Oligosaccharide assembly by one-pot multi-step strategy

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Saccharide synthesis is a formidable task for synthetic chemists. Although in recent years many advances have been made in this area, development of more convenient and efficient strategies for oligosaccharide synthesis is still in great demand. This review focuses on one of these new strategies—the one-pot sequential glycosylation approach as a potent tool for oligosaccharide assembly.

Introduction

The multifaceted biological importance of oligosaccharides and glycoconjugates has made them very popular synthetic targets in modern synthetic chemistry.[1] In the past few decades, great

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progress has been made in the construction of complex oligosaccharides based on a variety of synthetic strategies.[2] Among these strategies, the "one-pot glycosylation" is very attractive and shows great promise for assembly of oligosaccharide libraries and automated synthesis.

The one-pot glycosylation method refers to one in which several glycosyl donors are allowed to react sequentially in the same flask, resulting in a single main oligosaccharide product. This procedure integrates several glycosylation steps into one synthetic

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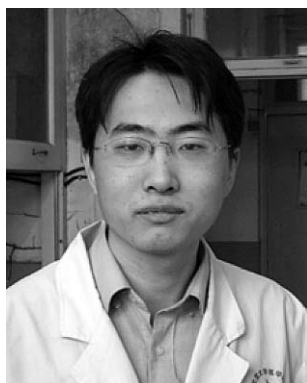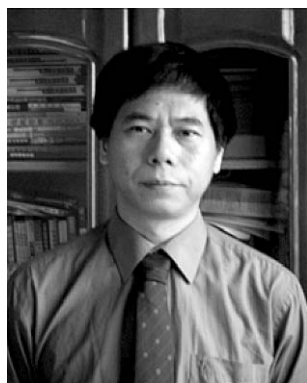
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operation to furnish target oligosaccharides in a short period of time without the need for protecting group manipulation and intermediate isolation, and therefore is much more efficient since traditional synthesis of saccharides requires multiple protection and deprotection steps and time-consuming purification processes using column chromatography. Complete consumption of the formed intermediate oligosaccharides is very important for a successful one-pot glycosylation, and the resulting high yield together with good stereoselectivity in every glycosylation steps ensure that only the target oligosaccharide will be produced as the major product, thus simplifying the final isolation operation and providing high efficiency of this protocol.

This review will introduce an outlined advance in one-pot oligosaccharide synthesis. The multi-enzyme one-pot method will also be covered as another aspect of the one-pot glycosylation strategy.

The chemical strategy for one-pot glycosylation

One-pot glycosylation for chemical synthesis of oligosaccharides relies on the reactivity disparity of glycosyl donors/acceptors. Although many variations of the one-pot strategy have been developed, there are three major concepts these protocols are based on, namely, "chemoselective principle", "orthogonal glycosylation", and "pre-activation strategy".

The chemoselective strategy uses the nature of the protecting groups' influence on the reactivity of donors and acceptors. It has long been recognized that electron-donating groups activate (arm) the leaving group in the glycosylation reaction whereas electron-withdrawing groups deactivate (disarm) the leaving group.[3] This armed–disarmed concept makes chemoselective glycosylations possible. Thus, in the chemoselective one-pot glycosylation, the most reactive donor is condensed with the less reactive donor to provide a new glycoside which can subsequently glycosylate the least reactive donor (Scheme 1a).

The orthogonal glycosylation strategy is based on selective activation of one leaving group over another.[4] In this approach different anomeric groups (*e.g.* thioglycoside and fluoride) are exploited either as an anomeric protecting group or as a leaving group, since they are mutually stable to the conditions used to activate the other anomeric functionality (Scheme 1b).

The recently established pre-activation strategy employs the activation of a glycosyl donor in the absence of a glycosyl acceptor to provide a reactive intermediate which is immediately treated with a second building block (glycosyl acceptor) to yield a coupling saccharide with an identical activatable aglycon at the reducing end.[5] This process can be repeated in the same reaction flask to extend the sugar sequence (Scheme 1c).

One-pot glycosylation based on the chemoselective strategy

Chemoselective glycosylation can be influenced by the reactivity of both glycosyl donors and glycosyl acceptors. The reactivity of glycosyl donors varies in different monosaccharide types. For example, the reactivity order of thioglycosides having the same substituents in the sugar ring appears to be: fucose > galactose > glucose > mannose.[6] The stereo- and inductive effects in the stabilization of the oxocarbenium ion intermediate might
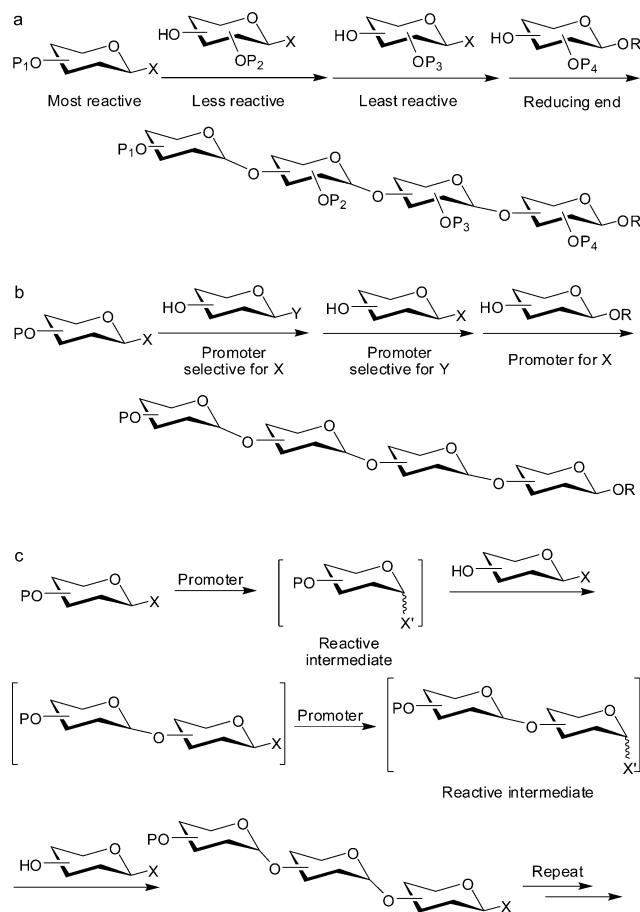

Scheme 1 The chemical strategies for one-pot glycosylation. a) One-pot glycosylation based on the chemoselective strategy. b) One-pot glycosylation based on the orthogonal strategy. c) One-pot glycosylation based on the pre-activation strategy.

explain the higher reactivity of galactosides than glucosides.[7] Protecting groups have great effects on the reactivity of glycosyl donors. The armed–disarmed concept, firstly described by the Fraser-Raid laboratory,[8] indicates that an electron-donating C(2) substituent activates and an electron-withdrawing C(2) substituent deactivates the anomeric centre, since the electron-withdrawing groups affect the positive charge distribution on the anomeric center, therefore destabilizing the oxocarbenium transition state, and they also decrease the nucleophilicity of the anomeric function and thereby lower the rate of attack of the leaving group on the electrophilic promoter species. This phenomenon allows activated donors to selectively couple with deactivated acceptors without the self-coupling of deactivated acceptors. Later, Ley and co-workers provided intermediate levels of reactivity tuning by using diacetal protected thioglycoside glycosyl donors ("semi-disarmed" effects).[9] The study by the Wong group revealed that the type and position of protecting groups contribute to anomeric reactivity.[6] For example, different protecting groups at the 2-position deactivate the specific galactosyl core in the order –N$_3$ > –OClAc > –NPhth > –OBz > –OBn. The degree of deactivation of thiogalactoside by the influence of benzoyl group positions was observed in the order 4 > 3 > 2 > 6. They also found that glycosylating the free hydroxyl leads to a slight deactivation of the acceptor. Other than the 2-position protecting group manipulation,

the Boons group regulated thioglycoside reactivity *via* steric modulation of the anomeric thioether substituent.[10] In addition, glycosylation conditions (*e.g.* solvent, reaction temperature) can sometimes affect glycosyl reactivity.

