,**b** as a glycosyl acceptor (Scheme 7.25). Thus, **46** and **49** (2.6 equiv) in CH_2Cl_2 were treated with DDQ (2.9 equiv) in the presence of 4Å molecular sieves (r.t. 3 h) to afford mixed acetal **50** [**a**: δ_H 5.58 (s, H-1^{Man}), 5.46 and 4.94 (s, acetal CH), **b**: 5.87 (dd, J = 54 and 7 Hz, H-1^{GlcN}), 5.63 (d, J < 1 Hz, H-1^{Man}), 5.35 and 4.80 (s, acetal CH)], which was precipitated from *t*-butyl methyl ether (TBME), collected by filtration and washed with TBME. Judging from ^{1}H NMR, it was apparent that DDQ-mediated mixed acetal formation was highly efficient, even in the polymer-supported case, to give **50** with excellent quality. Of additional note was the stereochemical homogeneity of the acetal carbon, which was obvious from ^{1}H NMR analysis. The configuration was assigned to be *S*, in analogy with nonpolymeric counterparts. IAD was promoted by the action of MeOTf-MeSSMe (4 equiv each) in the presence of 2,6-di-*t*-butyl-4-methylpyridine (DBMP, 4 equiv) in 1,2-dichloroethane at 40°C. In accordance with our initial expectations, the TLC profile (hexane:ethyl acetate 3:1) of the reaction mixture (Fig. 7.2) showed the nearly exclusive formation of the desired product, except for the presence of polymer-bound materials that remained at the origin. After very simple processing, β-mannosides **51a**,**b** were obtained in pure form. In a similar manner, **49a**,**b** were also stereoselectively β-mannosylated. The compatibility with the orthogonal glycosylation strategy was verified using fluoride **49b** as an acceptor. The product **51b** was immediately acetylated and used as the glycosyl donor for the further coupling with **24** to complete the βMan1→4βGlcNAc1→4GlcNAc sequence **52**, commonly found in Asn-linked glycans. Since the mannose residue of the compound **52** is 4,6-*O*-benzylidene and 3-*O*-silyl protected, all hydroxyl groups can be distinguished and further elongation of glycan chain at C3, C4, and C6 of Man can be performed based on the well established methodologies.

7.3 CONCLUSIONS

p-Methoxybenzyl-assisted intramolecular aglycon delivery provides a highly efficient protocol for the preparation of β-mannosides. In addition to the complete stereoselectivity and high yield, our method has proved to be flexible enough to find application in the synthesis of naturally occuring *N*-linked glycans. With the best donor **35**, the trisaccharide core sequence of Asn-linked glycans (i.e., βMan1→4βGlcNAc1→4βGlcNAc) can be synthesized in an unprecedented high yield. In particular, the first chemical synthesis of the complex-type undecasaccharide **40** was achieved recently in a fully stereocontrolled manner. The polymer-bound version of this transformation is featured by its "gatekeeper" function, which would dramatically simplify the isolation process of the correctly formed product. The yield of the β-mannoside currently obtainable by the polymer-supported approach is lower than with the original non-polymeric approach. Since it was revealed that the efficiency of our PMB assisted β-mannosylation is sensitive to the 4,6-*O*-protecting group pattern (i.e., cyclohexylidene > TIPDS > benzylidene ≅ isopropylidene > benzyl), further optimization would be possible in the polymer-supported version. In particular, further systematic studies concerning choices of activation conditions

(promoter, solvent, concentration, etc.), leaving group, protecting pattern, and the nature of polymer support and linker should allow this method to stand as a practical tool in β-Man-containing oligosaccharides.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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8 Tools for "On-Bead" Monitoring and Analysis in Solid-Phase Oligosaccharide Synthesis

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8.1 INTRODUCTION

A host of analytical techniques, including thin-layer chromatography and NMR spectroscopy, are available to the researcher in the field of solution-phase organic chemistry. These methods enable rapid assessment of the progress of the reaction in question.

The development of conditions for solid-phase synthesis in general has been hampered by the lack of tools that allow for reaction monitoring on the solid support. For a long time reaction development required that after each step a part of the sample be separated and the product cleaved from the resin followed by workup and analysis using traditional solution-phase methods. This practice was time-consuming, expensive, and wasteful, particularly in the context of multistep syntheses. An improvement in terms of time economy was made when mass spectrometric methods, in particular matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry, were introduced for the fast analysis of crude cleaved oligosaccharide products.[1] For more detailed analytical information, however, workup including chromatography of semipreparative samples (e.g., HPLC) was still necessary. Fast and nondestructive methods that would allow for the analysis of intermediates and products while still attached to the bead are clearly desirable.

In order to meet these demands, a number of on-bead analytical techniques[2] were adopted. The methods briefly outlined below have an immense impact on the development of new methods for solid-phase oligosaccharide synthesis by allowing direct monitoring of the reactions as they unfold.

8.2 IR SPECTROSCOPIC METHODS

An important tool for the fast characterization of intermediates and products in solution-phase synthesis are vibrational spectroscopic techniques such as Fourier transform infrared (FTIR) or Raman spectroscopy. These concepts have also been successfully applied to solid-phase organic chemistry. A single bead often suffices to acquire vibrational spectra that allow for qualitative and quantitative analysis of reaction products,[3] reaction kinetics,[4] or for decoding combinatorial libraries.[5]

FTIR microspectroscopy is a fast, nondestructive method that is extremely useful when reactions involving groups with characteristic absorption bands, such as esters and terminal double bonds, are monitored. This method has proved very helpful in the development of reaction conditions in solid-phase oligosaccharide synthesis since spectra can be acquired in less than a minute on various resins. Success and completion of glycosylation reactions and *O*-deprotection reactions on the resin were observed by monitoring the carbonyl band of ester protecting groups in our[6,7] and other[8] laboratories. Conditions for reductions such as of resin-bound anomeric disulfides to the corresponding thiols[9] and of 2-deoxy-2-azidoglycosides to the deprotected aminosugar derivative[10] have also been developed with the information provided by on-bead IR spectroscopy. Similarly, the reduction of TentaGel-bound 2-deoxy-2-azido glucuronic acid derivatives to the corresponding amines has been monitored by *diffuse reflectance infrared Fourier transform spectroscopy*[11] (DRIFTS) as illustrated in Figure 8.1.[12] The disappearance of the azide absorption band at 2108 cm^{-1} marks completion of the reduction. This method holds potential for synthesis automation and for library screening since automated DRIFTS devices are commercially available.

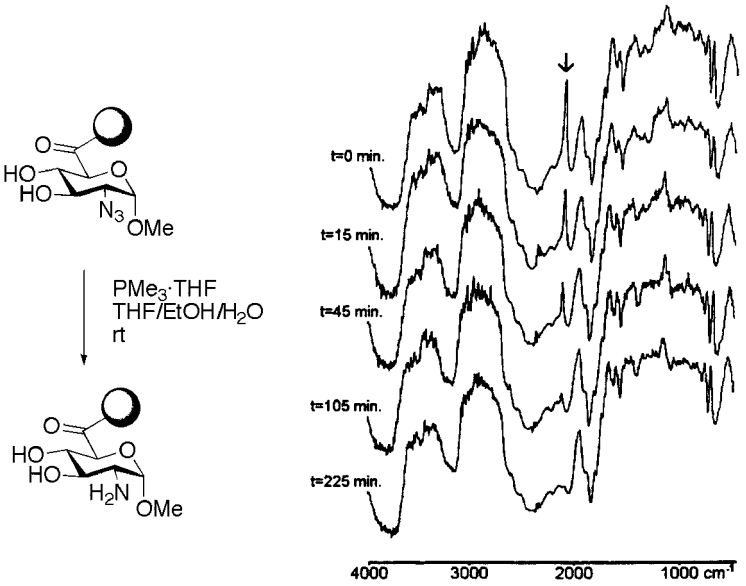


Figure 8.1 Time-resolved DRIFTS spectra of the reduction of a resin-bound 2-deoxy-2-azidoglucuronic acid derivative to the corresponding amine.[12]

FTIR methods have also been used to monitor the completion of cleavage reactions of carbohydrates from the resin. Cleavage based on ring-closing metathesis (RCM) was monitored by the disappearance of a terminal double-bond stretching band,[13] while progress of the cleavage of an acyclic acetalic linkage to Wang aldehyde resin was established by the reappearance of the carbonyl stretching band.[14]

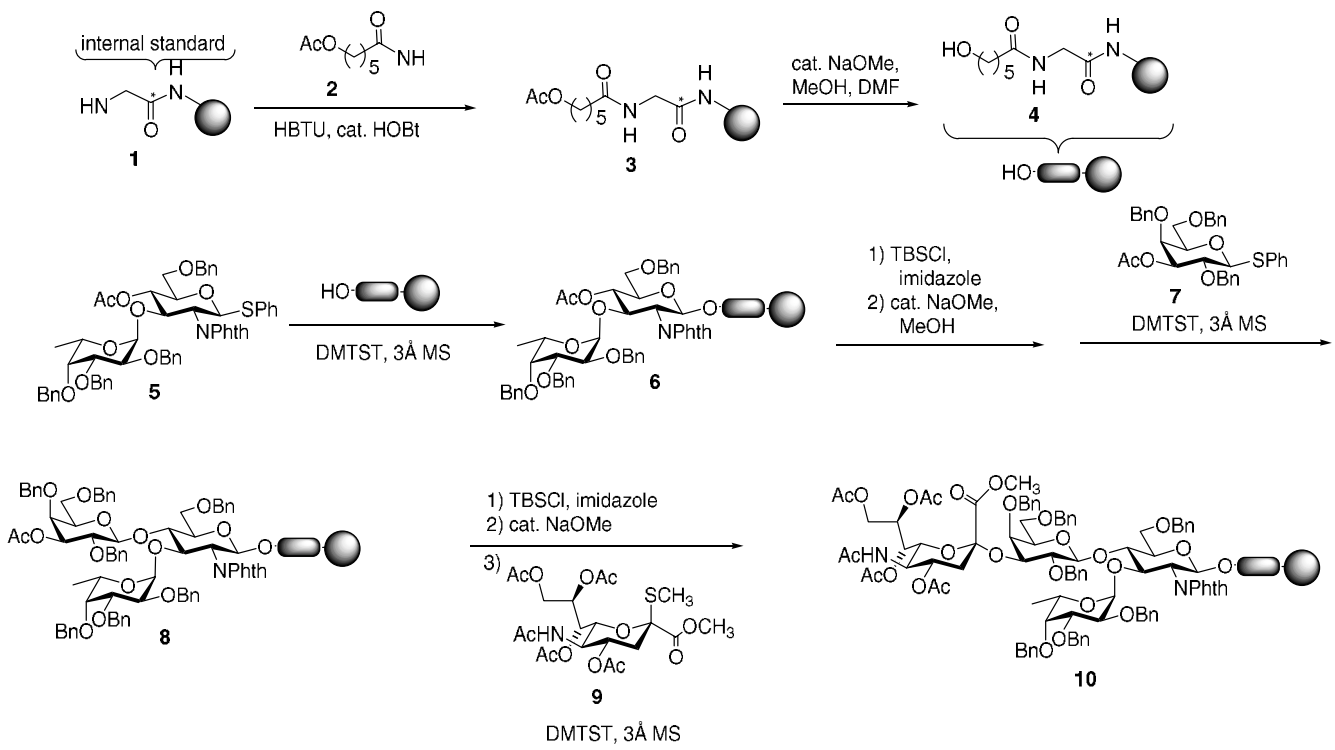
FTIR-based methods proved very useful in the development and monitoring of solid-phase reactions for oligosaccharide synthesis, but are limited to reactions involving IR-active functional groups. Furthermore, these methods fail to provide structural information needed for complete on-bead characterization of the growing oligosaccharide chain. In particular, information regarding the selectivity of glycosidic linkage formation or purity is crucial. In conventional solution-phase organic syntheses, this information is routinely obtained by NMR spectroscopic methods. In the following section we focus on applications of NMR spectroscopy to solid-phase oligosaccharide synthesis.

8.3 NMR SPECTROSCOPIC METHODS

NMR spectroscopy is an invaluable tool for the generation of detailed structural information in organic synthesis. However, conventional NMR spectra acquired on a gel of the resin in a regular NMR tube exhibit very broad signals. Gel-phase ^{13}C NMR spectroscopy[15] has been used in the development of solid-phase oligosaccharide synthesis[1a] on better swelling PEG-polystyrene composites, although it was not successful when applied to Merrifield's resin. This limitation notwithstanding, the use of a standard broadband decoupled pulse sequence with TentaGel-bound monosaccharides revealed semiquantitative information on the stereochemical outcome of several glycosylation reactions dependent on the reaction solvents and participating neighboring groups, which was obtained from the chemical shifts and intensities of the respective anomeric carbon signals.

A conventional high-field NMR-spectrometer was also used in a study involving gated decoupling ^{13}C NMR spectroscopy[16] for quantitative monitoring of solid-phase oligosaccharide synthesis.[17] This pulse sequence was applied in the presence of a relaxating agent in order to suppress intensity differences due to nuclear Overhauser effect (NOE) at carbon centers with adjacent hydrogen. Carbon-13-enriched protecting groups were employed to improve the signal-to-noise ratio when monitoring the carbon signals during the synthesis of a sialyl Lewis^x tetrasaccharide on a TentaGel support (Scheme 8.1).

Quantitative monitoring was achieved by comparison of the protecting group signal with the signal of a ^{13}C-enriched glycine that had been incorporated into the linker as internal standard. Coupling efficiency at each of the four coupling and deprotection steps was monitored by comparison of the internal standard with a ^{13}C-labeled protecting group incorporated via a thioglycoside donor (Fig. 8.2). Yields determined by this method were in good accordance with those obtained after cleavage from the support. While this analysis allows for the very effective and nondestructive assessment of the reaction yield and necessitates no special probe head,



Scheme 8.1 Synthesis of a sialyl-Lewis^{x} tetrasaccharide employing ^{13}C-enriched protecting groups for the quantitative reaction monitoring using gated decoupling NMR spectroscopy.

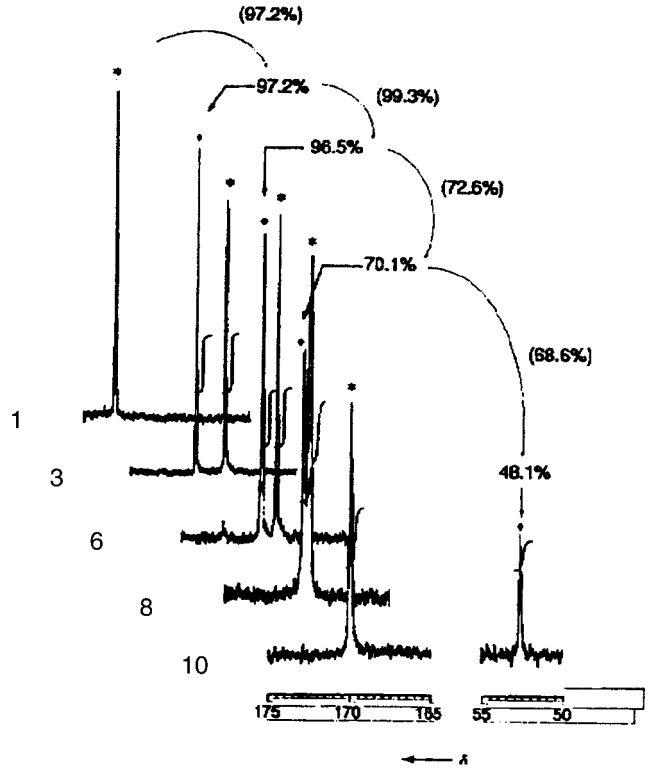


Figure 8.2 Parts of the ^{13}C spectra of the functionalized resins **1**, **3**, **6**, **8**, and **10**. Yields were calculated from the signal intensities.[17]

it requires the use of ^{13}C-enriched protecting groups. Furthermore, no information about the anomeric composition of the generated oligosaccharide was obtained.

For real on-bead analysis that goes beyond mere monitoring, it is desirable to obtain structural information from one- and two-dimensional proton NMR experiments. Purity, connectivity, and the presence or absence of functional groups can ideally be assessed without requiring isotopic labeling. High-resolution magic-angle spinning NMR (HR-MAS) techniques have been developed,[18] which allow high-resolution ^{1}H spectra to be obtained from resin-bound molecules. The major difficulty in using ^{1}H spectroscopy to analyze a resin-bound molecule is the intrinsic linewidth.[19] Solid-state NMR techniques are not sufficient to reduce the proton linewidths to the point where *J* couplings are observable. HR-MAS employs a solvent to swell the resin, thereby imparting mobility, which allows averaging of dipolar and chemical shift anisotropy (CSA) effects that are responsible for the large linewidths in solid-state spectra. This produces a spectrum (Fig. 8.3a) that is still broad by solution standards, but that definitely begins to show structural features (based on chemical shift). By spinning the slurry at the magic angle at rates of 1–2 kHz, residual dipolar and CSA effects, as well as magnetic susceptibility differences (due to the

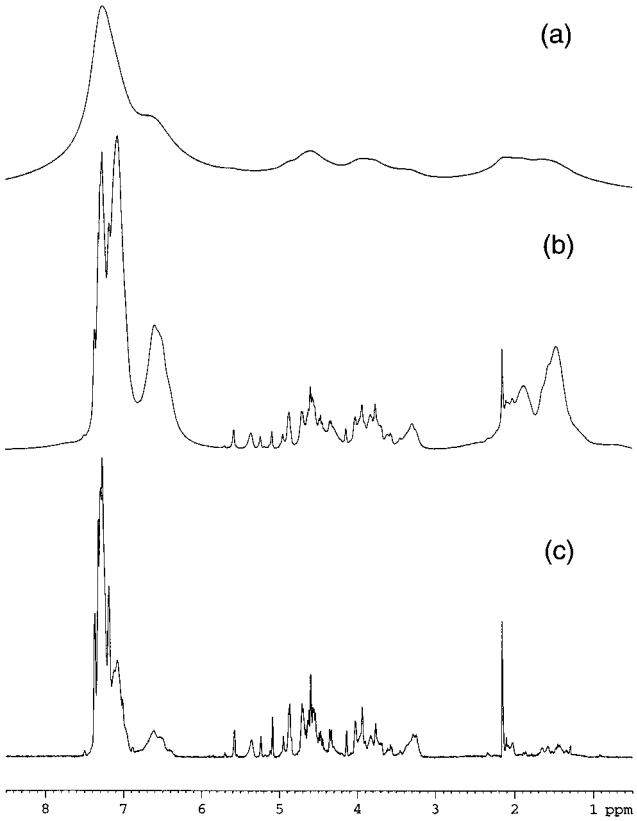


Figure 8.3 Illustration of HR-MAS techniques applied to a resin-bound trisaccharide: (a) static ^{1}H spectrum of the solvent swollen sample; (b) ^{1}H spectrum with magic-angle spinning at 3.5 kHz; (c) ^{1}H spectrum with MAS and spin echo pulse sequence.

heterogeneous nature of the sample) are significantly reduced, narrowing ^{1}H linewidths to the same order as ^{1}H–^{1}H J couplings (Fig. 8.3b). Employing a spin-echo pulse sequence (Fig. 8.3c) allows the discrimination of signals based on T_2 relaxation rates, which results in a spectrum where most of the resin resonances have been significantly attenuated and the ^{1}H–^{1}H J couplings of the resin-bound moiety are visible.

In addition to well-resolved one-dimensional (1D) ^{1}H and ^{13}C spectra, which are usually sufficient for monitoring synthetic steps, HR-MAS techniques can be applied to two-dimensional (2D) homonuclear and heteronuclear experiments, which allow a wealth of structural information to be obtained. ^{1}H,^{13}C HMQC (heteronuclear multiple quantum coherence) spectra are particularly useful in the analysis of solid support-bound oligosaccharides, since the anomeric protons exhibit characteristic resonances. Such a spectrum of a polymer-bound trisaccharide glycal is shown in Figure 8.4.

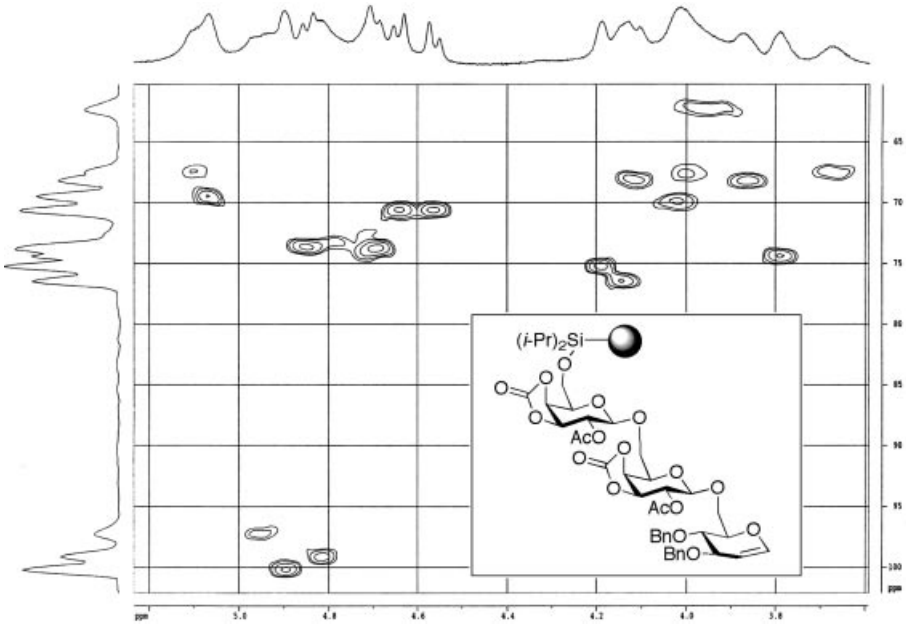


Figure 8.4 Example of an HR-MAS HMQC spectrum of a resin-bound trisaccharide glycal.[20]

We have characterized a resin-bound pentasaccharide by HR-MAS techniques. A comparison of the solution spectrum of the resin-cleaved pentasaccharide with the HR-MAS spectrum of the resin-bound pentasaccharide is shown in Figure 8.5. It is immediately obvious that the HR-MAS technique provides data of a quality similar to that of the solution technique, but in both cases, only four of the five anomeric protons are visible. However, a 2D homonuclear total correlation spectroscopy (TOCSY) spectrum (Fig. 8.6) of the resin-bound pentasaccharide allowed us to clearly observe the overlapped anomeric protons (demonstrating a resolution of 4.4 Hz).

The power of the HR-MAS method for on-resin analysis has been further underscored in the development of new linkers. Without this method, only indirect analytical data after removal from the resin was available. Direct assessment of the resin-bound linker greatly facilitated the introduction of a 4,5-dibromo octane-1,8-diol linker that was converted into an octane-1,8-diol linker cleavable by olefin metathesis at the end of the synthesis.[6] The disappearance and reappearance of the olefinic protons as well as the growing oligosaccharide chain was clearly visible in the ^{1}H spectrum (Fig. 8.7).[7]

Most of the methodology used so far in solid-phase oligosaccharide synthesis was developed before the analytical techniques outlined above had been adopted. Now that a closer insight into the reactions has become available, one may expect exciting

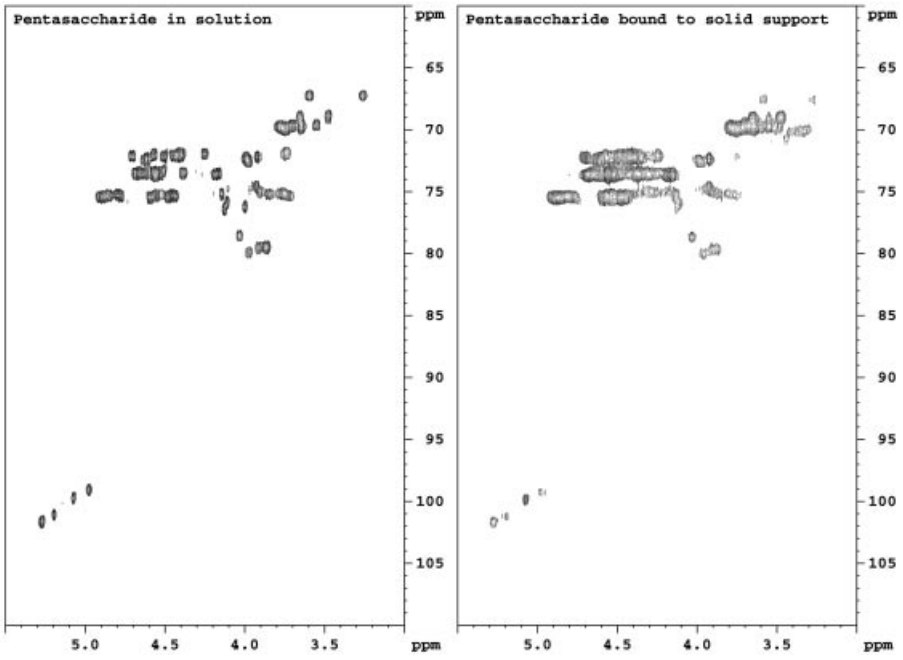


Figure 8.5 Comparison of $^{1}H,^{13}C$ HMQC spectra obtained on resin-cleaved and resin-bound samples of a pentasaccharide.

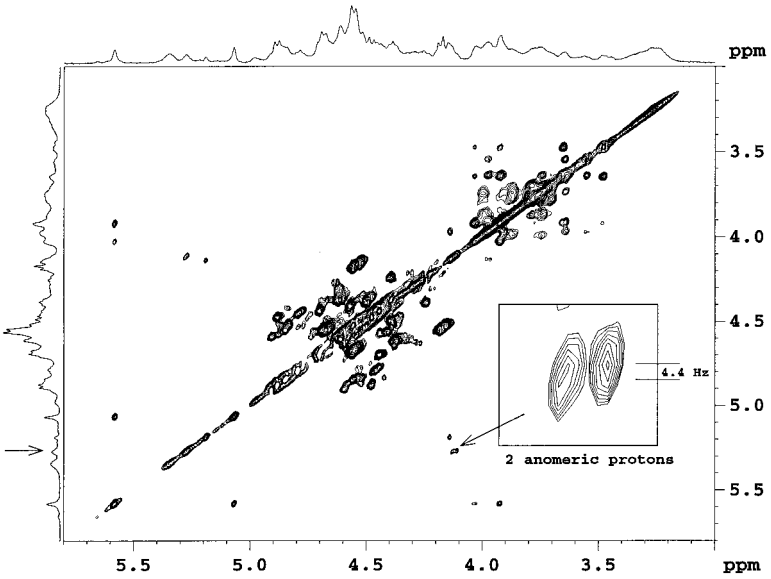


Figure 8.6 $^{1}H,^{1}H$ TOCSY spectrum of resin-bound pentasaccharide.

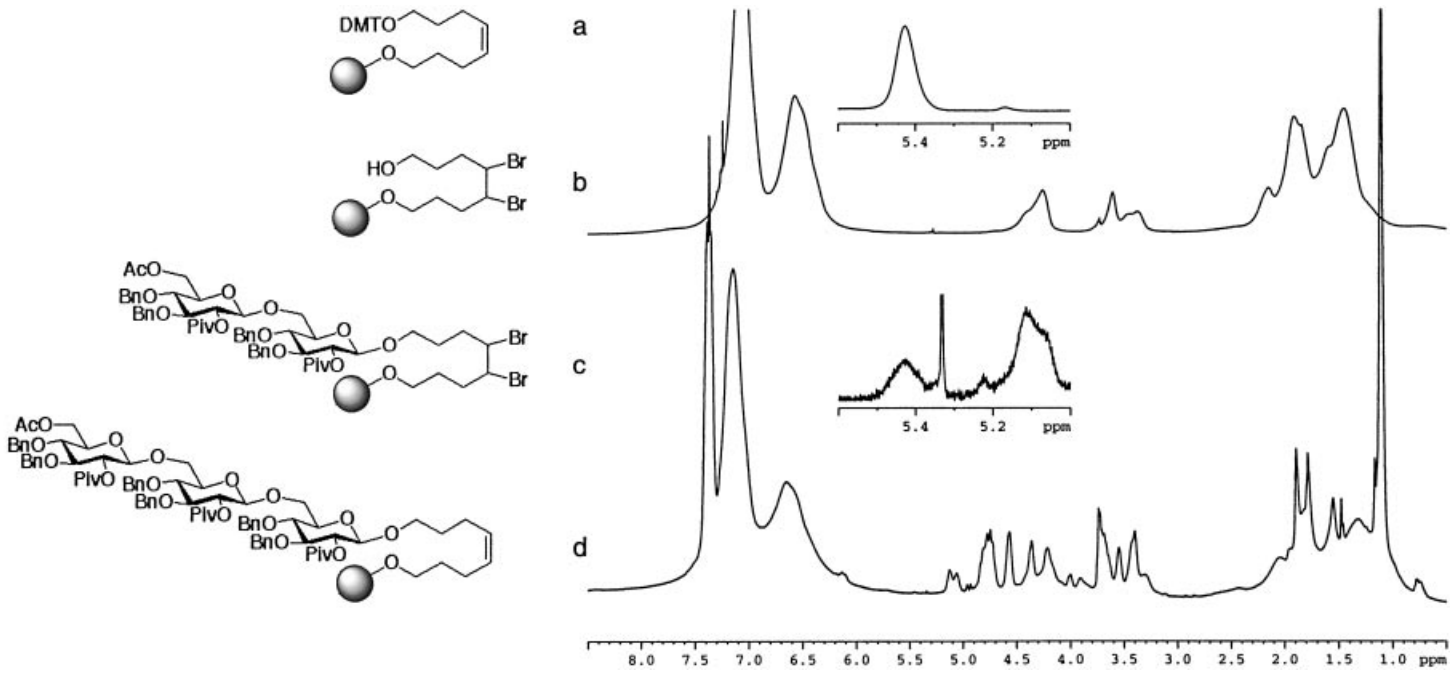


Figure 8.7 HR-MAS spectra of resin-bound intermediates in the solid-phase synthesis of a trisaccharide using thiodonors.[7] Traces *a* and *b* have been recorded using spin echo pulse sequences to suppress resonances of the resin. Traces *c* and *d* have been recorded using a standard proton experiment.

progress in future studies in this field that will facilitate the development of standardized procedures for the construction of an automated oligosaccharide synthesizer.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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9 Polyethyleneglycol ω-Monomethylether (MPEG)-Supported Solution-Phase Synthesis of Oligosaccharides

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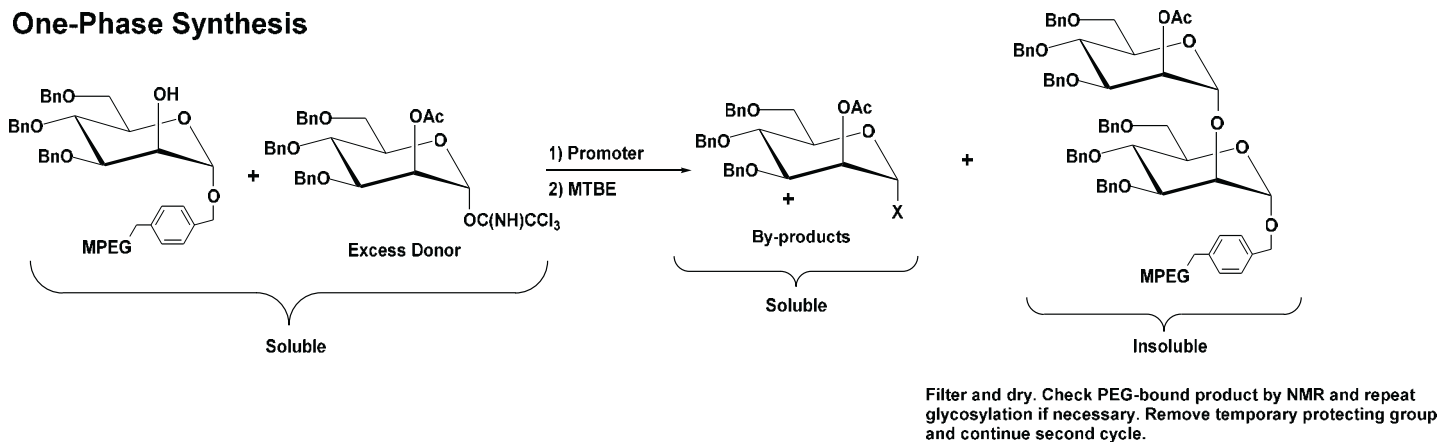
9.1 INTRODUCTION

It is now accepted that carbohydrates, including oligosaccharides, usually in combination with lipids and proteins, display diverse biological activities, and that the importance of most of them is not fully understood yet.[1] It is also agreed to that to improve this understanding, reasonably large quantities of pure oligosaccharides are needed.[2,3] These oligosaccharides can be made chemically, but this is a time-consuming process because chromatography must be used in most cases. Polymer-supported methods decrease considerably the time spent on purification. The polymer support is usually solid, therefore it is called *solid-state synthesis.* Normally quantities of oligosaccharides assembled on solid support are quite small, perhaps because it is difficult to handle large-scale two-phase reactions, and because some solid supports are relatively fragile. It appears to be easier to make larger quantities when all reaction components are in solution; that is, the polymer support should be dissolved in the reaction solvent at the time of its use as a part of a reactant. Reactions usually proceed faster when all reaction components are dissolved (unless a catalyst participates in the reaction). After the reaction is completed, the polymer with the oligosaccharide product is then rendered solid, to remove soluble impurities as with solid supports. The most important among the soluble but precipitable polymers is polyethyleneglycol, mostly its ω-monomethylether (MPEG). MPEG is particularly useful for syntheses of combinatorial oligosaccharide libraries, in which case the polymer support is essential to purify the desired intermediates or products as groups. PEG support has been used to synthesize larger quantities of short oligopeptides and oligonucleotides,[4] and as a general aid in organic synthesis.[5]

The solid-phase synthesis of oligosaccharides started in the early 1970s in Schuerch's group[6,7] with unsatisfactory results; among the problems were decreased reaction rate and lower yields compared to solution strategies, incomplete coupling, and poor anomeric specificity. These problems seemed to be attributed to the solid phase rather than its incompatibility with certain aspects of Koenigs–Knorr chemistry. It was suggested at that time that the use of a soluble polymer could improve the results,[8] but it took another 20 years until polyethyleneglycol as soluble support was examined.[9] It was also shown at this time[9] that the Koenigs–Knorr reaction (promoted by silver triflate) and trichloroacetimidate chemistry gave comparable results on MPEG, apparently confirming the assumption that the solid support (two-phase system) caused the failure of glycosylations. This conclusion became disputed when more data on two-phase synthesis were collected, and now it appears[10] that there are no significant differences between glycosylations performed in solution without a polymer support, with MPEG as a soluble polymer support, or using a solid polymer support,[11] although some variations were observed.[12] Insoluble catalysts are much more likely to be effective in a solution, that is, with a soluble support, as exemplified by the removal of boronate diester from 4,6-positions of galactose by Amberlite IRA-743.[13]

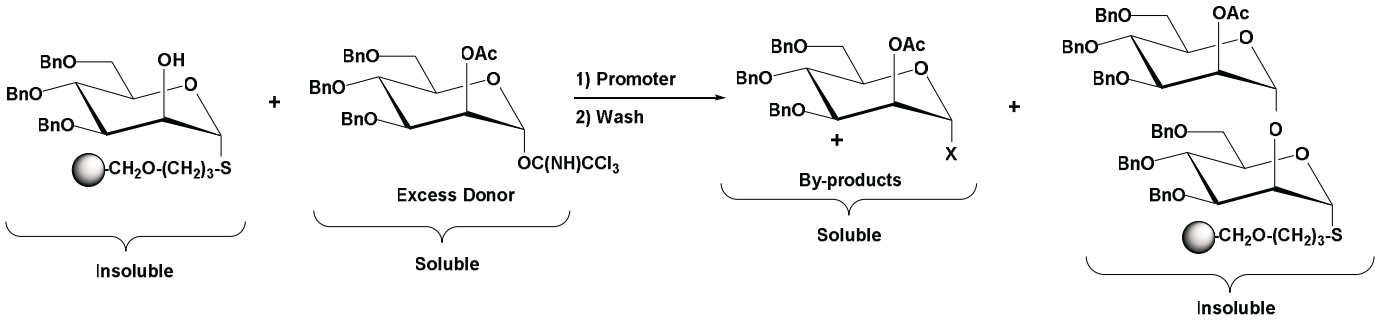
The difference between the solid-state (two-phase) synthesis and solution-phase (one-phase) synthesis pertains to the reaction stage. In the isolation stage, the products are supported by a solid polymeric support. Both one-phase and two-phase designs are depicted in Scheme 9.1; oligosaccharide chains can be extended (A) from the reducing end to the nonreducing terminus (using polymer-supported acceptors), as well as (B) from the nonreducing end to the reducing terminus (using polymer-supported donors) (Scheme 2). Both strategies (A and B) share problems attributable to incomplete glycosylations and/or donor decomposition. Problems caused by incomplete glycosylation tend to increase as the oligomer becomes longer; assuming that a small portion (probably ~ 3–5%) of polymer-supported monosaccharide M1 acceptor fails to be glycosylated to a disaccharide D1, the disaccharide D1 in the subsequent step is extended to a trisaccharide T1, but a portion of the previously unglycosylated monosaccharide M1 gives disaccharide D2. These second round glycosylations are again incomplete. After the third glycosylation the following compounds will be polymer-bound in addition to the desired tetrasaccharide: the monosaccharide M1; disaccharides D1, D2, and D3; and trisaccharides T1 and T2. After each additional glycosylation the mixture becomes even more complicated, but all impurities originating from the donors are absent. The mixture is detached from the polymer support, and the desired product must be separated from the truncated sequences (= failures). To minimize formation of truncated sequences, excess glycosylation reactants are recommended to be applied in portions (Scheme 9.2), as is practiced to obtain "difficult sequences" in polymer-supported oligopeptide syntheses. Incomplete anomeric specificity causes further complications for oligosaccharide syntheses.

Starting the synthesis from the nonreducing terminus, that is, using a polymer-bound donor, presents a similar problem of truncated sequences. Donors are

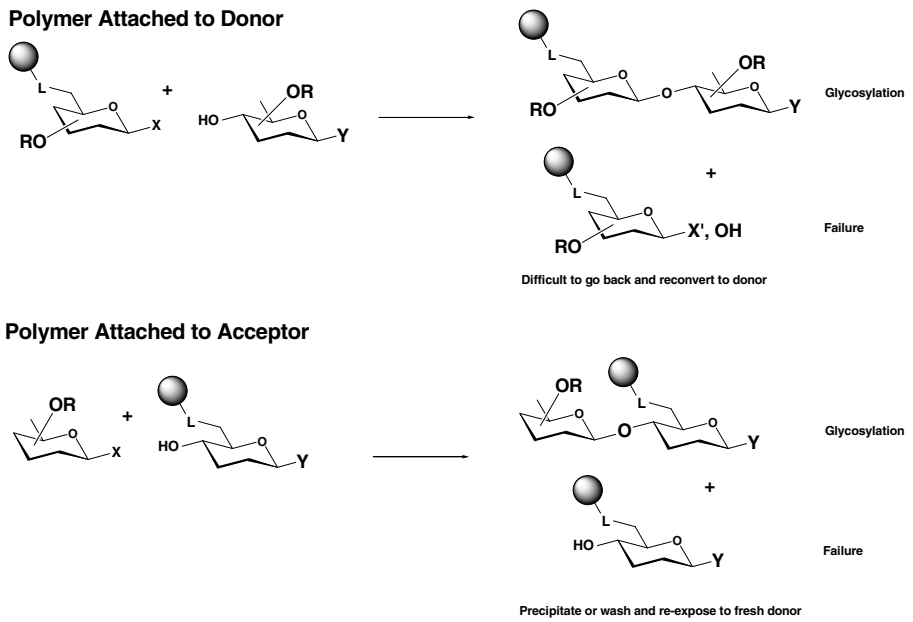


Scheme 9.1 Comparison of one-phase and two-phase polymer-supported synthesis of oligosaccharides. Continued next page.

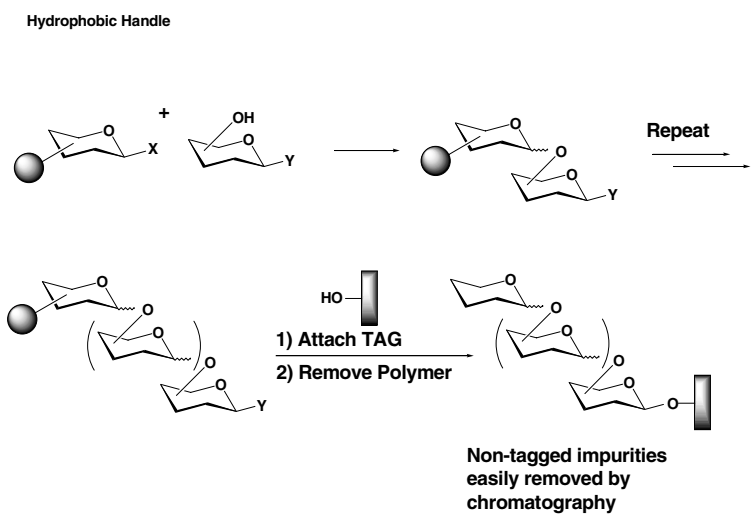
Two-Phase Synthesis



Scheme 9.1 *Continued.*



Scheme 9.2 Comparison of polymer-supported synthesis of oligosaccharides between the attachment of a polymer to a glycosyl donor and a glycosyl acceptor.



Scheme 9.3 Polymer-supported synthesis of oligosaccharides employing a hydrophobic handle attached to the growing chain in the last step of the synthetic sequence. The handle permits separation of the majority of failure sequences accumulated during the synthesis.

by definition quite reactive, and a portion of a donor may be transformed as a side reaction into molecules that do not react with acceptors. As these molecules are fastened to the polymer, they are carried through the synthetic sequence unchanged and must be removed by chromatography at the end of the synthesis (Scheme 9.2). A solution to this problem was suggested by Ogawa (Scheme 9.3).[14] The monosaccharide added last to the sequence is equipped with a hydrophobic group, such as 2-(trimethylsilyl)ethyl, which, after detachment from the polymer, permits isolation of the sequence containing the hydrophobic group by reverse-phase chromatography (previously utilized in carbohydrate synthesis by Hindsgaul's group),[15] thus separating the desired sequence from most failure sequences.

9.2 POLYETHYLENEGLYCOL ω-MONOMETHYLETHER (MPEG)

Oligopeptides and oligonucleotides are usually prepared by solid-phase (two-phase) techniques employing automated synthesizers in micromolar quantities, because of the combination of loading capacity (the loading capacity is a number of anchoring sites in millimole per gram of a support) of solid polymers and the limit of the support volume (mass) practically usable. For syntheses of these oligomers on larger scale, PEG-supported designs arose as methods of choice since PEG has sufficient mechanical and chemical stability that allow repeated contacts with the reagents. The same reasoning applies for syntheses of larger quantities of shorter sequences of oligosaccharides. Polyethyleneglycol ω-monomethylether (MPEG) of MW = 5000–6000,[16] has a loading capacity of approximately 0.2 mmol/g, which does not differ much from the loading capacities of polystyrene-based supports. MPEG is soluble in most solvents, including water, and thus the polymer-bound synthon can be dissolved under many reaction conditions. After completion of the reaction, ether such as methyl *t*-butylether (MTBE) is poured into the reaction mixture and the polymer-bound product precipitates because MPEG is insoluble in ethers. The remaining components of the reaction mixture can be removed from the desired MPEG-bound product by simple filtration (Scheme 9.1) because of their solubility in the precipitating solvent. If such impurities are not soluble, or if they are only partially soluble in the precipitating solvent, the purification effects of the precipitation is compromised. To improve the purification efficacy of the precipitation, isopropanol was introduced by Janda's group for nonoligosaccharide applications.[17] Isopropanol is expected to play an increasing role in syntheses of oligosaccharides as MTBE, which is a very inexpensive precipitating agent since it is mass produced as a gasoline additive, may be banned in the United States in the near future because of its toxicity and low biodegradability. Its high water solubility is responsible for extensive contamination of freshwater sources.

