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Factors Affecting Stereocontrol during Glycosidation of 2,3-Oxazolidinone-Protected 1-Tolylthio-*N*-acetyl-D-glucosamine

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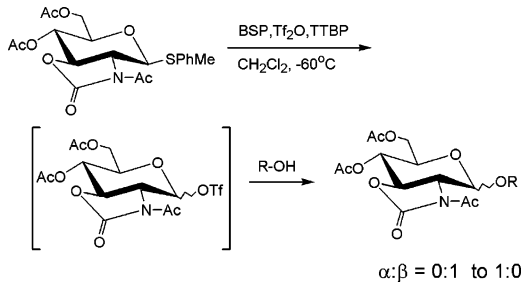

It is demonstrated that a ring-fused 2,3-oxazolidinone-protected derivative of 1-tolylthio-*N*-acetyl-D-glucosamine undergoes high-yield glycosidation under mild donor activation conditions. Stereoselective formation of α-linked or *β*-linked glycosides is dependent on reactivity of acceptor alcohols, where rate of glycosidation correlates to stereochemical outcome. Evidence for the role of glycosyl triflate intermediates and the *N*-acetyl substituent of the 2*N*,3*O*-oxazolidinone ring in stereochemical control is presented.

Variably substituted 2-amino-2-deoxy-D-glucopyranoside residues (D-glucosamine residues) are an integral structural component of numerous biologically important prokaryotic and eukaryotic glycoconjugates.[1] Protecting groups for the amine moiety of 2-amino-2-deoxy-D-glucopyranosyl donors play a major role in controlling anomeric stereochemistry during the introduction of these residues into glyconjugates structures. Substituted 2-amino moieties that impart neighboring group participation during glycoside bond formation typically promote efficient formation of *β*-linked (1,2-*trans*) glycosides.[2] In contrast, formation of α-linked (1,2-*cis*) glycosides of D-glucosamine derivatives is often plagued by limited stereoselectivity and modest yields.[2,3]

We previously reported ring-fused 2,3-oxazolidinone derivatives of phenyl 2-amino-2-deoxy-1-thio-D-glucopyranoside as glycosyl donors for the stereoselective synthesis of α-linked glycosides of D-glucosamine.[4] Protection of the 2-*N* and 3-*O* positions via the oxazolidinone ring also facilitates differentiation of the 3-hydroxyl moiety from other hydroxyl groups. Limitations of 2,3-oxazolidinone-protected donors have been observed.[4,5] Many 2,3-oxazolidinone-protected thioglycoside donors are difficult to activate, requiring phenylsulfenyl triflate (PST) for efficient activation at the low temperatures required for stereocontrol during glycosidation.[6] Complete activation of the oxazolidinone-protected thioglycosides requires at least 2 equiv of PST because 1 equiv is lost to *N*-sulfenylation, which also necessitates treatment of glycosylation products to remove the phenylsulfenyl adduct.[4] Finally, *N*-glycosylation has been observed during use of these donors in stepwise oligosaccharide synthesis.[5]

In connection with ongoing syntheses of oligosaccharides containing D-glucosamine residues, we envisioned substitution of the oxazolidinone nitrogen as a promising strategy to overcome inherent limitations of 2,3-oxazolidinone-protected D-glucosaminyl donors. Substitution of the oxazolidinone nitrogen precludes *N*-glycosylation and blocks reaction with donor activating reagents. Molecular models clearly show that a 2-*N*-acyl group appended to the 2,3-oxazolidinone ring will not provide anchimeric assistance during glycosidation. Coordination of an *N*-acyl carbonyl oxygen to the anomeric carbon (stabilization of oxacarbenium intermediate) is not possible here due to high torsional strain of the bicyclic system.

A thioglycoside derivative of 2-amino-2-deoxy-D-allopyranose bearing ring-fused *N*-acetyl-2*N*,3*O*-oxazolidine protection was previously shown to afford *β*-linked glycosides; however, the *N*-acetyl group in this 2,3-*cis*-fused allopyranosyl donor likely provides direct hindrance to acceptor attack from the α-face.[7] Energy minimization studies of 2-*N*-acyl-2*N*,3*O*-oxazolidinone derivatives of D-glucosamine demonstrate that substituents on the oxazolidinone nitrogen assume a pseudoequatorial orientation regardless of configuration at the anomeric center. This leaves the α-face of the pyranose ring open to nucleophilic attack at the anomeric center while impeding *β*-face attack on the anomeric center by sterically hindered acceptors. Considering these factors, we anticipated ring-fused 2,3-oxazolidinone protection of 1-tolylthio-*N*-acetylglucosamine derivatives would afford α-selective glycosyl donors. This expectation was consistent with the known α-selective glycosidation of *N*-unsubstituted oxazolidinone-protected thioglycoside donors.[4,5] To this end, novel ring-fused *N*-acetylglucosamine donor **2** was prepared by acetylation of thioglycoside **1**.[8]