In 1993, Kahne and Raghavan described the first sequential glycosylation using a one-pot procedure.[11] Their investigation was based on the reactivity disparity between both the two sulfoxide donors (**2** > **1**) and the acceptors (**3** with OH > **2** with TMS). Thus, the mixture of three reactants was treated with the promoter (TfOH), rendering the one-pot synthesis of ciclamycin 0 trisaccharide **4** (Scheme 2).
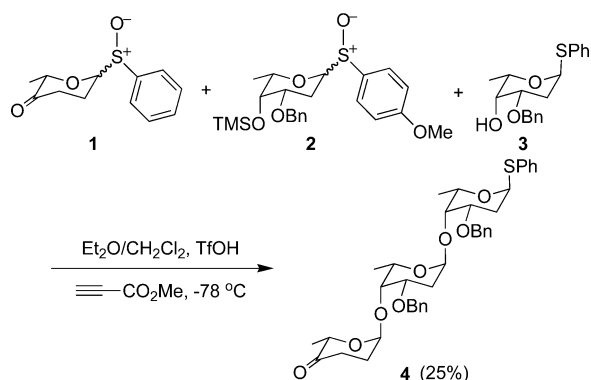

Scheme 2 One-pot synthesis of ciclamycin 0 trisaccharide **4**.

Subsequently the Ley group prepared the trisaccharide unit **5** of a group B *Streptococci* polysaccharide antigen by a facile one-pot two-step synthesis (Scheme 3).[12] In their exercise, a cyclohexane-1,2-diacetal (CDA) protecting group was introduced into thioglycosyl acceptors to perform reactivity tuning *via* the torsional disarmed effect, which hampered the formation of the flattening cationic transition state. Furthermore, they also utilized both seleno- and thioglycosides in combination with the CDA protecting group to provide different levels of reactivity, leading to one-pot synthesis of linear and branched oligosaccharides.[13]
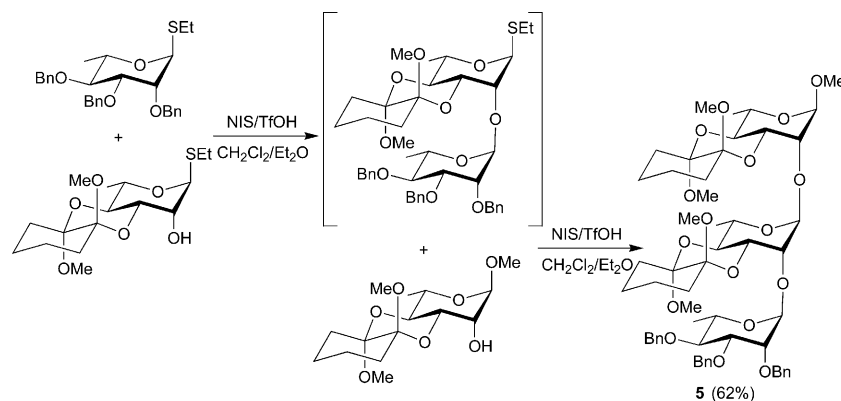
As the synthesis of an increasing number of complex oligosaccharides was carried out using the one-pot method, the need for more precise data on the relative reactivity of glycosyl donors became more apparent. The knowledge of quantification of the glycosyl donor reactivities was essential to researchers. To tackle this problem, the Ley group quantified the influence of protecting

groups, sugar skeletons and anomeric leaving groups on the reactivity of some glycosyl donors by NMR measurements.[14] The Wong group later made a great contribution to this effort. Hundreds of saccharide building blocks were synthesized. A general procedure for the quantitative measurement of relative reactivity of various thioglycoside donors and donor–acceptors was also established.[6] The relative reactivity values (RRVs) were measured by the use of the designed competition experiments performed by HPLC analysis. In this manner, they generated a relative reactivity database of thioglycosides.

Based on the reactivity database, they developed the "OptiMer" computer program for use as a database search tool and a guide for the selection of suitable building blocks for the one-pot assembly of a desired oligosaccharide or a library of individual oligosaccharides.[6] For example, as outlined in Scheme 4, once
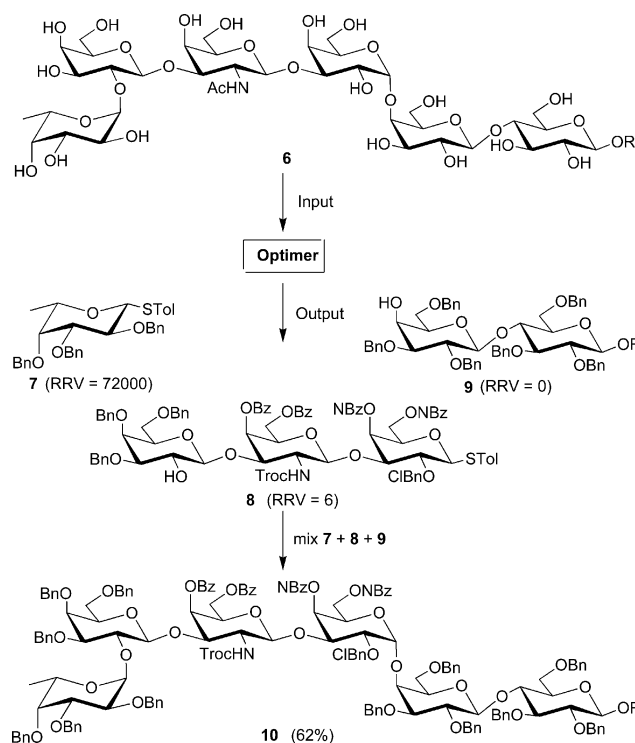
Scheme 4 Programmable one-pot synthesis of Globo H hexasaccharide **10**.

Scheme 3 Ley's one-pot synthesis of trisaccharide **5**.

