Thioglycosides in sequential glycosylation strategies

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This *tutorial review* surveys the use of thioglycosides in the development of sequential glycosylation methodologies, with a focus on chemoselective, orthogonal and iterative glycosylation strategies reported since the beginning of this century. Both fundamental aspects of glycosidic bond formation and ingenious state-of-the-art methodologies are presented.

1. Introduction

The multitude of biological functions performed by oligosaccharides and glycoconjugates stems from the infinite diversity in their molecular structure. The biological importance of oligosaccharides and glycoconjugates in combination with the synthetic challenges they pose has made them very popular synthetic targets, and synthetic carbohydrate chemistry has long been a central theme in organic chemistry. Over the years a variety of methods have been introduced for the construction of the interglycosidic bond, each having their distinct advantages and disadvantages. Amongst the most popular glycosylation donors are glycosyl trichloroacetimidates, fluorides, sulfoxides as well as *n*-pentenyl and thioglycosides.[1] Since traditional oligosaccharide synthesis is a time-consuming process (due to the extensive need for protecting group manipulation and purification steps), the development of new and efficient strategies for the assembly of oligosaccharides remains a field of intensive research. In order to streamline the oligosaccharide assembly process several elegant sequential glycosylation procedures have been developed. In most of these strategies thioglycosides play a crucial role.

The success of thioglycosides in oligosaccharide synthesis originates from the stability of the anomeric thio function towards a wide range of reaction conditions applied for the introduction of protecting and functional groups. In addition, the thio function can be activated using a range of electrophiles to provide a glycosylating species. The most commonly employed activators are depicted in Fig. 1, and are *N*-iodosuccinimide (NIS)–trifluoromethanesulfonic acid (TfOH), iodonium di-*sym*-collidine perchlorate (IDCP), methyl trifluoromethanesulfonate (MeOTf), phenylselenyl triflate (PhSeOTf), dimethylthiomethylsulfonium triflate (DMTST), and the recently introduced sulfonium triflate activators 1-benzenesulfinyl piperidine–triflic anhydride (BSP–Tf_2O), and diphenyl sulfoxide–Tf_2O (Ph_2SO–Tf_2O).

The thioacetal function thus conveniently combines the role of an anomeric protective group and that of an efficient leaving group. These characteristics make thioglycosides particularly suitable to be exploited in chemoselective, orthogonal and iterative glycosylation strategies, schematically depicted in Fig. 2. In a chemoselective glycosylation a thio donor of high reactivity is condensed with a thioglycoside of lower reactivity to provide a new thioglycoside which can immediately be used in another glycosylation event (Fig. 2A). An orthogonal condensation employs two glycosides with different anomeric leaving groups (*e.g.* an aryl/alkyl sulfide and a fluoride), which are mutually stable to the conditions used to activate the other anomeric functionality (Fig. 2B).[2] Recently developed iterative glycosylations (Fig. 2C) exploit the pre-activation of a thioglycoside to provide the reactive glycosylating species which is subsequently treated with a second thioglycoside to provide the thiodisaccharide. The latter can again be pre-activated in continuation of the glycosylation sequence. In the following sections these glycosylation strategies are discussed in more detail, with a focus on the developments reported since the beginning of this century.

2. Chemoselective glycosylations

A whole range of factors affects the reactivity of a donor glycoside to a smaller or larger extent. The influence of protecting groups on the reactivity of donor (and acceptor) glycosides has long been recognized and this realization has formed the basis for the development of the state-of-the-art chemoselective glycosylations we know today. Protecting groups exert their influence on crucial events during the glycosylation reaction. For instance, they affect the nucleophilicity of the anomeric thio function and thereby the rate of attack of the thio group on the electrophilic promoter species. Furthermore they determine the stability of the partial positive charge on the anomeric center, which develops upon expulsion of the anomeric leaving group. A donor glycoside is deactivated by electron-withdrawing protecting groups (such as acyl functions), since they decrease the nucleophilicity of the anomeric thio function and destabilize the carbocationic transition state. Fraser-Reid eloquently introduced the armed–disarmed concept to denote the influence of the protecting groups on the reactivity of the anomeric *n*-pentenyl group: acylated *n*-pentenyl glycosides were termed *disarmed* glycosides and their benzylated counterparts were labeled as *armed* glycosides.[3] The Fraser-Reid laboratory disclosed that

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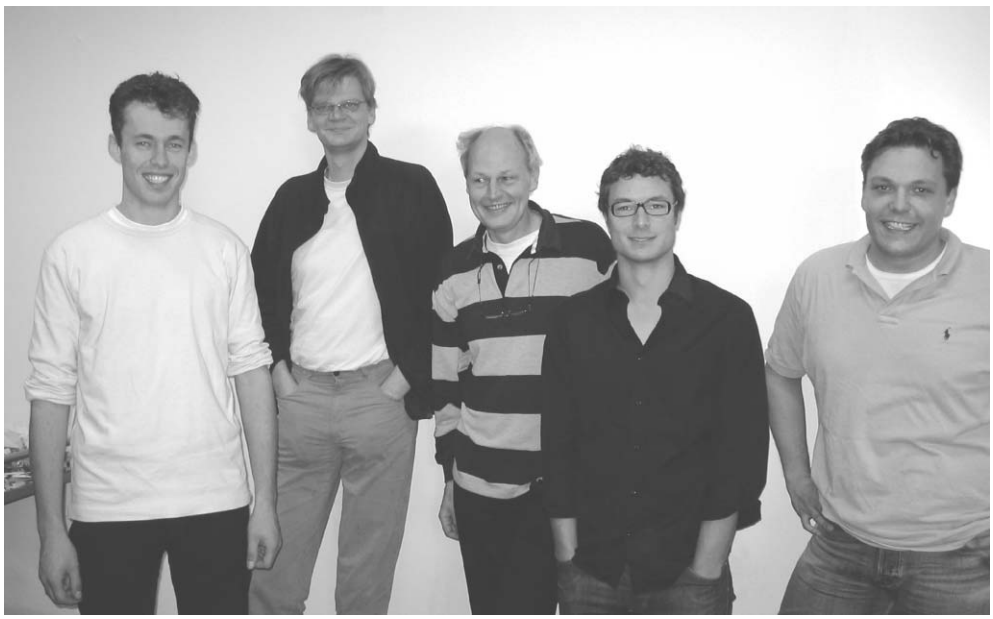
an armed *n*-pentenyl glucoside could selectively be condensed with a disarmed *n*-pentenyl glucoside using the mild iodonium promoter IDCP, capable of activating only the most reactive donor glycoside, thereby introducing the first chemoselective glycosylation. After the serendipitous discovery that IDCP could also activate armed ethyl thiorhamnosides,[4a] van Boom and co-workers translated this principle to the use of thioglycosides.[4] Ever since, the armed–disarmed concept has evolved from a system in which the reactivity of the donor was termed either reactive or unreactive, into a system in which the relative reactivity scale is regarded as a continuum.[5]

Different protecting groups on different positions in the donor glycoside all have a specific influence on the reactivity of the system. For example, the deactivating power of different protecting (and functional) groups at the 2-position of a specific galactose core were established to be in the order of $-N_3 > -O(ClAc) > -NPhth > -OBz > -OBn$.[5b] The disarming effect of the benzoate group was shown to depend on its position in the carbohydrate skeleton and the observed influence of the positions was shown to be in the order of $4 > 3 > 2 > 6$ for a given galactoside.[5b] Besides by electronic effects, protecting groups can also conformationally deactivate the donor glycoside.[6] The benzylidene, butane-2,3-diacetal (BDA) and the related cyclohexane-1,2-diacetal (CDA) protecting group may torsionally disarm the glycoside by locking the conformation of the sugar ring, thereby hampering the formation of the flattening cationic transition state. The chemical identity in terms of configuration and conformation



Leendert van den Bos, Herman Overkleeft, Gijs van der Marel, Jeroen Codée and Remy Litjens

Leendert van den Bos (01-03-1979) graduated as a synthetic organic chemist at Leiden University in 2002. He started his PhD research under the guidance of Gijs van der Marel and Herman Overkleeft and is currently working on the development of synthetic strategies towards zwitterionic capsular polysaccharides. He recently developed an independent strategy to the chemoselective oxidation of partially unprotected thioglycosides towards the corresponding thioglucuronides and their application as donor and acceptor in oligosaccharide synthesis.

Herman Overkleeft (12-04-1969) received his PhD education at the University of Amsterdam under the guidance of Upendra Pandit. After receiving his PhD degree on the subject of the synthesis and application of iminosugar glycosidase inhibitors (1997), he moved to Leiden University for a two-year postdoctoral research stay in the group of Gijs van der Marel and Jacques van Boom. From 1999 to 2001 he was a postdoctoral fellow at Harvard Medical School, Department of Pathology, where he worked with Hidde Ploegh in the emerging area of chemical biology. In July 2001 he was appointed the chair in bioorganic chemistry at Leiden University, where he currently is. His research interests include bioorganic chemistry, glycobiology and organic synthesis.

