Carbohydrates

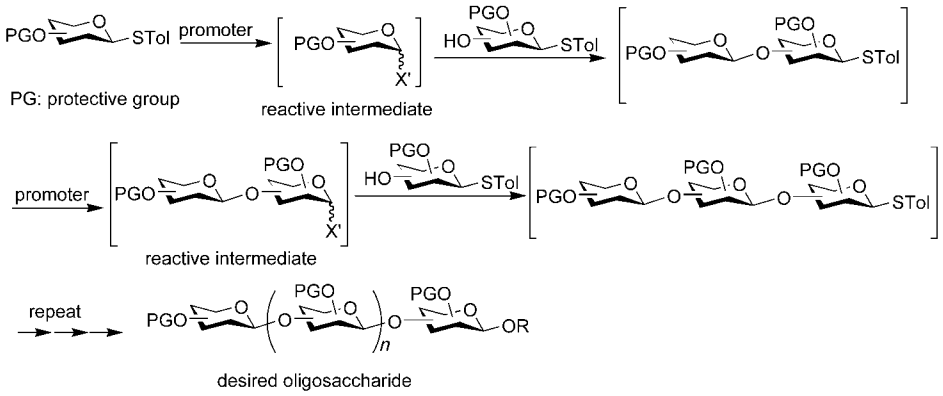
Iterative One-Pot Synthesis of Oligosaccharides**

Xuefei Huang, Lijun Huang, Haisheng Wang, and Xin-Shan Ye**

Traditional oligosaccharide synthesis is a time-consuming process, primarily due to tedious protective group manipulation and intermediate separation. To reduce the number of synthetic and purification steps, many innovative methodologies have been developed, such as automated solid-phase synthesis,[1] orthogonal glycosylation,[2] iterative glycosylation,[3] and chemoselective glycosylation,[4] among which the reactivity-based one-pot method is particularly noteworthy.[5] The reactivity-based one-pot method refers to one in which glycosyl donors with decreasing anomeric reactivities are allowed to react sequentially in a single reaction flask. Large oligosaccharides can be assembled in this fashion without tedious purification of intermediates or adjustment of anomeric leaving groups, as witnessed by total syntheses of complex oligosaccharides such as fucosyl GM1,[5a] Le^Y,[5c] and Globo H,[5d] as well as assembly of oligosaccharide libraries.[5b,e] However, to obtain building blocks with suitable anomeric reactivities, extensive protective group manipulations and/or aglycon adjustments must be carried out.[5] This excessive synthetic manipulation on building blocks complicates the synthetic process and decreases overall efficiency.

To overcome limitations of existing approaches, we have investigated the possibility of designing a general one-pot method independent of differential glycosyl donor reactivities. This can be achieved by pre-activating the donor,[6] which generates a reactive intermediate in the absence of the acceptor (Scheme 1). Upon 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. This process can be repeated *in the same reaction vessel* allowing rapid assembly of oligosaccharides. Several prerequisites, however, must be satisfied for a successful iterative one-pot synthesis: 1) the promoter utilized must be stoichiometric in activation of a wide range of glycosyl donors and be completely consumed by the donor to prevent activation of following building blocks; 2) the intermediate generated after pre-activation must be stable till addition of acceptor, yet reactive for rapid high-yielding glycosylations; and 3) side products formed from activation must not interfere with glycosylations.

After much experimentation examining the effects of various promoters,[7] aglycon leaving groups,[8] and additives,[9]



Scheme 1. Iterative one-pot synthesis of oligosaccharides.

general reaction conditions were established by using *p*-tolyl thioglycosides as building blocks, *p*-toluenesulfenyl triflate (*p*-TolSOTf),[10] formed in situ from *p*-toluenesulfenyl chloride (*p*-TolSCl) and silver triflate (AgOTf), as the stoichiometric promoter, in the presence of the dehydrating reagent MS-AW300. Chemoselective glycosylation of donors was observed independent of the reactivities of donors and acceptors, producing disaccharides bearing an anomeric *p*-thiotolyl moiety in satisfactory yields (Table 1).

