14 Combinatorial Carbohydrate Chemistry

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CONTENTS

14.1 INTRODUCTION

Early breakthroughs in peptide and oligonucleotide synthesis played an important role in providing pure samples that were used as chemical probes to understand their roles in biological processes. Solid-phase synthetic methods developed for these two classes of biopolymers could be successfully transferred to automation, providing easy access to a wide variety of derivatives. This turned out to be crucial in understanding various cellular responses related to proteins and DNAs/RNAs. During this period, a third class of natural biopolymers — carbohydrates and carbohydrate conjugates — were found to play important roles in intra- and intercellular events [1]. Owing to the high degree of difficulty involved in obtaining their pure derivatives, it was not easy to understand the exact nature of their involvement in biological events. In general, the field of "glycobiology" severely suffers from the lack of pure samples for use as biological probes.

In recent years, interest in chemical glycobiology has grown significantly because it is now well established that carbohydrates and carbohydrate conjugates are involved in the modulation of protein functions, fertilization, chronic inflammation, immune responses, and cancer metastases. Carbohydrate derivatives (e.g., glycolipids and glycoproteins) that are present on host cell surfaces provide specific binding sites for the attachment of bacterial and viral pathogens, leading to infectious diseases. Several groups have succeeded in identifying specific complex carbohydrate conjugates as tumor markers with unique structural features. These moieties have become highly attractive, challenging synthetic targets in developing chemically well-defined synthetic vaccines for cancer, and in the design of agents that could specifically deliver anticancer drugs to tumor cell surfaces [2].

Unlike polypeptides and nucleic acids, which are linear biopolymers, carbohydrates are nonlinear molecules and these derivatives present tremendous challenges in developing their total syntheses. In addition to their branched nature, the linkages between two glycoside moieties could present significant synthetic challenges as they can exist in both α- or β-anomeric configurations. Another daunting task with carbohydrates is the use of efficient orthogonal protection–deprotection strategies, as well as difficult stereoselective glycosyl coupling reactions. All of these factors have attributed to slow, tedious and laborious syntheses of carbohydrate derivatives [3]. These challenges need to be addressed before efficient automated processes for the synthesis of complex carbohydrates and their conjugates can be developed. During the past two decades, tremendous progress has been made in developing solid-phase syntheses of complex carbohydrates and carbohydrate conjugates [4]. In most cases, the solid-phase methods are not general in nature, and several carbohydrate-derived coupling reactions do not produce the required products in stereoselective manners.

The lack of chemical stability and bioavailability associated with peptides and nucleic acids means that the field of peptide and nucleic acid mimics has grown significantly. There are several solid-phase synthesis methods that allow rapid access to these derivatives with relative ease. The design and synthesis of agents that are relatively simple and mimic the structural and functional aspects of peptides and nucleic acids, has been an attractive strategy in the search for drug-like candidates. Similarly, the development of small molecule mimics of complex carbohydrates and glycoconjugates has triggered parallel developments in the design and synthesis of inhibitors of oligosaccharide functions [5]. In many cases, it has been established that, despite the complexity of the oligosaccharide moieties of carbohydrate conjugates, the terminal sugars (two to four residues) and their conformations are critical for biological activities. In cases like this, where the chemical complexity of the synthetic target(s) is relatively simple, the use of revolutionary synthetic strategies such as combinatorial chemistry allows rapid access to potential carbohydrate mimics.

Organic synthesis has always been a limiting factor when it comes to searching for bioactive compounds with drug-like properties. To meet the growing demand for the economical synthesis of large numbers of diverse chemical compounds in a relatively short time, solid-phase synthesis and combinatorial synthesis are emerging technologies in the arena of medicinal chemistry [6]. Over the years, solid-phase synthesis has continued to undergo refinement, and has been successfully extended to the synthesis of small organic molecules [7]. Although this field has existed for nearly 10 years, there are still major challenges to overcome before the benefits of combinatorial chemistry are broadly realized [8]. Nevertheless, this area of research is an important part of most drug discovery-based research programs. The branched nature of complex carbohydrates and the lack of highly stereoselective solid-phase reactions to obtain carbohydrate derivatives have precluded the rapid and efficient generation of oligosaccharide libraries either by solution or solid-phase synthesis [9].

In recent years, several groups have reported novel combinatorial approaches to the synthesis of oligosaccharides and carbohydrate mimics. In most cases, the development of efficient solid-phase reactions has been crucial to the library synthesis, as is discussed in this chapter.

14.2 SOLUTION-PHASE LIBRARY SYNTHESIS OF CARBOHYDRATES

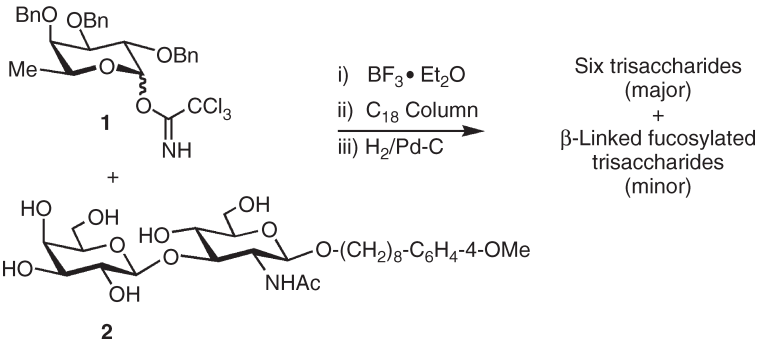
The polyvalent nature, the requirement for several orthogonal protection and deprotection steps, and the lack of a general glycosylation protocol make the rapid assembly of carbohydrates a challenging process. During the last few years, these challenges have continued to be addressed, resulting in novel approaches for the rapid assembly of oligosaccharides leading to combinatorial oligosaccharide libraries.

14.2.1 Hindsgaul's Random Glycosylation

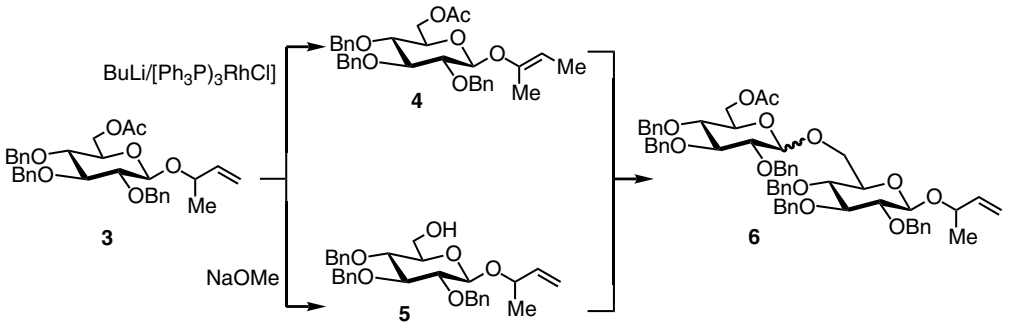
One of the daunting tasks in carbohydrate chemistry is the appropriate use of protecting groups and their deprotection. This is certainly not simple when it comes to complex carbohydrate synthesis in solution, let alone the solid-phase attempts. With this in mind, Hindsgaul et al. [10] undertook the challenge of working with fully deprotected glycosyl acceptors. If successful, their approach could provide several coupled derivatives in one step. The next hurdle to overcome was the development of an efficient purification method. In early attempts, an example of which is shown in Scheme 14.1, the perbenzylated trichloroacetimidate donor **1** was reacted with the unprotected disaccharide acceptor **2** with a hydrophobic group at the anomeric position. The use of the hydrophobic group facilitated purification. To their surprise, unlike the conventional thinking that hydroxyl groups on carbohydrates possess different reactivity profiles, six trisaccharides were formed in nearly equal yields as major products, while β-linked fucosylated trisaccharides were also formed in minor quantities. Interestingly, only 30% of the disaccharide acceptor was fucosylated. The trisaccharides were separated by high pressure liquid chromatography (HPLC) and then characterized by nuclear magnetic resonance (NMR). The outcome of this study demonstrated that this method faced problems of uncontrolled glycosylation reactions, low yields and a requirement for extensive purifications at the end.

14.2.2 Boons's Latent-Active Glycosylation

Using solution synthesis, Boons et al. [11] developed a novel glycosylation strategy that utilized latent-active reactivity principles. In this approach, the major building block, 3-buten-2-yl glycoside **3** (Scheme 14.2) was converted into a glycosyl donor as well as an acceptor. For example, in one case, the terminal olefinic moiety in glycosyl derivative **3** was subjected to isomerization using BuLi/$(Ph_3P)_3RhCl$, giving the corresponding glycosyl donor **4**. Alternatively, the acetate group at C-6 in **3** was subjected to deacetylation (NaOMe), producing the glycosyl acceptor **5**. The glycosylation coupling reaction between **4** and **5** provided the disaccharide **6** in ca. 90% yield with no control over the stereochemistry at the anomeric position. This method was efficiently utilized



SCHEME 14.1 Hindsgaul's random glycosylation.

