Combinatorial Carbohydrate Synthesis

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Abstract: Combinatorial chemistry has contributed to the preparation of biologically important oligosaccharide and glycoconjugate libraries, with a view of improving our understanding of the complex interaction between the carbohydrates and their protein-based receptors. The combinatorial carbohydrate synthesis strategies, developed in the past decade, provide access to small and medium size libraries. This mini review critically examines both the solution phase and solid phase strategies that have been reported to date.

Keywords: Combinatorial chemistry, Carbohydrates, Solution phase synthesis, Solid phase synthesis, Glycoconjugates.

1. INTRODUCTION

Glycoconjugates play important roles in cellular recognition, adhesion, cell-growth regulation, cancer cell metastasis, and inflammation [1, 2]. Cell surface carbohydrates also serve as attachment sites for infectious bacteria, viruses and toxins, resulting in pathogenesis [3]. The synthesis of structurally defined glycoconjugates should provide the opportunity to probe and intervene in critical biological processes. Chemical synthesis of carbohydrates normally includes laborious, protecting group manipulations to differentiate several hydroxyl groups of similar reactivity, complicated stereoselective glycosylations and long overall routes [4]. In most cases, purification by chromatography is necessary after each step. The overall synthesis inevitably becomes time consuming and technically demanding. Complex carbohydrate and glycoconjugate synthesis remains much more complicated than that of other biomolecules and advancing glycoconjugate synthetic technology to the automated level used in protein and nucleic acid synthesis remains a daunting challenge.

Combinatorial chemistry allows efficient preparation of large numbers of structurally distinct molecules and is increasingly being applied for preparation of chemical libraries in medicinal chemistry [5, 6]. This method has evolved to meet the growing demand for the economical synthesis of large numbers of diverse chemical compounds in a relatively short time. Combinatorial synthesis of libraries has developed into a useful method for the rapid identification of lead compounds for drug discovery. Combinatorial synthetic technologies, in both solution and solid phase, have revolutionized protein and nucleic acid science. In recent years, new solution and solid phase strategies have also been developed to prepare oligosaccharide libraries for biological investigation, and the use of monosaccharides as scaffolds in the generation of combinatorial libraries has also been described. The readers attention should be directed towards a number of excellent

reviews on the combinatorial chemistry of carbohydrates [7-12]. The current review highlights the most recent methods in combinatorial carbohydrate synthesis and includes a description of on-resin analytical methods.

## 2. SOLUTION PHASE COMBINATORIAL SYNTHESIS

Random Glycosylation

The first approach to oligosaccharide libraries in solution phase was reported by Hindsgaul and co-workers [13]. They investigated a "random glycosylation strategy". This strategy eliminated the need for numerous synthetic steps to construct orthogonally protected sugar building blocks and reduced both the time and the cost of synthesis. Perbenzylated fucosyl trichloroacetimidate glycosyl donor compound (**1**) and unprotected disaccharide acceptor compound (**2**) were coupled to give the six possible α-linked trisaccharides with minor amounts of β-fucosylated compounds (Fig. **1**). All six regioisomers were obtained in almost equimolar amounts. This suggests that under the reaction conditions used, all of the hydroxyl groups in compound (**2**) have similar reactivity. The mixtures obtained were used as substrates to screen for glycosyl transferase activities [14]. Although the random glycosylation strategy is the most direct manipulation, low conversion and purification difficulties limit the widespread use of this methodology.
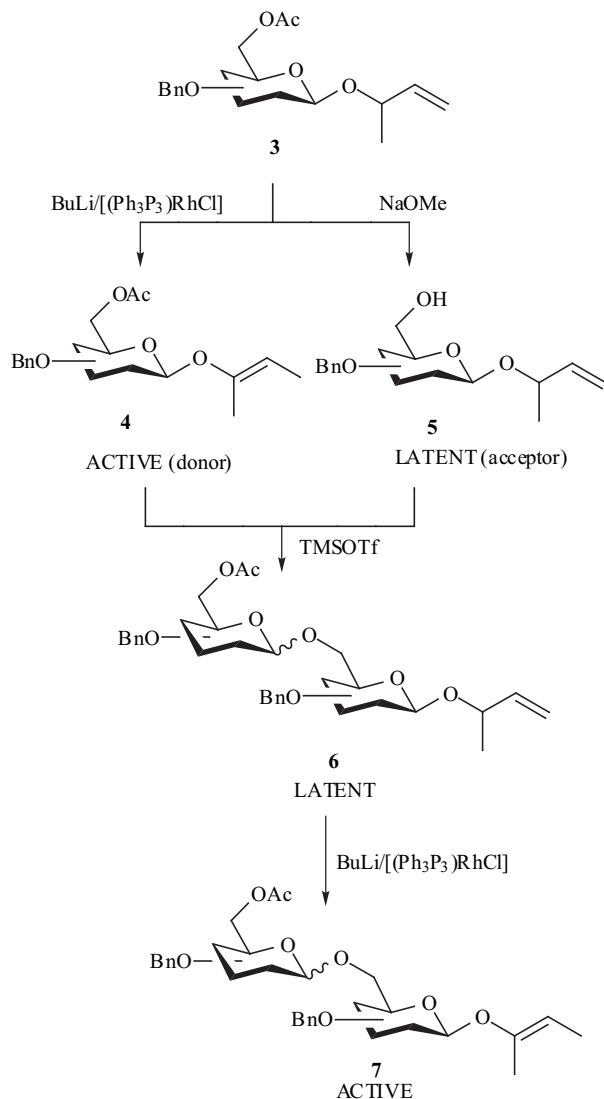
Active and Latent Glycosylation Strategy

One important approach to solution phase oligosaccharide synthesis was reported by Boons *et al.* [15], which relies on the "armed/disarmed" approach first described by Fraser-Reid and co-workers [16]. In this strategy, 3-buten-2-yl-glycoside building block compound (**3**) was used both as latent glycosyl acceptor following deacetylation with NaOMe and as a glycosyl donor by isomerization with BuLi/[(Ph$_3$P)$_3$RhCl] (Fig. **2**). Donor and acceptor were coupled to give disaccharide compound (**6**), which could then convert into a glycosyl donor compound (**7**). Both linear and branched trisaccharide model libraries were prepared using this strategy [17].

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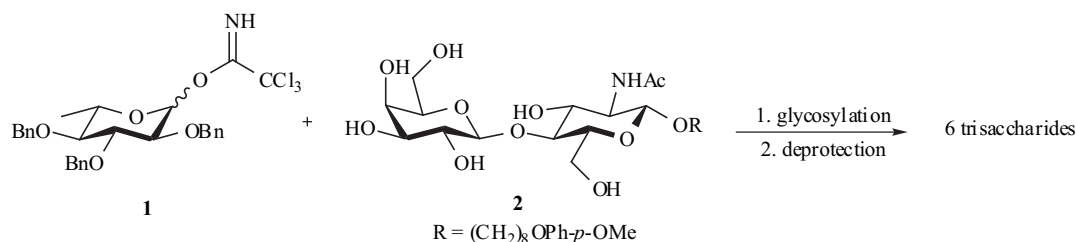
**Fig. (1).** Solution phase random glycosylation strategy.

