Chemical O-Glycosylations: An Overview

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Dedicated to Prof. Dr. Richard R. Schmidt, University of Konstanz, Germany

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Chemical O-glycosylation

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The development of glycobiology relies on the sources of particular oligosaccharides in their purest forms. As the isolation of the oligosaccharide structures from natural sources is not a reliable option for providing samples with homogeneity, chemical means become pertinent. The growing demand for diverse oligosaccharide structures has prompted the advancement of chemical strategies to stitch sugar molecules with precise stereo- and regioselectivity through the formation of glycosidic bonds. This Review will focus on the key developments towards chemical O-glycosylations in the current century. Synthesis of novel glycosyl donors and acceptors and their unique activation for successful glycosylation are discussed. This Review concludes with a summary of recent developments and comments on future prospects.

1. Introduction

Carbohydrates, or sugars, have been deemed an important weapon in solving many of nature’s mysteries. They are the most diverse and the most abundant biomolecules on earth. Apart from their energy-storage functions, they were only assumed to serve as the structural and protective elements in the plant, bacterial, and animal cell walls. It was only in the later part of the 20th century that the involvement of carbohydrates in various other biological processes became apparent. The renaissance witnessed over the past few years in the field of glycobiology shows that glycoconjugates have enormous significance in various biochemical processes, such as molecular recognition, cell–cell interaction, immunological recognition, transmission of biological information, and so forth. Proper scientific knowledge of the mechanism of these biological processes, in turn, leads to the study of various new applications in biomedical research. These applications invariably lead to the advancement of modern day science, thereby increasing its fundamental interest to scientists. Thus, the demand for homogenous carbohydrate samples and their derivatives for biological research have increased extensively over the past decade.

In nature, carbohydrates exist as polysaccharides, glycoconjugates, or glycosides. But, the isolation of pure carbohydrate samples in sufficient amounts is difficult and cumbersome. In such cases, chemical syntheses of the relevant glyco-structures become pertinent. For the chemical synthesis of complex carbohydrate molecules, the main challenge is to build glycosidic linkages connecting the monomeric units with proper stereo- and regiochemical orientation. Over the last few decades, many new and sophisticated procedures have been established for the successful synthesis of complex oligosaccharides. Thus, development of new methodologies for chemical glycosylation has emerged as an active area of research. More and more intriguing glycosidic bond syntheses are being standardized. However, achieving complete stereocontrol over glycosylation remains an eluding area of carbohydrate chemistry.

Hence, optimizing the reaction conditions has long been the basic theme in carbohydrate synthesis.

Various types of novel glycosyl donors for glycosylation reactions have been synthesized and implemented. Different activation strategies have been developed for the successful assembly of oligosaccharides from protected building blocks. Solid-phase oligosaccharide synthesis has also made significant progress and is of high interest, as the process avoids the necessity to purify the intermediates. Automated oligosaccharide synthesis has also been developed, which has given a new edge in the field of synthetic oligosaccharide chemistry.

Fundamental concepts of glycosylation have been covered in a wide range of Review articles. In this Review, we aim to indicate all of the different types of donors and their activation protocols, as well as the different types of O-glycosylation processes developed after the onset of the 21st century. We will primarily stick to all newly developed methodologies of O-glycoside formation reported in the 21st century.

2. General Aspects

2.1. Historical Background

Glycosylation, with all of its complexity, has long been a topic of research. The end of the 19th century witnessed the chemical formation of the first aryl and alkyl glycosidic bond formulated by Michael (p-methoxy phenyl β-D-glucopyranoside) and Fischer (methyl α-D-glucopyranoside), respectively. Following their lead, Koenigs and Knorr formulated a more controlled and modified glycosylation protocol. New methodologies are still being developed with more advanced variations in the mechanistic pathways. Later Zemplén and Gerecs and subsequently Helferich and Wedemeyer illustrated the principle of the metal(II) salt-induced removal of leaving groups. With the general standardization of glycosyl protocols, efforts were made to control the stereochemical outcome of glycosylations. The requirement for the efficient syntheses of the two anomeric stereoisomers, 1,2-cis and 1,2-trans glycoside led to more advanced and detailed studies to introduce variation in the mechanistic pathways. The works of Lemieux et al. as well as Ness and Fletcher instigated the importance of the nature of the protecting groups, especially at the C-2 position for its correlation with the stereoselective outcome of the glycosylations. This observation was further documented by Hashimoto et al and Fraser-Reid et al. whereby the concept of the ‘armed–disarmed’ approach was initiated, claiming other linkage at the C-2 position to be arming, leading to the 1,2-cis
glycoside, whereas an acyl linkage at the same position yielded the 1,2-trans glycoside by virtue of the disarming property and neighboring group participation. Roy et al. introduced the ‘active’ and ‘latent’ thioglycoside donors that further expanded the concept of selective anomeric reactivity. The latent-active glycosylation strategy was further utilized by Boons and Isles to form trisaccharide libraries, using vinyl and allyl donors with selective reactivity. Furthermore, Ogawa and co-workers investigated the possibility of using orthogonal glycosyl donors and implemented the ‘orthogonal glycosylation strategy’ in the synthesis of various oligosaccharides.

The search to find the ideal conditions for an effective glycoside formation revealed various modifications in the already established protocols. Varied promoter systems were implemented, newer activating groups established, and more versatile protecting groups were introduced. With further advanced control on the glycosidic linkages and reaction conditions, one-pot glycosylation also came into vogue. More advances in computational chemistry and subsequent kinetic studies aided in solving the mystery behind the mechanism of glycosylation to a considerable degree. But, despite all of these efforts in the refinement of reaction conditions, the recognition of a single general procedure to describe chemical glycosylation in its entirety is yet to be accomplished.

2.2. Anomeric Effect

It was between 1955 and 1958 that Edward and Lemieux first defined the ‘anomeric effect’, based on the stereochemistry of the C-1 carbon of the pyranose ring, that is, the anomeric carbon. It is also referred to as the Edward–Lemieux effect. It was originally defined as the tendency of an electronegative substituent attached to the anomeric carbon to reside in the axial position. However, further studies have revealed various physical interpretations behind it.

A popular and widely accepted theory is based on molecular orbital interaction, employing the hyperconjugation of the nonbonding electron pair on the ring oxygen atom with the vacant σ* orbital of the C–X bond, thereby stabilizing the axial configuration. Studies have, thus, led to the hypothesis that the dipole–dipole interaction and electrostatic interaction form the bulk of the reasoning behind the conformational preferences of the carbohydrates; whereas, the hyperconjugation is only a minor factor. In view of the explanations, the axial stereoisomer represents the thermodynamical-
ly controlled product, whereas the equatorial isomer represents the kinetically controlled product (except for sugars with axial 2-OH groups like β-mannose and l-rhamnose, where the axial product is both kinetically and thermodynamically controlled) (Figure 1c).

2.3. Mechanistic Pathways

For proper, methodical chemical synthesis of glycosides, the most important phenomenon involved is called glycosylation. Chemical glycosylation is the coupling of the two monomeric sugar units, with each other or with other aglycons, through the formation of a new linkage, known as the glycosidic bond. The linkage may be of different types depending on the linking heteroatom. Herein, we primarily deal with O-glycosides. However, other heteroatomic glycosides such as C, S, and N glycosides are also widely known in literature. Complete categorization of chemical glycosylation as an S_{N}2 or S_{N}1 reactions was tedious, as each theory brought forth many evidences in their support.

For a more generalized mechanistic pathway (Scheme 1), the donor 7 is first pre-activated with a leaving group attached to its anomic hydroxyl group. The addition of an electrophilic promoter activates the leaving group of the donor to form complex 8. The next step initiates the formation of oxacarbenium ion 9 in its flattened half-chair conformation. After this, nucleophilic attack by acceptor 10 occurs, thereby leading to the required glycoside formation 11. However, the attack of the acceptor can occur via two pathways, owing to the structural property of the oxacarbenium intermediate. The attack of the glycosyl acceptor from the bottom of the sugar ring yields the formation of alpha (α) or 1,2-trans glycoside 11b. Attack of the glycosyl acceptor from the top of the sugar ring yields beta (β) or 1,2-trans glycoside 11a. Furthermore, manipulation of the stability of the intermediate oxacarbenium ion remains responsible for the anomerization of the compound.

Presence of a participating acyl group in the C-2 position governs the stereochemistry of the glycosidic bond formed. The oxacarbenium ion formed owing to the departure of the leaving group further (Scheme 2) interacts with the acyl group in the C-2 position to form the acyloxonium ion complex 14 by virtue of neighboring group participation. This enables the acceptor 10 to attack the anomic carbon from only one side, that is, the 1,2-trans side to form the 1,2-trans glycoside 15.

For the formation of 1,2-cis glycoside 5, the presence of a non-participating group is required in the C-2 position of the donor molecule. Thus, increasing the stability of the oxacarbenium ion and its various electronic and steric factors may lead to the formation of the more thermodynamically controlled product, caused by the action of the anomeric effect described above (Figure 1c).

Thus, starting from Michael's vision of glycosylation, where the glycosyl acceptor was converted into its corresponding salt, to Fischer's use of hemiacetals as the donor, we have come a long way via Fraser-Reid's idea of ‘armed–disarmed’ glycosylation. However, the details of the newer and the more developed concepts in decoding the mystery behind the mechanism of chemical glycosylations are beyond the scope of this Review.

3. Glycosylations

3.1. Glycosyl Halides

3.1.1. Glycosyl Bromides and Chlorides

In 1901, Koenigs and Knorr and Fischer and Armstrong independently introduced the concept of glycosyl halides as donors, where glycosyl chlorides and bromides were reacted with alcohols in the presence of AgCO₃ or Ag₂O. Since then, there have been extensive studies on the preparation of glycosyl halides, including the treatment of free sugar units with AcBr⁻[35] (AcBr–AcOH,[36] treatment of per-acetylated sugars with HBr–AcOH[37] (BiBr₃–Me₅SiBr,[38] and subsequent conversion of the sugar hemiacetals under modified Mitsunobu conditions with PPh₃/N-halosuccinimide[39] or PBr₅.[40] However, the harsh conditions in the established protocols often cause difficulties in extensive oligosaccharide synthesis, where acid-labile protecting groups in substrates are a general phenomenon.
Hence, in the search for more environmentally friendly and greener methodologies, photocatalytic synthesis came into vogue.\(^{[41]}\) Likewise, while Mizuno and co-workers established the photo-irradiative phase-vanishing method for α-glycosyl bromide synthesis\(^{[42]}\) by applying UV light (irradiation at 352 nm), Xue and co-workers introduced the more versatile visible-light-mediated halide synthesis.\(^{[43]}\) However, in the latter case, an additional photocatalyst, tris(2,2’-bipyridyl)ruthenium(II) chloride [Ru(bpy)_3Cl_2], was essential. Various protocols for the activation of glycosyl bromides are in use for the synthesis of 1,2-cis glycosides with the help of Ag salts (AgOTf, Ag_2CO_3, AgClO_4, etc.) or Hg salts [Hg(CN)_2, HgBr_2, HgCl_2, etc.].\(^{[44]}\) The versatile use of Ag_2O for glycosyl bromide activation was shown by Beejmohun et al. in the synthesis of monolignol glucosides.\(^{[45]}\)

Owing to the restrictions offered by glycosyl halides in terms of their moisture stability and handling, glycosylations with glycosyl halides as donors require optimized low temperatures and inert conditions. Requirement of such conditions was usually managed with various limitations. However, the present century saw the emergence of room-temperature ionic liquids (RTILs), which came as a ready solution for all of the constraints. Malhotra and co-workers\(^{[46]}\) implemented a series of RTILs and molten halide salts for the glycosylation of acetabromo-α-D-galactose with p-nitrophenol. By virtue of their unique ionic combination and their probable role in the glycosylation pathway, ILs hold the promise of being an extensively used solvent and activator in the future. It has been shown that the activation of glycosyl bromides neat in ILs in the presence of Ag_2CO_3 gave β-products in moderate yields.

In terms of reactivity, glycosyl bromides have lower reactivity than glycosyl iodides.\(^{[47]}\) However, glycosyl bromides exhibit less stability than glycosyl chlorides. In 2006, Oscarson and co-workers reported the total synthesis of a tetrasaccharide O139 through the activation of glycosyl bromide over its corresponding thioethyl glycoside acceptor by using AgOTf as the promoter.\(^{[48]}\) This selective activation process, in turn, marks the initial steps towards more convenient one-pot oligosaccharide synthesis. This method also promotes the selective activation of glycosyl chlorides with the help of AgOTf in the presence of thioglycosides.\(^{[49]}\) Recently, Nitz and co-workers reported the activation of unprotected glycosyl chloride donors by using p-toluene sulfonylhydrazide and it has been proven to be more reactive than its protected counterpart.\(^{[50]}\)

### 3.1.2. Glycosyl Iodides

The limited shelf-life of glycosyl iodides has always rendered them disadvantageous as donors for glycosylations. This restriction requires the in situ formation of glycosyl iodides and their subsequent involvement in glycosylation. In this context, many research groups have demonstrated reliable methods for generating glycosyl iodides from per-O-acetylated sugars through treatment of trimethylsilyl iodide (TMSI)\(^{[51]}\) or HI equivalents.\(^{[52]}\)

The instability of TMSI was addressed by Koreeda and co-workers who utilized the possibility of the synthesis of anhydrous HI by the reaction of solid iodine with a thiol component in an organic solvent.\(^{[52a]}\) In line with this, Field and co-workers also generated TMSI in situ by reacting hexamethyldisilane (HMDS) with iodine.\(^{[53]}\) The same group further modified their established procedures in 2004, by presenting the synthesis of per-O-acetylated glycosyl iodides 16 from unprotected free sugars 15 in significant yields (Scheme 3).\(^{[54]}\) This protocol, equipped with a no-solvent system and inexpensive reagents, proved a versatile method, enabling the effective formation of glycosyl iodides.