Jeroen Codée (30-07-1975) studied chemistry at Leiden University, where he received his masters degree in synthetic organic chemistry in 1999. He continued his education as a PhD student at Leiden University, under the guidance of Jacques van Boom and Stan van Boeckel and elaborated the subject of oligosaccharide synthesis with a focus on thioglycosides and glycosaminoglycans. He defended his thesis on September 29th, 2004 and is currently a postdoctoral fellow at the Eidgenössische Technische Hochschule Zürich with Peter Seeberger. His research interests include glycobiology, carbohydrate chemistry and automated synthesis.

Remy Litjens (03-05-1974) studied chemistry in Leiden and continued to do his PhD research at the same university. Recently he was granted his PhD degree on his thesis describing the use of sulfonium salt activation in oligosaccharide synthesis. Together with Jeroen Codée and Leendert van den Bos he developed and applied several new chemoselective and orthogonal glycosylation strategies based on the use of sulfonium activator systems in combination with thioglycosides and hemiacetals. Remy is currently working as a research associate at the Lead Discovery Unit of Organon Oss, The Netherlands.

Gijs van der Marel (03-04-1952) got his training at Leiden University, where he graduated in 1977. He did his PhD studies on the subject of DNA oligonucleotide synthesis together with Jacques van Boom and received his PhD degree in 1981. He continued his career at Leiden University, first as Assistant Professor, then as Associate Professor and, since January 2005, as Full Professor in Organic Synthesis. His research is focused on synthetic aspects of biopolymers, primarily nucleic acids, peptides and carbohydrates, their hybrid structures and their synthetic analogues.

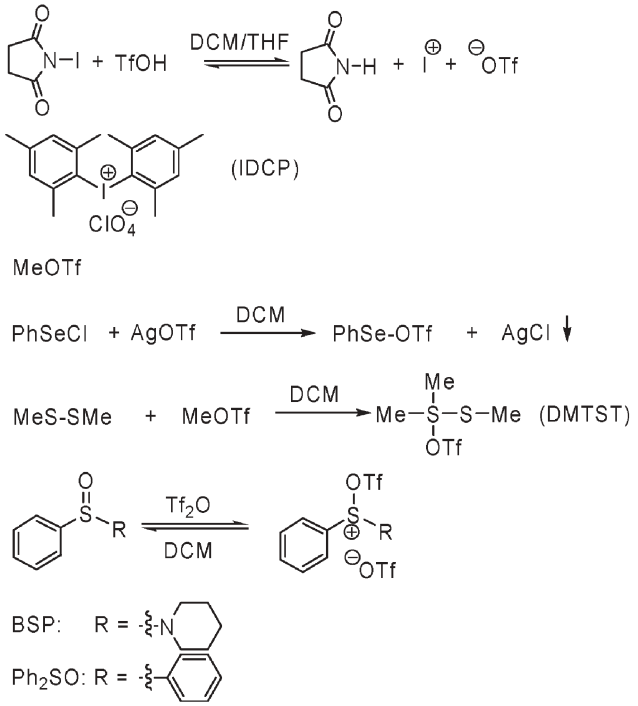


Fig. 1 Electrophilic reagents for the activation of thioglycosides.

of a monosaccharide also has a significant effect on its reactivity. Differences in ground-state energies and in steric and electronic interactions of the substituents during oxocarbenium ion formation lead to the relative reactivities in the order of galactose > mannose > glucose.[5]

The influence of the protecting groups and monosaccharide types was first quantified by Ley and co-workers to obtain a more precise overview of the relative reactivities of different ethyl thioglycosides.[5a] Subsequently, the Wong laboratory took up the mammoth task of quantifying the reactivity of hundreds of different *S*-tolyl mono-, di- and trisaccharides.[5b,7] The established relative reactivity values (RRVs) were exploited in the one-pot assembly of several complex oligosaccharides.[8] For example, the Lewis Y carbohydrate hapten **4** (Scheme 1) was synthesized using three building blocks in decreasing order of reactivity in a one-pot four-step manner.[8a] In the first (double) glycosylation event the very reactive *S*-tolyl fucoside **1** was condensed with the bridging disaccharide **2** under the influence of NIS and a catalytic amount of TfOH. The resulting tetrasaccharide was next reacted with the terminal lactosamine **3** to provide the target hexasaccharide **4** in 44% yield. It should be noted that the first condensation was executed at low temperature to prevent the formation of undesired fucosyl succinimide by-products. In one-pot glycosylations employing the NIS–TfOH promoter system, succinimide accumulates during the glycosylation sequence and competes with the acceptor hydroxyl functions for the activated donor glycoside. The application of different electrophilic activator systems, such as IDCP or DMTST, has been shown to circumvent this complication.

The same strategy was applied for the synthesis of a series of oligolactosamines (Scheme 2).[8e] A set of *S*-tolyl lactosamine building blocks was designed with a varying reactivity profile to be implemented in a reactivity-based one-pot assembly. Thus, saccharides **5**, **6** and **7** were constructed and their RRVs were determined to be 1.3×10^4, 246, and 0 respectively. These values clearly show the dramatic influence of the protecting group at the C-3 hydroxyl: changing the electron-donating benzyl group for an electron-withdrawing acyl group causes a >53-fold reactivity difference. Assembly of octamer **8** started with coupling of the most reactive lactosamine **5** and the less reactive **6** under influence of NIS–TfOH at −35 °C. After complete consumption of **5**, the dilactosamine building block **7** was added followed by an additional amount of NIS to provide the octamer **8** in 35% yield. The relatively low yield was attributed to extensive chromatographic purification.

Since a chemoselective glycosylation sequence is executed in the order of decreasing donor reactivity this imposes a demand on the protecting groups. To deactivate the donor glycosides at the end of the sequence one has to rely on disarming acyl

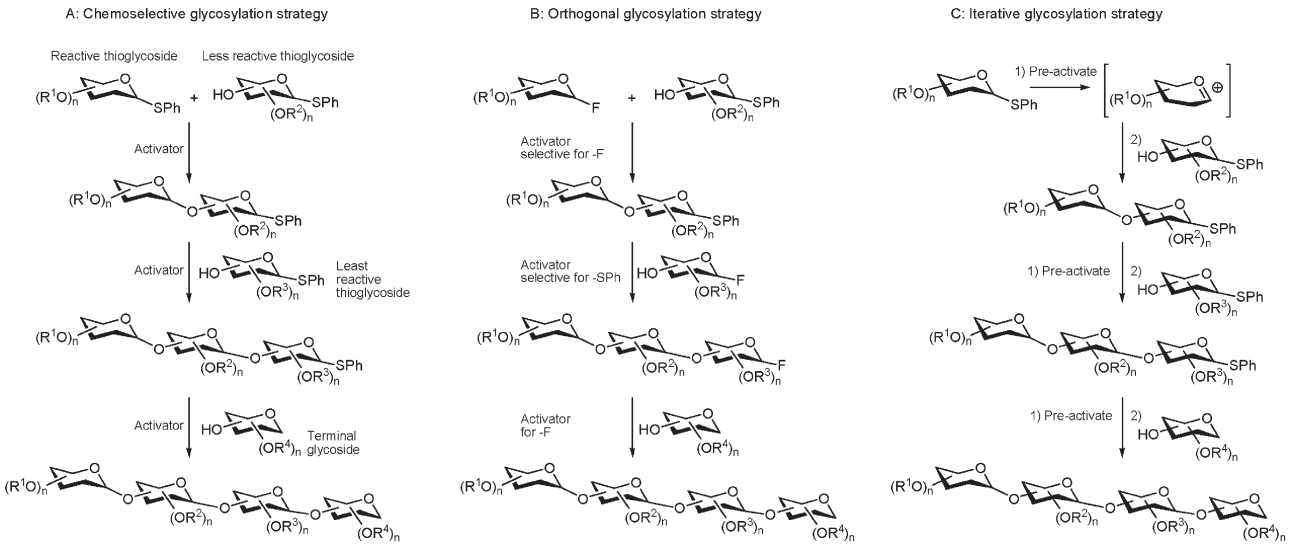
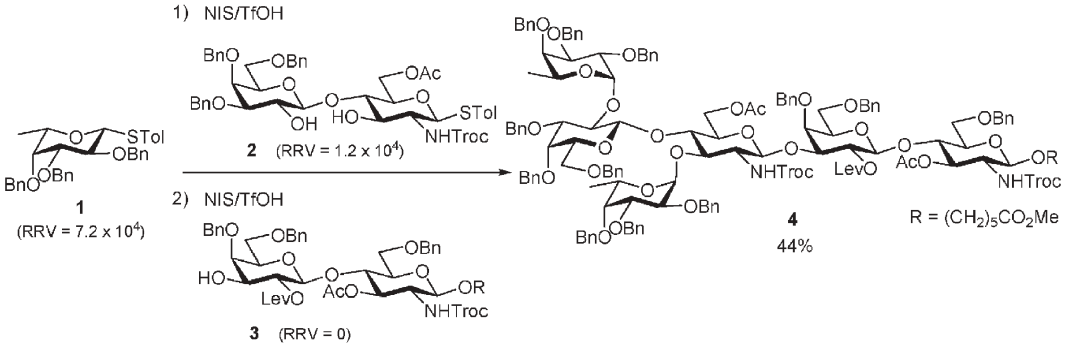
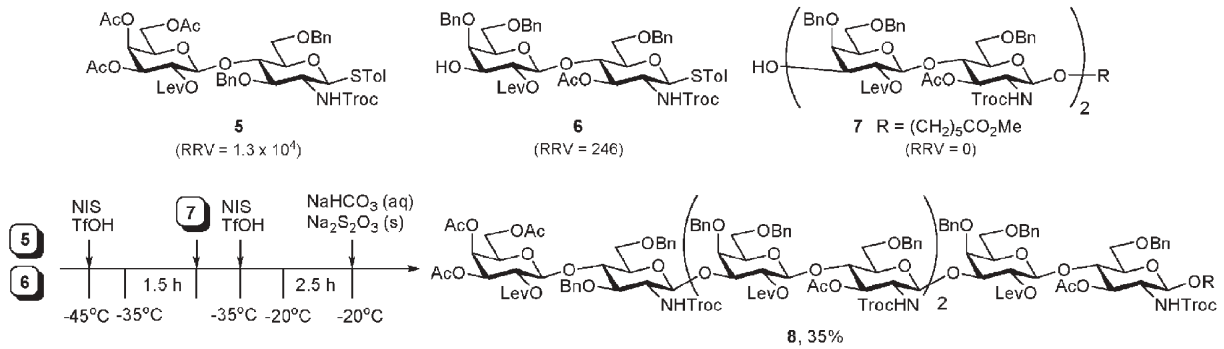