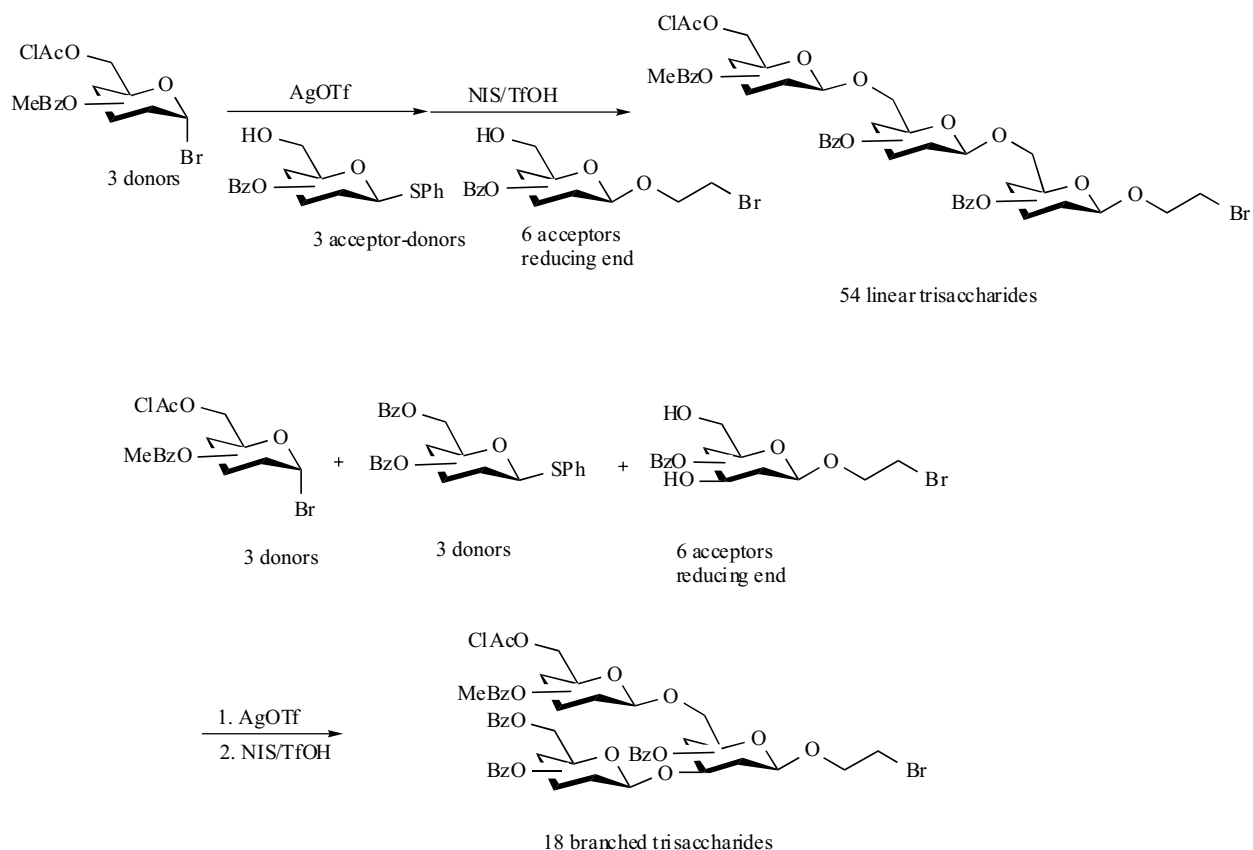**Fig. (2).** Active-latent glycosylation approach in solution phase.

deprotection and glycosylation with thioglycosyl donor steps were repeated to generate a protected very bulky oligosaccharide compound (**10**). Most reactions were both rapid and efficient, and this strategy should be applicable to solid phase synthesis.
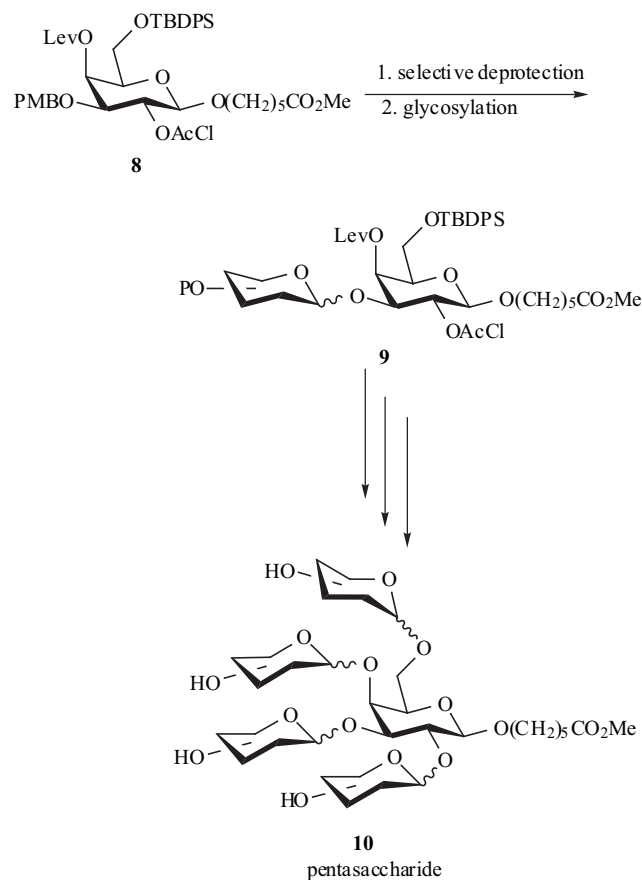
Fig. (3). Orthogonal protection-deprotection strategy.

One-Pot Glycosylation Technology

Wong and co-workers [19] pioneered the approach of programmable one-pot oligosaccharide synthesis by creating a database of thioglycosides as glycosyl donors, evaluating and mapping 50 different p-methylphenyl thioglycosides. The relative reactivity values (RRV) for each compound was determined using direct competition assays. A computer program developed by this group, called OptiMer, searches through the databases of characterized donors and identifies sets of monomer donors. Using the OptiMer synthesis planning methodology, a small library of 33 tri and tetrasaccharides and a tumor-associated Globo H hexasaccharide was synthesized based on the combination of

Orthogonally Protected Carbohydrates

An efficient orthogonal protection-deprotection strategy was demonstrated in solution phase combinatorial synthesis by Wong *et al.* [18]. Fully characterized 45-member oligosaccharide library of highly branched glycosides was synthesized using the well designed building block compound (**8**) containing four selectively removable protecting groups. The four chosen protecting groups (P), chloroacetyl (ClAc), p-methoxybenzyl (PMB), levulinyl (Lev) and tert-butyldiphenylsilyl (TBDPS) could be selectively removed in high yields (Fig. **3**). Selective

Fig. (4). One-pot glycosylation strategy.

orthogonal protection and one-pot glycosylation strategies using thioglycosides as glycosylation reagents [20, 21].

In a second approach, Takahashi *et al.* reported a one-pot, two step glycosylation as a potential method to synthesize solution phase oligosaccharide libraries on Quest 210™ manual synthesizer [22]. In this approach, parallel synthesis of 54 linear trisaccharides was carried out using three bromo glucoside donors. These donors were coupled under AgOTf activation with three thiophenyl donor-acceptor glycosides to give a mixture of disaccharides that were coupled with six 2-bromoethyl glycosides (Fig. **4**). For the synthesis of an 18-membered library of trisaccharides, three glycosyl bromides and three thiophenyl glycosides were used on a manual synthesizer as glycosyl donors with six 2-bromoethyl glycosides each bearing two free hydroxyl groups. All members of these libraries were isolated in good yields.

3. SOLID PHASE COMBINATORIAL SYNTHESIS

Solid phase synthesis is a methodology in which synthetic transformations are conducted with one of the reactant molecules attached to an insoluble matrix, referred to as a solid support. This approach was originally developed by Merrifield who first used the term 'solid phase peptide synthesis' to describe the preparation of a peptide on a polymer, which remained insoluble during the synthesis [23]. Solid-phase oligosaccharide synthesis has been studied since 1970's [24]. Application of solid phase methodology facilitates the generation of products that are physically separated by attachment to the resin, thus facilitating the removal of reagents and side products by simple filtration and washing steps. Besides the convenience of purification, solid supports also allow the use of excess solution phase reagents, to drive reactions to completion, since they can easily be removed. Unfortunately, the limited capacity of solid supports has restricted the exploitation of solid phase methodology to research applications. Work continues to increase the loading levels obtainable to make solid phase synthesis more useful for commercial applications.

Solid Supports

Most solid phase oligosaccharide syntheses have been carried out on Merrifield resin (gel-type, 1 or 2% cross-linked polystyrene (PS)). This resin has high capacity, stability across a broad range of reaction conditions, but requires swelling in polar solvent. By the addition of poly (ethylene glycol) (PEG), a chain graft-type PEG-PS resin has been prepared. The first generation of PEG-grafted PS resin, TentaGel resin has excellent chemical properties with respect to swelling, pressure resistance and stability. TentaGel swells both in aqueous systems and in organic solvents. In recent years, new graft-type PEG-PS with twice the loading capacity of TentaGel, called ArgoGel has been introduced for solid phase organic synthesis [25]. The hydroxyl substituted copolymer, ArgoGel-OH (AG-OH), has now been further elaborated to the chloro (AG-Cl) and amino (AG-NH$_2$) substituted resins, but the new supports have not yet found common use in carbohydrates synthesis.

Controlled pore glass (CPG) found its way into the solid phase synthesis literature early in the advent of DNA

synthesis. Although CPG has been used extensively in oligonucleotide synthesis, it has seldom been used in oligosaccharide synthesis [26, 27]. In contrast to polystyrene, only the surface of CPG is functionalized, thus resulting in lower loading but surface reactions might make for easier access to reagents. CPG has some limitations including as relatively low functional group loading, a high surface polarity, an incompatibility with fluoride reagent and a high cost [28].

