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[13] Treatment of (*E*)-**4** (*E*/*Z* = 46.5:1) with Bu_3SnH (1 equiv) in benzene under reflux for 2 h gave rise to an *E*/*Z* mixture (*E*/*Z* = 2:1) of **4**, suggesting that (*Z*)-**4** is a thermodynamic product.

[14] TDTH was found to be a slower reducing agent than Bu_3SnH. For example, reduction of benzyl *p*-iodophenyl ether with Bu_3SnH and TDTH (1.1 equiv) in CH_2Cl_2 at −78 °C under the influence of catalytic Et_3B (0.2 equiv) for 20 min afforded benzyl phenyl ether in 88% and 40% (recovery of *p*-iodophenyl ether in 56%) yields, respectively.

[15] Attempted reduction of the two different aldehydes with $(Me_3Si)_3SiH$ in the presence of Me_2AlCl resulted in recovery of most of these aldehydes.

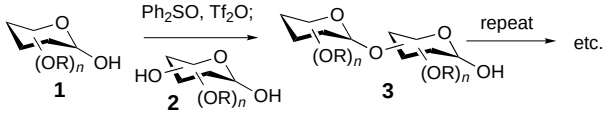
Chemoselective Iterative Dehydrative Glycosylation**

Hien M. Nguyen, Jennifer L. Poole, and David Y. Gin*

The development of strategies for efficient construction of complex oligosaccharides has been a long-standing challenge in organic synthesis.[1] This is a direct result of the immense structural diversity and biological importance of complex glycoconjugates in nature. In traditional approaches to the assembly of oligosaccharides, a preformed glycosyl donor incorporating an anomeric latent leaving group is coupled with a nucleophilic glycosyl acceptor, and the resulting disaccharide then undergoes either a selective deprotection or anomeric derivatization step prior to the subsequent coupling event. In order to streamline this process, a number of elegant chemoselective and orthogonal glycosylation strategies have been developed with the goal of circumventing deprotection and anomeric derivatization steps in the iterative glycosylation sequence. Within this context, two key strategies, which use well-established glycosylation methods, have been explored. One approach employs carbohydrate coupling partners with identical anomeric latent leaving groups; the reactivities of each of the leaving groups are differentiated by varying the electronic nature of proximal protective groups.[2] The success of this approach to oligosaccharide synthesis thus relies on intricate selection of protective groups to establish a suitable reactivity hierarchy among the carbohydrate building blocks.[2e] In an alternative strategy, the anomeric latent leaving groups in the carbohydrate coupling partners are mutually distinct, possessing chemically orthogonal reactivities; successive glycosylations are performed with a number of different reagents that are specific for a certain latent leaving group.[3]

We now report a novel approach to iterative oligosaccharide synthesis which employs a chemoselective glycosylation strategy that: 1) is not dependent on the careful selection and placement of specific protective groups in the carbohydrate coupling partners to electronically influence anomeric reactivity; 2) avoids the need for C1-derivatized carbohydrate building blocks with chemically distinct anomeric latent leaving groups; and 3) requires only a single glycosylation method to effect iterative one-pot anomeric bond constructions.

We have recently established a dehydrative glycosidic coupling method whereby a variety of nucleophilic acceptors can be directly glycosylated with C1-hydroxy donors using diphenyl sulfoxide and triflic anhydride.[4, 5] The fact that our dehydrative coupling is not plagued by self-condensation of the C1-hydroxy donor led us to investigate the possibility of selective glycosylation of an alkyl hydroxy group in the presence of a free hemiacetal functionality. In this context, our dehydrative glycosylation reaction can serve as the basis for a new approach to iterative chemoselective glycosylation in which a hemiacetal donor **1** is activated with diphenyl sulfoxide and triflic anhydride (Scheme 1). A nucleophilic



Scheme 1. Chemoselective iterative dehydrative glycosylation. Tf = trifluoromethanesulfonyl.