Introduction of one equivalent of *p*-TolSCl to a mixture of armed donor **1**, AgOTf, and MS-AW300 in diethyl ether at −60 °C led to instantaneous complete activation of the glycosyl donor.[11] Addition of the acceptor **4** to the pre-activated donor rapidly formed disaccharide **9** in just a few minutes, which was isolated in 87 % yield as the α anomer due to the anomeric effect (Table 1, entry 1). The *p*-tolyl disulfide generated from activation did not perturb the glycosylation.

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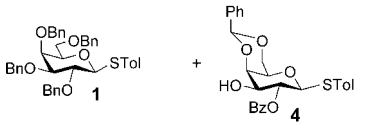
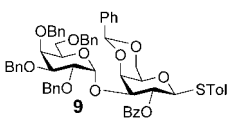
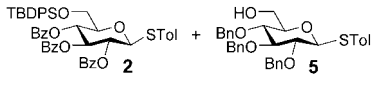
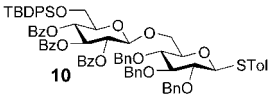
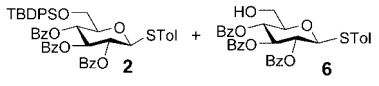
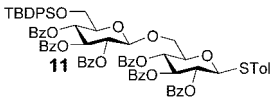
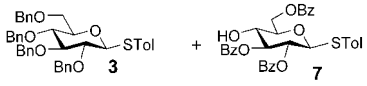
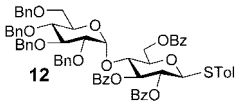
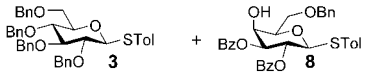
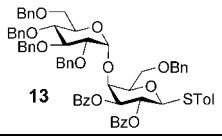
Table 1: Results of chemoselective glycosylations of thioglycoside donors.

Donor + AgOTf (2 equiv) $\xrightarrow[Et_2O,\ -60\ ^\circ C]{p\text{-TolSCl (1 equiv), MS-AW300}}$ Acceptor → Product

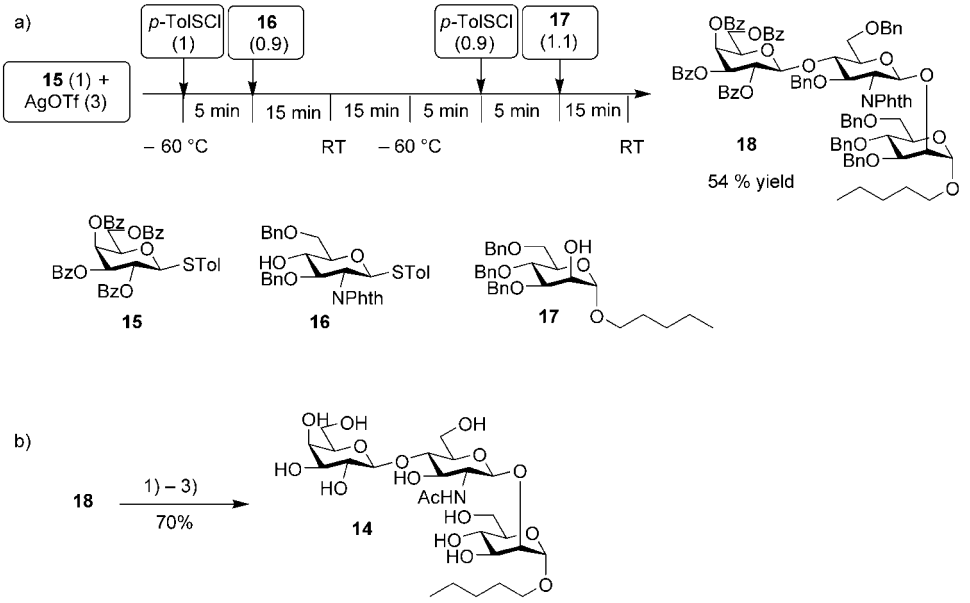
Entry	Donor + Acceptor	Product	Yield [%]
1			87
2			69
3			72
4			67
5			65

