Highly Selective *β*-Mannosylations and *β*-Rhamnosylations Catalyzed by a Bis-thiourea

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Abstract

We report highly *β*-selective bis-thiourea-catalyzed 1,2-*cis*-*O*-pyranosylations employing easily accessible acetonide-protected donors. A wide variety of alcohol nucleophiles, including complex natural products, glycosides, and amino acids were *β*-mannosylated and rhamnosylated successfully using an operationally simple protocol under mild and neutral conditions. Less nucleophilic acceptors such as phenols were also glycosylated efficiently in excellent yields and with high *β*-selectivities.

TOC graphic

ROH = Complex alcohol and phenol derivatives
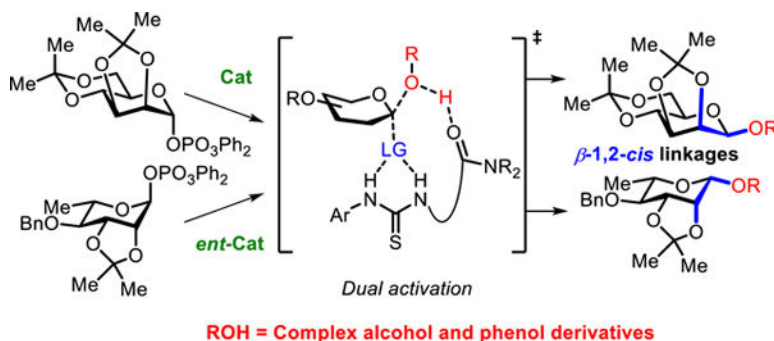
INTRODUCTION

Pyranosides bearing *β*–1,2-*cis*-*O*-glycosidic linkages are found in many biologically important oligosaccharides that play crucial roles ranging from structural support to cellular recognition.[1] For example, *β*-mannosides are key constituents of plant primary cell wall[2] and are found at the branch-points of asparagine-linked glycopeptides, the structure of which

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is known to define unique antibody-effector function for a given isotype.[3] β-Rhamnosides, although largely xenobiotic to humans, are the antigenic components of many pathogenic bacterial polysaccharides,[4] and improved understanding of their immunological effects may be of value in the development of therapeutics.[4a] The construction of well-defined β–1,2-*cis*-linkages is therefore an important objective in synthetic chemistry, but one that presents fundamental challenges. β–1,2-*cis*-*O*-Glycosides are intrinsically less stable than their α-diastereomers as a result of diminished anomeric stabilization[5] and increased steric congestion. These effects are also manifested in glycosylation pathways, resulting in a strong kinetic preference for α-products in typical mannosylation and rhamnosylation reactions (Figure 1A).

Significant efforts have been devoted to overcoming the inherent α-selectivity and developing β–1,2-*cis* glycosylation methods.[6] Successful approaches include intramolecular aglycone delivery,[7] hydrogen-bond-mediated aglycone delivery,[8] the use of 1,2-anhydropyranose donors with borinic and boronic acid catalysts,[9] protecting-group control such as with 4,6-benzylidene acetals[10] and 2,6-lactones,[11] and anomeric *O*-alkylation[12] (Figure 1B). Each of these strategies employs donors with specific protecting group schemes requiring multi-step syntheses. Although the strongly Lewis acidic or oxidizing reaction conditions required for these couplings are generally compatible with carbohydrate substrates and can therefore be applied to oligosaccharide synthesis,[13] they display poor tolerance toward a wide variety of common functional groups and therefore have limited utility for the glycosylation of complex acceptors.[14,15] Finally, no general methods for 1,2-*cis* glycosylation of less reactive nucleophiles such as neutral phenols have been identified to date. In particular, electron-rich *O*-aryl glycosides are difficult to access as they are prone to Fries-type rearrangement pathways to afford *C*-glycosides under Lewis acidic conditions.[16]

The use of hydrogen-bond-donor and chiral phosphoric acid organocatalysts in glycosylation reactions has been reported by several groups.[14,17] With the goal of developing a mild, functional-group-tolerant, and broadly applicable method for β–1,2-*cis* glycosylations employing easily accessible donors, we hypothesized that our recently reported stereospecific approach catalyzed by precisely designed bis-thiourea derivatives could be applied in this most challenging context (Figure 1C).[18] While construction of β–1,2-*trans* linkages[18a,b] and β–1,2-*cis*-*O*-furanosyl[18c] linkages has been demonstrated using the appropriate α-glycosylphosphate donors with linked bis-thiourea catalysts such as **1**, very limited success has been achieved as yet in the application of this strategy to β–1,2-*cis*-*O*-pyranosyl linkages. Herein, we describe the discovery of remarkable protecting group effects in catalytic glycosylations of mannosyl and rhamnosyl phosphate derivatives, leading to the development of highly selective, operationally simple, and general protocols for β–1,2-*cis* glycosylations of amino acids, carbohydrates, complex natural products, and pharmaceuticals.