MPEGs of different average molecular weights are commercially available.* MPEG of average MW = 5000 are most frequently used, but MW = 2000 and 12,000 were used as well. Depending on size, MPEG forms rigid rodlike linear structures

*Suitable MPEG was obtained from Fluka AG, Buchs, Switzerland.

stabilized by intramolecular interactions.[18] It is soluble in most apolar solvents (benzene, toluene, methylene chloride, chloroform, acetonitrile, acetone) as well as in water, and insoluble in hexane, diethylether, and methyl-*t*-butylether, isopropanol and cold ethanol. MPEG can be dissolved in hot tetrahydrofurane and ethanol; therefore, it is commonly purified by precipitation from ethanol. Otherwise, it is purified by precipitation with ether from its solutions in the organic solvents quoted above. Although MPEG is strongly hygroscopic, the adsorbed water can be almost completely removed by azeotropic distillation (e.g., with toluene or benzene); wet MPEG precipitates with ether only reluctantly. Methyl-*t*-butylether is safer to use for the precipitation than diethylether since its boiling and flash points are higher.* Recovery of MPEG derivatives is usually no less than 95%. This recovery is lower when MPEG is complexed with metal cations (e.g., mercury, chromium, cerium). Therefore, many common metal-containing reagents cannot be used with substrates containing MPEG. MPEG also precipitates less easily and is more difficult to filter if it carries higher-molecular-weight synthons. Precipitation with alcohols (isopropanol, methanol) gave similar results, although isopropanol may provide better purification results since it may dissolve contaminants better than ethers.

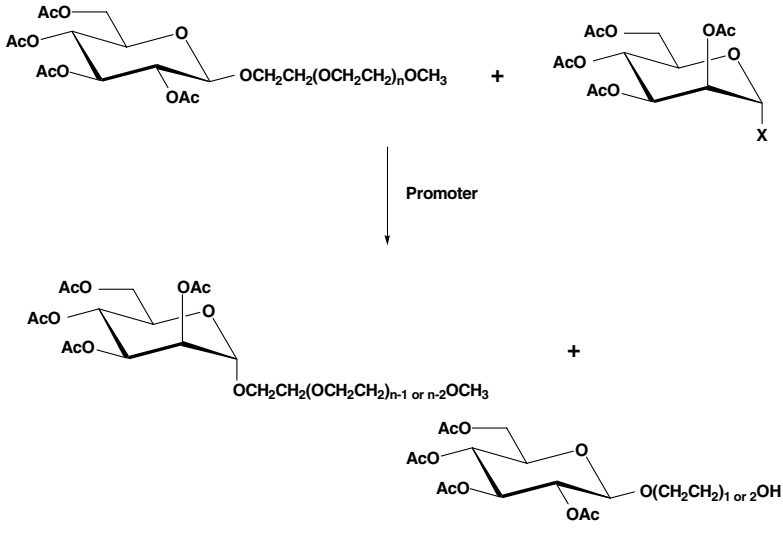
To improve precipitability of MPEG when the synthesized oligosaccharide extends beyond five monosaccharides and its protected form approaches MW 5000, MPEG of average MW = 12,000 can be used. Also, shorter MPEG of average MW = 2000 was used in some cases, and instead of precipitation, a filtration through a silica gel column was used for purification.[19] Although MPEG is stable enough under many reaction conditions that it can be considered a protecting group, some conditions cause its partial degradation.[16,20] For instance, pyridinium salts peel off one or more ethylene glycol units. Furthermore, MPEG cannot be located directly on the anomeric carbon. Treatment of an MPEGyl glycoside (e.g., 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside) with a glycosylating agent such as acetobromomannose causes a glycosyl exchange to take place and MPEGyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannoside is formed, and soluble glycolyl or (bis)glycolyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucoside can be isolated from the mother liquors[21] after the MPEG-bound saccharide is precipitated (Scheme 9.4).

The solubility of MPEG in organic solvents allows monitoring of the progress of the synthesis by high-field ^{1}H NMR spectroscopy using the signal of its terminal OCH_3 group ($\delta = 3.380$ ppm) as internal standard. Since the glycol peak of MPEG at $\delta = 3.640$ ppm is very strong, it is usually suppressed by irradiation for 3.0 s to increase the dynamic range and obtain a good-quality spectrum. However, this signal is strong enough to be a baseline-separated singlet even without the above presaturation, and its integration also serves to estimate MPEG loading.[22] The ^{1}H NMR spectra are commonly of such a good quality that structural information to identify the product can be usually obtained from its spectrum.

PEG has been converted into a solid support by grafting functionalized polyethylene glycol chains onto polystyrene to form constructs such as TentaGel**

**tert*-Butylmethyl ether of sufficient quality and degree of dryness for this purpose is available inexpensively in bulk quantities from ARCO (Atlantic Richfield) Chemicals.

**Rapp Polymere GmbH, 7072 Tübingen, Germany.



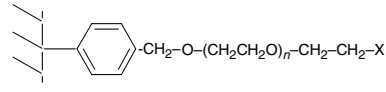
Scheme 9.4 Illustration of the instability of polyethylene glycol directly attached to the anomeric carbon.

TABLE 9.1 Polymer Supports Based on PEG, Showing the Functionality to Which Linkers Are Attached[a]

Polyethylene glycol methyl ether:

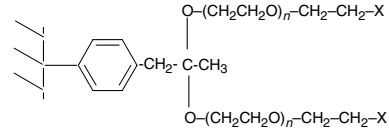
$CH_3O–(CH_2–CH_2–O)_n–CH_2–CH_2–OH$

TentaGel:



X = OH, NH_2, others

ArgoGel



X= OH, NH_2, others

[a]In both TentaGel and ArgoGel, swelling in organic solvents, polyethylene glycol is grafted on 1–2% crosslinked polystyrene–divinylbenzene with loading capacity 0.3 mmol/g/. ArgoGel is claimed to be chemically more stable.

and ArgoGel (Table 9.1).* These chains are highly mobile and can be considered to be in "solution."[23] Such supports have been extensively used.[24] For instance, TentaGel-N, linked via the succinoyl linker to a monosaccharide acceptor through an amidic bond, has utilized trichloroacetimidate chemistry to prepare disaccharides[25] and trisaccharides.[26] However, the delicate nature of these supports will probably not allow their use for large-scale syntheses of oligosaccharides.

9.3 LINKERS

The first member of the growing oligosaccharide chain is bound to MPEG via a linker. The linker provides a stable bond between MPEG and the first saccharide and it also allows for an easy release of the carbohydrate from the polymer support. The linker is also required to keep the growing chain at a distance from MPEG since too short a span between the carbohydrate chain and MPEG could create interactions affecting the course of the synthesis.

Although a limited number of linkers have been used for MPEG-supported syntheses (Table 9.2), more can and will be designed. The choice of a linker is determined by the reactions employed in the reaction sequence and on the form of the final oligosaccharide, which further depends on the eventual use of the oligosaccharide and the possible utilization of the linker or a part of it. As in any organic chemical synthetic scheme, reagents and protecting groups must act in harmony; MPEG linkers can be regarded as special protecting groups. The following examples will illustrate such criteria.

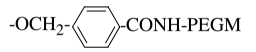
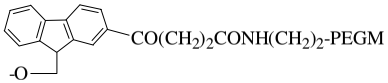
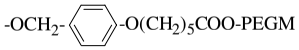
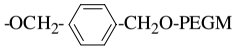
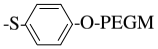
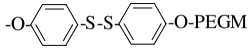
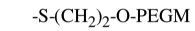
9.3.1 Ester Linkers

Succinoyl diester, the best example of this class of linkers, has been extensively used to connect small molecules with polymers, including oligopeptides and oligonucleotides in polymer-supported solution syntheses of these oligomers. Therefore, it was attractive to use it also as a tether in MPEG-supported syntheses of oligosaccharides. The major disadvantage of succinoyl diester is its base lability. Since it is essential that both the protective groups and the linker are stable during the synthetic sequence, it is obvious that the base lability of ester linkages prohibits temporary protection with ester groups. Succinoyl ester is also unstable in mild acidic environments when on anomeric carbons, and prone to migration to suitably disposed neighboring groups, for instance, 2-*O*-SuMPEG $\rightarrow$ 3-*O*-SuMPEG in acceptor β-mannosides with unprotected C3 hydroxyls.[27]

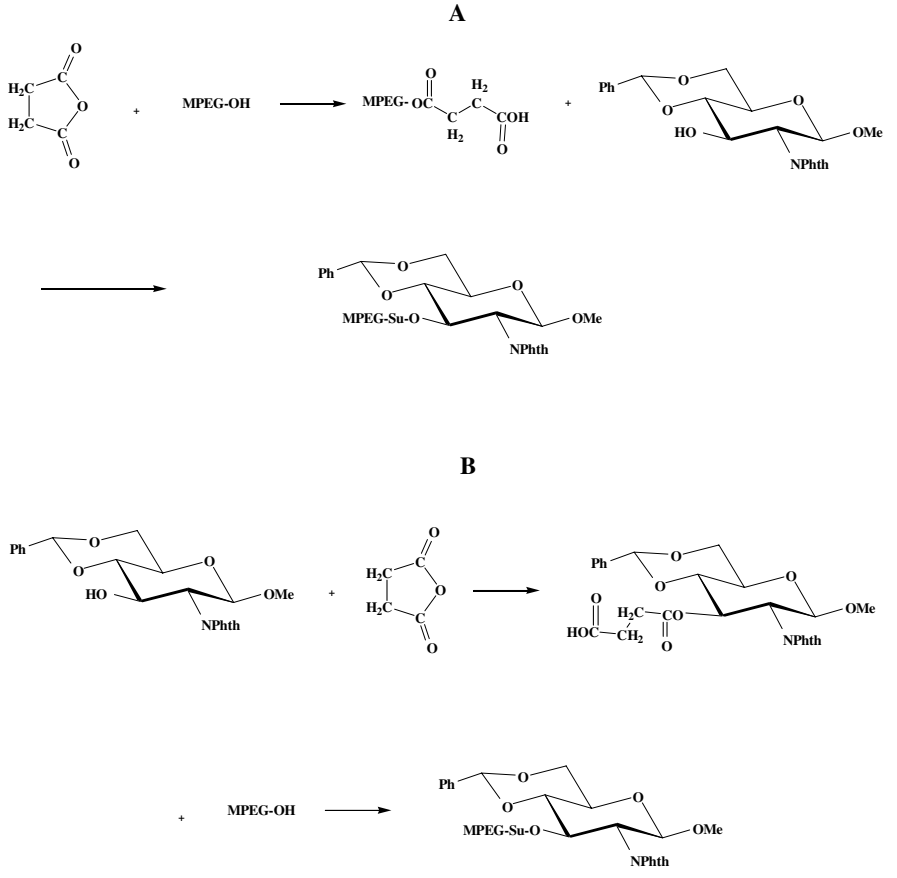
Despite these restrictions, the acceptor-bound succinoyl MPEG[28] was found to permit glycosylations with glycosyl trichloroacetimidates promoted by boron trifluoride, triflic anhydride, and trimethylsilyl or triethylsilyl triflates, with glycosyl halogenates promoted by silver triflate, and with thioglycosides promoted by the iodonium ion. It is compatible with long-term ester protection, with allylic,

*Argonaut Technologies, San Carlos, CA, USA.

TABLE 9.2 Major Linkers Used in Single-Solution Oligosaccharide Synthesis[a]

Linker	Structure	Donor(s) Used[a]	Removal	Entry
Succinoyl	$-OCO(CH_2)_2CO$-PEGM	A, B	Base-catalyzed (DBU; NaOMe)	1
Thioethanol	$-O(CH_2)_2S$-PEGM	B	Dioxirane oxidation then NaOMe/MeOH	2
Phenylacetamide		A	Hydrogenolysis	3
Fluorene		C	TEA/DCM	4
Benzylphenol		D, E	(1) NaOMe/MeOH for PEG Removal (2) Hydrogenolysis for complete removal	5
DOX		A	(1) Reductive PEG removal (2) Hydrogenolytic PEG-DOX removal (3) Acetolytic PEG removal with $Sc(OTf)_3/Ac_2O$	6
Thiophenol		A	NBS/aqueous acetone	7
Oxyphenyldisulfide		A	$HS(CH_2)_3SH/H_2O$	8
Hydroxyethylmercaptan		A	NBS/aqueous acetone	9
1,4-Thiobutylthiourea		A	NBS/aqueous acetone	10

[a]Chemical structure, glycosyl donor(s), and removal method(s) are displayed in this table; literature citations are shown for each entry number (in parentheses): (1) 9, 12, 29, 31, 32, 62; (2,3) 19; (4) 33; (5) 14; (6) 34, 35, 58, 61; (7–10) 38.
(*Abbreviations*: donors—A = trichloroacetimidate; B = bromide; C = sulfoxide; D = thioalkyl/aryl; E = fluoride).

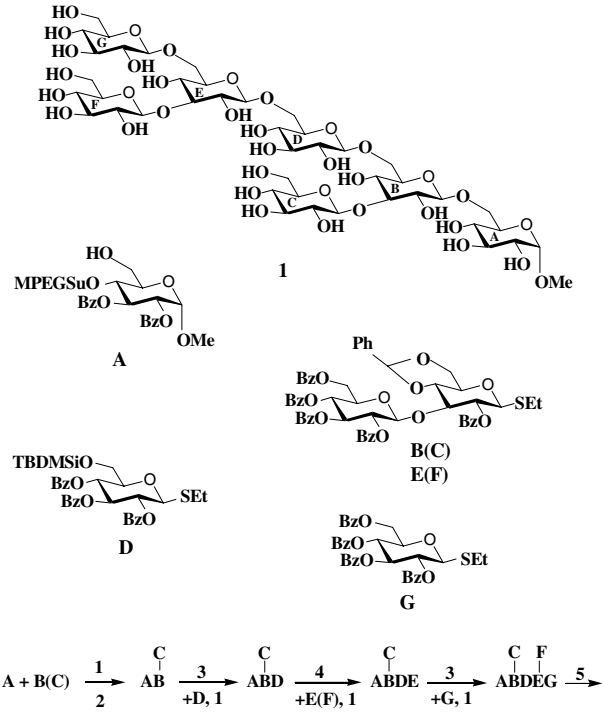


Scheme 9.5 Attachment of the succinoyl linker: (a) in one step as polymer-supported hemiester; (b) in a two-step sequence: (a) attaching to the acceptor succinoyl (using succinyl anhydride) first, followed by (b) the attachment of MPEG-OH to the free carboxyl of the succinoyl formed in step a.

benzylic-type (including benzylidene-type acetals), and certain silicon-based protecting groups, and deprotection with hydrogenolysis.

To synthesize an oligosaccharide, MPEG is bound to a hydroxyl of the first monosaccharide through the succinoyl diester linker (Scheme 9.5). Next, another hydroxyl of this monosaccharide is deprotected and glycosylated, temporary protection on the second monosaccharide is removed and the abovementioned process is repeated until the required oligosaccharide is synthesized. Although incomplete glycosylation can be minimized by using glycosylating agents in excess, it is probably more efficient to add an excess of the glycosylation agent in several portions.

The synthesis of the methyl heptaglucoside **1** (Scheme 9.6) is an excellent example of a regioselective glycosylation of the primary hydroxyl in 4,6-diol using succinoylated MPEG and iodonium promoted glycosylations with thioglycoside



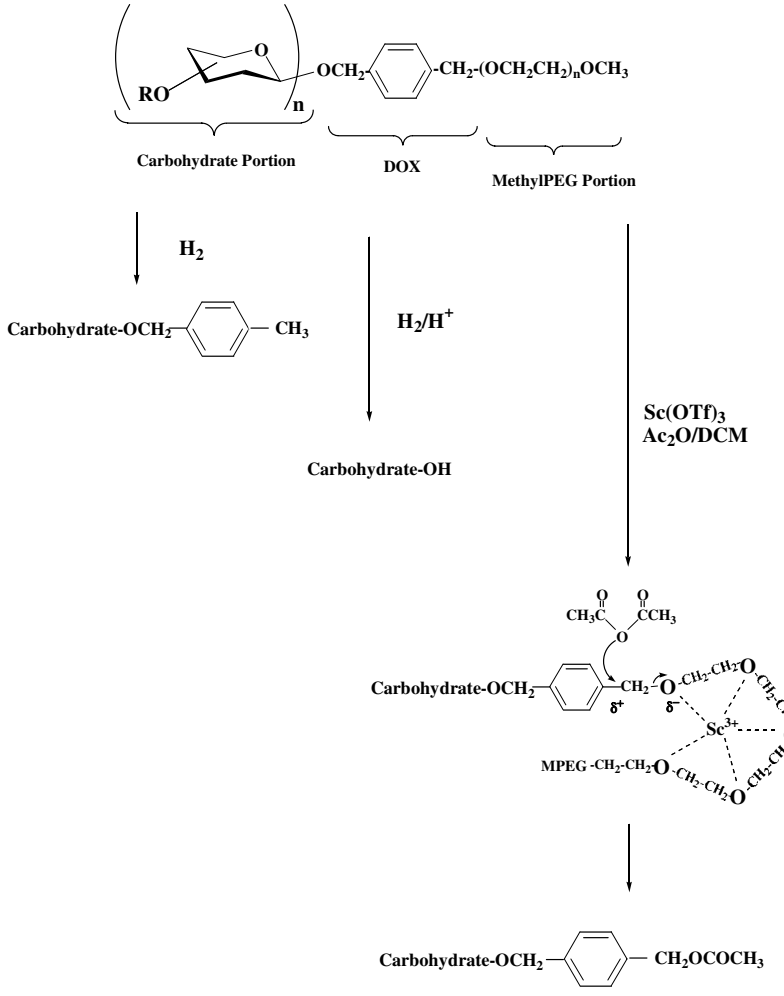
Scheme 9.6 Synthesis of methyl heptaglucoside **1**. The letters indicate intermediates used in the synthetic sequence displayed at the bottom of the scheme. The numbers describe reaction conditions or reagents: (1) *N*-iodosuccinimide, trifluoroacetic acid; (2) removal of benzylidine; (3) acetylation; (4) removal of t-butyldimethylsilyl; (5) sodium methoxide/methanol.

donors.[29] The synthesis verified that the iodonium ion–promoted glycosylations are compatible with the succinoyl tether. After the glycosylation, the unreacted C4 hydroxyl group was capped by acetylation. A comparison with a convention solution synthesis[30] confirmed, as expected, that the MPEG-supported synthesis was significantly faster. Another example of a synthesis using MPEG-Su-bound acceptor is the synthesis of heparan sulfate–like oligomers up to a dodecamer.[31] MPEGSu can also be linked to a donor as shown in the synthesis of an oligomer of *N*-acetylneuraminic acid.[32]

Succinoyl diester has been further modified by linking to 9-hydroxymethylfluorene (Table 9.2) and using MPEG terminating with an amino group. This conversion of one carboxyl into an amide renders MPEG-linker bond base-resistant, while base sensitivity of the ester linkage (from the second succinoyl carboxyl) through 9-hydroxymethylfluorene to a carbohydrate becomes elevated. This modification makes it possible to distinguish between less base-labile long-term protecting groups on the oligosaccharide moiety, such as pivaloyl, while removing the oligosaccharide from the polymer linker with a mild base.[33]

9.3.2 Ether Linkers

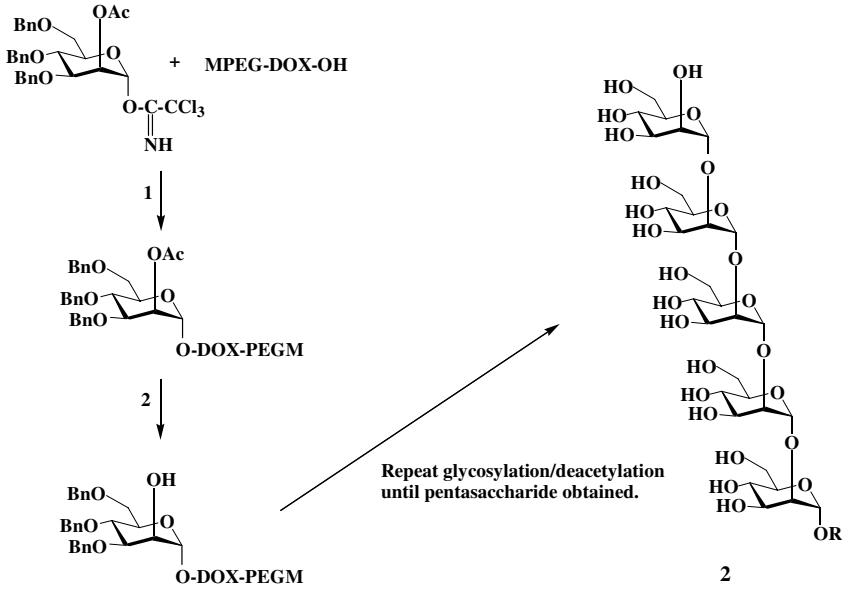
The base lability of succinoyl diester linker severely limits the selection of protecting groups available for an oligosaccharide synthesis, so a more versatile tether was required. Diether bonds of benzylphenol or dibenzyl of 1,4-di(hydroxymethyl)-benzene satisfy this requirement because they are stable to both bases and to acids. A sufficient acid stability is important since the formation of a glycosidic bond is an acid-catalyzed reaction, not surprisingly, as it is an acetal functionality. For instance, *DOX*,[34] the "dibenzyl" linker α,α′-*D*i*O*xy*X*ylyl diether, $-OCH_2C_6H_4CH_2O-$, is not limited by restriction of the succinoyl linker: (1) when bound via a hydroxyl or as an



Scheme 9.7 The attachment of α,α′-dioxyxylyl-(DOX) linker and different methods for its removal.

O-glycoside, it is stable under many reaction conditions, including glycosylation; (2) it is easily removable (Scheme 9.7) from a finished oligosaccharide by hydrogenolysis under mild acidic conditions; (3) using suitable hydrogenolytic conditions, MPEG *only* is removed and DOX is converted into 4-methylbenzyl protecting group; (4) using scandiumtriflate/acetanhydride,[35] DOX can be converted into a 4-(acetoxymethyl)benzyl protecting group by removal of MPEG only; and (5) it is easily prepared from α,α′-dichloro-*p*-xylene.

The MPEG-DOX assembly is readily synthesized from MPEG-OH and α,α′-dichloro-*p*-xylene by Williamson ether synthesis yielding MPEG-DOX-Cl, and, after hydrolysis, the alcohol MPEG-DOX-OH.[34] In a subsequent glycosylation of MPEG-DOX-OH with a glycosyl donor, a saccharide is attached at the anomeric position (an impossible connection through succinoyl tether[9]) to create an MPEG-supported acceptor. In a practical example (cf. Schemes 9.1 and 9.8), the MPEG-DOX-OH is glycosylated by 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyltrichloroacetimidate. The 2-*O*-acetyl group becomes the hydroxyl acceptor for the next glycosylation, and it also provides steric control of glycosylation via neighboring-group participation. Repetition of the glycosylation and hydrolytic steps gave the expected protected MPEG-DOX-bound pentasaccharide. Standard deprotection, including the removal from the polymer support, yielded the desired pentamannoside [Manp(α1→2)]$_4$Manp (**2**) (Scheme 9.8). Target oligosaccharides are often contaminated with impurities originating from deprotection steps that usually can be removed by chromatography of peracetylated oligosaccharides. Peracetylated oligosaccharides are commonly more suitable for final purification by



Scheme 9.8 The synthesis of methyl pentamannoside **2**. The numerals indicate reagents used in the synthetic sequence: (1) trimethylsilyl triflate, methylene chloride, 4 Å molecular sieves; (2) DBU, methanol. The final deprotection conditions shown in Scheme 9.7 were employed.

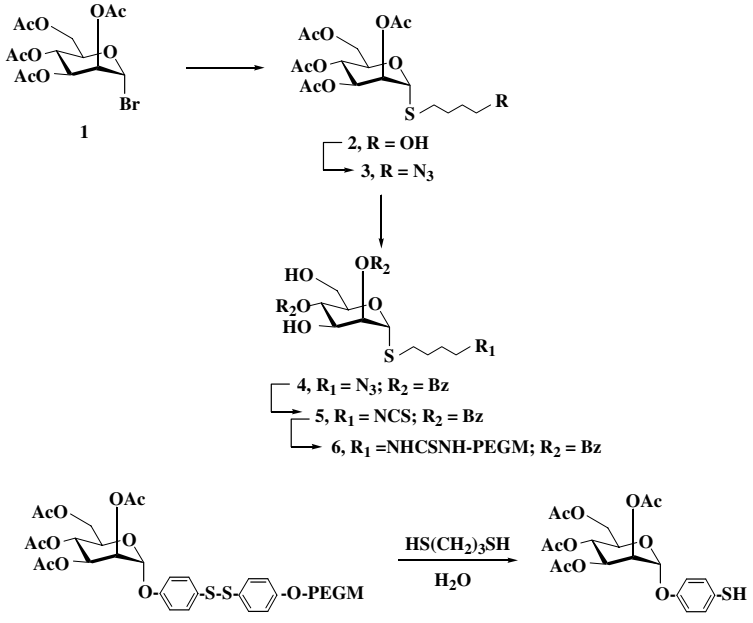
chromatography than deprotected compounds; after deacetylation, pure oligosaccharides are obtained readily.[36]

The attachment of MPEG-DOX to a carbohydrate hydroxyl other than the anomeric one is achieved by the reaction of such hydroxyl with MPEG-DOX-Cl by Williamson ether synthesis.[34] Syntheses of oligosaccharides on MPEG, tethered with DOX and related 4-oxybenzyloxy diethers, can be performed even more speedily using orthogonal glycosylation[11] and multicomponent carbohydrate[37] coupling strategies.

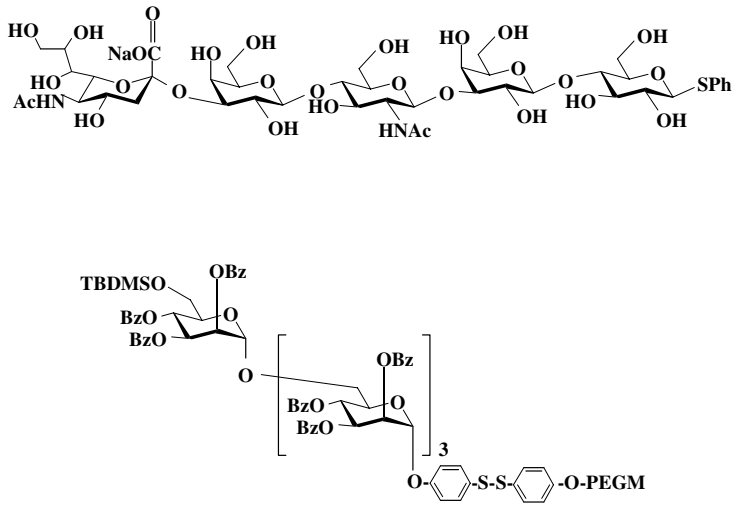
An interesting linker is based on bis(4-hydroxyphenyl)disulfide **3** (Scheme 9.9). Both MPEG and the carbohydrate are linked as ethers, and for removal from MPEG, the linker's disulfide bond is reductively split by propanedithiol. The carbohydrate is freed as a glycoside of 4-hydroxyphenylmercaptan.[38]

9.3.3 Thioether linkers

Thioglycosidic bonds are admirably stable under most conditions used for oligosaccharide synthesis and yet can be cleaved with many reagents, such as mercuric trifluoroacetate, *N*-bromosuccinimide with 1,6-di-*t*-butylpyridine or acetone, dimethylmethylthiosulfonium triflate or tetrafluoroborate, or oxidatively using dioxirane followed by NaOMe in methanol. Therefore, it is surprising that they were employed first exclusively in two-phase reactions with crosslinked polystyrene utilizing thioglycoside,[39] or sulfide-based linkers.[40] The thioglycoside linkers were first employed in one-phase synthesis only in 2000: 4-thiophenol, mercaptoethanol,



Scheme 9.9 The attachment of thiobutylisothioisocyanante linker and its removal with propane dithiol (the bottom part of the scheme).



Scheme 9.10 Linkers containing sulfur shown with a pentasaccharide and the tetramannoside (the latter before its deprotection).

and 4-mercaptobutylthiourea (Table 9.2; see also Schemes 9.9 and 9.10).[38] This type of linker will probably become one of the most important tethers in the future.

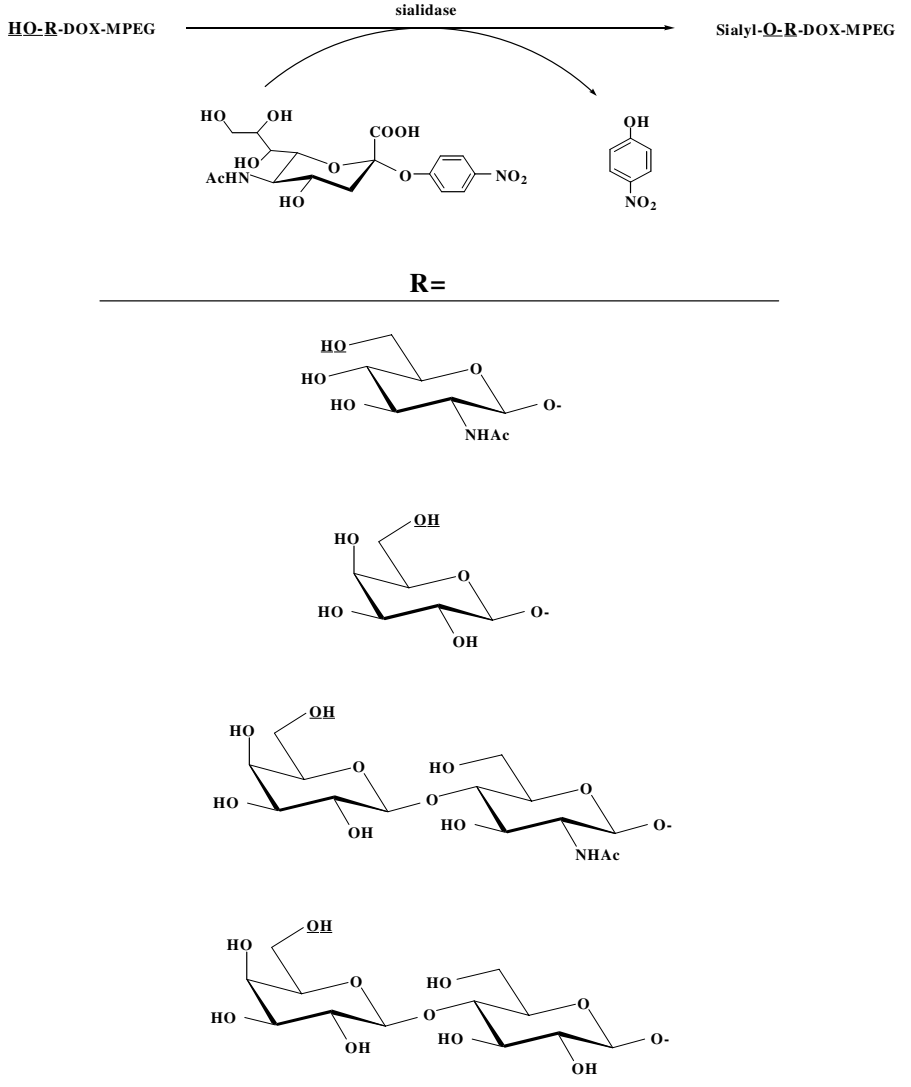
9.3.4 Other Linkers

Many other linkers besides those listed above have been developed for two-phase synthesis of oligosaccharides on insoluble supports, and it can be expected that at least some of them will be tested on soluble supports. It should be kept in mind that MPEG-supported syntheses can be easily scaled up; therefore, any relationship between both types of polymer supports will be cooperative rather than mutually exclusive. Such linkers will most probably include dialkyl- or diaryl-silyl linkers,[10,41–43] and linkers cleavable by photolysis such as the *o*-nitrobenzyl group and its modifications.[44–46]

9.4 MPEG-SUPPORTED SYNTHESES USING ENZYMES

Oligosaccharide syntheses employing enzymatic reactions would in principle greatly benefit from being performed on a polymer support since the support might effectively facilitate isolation of the final product. Presumably, a water-soluble polymeric support will be preferable to any insoluble support since reaction rates could otherwise become too slow. Glycosidases as synthetic enzymes would be the best candidates to study this type of the enzymic approach to oligosaccharide synthesis.

One problem identified at the outset was that MPEG or a monosaccharide DOX-MPEG as acceptors had to be presented to a glucosidase in a concentration, which renders the solution quite viscous at 37°C, and consequently the reaction becomes very slow.[47] Below this acceptor concentration threshold, reaction yields were very low. Enzymes from thermophilic bacteria working at higher temperatures

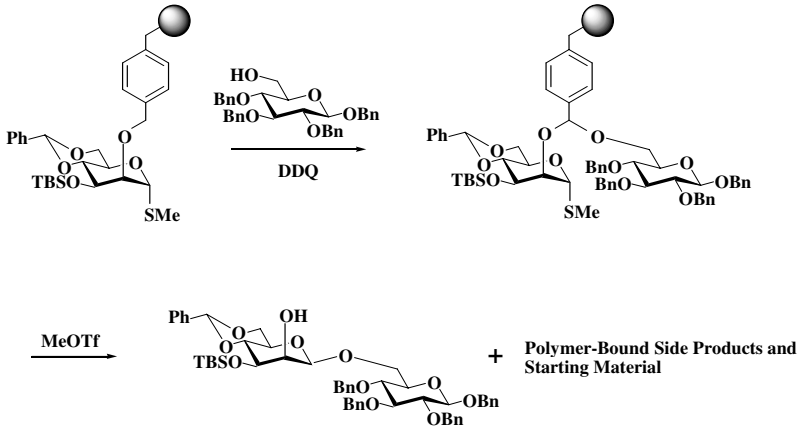


Scheme 9.11 Enzymatic transsialylation of four MPEG-DOXyl glycosides with sialidases from *Vibrio cholerae* and *Salmonella typhimurium*. *V. cholerae* sialylated in position 6 of the terminal monosaccharide units with yields of 12–17%, whereas *S. typhimurium* sialylated position 3 of the same monosaccharide units (yields 14–24%).

may ease this density problem. This acceptor concentration requirement may differ for different enzymes. A report from Thiem's group indicates that sialidases from *Vibrio cholerae* and *Salmonella typhimurium* transfer Neu5NAc from its *p*-nitrophenylglycoside to both MPEG-DOX-OH, and carbohydrates such as galactose, Glc2NAc, lactoseamine, and lactose regiospecifically in 14–24% yields. Enzyme from *V. cholerae* transfered sialyl residue to O6 of the galactose unit, whereas the transfer by the enzyme from *S. typhimurium* was directed to O3 of galactose (Scheme 9.11).[48]

9.5 USE OF MPEG IN MECHANISTIC STUDIES

As it is well known, few glycosylations are high-yield reactions. MPEG-DOX-OH employed as a nucleophile allows for rapid identification of reaction products because these products can be readily precipitated and identified by NMR spectroscopy. Therefore, it is possible to identify competing reactions and take steps to avoid them. It was established that the trans (β) anomeric specificity of some 2-acetamido glycosylating agents does not require oxazoline intermediacy, and that formation of oxazolines represents a side reaction. It was further found that oxazolines cannot be an obligatory intermediate since MPEG decomposes under the conditions required by oxazoline intermediacy.[49] The origins of acyl transfer from 2-acyloxyglycosylating agents to acceptors[50] have been studied in combination with calculations based on density functional theory in order to eliminate this very common side reaction.[51] Novel reactants in glycosylation reactions can be efficiently evaluated using MPEG-DOX-OH as illustrated by the investigation of dimethylboron triflate as a promoter.[52] The formation of the β-mannoside linkage by intramolecular aglycon delivery[53] was modified to be performed on MPEG support; in the last step of the

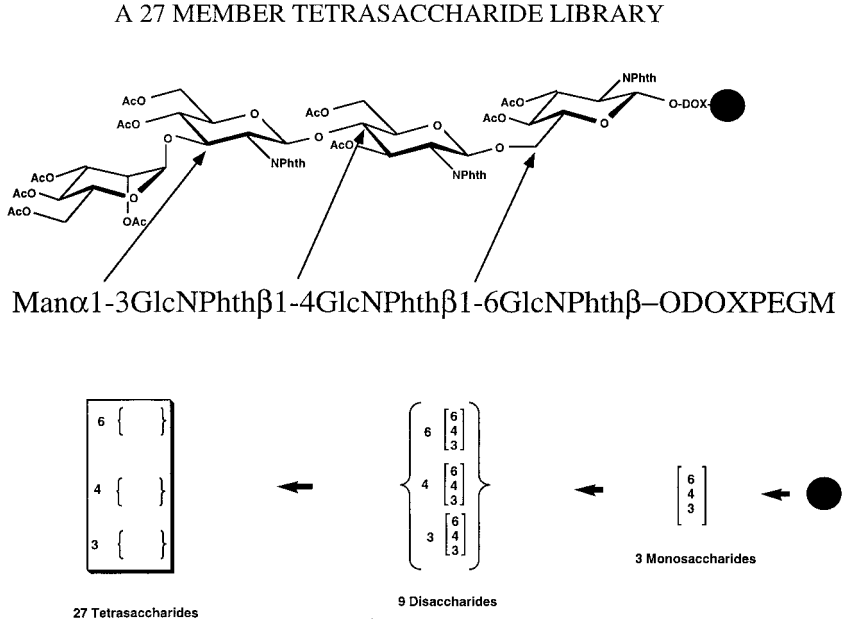


Scheme 9.12 "Reverse" use of a polymer support for the synthesis of β-mannoside linkage. The synthetic helper arm (MPEG), together with other polymer-bound by products, is removed from the desired product by simple precipitation.

synthesis the β-mannoside remains in solution after precipitation of MPEG-bound byproducts (Scheme 9.12).[54]

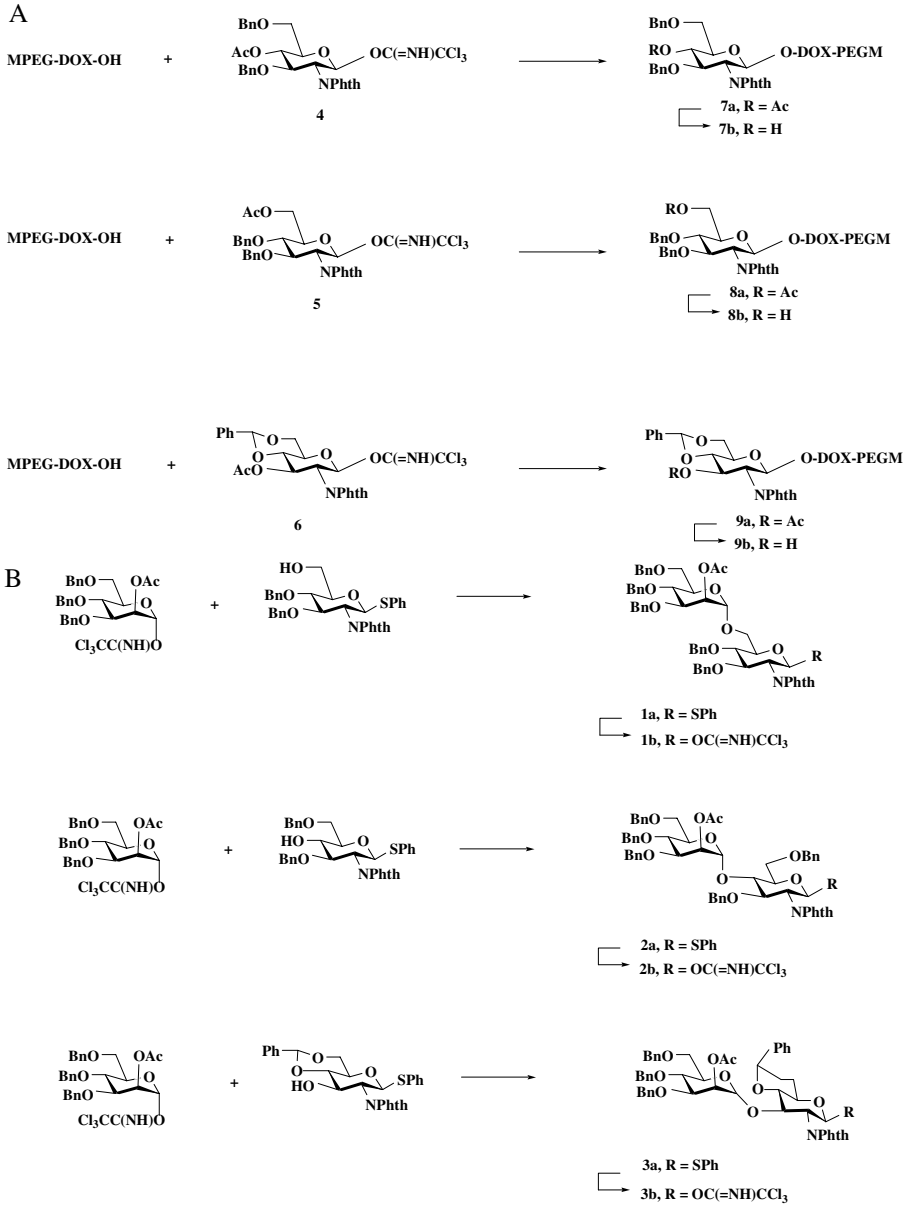
9.6 MPEG AND COMBINATORIAL LIBRARIES

Polymer-supported strategies of oligosaccharide synthesis are remarkably suitable[55,56] for preparations of combinatorial oligosaccharide libraries. All library "books" at any synthetic step are purified together; thus, it is unlikely that individual "books" could be lost during the process. Kahne's group has shown that libraries on TentaGel beads (i.e., on immobilized PEG)[57] are remarkably useful for screening for biological activities. So far, there is only one example of one-phase MPEG-DOX supported synthesis of a tetrasaccharide library (Scheme 9.13).[58] The purpose of the synthesis was to evaluate how incomplete glycosylations may influence "clarity" of a



Scheme 9.13 The synthesis of a tetrasaccharide library (I). One anomeric form of each glycosidic linkages was formed. Although only one glycosidic linkage is shown in the structural formula, the arrows indicate that all hydroxyl groups on these monosaccharide units form glycosidic linkages (e.g., GlcNAc-to-Glu are 1→3, 1→4, and 1→6). This is shown in a different way at the bottom of the scheme. A particular bracket identifies all the acceptor hydroxyls involved in glycosidic linkages; horizontal arrows point towards anomeric carbons; the starting acceptor is MPEG-DOX-OH (the black circle). The number under a bracket identifies how many compounds (i.e., glycosidic linkages) are contained in the particular bracket. Thus the brackets represent imaginary flasks (or, for 27 compounds, it is a rectangle). D-Mannose terminates all hydroxyls on all terminal units the same way.

library. Thus, monosaccharides from which the 27-member library was constructed (Scheme 9.14) are likely to form exclusively (or nearly exclusively) only one anomer in glycosylation reaction to limit the number of unwanted isomers. Furthermore, no 2-acyl transfer to acceptors is possible with these donors. The disaccharide glycosyl

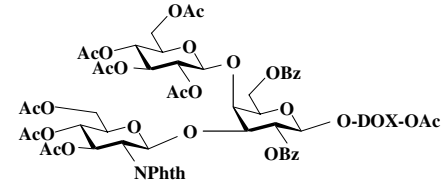


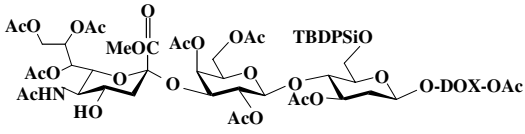
Scheme 9.14 The synthesis of a tetrasaccharide library (II). General design of library construction and monosaccharide donors are shown in part A. Disaccharide donors are constructed as shown in part B.

donors **1b–3b** were prepared as portrayed in Scheme 9.14B. Monosaccharide donors **4–6** (Scheme 9.14A) were first anchored to MPEG-DOX in individual reactions to determine the yields and spectroscopic characteristics of **7a–9a**. The same reaction was then performed with the mixture of donors **4–6** to give a mixture of **7a–9a.** PEGylated products **7a–9a**, both individually and combined, were then deacetylated to expose appropriate hydroxyls in **7b–9b**, and glycosylated with donors **4–6**. Combined donors **4–6** glycosylated combined acceptors **7b–9b**, giving a disaccharide library containing all structural permutations on carbons 3, 4, and 6 of the first accepting monosaccharide. This PEG-DOX supported disaccharide library was again deacetylated in a manner analogous to deacetylation of **7a–9a**, and glycosylated with donors **1b–3b**, to obtain the tetrasaccharide 27-member library. The last glycosylation was again investigated with individual selected acceptors, to evaluate the course of the reaction. It was noted that the employed donors did not differ significantly in their reactivity toward the same accepting hydroxyl; C4 and C6 hydroxyl groups were approximately equal in their acceptance of any donor, but the C3 hydroxyl group showed somewhat reduced acceptance of any of the donors. The difficulty in analyzing the complete composition using current analytical or spectroscopic methods were also noted.