Fig. 2 Chemoselective, orthogonal and iterative glycosylation strategies.



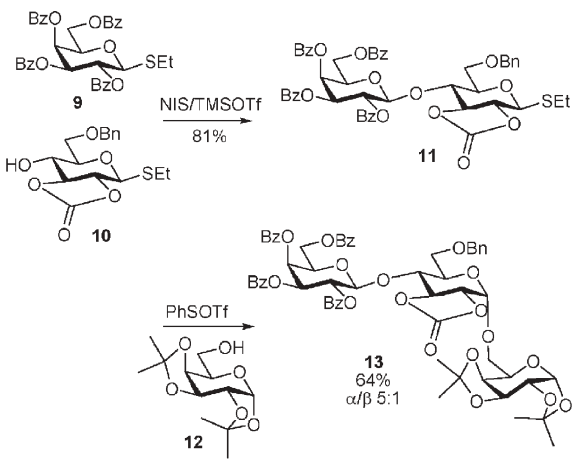
Scheme 1 One-pot synthesis of the Lewis Y hexasaccharide **4**.



Scheme 2 Chemoselective assembly of oligolactosamines.

protecting groups. As a consequence, the interglycosidic linkages formed at the end of the sequence will be 1,2-*trans* as a result of anchimeric assistance by the neighboring acyl protecting group. To elude this caveat Zhu and Boons introduced the 2,3-cyclic carbonate function as a non-participating disarming protecting group.[9] This made it possible to use a disarmed thioglycoside at the end of a glycosylation sequence that allows the construction of a 1,2-*cis* glycosidic linkage. Thus, the disarmed perbenzoylated galactoside **9** was condensed with the highly disarmed cyclic carbonate protected thioglucoside **10** to provide thiodisaccharide **11** (Scheme 3). In the next glycosylation event phenyl sulfenyl triflate (PhSOTf) was required to activate the unreactive disaccharide and give a productive glycosylation (NIS–TfOH and DMTST failed to efficiently activate **11**). After careful tuning of the solvent system (toluene–1,4-dioxane 1:3 v/v) a satisfactory α-selectivity was achieved.

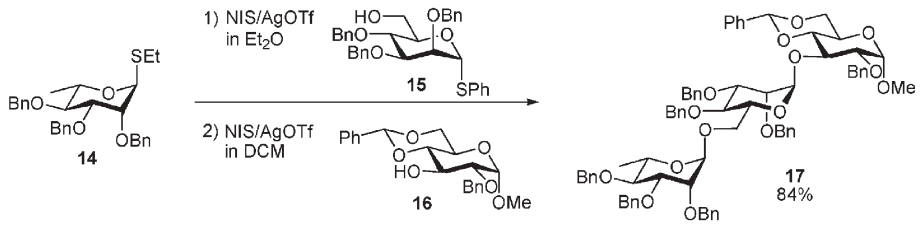
Recently, the effect a solvent has on the rate of a glycosylation was elegantly exploited in a set of one-pot syntheses by Lahman and Oscarson (Scheme 4).[10] They showed that ethyl thiorhamnoside **14** could be selectively activated using NIS–AgOTf in diethyl ether in the presence of the somewhat less reactive phenyl thiomannoside **15**, leading to the construction of the rhamnose-mannose disaccharide which was condensed with the terminal methylglucoside **16** under the action of additional promoter dissolved in dichloromethane, to give trimer **17**. Remarkably, execution of the first glycosylation in dichloromethane gave a complex product mixture, while the second condensation did not proceed in diethyl ether.



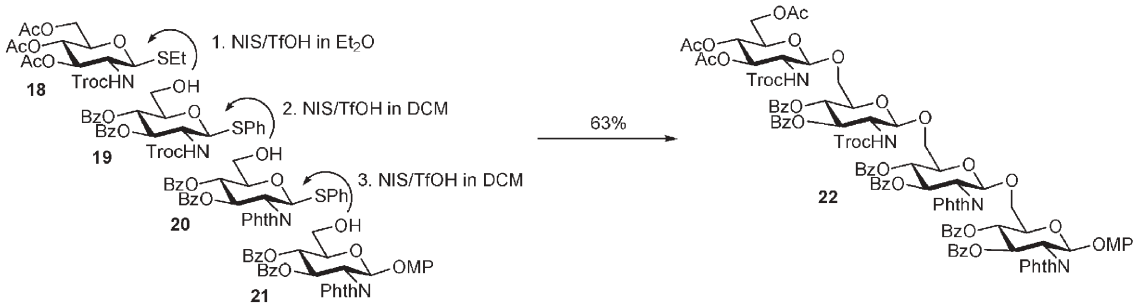
Scheme 3 Non-participating 2,3-cyclic carbonates in chemoselective glycosylations.

Fridman *et al*. used these findings in their one-pot three-step assembly of oligoglucosamines (*e.g*. tetramer **22**, Scheme 5).[11] A profitable difference in reactivity of thioglycosides **18** and **19** could be attained by very subtle variations in protecting (acetyl *vs*. benzoyl) and anomeric (thioethyl *vs*. thiophenyl, *vide infra*) groups and the use of diethyl ether as a solvent. It is also worthy to note that the Troc-protected disaccharide donor, produced in the first coupling step, could be selectively activated over the phthalimide protected thioglycoside **20**.

Another means to control the reactivity of thioglycosides is found in the use of different anomeric thio functions.[12] The



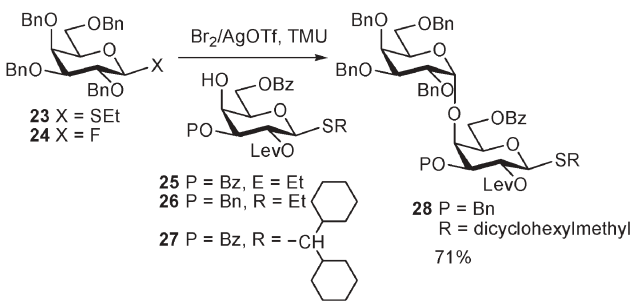
Scheme 4 Solvent effects in chemoselective glycosylations.