RESULTS AND DISCUSSION

Bis-thiourea **1**, the enantiomer of which had been identified previously as an effective catalyst for stereospecific β–1,2-*trans*-pyranosylation reactions,[18b] was evaluated in the mannosylation of the model acceptor **3a** (Figure 2A).[19] Variation of the protecting groups on

the mannosyl diphenylphospate donor resulted in pronounced effects on anomeric selectivity.[20] Simple alkyl protecting schemes such as perbenzyl (**2a**) or permethyl (**2b**) resulted in largely unselective coupling reactions (1:1 to 1:2 α:β). Incorporation of the 4,6-benzylidene acetal protecting group (as in **2c**) pioneered by Crich for *β*-selective mannosylations under strong Lewis acid conditions again led to an unselective reaction in the presence of catalyst **1**. However, a dramatic increase in *β*-selectivity was observed under catalytic conditions when the C2 and C3 substituents were bridged by an acetonide protecting group. Thus, coupling reactions with donors **2d**-**f** all afforded high *β*-selectivity (1:16–32 α:β) with the relatively unhindered acceptor **3a**.[21] Mannosylation of the more sterically congested secondary nucleophile **3b** was found to be substantially more *β*-selective with **2f** than with **2d** (1:24 vs 1:10 *α:β*, Figure 2B). The bis-acetonide-protected mannose derivative **2f** also offers important practical advantages, being prepared in a simple two-step sequence from D-mannose, and affording glycosylation products that may be selectively or fully deprotected under mild conditions (*vide infra*).[22] As observed previously in related bis-thiourea-catalyzed glycosylations of glycosyl phosphates, the inclusion of 4 Å molecular sieves is crucial for minimizing undesired hydrolysis of donors and sequestering the phosphoric acid by-product, which has been shown to promote glycosyations unselectively.[18b,c] Effects of varying the stoichiometry of donor **2f** and acceptor **3c** were also investigated (Figure 2C). While slightly higher conversions were obtained when the stoichiometry of either **2f** or **3c** was increased, those improvements were offset by observable decreases in *β*-selectivity. Accordingly, subsequent scope studies (Figures 4 and 5) were carried out with equimolar ratios of donor and acceptor.

We sought to elucidate the origin of the remarkably beneficial effect of the 2,3-acetonide protecting group on *β*-selective mannosylations with catalyst **1** through density functional theory computations (Figure 3).[23] B3LYP/6-311+G(d,p)//B3LYP/6-31G(d) were selected because they have been previously applied to compute the transition states of glycosylation reactions and geometries and energies of oxocarbenium ion intermediates.[24] Oxocarbenium ion intermediates were computed in isolation due to the observation of undesired ion-pair collapse into glycosyl phosphates when computations were performed in the presence of diphenyl phosphate anions. Crich has proposed that 4,6-benzylidene acetal protection of mannose serves to destabilize the corresponding oxocarbenium ion species relative to acyclic protection schemes (e.g. **5b** vs **5a**), thereby favoring stereospecific glycosylation pathways via transient *α*-mannosyl triflate intermediates.[10d–e,25] Computational modeling of the oxocarbenium ions **5a** and **5b** fully supports that hypothesis, with the 4,6-benzylidene-protected derivative **5b** calculated to be destabilized by 4.4 and 3.2 kcal/mol ($^4H_3$ and $B_{2,5}$ conformation respectively relative to the unbridged analog **5a**. The oxocarbenium ion **5c** derived from 4.6-acetonide-protected donor is conformationally similar to **5b**, and displays a similar destabilizing effect. However, a similar analysis of 2,3-acetonide-protected oxocarbenium ion **5d** revealed that it was instead stabilized by 1.3 kcal/mol relative to the unbridged analog **5a**. Thus, the dramatic increases in *β*-selectivity resulting from 2,3-acetonide protection cannot be ascribed to destabilization of an *α*-selective S_N1-type pathway.

Recently, high β-selectivity has been demonstrated in mannosylations utilizing 4,6-benzylidene acetal-protected donors in systems in which the formation of transient α-mannosyl triflates is not possible.[26] In those cases, the stereoselectivity has been ascribed to preferential axial attack of nucleophiles on the energetically accessible $B_{2,5}$ conformation of the oxocarbenium ion intermediates,[26b,27] a similar conformation to that calculated for **5d** and **5e**. However, if this mode of stereoselectivity were operative in reactions catalyzed by bis-thiourea **1**, comparable levels of β-selectivity might be expected with **2c** and **2f** because of the similarity in the conformations of **5b_$B_{2,5}$** and **5e**. In fact, dramatically higher β-selectivity is observed with donor **2f** (α:β =1:32 vs 1:1 for **2c**, Figure 2A). Furthermore, the divergent selectivities observed with **2d–f** in **1**-catalyzed and trimethylsilyl triflate (TMSOTf)-promoted reactions (1:16–32 vs 3–8:1 α:β, Figure 2A) appear to be inconsistent with stereoselective S_N1 addition to $B_{2,5}$ oxocarbenium ion intermediates in both pathways. We propose instead that the enhancement in β-selectivity is achieved by acceleration of a stereospecific S_N2-type pathway promoted by the bis-thiourea catalyst **1**, consistent with the observation that increased reactivity is observed with 2,3-acetonide protection **2d** relative to unbridged analogs **2a–b** (39% vs 11%, 15% conversion respectively). However, the basis for the acceleration effect resulting from the interplay between protecting group and catalyst remains to be determined, and the possibility that reactions proceed through a catalyst-promoted β-selective S_N1 pathway cannot be strictly ruled out. Further examination of the mechanism of substitutions catalyzed by **1** and related bis-thioureas is the focus of continuing investigation.[28]