acceptor **2**, which incorporates a free alkyl hydroxy group as well as an unprotected C1-hemiacetal functionality, is then introduced. Ideally, the alkyl alcohol is chemoselectively glycosylated in the presence of the hemiacetal hydroxy group to generate, in a one-pot procedure, the hemiacetal-terminated disaccharide **3**, which is immediately poised for another coupling iteration. Thus, the key issue of chemoselectivity in this approach pertains only to the relative nucleophilicities of the alkyl hydroxy group versus the hemiacetal hydroxy group

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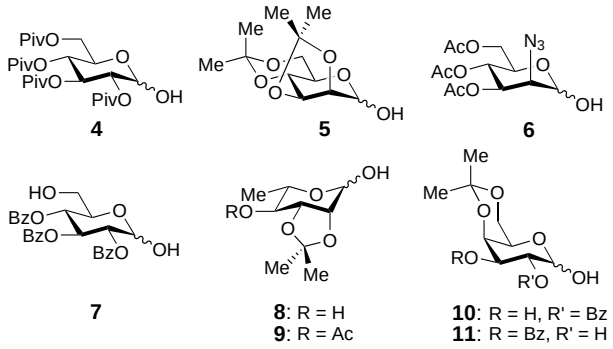
within the acceptor substrate itself, a feature that heretofore has not been successfully exploited in oligosaccharide synthesis with traditional chemical glycosylation methods. As a result, the versatility of this iterative process is not limited to the number of available orthogonal latent leaving groups or activating agents, nor is it limited by the need for extensive manipulation and selection of protective groups to adjust relative reactivities among carbohydrate coupling partners.[6]

This concept for chemoselective glycosylation (Scheme 1) can only be realized with the use of a controlled dehydrative coupling procedure that does not lead to indiscriminate oligomerization of the carbohydrate diol acceptor. Using the reagent combination of Ph_2SO and Tf_2O as the dehydrating agent, the critical question of chemoselectivity was first addressed by performing a variety of glycosidic couplings with several hemiacetal donors (**4**–**6**, and **9**, Scheme 2) and diol

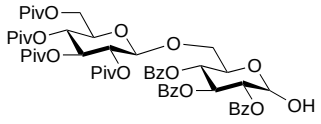
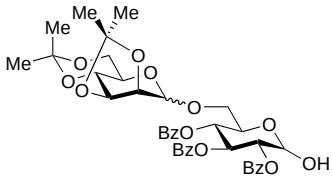
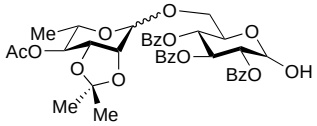
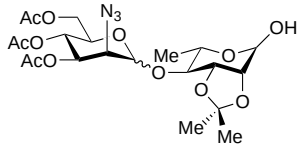
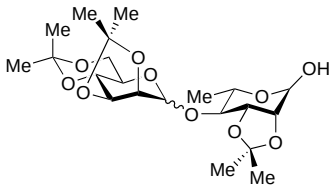
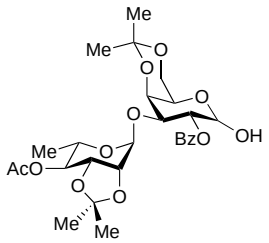
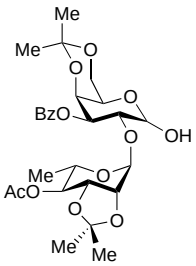


Scheme 2. Carbohydrate coupling partners. Piv = pivaloyl, Ac = acetyl, Bz = benzoyl.

carbohydrate acceptors (**7**, **8**, **10**, and **11**) incorporating an unprotected C1-hemiacetal functionality. For example, 2,3,4,6-tetra-*O*-pivaloyl-D-glucopyranose (**4**, Table 1, entry 1) was coupled with 2,3,4-tetra-*O*-benzoyl-D-glucopyranose (**7**) to afford the corresponding 1,6-β-linked disaccharide as the sole disaccharide product. Likewise, entries 2 and 3 clearly demonstrate that the primary hydroxy group within a carbohydrate can be selectively glycosylated in the presence of the free C1-hemiacetal; no evidence of 1,1′-linked disaccharides was detected in these reactions.