To explore the scope of the current method, glycosylations with poorly nucleophilic acceptors are examined, which are known to be challenging due to reduced glycosylation rates and/or competition of other more nucleophilic compounds in the reaction mixture.[5f,12] With our protocol, donor **2** reacted readily with a disarmed acceptor **6** to give disaccharide **11** in a few minutes in 72% yield (Table 1, entry 3). Two poorly nucleophilic acceptors **7** and **8**[12c] were also glycosylated smoothly by pre-activated donor **3** in 67% and 65% yields, respectively (Table 1, entries 4 and 5).[13]

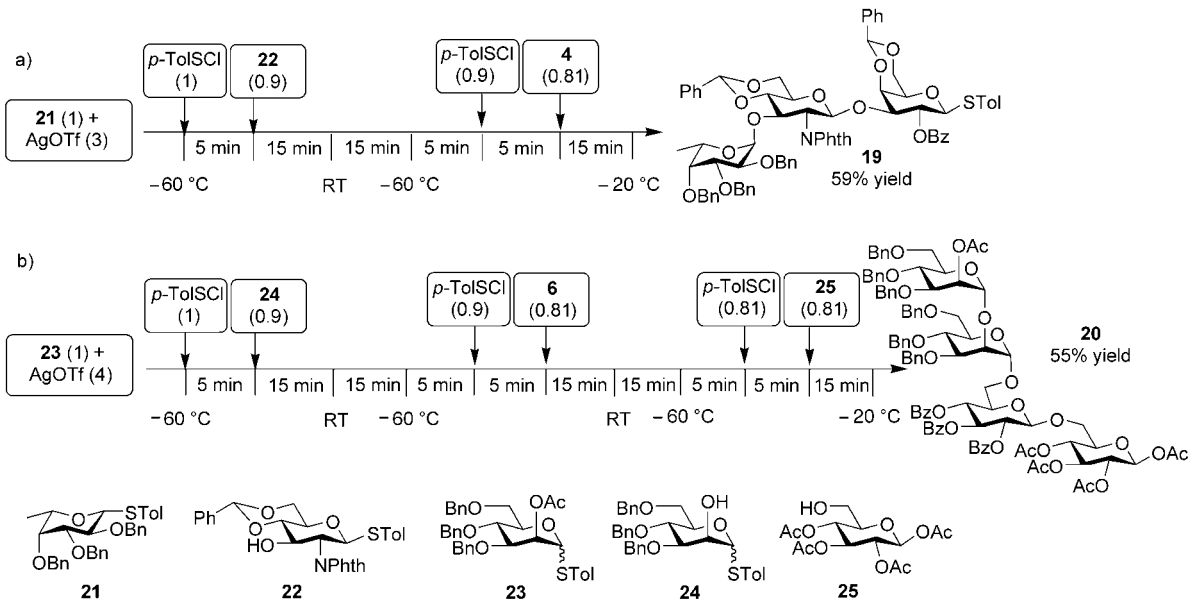
We next focused on applying this strategy to the one-pot synthesis of oligosaccharides. A sub-stoichiometric amount (0.9 equiv) of acceptor was utilized for each coupling to ensure complete consumption of the acceptor. The *p*-TolSOTf is a powerful promoter capable of stoichiometrically activating highly disarmed donors as well. Disarmed donor **2** with the participating benzoyl moiety at C-2 reacted smoothly with the more reactive armed acceptor **5** to give disaccharide **10** in 69% as the β anomer (Table 1; entry 2). No products due to the self-coupling of acceptor **5** were isolated. This reversal of reactivity, that is, the less reactive donor is selectively glycosylated with the more reactive acceptor, is not possible with the traditional reactivity-based approach.

reaction mixture was warmed up to room temperature after each glycosylation to decompose the slight excess of activated donor that had been present to prevent the formation of deletion sequences.

The trisaccharide motif **14** occurs in complex *N*-glycoprotein structures containing the demanding Gal-β1,4-GlcNAc and GlcNAc-β1,2-Man linkages. Because our strat-



Scheme 2. One-pot synthesis and deprotection of trisaccharide **18**. The values given in parentheses denote the number of equivalents of each reagent. Reagents and conditions: 1) ethylenediamine, EtOH, reflux; 2) Ac_2O, MeOH; 3) H_2, Pd/C.