Field and co-workers later studied the stability, reactivity, and characterization of glycosyl iodides as donors.\(^{[55]}\) Over extensive studies, it has been observed that, although glycosyl iodides are usually less effective for glycosylations, many research groups have illustrated its unique advantages over other glycosyl halides in terms of efficiency and stereospecificity.\(^{[56]}\) Thus, by using these glycosyl iodide donors, various glycosides have been synthesized. Here, the work of Gervay-Hague and group deserve special mention. They have illustrated how glycosyl, galactosyl, and mannosyl iodides can react with oxa (20) and thio cycloalkanes to yield O-glycosides, 21 and 22, respectively, with high β-selectivity (Scheme 4).\(^{[57]}\) They also demonstrated the selectivity with decreasing temperature, where the β-isomer was dominant at lower temperatures (Table 1).\(^{[58]}\) The most noteworthy point in this study was the realization that reactions did not require pre-activation of the donor, which is exclusive for glycosyl iodides.

Glycosyl iodides can also be selectively activated in the presence of other donor systems. In this respect, Gervay-Hague and co-workers demonstrated iterative oligosaccharide synthe-

\[\text{Table 1. Dependence of } \beta \text{-selectivity with decreasing temperature.} \]

<table>
<thead>
<tr>
<th>No.</th>
<th>Donor</th>
<th>Temp. [°C]</th>
<th>Time</th>
<th>Yield [%]</th>
<th>α/β ratio</th>
</tr>
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<tr>
<td>1</td>
<td>Glc</td>
<td>18</td>
<td>40</td>
<td>2 h</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>Glc</td>
<td>18</td>
<td>0</td>
<td>5 h</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>Glc</td>
<td>18</td>
<td>−60</td>
<td>3 days</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>Glc</td>
<td>19</td>
<td>40</td>
<td>5 min</td>
<td>83</td>
</tr>
<tr>
<td>5</td>
<td>Glc</td>
<td>19</td>
<td>0</td>
<td>30 min</td>
<td>74</td>
</tr>
<tr>
<td>6</td>
<td>Glc</td>
<td>19</td>
<td>−60</td>
<td>12 h</td>
<td>73</td>
</tr>
</tbody>
</table>

\[\text{Scheme 3. Preparation of glycosyl iodide from unprotected free sugars.} \]

\[\text{Scheme 4. Synthesis of oxacycloalkane with glycosyl iodides.} \]
sis by selective activation of glycosyl iodides\textsuperscript{[59]} over per-O-acyl-
tylated glycosyl donors with TBAI/DIPEA. Selective activation of

glycosyl iodides over thioglycosides was also implemented,

where glycosides were formed though the activation of glyco-
syl iodides in the presence of triphenylphosphine oxide

(Ph$_3$PO).\textsuperscript{[60]} In another interesting example, Lam and Gervay-

Hague\textsuperscript{[61]} illustrated the double glycosylation of an orthogonal-
ly protected acceptor 23 with mannosyl iodide 24 by using

AgOTf to yield the desired glycoside 25 (Scheme 5).

Citing more studies in terms of glycoside synthesis,\textsuperscript{[62]} Castil-

lon et al. converted per-O-acetylated iso-globotrihexose to a

glycosyl iodide and coupled it with stannylceramide in the

presence of TBAI.\textsuperscript{[63]} In 2014, Gervay-Hague and co-workers ac-
tivated glycosyl iodides with I$_2$ to their corresponding reactive
glycosyl donors and proceeded towards glycosylation to yield

various glycoconjugates.\textsuperscript{[64]} Though there has been much

study of glycosylations with typically benzyl-protected glycosyl

iodides,\textsuperscript{[65]} Stachulski and co-workers presented a detailed study of
glycosylation reaction of ‘disarmed’ glycosyl iodides with various types of alcohols promoted by the NIS–I$_2$–TMSOTf

system.\textsuperscript{[66]} Various types of ‘non-heavy-metal’ salts were also
demonstrated as effective catalysts in the formation of $\alpha$-glyc-
osides. Thus, it is evident that the promoter systems required

towards glycosyl iodides are widely varied. Glycosylations with gly-

cosyl iodides have been reviewed extensively by Murakami

et al.\textsuperscript{[67]}

3.1.3. Glycosyl Fluorides

Mukaiyama et al.\textsuperscript{[68]} asserted the activation of glycosyl fluorides

by SnCl$_2$–AgClO$_4$ and their role as glycosyl donors. Their versa-
tility resides in their readily accessible nature and their higher

termal and chemical stability compared to other glycosyl halide

counterparts. After the onset of the 21st century, the

synthesis of glycosyl fluorides has been broadly developed and

used for glycoside formation.\textsuperscript{[69]} The IP$_2$BF$_4$-catalyzed synthesis

ger of glycosyl fluorides from thioglycosides was reported in 2007,\textsuperscript{[70]}

which provided the best output being activated by TFOH, which mechanistically formed a co-ordination complex

with the pyridine moiety to liberate the iodonium species. More successful illustrations of glycosyl fluoride synthesis from

thio-, seleno-, telluro-, and n-pentenyl glycosides have been re-

ported.\textsuperscript{[71]} Kanie and co-workers used N,N-diethylamino-

sulfur trifluoride (DAST) in the absence of N-bromosuccinimide

(NBS).\textsuperscript{[72]} Dimethyl(methylthio)sulfonium trifluoromethanesuf-

fionate (DMTS) was used as a substitute for NBS to give excellent

yields. Glycosyl fluorides have also been widely used for intra-
molecular aglycon delivery (IAD) in the synthesis of $\beta$-manno-

sides (Scheme 6).\textsuperscript{[73]}

For the next generation of oligosaccharide synthesis, selec-
tive activation of donors based on their activating groups is a

major area of research. There has been much research aimed

toward selective activation of glycosyl fluorides.\textsuperscript{[74]} Mukaiyama

et al. selectively activated glycosyl fluorides over thioglycosides

by using catalytic amounts of TFOH, HClO$_4$, or CF$_3$SO$_2$H.\textsuperscript{[75]}

Interestingly, in a report by Yang and Yu in 2014,\textsuperscript{[76]} there is an il-

lustration of the use of glycosyl fluoride as an acceptor in the

presence of a thioglycoside donor for the synthesis of disac-

charide in its fluoride form, which could subsequently be acti-

vated in the presence of a thioglycosides acceptor to form the

trisaccharide. Glycosyl fluorides can also be activated in the

presence of glycols with the help of Sn(OTf)$_2$.\textsuperscript{[77]} Thus, these

selective activation protocols are stepping stones for the for-

mulation of more versatile iterative and automated oligosacchar-

ide syntheses. Utilizing the different activation protocols, these

donors have helped in the broader aspect of natural product

syntheses.\textsuperscript{[8]}

3.2. Thioglycosides

Thioglycosides are the most versatile glycosyl donor in oligo-
saccharide synthesis. Although the anomic thioc functionality is

highly stable towards varied protecting-group manipulations, it

can be activated under extremely mild conditions and, hence,
can be used in varied types of glycosylation conditions.
The soft sulfur atom provides easy and selective reactivity with

soft electrophiles. Various modifications have been made to
the nature of thioglycosides over the years and many novel and mild promoter systems have been developed. The present century has seen modification in the use of harsh thiophilic promoters like Hg$^{2+}$, Cu$^{2+}$, and Ag$^{+}$ salts to halonium ions, which have more thiophilic character. They revealed the promise of being activated under highly moderate conditions. Starting with the use of NBS$^{[70]}$ to activate armed thioglycosides, much progress has been made in utilizing bromonium and iodonium salts for the activation process with promoters like NOBF$_4$,$^{[78]}$ NBS/TfOH,$^{[90]}$ Br$_2$/AgOTf,$^{[81]}$ NIS/HOTf or AgOTf,$^{[82]}$ IDCP (iodonium dicollidine perchlorate),$^{[83]}$ IDCT (iodonium sym-collidiniumtriflate),$^{[84]}$ I$_2$,$^{[85]}$ PhIO/Tf$_2$O,$^{[86]}$ and iPr$_2$BF$_3$.$^{[70]}$

In an attempt to develop more versatile promoter systems, Ye and co-workers implemented the ‘soft’ nature of the bromonium species to report a bromodimethylsulphonium bromide (BDMS)/AgOTf$^{[87]}$ system for the activation of thioglycosides. The low cost and easy accessibility of the BDMS (32) catalyst made it an effective glycosylating promoter (Figure 2). In a similar attempt, the same group went on to establish an O,O-dimethylthiophosphonosulfenyl bromide (DMTPSB) activation system for the pre-activation of thioglycosides and subsequent glycosylations (Scheme 7).

![Figure 2: Bromodimethylsulphonium bromide (BDMS); O,O-dimethylthiophosphonosulfenyl bromide (DMTPSB).](image)

Scheme 7. Synthesis of a disaccharide with DMTB5B/AgOTf as the promoter.

The presence of bromine was also shown to activate β-ethyl thioglycoside 37 to provide disaccharide 39 as the exclusive α-linked diastereomer (Scheme 8).$^{[89]}$ Interestingly, the mechanistic rationale behind such activation lies in the in situ generation of glycosyl bromide, which acts as the glycosyl donor reacting with the acceptor.

Similarly, Crich and Smith reported the use of S-(4-methoxyphenyl) benzenethiosulfinate (MPBT, 40) and trifluoromethane-sulfonic anhydride (Tf$_2$O)$^{[90]}$, which react with each other to form an electrophile 41, aiding the in situ conversion of thioglycosides to their corresponding triflates. These glycosyl triflates were further implemented for the generation of the challenging β-mannoside linkages (Scheme 9). 1-Benzesulfonyl peridine (BSP) and Tf$_2$O$^{[91]}$ also served the same purpose, and it was further used for double glycosylation, using carbohydrate diol as the acceptor 43 (Scheme 10). Later, van der Marel and co-workers proved the versatility of the system successfully applied the system.$^{[92]}

Van der Marel et al.$^{[93]}$ also introduced the diphenyl sulfoxide (DPS)–triflic anhydride combination for thioglycoside activation in β-mannoside synthesis. They first activated a relatively armed thioglycoside 45 with the BSP/Tf$_2$O activator system and coupled it with a thio acceptor 47 to yield disaccharide 48 in its thio form (Scheme 11a). (N-piperidinophenyl-S-thiophenyl)sulfide triflate (46) is formed as a by-product. After quenching the by-product with triethylphosphite, thio-disaccharide 48 was activated with a more versatile DPS/Tf$_2$O activator system for its coupling with another monosaccharide 49 for successful glycosylation (Scheme 11b). This example extensively illustrates the difference in reactivity between the two activator systems. Similar pre-activation of thioglycosides have also been achieved by benzenesulfonfylmorpholine (BSM)–Tf$_2$O$^{[94]}$ as well as Me$_2$S–Tf$_2$O$^{[95]}$ and was further utilized for glycosylation through both primary and secondary hydroxyl groups.

Thioglycosides, owing to their unique reactivity pattern, are promising candidates for one-pot oligosaccharide synthesis. To establish this system, Wong and co-workers implemented the novel reagent N-(phenylthio)caprolactam for thioglycoside activation at room temperature.$^{[96]}$ N-(Phenylthio)caprolactam was first reacted with Tf$_2$O, which further proceeded for thio-
glycoside activation. In a similar example, instead of Tf$_2$O, TMSOTf was used for the pre-activation of N-(p-methylphenylthio)-e-caprolactam by Ghosh and co-workers.[97] They also reported the use of trichloroisocyanuric acid (TCCA),[98] an easily available, inexpensive, and shelf-stable reagent, in combination with catalytic amounts of TMSOTf for the efficient activation of the thioglycosides.

In 2000, Takeuchi et al.[99] introduced the activation of disarmed thioglycosides through the combination of trityltetraakis(pentafluorophenyl)borate [TrB(C$_6$F$_5$)$_4$], iodine, and DDQ. On a similar hypothesis, they also put forward another activator system, PhthNSET in the presence of TrB(C$_6$F$_5$)$_4$. [100] The same complex was used in the presence of NIS and NBS for successful glycosylations.[101]

Various lanthanide(III) salts were also introduced for the activation of the thioglycoside donors. Employing the concept of lanthanide-ion co-ordination, Chung and Park reported a comparative study by using various lanthanide(III) salts to envisage the noncovalent approach of the IAD strategy in the process of the formation of β-mannosides.[102] The Lewis acidity and high co-ordination numbers of the lanthanide salts were considered to be highly favorable for the desired co-ordination. Yb$^{	ext{III}}$ and Eu$^{	ext{III}}$ were especially effective (Scheme 12) in the presence of NIS–TfOH as the activator system (Table 2).