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given the Globo H hexasaccharide sequence **6**, the program searched the in-house database to present the best combination of available characterized building blocks (**7**, **8** and **9**). Then the three building blocks were used sequentially in one-pot synthesis to give the protected oligosaccharide product **10**.[15] Programmable one-pot synthesis facilitates the convenient assembly of oligosaccharides and has been applied with success in the synthesis of a large number of oligosaccharides, including the colon cancer antigen Le[y],[16] sLe[x],[17] fucosyl GM$_1$ oligosaccharide,[18] tumor-associated antigen N3 minor octasaccharide[19] and other biologically significant oligosaccharides.[20] This method also showed great potential for constructing biologically important oligosaccharide libraries, demonstrated by the rapid assembly of 33 linear or branched fully protected oligosaccharides using simply designed building blocks.[21]

Besides changing protective groups, another variable of glycosyl donors is the nature of the anomeric leaving group. Simple modification of the anomeric substituents can confer different levels of reactivity to common building blocks, which might facilitate efficient oligosaccharide one-pot synthesis. Thus, Huang and co-workers applied the concept of post-synthetic aglycon modification to their one-pot oligosaccharide synthesis.[22] By noticing the fact that glycosyl donors with electron-rich and sterically less hindered aglycons are usually more reactive than those with electron-poor and sterically more hindered ones, they selected *S*-(4-aminophenyl) thioglycoside as the key intermediate so that they were able to easily convert the amino function into substituents bearing various electron-withdrawing groups through simple manipulations, resulting in different levels of anomeric reactivities. With the derived building blocks (methoxy **11**, bromo **12**, and nitro **13**) in hand, the assembly of chitintetraose **14** was finished in the presence of three different thiophilic promoters in moderate yield (Scheme 5). In other instances, Sulikowski *et al.* utilized diethyl glycosyl phosphite and pinacol phosphate to synthesize a trisaccharide in a one-pot manner,[23] and very recently

Iadonisi *et al.* reported a one-pot assembly of trisaccharides employing glycosyl trichloro- and (*N*-phenyl)trifluoroacetimidates catalyzed by a low amount of ytterbium triflate [Yb(OTf)$_3$].[24]

The effect of solvents on the reactivity of donors during the glycosylation process was elegantly exploited in a set of one-pot syntheses by the Oscarson group.[25] As shown in Scheme 6, by adjusting the reactivity of donors and performing the first glycosylation in Et$_2$O (low glycosylation rate) and the second in CH$_2$Cl$_2$–Et$_2$O (higher glycosylation rate), trisaccharide **15** was synthesized in very high yield (84%). It is noteworthy that performing the first glycosylation in CH$_2$Cl$_2$ resulted in a complex product mixture, while the second glycosylation did not occur in Et$_2$O. Later, this solvent effect on reactivity, in combination with the armed–disarmed glycosylation strategy by the use of *N*-Troc and *N*-Phth protected thioglycosides, was once again successfully applied to the one-pot synthesis of glucosamine oligosaccharides by the Baasov group.[26]

Valverde and co-workers developed a new route for one-pot glycosylation which relies on the kinetic acceleration of an intramolecular *versus* an intermolecular glycosidic coupling, rather than on large disparities between the reactivity of different glycosyl donors. In their one-pot procedure, the intramolecular glycosylation of **16** occurred selectively on the 6-OH, followed by the second coupling on the 3-OH with the newly added donor **17** yielding the branched trimannoside **18** (Scheme 7).[27]

In order to solve the problem that reactive glycosyl donors cannot carry reactive hydroxyl groups due to the self-coupling issue, a new approach, in which a *p*-methoxybenzyl ether is used as an *in situ*-removable temporary protecting group for a reactive hydroxyl group, was applied to the one-pot two-step glycosylation process by Nilsson *et al.*[28] As displayed in Scheme 8, at −45 °C, a *p*-methoxybenzyl ether is stable enough to withstand the NIS/TfOH conditions and can thus block a highly reactive hydroxyl group. When the first coupling reaction between **19** and **20** was complete, the temporary *p*-methoxybenzyl group was cleaved by simply
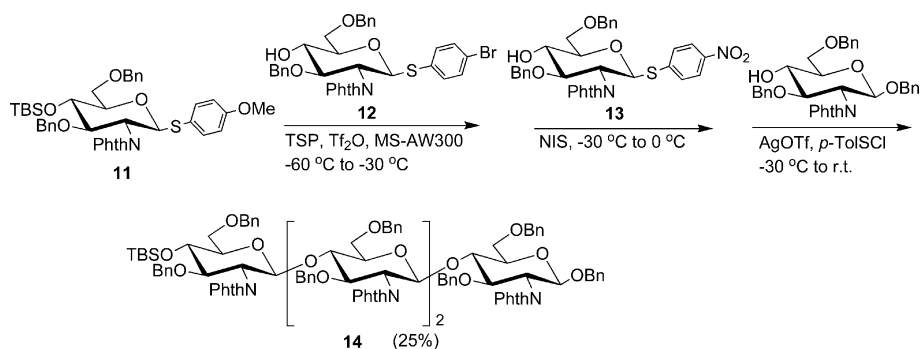
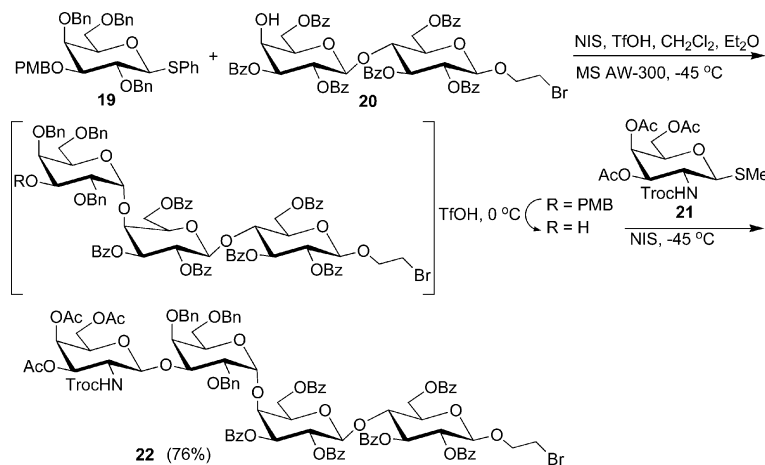**Scheme 5** One-pot synthesis of chitintetraose **14**.
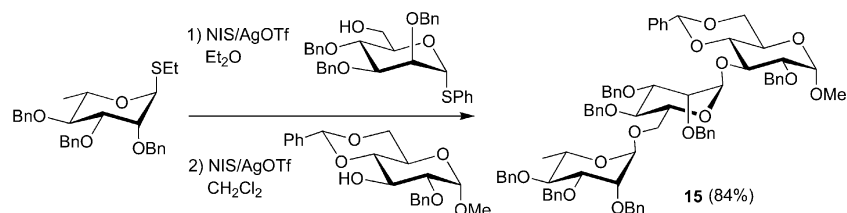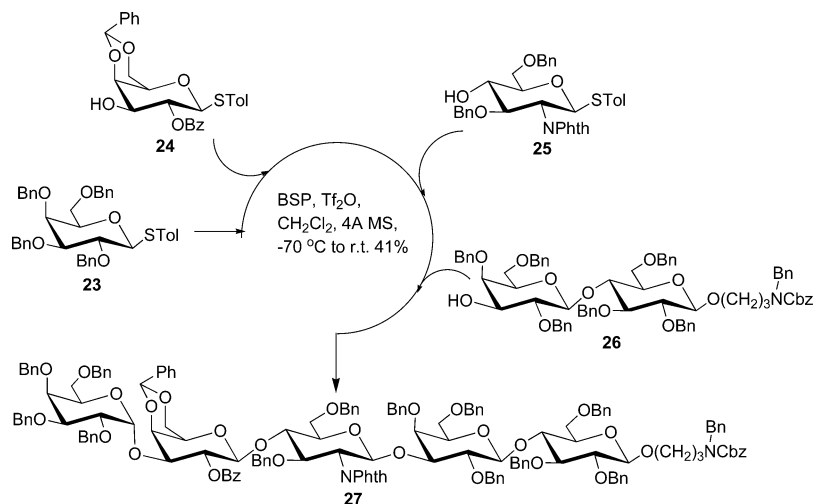