(1) (a) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683−720. (b) Casu, B.; Lindahl, U. *Adv. Carbohydr. Chem. Biochem.* **2001**, *57*, 159−206. (c) Davis, B. G. *Chem. Rev.* **2002**, *102*, 579−601.

(2) Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92,* 1167−1195.

(3) Demchenko, A. V. *Curr. Org. Chem.* **2003**, *7*, 35−79.

(4) Benakli, K.; Zha, C.; Kerns, R. J. *J. Am. Chem. Soc.* **2001**, *123*, 9461−9462.

(5) Kerns, R. J.; Zha, C.; Benakli, K.; Liang, Y. *Tetrahedron Lett.* **2003**, *44*, 8069−8072.

(6) PST is a costly and difficult reagent to employ. It must be prepared in situ from benzenesulfenyl chloride and silver triflate, both of which are unstable and/or costly. See: (a) Martichonok, V.; Whitesides, G. M. *J. Org. Chem.* **1996**, *61,* 1702−1706. (b) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1998**, *120,* 455−456. Other milder thioglycoside activating reagent systems such as BSP/Tf_2O and diphenyl sulfoxide/Tf_2O either have been insufficient at promoting glycosylation of *N*-unsubstituted 2,3-oxazolidinone protected donors or form detrimental adducts with the oxazolidinone nitrogen, sequestering activating agent, and/or resulting in degradation of donor.

(7) Takahashi, S.; Terayama, H.; Kuzuhara, H. *Tetrahedron Lett.* **1994**, *35,* 4149−4152.

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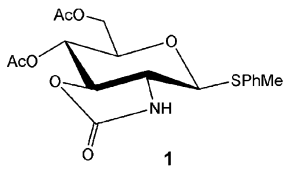
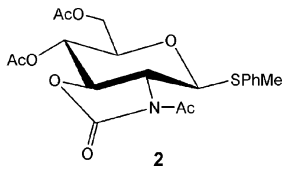


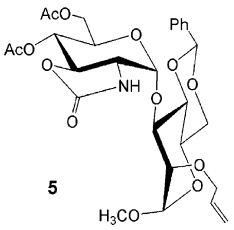
1



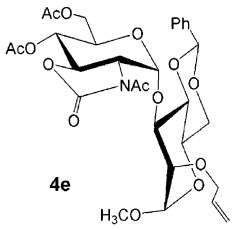
2

Glycosidation of **2** using PST activation as previously employed for glycosidation of **1** afforded complex reaction mixtures containing low yields of glycoside product. In contrast, activation of **2** using a combination of 1-benzensulfinylpiperidine (BSP), 2,4,6-tri-*tert*-butylpyrimidine (TTBP) and triflic anhydride (Tf_2O) in anhydrous dichloromethane at −60 °C as previously reported by Crich and co-workers showed complete activation, loss of **2** by TLC, within 30 min.[9] It is notable that these later activation conditions are insufficient for promoting glycosidation of **1**.[6] Encouraged by this observation, BSP/TTBP/Tf_2O activation was employed in the glycosylation of glucuronic acid derivative **3i** (Table 1, entry 9). To our surprise, coupling of activated **2** proceeded very slowly, affording a 77% yield of disaccharide **4i** as an anomeric mixture (α:*β*/2.8:1) after 48 h. Subsequent studies to understand this result led us to evaluate the coupling of **2** with a range of diverse acceptor alcohols (Table 1). All coupling reactions proceeded smoothly, but a clear trend emerged. Stereochemical control of the glycosidation reactions to preferentially yield α-linked versus *β*-linked products correlates to acceptor reactivity, steric hindrance, and reaction rate (Table 1).