The scope of catalytic, β-selective mannosylation reactions with the bis-acetonide donor **2f** was explored, with particular attention to cases where the mild and neutral conditions could provide a unique advantage over traditional Lewis acid-promoted glycosylation protocols (Figures 4 and S1). Sugar derivatives were found to be excellent substrates for the methodology, with both primary (6-OH-galactose **3c** and 6-OH-glucose **3d**) and secondary glycosyl acceptors (2-OH-galactose **3e** and 4-OH-rhamnose **3f**) undergoing mannosylation with high β-selectivity.[29] The β-mannoside of C4-linked *N*-acetylglucosamine derivative **3g** is of particular interest as it is a conserved branch-point on all *N*-glycans.[3a] A wide variety of other alcohols containing Lewis basic functional groups including esters, carbamates, and tertiary amines (**3a**, **3h–i**, and **S3f**) were also found to undergo β-mannosylations selectively and cleanly, with no decomposition of nucleophiles observed.

Substrates bearing multiple hydroxyl groups such as deoxycholic acid octyl ester (**S3g**), pleuromutilin (**3j**) and FK-506 (**3k**) were found to undergo glycosylation at the least hindered position with high site-selectivity.[30,31] Highly sterically congested secondary alcohols and tertiary alcohols underwent mannosyation with reduced level of selectivity and reactivity (Figure S1).

Phenolic acceptors were found to display excellent reactivity toward **2f** under catalysis by bis-thiourea **1** to afford β-mannosylation products (Figures 4 and S1). A wide assortment of phenol derivatives, including sterically hindered substrates such as clofoctol (**3l**) and α-tocopherol (**3m**) were mannosylated with high β-selectivity. Excellent functional group tolerance was again demonstrated, such as in the glycosylation of the β-lactam-containing

amoxicillin derivative **3n**. Fries-type rearrangement of *O*-aryl glycosides to *C*-glycosides, which is common with electron-rich phenol derivatives (e.g. **3o** and **3q**) under Lewis acidic conditions,[16] was not observed in any reactions promoted by **1**.

Mannosylations with donor **2f** using catalyst **1** were compared to reactions promoted by the Lewis acid TMSOTf.[32] In all the examples illustrated in Figure 4, the reactions catalyzed by **1** afforded the mannosylation products cleanly and with high *β*-selectivity. In contrast, TMSOTf-promoted reactions yielded *α*-anomers as the major glycosylation products in almost all cases, consistent with the expected intrinsic preference for *α*-product in mannosylations proceeding via oxocarbenium ion intermediates. Functionally complex acceptors such as FK-506 (**3k**) were unstable to the Lewis acid activation conditions, while substrates possessing Lewis basic functionality such as quetiapine (**3h**) afforded none of the desired glycosylation products. Other sensitive substrates such as amoxicillin (**3n**) and serotonin (**3o**) underwent mannosylation in low yield and anomeric selectivity under TMSOTf conditions, with formation of multiple side products.

We sought to explore whether the 2,3-acetonide protection strategy applied successfully in *β* −1,2-*cis* mannosylations catalyzed by **1** might be extended to other interesting glycosyl donors. Rhamnose is the C6-deoxy analogue of mannose, and it presents another long-standing challenge as a coupling partner in *β*–1,2-*cis* glycosylation reactions. Because the C6 hydroxyl of mannose is lacking, the 4,6-benzylidene acetal protecting group strategy pioneered by Crich for *β*-mannosylations is not applicable. Alternative strategies for *β*-rhamnoside synthesis have been identified, but the reported successful examples have been limited to simple alcohols as nucleophilic coupling partners.[7e,8b,9c,23f,33] The 2,3-acetonide-protected L-rhamnose phosphate donor **6** was evaluated with **3a** as a model acceptor (Figure S14). L-Rhamnose is pseudoenantiomeric with D-mannose, and we observed that a large improvement in *β*-selectivity was achieved by using the enantiomer of catalyst **1** (1:7 *α*:*β* with catalyst **1** vs 1:32 *α*:*β* with *ent*-catalyst **1**, Figure S14). Such stereochemical matching points to precise catalyst-substrate interactions in the catalytic glycosylation event and is consistent with observations we have made previously in less challenging glycosylation reactions.[18b,c] The nucleophile scope in *β*-rhamnosylations catalyzed by *ent*-**1** was examined and proved to be similarly broad to that documented above in *β*-mannosylations (Figures 5 and S2). Thus, excellent functional group compatibility was demonstrated with substrates bearing Lewis basic groups such as tertiary amines (**3h**), imide (**3i**), and carbamates (**3o–p**), as well as with complex natural products (e.g. **3k** and **3t**).[34] Excellent *β*-selectivity and reactivity were observed in the rhamnosylation of phenols as well. Reactions promoted by TMSOTf displayed limited functional group tolerance and generally favored the *α*-rhamnosylation products in cases where glycosylation product was obtained.

