Combinatorial Chemistry toward Understanding the Function(s) of Carbohydrates and Carbohydrate Conjugates

Angela Barkley and Prabhat Arya*[a]

Abstract: Combinatorial chemistry has contributed significantly to understanding the structure–function relationships of biologically important molecules such as proteins and nucleic acids. However, carbohydrates and carbohydrate conjugates, which have been identified as key modulators of several biological functions have not enjoyed the same measure of success. The complexity and synthetic challenges of carbohydrate conjugates have resulted in a number of conceptual approaches to rapidly access sufficient quantities of these biomolecules. This article summarizes these combinatorial approaches and also highlights fully automated library synthesis of artificial glycopeptides with the goals of understanding their biological roles.

Keywords: artificial glycopeptides · carbohydrates · combinatorial chemistry · glycoconjugates · glycomimetics

Introduction

Glycoconjugates play pivotal roles in biological processes. These processes range from cell growth and differentiation, cell–cell communication, modulation of protein function to pathological processes namely cancer metastasis, lysosomal storage diseases, chronic inflammation, and microbial infections.[1] Such diversity in biological activity has been attributed primarily to the oligosaccharide moiety of these glycoproteins and glycolipids. Moreover, it has been demonstrated that the same oligosaccharides can mediate a variety of functions. Therefore, understanding the structure–function relationships of these molecules at a molecular level is a non-trivial undertaking. This is further complicated by the chemical diversity of oligosaccharides. Securing sufficient quantities of these complex glycans for probing biological functions have been a major challenge at the glycochemistry/glycobiology interface. Nonetheless, the technological advances of the past century have laid the foundation for exploiting the diagnostic and therapeutic potential of this class of biomolecules.

Advances in analytical techniques, NMR spectroscopy, and mass spectrometry expedited the isolation and structural determination of oligosaccharides.[1, 2] While this made the chemical diversity of oligosaccharides rapidly accessible, it further highlighted the urgent need for access to sufficient quantities of these molecules to understand the mechanism of action at a molecular level. However, the chemical complexity of these biomolecules makes them very challenging synthetic targets. These polyhydroxy compounds contain an array of monosaccharide units and have a variety of glycosidic linkages between them. Each glycosidic linkage can exist in the α- or β-anomeric configuration. Therefore, carbohydrate synthesis requires many orthogonal protection and deprotection schemes and involves difficult glycosyl coupling reactions.[3] Nonetheless, many groups have risen to this chemical challenge and several synthetic approaches leading to oligosaccharides and glycoconjugates have been reported.[4] Despite these advances, synthesis of these biomolecules remains time consuming and expensive. Furthermore, the natural oligosaccharides may lack the chemical stability and bioavailability for detailed biological studies. This has fuelled parallel developments in synthetic glycoconjugate mimics and inhibitors of oligosaccharide functions.[5] It is well established that despite the complexity of the oligosaccharide moieties of glycoconjugates, the terminal sugars (two to four residues) and their conformation are critical for biological activities. This not only reduces the chemical complexity of the synthetic target(s), but also makes possible the use of revolutionary synthetic strategies such as combinatorial chemistry, for rapid access to potential carbohydrate mimics.

Combinatorial chemistry, a multi-dimensional strategy, has evolved to meet the growing demand for economical synthesis of large numbers of diverse chemical compounds in a relatively short time. In this approach, a large array of building blocks is chemically assembled to give all possible combinations, either in solution or more commonly, on a solid support. The collection of compounds can be generated using

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a "split-pool" or "parallel" synthetic strategy. This diverse collection of compounds, a chemical library, is then screened for biological activity. Combinatorial libraries have now been added to the repertoire of strategies used in the pharmaceutical sector for lead discovery and lead optimization, as many aspects of this evolving technology have been well reviewed in the literature.[6]

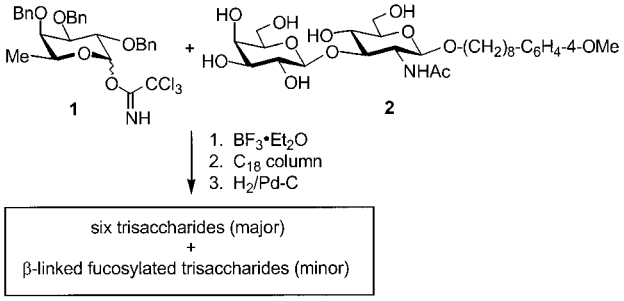
Merrifield's conceptual solid-phase approach to peptide synthesis in 1963 laid the foundation for the first set of combinatorial libraries, peptide libraries.[7] Solid-phase synthesis has been refined over a thirty-year period and has been successfully extended to the synthesis of small organic molecules. Today combinatorial libraries are commonly used for the elucidation of structure–function relationships.[8]

While combinatorial peptide and oligonucleotide libraries have been invaluable in the generation of bioactive peptides such as opioid peptides, antimicrobials, monoclonal antibodies, and oligonucleotide primers, mutagenic agents, and potential therapeutic agents, combinatorial carbohydrate libraries have yet to attain such prominence.[9] Of course, peptides/proteins and oligonucleotides are linear polymeric derivatives. They have a diversity of building blocks, amino acids or nucleotides, connected to each other by a common linkage, amide bond (proteins) or 3′–5′ phosphodiester bond (oligonucleotide). This made automated solid-phase synthesis and subsequently combinatorial library synthesis a very facile process. The chemical nature of carbohydrates has precluded the rapid and efficient generation of oligosaccharide libraries either in solution or on solid support. In this concept article, several novel strategies adopted in the generation of combinatorial libraries of oligosaccharides and glycomimetics are highlighted.