Scheme 6 One-pot synthesis of trisaccharide **15**.
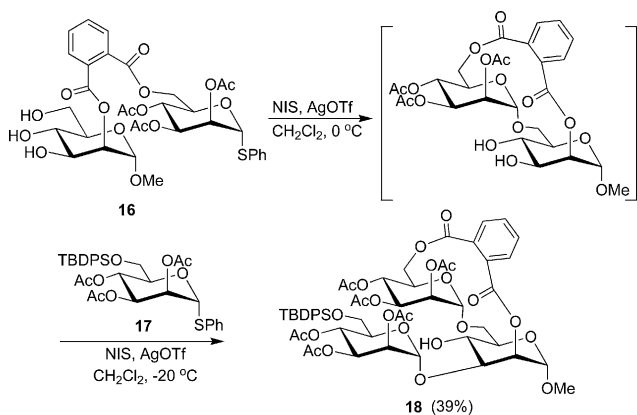
Scheme 7 One-pot synthesis of trimannoside **18**.

elevating the temperature from −45 °C to 0 °C to expose the reactive hydroxyl group for the next glycosylation. Then the donor **21** was added to the reaction mixture at −45 °C to carry out the second glycosylation. In this one-pot manner, the globotetraose (Gb4) tetrasaccharide **22** was synthesized successfully.

To date, most glycosyl donors used in one-pot synthesis are thioglycosides and the application of recently developed thioglycoside activator systems to one-pot glycosylation follows a rational line. Kondo *et al.*'s one-pot two-step synthesis of Le^x derivatives employed a thiophenyl group as the leaving group and NIS/TfOH as the activator.[29] Another type of promoter, trityl tetrakis(pentafluorophenyl)borate/*N*-(ethylthio)phthalimide [TrB(C$_6$F$_5$)$_4$/PhthNSEt] for one-pot glycosylation of armed-disarmed thioglycosides was reported by Mukaiyama *et al.* in 2000.[30] Wong and co-workers introduced the 1-benzenesulfinyl piperidine and triflic anhydride (BSP/Tf$_2$O) system into the one-pot synthesis of fucose GM1.[18] And this activator system was later used in our laboratory to successfully perform the four-component one-pot sequential synthesis of biologically important α-Gal pentasaccharide derivative **27** with four readily available common saccharide building blocks (**23**, **24**, **25** and **26**), as is illustrated in Scheme 9.[31] Furthermore, Wong *et al.* applied *N*-(phenylthio)-ε-caprolactam as a new promoter for the activation of thioglycosides at room temperature, which has been employed in a one-pot trisaccharide synthesis.[32]

Other types of donors were also exploited. Du *et al.* furnished the synthesis of a saponin having a 2,4-branched trisaccharide

Scheme 8 One-pot synthesis of Gb4 **22**.

Scheme 9 Four-component one-pot sequential synthesis of α-Gal pentasaccharide derivative **27**.

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moiety in one-pot fashion by using trichloroacetimidates as glycosyl donors.[33] Recently Mukaiyama and Chiba developed armed–disarmed glycosyl *p*-trifluoromethylbenzylthio-*p*-trifluoromethylphenyl formimidates (glycosyl thioformimidates) (activated by TfOH) for one-pot glycosylations.[34]

## One-pot glycosylation based on the orthogonal strategy

Compared with the chemoselective glycosylation, the clear advantage of an orthogonal strategy is that it allows the condensation of building blocks, independent of their relative reactivities. This strategy has been applied in the early investigation on one-pot glycosylation. The first example of orthogonal one-pot glycosylation was reported by the Takahashi group. The difference in reactivity between glycosyl donors was obtained by using various types of anomeric leaving groups. By varying the activating

agent, the orthogonal glycosyl donors (trichloroacetimidate **28**, thioglycosides **29** and **32**, bromide **31**) can be selectively activated and coupled with glycosyl acceptors sequentially. This method led to the one-pot construction of the linear trisaccharide **30**[35] and the branched trisaccharide **33**[36] (Scheme 10).

In 2002 Takahashi *et al.* described an efficient synthesis of the phytoalexin elicitor heptasaccharide **39** by a one-pot procedure (Scheme 11).[37] The synthesis comprised six sequential glycosylation steps with high chemo- and regioselectivity using seven independent building blocks and sequential removal of acyl and benzyl ether-type protecting groups. Among the glycosyl donors, four selective activatable leaving groups (glycosyl bromide **31**, ethylthioglycosides **34** and **37**, glycosyl fluoride **35**, as well as phenylthioglycosides **36** and **38**) were involved for the coupling of seven building blocks. Interestingly, all the experimental processes including both glycosylation and deprotection were performed on a parallel manual synthesizer (Quest 210). This can be viewed
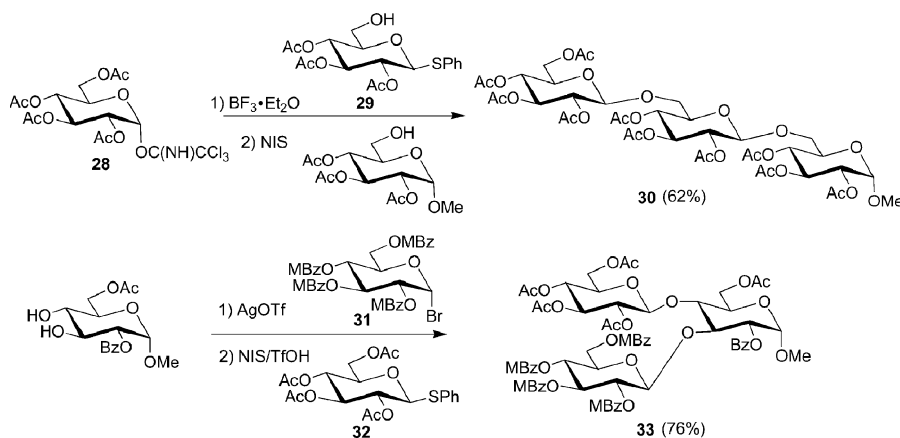
Scheme 10 One-pot synthesis of trisaccharides **30** and **33**.
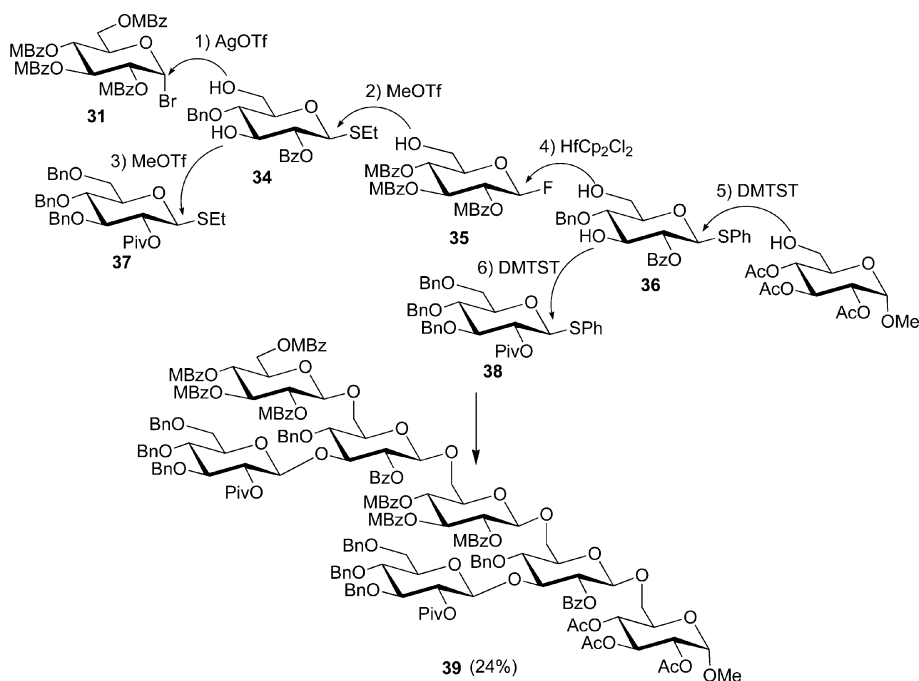
Scheme 11 One-pot synthesis of the phytoalexin elicitor **39**.