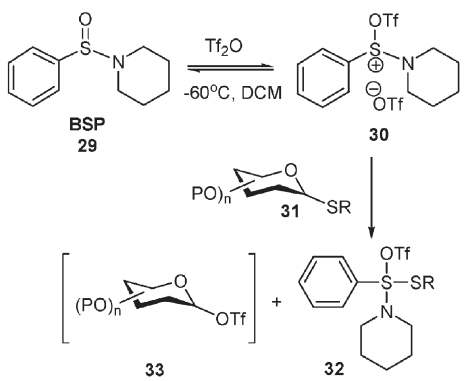
Scheme 5 One-pot synthesis of oligoglucosamines.

substituent on the sulfur atom can enhance or reduce its reactivity through both steric and electronic effects. It is now well established that a thioethyl group is slightly more reactive than the corresponding thiophenyl group in NIS or DMTST mediated glycosylations.[13] A more dramatic increase in reactivity is observed for the *p*-methoxyphenylthio function, which is significantly more nucleophilic because of its electron-donating *p*-methoxy substituent.[14] The bulky dicyclohexylmethanethiol group was introduced by the Boons laboratory as an anomeric thio function of low reactivity, obviously originating from steric factors.[15] An illustrative example in which this group was employed is shown in Scheme 6.[15b] In their synthesis of a heptasaccharide, a structural element of the jelly coat glycoprotein of *Xenopus laevis*, Geurtsen and Boons found that the reactivity difference of the two ethyl thioglycosides **23** and **25** was, quite surprisingly, not large enough to accomplish a productive condensation (1,1-linked disaccharides and trisaccharides were found as side products). Changing the OH-3 protecting group (Bz to Bn, **25** to **26**) to enhance the nucleophilicity of the proximal hydroxyl did not improve the outcome of the coupling reaction. An orthogonal strategy (*vide infra*) in which an anomeric fluoride (**24**) was employed led to substantial aglycon transfer and the isolation of the ethyl thioglycoside **23**. Replacement of the anomeric ethylthio by the sterically demanding dicyclohexylmethylthio group gave acceptor **27**, the reactivity of which proved to be sufficiently low to allow a productive coupling with **23**. The desired disaccharide **28** was obtained in good yield and with excellent stereoselectivity.

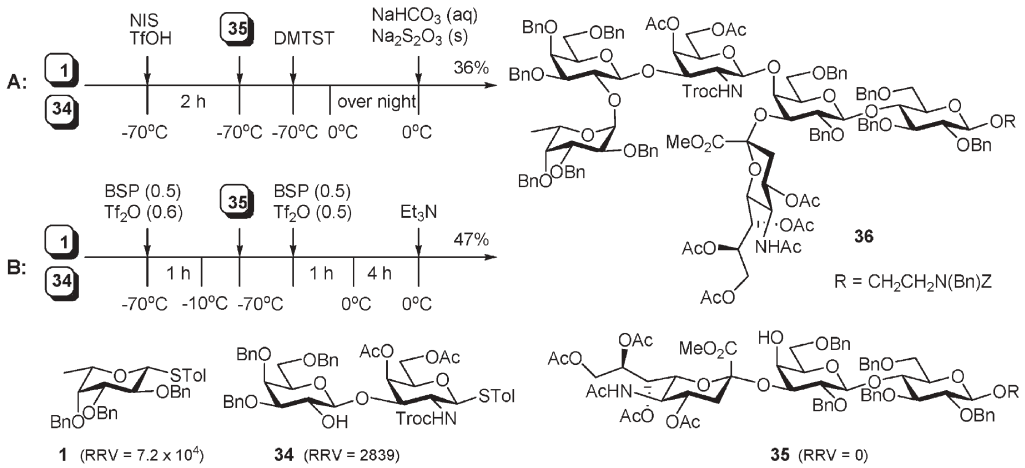
The development of novel activator systems for the activation of thioglycosides continuously pushes the glycosylation field forward and as a consequence opens up novel possibilities in the area of chemoselective condensations. Wong and co-workers used the recently introduced BSP (**29**)–Tf_2O activator system (Scheme 7)[16] in the assembly of the fucose GM_1 oligosaccharide **36** (Scheme 8B).[8c] In this synthesis they found that the transiently formed (*N*-piperidinyl)phenyl-(*S*-thiotolyl)sulfonium triflate (**32**, R = Tolyl) could also be employed for the activation of thioglycosides. Consequently, they managed to completely activate the thioglycosidic donors using 0.5 equivalents of the activator **30** at low temperature in both glycosylation events. The assembly of the complex fucose GM_1 hexasaccharide **36** was accomplished in 47% yield, which



Scheme 6 Tuning the reactivity of the anomeric thio function.



Scheme 7 The Crich BSP–Tf_2O activator system.



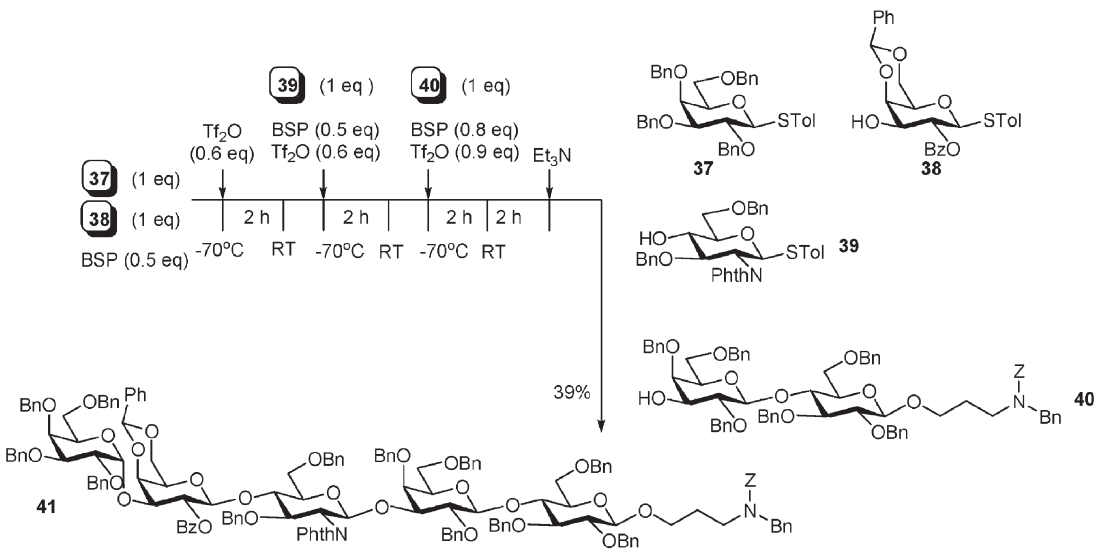
Scheme 8 One-pot assembly of the fucose GM_1 oligosaccharide **36**.

was an improvement in comparison to the synthesis employing the NIS–TfOH and DMTST activator systems (Scheme 8A, 36% yield). The latter condensation strategy suffered from the competitive formation of succinimide side products as mentioned previously.

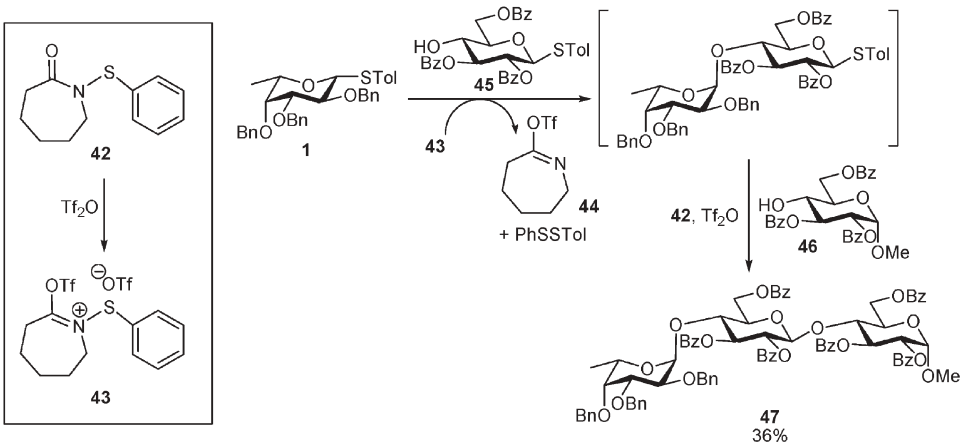
Using the same activator system (0.5 to 0.8 equivalents) in combination with *S*-tolyl glycosides, Ye and co-workers assembled an α-Gal pentasaccharide as depicted in Scheme 9.[17] Note that in both Wong's GM1 assembly and Ye's α-Gal synthesis, the linker amino functionality is doubly protected. Also no additional base (generally added to scavenge the triflic acid which is released upon productive coupling) is included in the reaction mixtures. It has been shown in other cases that a (mono) carbamate protected amine can cause side reactions involving the electrophilic promoter species, or its by-product.[18]

To circumvent the formation of reactive by-products in the activation of thioglycosides using the BSP–Tf_2O activator system, Wong and co-workers identified *N*-(phenylthio)-ε-caprolactam (**42**) in combination with triflic anhydride as a novel thiophilic activator species (Scheme 10).[19] It was proposed that **42** is transformed into *O*-triflate species **43** when exposed to Tf_2O. This promoter is then capable of activating thioglycosides, thereby producing triflyl imidate **44** and a disulfide as inert by-products. A one-pot glycosylation sequence was reported for the preparation of trisaccharide **47** using this novel activator system (Scheme 10). The reactivity of activator **43** is considerably lower than the reactivity of its BSP-congener **30**, thereby necessitating higher reaction temperatures (as high as room temperature).

Although the scope and limitations of these sulfonium-based activator systems (including the analogous Ph_2SO–Tf_2O couple) will have to be established in the assembly of more complex oligosaccharides, they hold great promise for the future. Their similar mode of operation in combination with the difference in reactivity opens up possibilities to fine-tune the activator to the reactivity of a given glycosylation system. Moreover, they have recently also been used in a selection of iterative sequential glycosylation strategies, as is outlined in Section 4.