Scheme 3. One-pot syntheses of oligosaccharides **19** and **20**. The values given in parentheses denote the number of equivalents of each reagent.

egy does not require tuning of reactivities, this allowed us to select the readily available building blocks **15**–**17** (Scheme 2). Pre-activation of the disarmed thiogalactoside **15** (1 equiv) by *p*-TolSOTf (1 equiv) at −60 °C was followed by addition of the more reactive armed glucosamine building block **16** (0.9 equiv) (Scheme 2a). The reaction mixture was warmed up to room temperature for 15 min, and then cooled down back to −60 °C. Activation of the newly formed disaccharide with *p*-TolSOTf followed by addition of acceptor **17** produced the trisaccharide **18**[14] within one hour in 54% yield. The progress of the reaction was readily monitored by TLC. The trisaccharide **18** was then deprotected in 70% yield by using a three-step sequence to give **14** (Scheme 2b). Trisaccharide **14** has also been synthesized by employing automated solid-phase methodology using an excess of each glycosyl donor (12 equiv) in 37.2% overall yield for assembly and deprotection.[1a] Our iterative one-pot method has the advantages of using a near-stoichiometric amount of building blocks, ease in reaction monitoring, and simplicity of operation while achieving similar overall synthetic efficiencies in the syntheses of medium-sized oligosaccharides.

To illustrate the generality of our methodology, trisaccharide **19**[14] and tetrasaccharide **20**,[14] which contain biologically relevant glycosidic linkages such as Fuc-α1,3-GlcNAc, GlcNAc-β1,3-Gal, Man-α1,2-Man, Man-α1,6-Glc, and Glc-β1,6-Glc, were assembled as outlined in Scheme 3. One-pot sequential coupling of fucosyl thioglycoside **21**, glucosamine **22** and thiogalactoside **4** produced trisaccharide **19** in 59% yield in only one hour (Scheme 3a). With its anomeric *p*-thiotolyl moiety, the trisaccharide **19** can be immediately utilized as a donor in the synthesis of Le^x oligosaccharides without any aglycon modifications. Consecutive condensation of building blocks **23**, **24**, **6**, and **25** promoted by *p*-TolSOTf led to formation of tetrasaccharide **20** in 55% yield in less than two hours (Scheme 3b). It should be noted that both α and β linkages were constructed in one pot within the same oligosaccharide with excellent stereoselectivities.

In summary, a new iterative one-pot glycosylation approach is developed for the efficient assembly of oligosaccharides, which is based upon pre-activation of a *p*-tolyl thioglycoside donor, followed by sequential addition of building blocks in a single reaction flask. This strategy obviates the need for the extensive adjustment of protective groups required for traditional reactivity-based one-pot synthesis, significantly reducing the amount of time needed for preparing building blocks. Furthermore, a single glycosylation protocol was found applicable for the assembly of a wide range of oligosaccharides. This will be particularly advantageous for designing oligosaccharide libraries. Studies are ongoing to further explore the scope of this method and apply it to syntheses of complex oligosaccharides as well as carbohydrate libraries.

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[6] While pre-activation of donors in the absence of an acceptor has been reported before (for several examples see ref. [3a,c, 4a, and 10a,b], the conditions utilized are not applicable to iterative one-pot synthesis due to the need for excess promoter, excess acceptor, extra rearrangement steps, additional quenching reagent, or intermediate aglycon adjustments.

[7] Other promoters such as N-iodosuccinimide (NIS)/TMSOTf, dimethyl (methylthio) sulfonium triflate (DMTST), *p*-nitrophenylsulfenyl chloride/AgOTf, phenylselenyl bromide/AgOTf, and the newly developed 1-benzenesulfinyl piperidine/Tf_2O, were also examined. None of them gave desired oligosaccharides consistently in satisfactory yields.