9.7 OTHER APPLICATIONS

It would be of interest to evaluate different polymer-supported synthetic strategies for the preparation of the same oligosaccharide. To date, two oligosaccharides, (**1**, see p. 186) and (**2**, see p. 188) have been synthesized using both the one-phase system on MPEG (cf. Schemes 9.5–9.8) and the two-phase system using crosslinked polystyrene. Since different chemistries were used in one example[46a] and the pentasaccharide synthesized using the two-phase design was not deprotected,[40a] such a comparison is difficult to make.





Scheme 9.15 Further examples of oligosaccharides prepared using one-phase polymer-supported design.

Despite the lack of direct comparison between the two general kinds of polymer-supported designs, MPEG-DOX methodology has been used to synthesize oligosaccharides (Scheme 9.15) related to the development of vaccines against *Neisseria meningitidis*[59] and IA group B *Streptococcus pneumoniae*.[60] The combination of MPEG-supported methodology with enzymatic sialylation with sialyl transferase to produce sialylated oligosaccharide donors, and sialylated oligosaccharides, has been reported.[59,61]

9.8 CAPPING

"Capping," or protection of the unreacted hydroxyl to prevent it from reacting in the next condensation step, is a very important operation in oligonucleotide automatic synthetic protocols. In oligosaccharide synthesis capping is not done regularly, although unreacted hydroxyls are sometimes acetylated.[29] It is more useful to repeat the glycosylation step, in analogy with syntheses of "difficult sequences" of oligopeptide synthesis, or strategies involving a hydrophobic handle (Scheme 9.3).

9.9 OUTLOOK

The polymer-supported syntheses of protected oligosaccharides in solution (one-phase syntheses) have attracted considerable attention of organic chemists. It appears to be a suitable methodology for the synthesis of oligosaccharides in quantities required for biomedical applications. For instance, MPEG-supported synthesis was used to prepare fragments of the capsular polysaccharide of *Haemophilus influenzae*.[62] In Whitfield's group, MPEG-DOX methodology is used for oligosaccharides related to the development of vaccines against *Neisseria meningitidis* and IA group B *Streptococcus pneumoniae*. It is clear that the methodology, while not perfect, has already shown its value. It will become more widely used, as better and more efficient reaction conditions are discovered and the essential components become less expensive.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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10 Two-Direction Glycosylations for the Preparation of Libraries of Oligosaccharides

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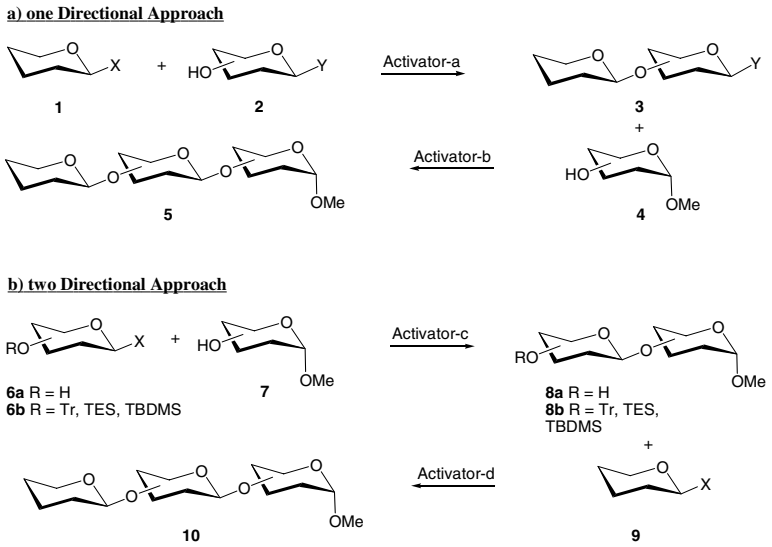
10.1 TWO-DIRECTIONAL GLYCOSYLATIONS IN SOLUTION

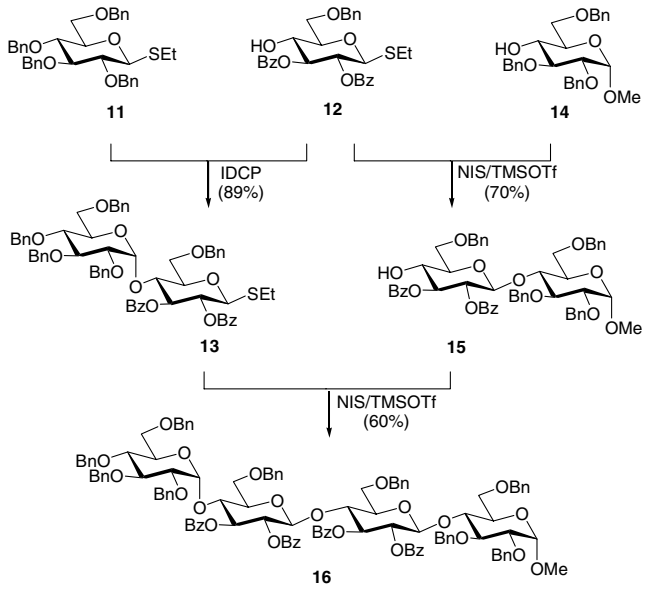
Traditional strategies for oligosaccharide assembly are characterized by protecting group manipulations between each glycosylation step. Such manipulations increase the linearity and decrease the efficiency of oligosaccharide assembly. Chemoselective, orthogonal, and two-directional glycosylation strategies condense the process of oligosaccharide synthesis by removing the need for unmasking procedures.[1] In chemoselective or orthogonal glycosylation strategies, a glycosylation product (3, Scheme 10.1a) is immediately used as a glycosyl donor in a subsequent coupling. Such a sequential assembly of monosaccharides can use donors (**1**) and acceptors (**2**) that have the same type of anomeric group (X = Y = SR, pentenyl, F) but with different anomeric reactivities.[2–15] In these cases, the anomeric reactivity is primarily controlled by the nature of protecting groups. Orthogonal glycosylations use donors and acceptors that have different anomeric groups (e.g., X = F and Y = SR) that can be activated without effecting the other one.[16–24] These synthetic approaches are attractive since no—or very few—protecting group manipulations are involved during the assembly of a complex oligosaccharide. Undeniably, however, the overall efficiency of chemoselective and orthogonal glycosylations is compromised by the linear nature of the glycosylation sequence and by the fact that the growing oligosaccharide chain acts in each reaction as a glycosyl donor.

To address the above mentioned problem, we have developed a two-directional glycosylation strategy (Scheme 10.1b) in which a partly protected glycosyl donor **6a** is coupled with a glycosyl acceptor **7**, and the product bearing free hydroxyl **8a** can be used in subsequent glycosylations.[25,26] In combination with the chemoselective and orthogonal glycosylation approaches (e.g. **9** = **3**) discussed above, this methodology makes it possible to prepare a range of tetra-, penta-, and hexasaccharides without protecting group manipulations between glycosylation steps. The chemical versatility



Scheme 10.1 One- and two-directional glycosylation strategies.

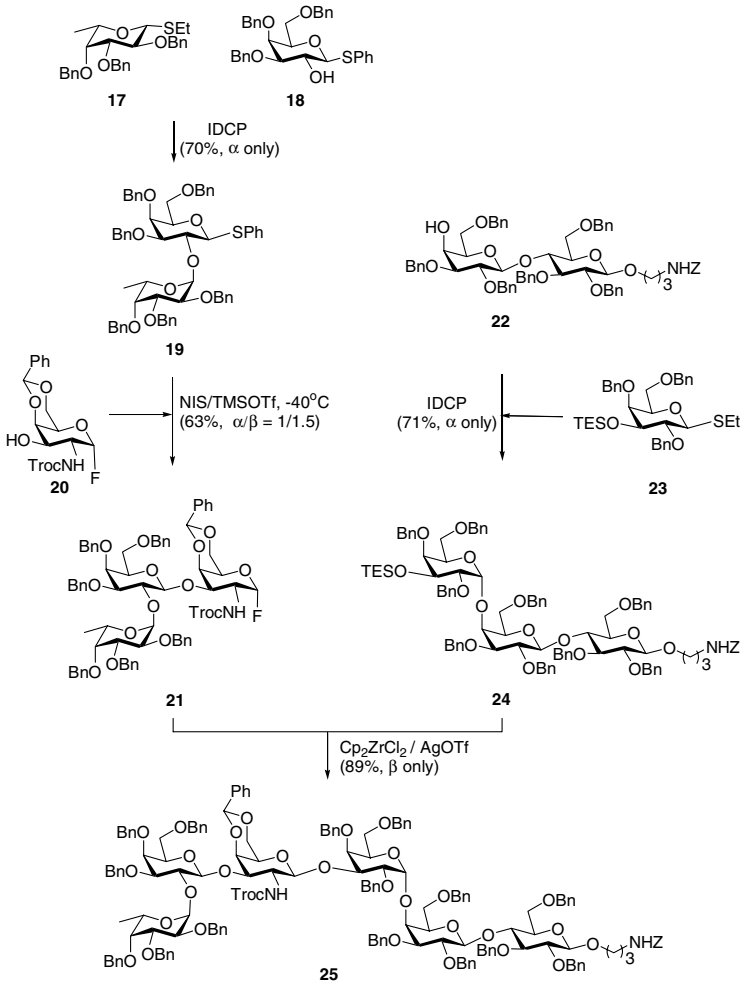
of the approach is illustrated by the preparation of tetrasaccharide **16** (Scheme 10.2). The disaccharide donor **13** was obtained by a chemoselective glycosylation between **11** and **12** that exploits the higher reactivity of benzylated—compared to benzoylated—thioglycosides. The reactivity of the C4 hydroxyl of the partially



Scheme 10.2 Preparation of a tetrasaccharide by a two-directional approach.

benzylated methyl glucoside **14** is much more reactive than the C4 hydroxyl of the partially benzoylated thioglucoside **12**. Thus, NIS/TMSOTf-promoted glycosylation of **12** with **14** proceded with high regioselectivity to give disaccharide **15** in a yield of 70%. This reaction benefited from two benzoyl groups at C2 and C3 of glycosyl donor **12** that deactivate the C4 hydroxyl group sufficiently to suppress self-condensation and oligomerization of **12**. Next, chemoselective coupling of disaccharides **13** with **15** in the presence of NIS (*N*-iodosuccinimide)/trimethylsilyl triflate (TMSOTf) gave tetrasaccharide **16** in a good yield (60%). The versatility of common building block **12** is elegantly shown as it acts as a glycosyl acceptor in the preparation of disaccharide donor **13**, whereas for the preparation of disaccharide acceptor **15**, it functions as a glycosyl donor. Thus, tetrasaccharide **16** can be assembled from the monosaccharides **11, 12**, and **14** in a convergent manner without the need to resort to protecting group manipulation.

A prerequisite of the two-direction glycosylation strategy discussed above is that the hydroxyl of the acceptor is sufficiently more reactive than the hydroxyl of the glycosyl donor. In some cases, this requirement imposes problems. Fortunately, this shortcoming could be addressed by the application of tritylated and silylated thioglycosides.[27–29] Trityl and silyl ethers are sufficiently stable under iodonium-ion-promoted glycosylation conditions; therefore, thioglycosides protected with these functionalities can act as glycosyl donors (Scheme 10.1b, **6b** + **7** $\rightarrow$ **8b**). On the other hand, under a different set of glycosylation conditions, these protecting groups act as efficient glycosyl acceptors (**8b** + **9** **10**). An example of this approach[29] is depicted in Scheme 10.3, where the triethyl silyl (TES)-protected thioglycoside **23** first acts as a donor and in the next step as an acceptor. Trisaccharide **21** was prepared by a combination of a chemoselective and orthogonal glycosylation. Thus, IDCP-mediated glycosylation of the armed thioglycosyl donor **17** with semidisarmed glycosyl acceptor **18** in DCM/diethyl ether gave disaccharide **19** as a single anomer in a good yield (70%) (Scheme 10.1). The observed chemoselectivity is based on the fact that 6-deoxysugars are more reactive glycosyl donors than are their 6-hydroxy counterparts. Furthermore, ethyl thioglycosides are considerably more reactive than analogous phenyl thioglycosides.[30] The anomeric thiophenyl moiety of **15** could, however, be activated with the more thiophilic reagent NIS/TMSOTf, and reaction with the glycosyl fluoride **20** gave trisaccharide **21** as a separable mixture of anomers. The trisaccharide **24** was prepared by an iodonium dicollidine perchlorate (IDCP)-mediated glycosylation of disaccharide acceptor **22** with TES-protected thioglycosyl donor **23** to give trisaccharide **24** as the α-anomer only. Careful analysis of the crude reaction mixture showed that no hydrolysis of the TES ether had occurred. The fully protected hexasaccharide **25** was obtained by coupling trisaccharides **21** with **24** in the presence of Cp_2ZrCl_2/AgOTf. The reaction was almost instantaneous, and the product was isolated in a yield of 89%. No desilylated acceptor was observed, and the reaction was significantly slower when the coupling was performed with a trisaccharide acceptor, which had a free hydroxyl. These results indicated that a TES moiety activates the glycosyl



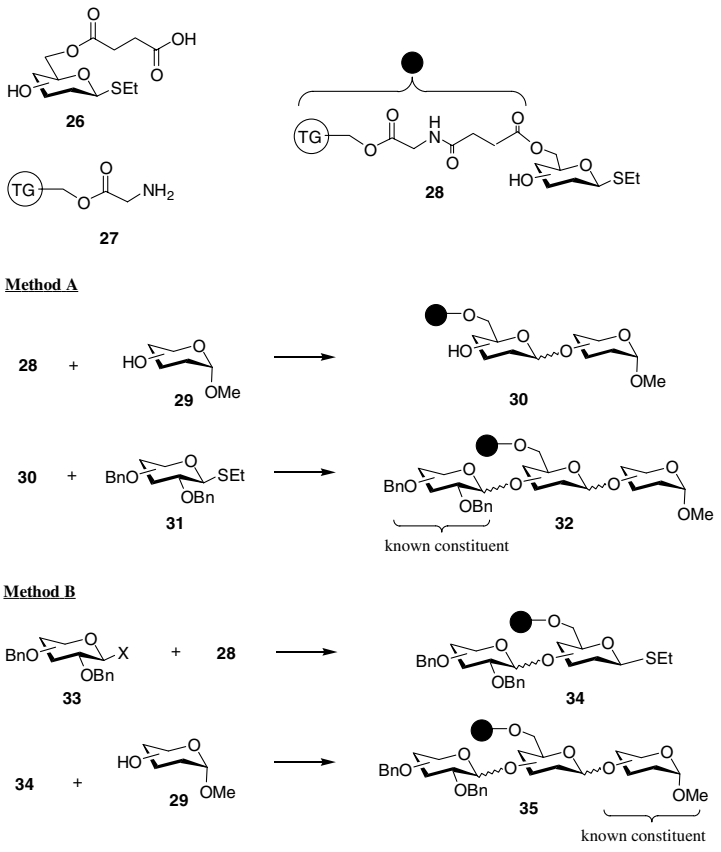
Scheme 10.3 Preparation of the tumor-associated antigen globo-H by a two-direction glycosylation approach.

acceptor in a Cp_2ZrCl_2/AgOTf-mediated glycosylation. The same protecting group, however, is perfectly stable in an IDCP-mediated glycosylation. Deprotection of **25** gave the tumor-associated antigen globo-H, which is modified with an artificial spacer for controlled conjugation to carrier proteins.

10.2 TWO-DIRECTIONAL GLYCOSYLATIONS ON SOLID SUPPORT

Encouraged by the success of the solution-phase approaches, we introduced a two-directional approach for oligosaccharide assembly on solid support. Two

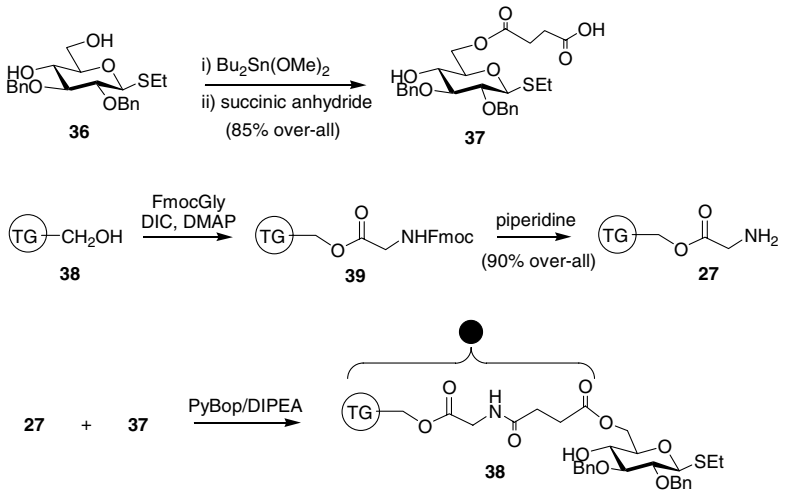
strategies are commonly used for the preparation of oligosaccharides on solid support.[31] In the first one, a glycosyl acceptor is immobilized to a polymer and then glycosylated with a glycosyl donor in solution. In the next step, a protecting group of the immobilized disaccharide is removed and the resulting acceptor is glycosylated with a donor in solution. In an alternative strategy, a glycosyl donor is attached to the solid support and acceptors in solution are added. We have introduced a more versatile strategy where immobilized saccharides can act as both donors and acceptors.[32] In such a strategy, an immobilized saccharide can first act as a glycosyl acceptor and in the next glycosylation, as a glycosyl donor. This approach requires glycosyl donors that can be orthogonally activated, and it was found that thioglycosides and anomeric trichloroacetimidates provide such a set. Alternatively, a two-directional approach can be conducted, using the immobilized saccharide first as a glycosyl donor and in the next glycosylation step as a glycosyl acceptor. The strategic details of the approach are summarized in Scheme 10.4. Thioglycosyl building blocks **26** are immobilized through the reliable formation of an amide linkage between the succinic acid moiety of a saccharide and the amine group of glycine-derivatized polymer **27**. Removal of a



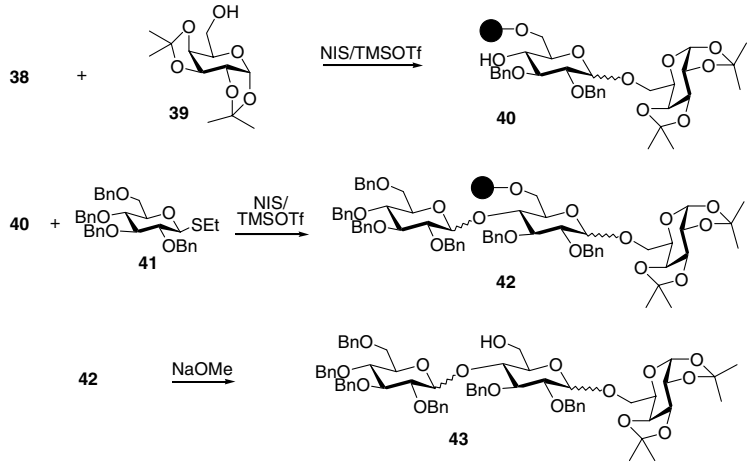
Scheme 10.4 A two-direction glycosylation strategy on solid support.

product from solid support, however, can be achieved simply by base-mediated cleavage of the ester linkage between the carbohydrate and the succinoyl residue. Glycine-derivatized TentaGel hydroxyl resin **27** was selected as the solid support to take the advantage of the high flexibility and mobility of its polyethylene glycol moieties, ensuring high reactivities of the immobilized compounds. A problem of solid-supported oligosaccharide synthesis is a lack of a reliable method for the stereoselective introduction of 1,2-*cis*-glycosides. We addressed this problem by performing the glycosylations under conditions that give reliably mixtures of anomers. Thus, at the end of a synthetic sequence, a particular oligosaccharide is obtained as a mixture of all possible anomers. Such a mixture can be used for biological screening, and only when a positive result is obtained, the compound should be prepared as a single anomer. We have established[33] that NIS/TMSOTf mediated glycosylations of thioglycosides in DCM at room temperature give consistently mixtures of anomers.

A thioglycoside was immobilized by the following sequence of reactions. Treatment of the dibutyltin acetal of diol **36** with succinic anhydride afforded **37** in an excellent yield of 85%. Attachment of Fmoc protected glycine to TentaGel hydroxyl resin (**38**, 0.37 mmol/g resin) under standard conditions followed by removal of the Fmoc group by treatment with piperidine gave polymer **27**. Compound **37** was immobilized by amide bond formation with **27** in the presence of benzotriazole-1-yl-oxy-tris(pyrrolidinophosphonium)hexafluorophosphate (PyBOP) to give polymer-bound monosaccharide **38** (Scheme 10.5). Compound **38** was coupled with **39** in the presence of NIS/TMSOTf to give the desired polymer-bound disaccharide **40**, which, after washing, was used as an acceptor and glycosylated with fully benzylated thioglycosyl donor **41** to give the trisaccharide **42** (Scheme 10.6). The latter coupling was repeated following an incomplete reaction (the 4′-OH of **40** is of



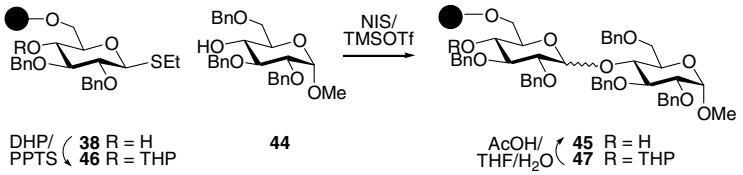
Scheme 10.5 Immobilization of a thioglycoside.



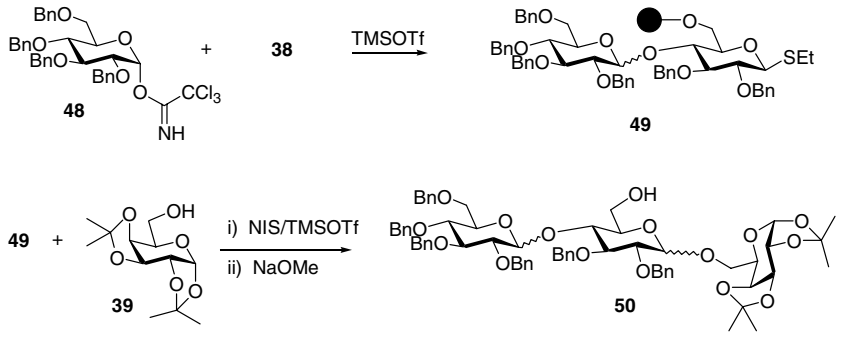
Scheme 10.6 Glycosylations on solid support.

low reactivity). Completion of each reaction step was ascertained by TLC and MALDI-TOF MS analysis of crude product that was cleaved by base treatment from a small amount of beads. Immobilized **42** was released from the solid support by treatment with MeONa/MeOH in 1,4-dioxane to give trisaccharide **43** (~65% overall yield based on loading of resin). NMR and MS analysis showed that no oligomerization had occurred and that all glycosidic linkages were formed as mixtures of anomers (Glc(1→4)Glc: α/β ~ 2/1 and Glc(1→6)Gal: α/β ~ 1/1). The results demonstrated that NIS/TMSOTf-mediated glycosylation of thioglycosides is compatible with the solid support and linker system used. It was observed that the glycosylation rates are significantly decreased (4 h) compared to similar reactions in solution (5–10 min). However, this feature did not affect the reliability of the glycosylation approach.

The preparation of trisaccharide **43** required a regioselective glycosylation of the primary hydroxyl of **39** without glycosylation of the secondary hydroxyl of donor **40**. The use of glycosyl acceptor **22**, which has an unreactive 4-hydroxy group, proved more challenging (Scheme 10.7). Unfortunately, coupling of **38** with **44** in the presence of NIS/TMSOTf gave **45**, which was contaminated with a small amount of oligomeric products (5–10%). A similar result was obtained when the more rigid



Scheme 10.7 Solid-supported glycosylation of a hindered saccharide alcohol.



Scheme 10.8 An immobilized monosaccharide that first acts as acceptor and then as donor.

Merrifield resin (1% crosslinked) was employed as the solid support. The problem of oligomerization was prevented by protection of the hydroxyl of **38** as a THP ether by treatment with 3,4-dihydro-2H-pyran (DHP) in the presence of pyridinium *p*-toluenesulfonate (PPTS) to give **46**. Immobilized **46** was successfully coupled with **24** to give disaccharide **47**. The THP group of **47** was easily removed by treatment with acetic acid/water to yield **45**.

Immobilized **38** also proved to be an appropriate glycosyl acceptor when coupled with a full benzylated trichloroacetimidate donor (Scheme 10.8). Thus, TMSOTf mediated coupling of **38** with an excess of **48** gave resin-bound **49**, which was further coupled with acceptor **38** to give trisaccharide **60** (60% overall yield based loading of the resin).

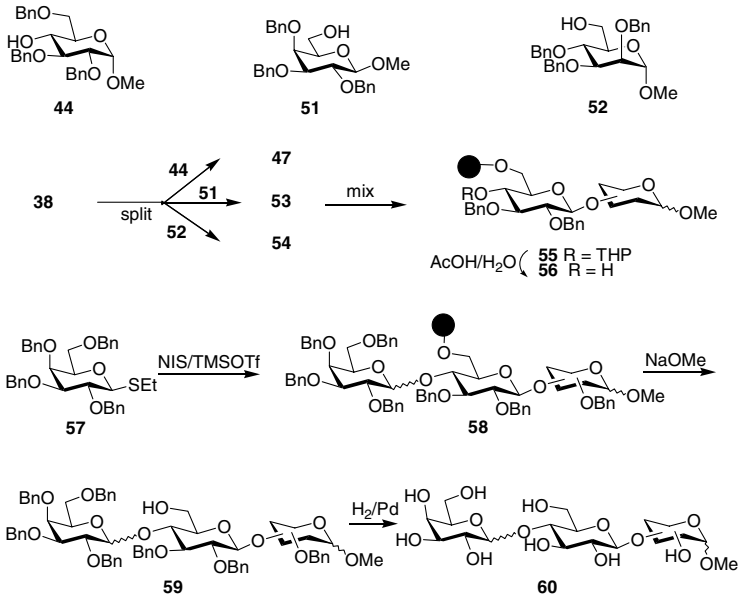
10.3 BIDIRECTIONAL SYNTHESIS OF CARBOHYDRATE LIBRARIES

Modern glycosylation strategies and the use of polymer-supported synthesis may increase the speed of oligosaccharide synthesis. It should, however, be realized that the preparation of this class of compounds is still laborious and even in the hands of experienced carbohydrate chemists, the preparation of relatively simple tri- and tetrasaccharides usually takes 3–6 months. To realize the biomedical potential of saccharides, faster methods for oligosaccharide synthesis need to be developed. Combinatorial chemistry is an important technology for the creation of large numbers of compounds, and it is now well established that it offers a faster approach for the discovery of new drugs, catalysts, and materials than does conventional one-molecule-at-a-time synthesis.[34–40] The two-directional glycosylation strategy is very attractive for the preparation of libraries of oligosaccharides. In individual glycosylations, an immobilized thioglycoside can be coupled with a range of acceptors, and the resulting products can be mixed, and split, and each pool of resin-bound disaccharide acceptors, can be glycosylated with a range of donors to give, after cleavage of the resin and deprotection, libraries with known residues at the nonreducing end (Scheme 10.4, method A). When the thioglycoside acts first as an

acceptor and then as a donor, a mix–split approach will give a trisaccharide library with a known residue at the reducing end of the saccharides (Scheme 10.4, method B).

To demonstrate the proposed strategy, a relatively small library of 12 trisaccharides was prepared (Scheme 10.9). Three different glycosyl acceptors—**44**, **51**, and **52**—were coupled in individual reactions with **38** using NIS/TMSOTf as the promoter to give three different disaccharides as mixtures of anomers (**47**, **53**, and **54**, respectively). The beads were combined, the THP group removed by treatment with HOAc/THF/H_2O, and the mixture of disaccharide acceptors coupled with glycosyl donor **57** using NIS/TMSOTf as the promoter to give the trisaccharide library **58**. The trisaccharide library was released from the polymer after TLC and MALDI-TOF MS had shown that all the disaccharides had been consumed. The library was purified by size-exclusion column chromatography and the benzyl groups removed by hydrogenation over $Pd(OAc)_2$ to give the deprotected library **60** (55% overall yield, based on loading of the resin).

The quality of this library was examined by monosaccharide compositional analysis. A portion of the trisaccharide library was treated with aqueous trifluroacetic acid at 100°C for 4 h. Analysis of the resulting mixture of monosaccharides on a Dionex HPLC system with a PA1 column and pulsed amperometric detection (PAD) showed that galactose, glucose, and mannose were present in approximately the required ratio.



Scheme 10.9 Synthesis of a trisaccharide library on solid support using a two-directional glycosylation approach.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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11 Carbohydrate Libraries in Solution Using Thioglycosides: From Multistep Synthesis to Programmable, One-Pot Synthesis

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This chapter describes the efforts in one of our laboratories to produce carbohydrate libraries in solution from a pool of common thioglycoside building blocks. Before describing our work in detail, we trace the history of scientific advances that have set the stage for these efforts, collectively referred to as "one-pot strategies." The use of orthogonal protection/deprotection strategies is also discussed, as it provides a convenient route to the starting materials for one-pot syntheses.

11.1 INTRODUCTION

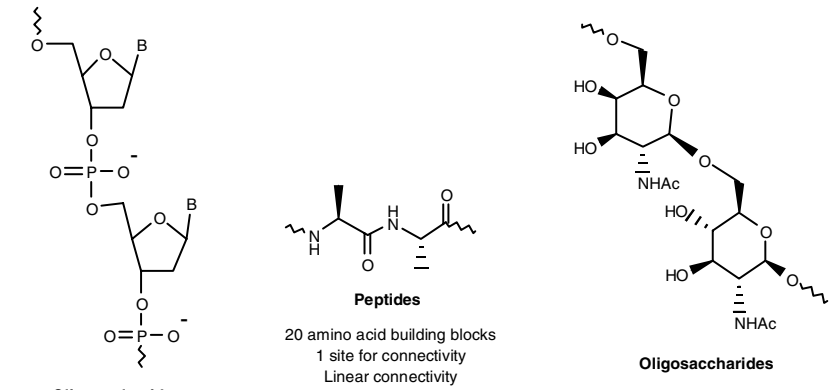
11.1.1 Libraries of Carbohydrates: Motivations and Challenges

Of the three major classes of biological polymers of nature, only the preparation of carbohydrates remains to be reduced to trivial synthetic protocols. Merrifield conquered polypeptides with solid-phase techniques winning decisive victory in 1963 with the synthesis of bradykinin.[1] The work of Letsinger and Caruthers heralded the dawn of the convenient syntheses of oligonucleotides.[2] The syntheses of both of these polymers are aided by their structure, and by the nature of their chemical bonds; that is, both products are linear polymers in which the bond connecting the monomeric units does not give rise to stereoisomers. Only a single, common protecting group must be removed between monomer couplings [e.g., Boc or Fmoc for the α-amino

group in peptide synthesis and the dimethoxytrityl (DMT) group for the 5′-OH in nucleotide synthesis]. Carbohydrates, on the other hand, exist as linear or branched polymers connected by a glycosidic linkage adopting either an α or β geometry, and after each glycosidic bond formation, selective deprotection of one of up to four sites is necessary, thus requiring the use of different reagents and different conditions, making automatic solid-phase synthesis more complicated.

Unlike amino acids or nucleotides, the structure of a pyranose sugar varies significantly, and these differences give rise to different chemistry; it is well appreciated that the substituents on the pyranose ring affect the rate and the stereochemical outcome of the glycosidic bond-forming reaction. The electronic and/or steric origins of these effects are only beginning to be understood. Peptides and oligonucleotides do not suffer from the same delicacies. So, while the side chains of amino acids are of markedly different character, these differences do not influence the rate of reaction (when these groups are protected), probably because they are remote from the bond-forming site. Consistent with this fact, and with the possible exception of proline, all amino acids can be condensed in the presence of a common activating agent at similar rates. The phosphoramidite groups of any nucleotide react with similar proficiency.

The number of carbohydrate building blocks is large, and their synthesis is further complicated by the number of different linkages that can be formed. Consider a random tetramer of each of these species when choosing from the four major nucleotides, the 20 natural amino acids, or nine common hexoses (a substantial underestimate given natural diversity). One sees that 256 oligonucleotides and 16,000 tetrapeptides are available. Limiting the number of carbohydrate building blocks to nine, but offering four sites for attachment with either α- or β-isomers and branching, more than 10 million tetrasaccharides are available. (See Scheme 11.1.)



Scheme 11.1 Tetrasaccharide yield in a carbohydrate library following limitation of the number of building blocks.

11.1.2 Libraries of Carbohydrates: Strategies

The success of solid-phase techniques in conquering the synthesis of polypeptides and oligonucleotides has led many to consider this strategy for carbohydrates. These strategies are discussed at length in other chapters of this monograph, and come with their own set of challenges. We concern ourselves with solution phase protocols. For either method to be useful, however, certain general criteria must be met.

Access to Suitably Protected Monomers Both strategies require suitably protected monomers for polymerization. The potential for a variety of linkages suggests that for a hexose-like glucose, at least four different monomer building blocks are required given that the reducing end and either the 2′, 3′, 4′, or 6′ hydroxyl oxygen will be engaged in glycosidic bonds in a linear polymer. Having access to these molecules from a common intermediate reduces the number of reagents necessary.

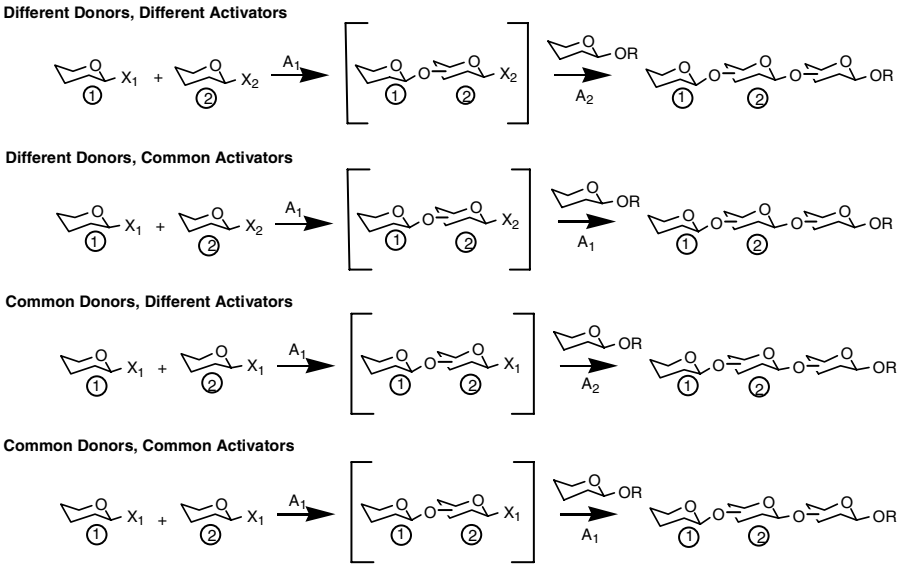
Selective Transformation Glycosidic bond formation is complicated by the potential for both α- and β-epimeric products. As most of these reactions proceed through an oxonium ion intermediate, the α/β ratio is derived from substituents on the pyranose ring. The number of required building blocks doubles even with the oversimplification that the stereoselectivity is derived solely from the nature of C2 substituent.

Removal of Side Products Using solid-phase protocols, unreacted reagents, consumed reagents, and the products of protecting group manipulations are washed away. Solution-phase strategies will be limited to a single transformation per step with subsequent purification of the product (a multistep route) unless the byproducts either (1) are not produced or (2) do not interfere with subsequent transformations.

11.1.3 One-Pot Methods

For the purposes of this chapter, a "one-pot method" is any strategy in which multiple glycosylation reactions are performed in the same reaction vessel without intermediate purification steps. Typically, these strategies rely on the sequential addition of glycoside donors coupled with the delivery of new or additional equivalents of activating agent. These strategies can be divided into four groups as identified above. In practice, however, many investigators rely on a combination of these strategies dictated by the complexity of the target, and the availability of reagents. In this scheme (see Scheme 1.2), donors are numbered 1–3. The activators, A_n, and anomeric leaving groups, X_n, are distinguished by subscripts.

11.1.3.1 Different Anomeric Groups, Different Activators Combining different anomeric groups and activators into the same reaction vessel is based on glycosylation methodology developed since the mid 1980s.[3,4] Successful implementation of this strategy relies on suitable combinations of orthogonal reagents such that the byproducts of the consumed activators and donors of the first glycosylation do not interfere with the reagents utilized in the second glycosylation reaction. With the



Scheme 11.2 Performing glycosylation reactions using a one-pot method.

identification of suitable reagent combinations, typically a glycosyl halide or tricholoroacetimidate in the first step, and a thioglycoside in the second step, the wealth of literature describing α/β preferences of particular substrates can be used advantageously in designing the appropriate strategy. The work of Tatsuta and Chenault described in the following sections are examples of this strategy.

11.1.3.2 Different Anomeric Groups, Common Activators For a common activator to afford selective oxonium ion formation, the leaving groups (X_1 or X_2) of the donor must display significant differences in reactivity toward the activating agent. These differences can result from steric or electronic effects. For example, Kahne employs thioaryl glycosides (activated with TfOH) in which the electronic properties of the leaving group, X_n, control selectivity. Ley has introduced selenophenyl glycosides as more reactive analogues of thioethyl glycosides, and both donors are activated by NIS/TfOH. Alternatively, Boons controls selectivity with steric bulk of the leaving group. Thioethyl glycosides and dicyclohexylmethane thioglycosides can both be activated with NIS/TMSOTf, but the bulkier $(Chx)_2CH_2S-$ group reacts much more slowly with the electrophilic iodine than its thioethyl counterpart.

11.1.3.3 Common Anomeric Groups, Different Activators To our knowledge, there are relatively few examples of common anomeric groups requiring different activators. Often, this protocol is introduced when the less reactive donor reacts too

sluggishly during the second glycosylation reaction and a stronger activator is added to accelerate the reaction rate. Alternatively, in our work with thiotoluyl glycosides, the succinimide byproduct of NIS/TfOH activation in some cases reacts faster with the oxonium ion than the acceptor. DMTST produces no such byproducts, and the reaction proceeds cleanly, albeit more slowly. This retardation of rate is overcome in part by the fact that DMTST is used for the most reactive donors, while the stronger NIS reagent is used later in the scheme with less reactive donors, thus keeping the concentration of succinimide to a minimum.

11.1.3.4 Common Anomeric Groups, Common Activators Both conformational and electronic factors have been exploited to obtain selectivity in one-pot glycosylations using common anomeric groups and activators. Selectivity results from differences in the stability of oxonium ion intermediate between the two prospective glycosyl donors.

Electronics Electron-withdrawing groups destabilize the cationic intermediate. These effects are most profound at the 2′ position of the pyranoside, and extend from Paulsen's original investigation of glycosyl halide hydrolysis in which 2′-benzylethers were found to be more reactive than 2′-benzoylesters. The pioneering efforts of Fraser–Reid and coworkers also exemplify this strategy. The introduction of "armed" and "disarmed" *n*-pentenyl glycosides, which are both activated with bromonium or iodonium ion, react selectively as a result of the substituents on the pyranoside.

Conformation Groups that favor a flattening of the pyranose, a consequence of oxonium ion formation, increase the rate of reactivity. Ley uses 3,4-spiroketals to enforce chairlike structure on the pyranose ring, thus deactivating them toward reaction.

11.2 AN ABBREVIATED HISTORY

For our work to be put in perspective, and to help illuminate the paths of future inquiry, we look at important observations and studies that laid the foundations for our work. The discussion presented in the following paragraphs is not intended as a thorough overview of the field, but rather presents highlights along the road to the development of programmable, one-pot glycosylations. The information is not exhaustive, nor have contributions from all laboratories engaged in these efforts been sought for inclusion. The choice of inclusion is based solely on those papers that have most affected our approach to this problem.

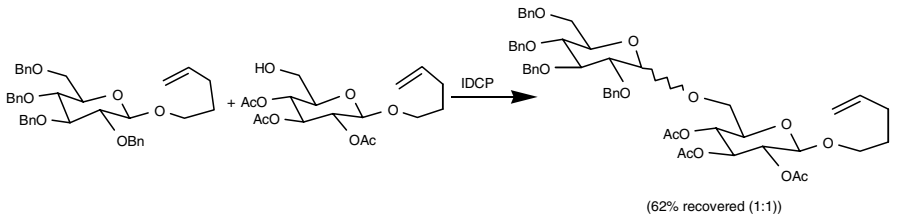
11.2.1 Paulsen and the Hydrolysis of Anomeric Halides

The origins of this field are often traced to observations made by Paulsen[5] in the 1970s with regard to the differences in rate at which anomeric halides are hydrolyzed.

Specifically, Paulsen recognized that glycosyl halides bearing C2-benzylethers were much more prone to hydrolysis than the corresponding C2 esters. Similarly, Paulsen noted that the glycosyl iodides were more prone to hydrolysis than glycosyl bromides, and these bromides were more reactive than glycosyl chlorides.

11.2.2 Fraser-Reid and Armed/Disarmed Isopentenyl Glycosides

Consistent with Paulsen's observations, Fraser-Reid[6,7] and coworkers in the late 1980s described the ability of a C2-protecting group to "arm" or "disarm" an *n*-pentenyl glycoside for oxonium ion formation. Electrophilic iodine activates the donor bearing a 2′-ether (the "armed" glycoside) selectively over that bearing the 2′-ester (the "disarmed" glycoside). Fraser-Reid has extended these strategies to temporarily "sidetracking" of armed donors,[7] but not yet into the one-pot syntheses of polysaccharides. (See Scheme 11.3.)