The acetonide-protected glycosylation products are readily unmasked under mild conditions.[22c] Thus, both acetonides on disaccharide **4d** were hydrolyzed in a 4:1 acetic acid:water mixture at room temperature, with the *β*-mannoside product isolated in 81% yield with no erosion of anomeric purity (Figure 6A). Similarly, mannosylated FK-506 (**4k**) was also deprotected in 86% yield with no detectable anomerization, demonstrating how even highly sensitive functional groups are tolerant to the mildly acidic hydrolytic conditions. The potential practicality of the mannosylation procedure was demonstrated in a multi-

millimole-scale synthesis of methyl β-D-mannopyranoside **8c**. The shortest prior reported stereoselective synthesis of **8c** required 7 steps from D-mannose.[35] Using the approach outline herein and in Figure 6B, **8c** was prepared in 2 steps from **2f** and isolated in >95% purity without column chromatography (4 steps from D-mannose). The glycosylation step was performed on 2.5 mmol scale using 1 mol% catalyst **1** to provide the acetonide protected-mannoside quantitatively,[36] and that material was subjected to the deprotection protocol directly to afford the fully deprotected β-mannoside **8c** in 1:99 $\alpha:\beta$ selectivity.

CONCLUSION

We have demonstrated that β-mannosides and β-rhamnosides can be mildly and selectively accessed under catalyst control using readily accessible 2,3-acetonide-protected glycosyl phosphate donors. The catalytic protocol is compatible with extensive functional group complexity on the nucleophilic coupling partner, so we anticipate this method may enable exploration of the effect of specific *O*-glycosylation on a wide range chemical matter, although the application of the current methodology to highly sterically congested nucleophiles is still limited. Work is currently underway to elucidate the origins of selectivity imparted by the 2,3-acetonide protecting group in connection with the bis-thiourea catalyst, and to further illuminate the scope of potential coupling partners in these catalytic glycosylation reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

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(19). A systematic evaluation of dimeric hydrogen-bond donors led to the identification of **1** as the optimal catalyst for the β-mannosylation reactions. *Ent*-**1** and catalysts with less rigid structures or different arylpyrrolidine components were found to be less β-selective and less reactive (see Figures S3–S5 for details).**11**

(20). (a)A screen of phosphate leaving groups revealed the diphenyl derivatives as optimal. The more electron-withdrawing bis-(4-cholorophenyl) phosphates were found to be more reactive but less β-selective in the presence of **1** (Figure S12).**1**(b)Comparable conversions and selectivities were obtained in di-*n*-butyl ether (see Figures S6–S7). Diisopropyl ether was selected because of its greater ability to solubilize acceptors.

(21). Higher reactivity of **2f** was achieved by increasing reaction temperature to 50 °C, albeit with slightly lower β-selectivity (85% conversion, 1:19 α:β). α- and β-Configurations were assigned based on 3JH1-H2 (0 Hz for α-products and 2.5–3.0 Hz for β-products) and 1JC1-H1 (ca. 170 Hz for α-products and ca. 160 Hz for β-products).**2f**

(22). For selective hydrolysis of 4,6-acetonides, see(a)Combemale S; Assam-Evoung J-N; Houaidji S; Bibi R; Barragan-Montero V. Gold Nanoparticles Decorated with Mannose-6-phosphate Analogues. Molecules 2014, 19, 1120–1149. [PubMed: 24445341] (b)Neogi A; Majhi TP; Achari B; Chattopadhyay P. Palladium-Catalyzed Intramolecular C–O Bond Formation: An Approach to the Synthesis of Chiral Benzodioxocines. Eur. J. Org. Chem 2008, 2008, 330–336. For deprotection of bis-acetonide, see(c)Cocinero EJ; Stanca-Kaposta EC; Scanlan EM; Gamblin DP; Davis BG; Simons JP Conformational Choice and Selectivity in Singly and Multiply Hydrated Monosaccharides in the Gas Phase. Chem. Eur. J 2008, 14, 8947–8955. [PubMed: 18720336]

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(28). The bis-acetonide-protected oxocarbenium intermediate **5e** was found to be destabilized by 2.8 kcal/mol relative to **5d**, suggesting that the high *β*-selectivities obtained with donor **2f** even with relatively unreactive, hindered nucleophiles result from a combination of acceleration of *β*-selective and deceleration of *α*-selective pathways arising from **5e**.**5e5d2f5e**

(29). Chromatographic separation of **4b** from **3b** was challenging, so accurate isolated yield could not be determined. Thus, **3b** was not included in Figure 4.**4b3b3b**

(30). (a)Chamni S; He Q-L; Dang Y; Bhat S; Liu JO; Romo D. Diazo Reagents with Small Steric Footprints for Simultaneous Arming/SAR Studies of Alcohol-Containing Natural Products via O–H Insertion. ACS Chem. Biol 2011, 6, 1175–1181. [PubMed: 21894934] (b)McPherson M; Yang Y; Hammond PW; Kreider BL Drug Receptor Identification from Multiple Tissues Using Cellular-Derived mRNA Display Libraries. Chem. Biol 2002, 9, 691–698. [PubMed: 12079780]

