Solid-Phase Synthesis of Oligosaccharide Drugs: A Review

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Abstract: Solid- phase synthesis is an approach, where synthetic transformation in carbohydrates are carried out with one of the reactant molecule attached to an insoluble material referred as polymeric support *via* linker. In recent years, it has been extensively accepted as fast and accurate method for the synthesis of biologically active oligosaccharides. This review attempts to focus on various methods of oligosaccharide synthesis by solid- phase including their applications.

Key Words: Carbohydrate, oligosaccharide, biologically active, solid- phase synthesis, linkers, protecting groups, glycosylating agents.

1. INTRODUCTION

Oligosaccharides play an important role in many biochemical recognition processes and synthetic analogs to these natural biopolymers could be used to study the influence or even control to these biochemical processes. Hence, they hold great potential as therapeutic agents. The limited availability of complex oligosaccharides is a reason for the major impediment in the study of carbohydrates.

In the recent years, development of efficient and yet simple procedures for the synthesis of oligosaccharides has been a major goal of carbohydrate chemistry. It is highly desirable to establish methodology for rapid oligosaccharide assembly. Several methods and strategies have been developed so far, which include synthesis in the solution phase leading to stereo controlled and high yield reactions. Chemical and enzymatic synthesis of oligosaccharides on soluble or insoluble polymeric support has also been reported. The advantages associated with the use of an insoluble polymeric support justify further efforts to improve the solid phase strategy.

The solid- phase synthesis has been demonstrated to be extremely valuable for routine preparation of oligopeptides and oligonucleotides. The potential usefulness of this procedure for the synthesis of other biomolecules is obvious. In past few years, the interest in solid-phase synthesis has increased dramatically due to the excitement engendered by the concept of combinatorial chemistry. The Solid-phase synthesis of oligosaccharides, when carried out combinatoriaLy, offers the possibility of easy access to a very large number of well-defined oligosaccharides. This can be used as potential tools in glycobiology [1-5].

2. PRINCIPLES OF OLIGOSACCHARIDE SYNTHESIS

A number of methods for the synthesis of oligosaccharides have been developed in the recent years which involve stereo controlled and high yield reactions [6]. One of the key difficulty in synthesizing oligosaccharides is ensuring the stereochemistry of the glycosidic linkages (anomeric centre) – the carbon-oxygen-carbon bridges between the sugar units and presence of multiple reactive sites Fig. (**1**) [7]. A glycosyl donor couples with acceptor at the anomeric centre resulting in either α or β stereochemistry [5]. Oligosaccharides can be synthesized chemically or enzymatically depending upon the reagent used.

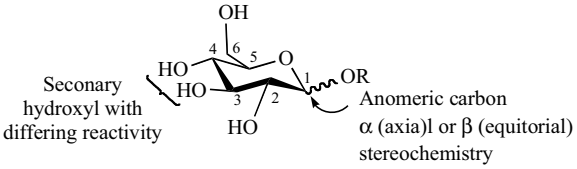

Fig. (1). Monosaccharide contains multiple reactive sites and an anomeric carbon.

2.1. Chemical Synthesis

Chemical synthesis, also called classical multi-step approach is advantageous as it provides homogeneous compounds in substantial quantity. Though the method provides almost unlimited flexibility to synthesize various structures including non-natural ones, it requires multi-step transformations, complicated protecting group pattern and tedious chromatographic purification after each step, hence making it time consuming and technically not demanding for large scale production [8].

2.2. Enzymatic Synthesis

This method is an alternative to chemical oligosaccharide synthesis and includes both glycosyltransferases (catalyst) and glycosidase where nucleotide sugars are the donors [9]. Glycosyltransferases show high regio- and stereo- selectivity along with elimination of protection and deprotection steps [10]. On combination with solid-phase techniques, the method offers particularly a simple way to synthesize oligosaccharides on a laboratory scale [8]. Availability of the glycosyl donor, substrate tolerance and cost factor are the limitations which hampers its use on large scale [10].

The substrate specificity of the glycosyltransferases can be modified, improved or changed in order to create new

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enzymes with new specificity for unnatural acceptor and/or donor substrates. The recent advent of glycosynthases – specifically mutated glycosidases that efficiently synthesize oligosaccharides but do not hydrolyze them represents a promising solution to these problems [11].

3. SOLID- PHASE SYNTHESIS OF OLIGOSACCHARIDES

Solution-phase synthesis is a slow process due to the need for iterative coupling and deprotection steps, with purification at each step [12-14]. Micro sequencing technique and amplification technologies similar to those used for peptides and oligonucleotides are now available. Library-encoding methods are also being developed simultaneously for some small molecules and applied to the complex molecules as well. A promising solution to all these problems is the use of solid- phase synthesis. The solid-phase synthesis techniques have proven to be useful methods for generating oligopeptides, oligonucleotides and, more recently, the oligosaccharides [15-17]. The Solid-phase oligosaccharide synthesis is also suitable for rapid and efficient generation of the constituents for a carbohydrate-based library. A comprehensive carbohydrate-based combinatorial capability requires the ability to link, attach, and modify sugars Fig. (**2**) [7].

3.2. Advantages of Solid- Phase Synthesis

The solid- phase synthesis includes the use of excess reagents to drive the reaction to completion. The complete automation of the synthesis process is also reported [3]. The ease of separation of resin-bound products eliminates the steps of tedious work-up and purification [8]. This Single donor approach reduces the number of glycosyl donor and simplifies the optimization of the chemistry required to synthesize an oligosaccharide library on the solid.

3.3. Problems in Solid- Phase Synthesis

The solid- phase synthesis of oligosaccharide is hampered by stereochemistry and concentrated functional group complexity because of the presence of multiple reactive sites on a monosaccharide. All these reasons emphasize the need of site- selective, stereo- selective and region- selective glycosylation.

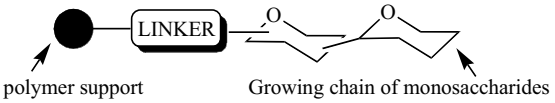

Fig. (3). Generalized scheme for polymer supported synthesis.

3.4. General Scheme: Fig. (3)

4. BASIC REQUIREMENTS FOR THE SOLID- PHASE SYNTHESIS

4.1. Support

Soluble and insoluble polymers, both having their own characteristic properties, are being used as a support in solid-phase synthesis.

4.1.1. Insoluble Support

Most of the oligosaccharide syntheses have relied on Merrifield's resin [polystyrene (PS), cross linked with 1% divinylbenzene] [18]. TentaGel is also used, due to its better swelling property Fig. (**4**) [27]. Recently S-PEG polymers (e.g., Agro Gel) have been introduced. Some novel type of PEG based resins that enhance both loading capacity and swelling property in a wider range of solvents, are commonly used in enzymatic and chemical reactions. These resins consist of primary and secondary (POE-POP) or exclusively of primary (SPOCC). These are used in the solid-phase syntheses.

Controlled pore glass (CPG) Fig. (**4**) found its way into the solid- phase synthesis literature early in the advent of DNA synthesis. Though CPG has been used extensively in oligonucleotide syntheses, it has seldom been used in oligosaccharide synthesis [19, 20].

Insoluble supports often require extensive reaction development where as soluble supports provide an advantage of easy work-up of solid- phase synthesis. MPEG are commonly used supports. Recently, a polydispersed soluble hy-

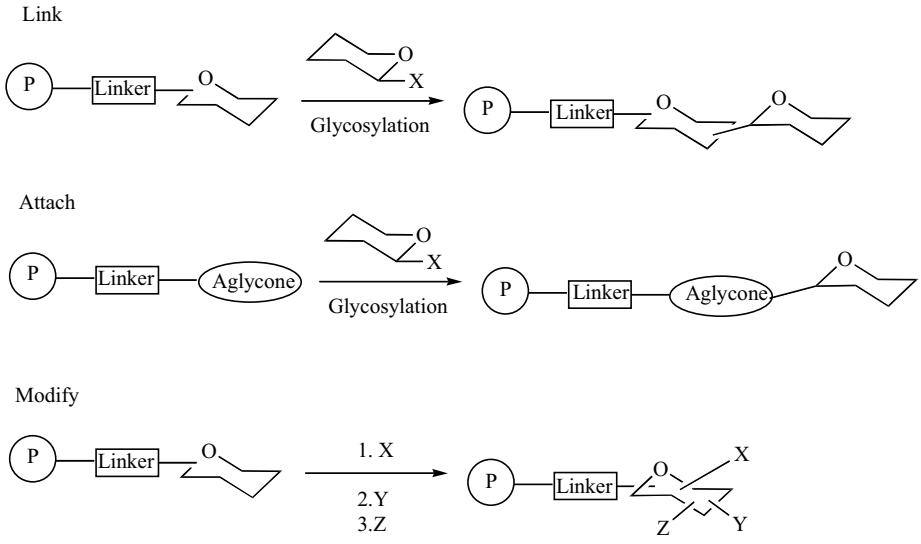

Fig. (2). A comprehensive carbohydrate-based combinatorial library.

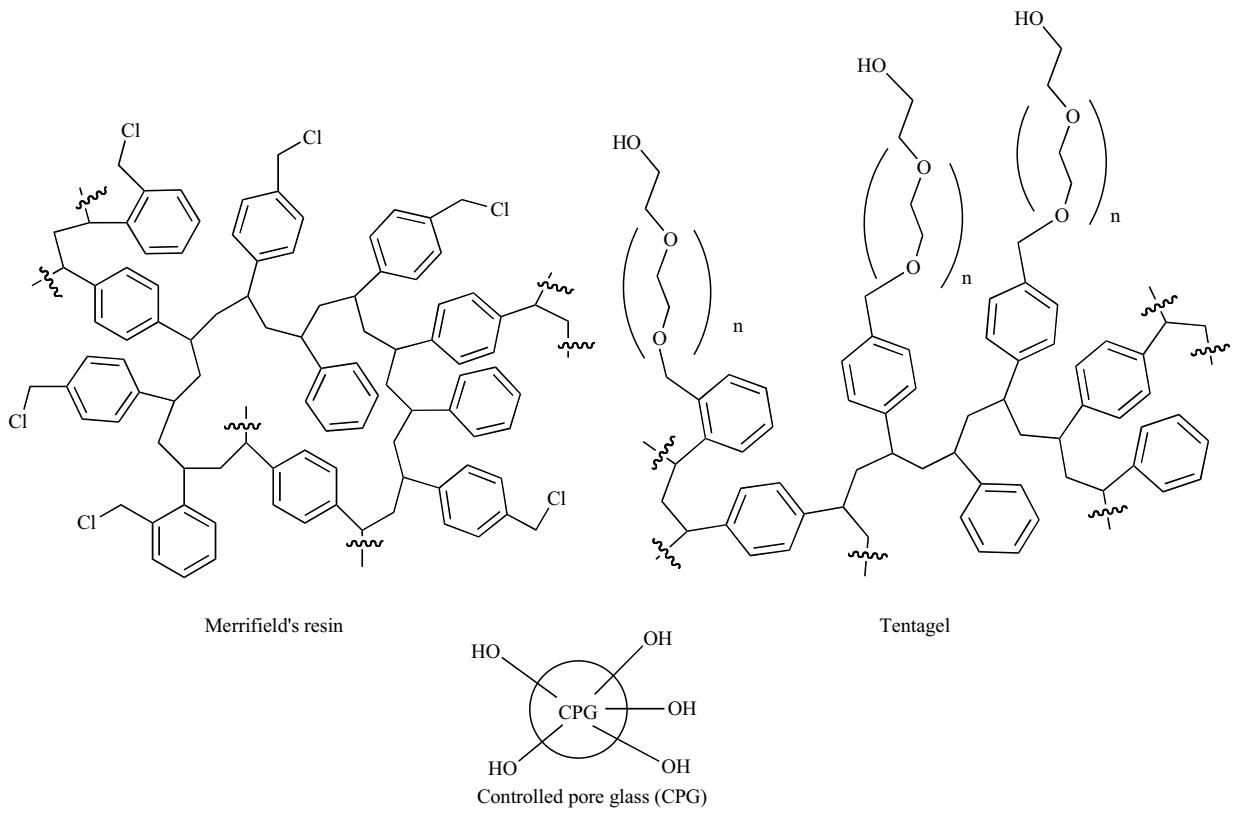

Fig. (4). Insoluble supports.