It is notable that secondary alcohols on carbohydrate acceptors are also preferentially glycosylated in the presence of a C1-hemiacetal. For example, the C4- and C3-hydroxy groups of **8** and **10**, respectively, are glycosylated with the carbohydrate donors **5**, **6**, and **9** to generate the hemiacetal-terminated disaccharides in good yield with complete chemoselectivity (entries 4–6). The C2,C1-diol **11** was anticipated to be a challenging substrate in this glycosylation strategy. Not only is the preferred reaction site in **11** a secondary alcohol, but this group is also flanked by both the competing C1-hydroxy nucleophile and the electron-withdrawing acyl group at C3, which can inductively attenuate the nucleophilicity of the C2-hydroxy group. Even with this "difficult" diol acceptor, the glycosylation of **11** with **9** (entry 7) proceeded with good chemoselectivity (5:1 ratio), to afford the desired 1,2′-linked disaccharide as the major product.

Table 1. Results of chemoselective dehydrative glycosylation.

Entry	Donor + acceptor	Product	Yield [%] (α:β)[a]
1	**4** + **7**		71 (β)
2	**5** + **7**		85 (73:27)
3	**9** + **7**		81 (71:29)
4	**6** + **8**		77 (90:10)
5	**5** + **8**		96 (86:14)
6	**9** + **10**		86 (α)
7	**9** + **11**		68[b] (α)

[a] α:β ratios refer to the anomeric distribution in the newly formed glycosidic bond. [b] The corresponding Rhamα1−1′α/βGal disaccharide was also isolated in 14% yield.

The efficacy of this approach lies in its ability to function in iterative single-pot glycosylations for the preparation of oligosaccharides. In an illustrative example, the 1,4-α-linked tetrasaccharide **14** (Scheme 3), which is the carbohydrate moiety within the antiserotonic resin glycoside merremoside d,[7] was prepared by two routes. In the first, the rhamnopyranose donor **9** was iteratively coupled with the diol acceptor **8** in a three-step sequence (**9** → **12** → **13** → **14**) to

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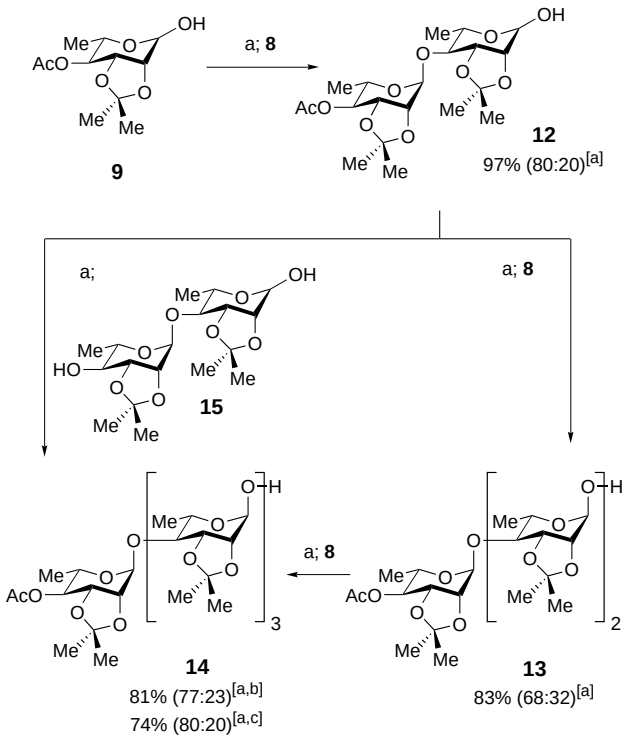

