Glycosylation

Iterative Glycosylation of 2-Deoxy-2-aminothioglycosides and Its Application to the Combinatorial Synthesis of Linear Oligoglucosamines**

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The development of new methods for oligosaccharide synthesis is a major focus in synthetic carbohydrate chemistry owing to the multifaceted role of complex oligosaccharides and glycoconjugates in biology.[1,2] As oligosaccharides consist of several anomeric C–O bond linked monosaccharides, the synthesis would necessarily require iterative glycosylation. Although many methods have been developed to enhance the efficiency of the iterative process,[3] the most straightforward method is the use of one set of glycosylation conditions with a single anomeric substituent for both glycosyl donors and acceptors. However, this type of reaction has been limited to the glycal assembly method.[4] Recently, new examples have appeared from Gin and co-workers,[5] and from our own laboratory.[6]

In a previous paper, we reported that β-bromoglycosides generated from selenoglycosides could serve as glycosyl cation equivalents and couple with selenoglycosides that bear hydroxy groups to give new selenoglycosides, which could then be used in the next glycosylation reaction under the same reaction conditions. Although this method is suitable for the iteration, it suffers from the low reactivity of the β-bromoglycoside as a result of the strong covalent character of the carbon–bromine bond. To overcome this, we decided to use reactive glycosyl cation intermediates or their equivalents. After the pioneering work by Crich and co-workers, covalently bonded glycosyl cation equivalents, such as glycosyl triflate intermediates, were recognized as discrete intermediates with considerable stability.[7] Therefore, we envisaged modulating the reactivity of glycosyl cations and their equivalents by changing the counteranion species X, from the covalently bonded **2** to the ionically bonded **2′** (Scheme 1). We were especially interested in the formation of the β-glycoside bond of glucosamine derivatives, which is a common structural unit in many biologically active oligosaccharides.[8,9] We report herein a new iterative glycosylation strategy that makes use of thioglycosides of the *N*-phthaloyl

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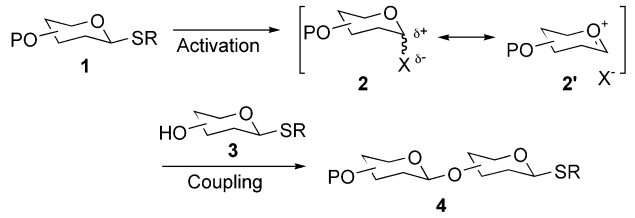
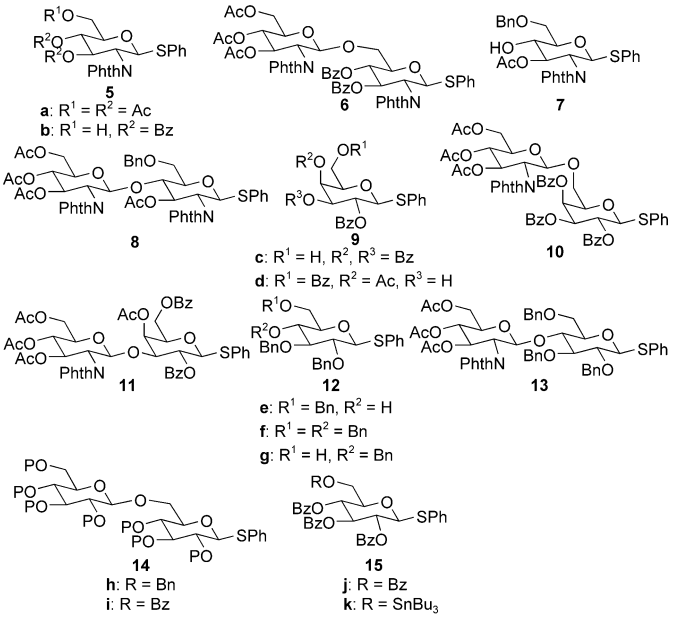


Scheme 1. Strategy for iterative glycosylation.