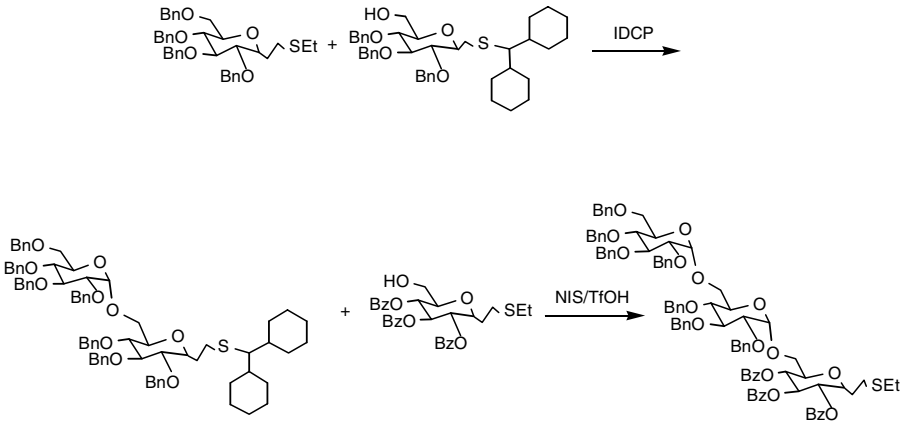
Scheme 11.3 Using a C2-protecting group for arming/disarming of an *n*-pentenyl glycoside for oxonium ion formation.

11.2.3 Boons and Hindered Thioglycosides

Boons[8] and colleagues introduced dicyclohexylmethane thioglycosides as hindered donors that are less reactive than thioethyl glycosides when either IDCP or NIS/TMSOTf is used as an activating agent. Boons continued his studies with doubly disarmed thioglycosides by varying the C2-protecting group. Consistent with Fraser-Reid's findings, Boons and coworkers report that the bulky thioglycoside bearing a 2′-benzyl ether reacts more rapidly than does the thioethyl glycoside bearing a benzoylester group at the 2′ position. Taking advantage of this strategy, pentasaccharides have been produced in a multistep strategy without a single protecting group manipulation. (See Scheme 11.4.)

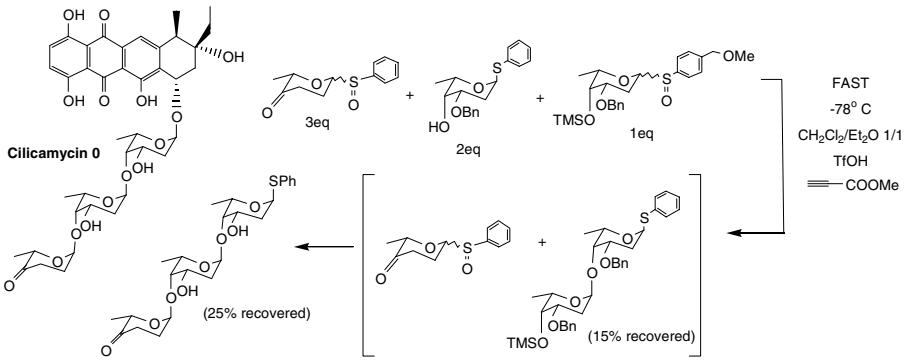
11.2.4 Kahne and Differential Glycoside Activation

In 1993, the first of many reports of one-pot glycosylation reactions appeared. Raghavan and Kahne[9] used TfOH (or triflic anhydride) to sequentially activate phenylsulfoxide glycosides that differed in their substitution pattern at the para



Scheme 11.4 Formation of pentasaccharides in a multistep strategy without protecting group manipulation.

position of the phenyl ring. Kahne reports that activation rates decrease in the order OMe > H > NO_2. The one-pot synthesis of the trisaccharide portion of ciclamycin 0 was effected in 25% overall yield. The reducing end of this trisaccharide bears a thiophenyl glycoside that can presumably be activated for installation onto the aglycone. (See Scheme 11.5.)

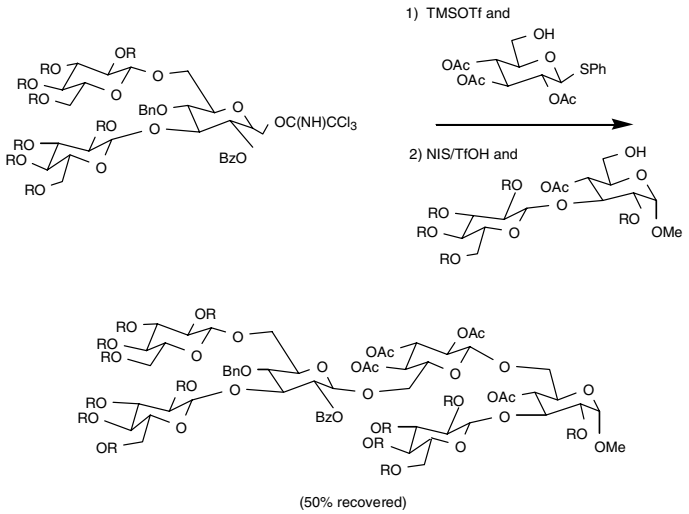


Scheme 11.5 Phenylsulfoxide glycoside activation.

11.2.5 Takahashi and Varied Donor Glycosides

In 1994, Takahashi and coworkers[10,11] described another one-pot synthesis of trisaccharides using sequential additions of various glycoside donors and their corresponding activators.[10] The first transformation relies on the activation of a

glycosyl halide with AgOTf in the presence of a thiophenyl glycoside acceptor. Addition of NIS activates the resulting thiophenyl glycoside disaccharide for reaction with the new acceptor that is added subsequently. While thiophenyl glycosides are almost exclusively used as the second donor, Takahashi and coworkers have investigated trichloroacetimidates or glycosyl fluorides as substitutes for glycosyl bromides as the first donor. Yields for different trisaccharide products varied within 40–85%. Takahashi extended these studies to larger targets using more complex building blocks.[11] The hexasaccharide product shown in Scheme 11.6 is furnished in a one-pot strategy using a trisaccharide and a trichloroacetimidate activated with TMSOTf in the presence of a thiophenylglycoside. Subsequent addition of the suitably protected disaccharide and NIS/TfOH as an activator yields the desired product in 50% yield.



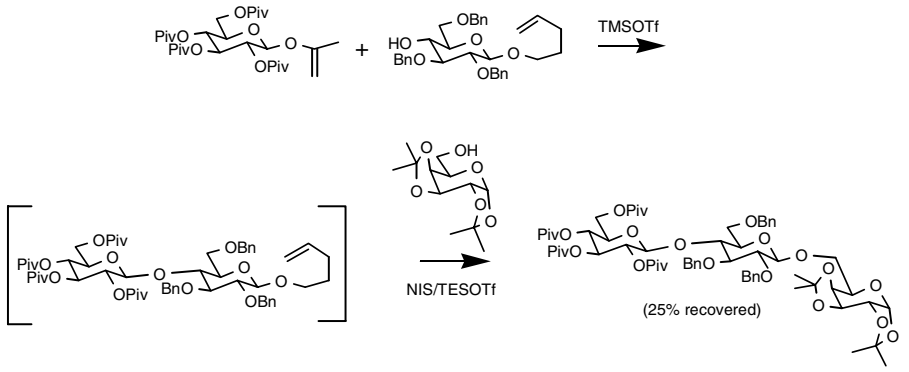
Scheme 11.6 Hexasaccharide production in a one-pot strategy.

11.2.6 Chenault and Varied Glycoside Donors

Building on the observations of Kahne and Takahashi, Chenault and Castro relied on the selective activation of an isopropenyl glycoside donor with TMSOTf, followed by subsequent activation of a second *n*-pentenyl glycoside with NIS/TESOTf.[12] This one-pot glycosylation offers proof of the concept that Fraser-Reid's glycosides can be incorporated into one-pot syntheses. (See Scheme 11.7.)

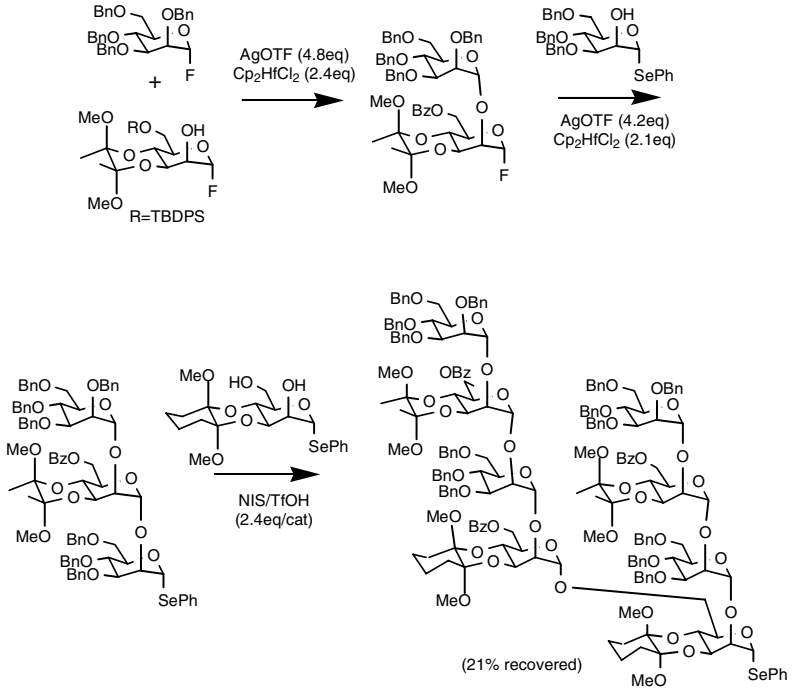
11.2.7 Ley and a Universal Approach

Ley and coworkers[13–18] are responsible for setting much of the stage on which our work is played. Showcased by the synthesis of the heptasaccharide shown in Scheme



Scheme 11.7 A one-pot glycosylation reaction.

11.8, Ley and coworkers have (1) explored the use of cyclic 3,4-diketals to influence the rate of oxionium ion formation (common activator, common donor), (2) utilized thiophenyl and selenophenyl donors to achieve targets (different donors, common activator), and (3) adopted most encompassing approaches to one-pot syntheses.



Scheme 11.8 One-pot synthesis of heptasaccharide.

We comment briefly on some of Ley's advances here, while introducing other insights from his group in later sections of the text.

Cyclic Diketals (Common Activator, Common Donor)[13–15] Ley and Priepke recognized that the reactivity of a thioethyl glycoside was reduced by the cyclohexane-1,2-diacetal (CDA) protecting group. Taking advantage of this group, a CDA-protected glycoside and a perbenzylated thioethyl glycoside could be treated with NIS and TfOH to yield the disaccharide intermediate. Addition of the second acceptor and additional NIS/TfOH resulted in the trisaccharide in 62% overall yield. Unlike previous strategies, nearly stoichiometric amounts of each monosaccharide were used.

Thiophenyl Glycosides and Selenophenyl Glycosides (Different Donors, Common Activator)[16] Changing from thioethyl glycosides to selenophenyl glycosides increases the reactivity by approximately 20-fold in the mannoside series. Both of these donors can be activated by NIS/TfOH.

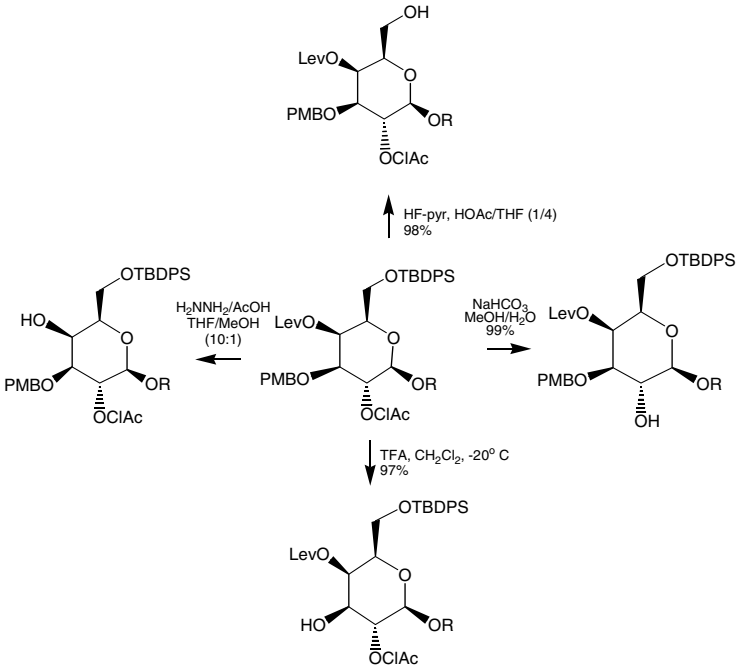
Universal Strategies[16–18] The synthetic route shown above demonstrates Ley's approach to the one-pot syntheses of complex carbohydrates. Applying Takahashi's different activators–different donors strategies, Ley begins with two glycosylfluorides (common activator, common donor) that react selectively, as one is activated with 2′-benzylether while the other is deactivated by the diketal substituent. Ley then adds an orthogonal donor, a selenophenyl glycoside (itself unreactive under AgOTf activation), to generate the trisaccharide. The fourth donor, a selenophenyl glycoside (deactivated with free hydroxyls and a 3,4-CDA group), is incorporated on the reducing end of the tetrasaccharide, as the addition of a source of electrophilic iodine selectively activates the trisaccharide.

11.3 WORK FROM THE WONG LABORATORY

Our initial route for the preparation of linear and branched carbohydrate libraries utilized a multistep strategy employing an orthogonally protected carbohydrate core. This core could then be selectively deprotected, and glycosylated in an iterative fashion. Synthesis of polysaccharides using this core required purification at every step, however, which quickly became burdensome for the 45-member library that was prepared. So, we turned our attention to one-pot methods. These one-pot methods use thiotoluyl glycosides, reagents that have acceptable shelf lives and ones that can be activated with a variety of reagents.[3,4] Our work in both areas is addressed in the following sections.

11.3.1 Orthogonal Protecting Groups

We have demonstrated an orthogonal protection/deprotection strategy for preparing a carbohydrate core that could be iteratively deprotected and glycosylated to form



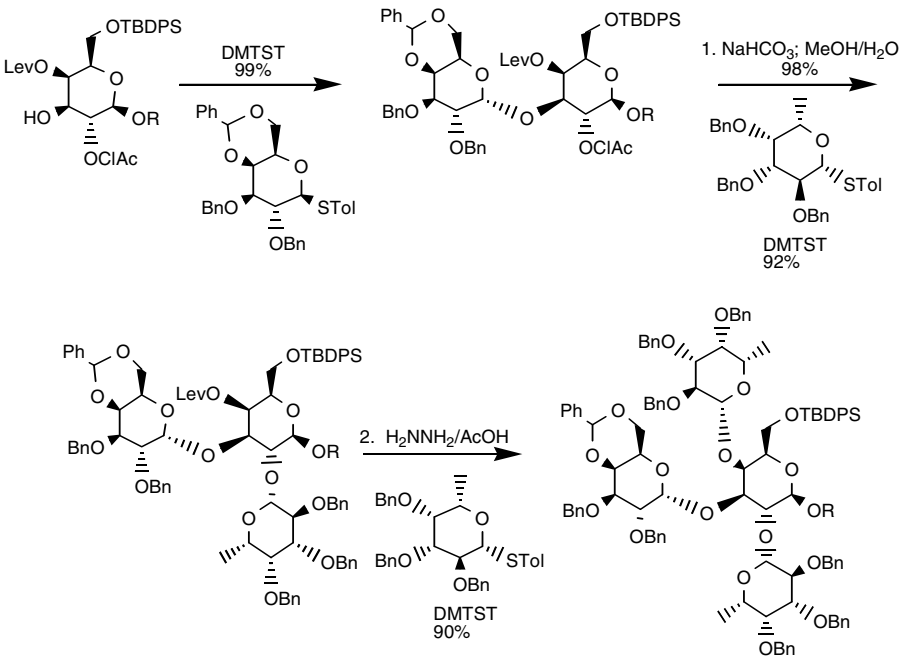
Scheme 11.9 Orthogonal deprotection strategy for the preparation of carbohydrate cores.

libraries of linear and branched carbohydrates (Scheme 11.9).[19] The common galactose intermediate is available in five steps from thiotoluylgalactose through sequential reaction: TBDPSCl and imidazole (100%), PMB-Cl with Bu_4NI (50%), chloroacetylchloride and triethylamine (52%), and levulinic acid with DCC/DMAP (83%). Glycosylation of the thioglycoside is effected with NIH/TMSOTf followed by addition of $HgBr_2$. An example of the method used for the preparation of one of the library members is shown in Scheme 11.10.

The generality of this strategy was investigated by the preparation of a small carbohydrate library of tri-, tetra-, and pentasaccharides (Table 11.1). This protocol quickly becomes burdensome if libraries (rather than single targets) are desired since it requires isolation of every intermediate. We pursued one-pot methods as a practical and more efficient alternative. (See Scheme 11.11.)

11.3.2 Programmable One-Pot Synthesis

We use the term "programmable" to describe the rational (and ideally, computer-aided and automated) approach to polysaccharide synthesis.[20] To reduce the synthesis of complex carbohydrates to routine, we envision a four-step protocol: (1) the sequence of interest is keyed into a computer, (2) the computer selects appropriate reagent combinations, (3) a laboratory worker (human or robotic) prepares the reagent



Scheme 11.10 Application of an orthogonal protection/deprotection strategy in preparation of a tetrasaccharide.

containers for the delivery to the reaction vessel, and (4) the synthesis is executed and a crude reaction product is delivered. Subsequent purification affords the oligosaccharide of interest. Developing libraries of reagents and understanding the relative reactivities of these reagents are paramount to the success of this strategy.

11.3.2.1 Strategy Our strategy for one-pot methods rested on using common anomeric groups, and a common activator. We chose *p*-methylphenyl thioglycosides (STol) as our donor species for three reasons:

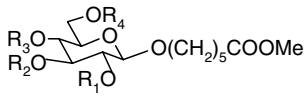
1. Thioglycosides can be activated by a wealth of strategies,[3,4] although our investigations center on the use of electrophilic iodine (*N*-iodosuccinimide).
2. The thiotoluoyl group offers a convenient spectroscopic handle.
3. The reagents have significant shelf lives.

Our goals for assembly of carbohydrate libraries included (1) methods that did not rely on protecting group manipulations during synthesis and (2) the preparation of a significant pool of reagents. Additionally, we desired building blocks that afforded simple, branched carbohydrates as well. These building blocks should also allow for both α and β linkages to be prepared. To accomplish this strategy under competitive reaction (one-pot)

TABLE 11.1 Preparation of a Carbohydrate Library

Number	R^1	R^2	R^3	R^4	%
1	L-Fuc(α1,2)	D-Gal(α1,3)	Lev	TBDPS	87
2	L-Gal(α1,2)	D-Gal(α1,3)	Lev	TBDPS	60
3	L-Gal(β1,2)	D-Gal(α1,3)	Lev	TBDPS	33
4	D-Man(α1,2)	D-Gal(α1,3)	Lev	TBDPS	78
5	L-Fuc(α1,2)	D-Gal(α1,3)	Lev	TBDPS	53
6	L-Fuc(β1,2)	D-Gal(α1,3)	Lev	TBDPS	38
7	D-$GalN_3$(α1,2)	D-Gal(α1,3)	Lev	TBDPS	72
8	D-$GalN_3$(β1,2)	D-Gal(α1,3)	Lev	TBDPS	14
9	L-Fuc(α1,2)	D-$GalN_3$(α1,3)	Lev	TBDPS	29
10	L-Fuc(β1,2)	D-$GalN_3$(α1,3)	Lev	TBDPS	5
11	D-Man(α1,2)	D-$GalN_3$(α1,3)	Lev	TBDPS	34
12	ClAc	D-Gal(α1,3)	L-Fuc(α1,4)	TBDPS	65
13	ClAc	D-Gal(α1,3)	Lev	L-Fuc(α1,6)	59
14	ClAc	D-Gal(α1,3)	Lev	L-Fuc(β1,6)	22
15	ClAc	D-Glc(α1,3)	L-Fuc(α1,4)	TBDPS	59
16	ClAc	D-Glc(α1,3)	Lev	L-Fuc(α1,6)	67
17	ClAc	D-Glc(α1,3)	Lev	L-Fuc(β1,6)	22
18	L-Fuc(α1,2)	D-Glc(α1,3)	Lev	TBDPS	76
19	L-Fuc(α1,2)	D-Glc(α1,3)	Lev	TBDPS	13
20	L-Fuc(α1,2)	D-Man(α1,3)	Lev	TBDPS	35
21	L-Fuc(α1,2)	PMB	Lev	L-Fuc(α1,6)	57
22	L-Fuc(α1,2)	PMB	Lev	L-Fuc(β1,6)	16
23	L-Fuc(α1,2)	PMB	D-Glc(1,4)	TBDPS	65
24	L-Fuc(α1,2)	L-Fuc(α1,3)	Lev	TBDPS	85
25	ClAc	D-Gal (α1,3)	Lev	D-Glc(β1,6)	26
26	ClAc	D-Gal (α1,3)	Lev	D-Glc(α1,6)	52
27	L-Fuc(α1,2)	PMB	Lev	D-Glc(α1,6)	60
28	ClAc	PMB	D-Glc(1,4)	D-Glc(1,6)	66
29	L-Fuc(α1,2)	D-Gal (α1,3)	L-Fuc(α1,4)	TBDPS	74
30	L-Fuc(α1,2)	D-Gal (α1,3)	L-Fuc(α1,4)	L-Gal(α1,6)	45
31	L-Fuc(α1,2)	D-Gal (α1,3)	L-Fuc(α1,4)	L-Gal(β1,6)	18
32	D-Gal(α1,2)	PMB	D-Gal(α1,4)	TBDPS	76
33	ClAc	L-Fuc(α1,3)	D-Gal(α1,4)	TBDPS	54
34	ClAc	L-Fuc(α1,3)	D-Gal(β1,4)	TBDPS	14
35	L-Fuc(α1,2)	PMB	D-Gal(α1,4)	TBDPS	61
36	L-Fuc(α1,2)	PMB	D-Gal(β1,4)	TBDPS	15
37	ClAc	PMB	D-Gal(α1,4)	L-Fuc(α1,6)	37
38	ClAc	PMB	D-Gal(α1,4)	L-Fuc(β1,6)	19
39	L-Fuc(α1,2)	PMB	L-Fuc(α1,4)	TBDPS	46
40	L-Fuc(β1,2)	PMB	L-Fuc(α1,4)	TBDPS	12
41	L-Fuc(α1,2)	PMB	L-Fuc(β1,4)	TBDPS	12
42	L-Fuc(β1,2)	PMB	L-Fuc(β1,4)	TBDPS	9
43	L-Fuc(α1,2)	D-Gal (α1,3)	ClAc	TBDPS	83
44	Lev	D-Gal (α1,3)	D-Gal(β1,4)	TBDPS	46
45	Lev	PMB	D-Gal(β1,4)	D-Gal(β1,6)	49

conditions, we use a strategy in which the reactivity of donors decreases over the course of the reaction; that is, we intended to begin at the nonreducing end and perform glycosylations up to the reducing end. This strategy is shown schematically as applied to the synthesis of linear and simple branched targets (Scheme 11.12).

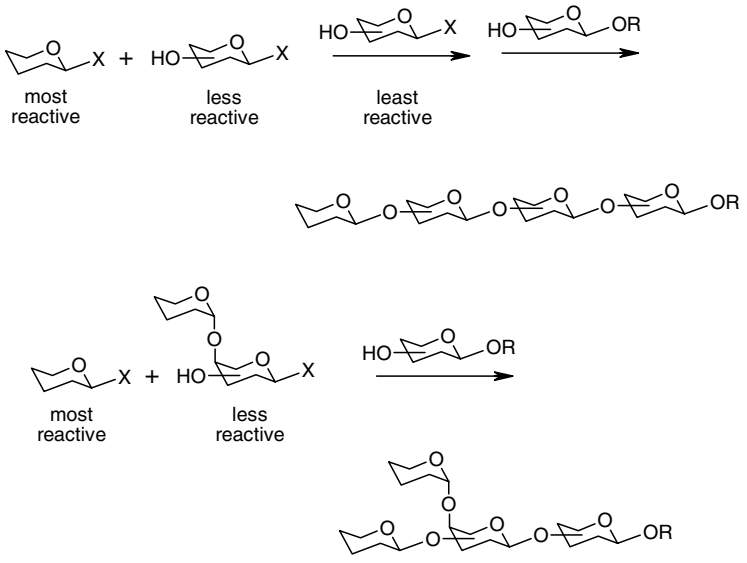


Scheme 11.11 A structure formed using a one-pot method with orthogonal protecting groups.

For this strategy to be successful, data describing the relative reactivities of a variety of donors are needed. In general, most one-pot syntheses had been carried out previously with the general knowledge of the reactivity difference between ether and ester protecting groups; where no reactivity number was involved in the design of the synthesis. We envisioned that a greater diversity of targets could be prepared if reactivity values could be collected and used. The compiled data from our group are presented in the following pages. We calculated relative reactivity values that compare the most reactive donors (high values) to the least reactive donor (1.0). Additional detail is provided in the following section.

11.3.2.2 Routes to Relative Reactivity Data Relative reactivity relationships describe the ratio of products between two glycosyl donors for an acceptor. Ley first constructed such relationships for fully protected mannoside and rhamnoside donors to rationalize the results of his one-pot syntheses employing cyclic diketals.[16]

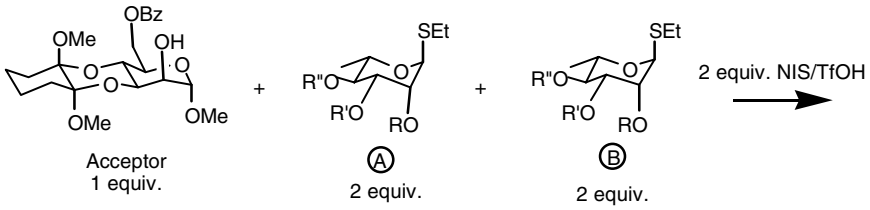
Ley showed that these relationships need not be measured for every potential combination of donors, but rather the multiplicative relationship between these donors held with only small discrepancies. Throughout his work, Ley reports deactivation factors, which describe the decreased reactivity of a donor in comparison with the



Scheme 11.12 Synthesis of linear and simple branched targets.

perbenzylated species. As such, larger numbers imply lesser reactivity. Alternatively, we normalize reactivities to the least reactive donor; hence larger numbers in our series represent greater reactivity. Nevertheless, competition experiments are used to determine these reactivity differences. Details of these experiments vary, as Ley looks at product formation using NMR, while we look at reagent consumption using HPLC.

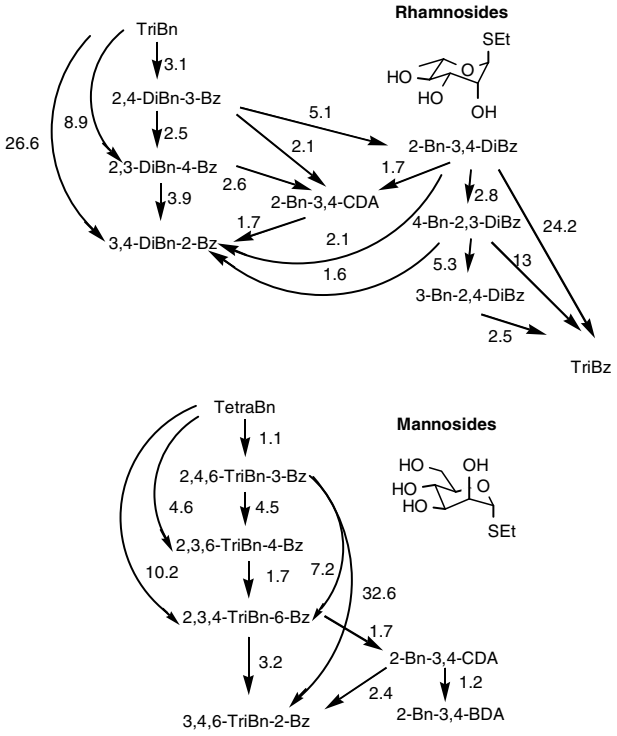
Competition Experiments of Ley Ley examined product distributions to obtain relative reactivity values (RRVs) for both rhamnose and mannose sugars with benzyl, 3,4-cyclic diacetals, and benzoyl groups. Using a limiting amount of acceptor (1 equiv), two donors were added in excess (2 equiv each), followed by the addition of activator (NIS/TfOH, 2 equiv) (Scheme 11.13). After quenching the reaction, the product distribution was analyzed by ^{1}H-NMR.



Scheme 11.13 Production distribution in a reaction with rhamnose and mannose sugars with benzyl, 3,4-cyclic diacetals, and benzoyl groups.

Ley introducted deactivation factors (DFs) to describe the effect that a protecting group had at a position on the rate of reaction. These DFs, although carbohydrate-specific, were shown to be predictive of the RRVs for these sugars as determined by direct competition experiments. For the protecting groups used, Ley observed that the extent of deactivation of a protecting group is *independent* of other protecting groups on the pyranose. In our studies involving larger numbers of building blocks, we found that this observation does not necessarily hold true; that is, the effect of a protecting group is not always predictive of reactivity. From these data Ley obtained the following relationships (see also Scheme 11.14).

Competition Experiments of Wong RRVs were obtained by monitoring the disappearance of donor with respect to a standard. These rates are obtained by HPLC identification of the starting materials and did not rely on identification of the product. Each donor (0.01 M, 0.01 mmol, each) was added to an excess of acceptor, methanol (0.05 M, 0.05 mmol), in dichloromethane. We use methanol as an acceptor to eliminate the steric effect on the glycosylation reaction. Activation was accomplished by adding a solution of NIS in acetonitrile (0.01 mmol) followed by TfOH (0.001 mmol) (see Scheme 11.15). After 2 h, the reaction was worked up with saturated sodium thiosulfate, then sodium bicarbonate, and the organic phases evaporated to dryness. The resulting residue was suspended and subjected to HPLC analysis. To

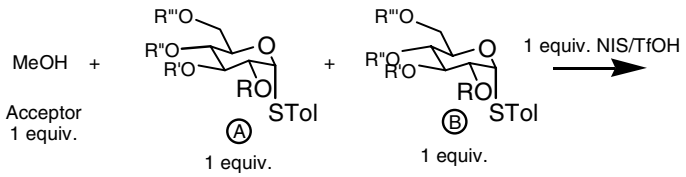


Scheme 11.14 Reactions with rhamnose and mannose sugars demonstrating relative extent of protecting group deactivation.

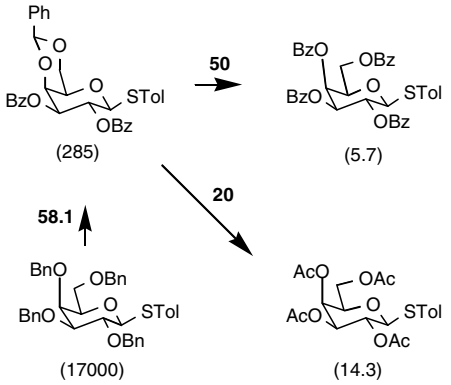
accommodate the wide range in relative reactivities, four reference molecules were selected as shown. From the competition experiments, the reactivity coefficients (Schemes 11.16–11.18) were tabulated.

11.3.2.3 Lessons Learned The quantification of reactivity for the 50 donors explored revealed some interesting trends, which are reported on in the following paragraphs:[20]

1. *Pyranosides show reactivities that differ as a function of sugar.* Comparison of commonly protected pyranosides (i.e., perbenzylated) showed that, in general,



Scheme 11.15 Product distribution using methanol as an acceptor to eliminate the steric effect on a glycosylation reaction.



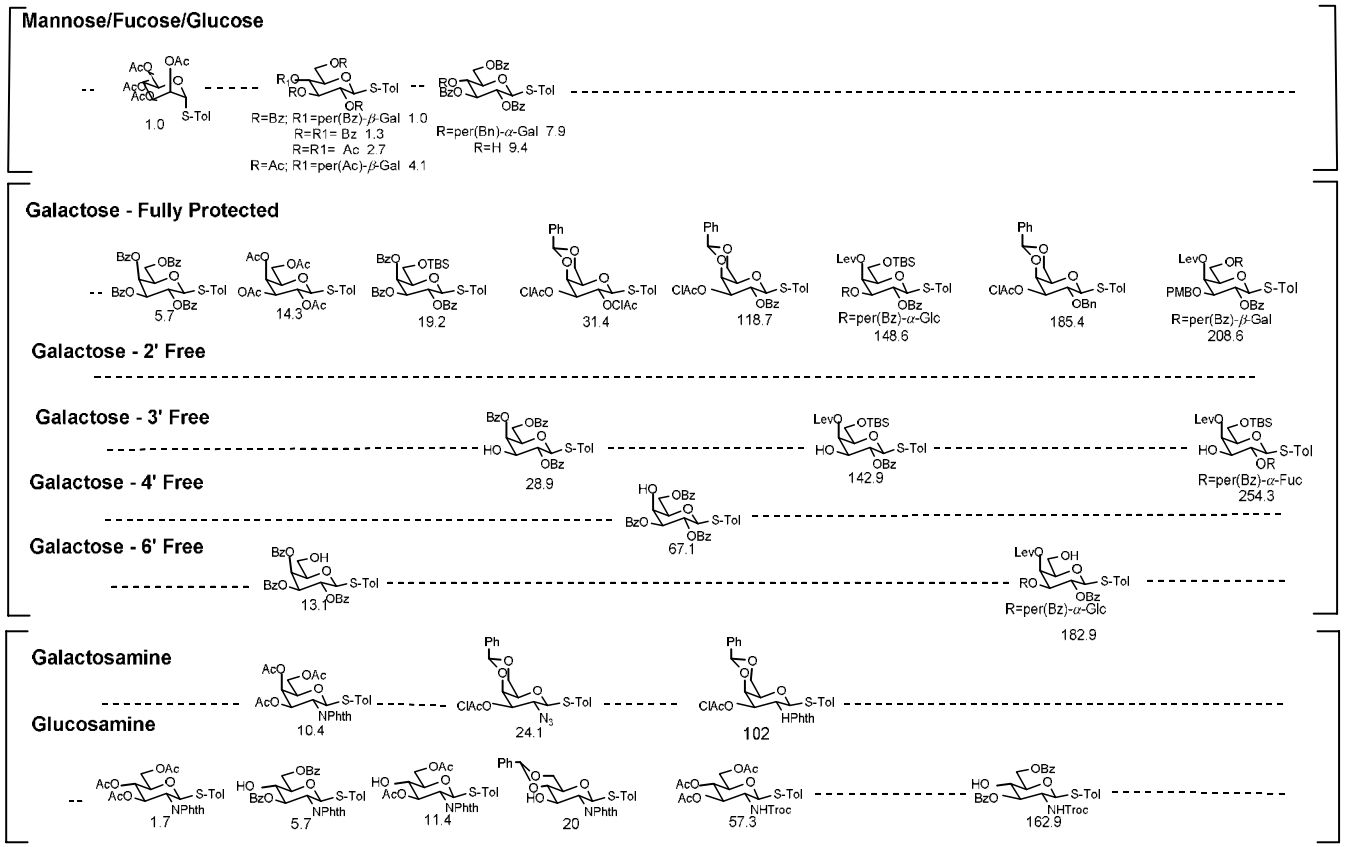
Scheme 11.16 Monitoring the relative reactivity values of four reference molecules.

reactivity decreases in the order fucose > galactose > glucose > mannose. These differences in reactivity are not great. Fucose is approximately four-fold more reactive than galactose which is approximately sixfold more reactive than glucose. These observations are not new, but rather are consistent with the rates of hydrolysis of their corresponding glycosyl halides and glycosides.[21,22]

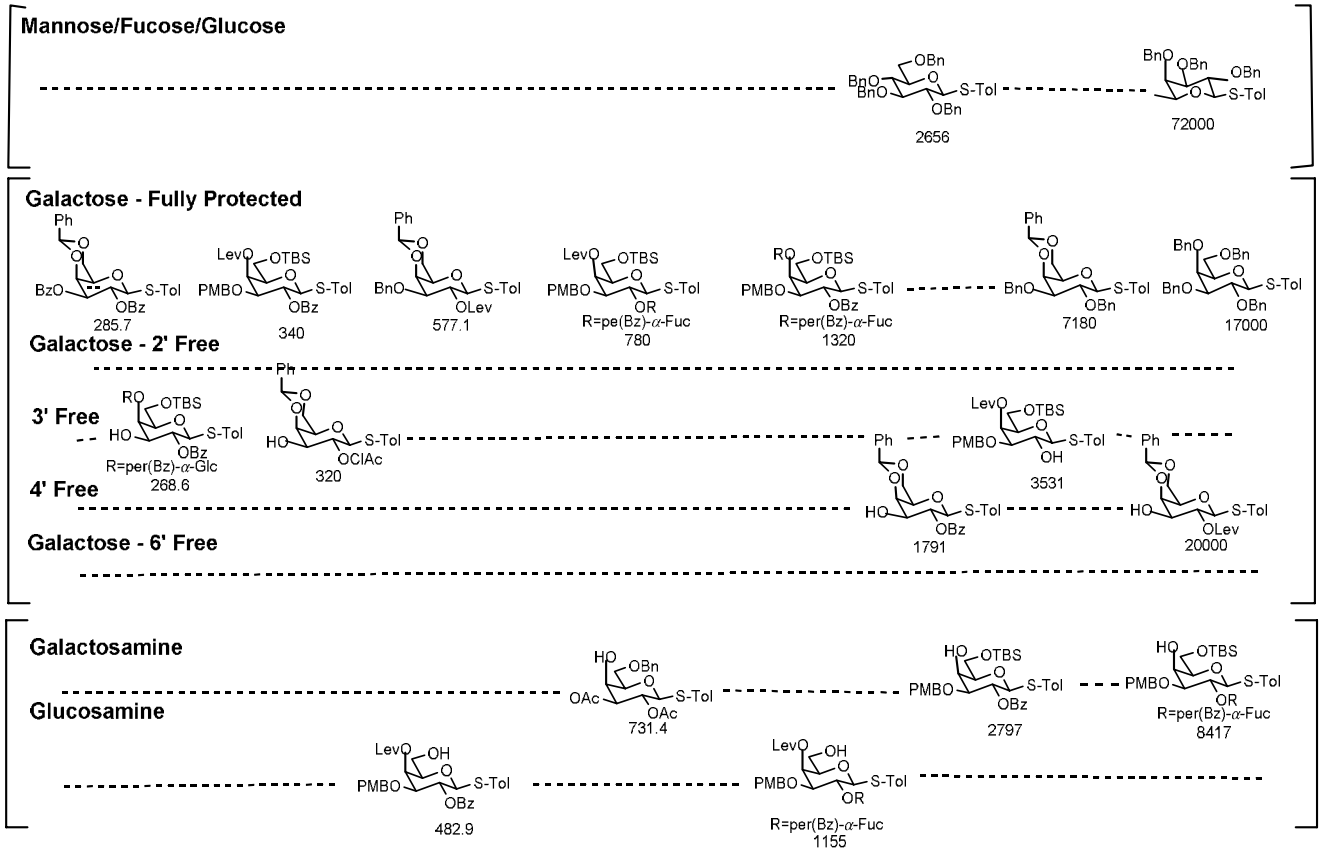
2. *^{1}H NMR is predictive of reactivity within a series.* Within a set of common donors (i.e., galactose) in which the C2 position is constant, the ^{1}H chemical shift of the anomeric carbon appears to be a good predictor of relative reactivity. Little correlation is found when the C2 position is varied, or between different donor pyranoses. The extent to which this correlation will prove useful remains in question. We envision that its primary applicability may be in trouble shooting a failed synthesis when a complex donor does not behave as predicted. Checking the ^{1}H NMR of the advanced intermediate may quickly reveal that the RRV value for the pyranose of interest is not as expected, and hence the reactivity is not as expected.

3. *The reactivity of aminosugars can be tuned by choice of the N-protecting group.* We investigated the ability of different *N*-protecting groups on glucosamine and galactosamine in order to generate donors that could be used early in a synthetic protocol (reactive) or later (less reactive). Although we were not surprised, we found that the nature of the protecting group played some role. Aminosugars bearing phthalamide groups showed very little reactivity (1.0–3.5) in comparison with those bearing Troc (trichloroethoxycarbonyl) groups (28.6). Given the large effect of the C2 group on overall reactivity of a donor, the range of reactivities, 1–28.6, is small and may greatly determine where these molecules can be incorporated into targets. Increasing these reactivities through alternate C2 groups or different protecting groups on hydroxyl groups should be investigated. Otherwise, alternate strategies may have to be employed for some targets.

4. *A general trend in protecting group effects exists.* For galactose, the most thoroughly explored series of donors, we find that the substituent plays a significant



Scheme 11.17 Tabulation of the relative reactivity values of various carbohydrates.



Scheme 11.18 Further tabulation of the relative reactivity values of carbohydrates.

role in deactivating the pyranose. Reactivity is most reduced by $-N_3$ > ClAc > NPhth > OBz > OBn.

5. *The position of the pyranoside that most greatly affects the reactivity varies from carbohydrate to carbohydrate.* While Ley reported that for mannose, the C2 position had the greatest effect on reactivity (followed by C2 > C6 > C4 > C3), we find for galactose, the order is C4 > C3 > C2 > C6.

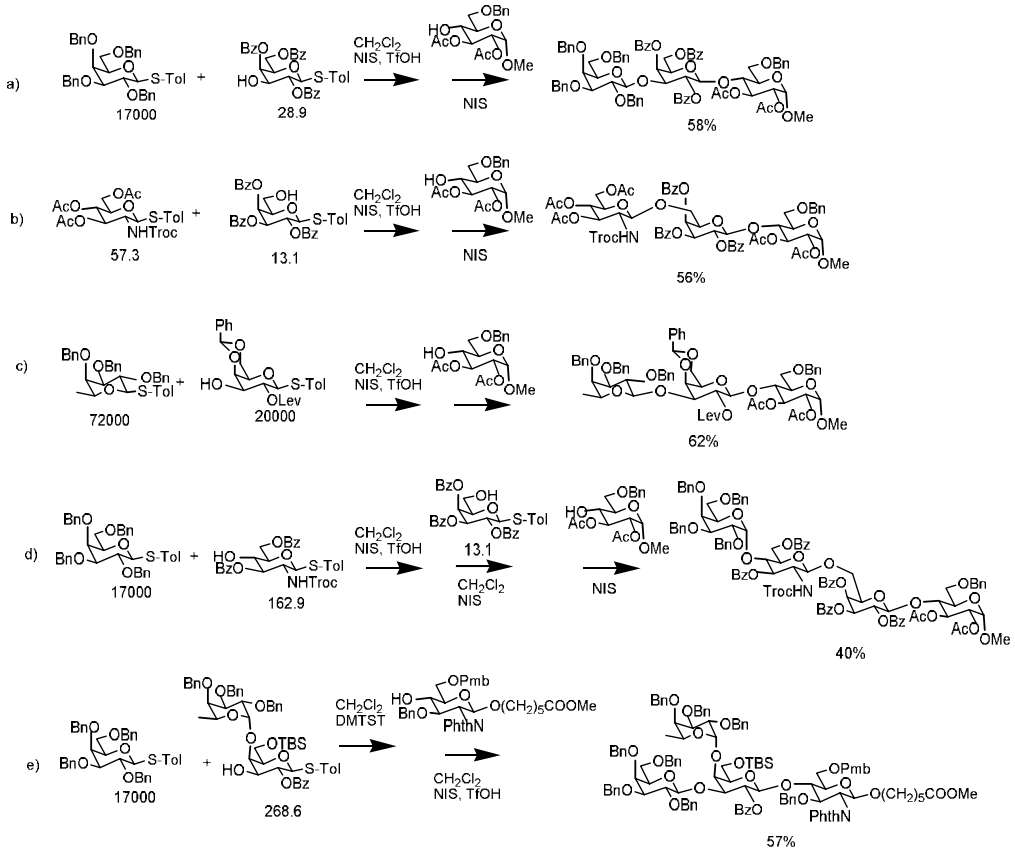
6. *The magnitude of any effect is attenuated by its position on the pyranoside.* While the substituents affect the reactivity in a predictable manner, the magnitude of this effect depends on the position of the group in most cases (similarly observed by Ley). This trend is most easily observed in the tribenzylated thiogalactoside bearing one free hydroxyl group. Reactivity increases as the hydroxyl group is available at the C6 (2.3) < C2 (3.1) < C3 (5.1) < C4 (11.8) positions. It seems likely that steric factors as well as electronic factors are playing a role.