per branched polyester is also introduced Fig. (**5**). These are used in solution phase synthesis [21].

4.2. Linkers

The linker used to attach the first monosaccharide to the solid support and is of crucial importance. The chemical nature of this anchor determines all other protecting-group and coupling manipulations. Therefore, any protecting group used in carbohydrate synthesis, in principle, may serve as a linker for tethering a molecule to a solid support. These are stable to a wide range of reaction conditions, but can be cleaved under well-defined conditions, thereby liberating the molecule from the solid support. Preferably, the linkers are used to attach to the solid support which are either unprotected, partially-protected or fully-protected monosaccharides, oligosaccharides, or glycoconjugates [22].

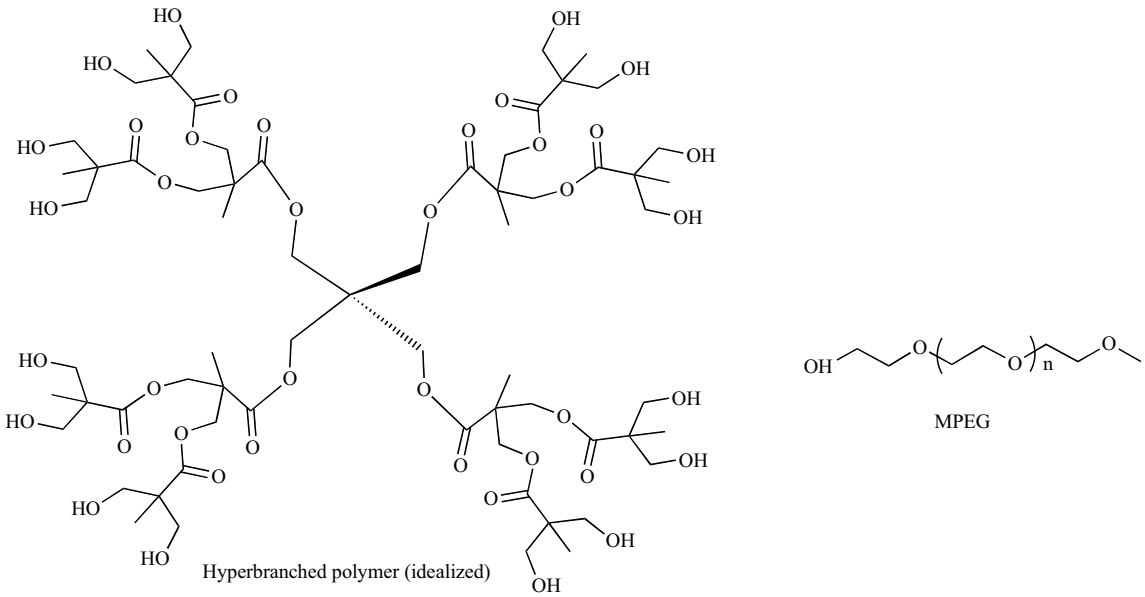

Fig. (5). Soluble support.

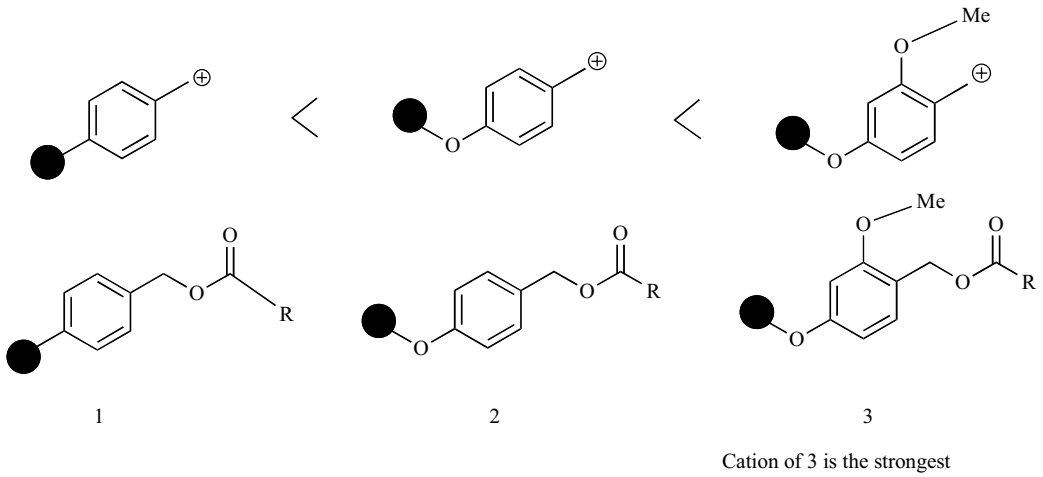

Fig. (6). Groups showing strength of formed cation.

4.2.1. Silyl Ether Linkers

Silyl linkers are of great promise because of their orthogonally cleavable property by fluoridolysis. Several fluoridolyzable silyl linkers have been utilized for the syntheses of oligopeptides and oligosaccharides [23].

4.2.2. Acid Labile Linkers

Strong acid is one of the most common cleavage conditions used in solid- phase synthesis. Volatile acids, such as I-IF or much more commonly used trifluoroaceticacid (TFA), allow easy removal of excess cleavage reagent by evaporation. The ability of an acid labile linker is dependent on the relative stability of the protonated linker versus the cation formed upon cleavage Fig. (**6**) [22].

4,5-dibromooctane-1,8-diol linker is an acid and base labile linker used for the synthesis of trisaccharide Fig. (**7**) [24].

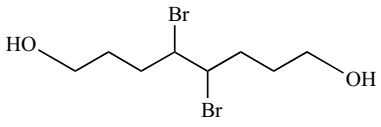

Fig. (7). Acid labile linkers.

4.2.3. Base Labile Linkers

A base-labile succinoyl linker, commonly used in automated DNA synthesis. Earlier this used to be employed in oligosaccharide syntheses on soluble supports. It has also been used to improve the synthesis of polylactosamine oligosaccharides [25]. The term 'base labile' includes two types of cleavages. The more common type actually involves nucleophilic addition/elimination, usually on an ester, whereas the less common type involves a base catalyzed reaction, such as elimination or cyclization [22]. The true base labile linker is demonstrated by the tertiary amine linker shown in Fig. (**8**).

4.2.4 Thioglycoside Linkers

Thioglycosides are stable to a wide range of activating conditions. They can be easily activated by thiophiles Fig. (**9**) [26].

4.2.5. Photo Cleavable Linkers

Photo labile linkers are not commonly used. The most common type is based on the nitro benzyl moiety Fig. (**10**) [27].

3-azidomethyl-4-hydroxybenzyl alcohol is a linker cleavable under neutral conditions and can be used in solid-phase synthesis of base-labile compounds. The linker is comprised of a moiety and the azidomethyl group in the linker and is readily converted to an amino methyl group by treatment with a phosphine reagent in the presence of water leading to an intramolecular cyclization to release the compounds. Using the linker, a base-labile dinucleoside methyl phosphate was synthesized on a highly cross-linked polystyrene (HCP) support and cleaved successfully from the resin without decomposition of the product Fig. (**11**) [28].

Linkers are also differentiated on the basis of their cleavage from polymer support e.g. linkers cleaved by oxidation, hydrogenation or olefin methasis.

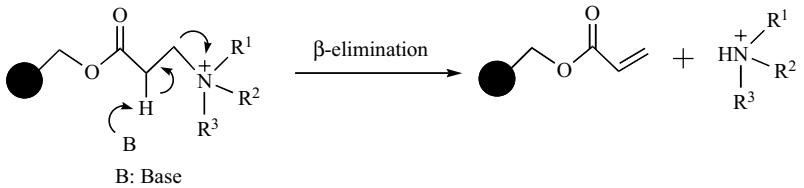

Fig. (8). Base labile linkers.

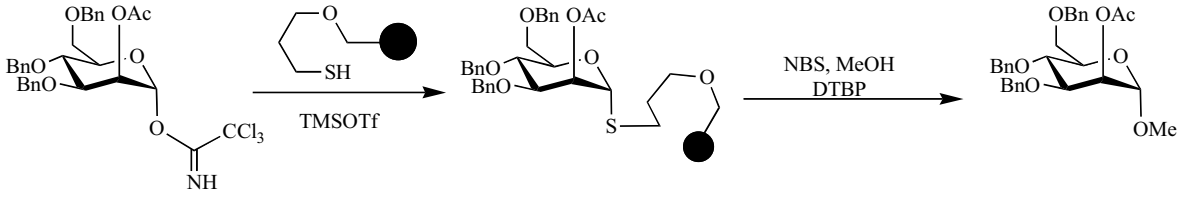

Fig. (9). Thioglycoside Linker.