Scheme 9 One-pot BSP–Tf_2O mediated assembly of an α-Gal pentasaccharide.



Scheme 10 *N*-(Phenylthio)-ε-caprolactam–Tf_2O as an activator system.

3. Orthogonal glycosylation strategies

The stability of thioglycosides not only allows their use in a chemoselective fashion but also opens up pathways for orthogonal glycosylations in which they are condensed with other glycosyl donor types. The clear advantage of an orthogonal strategy as compared to a chemoselective glycosylation sequence is that the orthogonal strategy allows the condensation of building blocks, independent of their relative reactivities. Whereas the chemoselective approach commands the use of the most reactive building block as a donor, in an orthogonal glycosylation strategy a disarmed donor of one type can be condensed with an armed donor of another. Evidently a variety of possibilities exists for an orthogonal glycosylation, most of which actually employ a thioglycoside as one of the reaction partners. The beginning of this century has witnessed the advent of a variety of novel glycosyl donor types which can be used in combination with thioglycosidic acceptors. The Mukaiyama laboratory reported orthogonal glycosylations using anomeric carbonates [*e.g.* **48**, activated by $TrB(C_6F_5)_4$],[20a] 6-nitro-2-benzothiazoates (*e.g.* **49**, activated by protic acids),[20b] and phosphinites (*e.g.* **50**, activated by MeI)[20c] (see Fig. 3). Novel activator systems for the use of anomeric fluorides (several protic acids)[20d] and bromides (phosphine oxides)[20e] in combination with thioglycosides were also introduced. Although some creative new donor and activator species were presented, the scope of these novel glycosylation methods remains to be established.

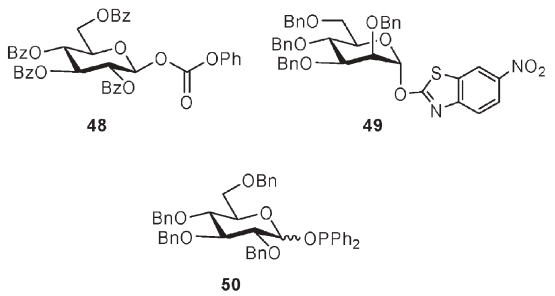
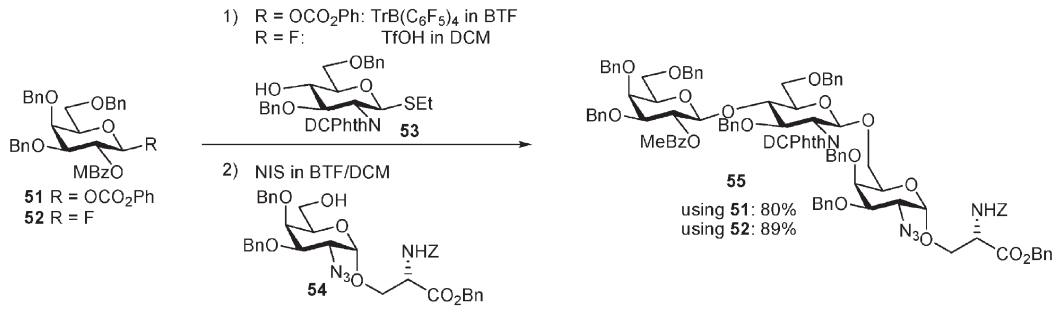


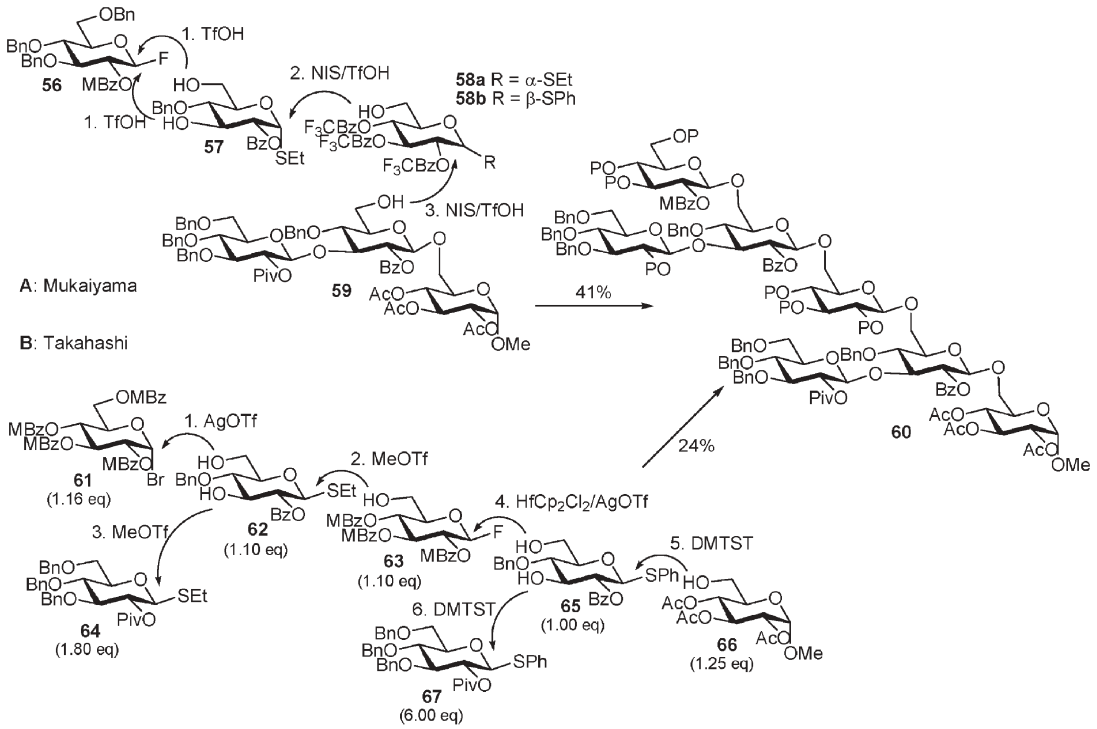
Fig. 3 Glycosyl donors for orthogonal glycosylations introduced by Mukaiyama and co-workers.

The pioneering studies of the Mukaiyama laboratory using anomeric fluorides and carbonates culminated in the one-pot assembly of a mucin related F1α antigen.[21] After the evaluation of a vast number of reaction conditions (several solvent systems, promoters and reaction temperatures) the trisaccharide **55** was assembled as is depicted in Scheme 11. In the first step phenylcarbonate **51** or fluoride **52** was coupled with ethyl thioglucoside **53** in the presence of $TrB(C_6F_5)_4$ or TfOH, respectively, in trifluoromethyl benzene (BTF) or dichloromethane as solvent. The second condensation involved the consecutive addition of the terminal glycosyl amino acid **54** and NIS to provide the target trisaccharide **55** in high yield (80% and 89% respectively). The Mukaiyama laboratory also combined an anomeric fluoride donor and two thioglycosides in the synthesis of a phytoalexin elicitor heptasaccharide (**60**),[22] which is often used as a model compound to demonstrate the effectiveness of novel methodologies in oligosaccharide synthesis. In an one-pot synthesis (Scheme 12A) it was found that the subtle difference in reactivity of the α-*S*-ethyl and the β-*S*-phenyl glucoside **58** meant the difference between failure and success: after the first orthogonal condensation of fluoride **56** and ethyl thioglycoside **57**, the slightly less reactive α-ethyl thioglycoside **58a** was required in the next NIS–TfOH mediated coupling step, since the more reactive β-phenyl thioglucoside **58b** only engaged in self condensation. The resulting intermediate thiotetrasaccharide was finally condensed with the terminal triglucoside **59** to provide the fully protected phytoalexin elicitor **60** in 41% yield.

The most impressive example of the potential of orthogonal glycosylation technology to date has been delivered by Takahashi and co-workers, who synthesized the same phytoalexin elicitor heptamer **60** *via* a one-pot six-step glycosylation protocol (Scheme 12B).[23] The reaction sequence involved *i)* the regioselective glycosylation of the primary alcohol of **62** using α-bromide **61** in combination with AgOTf; *ii)* glycosidation of the resulting thiodisaccharide with acceptor **63** without self-condensation under the influence of a large excess of MeOTf (10.0 equivalents); *iii)* glycosylation of the C3-hydroxyl using the second thioglucoside **64**; *iv)* $HfCp_2Cl_2$–AgOTf mediated coupling of the branched tetraglucoside to the third thioglycosidic building block **65**; *v)* condensation with the terminal



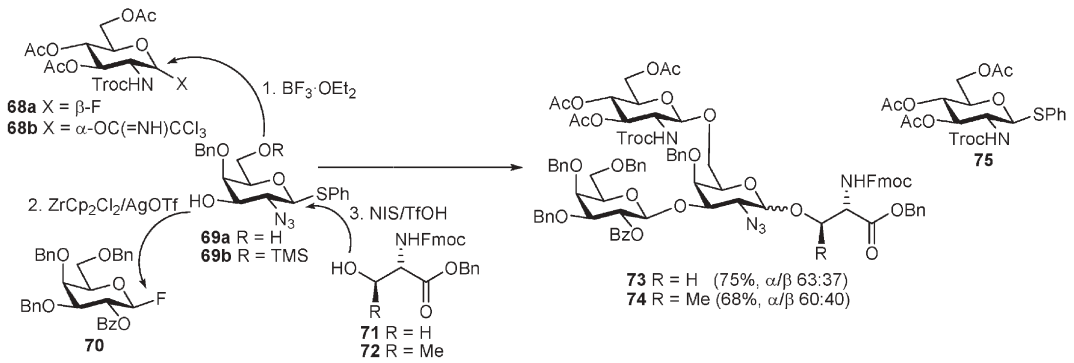
Scheme 11 One-pot synthesis of mucin related F1α antigen **55**.