Linkers

In syntheses on solid phase, a critical step is the attachment of the sugar molecule to the support. This is usually achieved through the introduction of a cleavable linker that contains a heterobifunctional protecting group. This linker represents a key building block and the correct choice of linker is often critical in planning a synthetic strategy. The ideal linker should be stable to the chemistry used in the synthesis, inexpensive and readily available. The attachment of starting material needs to be achieved in high yield and the cleavage of product should be efficient and utilize conditions that do not damage the structure of the final product. A great number of linkers have been developed. These facilitate multi-step organic syntheses and permit the use of a broad range of reagents, and allow cleavage in a very selective manner [29-32]. In oligosaccharide synthesis, silyl ethers are frequently used as temporary protecting groups for hydroxyl moieties. Danishefsky and co-workers employed a donor-bound strategy, using 6-*O*-diphenyl arylsilane linker, for the solid phase synthesis of a β-(1→6)-linked tetrasaccharide [33]. Acid and base-labile linkers are also commonly used in solid phase peptide synthesis. Ogawa and co-workers used an acid-labile Wang resin, which is cleavage from resin with triphenylmethylboron tetrafluoride (TrBF$_4$), for the synthesis of oligosaccharides [34]. Kahne and co-workers have employed thiol-based linkers in the solid phase synthesis of

a diverse library of di and trisaccharides [35]. In this approach cleavage of tri and tetrasaccharides was performed using *N*-bromosuccinimide (NBS) in THF/MeOH [36]. A number of methods have been developed for the cleavage of the linker from the product. These include cleavage by oxidation, hydrogenation and photolysis. For example, to construct complex oligosaccharides, Nicolaou and co-workers used a photolabile linker by including a 4-oxybenzoic acid spacer [37]. Such linker systems offer the wide possibilities for the final diversification of a synthesized oligosaccharide library.

Solid Phase Oligosaccharide Libraries

Parallel Solid-Phase Synthesis

The solid-phase synthesis of encoded oligosaccharide libraries was first reported by Kahne and co-workers [35]. They used a split and mix strategy from the monomers. Six distinct acidic carbohydrate monomers were attached separately to amino functionalized TentaGel resin through an anomeric thioether linker, and then 12 different monosaccharide and disaccharide sulfoxide donors were coupled to mixtures of beads containing all six monomers (Fig. **5**). Controlling of both α and β anomeric stereochemistry, 72 different di and trisaccharides were formed. Additional diversity was achieved by reduction of the azide and the acylation of the each oligosaccharide amine using 20 different acylation reagents. A targeted library of 1269 di and trisaccharides was produced and on removal of protecting groups the resin-bound oligosaccharide library was screened for binding to *Burhinia purpurea* lectin.

Two-Directional Glycosylation Strategy

Boons and Zhu described a highly efficient approach to the synthesis of solid-phase trisaccharide library [38]. A thioethyglycosyl building block was first immobilized on
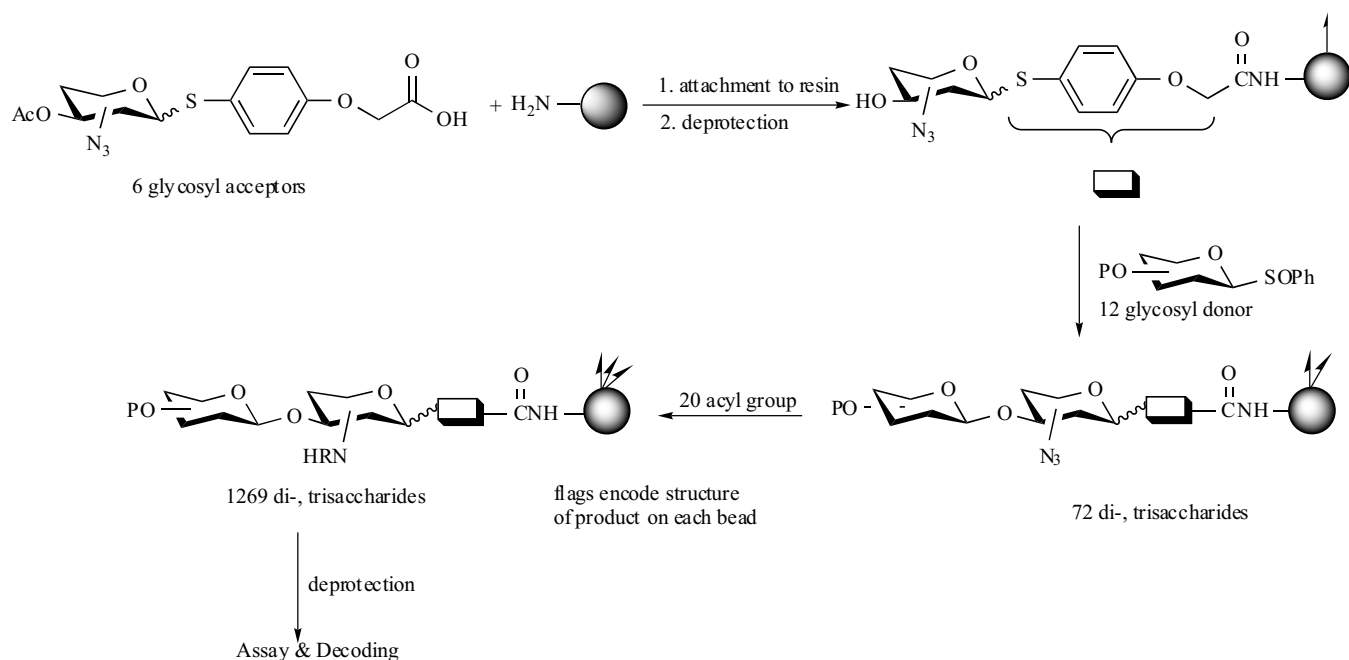

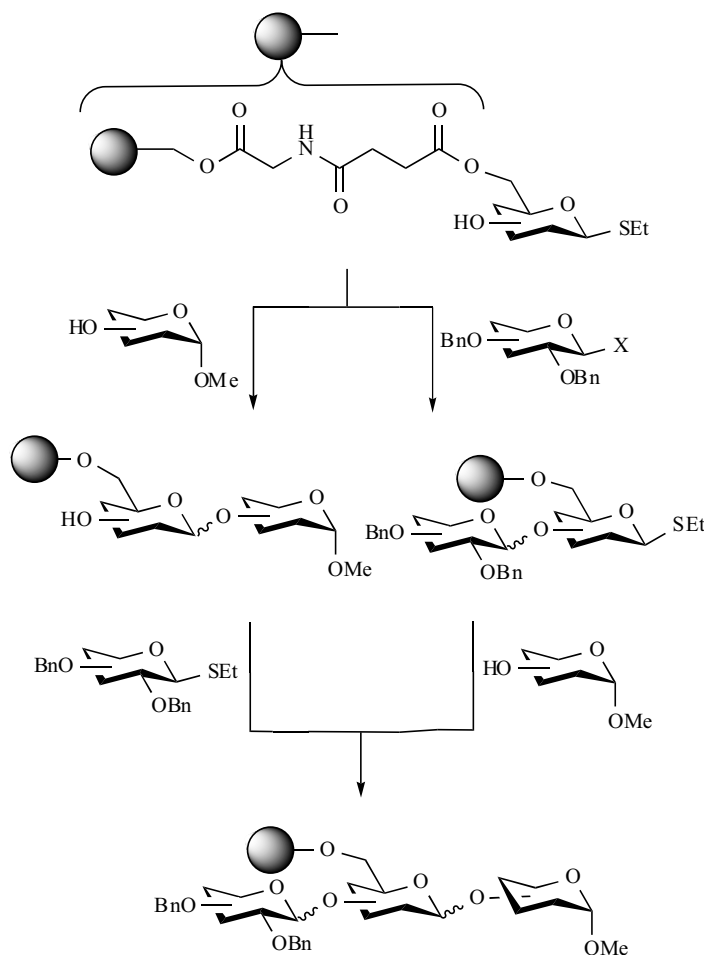**Fig. (5).** Parallel solid phase encoded oligosaccharide library.

Fig. (6). Two-directional strategy for solid phase trisaccharide library.

the solid support by amide formation between a succinate half-ester at C6 of the saccharide and glycine-derivatized TentaGel resin. By immobilization through the C6 of thioethyl glycoside it could be used both as donor and acceptor (Fig. **6**). For the synthesis of trisaccharide library, the immobilized thioethyl glycosides were coupled with three different glycosyl acceptors, on NIS/TMSOTf activation. The resins were combined, a protecting group removed, and the beads were glycosylated with a protected galactosyl thioglycoside donor to afford a library with 12 trisaccharides.