Scheme 3. Chemoselective iterative dehydrative glycosylation. Reagents: a) Ph_2SO, Tf_2O, 2,4,6-tri-*tert*-butylpyridine, $-78 \rightarrow -40\,^{\circ}C$. [a] Ratio refers to the anomeric distribution (α:β) in the newly formed glycosidic bond. Only the major (α) anomer was subjected to the subsequent glycosylations. [b] Yield derived from **13**+**8** (three-step route). [c] Yield derived from **12**+**15** (two-step route).

afford the tetrasaccharide **14**. The second route (**9** → **12** → **14**) highlights the adaptability of this strategy to the block synthesis of oligosaccharides, in which the C4,C1′-dihydroxy disaccharide acceptor **15**[8] is chemoselectively glycosylated with the hemiacetal donor ***12α*** to afford tetrasaccharide **14**. In both of these synthetic sequences, it is worth noting that: 1) each one-pot glycosylation proceeds with complete chemoselectivity; 2) all of the carbohydrate acceptor building blocks incorporate the same protective group (that is, isopropylidene ketal); and 3) a single coupling method is used to construct all of the glycosidic bonds.

A new approach to iterative chemoselective glycosylation is reported for the efficient preparation of oligosaccharides. The strategy employs a controlled dehydrative glycosylation method that allows one to capitalize on the inherent difference in nucleophilicity between an alkyl hydroxy group and a free hemiacetal functionality within carbohydrate acceptors. Not only does this approach require just a single glycosylation method for each coupling event, but it also obviates the need for the extensive protective group differentiation that is traditionally required for chemoselective glycosylation. Efforts are currently underway to adapt this strategy to polymer-supported oligosaccharide synthesis.