(Phth)-protected glucosamine (GlcN) derivative **5** as both the glycosyl donor and acceptor.

We initially examined the effect of the counteranion X on iterative glycosylation, with thioglycosides **5** as the donors and acceptors (Table 1). Representative results are as follows. The thioglycoside **5a** was treated with 1-benzenesulfinyl piperidine (BSP) and triflic anhydride,[10] and the resulting "glycosyl triflate" intermediate was treated with **5b** at $-60\,^{\circ}C$ for 15 min to give the desired disaccharide **6** in 82% yield (Table 1, entry 1). The involvement of the α-glycosyl triflate intermediate was suggested by the signal for the anomeric proton at $\delta = 6.19$ ppm with $^{3}J_{H\text{-}H} = 3.0$ Hz in the ^{1}H NMR and the signal for the anomeric carbon atom at $\delta =$ 104 ppm in the ^{13}C NMR in CD_2Cl_2 at $-75\,^{\circ}C$. Although the intramolecular participation of the Phth-group has been proposed,[8] no such intermediate was observed by NMR spectroscopic analysis. The glycosyl triflate intermediate was stable at this temperature, but decomposed rapidly above $-50\,^{\circ}C$.

Table 1: Glycosylation of thioglycosides.



Entry	Donor	Acceptor	Method[a]	Product	Yield [%]
1	**5a**	**5b**	A	**6**	82
			B		62
			C		82
2	**5a**	**7**	A	**8**	84
3	**5a**	**9c**	A	**10**	76
4	**5a**	**9d**	A	**11**	46
5	**5a**	**12e**	A	**13**	62
6[b]	**12f**	**12g**	A	**14h**	61[c]
7	**15j**	**15k**	D	**14i**	66

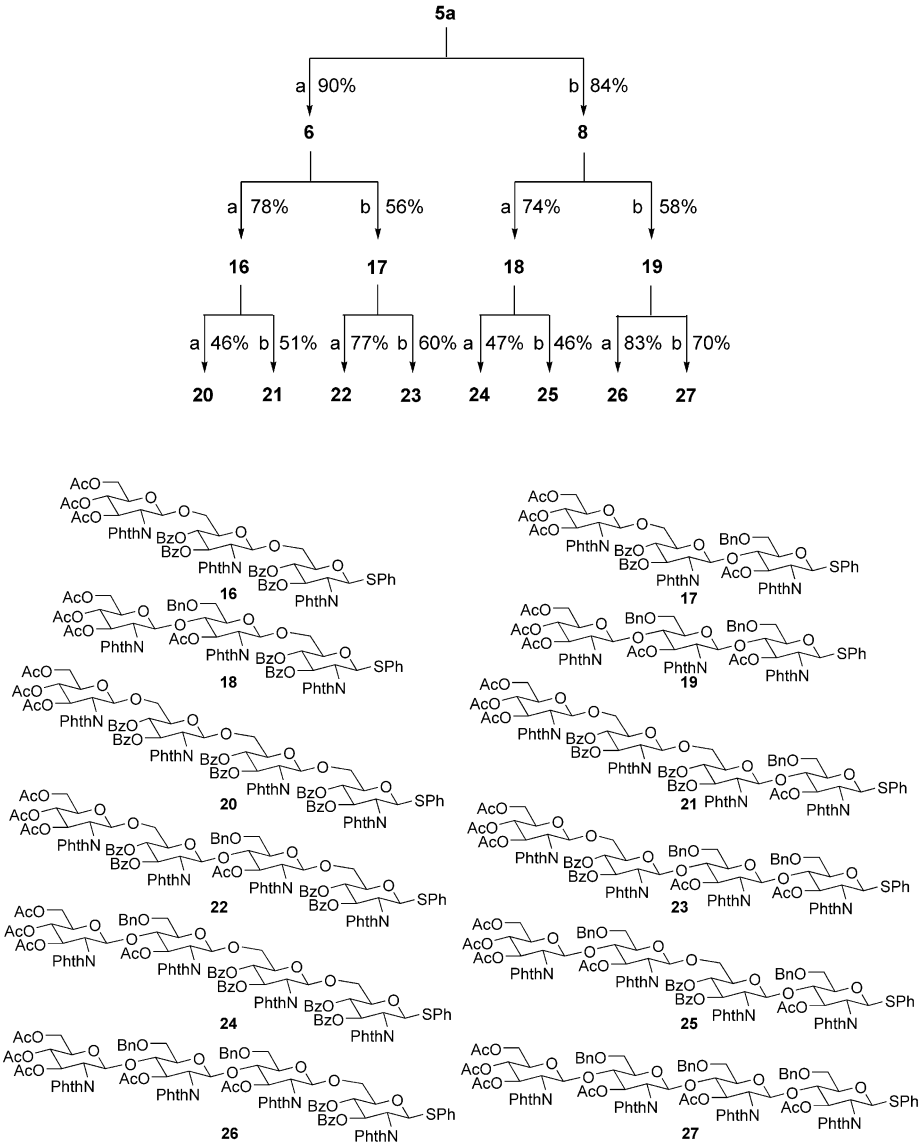
[a] A: BSP/Tf_2O; B: PhSCl/AgOTf; C: PhSCl/$AgN(Tf)_2$; D: PhSCl/$AgSbCl_6$. The reaction was carried out in CH_2Cl_2. [b] The reaction was carried out in toluene/CH_2Cl_2 mixture. [c] Products formed as a 39:61 mixture of α and β isomers.