Acceptor alcohols that react rapidly with activated **2** afford *β*-linked glycosides in high yield and good to excellent stereoselectivity (entries 1−7, Table 1). A definitive distinction between α- and *β*-anomers was initially unclear because the ^{1}H NMR coupling constants for anomeric protons in these *β*-glycosides are of intermediate values ($J_{1,2}$ = 6.4−6.9 Hz). To confirm these disaccharide products were *β*-linked thioglycoside donor **1** was coupled to acceptor **3e** using PST activation to provide α-linked disaccharide **5** ($J_{1,2}$ = 2.7 Hz).[4] Acetylation of oxazolidinone **5** provided α-linked *N*-acetyl derivative **4e,** which was identical in all respects to the minor isomer (α) obtained during glycosidation of **3e**.[10]



5



4e

The observation that rapid glycosidation of donor **2** affords *β*-linked glycosides was contrary to our expectation and opposite to results previously obtained for glycosidation of **1**. In contrast, acceptor alcohols that react more slowly with **2** provided increased proportions of α-linked glycosides (Table 1). Assuming a single reactive intermediate for donor **2**, the disparity in reaction rates and stereochemical preferences cannot be explained by simply invoking steric factors and the relative reactivity of acceptor alcohols. Crich and co-workers previously reported glycosyl triflate intermediates during BSP/Tf_2O activation of thioglycosides.[11] Glycosyl triflate was shown to be predominantly α-linked regardless of donor configuration. A dynamic system where α-triflate is in equilibrium with its less stable but more reactive *β*-anomer was proposed.[11] Here, this putative triflate equilibrium and relative reactivity of α- and *β*-triflates in combination with steric hindrance of the *N*-acetyl substituent offer a likely explanation for the relationship between reaction rates and configuration of glycoside products.

To test this explanation, ^{1}H and ^{19}F NMR studies were performed to determine the presence of triflate intermediates upon activation of **2** (Figure 1). As shown, ^{1}H NMR demonstrates that α-triflate begins to form immediately upon activation of **2**. A consistently proportionate signal that we attribute to *β*-triflate also appears to be present, but to a much smaller extent. These results are consistent with previous observations.[9,11] Molecular models of the α-triflate and *β*-triflate clearly show that the *N*-acyl moiety affords steric hindrance to *β*-face attack on the α-triflate by hindered nucleophiles (data not shown). These results suggest that the more reactive and sterically less hindered alcohols (**3a**−**e**) rapidly attack the equilibrium-predominant α-triflate to preferentially yield *β*-linked glycosides. Sterically crowded acceptor alcohols that are also inherently less reactive, such as C-4-OH hexopyranoside acceptors **3h**−**j**, do not readily react with the α-triflate. These hindered alcohols preferentially react with the more reactive *β*-triflate from the less hindered α-face of **2**. Glycosylation of these less reactive acceptors by the equilibrium-limited *β*-triflate correlates to prolonged reaction times.[12]

In summary, this study demonstrates that a ring-fused 2,3-oxazolidinone derivative of 1-tolylthio-*N*-acetyl-D-glucosamine undergoes efficient glycosidation under mild donor activation conditions, conditions that do not promote glycosidation of the corresponding donor possessing an *N*-unsubstituted ring-fused 2,3-oxazolidinone moiety. Stereochemical control of glycosidation is dependent on the relative reactivity and steric hindrance of acceptor alcohols, as is often the case. However, evidence presented here indicates the correlation of reaction rate to stereochemical outcome is mediated by an equilibrium between α and *β* anomeric triflates in combination with

(8) 1-Tolylthio donor **1** was prepared in a manner identical to that previously reported for synthesis of the corresponding 1-phenylthio derivative (see ref 4).

(9) Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123,* 9015−9020.

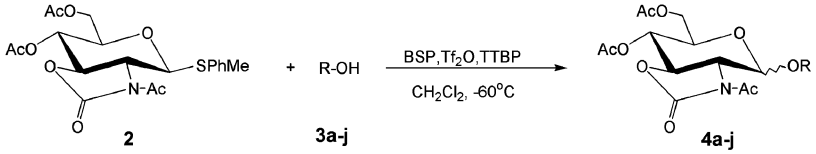
(10) Complete hydrolysis and peracetylation of **4a** was also undertaken to demonstrate formation of *β*-linked glycoside (**6**). NMR revealed a typical $^4C_1$ chair conformation with $J_{1,2}$ = 8 Hz, which is consistent in all respects to reports for known **6**. (See: Pertel, S. S.; Chirva, V. Y.; Kadun, A. L.; Kakayan, E. S. *Carbohydr. Res*. **2000**, *329*, 895−899.)