Experimental Section

Preparation of **12** as a representative chemoselective glycosylation procedure: Trifluoromethanesulfonic anhydride (200 μL, 1.2 mmol, 1.4 equiv) was added to a solution of 4-*O*-acetyl-2,3-*O*-isopropylidene-L-rhamnopyranose (**9**, 200 mg, 0.81 mmol, 1 equiv), 2,4,6-tri-*tert*-butylpyridine (570 mg, 2.3 mmol, 2.8 equiv), and diphenyl sulfoxide (470 mg, 2.3 mmol, 2.8 equiv) in a mixture of toluene and dichloromethane (24 mL, 3:1) at $-78\,^{\circ}C$. The reaction mixture was stirred at this temperature for 10 min and then at $-40\,^{\circ}C$ for 85 min. A solution of 2,3-*O*-isopropylidene-L-rhamnopyranose (**8**, 320 mg, 1.5 mmol, 2.0 equiv) in a mixture of toluene and dichloromethane (24 mL, 1:1) was then added through a cannula at $-40\,^{\circ}C$. The resulting mixture was stirred at this temperature for 1 h, and then at $0\,^{\circ}C$ for 30 min before the addition of excess triethylamine (1.10 mL, 7.7 mmol, 9.5 equiv). The reaction mixture was diluted with dichloromethane (500 mL) and was washed sequentially with saturated aqueous sodium bicarbonate solution (2×200 mL) and saturated aqueous sodium chloride solution (2×200 mL). The organic layer was dried (sodium sulfate), filtered, and concentrated. The residue was purified by silica gel flash column chromatography (ethyl acetate/benzene (1:3)) to afford the readily separable disaccharides ***12α*** (270 mg) and ***12β*** (73 mg) as viscous oils (4.1:1 α:β, 97% total).[9] ***12α***: $R_f = 0.55$ (ethyl acetate:hexane (1:1)); ^{1}HNMR (500 MHz, $CDCl_3$): $\delta = 5.32$ (d, 1H, $J = 2.4$ Hz, H1′), 5.23 (s, 1H, H1), 4.84 (dd, 1H, $J = 10.0$, 7.8 Hz, H4), 4.74 (dd, 1H, $J = 5.9$, 3.7 Hz, H3′), 4.57 (d, 1H, $J = 5.9$ Hz, H2′), 4.12 (dd, 1H, $J = 7.8$, 5.4 Hz, H3), 4.06 (d, 1H, $J = 5.4$ Hz, H2), 4.08–4.02 (m, 1H, H5′), 3.94 (dd, 1H, $J = 8.5$, 3.7 Hz, H4′), 3.85 (dq, 1H, $J = 10.0$, 6.4 Hz, H5), 3.12 (d, 1H, $J = 2.7$ Hz, OH), 2.08 (s, 3H), 1.54 (s, 3H), 1.42 (s, 3H), 1.42 (s, 3H), 1.33 (s, 3H), 1.30 (d, 3H, $J = 6.1$ Hz), 1.29 (s, 3H), 1.13 (d, 3H, $J = 6.4$ Hz); ^{13}CNMR (126 MHz, $CDCl_3$): $\delta = 170.2$, 112.5, 109.6, 101.2, 98.1, 85.4, 82.9, 79.7, 76.2, 75.7, 74.5, 73.2, 64.2, 27.7, 26.5, 26.1, 24.8, 21.0, 19.4, 16.7; FT-IR (neat film) 3441, 2985, 2939, 2907, 1743, 1454, 1375, 1222, 1134, 1076, 1046, 1026, 856 cm^{-1}; HR-MS (FAB): *m*/*z*: calcd for $C_{20}H_{32}O_{10}Na$ [*M*Na$^+$] 455.1892, found 455.1893. ***12β***: $R_f = 0.38$ (ethyl acetate:hexane (1:1)); ^{1}HNMR (500 MHz, $CDCl_3$): $\delta = 5.34$ (d, 1H, $J = 2.4$ Hz, H1′), 5.09 (dd, 1H, $J = 10.0$, 7.1 Hz, H4), 4.93 (dd, 1H, $J = 5.9$, 3.4 Hz, H3′), 4.91 (d, 1H, $J =$ 2.4 Hz, H1), 4.57 (d, 1H, $J = 5.9$ Hz, H2′), 4.27–4.22 (m, 2H, H2, H5′), 4.19–4.16 (m, 2H, H3, H4′), 3.46 (dq, 1H, $J = 10.0$, 6.4 Hz, H5), 2.56 (d, 1H, $J = 2.2$ Hz, OH), 2.09 (s, 3H), 1.57 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H), 1.33 (d, 3H, $J = 6.1$ Hz), 1.31 (s, 3H), 1.21 (s, 3H); ^{13}CNMR (126 MHz, $CDCl_3$): $\delta = 169.8$, 112.1, 111.2, 100.9, 96.6, 85.5, 82.8, 79.7, 74.7, 74.1, 72.6, 69.6, 27.3, 26.2, 26.1, 24.7, 21.0, 17.6, 17.1; FT-IR (neat film) 3453, 2984, 2938, 2880, 1742, 1456, 1374, 1232, 1078, 855 cm^{-1}; HR-MS (FAB): *m*/*z*: calcd for $C_{20}H_{32}O_{10}Na$ [*M*Na$^+$] 455.1892, found 455.1893.