The glycosyl triflate intermediate prepared by treatment of **5a** with benzenesulfenyl triflate (PhSOTf), which was prepared in situ from benzenesulfenyl chloride and silver triflate,[7b] afforded the same disaccharide in 62% yield. A different counteranion of the silver salt in the formation of the "glycosyl cation" intermediate resulted in a pronounced effect on disaccharide synthesis. Thus, while the use of silver bis[(trifluoromethyl)sulfonyl]amide (triflimide, NTf_2) afforded the same disaccharide in 82% yield,[11] other silver salts, such as AgOTs, $AgBF_4$, $AgPF_6$, or $AgSbF_6$ resulted in low coupling efficiency (> 10% yield).

We next examined the generality in terms of glycosyl acceptors. Although the hydroxy group at C4 of glucosamine is known to be relatively unreactive, the glycosylation of **5a** with **7** afforded the desired disaccharide **8** in good yield (Table 1, entry 2). The glycosyl acceptors are not limited to glucosamine derivatives; galactose **9** and glucose **12** derivatives, which bear hydroxy groups, coupled with the glycosyl triflate intermediate with high efficiency (Table 1, entries 3–5). The current method enables the construction of β-GlcN-1,6-Gal, β-GlcN-1,3-Gal, and β-GlcN-1,4-Glc structures in **10**, **11**, and **13**, respectively, which are found in many important biologically active compounds such as proteoglycans, glycolipids, and blood group substances.

The current method enables the use of hitherto impossible glycosyl donor/acceptor combinations by selective activation of glycosyl donors prior to the addition of acceptors. For example, the activation of **12e** is estimated to take place approximately 1500 times faster than the activation of **5a** when these two glycosides are activated concurrently,[2e] and thus **5a** cannot serve as glycosyl donor in the presence of **12e** under conventional chemoselective glycosylation methods.[3h] The present strategy, however, enables the use of **5a** as donor and **12e** as acceptor to give **13** with high coupling efficiency (Table 1, entry 5). Furthermore, while glycosides **12f** and **12g** are estimated to have a similar reactivity based on the armed/disarmed glycosylation method,[3b] the coupling of **12f** and **12g** was successful and gave the desired disaccharide **14h** (Table 1, entry 6). The coupling of **15j** and **15k**, both of which have similar reactivity, also took place selectively to give **14i** (Table 1, entry 7). In all cases, we could not detect side products derived from the activation of the glycosyl acceptors.