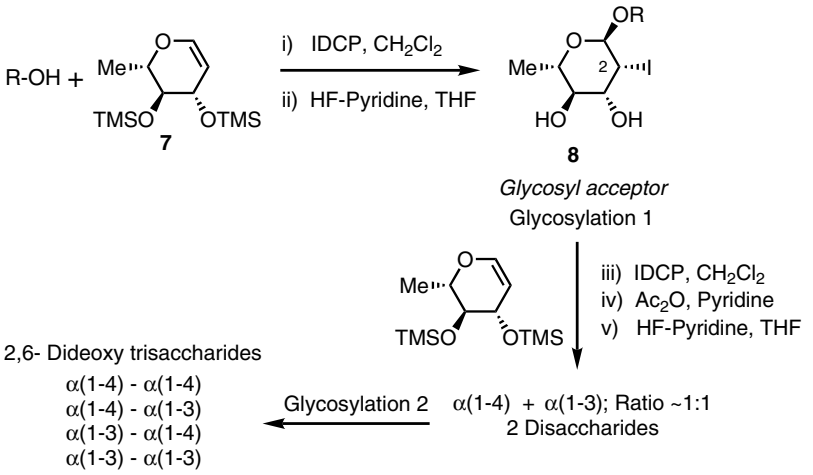


SCHEME 14.2 Boons's latent-active glycosylation.

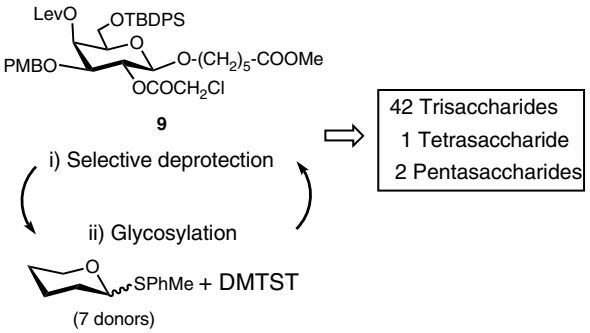
in the solution-phase synthesis of linear and branched trisaccharide libraries. The purification of saccharide mixtures was achieved by chromatography.

14.2.3 Ichikawa's Stereoselective (and Nonregioselective) Glycosylation

In a different approach, Ichikawa et al. [12] used a method that involves the "divergent" synthesis of an oligosaccharide library, employing stereoselective glycosylation combined with random glycosylation strategies. In their studies, the glycosyl acceptor had hydroxyl groups at C-3 and C-4 available for coupling reactions. In one example, the monosaccharide, 6-deoxy-3,4-di-*O*-trimethylsilyl-L-glucal **7** (Scheme 14.3) was converted into the corresponding iodo derivative **8** following desilylation of both hydroxyl groups. This glycosylation acceptor (with two hydroxyl groups) was reacted with glucal **7** as a donor. Surprisingly, the glycosylation reactions were stereoselective and α-(1→4)- and β-(1→3)-linked disaccharides were formed in near equal yields. The stereochemistry of the α-anomeric glycosidic linkage was controlled by a glycosylation reaction under iodonium ion-catalyzed conditions. By repeating the above steps, these two disaccharides were converted into the corresponding acceptors and then subjected to repeated glycosylation reactions. As before, a mixture of eight trisaccharides was formed in ca. 70% yield. All of them have an iodo group at the C-2 position that could be further reduced or subjected to substitution to enhance the library diversity.



SCHEME 14.3 Ichikawa's stereoselective (nonregioselective) glycosylation.



SCHEME 14.4 Wong's orthogonally protected building-block approach.

14.2.4 Orthogonal Protection in Library Synthesis

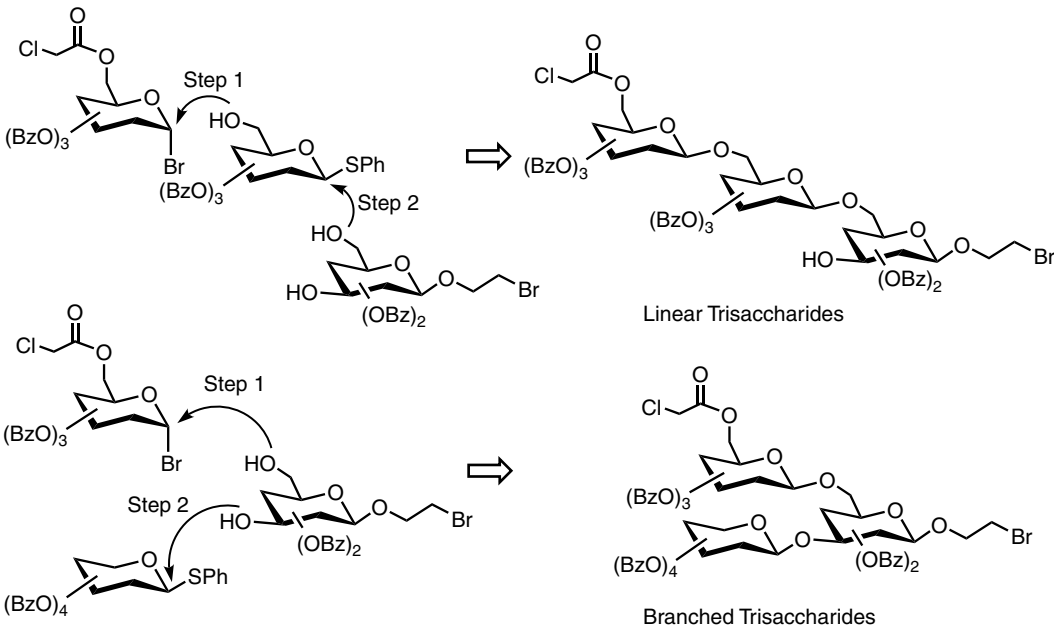
With the goals of developing methods that could provide stereochemically well-defined oligosaccharide derivatives, Wong et al. [13] relied upon orthogonal protecting groups on glycoside acceptors and thioglycosides as donors. In their approach, they utilized the monosaccharide building block **9** with four selectively removable hydroxyl protecting groups (Scheme 14.4). These groups were chloroacetyl (ClAc) at C-2, *p*-methoxybenzyl (PMB) at C-3, levulinoyl (Lev) at C-4 and *tert*-butyldiphenylsilyl (TBDPS) at C-6. The anomeric hydroxyl at C-1 was protected by the hydrocarbon chain with a terminal carboxymethylester group. At a given time, following the selective deprotection of the hydroxyl group, this was subjected to coupling with seven thioglycoside donors in the presence of dimethyl(methylthio)sulfonium triflate (DMTST). This allowed the solution synthesis of 45 derivatives in total. Out of these, 42 derivatives were found to be trisaccharides. Of the remainder, one was a tetrasaccharide and two were pentasaccharides.

Moreover, the researchers developed a computerized database, *OptiMer*, with the anomeric reactivity values for orthogonally protected thioglycosides [14]. This database turned out to be crucial in predicting the reactivity profiles of different thioglycosides and led to the development of one-pot glycosylation assembly strategies. The solution-phase chemoselective one-pot glycosylation approach may prove to be a valuable strategy for combinatorial solid-phase oligosaccharide library synthesis. The use of Wong's OptiMer database for selection of glycosyl donors and acceptors would certainly rival solid-phase approaches for the rapid synthesis of oligosaccharide libraries.

In a different approach to one-pot oligosaccharide synthesis, the reactivity difference between hydroxyls of the glycosyl acceptor was taken advantage of (Scheme 14.5) [15], in combination with the one-pot activation of thioglycosides and glycosyl bromides. Regioselective glycosylation of 3,6-diol acceptors, first with glycosyl bromides at O-6, then a second glycosylation of the resulting disaccharides with free O-3, afforded a 54 compound library of (1→3,6)-branched trisaccharides. Alternatively, sequential glycosylation of O-6 unprotected thioglycosides with glycosyl bromides, followed by one-pot activation of the thioglycoside, gave a library of 18 (1→6)-linked trisaccharides [15].

14.3 SOLID-PHASE LIBRARY SYNTHESIS OF CARBOHYDRATES

Solid-phase approaches may ease the product isolation and purification in the generation of larger oligosaccharide libraries. However, solid-support oligosaccharide synthesis requires thorough



SCHEME 14.5 Takahashi's one-pot glycosylation approach.