(31). The anomeric configuration of **4k** was assigned based on $^1J_{C1\text{-}H1}$ (158.2 Hz) of deprotected compound **8b**. No other glycosylated FK-506 derivatives were detectable in the crude NMR spectra or isolated upon chromatographic purification.**4k**$^1J_{C1\text{-}H1}$**8b**

(32). The TMSOTf-promoted glycosylation reactions were carried out using the procedures reported by Seeberger and Hashimoto:(a)Plante OJ; Palmacci ER; Andrade RB; Seeberger PH Oligosaccharide Synthesis with Glycosyl Phosphate and Dithiophosphate Triesters as Glycosylating Agents. J. Am. Chem. Soc 2001, 123, 9545–9554. [PubMed: 11572674] (b)Tsuda T; Nakamura S; Hashimoto S. A Highly Stereoselective Construction of 1,2-*trans*-*β*-Glycosidic Linkages Capitalizing on 2-azido-2-deoxy-D-Glycosyl Diphenylphosphates as Glycosyl Donors. Tetrahedron 2004, 60 10711–10737.

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benzoate Donors. Org. Biomol. Chem 2016, 14, 1536–1539. [PubMed: 26705552] (d)Heuckendorff M; Pedersen CM; Bols M. Rhamnosylation: Diastereoselectivity of Conformationally Armed Donors. J. Org. Chem 2012, 77, 5559–5568. [PubMed: 22639871] (e)Elferink H; Pedersen CM L-Rhamnosylation: The Solvent is the Solution. Eur. J. Org. Chem 2017, 53–59. For a recent review, see:(f)Rai D; Kulkarni SS Recent Advances in β-L-Rhamnosylation. Org. Biomol. Chem 2020, 18, 3216–3228. [PubMed: 32270840]

(34). The anomeric configuration of **7k** was assigned based on $^{1}J_{C1\text{-}H1}$ (158.2 Hz). We did not isolate any other glycosylated FK-506 derivatives after chromatographic purification, and no discernible peaks corresponding to other glycosylated FK-506 derivatives were present in the crude NMR. We therefore reported the selectivity as <1:50 α:β.**7k**$^{1}_{C1\text{-}H1}$

(35). The stereoselective synthesis utilized 4,6-benzylidene acetal strategy for β-mannosylations. Crich D; Banerjee A; Yao Q. Direct Chemical Synthesis of the β-d-Mannans: The β -(1→2) and β -(1→4) Series. J. Am. Chem. Soc 2004, 126, 14930–14934. [PubMed: 15535720]