Other Basic Strategies Used in Solid Phase Combinatorial Carbohydrate Synthesis

Orthogonal Glycosylation Strategy

In addition to the many methods developed for the preparation of oligosaccharides on solid support, a number of innovative approaches address very specific challenges of carbohydrate chemistry in the solid phase. Ogawa and co-workers developed an orthogonal glycosylation strategy by the combined use of phenylthioglycosides and glycosylfluorides as both donors and acceptors in solution phase [39]. They demonstrated the applicability of this strategy to the synthesis of extended blood group B determinant with four different types of glycosidic linkages [40]. Using PEG as a soluble polymer, an orthogonal

glycosylation strategy was applied also "solid phase" carbohydrate synthesis (Fig. **7**). The use of soluble polymeric supports in place of insoluble matrices allows convenient product recovery and isolation without imposing the mass transport limitations associated with two-phase reactions. Methyl thiomannoside compound (**11**) was attached to monomethyl poly(ethylene glycol) (MPEG) as a soluble polymer through an ester linkage and coupled with mannosyl fluoride compound (**13**) by activation with dimethylthiosulfonium triflate (DMTST) generated *in situ* to stereoselectively afford support-bound disaccharide compound (**14**). Disaccharide-PEG compound (**14**) was converted into trisaccharide compound (**16**) by coupling with 2-(trimethylsilyl)ethyl (SE) glycoside compound (**15**) under Suzuki conditions. After cleavage from the solid support, reversed-phase chromatographic separation gave desired oligosaccharide compound (**17**) in 40% overall yield [41].

The application of thioglycosides to the synthesis of highly branched oligosaccharides on solid phase was reported by Nicolaou and co-workers in the synthesis of a heptasaccharide with phytoalexin elicitor activity [42]. A polystyrene support, equipped with a photolabile *o*-nitrobenzyl linker, and thiomethyl and thiophenyl glycosides were utilized in this synthesis. This methodology has several disadvantages including the anomeric mixture obtained at each cleavage step and the requirement to

reactivate the cleavage product. To solve these problems, this group developed a new block-type solid phase synthetic strategy for the construction of complex oligosaccharides

with a photolabile linker included 4-oxybenzoic acid spacer and thioglycoside donor [37]. The key trisaccharide building block compound (**18**), which can either be cleaved with
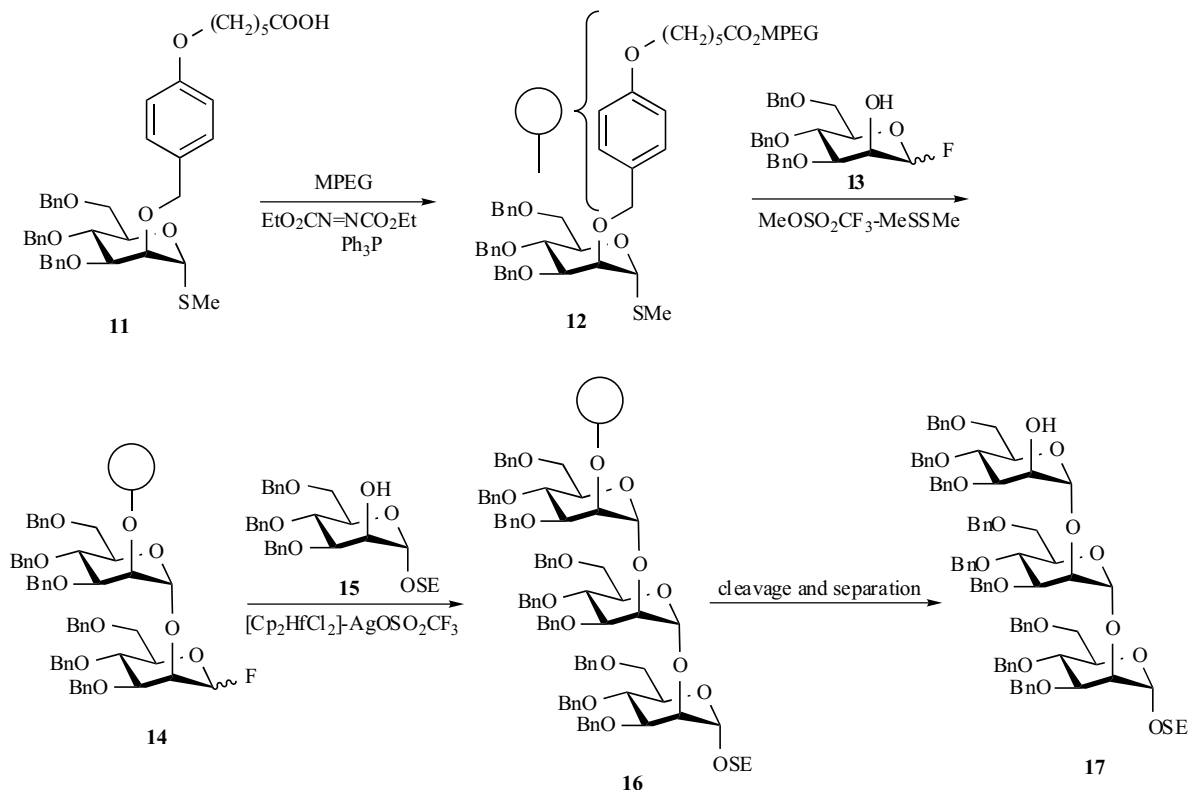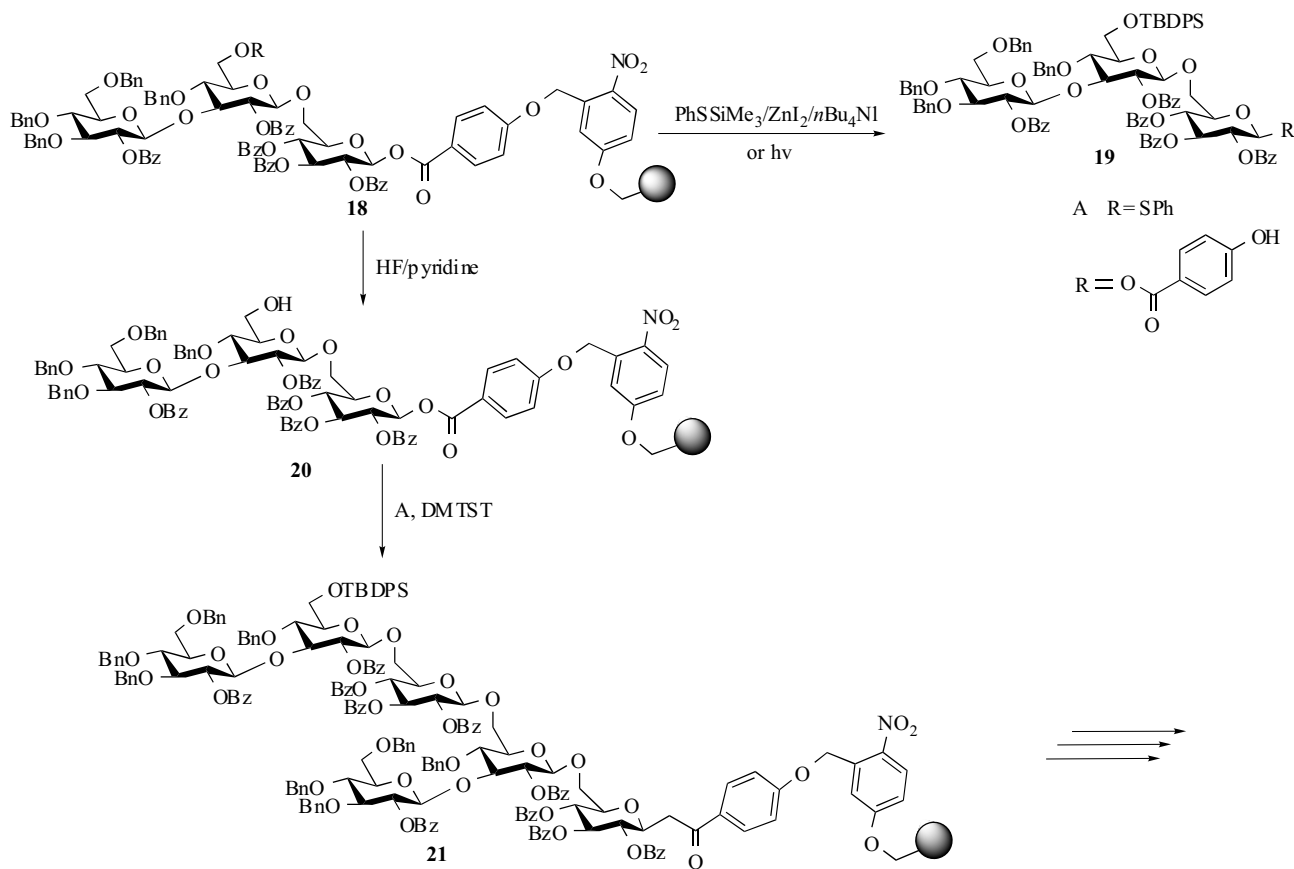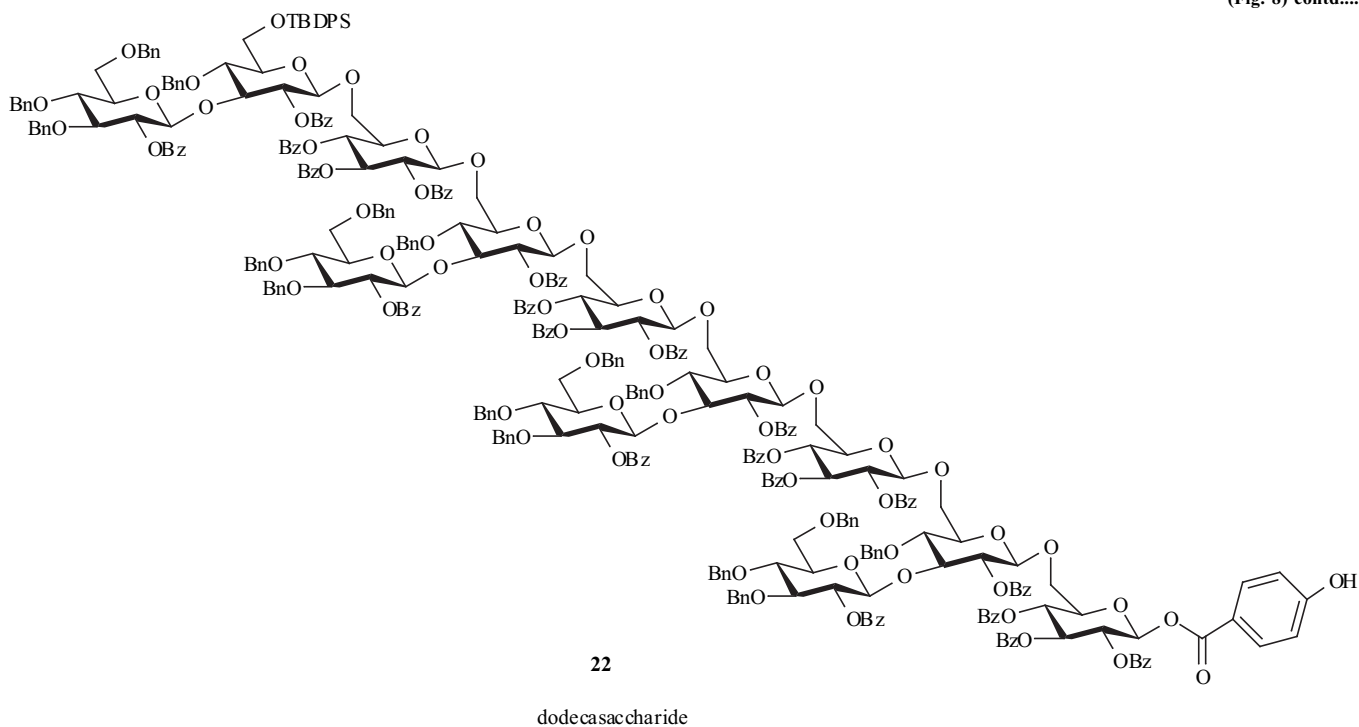