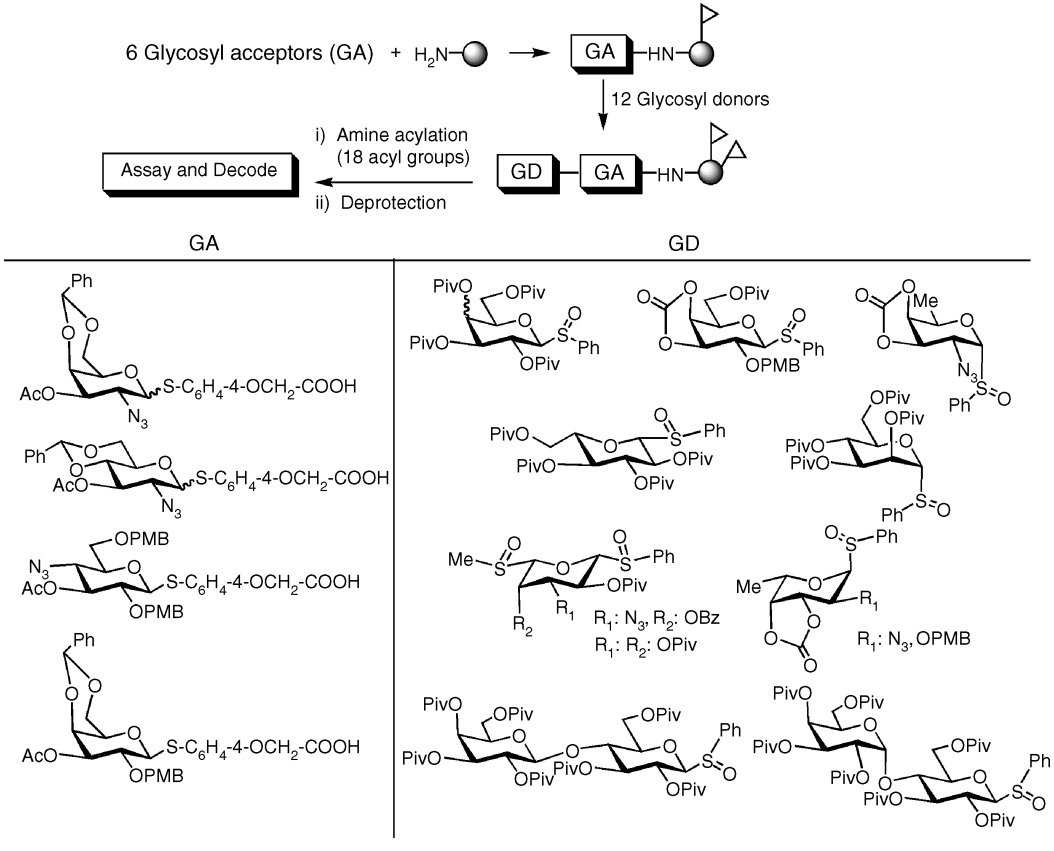
optimization of steps for adapting solution synthesis to the solid support. This can be attributed to the additional challenges of solid support-derived approaches together with the inherent nature of oligosaccharides. Because of these challenges, very few oligosaccharide libraries have been synthesized successfully on solid support.

14.3.1 Kahne's Split-Mix Approach to Glycosylation

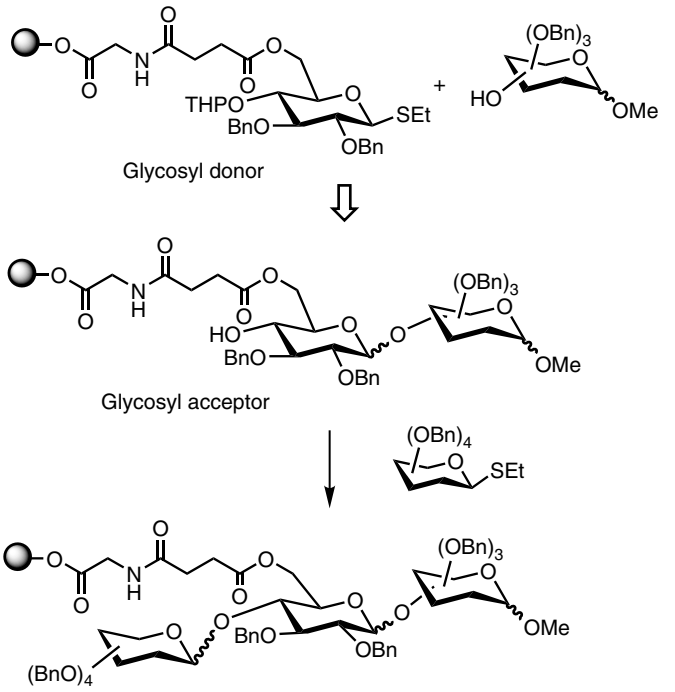
Unlike the solution-phase approaches discussed above, Kahne et al. [16] decided to explore solid-phase split-mix strategies for the synthesis of carbohydrate libraries. In order to obtain efficient glycoside coupling reactions that are stereoselective in nature, the researchers utilized the well-established sulfoxide derivatives as glycosyl donors. In the past, these types of glycosyl donors have been found to be readily activated at low temperatures. This appears to be independent of the nature of the protecting groups on the glycosyl donor. This method results in high yields of glycoside coupling reactions with a high degree of stereoselection. Scheme 14.6 shows this novel coupling method in combination with the split-mix approach and a library of approximately 1300 di- and tri-saccharides was synthesized in three steps. For the library synthesis by a split-mix strategy, the Still encoded method was utilized for deconvolution. The six glycosyl acceptors were anchored onto TentaGel amine resin and then coupled with 12 glycosyl donors by the sulfoxide method. After three steps, which included amine acylation and deprotection of the glycoside hydroxyl groups, a coded library anchored onto a solid support was obtained. The one bead–one compound library was tested to study the binding of glycoside derivatives to the lectin *Bahuinia purpurea* using a colorimetric assay. The anchoring of the ligand on the bead may mimic the presentation of the ligand on cell surfaces. Thus, this approach may prove to be valuable when searching for new cell surface antiadhesive agents.

14.3.2 Boons's Two-Directional Approach

Following solid-phase efforts from Kahne's group, Boons et al. [17] reported a small glycoside library that was synthesized using a two-directional split-mix method. As shown in Scheme 14.7,



SCHEME 14.6 Kahne's split-mix glycosylation.

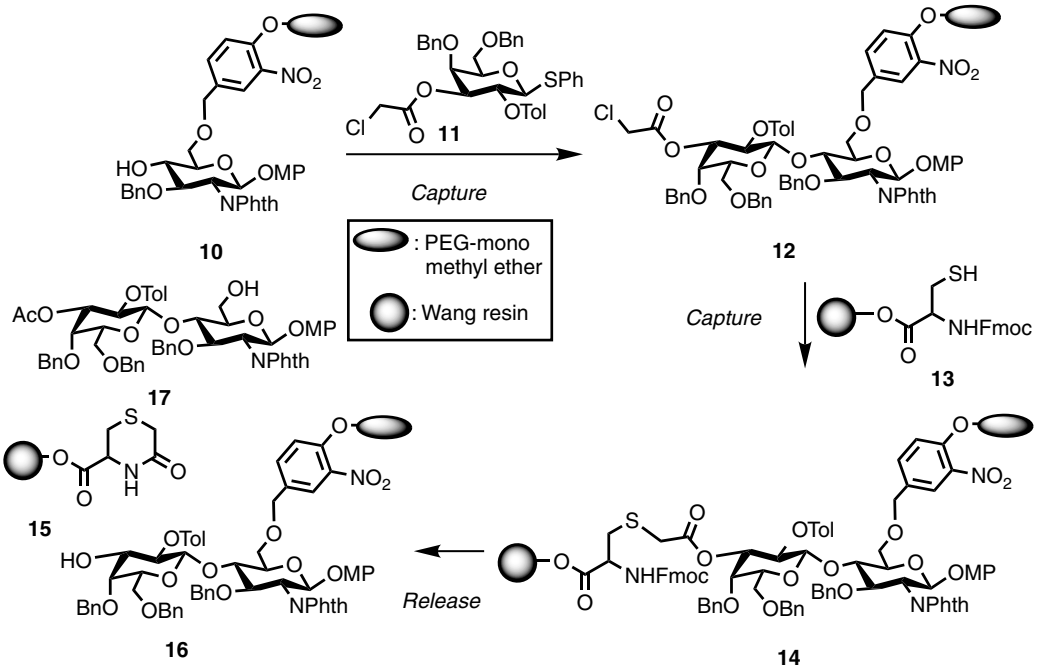


SCHEME 14.7 Boons's two-directional glycosylation.

the thioethylglycoside derivative, useful as both a donor and an acceptor, was immobilized onto TentaGel resin with glycine as the terminal amino group. The thioglycoside donor was anchored onto the resin via the succinamyl linker. In order to eliminate the formation of oligomeric side products during *N*-iodosuccinimide (NIS)/trimethylsilyl trifluoromethanesulfonate (TMSOTF)-based glycosylation reactions, the hydroxyl group at C-4 on the thioglycoside donor was protected as the tetrahydropyranyl (THP) ether. Following the coupling, the group was easily removed on solid phase. Subsequent glycosylations with additional donors were then carried out. The glycoside coupling reactions on solid phase were not stereoselective and resulted in mixtures. A small, 12-compound library was synthesized using this approach. The trisaccharides were purified by chromatography following cleavage from the support.