Thus, NIS–NBS-based activator systems that were introduced long ago saw a number of important modifications after the onset of the 21st century. Mukaiyama and co-workers used TrOTf$^{	ext{I}}$[103] in conjunction with NIS for the convergent synthesis of oligosaccharides. Simultaneously, Li and co-workers used Me$_3$SiOTf with both NIS and NBS for the activation of phenyl and ethyl thioglycosides to introduce spacer arms.[104] Moreover, the catalyst system involved for effective glycosylations demands highly moisture tolerant properties. In that respect, Mukherjee and Mukhopadhyay reported the use of La(OTf)$_3$ as the Lewis acid activator with NIS for the activation of disarmed thioglycoside donors,[105] which, by virtue of its solid nature and moisture-tolerant capability, became more effective than other traditional reagents like TfOH or TMSOTf.[106] Similarly, supported acid catalysts became interesting alternatives to traditional Lewis acid promoters. HClO$_4$–silica was used for the activation of ‘disarmed’ thioglycosides in conjunction with NIS in 2005 by Field and co-workers,[107] which promised to be a better protic acid source under milder and safer reaction conditions. Later, Mukhopadhyay and co-workers introduced a better and environmentally friendly H$_2$SO$_4$–silica system for the same purpose.[108] The good availability of H$_2$SO$_4$–silica and its effective desiccant properties were reasons for its wide use for various bacterial and plant oligosaccharide syntheses in moderate to high yields (Figure 3).[109] Specific acid-washed

<table>
<thead>
<tr>
<th>No.</th>
<th>Ln(OTf)$_3$ (equiv)</th>
<th>Activator</th>
<th>Solvent</th>
<th>Temp [°C]</th>
<th>Time</th>
<th>Yield [%]</th>
<th>α/β</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Yb (1)</td>
<td>Tf$_2$O</td>
<td>CH$_2$Cl$_2$</td>
<td>–78–RT</td>
<td>10 h</td>
<td>63</td>
<td>1:2</td>
</tr>
<tr>
<td>2</td>
<td>Yb (1)</td>
<td>Tf$_2$O</td>
<td>CH$_3$CN</td>
<td>–40–RT</td>
<td>12 h</td>
<td>63</td>
<td>1:2.8</td>
</tr>
<tr>
<td>3</td>
<td>Yb (1)</td>
<td>NIS</td>
<td>CH$_2$Cl$_2$</td>
<td>RT</td>
<td>20 min</td>
<td>47</td>
<td>1:1:5</td>
</tr>
<tr>
<td>4</td>
<td>Yb (1)</td>
<td>NIS–TfOH (cat.)</td>
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<td>8.5 h</td>
<td>59</td>
<td>1:2:9</td>
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<td>5</td>
<td>Eu (1)</td>
<td>Tf$_2$O</td>
<td>CH$_2$Cl$_2$</td>
<td>–78–RT</td>
<td>10 h</td>
<td>76</td>
<td>1:1:5</td>
</tr>
<tr>
<td>6</td>
<td>Eu (1)</td>
<td>Tf$_2$O</td>
<td>CH$_3$CN</td>
<td>–40–RT</td>
<td>10 h</td>
<td>82</td>
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<td>7</td>
<td>Eu (1)</td>
<td>NIS</td>
<td>CH$_2$Cl$_2$</td>
<td>RT</td>
<td>20 min</td>
<td>48</td>
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<td>8</td>
<td>Eu (1)</td>
<td>NIS–TfOH (cat.)</td>
<td>CH$_2$Cl$_2$</td>
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<td>6 h</td>
<td>64</td>
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<tr>
<td>9</td>
<td>La (1)</td>
<td>Tf$_2$O</td>
<td>CH$_2$Cl$_2$</td>
<td>–78–RT</td>
<td>12 h</td>
<td>51</td>
<td>1:7:1</td>
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</table>
molecular sieves (AW-300 MS) along with NIS have also been shown to function as glycosylation promoters by Yao and Lee.\textsuperscript{[110]} Owing to its ease of handling and storage, and its capacity to scavenge water, AW-300 MS proved beneficial as a Lewis acid in the NIS-mediated glycosylation of thioglycosides.

For expeditious oligosaccharide synthesis, various modifications in the protecting groups and the catalyst in the presence of NIS/NBS-based promoters have been widely investigated. Périon et al. have used both Sn(OTf)\(_2\) and Cu(OTf)\(_2\) to perform critical glycosylation with the fucosyl donor by varying the protecting group in its C-4 position.\textsuperscript{[111]} In another example, a stoichiometric amount of NBS was used with Bi(OTf)\(_3\) for the activation of alkyl and phenyl thioglycoside donors by Valerio et al.\textsuperscript{[112]}

Introducing a novel promoter-free glycosylation technique, Pohl and co-workers efficiently used sub-stoichiometric amounts of triphenyl bismuth ditriflate [Ph\(_3\)Bi(OTf)\(_2\)] to activate various thiopropyl glycosides.\textsuperscript{[113]} The highly robust nature and solubility of bismuth(V) salts made it a highly advantageous glycosylation catalyst, employing just catalytic amounts of the promoter. This glycosylation protocol opened more doors to investigate elegant glycosylations without the involvement of any co-promoter. In sync, Vibhute et al. recently showed that only 3 mol% of AuCl\(_3\) catalyst was sufficient to trigger thioglycoside activation.\textsuperscript{[114]}

Light-induced activation of thioglycoside donors has also been investigated.\textsuperscript{[115]} In 2013, Bowers and co-workers demonstrated the efficient use of visible light for mediating O-glycosylation through the activation of thioglycosides.\textsuperscript{[116]} Ir\(\text{[dF(CF}_3\text{)]ppy}_2\text{[dtbbpy]}\)PF\(_6\) was used as the catalyst to oxidize an electron-rich thiol glycoside, yielding an oxocarbenium ion intermediate. Then, effective glycosides were obtained in the presence of bromotrichloromethane and a non-nucleophilic and protic solvent, hexafluoroisopropanol (HFIP) (Scheme 13). The proposed mechanism involved the unique visible-light-activated single-electron oxidation of sulfur. In a similar mechanism, photosensitizer [Ru(bpy)\(_3\)]\(^{2+}\) was used along with DTBMP for the activation of thioglycosides.\textsuperscript{[117]} Recently, glycosylations avoiding the use of photosensitizers were also reported, where UV irradiation was used to cleave the C–S bond\textsuperscript{[118]} and Cu(OTf)\(_2\) was oxidized to generate the species capable of undergoing glycosylation. In a similar proposal, O-glycosylation of unprotected deoxythioglycosides was also performed with alcohols in the presence of DDO under long-wavelength UV irradiation through the single electron transfer (SET) mechanism.\textsuperscript{[119]} During the process, a boronic acid was used as a temporary protecting group for the glycosyl donors to prevent self-coupling or formation of 1,6-anhydro glycoside.

Bennett et al. reported the use of air- and water-stable iodonium salt phenyl(trifluoroethyl)-iodoniumtriflimide to activate a wide array of both armed and disarmed thioglycosides for glycosylation at room temperature.\textsuperscript{[120]} A special advantage of this process is that low-temperature reactions or additives used to remove water from the reaction, such as molecular sieves, are not necessary. Electrochemical O-glycosylation of primary alcohols with thioglycoside donors have been reported by Nakamia and co-workers\textsuperscript{[121]} in the presence of a small amount of sodium trifluoromethansulfonate as the supporting electrolyte (Scheme 14).

![Scheme 13. Light-induced activation of thioglycoside donor.](image)

**Scheme 13. Light-induced activation of thioglycoside donor.**

**Scheme 14. Electrochemical O-glycosylation.**

### 3.3. Glycosyl Imidates

Glycosyl imidates represent another versatile leaving group in addition to the thioglycosides, whose extensive use in the domain of oligosaccharide glycosylations is noteworthy.

#### 3.3.1. O-Imidates

Since its inception by Schmidt et al. in 1980,\textsuperscript{[122]} glycosyl trichloroacetimidates and trifluoroacetimidates have become widely used glycosylating reagents. Glycosyl trichloroacetimidates can be readily prepared through the base-promoted addition of an anomic hydroxyl group to trichloroacetanitride, using either inorganic (NaH, K\(_2\)CO\(_3\), Cs\(_2\)CO\(_3\), etc.) or organic (DBU) bases. Imidate-based glycosylations require strong Lewis acids like TMSOTf\textsuperscript{[123]}\(\text{BF}_3\cdot\text{Et}_2\text{O}\textsuperscript{[124]}\), \(\text{TBDMSCOTf}\textsuperscript{[125]}\), \(\text{TiF}_4\textsuperscript{[126]}\), \(\text{ZnBr}_2\textsuperscript{[127]}\), or \(\text{AgOTf}\textsuperscript{[128]}\), and moisture-stable activating reagents such as \(\text{I}_2\text{/Et}_2\text{SiH}\textsuperscript{[129]}\). In special cases, such as fructofuranosides, where the synthesis of the trichloroacetimidate donor proved to be challenging, N-phenyltrifluoroacetimidate was established as an efficient substitute.\textsuperscript{[130]} The leaving group showed effective \(\alpha\)-selectivity when performing glycosylations with various flavonoids.\textsuperscript{[131]} Their versatility has also been effectively established in the syn-
Versatility of BF₄⁻ Et₂O  

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To activate armed glycosyl trichloroacetimidates, leading to the selective formation of 1,2-trans glycosides in high yields, has been widely studied.  

However, with more recent studies in 2012, Cloninger and co-workers have established that In₃ salts serve as even better and more effective promoters than BF₄⁻ Et₂O for performing glycosylations with glycosylacetimidate donors.  

To establish the novelty of the salts, InCl₃, InBr₃, and In(OTf)₃ were reacted with various protecting-group-manipulated glycosyltrichloroacetimidate donors, and all of them led to the formation of the glycosylated products in high yields.

Use of iodine for the activation of armed and disarmed acetimidates was reported by Iadonisi and co-workers. Addition of a catalytic amount of triethylsilane to trigger the in situ generation of HI was the key step for such an activation strategy. The same group previously established the use of catalytic amounts of Sm(OTf)₃ to activate armed glycosyl trichloroacetimidates. In analogous conditions, salts of other lanthanides like Sc(OTf)₃, Tb(OTf)₃, and Yb(OTf)₃ were also used to activate trichloroacetimidate donors and promote glycosylations at room temperature. A catalytic amount of Yb(OTf)₃ was also efficient for performing one-pot glycosylation of glycosyltrichloroacetimidate donors with suitably protected thioglycosides activation, which proved trichloroacetimidates to have more reactivity than glycosyl trifluoroacetimidates.

For instance, the readily accessible glucopyranoside 60 was reacted with (N-phenyl)trifluoroacetimidoyl chloride in the presence of a slight excess of K₂CO₃ in acetone to yield the trifluoroacetimidate derivative 61, which was then reacted with a trichloroacetimidate glycoside 62 in the presence of a catalytic amount of Yb(OTf)₃, where the more reactive compound, 62, was readily consumed to yield disaccharide 63. In the same pot, acceptor 64 was added with extra catalyst at room temperature to give the required trisaccharide 65 (Scheme 15a,b). To reduce the toxicity involved in the use of lanthanide salts, they also reported the alternate use of Bi(OTf)₃ for the activation of perbenzylatedtrichloroacetimidate donors, which acted as good activators, both for armed and disarmed trichloroacetimidates. Notably, in this case, high β-stereoselectivity was obtained. Subsequently, Vankar and co-workers studied the application of gold catalysts in glycosylations and modified it to use AuCl₃ and the AuCl₃–phenylacetylene combination, which acted as good activators, both for armed and disarmed trichloroacetimidates. Notably, in this case, high β-stereoselectivity was observed. Recently, further investigation into gold catalysts by Peng and Schmidt has demonstrated the successful synthesis of glycosides with trichloroacetimidate donors via an intramolecular dual catalysis pathway, where AuCl₃ acted as an initiator for the formation of catalyst–acceptor adducts. Interestingly, they obtained high β-stereoselective products even with the use of armed glycoside donors.

A commercially available cationic Pd³⁺ reagent, Pd(CH₃CN)₂(BF₄)₂, was used by Nguyen and co-workers as the Lewis acid, because of its vacant d orbitals. The easily accessible Pd salt has, thus, been used for stereoselective glycosylations with trichloroacetimidate donors to give exclusive α-products. The use of a larger mol% of the Pd³⁺ source gave...
a higher yield, whereas the use of additives like DTBP with the same amount of PdII source did not show much difference in the yield (Table 3). To facilitate the synthesis of β-glycoside products, Baati and co-workers used TMSNTf2 as an efficient promoter in the glycosylation reaction when using per-methacrylated Schmidt reagents.[149]

### Table 3. Efficiency of PdII salts in glycosylation.

<table>
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<th>Entry</th>
<th>Pd (II) source</th>
<th>PdII [mol %]</th>
<th>Additive</th>
<th>Time [h]</th>
<th>Yield [%]</th>
<th>α/β</th>
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</thead>
<tbody>
<tr>
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<td>Pd(CH3CN)2BF4</td>
<td>3</td>
<td>none</td>
<td>5</td>
<td>70</td>
<td>α only</td>
</tr>
<tr>
<td>2</td>
<td>Pd(CH3CN)2BF4</td>
<td>5</td>
<td>none</td>
<td>3</td>
<td>85</td>
<td>β only</td>
</tr>
<tr>
<td>3</td>
<td>Pd(CH3CN)2BF4</td>
<td>5</td>
<td>DTBP</td>
<td>4</td>
<td>83</td>
<td>α only</td>
</tr>
</tbody>
</table>

Photoinduced reactions have attracted much interest in the last few years. Organic photoacids have been implemented for the activation of trichloroacetimidates under photoirradiation.[150] An organic photoacid excited upon photoirradiation subsequently activates glycosyl imidates and facilitates coupling with corresponding alcohols as acceptors.