Fig. (7). Orthogonally glycosylation strategy.

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dodecasaccharide

Fig. (8). Nicolaou's block-type glycosylation strategy.

PhSSiMe$_3$/ZnI$_2$/nBu$_4$NI to yield trisaccharide donor compound (**20**), or released from solid support under photolytic conditions to afford fully protected trisaccharide β-anomer compound (**19**), was synthesized on Merrifield resin (Fig. **8**). Building block compound (**18**) was coupled with compound (**19**) in the presence of DMTST to give

compound (**21**). Fragment couplings using compound (**19**) and cleavage from the resin furnished the dodecasaccharide target compound (**22**) in 10% overall yield.

Schmidt and co-workers developed an approach using *O*-glycosyl trichloroacetimidate, 9-fluorene methyloxycarbonyl

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1. attachment to resin
2. selective deprotection

24

25

O-Glycosyl trichloroacetimidate donor

26
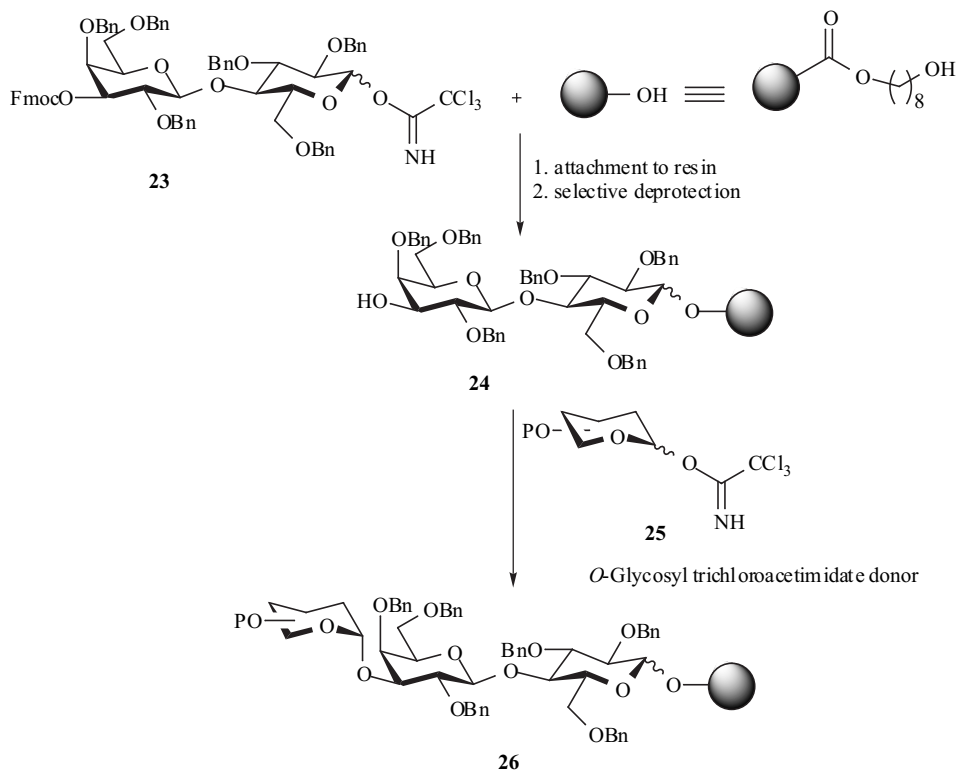
Fig. (9). Using Fmoc protection group solid phase glycosylation.

(Fmoc) protected donor, oligosaccharides for synthesis [43]. Lactose derivative oligosaccharide was converted to an anomeric mixture of trichloroacetimidate glycosyl donor in a good yield. To show the utility of glycosyl donor as a building block, it was employed in a solid phase glycosylation reaction (Fig. **9**). Lactosyl donor compound (**23**) was attached to carboxypolystyrene resin through ester type linker. After removing of the Fmoc group, disaccharide moiety compound (**24**) was used as acceptor and elongated with fucosyl donor compound (**25**). This strategy was applied to synthesis of a set of tri and tetrasaccharides [44] and a human milk branched hexasaccharide [45].

4. GLYCOCONJUGATE LIBRARIES

Carbohydrates can be attached to other non-sugar aglycones to produce glycoconjugates, *i.e.* proteoglycans, glycoproteins and glycolipids. These glycoconjugates play important roles in specific biological processes. The role of the carbohydrate portion of the glycoconjugate relates to

their function as carriers of information; through biological interactions that regulate and/or effect physiological and biological processes. Glycoconjugates interact with other proteins on cell surfaces, hormones, bacteria, viruses through their carbohydrates and their recognition depends on specific structural features within the carbohydrate moiety. Glycoconjugates can be present in its natural *N*- or *O*-linked form. In addition, *S*- and *C*-glycosides have been developed as stable mimetics to the often hydrolytically unstable *O*-glycosidic bond. Several approaches have been used for the generation of biologically active glycoconjugate libraries.