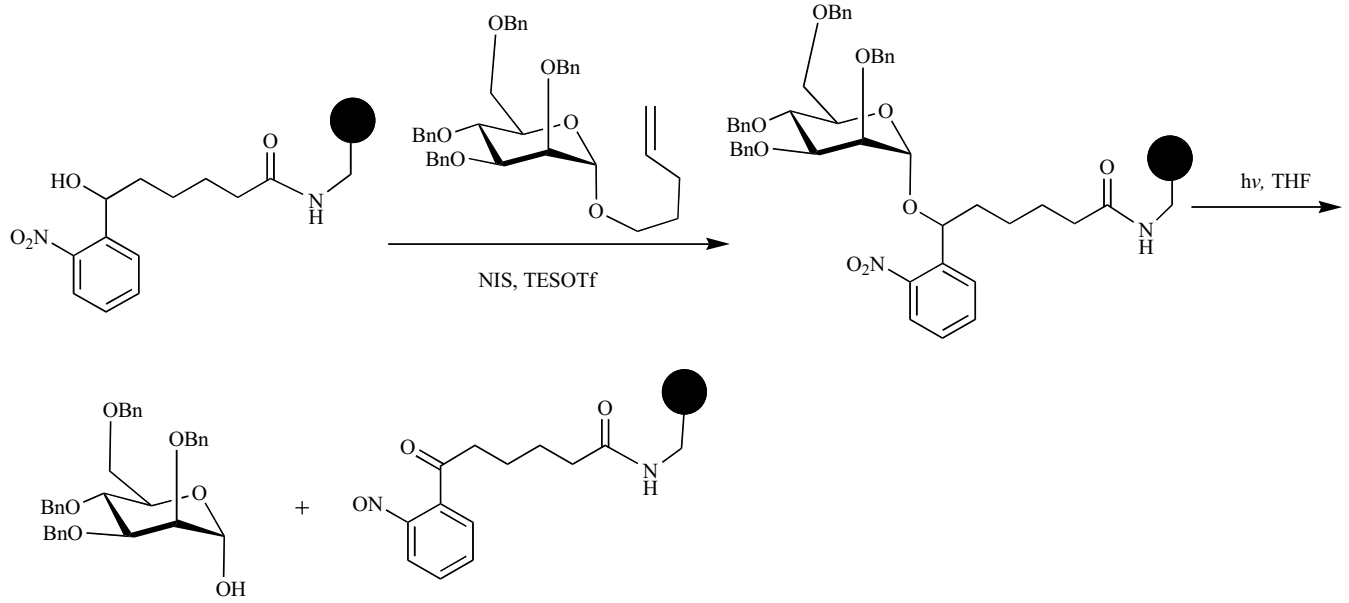

Fig. (10). Photo cleavable linker.

4.3. Protecting Groups

The multitude of hydroxyl groups present in the carbohydrates requires efficient orthogonal protecting groups that allow selective manipulation of a particular functional group of interest. Hence, careful synthetic planning with respect to the protecting group is required to be given prime importance.

4.3.1. Benzyl Ethers

Commonly used as a permanent protection. They are easily removed by catalytic hydrogenation, and provide potential selective cleavage [29].

4.3.2. Base Labile Protecting Groups

The most common base-labile group used for the protection of hydroxyl groups is the acetyl group. The most usual

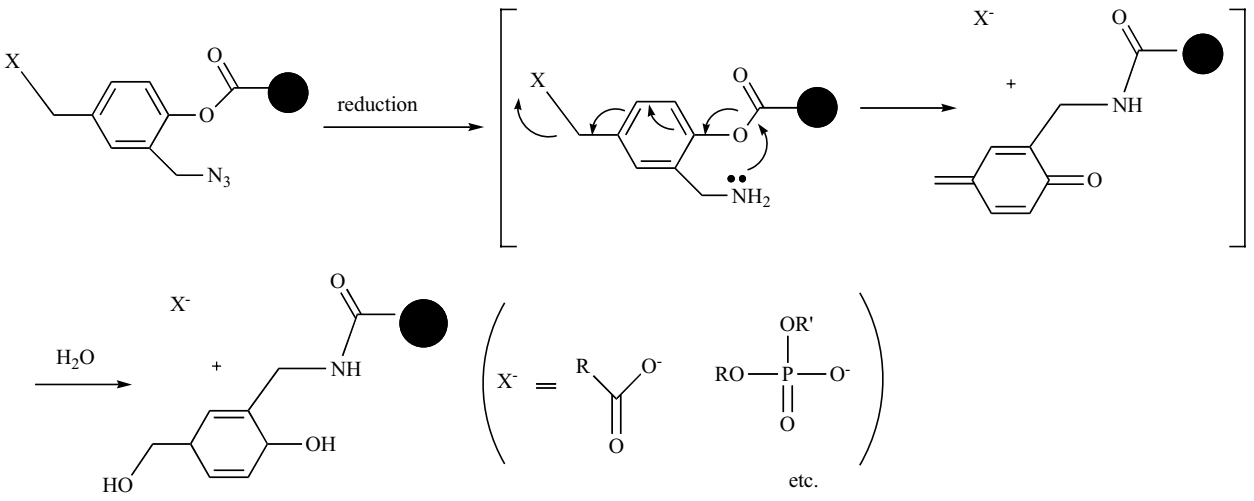

Fig. (11). Reaction mechanism for the cleavage of attached compounds from the linker.

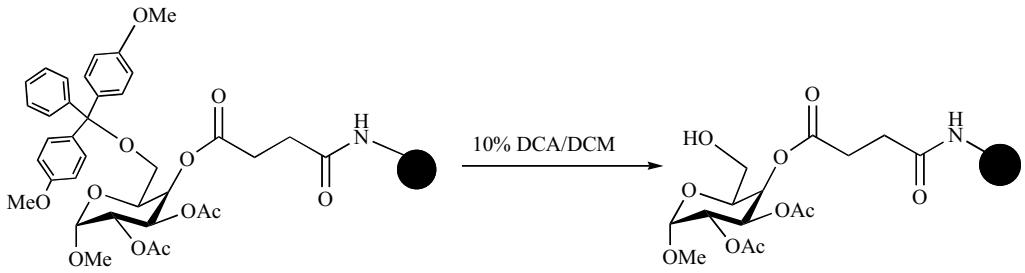

Fig. (12). Example of Acid labile protecting groups.

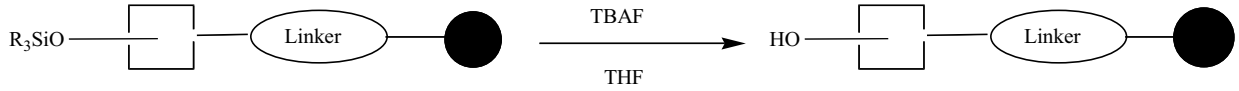

Fig. (13). Model reaction for Silyl ether protecting groups.

method of deprotection is the use of NaOMe-promoted hydrolysis. Benzoyl and pivaloyl esters are equally useful [30].

4.3.3. Acid Labile Protecting Groups

They have been used less frequently than base labiles because in many glycosylating reactions, acid conditions lead to loss of temporary protecting groups during coupling. The trityl family of protecting groups is widely used for the protection of hydroxyl groups in solid- phase organic synthesis (SPOS). The most widely used of these is the dimethoxytrityl (Dmt) group Fig. (**12**) [31].

4.3.4. Silyl Ether Protecting Groups

The silyl family of hydroxyl protecting groups has also found widespread use in solid-phase organic synthesis. They are usually introduced as preprotected building blocks Fig. (**13**).

Cleavage is performed most commonly with tetra butyl ammonium fluoride (TBAF). The use of hydrogen fluoride (HF) is reported, though it is usually confined to the instances where the linker used is not acid-labile. Some of the examples of Silyl ether protecting groups are ter-butyl dimethylsilyl (TBDMS), tri isopropyl silyl (TIPS), tri-ethyl silyl ether (TES) [30].

4.3.5. 2-(Allyloxy) Phenyl Acetic Acid

A new protecting group for oligosaccharide synthesis is shown in Fig. (**14**). It is orthogonal with the levulinoyl and acetyl ester and is capable of controlling the anomeric selectivity of glycosylations by neighboring group participation [32].

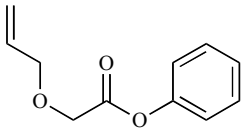

Fig. (14). 2-(Allyloxy). phenyl acetic acid.

4.4. Glycosylating Agents

Glycosylating agents are used for the formation of glycosidic linkages. Glycosylating agents developed during the past year have also proven useful.

4.4.1. Glycosyl Trichloroacetimidates

Glycosyl Trichloroacetimidates has been found to be extremely successful in solution-phase oligosaccharide synthesis than in solid- phase synthesis in terms of reactivity and selectivity. These donors can be activated under very mild conditions by catalytic amounts of TMSOTf and other triflates including dibutylboron triflate (DBBOTf) Figs. (**15,16**) [33].

4.4.2. Glycosyl Sulfoxide

Anomeric glycosyl sulfoxides are highly reactive glycosylating agents upon activation with Lewis acid promoters Fig. (**17**). Trifilic anhydride was used most commonly to induce facile reactions at -78 ^{o}C and fashion even difficult linkages with hindered acceptor [34, 35].

4.4.3. 1, 2-Anhydrosugars

1, 2-Anhydrosugars Fig. (**18**) are readily derived from glycal precursor and have been activated to fashion a variety of glycosidic linkages. The glycal assembly method has been the basis for extensive synthetic studies under the donor bound paradigm. Support bound glycal were readily converted to the corresponding anhydrosugars by epoxidation with dimethyl dioxirane (DMDO). This method has been very succefully used in the solid- phase synthesis of oligosaccharides [36, 37].

4.4.4. Thioglycosides

Thioglycosides are readily prepared from anomeric acetates or 1, 2-anhydrosugars and have been frequently used as glycosyl donor. Thioglycosides can be prepared on the large scale, stored even at room temperature over prolonged period of time, and can be selectively activated with a range of thiophillic promoters such as dimethylthiosulfonium triflate (DMTST), methyl triflate and trific acid [16]. With the use of thioglycosides, it has been possible to achieve the α- or β-selectivity in glycosylation reactions [38]. High toxicity of the activators is one of the drawbacks of thioglycoside donors on the solid support. Alkyl (aryl) thio groups appear to offer efficient temporary protection of the anomeric centre of a saccharide. In the earlier instances they were not used directly as glycosylating agents, but effectively converted to

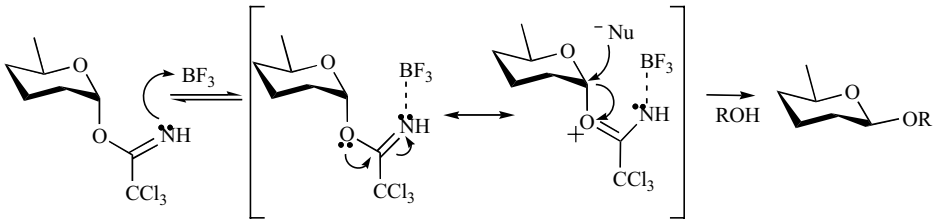

Fig. (15). Mechanism of reaction using glycosyl trichloroacetimidate as glycosylating agents.