Combinatorial Oligosaccharide Libraries

The polyvalent nature of carbohydrates and the lack of a general method to form glycosidic linkages have resulted in unique approaches for the generation of oligosaccharide libraries.[10] The challenge to gain access to monosaccharide building blocks continues, which can be readily synthesized and assembled in a controlled combinatorial fashion. Having generated the library, purification and analysis are equally important. Both solution- and solid-phase strategies have been developed in the search for libraries of oligosaccharides for biological investigation.

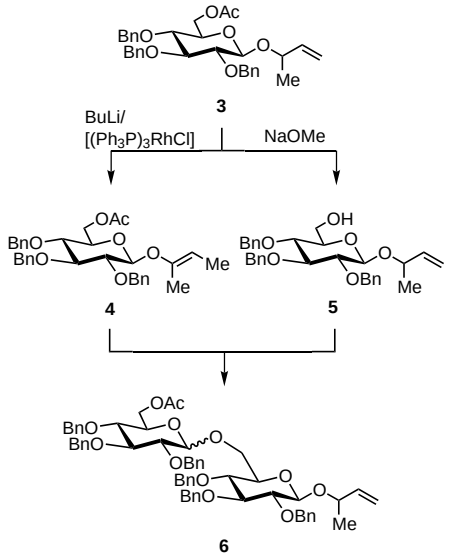
Random glycosylation: The first oligosaccharide library synthesized consisted of di- and tri-saccharides produced by a random glycosylation strategy (Scheme 1) in solution.[11] As reported by Hindsgaul et al., this approach circumvented the need for numerous orthogonally protected monosaccharide building blocks. A fully benzylated glycosyl donor **1** activated with the trichloroacetimidate group and disaccharide acceptor **2**, as a *p*-methoxyphenoxyoctyl glycoside with six unprotected hydroxyl groups, were coupled for three hours at room temperature to give a mixture of all six possible trisaccharides in a single step. In this reaction only about 30% of the disaccharide acceptor was fucosylated and interestingly, all



Scheme 1. Hindsgaul's random glycosylation.

the OH groups showed similar reactivity. Chromatographic separation by HPLC and NMR confirmed the presence of the trisaccharides. Using this strategy, Hindsgaul's group further investigated a fucosyl-transferase enzyme present in human milk using a disaccharide mixture in which active compounds were present in less than 5%.[12] However, the uncontrolled glycosylation reaction, low yields and the need for extensive purification limits the widespread applicability of this methodology.

Latent active glycosylation strategy: An alternative solution-phase approach was developed by Boons et al.[13] In this novel latent-active glycosylation approach (Scheme 2), one



Scheme 2. Boons's latent-active glycosylation.

major building block, 3-buten-2-yl glycoside **3** which can be converted into a glycosyl donor and acceptor was used.[14] Isomerization of compound **3** with BuLi/$[(Ph_3P)_3RhCl]$ gives the glycosyl donor **4** whereas deprotection of the acetate group of **3** gives the glycosyl acceptor **5**. Coupling of compounds **4** and **5** gives the disaccharide **6** in 89% yield as an anomeric mixture. Using this methodology building blocks containing other selectively removable groups such as *p*-methoxybenzyl ether were prepared and used for the solution-phase synthesis of mixtures of linear or branched trisaccharide libraries.[15] The libraries were readily purified by gel-filtration chromatography and contained over 80% of the expected products.

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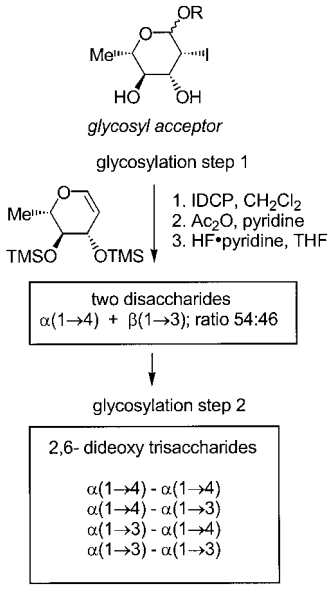
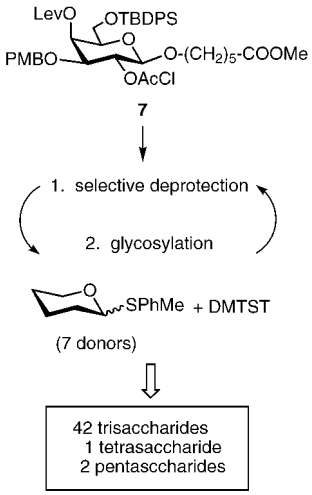
Stereoselective, non-regioselective glycosylation: Ichikawa's group has developed a "stereoselective, yet non-regioselective" glycosylation approach toward solution-phase combinatorial oligosaccharide synthesis.[16] Only one monosaccharide building block, 6-deoxy-3,4-di-*O*-trimethylsilyl-L-glucal was utilized in the synthesis of a small library of 2,6-dideoxy trisaccharides in the search for antitumor agents (Scheme 3). The stereoselectivity of the glycosidic linkage (α-anomer) was controlled by performing the glycosylation



Scheme 3. Ichikawa's 2,6-deoxy-based trisaccharide library synthesis.