Glycohybrids Technology

Hindsgaul and co-workers reported the synthesis of a small library of *S*-linked galactopyranosides in solution phase and screening as inhibitors of *E. coli* β-galactosidase [46]. The carbohybrid library was synthesized through the S-alkylation of a 1-thio-glycoside building block compound (**27**) using Michael acceptors and α-chloroketones, followed

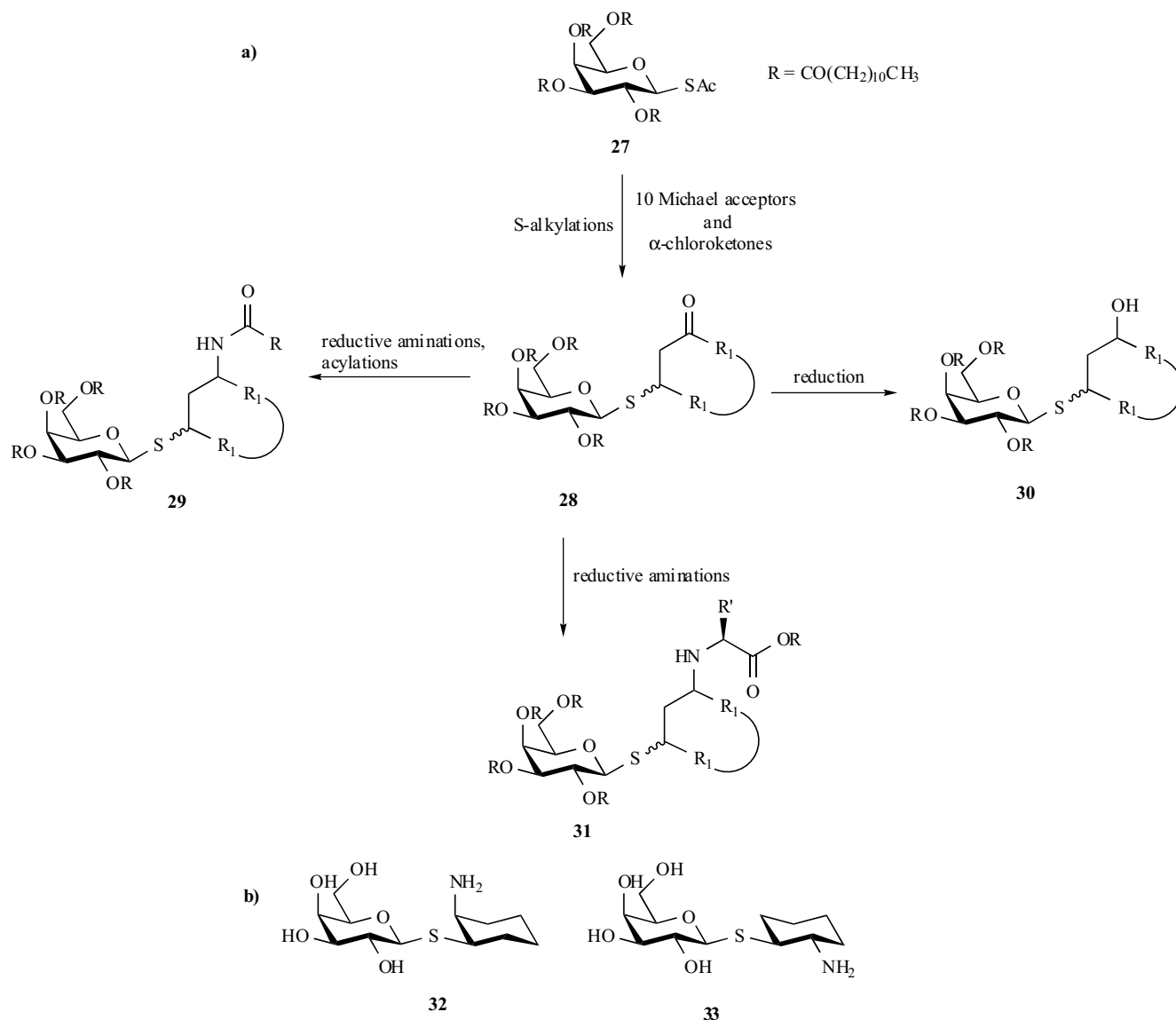**Fig. (10). a)** Synthesis of glycohybrid library **b)** Most potent plant lectin-binding compounds.
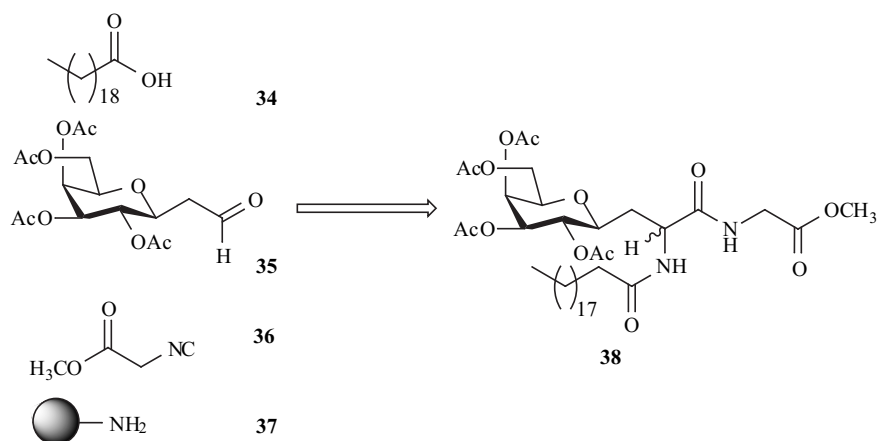
Fig. (11). sLe^x glycomimetics.

by ketone reduction, reductive amination and acylation (Fig. **10a**). A 1-thio-β-D-galactoside library was afforded as a mixture of four diastereomers of each member. Using ten Michael acceptors and α-chloroketones, and six amino acid esters, they expanded their library and screened it for binding to plant lectins and in a hemagglutination inhibition assay. Compound (**32**) and (**33**) were found to be most potent inhibitors (Fig. **10b**) [47].

Four Component Ugi Condensation Reaction

The Ugi reaction is multiple component chemistry that combines a carboxylic acid, aldehyde, isocyanide and amine, resulting in α-acylaminoamide. Using this four-component solid phase condensation strategy, a glycoconjugate library was reported by Armstrong and co-workers [48]. They synthesized tripeptides by condensation of aldehyde- and/or carboxylic acid-functionalized *C*-glycoside as the aldehyde or

acid component, Rink resin carrying amino substituent and isocyanide (Fig. **11**). The products were obtained in high purity. This research group was also reported a 96-membered library of sialyl Lewis X (sLe^x) blood group glycomimetics. Eight diacids, two isocyanides, five Rink resin-bound amino acids and a *C*-fucose aldehyde were used to construct this library.

sLe^x Mimetics

A solid phase synthesis of heterocyclic β-turn mimetics of sLe^x, the natural carbohydrate ligand of selectins, was reported by Kondo and co-workers [49]. To obtain this target compound, a derivatized support-linker, a branched alkyl, an Fmoc-protected serine derivative and a α-bromo acid were condensed in several steps. The support-linker was then removed, and a heterocyclization reaction was carried out to afford a heterocyclic β-turn mimetic containing sugar
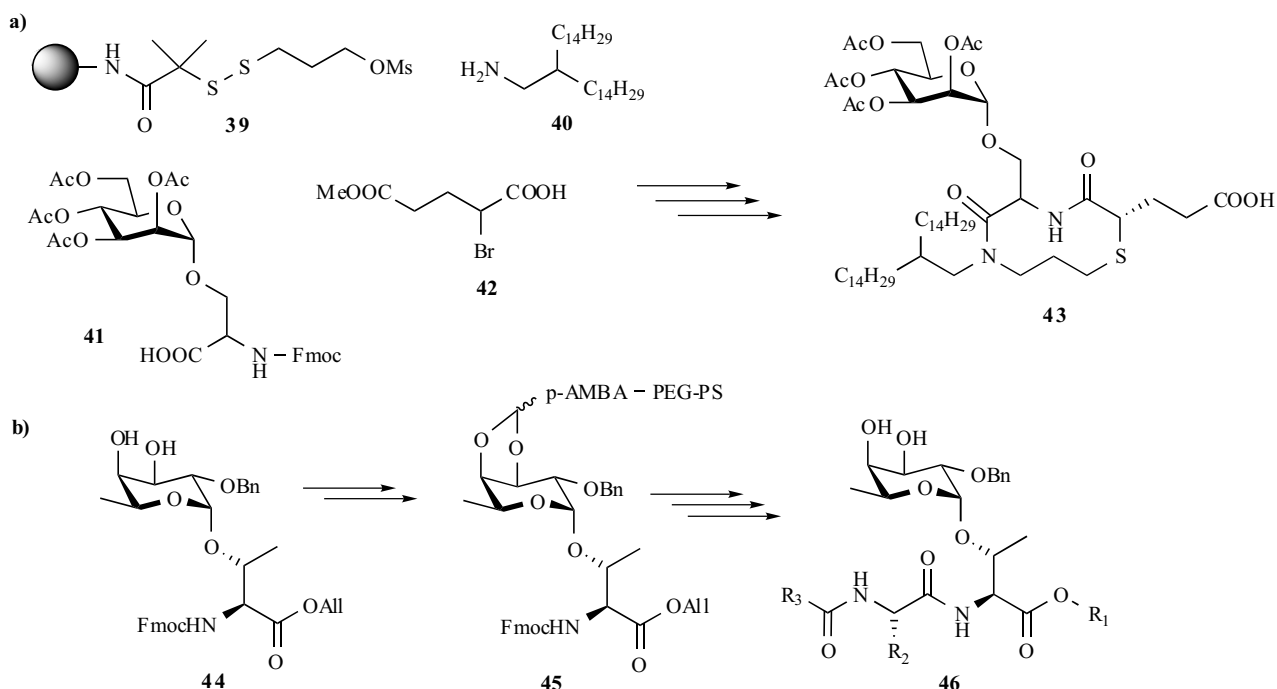

Fig. (12). a) Heterocyclic β-turn mimetics of sLe^x **b)** Fucosylated amino acid building block approach for the parallel synthesis of fucopeptides as sLe^x mimetics.

molecule. The inhibitory activity of compound (**43**) against selectin/sLe^x binding was determined *in vitro* using ELISA (Fig. **12a**).