as an initial form for the automatic oligosaccharide synthesis. Subsequently, the same group reported the synthesis of core 2 class glycosyl amino acids by a one-pot glycosylation approach using orthogonal anomeric substituents in a combination of *in situ*-removable temporary protecting groups.[38] Very recently, Takahashi *et al.* introduced the one-pot strategy to the synthesis of branched and linear sialo-containing glycosyl amino acids.[39] On the basis of their previous work, Takahashi *et al.* constructed a library of 54 linear and 18 branched trisaccharides by the strategy of solution-phase one-pot glycosylation performed on a manual Quest 210 synthesizer.[40]

Based on their previous work, the Ley group combined the orthogonal glycosides (fluoroglycoside, seleno- and thioglycoside) with the disarmed 1,2-diacetal protecting groups, affording three reactivity levels from five building blocks. A four-step one-pot synthesis was realized for the assembly of the linear pentamannoside **40** (Scheme 12).[41]

The Fraser-Reid group disclosed a synthetic approach,[42] in which nuanced activation of *n*-pentenyl, thioglycoside, and trichloroacetimidate donors by lanthanide salts in combination with the donor–acceptor "match" concept can facilitate oligosaccharide synthesis. As exemplified in Scheme 13, a one-pot double-
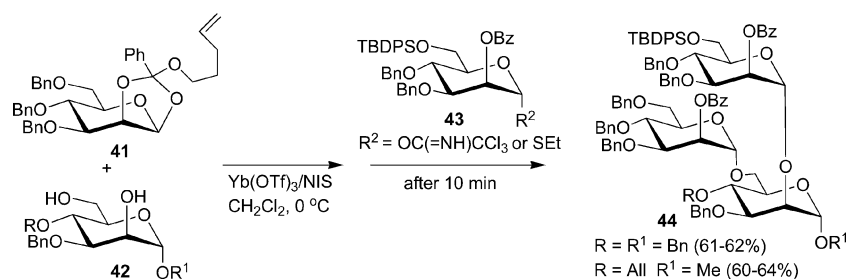
differential glycosylation process was carried out, in which a mannose acceptor-diol **42** was firstly chemo- and regioselectively glycosylated at the 6-OH position with an *n*-pentenyl ortho ester **41** under the agency of Yb(OTf)$_3$/NIS followed by *in situ* addition of a 2-*O*-benzoylated saccharide building block **43** (trichloroacetimidate or ethyl thioglycoside) to effect stereoselective glycosylation at the remaining 2-OH position yielding branched trisaccharides **44**.

Mukaiyama and Kobashi have reported the use of phenylcarbonate paired with thioglycoside in the one-pot assembly of a mucin related F1α antigen **48**.[43] In the first glycosylation phenylcarbonate **45** was coupled with ethyl thioglycoside **46** in the presence of TrB(C$_6$F$_5$)$_4$ using trifluoromethyl benzene (TFB) as the solvent. In the second condensation the terminal glycosyl amino acid **47** and NIS were consecutively added providing the target trisaccharide **48** in excellent yield (Scheme 14). They also combined one fluoride donor and two thioglycosides in the preparation of the phytoalexin elicitor heptasaccharide.[44]
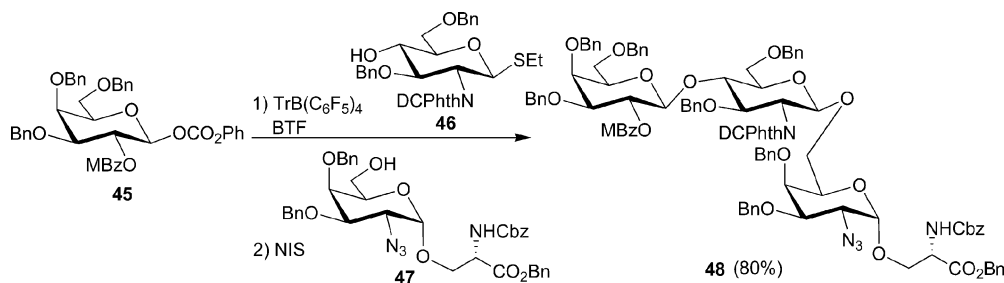
Recently, Demchenko *et al.* reported a leaving group differentiated one-pot synthesis using their previously developed two thioglycosides, *S*-benzoxazolyl (SBox)[45] and *S*-thiazolyl (STaz, 4,5-dihydrothiazol-2-yl)[46] glycosides as orthogonal donors.[47] It
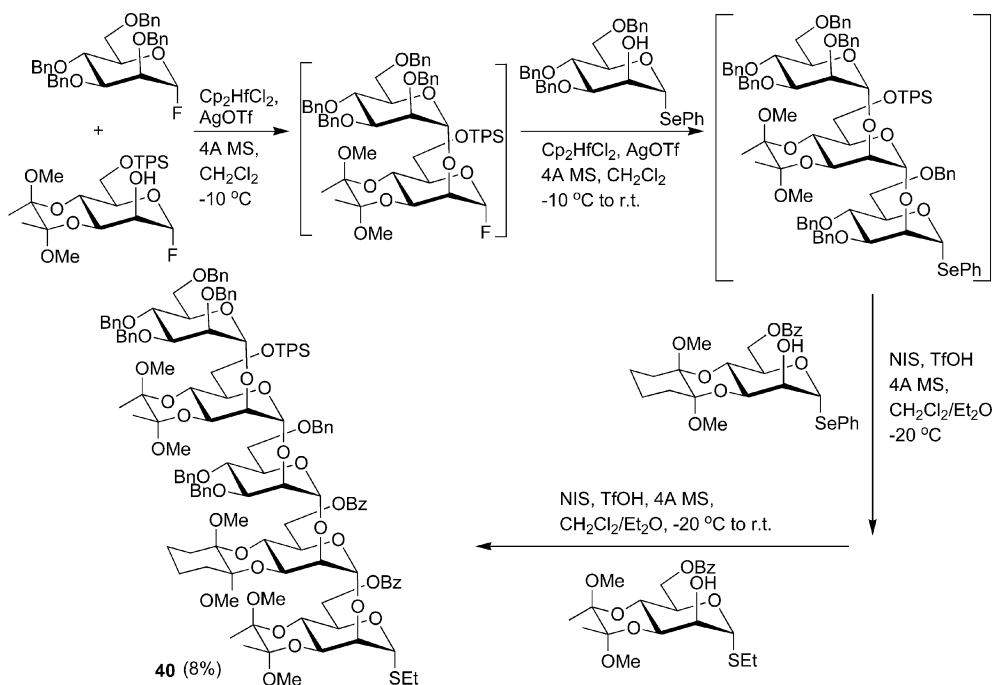
Scheme 12 One-pot synthesis of pentamannoside **40**.