In addition to trichloro- and N-phenyl trifluoroacetimidate, trichloroacetyl carbamate glycosyl donors have also been reported to be effective glycosyl donors. Glycosyl trichloroacetimidate carbamate synthesized upon reaction of the hemiacetal 71 with trichloroacetyl isocyanate at 0 °C was utilized as the donor for performing glycosylation with various acceptors, using a catalytic amount of TMSOTf as the promoter (Scheme 17).[151] Many other electrophilic promoters have been used for the activation of such donors. Interestingly, Zn(OTf)2 was unable to activate the imidate donor, whereas the glycosylation with TfOH and Sc(OTf)3 was seen to proceed moderately. The same reaction was slow with other Lewis acids such as Mg(ClO4)2 and Cu(OTf)2, even at room temperature. However, the reaction provided high yields of glycosylated products with TMSClO4.[152] Selective activations in different promoter systems invariably hold promise for the use of such imidate donors in iterative one-pot glycoside synthesis.

O-Glycosyl trichloro cyano acetimidates have also been popular as powerful glycosyl donors.[153] To increase the donor properties of the glycosylimidate, one chlorine group of the trichloroacetitrile was replaced with a more electronegative cyano group, that is, trichloromalonitrile was used as the activating agent.

#### 3.3.2. S-Imidates

Thioimidates have been developed as versatile building blocks for selective glycoside formation. Special mention is credited to Demchenko and co-workers, who significantly contributed towards the development and establishment of various thioimidate donors.[154]

Demchenko and co-workers introduced S-benzoxazolyl (SBox) as a novel thioimidate donor.[155] SBox proved to be a stable and efficient glycosyl donor for performing several types of glycosylations under mild conditions. Both 1,2-cis and 1,2-trans glycoside formations have been reported with SBox-protected thioglycosides, 75 (Figure 4). The synthesis of SBox glycosides and the probable mechanism for its activation was reported by the same group.[156] Standard thiophilic promoters like NIS/TFOH, AgOTf,[157] Cu(OTf)2,[158] MeOTf,[159] and so forth have been used to activate this class of donors.[156] SBox glycosides also showed selective activation over thioglycosides and penteny glycosides.[157] Several glycosides with different protecting groups were also tested for activation,[156] which revealed that per-benzylated glycosides showed the optimum activation. Demchenko and co-workers gave a detailed analysis about the effects and logistics behind variable activation patterns observed by armed and disarmed SBox glycosides. Armed glycosides could be chemoselectively activated over its disarmed counterpart[160] to give trans-glycosides. Oligosaccharides formed by using armed SBox donors showed high α-stereoselectivity.

However, SBox glycosides showed certain instability towards some extreme reaction conditions when being activated by triflic acid (TFOH). To bypass this limitation, Demchenko and co-workers later introduced S-thiazolinyl (STaz) glycosides, 76 (Figure 4), as building blocks for chemoselective and orthogonal oligosaccharide synthesis.[162] SBox and STaz thioimidates were also implemented in solid-phase glycosylation strategies (Scheme 18a,b).[163] where the thioimidates were utilized as donors for glycosylations with resin-bound acceptors after being activated by promoters like AgOTf, TMSOTf, NIS/TMSOTf, MeOTf, and so forth.[164] STaz glycosides proved to be highly stable towards extensive protecting-group manipulations.[165] These glycosides also showed activation in line with Fraser-
Reid’s armed–disarmed activation strategy. Armed STaz glycosides could be chemoselectively activated over their disarmed counterparts.[31b] Demchenko and co-workers also explored other analogs of silver salts to activate the STaz glycosides, and they successfully reported the application and versatility of silver tetrafluoroborate, AgBF$_4$, as an excellent promoter for the activation of such donors. The more popular thiophilic promoter AgOTf was also found to be effective towards the activation of STaz donors.

S-glycosyl O-methyl phenylcarbamothioates (SNea carbamothioates) (77) (Figure 4) were introduced as the next-generation glycosyl thioimidate donor. SNea leaving group was illustrated to represent a bridging compound between acyclic thiocyanates (SCN) and cyclic SBox glycosides. These donors showed similar properties to SBox glycosides and extensive studies were performed to compare the activation properties of the two. Activation of SNea donors with promoters like Cu(OTf)$_2$, AgOTf, or MeOTf afforded the a-glycosides in significant yields.[168] Other thioimidate S-benzothiazolyl (SBaz) glycosides, 84 (Figure 5), have also been commonly used for glycosylation. This derivative was first introduced for glycosylation by Mukaiyama et al.[169] In 2011, Hasty and Demchenko introduced another variant of thioglycoside donor, S-benzimida zolyl (SBiz) glycosides, 85 (Figure 5), suitable for extensive oligosaccharide synthesis.[170]

Glycosyl dithiocarbamates (DTCs) have been used for glycosylations to give β-selective products upon being activated by Cu$^+$ or Cu$^2+$ triflates.[171] They have been specifically used for glycosides with no protecting group in the C-2 position.

3.4. Alkenyl Glycosides

Although thioglycosides are established as highly versatile donors that facilitate glycosylation reactions, the use of thioglycosides sometimes becomes highly unwanted because of the obnoxious smell of the thiols. Alkenyl glycosides fit perfectly in this void. Pent-n-etyl glycosides (NPGs) 86 pose as the most apt alternative in terms of their versatility and environmentally friendly nature. Similar to thioglycoside activation, activation of n-pentenyl glycosides is achieved either by bromonium (NBS) or iodonium (NIS) ions. The subsequent cyclization leads to the transition state, that is, the tetrahydrofuranyl oxonium ion (88) (Scheme 19). The increased leaving-group prop-

![Scheme 18.](image)

**Figure 5.** SBaz (84) and SBiz (85) glycosides.

![Scheme 19.](image)

**Figure 6.** gem-Dimethyl analogs of n-pentenyl glycosides.
The enhanced leaving-group property of the halomethyl furan moiety aids in the easy conversion of intermediate 96 to 97. Furthermore, the attack of acceptor 98 effectively forms the required glycoside 99. Interestingly, the released moiety, which is highly non-nucleophilic, fails to compete with the sugar nucleophile, which attacks the intermediate, dioxolenium ion 97, and forms the required glycoside 99.

There have been various reports establishing the activation of $n$-pentenyl orthoesters (NPOEs) by lanthanide triflates to form the glycosylated product. In an exemplary illustration, Fraser-Reid and co-workers showed that NPOE 100, upon being activated by NIS followed by Yb(OTf)$_3$ (Scheme 21), produced highly regioselective glycosylated product 102 after reacting with the acceptor 101. The $n$-pentenyl intermediate, 103, formed as a by-product, however, remains inactivated. This $n$-pentenyl glycoside 103 can be activated by Sc(OTf)$_3$, which goes on to form the doubly glycosylated product 104 along with the normal glycoside 102. Thus, NPOE glycosides prove to be better donors than $n$-pentenyl glycosides, as they overcome reactivity restrictions faced in case of the latter.

The implementation of NPOEs for glycosylation was also evident in performing selective glycosylations with more than one free hydroxyl groups in the synthons. This facilitated the possibility of performing iterative glycosylations in the synthesis of larger oligosaccharide building blocks. In a comparative study, it was illustrated that $n$-pentenyl glycosides gave a mixture of products, whereas NPOE yielded strictly the equatorial product in a clean reaction with a higher conversion ratio. Mathew et al. also demonstrated that NPOEs provided better yields upon being irradiated under microwave conditions. A detailed comparison between the activation of NPOEs under thermal and microwave conditions shows a distinct increase in the yield under the latter conditions. Microwave irradiation features the reaction mixture reaching the target temperature rapidly and maintaining the temperature throughout the entire reaction time, which becomes instrumental in obtaining better yields.

Fraser-Reid and co-workers also introduced mannopyranoside-derived methyl orthoesters as possible donors for glycosylation. They underwent easy activation with the help of BF$_3$·Et$_2$O and demonstrated proceedings analogous to that of NPOEs. It was also observed that the iodonium-ion activation and the stoichiometry of the Lewis acid had significant roles in the regioselectivity of the product formed.

3.5. Alkynyl Glycosides

Propargyl glycoside remains the most prominent alkynyl glycoside used in oligosaccharide synthesis to date. The versatility of the propargyl glycosides led to their introduction as a probable glycosyl donor suitable for oligosaccharide synthesis. The alkynophilicity of gold catalysts has been utilized by using Au$^{III}$ halides as the catalyst to activate propargyl glycosides. Optimization of reaction conditions showed that, through AuCl$_3$ activation, complete conversion of the starting material occurred at 60°C after approximately 6 h. However, upon coupling with various aliphatic, alicyclic, steroidal, and sugar alcohols, the stereoselectivity of the product was a 1:1 $\alpha$/$\beta$ mixture. Other alkyne-activating promoters like PtCl$_2$, Co$_2$(CO)$_8$, and RuCl$_3$, however, failed to give the product and resulted in decomposition of the donor. Building upon the concept of activation of propargyl glycosides with gold catalysts, the possibility of using the ‘armed–disarmed principle’ to build higher analogs of oligosaccharides was also explored. They coupled the armed per-benzylated propargyl mannopyranoside 103 with disarmed per-benzoylated mannopyranoside 104 to get the desired disaccharide 105 (Scheme 22a), where the armed propargyl monosaccharide 103 acted as the donor, as anticipated. However, when the disarmed disaccharide formed, 105 was further utilized for sequential glycosylations by con-
verting it to its armed counterpart 106; it was observed that instead of the desired trisaccharide, 107, disaccharide 108 was formed along with the anhydro sugar, 109 (Scheme 22b). The reason behind such unnatural phenomenon was also explained by a mechanistic rationale (Scheme 23), stating the concept of ‘double-activation’ of the armed glycosyl donor, 106, as the probable cause. Upon reacting with further disarmed aglycon, higher oligosaccharides were synthesized effectively. Hotha and co-workers also reported glycosylation with propargyl glycosides at ambient temperature.[183]

Interestingly, gold catalyst AuBr₃ alone led to a very slow reaction where propargyl furanosides were used as the donor, and the disaccharides were also formed in a low yield. Addition of AgOTf along with AuBr₃ in turn, improved the reaction considerably,[184] making it more spontaneous and high yielding. Benzylated propargyl furanoside 113, acting as the armed donor, was also coupled with benzoylated propargyl pyranoside 114 (Scheme 24) to give product 115 in line with the ‘armed–disarmed’ concept.

Apart from propargyl glycosides, reports of the implementation of S-but-3-ynyl glycoside donors and derivatives have also been revealed.[185] These donors were used for gold-catalyzed glycosylations for the synthesis of 2-deoxy glycosides. Another set of alkynyl derivatives, ω-(methyltosylaminoethynyl)benzyl glycosides, was also developed as donors suitable for expeditious oligosaccharide synthesis and for the introduction of various aglycons.

Similar to the concept of alkenyl orthoesters, alkynyl orthoesters were developed as a versatile leaving group aiding in glycosylations. Propargyl orthoesters were glycosylated with

Scheme 22. a) Selective glycosylation with propargyl glycosides. b) Unnatural inter-glycosidic bond cleavage.

Scheme 23. Mechanistic rationale.
various aglycons through gold (III) activation to give the highly stereoselective 1,2-trans glycosylated product in moderate-to-high yields. However, Hotha and co-workers revealed that the propargyl orthoesters, when coupled with carbohydrate-derived secondary alcohols as the acceptor gave comparatively lower yields when compared with coupling with primary carbohydrate alcohols. The same group also reported the selective activation of the propargylox group of the propargyl orthoesters in the presence of the competing propargyl glycosides by AuBr₃ activation.

With a similar intention to develop new and modified glycosylation strategies, Li et al. failed to activate 4-pentynyl or 5-hexynyl glycosides by gold (III) catalysis. To overcome the limitation, they introduced ortho-alkynyl benzoate glycosides, a more active alkynyl group that can be activated by gold catalysts and illustrated the detailed mechanistic insight towards the glycosylation procedure. This protocol for activation has been implemented for the synthesis of various saponins and higher oligosaccharides. O-Hexynyl benzoate glycosides were glycosylated with various aglycons on being activated by Ph₃PAuOTf at room temperature to get high β-selective products (Scheme 25). The same alkynyl benzoate glycoside was also used for its coupling with acid alcohols. A chemoselective protocol has also been employed, where it was observed that Ph₃PAuOTf activation in the presence of BF₃·Et₂O and DBU gave exclusive coupling with the acid alcohols, whereas the same coupling through Ph₃PAuOTf in the presence of DTBP led to the exclusive formation of alcohol orthoester in high yields (Scheme 26). Further studies revealed that the loading of gold catalysts could be lowered to 0.01 equivalents or 0.5 mol% with the external assistance of an additional strong protic acid (10 mol% or 0.1 equiv HOTf).