11.3.2.4 OptiMer With the reactivities of many donors and donor-acceptors (e.g., the building blocks with one hydroxyl group unprotected) available, one can use the database to conduct one-pot synthesis of a desired oligosaccharide. According to our experience, to achieve a high-yielding coupling and reduce byproduct formation, the reactivity difference for each coupling should be larger than 10. In order to optimize the reaction to create greater diversity, the one-pot synthesis needs to rely on a computer program to select the appropriate building blocks. Our first version of the computerized database and search engine, OptiMer, was created using FileMaker Pro 4.0 (Filemaker Inc.). The database contains the name of the residue, the position of unprotected hydroxylgroups, and whether the C2 substituent directs the glycosylation to α or β positions. The database also stores the reference for its preparation and a picture of the compound. The program is designed to select the 100 best combinations of reagents and sort this list by expected yield for the transformation. Details of the strategy used to predict yields can be found in the experimental section of the lead reference.[20] In short, yield is related to the ratio of relative reactivities of the two donors such that

$$(\text{Yield})^{k_1/k_2} = 1 - y$$

in which k_1 and k_2 refer to the rates at which donor and electrophile react (the relative reactivity values) to form the oxonium ion. This relationship allows the computer to solve numerically for yields during its generation of synthetic strategies. To evaluate the utility of both the relative reactivity data and the implementation of OptiMer, we pursued the one-pot synthesis of the five oligosaccharides (Scheme 11.19). These reactions proceeded in excellent yields.

11.3.2.5 Applications Using these protocols, an oligosaccharide library was prepared (Table 11.2). The synthesis of a tetrasaccharide in general, takes 3–15 min, and with the aid of the computer program, a library of individual oligosaccharides can be assembled rapidly.



Scheme 11.19 One-pot syntheses of tri- and tetrasaccharides using relative reactivity data.

TABLE 11.2 Preparation of an Oligosaccharide Library

L-Fucα1,2Galα1,6GlcN	L-Fucβ1,4Galβ1,6GlcN	Galα1,3Galβ1,4Glc
L-Fucβ1,2Galα1,6GlcN	Galα1,3Gal(α1,6GlcN)(L-Fucα1,2)	Galα1,3Galβ1,6GlcN
L-Fucα1,2Galβ1,6GlcN	—	Galα1,3Galα1,6GlcN
L-Fucα1,3Galβ1,3GlcN	GlcNβ1,6Galβ1,4Glc	Galα1,6Galβ1,3GlcN
L-Fucα1,3Galβ1,4GlcN	Galα1,3Galβ1,3GlcN	Galα1,6Galβ1,4GlcN
L-Fucα1,3Galα1,4Glc	Galα1,3Galβ1,4GlcN	Galβ1,6Galβ1,4GlcN
L-Fucα1,3Galβ1,6GlcN	Galα1,3Gal(α1,4GlcN)(L-Fucα1,4)	Galα1,6Galβ1,6GlcN
L-Fucβ1,3Galα1,6GlcN	—	Galβ1,6Galβ1,6GlcN
L-Fucα1,4Galβ1,3GlcN	Glcα1,3Gal(β1,4GlcN)(L-Fucα1,6)	Glcα1,3Galβ1,6GlcN
L-Fucα1,4Galβ1,4GlcN	—	Glcα1,3Galβ1,3GlcN
L-Fucα1,4Galα1,6GlcN	L-Fucβ1,4Galβ1,6GlcN	Glcα1,3Galβ1,4GlcN

11.4 FUTURE DIRECTIONS

Correlations between Pyranosides Our work has emphasized galactoside donors with a limited number (~50) of fucose, glucose, *N*-acetylglucosamine, and *N*-acetylgalactosamine donors. More building blocks based on these sugars and other sugars such as mannose and sialic acid donors have been investigated (a total of ~200 building blocks) and will be reported in the near future. Ley has focused on mannosides and rhamnosides. The utility of these one-pot procedures will be judged in part by the diversity of structures accommodated, so correlating Ley's efforts with our own is an important step toward this goal. Additional examples of lesser and unrepresented pyranosides also need to be introduced.

Thiotoluyl Glycosides versus Selenophenyl Glycosides Ley's efforts of increasing the reactivity by substituting selenophenyl glycosides for thioethyl glycosides will likely prove a general paradigm for increasing reactivity. Correlating thioethyl glycosides, thiotoluyl glycosides, and selenophenyl glycosides could greatly increase the capabilities of these libraries, especially because all these groups can be activated with NIS/TfOH. We are most interested in whether this 20-fold increase in activity that Ley observes is general across our series of donors. This increase would be greatly valuable in increasing the reactivity range of the aminoglycosides (glucosamine and galactosamine), which display a reactivity value of 28.6 when Troc protecting groups are employed.

Alternative Activation Strategies Most of our efforts have focused on the use of NIS/TfOH as the activation strategy of choice. Initial experiments with DMTST suggest that the rates are slower, but the relative reactivity difference remains approximately the same. Other activation reagents may be exploited to see the extent to which these activators affect the rate and relative reactivity. An excellent recent review[3] by Toshima and Tatsuta compiled the strategies documented for thioglycoside activation (Table 11.3). It should also be noted that all the work done so far is based on the assumption that the acceptor reactivity is not affected by steric effects, which are difficult to measure. While the concept of programmable one-pot

TABLE 11.3 Thioglycoside Activation Strategies

Reagent	Principal Investigator(s)	Ref.
$HgSO_4$	Ferrier	23
$HgCl_2$	Ferrier, Wiesner	23,24
PhHgOTf	Garegg	25
$Hg(OBn)_2$	van Cleve	26
$Hg(NO_3)_2$	Hanessian	27
$Cu(OTf)_2$	Mukaiyama	28
$Pd(ClO_4)_2$	Woodward	29,30
$CuBr_2$-Bu_4NBr-AgOTf	Ogawa	31–35
PhSeOTf	Ogawa	36–38
Br_2	Koto, Zen	39
AgOTf,Br_2	Kihlberg	40
NBS	Nicolaou	41
NBS	Roush	42
NIS-HOTf	Fraser-Reid, Van Boom	43,44
NBS-HOTf	Sasaki	45,46
IDCP	Van Boom	47–50
$NOBF_4$	Pozsgay, Jennings	51,52
MeI	Mereyala	53–57
MeOTf	Lonn	58,59
MeSOTf	Garegg	60,61
DMTST	Fugedi	62,63
DMTST	Hasegawa	64–70
XSCN	Kochetkov	71–74
1-phenyltetrazol-5-ylthio/$TrClO_4$	Ogura	75
TMSOTf	Ito	76
Electrochem	Sinay, Balavoine	77–79
[O], Tf_2O	Kahne	80
[O], MgBr,Et_2O,,	Ley	81,82
Selectfluor	Wong	83

synthesis is concerned with oligosaccharide synthesis, the strategy and principle could be applied to other types of organic reactions.

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The body of the work described in this chapter was accomplished by highly skilled coworkers in the Wong laboratory at the Scripps Research Institute. Drs. Xin-Shan Ye and Zhiyuan Zhang are solely responsible for the initial, impressive, synthetic and analytical efforts in the development of orthogonal protecting groups and their application to multistep syntheses. Together with Dr. Ralf Wishnat, the use of these building blocks in a programmable, one-pot strategy followed. Dr. Timor Baasov, on sabbatical leave from the Technion (Haifa, Israel), lent both synthetic and intellectual contributions to this project. Dr. Ian Ollmann was responsible for the computer program, OptiMer, which was used to choose the appropriate building blocks for a desired product.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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12 Carbohydrate Libraries by the Random Glycosylation Approach

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12.1 INTRODUCTION

Carbohydrates hold the potential to become an important class of pharmaceuticals since they have a wide range of functions involved in cellular interactions.[1] Despite such diverse functions, only few oligosaccharide-based pharmaceuticals have been explored to date.[2,3] Efforts directed toward the synthesis of complex glycoconjugates have made it possible to control the stereo- and regiospecificity of the glycosylation reactions using solution-phase organic synthesis. The state-of-the-art synthesis of oligosaccharides (Fig. 12.1),[4] however, cannot be directly applied to a current trend of combinatorial chemistry and in high-throughput screening to discover pharmaceutical leads. This is due mainly to the lack of established solid-phase synthetic methods, where overall efficiency and simplicity are required. Oligosaccharide synthesis is, given the nature of carbohydrates, in general, multifunctional and far from simple. *Multifunctionality* means that more than three hydroxyl groups exist even on a monosaccharide, all of which have the potential to be glycosylated by other sugars and anomeric stereoisomers that are formed during glycosylation. Therefore, in order to control stereo- and regiospecificity, orthogonal protecting group manipulations and the introduction of special protecting groups at the C2 position are necessary.

Combinatorial chemistry has been used with great success to create libraries in the development of inhibitors in the field of peptide and nucleic acid recognition.[5,6] The basic strategy of a library approach is to synthesize large sets of molecules at a time, even as complex mixtures, and then determine whether any of the compounds is inhibitory. The active compound must be subsequently identified. This strategy stands in contrast to the extremely laborious and expensive process of traditional medicinal chemistry, where individual molecules are carefully synthesized and evaluated. The

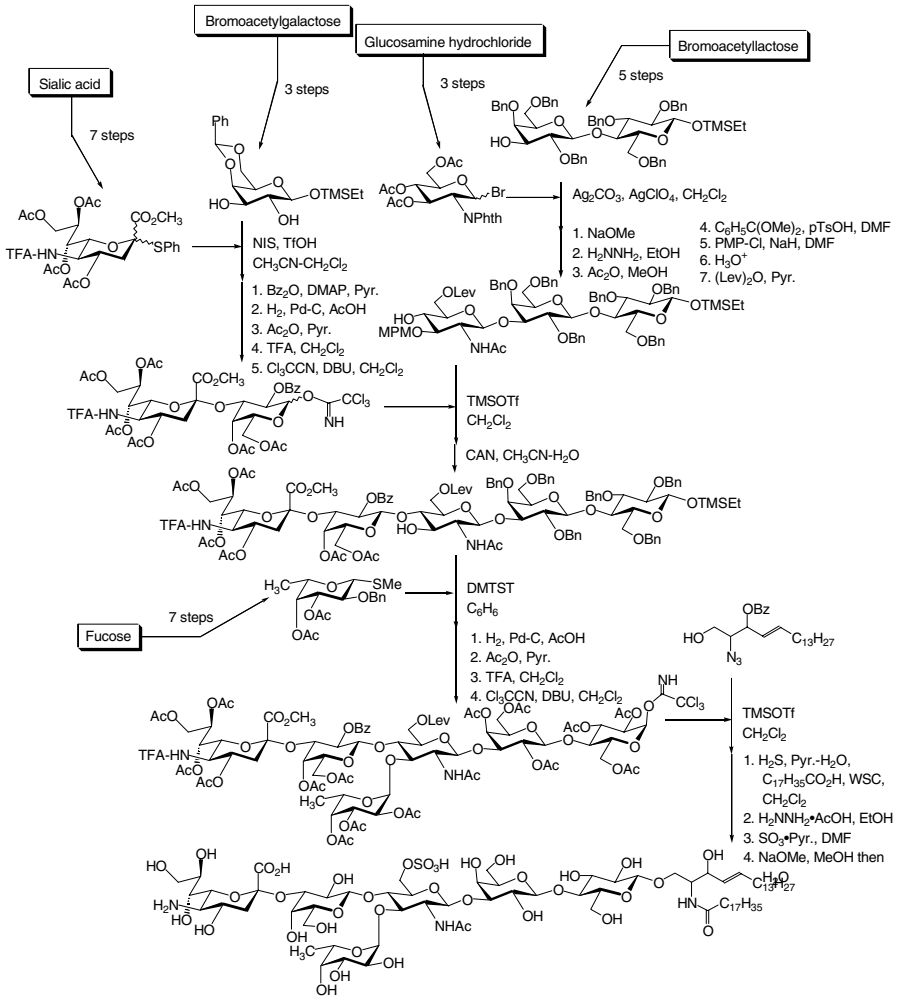


Figure 12.1 Oligosaccharide synthesis.

principles of combinatorial chemistry have been extended beyond the biological oligomers to the assembly of small organic molecules and analogs.[7,8] There are, however, only few reports of a combinatorial chemistry approach for the preparation of oligosaccharide libraries.[9–15]

There are, in principle, two possible approaches to create a library of molecules. One strategy is based on the synthesis of structurally defined molecules independent of the method of synthesis, while the other strategy relies on the creation of a mixture of compounds. The former strategy is more straightforward and seems to be less problematic to assay since only one molecule is involved in each assay. The advantage of the latter strategy, on the other hand, is the greater number of compounds that can be obtained in the same number of reactions. In the case of peptides for example,

multiple units (*X*,*Y*,*Z*) where each unit consists of multiple compounds ($x_1, x_2, \ldots, x_n; y_1, y_2, \ldots, y_n; z_1, z_2, \ldots, z_n$) can be used to create a library of mixtures (*X*–*Y*–*Z*) (Fig. 12.2). Although the number of compounds in a mixture could be close to the theoretical maximum, more focused libraries may be advantageous to avoid difficulties in assaying the mixtures.[16]

In order to obtain a "good mixture" with equal distributions of all compounds, it is essential that the reactivities of individual molecules or functional groups in a reaction are equal. A possible problem is the fact that an observed activity might be the result of a synergistic effect of multiple compounds in the assay, or the activity could be affected by the presence of inhibitors. Mindful of these limitations a mixture library of oligosaccharides can be synthesized. Since carbohydrates have multiple hydroxyl groups in a molecule, one may be able to obtain a completely random mixture of products consisting of all possible positional isomers if all hydroxyl groups are equally reactive during the glycosylation reaction.

The natural mammalian sugar monomers, Glc, Gal, Man, Xyl, GlcNAc, GalNAc, Fuc, GlcA and NANA (Fig. 12.3) all carry at least three hydroxyl groups that can undergo glycosylation. Extensive branching and other functionalization such as sulfation or phosphorylation can therefore occur. New stereocenters are formed at the anomeric position during glycosylation reactions. These difficulties, inherent properties of this class of molecules, cannot be circumvented, and render the preparation of useful oligosaccharide libraries a formidable challenge. Oligosaccharides are quite different from the other linear biopolymers, nucleic acids, and peptides. The combinatorial number of trisaccharides possibly formed using nine carbohydrates is 119,736, whereas 20 amino acids or 4 nucleotides can form 8000 and 64 trimers, respectively. It is easily understood that the generation of oligosaccharide

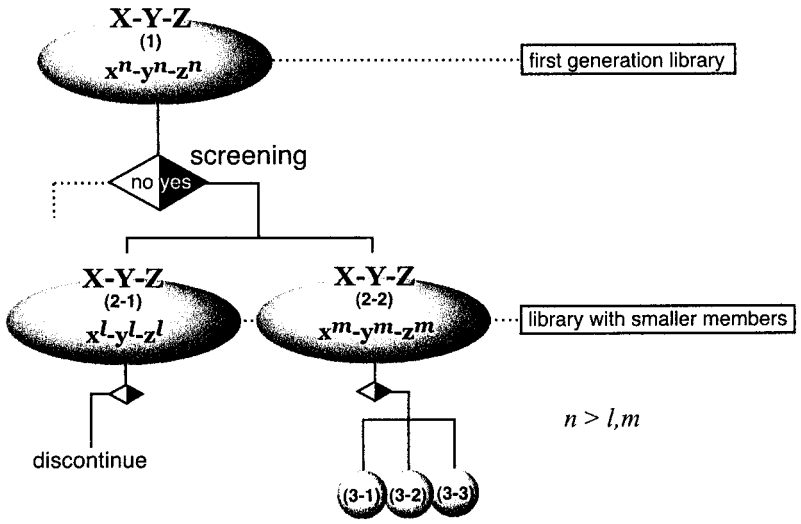


Figure 12.2 Creating a molecule library using multiunit mixtures from the three basic Casrtesian coordinates (*X*, *Y*, *Z*).

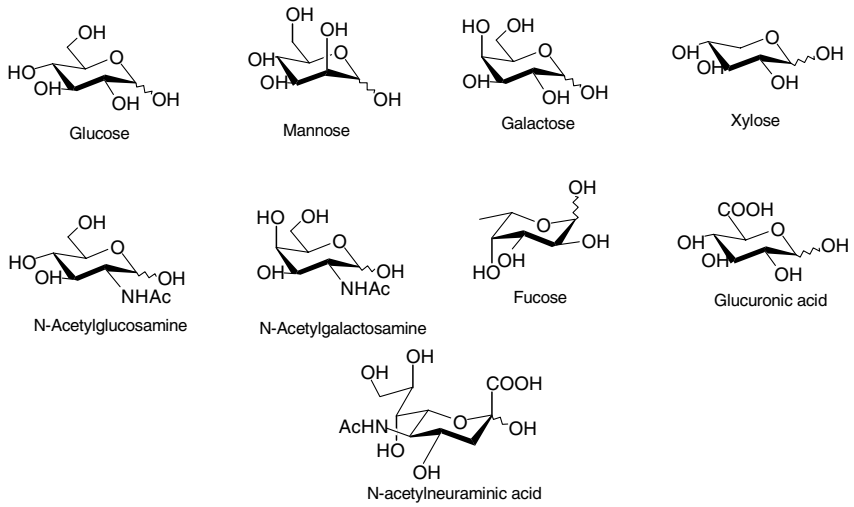


Figure 12.3 Structures of the nine natural mammalian sugar monomers.

libraries is therefore difficult. This number impressively underscores the enormous amount of information an oligosaccharide can contain in a limited space, thus explaining why this class of molecules is used to deliver information in cell-cell interactions.

12.2 STRATEGIES FOR OLIGOSACCHARIDE LIBRARY GENERATION

Before creating an oligosaccharide library, an important question has to be asked, which is how many carbohydrate residues are involved in the carbohydrate recognition processes. Many systematic studies on the binding of oligosaccharides with proteins have been performed[17,18] and the general trend to emerge is that the size of the ligand bound by a protein is usually only a tri- or tetrasaccharide, or even smaller.

12.2.1 Libraries of Structurally Defined Compounds

The synthesis of libraries of structurally defined compounds can potentially be achieved either by split–mix synthesis or by parallel synthesis of individual compounds. The synthesis requires a reliable methodology of oligosaccharide synthesis, where stereochemistry and regioselectivity have to be achieved unlike other library approaches. Development of synthetic methodologies that can provide access to any oligosaccharide structure is underway.

Nevertheless, the strategy is most straightforward and the starting point leading to the parallel synthesis of defined compounds is to have all synthetic intermediates with all the hydroxyl groups suitably protected. Figure 12.4 illustrates such a strategy,

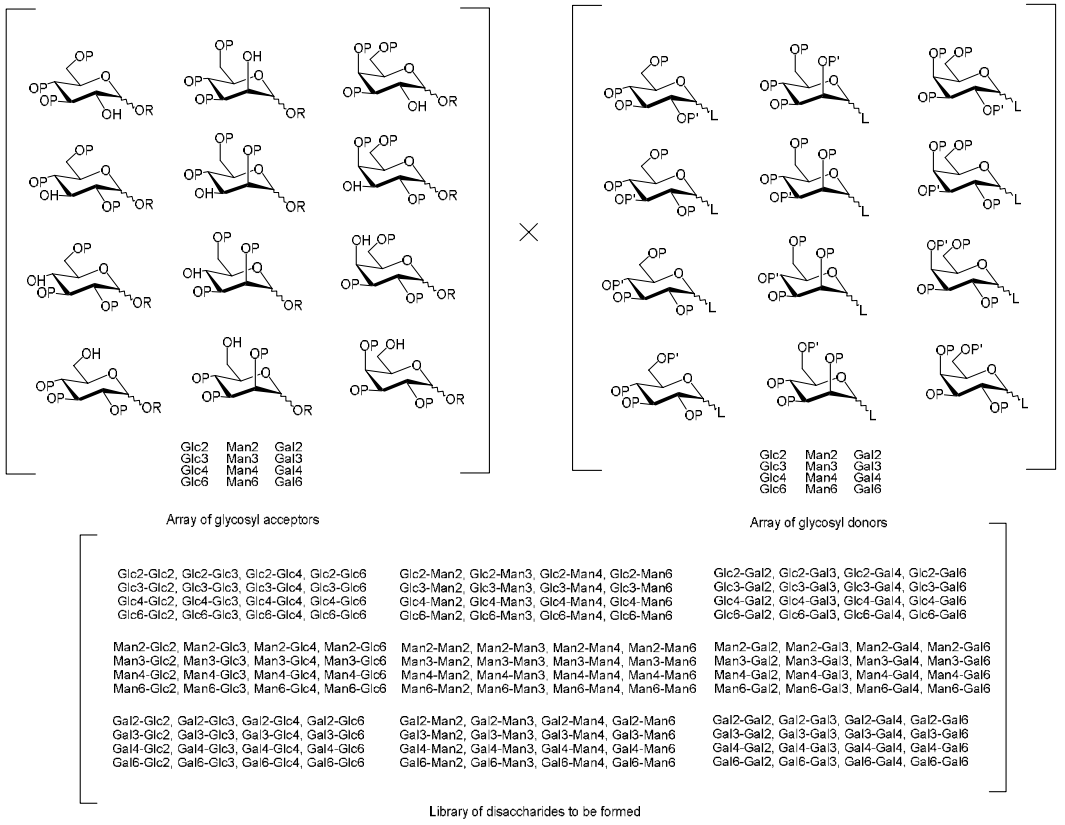


Figure 12.4 Individual precoupling syntheses of all glycosyl donors and acceptors containing galactose, glucose, and mannose.

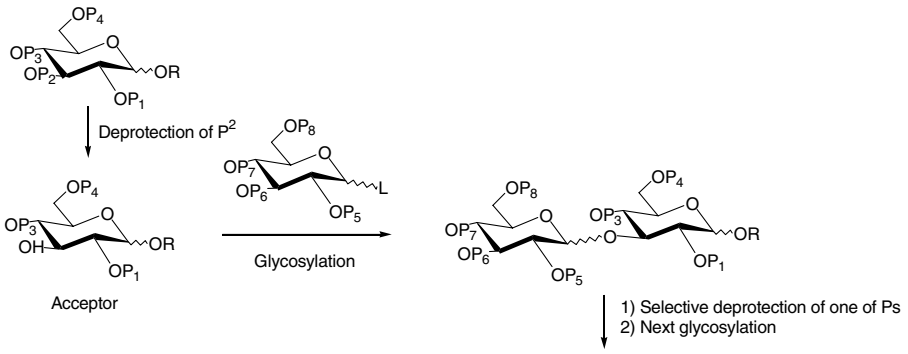


Figure 12.5 Branching of starlike pentasaccharides.

where all the glycosyl donors and acceptors consisting of galactose, glucose, and mannose are individually synthesized prior to the coupling reactions. Regardless of the method used to control stereospecificity of each glycosylation reaction, $(4 \times 3 \times 2)^n$ individual compounds are formed after n cycles of coupling and deprotection.

An alternative idea for the preparation of oligosaccharide libraries is based on orthogonal protection[19,20] where orthogonally protected synthetic units are required to generate all possible regioisomers. This orthogonal strategy would require the handling of an unmanageable number of protecting groups because even at the disaccharide stage, up to seven different orthogonal protecting groups would be required. Furthermore, many combinations of these protecting groups would have to be installed on the same monosaccharide to enable the construction of a complete set of trisaccharides. An approach emerged using orthogonal protection to synthesize highly branched starlike pentasaccharides, where the central sugar unit carrying four orthogonal protecting groups served as glycosyl acceptor after deprotection of one of the protecting groups (Fig. 12.5).[14]

12.2.2 Creating a Mixture Library by Random Glycosylation

Knowing the current difficulties in creating a structurally defined oligosaccharide library, one may find a way to obtain a mixture library. One of the ideas to create such a library is to use an acceptor molecule that does not carry any protecting group. In this strategy only the glycosyl donor but not the glycosyl acceptor is protected (Fig. 12.6). In principle, several glycosylation reactions can be performed with or without protecting group manipulations in order to control the type of library (linear, branched, or both). Before attempting such an approach, one has to answer the question as to whether the reactivities of all hydroxyl groups are equal. Traditional organic chemistry suggests that reactivities of hydroxyl groups differ, a feature that is essential to achieve selective reactions in total synthesis of natural products.[21–26]

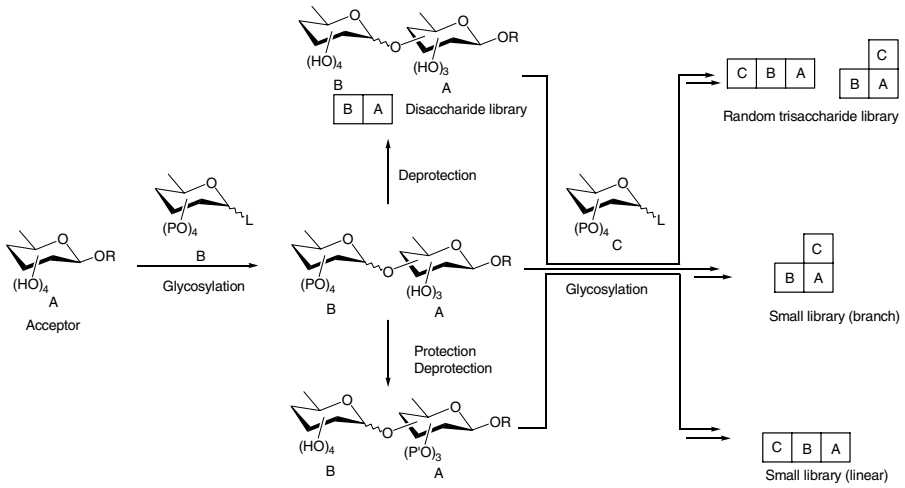


Figure 12.6 Protection of a glycosyl donor but not the glycosyl acceptor.

12.3 ATTEMPTS TO CREATE A RANDOM MIXTURE

The difference in reactivity of hydroxyl groups is often influenced by the presence of nearby bulky protecting groups, and the results may not reflect the inherent reactivities of each hydroxyl group in oligosaccharides toward glycosylation. Intra- and inter-molecular hydrogen bonding and/or aggregation of molecules may also be involved in such reactions essentially carried out in an apolar solvent. The results of performing glycosylation reactions on totally unprotected acceptors, which requires the use of polar solvents for solubility, could hardly have been anticipated.

The initial results are summarized in Figure 12.7.[10] β-GlcNAc glycoside **1** equipped with a hydrophobic aglycon was used as the acceptor to facilitate product isolation by C-18 reverse-phase chromatography (Fig. 12.7A).[27] Treatment of **1** with tetra-*O*-benzyl-galactopyranosyl trichloroacetimidate **2**[21] afforded in DMF using BF_3 etherate as the catalyst, at ~25% conversion, the deprotected disaccharide product mixture in the indicated ratios. Separation of acceptor that contains no benzyl group, disaccharides having four benzyl groups and trisaccharides having eight benzyl groups could be conveniently accomplished by reverse-phase chromatography using a step gradient. Hydrogenolysis of the resulting disaccharide fraction then gave the product mixture, which was adsorbed onto a C-18 column[27] and then washed with water to remove byproducts formed by galactosyl donor degradation. The disaccharides were eluted with methanol and characterized by NMR spectroscopy after further partial purification. The anticipated high selectivity towards the C6 hydroxyl group was not observed, and all the regioisomers were present. Glycosylation using *O*-benzyl ethers as protecting groups on a donor is known to yield α-linked compounds as the major products. Remarkably, all three α-linked products were present in similar amounts (20%, 20%, and 30%). These early results showed

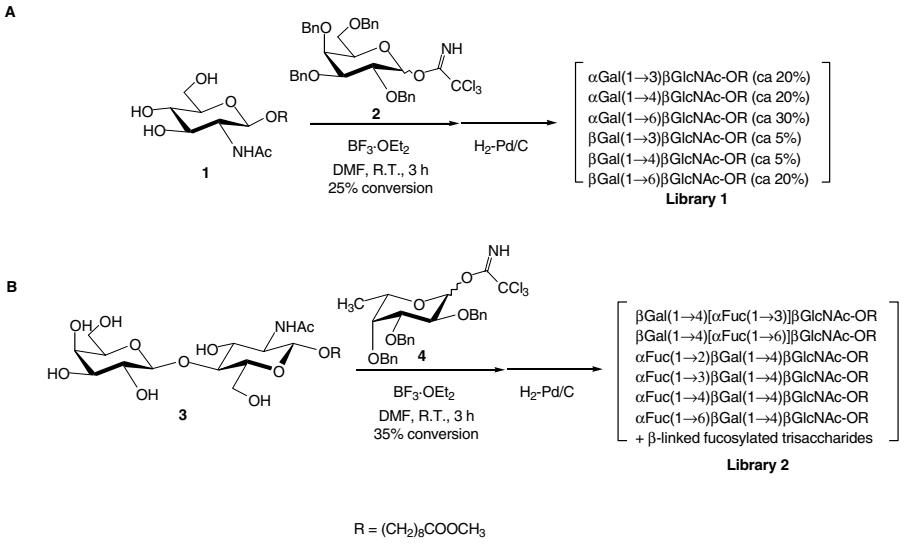


Figure 12.7 Glycosylation reactions performed on completely unprotected acceptors.

that a mixture containing all six possible disaccharides was formed during the galactosylation as planned. However, the common β-(1→3)- and β-(1→4)-linked disaccharides were barely detectable as they were formed in only about 5% yield.

The potential of random glycosylation was further explored using the β-(1→4)-linked LacNAc sequence **3** as the acceptor for a random fucosylation reaction employing tri-*O*-benzyl fucosyl trichloroacetimidate **4**[21] as donor (Fig. 12.7B). This acceptor has two primary and four secondary hydroxyl groups, which can potentially be fucosylated to furnish 12 different fucosylated trisaccharides (library 2). The product mixture was indeed complex and very difficult to analyze, but the ^{1}H NMR spectrum showed the presence of the six α-linked products. One of the products had the signal for H-1 (Fuc) at δ 5.32 ppm (J ~ 4 Hz), exactly as expected for the H-type 2 trisaccharide that had previously been synthesized[28] and was available for comparison. The intensity of the signal suggested that this trisaccharide constituted about 5–8% of the trisaccharide mixture.

12.4 PROTEIN BINDING TO RANDOM GLYCOSYLATION LIBRARIES

It was also important to answer the question of whether an oligosaccharide present in a synthesized random library could be specifically recognized and bound by a protein.[10] The galactosylated-GlcNAc mixture, library 1 (Fig. 12.7A) appeared to contain minor quantities (~ 5%) each of βGal(1→3)GlcNAc-OR **3** and βGal(1→4)GlcNAc-OR **4**. These two disaccharides have been shown to be acceptors for fucosyltransferases present in human milk producing the Le^a and Le^x

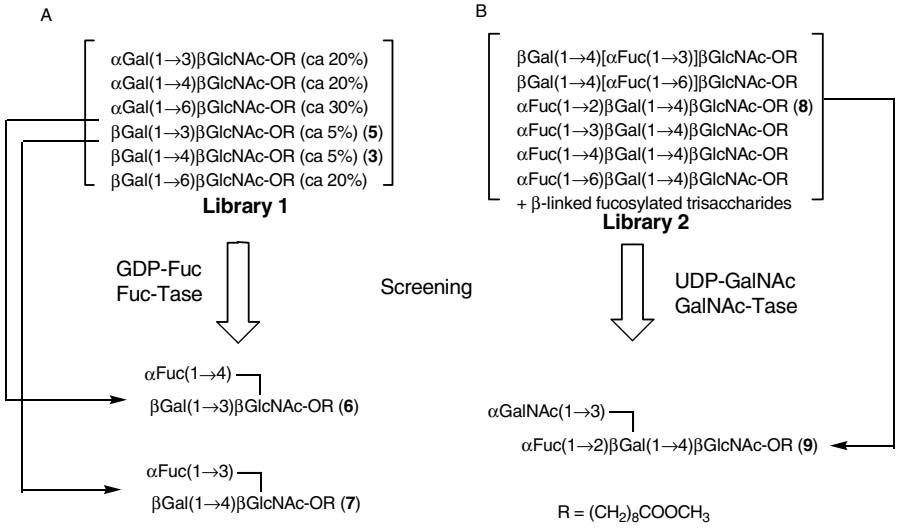


Figure 12.8 Protein binding to two random glycosylation libraries.

trisaccharides, respectively.[29] Incubation of the library with partially purified human milk enzymes in the presence of GDP-[^{3}H]-Fuc resulted in the incorporation of labeled fucose into the library as assayed by a Sep-Pak assay (Fig. 12.8A).[27] The enzymes recognized their substrates in the library mixture and transferred fucosyl residues to them. On the basis of NMR evidence that the H-type 2 sequence **8** was indeed present in the fucosylated LacNAc-library (library 2, Fig. 12.8B), incubation of the library with human serum from a blood group A donor, in the presence of tracer labeled UDP-[^{3}H]-GalNAc was attempted. This serum contained the blood group A GalNAc-transfer enzyme for which the presence of the αFuc(1→2)βGal sequence present in **8** is an absolute requirement in order to transfer GalNAc.[30] Disaccharide **3** does not function as an acceptor for this enzyme. Incubation of the fucosylated library as potential acceptor resulted again in the readily detected incorporation of GalNAc under standard conditions. It was therefore evident that the trisaccharide library could be used in biological screening experiments, which indicated that the H-type 2 trisaccharide **8** was present and could be converted to the blood group A–active tetrasaccharide **9**.

12.5 COMPOUND DISTRIBUTION DEPENDS ON REACTION CONDITIONS

On the basis of the early results described above, random glycosylation as an approach to oligosaccharide library preparation has been further pursued. The product distributions in these early reactions were difficult to determine, and in subsequent work, the aglycon of the acceptor was changed to the *p*-methoxyphenoxy octyl

group.[31] This group retained the desirable hydrophobic properties that facilitated the removal of reaction byproducts from the desired materials and in addition contained a chromophore that permitted quantitation of peaks in HPLC.

To facilitate the analysis of random glycosylation reactions, all six possible products (**13**–**18**) were synthesized as reference standards to simplify the determination of product distributions.[11] These standards could be separated on a PAC column permitting direct analysis of mixtures by HPLC. A typical chromatogram of a separation of a random glycosylation is shown in Figure 12.9.

For the galactosylation reactions, 2,3,4,6-tetra-*O*-benzylgalactopyranosyl trichloroacetimidate **2** and dibenzylphosphite **10** were used as donors (Table 12.1). Under these conditions, the β-(1→3)- and β-(1→4)-linked disaccharides **16** and **17** were formed in only minor amounts (entries 1 and 2). To increase the proportion of the β-anomers, the peracetylated galactopyranosyl trichloroacetimidate **11** was used as the donor. Reactions were poor in DMF, but in dioxane, a clean mixture of products was obtained. Two equivalents of donor were required to give a conversion of 20–30% of **1** to disaccharides **13**–**18**. Peaks eluting when the column was washed with more polar solvent mixtures suggested that only traces of trisaccharides were formed, but the identity of these peaks was not investigated.

Using **11** as the donor at either 20 or 50°C (entries 4 and 5), all six possible disaccharides were formed; the α(1→6) anomer **15** was the least favored. A similar

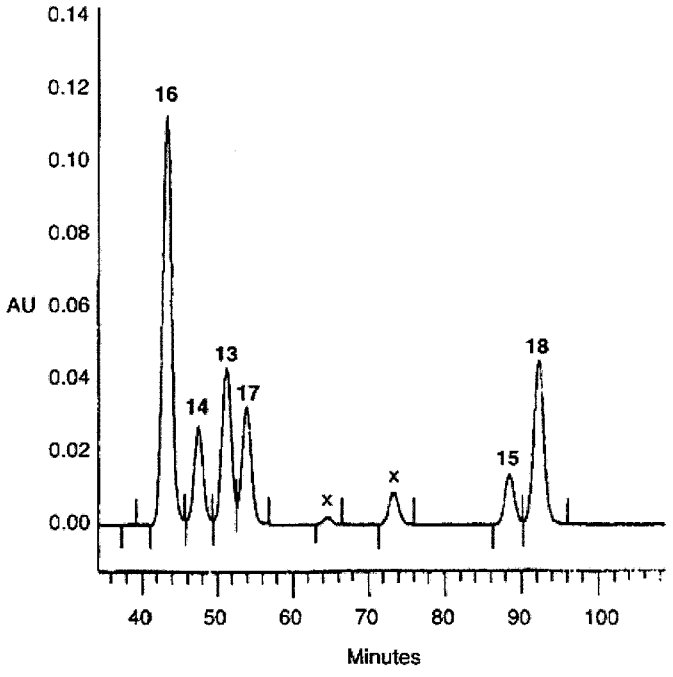
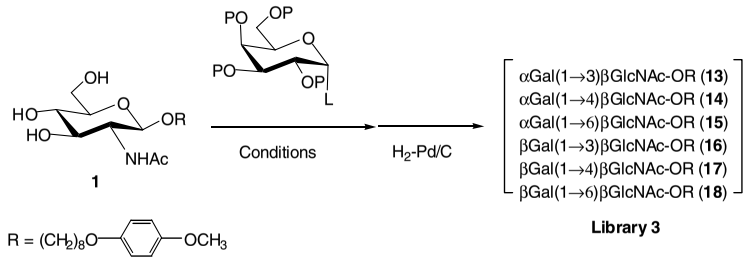


Figure 12.9 Chromatogram of the separation of a random glycosylation.

TABLE 12.1 Use of 2,3,4,6-Tetra-*O*-benzylgalactopyranosyl Trichloroacetimidate and Dibenzylphosphite in Galactosylation Reactions



Donor							Product Distriubution					
						Temperature	**13**	**14**	**15**	**16**	**17**	**18**
Entry		P	L	Promoter	Solvent	(°C)	α(1→3)	α(1→4)	α(1→6)	β(1→3)	β(1→4)	β(1→6)
1	**2**	Bn	Imidate	$BF_3{\cdot}OEt_2$	DMF	20	15	17	35	~2	<2	30
2	**10**	Bn	Phosphite	TMSOTf	DMF	–20	23	11	51	<2	<2	15
3	**11**	Ac	Imidate	$BF_3{\cdot}OEt_2$	Dioxane	0	22	10	5	32	7	22
4	**11**	Ac	Imidate	$BF_3{\cdot}OEt_2$	Dioxane	20	22	11	6	30	11	20
5	**11**	Ac	Imidate	$BF_3{\cdot}OEt_2$	Dioxane	50	13	10	5	42	10	19
6	**11**	Ac	Imidate	$BF_3{\cdot}OEt_2$	Dioxane	80	24	12	2	45	13	2
7	**12**	Ac	Phosphite	TMSOTf	Dioxane	20	6	8	7	16	26	36

result was observed when phosphite **12** was used as the donor (entry 7). It is surprising that so much α-anomer was formed in a reaction that yields β-glycosides almost exclusively when using partially protected acceptors in nonpolar solvents. In addition, the product of galactosylation of the primary C6 hydroxyl group, intuitively the least hindered and most reactive alcohol, did not dominate the reaction products, whereas the β-(1→3) disaccharide **16** was obtained as the major product. The possibility that the β-(1→6) product **18** might have formed rapidly but then been selectively destroyed was eliminated by determining that the product ratios did not change significantly when the reaction was run for 10 or 30 min, 3 or 14 h even though very little product had formed during the shorter reaction times. The possibility that coeluting UV-absorbing noncarbohydrate impurities might have caused an overestimation of the amount of β-(1→3) disaccharide **16** was eliminated for the reaction products of the experiment summarized in entry 5 where **16** is clearly the major product (42%). The first four peaks of the chromatogram (Fig. 12.9) were collected as a single fraction, as were the last two peaks, and the relative intensities of the anomeric signals in the ^{1}H NMR spectra of these fractions, assigned by comparison with the authentic standards, independently confirmed the results of Table 12.1. Replicates of runs yielded product distributions within 10% of each other.

The results in Table 12.1 show that the anomeric configuration of the products of random galactosylation can be biased toward the α-glycosides by use of **2** or **10** as donor, or all of the products can be obtained in a single run using **11** or **12** as donor. While all disaccharides are clearly present in significant amounts in the product, they are not formed in equal amounts, and it is hoped that further studies on galactosyl and other donors will lead to an improvement in the product distributions.

12.6 CREATING RANDOM OLIGOSACCHARIDE LIBRARIES

Using the *p*-methoxyphenoxy octyl group as the aglycon, three sets of mixtures have been synthesized by random fucosylation of disaccharides βGal(1→3)βGlcNAc-OR **16**, βGal(1→4)βGlcNAc-OR **17**, and βGal(1→6)βGlcNAc-OR **18** (Fig. 12.10), where reaction conditions used were the same as those investigated for library 2.[9] The ^{1}H NMR spectra of these trisaccharide libraries thus obtained are shown in Figure 12.11. The starting disaccharides contained only β linkages, so the new doublets (J = 3–4 Hz) appearing in the region δ = 4.7–5.3 are from the H1 atoms of the α-fucose residue. The fast-atom bombardment (FAB) mass spectra confirmed that the products were the expected trisaccharides. Integration of the H1 signals revealed that libraries 4 and 5 contained about 20% of β-linked fucose, while library 6 contained nearly 5%. Only library 4, produced by random fucosylation of βGal(1→3)βGlcNAc-OR, was analyzed in detail through separation of the individual isomers by repeated chromatography at both the protected and deprotected stages, followed by ^{1}H NMR analysis to determine the anomeric configuration of the sugar residues. Methylation analysis was used for assigning the position of attachment of the new fucosyl units. The distribution of the α-fucosylation on disaccharide **16** was thus shown to be 12% α-(1→4), 22% α-(1→6), 19% α-(1→2′), 23% α-(1→3′), 8% α-(1→4′), and 16%

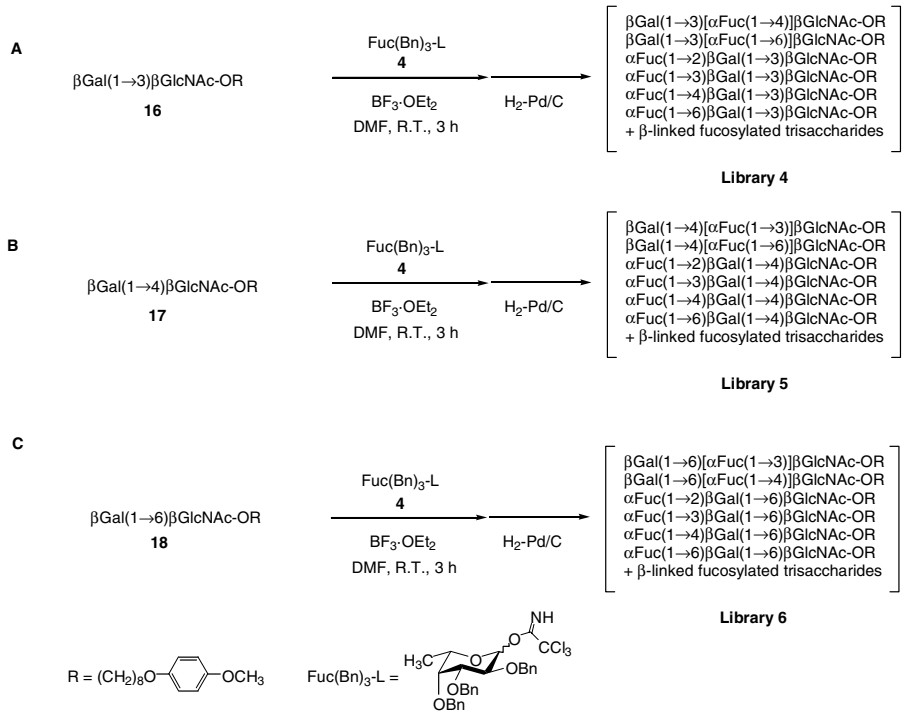


Figure 12.10 Creating random oligosaccharide libraries using the *p*-methoxyphenoxy octyl group as the aglycon.