Scheme 12 Mukaiyama's and Takahashi's one-pot syntheses of phytoalexin elicitor **60**.

glucose moiety **66** using a large excess of DMTST and finally *vi)* formation of the second β-(1→3)-glucosidic linkage using thioglucoside **67**. The heptasaccharide **60**, being the largest oligosaccharide amongst the reaction products, was obtained after silica gel chromatography and size exclusion chromatography in a very rewarding 24% yield based on thioglucoside **65**. Evidently, this *tour de force* could only be achieved after substantial experimentation and is not yet a routine operation, but it serves well to demonstrate the potential of an orthogonal glycosylation strategy.

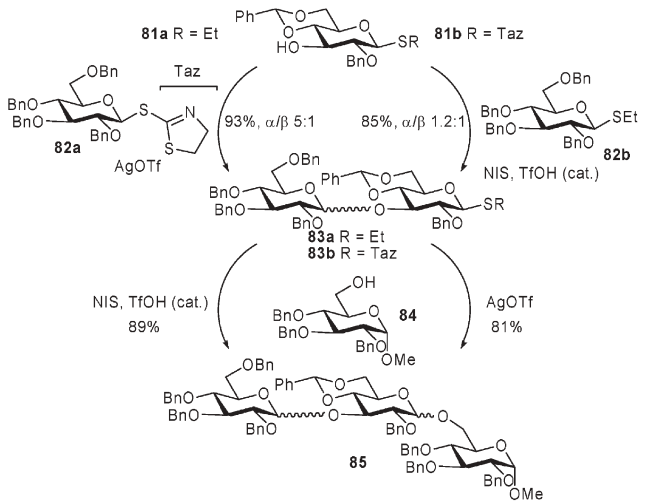
Last year, the Takahashi laboratory reported the one-pot syntheses of two core 2 class glycosyl amino acids (**73** and **74**, Scheme 13).[24] In these syntheses a glucosamine donor was condensed with the primary hydroxyl function of phenyl thiogalactosazide **69**, also bearing a free hydroxyl at its 3-position. Due to the presence of the axial 4-alkoxy substituent, the difference in reactivity of the C3 and C6 hydroxyl functions in **69a** was not large enough to allow a regioselective glycosylation in this step. Therefore, the nucleophilicity of the primary alcohol function was enhanced by transforming it into the corresponding trimethylsilyl ether (**69b**). Several conditions were examined to optimize this regioselective condensation using β-fluoride donor **68a**. A stoichiometric amount of $BF_3{\cdot}OEt_2$ was shown to be the promoter system of choice, since substoichiometric amounts led to small amounts of trisaccharide side-product. It is of interest to note that the use of the α-imidate congener of donor **68a**, *i.e.* **68b**, led to a substantial amount of aglycon transfer to furnish thioglycoside **75**. Having established the optimal conditions for the first coupling step, the glycosyl amino acids **73** and **74** were finally obtained in the following fashion. Regioselective glycosylation of **69b** with fluoride **68a** under the agency of $BF_3{\cdot}OEt_2$ and subsequent $ZrCp_2Cl_2$–AgOTf mediated condensation using galactoside **70** led to the assembly of the glycon part of the target compounds. Final glycosidation of the thiotrisaccharide with amino acids **71** and **72** in the same pot gave the glycosyl amino acids in very satisfactory yields as separable α/β mixtures.



Scheme 13 One-pot synthesis of core 2 class glycosyl amino acids.

The orthogonal glycosylations presented above are all based on the condensation of two reaction partners bearing anomeric functional groups which are activated by mutually distinct promoter systems. Demchenko and De Meo recently reported the selective coupling of thioglycosides with *n*-pentenyl glycosides, which are typically activated by the same electrophilic activator systems (*e.g.* NIS, IDCP, NBS). They showed that MeOTf can be used to selectively activate the thioglycoside in the presence of the *n*-pentenyl group.[25] Exploiting this "semi-orthogonality", a tetrasaccharide (**80**) was assembled by first coupling the disarmed thiodisaccharide **76** with armed *n*-pentenyl glucoside **77** (Scheme 14). Evidently such a condensation is not possible using the 'classic' iodonium or bromonium reagents. The ethyl thiotetrasaccharide **80** was obtained by condensation of the armed *n*-pentenyl trisaccharide **78** and disarmed thiogalactoside **79**.

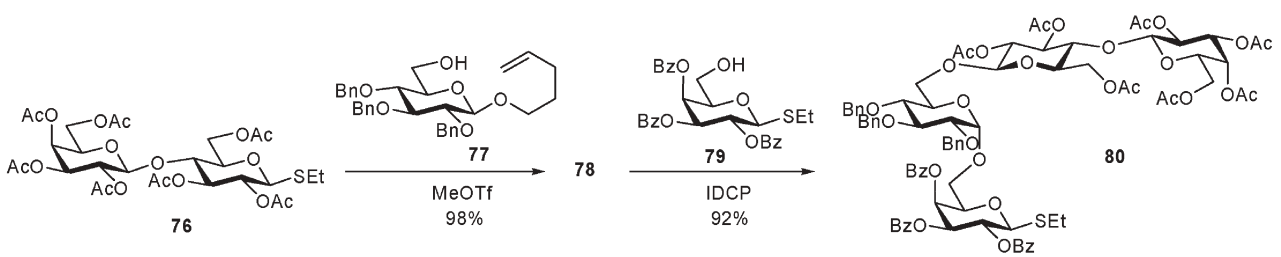
The same laboratory also disclosed a set of thioimidoyl donors, based on the generic leaving group $SCR^1{=}NR^2$. The firstly introduced *S*-benzoxazolyl (SBox)[26a] glycosides proved to be proper glycosyl donors but were unstable to some commonly used reagents, such as NaH and TfOH. Therefore, the Demchenko laboratory introduced the *S*-thiazolyl (STaz)[26b] glycosides, which can be obtained from glycosyl halides, acetates or 1,2-anhydro sugars. This class of glycosyl donors can be activated by MeOTf, AgOTf, $Cu(OTf)_2$, or NIS in combination with a stoichiometric amount of TfOH. Virtually no activation was observed using NIS–catalytic TfOH. This opened up the way to exploit these glycosides in an orthogonal glycosylation sequence as depicted in Scheme 15. Trisaccharide **85** was procured following both orthogonal synthetic routes: activation of STaz donor **82a** over *S*-ethyl glucoside **81a** or selective coupling of thioglucoside **82b** to STaz acceptor **81b** both led to the isolation of the disaccharide



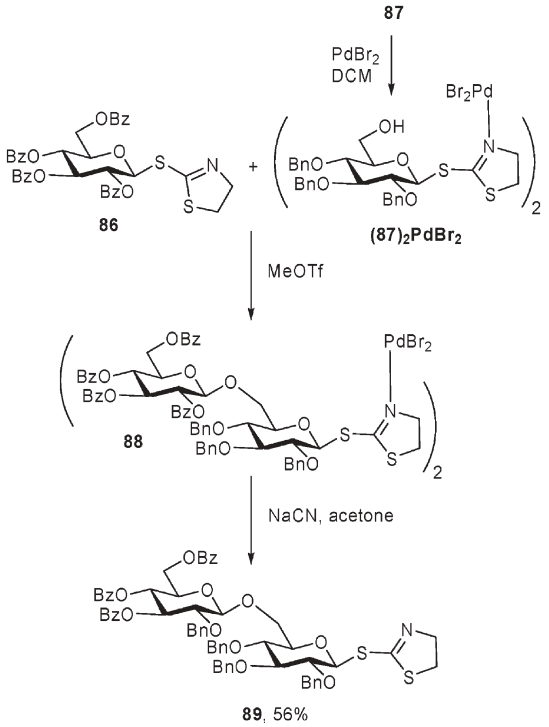
Scheme 15 STaz glycosides in an orthogonal glycosylation strategy.

83 in comparable yields, but differing α/β selectivities. Ensuing condensation with the highly nucleophilic tri-*O*-benzyl methyl glucoside **84** provided the trisaccharide **85** (α/β not determined).