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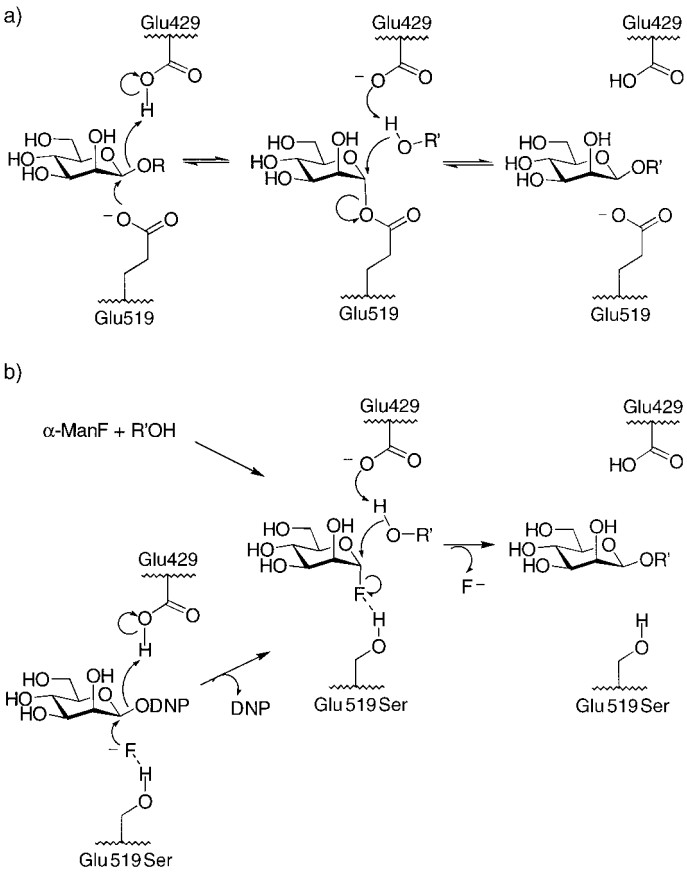
[9] Excess **8** (0.8 equiv) could be recovered in pure form.

β-Mannosynthase: Synthesis of *β*-Mannosides with a Mutant *β*-Mannosidase**

Oyekanmi Nashiru, David L. Zechel, Dominik Stoll, Taraneh Mohammadzadeh, R. Antony J. Warren, and Stephen G. Withers*

The *β*-D-mannopyranoside linkage is found in a number of biological structures, most notably in the core trisaccharide of N-linked glycoproteins,[1] as well as within the antigenic polysaccharides of yeasts,[2] *Salmonella*,[3] and glycolipids.[4] This is arguably the most difficult glycosidic bond to synthesize chemically and has inspired numerous approaches.[5] Despite these successes, each method requires a number of protection and activation steps prior to glycosylation, and few methods can achieve complete anomeric stereoselectivity.[5f,h]

Enzymatic synthesis of *β*-mannosides avoids both of these problems.[6] Successful transfer of *β*-mannosyl residues to a variety of acceptor sugars has been achieved with retaining *β*-glycosidases,[7] which function through the mechanism shown in Scheme 1a (R′ = glycoside acceptor). However, the yields



Scheme 1. a) Hydrolysis (R′ = H) and transglycosylation (R′ = glycoside acceptor) mechanisms of the retaining *Cellulomonas fimi* *β*-mannosidase (Man2a). b) Mechanism of the Man2a Glu519Ser mutant acting as a mannosynthase (upper pathway) and catalyzing the in situ generation of *α*-mannosyl fluoride (*α*-ManF; lower pathway). R′OH = glycoside acceptor. DNP = 2,4-dinitrophenyl.

of these transglycosylation reactions are inherently modest as the product formed is a substrate for the glycosidase used, resulting in hydrolysis. Further, the approach requires the preparation of a *β*-mannoside as a glycosyl donor, thereby minimizing advantages. Substantially better yields have been obtained with recombinant yeast and *Salmonella* *β*-mannosyltransferases (60 – 90 %),[8] but these enzymes are limited by their requirement for a complex acceptor. In contrast to the above, glycosynthases[9] are new enzymatic catalysts for oligosaccharide synthesis that are derived by mutating the catalytic nucleophiles of retaining glycosidases.[10] Here we report the successful construction of a mannosynthase from a retaining *β*-mannosidase and its use in the synthesis of *β*-mannosides with the readily accessible donor, *α*-D-mannosyl fluoride (Scheme 1b, upper pathway).

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