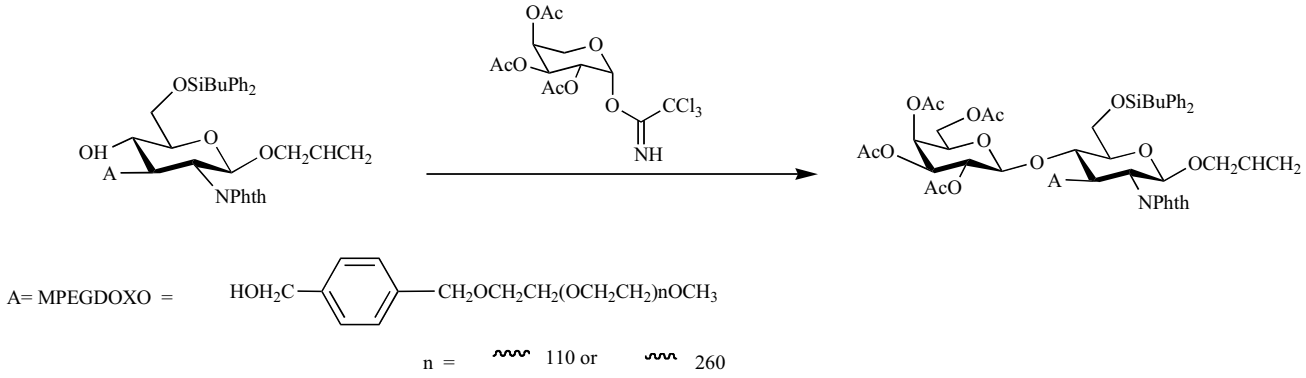

Fig. (16). Example of glycosyl trichloroacetimidate.

the classical glycosyl donors namely glycosyl bromide, chlorides using the respective halogen Fig. (**19**).

4.4.5. Glycosyl Fluorides

Glycosyl fluorides as glycosylating agents have the advantage of being more stable than the corresponding chlorides or bromides and are easier to handle. Promoters used for the activation of fluorides as glycosylating agents are tin (II) chloride in combination with silver perchlorate, lithium perchlorates, titanium fluorides, lanthanum perchlorates, and a number of silver compounds in combination with hafnocene and zirconecene complexes [16, 39].

4.4.6. N-pentyl Glycosides

These reagents are believed to operate by addition of iodide ion to the double bond of the n-pentyl group to gener-

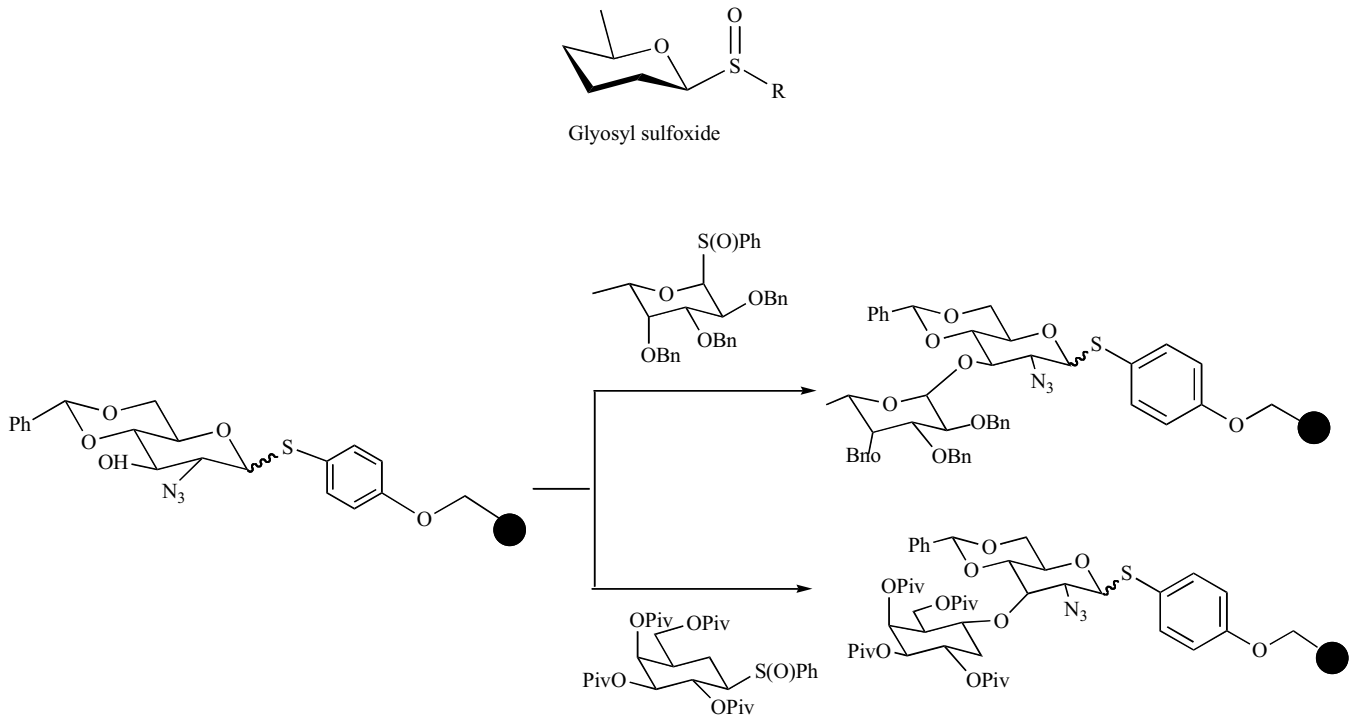

Fig. (17). Example showing glycosyl sulfoxide as glycosylating agents.

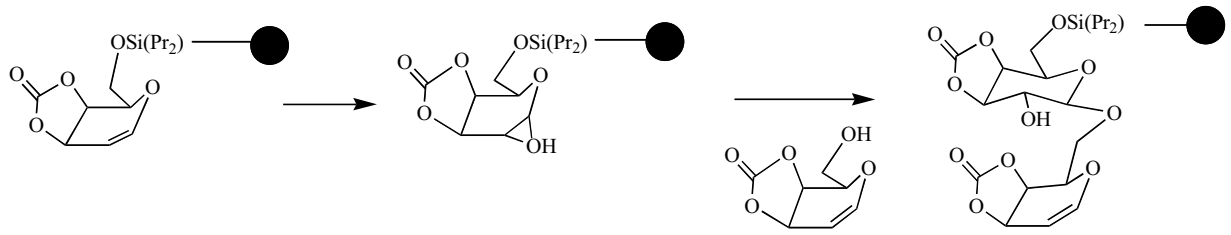

Fig. (18). Example showing use of 1, 2-Anhydrosugars.

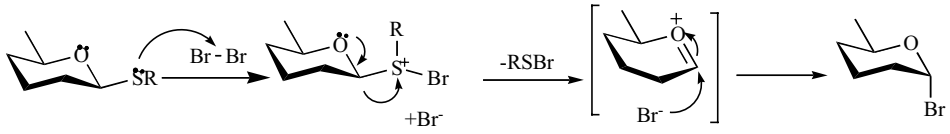

Fig. (19). Mechanism of conversion of thioglycoside into donors.

ate a cyclic iodonium ion which is attacked by the anomeric oxygen, leading to activation. They are also relatively simple to produce, by Fischer glycosidation reaction of pent-4-enyl alcohol with a free sugar under acidic conditions Fig. (**20**). High average coupling yields exceeding 90% and excellent α- or β-selectivity were achieved with the acceptor bound strategy Fig. (**21**) [40].

4.4.7. Glycosyl Phosphates

Glycosyl phosphates have been recently applied to the solid- phase synthesis of oligosaccharides, introducing a new and straight forward route for the preparation of these donors from glycal precursor. Glycosyl phosphates are extremely reactive glycosylating agents activated at low temperatures. Following solution phase studies, the compatibility of this methodology with solid- phase was explored. Using octenediol linker β-(1-4)-linked trisaccharide was readily assembled in 53% overall yield Fig. (**22**) [42-44].

5. METHODOLOGY

In solid- phase synthesis of oligosaccharides, the creation of a new glycosidic bond by coupling of the glycosyl acceptor with the glycosyl donor is the central feature of any such reaction. Selective deprotection of a temporary protecting

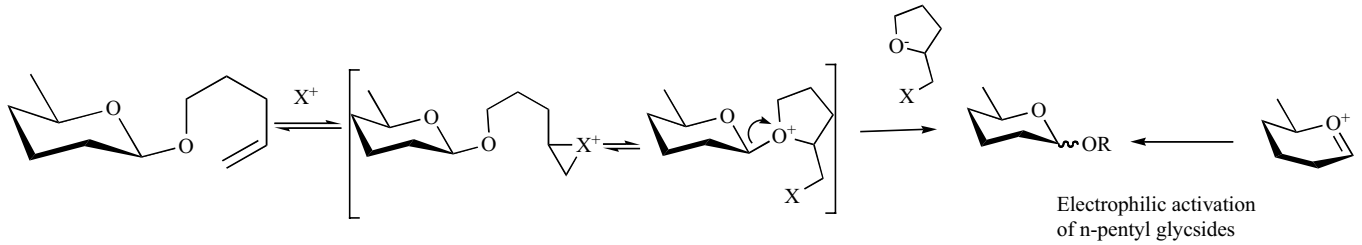

Fig. (20). Mechanism of reaction of N-pentyl glycosides.

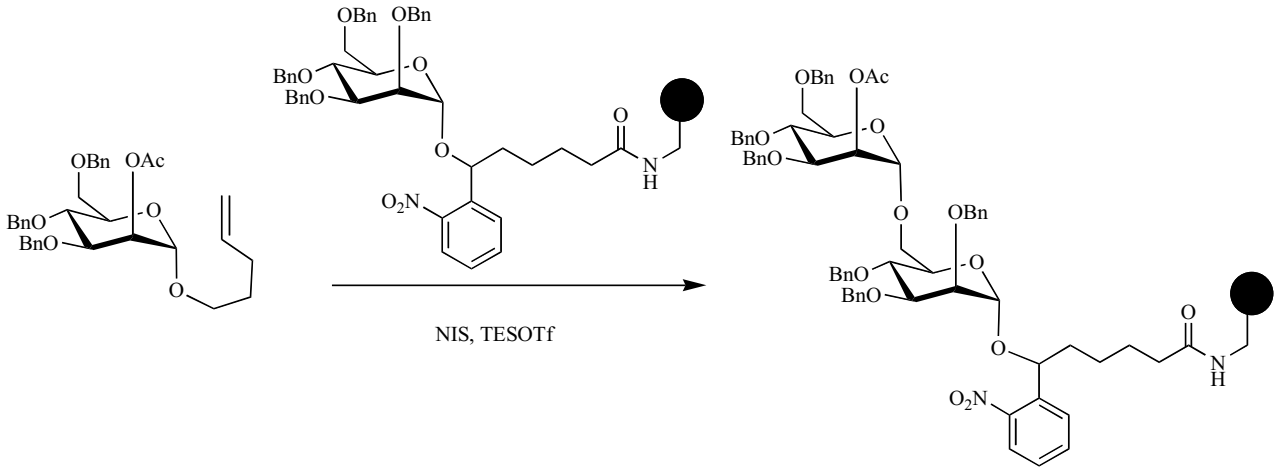

Fig. (21). Synthesis of disaccharide using N-pentyl glycosides as glycosylating agent [41].