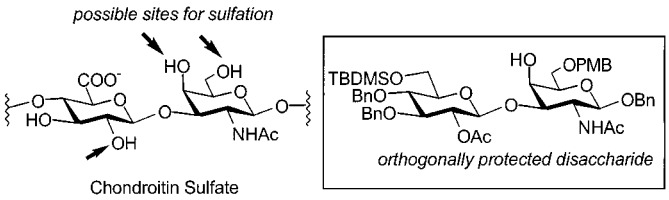
reaction under iodinium ion-catalyzed conditions. The glucal was first coupled to 6-trifluoroacetamidohexanol in the presence of iodinium di(*sym*-collidine)perchlorate (IDCP) which generated the α-glycoside and an iodo-group at the 2-position. Subsequently, the glycosyl acceptor having two free hydroxyl groups was obtained by removal of the silyl groups. After two cycles of glycosylation (Scheme 3) under IDCP catalysis, regioisomeric linear trisaccharides were obtained in 73% yields. Since each glycosylation reaction generated an iodo-group at the 2-position, the mixture can undergo further modification.

Orthogonally protected carbohydrates: Wong et al. have utilized a versatile central monosaccharide building block with four selectively removable protecting groups to generate an oligosaccharide library with a high degree of regio- as well as stereoselectivity (Scheme 4).[17] The key compound is a monosaccharide glycosyl acceptor **7** with a chloroacetyl (ClAc), *p*-methoxybenzyl (PMB), levulinoyl (Lev), and *tert*-butyldiphenylsilyl (TBDPS) group, in which every protecting group can be removed selectively in high yields. In this synthesis, seven thioglycoside donors were coupled in the presence of (dimethylthio)methylsulfonium triflate (DMTST) with the selectively deblocked glycosyl acceptor. They demonstrated efficient orthogonal protection–deprotection schemes in the parallel solution synthesis of a library of 45 oligosaccharides.



Scheme 4. Wong's orthogonally protected building block approach.

Lubineau and Bonaffé[18] have developed a split-pool library approach for the synthesis of all sulfoforms of chondroitin sulfate (CS) disaccharide. An orthogonally protected disaccharide was central to the success of the synthesis (Scheme 5). Since natural chondroitin sulfates of glycosaminoglycans are chemically modified upon enzymatic or chemical degradation, the synthesis of sulfated CS will undoubtedly



Scheme 5. Bonnaffe's combinatorial approach to chondroitin sulfate disaccharides.

contribute to exploring the biological functions of these glycoconjugates. In the synthesis of eight sulfated disaccharides, the authors demonstrated that sulfate esters are effective protecting groups in the crucial C-6 oxidation of a glucosyl to a glucuronyl. The mixture of disaccharides were readily purified and analyzed.

One-pot glycosylation: The reactivity of the *p*-methylphenyl thioglycoside of different monosaccharides with different protecting groups (e.g. electron-donating or electron-withdrawing leaving groups) has been quantitatively evaluated by Wong's group in the search for a facile strategy for oligosaccharide synthesis.[19] This has led to the development of a computerized database of anomeric reactivity values for orthogonally protected thioglycosides.[20] This database was then used for the selection of glycosyl donors and acceptors for the one-pot, parallel solution synthesis of a library of oligosaccharides.

Takahashi's group has also synthesized a library of 72-trisaccharides by solution-sphase one-pot glycosylation.[21] In this approach a combination of bromo glycosides, phenylthio glycosides and 2-bromoethyl glycosides of glucose, galactose, and mannose in the presence of selective activating agents