(36). The reaction proceeded to 94% conversion after 22 h.
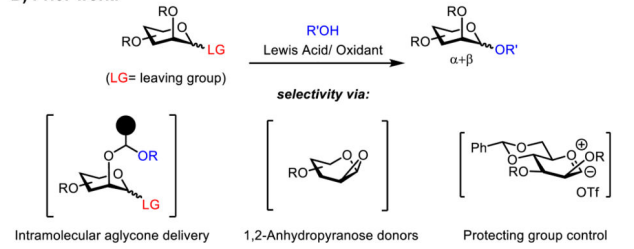
Figure 1.
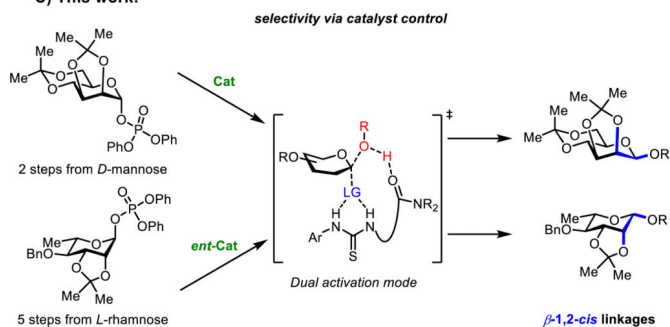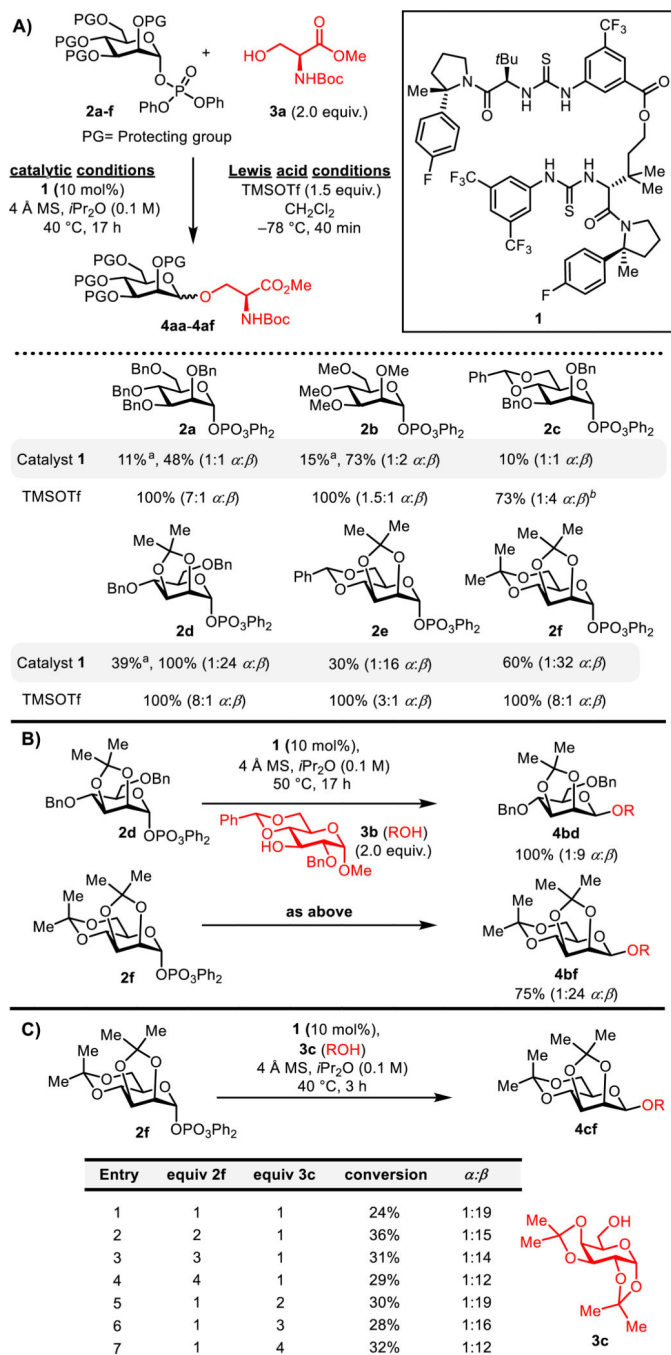(A) Challenges associated with β–1,2-*cis*-pyranosyl glycosidic bond construction. (B) Traditional approaches to achieving selectivity in glycosylation. (C) Catalyst-controlled approach to β–1,2-*cis* linkages with a bis-hydrogen-bond donor.
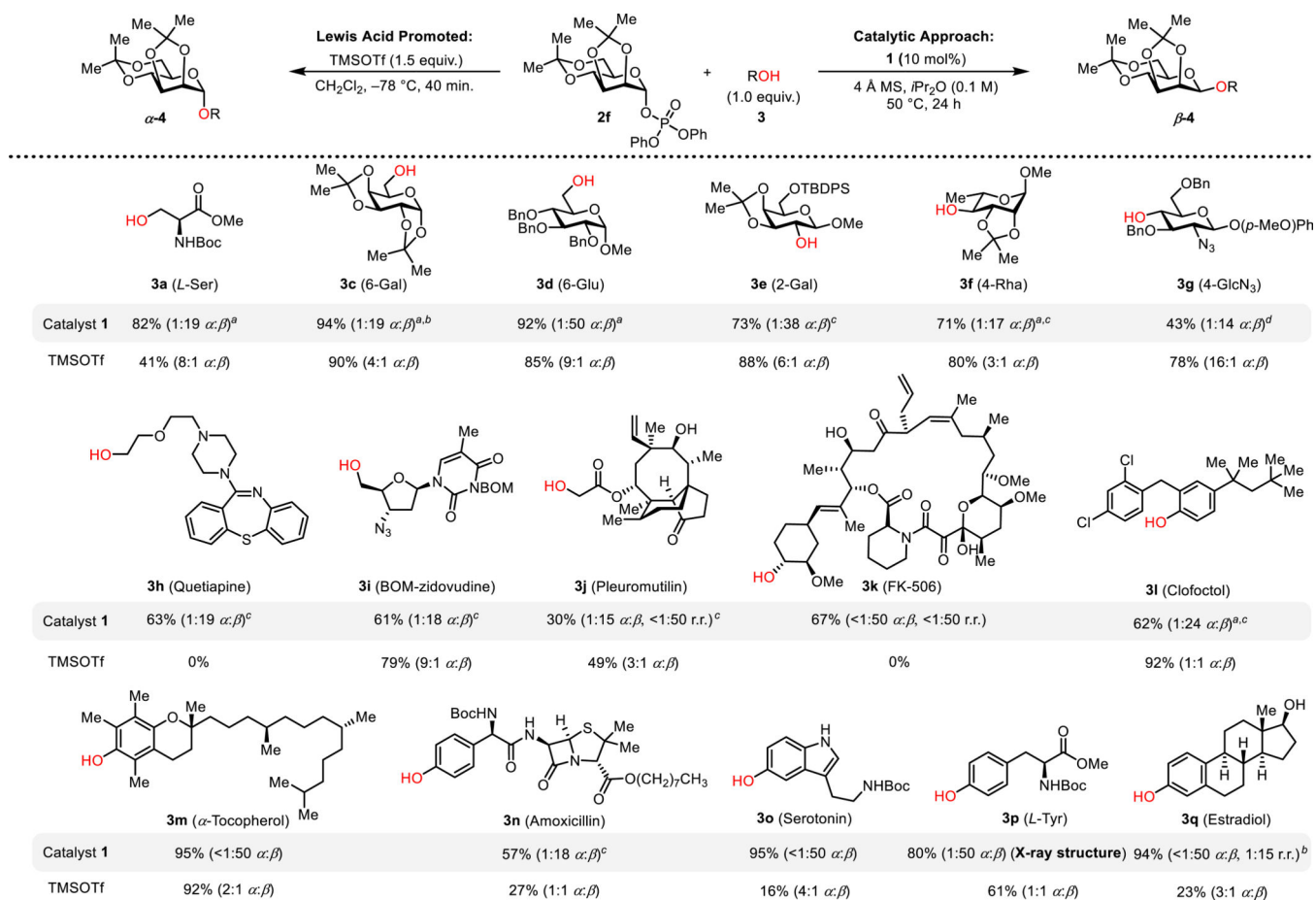
Figure 2.
(A) Protecting-group effects on bis-thiourea-catalyzed and TMSOTf-promoted mannosylations of primary alcohol **3a**. (B) Protecting-group effect on mannosylation reactions with secondary alcohol nucleophile **3b** catalyzed by **1**. (C) Stoichiometry optimization. Conversions were determined by ^{1}H NMR analysis of crude product mixtures