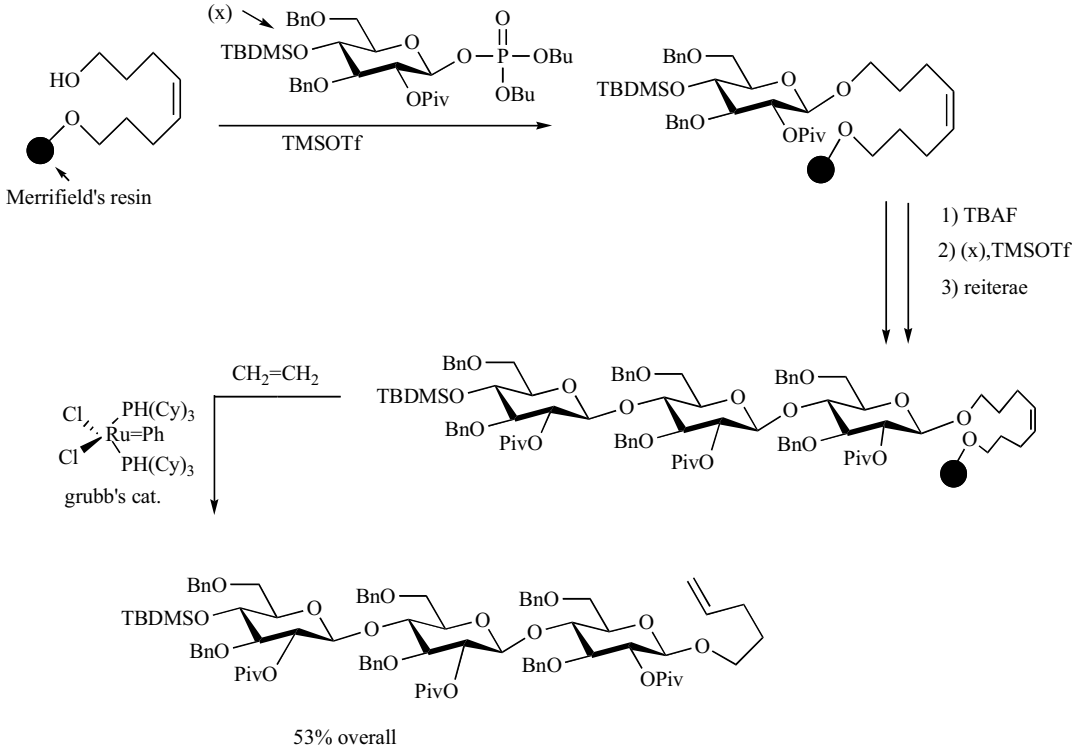

Fig. (22). Reaction of glycosyl phosphates as glycosylating agent.

group to liberate the free hydroxyl group will be subjected to the next coupling with a glycosyl donor [45]. Thus three general synthetic strategies are available at present.

5.1. Donor-Bound Glycosylation Strategy

When the non- reducing ends of the first carbohydrate moiety is attached to the polymeric support *via* an anchoring group, a glycosyl donor is immobilized Fig. (**23**).

The main drawback of the donor-bound strategy was that, most side reactions during glycosylations involve the glycosyl donor and any side reaction in the donor attached to the resin will provoke termination of chain elongation. The consequence is a reduction of the overall yield. However, an impressive array of complex oligosaccharides has been synthesized by Danishefsky and co-workers using the glycal assembly method under this strategy. In the following example a tetrasaccharide was synthesized using the same strategy Fig. (**24**).

5.2. Acceptor-Bound Glycosylation Strategy

The glycosyl acceptor is immobilized by fixing the anomeric position to the support [46]. Excess of donors are used. The overall yield is good and side products are washed away after each coupling Fig. (**25**). For this reason, the acceptor-bound approach has generated an immense interest in the solid-phase oligosaccharide synthesis. Acceptor-Bound Glycosylation by trichloroacetimidate method was shown in Fig. (**26**).

5.3. Bidirectional Strategy

Variations of above mentioned strategies lead to bidirectional synthesis plan. Elongation of the growing oligosaccharide in both directions requires two sets of orthogonal glycosyl donors Fig. (**27**) [47].

In the reaction scheme Fig. (**28**), first the acceptor containing a potential leaving group is bound to the resin. Reaction with the donor is performed under different conditions.

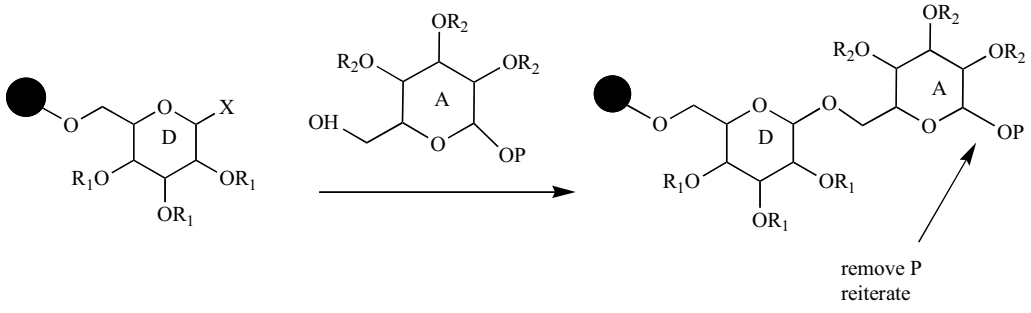

Fig. (23). Donor-bound glycosylation.

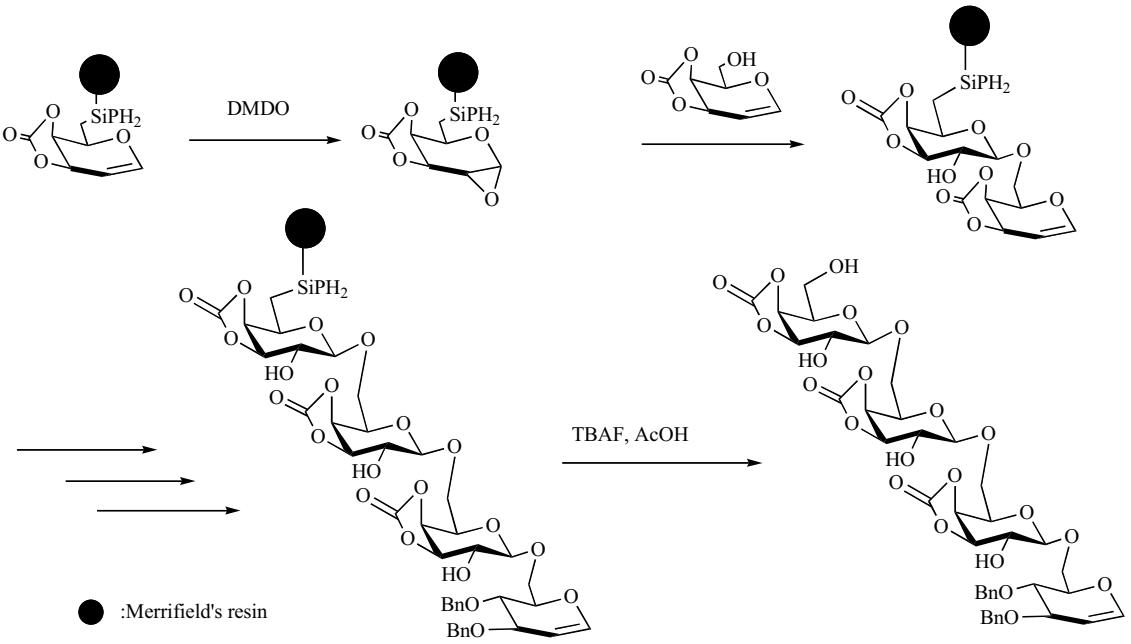

Fig. (24). Donor-bound glycosylation: 1,2-anhydroglycal method.

Then an acceptor is made to react with the initial anomeric leaving group. The choice of support matrix has an immense impact on the overall synthetic strategy, the choice of reagents, and the reaction conditions, also the price and the availability plays very important role in the selection.

At the final stage of oligosaccharide synthesis, all protecting groups are removed. In solid-phase oligosaccharide synthesis, additional operations are required for attachment of the first carbohydrate residue to the solid support *via* a linker molecule and for capping of the residual hydroxyl group after each step.

6. ENZYME BASED SOLID PHASE SYNTHESIS

Enzyme technology has also been applied to the solid-phase synthesis of glycopeptides Fig. (**29**). The use of enzymes to construct glycopeptides has focused primarily on

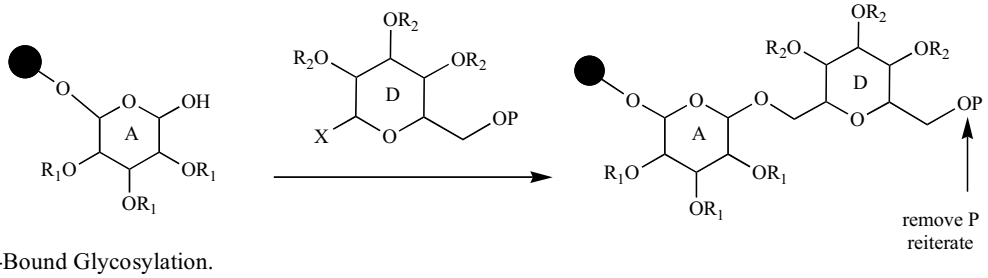

Fig. (25). Acceptor-Bound Glycosylation.