Interestingly, the STaz glycosides were also employed in a novel active–latent type glycosylation strategy, in which one of the STaz coupling partners was temporarily deactivated by complexation in a stable Pd(II) complex (Scheme 16).[27] For example, it was shown that when the armed STaz glucoside **87** was engaged in the palladium complex **$(87)_2PdBr_2$** it could be condensed with disarmed STaz donor **86** to provide the STaz-dimer-Pd complex **88**. Several coupling combinations were possible, and different promoters [*i.e.* MeOTf, AgOTf, $Cu(OTf)_2$, or NIS–TfOH] could be used. The STaz



Scheme 14 Demchenko's "semi-orthogonal" glycosylation strategy.



Scheme 16 Temporary deactivation of STaz glycosides by complexation to $PdBr_2$.

disaccharides (*e.g.* **89**) could be liberated from the Pd complex by treatment with NaCN in acetone. It was observed that when AgOTf was used as a promoter in combination with electron-rich (*i.e.* benzylated) STaz-$PdBr_2$ acceptors, the yields of the glycosylations were sometimes compromised due to ligand exchange, forming a drawback of the methodology. Thus, this strategy presents an elegant new glycosylation approach, the general applicability of which has to be established in more challenging condensation reactions, employing larger and more diverse building blocks.

4. Iterative glycosylation strategies

The most straightforward way to assemble an oligosaccharide would be to use one type of glycosyl donor (and acceptor) in combination with a single set of glycosylation conditions (as in a chemoselective strategy), which is independent of the substituent pattern of the coupling partners (as in an orthogonal strategy). Ideally, the full assembly should be accomplished in a one-pot fashion, thereby eliminating intermediate work-up procedures and purification steps. Such an iterative procedure has previously been disclosed using Danishefsky's glycal methodology,[28] Gin's chemoselective dehydrative glycosylations,[29] and the strategy developed by Yamago and co-workers, which is based on the pre-activation of selenoglycosides with bromine.[30]

Pre-activation of a stable glycosyl donor leads to the formation of a reactive glycosylating species, which can be treated with a suitable acceptor in the ensuing glycosylation event. If the acceptor glycoside bears the same anomeric substituent as the first donor building block the process can be repeated to provide an iterative glycosylation sequence. Pre-activation of thioglycosides was first reported by Crich and co-workers.[31] They pre-activated thiomannosides to provide a reactive α-mannosyl triflate, which could undergo S_N2-type substitution, leading to the stereoselective formation of the challenging β-mannosidic bond (Fig. 4). The group of van Boom/van der Marel applied the pre-activation of thioglycosides by the BSP–Tf_2O reagent combination in a set of condensations with thioglycosidic acceptors.[32] In these studies they noted that the initial productive condensation reactions were followed by deterioration of the thiodisaccharides, as a result of activation by the transiently formed **32**. Timely quenching of this species by triethyl phosphite proved to provide a profitable glycosylation (Scheme 17). Attempts to decrease or even reverse the difference in reactivity of the donor and acceptor, to fulfil the requirements for the envisaged iterative protocol, failed, which was partly attributed to the reactivity of the activator's side product **32**. For the activation of highly disarmed thioglycosides, the Ph_2SO–Tf_2O reagent combination was introduced.[32a] The latter activator proved more effective in the activation of 4,6-*O*-benzylidene protected glucosazides and mannosazides than the corresponding BSP–Tf_2O promoter. In Scheme 17 the assembly of a trisaccharide featuring a β-mannosaminic linkage is outlined: treatment of *S*-phenyl galactoside **90** with BSP–Tf_2O and subsequent addition of mannosazide **91** led, after $(EtO)_3P$-quenching of **32**, to the isolation of the thiodisaccharide **92**, which was elongated in the next Ph_2SO–Tf_2O-mediated glycosylation event to stereoselectively provide trimer **94**.

To prevent the formation of reactive by-products such as **32**, the van Boom/van der Marel group developed an alternative glycosylation sequence which is based on the combination of 1-hydroxyl donors[33] and thioglycosides, which can both be activated by the sulfonium activators Ph_2SO–Tf_2O or BSP–Tf_2O.[34] Pre-activation of a lactol donor, by the former activator system, and ensuing glycosylation with a thioglycosidic acceptor furnished the desired thiodisaccharide and diphenyl sulfoxide as a benign side product. Notably, the regenerated diphenyl sulfoxide could be used for the *in situ* activation of the thiodisaccharide, upon addition of a second equivalent of Tf_2O. Following this protocol, a hyaluronan trisaccharide and the α-Gal epitope **98** were assembled in a one-pot fashion (Scheme 18).

The scope of this methodology was further broadened by the incorporation of 1-thio glycuronic acids in the glycosylation

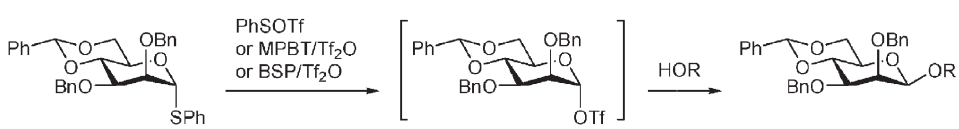
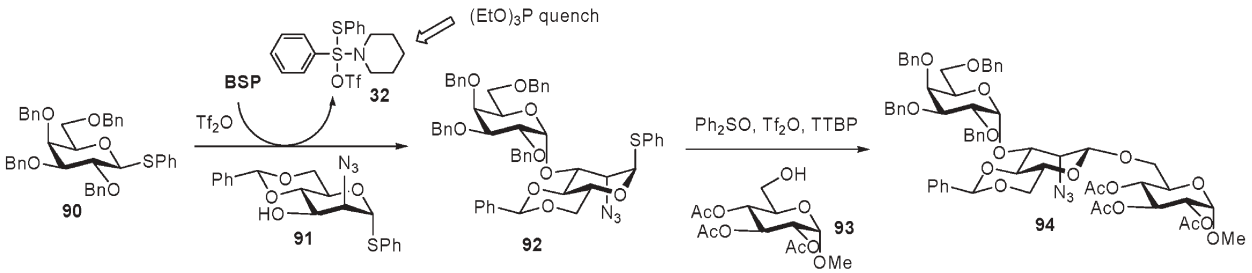
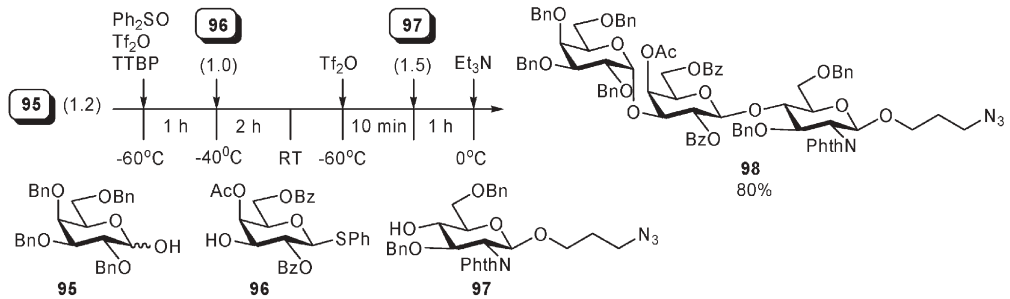


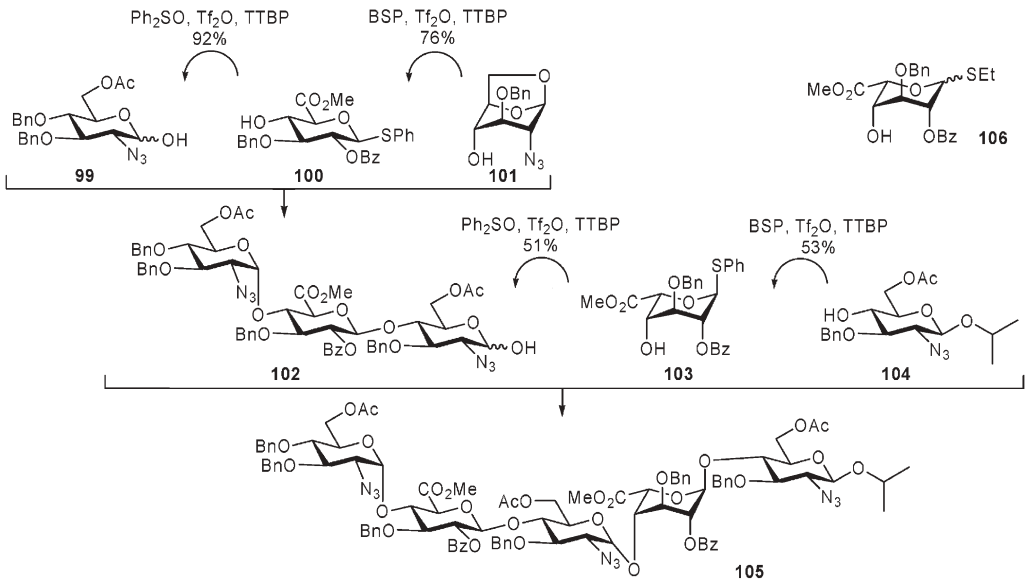
Fig. 4 Pre-activation of thiomannosides by the Crich laboratory to provide β-mannosides.