with mesitylene as an internal standard. Selectivities were determined by ^{1}H NMR analysis of crude product mixtures. [a]Conversions after 3 h. Selectivies remained constant over time. [b]Reaction run at –40 °C.
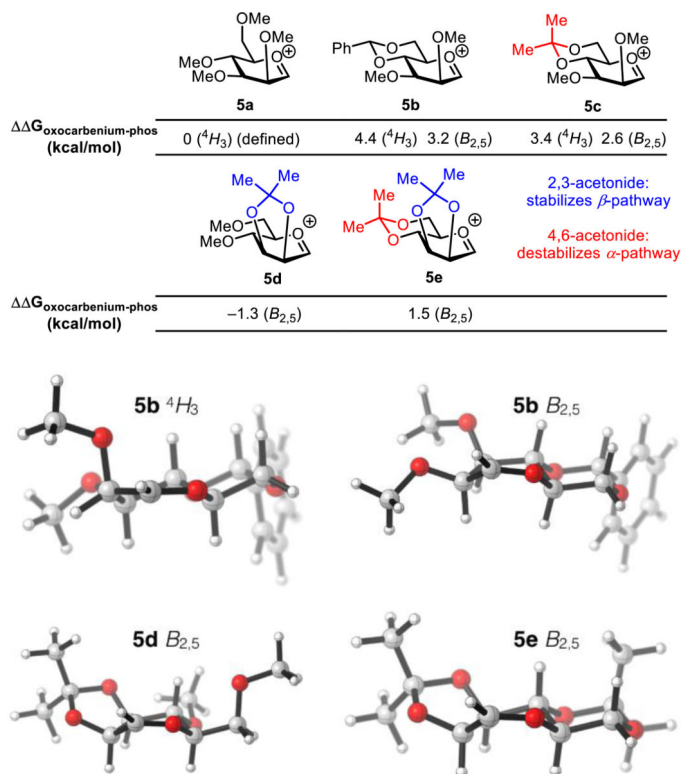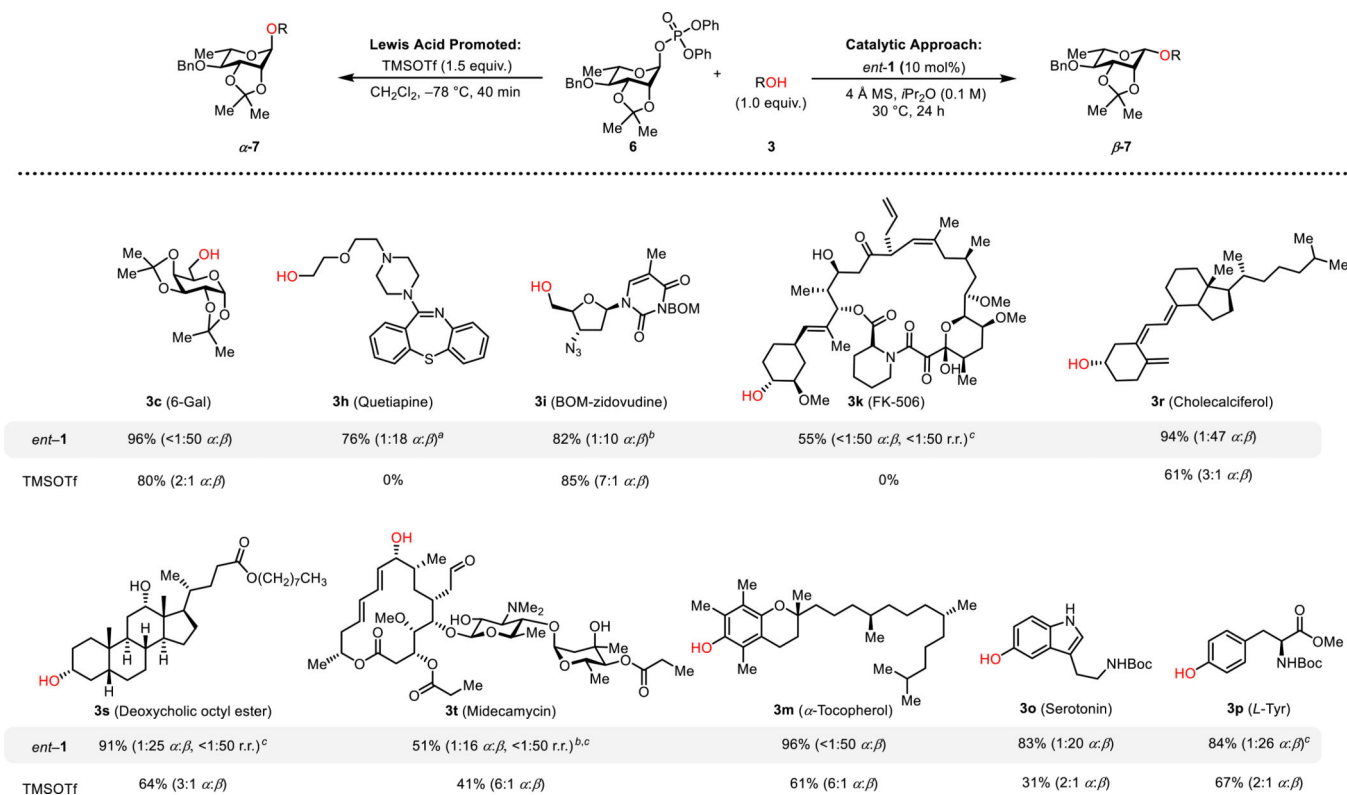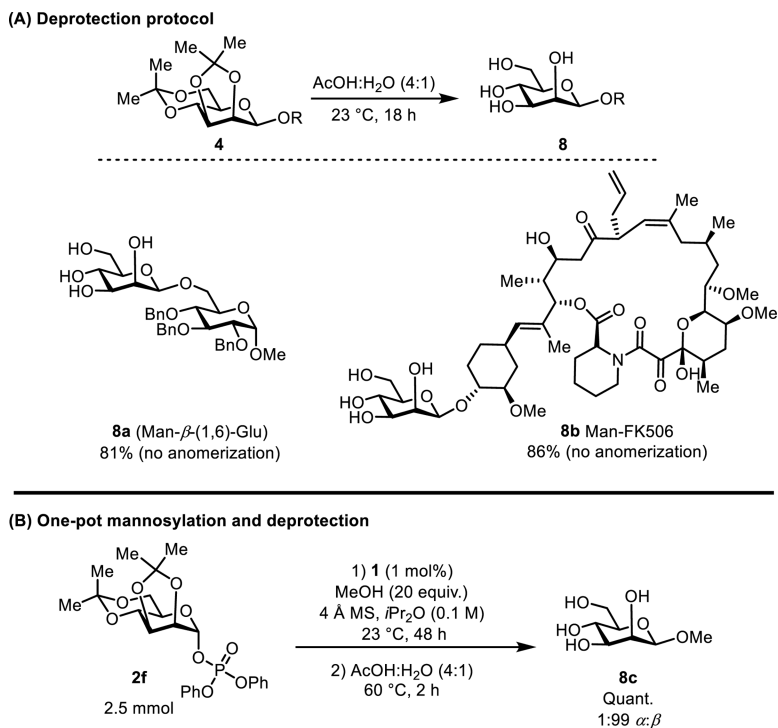
Figure 3.
Computed energies of oxocarbenium ion intermediates relative to the corresponding α-mannosyl diphenyl phosphates. Calculations were performed at B3LYP/6–311+G(d,p)//B3LYP/6–31G(d) with D3BJ dispersion corrections and PCM (H_2O). Free energy corrections were determined at the B3LYP/6–31G(d) level of theory at 298.15 K using Grimme's quasi-harmonic free energy correction. Similar trends were observed in ether solvation model and other levels of theory (see SI for full details).