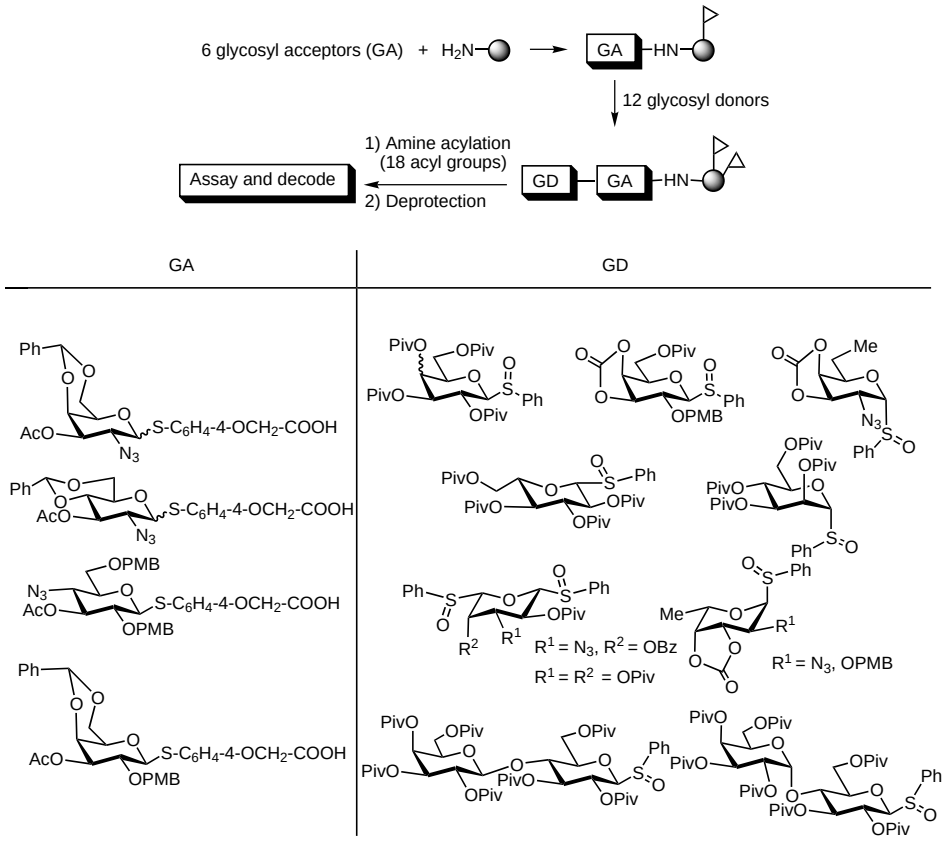
were rapidly assembled on a QUEST 210 manual synthesizer in good yields (64% to 99%).

The above chemoselective one-pot glycosylation approach may prove to be a very powerful strategy in the future generation of combinatorial oligosaccharide libraries. Furthermore, the use of Wong's Optimer database[20] for selection of glycosyl donor and acceptors, and Takahashi's manual synthesizer approach would certainly rival solid-phase approaches for the rapid synthesis of oligosaccharide libraries. Standard work-up and purification for larger libraries may be more challenging for routine library synthesis. In this respect, solid-phase approaches may simplify product isolation and purification in the generation of larger oligosaccharide libraries. However, solid support oligosaccharide synthesis requires an initial investment in optimization steps for adapting solution synthesis to a solid support. This approach also embodies additional challenges.[22] The resin, linker, and the screening techniques to be used must be considered in planning the library. It is therefore not surprising that very few oligosaccharide libraries have been successfully synthesized on the solid support so far.

Anomeric sulfoxides: For a successful solid-phase synthesis, glycosylation reactions must be stereospecific and high yielding. To achieve this, Kahne's group used anomeric sulfoxides as glycosyl donors.[23] Previous studies had demonstrated that these sulfoxides were readily activated at low temperatures regardless of the protecting groups on the glycosyl donor and acceptor pairs. Moreover, nearly quantitative yields (~90%) of the glycosylated products were obtained on solid phase. This novel coupling procedure was used to produce a library of 1300 di- and trisaccharides (Scheme 6) in only three steps. The monomers used were appropriately protected to ensure diversity in glycosidic linkages. An encoded split-mix library approach on TentaGel resin was used. Six glycosyl acceptors were attached separately to the resin. This was pooled and divided into twelve parts, each of which was coupled separately with one of twelve glycosyl donors. Again, the beads were pooled, the azido group was reduced to amine and the beads were divided into eighteen parts. Each set of beads were N-acylated with different reagents. All the beads were combined again and fully deprotected. This on-bead library (10 mg) was then screened against *Bauhinia purpurea* lectin using a colorimetric assay. Only 25 beads stained purple, a positive interaction with the lectin. Of these, 13 contained the same core disaccharides and beads containing the natural ligand, included in the library, did not stain whereas in solution they were all inhibitors. Thus, on-bead assay presented the carbohydrate ligands in unique orientations. This further highlighted the importance of not only the specificity of the sugar but also its presentation for carbohydrate – protein interactions.

Two-directional solid-phase approach: Zhu and Boons synthesized the second solid-phase library, a small trisaccharide library of 12 compounds (Scheme 7).[24] In this synthesis a thioethyl glycoside that can act as a donor or acceptor was immobilized on glycine-derivatized TentaGel resin thorugh a succinimidyl linker. The key to this approach was the use of the tetrahydropyranyl group (THP) on the immobilized thioglycoside, which eliminated the formation of oligomeric side products during *N*-iodosuccinimide/trimethylsilyl trifluoromethanesulfonate glycosylation. The immobilized thioglycoside was glycosylated separately with three different glycosyl acceptors, the resin was pooled, and the THP group removed. The anomeric mixture of disaccharide acceptors was coupled with a perbenzylated thioglycoside donor to give a mixture of trisaccharides. The trisaccharides were cleaved from the resin, purified by gel chromatography, and then fully deprotected.

Thus far, only a few solid-phase oligosaccharide libraries have been reported. The challenges of well-planned orthogonal protecting groups and high yielding stereospecific



Scheme 6. Kahne's di- and trisaccharide library using split-mix synthesis and screening on the solid phase.