Scheme 17 Iterative glycosylations using sulfonium triflate activators.



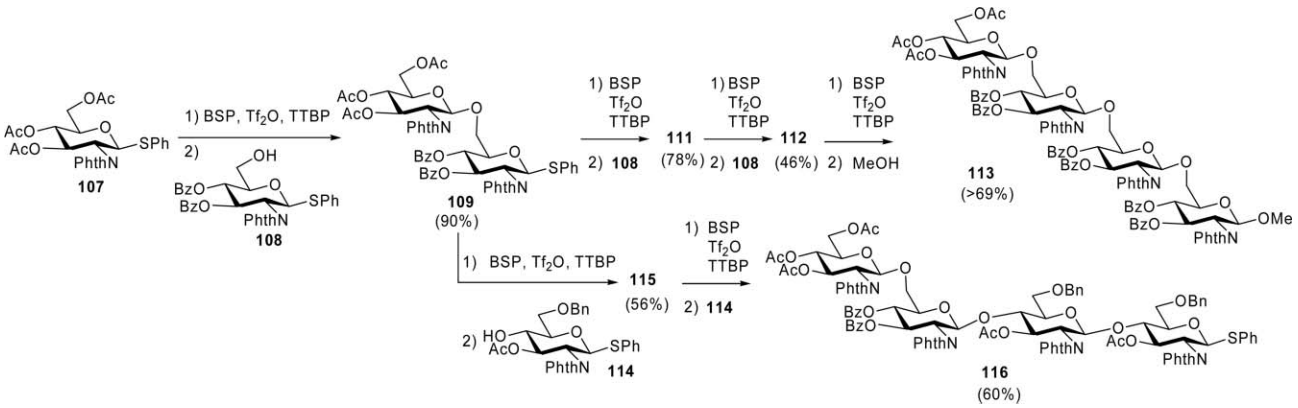
Scheme 18 One-pot assembly of the α-Gal epitope.



Scheme 19 Sequential glycosylation strategy towards the synthesis of heparin oligosaccharides.

sequence. Up to now, this class of thioglycosides has received little attention, probably as a result of problems associated with their synthesis and their low donor reactivity. On the basis of a regio- and chemoselective oxidation method for the construction of these 1-thio uronic acids,[35] a modular approach towards the synthesis of heparin/heparin sulfate oligosaccharides was disclosed.[36] In contrast to previously reported modular heparin assemblies, relying on dimer building blocks, the approach depicted in Scheme 19 used monomeric synthons in order to attain a maximum degree of flexibility in the assembly of H/HS fragments. Efficiency in the assembly strategy of the oligomers was warranted by the application of the above outlined iterative sequential glycosylation strategy, which precludes excessive synthetic steps at the oligosaccharide level.

In the first coupling event 1-hydroxyl glucosamine donor **99** was, after pre-activation with Ph_2SO–Tf_2O, condensed with 1-thio glucuronic acid **100**. Activation of the resulting thiodisaccharide using the same sulfonium triflate species was very efficient, however, rather surprisingly no ensuing coupling to acceptor **101** took place. The BSP–Tf_2O system on the other hand, proved to be capable to promote the formation of the desired β-glucuronic acid linkage. Elongation of trisaccharide **102** was accomplished in a similar fashion using 1-thio uronic acid **103** and terminal glucosamine **104** to furnish the heparin pentamer **105**. Interestingly, it was shown (in an



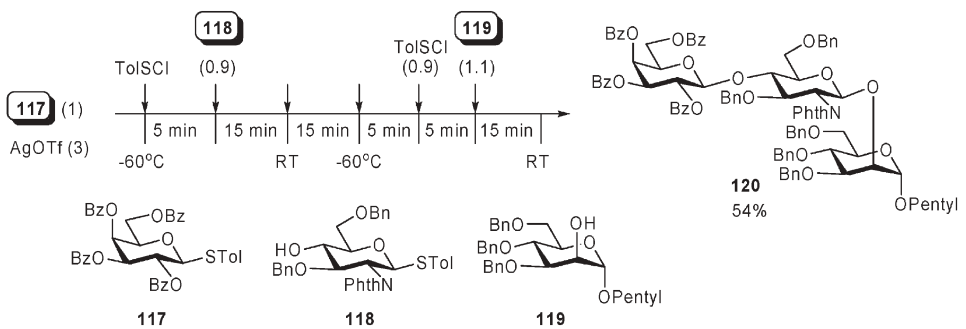
Scheme 20 Iterative assembly of a set of oligoglucosamines.

analogous coupling reaction) that the use of α/β-*S*-ethyl iduronic acid acceptor **106** led to substantial aglycon transfer: the anomeric ethylthio function proved to be too nucleophilic and competed with the OH-4 for the activated donor species. In similarity to Mukaiyama's phytoalexin elicitor synthesis (Scheme 12A), the small reactivity difference between the α/β-*S*-ethyl and the α-*S*-phenyl meant the difference between failure and success.

Yamago and co-workers recently exploited the BSP–Tf_2O pre-activation of a set of thioglycosides in a truly iterative operation to assemble a library of tri- and tetraglucosamines (Scheme 20).[37] Besides BSP–Tf_2O they also scanned PhSCl–AgOTf, PhSCl–$AgNTf_2$ and PhSCl–$AgSbCl_6$ for applicability and found the BSP–Tf_2O reagent system to be superior. Notably, they were able to condense disarmed donors with armed acceptors using a slight excess of the activator, and reported that no side products were observed from the activation of the acceptors. Also no deterioration of the thioglycosidic products was observed, since short reaction times and low temperatures (the reactions were quenched at −60 °C) were used. For example, tetramer **113** was constructed by the combination of building blocks **107** and **108**, both of similar reactivity. Tetramer **116** was assembled by iterative coupling of building block **114** to dimer **109**. Obviously, these disarmed donor-armed acceptor combinations are beyond the scope of a "classic chemoselective" glycosylation.

In contrast to the above described findings of Yamago and co-workers, Huang *et al*. reported that in their iterative one-pot assembly protocol, *p*-toluenesulfenyl triflate (formed *in situ* from *p*-TolSCl and AgOTf) proved to be a superior activator to *i.a*. the BSP–Tf_2O system.[38] The use of *p*-TolSCl–AgOTf as a promoter in the iterative one-pot synthesis of trisaccharide **120** is displayed in Scheme 21. Pre-activation of disarmed galactoside **117** using a stoichiometric amount of *p*-TolSOTf was followed by addition of the more armed glucosamine **118**. After a complete reaction, the intermediate disaccharide was pre-activated using the same activator. Subsequent addition of the terminal mannoside **119** led to the formation of trisaccharide **120** in 54% yield.

It is clear that the pre-activation of thioglycosides has opened up the way for the development of several efficient sequential oligosaccharide assembly protocols. Since these methods do not require the tuning of the building blocks in an armed–disarmed fashion, building blocks can be used that are more readily synthesized. Additionally, the saccharide synthons can be designed to streamline global deprotection at the end of the assembly or to match the reactivity of the coupling partners. It should be noted however that, although the results obtained with the iterative strategies bode well for future applications, there are several incongruities that need to be cleared before these methodologies can actually be applied to any given oligosaccharide. Firstly, the behaviour of sulfonium triflate **32** in the activation of thioglycosides seems to be somewhat inconsistent. Secondly, a number of examples have appeared in which pre-activated thioglycosides unexpectedly proved to be reluctant to react with their designated



Scheme 21 One-pot iterative glycosylation using *p*-TolSOTf as a stoichiometric pre-activator.

acceptors.[36,38,39] Lastly, aglycon transfer (which is difficult to detect when thioglycosides bearing the same anomeric substituent are used) has to be prevented.

Conclusions

Over the years thioglycosides have proven to be a very valuable class of glycosyl donors and they have been at the forefront of the development of novel glycosylation strategies, paving the way for efficient and streamlined oligosaccharide assemblies. The recently emerged thioglycoside-based iterative glycosylation procedures, in conjunction with the development of a set of activator reagents, tuned to the reactivity of a given condensation system, allude to a general glycosylation method that should be applicable for the rapid assembly of a very wide range of oligosaccharides. However, oligosaccharide synthesis is by no means routine yet, and many fundamental obstacles remain, including the stereoselective formation of 1,2-*cis*-glycosidic bonds and matched–mismatched (both from a steric and electronic point of view) donor–acceptor pairs. Undoubtedly, the intrinsic diversity of carbohydrates and glycoconjugates will continue to put novel methodologies to the test and inspire researchers to come up with ever more ingenious and elegant synthetic solutions.

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