Figure 4.
Acceptor scope of β-mannosylations. Yields of reactions catalyzed by **1** reflect isolated yields of pure β-products. Yields of TMSOTf reactions reflect combined yield of the α- and β-products and were determined by ¹H NMR analysis of crude product mixtures with mesitylene as an internal standard. Selectivities were determined by ¹H NMR analysis of crude product mixtures. [a]2.0 equiv. nucleophilic coupling partners were used. [b]Reaction was run at 40 °C. [c]48 h reaction time. [d]63 h reaction time. Additional substrates are provided in Figure S1.

Figure 5.
Acceptor scope of *β*-rhamnosylations. Yields of reactions catalyzed by *ent*-**1** reflect isolated yields of pure *β*-products. Yields of TMSOTf reactions reflect combined yield of the *α*- and *β*-products and were determined by ¹H NMR analysis of crude product mixtures with mesitylene as an internal standard. Selectivities were determined by ¹H NMR analysis of crude product mixtures. [a]Reaction was run at 50 °C for 48 h. [b]48 h reaction time. [c]Reaction was run at 40 °C. Additional substrates are provided in Figure S2.

(A) Deprotection protocol

(B) One-pot mannosylation and deprotection

Figure 6.
(A) Protocol for bis-acetonide deprotection under mildly acidic hydrolytic conditions. (B) Multi-millimole-scale synthesis of methyl β-D-mannopyranoside. Yields reflect isolated yields of pure β-products. Selectivities were determined by ^{1}H NMR analysis of crude product mixtures.