There have been reports where glycosyl ortho-alkynyl benzoates were efficiently activated by using cheap and easily available molecular iodine. The mild iodine was also capable of selectively activating these donors over thioglycosides, which helped in performing various sequential glycosylations (Scheme 27). Activation of alkynoate glycosides using Hg(OTf)₂ as the promoter has also been reported.

### 3.6. Glycals

Glycals are highly important components in carbohydrate chemistry that can be effectively used for the formation of 2,3-unsaturated glycosides. The transformation of glycals into unsaturated-O-glycosides takes place in the presence of various catalysts like Lewis acids, metal salts, or other activat-
Special consideration was given to the use of metal salts and metal triflates as catalysts for the coupling of the glycosylations.

Starting with alkali metals, potassium dodecatungstocobaltate, $K_3CoW_12O_{40}·3H_2O$ (POM), was explored to understand the role of potassium as the catalyst. Various glycosylations with different acetylated glycols 130 and POM as the catalyst in varied solvents afforded $\alpha$-glycosides 132 as the major product in good-to-excellent yields (Scheme 29). Substantial optimization revealed acetonitrile as the best choice of solvent. Rafiee et al. also showed the probable mechanistic pathway that elegantly described the reason behind the effective use of perfect outer-sphere one-electron oxidants for such glycosylations.\textsuperscript{[209]}

The catalyst showed high yields, even after being recovered four times, thus proving the high efficiency of the alkali metal as the catalyst.

Proceeding to the more versatile transition metals, iron(III) chloride was established as an efficient catalyst for Ferrier rearrangement by Bettadaiah and Srinivas\textsuperscript{[206]} where it was used for the glycosylation of tri-O-acetyl-$\beta$-glucal with butan-1-ol, yielding the $\alpha$-isomer as the major product. Among the reaction conditions tested, refluxing acetonitrile was found to be best. In such optimized conditions, the loading of the catalysts could be lowered to 0.001 mol equivalents.\textsuperscript{[207]} FeCl$_3$ has also been used in ionic-liquid medium. The versatility of ionic liquid resides in the phenomenon of their adjustable solubility. [bmim][FeCl$_3$] is a FeCl$_3$-based ionic liquid prepared by mixing anhydrous FeCl$_3$ with 1-butyl-3-methylimidazolium chloride, [bmim][Cl]. This ionic medium was further utilized as the catalyst for the reaction of glucals with various alcohols (Scheme 30).

A comparative assessment has also been shown for the Ferrier reaction by using CAN (ceric ammonium nitrate) and Fe$_2$(SO$_4$)$_3$·xH$_2$O, respectively.\textsuperscript{[208]} The latter was shown to yield exclusive 2,3-unsaturation-$\alpha$-glucosides with both per-acetyl and per-benzyl glucals. From the schematic study with various iron(III) salts by Wang and Chen in 2012, it is evident that ferric triflate is the most versatile iron-based Lewis acid for performing the glycosylation of peracetyl glycols with alcohols in terms of selectivity and yield (Table 4). The use of only 10 mol\% of the catalyst in dichloromethane as the solvent gave a maximum of 87\% yield with the $\alpha$-isomer as the major product.\textsuperscript{[206]}

The inexpensive ZnCl$_2$ was also shown to catalyze Ferrier glycosylation when using $\text{S}_2\text{O}_8$ active nucleophiles. The catalyst provided good-to-excellent yields upon reaction of 3,4,6-tri-O-acetyl-$\beta$-glucal 133 with allylic, benzylic, and tertiary alcohols in dichloromethane medium.\textsuperscript{[206]} Liu and co-workers illustrated that ZnCl$_2$ impregnated with activated alumina can be optimally utilized for the preparation of 2,3-unsaturated glycosides. They have shown the use of this catalyst system both in the presence and absence of solvent system.\textsuperscript{[206]} The same catalyst system was also used in the subsequent year for Ferrier azaglycosylation.\textsuperscript{[211]} More recently, Zn(OTf)$_2$ has been estimated to be a cheaper, milder, and more efficient catalyst for the stereoselective synthesis of $\alpha$-glycosides with a variety of O and S nucleophiles adorned with a wide range of functionalities.\textsuperscript{[212]}

In 2014, Srinivas and co-workers reported the use of the otherwise unreactive ZnBr$_2$ in the $\alpha$-glycosylation reaction of 3,4,6-tri-O-acetyl glucal 133 with a variety of primary, secondary, tertiary, allyl, and benzyl alcohols under microwave conditions.\textsuperscript{[188]}

In another noteworthy set of experimental results, Et$_2$Zn was used to activate the alcoholic acceptor. However, the synthesis of 2,3-unsaturated glycosides was achieved by reacting the substituted glucal with the Zn-salt-activated alcohol in the presence of a Pd catalyst.\textsuperscript{[213]} Special consideration was given to the fact that, in such reactions, the anomeric stereoselectivity was governed by the reagent and solvent system rather than the protecting group. Addition of di(tert-butyl)-2-biphenylphosphine, DTBBP yielded the $\beta$-product, whereas the employment of P(OMe)$_3$ produced the $\alpha$-isomer as the major product.\textsuperscript{[206]}

### Table 4. Comparative study of Fe$^{II}$ catalysts in Ferrier rearrangements.

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<tr>
<th>No.</th>
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<th>Yield [%]</th>
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<tr>
<td>2</td>
<td>Fe$_2$(SO$_4$)$_3$</td>
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</tr>
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</tr>
<tr>
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<td>30</td>
<td>88</td>
<td>10:1</td>
</tr>
</tbody>
</table>

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**Scheme 28.** Ferrier reaction.

**Scheme 29.** Use of potassium as the catalyst in the Ferrier(I) rearrangement.

**Scheme 30.** Ionic-liquid-induced Ferrier glycosylation.

---

**Table 4. Comparative study of Fe$^{II}$ catalysts in Ferrier rearrangements.**
product (Table 5). Nguyen and co-workers also implemented the use of Pd\(^2\)/L system for the direct construction of the α-glycoside from the glycal through the formation of glycal tri-chloroacetimidates (Scheme 31).\(^{[214]}\) Following extensive work with several ligand systems, Pd(PhCN)\(_3\)Cl\(_2\) was reported to give the maximum stereoselectivity in the presence of DTTBP providing the α-isomer in 84% yield. Furthermore, many derivatives of 2,3-unsaturated glycosides have been prepared by using the same concept of implementing a Pd\(^2\)/L catalyst to activate the glycal π system.\(^{[215]}\) Recently, Liu and co-workers employed decarboxylative strategy by using Pd(II) and DtbBP \([1,1’-\text{bis(di-tert-butylphosphino)}\text{ferrocene}]\) as the ligand in the presence of the base, Cs\(_2\)CO\(_3\), for the formation of 1,2-unsaturated O-glycosides.\(^{[216]}\) Higher base loading (2.0 equiv) led to the effective formation of the β-glycoside. Excellent α-selectivity was obtained by coupling carbohydrate acceptors with glycals when being activated by Re\(^{3+}\)-oxo complex, ReOCl\(_3\)(SMe\(_2\))(Ph\(_3\)PO).\(^{[217]}\)

Similarly, with other variety of donors, the high alkylnophilicity and oxophilicity of Au catalysts were utilized to perform Ferrier glycosylation. Later, depending on the same phenomenon, Balamurugan and Koppolu used AuCl\(_3\) as the catalyst under mild reaction conditions to synthesize 2,3-unsaturated glycoside with high α-stereoselectivity.\(^{[218]}\) When glycal 140 was reacted with propargyl alcohol in the presence of AuCl\(_3\) in acetonitrile medium, intermediate 141 was formed in situ, which was subsequently subjected to glycosylation with acceptors to give the product in moderate-to-good yield with the α-isomer predominating (Scheme 32). Thus, wide studies of glycosylations with gold catalysts have established their versatility in the successful formation of products with a wide range of nucleophiles.

Studies have also been done with other transition-metal salts. Copper(II) triflate has been reported to give 2,3-unsaturated product under milder conditions.\(^{[219]}\) Kashyap and co-workers introduced RuCl\(_3\).\(_3\)H\(_2\)O as an efficient Lewis acid catalyst for such reactions.\(^{[220]}\) Niobium(V) chloride was also implemented as an efficient catalyst for the synthesis of unsaturated glycosides from per-O-acetylated glycal\(^{[221]}\) by virtue of its high stability, moisture tolerance, and economic viability. In a corresponding report, the phenomenon of water solubility of scandium(III) triflate was utilized to perform Ferrier glycosylations in aqueous medium. In non-aqueous medium, only 5% w/w of Sc(OTf)\(_3\) proved to be adequate to complete the reaction within a maximum time period of 3.5 h with a wide variety of aryl and alkyl alcohols.\(^{[222]}\) A variety of yttrium salts was also explored, of which Y(OTf)\(_3\) was found to be the most efficient, 10 mol% of which was optimum to bring the reaction to completion.\(^{[223]}\) Among other transition-metal salts, TiCl\(_4\)(OTf),\(^{[224]}\) organozinc reagents,\(^{[225]}\) and zirconium(IV) chloride\(^{[226]}\) have also been used as highly efficient catalysts for type I Ferrier rearrangement.

 Proceeding to the use of lanthanides as catalysts for Ferrier rearrangements, Yadav et al. illustrated Dy(OTf)\(_3\)-catalyzed glycosylation of glycal with alcohols, phenols, and hydroxy-α-amino acids in 1-butyl-3-methylimidazolium hexafluorophosphate, ([bmim][PF\(_6\)]) ionic liquid.\(^{[225]}\) High yields of unsaturated glycopyranosides with the α-isomer predominating were obtained in mild, solvent-free conditions by glycosylation of glycal mediated by inexpensive, versatile, and environmentally friendly lanthanum(III) nitrate catalyst.\(^{[227]}\) However, this catalyst failed to provide a significant yield with solvents like CH\(_2\)Cl\(_2\), THF, diethyl ether, and so forth, but on using nitromethane as the solvent, the yield improved significantly (Table 6).\(^{[228]}\) The good stability of the catalyst and milder reaction conditions made Er(OTf)\(_3\) one of the most efficient catalysts for triggering Ferrier glycosylation (Scheme 33). In almost all the cases, the α-anomer was found to be predominant. A recent addition in Ferrier glycosylation is the electrochemical generation of zirconium catalyst, which provided a novel protocol for the effective glycosylation of acetylated glycal.\(^{[229]}\)

Basic metals, such as aluminum, have also been used as catalysts. The use of aluminum triflate as a potential catalyst in Ferrier reaction has also been widely studied. In 2002, it was revealed that tri-O-benzyl-d-glucal catalyzed by Al(OTf)\(_3\) gave both 2-deoxy glycosides and Ferrier-rearranged pseudoglycal products at 60 and 0 °C, respectively.\(^{[230]}\) The versatility of the aluminum salt was verified when a wide range of acceptors were used ranging from alkyl alcohols to phenolic alcohols. Al(OTf)\(_3\) was also used to show the temperature dependence in Ferrier rearrangement. Williams and co-workers noticed that different temperatures yielded different products upon reacting glycal with alcohols in the presence of an Al catalyst.\(^{[231]}\)
Table 6. Comparative study of glycosylations with glucals in various solvents.

<table>
<thead>
<tr>
<th>No.</th>
<th>Solvent</th>
<th>Time [h]</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH$_2$Cl$_2$</td>
<td>48</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Et$_2$O</td>
<td>48</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>THF</td>
<td>48</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>CH$_2$Cl$_2$(dry)</td>
<td>48</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>CH$_2$Cl$_2$(dry)</td>
<td>12</td>
<td>46</td>
</tr>
<tr>
<td>6</td>
<td>Et$_2$O</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>CH$_2$CN (dry)</td>
<td>12</td>
<td>58</td>
</tr>
<tr>
<td>8</td>
<td>CH$_3$NO$_2$ (dry)</td>
<td>2</td>
<td>90</td>
</tr>
<tr>
<td>9</td>
<td>CH$_3$NO$_2$ (dry)</td>
<td>6</td>
<td>90</td>
</tr>
</tbody>
</table>

Scheme 33. The use of erbium triflate as a catalyst.

At 60°C, 2-deoxy-1-O-glycosides were obtained, whereas on lowering the temperature to 0°C, Ferrier product 2,3-unsaturated-O-glycosides were obtained. Both products were obtained in high α-selectivity. Sequential glycosylation has also been illustrated to being catalyzed by AlCl$_3$.[232] In this reaction, derivatives of 3-hydroxy galactal 146 were reacted with diethyl phosphorochloridite and Et$_2$N, which provided the corresponding 3-O-diethoxyphosphanyl-α-galactal, 147. It subsequently underwent Ferrier rearrangement with various nucleophiles in the presence of AlCl$_3$, to give the required 2,3-unsaturated glycosides 148 (Scheme 34). Indium is another basic metal that has been frequently reported to catalyze the Ferrier reaction with glucals. In an interesting finding, Boga and Balasubramanian reported a Ferrier glycosylation where glucals were reacted with varied nucleophilic acceptors catalyzed by 20 mol% of highly anhydrous InCl$_3$.[234a] They, however, continued the reaction for a longer time to observe the conversion of O-glycoside to the corresponding C-glycoside.[233] It was found to provide a better yield compared to the other metal-based catalysts. Unactivated glucals with no protecting group in the C-3 position have also been successfully activated to undergo Ferrier rearrangement by InCl$_3$.[234] In a similar protocol, basic BiCl$_3$[235] has also been used. Bismuth(III) triflate was implemented as a probable catalyst for Ferrier rearrangement both in the presence and absence of silica.[236] Various types of alkyl and aromatic alcohols have been illustrated to undergo the glycosylation to stereoselectively produce the α-isomer as the major product in a considerably high yield.