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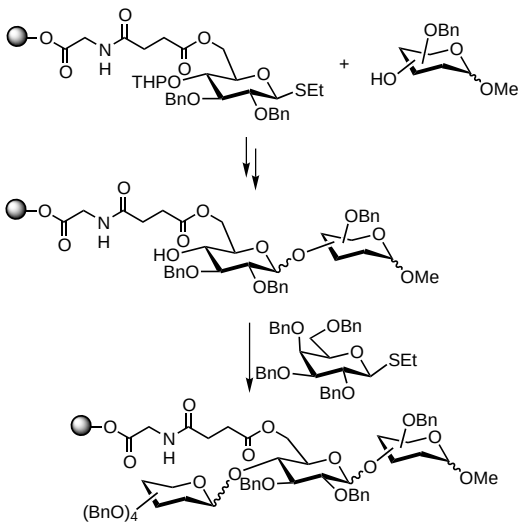


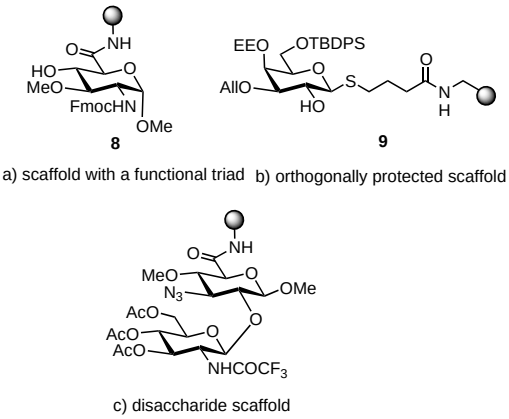
Scheme 7. Boons's two directional approach for solid-phase synthesis of trisaccharide library.

glycosidic bond formation on solid support continue to stimulate chemists to devise novel approaches. A number of these innovative strategies would certainly impact future solid-phase oligosaccharide library generation. They include the following: the use of soluble polymer-based liquid phase glycosylation;[25] solid-supported chemical-enzymatic synthesis;[26] the widely applicable and high yielding trichloroacetimidate glycosylation;[27] novel linkers such as a new thiol linker for α-mannose and α-fucose glycosides[28] and a ring closing metathesis based linker that generates *O*-allyl glycosides upon cleavage from the resin;[29] the use of glycosylating agents such as *n*-pentenyl glycosides;[30] the synthesis of β-(1 $\rightarrow$4)- and β-(1 $\rightarrow$6)-linked oligosaccharides using glycosyl phosphates in combination with a versatile octenediol linker;[31] the glycal assembly method for the synthesis of polymer bound thioethyl glycosyl donors for the synthesis of β-linked oligosaccharides;[32] the synthesis of thio-oligosaccharides by nucleophilic substitution of triflate activated glycosides by resin-bound sugar-1-thiolate containing unprotected hydroxyl groups,[33] and the use of a novel photocleavable aglycon linker[34] are very promising approaches for the rapid access to oligosaccharides.

Combinatorial Libraries Using Carbohydrate Scaffolds

Oligosaccharide library synthesis has been hampered by the polyfunctional nature of carbohydrates. This same feature places carbohydrates in a distinctive class of privileged template structures for displaying chemical diversities toward drug discovery efforts.[35] The advantageous use of the polyfunctional nature of carbohydrate units as scaffolds for displaying diversity represents a unique approach to combinatorial libraries that are not limited to glycoconjugate investigation. Previous work had demonstrated the validity of this approach in the design of nonpeptide somatostatin mimics.[36] Sofia et al. reported the first such solid-phase library containing three sites of diversity (Figure 1).[37] The important features of the scaffold was the use of a functional triad that included a carboxylic acid moiety, a free hydroxyl group, and a protected amino functionality on the glucoside **8**. This derivatized monosaccharide was then coupled to an amino acid functionalized trityl TentaGel resin. Using the IRORI radiofrequency tagged split-pool methodology[38] with glucoside **8**, sixteen 48-member libraries were prepared from eight amino acids, six isocyanates, and eight carboxylic acids. The libraries were analyzed by LC/MS in greater than 80% purity. These libraries were referred to as "universal pharmacophore mapping libraries".

Unlike Sofia's approach, Kunz et al. initially used an orthogonally protected thioglucoside as a scaffold.[39] The protecting groups included *tert*-butyldiphenylsilyl (TBDPS), 1-ethoxy ethyl (EE), and the propyl moiety. An important feature of the scaffold was the use of a functionalized thioglycoside, which not only served as a glycosyl donor but also as a linker for immobilizing the compound on aminomethyl polystyrene resin. Diversity was introduced at positions 2 and 6 after selective deprotection and alkylation. An anomeric mixture of methyl glycosides was obtained in yields of 30 to 80%. This combinatorial methodology was extended to a galactopyranose scaffold **9**, which contains five sites of diversity (Figure 1b).[40] Instead of the propyl group at position 3, the *O*-allyl group was introduced. Using sequential deprotection and alkylation protocols, an array of structurally diverse compounds were successfully synthesized.