The most striking feature of the current method is the existence of the unreduced phenylsulfanyl group, which can be directly used for the next glycosylation reaction under the same reaction conditions. This feature was demonstrated in the combinatorial synthesis of oligoglucosamine (Scheme 2). Thus, the disaccharides **6** or **8**, activated with BSP and Tf_2O followed by coupling with either **5b** or **7**, afforded the isomeric triglucosamines **16**,**17**, **18**, and **19** in good yields. Repetition of the same reaction sequence with the triglucosamines as the glycosyl donors gave the isomeric tetraglucosamines **20**–**27**. The tetra-β-GlcN-1,4-GlcN structure in **27**,



Scheme 2. Reaction conditions: a) Donor (1.0 equiv), BSP (1.1 equiv), Tf_2O (1.4 equiv), CH_2Cl_2, $-60\,^{\circ}C$; then **5b** (1.5 equiv), $-60\,^{\circ}C$. b) Donor (1.0 equiv), BSP (1.1 equiv), Tf_2O (1.4 equiv), CH_2Cl_2, $-60\,^{\circ}C$; then **7** (1.5 equiv), $-60\,^{\circ}C$. BSP = 1-benzenesulfinyl piperidine; Tf = trifluoromethanesulfonyl.

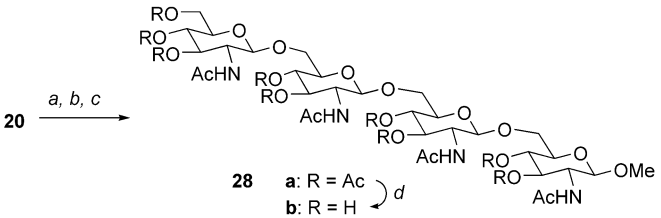
which is a fundamental sugar skeleton of Nod factors,[12] could be easily prepared under a single set of conditions.

The reactive phenylsulfanyl groups in these tetraglucosamines can be used for further elongation of oligosaccharides or for the synthesis of a simple reducing-end glycoside by the glycosylation with alcohol. For example, activation of **20** followed by treatment with methanol afforded the corresponding *O*-glycoside, which was transformed into free glycoside **28b** by standard deprotection procedures (Scheme 3).

In summary, we have developed a new iterative glycosylation to carry out β-glycosidic bond formation of glucosamine derivatives with the corresponding thioglycosides as both donors and acceptors. Because thioglycosides are stable and readily available, the current method offers practical advantages for the rapid assembly of oligosaccharides. As oligosaccharides can be assembled under a single set of conditions, the current method would be suitable for the automated synthesis of oligosaccharides. Furthermore, the fundamental principle described herein should be applicable to a wide variety of oligosaccharide structures. Such possibilities are now under active investigation.