α-(1→6′). The statistical distribution would be 17% of each isomer. Figure 12.11 also shows the presence of the characteristic[32] downfield-shifted quartet (δ = 4.8) for H5 of fucose α-linked to C4 of the GlcNAc residue in the Lewis[a] blood group trisaccharide βGal(1→3)-[αFuc(1→4]βGlcNAc-OR that had formed as 12% of the product mixture. Library 5, produced by random fucosylation of βGal(1→4)βGlcNAc-OR **17** and processed in the same way, gave a strikingly similar pattern in the ^{1}H NMR spectrum. The identity of the individual isomers was not established, but since only six α-fucosylated trisaccharides are possible, and six α-doublets are seen, they are all present in the mixture in comparable amounts. The characteristic[32] H5 quartet of the α-linked fucose residue in the blood group Lewis[x] structure βGal(1→4)-[αFuc(1→3)]βGlcNAc-OR is also seen near δ = 4.8. Finally, the ^{1}H NMR spectrum of library 6, produced by random fucosylation of β Gal(1→6)βGlcNAc-OR **18**, again confirmed the presence of all six possible α-linked fucose trisaccharides.

Chemical intuition, based on numerous literature precedents[21–26] demonstrating highly varying hydroxyl group reactivities, would have predicted that the major products in the above fucosylation reactions would be those formed by glycosylation

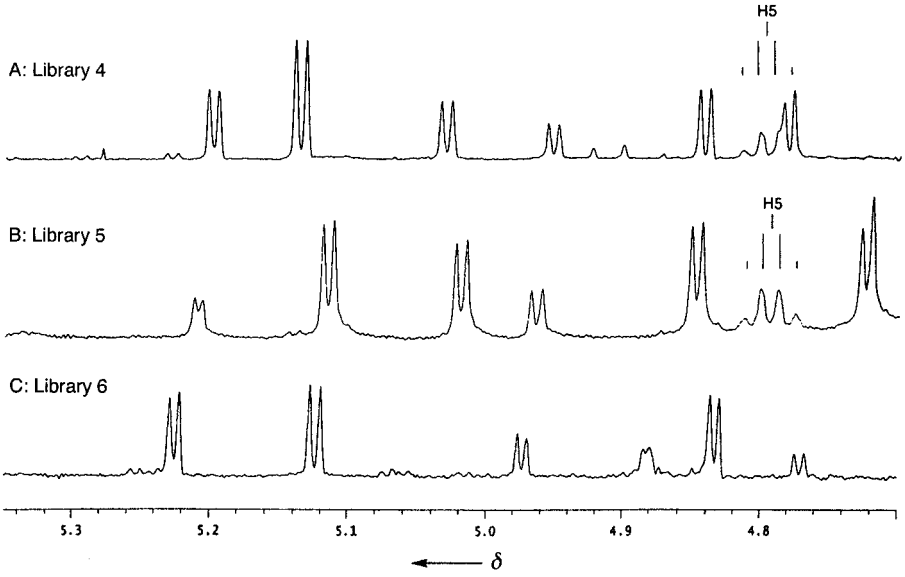


Figure 12.11 ^{1}H NMR spectra of trisaccharide libraries obtained by random fucosylation of disaccharides **16**–**18**.

of the least hindered primary alcohols, 6-OH or 6′-OH. This was clearly not the case in any of the three examples. The greatest difference between the major and the minor α-fucoside, based on integration of the anomeric signals, is only 3:1, and all isomers are present in at least half the expected statistical amount. The mixtures produced were confirmed to contain the kinetic product distribution, since running the reactions at much lower conversion (10%) to trisaccharides, or leaving the reaction for much longer (16 h) yielded the same distribution of products. It is astonishing that all the hydroxyl groups on the acceptor disaccharides showed such similar reactivity. The explanation is undoubtedly complex but will include the fact that since no protecting groups are used, no added steric hindrance prevents access of the glycosyl donor to any of the hydroxyl groups. In addition, the reaction is taking place in DMF or dioxane, a very polar hydrogen-bond-accepting solvent, and the reacting molecules are very likely involved in very complex arrays of inter- and intramolecular hydrogen bonds.

12.7 OTHER APPROACHES TO OLIGOSACCHARIDE MIXTURE LIBRARIES

A similar approach to create an oligosaccharide library was reported using a glycal and diols as the donor and acceptor to produce stereospecific regiorandom mixture of glycosides (Fig. 12.12).[15] α-Specific glycosylations were achieved via addition of

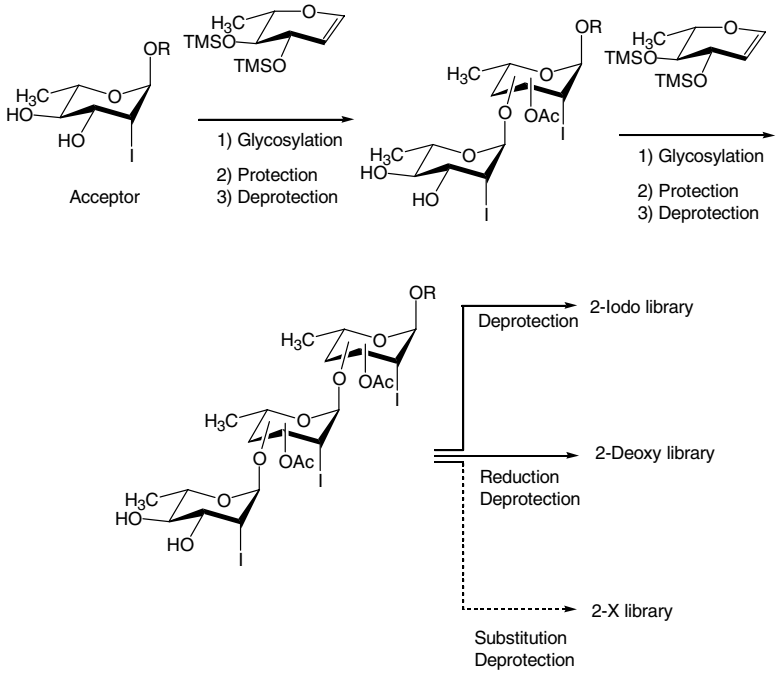


Figure 12.12 Formation of an oligosaccharide library using glycal and diols as donor and acceptor, respectively, to produce a stereospecific regiorandom glycoside mixture.

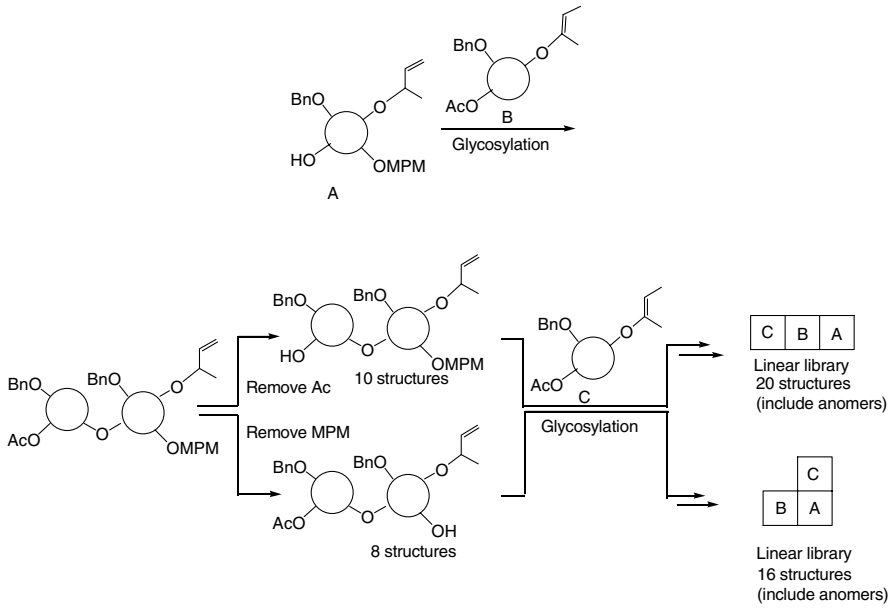


Figure 12.13 A disaccharide array resulting from individual coupling reactions of various glycosyl donors and acceptors.

iodonium ion to the double bond from the β face and subsequent attack of an alcohol from the other face. Equal distribution of regioisomers was also achieved. Two cycles of the random glycosylations were carried out after blocking the hydroxyl group, in order to avoid formation of branched structures and deprotection of silyl groups on the newly added sugar. Again, a nearly equal distribution of products was observed. These iodinated sugar residues can be subjected to further transformations such as radical reduction and S_N2 reactions to increase library diversity.

Another approach similar to that of peptide mixture libraries has been addressed (Fig. 12.13).[13] Individual coupling reactions of several glycosyl donors and acceptors resulted in an array of disaccharides. The strategy employs limited use of an orthogonal protection scheme in order to determine whether either linear or branched oligosaccharides were to be formed. Each acceptor used for the synthesis contains a single hydroxyl group. These disaccharides were then mixed and subjected to the orthogonal deprotection of acetyl and methoxybenzyl groups. The resulting mixture of disaccharides containing one hydroxyl group each, was coupled with a glycosyl donor to give a trisaccharide mixture library.

12.8 CONCLUSIONS

Random glycosylation has proved remarkably effective for the generation of mixture libraries of oligosaccharides. It was found that all possible regioisomers were formed through a random process, although equal distributions of all formed glycosides were not accomplished. Several examples demonstrated the potential of direct assays of the random library using glycosyltransferases. Further developments are needed in order to identify the structure(s) of the hit(s) without going through methylation analysis. For this, mass spectral analysis may be used to obtain positional information through fragmentation, but anomeric configuration has to be determined separately. Repeated glycosylation reactions and functionalization such as sulfation and phosphoryation, which expand the members in a library dramatically, remain to be investigated. The preparation of libraries that consist of defined sugar units but not anomeric and positional formulation was accomplished in the simplest and most expedient manner. It is therefore our hope that it will become possible to find new oligosaccharide structures and to reveal new functions of such compounds using oligosaccharide libraries generated by random glycosylations.

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Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries
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13 Solid-Phase Synthesis of Biologically Important Glycopeptides

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13.1 INTRODUCTION

Glycoproteins play important roles in various biological processes such as cell–cell recognition, regulation of cell growth, cell adhesion, immunodifferentiation, metastasis of cancer cells, and inflammation.[1–3] The carbohydrate portions of glycoproteins are known to influence the properties of the parent proteins in different ways; they enhance their thermal stability and protect them against proteolysis, they influence the protein conformation, and they modify their physicochemical properties such as solubility, electrical charge, mass, size, and viscosity in solution. Depending on their different functions, saccharides are bound to different amino acids via varied forms of glycosylation. The interest in the molecular mechanisms of biological interactions has resulted in an increased attention to glycoproteins since 1990 or so. Specifically glycosylated proteins are not easily available by gene technological methods, because they are products of posttranslational enzymatic glycosylation. Therefore, chemical synthesis of exactly defined model glycopeptides is required as a valuable tool for the study of structure–function relationships. Besides numerous successful examples for solution-phase glycopeptide synthesis,[4] synthesis on solid phase receives increasing attention. Several approaches have been developed:

1. Sequential solid-phase glycopeptide synthesis using glycosylated amino acid building blocks
2. Glycosylation of preformed peptides on solid phase
3. Convergent glycopeptide synthesis with polymer-bound carbohydrates

13.2 BUILDING-BLOCK APPROACH

The most effective and general route to synthetic glycopeptides includes the employment of glycosylated amino acids in stepwise solid-phase syntheses based on

Core 1: Gal(β1→3)GalNAcα-Ser/Thr

Core 2: GlcNAc(β1→6)[Gal(β1→3)]GalNAcα-Ser/Thr

Core 3: GlcNAc(β1→3)GalNAcα-Ser/Thr

Core 4: GlcNAc(β1→6)[GlcNAc(β1→3)]GalNAcα-Ser/Thr

Core 5: GalNAc(α1→3)GalNAcα-Ser/Thr

Core 6: GlcNAc(β1→6)GalNAcα-Ser/Thr

Core 7: GalNAc(α1→6)GalNAcα-Ser/Thr

Core 8: Gal(α1→3)GalNAcα-Ser/Thr

Scheme 13.1 Characteristic core regions found on mucins.

the Fmoc strategy. The synthesis of the suitably protected glycosylamino acid building blocks constitutes the crucial precondition of such a strategy.[5]

13.2.1 *O*-Glycopeptides

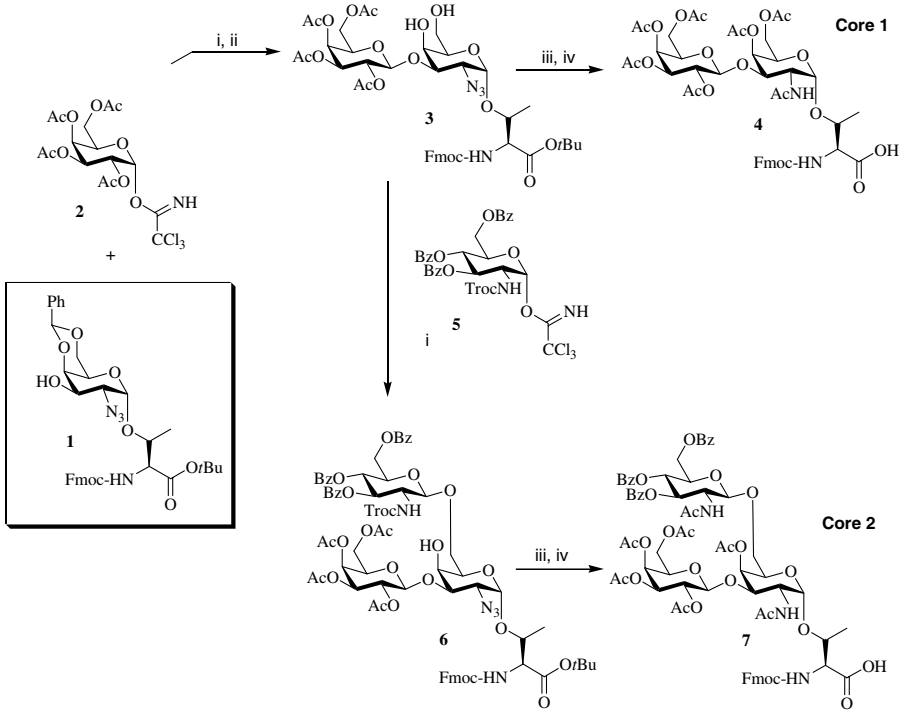
13.2.1.1 Glycopeptides Containing the GalNAc-α-Ser/Thr Linkage The *N*-acetylgalactosamine-α-Ser/Thr linkage was first discovered in mucins.[6] Mucins are high-molecular-mass *O*-glycoproteins with a high degree of glycosylation and structural heterogeneity.[7,8] Their apoproteins consist of tandem repeats with high contents of the hydroxyl amino acids threonine and serine. Eight different core regions of mucins containing GlcNAc, Gal, and GalNAc residues have been identified. They all have the central GalNAc-α-O-Ser/Thr linkage in common (Scheme 13.1).

Different routes to the synthesis of Fmoc-protected glycosylamino acids have been developed.[9–11] On the basis of this methodology numerous solid-phase syntheses of glycopeptides have been carried out. Scheme 13.2 shows an example in which glycosylamino acid building blocks containing the core 1, core 2, and core 4 oligosaccharide structures have been used in the synthesis of glycopeptide sequences of repeating units of the mucins MUC2 and MUC3.[12] The key building block in these syntheses is conjugate **1**.

The galactosylamine threonine conjugate **1** was glycosylated with the galactosyl trichloroacetimidate **2**[13] in the presence of catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf)* followed by removal of the benzylidene protecting group using acetic acid to give disaccharide conjugate **3**. Conversion of the

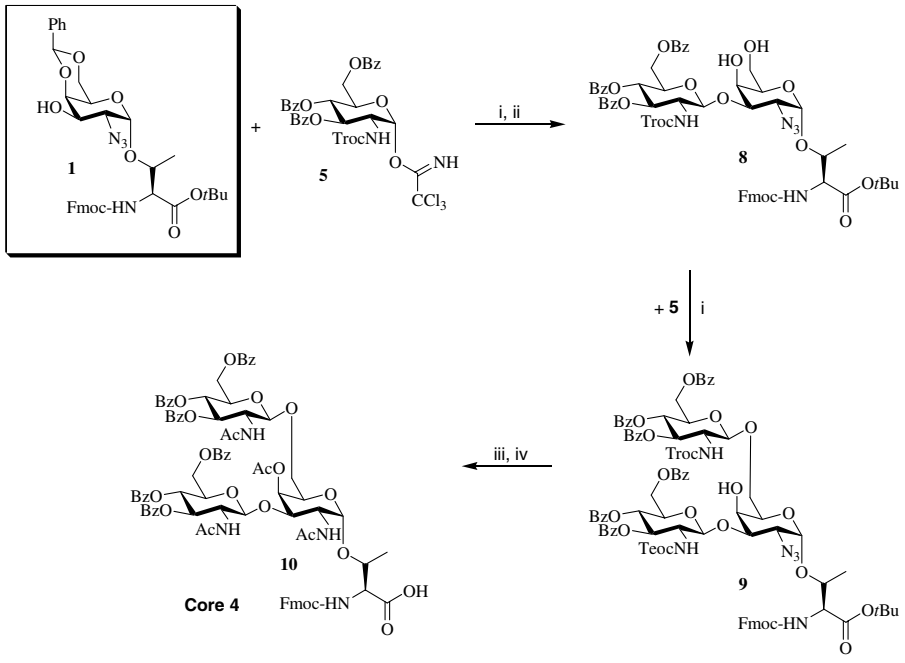
*See Abbreviations list at the end of this chapter.

azido into the acetamido group was accomplished with activated zinc in acetic acid anhydride, acetic acid, and THF. Subsequent *O*-acetylation followed by removal of the *tert*-butyl group using TFA afforded core 1 building block **4** carrying acetyl protecting groups in the carbohydrate portion and the Fmoc amino protecting group. Intermediate **3** was applied to the synthesis of core 2 building block **7**. Regioselective glycosylation of diol **3** with the glucosamine trichloroacetimidate **5** in which the 2-amino group was protected with the trichloroethoxycarbonyl[14] (Troc) group gave the trisaccharide conjugate **6**.[15] Treatment of **6** with activated zinc in acetic acid anhydride, acetic acid, and THF resulted in the simultaneous conversion of the azido function into the acetamido group, the cleavage of the Troc group and the subsequent acetylation of the amino group. Removal of the *tert*-butyl group with TFA afforded core 2 building block **7**.



Scheme 13.2 Synthesis of core 1 and core 2 building blocks.

Conjugate **1** also served as the precursor in the synthesis of the core 4 building block **10** (Scheme 13.3). Glycosylation of **1** with glucosamine trichloroacetimidate **5** yielded the disaccharide conjugate **8** after removal of the benzylidene group with acetic acid. Diol **8** was regio- and stereoselectively glycosylated with **5** to give



i, TMSOTf; ii, AcOH (80%); iii, Zn, Ac_2O, AcOH, THF; Ac_2O, pyridine; iv, TFA (95%)

Scheme 13.3 Synthesis of the core 4 building block.

trisaccharide conjugate **9**. Its azido function and the Troc-protected amino functions were simultaneously converted into the acetamido functions. Cleavage of the *tert*-butyl group using TFA gave core 4 building block **10** suitable for solid-phase glycopeptide syntheses. The synthesized building blocks were incorporated at the different positions in the syntheses of two partial sequences of the repeating units of the two human intestinal mucins MUC2 and MUC3 (positions indicated by * in Scheme 13.4) by multiple-column solid-phase synthesis (MCPS) in a manual 20-column multiple synthesizer.[16] Wang resin[17] served as the polymer support. Coupling of the glycosylated amino acids was performed using *O*-(1-benzotriazole-1-yl)- *N,N,N′,N′*-tetramethylammonium tetrafluoroborate (TBTU)[18] as coupling reagent. The nonglycosylated amino acids were applied as their Fmoc/Pfp esters using Dhbt-OH as activating agent which allows a visual control of peptide bond formation.[19] Side chains of threonine and serine were protected as their *tert*-butyl ethers; those of glutaminic acid, as their *tert-butyl* esters; and histidine was *tert*-butyloxycarbonyl-protected. Final detachment of the synthesized glycopeptides from the resin using TFA proceeded with simultaneous removal of the acid-sensitive side-chain protecting groups. It was followed by removal of the *O*-acetyl and -benzoyl protecting groups by transesterification with sodium methanolate/methanol.

MUC2:TT*T*VT*PT*PT*G

MUC3:TET*T*SHST*PG

Scheme 13.4 Partial sequences of MUC2 and MUC3.

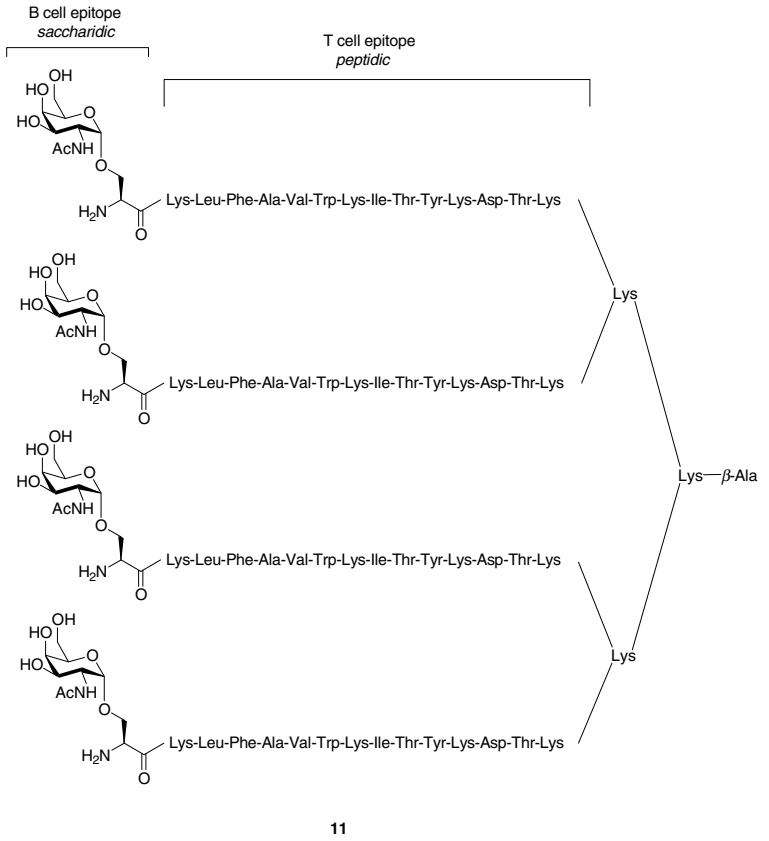
13.2.1.2 Glycopeptides Carrying Tumor-Associated Antigens In cancer cells, glycoproteins show glycosylation patterns different from those of glycoproteins in normal cells. The carbohydrate side chains are incompletely developed. Different tumor-associated carbohydrate structures such as the T_N (GalNAcα-*O*-Ser/Thr), T_F or T(Gal(β1→3)GalNAcα-*O*-Ser/Thr), SialylT_N(NeuNAc(α2→6)GalNAcα-*O*- Ser/Thr), and the two regioisomeric SialylT Gal(β1→3)[NeuNAc(α2→6)]GalNAcα-*O*-Ser/Thr) and NeuNAc(α2→3)[Gal(β1→ 3)]GalNAcα-*O*-Ser/Thr antigens have been identified.[20] They are the result of premature completion of glycosylation in cancer cells, due to changes in the expression and reactivity of glycosyltransferases.[21]

The tumor-associated T_N antigen structure was used in the synthesis of glycopeptides that then were coupled to carrier proteins in order to obtain synthetic antigens.[9b] As an example, a multiple-antigen glycopeptide (MAG) was synthesized on solid phase and subjected to immunological evaluation.[22] The presentation of peptidic epitopes to the immune system can be achieved by means of a polylysine core.[23] To introduce an efficient antibody response, a conjugate of a B-cell epitope (T_N antigen) and a T-cell epitope, the T-cell epitope of the VP1 protein of polio virus type 1,[24] was presented by the multiple antigen glycopeptide **11** (Scheme 13.5).

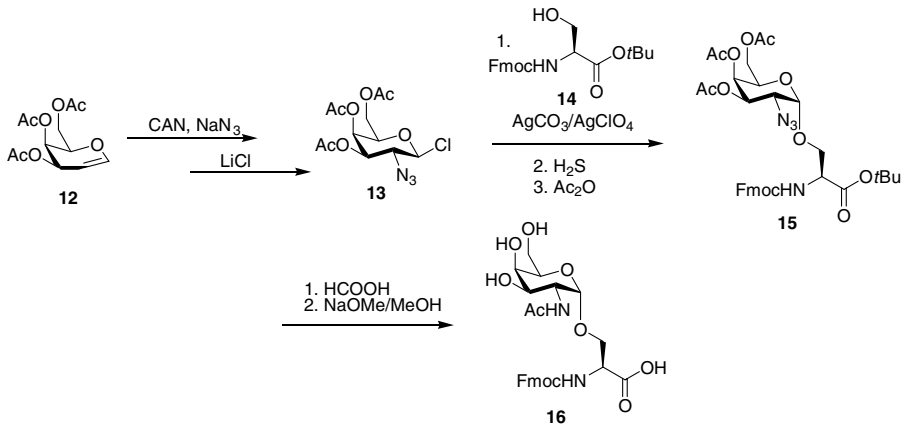
The synthesis of the T_N antigen building block **16** was performed starting from tri-*O*-acetyl-D-galactal **12** (Scheme 13.6).[25] Glycosylation of Fmoc serine *tert*-butyl ester **14** with the azidogalactosyl chloride **13** using $Ag_2CO_3/AgClO_4$[26] as promotors was followed by reduction and acetylation of the 2 position to give compound **15**. Cleavage of the *tert*-butyl ester was performed in formic acid, and the acetyl groups of the sugar moeity were removed by NaOMe/MeOH.

For the synthesis of the multiple antigen **11**, a β-alanyl spacer was attached to Wang resin[17] and the lysine core was constructed by coupling successively two levels of FmocLys(Fmoc)OH using TBTU as the carboxyl activating agent. The conjugate was sequentially elongated at the four amino functions in order to assemble the T-cell epitope sequence. Finally, Fmoc-protected glycosyl serine **16**, deprotected in its carbohydrate portion, was coupled to the *N* termini of this construct. After Fmoc removal, the conjugate was detached from the resin using TFA/water/ethanedithiol 95/2.5/2.5 with simultaneous cleavage of the acid-labile amino acid side chain protecting groups. Preliminary immunological tests showed that the MAG system **11** is recognized by the monoclonal anti-T_N antibodies 83D4 (IgM) and MSL128 (IgG).[22]

The synthesis of a glycopeptide from the homophilic recognition domain of epithelial cadherin 1 (E-CAD1) carrying the T_N structure was performed using the novel condensing reagent *N,N-N′,N′*-bis(tetramethylene)-*O*-pentafluorophenyl-uronium hexafluorophosphate PfPyU **18**.[27] The cadherins constitute a family of about 30 cell surface glycoproteins that are involved in the Ca^{2+}-dependent adhesion of

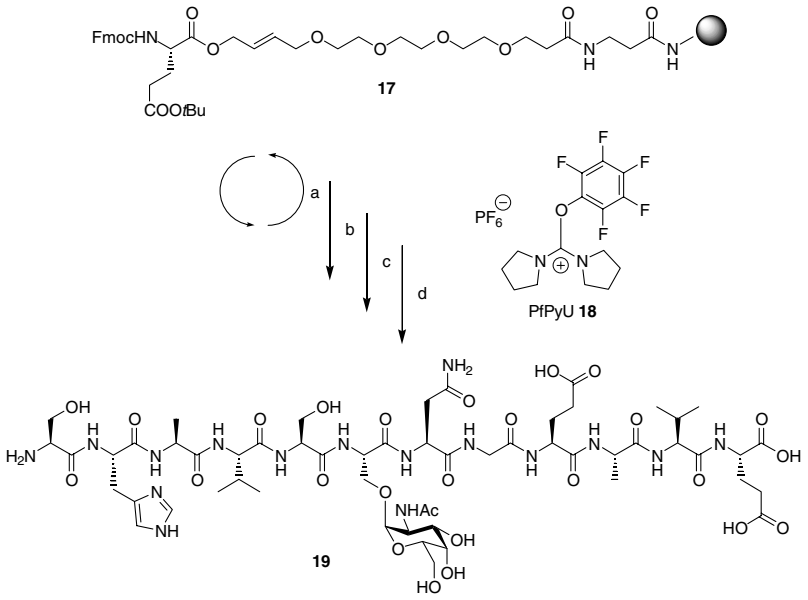


Scheme 13.5 Schematic representation of the multiple-antigen glycopeptide **11**.



Scheme 13.6 Synthesis of the T_N-antigen building block.

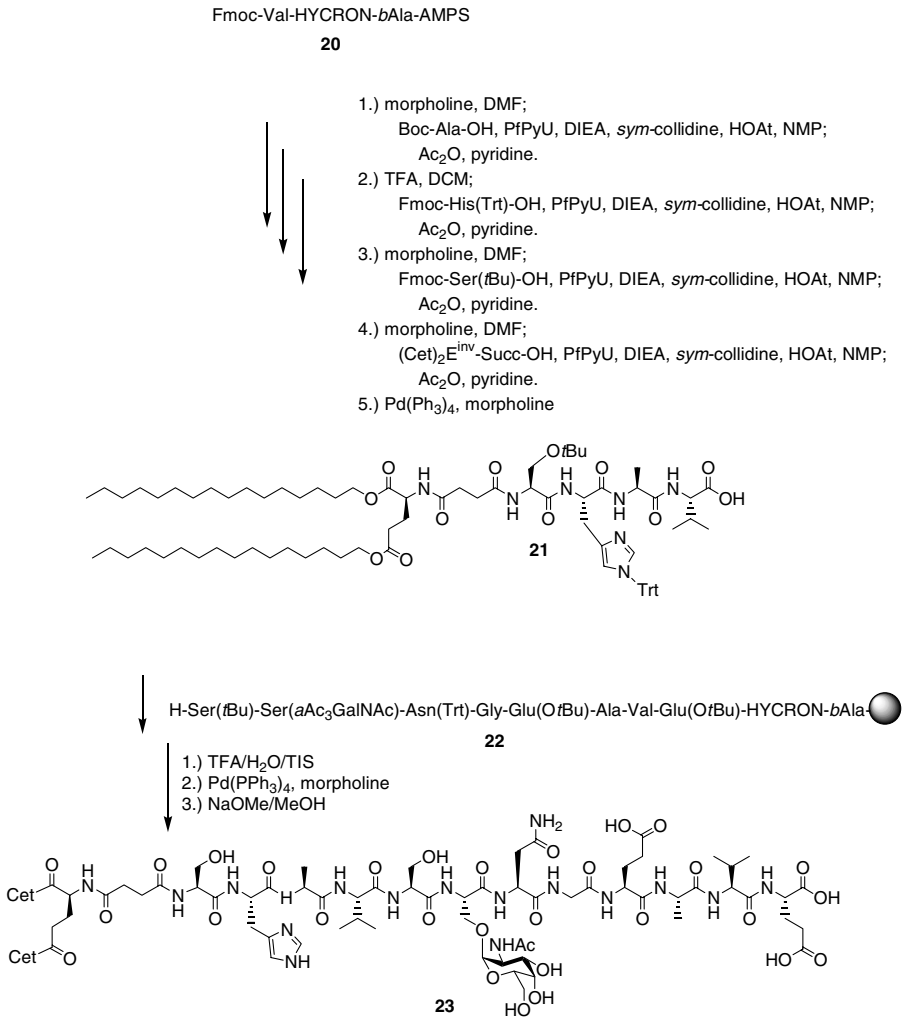
cells.[28] They play an important role in the morphogenesis of cells and are found on every tissue-forming cell type. First attempts to synthesize the E-CAD1 sequence SHAVSSNGEAVE using TBTU as the coupling reagent gave only small amounts of the target compound, presumably because of a backfolding effect of the β-sheet-forming sequence. Comparative coupling reactions of Fmoc-Ala-OH to H-Val-O*t*Bu showed that the coupling rate induced by PfPyU is 8 times higher than that induced by TBTU. The synthesis of a partial sequence of the homophilic recognition region of E-CAD1 containing α-GalNAc at serine within the turn structure using PfPyU as acylating reagent and β-alanyl-TentaGel S[29] loaded with the allylic HYCRON[30] anchor conjugate of Fmoc-Glu(O*t*Bu)-OH as the solid support gave a high yield of desired compound **19** (Scheme 13.7).



a) morpholine/DMF; 3 eq. Fmoc-Xaa-OH, PfPyU, *sym*-collidine, iPr$_2$NEt, NMP; b) cat. Pd^0(PPh$_3$)$_4$, *N*-methylaniline, DMF/DMSO (1:1); c) TFA/H$_2$O/TIS (95/2.5/2.5); d) NaOMe/MeOH, pH = 9.5. = TentaGel S.

Scheme 13.7 Solid-phase synthesis of a glycopeptide from the homophilic recognition domain of epithelial Cadherin 1 using the new coupling reagent PfPyU.

The PfPyU/HYCRON methodology also proved efficient in the synthesis of a lipoglycopeptide of the E-CAD1 recognition region sequence. The synthesis of the glycododecapeptide amphiphile was performed using a convergent solid-phase approach.[31] A fragment of the N-terminal tetrapeptide with the lipophilic tail group was synthesized first (Scheme 13.8).

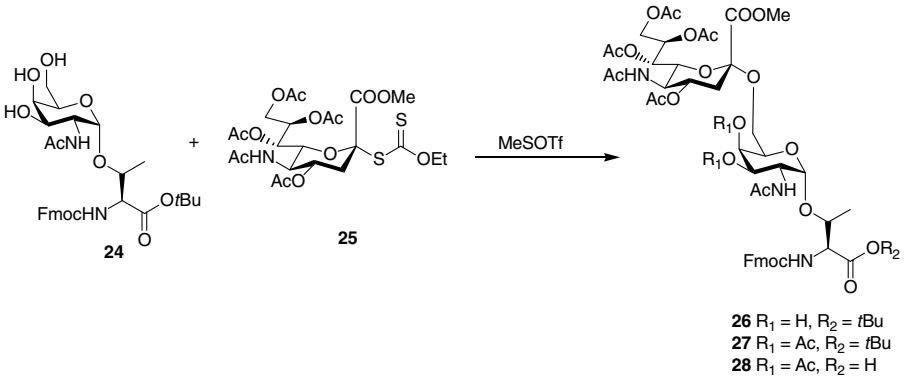


Scheme 13.8 Synthesis of a lipoglycopeptide by means of fragment condensation methodology.

The allylic HYCRON anchor conjugate **20** of Fmoc-Val-OH and β-alanyl-polystyrene was used as the starting material, and the polymer-bound SHAV tetrapeptide was synthesized according to the Fmoc methodology. However, the second amino acid alanine was coupled as the Boc-protected derivative in order to minimize diketopiperazine formation on the stage of the amino-deprotected resin-linked dipeptide. Polymer-bound tetrapeptide was then coupled with the lipophilic succinylglutaminic ester derivative.[32] Owing to the very mild cleavage of the HYCRON anchor by Pd(0)-catalyzed allyl transfer to morpholine leaving acid- and base-sensitive protecting groups unaffected, the fully protected conjugate **21** was

obtained in pure form. Fragment **21** was now coupled to the E-Cadherin octapeptide fragment H-S(*t*Bu)S(αAc$_3$GalNAc)N(Trt)GE(O*t*Bu)AVE(O*t*Bu) linked to the HYCRON resin **22** using a 1.7-fold excess and PfPyU/*sym*-collidine/diisopropylethylamine as the activating reagent. After cleavage of the acid-labile protecting groups using TFA/H_2O/triisopropylsilane (95:2.5:2.5), the obtained lipoglycopeptide was detached from the resin by Pd(0)-catalyzed allyl transfer to morpholine as trapping agent. Final removal of the *O*-acetyl groups from the carbohydrate moiety by Zemplén transesterification yielded the target compound **23**. The synthesis demonstrates both the versatility of the allylic HYCRON system, which can be used to generate protected peptide fragments for fragment condensation reactions, and the efficiency of PfPyU as condensing reagent.

13.2.1.3 Glycopeptides Containing Sialic Acid The sialyl-T_N structure is considered to be one of the most important tumor-associated antigens of epithelial tumors.[33,34] It is found on mucins[35] on glycophorin[20a,36] and on the envelope glycoprotein gp120 of the human immunodeficiency virus HIV.[37,38] The solid-phase synthesis of a sialyl-T_N glycoundecapeptide of the MUC1-repeating unit has been described.[39] The sialyl-T_N-threonine building block **28** was obtained by a stereo- and regioselective sialylation of galactosamine threonine conjugate **24** with the sialyl xanthate **25**[40] and methylsulfenyl triflate as the promoter in a yield of 32% (Scheme 13.9).



Scheme 13.9 Synthesis of the sialyl-T_N building block **28**.

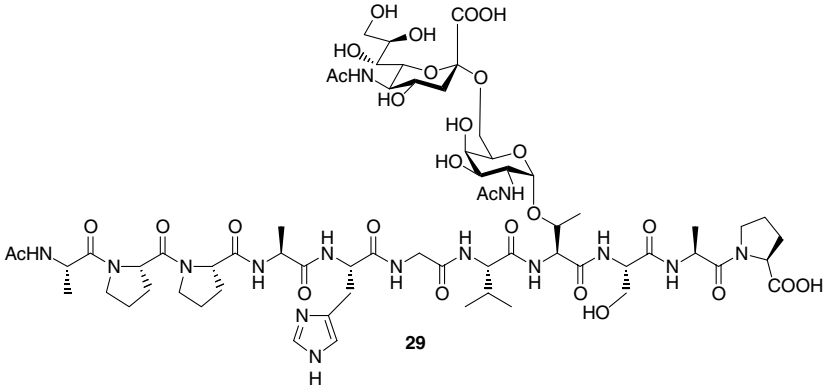
O-Acetylation and subsequent cleavage of the *tert*-butyl ester with TFA gave building block **28**. Solid-phase glycopeptide synthesis was performed using an aminomethylpolystyrene (AMPS) resin modified with the HYCRON anchor. Coupling of the Fmoc amino acids was performed with TBTU/HOBt and a 4.2-fold excess of the corresponding *N*-protected amino acids. After final *N*-acetylation of the terminal amino acid, the completely protected glycopeptide was cleaved from the resin by Pd(0)-catalyzed allyl transfer to morpholine. Cleavage of the acid-labile

amino acid side-chain protecting groups was achieved using TFA. In the case of sialic acid–containing glycopeptides, the deprotection of the carbohydrate moiety is the crucial step. Systematic investigations showed that the application of NaOH in aqueous MeOH at pH 10 results in the removal of the acetyl groups whereas the cleavage of the neuraminic acid methyl ester requires a careful treatment with 5 mM aqueous NaOH at pH 11.5. Under these conditions the saponification of the NeuNAc methyl ester proceeded without side reaction and the glycoundecapeptide **29** was obtained in an overall yield of 23% (Scheme 13.10). At a pH lower than pH 11 no hydrolysis of the methyl ester took place, at pH higher than pH 11.5 a number of side reactions including the β-elimination of the carbohydrate moiety occurred.

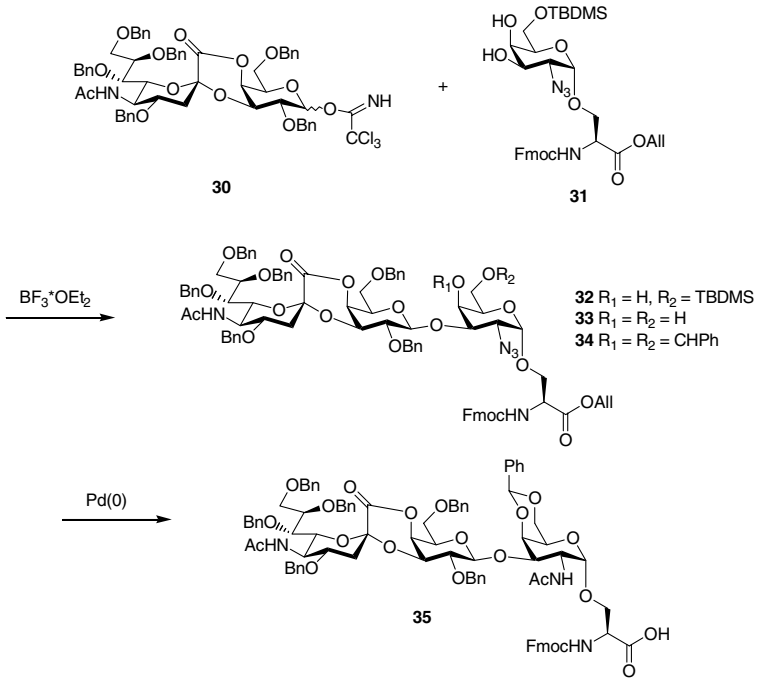
Independently and at the same time the incorporation of a sialyl-T_N-antigen building block in the synthesis of a fragment from the HIV gp120 glycoprotein was described.[41]

The solid-phase synthesis of the B-chain of human α2HS glycoprotein carrying the (2→3) sialyl-T-antigen has been reported.[42] The sialyl-T serine building block **35** suitable for solid-phase synthesis was constructed starting from preformed sialyl-galactose disaccharide **30** (Scheme 13.11). The problem of selective protection and deprotection of the sialic acid carboxylic function was solved by formation of the lactone structure in **30** as has earlier been shown in the synthesis of sialyl Lewis x glycopeptides.[43]

Glycosylation of the Fmoc-protected glycosyl serine allyl ester **31** with the trichloroacetimidate **30** using $BF_3 \bullet EtO_2$ as the promotor yielded the sialyl-T-antigen serine conjugate **32**. Cleavage of the silyl ether with 80% aqueous TFA and subsequent benzylidenation gave compound **34**. The azido function was converted to the acetamido group using thioacetic acid–pyridine.[44] Finally, the allyl ester was cleaved by Pd(0)-catalyzed allyl transfer[45] to give the desired building block **35**. The stepwise synthesis of the 27 amino acid–containing glycopeptide was performed on a Wang resin using DCC/HOBt as activating agent. The side-chain functional groups of the nonglycosylated amino acids were protected with Trt groups for cysteine,



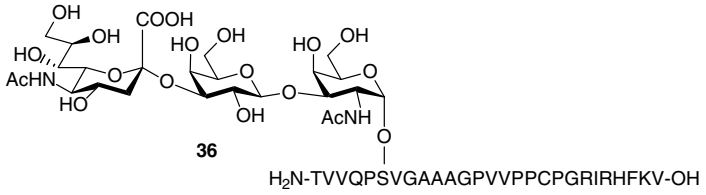
Scheme 13.10 A sialyl-T_N-glycoundecapeptide of the MUC1 repeating unit.