a) scaffold with a functional triad b) orthogonally protected scaffold

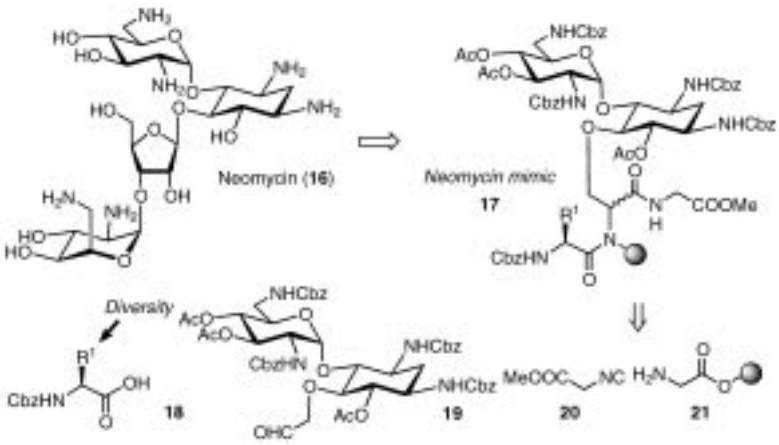
c) disaccharide scaffold

Figure 1. Carbohydrate-based scaffolds.

Silva's group has recently reported the synthesis of a unique β-linked disaccharide scaffold that was employed in the solid-phase synthesis of a 48-member library (Figure 1c).[41] Central to this approach was the use of phenylsulfenyl 2-deoxy-2-trifluoroacetamido glycopyranosides as glycosyl donors in the synthesis of the β-linked disaccharide.

These scaffolds may provide important small molecules for probing a variety of biological processes. No biological data has been presented. Other motifs have been investigated especially in the search for potent aminoglycoside mimics. An aminoglucopyranoside core containing a 1,3-hydroxyamine motif at the anomeric position has also been used as a privileged template for design of RNA binders using a parallel solution phase approach.[42] Unlike Sofia's use of a scaffold with no a priori information, Wong's use of this aminoglucopyranoside core represented a rational approach for

small-molecule derivatives of aminoglycoside antibiotics based on available structural information. Small-molecule mimics of glycoconjugates are therapeutically more relevant than biologically active oligosaccharides or even aminoglycoside antibiotics since important pharmacokinetic and pharmacodynamic properties can be incorporated in the structure. Therefore, it is not surprising that combinatorial glycomimetic library generation is a very dynamic and rapidly expanding field.



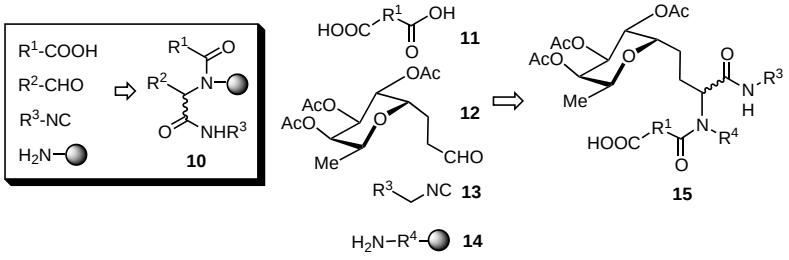
Scheme 9. Wong's neomycin mimics by Ugi four-component condensation.

Combinatorial Glycomimetic Libraries

To overcome the many challenges of complex oligosaccharide libraries, small glycoconjugates including glycopeptides have been exploited as functional mimics of oligosaccharides. These glycomimetics, in addition to being more readily accessible, may contain diverse aglycon scaffolds with an array of hydrophobic and/or charged functionalities upon which pertinent sugar moieties are displayed. Furthermore, the glycoside moieties may be present in its native form (*O*- or *N*-linked) or as stable isoteres such as *C*-linked and *S*-linked glycosides. A number of conceptual approaches have been successfully used for the rapid generation of libraries for biological studies.

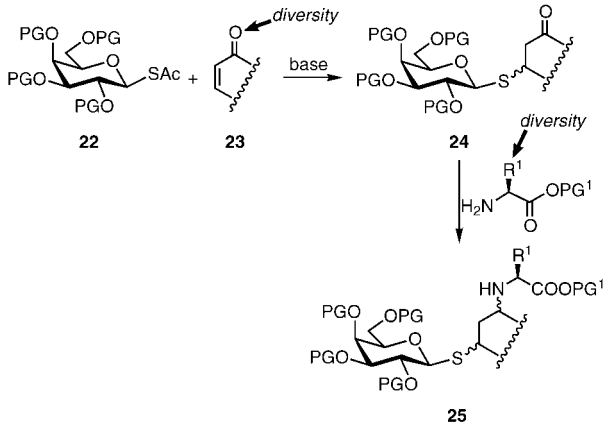
Multiple component reaction (MCR): Ugi's novel four component condensation reaction of an amine, aldehyde, isocyanate, and carboxylic acid to give the glycomimetic **10** has been successfully adapted to the solid phase.[43] This powerful strategy (Scheme 8) has been used for rapidly generating solid-phase combinatorial libraries of C-glycosides **15**.[44] Using eight diacids, a C-fucose aldehyde **12**, two isocyanides, and Rink amine resin derivatized with five different amino acids, Armstrong's group synthesized a focussed library of sialyl Lewis x mimetics **15** with high purity.