Addition of glycols to the glucals through Ferrier glycosylation using tellurium salts[237] was also reported for the expedient synthesis of various macromolecules. Thus, metal-catalyzed Ferrier reaction has been extensively studied in the present century. However, more protocols to promote the formation of β-selective products are still in demand.

Advancing from metal-based Ferrier glycosylations, immediate interest is directed towards the development of metal-free strategies. In 2008, De et al. showed the effectiveness of metal-free synthesis of 2,3-unsaturated glycosides from corresponding glucals.[238] They showed that the glucal reacted with various nucleophilic alcohols in a greater amount (20 equiv) to give the corresponding 2,3-unsaturated product without the addition of any metal or Lewis acid catalyst, provided HFIP is used. The solvent-free rearrangement reaction was further facilitated by the use of Montmorillonite K-10 as the catalyst.[239] Application of microwave heating was also reported to be advantageous over standard heating conditions, leading to less effective reaction time. In both cases, exclusive α-products were obtained with a cleaner and milder method.

Montmorillonite K-10 clay-supported Bi(OTf)$_3$[240] has also been illustrated to achieve the synthesis of various types of sialic acid through Ferrier transformation. Other versatile catalyst systems, for example HClO$_4$—silica,[241] CF$_3$SO$_2$H—silica,[242] NaH$_2$PO$_4$—silica,[243] and H$_2$SO$_4$—silica,[244] have been used for the synthesis of a wide range of pseudoglycosides. In the context of organic acid catalysts, when acetic acid failed to yield any desired unsaturated glycoside, Liu and co-workers decided to test the effectiveness of camphorsulfonic acid (CSA)[245] as a probable catalyst for triggering the formation of pseudoglycosides. Both (R) and (S)-CSA, in their optically pure forms, were reported to give the desired product, the (S)-isomer being the more efficient one.

Various other catalyst systems have been developed over the decade, promoting the formation of 2,3-unsaturated O- and C-glycosides from glucals. Notable ones are cyanuric chloride-catalyzed Ferrier reaction,[246] bromodimethylsulfoxonium (BDMS)-mediated rearrangement,[247] and 3,5-dinitrobenzoic acid (DNBA)-mediated synthesis of O and S-glycoside.[248] The incorporation of potassium alkynyltrifluoroborate group to the glycal has also been implemented by BF$_3$Et$_2$O-mediated[249] Ferrier glycosidation.

The vinyl oxirane chemistry was implemented by Crotti and co-workers who mastered the synthesis of the oxirane or epoxide from the corresponding glycal and further used it for subsequent catalyzed or uncatalyzed[250] glycosylation with a wide range of nucleophiles under different reaction conditions.[251] This glycosylation protocol led to yield the β-isomer as the major product, whereas the use of benzene as the solvent gave the β-product exclusively.[252] However, the synthesis of α-derivatives has also been reported by using the same protocol.[253] The addition of organolithium reagents[254] onto the oxiranes by using the same procedure also gave efficient reactions to give the desired product.
3.7. Deoxy Sugars

3.7.1. 2-Deoxy Sugars

2-Deoxy sugars with the OH group in the C-2 position replaced by a hydrogen atom are widely distributed in various natural products. The wide biological importances of the 2-deoxy sugars have made them a highly novel synthon in chemical glycosylations. However, the formation of glycosidic linkages with these 2-deoxy glycosides pose quite a challenging task, owing to the absence of any directing group in the C-2 position. Moreover, the absence of any electron-withdrawing group in the strategic C-2 position makes the moieties highly acid labile, leading to hydrolysis of the compound. Various analyses have been performed over the years to control the stereoselectivity of the glycosides formed at the anomeric position of the 2-deoxy sugars. Many of the earlier developed protocols have been extensively discussed in various Reviews.\(^\text{[255]}\)

Employing direct methodology for the stereoselective synthesis of natural 2-deoxy glycosides, Lear et al.\(^\text{[246]}\) established the use of the mild activating system, AgPF\(_6\)/DTBMP (2,6-di-tert-butyl-4-methylpyridine). Amongst the various Ag catalysts, this promoter system was found to give the maximum \(\alpha\)-stereoselectivity under optimized conditions. Recently, in the entire domain of glycosylation, pre-activation of the donors is much in demand. In the case of glycals, this has also been established to give better products. As an illustration, Ye and co-workers first activated 2-deoxy thioglycosides 149 by using BSM–TF\(_2\).\(^\text{[257]}\) Subsequently, addition of acceptor 151 to the same reaction mixture gave the 1,2-cis-glycosylated product 152 with high stereoselectivity (Scheme 35). 2,6-Dideoxy thio-

glycosylations based on pre-activation protocol with 2-deoxy and 2-dideoxy glycosides.

sugars 153 were also observed to give similar results. In a side reaction, when the mixture of donor and acceptor was directly exposed to the promoter system, the yield diminished drastically, indicating the importance of pre-activation of the donor.

For the synthesis of higher analogs of 2-deoxy oligosaccharides, Issa and Benett established the use of potassium hexamethyldisilazane (KHMDS)/\(p\)-tolenesulfonic anhydride\(^\text{[258]}\) as an effective promoter system, leading to the exclusive synthesis of \(\beta\)-linked 2-deoxy-sugar disaccharides from hemiacetal donors. The reaction was presumed to go through a \(S_n2\)-pathway via an in situ glycosyl sulfonate intermediate to accomplish a highly \(\beta\)-stereoselective glycosylation.

Glycosylation of glycals mediated by metal catalysts is, in itself, a wide area of research. Zhu and co-workers investigated the role of 2-deoxy 5-but-3-ynyl thioglycosides as an effective glycoside donor\(^\text{[185]}\) for performing O-glycosylation through the homogeneous cationic gold(I)-catalyzed selective activation of the alkyne group attached with the more nucleophilic thio functionality. The reaction conditions were optimized by the use of 5 mol% (4-CF\(_3\)--Ph)\(_3\)PAuCl along with 10 mol% AgOTf, which gave almost quantitative yield. Coming to organoboron-catalyzed glycosylations, \(\beta\)-acetylated 2-deoxy as well as 2,6-dideoxy chlorides were reported to show high regio- and \(\beta\)-stereoselectivity on activation with diphenylborinate 156 (Figure 7) in moderate-to-good yields. However, the require-

Figure 7. 156: Borinic acid pre-catalyst; 157, 158: Polymer-supported haloate complexes.

3.7.2. 2-Amino-2-deoxy Sugars

By virtue of their structures and reactivities, 2-amino-2-deoxy sugars are widely distributed in various living organisms as glycoconjugates\(^\text{[2, 264]}\). Previous Reviews\(^\text{[265]}\) have widely explained the strategy behind the introduction of the participation and non-participating group at the C-6 position to direct the glycosylation to the required stereochemistry. 2-Amino-2-deoxy sugars have been widely used in oligosaccharide syntheses.\(^\text{[258]}\) Repeating sequence of \(\alpha\)-GalNAc was found to be effectively
synthesized by the introduction of oxazolidinone group.\[267\] The N-acetyloxazolidinone-protected thioglycosides both as the glycosyl donor 159 and acceptor 160 was effective (Scheme 36) when activated with Ph$_2$SO--Tf$_2$O promoter system to give exclusive α-stereoselective product 161 in good yields. The synthesis of various important core glycoside parts of GPI anchors, heparin, heparin sulfate, and heparosan had also been successfully accomplished by Hung and co-workers.\[268\] In 2008, anhydrous FeCl$_3$-catalyzed direct formation of α-N-acetyl-glycosaminides was also successfully achieved by Wei et al.\[269\] and it was effectively implemented in the large-scale preparation of fluorogenic T$_2$-antigen probes.

Recently, glycosides with a phthalimido group in the C-2 position were found to be activated by the hypervalent iodine compound, phenyliodine bis(trifluoroacetate) (PIFA) in the presence of triflic acid.\[270\] Proper optimization of the reaction conditions of this metal-free glycosylation afforded the coupled product in significant yields. Direct glycosylation with GlcNAc has also been reported by Jensen and co-workers\[271\] through the activation of thioglycosides 166 or pentenyl glycosides 167 by using various metal triflates (Table 7). However, when glycosylated with carbohydrate acceptors, the yields obtained were poorer than simple acceptors like 1-octanol or (−)-menthol. 2-Deoxy-2-N-sulfate glycosides 162 were also successfully utilized (Scheme 37) to give the desired β-glycosylated products 163 in excellent yields\[272\] on being protected with 2,2,2-trichloroethyl (TCE) group, after which it could be easily deprotected by reacting Zn in the presence of ammonium chloride in methanol. A Yb(OTf)$_3$-mediated synthesis of N-acetyl glucosamine derivatives\[273\] has also been established by Crasto and Jones, which gave β-glycosylated product in high yields. Direct synthesis of 1,2-amino glycosides with p-methoxybenzylidene group in the C-2 position was achieved by Mensah and Nguyen by using nickel catalysts.\[274\] Optimization

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**Table 7. A comparative study of metal-catalyzed glycosylations with 2-amino-2-deoxy donors with different acceptors.**

<table>
<thead>
<tr>
<th>No</th>
<th>Donor</th>
<th>Acceptor</th>
<th>Product</th>
<th>Activation</th>
<th>Temp</th>
<th>Time</th>
<th>Yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>166</td>
<td>1-octanol (1 equiv)</td>
<td>168</td>
<td>Sc(OTf)$_3$, NIS</td>
<td>RT</td>
<td>16 h</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>167</td>
<td>1-octanol (1 equiv)</td>
<td>168</td>
<td>Cu(OTf)$_3$, NIS</td>
<td>RT</td>
<td>16 h</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>167</td>
<td>1-octanol (1 equiv)</td>
<td>168</td>
<td>Zn(OTf)$_3$, NIS</td>
<td>RT</td>
<td>16 h</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>167</td>
<td>(−)-menthol (1 equiv)</td>
<td>167</td>
<td>Cu(OTf)$_3$, NIS</td>
<td>RT</td>
<td>25 h</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>167</td>
<td>171</td>
<td>Cu(OTf)$_3$, NIS</td>
<td>RT</td>
<td>5 days</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>167</td>
<td>173</td>
<td>Cu(OTf)$_3$, NIS</td>
<td>RT</td>
<td>2 days</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

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**Scheme 36.** The use of N-acetyloxazolidinone protected thioglycosides as both donor and acceptor.

**Scheme 37.** Use of 2-deoxy – 2-N-sulfate glycosides in glycosidations.
of the reaction showed that Ni(4-FPhCN)$_2$(OTf)$_2$ gave the best stereoselectivity with maximum effectiveness within a short reaction time.

Thus, for amino sugars, the stereochemistry of the products largely depended on the directing properties of the protecting group in the C-2 position. Modifications in the nature of the protecting group are still an active area of research.

### 3.8. Other Types of Glycosylation

#### 3.8.1. Protecting-Group-Free Glycosylation

Until now, most of the glycosylating protocols described dealt with O-protected glycosyl donors, aiding in the synthesis of varied glycoconjugates and higher oligosaccharides. However, there has been much discontent about glycosylations performed with unprotected glycosyl donors with activated anomeric center. This is largely because accomplishing glycosylations without protecting the hydroxyl groups brings forth a number of advantages, the prime issue being the reduction in the protecting-group manipulation steps of the complex oligosaccharide synthesis. It also opens the scope for iterative glycosylations, which lead to the continued coupling of the sugar fragments. Moreover, these hydroxyl glycosides also promises to overcome the issue of reduced reactivity with the acyl-protected glycosides. The free hydroxyl groups also leave the opportunity for its ready use in various antibodies and therapeutics.

Despite all of the advantages, performing glycosylations with unprotected glycosyl donors present some major hurdles. Similar to 2-deoxy glycosides, the absence of any directing group in the C-2 position makes it highly difficult to control the stereochemistry of the formed glycosidic bond in the anomeric center. Moreover, the easy accessibility of more than one reactive hydroxyl groups requires the application of highly selective reagents capable of performing the coupling in the desired position. Hence, studies to optimize the reagents and the conditions to attain the best possible output are underway. Describing all glycosylation protocols developed in this field would require a complete detailed Review in its own right. The strategies of the past century have already been extensively reviewed. However, we aim here to describe the ones that show the most significant promise and can contribute to the challenges in performing regular glycosylations.