[8] Thioglycosyl donors of varying electron-withdrawing power and steric sizes, such as *p*-methoxyphenyl, *p*-nitrophenyl, *p*-trifluoromethylphenyl, *o*-fluorophenyl, 2,6-dimethylphenyl, *i*Pr, and *t*Bu have been examined, and have been found to give lower yields compared with those obtained by using *p*-tolyl thioglycoside donors. Other excellent donors such as glycosyl sulfoxide, *n*-pentenyl glycoside, glycosyl fluoride, and glycosyl iodide may be potentially utilized in iterative one-pot synthesis as well.

[9] Additives such as tetrabutylammonium triflate, lithium perchlorate, and 2,6-di-*tert*-butyl 4-methylpyridine did not lead to higher yields.

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[11] Although Crich and co-workers have convincingly demonstrated the presence of α-glycosyl triflate as the dominant intermediate after pre-activation of several thioglycosides through a series of outstanding low-temperature NMR experiments, the identity of kinetically reactive species remained to be illustrated. For references regarding the reactive intermediate, see: ref. [10a,b], and A. A. H. Abdel-Rahman, S. Jonke, E. S. H. El Ashry, R. R. Schmidt, *Angew. Chem.* **2002**, *114*, 3100; *Angew. Chem. Int. Ed.* **2002**, *41*, 2972.

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[13] Pre-activated donor **2** did not react with acceptors **7** or **8**, and we are currently investigating the reasons for this.