Wong's group has also used this methodology on a soluble polyethyleneglycol (PEG) polymer for the generation of mimetics of the aminoglycoside antibiotic **16** (Scheme 9).[45] In this library, the neamine moiety **19** (Cbz: benzoxycarbonyl) which is critical for inhibition of HIV RNA transactivator protein was kept constant and diversity was introduced in the amino acid group **18**. Using Ugi's versatile approach, carbohydrate building blocks containing aldehyde, amino, carboxylic, and isocyanide groups can be readily incorporated into small glycomimetics and used as small probes for carbohydrate–receptor interaction as well as therapeutically useful lead compounds.[46]



Scheme 8. Armstrong's glycomimetics by Ugi four-component condensation.

Glycohybrids: In another approach, a 1-thio-β-D-galactopyranoside library was prepared in solution using solid-phase extraction techniques for purification (Scheme 10). A building block such as **22** containing *O*-laurates (PG) as hydrophobic tags which facilitated reverse-phase C18 silica purification of the glycohybrids was used. This thio-glycoside building block underwent Michael addition reactions followed by derivatization of the carbonyl group with several amino acids. A library of an easily separable mixture of thirty compounds, **25**, each present as four diastereomers was produced. This library was screened for inhibitors of β-galactosidase from *E. coli*. One of the members was a better inhibitor than their reference compound.[47]



Scheme 10. Hindsgaul's glycohybrids.

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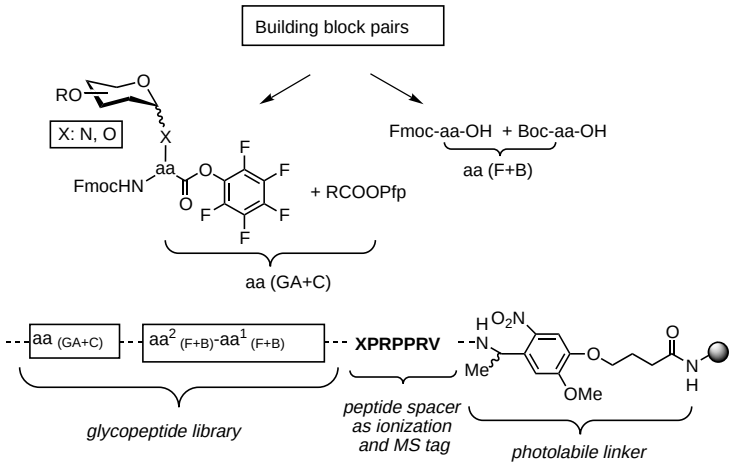
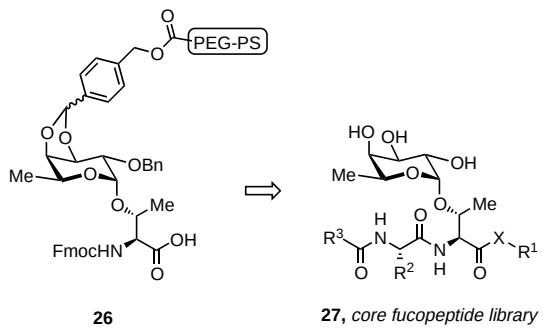
Glycosylated amino acid building blocks: The glycosylation of *N*-fluoren-9-yl-methoxycarbonyl (Fmoc) amino acid pentafluorophenyl esters (OPfp) (Scheme 11) has provided a range of building blocks for assembly of glycopeptides by multiple column solid-phase peptide synthesis. A variety of solid supports have also been used to produce parallel arrays of glycopeptides with native and isosterically substituted glycosidic linkages.[5, 10, 48] St. Hilaire and Meldal have reported an elegant strategy for combinatorial glycopeptide libraries that afforded unambiguous characterization of active compounds (Scheme 11).[49] An encoded one-bead-one-compound heptaglycopeptide library consisting of 300000 members were rapidly synthesized on a PEGA resin containing a photolabile linker by the split-mix technique. The glycopeptide-resin was then screened against a fluorescently labeled lectin from *Lathyrus odoratus*. Active compounds on fluorescent beads were determined by irradiation of the linker using the MALDI/TOF-MS laser with concurrent analysis of the ladder of terminated fragments, which directly gave the sequence and structure of the glycopeptide. The importance of the terminal mannose unit for lectin recognition was demonstrated since all the active glycopeptides contained this moiety.



Scheme 11. Encoded glycopeptide library.