14.3.3 Ito's Capture and Release Strategy

The development of efficient and reliable glycosidic coupling reactions on solid phase is an ongoing challenge and results are still far from optimal. In most cases, it has been observed that these reactions on heterogeneous supports are sluggish and not very stereoselective. To overcome these hurdles, the use of soluble polyethylene glycol (PEG) polymers is becoming an attractive alternative [18]. The short-chain PEG polymers dissolve in certain organic solvents. This allows the study of glycosidic reactions in solutions. Following the coupling reaction, the product coupled with the soluble support could be separated by precipitation using an appropriate solvent. To explore the use of two types of supports, Ito et al. [19] recently developed a solid-phase capture and release strategy for the synthesis of oligosaccharides on a soluble polymer support. Scheme 14.8 shows the glycosyl acceptor **10**, bound to a low molecular weight PEG support that was glycosylated with thioglycoside donor **11** with a chloroacetyl group at C-3. Following the coupling reaction, the PEG-bound component was recovered by filtration through a pad of silica gel to remove excess donor **11** and the side products. The coupled product **12**, combined with the



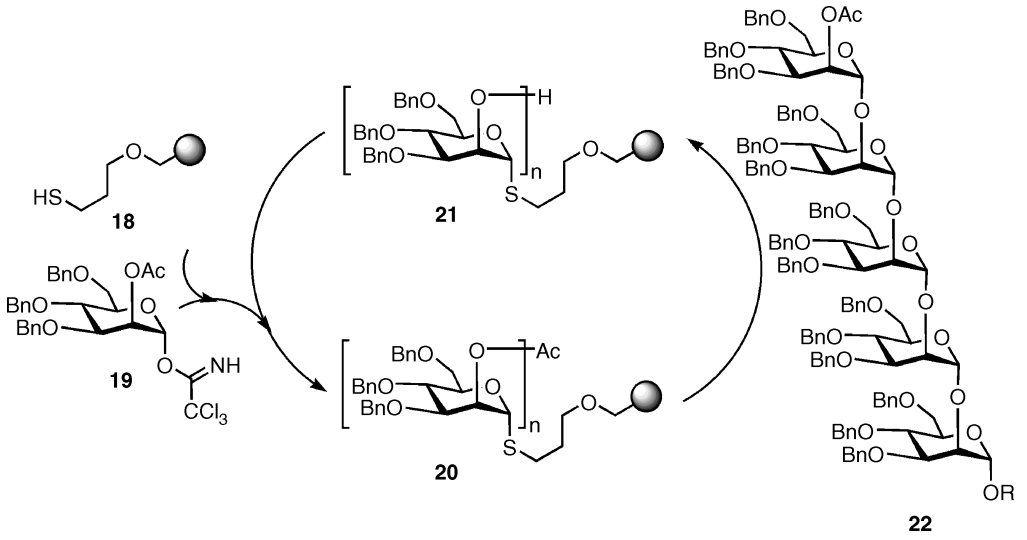
SCHEME 14.8 Ito's capture–release approach to oligosaccharides.

unreacted acceptor **10**, was subjected to a chemoselective reaction on a polystyrene-based solid support. Thus, the reaction of the chloroacetyl group from the PEG-based support with resin bound thiol **13** resulted in derivative **14** with mixed supports. After fluorenylmethyoxycarbonyl (Fmoc) removal, the free amine undergoes spontaneous intracyclization releasing the PEG-bound disaccharide **16** and resin-supported product, **15**. These two were easily separated and the released PEG-bound product **16** was further cleaved, giving the disaccharide derivative **17**. Disaccharide **16** was subjected to two additional cycles of the capture and release strategy for the synthesis of tetrasaccharides. By combining the use of two types of supports, the authors were able to develop an elegant approach to the synthesis of oligosaccharides.

Using the trichloroacetamidate glycosylation coupling and alkyl thiol-based resin, Schmidt et al. [20] developed a method that enables repetitive glycosylation and deprotection reactions in high yields. In this repetitive solid-phase glycosylation approach (Scheme 14.9), the fully protected 2-*O*-acetyl-D-mannosyl trichloroacetamidate donor **19** was coupled to the thiol polymer **18** using TMSOTf, giving the α-glycosylated product **20** exclusively. The *O*-2-acetyl group in mannose led to α-product formation owing to neighboring group participation. In addition, the *O*-acetyl group provides the required temporary protection for chain extension. Deacetylation of **20** was carried out under standard conditions giving **21**, which was then glycosylated with **19**. The repetitive glycosylation of **21** with **19** and deacetylation was continued for up to five cycles. Final cleavage from solid support using NBS provided methyl α-(1→2)-D-mannopentaoside **22** in high yields. The product was analyzed by MALDI-TOF.

14.3.4 Linkers in Solid-Phase Synthesis

Fraser–Reid et al. [21,22] reported the synthesis of polymer supported oligosaccharides using *n*-pentenyl glycosides, while Seeberger et al. [23] accomplished the synthesis of β-(1→4)- and β-(1→6)-linked oligosaccharides using glycosyl phosphates combined with an octenediol linker.

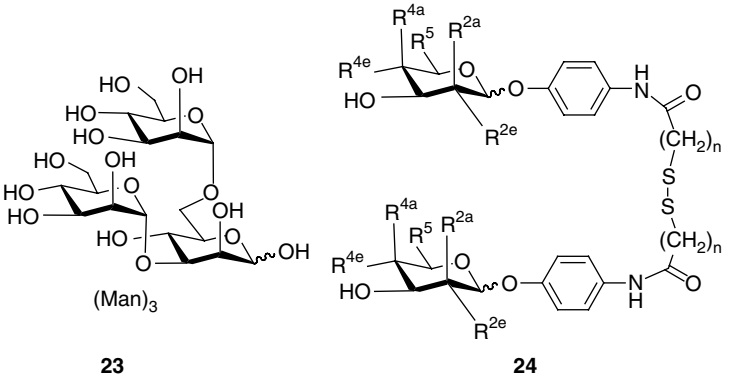


SCHEME 14.9 Schmidt's repetitive solid-phase glycosylation.

Hindsgaul et al. [24] achieved the synthesis of thio-oligosaccharides by nucleophilic substitution of triflate-activated glycosides from resin-bound glycoside-1-thiolates containing unprotected hydroxyl groups. Using the photo-cleavable aglycone linker, Nicolaou et al. [25] reported the synthesis of heptasaccharides and dodecasaccharides on solid phase.

14.4 DYNAMIC COMBINATORIAL CHEMISTRY

Lehn's [26] dynamic combinatorial approach toward carbohydrate library generation differs fundamentally from the solution and solid-phase library approaches discussed above. This dynamic combinatorial library (DCL) strategy involves the transient formation of compounds *in situ* using reversible reactions. These compounds are generated in the presence of a receptor (adaptive combinatorial library), which then selects for ligand(s) possessing the highest affinity. Alternately, the receptor can be added after equilibration is achieved (pre-equilibrated dynamic library). Lehn et al. developed a dynamic combinatorial library against the lectin concanavalin A (conA). The natural ligand for this lectin is the branched trisaccharide **23** (Scheme 14.10). To generate an



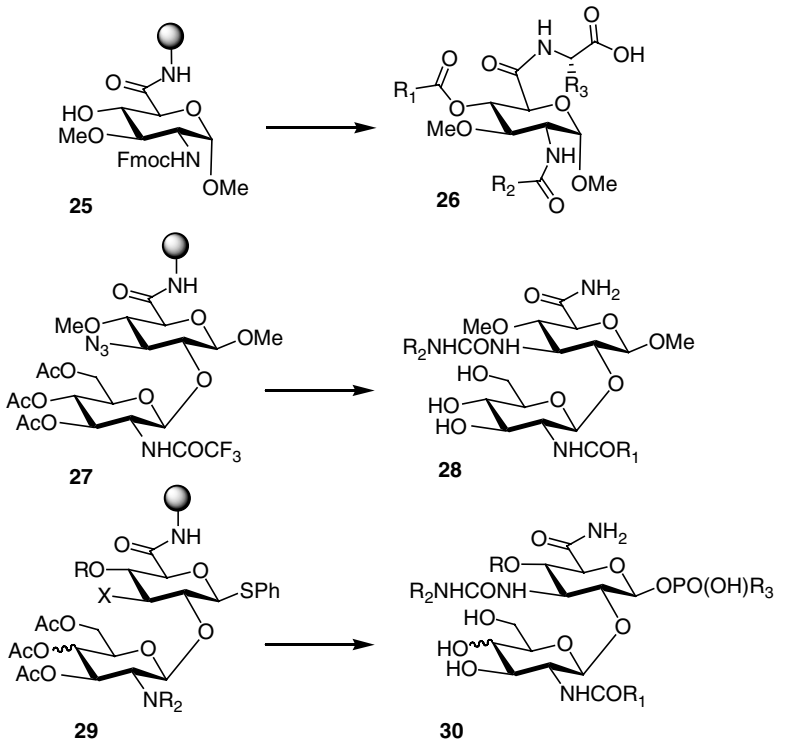
SCHEME 14.10 Lehn's dynamic combinatorial approach.

appropriate spacer between two monosaccharide units, a chemoselective thiol disulfide reaction was selected. The monosaccharides of D-mannose, D-galactose, L-arabinose, and D-xylose were derivatized with the phenylamido group and **24** was attached to two linkers differing only by a CH_2 group. Both homodimers (pH > 7) and heterodimers (in the presence of dithiothreitol) were generated. The libraries were produced either in the presence of conA, or conA was added after pre-equilibration where conA was immobilized onto sepharose beads for isolation of the bound compound. A *bis*-mannose derivative was preferentially selected by the receptor and demonstrated the potential for such simultaneous library generation and screening capabilities of this approach.

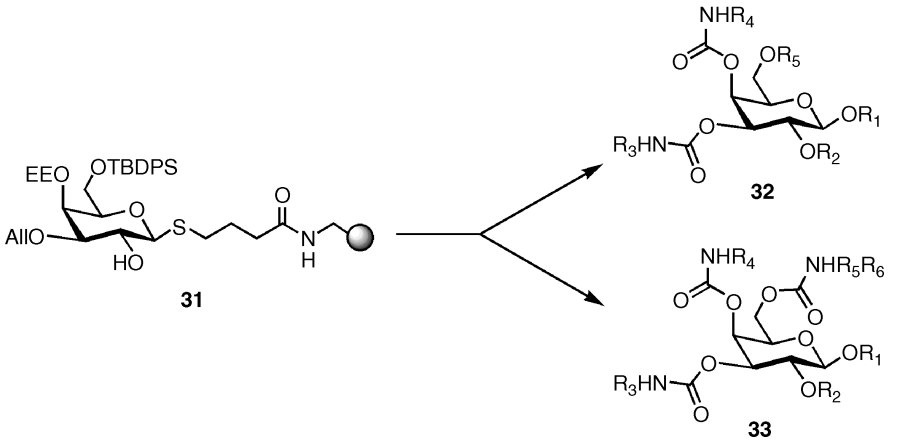
14.5 CARBOHYDRATE SCAFFOLDS IN COMBINATORIAL CHEMISTRY

The complex and polyfunctional nature of carbohydrates has made the development of automated methods for oligosaccharide libraries extremely slow. However, carbohydrates can serve as excellent library scaffolds [27]. Several chiral hydroxyl groups, upon appropriate modifications and utilization, could be utilized further in the display of chemical diversities. This approach could provide a novel entry to different classes of compounds to be explored for drug-like properties.