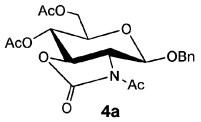
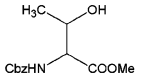
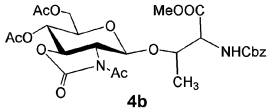
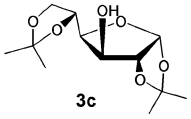
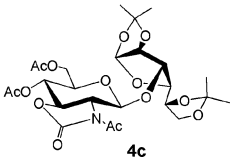
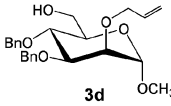
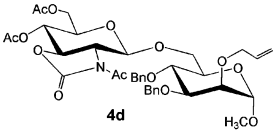
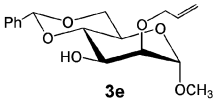
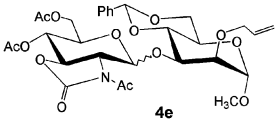
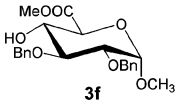
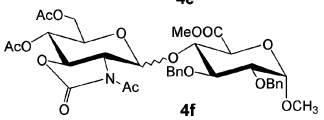
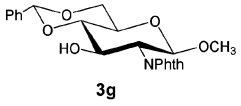
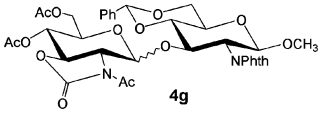
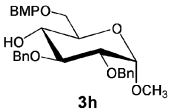
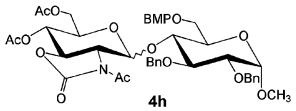
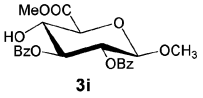
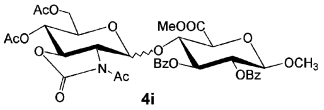
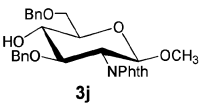
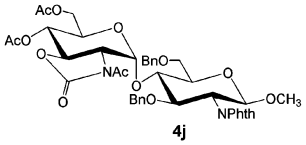
(11) (a) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119,* 11217. (b) Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64,* 4926−4930.

(12) An alternative explanation for the slow formation of α-linked glycosides would be the gradual trapping of oxacarbenium cation intermediate upon dissociation of glycosyl triflate, thus affording a prototypical glycosaminyl donor bearing a nonparticipating C-2 substituent to provide α-linked glycoside.

(13) All acceptors in this study were purchased from commercial sources except for monosaccharides **3f**, **3h** and **3i**, which are known and were prepared based on previous procedures. (a) Wacek, A.; Leitinger, F.; Hochbahn, P. *Monatsh. Chem.* **1959**, *90*, 562−567. (b) Coutant, C.; Jacquinet, J.-C. *J. Chem. Soc., Perkin Trans. 1* **1995**, *12*, 1953−1981.

TABLE 1. Glycosidation of Thioglycoside Donor 2 under BSP/Tf_2O Activation Conditions



Entry	Acceptor [13] (R-OH)	Product	Anomeric Ratio (α:β)	Isolated Yield	Reaction Time
1	 3a	 4a	β only	90%	1h
2	 3b	 4b	β only	83%	1h
3	 3c	 4c	β only	95%	1h
4	 3d	 4d	β only	90%	4h
5	 3e	 4e	1:20	82%	12h
6	 3f	 4f	1:7	75%	12h
7	 3g	 4g	1:4.5	75%	24h
8	 3h	 4h	1.6:1	65% (92%)[a]	48h
9	 3i	 4i	2.8:1	77%	48h
10	 3j	 4j	α only	81%	48h

[a] Yield based on recovery of unreacted acceptor.

steric affects of the *N*-acetyl substituent on the oxazolidinone ring.

While it is tempting to conclude that substitution of the oxazolidinone ring with sterically and electronically disparate functional groups might be utilized to more stringently control stereochemical outcome of these glycosidation reactions, it is yet unclear how such substitutions will ultimately alter trapping of the presumed oxacarbenium intermediate by triflate anion. Indeed, it is now not clear why activation of *N*-unsubstituted 2,3-