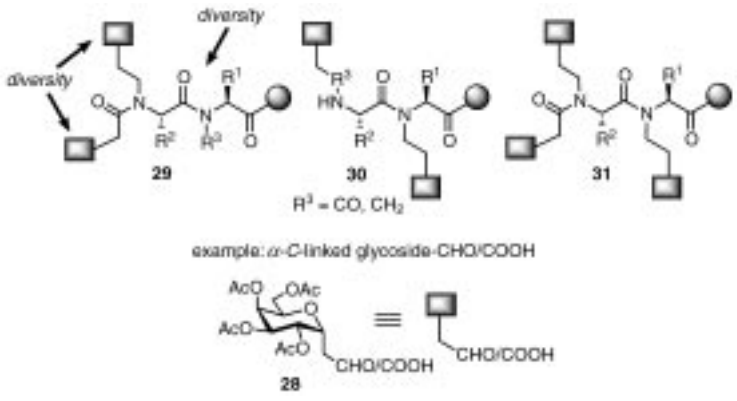
Wong et al. used a fucosylated amino acid building block approach (Scheme 12).[50] The fucose moiety of fucosylated threonine derivative was immobilized through a *p*-(acyloxymethyl)benzylidene acetal (*p*-AMBA) on a carboxyl-functionalized PEG-PS resin **26**. This was used to generate a fucopeptide library of sialyl Lewis x mimetics using parallel synthesis. In this library, the critical hydroxyl groups of the fucose moiety required for recognition of sialyl Lewis x by E-selectin was invariant and diversity was introduced at both the N- and C-termini of the glycopeptide. The cleaved and purified fucopeptides showed moderate binding when assayed against E- and P-selectins.



Scheme 12. Sialyl Lewis x mimics.

Automated, multistep approach to neoglycopeptide libraries: A versatile, fully automated multi-step solid-phase strategy has also been developed for the parallel synthesis of neoglycopeptide libraries (Scheme 13). Instead of using a glycosylated amino acid building block, which limits the choices of attached amino acids, C-glycoside **28** building blocks protected as acetates are used.[51] The C-glycoside can be in either the α- or β-configuration or even as a mixture of anomers and contain an aldehyde or carboxylic acid functionality. These building blocks can then be independently incorporated on a peptide/pseudo-peptide scaffold. Furthermore, the chain length of the C-glycoside can be varied and the carbohydrate moiety can be synthesized in either the pyranose or furanose form. In addition, these types of carbohydrate building blocks are not limited to monosaccharide derivatives since disaccharides can also be used.

Using this approach, libraries of neoglycopeptides are readily synthesized for probing carbohydrate–protein inter-



Scheme 13. Programmed approach to neoglycopeptides.

action. A number of "working models" have been developed for these libraries which addresses the multivalent presentation of carbohydrates **29**, **30**, and **31** (Scheme 13) while the dipeptide scaffold may contribute to secondary interactions with the biological target.[52] Initially, the neoglycopeptides were synthesized by a convergent strategy on a peptide synthesizer.[53] Since then, the synthesis has been successfully transferred to a fully automated multiple organic synthesizer and has been further optimized.[54] This fully automated methodology involves coupling an amino acid to an insoluble support such as Rink amide MBHA resin or TentaGel derivatized Rink amide resin. After removal of the protecting group on the amino acid, the sugar aldehyde undergoes reductive amination (Scheme 13, models **30** and **31**) with the resin bound amino group followed by amide bond formation with a second amino acid. After the amino group is deprotected it can undergo either reductive amination with any sugar aldehyde or coupling with any sugar acid or both. Using this approach, a parallel 96-compound library was recently synthesized using 24-dipeptides and two sugars, α-C-linked mannose- and glucose-aldehyde derivatives.[55] The choice of the dipeptide and sugars are reflected in the biological mechanism under investigation. This library provided chemical probes for studying protein folding and trafficking especially of N-linked glycoproteins[56, 57] as well as enzyme systems that convert a glucose moiety to rhamnose prior to incorporation of the rhamnose unit during biosynthesis of the mycobacterium cell wall.[58] Therefore, negatively charged amino acids, polar amino acids as well as hydrophobic residues were included in the 24 dipeptides that contained a diversity of two monosaccharides. A number of potential glycoside-based inhibitors containing at least one negatively charged amino acid residue were identified and detailed biological studies are in progress. Work is ongoing in the generation of a number of libraries including those with the nucleoside moiety at the C-terminal end of the model compounds (**29**, **30**, **31**) as chemical probes for investigating various glycosyl transferase-based reactions using high throughput assays.

Future Directions

The field of combinatorial carbohydrate based libraries is only a few years old. During this formative period, chemists have devised novel strategies to rapidly synthesize biologically relevant carbohydrates. The conceptual approaches described in this paper addressed many of the challenging aspects of carbohydrate library synthesis. Although many of the libraries were small (less than 50 compounds), they nonetheless demonstrated the principles of the varied strategies. Many groups are actively involved in this dynamic field in the synthesis of atypical monosaccharide building blocks and development of new solid supports and linkers as well as analysis and screening of solid-phase carbohydrate-based libraries. These approaches will certainly contribute to innovative library generation of oligosaccharides as well as of glycomimetics. Moreover, there is a trend towards fully automated and flexible approach to glycommimetic libraries, which may play a crucial role in rapidly identifying small-molecule inhibitors especially for carbohydrate processing enzymes. Such a parallel solid-phase approach eliminates the need for additional deconvolution of split-mix libraries as well as problematic identification of active compounds. Thus, glycobiology continues to be the driving force behind innovative chemical approaches to the study of the least exploited of the biomolecules, carbohydrates, and carbohydrate conjugates.

Acknowledgement

This work is funded in part by the National Research Council, publication No. 43852. One referee and Dr. Karla Randell are thanked for providing several very useful suggestions.

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