[14] Compound **18**: ^{1}H NMR (599.87 MHz, $CDCl_3$): $\delta = 0.80$ (t, $J =$ 7.2 Hz, 3H), 1.10–1.26 (m, 4H), 1.36 (quin, $J = 7.2$ Hz, 2H), 2.94 (dd, $J = 6.6$, 10.8 Hz, 1H), 3.11 (dt, $J = 7.2$, 9.0 Hz, 1H), 3.38–3.52 (m, 5H), 3.55 (d, $J = 10.2$ Hz, 1H), 3.70–3.76 (m, 2H), 3.94–4.08 (m, 4H), 4.22–4.43 (m, 8H), 4.65 (d, $J = 12.0$ Hz, 1H), 4.72 (d, $J = 12.0$ Hz, 1H), 4.73 (d, $J = 12.0$ Hz, 1H), 4.74 (d, $J = 10.8$ Hz, 1H), 4.98–5.02 (m, 2H), 5.17 (d, $J = 7.8$ Hz, 1H), 5.43 (dd, $J =$ 3.6, 10.8 Hz, 1H), 5.79 (dd, $J = 7.8$, 10.8 Hz, 1H), 5.85 (d, $J =$ 3.6 Hz), 6.78–6.82 (m, 2H), 7.06–7.68 (m, 39H), 7.74–7.78 (m, 2H), 7.89–7.94 (m, 4H), 8.06–8.42 ppm (m, 2H); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 14.20$, 22.62, 28.41, 29.21, 55.77, 60.64, 61.97, 67.89, 68.32, 68.43, 70.24, 70.62, 70.83, 71.40, 71.92, 71.97, 73.08, 73.75, 73.87, 74.49, 74.94, 75.07, 76.80, 77.25, 78.03, 78.37, 97.16, 100.82, 123.32, 127.28, 127.49, 127.56, 127.64, 127.74, 127.98, 128.06, 128.12, 128.38, 128.45, 128.48, 128.52, 128.55, 128.61, 128.68, 129.17, 129.32, 129.71, 129.98, 130.06, 132.02, 133.47, 133.51, 133.68, 138.25, 138.69, 138.76, 138.81, 165.22, 165.53, 165.69, 166.26 ppm; HRMS $C_{94}H_{91}NNaO_{21}$ [M+Na^+] calcd 1952.5981 found 1952.5961. Compound **19**: ^{1}H NMR (599.87 MHz, $CDCl_3$): $\delta = 0.81$ (d, $J = 6.6$ Hz, 3H), 2.27 (s, 3H), 3.38 (d, $J = 1.8$ Hz, 1H), 3.52–3.65 (m, 6H), 3.79 (d, $J =$ 9.6 Hz, 1H), 3.93 (q, $J = 6.6$ Hz, 1H), 4.01–4.08 (m, 3H), 4.23 (d, $J = 11.4$ Hz, 1H), 4.28–4.38 (m, 4H), 4.42–4.47 (m, 2H), 4.66 (d, $J = 3.0$ Hz, 1H), 4.68 (d, $J = 9.6$ Hz, 1H), 4.73 (d, $J = 11.4$ Hz, 1H), 5.31 (t, $J = 9.6$ Hz, 1H), 5.48 (s, 1H), 5.51 (s, 1H), 5.55 (d, $J = 9.6$ Hz, 1H), 6.88–6.92 (m, 2H), 6.93–6.96 (m, 2H), 7.08–7.50 (m, 32H), 7.62–7.66 ppm (m, 2H); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 16.67$, 21.46, 55.50, 66.27, 67.45, 68.77, 68.99, 69.37, 70.25, 72.62, 73.32, 74.88, 75.61, 76.28, 76.33, 78.13, 79.68, 80.06, 82.08, 86.02, 99.77, 100.16, 101.19, 101.33, 122.92, 126.19, 126.80, 127.49, 127.58, 127.66, 127.67, 127.71, 127.89, 128.01, 128.27, 128.38, 128.43, 128.49, 128.72, 129.68, 129.84, 129.86, 132.81, 133.45, 134.19, 137.21, 137.94, 138.26, 138.54, 138.71, 139.10, 164.55 ppm; HRMS $C_{75}H_{71}NNaO_{16}S$ [M+Na^+] calcd 1296.4391 found 1296.4384. Compound **20**: ^{1}H NMR (599.87 MHz, $CDCl_3$): $\delta = 1.91$ (s, 3H), 1.92 (s, 3H), 1.97 (s, 3H), 1.98 (s, 3H), 2.10 (s, 3H), 3.46–3.92(m, 17H), 4.32–4.53 (m, 8H), 4.60 (d, $J = 10.8$ Hz, 1H), 4.68 (d, $J = 12.0$ Hz, 1H), 4.78–4.85 (m, 4H), 4.87 (t, $J = 9.0$ Hz, 1H), 4.93 (dd, $J = 8.4$, 9.6 Hz, 1H), 4.99 (d, $J =$ 1.8 Hz, 1H), 5.10 (t, $J = 9.6$ Hz, 1H), 5.44 (dd, $J = 7.8$, 9.6 Hz, 1H), 5.46–5.49 (m, 1H), 5.50 (d, $J = 9.6$ Hz, 1H), 5.57 (d, $J =$ 8.4 Hz, 1H), 5.80 (t, $J = 9.6$ Hz, 1H), 7.08–7.42 (m, 42H), 7.48–7.52 (m, 1H), 7.78–7.82 (m, 2H), 7.87–7.90 (m, 2H), 7.96–8.02 ppm (m, 2H); ^{13}C NMR (100.5 MHz, $CDCl_3$): $\delta = 20.76$, 20.91, 21.38, 66.93, 67.46, 68.80, 68.92, 69.05, 69.27, 70.40, 70.59, 71.83, 72.05, 72.08, 72.88, 73.16, 73.35, 73.57, 74.27, 74.47, 74.50, 74.89, 75.18, 75.24, 77.44, 78.33, 79.69, 91.80, 99.22, 99.76, 101.06, 127.56, 127.63, 127.70, 127.74, 127.77, 127.83, 127.99, 128.09, 128.30, 128.38, 128.45, 128.48, 128.52, 128.55, 128.64, 129.17, 129.26, 129.59, 129.98, 130.17, 133.33, 133.35, 133.49, 138.25, 138.55, 138.64, 138.71, 138.77, 138.87, 165.22, 165.36, 166.01, 168.94, 169.38, 169.61, 170.22, 170.32 ppm; $^1J(^{13}C,^1H)$: 166.4 Hz ($\delta = 91.80$ ppm, β linkage), 171.8 Hz ($\delta = 99.22$ ppm, α linkage), 172.3 Hz ($\delta = 99.76$ ppm, α linkage), 163.9 Hz ($\delta = 101.06$ ppm, β linkage); HRMS $C_{97}H_{100}NaO_{29}$ [M+Na^+] calcd 1751.6248 found 1751.6301.