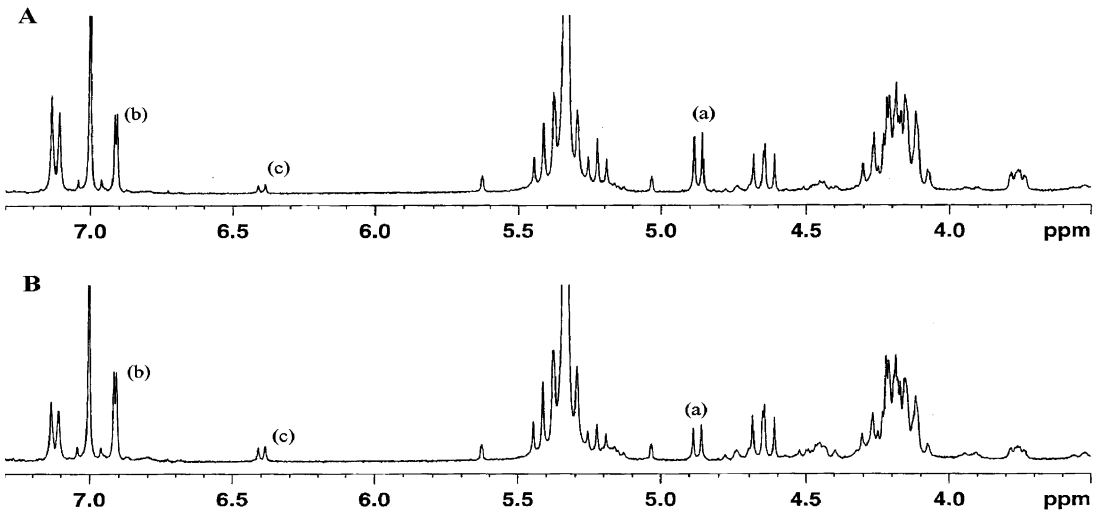


FIGURE 1. ^{1}H NMR spectra showing formation of glycosyl triflate at −60 °C. (A) A decrease in the anomeric signal of **2** is observed upon addition of 0.6 equiv of Tf_2O to a solution of donor **2**, BSP, and TTBP (a), with concomitant appearance of signals correlating to the anomeric proton of α-triflate (b) and β-triflate (c) intermediates. (B) Further addition of Tf_2O affords a continued increase in signals for the anomeric triflates with a concomitant decrease in the anomeric signal for thioglycoside **2**.

oxazolidinone-protected glucosaminyl donors with PST (e.g., **1**) rapidly affords *N*-sulfenylated α-linked glycosides with sterically hindered alcohols. Future studies are anticipated to reveal how donor reactivity and stereoselectivity of glycosylation might be more stringently controlled by altering the steric and electronic nature of the *N*-substituent on the oxazolidinone ring of this exciting new type of donor. Future studies evaluating different substituents on the oxazolidinone ring are also expected to reveal orthogonal protection/deprotection strategies for 2*N*,3*O*-oxazolidinonyl-protected sugars, which will facilitate synthesis of glycoconjugates containing variably substituted D-glucosamine residues.

Experimental Section

General Procedure for BSP/Tf_2O Glycosylation Reactions. Tf_2O (1.3 equiv) was added to a stirred solution of **2** (1.2 equiv), BSP (1.3 equiv), TTBP (2.4 equiv), and activated, powdered 3 Å molecular sieves in CH_2Cl_2 (5 mL) at −60 °C under nitrogen atmosphere. The reaction mixture was stirred for 5 min, after which time a solution of the acceptor alcohol **3a**−**j** (1 equiv) in CH_2Cl_2 (3 mL) was added. The mixture was stirred at −60 °C for 1−48 h and then quenched by the addition of saturated aqueous $NaHCO_3$. The organic layer was washed with brine, dried, and concentrated. Glycosides were purified by silica gel chromatography.

Separation and characterization data for representative glycosidations reported in Table 1 are presented below. Data for the remaining reactions in Table 1 are provided in the Supporting Information.

Coupling of 2 with 3a To Give 4,6-di-*O*-acetyl-1-*O*-benzyl-2-deoxy-2-*N*-acetyl-β-D-glucopyranosid[2,3-*d*]-1,3-oxazolidin-2-one (4a). The reaction was quenched after 1 h and separated by flash chromatography (ethyl acetate/hexanes, 1:1.5): yield = 90%; R_f = 0.58 (ethyl acetate/hexanes = 1:1); ^{1}H NMR ($CDCl_3$) δ7.41−7.28 (m, 5H, Ar−H) 5.20 (d, 1H, H-1, J = 6.6 Hz), 5.14 (dd, 1H, H-4), 4.88 (d, 1H, −CH_2Ph), 4.68 (d, 1H, −CH_2Ph), 4.55 (dd, 1H, H-3), 4.28 (m, 2H, H-5, H-6_a), 4.15−3.95 (m, 2H, H-6_b, H-2), 2.52 (s, 3H, −$COCH_3$), 2.15 (s, 3H, −$COCH_3$), 2.10 (s, 3H, −$COCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.4, 170.3, 169.6, 153.1, 136.5, 128.4, 128.1, 128.0, 99.6, 77.6, 74.9, 71.0, 70.2, 64.1, 60.5, 24.5, 20.7; HRESI MS calcd for $C_{20}H_{23}NO_9$ $[M + Na]^+$ 444.1271, found 444.1261.