Scheme 13 One-pot synthesis of trisaccharides **44**.

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Scheme 14 One-pot assembly of F1α antigen **48**.

was found that either SBox or STaz derivatives can be selectively activated over *S*-ethyl or *O*-pentenyl glycosides in the presence of AgOTf and that STaz derivatives withstand NIS/TfOH, the conventional activation conditions for thioglycosides. Thus, their one-pot synthesis of the tetrasaccharide **53** was executed in good yield in the activation sequence of SBox **49** + SEt **50** + STaz **51** (Scheme 15).

Chenault and Castro reported a method of selective activation of orthogonal isopropenyl and *n*-pentenyl glycosides in the one-pot synthesis of a trisaccharide.[48] And Yu *et al.* also used an orthogonal imidates *vs.* thioglycosides strategy to synthesize a group of natural diosgenyl saponins in one-pot fashion.[49]

In many cases, poor stereoselectivity of glycosidic bond formation barricades the application of the one-pot glycosylation method. In general, neighboring group participation by C-2 esters will often give 1,2-*trans*-glycosides, whereas 1,2-*cis*-glycosides are often obtained under the control of nonparticipating functionalities (*e.g.* ethers or azide). The Boons group has recently provided an alternative approach for the stereoselective introduction of 1,2-*cis*-glycosides through an elaborate neighboring group participation effect.[50] The glycosyl donors they used bear a C-2 (1*S*)-phenyl-2-(phenylsulfanyl)ethyl functionality, of which the nucleophilic phenylsulfanyl moiety performs neighboring group participation to give a quasi-stable anomeric sulfonium ion as a *trans*-decalin. Subsequent displacement of the equatorial sulfonium ion by a sugar hydroxyl leads exclusively to the formation of a 1,2-*cis*-glycoside (Scheme 16a). This novel glycosylation strategy in combination with traditional neighboring group participation also allowed the one-pot two-step synthesis of trisacchairde **57** (Scheme 16b). In their protocol the galactosyl donor **54** bearing a

C-2 *S*-auxiliary group directed the formation of the α-glycosidic bond, whereas the C-2 benzoyl group of **55** afforded the β-glycosidic linkage.

Very recently, Manabe *et al.* reported the use of *N*-benzyl 2,3-*trans*-oxazolidinone protected glycosyl donors for the 1,2-*cis* glycosidic bond formation for 2-amino-2-deoxy glycoside synthesis.[51] Based on the high α-selectivities, they prepared a trisaccharide with two 1,2-*cis* glycosidic linkages in a one-pot operation.

One-pot glycosylation based on the pre-activation strategy

Although the reactivity-based one-pot strategy has greatly facilitated the glycosylation reaction process, extensive protecting group manipulations and/or aglycon adjustments are still needed for designing building blocks with suitable anomeric reactivities. This excessive synthetic operation on building blocks complicates the synthetic process and decreases overall efficiency. Thus, an ideal way to assemble an oligosaccharide should integrate the advantages of both the chemoselective strategy (activation under a single set of glycosylation conditions) and the orthogonal strategy (independence of relative activity). The pre-activation strategy is certain to be a good choice, since it does not require the tuning of the building blocks in an armed–disarmed fashion. In 2003 Van der Marel and co-workers reported a new glycosylation procedure in which the Ph₂SO/Tf₂O system was employed to mediate dehydrative condensation of 1-hydroxyl donors with thioglycosides affording the thiodisaccharides in good yield, which in turn were able to be activated by the same activator system to furnish trisaccharides.[52] Following this protocol, the α-Gal epitope
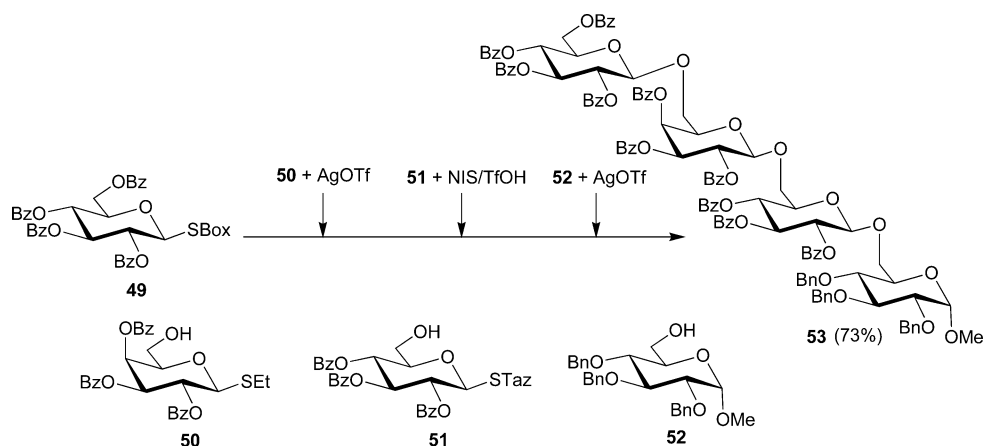
Scheme 15 One-pot synthesis of the tetrasaccharide **53**.
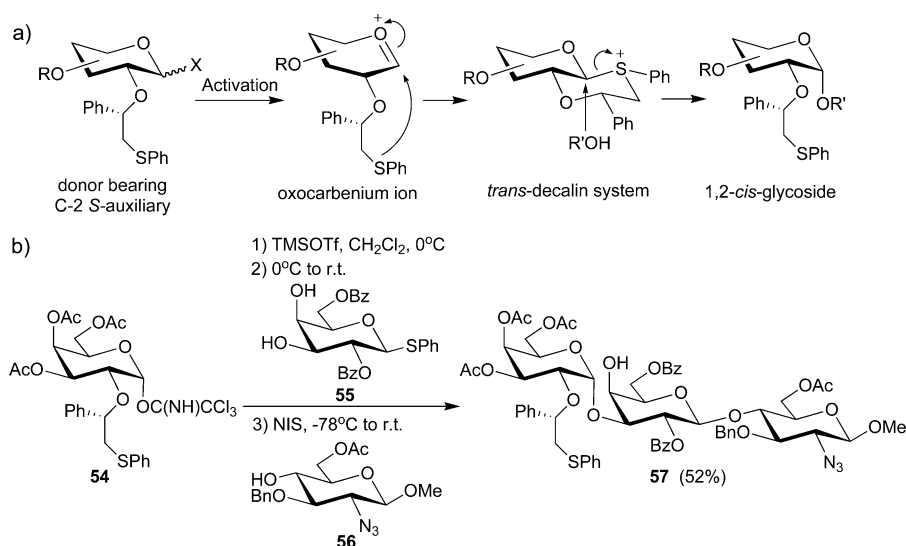
Scheme 16 Neighboring group participation by C-2 *S*-auxiliary to 1,2-*cis*-glycosides (a) and one-pot synthesis of trisaccharide **57** (b).