With these goals, the first solid-phase library synthesis that utilized carbohydrate scaffolds was reported by Sofia et al. [28] in 1998. An important feature of their approach was that the scaffold **25** (Scheme 14.11) had three distinct functionalities: (i) the carboxylic acid, (ii) the free hydroxyl group, and (iii) the protected amino group. This derivative was loaded onto amino acid functionalized trityl-based TentaGel resin. From **26**, several libraries were synthesized using the IRORI radiofrequency tagged split-mix method [29]. The library was generated from the sugar



SCHEME 14.11 Carbohydrate-based small molecule scaffolds.

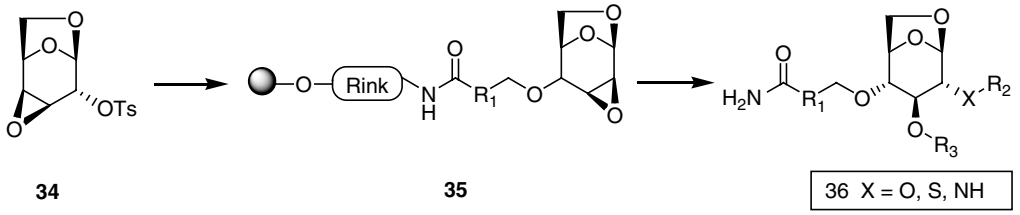


SCHEME 14.12 Kunz's orthogonally protected scaffold.

scaffold via modification of the above described functionalities as diversity points, including (i) functionalization of amino group at C-2, (ii) esterification of the hydroxyl group at C-4, and (iii) amino acid diversity from the −COOH group at C-6. The libraries were analyzed for purities by high pressure liquid chromatography-mass spectrometry (HPLC-MS). In another example, they achieved the solid-phase synthesis of a library of β-disaccharides on Rink amide resin using the disaccharide scaffold **27**. The key feature of this approach was the use of phenylsulfenyl 2-deoxy-2-trifluoroacetamido-glycopyranosides as glycosyl donors in the synthesis of β-linked disaccharides. Disaccharide **27** was derivatized using six isocyanates and eight carboxylic acids to generate a 48-membered library **28**. The glycoside donor discussed above and its galactosamine counterpart were used in the synthesis of meonomycin A disaccharide templated libraries **29**. A combinatorial library of 1300 disaccharides **30** was made by introducing diversities in **29** at C-1, C-3, and C-2′. The library was screened for inhibition of bacterial cell-wall biosynthesis and bacterial growth, and novel classes of potent inhibitors for both processes were identified.

Kunz et al. [30] used an orthogonally protected thioglycoside **31** (Scheme 14.12) as a scaffold. The protecting groups utilized were *tert*-butyloiphenyl silyl (TBDPS), 1-ethoxyethyl (EE) and allyl (All) groups. An important feature of the scaffold was the use of functionalized thioglycosides, which not only served as glycosyl donors but also as linkers for immobilization onto aminomethyl polystyrene resins. This combinatorial method was extended to the galactopyranose scaffold **31** containing five diversity sites. An array of structurally diverse compounds **32** and **33** were successfully synthesized using sequential deprotection and derivatization protocols.

The solid-phase synthesis of 1,6-anhydro sugar derivatives, starting with the 2-*O*-*p*-toluenesulfonyl-β-D-galactopyranoside derivative **34** (Scheme 14.13), was reported by a



SCHEME 14.13 Solid-phase synthesis of levoglucosan derivatives.

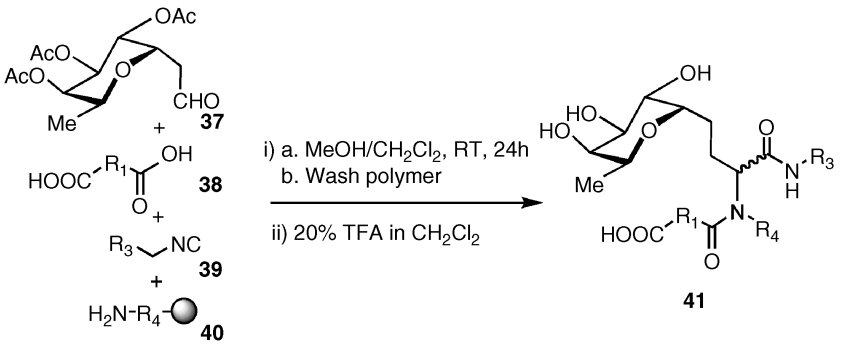
Novartis group [31]. The scaffold **35** was derivatized at the C-2, C-3, and C-4 positions to several diversity sites (e.g., ether, thioether, and amino groups). These groups were further derivatized in the library synthesis of **36**. An important feature was the utilization of stereo- and regioselective epoxide ring-opening reactions. In addition, no protection/deprotection reactions were required. The opening of epoxides by oxygen, sulfur and nitrogen nucleophiles was studied in this approach.

14.6 CARBOHYDRATE/GLYCOCONJUGATE-LIKE COMPOUNDS (GLYCOMIMETICS) BY COMBINATORIAL CHEMISTRY

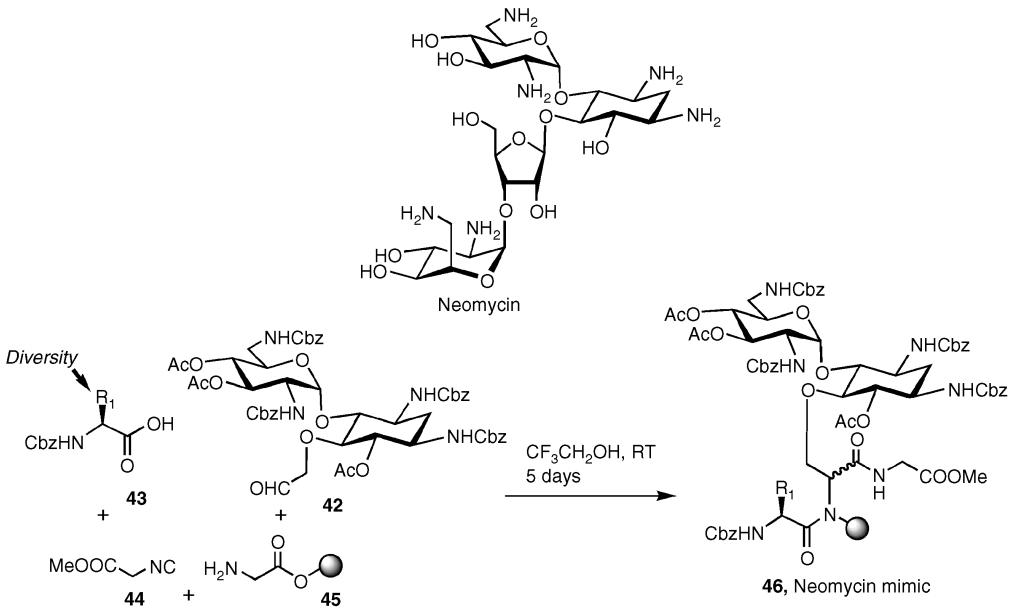
14.6.1 Multiple Component Condensations (MCC)

In combinatorial chemistry, the development of multicomponent reactions leading to product formation is an attractive strategy because relatively complex molecules can be assembled with fewer steps and in shorter periods. For example, the Ugi multicomponent reaction involving the combination of an isocyanide, an aldehyde, an amine, and a carboxylic acid results in the synthesis of α-acyl amino amide derivatives [32]. The scope of this reaction has been explored in solid-phase synthesis and it allows the generation of a large number of compounds with relative ease. This reaction has been employed in the synthesis of a library of *C*-glycoside conjugated amino amides [33]. Scheme 14.14 shows that, on reaction with carboxylic acids **38**, isocyanides **39**, and Rink amide resin derivatized with different amino acids **40**, the *C*-fucose aldehyde **37** results in the library synthesis of *C*-linked fucosyl amino acids **41** as potential mimics of sialyl Lewis^x.

A similar Ugi approach was utilized by Wong et al. [34] in the library synthesis of artificial glycopeptide-based small-molecule mimics of the aminoglycoside antibiotic neomycin. They utilized a soluble PEG polymer in the synthesis. According to the researchers, the use of a neamine moiety **42** (Scheme 14.15) that has the terminal saccharide unit is critical because it is the major glycoside component involved in the binding of human immunodeficiency virus (HIV)–ribonucleic acid (RNA) transactivator protein. Thus, in their plan, the crucial glycoside component was kept constant and the diversity was introduced as amino acid residues **43**. The four-component condensation reaction with **44** and **45** provided the neomycin mimic library **46**. The use of the PEG linker facilitated the Ugi reaction on the soluble support and the products could be easily separated by precipitation. One of the limiting factors in this Ugi approach is the lack of stereocontrol at the newly formed stereogenic center.