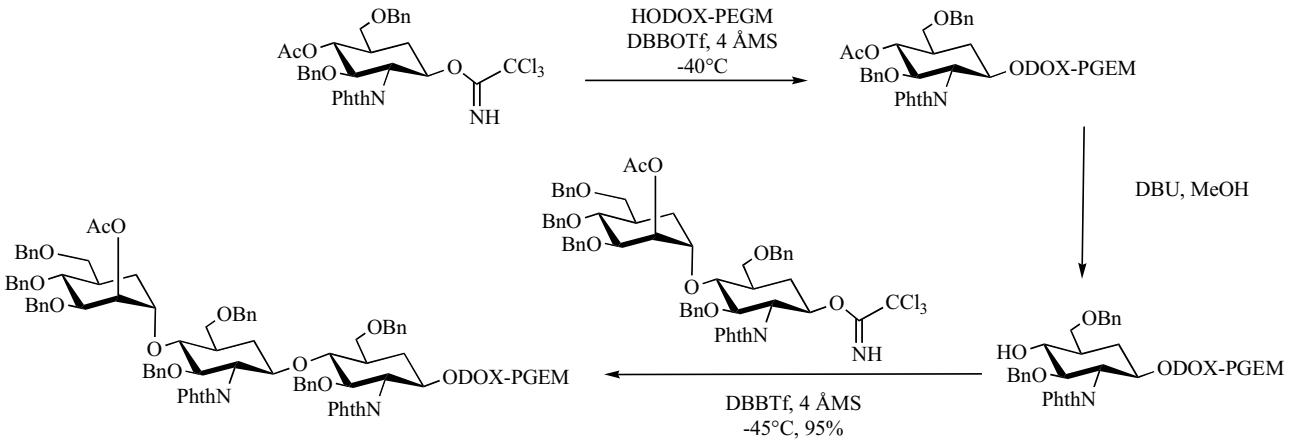

Fig. (26). Acceptor-bound glycosylation: trichloroacetimidate method.

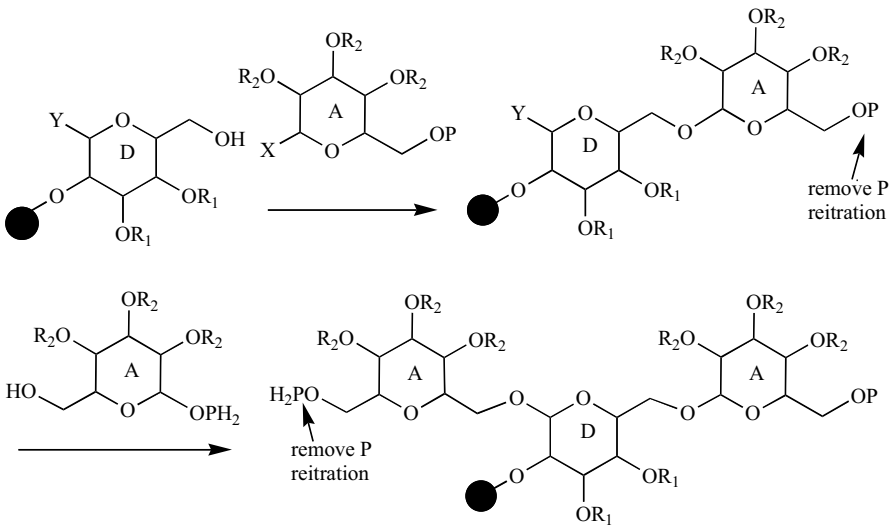

Fig. (27). Bidirectional strategy.

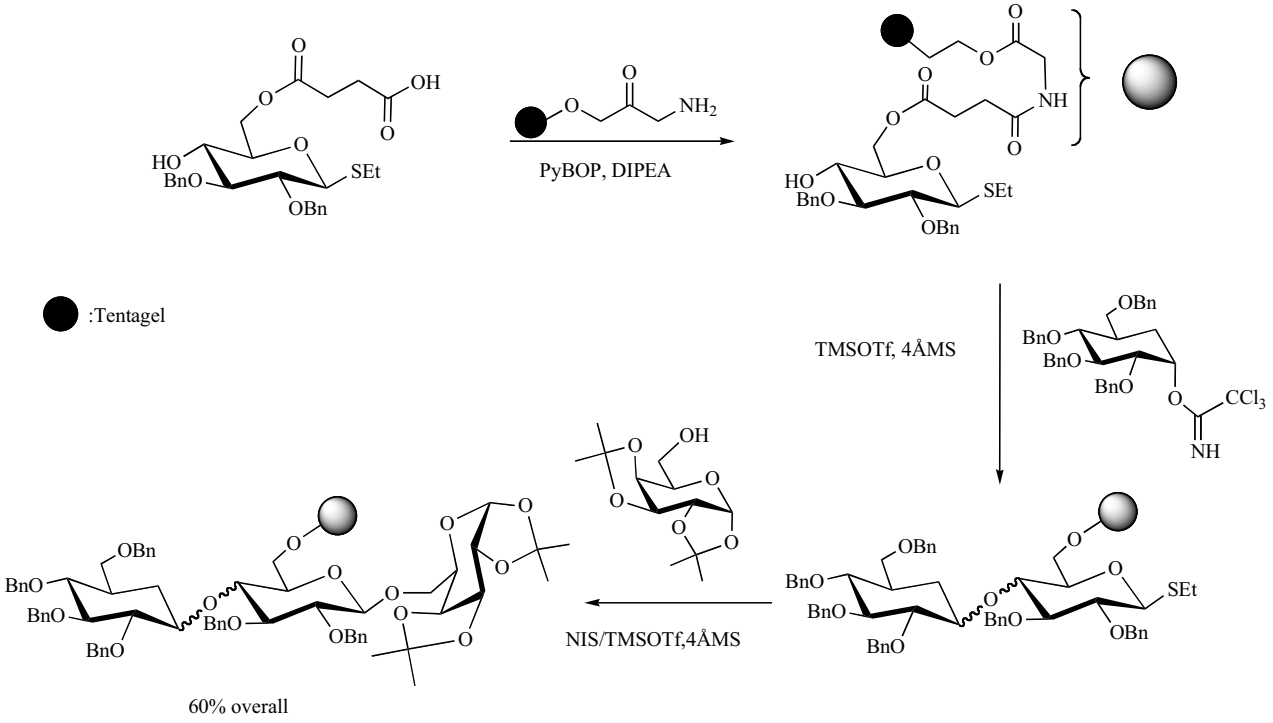

Fig. (28). Example of bidirectional strategy [48].

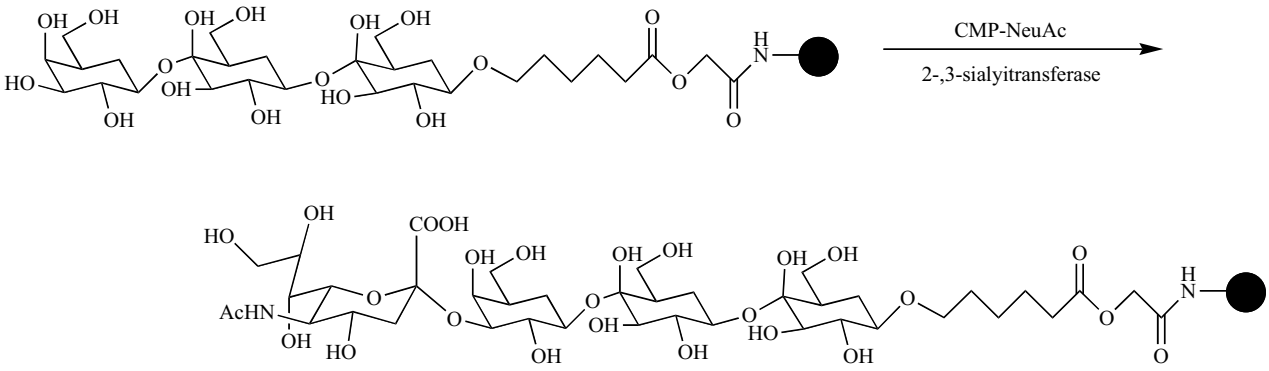

Fig. (29). Example of enzyme based solid phase synthesis.

the use of glycosyltransferases to extend the saccharide unit from a preformed glycopeptide fragment. Advantages associated with enzyme technology are the ability to provide efficient and stereo specific glycosidic bond formation with co-substrate, and the elimination of the need to use protecting groups on the carbohydrate moieties. The enzyme-compatible resins have been developed, reducing the general problem of the accessibility of the interior of polymeric resin beads to the enzyme. The enzyme-based solid-phase chemistry is still limited by low reaction rates and the availability of glycosyltransferases and their sugar-phosphate-nucleotide co-substrate [49, 50].

7. APPLICATIONS OF SOLID- PHASE SYNTHESIS

7.1. Synthesis of Heparin-Like Oligosaccharides on Polymer Supports

Heparan sulfate glycosaminoglycans regulate important biological functions by interacting with a variety of heparin-binding proteins Fig. (**30**). Heparan sulfate glycosaminoglycans constitute a family of closely related linear polysaccharide species which include heparin consisting of unsulfated and various sulfated sequences of alternating 1→4 linked D-glucosamine and L-iduronic or D-glucuronic acid units. These sequences are distributed in different domains along the linear polysaccharide chain. In order to facilitate the elucidation of the molecular basis of glycosaminoglycan-protein interactions, synthesis of heparin like oligosaccharides on polymer supports was done. A completely stereo selective strategy for the synthesis of these oligosaccharides in solution has been extended to the solid- phase using an acceptor-bound approach. Both a soluble polymer support and a polyethylene glycol-grafted polystyrene resin have been used and different strategies for the attachment of the acceptor to the support have been explored. The attachment of fully protected disaccharide building blocks to a soluble support was carried out through the carboxylic group of the uronic acid unit by a succinic ester linkage and the use of trichloroacetimidates as glycosylating agents and of a functionalized Merrifield type resin for the capping process was allowed for the construction of hexasaccharide and octasaccharide fragments containing the structural motif of the regular region of heparin. This strategy may facilitate the synthesis of glycosaminoglycan oligosaccharides by using the required building blocks in the glycosylation sequence [51-54].

7.3. Automated Solid-Phase Synthesis of a Branched Leishmania Cap Tetrasaccharide

The preparation of fully synthetic and semi synthetic immunogens that target leishmaniasis is based on the unique tetrasaccharide cap of the parasite's cell-surface lipophosphoglycan. In principle, any unique feature the parasite exposes on its cell surface may be used for the development of a leishmaniasis vaccine. Lipophosphoglycans which are ubiquitous on the cell surface of *Leishmania* parasites are composed of a glycosylphosphatidylinositol (GPI) anchor, a repeating phosphorylated disaccharide, and different cap oligosaccharides Fig. (**31**). In addition to establish a reliable new route for the synthesis of the *Leishmania* captetrasaccharide, Michael and Seeberger developed novel synthetic strategies that would provide advantage to the solid-phase paradigm. Solid-phase synthesis demonstrated that branched oligosaccharides of biological interest are accessible in good overall yields using glycosyl trichloroacetimidates and glycosyl phosphate donors in concert Fig. (**32**) [55, 56].

7.4. A Novel and Efficient Synthesis of a Dimeric Le^x Oligosaccharide on Polymeric Support

The synthesis of a dimeric Le^x oligosaccharide constitutes the preparation of the most complex structure using soluble polymeric supports. Also, this is the first example of a one-pot multistep glycosylation sequence performed on polymeric support using highly efficient strategy for the polymer supported synthesis of the dimeric Lewis antigen $Lewis^x$-$Lewis^x$ Fig. (**33**) [57].