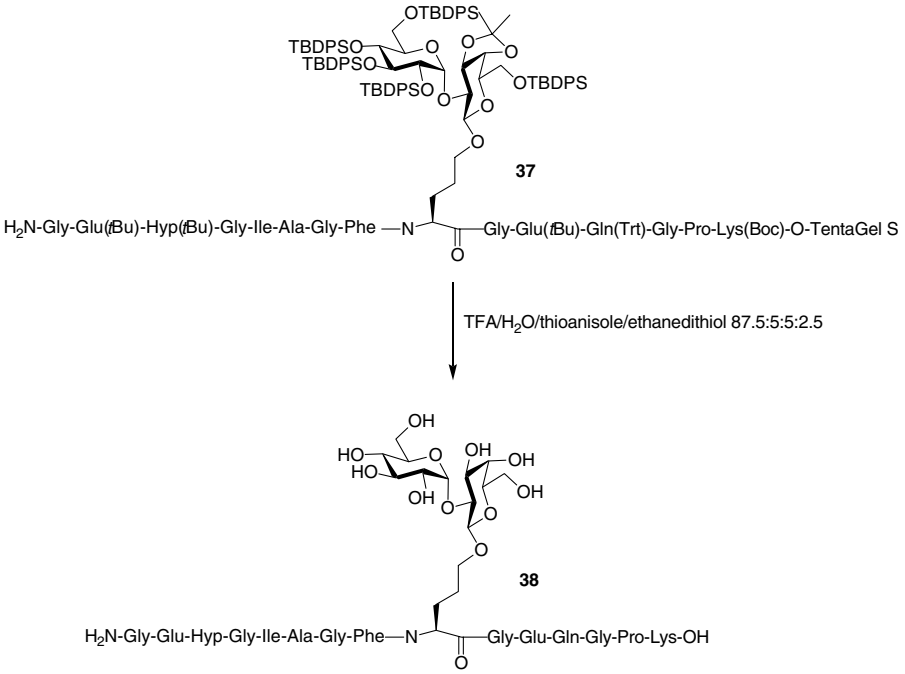
Scheme 13.11 Synthesis of a sialyl-T building block.

glutamine, and histidine; Boc for lysine; and the Pmc group[46] for arginine. Detachment of the glycopeptide from the resin using TFA/H_2O/thioanisole/1,2-ethanedithiol/phenol yielded the desired glycopeptide as well as mono- and dibenzylated byproducts. The combined fractions were thus treated with 1 M TMSOTf/TFA[47] in the presence of thioanisole and then with aqueous ammonium fluoride for complete deprotection to yield the target compound **36** (Scheme 13.12). The usual debenzylation procedure by Pd/C-catalyzed hydrogenation was unsuccessful because of the simultaneous desulfurization of the cysteine residue.

Besides acetyl, benzoyl, and benzyl protecting groups for the carbohydrate hydroxyl groups, silyl, isopropylidene, and *p*-methoxybenzyl groups have also been employed in solid-phase glycopeptide synthesis. The synthesis of a glycopeptide



Scheme 13.12 B chain of human α2HS glycoprotein carrying the sialyl-T-antigen.



Scheme 13.13 Synthesis of a type II collagen analog using acid-labile carbohydrate protection groups.

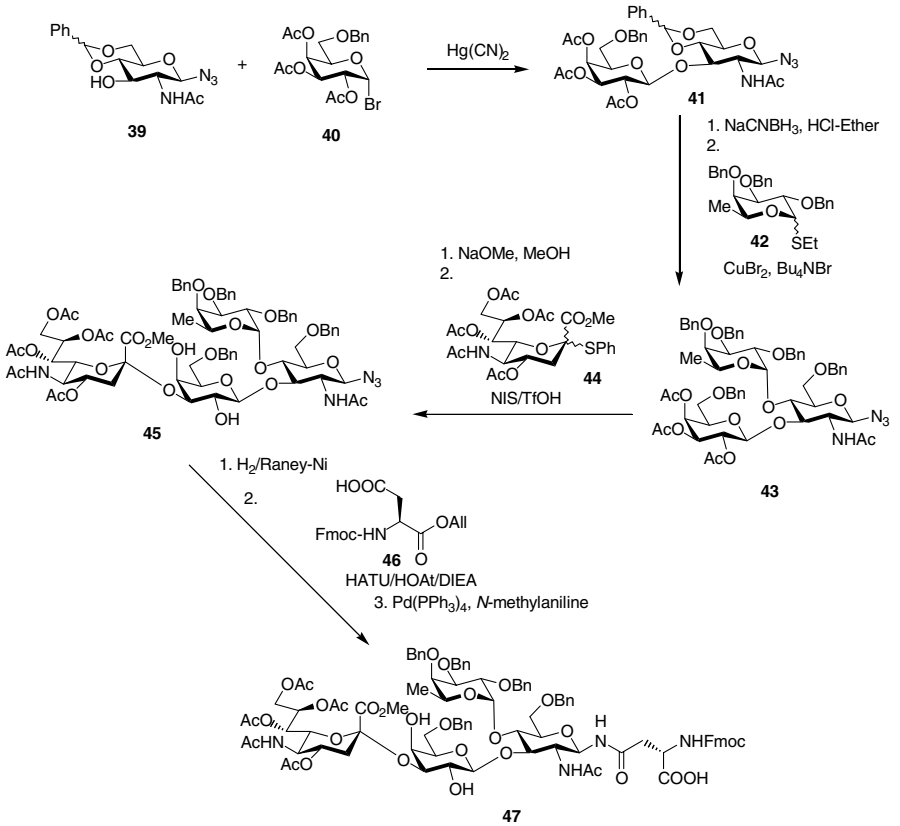
related to the immunodominant fragment of type II collagen was achieved employing acid-labile silyl and isopropylidene protecting groups (Scheme 13.13).[48,49] The solid-phase synthesis was performed using crosslinked polystyrene grafted with poly(ethylene glycol) chains (TentaGel S)[29] carrying an acid labile 4-alkoxybenzyl alcohol linker as the solid support. Coupling reactions were performed using DIC/HOBt for the unglycosylated amino acids and DIC/HOAt for the glycosylated amino acid.

The detachment of the glycopeptide from the resin and deprotection of the amino acid side chains as well as the removal of the acid-labile carbohydrate protecting groups were simultaneously carried out using TFA/H_2O/thioanisole/ethanedithiol (87.5:5:5:2.5). This treatment proceeded without affecting the glycosidic bonds and furnished the target molecule **38**.

13.2.2 *N*-Glycopeptides

13.2.2.1 N-Glycopeptides Carrying the Sialyl Lewis A Structure The regioisomeric tetrasaccharide structures sialyl Lewis A (sLe^a) (NeuAcα(2→3)Galβ(1→3)[Fucα(1→4)]GlcNAc) and sialyl Lewis X (sLe^x)[50] (NeuAcα(2→3)Galβ(1→4)

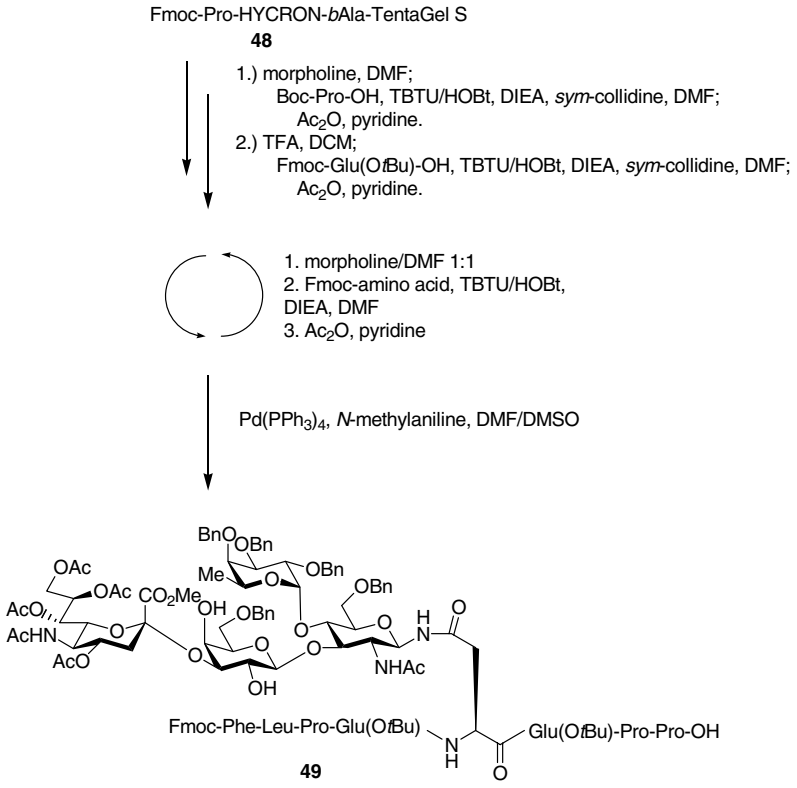
[Fucα(1→3)]GlcNAc) occur in saccharide side chains within the *N*-terminal domain of various glycoproteins on the surface of leukocytes, for example PSGL-1 (P-selectin–glycoprotein–ligand-1).[51] Within inflamed tissues, cytokines are released, which induces the expression of E- and P-selectins on the endothelial cells. The recognition of sLe^a and sLe^x by E- and P-selectin induces leukocyte adhesion on the vascular endothelial surface. This primary binding is followed by stronger protein–protein interactions (e.g., between ICAM-1 and LFA-1) and subsequent diapedesis, the passing of the leukocyte through the endothelial wall, and its invasion into the site of infection. The prevention of the first sLe^a/sLe^x-selectin interaction at the beginning of the inflammatory adhesion cascade has been thoroughly investigated in order to develop a therapy of acute and chronic inflammation processes.[52] The synthesis of a glycopeptide with the sLe^a structure bound to a sequence derived from the *N*-terminal region of the surface glycoprotein PSGL-1 was performed using the building-block approach.[53] The complex sLe^a building block **47** (Scheme 13.14) was



Scheme 13.14 Synthesis of a sialyl Lewis A buiding block for solid-phase glycopeptide synthesis.

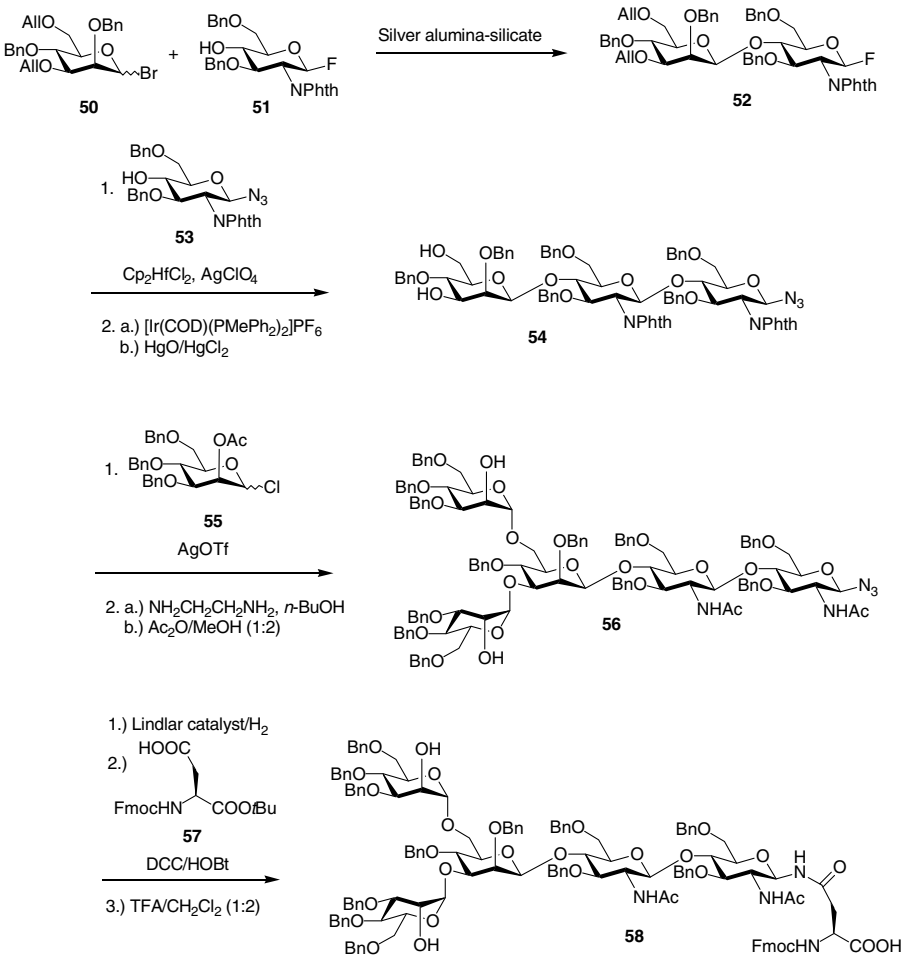
synthesized starting with the coupling of galactosyl bromide **40** to a glucosamine azide **39** by activation with $Hg(CN)_2$.

Neighboring-group participation at the 2 position of the galactosyl bromide selectively furnished the β-glycoside **41**. Regioselective opening of the benzylidene group with $NaCNBH_3$ in HCl/ether[54] was followed by fucosylation to trisaccharide **43** employing ethylthiofucoside **42** as the donor and a variation of Lemieux's in situ anomerization method described by Ogawa.[55,56] After cleavage of the acetyl groups with NaOMe/MeOH, *N*-acetyl neuraminic acid was introduced in a regio/stereoselective (2→3) sialylation procedure furnishing sialyl Lewis A azide **45**. Tetrasaccharide **45** was converted into the *N*-glycosylamino acid **47** by reduction with Raney nickel/H_2[43] and subsequent coupling of the glycosylamine to the Fmoc-aspartic acid α-allylester **46**. The *C*-terminal allyl ester was cleaved by Pd(0)-catalyzed allyl transfer to *N*-methyl aniline[45] to give building block **47** ready for solid-phase glycopeptide synthesis. Octaglycopeptide **49** was synthesized using the HYCRON/TBTU methodology (Scheme 13.15).



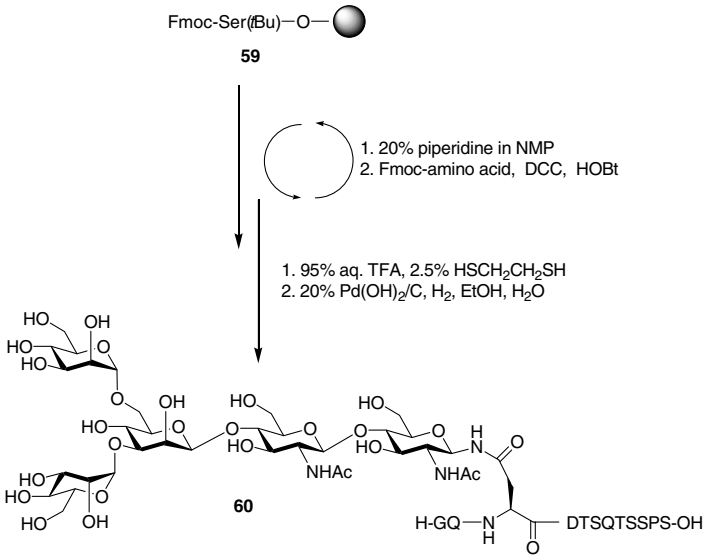
Scheme 13.15 Solid-phase synthesis of a sequence from PSGL-1 carrying sialyl-Lewis A.

13.2.2.2 A Glycopeptide Carrying an N-Linked Pentasaccharide Core CD52 is a GPI-anchored glycoprotein expressed on human lymphocytes. Monoclonal antibodies against CD52 are effectors of the complement-mediated lysis of cells. They have been used both in vivo and in vitro for the control of graft–host disease and for the prevention of bone marrow transplant rejection.[57] CD52 is composed of a peptide containing only 12 amino acid residues and a *N*-glycosylated pentasaccharide chain.[58] The synthesis of target compound **60** was performed using Fmoc amino acids and a carbohydrate building block **58** protected with benzyl ethers. Building block **58** was prepared starting from the monosaccharide building blocks **50** and **51** (Scheme 13.16).



Scheme 13.16 Synthesis of an Asn-Core pentasaccharide buiding block.

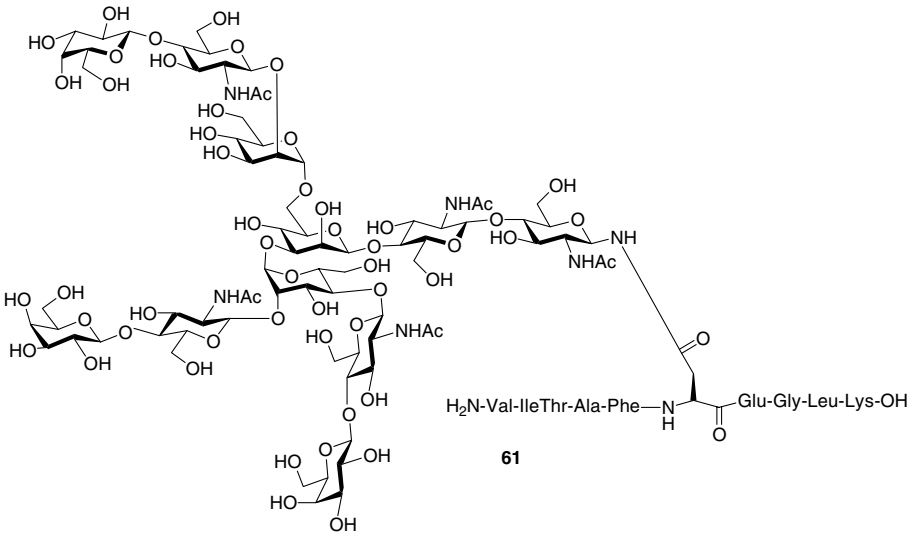
For glycosylation of the glucosaminyl fluoride **51**, the mannosyl bromide **50** was selectively activated by silver alumina–silicate. After purification, the disaccharide fluoride was directly used for a further glycosylation of a glucosamine azide **53** using Cp_2HfCl_2 and $AgClO_4$ as the activating reagents. The trisaccharide was deallylated by an iridium-catalyzed isomerization[59] and subsequent cleavage of the propenyl ether with $HgO/HgCl_2$ to yield the β-mannosyl chitobiosamine derivative **54**. α-Mannosylation of the two hydroxyl groups of **54** was achieved using mannosyl chloride **55** in the presence of silver triflate. Cleavage of the phthaloyl groups by ethylendiamine in *n*-butanol was followed by *N*-acetylation using Ac_2O/MeOH (1:2). The azido function of **56** was reduced with H_2 in the presence of Lindlar catalyst and the amine coupled to the Fmoc-amino acid **57** using DCC/HOBt as the condensing reagent. Cleavage of the *tert*-butyl ester gave building block **58** carrying exclusively benzyl protecting groups in the carbohydrate portion. Pentasaccharide asparagine conjugate **58** was then used in the solid-phase synthesis of target structure **60** (Scheme 13.17).[58c]



Scheme 13.17 Solid-phase synthesis of CD52 glycopeptide.

The synthesis was performed on a 4-hydroxymethyl-phenoxymethylcopolystyrene-1% divinylbenzene resin. Deprotection of the amino acids was achieved using 20% piperidine in *N*-methylpyrrolidone. Condensation reactions were carried out using DCC/HOBt as coupling reagents. After stepwise synthesis, the glycopeptide was released from the resin with concomitant cleavage of the acid-labile amino acid side-chain protecting groups using 95% TFA. Final deprotection of the carbohydrate moiety by hydrogenolysis catalyzed by 20% $Pd(OH)_2/C$ gave target compound **60**.

13.2.2.3 Synthesis of Glycopeptides Using Saccharide Units Isolated from Natural Sources Another approach to the synthesis of *N*-linked glycopeptides consists of the use of oligosaccharide building blocks that have been isolated from the natural sources fetuin and ribonuclease B by appropriate degradation and separation of the fragments.[60] Such oligosaccharides can be obtained from the natural glycoproteins by a mild hydrazinolysis procedure that yields the unreduced forms of the carbohydrate moieties. Conversion of the carbohydrates to the β-glycosyl amines was achieved by treatment with a saturated solution of $(NH_4)HCO_3$, a method introduced by Kochetkov et al.[61] Subsequent coupling to a suitable protected aspartic acid derivative gave the desired glycosyl asparagine conjugates. After carboxy deprotection, these building blocks could be used in solid-phase synthesis without protection of the carbohydrate hydroxyl functions to give glycopeptide structures such as **61** (Scheme 13.18). This approach enables an access to complex carbohydrate structures that still are not easily available by purely synthetic means.



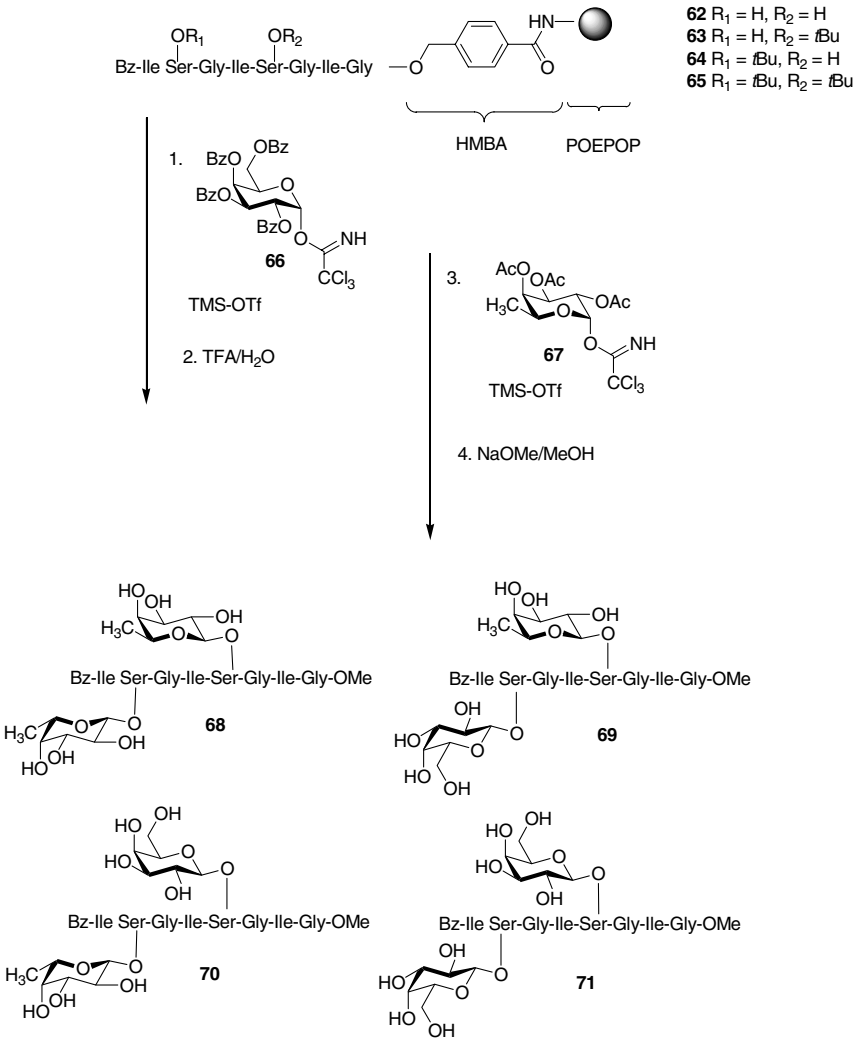
Scheme 13.18 *N*-linked glycopeptide with a triantennary structure.

13.3 DIRECT SOLID-PHASE GLYCOSYLATION OF SOLID-SUPPORT-BOUND PEPTIDES

A convergent method for the synthesis of glycopeptides consists of the direct coupling of oligosaccharides to preformed peptides. A solution-based synthesis of *N*-linked glycopeptides by acylation of β-glycosyl amines with partially protected aspartic acid–containing peptides has previously been reported.[62] More recently, the direct *O*-glycosylation of peptides on solid phase has been published.[63] Four different bisglycosylated *O*-glycopeptides were obtained by glycosylation of a set of peptide

structures with different side-chain protection patterns bound to POEPOP (polyoxyethylenpolyoxypropylen)—a polyethylenglycol crosslinked resin—carrying the hydroxymethylbenzoic amide (HMBA) linker (Scheme 13.19).

Glycosylation of the free hydroxyl-functions of the polymer-bound peptides **62**–**65** was achieved by the trichloroacetimidate glycosylation procedure.[13] After glycosylation using the first donor, the perbenzoyl-D-galactosyltrichloroacetimidate **66**, the remaining *tert*-butyl ethers were cleaved and the hydroxyl functions thus set free were now glycosylated with the peracetyl-L-fucopyranosyl trichloroacetimidate **67**. The four glycopeptides were detached from the resin by treatment with



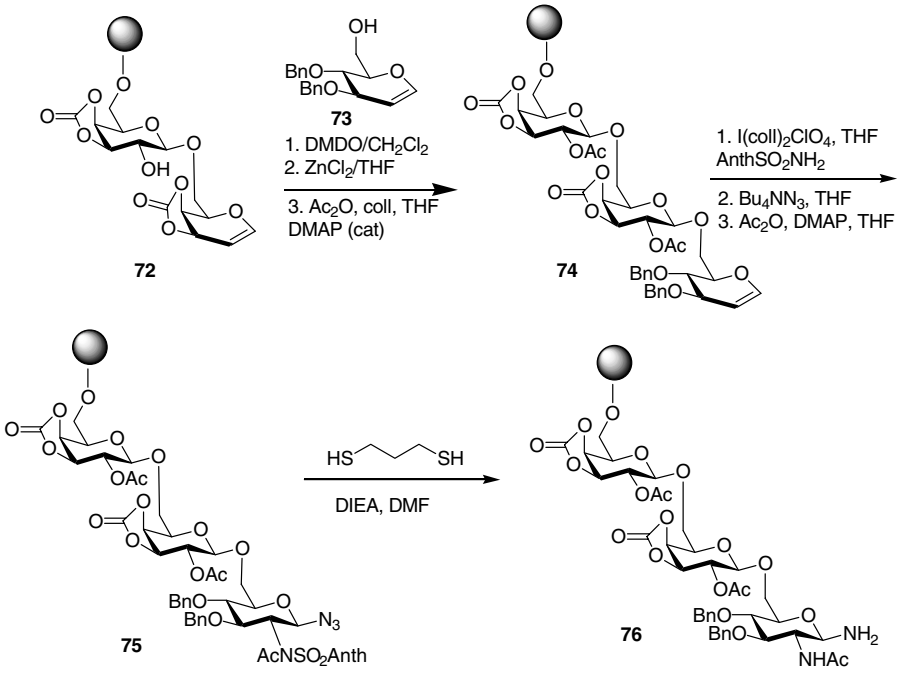
Scheme 13.19 Direct glycosylation of preformed peptides on solid phase.

NaOMe/MeOH with concomitant cleavage of the acetyl and benzoyl groups to furnish structures **68**–**71**. This method takes advantage of the fact that the *C*-terminal amino acid is glycine. It offers the possibility of synthesizing glycopeptide libraries by means of solid-phase combinatorial chemistry. The optimal choice of the solid support is important. Previous attempts of direct solid-phase glycosylations of peptides bound to resins such as PEGA, TentaGel, Polyhipe, and Macrosorb resins via a rink linker or on polystyrene via the Wang linker failed.[15]

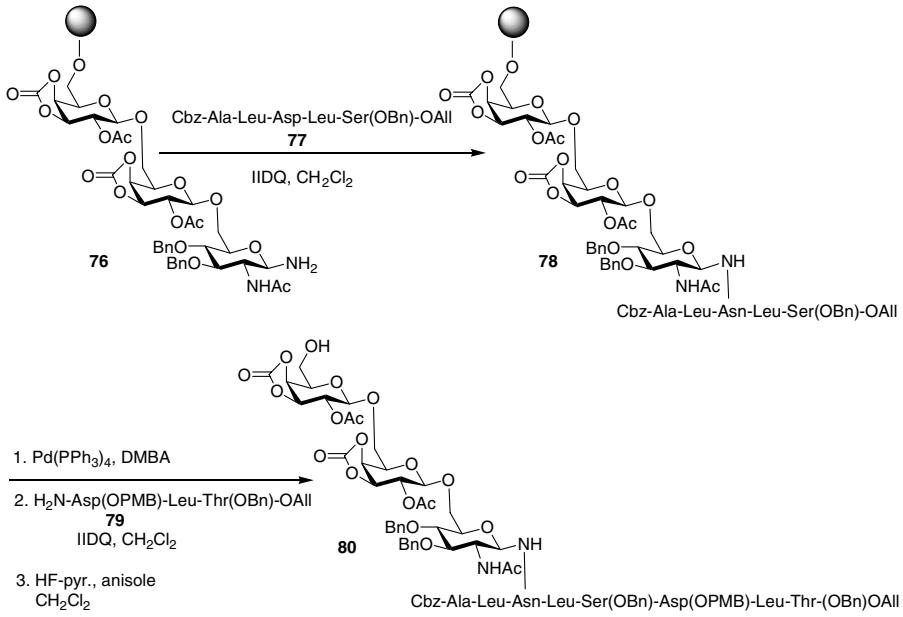
13.4 CONVERGENT GLYCOPEPTIDE SYNTHESIS WITH POLYMER-BOUND CARBOHYDRATES

The convergent synthesis of glycopeptides in which the carbohydrate moiety is attached to the solid support proved an efficient alternative methodology.[64] The carbohydrate structure itself was synthesized on solid support via glycal assembly and anomeric amine synthesis (Scheme 13.20).

The galactosyl glycal **72** was bound to solid phase via a silyl ether linker. Epoxidation of the glycal using 2,2-dimethyldioxirane and subsequent electrophilic activation of the epoxide resulted in the glycosylation of 3,4-di-*O*-benzyl glucal **73.** After acetylation of the product, the polymer-linked trisaccharide **74** was obtained.



Scheme 13.20 Solid-phase glycal assembly and anomeric amine synthesis.



Scheme 13.21 Solid-phase synthesis of carbohydrate bound glycopeptides.

The conversion into the polymer-bound *N*-acetylglucosamine **76** suitable for coupling with a preformed peptide was achieved after iodosulfonamidation and a nucleophilic sulfonamide rearrangement induced by reaction with tetrabutylammonium azide. After *N*-acetylation of the sulfonamide, the acetylated sulfonamide was cleaved by 1,3-propanedithiol and Hünig's base. Under these conditions concomitant reduction of the anomeric azide took place and furnished **76**. Glucosamine **76** was now coupled to preformed peptides containing an aspartic acid residue. Using this strategy, asparagine *N*-glycopeptides such as **78** have been successfully synthesized (Scheme 13.21).

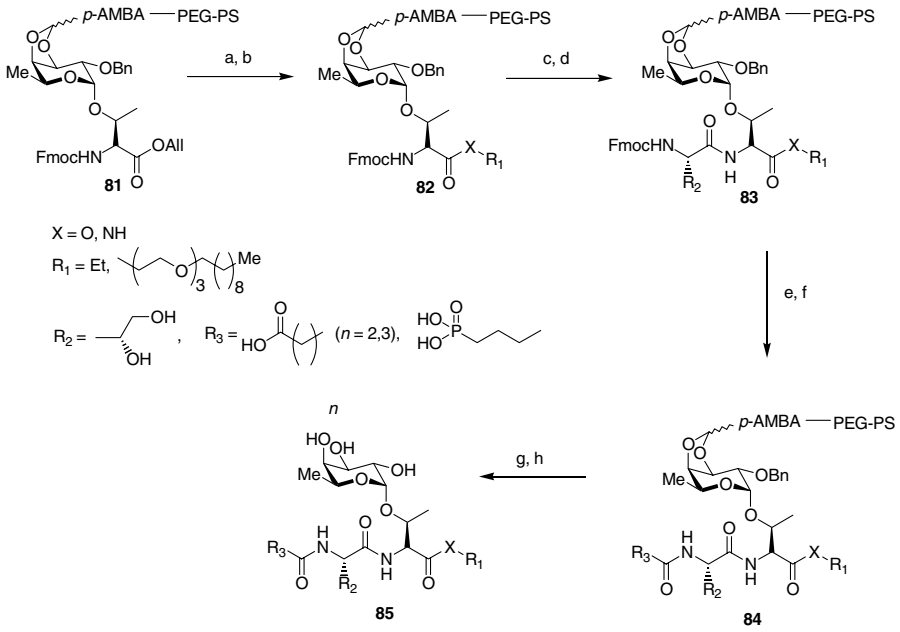
The orthogonal protecting group pattern of **78** allowed extension of the peptide chain on the solid support by fragment condensation with further peptide fragments. After Pd(0)-catalyzed cleavage of the allyl ester,[65] the solid-phase-bound acid was coupled to the *N*-terminally deblocked tripeptide H-Asp(OPMB)-Leu-Thr(OBn)-OAll **79** to form the polymer-bound trisaccharide octapeptide, which, after release from the resin by treatment with HF/pyridine, gave the free glycopeptide **80**.

A similar approach was used in the synthesis of the sialyl Lewis X mimetics of type **85** (Scheme 13.22).[66] Protein crystallization,[67] conformational studies of sLe^x in solution[68] and in bound form to E- and P-selectin[69] as well as the study of structure–function relationships[70,71] gave information about the functional groups of the sLe^x-epitope essential for the binding to the selectins. Synthesized mimetics must contain the three essential hydroxyl functions of the fucose. Sialic acid, galactose, and

glucosamine were substituted by *N*- and *C*-terminal substitutions of threonine, which was itself bound to the fucose moiety by an α-glycosidic linkage. A fucosyl threonine building block was coupled to the solid support through its 3,4-*cis*-diol as an acetal. The combination of a *p*-(acyloxymethyl)benzylideneacetal anchor (*p*-AMBA) and a polyethylenglycol blockcopolymer (PEG-PS)[72] proved efficient for the syntheses illustrated in Scheme 13.22.

Cleavage of the glycosylamino acid allyl ester **81** by Pd(0)-catalyzed allyl transfer to dimedone[73] was followed by esterification or, alternatively, amidation of the carboxyl group. Further *N*-terminal deprotection and condensation steps resulted in the solid-phase-bound derivatives **84**. Acid-catalyzed cleavage from the solid support and final hydrogenolysis of the benzyl protecting group resulted in a library of potential sLe^x mimetics **85**.

The few examples outlined in this chapter illustrate the different methods and the efficiency of solid-phase glycopeptide synthesis. Despite these successful examples and those in the literature, the numerous and varying glycosylation patterns occurring in natural glycoproteins demand not only an optimal choice among the existing methods but also the further extension of the tool box for the synthesis of these complex biologically important structures. In other words, the synthesis of glycopeptides still remains a challenging task in organic synthesis.



a.) $Pd(PPh_3)_4$, dimedone; b.) R_1OH, 2,6-dichlorbenzoylchloride, pyridine or R_1NH_2, HBTU, HOBt, NMM; c.) morpholine/DMF; d.) $FmocNHCHR_2CO_2H$, HBTU, HOBt, NMM; e.) piperidine/DMF; f.) R_3CO_2H, HBTU, HOBt, NMM; g.) aqueous AcOH (80% + 2% TFA); h.) H_2, 10% Pd/C.

Scheme 13.22 Parallel synthesis of fucopeptide library.

ABBREVIATIONS

p-AMBA—*p*-(acyloxymethyl)benzylideneacetal
AMPS—aminomethyl polystyrene
Anth—anthracene
Boc—*tert*-butyloxycarbonyl
DCC—dicyclohexylcarbodiimide
Dhbt-OH—3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine
DIC—diisopropylcarbodiimide;
DIEA—diisopropylethylamine
DMAP—4-dimethylaminopyridine
DMBA—1,3-dimethylbarbituric acid
DMDO—2,2-dimethyldioxirane
E-CAD1—epithelial Cadherin 1
HATU—*O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophos- phate
HMBA—hydroxymethylbenzoic acid
HOAt—1-hydroxy-7-azabenzotriazole
HOBt—1-hydroxybenzotriazole
HYCRON—(*E*)-17-hydroxy-4,7,10,13-tetraoxy-15-heptadecenoyl
ICAM-1—intercellular adhesion molecule 1
LFA-1—leukocyte function associated antigen 1
MAG—multiple-antigen glycopeptide
PEG-PS—polyethylenglycol polystyrene
PfPyU—*N,N-N′,N′*-bis(tetramethylene)-*O*-pentafluorophenyluronium hexafluorophosphat
Pmc—2,2,5,7,8-pentamethyl-chromane-6-sulfonyl
$SLe^{a/x}$—sialyl Lewis A/X
TBTU— *O*-(1-benzotriazole-1-yl)-*N,N,N′,N′*-tetramethylammonium tetrafluoroborate
TIS—triisopropylsilane
TMSOTf—trimethylsilyl trifluoromethanesulfonate
Troc—trichloroethoxycarbonyl
Trt—trityl

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14 Preparation and Screening of Glycopeptide Libraries

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14.1 PARALLEL ARRAYS VERSUS LIBRARIES OF COMPOUNDS

Combinatorial methodology has been a driving impetus in many areas of research and has gained considerable ground since its introduction in 1989 or 1990. This progress, however, has not been reflected in the field of oligosaccharides primarily because of the difficulty in their synthesis and analysis. As an alternative, the readily available glycopeptide libraries have been exploited as potential functional mimetics of oligosaccharides. These libraries can be essential tools in the development of

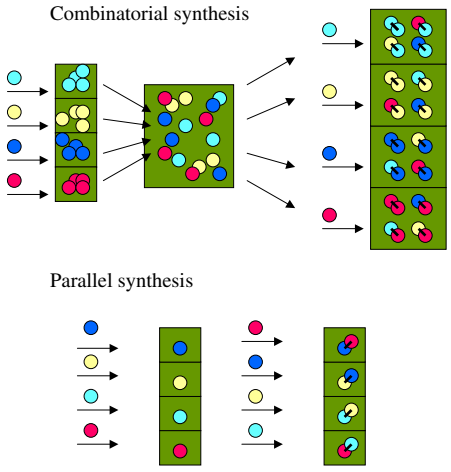


Figure 14.1 The conceptual difference between the synthesis of a combinatorial library with exponential increase in number of compounds and the synthesis of parallel arrays of discrete compounds.

carbohydrate-based therapeutics, such as tumor vaccines, xenotransplantation aids, and antiinfectives.

Here, we present an overview of the aspects of the design, synthesis, analysis, and screening of combinatorial solid-phase glycopeptide libraries as compared to parallel glycopeptide arrays.

Combinatorial libraries have been defined as assemblies of compounds that are derived through a real combinatorial step leading to exponentially increasing numbers of products relative to the number of reagents used (Fig. 14.1). This staggering increase in products may be achieved either by reacting mixtures of reagents to form product mixtures or by introducing mixing of intermediates in solution or compartmentalized on solid phase, such as by the split–combine approach. Because of the ease with which this process is carried out on solid phase, the potential of solid-phase libraries by far exceeds that of solution libraries; therefore, we focus on solid-phase libraries in the present review.

Parallel synthesis of glycopeptides is not described in great detail since such synthesis was reviewed previously and may be regarded as an extension of conventional glycopeptide synthesis.

14.2 CARBOHYDRATE BINDING PROTEINS

Carbohydrate binding proteins (CBPs) located in cellular membranes or recirculating in the cytosol or serum are involved in a variety of important biological functions, including communication and intercellular adhesion, adhesion of bacteria or viruses, activation of the innate immune system, leukocyte rolling, hepatic clearing of aged serum proteins, and sorting of newly synthesized glycoproteins.[1,2] Mammalian CBPs have been divided into three major groups according to their mode of binding: the C-type, the S-type and the P-type lectins.[3]

The calcium-dependent C-type lectins include the E-, L- and P-selectins; the galectins are calcium-independent S-type lectins. The latter have a binding site sized for tight interaction with di- or trisaccharides. The former bind their ligands mainly through coordination of two vicinal hydroxyl groups of a single sugar moiety to a bound calcium ion in the carbohydrate recognition domain (CRD), and the surrounding sugars of the oligosaccharide ligand add to the binding specificity through relatively weak additional interactions. Because of the simple nature of this interaction, the specificity of selectin binding is quite broad and can be mimicked by simple monosaccharide bearing compounds.[4,5] However, the biological activity of ligands is structure-dependent and complicated by a range of factors such as multivalency and clustering.[6]

The calcium-independent receptors of the P-type involved in the clearing and sorting of glycoproteins include the mannose 6-phosphate receptors,[7] the hepatic Gal/GalNAc receptors,[8] and the Man/GlcNAc receptors isolated from liver extracts.[9] These receptors are truly multivalent and bind to the termini of bi-, tri- or tetraantennary *N*-linked oligosaccharides. Although most known receptors recognize

Gal/GalNAc or Man/GlcNAc, there are also receptors (e.g., the macrophage sialic acid receptor) for the recognition of sialylated complex glycans.[10]

The collectins, which are involved mostly in the innate immune system,[11] are large surface or serum proteins composed of bundles of structural collagen stalks with trimer heads containing a calcium dependent CRDs and may be considered C-type by structural similarity. The binding to simple mannose oligosaccharides is relatively weak, and activation of the complement cascade is effected only when the protein interacts multivalently with large polymannans on foreign cell surfaces, creating an activation template for complement processing enzymes.[12] Because of the unspecific nature of this multivalent interaction and the long distance between the sites of interaction (53 Å),[13] multivalent synthetic glycopeptides for these proteins are difficult to design and prepare. However, each CRD may be targeted with high-affinity monovalent ligands.

14.3 THE CARBOHYDRATE LIGANDS

Mammalian glycoproteins consist of 5–90% glycan structures, and these fall into several major groups. There are the *N*-glycosylations including high-mannose, complex, and hybrid oligosaccharides and *O*-glycosylations, including the mucins, the blood group determinants, and the proteoglycans (Fig. 14.2). Glycolipids represent another group of oligosaccharide signaling molecules. The high-mannose oligosaccharides are based on 1→2-α-mannosylation of the mannose pentasaccharide core, α-D-Man-(1→3)-{α-D-Man-(1→3)-[α-D-Man-(1→6)-]α-D-Man-(1→6)}-β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-β-D-GlcNAc-(1→N), and these oligosaccharides exist as a mixture of conformational populations around the 1→6 bonds as depicted in Figure 14.3. Either of these conformers may be the active one in contact with a receptor.

The complex antennary glycans are structures obtained by attachment of β-(1→4)-linked lactosamine unit to 2, 4, and 6 positions of terminal mannoses in a α-D-Man-(1→3)-{α–D-Man-(1→6)-}β-D-Man-(1→4)-β-D-GlcNAc-(1→4)-β-D-GlcNAc-(1→N) core. The terminal galactoses are often sialylated at the 3 or 6 position.