Coupling of 2 with 3j To Give Methyl (4,6-Di-*O*-acetyl-2-deoxy-2-*N*-acetyl-α-D-glucopyranosid[2,3-*d*]-1,3-oxazolidin-2-one)-(1−4)-3,6-di-*O*-benzyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranoside (4j). The reaction was quenched after 48 h and separated by flash chromatography (ethyl acetate/hexanes, 1:1.5): yield = 81%; R_f = 0.41 (ethyl acetate/hexanes = 1:1); ^{1}H NMR ($CDCl_3$) δ 7.72−7.66 (m, 4H, Ar-H), 7.43−7.34 (m, 5H, Ar-H), 7.08−6.90 (m, 5H, Ar-H), 6.27 (d, 1H, H-1, 2.7 Hz), 5.28 (dd, 1H, H-4), 5.02 (d, 1H, H-1′), 4.83 (d, 1H, −PhCH_2), 4.74 (d, 1H, −PhCH_2), 4.68 (d, 1H, −PhCH_2), 4.64 (dd, 1H, H-3) 4.44 (dd, 1H, H-3′), 4.32 (d, 1H, −PhCH_2), 4.26 (dd, 1H, H-2′), 4.18 (t, 1H, H-4′), 4.14 (m, 1H, H-6_a), 4.06 (m, 1H, H-5), 4.02 (t, 1H, H-6_b), 3.98 (t, 1H, H-6_a′), 3.86(m, 1H, H-2), 3.82 (m, 1H, H-6_b′), 3.64 (dd, 1H, H-5′), 3.40 (s, 3H, −OCH_3), 2.36 (s, 3H, −$COCH_3$), 2.15 (s, 3H, −$COCH_3$), 2.10 (s, 3H, −$COCH_3$); ^{13}C NMR ($CDCl_3$) δ 171.3, 170.5, 169.2, 152.8, 137.9, 137.8, 133.9, 131.6, 128.4, 128.1, 127.8, 127.6, 127.3, 127.1, 123.4, 99.1, 95.2, 79.9, 75.6, 74.9, 74.6, 73.8, 73.6, 70.5, 68.4, 68.1, 60.3, 56.6, 55.6, 29.7, 23.5, 20.7; HRESI MS calcd for $C_{42}H_{44}N_2O_{15}$ $[M + Na]^+$ 839.2639, found 839.2626.

Representative Procedure for NMR-Scale Activation of Thioglycoside 2 with BSP, TTBP, and Tf_2O at −60 °C. To a solution of thioglycoside **2** (4.4 mg, 0.01 mmol), BSP (2.1 mg, 0.01 mmol), and TTBP (5.0 mg, 0.02 mmol) in CD_2Cl_2 (0.8 mL) in a 5 mm NMR tube at −60 °C, under an argon atmosphere, was added 1.1 equiv of Tf_2O (0.011 mmol, 1.9 μL). The NMR tube was immediately transferred to the precooled NMR probe (−60 °C), and the ^{1}H and ^{19}F spectra were recorded. The α-glucosyl triflate [major component,^{1}H NMR δ 6.91 (H-1, d, $J_{1,2}$ = 2.4 Hz,); ^{19}F NMR δ 0.69] and β-glucosyl triflate [minor component,^{1}H NMR δ 6.41 (H-1, d, $J_{1,2}$ = 7.2 Hz,); ^{19}F NMR δ 0.69] were formed immediately. Other signals at δ −3.08 ($TTBPH^+OTf^-$) and δ 4.26 (Tf_2O) were observed in the ^{19}F NMR spectrum.

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Supporting Information Available: Experimental details and glycoside characterization for remaining glycosidations reported in Table 1. ^{1}H and ^{13}C NMR spectra for all new compounds. Additional spectra for low-temperature ^{1}H and ^{19}F NMR studies on glycosyl triflate intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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