A fucosylated amino acid building block approach was used for the parallel solid phase synthesis of fucopeptides as sLe^x mimetics [50]. Orthogonally protected α-fucoside compound (**44**) was immobilized on a carboxyl-functionalized PEG-PS resin through highly acid sensitive para-acyloxymethylbenzylidene acetal (p-AMBA) anchor group. The sugar containing compound (**45**) had variable functionalization of N- and C-termini of its peptide moiety. Fucopeptides were prepared by allyl cleavage and esterification or amidification at the C-termini. Removal of the Fmoc group on the N-terminus, amino acid coupling, cleavage of acetal group and removal of the benzylic protecting groups (Fig. **12b**) afforded fucosylpeptides compound (**46**), which were evaluated for binding E and P-selectins.

Hilaire *et al.* reported an excellent method for combinatorial random heptaglycopeptide libraries suitable for screening and structural analysis [51]. The 300,000-membered library was synthesized using the encoded ladder synthesis approach, linked to the PEGA solid support, and contained a photolabile amide linker using portion-mixing technique. Active compounds on fluorescent beads were identified by irradiation of the bead with the MALDI-TOF-MS laser, and analysis of the resulting spectrum yielded the sequence of the active glycopeptide. The resulting encoded, one-bead-one-glycopeptide library was screened in a solid phase fluorescence-binding assay for binding to C-type lectin from *Lathyrus odoratus*.

To circumvent the inherent incompatibility of carbohydrate and peptide chemistry in glycopeptide synthesis, glycosyl amino acids are used as building blocks in glycopeptide construction [52-54]. Enzymatic methods have also been employed for the elongation of glycopeptide glycans [55, 56]. Although solid phase synthesis is applied in glycopeptide chemistry because of its convenience and efficacy [57-59], problems associated with the removal of glycopeptides from solid support with strong acid, such as trifluoroacetic acid (TFA), and the deprotection of carbohydrates, are still waiting a solution. Wen and Guo have demonstrated a new strategy for the glycopeptide synthesis using *N*-glycosyl asparagines as building blocks and unprotected oligosaccharides as phase tags in solution phase synthesis and solid phase workup [60]. The synthesis of N-liked glycopeptides was carried out in a solution of *N*-methylpyrrolidinone (NMP), which is a commonly used solvent in peptide synthesis and can dissolve unprotected sugar. The product of each step was obtained through precipitation by adding diethyl ether to the reaction mixture.

5. CARBOHYDRATE SCAFFOLDS FOR COMBINA-TORIAL SYNTHESIS

Carbohydrates offer enormous potential as highly functionalized scaffolds for the use in combinatorial synthesis. Monosaccharides are readily available, often possess a rigid conformation, and thus, are very suitable for combinatorial synthesis. The use of sugar-derived skeletons as somatostatin (a cyclic tetradecapeptide) mimetics

demonstrated for the first time the use of sugars as such platforms [61]. Sofia and co-workers reported the solid phase synthesis on trityl-functionalized TentaGel, of a library based on trifunctionalized saccharide scaffolds [62]. Kunz and co-workers developed a strategy based on an orthogonal protection procedure for a glucose scaffold in solid phase synthesis [63]. Orthogonally protected thioglycoside compound (**47**), stable during protecting group manipulations and alkylation reactions, was immobilized on an amino-derivatized solid support, and followed by deprotection and alkylation steps. Cleavage from the resin in the presence of a large excess of alcohol yielded to anomeric mixture of scaffold compound (**50**). Sequential deprotection and alkylation protocols resulted in the synthesis of a structurally diverse array of compounds (Fig. **13**).
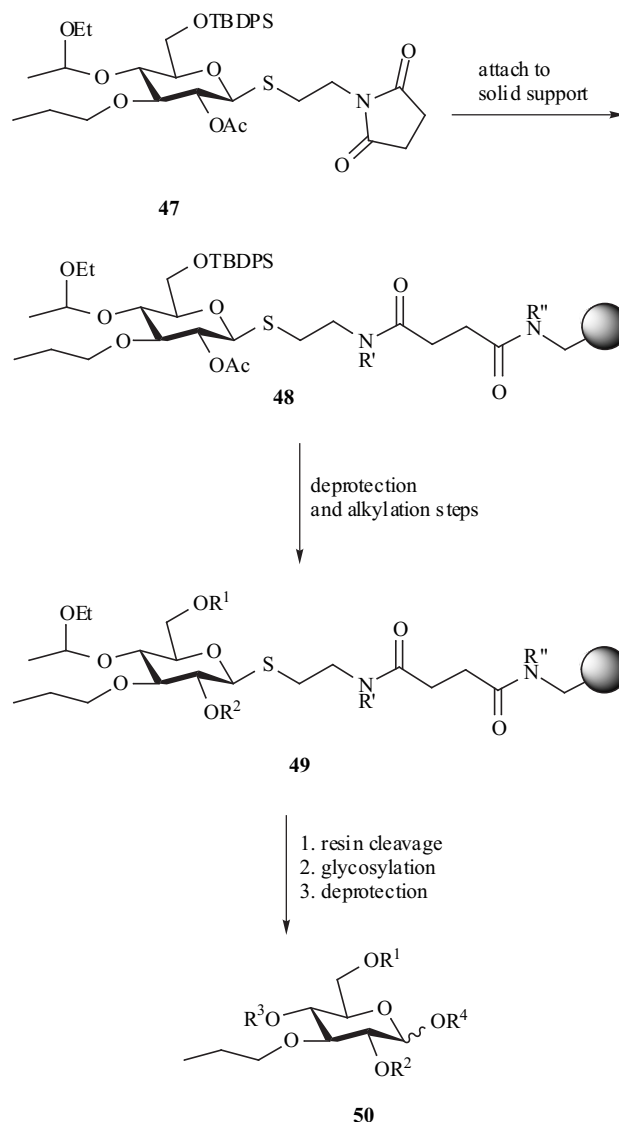
Fig. (13). Synthesis carbohydrate scaffolds based on orthogonally protection procedure.

In another approach, a new combinatorial library of selective Arg-Gly-Asp (RGD) peptide mimetics, based on chiral sugar scaffolds, was carried out in solution phase by molecular modeling with a particular emphasis on library stereodiversity [64]. D-Xylose was selected as a scaffold as it has three equatorial hydroxyl groups with the same reactivity

and converted to allyl glycosides. The α− and β-allyl glycosides formed were acetylated and benzylated to afford a mixture of mono- and di-benzylated derivatives. The carboxylic acid residue was introduced by alkylation with tert-butylbromoacetate. Further diversity was created by the introduction of a secondary amino group formation at anomeric position. Biological evaluation of this library was carried out on S180 sarcoma cells and an active RGD mimic compound (**51**) (Fig. **14**) having α-linked *N*-propyl substituent at anomeric position and carboxylic acid at position 4 was identified.
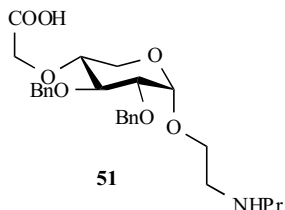

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Fig. (14). Arg-Gly-Asp (RGD) peptidic mimetic based on chiral sugar scaffolds.