SCHEME 14.14 Synthesis of glycomimetics by Ugi four-component condensation.

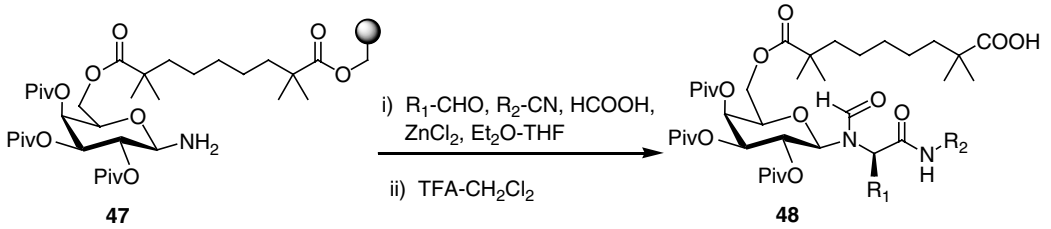


SCHEME 14.15 Wong's Ugi-multicomponent combinatorial synthesis of neomycin mimics.

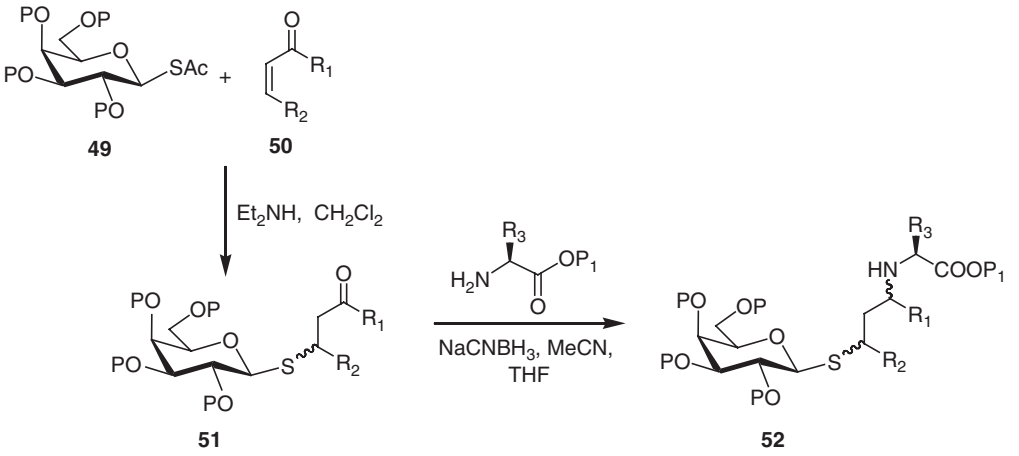
A recent report by Kunz et al. [35] describes the first stereoselective, combinatorial Ugi multicomponent synthesis on solid support. As shown in Scheme 14.16, *O*-pivaloylated-galactosamine **47**, linked to Wang resin via the α,α,α′,α′-tetramethyl azelaic acid spacer, was utilized in the library synthesis giving glycosylated amino acids **48** with a high degree of diastereoselection. The products were cleaved from the support upon acid treatment and were compared with the authentic samples prepared by solution synthesis.

14.6.2 Glycohybrids

To explore the concept of using terminal carbohydrate moieties as crucial elements, Hindsgaul et al. [36] developed a new class of compounds — glycohybrids. These compounds represent a unique combination of glycoside moieties combined with amino acids and do not occur in natural systems. Cognizant with the typical weak carbohydrate-protein bindings, the idea was to assist carbohydrate-based weak interactions by additional amino acids that may provide the extra contact points for specific bindings.



SCHEME 14.16 Kunz's stereoselective Ugi combinatorial synthesis on solid phase.



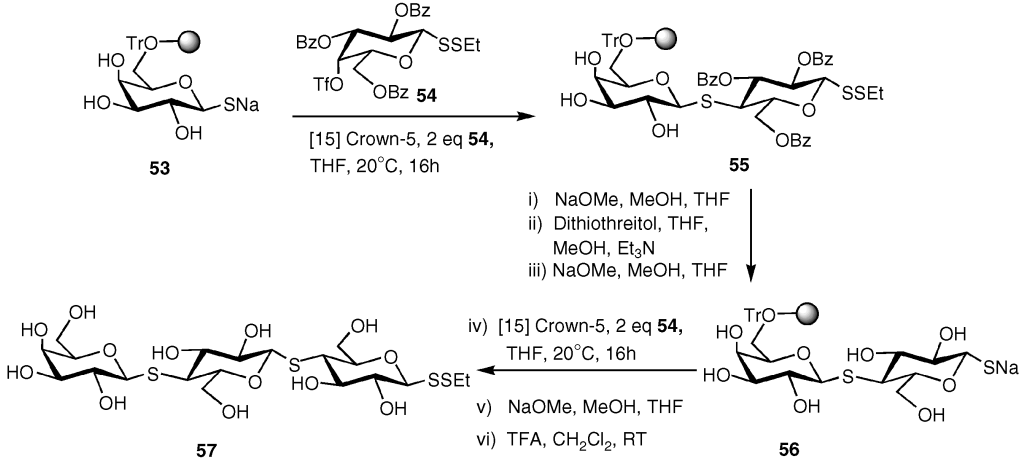
SCHEME 14.17 Hindsgaul's approach to glycohybrids.

A library of glycohybrids was synthesized by the reaction of 1-thio-β-D-galactopyranose derivatives with electron deficient olefins in a Michael-type manner. Following the addition, the plan was to use the carbonyl functionality in a reductive amination. This would allow the coupling of amino acids onto glycoside moieties. The researchers also used hydrophobic protecting groups on glycoside hydroxyls to facilitate reverse-phase purification after the library synthesis. In one example (Scheme 14.17), the 1-thiosugar with the hydroxyl groups protected as *O*-laurates, **49**, was reacted in a Michael fashion with various acceptors, **50**, giving the corresponding keto-derivatives, **51**, as diastereomeric mixtures in high yields. These were then reacted with several amino acid ester derivatives by reductive amination. Thus, a library of thirty compounds, **52**, in which each product contains a mixture of four diastereomers was produced. This library was screened for inhibitors of β-galactosidases.

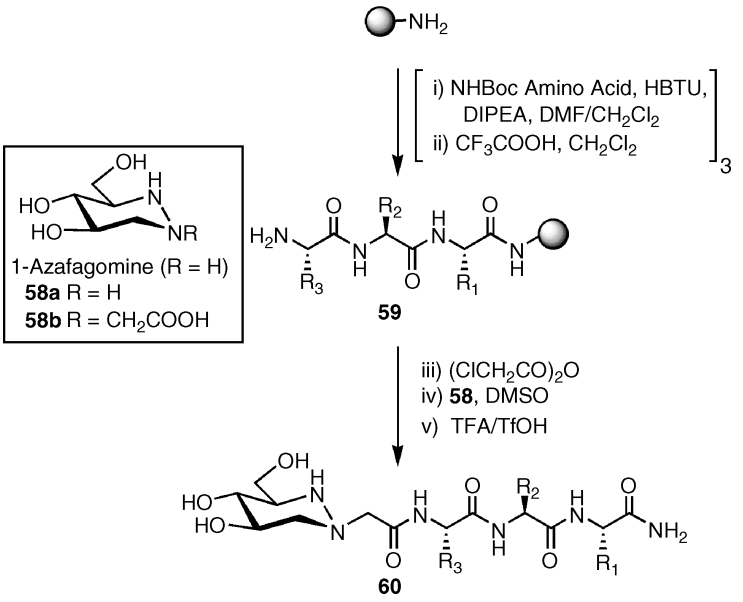
Thioglycosides were also utilized in the development of solid-phase syntheses of thio-oligosaccharides with free hydroxyl groups on glycosides. A key feature of this approach relied on the high reactivity of nucleophilic glycoside-1-thiolates that could preferentially react with triflate-activated glycosides with protected disulfides at C-1 in the presence of free hydroxyl groups. The required free thiolates were then generated from the protected unsymmetrical ethyl disulfide at C-1. As shown in Scheme 14.18, the immobilized thiolate **53**, via trityl-resin, was then glycosylated with triflate derived 1-dithioethylgalactoside **54** giving the immobilized disaccharide **55** [37]. Following debenzoylation (NaOMe in THF), the anomeric disulfide was then reduced with dithio-threitol (DTT) to obtain the fully deprotected disaccharide **56**, which was then subjected to glycosylation with the triflate derivative **54** in the presence of a crown ether. Thus compound **57** was obtained following the complete deprotection of hydroxyl groups as before and after cleavage from the resin.

14.7 GLYCOPEPTIDE-LIKE DERIVATIVES BY COMBINATORIAL CHEMISTRY

The endocyclic nitrogen-containing carbohydrate derivatives (also known as azasugars) are important because many of them are found to be selective inhibitors of carbohydrate processing enzymes, particularly glycosidases. With the goals of developing a combinatorial chemistry program that allows rapid access to azasugar based artificial glycopeptides, Bols et al. [38] synthesized a library of 125 compounds **60** containing a 1-azafagomine moiety **58** (Scheme 14.19). In their design, the tripeptide library **59** was coupled with the 1-azafagomine derivative possessing



SCHEME 14.18 Hindsgaul's solid-phase synthesis of thio-oligosaccharides.