Experimental Section

Typical procedure (**6**): Tf_2O (230.3 mg, 0.82 mmol) was added to a solution of **5a** (316.5 mg, 0.60 mmol), BSP (138.1 mg, 0.66 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (246.8 mg, 1.20 mmol), and molecular sieves (4 Å; ≈600 mg) in CH_2Cl_2 (6.0 mL) at $-60\,^{\circ}C$. After 30 min, a solution of **5b** (548.7 mg, 0.90 mmol) in CH_2Cl_2 (3.0 mL) was added. After 15 min, the reaction was quenched by the addition of Et_3N (ca. 0.60 mL), and the resulting mixture was warmed to room temperature, filtered, and washed with a saturated aqueous $NaHCO_3$ solution. After separation of the organic layer, the aqueous phase was extracted with ethyl acetate, and the combined organic extract was washed with saturated aqueous NaCl solution, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude oil. Purification by flash chromatography (silica gel: 70.0 g; eluent: EtOAc/hexane (55%)) afforded **6** (557.3 mg, 90%) as a white amorphous powder. IR (KBr): $\tilde{\nu}$ = 1779, 1717 (s), 1387, 1273, 1244, 1109, 1071, 1028, 720 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): $\delta = 1.87$ (s, 3H), 2.03 (s, 3H), 2.05 (s, 3H), 3.76 (dd, J = 11.3, 7.3 Hz, 1H), 3.78–3.82 (m, 1H), 4.01 (dd, $J = 11.0$, 2.0 Hz, 1H), 4.05–4.12 (m, 2H), 4.25 (dd, $J = 12.3$, 4.8 Hz, 1H), 4.35 (dd, $J = 10.5$, 8.5 Hz, 1H), 4.42 (t, $J = 10.3$ Hz, 1H), 5.13 (t, $J = 9.5$ Hz, 1H), 5.29 (t, $J = 9.5$ Hz, 1H), 5.50 (d, $J = 8.5$ Hz, 1H), 5.70 (d, $J = 10.5$ H, 1H), 5.74 (dd, $J = 10.5$, 9.0 Hz, 1H), 6.15 (t, $J = 9.8$ Hz,



Scheme 3. Reaction conditions: a) BSP (1.2 equiv), Tf_2O (1.5 equiv), CH_2Cl_2, $-60\,^{\circ}C$; then methanol (2.0 equiv), 15 min; b) N_2H_4 (420 equiv), CD_3OD, 60 °C, 11 h, c) Ac_2O (430 equiv), py (450 equiv), DMAP (2 equiv), room temperature, 1 h, 69% (three steps), d) NaOMe (56 equiv)/MeOH/H_2O, room temperature, 2 h, 65%. DMAP = dimethylaminopyridine.

1H), 7.20 (t, $J = 10.5$ Hz, 2H), 7.26–7.35 (m, 7H), 7.35–7.41 (m, 1H), 7.45–7.50 (m, 1H), 7.58–7.86 ppm (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 20.4$ (CH_3), 20.6 (CH_3), 20.7 (CH_3), 53.5 (CH), 54.4 (CH), 61.9 (CH_2), 68.8 (CH_2), 69.0 (CH_2), 69.8 (CH), 70.8 (CH), 71.8 (CH), 71.8 (CH), 77.3 (CH), 82.6 (CH), 98.3 (CH), 123.6 (CH), 128.2 (CH), 128.2 (CH), 128.4 (CH), 128.4 (C), 128.6 (C), 129.0 (CH), 129.7 (CH), 129.7 (CH), 131.1 (C), 131.1 (C), 131.5 (C), 132.8 (CH), 133.2 (CH), 133.4 (CH), 134.0 (CH), 134.1 (CH), 134.2 (CH), 165.1 (C=O), 165.5 (C=O), 166.7 (C=O), 167.8 (C=O), 169.5 (C=O), 170.1 (C=O), 170.7 ppm (C=O); HRMS (FAB): *m/z*: calcd for $C_{48}H_{46}O_{17}NS$ [M+H$^+$]: 940.2486; found: 940.2493.

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[11] The ^{1}H and ^{13}C NMR spectroscopic analyses suggested the formation of a 72:28 mixture of two β-glycosyl triflimide intermediates, in which the signal for the anomeric proton appeared at $\delta = 6.53$ (d, $^3J_{H\text{-}H} = 9.5$ Hz) and 6.39 ppm (d, $^3J_{H\text{-}H} = 10.0$ Hz) and the signal for the anomeric carbon atom at $\delta = 85.9$ and 86.4 ppm at $-75\,^\circ$C, respectively. Because the two peaks tended to coalesce upon warming, we tentatively assigned them as the rotamer of the carbon–nitrogen bond. The intermediates decomposed above $-20\,^\circ$C, before complete peak coalescence.

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