6. AUTOMATION OF OLIGOSACCHARIDE SYNTHESIS

While the synthesis of peptides and oligonucleotides was automated decades ago, there are no general synthetic procedures available for the synthesis of complex oligosaccharides [65, 66]. One important goal of research in modern oligosaccharide synthesis is the development of an automated oligosaccharide synthesizer. Seeberger and co-workers described the first fully automated chemical solid phase synthesis of oligosaccharides using an appropriately modified peptide synthesizer [67]. Trichloroacetimide mannoside donor was loaded to Merrifield's resin through olefinic linker and selectively deprotected with NaOMe. By repeating the coupling-deprotection steps respectively, linear oligosaccharides compound (**55**) was synthesized in high yield and in a fully automated process (Fig. **15**). This method has also been applied to a branched tetrasaccharide, which is part of the cell surface lipophosphoglycan of Leishmania parasites, by the stepwise assembly from monosaccharides [68]. Seeberger and co-workers also introduced a new approach to facilitate the purification of oligosaccharides prepared by automated solid phase synthesis. This approach, called as "cap-tag methods", allows the removal of incompletely elaborated product obtained during synthesis [69].

7. MONITORING OF THE REACTION PROGRESS

Development of the quantitative methods to monitor the reaction progress on solid phase is quite important in the determination and optimization of reaction conditions. Cleavage of the product from solid support and analysis by TLC, to determine the reaction progress, is often time-consuming and very difficult. To solve this problem, several new methods have been developed.

Gated Decoupled ^{13}C NMR Spectroscopy

Wong and co-workers developed a non-destructive and quantitative monitoring method for solid phase sLe^x tetrasaccharide synthesis using ^{13}C-enriched linker and
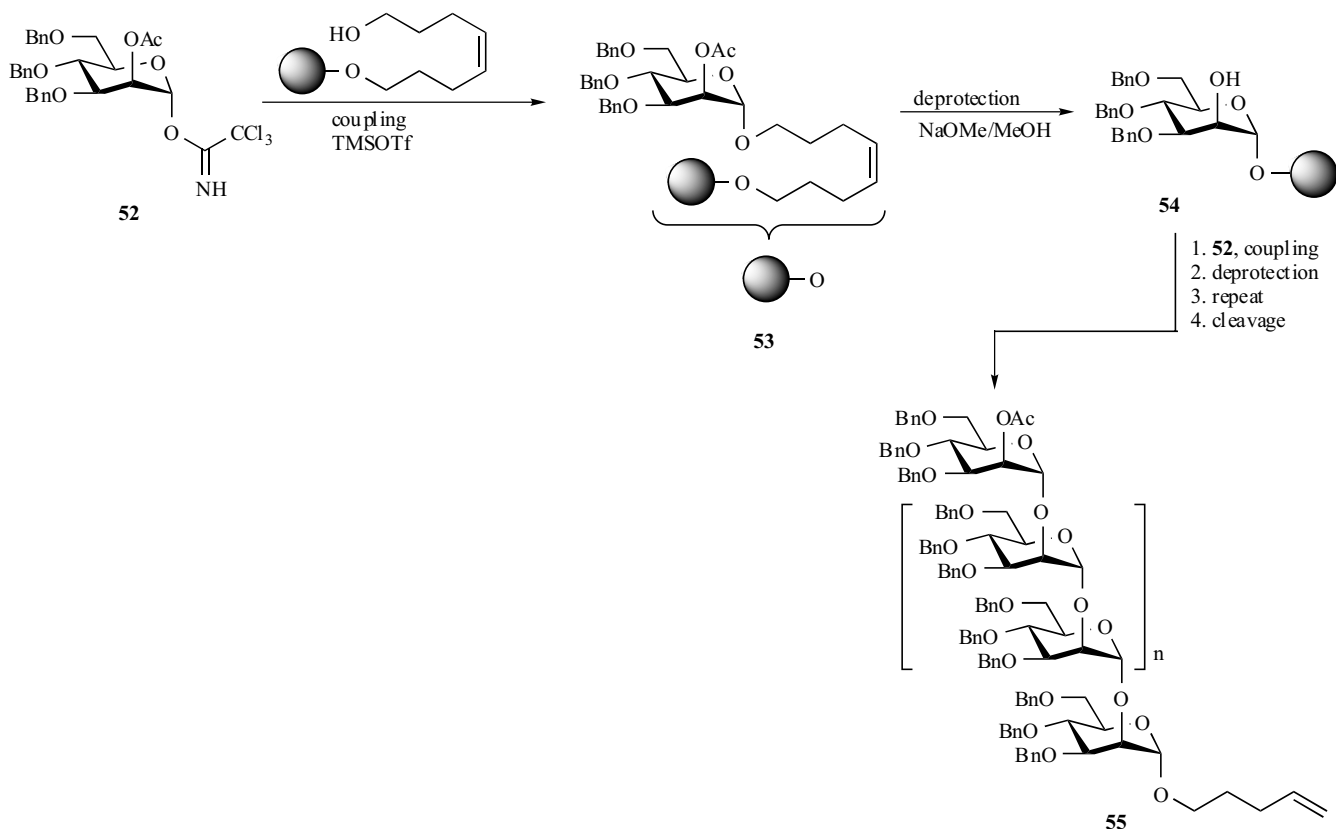

Fig. (15). Automated chemical synthesis of oligosaccharides.

protecting groups [70]. The ^{13}C-labeled acylsulfonamide linker was attached to the TentaGel resin as an internal integral standard and coupled with thioglycosyl donor bearing ^{13}C-enriched protecting group. By comparison of the protecting group and linker signal, quantitative monitoring was carried out on solid support. This method is particularly useful for a small quantity material and can be carried out in standard 5 mm NMR tubes.

High Resolution Magic Angle Spinning (HR-MAS) NMR Spectroscopy

HR-MAS has been used for the characterization of the crude product of solid phase trisaccharide synthesis [71]. Merrifield's resin-bound sugar molecule was examined by ^{1}H, ^{13}C and ^{1}H-^{13}C HMQC NMR. Spin-echo techniques were used to reduce the signal of the solid phase obtained in the ^{1}H NMR spectra. Several indications of the coupling reaction were identified using ^{1}H, ^{13}C NMR and the best results were provided by ^{1}H-^{13}C HMQC NMR.

Gel-Phase ^{19}F NMR Spectroscopy

^{19}F NMR spectroscopy for monitoring carbohydrate synthesis on solid support was developed by Kihlberg and co-workers [72]. ^{19}F NMR spectroscopy has several favorable properties, such as high sensitivity and the dispersion of ^{19}F resonances over a wide spectral range as a result of the high polarizability of the ^{19}F nucleus. Using a saccharide building block having fluorine-labeled protective groups and fluorinated linkers, they demonstrated that ^{19}F chemical shifts of compounds are useful for optimization of glycoside synthesis on ArgoGel resin.

8. CONCLUSION

In the area of combinatorial carbohydrate synthesis, many novel methodologies have been developed for the successful preparation of oligosaccharide and glycoconjugate libraries over past decade. Technology has also been developed for the monitoring of the reaction progress on solid supports and the identification of active carbohydrate ligands. Developments of the new functional linker systems and solid supports have also improved in the yield of target molecules. Recent advanced in solid phase oligosaccharide synthesis has also led to the development of an automated synthesizer. The oligosaccharide and glycoconjugate libraries obtained using the many technologies hold great promise particularly in the area of drug discovery. In addition, with advent of the post-genomic field of glycobiology, these libraries might lead to a better understanding of the biological importance of carbohydrate structural diversity.

ABBREVIATIONS

Ac	=	Acetyl
AG-Cl	=	ArgoGel-Cl
AG-NH$_2$	=	ArgoGel-NH$_2$
AG-OH	=	ArgoGel-OH
AgOTf	=	Silver triflate

Bn	=	Benzyl
Bz	=	Benzoyl
ClAc	=	Chloroacetyl
CPG	=	Controlled pore glass
DMTST	=	Dimethylthiosulfonium triflate
Fmoc	=	Fluorenylmethoxycarbonyl
HR-MAS	=	High Resolution-Magic Angle Spinning
Lev	=	Levulinyl
MALDI-TOF-MS	=	Martix assisted laser desorption/ionization time-of-flight mass spectrometry
MPEG	=	Monomethyl poly(ethylene glycol)
NBS	=	N-bromosuccinimide
NIS	=	N-iodosuccinimide
NMP	=	N-methylpyrrolidinone
P	=	Protecting groups
p-AMBA	=	para-Acyloxymethylbenzyliden acetal
PEG	=	Poly (ethylene glycol)
PMB	=	*p*-Methoxybenzyl
PS	=	Polystyrene
RGD	=	Arg-Gly-Asp
RRV	=	Relative reactivity value
SE	=	2-(Trimethylsilyl)ethyl
TBDPS	=	Tert-Butyldiphenylsilyl
TFA	=	Trifluoroacetic acid
TfOH	=	Trifluoromethanesulfonic acid
TMSOTf	=	Trimethylsilyltrifluoromethanesulfonate
TrBF$_4$	=	Triphenylmethylborontetrafluoride

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