SCHEME 14.19 Bols's approach to azasugar-based artificial glycopeptides.

a carboxyl group via an amide bond acetic acid linker. The tripeptide library was synthesized by a standard split/mix peptide library-synthesis approach prior to coupling with the azasugar moiety. Following cleavage from the support, the library was screened for the inhibition of β-glycosidase, α-glucosidase, and the glycogen phosphorylases. Several compounds were found to display β-glycosidase inhibition. Following the deconvolution of the library, it was found that several compounds caused inhibition, but the compound with three hydroxyproline residues as the peptide fragment showed the best activity.

14.7.1 Glycosylated Amino Acids as Building Blocks

The rapid assembly of glycopeptides by solid-phase synthesis has been achieved by use of many glycosylated Fmoc-protected amino acid pentafluorophenyl esters (OPfp) or free amino acids (Figure 14.1) [39]. A number of solid-support methods have been explored to generate parallel arrays of glycopeptides with native and isosteric substituted glycosidic linkages. In an elegant approach by St. Hilaire and colleagues [39], the synthesis of combinatorial glycopeptide libraries with unambiguous characterization of active compounds (Scheme 14.20) was achieved. The 30,000

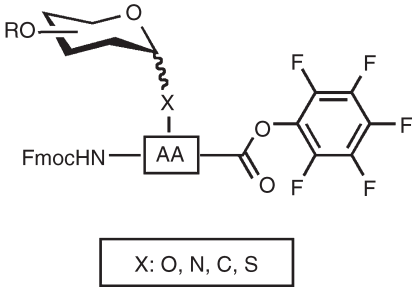
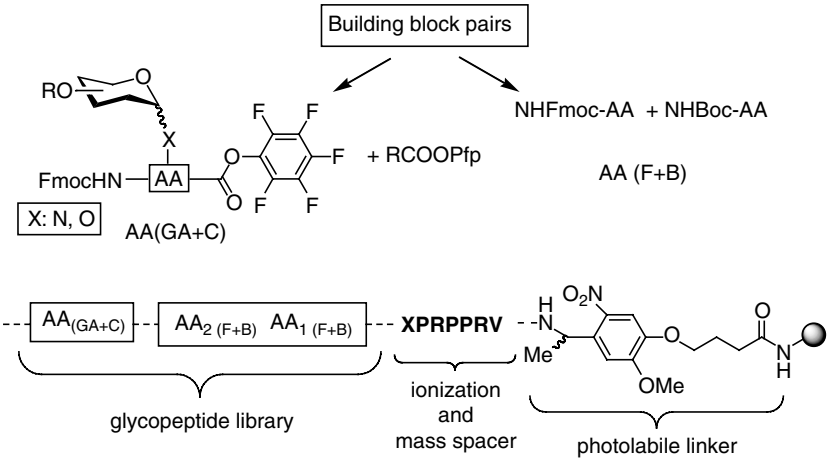


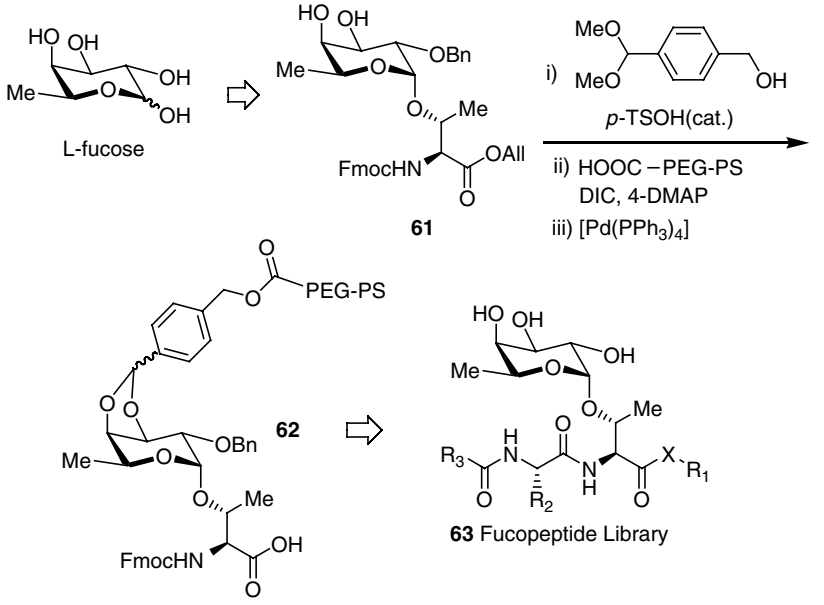
FIGURE 14.1 Glycosylated amino acids for glycopeptides.



SCHEME 14.20 Solid-phase synthesis of glycopeptide-based mimics.

membered heptaglycopeptide encoded library was synthesized by a split-mix approach on PEGA resin containing a photolabile linker. The glycopeptide-based resins were then screened against a fluorescent-labelled lectin from *Lathyrus odoratus*. The active compounds, on fluorescent beads, were identified by irradiation using a MALDI/TOF-MS laser with concurrent analysis of the ladder of terminal fragments, and directly gave the sequence as well as providing the structure of the glycopeptides. Significantly, all active glycopeptides contained the terminal mannose moiety, an important feature for the lectin recognition.

Following the building-block approach, artificial glycopeptides were synthesized by Wong et al. [40], as small-molecule mimics of sialyl Lewis^x. They utilized the fucosylated amino acid *O*-allyl carboxylic ester building block (Scheme 14.21). The properly protected, threonine



SCHEME 14.21 Wong's fucopeptide-based sialyl Lewis^x mimics.

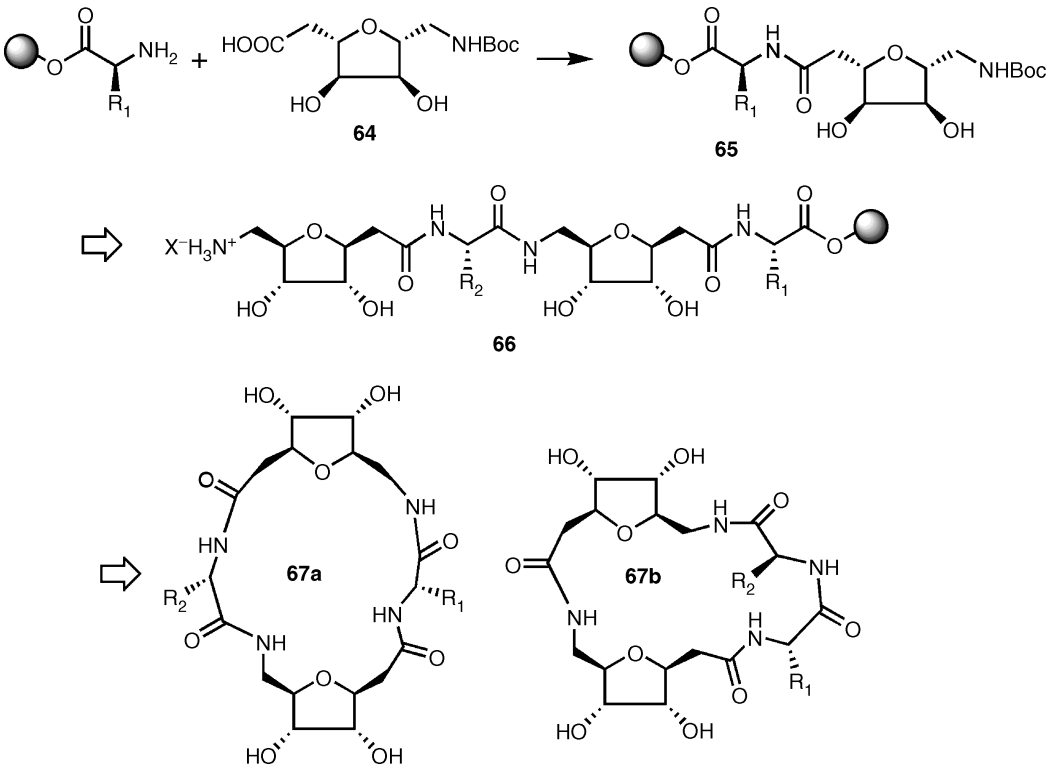
glycosylated fucose derivative **61** was immobilized onto the carboxy functionalized PEG-PS resin via an acid sensitive *p*-(acyloxymethyl)benzylidene acetal (*p*-AMBA). After removal of the allyl protecting group, the fucopeptide library **63** was obtained from the building block **62**. The library synthesis by parallel approach had the diversity at the amino acid side chain and both at the *N*-and *C*-termini of the polypeptide backbone. In this library, the fucose moiety was kept constant because it is the crucial glycoside section required for the recognition of sialyl Lewis^x by E-selectin. Following cleavage and purification, the derivatives showed moderate binding affinities with E- and P-selectins.