In an attempt to find a suitable robust donor capable of being activated in its unprotected state, Roy and Mukhopadhyay performed Fischer-type glycosylation to establish the use of H$_2$SO$_4$–silica to couple unprotected free sugars with different alcohols. Interestingly, Pfafe and Mahwald showed that, when variously functionalized alcohols were reacted with free d-ribose 175 catalyzed by 10 mol% of titanium-(IV) tert-butoxide and 50 mol% d-mandelic acid at room temperature, only coupled ribose furanosides (Scheme 38) were obtained. Addition of lithium bromide significantly enhanced the yield in acetonitrile medium. The same group in the following year introduced the concept of organo-catalyzed glycosylation of unprotected and unactivated glycosides. Here, traces of PPh$_3$ and CBr$_4$, were used as the catalyst with LiClO$_4$ as the essential additive. Surprisingly, this catalyst was successful in facilitating the formation of exclusive β-products. Recently, Bhattacharrya and co-workers also described the activation of both unprotected and unactivated glycosides by using 10 mol% of bismuth nitrate pentahydrate. The yields of the desired products were significantly increased when unprotected sugars were subjected to Fischer glycosylation by sulfamic acid. Sharma et al. reported the coupling of free unprotected and unactivated sugar molecules with alcohols on being mediated by NH$_4$Cl at 90 °C. The yields of the products obtained were claimed to be higher and the process less time consuming compared to the standard glycosylation protocols using unpro-

![Scheme 38. Reaction of d-ribose with different alcohols.](image)
tected glycosyl donors in the presence of common catalysts like Amberlite IR-120 (H\(^+\)) resin, InCl\(_3\), In(OTf)\(_3\), Sc(OTf)\(_3\), and HCl.

The use of ionic liquids for performing glycosylations with unprotected glycosyl donors has also been explored. RTILs such as 1-ethyl-3-methylimidazolium benzoate ([emim][ba]) in the presence of Amberlite IR-120 (H\(^+\)) resin or p-toluenesulfonic acid (TsOH) were used\(^{[242]}\) as promoters. These ionic liquids were reported to be successful in activating various unprotected sugar units for the desired oligosaccharide synthesis. Glycoconjugates derived from the glycosylations with this ionic liquid were also studied for their affinity towards lectins.\(^{[283]}\)

Auge and Sizun reported the use of ionic liquids in Lewis-acid-catalyzed glycosylations in 2009. They first explored the catalytic efficiency of various metal salts like InCl\(_3\), In(OTf)\(_3\), Sc(OTf)\(_3\), and Yb(OTf)\(_3\) at different temperatures. The studies revealed that even a catalytic amount of Sc(OTf)\(_3\) provided the optimum yield at 80 °C on continuing the reaction for a longer period. Considering this and predicting the mechanism following the oxocarbenium ion intermediate, Auge and Sizun attributed the increase in yield and regioselectivity by the use of ionic liquids.\(^{[284]}\) A variety of ionic liquids, varying in their ionic part, were tested, among which 1-butyl-3-methylimidazolium trifluoromethanesulfonate [bmim][OTf] gave encouraging results.

Optimization of the reaction was, thus, accomplished with different unprotected sugars\(^{[285]}\) and alcohol\(^{[286]}\) catalyzed by Sc(OTf)\(_3\) in the presence of 1 mol% of [bmim][OTf] as the solvent (Scheme 39). The results of the reactions with octanol as the acceptor alcohol (Table 8) showed that the reagent is capable of generating the desired product in subtle α-selectivity. Sc(OTf)\(_3\) as the catalyst has also been utilized for glycosylation of carbohydrates with different amino acids have also been accomplished in [bmim][OTf].\(^{[285]}\)

Unprotected alkynyl glycosides were also explored for activation by Mamidyala and Finn,\(^{[286]}\) who tried to optimize the reaction conditions by using different solvent systems and employing variable temperatures catalyzed by Au\(^{13}\) salts. Detailed studies revealed the versatility of refluxing acetonitrile as the solvent to achieve the best possible yield. The same protocol was also verified by applying it for the synthesis of a trisaccharide. However, the stereoselectivity obtained in the reaction did not show much promise.

A photoinduced protocol was also employed by Toshima and co-workers in 2013, who described a novel glycosylation protocol through photoinduced activation of unactivated deoxy thioglycosyl donors.\(^{[119]}\) Here, DDQ served as the SET reagent, whereas boronic acid was used as a temporary 1,3-diol protection, leaving the primary hydroxyl group available for reaction to prevent the corresponding self-coupling and the formation of anhydrosugars.

![Scheme 39. Reaction of unprotected sugars with alcohol.](image)

### Table 8. Different yields obtained on the reaction of free sugars with octanol.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Temp [°C]</th>
<th>Time [h]</th>
<th>Yield [%]</th>
<th>α/β</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-glucose</td>
<td>80</td>
<td>24</td>
<td>74</td>
<td>75.25</td>
</tr>
<tr>
<td>α-galactose</td>
<td>80</td>
<td>24</td>
<td>70</td>
<td>61.39</td>
</tr>
<tr>
<td>α-mannose</td>
<td>80</td>
<td>24</td>
<td>88</td>
<td>100.0</td>
</tr>
<tr>
<td>L-fucose</td>
<td>80</td>
<td>24</td>
<td>51</td>
<td>67.33</td>
</tr>
<tr>
<td>N-acetyl-glucosamine</td>
<td>110</td>
<td>24</td>
<td>60</td>
<td>79.21</td>
</tr>
</tbody>
</table>

Nitz and co-workers introduced different anomeric protecting groups that show promise to be activated in protecting-group-free conditions.\(^{[287]}\) N'-Glycosyltoluenesulfonohydrizides (GSHs)\(^{[186]}\) have also been used in the formation of various glycosyl O-phosphates\(^{[288]}\) upon being activated by NBS in DMF. Glycosylation of these GSHs with non-volatile alcohols were demonstrated to give moderate yields, but with poor stereoselectivity.\(^{[50]}\) However, the possibility of the formation of unprotected glycosyl chloride\(^{[187]}\) from GSH was explored in situ (Scheme 40), which can further undergo coupling with alcohols at a faster rate, giving better yields. But, in both cases, poor stereoselectivity (β-anomer predominating) limits the scope of such anomeric leaving groups.

Further efforts are still being made in improving the stereo-selectivity of glycosylations involving unprotected glycosyl donors. Atom economy and faster protocols show much promise towards expeditious oligosaccharide synthesis.

#### 3.8.2. One-Pot Glycosylation

Among the various progress achieved in oligosaccharide synthesis in the current century, the one-pot glycosylation strategy has emerged as an important strategy. One-pot glycosylations claim to be the shortest possible route for oligosaccharide synthesis. The strategies focus on an easy solution for issues related to purification and isolation of the intermediate products. However, one-pot iterative glycosylations demand the use of strategically synthesized sugars. Such glycosylation strategies may be accomplished by employing either ‘armed-disarmed’ concept\(^{[246]}\) or chemoselective activation of the glycosyl donor. Thus, it becomes highly evident that one-pot iterative glycosylations are primarily based on the reactivity difference of synthons at a particular reaction condition.

The present century has seen significant development in the domain of one-pot glycosylations.\(^{[289]}\) In this Review, we report some of the most distinct illustrations that prove the versatility...
and novelty of such a glycosylation protocol and make it clear that one-pot glycosylations are here to stay. In 2005, Demchenko and co-workers utilized the reactivity difference of various thioimidates in convergent oligosaccharide synthesis.\[298\] They illustrated the successful one-pot synthesis of a tetrasaccharide, utilizing the reactivity difference of SBox, STaz, and SET derivatives. Starting with an SBox and a thioglycoside, the first glycosylation was performed at room temperature in the presence of AgOTf as the catalyst, which selectively activated the SBox derivative to give the desired SET trisaccharide exclusively. It was followed by the addition of another STaz acceptor in the same reaction pot, and now the SET group was activated by using the NIS/TfOH promoter system to afford the desired STaz trisaccharides exclusively. Finally, the methyl glycoside acceptor was added along with AgOTf to afford the required tetrasaccharide (Scheme 41).

![Scheme 41. One-pot glycosylation exploiting reactivity differences of different thioglycosides donors.](image)

through the activation of STaz. The final yield of the product obtained was quite significant, which highlighted the versatility of the iterative sequence. The advantage of one-pot protocols over stepwise methodologies with leaving groups having different reactivities has also been illustrated by Cao and co-workers in a recent report of synthesis of a branched pentamannoside.

Fine tuning of the donors has also facilitated synthesis of various convergent oligosaccharides. Polat and Wong demonstrated the successful one-pot synthesis of the biologically relevant heparin-like oligosaccharide through pre-activation methods.\[296\] Ye and co-workers introduced a new promoter system, benzensulfanyl morpholine (BSM), to increase the efficiency of one-pot glycosylations through pre-activation methods.\[294\] For the synthesis of the trisaccharide, BSM was added for the complete activation of the thioglycoside in the presence of TfOH at \(-70\) °C. Further addition of methyl glycoside acceptor to BSM/TfOH promoter system followed by addition of acceptor provided trisaccharide (Scheme 43) within 1 h in moderate yield.

In different activation methods, polymer-assisted one-pot synthesis of trisialic acid has been effectively achieved by Tanaka et al. Ionic liquids have also facilitated one-pot syntheses. Galan et al. introduced the use of [bmim][OTf] as promoter in the presence of TfOH for region- and chemoselective glycosylation of glycosides with different reactivities.\[298\] Despite the progress of one-pot synthesis methods in the last two decades, there are still many limitations in its universal application. Studies and extensive investigations are being

![Scheme 42. Synthesis of a trisaccharide through one-pot glycosylation.](image)

In a different sequence, using chemically different functionality in the anomeric center, Ladonisi and co-workers modified the stepwise assembly of the biologically relevant antitumor PI-88 pentasaccharide to a more robust and convenient one-pot reaction strategy.\[138\] The product was obtained by the use of mild Lewis acid catalysts like Yb(Otf)\(_2\) and Bi(Otf)\(_3\). Similarly, tumor-associated carbohydrate antigen Globo-H hexasaccharide was synthesized through the pre-activation protocol in near-stoichiometric yield. Various other expedient oligosaccharide syntheses have been successfully achieved by using chemoselective one-pot strategies.\[294\] Ye and co-workers introduced a new promoter system, benzensulfanyl morpholine (BSM), to increase the efficiency of one-pot glycosylations through pre-activation methods.\[294\] For the synthesis of the trisaccharide, BSM was added for the complete activation of the thioglycoside in the presence of TfOH at \(-70\) °C. Further addition of methyl glycoside acceptor to BSM/TfOH promoter system followed by addition of acceptor provided trisaccharide (Scheme 43) within 1 h in moderate yield.

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made to implement such processes in the expeditious synthesis of more natural oligosaccharides. However, this strategy shows immense promise, having the potential to emerge as a powerful synthetic tool in the domain of carbohydrate synthesis.

### 3.8.3. Solid-Phase Synthesis

Solid-phase oligosaccharide synthesis (SPOS) is an emerging field in carbohydrate chemistry that has attracted overwhelming interest in the present century. This modern method of carbohydrate synthesis claims to overcome various shortcomings of solution-phase synthesis. Moreover, the need to perform purification at each step of a solution-phase synthesis limits the applicability of the process, to a large extent, whereas SPOS involves a protocol that removes the excess reagents and other solution-phase impurities by simple washing of the resin, thereby reducing the number of chromatographic steps involved. However, solid-support-based carbohydrate synthesis requires judicious planning and systematic implementation. The polymer support and the linker for its attachment to the sugar need to be planned very carefully so that it can withstand the conditions for the essential protecting-group manipulations. However, at the same time, it should be labile so that it can be cleaved as and when required. Solid-phase iterative strategies may be accomplished either by attaching the reducing or the non-reducing end of the sugar to the solid polymeric support, leaving the growing end subjected to glycosylation steps. Attainment of stereospecific glycosylations in high yields through rationally designed strategies has made SPOS a widely used protocol. Taking cue from the first solid-phase oligosaccharide synthesis by Fréchet and Schuerch\[299a\] in 1971 using Merrifield resin, rigorous attempts to develop smarter strategies have been observed in the later part of the last century\[299b, 300\]. Various Reviews\[301\] have revealed versatile and comprehensive overviews on SPOS. A study by Kanie et al. gives a broad comparative picture, as the group synthesized a wide range of fucosyl–galactosyl oligosaccharides in both the solution and solid phase.\[302\]

Monitoring the progress of reactions in SPOS is extremely essential. The crudest way is to take aliquots of the reacting resins and subject them to thin-layer chromatography after cleaving them from the resins. Moreover, knowledge of the exact time required for the total completion of the reaction is crucial, as it has been observed that both a shortened reaction time and exposure to excess time have been detrimental, increasing the amount of impurities in the product library. Thus, analysis of the on-support conversions and yields becomes inevitable for the development of SPOS. \(^1H\) and \(^{13}C\) NMR spectroscopy has been explored in combination with other methods; but, it requires some time consuming and expensive protocols. Hence, in early 2001, Kihlberg and co-workers implemented the use of gel-phase \(^{19}F\) NMR spectroscopy\[303\] by using fluorine-labeled protecting groups in combination with fluorinated linkers. No countering signals were observed, owing to the absence of any fluorine group in the resins. Hence, monitoring solid-phase oligosaccharide reactions by \(^{19}F\) NMR promised to provide much impact in the times to follow. By using the same protection protocols of fluorinated analogs, the same group utilized \(^{19}F\) NMR spectroscopy to monitor the solid-phase synthesis of \(\alpha\)-Gal trisaccharide epitopes \[207\] after loading them with ArgoGel resin (Scheme 44). \(^{13}C\) NMR spectroscopy has also been found to monitor SPOS successfully, where the gated decoupling technique was implemented.\[305\] Other methods for monitoring SPOS include single-bead FTIR microspectroscopy, but it leads to significant overlap of spectra from the presence of multiple components including starting material, the involved resin, along with the formed product. To overcome this shortcoming,
In this method, the removal or introduction of the organic functionalities in the ongoing reactions was displayed as positive or negative signals, respectively, thereby establishing itself as an effective and powerful analytical tool for SPOS.