(Le^x-Le^x) Lewis antigens are an important family of tumor associated antigens that offer promise for the development of cancer vaccines for breast, prostate, lung, colon, stomach, and ovarian cancer. These branched compounds

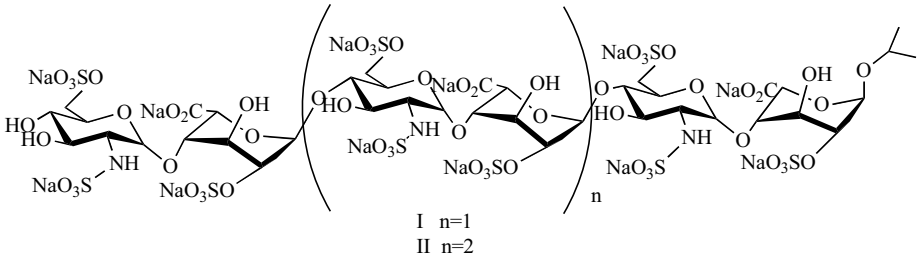

Fig. (30). Hexa and octasaccharide of the regular region of the heparin.

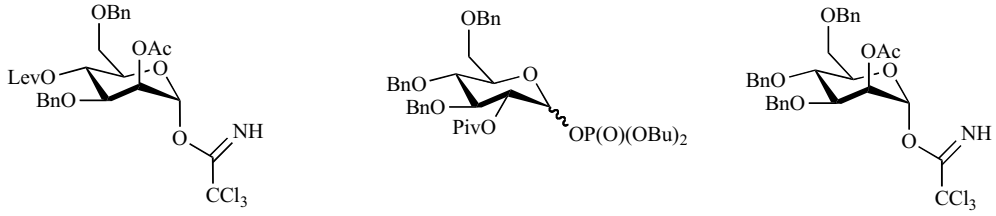

Fig. (31). Leishmania LPG.

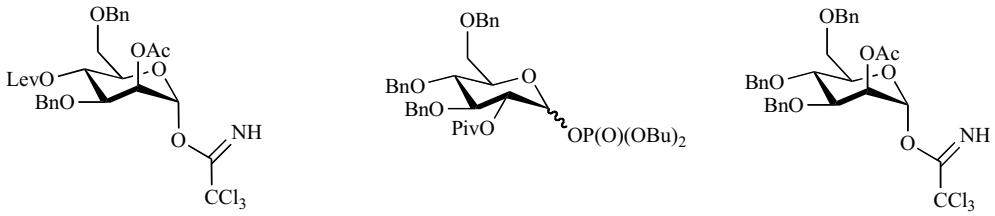

Fig. (32). Monosaccharide building blocks required for automated synthesis.

contain both α- and β-glycosidic linkages attached to hindered hydroxyls of a glucosamine moiety [58].

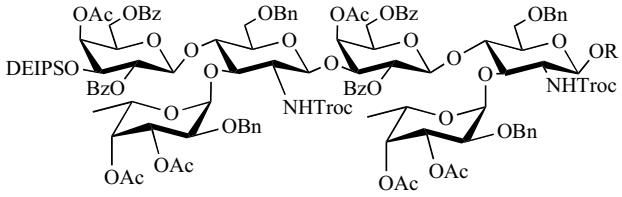

Fig. (33). Leishmania LPG Dimeric Le^x trisaccharide.

A strategy was adapted, whereby a polymer-bound Le^x trisaccharide was prepared, which could be converted into a glycosyl acceptor by selective removal of a temporary protecting group or into a soluble glycosyl donor by cleavage from the polymeric support followed by activation of the anomeric center. Coupling of the resulting glycosyl donor and acceptor followed by cleavage from the solid support should give the target hexasaccharide. This compound, which is branched and contains both α and β-glycosidic linkage, is one of the most complex saccharide ever synthesized on polymeric support [59].

7.5. Automated Solid-Phase Synthesis of Oligosaccharides

Traditionally, access to structurally defined complex carbohydrates has been very laborious. Although recent advancements in solid-phase synthesis have made the construction of complex oligosaccharides less tedious, a high level of technical expertise is still necessary to obtain the desired structures. A branched dodecasaccharide was synthesized through the use of glycosyl phosphate building blocks and an octenediol functionalized resin Fig. (**34**). The target oligosaccharide was readily obtained after cleavage from the solid support. Access to certain complex oligosaccharides has now become feasible in a fashion much like the construction of oligopeptides and oligonucleotides [60, 61].

One of the reasons why automated solid-phase synthesis of oligosaccharides is not currently carried out by a larger number of groups is the fact that the synthesis instrument is not yet commercially available.

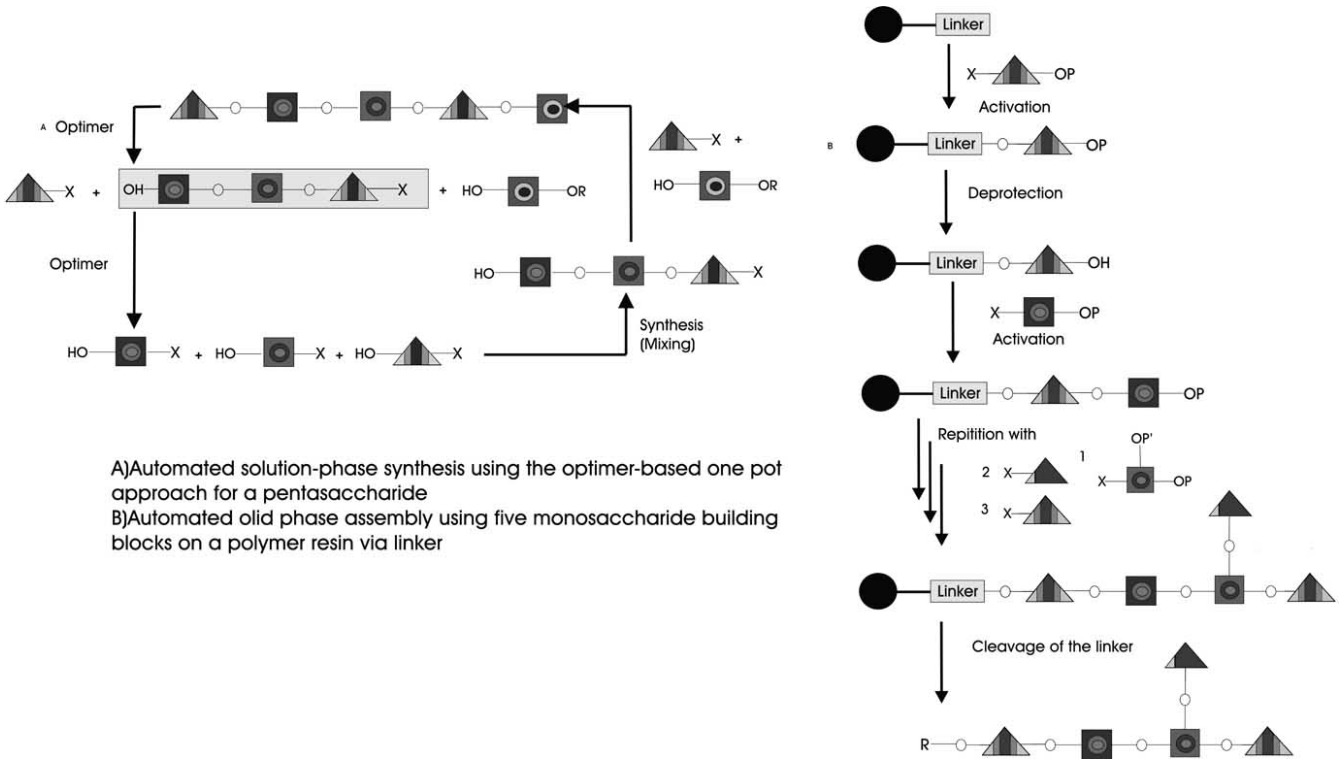

Fig. (34). Schematic representation of strategies for automated oligosaccharide assembly.

7.6. Synthesis of the Human Breast Tumor (Globo-H) Antigen Hexasaccharide Using the Programmable Reactivity Based One-Pot Strategy

The one-pot synthesis of the tumor-associated carbohydrate antigen Globo H was done by Burkhart F. *et al*., Fig. (**35**) and they also demonstrated the potential of the Optimer TM strategy for synthesis planning. An epitope found on the cell surface of breast, prostate, and ovarian cancer**,** is another promising approach towards the automation of part of the synthetic process [62-64].

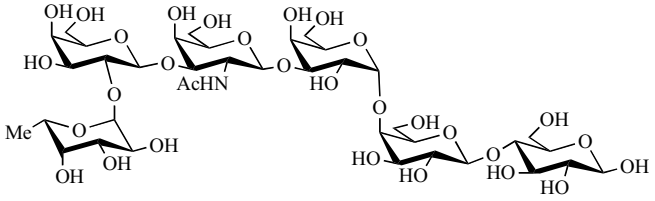

Fig. (35). Human breast tumor (Globo-H). antigen hexasaccharide.

7.7. Carbohydrate-Based Anticancer Vaccines

For the first time, the synthesis of a glycopeptide construct containing three different tumor-associated carbohydrate antigens (Globo H, Tn and Ley) was reported. Constructs of this type may offer the possibility to develop carbohydrate-based vaccines incorporating multiple antigens. Immunological evaluation of this compound is currently in progress [65].

7.8. Polymer-Supported and Chemoenzymatic Synthesis of the *Neisseria meningitidis* Pentasaccharide

The *Neisseria meningitides* pentasaccharide is shown in the Fig. (**36**). Its successful polymer-supported synthesis was carried out by Yan F.Y. *et.al*. The polymer polyethylene glycol monomethylether (MPEG) and the linker dioxyxylene (DOX) were used with a lactose-bound acceptor to improve the purification process [66]. This pentasaccharide is the upstream terminus of the *N. meningitides* lipo-oligosaccharide (LOS), and it has been shown to play an important role in the pathogenesis of disease caused by these organisms [67].

7.9. Solid-Phase Synthesis of Oligosaccharides and On-Resin Quantitative Monitoring Using Gated Decoupling 13C NMR

A general strategy for solid-phase oligosaccharide synthesis capable of nondestructive quantitative monitoring has been developed. The synthesis was carried out on TentaGel using thioglycosides as glycosylating agents and dimethylthiomethylsulfonium triflate as the activator. An acylsulfonamide linker was introduced to cleave the oligosaccharide from the resin. The solid-phase reactions were monitored quantitatively by using the inverse gated decoupling technique of ^{13}C NMR, where two ^{13}C-enriched markers were used to monitor the reactions: one was ^{13}C-enriched glycine incorporated as a part of the linker and as an internal standard, and the other was a ^{13}C-enriched acetyl group used as a protecting group of the glycosylation reagent [68].