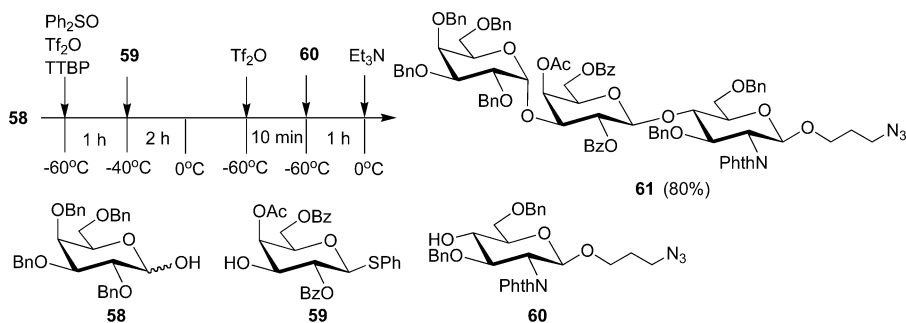trisaccharide derivative **61** was synthesized in a one-pot fashion (Scheme 17).

In 2004, for the first time Huang *et al.* established the concept of iterative one-pot synthesis of oligosaccharides based on the pre-activation strategy.[53] This concept refers to pre-activation of the glycosyl donors, which generates a reactive intermediate in the absence of the acceptor. After addition of the second building block to the pre-activated donor, a disaccharide will be formed with an identical activatable aglycon at the reducing end. Therefore, by repeating this process in the same reaction flask, rapid construction of oligosaccharides can be realized. In order to effectively accomplish such an iterative one-pot synthesis, several general requisites have to be provided: 1) the promoter utilized can activate a wide range of glycosyl donors in a stoichiometric amount and be completely consumed by the donor to prevent further activation of following building blocks; 2) the intermediate generated after pre-activation must be stable till the addition of the acceptor, but reactive for high-yielding glycosyl coupling; and 3) side products formed during the reaction process do not interfere with glycosylation.

In the proof-of-principle synthesis of tetrasaccharide **66**, *p*-toluenesulfenyl triflate (formed *in situ* from *p*-TolSCl and AgOTf) was used as the promoter, which proved to be a superior activator to the BSP/Tf$_2$O system. As shown in Scheme 18, pre-
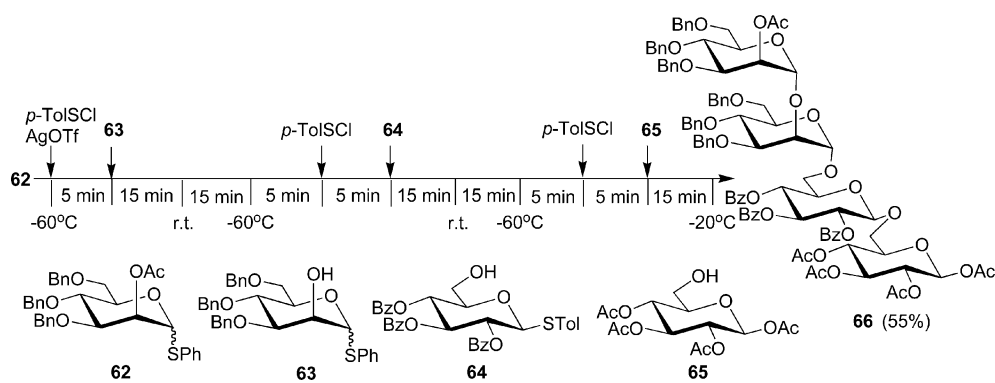
activation of the disarmed donor **62** using a stoichiometric amount of *p*-TolSOTf was followed by addition of the more reactive donor **63**. After the reaction was completed, the pre-activation procedure was repeatedly applied to the next two glycosylation steps involving building blocks **64** and **65** using the same activator. Thus, tetrasaccharide **66** was prepared in less than two hours. This approach is a significantly improved strategy compared with the traditional reactivity-based one-pot strategy. This approach has also been applied to the assembly of chitotetroses[54] and hyaluronic acid oligosaccharides;[55] the results are satisfactory.

Recently our group reported a new glycosyl coupling reagent, benzenesulfinyl morpholine (BSM), for the activation of thioglycoside donors, and applied it to pre-activation one-pot oligosaccharide assembly.[56] Compared with the known *p*-TolSCl/AgOTf system, the BSM/Tf$_2$O system has several advantages: 1) BSM is much more stable than *p*-TolSCl and has a much longer shelflife; 2) unlike *p*-TolSCl, BSM is odorless; 3) the BSM/Tf$_2$O system is a metal-free promoter. Thus, to demonstrate the feasibility of BSM/Tf$_2$O to pre-activation one-pot synthesis, pre-activated by this single promoter system, construction of tetrasaccharide **69** was achieved in less than two hours by the one-pot sequential coupling of four building blocks **23**, **24**, **67**, and **68** (Scheme 19).
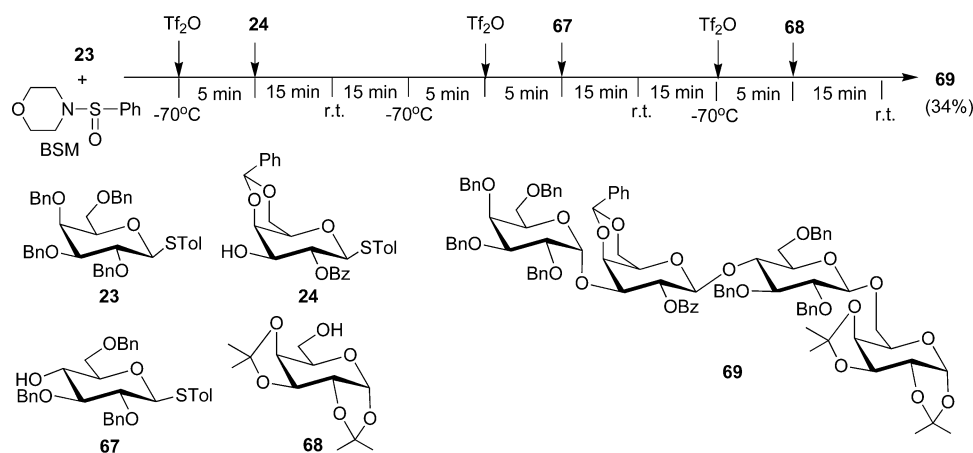
There is no doubt that the pre-activation one-pot synthesis has presented a new approach for efficient sequential oligosaccharide

Scheme 17 One-pot synthesis of α-Gal epitope **61**.

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Scheme 18 One-pot iterative synthesis of tetrasaccharide **66**.