In another method, Manabe and Ito utilized a colorimetric method to establish real-time monitoring of the on-resin oligosaccharide synthesis by employing disperse red (C_{31}H_{21}N_{3}O_{3}) and (p-nitrobenzyl)pyridine (PNBP) color tests. Owing to the complementary nature of the two tests, the progress of glycosylation reactions were successfully established by using these methods. The removal of the chloroacetyl group in the monosaccharide was established by the positive red color in the disperse red test and negative result in the PNBP test. Subsequently, the disappearance of the hydroxyl groups in the glycosylation reaction was indicated by the negative disperse red and strongly positive PNBP tests (Table 9).

In SPOS, studies have shown that the presence of longer spacer connecting the synthons with the resin helps the coupling more than glycosides with a shorter spacer. Longer spacers help in bringing the sugar part more into the solution phase and provide more accelerated reactive platform.

Resin-bound thioglycoside donors have successfully undergone glycosylation reactions with various acceptors under standard reaction conditions. Direct transfer of resin-bound (JandaJel) thioglycosyl donors to complex aglycons was accomplished by Bennett and co-workers. Optimization of reaction conditions implied benzenesulfinyl piperidine/triflic anhydride (BSP/TfO) as the best candidate of promoter in such a transformation. Glycosyl thioimidates have been successfully utilized for solid-supported glycosylation strategies. In 2007, Demchenko and co-workers performed glycosylations and, in one case, the solid-bound acceptor was coupled with the imidate (SBox or STaz) donor and, in another case, the glycosyl thioimidate was bound to the resin followed by its glycosylation with the acceptor. Normal activation protocols using TMSOTf or AgOTf were employed for the glycosylations.

To establish a versatile SPOS strategy, Boons and co-workers introduced polystryrene boronic acid (PSA) as the resin support that proved to be apt in its role. The ease of its preparation, the use of minimum amount of solvents for its loading, the cleavage, and its reusable nature made this resin widely used in oligosaccharide synthesis. When using PSA as the resin in oligosaccharide synthesis, protecting groups were selected, taking into consideration that the boronic ester linkage hydrolyzed in protic solvents. Hence, Fmoc protection was selected, as it is inert to protic solvents and could be cleaved by the treatment of triethylamine. Fmoc-protected thioglycosides donor was coupled with PSA-immobilized acceptor with the help of NIS/TMSOTf to obtain the resin-bound disaccharide. The Fmoc group was then selectively cleaved to obtain the disaccharide acceptor, with the solid support intact. This PSA-bound disaccharide was further coupled with thioglycoside donor to obtain the final trisaccharide. Eventually, the resin could be cleanly cleaved by the application of an acetone–water mixture, obtaining trisaccharide (Scheme 45), which can be further characterized by using spectroscopy. Capping reagents like benzoyl isocyanate have also been implemented, which aid in performing glycosylations by blocking the unconsumed acceptors and helping with cleaner SPOS. It helped in the purification of the formed glycosides, as it conveniently prevented the formation of unwanted sugar analogs with the unreacted counterparts.

Based on the convenient principles of solid-phase polymer-supported oligosaccharide synthesis, there have been examples of the synthesis of higher analogs of carbohydrates. Crich and Smith successfully applied a method for the synthesis of the unnatural β-mannoside linkage. Schmidt and co-workers also performed SPOS to synthesize a branched hexa-accharide, lacto-N-neohexaose derivative (found in human milk) by immobilization of linker-loaded polystryrene resin. Glycosyl trichloroacetimidates were used as the efficient donors, whereas Fmoc and Lev protecting groups were exploited.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PNBP test</th>
<th>Disperse red test</th>
<th>Remarks</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>209</td>
<td>positive</td>
<td>negative</td>
<td>positive PNBP test confirms presence of chloroacetyl group</td>
<td></td>
</tr>
<tr>
<td>210</td>
<td>negative</td>
<td>positive</td>
<td>positive disperse red test confirms presence of OH group</td>
<td></td>
</tr>
<tr>
<td>212</td>
<td>positive</td>
<td>negative</td>
<td>negative confirms of the presence of chloroacetyl group</td>
<td></td>
</tr>
</tbody>
</table>

Scheme 45. Glycosylations on polymer support.
Thus, from where it was left in the previous century, SPOS has seen much progress in recent years. More vivid study on SPOS methods reveals widespread use of various glycoside donors like sulfoxides, thioglycosides, trichloroacetimidates, pentenyl glycoside, and so forth for effective glycosylation methodologies. However, solid-phase synthesis still suffers certain limitations. These challenges open broader scopes of study to ascertain higher levels of stereochemistry, optimum, and suitable protecting-group manipulations as well as the efficient use of the reagents in the synthesis of the solid-supported glycosides. It is noteworthy that the present century promises to open new dimensions of solid-phase oligosaccharide synthesis with its innovative and successful implementation in the introduction of a new domain involving automated oligosaccharide synthesis.

### 3.8.4. Automated Oligosaccharide Synthesis

Building on solid-phase oligosaccharide synthesis, Seeberger et al. introduced the concept of automated oligosaccharide synthesis. Automated oligosaccharide synthesis is based on the same principle of SPOS, where the choice of the solid support and the linker plays a major role in the determination of the synthetic strategy. Moreover, protecting-group manipulations should also be planned accordingly, with the inertness of the solid support and the linker with the glycosides. Considering the various aspects, Seeberger first utilized peptides in the synthesizer to develop an automated method for glycoside synthesis. Automated synthetic procedures enable the number various manual routine functions to be reduced by employing iterative synthesis comprising of selective deprotection, glycosylation, removal of the resin, and deprotection in a programmed reactor. The reactor was designed to perform conversions at variable temperatures subject to external programming, thus simplifying the synthesis of complex oligosaccharides. The widely used temporary protecting group suitable for such programmed reaction strategies, owing to their capability of selective cleavage, include Fmoc, Lev, and Nap, whereas the permanent protecting groups include benzyl, benzoyl, benzylidine, or azide.

With systematic synthetic strategy, Seeberger and co-workers accomplished the first automated synthesis of two phytoalexin elicitors, a hexasaccharide 220 and a dodecasaccharide 219 (Figure 8). The widespread applicability of the glycosphingolipids, such as Globo-H and Gb-3, as probable antigens in a variety of cancers made them highly attractive biological targets. Automation of oligosaccharide synthesis has accomplished their synthesis in a linear fashion with glycosyl phosphates as the building blocks, facilitating the formation of the $\alpha$-galactosidic linkages immobilized on a solid support. LC–MS was successfully used to monitor reaction steps. Synthesis of the difficult $\beta$-mannosidic bond was also achieved by using an automated protocol. Codée et al. demonstrated the $\beta$-mannosylation by using carboxybenzyl glycosides on a solid support through automated synthetic cycles. Purification of the synthesized oligosaccharides through an automated strategy was also optimized by using the capping and tagging method of Seeberger and co-workers. The use of any scavenger resin or proper filtration through a fluorinated silica gel has proved to be the best choice for such a process.

Although many reactions have been accomplished with the peptide reactor turned carbohydrate reactor, Seeberger and co-workers developed the first fully automated solid-phase oligosaccharide synthesizer in 2012. The reactor not only allowed the iterative steps involved in the multistep synthesis, but is also equipped with various essential gadgets that facilitate the controlled syringe–pump driver reagent delivery and computerized temperature control ranging from $-50$ to $90^\circ C$.

The choice of linker has always been the most significant aspect in planning the strategy for performing automated
solid-support-based oligosaccharide synthesis. The limitations of their instability in different acidic and basic conditions prompted to develop photo-labile linkers as a suitable choice in such automated synthesis. A photolabile nitrobenzyl-ether-based linker has been used to synthesize two chondroitin hexasaccharide fragments of glycosaminoglycans in a continuous flow reactor, implementing a carefully planned protecting group manipulations and glycosylation strategy. Seeberger’s group carried out the synthesis of \(\beta\)-(1,3)-glucan dodecasaccharides by using both thio and phosphate glycosides as donors in the presence of photo-labile linker immobilized on Merrifield’s resin. Solid support was used for the immobilization, which utilized three equivalents of glycosyl phosphate in three repeats over 12 glycosylation cycles. TMSOTf was used as the Lewis acid promoter that could be conveniently added to the building blocks, and the attacking acceptor glycoside through strategically designed syringe pumps in the automated flow reactor (Scheme 46). In between each glycosylation steps, the temporary Fmoc protecting group had to be selectively removed. After the successful completion of the glycosylation sequences, the cleavage of the solid support was also performed in the same reactor by photoradiation. The use of fully automated synthesizers has also been used for the synthesis of sialyl oligosaccharides, oligoarabinofuranosides and galactans, and hyaluronan oligosaccharides that offer broad scopes in conveniently evaluating their biological properties. Higher oligosaccharides, such as a 30-mer polysaccharide of mannose were also synthesized by using an automated flow reactor where the purification was done by the catch-release method. The yields obtained through this protocol were significant, with an average of 96% in each step and the entire synthesis of the polysaccharide accomplished in less than a week.

Nullifying the requirement of a sophisticated automated oligosaccharide synthesizer, methods of automated oligosaccharide synthesis in solution phase were also developed. Jaipuri and Pohl effectively synthesized linear and branched mannoside fragments based on solution-phase automated iterative synthesis. The same group recently used the same protocol to synthesize \(\beta\)-1,4-mannuronate and \(\beta\)-1,4-mannan by using glycosyl trichloroacetimidates as the subsequent donors. Recently, Demchenko and co-workers introduced the concept of HPLC-based carbohydrate synthesis. They included an ormal HPLC setup in order to facilitate all standard reaction steps essential in automated synthesis of a pentasaccharide using repeated glycosylations with 225 and 226 as the synthons (Scheme 47). However, optimization of the various reaction conditions for the setup is under way. A suitable carbohydrate building block was rigorously optimized for performing glycosylations in an automated electrochemical assembly to synthesize a tetrasaccharide fragment from \(N,N,N\)-trimethyl-\(\alpha\)-glucosaminyl (TMG)–chitotriomycin. Thioglycosides have also been implemented in such an electrochemical assembly to synthesize poly-\(\beta\)-\(\alpha\)-(1-6)-N-acetylglucosamine (PNAG), using six thioglycosides in a sequential one-pot setup. Recently Seeberger’s group performed a gold-catalyzed solution-phase oligosaccharide synthesis in a continuous flow reactor. Glycosyl ortho-hexynylbenzoates were used as the donor for coupling with various sugar and non-sugar derivatives catalyzed by PPh\(_3\)AuOTf, furnishing the required glycosides/glycoconjugates in significant yields.

Automated glycoside synthesis in flow reactors is the latest addition in the chemistry of carbohydrate glycosylations based on the available instrumental facilities. This protocol claims to show much applicability in the following years by virtue of its ability to furnish complex higher analogs of polysaccharides in shorter time and in significant yields. However, the procedure still awaits further optimizations by solving the limitations it offers. The applicability of the process would facilitate interdisciplinary aid to the biologists in understanding the role of carbohydrates in the biological systems and in the development of carbohydrate-derived antigens, thereby facilitating the advancement of the domain of therapeutics.
4. Conclusions and Outlook

Significant development in the area of chemical O-glycosylation strategies is evident from the literature. These novel approaches offer a solid platform to deal with various complex oligosaccharides relevant for biological applications. The methods developed in the current century have helped us to get a better insight into the reactivity of glycosyl donors and acceptors. Novel promoter systems have enabled us to form glycosidic linkages with better stereoselectivity. Introduction of one-pot iterative synthesis has minimized the labor required for complex oligosaccharide synthesis. With the development of solid-phase synthesis and sophisticated automated glycosylation techniques, the future looks truly promising. However, the challenge of oligosaccharide synthesis is not yet over. Other than few ventures with protecting-group-free syntheses, chemical O-glycosylations still suffer from the requirement of cumbersome protecting-group manipulations that seriously defy the urge for atom economy. Moreover, the number of truly green reaction strategies is low in this domain. A sincere effort is needed to develop green and atom economic glycosylation strategies that can take us to a comparable domain of nature’s enzymatic means of oligosaccharide preparation. We sincerely hope that the present survey of chemical O-glycosylation strategies will help researchers to have a better understanding of the current scenario and pave the way for future developments in this important area.

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Keywords: catalysis • glycosylation reactions • promoters • protecting groups • stereoselectivity

[6] Although we have now learned to synthesize oligosaccharides, it should be emphasized that each oligosaccharide synthesis remains an independent problem, whose resolution requires considerable systematic and research and a good deal of know-how. There are no universal re-action condition for oligosaccharide synthesis—Hans Paulsen.