7.10. Solid-Phase Enzymatic Synthesis of a Sialyl Lewis^x Tetrasaccharide on a Sepharose Matrix

A radically different approach to oligosaccharide synthesis is by using enzymes (e.g., glycosyltransferases) acting as glycosylating catalysts. These give regio and stereo specific glycosylations, without protecting groups on the monosaccharides. Glycosyltransferase catalyzed glycosylations, in combination with solid-phase techniques offers a particularly simple way to synthesize natural oligosaccharides on a laboratory scale.

Thiopyridyl Sepharoses with different linker arm lengths were prepared from epoxy Sepharose. The thiopyridyl Sepharoses were reacted with the glucosamine derivative giving GlcNAcSepharoses with different linker lengths. A GlcNAcSepharose with a long linker was then used in solid-phase synthesis of a sialyl Le^x tetrasaccharide Figs. (**37**, **38**). The three required enzymes (galactosyl, sialyl and fucosyltransferase) and nucleotide sugars were reacted consecutively with the GlcNAcSepharose, leading to the cleavage from Sepharose with DTT, and the free sialyl Le x tetrasaccharide derivative was obtained in a 57% total yield after purification [69].

7.11. Automated Synthesis of an Oligosaccharide Malaria Vaccine

An automated synthesis process of hexasaccharide malarial toxin I is currently under development as a malaria vaccine candidate Fig. (**39**). Using a combination of automated solid-phase methods and solution-phase fragment coupling, the target glycosylphosphatidylinositol was assembled in a matter of days, compared with several weeks for a comparable solution-phase synthesis [70].

Tetrasaccharide was accessed on solid- phase using four readily available trichloroacetimidates mannose building blocks Fig. (**40**). The automated synthesis was carried out on an automated oligosaccharide synthesizer using octenediol functionalized Merrifield resin. Hewitt, Daniel and Seeberger demonstrated a new method for rapid access to malarial toxin.

7.12. Solid-Phase Synthesis of Human Salivary Mucin-Derived O-Linked Glycopeptides

Short glycopeptides derived from salivary mucin have been synthesized in order to delineate the O-glycosylation

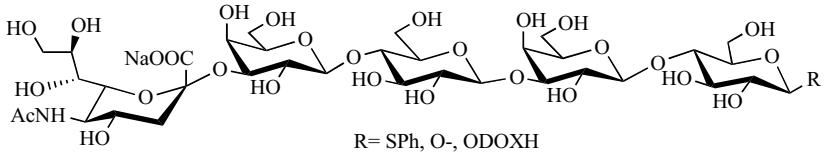

Fig. (36). *Neisseria meningitidis* pentasaccharide.

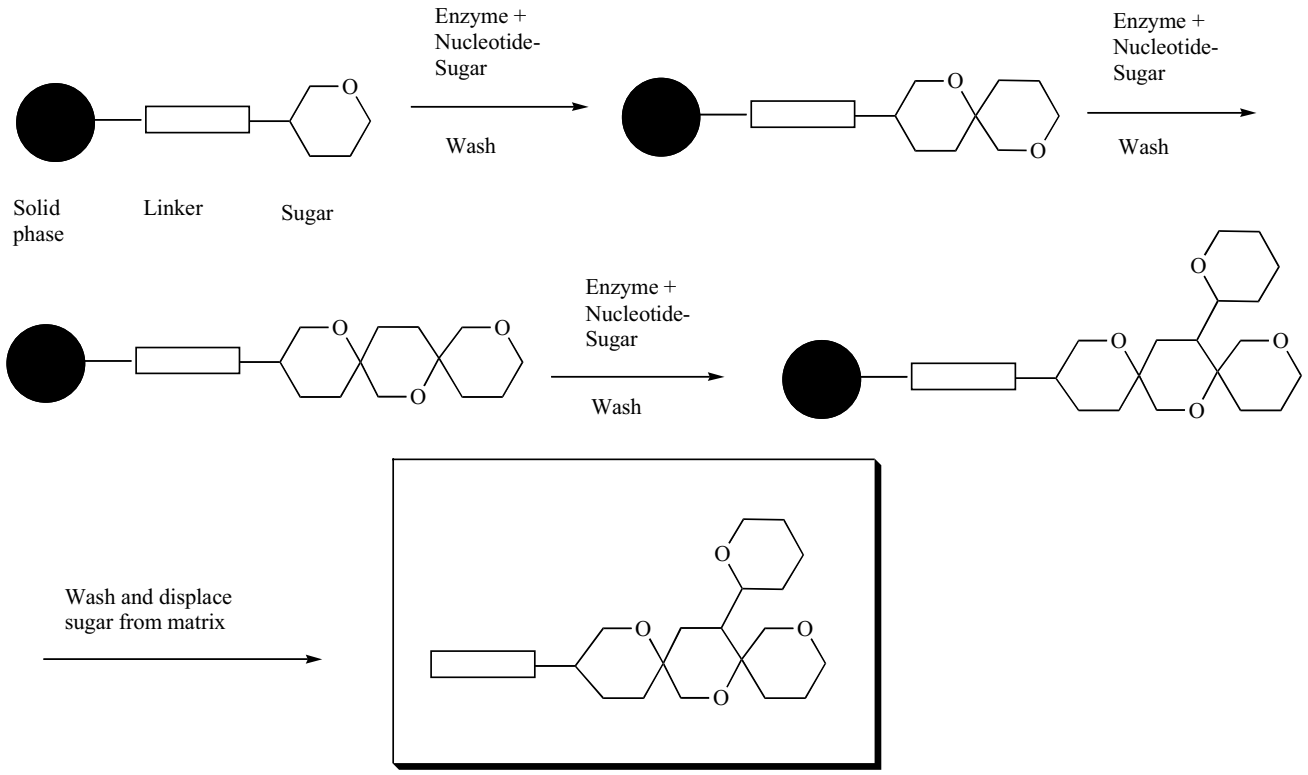

Fig. (37). Typical Solid-phase enzymatic oligosaccharide synthesis.

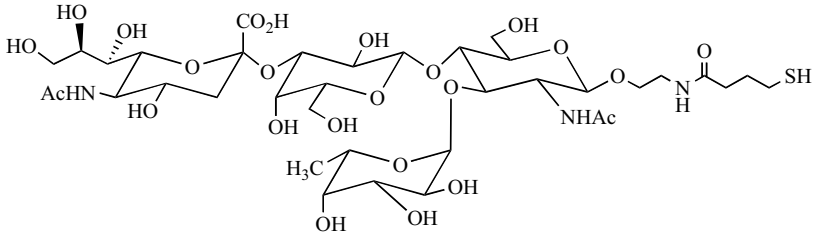

Fig. (38). Lewis x Tetrasaccharide.

pattern, that play an important role in the biological activity of mucin [71].

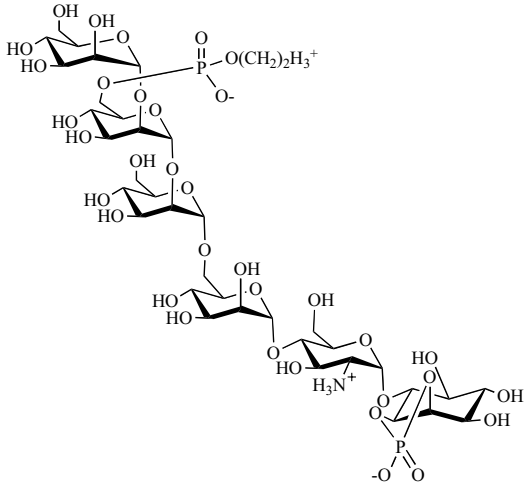

Fig. (39). Hexasaccharide GPI Malaria toxin I.

7.13. Solid-Phase Synthesis of Sialyl Tn Antigen

A disaccharide, namely sialyl Tn Fig. (**41**), is known as a tumor-associated antigen present in glycoproteins expressed

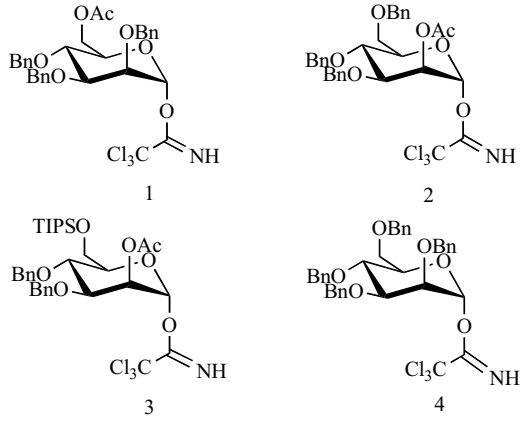

Fig. (40). Trichloroacetimidate mannose building blocks (1-4).

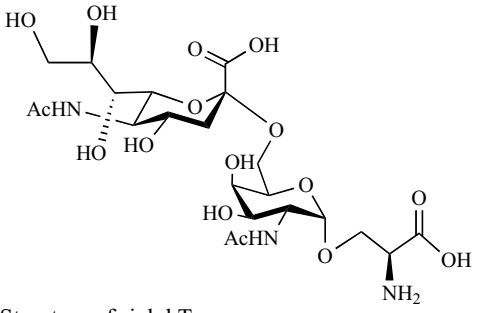

Fig. (41). Structure of sialyl Tn.

on the surface of cancer cells and is also found in the envelope glycoprotein gp 120 of the human immunodeficiency virus (HIV) [72]. A synthetic cancer vaccine [73] and a potential target for HIV immuno intervention based on sialyl Tn antigen has thus been a target of investigations [74].

Solid-phase synthesis of sialyl Tn antigen with Kenner's acyl sulfonamide linker was carried out. The acyl sulfonamide bond was found to be stable under glycosylation reactions using dimethyl (methylthio) sulfoniumtriflate (DMTST) as a promoter and basic conditions used for the removal of protecting groups. At the end of the synthesis, the sulfamyl group of the linker was activated by treatment with (trimethylsilyl) diazomethane to provide an N-methyl-N-acyl sulfonamide. The acyl group was displaced with hydroxide to give the corresponding precursors of sialyl Tn antigen and its anomeric isomers, which were deprotected to afford the target molecules [74].

CONCLUSIONS

Carbohydrates remain elusive and exciting targets for organic chemists. Solid-Phase methodology has deeply impacted the way for the synthesis of oligosaccharides. Automation has just begun to accelerate the field of glycobiology and the drug discovery process. We can expect sustained interest in this field to act as a driving force for innovative synthetic solutions and progress towards "full automation" new building blocks and methodologies to access all possible linkages, accelerated protocols for the coupling and deprotection of oligosaccharides, robotic systems capable of synthesizing multiple carbohydrates in parallel and highthroughput usage including combinatorial libraries and micro arrays.

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