The tetraantennary oligosaccharide illustrated in Figure 14.2 has been shown to present an umbrellalike structure as one of the major conformational populations.[14,15] The distance between terminal sugar residues interacting with receptors may vary from ~8 to ~30 Å. In the case of the phosphorylated high-mannose ligands for the MPR, the distance between phosphates is ~15 Å.[16]

The mucin oligosaccharides do not appear to have many mammalian receptors. However, bacteria in the gastrointestinal tract often display receptors that adhere to the mucin-coated epithelia.[17] Recognition of the aberrantly glycosylated forms present in malignant tissue is essential for the rejection of tumors by the immune system.[18–20] The most thoroughly investigated ligands are analogs of the SLe^x antigen[4,21,22] involved in leukocyte rolling, adhesion, and shedding by interaction with the E-, P-, or L-selectins for which the optimal ligands and mode of recognition for biological activity are still sought. The fucose residue, one hydroxyl group from the central

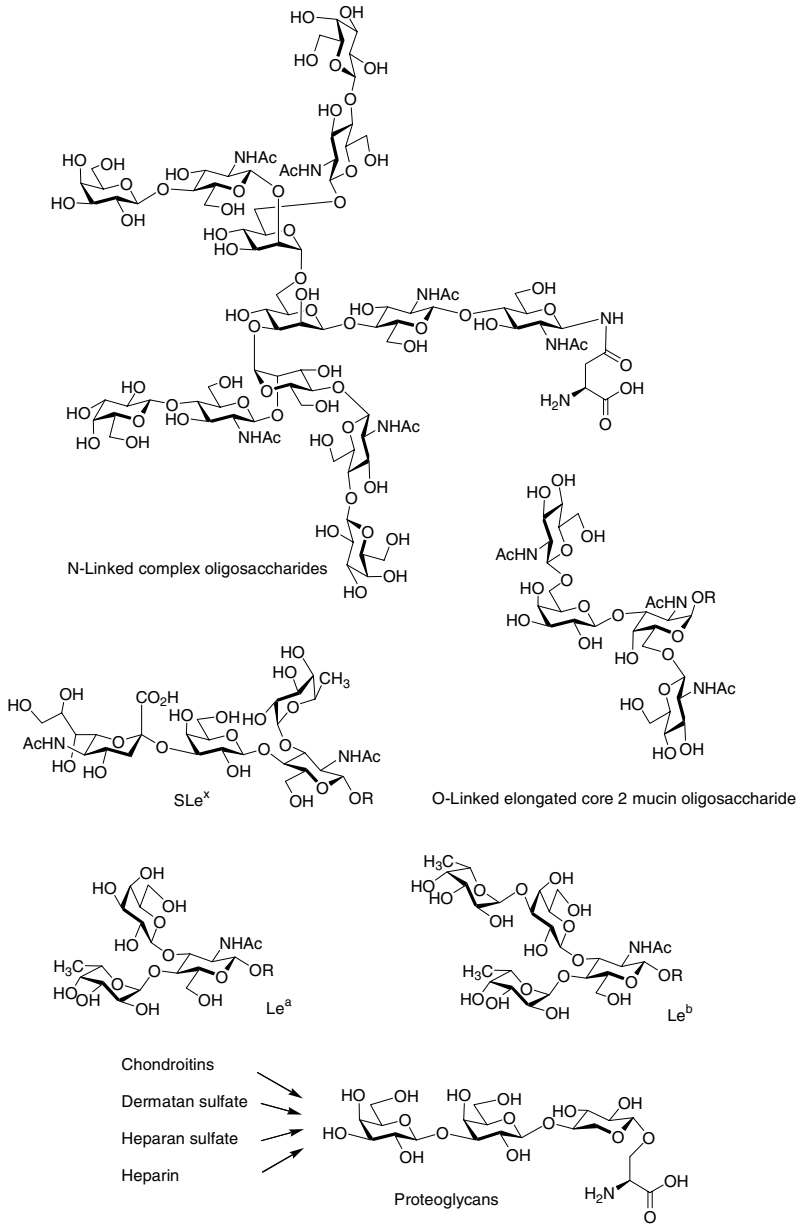


Figure 14.2 Representative oligosaccharide structures found on mammalian glycoproteins and glycolipids. The complex oligosaccharides may be bi-, tri-, or tetra-antennary; the branches may be more or less elongated with 1→4 linked lactosamine units, and they may or may not be sialylated. The SLe^x, Le^a, and Le^b structures represent the different blood group determinants often present on lipids, and the elongated core 2 structure is a mucin-type glycosylation. Proteoglycans have a common core to which a variety of linear acidic polysaccharides are attached.

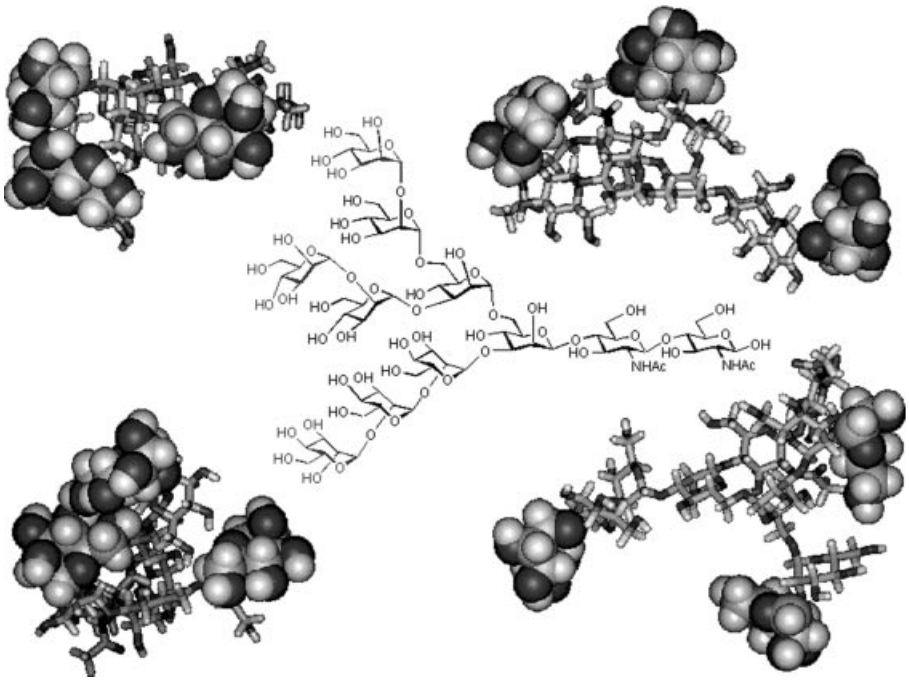


Figure 14.3 Structure models of four different conformer populations (2 for each 1→6 link) of a triantennary high-mannose undecasaccharide M9. Terminal mannoses are highlighted as CPK models.[12]

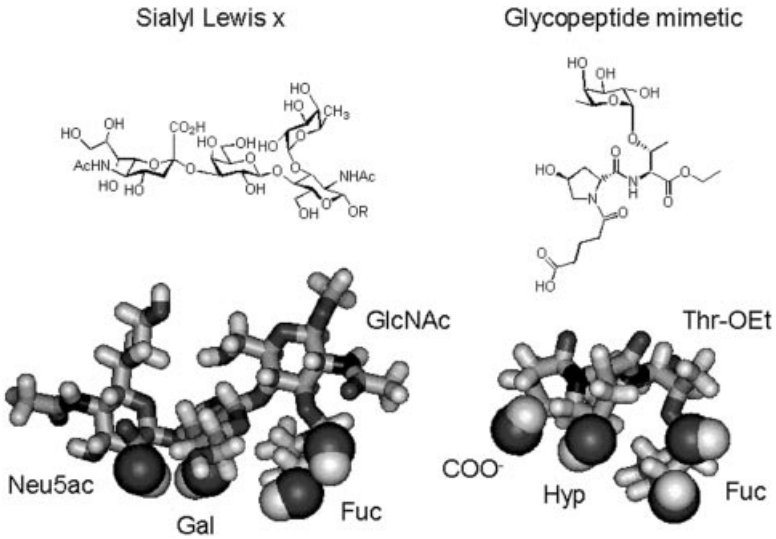


Figure 14.4 Bound conformation of the SLe^x tetrasaccharide compared to that of an efficient fucosyl glycopeptide mimetic compound.[81] Tightly bound hydroxyl groups are shown as spheres.

galactose unit and the carboxyl group of the sialic acid are essential for the interaction (Fig. 14.4).

The structural investigations of receptors and their carbohydrate ligands indicate that binding of the ligand to the receptor often involves only a few terminal residues. It is therefore anticipated that these receptors will bind to carbohydrate mimetics that present these essential functional groups in the optimal binding orientation. The use of glycopeptide combinatorial libraries that are either randomly generated or carefully designed on the basis of knowledge of the receptor and/or ligand will greatly facilitate the discovery of mimetics of complex oligosaccharide ligands.

14.4 SUPPORTS FOR SOLID-PHASE LIBRARIES

Parallel arrays as well as libraries of glycopeptides can be synthesized in solution or on solid phase. The advantages of using solid phase methods[23] are well documented, and detailed descriptions have been published.[24,25] The choice of solid support is crucial and depends on the types of reactions to be carried out as well as the screening methods to be employed. The two most commonly used types of solid supports are based on polystyrene (PS) (e.g., Merrifield,[23] including commonly used Wang resin[26] and TentaGel[27]/ArgoGel) or polyethylene glycol (PEG) (e.g., PEGA,[28] POEPOP,[29] POEPS-3,[30] and SPOCC[31]) (Fig. 14.5). Polystyrene resins are comprised of mainly 1–2% divinylbenzene crosslinked backbones with short linkers (Fig. 14.5a). Polystyrene based gels are generally unsuitable for direct on-bead screening either because of loss of enzymatic activity in the polymer or because of their poor swelling in polar media and consequent exclusion of biomolecules from the hydrophobic core of the polymer. This is also true for PS resins grafted with long PEG chains such as ArgoGel or TentaGel (Fig. 14.5b),[32] although swelling in water is improved, and some access to the interior has been achieved.[33,34] Furthermore, the polystyrene material absorbs light, thus interfering with some fluorescence assays, and the hydrophobic core may result in nonspecific protein binding.

PEG-based resins, on the other hand, are crosslinked with long-chain PEG macromonomers, which also present the amino or hydroxyl functional groups. Therefore, the mechanical and chemical properties of the resins are strongly influenced by the nature of the PEG chains.[24] Additionally, PEG chains are highly miscible with most solvents creating a quasi-homogenous reaction medium. PEG based resins swell tremendously in aqueous media, thus allowing biomolecules access to the entire bead.[35,36] These resins are therefore suitable for protein ligation,[37] enzymatic reactions, and screening for enzymatic activity and inhibition.[34,38–43]

Since PEG-based polymers confer the advantage of a support that is amenable to both synthesis as well as screening of enzymatic activity and protein binding, efforts have been made to generate such polymers with superior properties in both areas. They may be obtained by radical polymerization of long-chain PEG macromonomers to give PEG-polyacrylamide (PEGA; Fig. 14.5c) copolymer[28,35] or PEG-crosslinked oligostyrene (POEPS-3; Fig. 14.5d).[30] Two novel types of gel supports obtained by anion catalyzed bulk polymerization of PEG, derivatized with epichlorohydrin

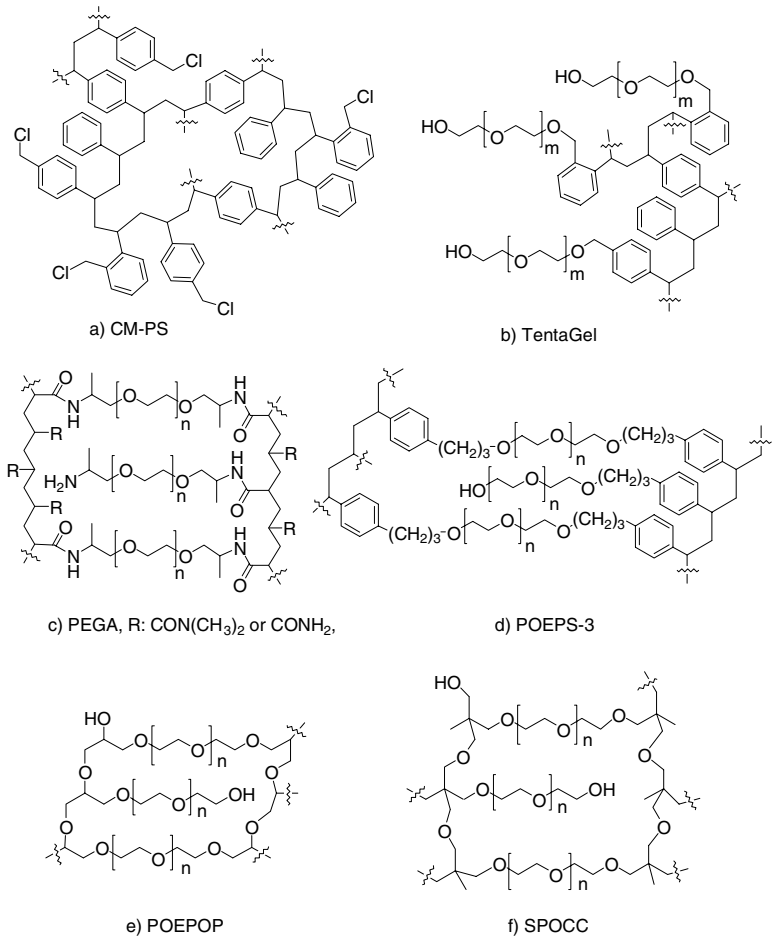


Figure 14.5 The chemical structure of TentaGel and PEG-based resins. The open structure and the inert nature of PEG in biological systems confer ideal properties for bioassays to the PEG-based resins.[24]

(POEPOP; Fig. 14.5e)[29] or by cation-catalyzed bulk polymerization of PEG, derivatized with oxetane (SPOCC, Fig. 14.5f)[31] have been introduced. The inert character of the polymers allows the application of harsh organic reactions. Because of the excellent swelling in aqueous buffers, all the resins mentioned above have been used in bioassays for enzymes. PEGA resin was further investigated and showed no nonspecific binding in protein-binding studies.[44]

For the solid-phase synthesis of glycopeptides, both polystyrene and PEG-based resins have been successfully used. Experiments that compare the rates of reactions on various resins have revealed that the rate of reaction completely depends on the nature of the reaction itself.[45] Some reactions perform better on hydrophobic resins, while others are better on hydrophilic resins.

As of this writing, there are presently only two examples of successful synthesis of glycopeptide libraries in the literature, and these have been generated using PEG-based resins (PEGA and POEPOP), thus enabling rapid solid phase screening of the library (see Sections 14.8 and 14.7.2).

14.5 ANALYTICAL TOOLS FOR GLYCOPEPTIDE LIBRARIES

For solution-phase libraries that are composed of mixtures of compounds, the difficulty of analysis escalates with increasing numbers of compounds. Typically, large mixtures of compounds are not analyzed before screening, whereas small ones may be analyzed for reaction completeness using mass spectrometry, HPLC, NMR, or combinations thereof. The identification and analysis of active compounds from these mixtures is painstakingly tedious, and often complete characterization is possible only after deconvolution procedures and resynthesis of the active compound. For solid-phase libraries, the methods currently developed are discussed below.

14.5.1 Tagging Techniques for Libraries

The number of methods for analyzing nonpeptide libraries is increasing, and the methods generally fall into two categories: direct methods, usually based on mass spectrometry and NMR spectroscopy; and indirect methods, employing encoding, chemical,[46–48] chemoluminescent,[49] or other procedures.[50–52] Many of the methods of chemical encoding are restricted by the additional synthetic effort required and the need to design orthogonal reaction conditions required for the two sets of syntheses. The currently most successful method utilizes a carbene insertion reaction to attach polyhalogenated aromatic tags that are photochemically released and then analyzed by GCMS.[48]

14.5.2 Analysis by Mass Spectrometry

The development glycopeptide libraries obtained by the split–mix method is severely hampered by the lack of concurrent development of a general, facile separation and characterization technology. Some headway has been made with chemical coding of the libraries, but very few direct methods of analysis exist. One promising method that could be applied to the direct characterization of both types of libraries is mass spectrometry. More specifically, post-source-decay matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (PSD-MALDI-TOF-MS) and CID-FAB/MS/MS have been used to characterize glycopeptides.[53–55]

The analysis of glycopeptides has been carried out using primarily PSD-MALDI-TOF-MS. Two strategies are employed: (1) the glycan portion is first cleaved enzymatically or by base-catalyzed β-elimination[54,56] and separately characterized by other means (MS or NMR) while the peptide is sequenced using PSD, or (2) the entire glycopeptide is fragmented by PSD and the spectra

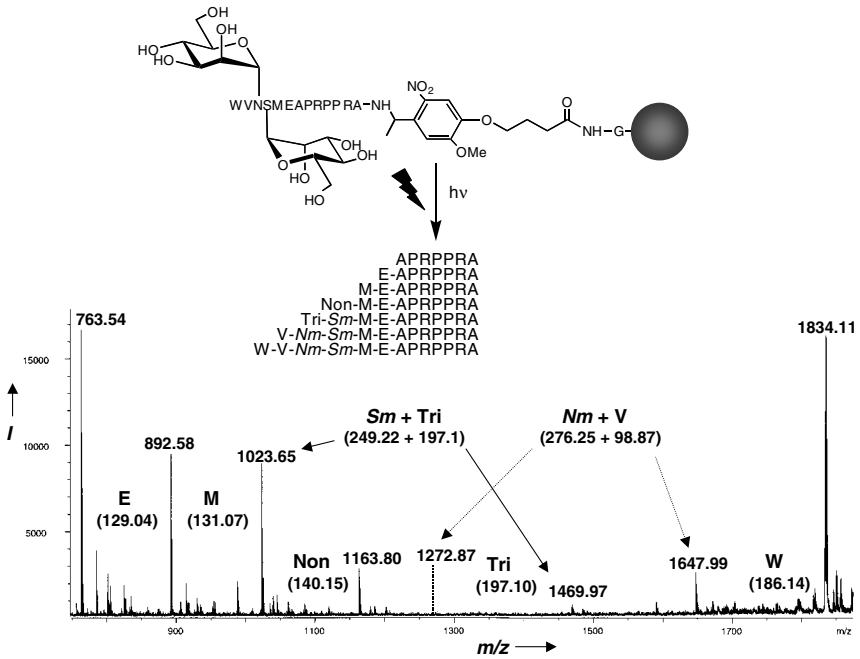


Figure 14.6 Representative mass spectrum showing "ladder" of a bisglycosylated glycopeptide obtained from screening of a glycopeptide library for the mannose binding protein. The nonanoic acid (Non) and tridecanoic acid (Tri) encode for Ser(Man) (Sm) and Asn(Man) (Nm), respectively.

analyzed.[53,55] All these spectral analyses are time-consuming and not suited for high-throughput screening. The only example to date of a facile, direct method of characterizing glycopeptide libraries generated using the building-block approach is one that combines chemical coding (to determine the glycan portion) with capping steps to generate fragments for the mass analysis (ladder synthesis).[44] An example of the mass spectrum of a diglycosylated peptide from a library screened by macrophage mannose binding protein is shown in Figure 14.6. The principle of this method and its application in the synthesis of a glycopeptide library are detailed in Section 14.7.2.

14.5.3 Structural Analysis of Compounds Linked to Single Beads by MAS-NMR

In the absence of highly efficient and effective tagging methods, nanoprobe magic-angle spinning (MAS) NMR spectroscopy is the analytical technique that holds the most promise for direct identification of single compounds bound to one polymer particle. Gel-phase NMR can give respectable ^{13}C NMR spectra of large amounts of resin-bound compounds, but for single beads, such analysis is not possible and proton spectra do not show useful resolution. The properties of the polymer have a major

influence on the quality of the spectra recorded, and in general, the more mobile and liquidlike the backbone of the polymer, the better the resolution of the ^{1}H NMR-spectra. Solid-phase spectra displaying a resolution indistinguishable from that of solution spectra may be obtained with PEG-based resins.[57,58]

Analysis has been performed using polystyrene-based beads,[59] and although the 1D ^{1}H MAS-NMR spectra generally showed little or no resolution of coupled resonances, useful 2D MAS-NMR spectra could be obtained.[60] Only known reaction products and known compounds on polystyrene or TentaGel have been analyzed so far.[61–64] More recently, the complete structural elucidation of an unknown peptide of eight residues on a single POEPOP bead (6 nmol) has been achieved.[58] The minimum amount of resin-bound material required for a complete structural elucidation on PEG-based resins at 500 MHz is 2–7 nmol of compound, depending on the complexity of the structure and the resin.[58] The time-consuming nature of NMR structural analysis also demands a more robust (no false positives) and quantitative biological assay in which the rank of the compound activities may directly quantified in order to limit the number of structural analyses necessary.[65] MAS-NMR spectroscopy combined with MALDI-MS has been used for the monitoring of solid-phase glycosylation reactions of peptide templates with the objective of analyzing single-bead glycopeptides from consecutive glycosylations of peptide template libraries.[66,67]

14.6 GLYCOPEPTIDES AS OLIGOSACCHARIDE MIMETICS

In the quest for carbohydrate-based therapeutics, the synthesis of large numbers of diverse oligosaccharides is an expensive, synthetically challenging, and time-consuming process. Whereas the use of oligosaccharide libraries would save time during synthetic operations and screening, the expense, synthetic challenge and analysis of active compounds from the library would still be problematic. Furthermore, it has been shown that small modifications of the natural oligosaccharide ligand rarely confer any increase in binding efficacy.[68–70] This has been attributed to the enthalpy–entropy compensation often found for analogous structures in the interaction with their receptors in aqueous media.[71–73] The free energy of binding of a receptor with any ligand of similar structure has been correlated to the preorganization of water molecules in the media close to the binding site.[74] If this is the case, only ligand structures, which allow significantly different interactions with both the receptor and the aqueous environment, are expected to have affinities significantly different from that of the natural ligand.

When glycopeptides were first introduced as mimetics of oligosaccharides,[75] it was envisaged that the saccharide moiety would provide the specificity of the binding by directing the ligand to the oligosaccharide binding site while the peptide would function as a scaffold for optimal orientation of the glycan portion. However, since peptide ligands generally bind with high affinity to peptide receptors, it is also expected that the glycopeptide could furthermore interact favorably with carbohydrate-binding receptors through the peptide scaffold, thus leading to increased

binding affinity.[76] The source of the enhanced affinity of peptides may lie in their ability to perform an induced fit because of the relatively limited flexibility around peptide bonds leading to fast on-rates for the binding. In the peptide architecture, this flexibility is obtained with the minimum entropic penalty. Conversely, the carbohydrates generally display conformational assemblies located closely around one global energy minimum conformation (or a multiple of 2 for each 1→6 linkage included; see Fig 14.3). In contrast to glycopeptides, highly flexible neoglycoconjugates[77] do not show high affinity, because of the large entropic penalty.[78,79]

The principle of glycopeptides mimicking oligosaccharides was consolidated through binding studies with an array of phosphorylated glycopeptides and the divalent mannose 6-phosphate receptor.[76,80] The structural similarity between the oligosaccharide and the most active glycopeptide ligand was supported by molecular dynamics (Fig. 14.7).

Similar results were later obtained with glycopeptides designed for binding to the selectins.[5] A glycopeptide mimic of the SLe^x tetrasaccharide containing fucose on a peptide scaffold had a greater than 10-fold increased binding affinity for E-selectin.[81] There was no significant further increase in affinity when the ligands were immobilized by polymerization in a multivalent arrangement in liposomes.[82] In

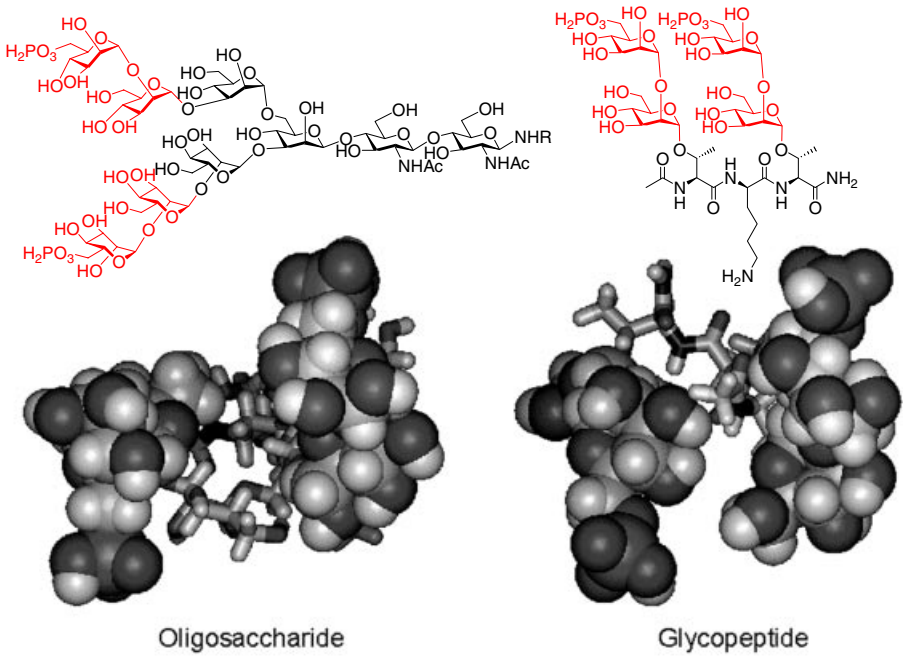


Figure 14.7 Models of phosphorylated high-mannose M7 and a glycopeptide mimic obtained by MD calculation showing similar conformations. They are seen from the point of interaction with the mannose-6-phosphate receptor. Disaccharide phosphates are emphasized as spheres.[76]

another example, a high-affinity divalent ligand adhesin containing αGal(1→4)αGal-linked via peptide bonds to an aromatic nucleus was prepared for the *Streptococcus suis*. The assay with structurally similar tetravalent ligands showed no significant increase in binding, indicating the interaction to be truly divalent.[17] Glycopeptide mimics of galactose or GlcNac containing oligosaccharides afforded 1.7 μM inhibitors of galactosidase[83] and GlcNAc-transferase inhibitors,[84] respectively. In another approach glycopeptide like azasugar inhibitors were prepared; however, reduction of inhibitory activity was found when compared with the parent azasugar without peptide moiety.[85]

Glycopeptides are excellent mimics of the complex oligosaccharides and may be utilized in a library format to identify high-affinity ligands.[86] The ease with which glycopeptides are synthesized using preactivated amino acids and glycosylated amino acid building blocks can—by careful assembly of a library—ensure the generation of a single compound in each bead. For the preparation of glycosyl amino acid building blocks, the glycosylation of Fmoc-amino acid-OPfp esters or free Fmoc-amino acids has proved a general and versatile method useful for the preparation of complex compounds for direct incorporation into the glycopeptide libraries.[87–92] Many other strategies have also been successfully employed for glycopeptide synthesis. However, these all require further manipulations of the glycosylated building blocks before use in peptide synthesis.[93] In addition to the relative ease of synthesis, facile characterization of active compounds makes libraries of glycopeptide very attractive alternatives to oligosaccharide libraries.

14.7 PARALLEL AND LIBRARY SYNTHESIS OF GLYCOPEPTIDES

14.7.1 Parallel Synthesis of Glycopeptide Arrays

When considerable a priori structural knowledge of a protein carbohydrate interaction is available, it is possible to synthesize a range of active glycopeptide analogs by biased design using a parallel synthetic approach. The requisite knowledge is the nature of the dominant sugars involved in the interaction and their spatial orientation in the receptor interaction. With such information available from X-ray crystal data or from transfer NOE-NMR experiments, a range of high-affinity ligands of the glycopeptide type have been developed for several receptors by parallel synthesis.

In an earlier study, 20 bidentate glycopeptide ligands for the mannose 6-phosphate receptor (MPR) were synthesized by parallel synthesis, and high-affinity ligands with approximately 20 fold increased affinity for the receptor were obtained.[94] The array of analogs contained glycosylated linear tri- to pentapeptides and cyclic hexa- to octapeptides. The glycans were phosphorylated 1→2- and 1→6-linked mannodisaccharides and phosphorylated mannosides. Glycopeptides were synthesized by solid-phase multiple-column peptide synthesis (MCPS)[95,96] using Fmoc-amino acid–OPfp esters and PEGA resin. The sugar hydroxyl of the glycosylated building blocks were protected as acetates or benzoates and the

phosphate with trichloroethyl groups. Deprotected glycopeptides were obtained in high yield and purity. Assaying the compounds demonstrated that a minimum of two disaccharides on a scaffold was required for the specific interaction with the receptor and clearly showed the necessity to have sufficient but not excessive flexibility in the scaffold. Two 6-P-α-Man-(1→2)-α-Man disaccharides on a linear tripeptide had the highest affinities for the receptor. Attempts to increase the affinity through cyclization of the peptide were unsuccessful.[97]

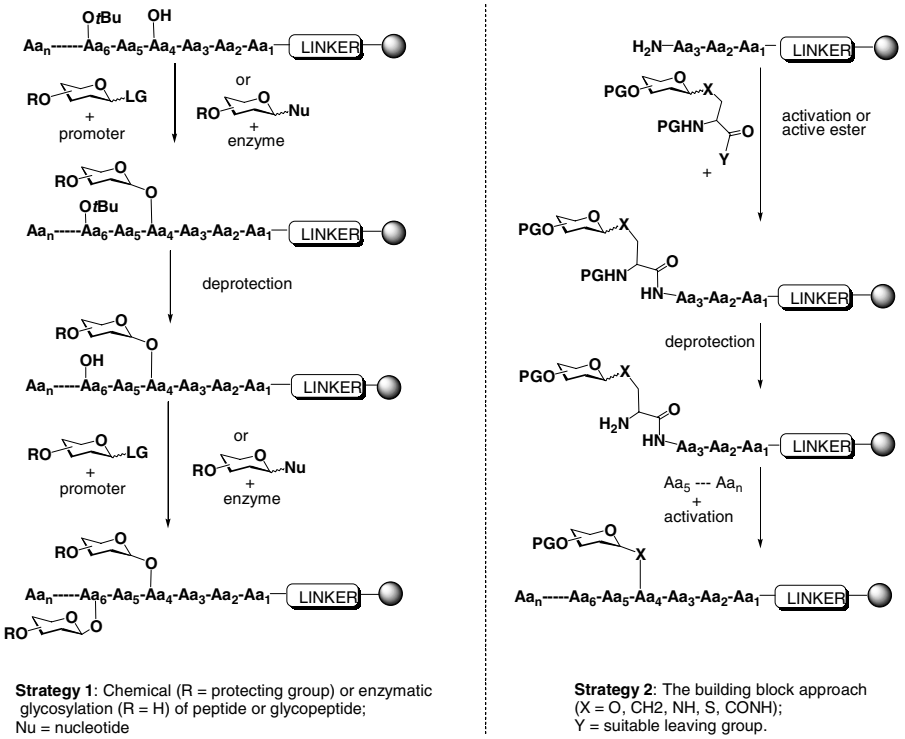
An array of 120 galactose-containing compounds (30 mixtures of four diastereomers) was prepared in parallel by base-catalyzed Michael addition of β-D-$(C_{12}H_{25}CO)_4$Gal-SAc to four different unsaturated ketones and an α-chloro ketone followed by reductive amination with six amino acids. The 30 products were purified by solid-phase extraction and tested as inhibitors of galactosidase. Galactose provided the specificity for the enzyme, while the affinity was obtained through interaction with the aglyconic scaffold. In this way, 1.7 μM inhibitors of the enzymatic activity were obtained.[83]

Parallel synthesis of 62 different fucosylated tripeptides resulted in two ligands with submicromolar affinity for the P-selectin; however, the desired activity for the E-selectin was not observed.[98] For the E-selectin selectivity, it was necessary to incorporate a hydroxyl group that mimics the 4-hydroxyl of the central Gal in SLe^x in addition to a Fuc-residue and a carboxylate to obtain ligands with > 10-fold increased activity over that of the SLe^x tetrasaccharide.[81] One of the best ligands was obtained from Thr(α-Fuc)-OEt, which was first *N*-acylated with a hydroxyl amino acid and then elongated with a di-acid to furnish the acid mimic of the sialic acid carboxylate (Fig. 14.4). This approach was further developed as a solid-phase method where the molecule was linked to a solid support through the invariable fucosyl moiety.[99]

In an elegant and different approach, an array of *C*-linked glycopeptide like mimics of SLe^x were synthesized in parallel by a four-component Ugi reaction.[100] Reaction of different anomeric two or three carbon extended *C*-glycosyl aldehydes or acids with resin bound amines, isonitriles and with other acids or aldehydes, respectively, yielded an array of *C*-linked analogs. The method is easy to perform in good yield on solid phase. However, this strategy can be used only for a mixed library and not for a one-bead-one-compound library. It is derived through a multicomponent reaction where all components are introduced in a single step and deconvolution strategies such as positional scanning or iterative synthesis are required to identify the components of the library. On the other hand, deconvolution is feasible with fast screening because the products are generated rapidly.

14.7.2 Preparation and Analysis of Solid-Phase Glycopeptide Libraries

While parallel synthesis of arrays of glycopeptides is readily achieved by implementation of the building-block approach (Scheme 14.1, Strategy 2),[101] glycopeptide library synthesis in a combinatorial manner via the split–mix method has yet to prove routine. The difficulty lies in the structural analysis of the vast number of compounds generated in picomolar quantities on a single bead. Whereas peptides on



Scheme 14.1 Strategies for glycopeptide library synthesis: Strategy 1: chemical or enzymatic glycosylation of peptide or glycopeptide; Strategy 2: the building-block approach. While enzymes have not yet been used in the solid-phase synthesis of glycopeptide libraries, several resin-bound glycopeptides have been glycosylated enzymatically.[36,114]

beads can be conveniently analyzed by solid-phase ladder sequencing[102] or Edman degradation, neither of these methods is suitable for glycopeptides because of the instability of the glycosidic bond under the acidic and basic conditions employed.

An early report described a pentaglycopeptide library containing three randomized positions and an Asn(GlcNAc) building block fixed at position 4.[103] However, the library members were not characterized, and screening results were not described. As of this writing, only one example of a combinatorially generated glycopeptide library suitable for screening and structural analysis has been reported.[44] Generation of a 300,000-member library and analysis of its components was made possible in part by the development of mass spectrometry (MS)-based techniques for identifying the sequence of the individual glycopeptides in the library. In this method, the synthetic history of the glycopeptide is captured on the beads by capping a small percentage of the growing oligomer chain in each synthetic step.[104] Thus, a series of related fragments rather than a single compound is generated on the bead (Fig. 14.6). The difficulty arises when this technique is applied for a glycopeptide library in which the

amino nucleophiles present significant reactivity differences. To account for amine reactivity differences, an in situ capping method based on Fmoc amino acids mixed with 10% of the Boc analog was developed.[86] The glycosylated Fmoc-amino acid–OPfp esters were encoded by capping with selected carboxylic acid–OPfp esters. The library was linked to the solid support via a photolabile linker (Fig. 14.6), which facilitated the immediate analysis of compounds released from the resin beads on an MS target by 20 min irradiation with a mercury lamp. The purity of the library was assessed by MALDI-TOF mass spectrometry by collection and analysis of a few beads. Most of the beads collected afforded spectra of the ladders, which could be easily deciphered using mass-difference assignment software.

In a departure from the building-block approach, glycopeptide libraries were also obtained by glycosylation of a preattached glycan or the hydroxyl group of an amino acid side chain of a peptide library (Scheme 14.1, Strategy 1). In preliminary studies,[105,106] good yields of (~35% of the initial resin loading) of glycopeptides containing di- and trisaccharides were obtained using 5–8 equiv of the perbenzoylated glycosyl trichloroacetimidate donor to glycosylate a known glycopeptide. Glycosylation was attempted on four resins: Polyhipe, TentaGel, PEGA1900, and Macrosorb-SPR250 but was successful only on PEGA and Polyhipe. Early attempts at direct solid-phase glycosylation of longer peptides were not very successful, although the presence of glycopeptide product could be demonstrated.[93,107]

Direct glycosylation of the amino acid side-chain hydroxyl were partially successful albeit low yield.[105,108] The failure of polar resins such as PEGA may be due to the many primary amides in the resin backbone, which interfere with the carbocation intermediate. Peptide amide bonds seem to show less interference. However, since polar resins are required for solid-phase bioassays, new types of polar resin containing only ether bonds were developed for the solid-phase glycosylation of peptides. Quantitative glycosylation of a known peptide was achieved on a PEG-based resin (POEPOP) containing only ether bonds[29] using 5–8 equiv of the peracetylated or benzoylated trichloroacetimidate.[67] In a "one-bead-one-compound" approach, two resin-bound peptides bearing protected and unprotected hydroxyl groups were first glycosylated with galactose and then with fucose after deprotection of the second hydroxyl group, affording a small library of four glycopeptides. The glycopeptides were cleaved off, separated, and characterized by mass spectrometry. While solid-phase glycosylation is undoubtedly a feasible alternative for the generation of truly random glycopeptide libraries with diversity in both the peptide and glycan portions, analysis of such libraries will present quite a challenge. One possibility is the use of fragmentation of the compounds by mass spectroscopy, or alternatively, MAS NMR as discussed in Section 14.5.

14.8 SCREENING OF GLYCOPEPTIDE LIBRARIES

An important requirement for the successful application of the combinatorial library approach to the drug discovery process, is the ability to utilize the library in high-throughput screening (HTS) procedures. HTS screening of glycopeptide

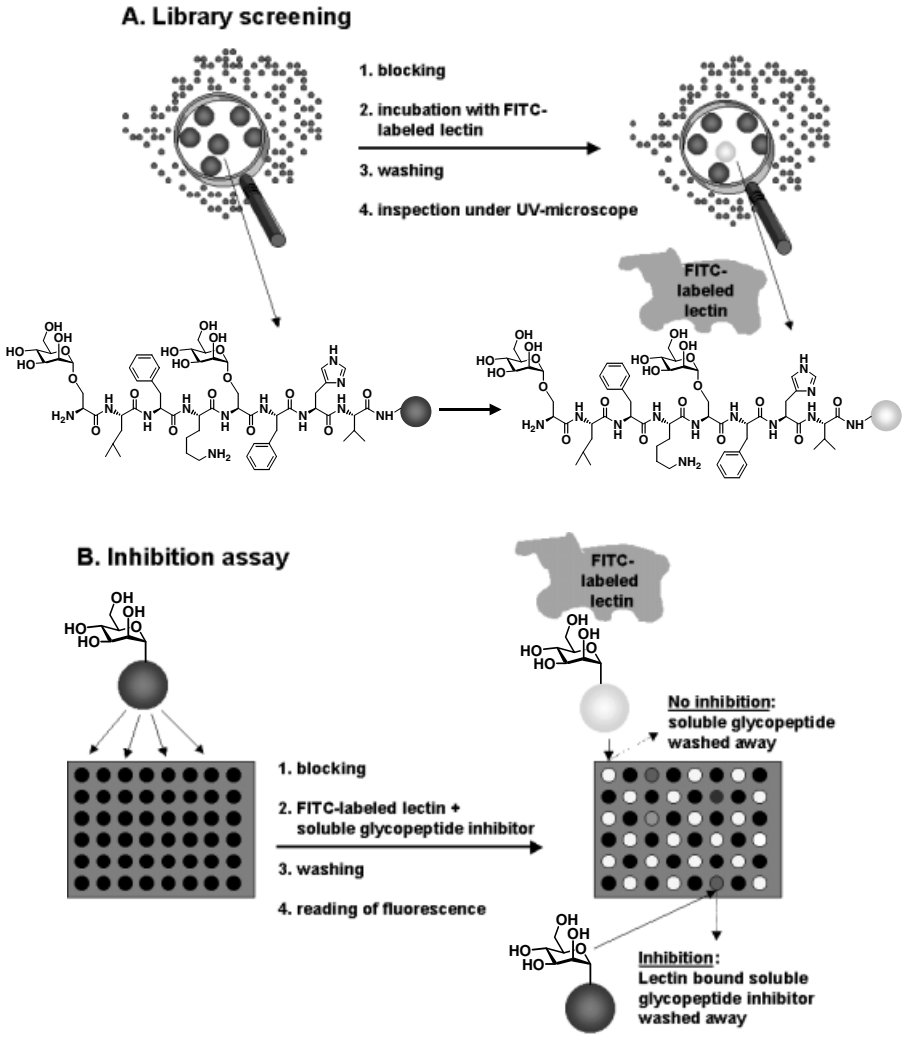


Figure 14.8 (A) Screening of a glycopeptide library using a fluorescent-labeled lectin and ligands bound to PEGA beads. The active compounds are analysed by mass spectrometry. (B) FITC-labeled lectin binding to resin bound mannose could be inhibited by soluble glycopeptides obtained from library screen. Percent inhibition was quantified by recording of lectin fluorescence. Only every second well of the microtiter plate was used and nonfluorescent beads indicated good inhibitors.[44]

libraries is dependent on the mode of library synthesis and can be achieved by screening mixtures of compounds in solution, discrete compounds in solution, or discrete resin-bound compounds. Screening mixtures of compounds is nontrivial, and the various methodologies that can be used for this purpose have been reviewed.[109] One method that has been used for screening an oligosaccharide "library" and could

be equally useful for glycopeptide libraries is based on NMR transfer nuclear Overhauser effects (tNOEs).[110,111] In one demonstration, the *Aleuria aurantia* agglutinin bound to α-L-Fuc(1→6)-β-D-GlcNAc-OMe in the presence of five or 14 other nonbinding oligosaccharides.[110] This methodology is limited by the number of compounds in the mixture that can be screened simultaneously, the difficulty in detecting low or very high affinity ligands, and the time required for analysis of spectra.

As discussed earlier in this review, the split–mix methodology facilitates the rapid production of a large number of compounds. Traditionally, the compounds were subsequently cleaved and then screened and analyzed in solution. More recently, because of the development of solid supports that are compatible with both organic and aqueous media, screening of the library can take place on the solid support itself. Active ligands are detected using immunostaining or colorimetric methods[47] or more directly by use of fluorescent–labeled receptor. In the binding studies with large libraries it is important to avoid having cascades of recognition events involved in the detection method employed since false positives may easily result.

Glycopeptide libraries have been screened in solid-phase assays using soluble receptors.[112] For example, the library generated by ladder synthesis on PEGA resin (Section 14.7.2) was incubated with the fluorescent-labeled lectin from *Lathyrus odoratus* (Fig. 14.8A).[44] The most fluorescent beads were collected and ligands identified by MALDI-TOF mass spectrometry. The most active compounds were glycopeptides containing only mannose [T(α-D-Man)ALKPTHV, LHGGFT(α-D-Man)HV, T(α-D-Man)EHKGSKV, GT(α-D-Man)-FPGLAV, and T(α-D-Man)-LFKGFHV] displaying up to a 25-fold increase in fluorescence compared to lectin binding to resin-bound mannose. Binding of the lectin to resin-bound mannose was inhibited by the active glycopeptides synthesized (Figure 14.8B), suggesting that the glycopeptides and the natural carbohydrate ligand bind to the same or to closely related binding sites of the lectin. Similar studies have been performed with the mannose-binding protein isolated from placenta (see Fig. 14.6).

Parallel arrays of resin-bound glycopeptides and resin-bound glycopeptide libraries have also been used in high-throughput screening of whole cells. A GlcNAc containing pentapeptide library attached to polystyrene resin was incubated with erythrocytes, and adherence was observed but not further investigated.[103] More recently, Tn-antigen containing glycopeptide dendrimers bound to TentaGel resin were used in rosetting tests and showed positive reaction with anti-Tn antibodies and Tn+ erythrocytes.[113] Immunization of animals with one of the most active glycopeptide dendrimers led to an amazing increase in the level of anti-Tn.

14.9 CONCLUSIONS

It is clear that protein–carbohydrate interactions are essential in numerous biological processes and that the development of carbohydrate mimetics that interfere with these processes would provide a powerful methodology for both modulation and amelioration of specific biological activity. Since the early 1990s, novel

methodologies have been presented for the synthesis of glycopeptide libraries that should provide rapid access to such carbohydrate mimics. The technology for the identification of active glycopeptide ligands isolated from solid-phase libraries for the carbohydrate binding proteins has been developed to a practical and useful level.

Glycopeptide libraries, in which the peptide portion confers additional favorable binding affinity, may easily be formed on solid phase and are not difficult to prepare by the building-block approach in a split–combine format, in high purity and hold a lot of promise. Currently, the most versatile method for glycopeptide library synthesis utilizes an in situ capping procedure with a mixture of Boc and Fmoc amino acids, while glycosylated amino acids are separately encoded by capping with carboxylic acids. The analysis of structures is facilitated by direct photolytic release from PEG-based supports for MALDI-TOF analysis. Application of this technique yields high-affinity ligands for carbohydrate binding proteins. Alternatively, generation of libraries of libraries may be achieved by direct glycosylation of glycopeptide libraries followed by analysis of single beads by MAS-NMR spectroscopy.

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