Scheme 19 Pre-activation one-pot synthesis of tetrasaccharide **69**.

assembly. However, it should be noted that, there are still several obstructions that need to be overcome before this strategy can be developed to be applicable to any complex oligosaccharide. Firstly, in some cases pre-activated donors do not react with acceptors; this demands improvement in coupling conditions and the development of new, more powerful promoters. Secondly, the regeneration phenomenon, which may result from aglycon transfer, tends to occur occasionally when thioglycosides bear the same anomeric leaving groups. Many researchers have made great efforts to minimize aglycon transfer of thioglycosides.[57] Recently, Li and Gildersleeve studied and presented the mechanism and affecting factors of aglycon transfer, and also modified and examined a number of thiophenyl aglycons, amongst which the 2,6-dimethylphenyl (DMP) aglycon was demonstrated to prevent the transfer of sulfur-containing aglycon most effectively.[58] However, more investigations are still required to unveil the mysterious nature of this side reaction.
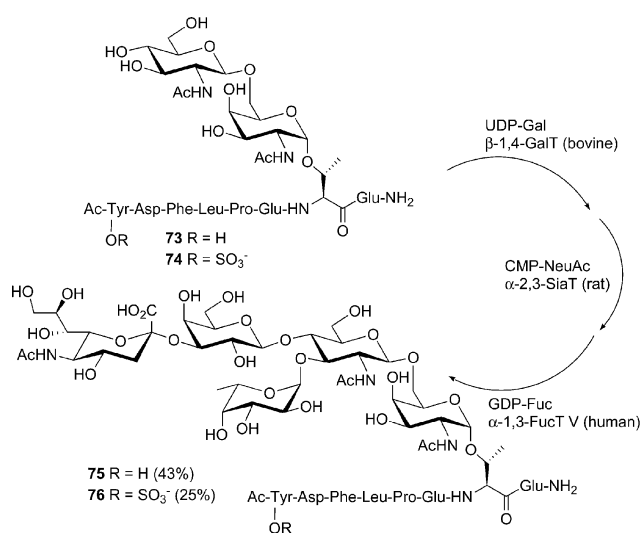
Multi-enzyme one-pot glycosylation

Enzymes that catalyze the formation of glycosidic linkages, such as glycosyl transferases and glycosyl hydrolases, have been utilized extensively in the construction of complex glycoconjugates. The enzymatic methods can dramatically reduce the protection–deprotection steps and provide great regio- and stereoselectivity. A glycosyltransferase based one-pot system coupled with regeneration of sugar nucleotides *in situ* was applied to the synthesis of an α-

Gal epitope by Wang *et al.*[59] Their operation involved two sequential enzymatic glycosylations using β-1,4-galactosyltransferase (β-1,4-GalT) and α-1,3-galactosyltransferase (α-1,3-GalT) that share the same common UDP-galactose donor. Tetrasaccharide **71** and pentasaccharide **72** were obtained in 53% and 35% yields respectively from the starting trisaccharide **70** (Scheme 20). In another instance Lin and co-workers utilized a multi-enzyme one-pot, three-step glycosylation strategy with β-1,4-galactosyltransferase (β-1,4-GalT), α-2,3-sialyltransferase (α-2,3-SiaT), and α-1,3-fucosyltransferase (α-1,3-fucT V) to assemble the sialyl Lewis X moiety of truncated PSGL-1 glycopeptides.[60] Unsulfated glycopeptide **73** and sulfated glycopeptide **74** were employed as substrates which were incubated with GalT and SiaT in the presence of UDP-Gal, CMP-NeuAc and alkaline phosphatase respectively, followed by the addition of FucT and GDP-Fuc and incubation, giving rise to the unsulfated and sulfated glycopeptides **75** and **76** (Scheme 21). The similar one-pot enzymatic glycosylation strategy using multiple glycosyltransferases was also employed to the construction of other complex oligosaccharides such as Lewis X,[61] SLe^x,[62] 6'-SLN,[63] and hyaluronic acid polymer.[64]

Another type of enzymatic one-pot glycosylation is exemplified by the synthesis of the sialylated Thomsen–Friedenreich (TF) antigen reported by Thiem and Gambert.[65] In their synthesis, galactosylation of the monosaccharide amino acid **77** with β-galactosidase from bovine testes and *p*-nitrophenyl β-galactoside (*p*NPβGal) as the donor gave the disaccharide amino acid **78**. This intermediate compound was immediately converted into the
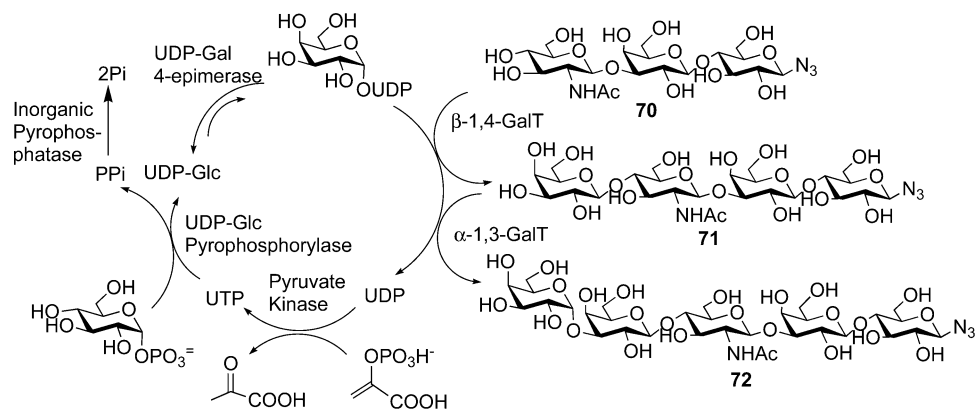
Scheme 20 One-pot enzymatic synthesis of α-Gal epitope.

Scheme 21 Multi-enzyme one-pot synthesis of glycopeptides **75** and **76**.

sialyl-TF trisaccharide **79** catalyzed by α-2,3-SiaT, and this also contributed to the shift of the reaction equilibrium to form **78** (Scheme 22). Other examples of enzymatic one-pot glycosylation with the combined use of glycosidases and glycosyltransferases include the synthesis of core 2 trisaccharide[66] and 6′-SLN.[67]
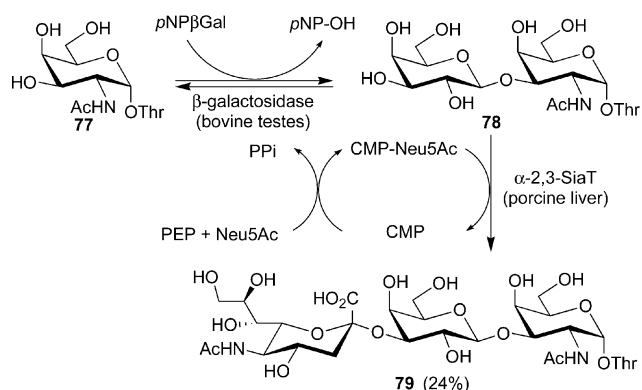
Scheme 22 β-Galactosidase and α-2,3-SiaT combined one-pot synthesis of sialyl-TF antigen.

Conclusion

In the past two decades, enormous progress has been achieved in oligosaccharide synthesis, amongst which the one-pot sequential glycosylation strategy is undoubtedly an important one. However, there are still some limitations that need to be overcome for wider utilizations of this technology. This methodology is required to be improved to be suitable for the assembly of more naturally occurring oligosaccharides which are larger and more complex than hexasaccharides. More reactivity tuning fashions need to be found to broaden the reactivity window and reduce the time required for building block preparation. It seems that the pre-activation strategy is a promising way for more efficient construction of oligosaccharides. In one word, the one-pot glycosylation strategy has made great progress in oligosaccharide synthesis, and it will surely be utilized in more extensive fields in carbohydrate chemistry and glycobiology in the future. For instance, this methodology as a useful synthetic tool could provide oligosaccharide sources for the fabrication of carbohydrate microarrays,[68] which are expected to play an important role in the study of functional glycomics, carbohydrate-based drug discovery, and the diagnosis of carbohydrate-related diseases. Compared with stepwise solid-phase synthesis, the one-pot approach requires protecting group manipulation only at the stage of building block synthesis and thus holds potential for automation for the assembly of a greater diversity of oligosaccharide and glycoconjugate structures, which make it a powerful tool for tackling many interesting problems in glycobiology.

Acknowledgements

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