14.7.2 Cyclic Artificial Glycopeptides

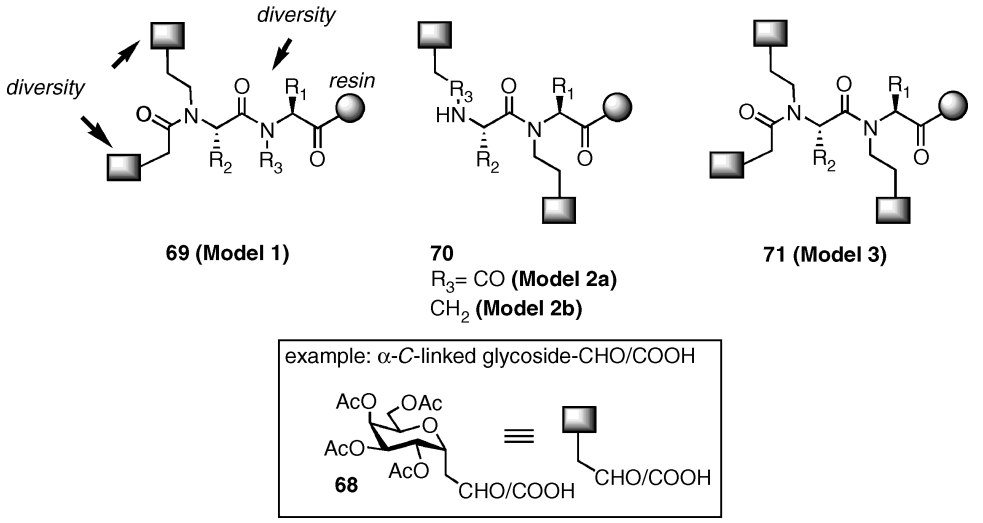
Parallel robot-assisted solid-phase syntheses of cyclic sugar, amino acid-based, artificial glycopeptides were accomplished by van Boom et al. [41]. These cyclic artificial glycopeptides resemble cyclodextrins in their structures. Scheme 14.22 shows that the linear derivative **66** was first synthesized using building block **64** on solid phase. The linear derivative **66** was then subjected to acid-catalyzed cyclization, giving the cyclic artificial glycopeptides **67a** and **67b**. Following purification, the products were characterized by NMR.

14.7.3 Automated Synthesis of Artificial Glycopeptides

With the goal of developing automated methods to the synthesis of artificial glycopeptides, Arya et al. [42] were interested in stable, artificial glycopeptides that possessed pertinent features of



SCHEME 14.22 Sugar amino acid-derived cyclic derivatives.

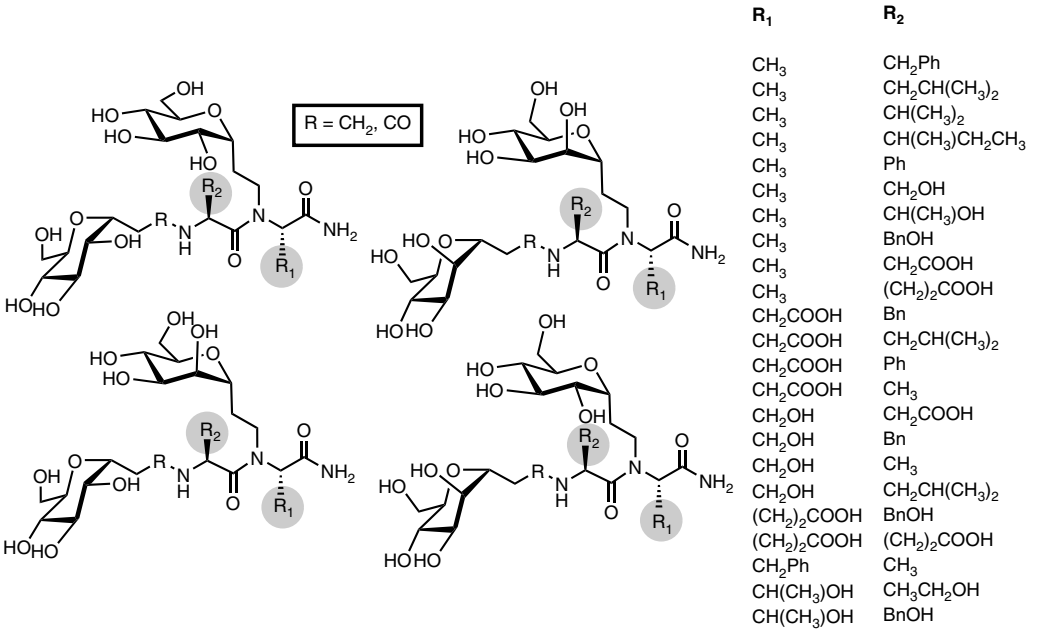


SCHEME 14.23 Arya's automated approach to artificial glycopeptides.

the underlying protein as well as the carbohydrate moiety or glycoform of the glycoconjugates. This idea led to the development of carbohydrate diversity on a dipeptide/pseudopeptide scaffold, which demonstrates the flexibility of this approach (see Model 1, Model 2 and Model 3, **69** through **71**, Scheme 14.23). The carbohydrates were incorporated as α- and β-linked *C*-glycosides, which are stable isosteres of native terminal sugars. The versatility of this approach is reflected by the fact that a variety of sugars can be independently incorporated as peracetylated ethanal- or ethanoic acid derivatives **68**. The building blocks (as carbon-linked sugar aldehydes and carbon-linked sugar acids) can be incorporated either at the *N* terminal moiety or at the internal amide nitrogen of a short peptide/pseudopeptide scaffold. This can be done in a highly flexible and controlled manner. Using this approach, libraries of artificial glycopeptides could be easily synthesized for probing carbohydrate–protein interactions. The libraries display two (i.e., homogeneous or heterogeneous) copies of carbohydrates, whereas the dipeptide scaffold may contribute to secondary interactions with the biological target.

Using TentaGel S RAM resin as solid support, the artificial glycopeptide libraries, based upon Model 2a and Model 2b, were successfully synthesized on a multiple organic synthesizer (MOS). The success of the methodology was dependent on the optimization of the reductive amination reaction of the acetylated *C*-glycoside ethanol derivatives with the amino group of the anchored amino acid. After several attempts at the synthesis of glycosyl amino derivative on solid phase, the reductive amination product could be obtained in high yields (70 to 95%) by using a relatively low excess of the glycoside derivatives. The next task — the coupling of the resulting secondary amine to the next amino acid — was accomplished after a series of optimization with HATU. Deacetylation of the sugar derivatives was achieved with hydrazine hydrate/*N,N*-dimethylformamide mixture at *p*H 9–10 for 4.5 h.

Using the method discussed above, four 96-compound artificial glycopeptide libraries were synthesized in a fully automated manner. The artificial glycopeptide libraries show a combination of two glycosides: (i) α-galactoside and β-galactoside, and (ii) α-glucoside and α-mannoside (Figure 14.2). The compounds in the artificial glycopeptide libraries were used to test the ability of these derivatives to inhibit the reglucosylation of *N*-linked glycoproteins by a glucose processing enzyme. Reglucosylation of *N*-linked glycoproteins appears to be a critical step in *N*-glycoprotein biosynthesis, protein folding and trafficking pathways [43]. In a different study, the artificial glycopeptide libraries were tested in enzyme systems that convert a glucose



R_1	R_2
CH_3	CH_2Ph
CH_3	$CH_2CH(CH_3)_2$
CH_3	$CH(CH_3)_2$
CH_3	$CH(CH_3)CH_2CH_3$
CH_3	Ph
CH_3	CH_2OH
CH_3	$CH(CH_3)OH$
CH_3	BnOH
CH_3	CH_2COOH
CH_3	$(CH_2)_2COOH$
CH_2COOH	Bn
CH_2COOH	$CH_2CH(CH_3)_2$
CH_2COOH	Ph
CH_2COOH	CH_3
CH_2OH	CH_2COOH
CH_2OH	Bn
CH_2OH	CH_3
CH_2OH	$CH_2CH(CH_3)_2$
$(CH_2)_2COOH$	BnOH
$(CH_2)_2COOH$	$(CH_2)_2COOH$
CH_2Ph	CH_3
$CH(CH_3)OH$	CH_3CH_2OH
$CH(CH_3)OH$	BnOH

FIGURE 14.2 Examples of artificial glycopeptide libraries prepared by automation.

moiety into rhamnose prior to incorporation of the rhamnose unit during the biosynthesis of the mycobacterium cell wall. The inhibition of this step may play an important role in the development of novel carbohydrate-derived therapies to combat *Mycobacterium tuberculosis* cell-wall biosynthesis [44]. Further, inhibition of this biosynthetic pathway may lead to the development of compounds with a specific action, because this particular biotransformation does not occur in mammalian systems. To date, few artificial glycopeptide derivatives as potential glycoside-based inhibitors containing at least one negatively charged, amino acid residue have been identified [45].

14.8 SUMMARY AND OUTLOOK

Our ability to rapidly access pure peptide and nucleic acid derivatives has played an important role in understanding the biological functions of these two classes of natural biopolymers. The field of glycobiology has not advanced to that level, mainly because of the difficulty of accessing carbohydrate-based derivatives. Over the years, significant progress has been made in carbohydrate-oriented solution and solid-phase synthesis, including combinatorial chemistry. Several combinatorial approaches covered in this chapter have the potential to make carbohydrate-based compounds accessible. However, some methods may require further improvement before they are accepted by the community. In the future, automated synthetic methods will probably grow and greatly impact the field of chemical glycobiology by adding to our understanding of the biological functions of carbohydrates and the use of carbohydrate-based chemical probes.

ACKNOWLEDGMENTS

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