Differential Carbohydrate Recognition of Two GlcNAc-6-sulfotransferases with Possible Roles in L-Selectin Ligand Biosynthesis

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Abstract: Two human GlcNAc-6-sulfotransferases, CHST2 and HEC-GlcNAc6ST, have been recently identified as possible contributors to the inflammatory response by virtue of their participation in L-selectin ligand biosynthesis. Selective inhibitors would facilitate their functional elucidation and might provide leads for antiinflammatory therapy. Here we investigate the critical elements of a disaccharide substrate that are required for recognition by CHST2 and HEC-GlcNAc6ST. A panel of disaccharide analogues, bearing modifications to the pyranose rings and aglycon substituents, were synthesized and screened for substrate activity with each enzyme. Both GlcNAc-6-sulfotransferases required the 2-*N*-acetamido and 4-hydroxyl groups of a terminal GlcNAc residue for conversion to product. Both enzymes tolerated modifications to the reducing terminal pyranose. Key differences in recognition of an amide group in the aglycon substituent were observed, providing the basis for future glycomimetic inhibitor design.

Introduction

Sulfation of carbohydrates in the Golgi compartment is a mechanism for modulating their activity on the cell surface. The enzymes that effect this, carbohydrate sulfotransferases, have only recently been identified as modulators of biological recognition events and therefore possible therapeutic targets.[1] The importance of sulfate as a regulatory element is highlighted by the discovery of sulfated oligosaccharides as key determinants for recognition by the leukocyte homing receptor L-selectin.[2−4] This receptor−ligand interaction mediates the transient rolling of lymphocytes on high endothelial venules in peripheral lymph nodes, and a similar interaction of leukocytes with vascular endothelium at sites of chronic inflammation.[5−10] Presented on mucin-like glycoproteins (*O*-linked to Ser or Thr) on the endothelial cell surface, the oligosaccharides share a sulfated sialyl Lewis x motif (6-sulfo sialyl Lewis x, highlighted in the dashed box in Figure 1) built from a sialylated core 2 GlcNAc residue (**1**, Figure 1).[2−4] The sulfate ester on the 6-hydroxyl group of GlcNAc has been demonstrated to increase the binding affinity of the oligosaccharide with L-selectin.[11−13] Moreover, immunohistological studies using an antibody specific for this unusual epitope indicate that its expression is restricted to sites of lymphocyte recruitment, including chronically inflamed tissues.[14,15] Due to the importance of the sulfate ester

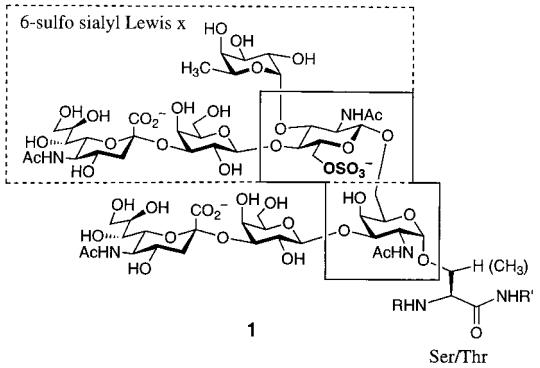

Figure 1. A sulfated oligosaccharide ligand for L-selectin characterized on mucin-like glycoproteins from peripheral lymph node high endothelial venules. Dashed box: 6-sulfo sialyl Lewis x, a critical motif for L-selectin recognition. Solid box: Disaccharide motif on which synthetic sulfotransferase substrates were based.

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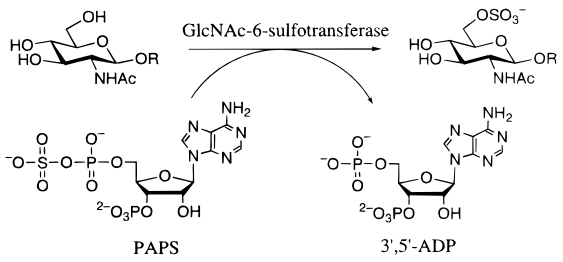

Figure 2.

for L-selectin binding and its possible correlation with inflammatory disease, the associated sulfotransferase represents a novel target for inhibition and study.

The notion of a specific GlcNAc-6-sulfotransferase responsible for sulfation of **1** in endothelial cells was supported by our previous observation, using an in vitro biochemical assay, of a GlcNAc-6-sulfotransferase activity restricted to lymphoid tissue.[16] Several groups have since reported the molecular cloning of human GlcNAc-6-sulfotransferases that are candidates for this activity. Three such enzymes have been recently described that constitute a new enzyme family and share approximately 30−50% sequence identity. The first, termed CHST2 by Uchimura et al., is broadly distributed in many tissues and when assayed in vitro prefers terminal GlcNAc oligosaccharides as substrates.[17,18] The second, termed HEC-GlcNAc6ST by Bistrup et al.[19] and LSST by Hiraoka et al.,[20] is highly restricted in expression to lymph node high endothelial venules. It requires a terminal GlcNAc residue for modification as well.[21] Both HEC-GlcNAc6ST and CHST2 have been shown to generate 6-sulfo sialyl Lewis x when transfected into COS cells,[19,20] as well as functional L-selectin ligands that support lymphocyte rolling.[20,22] A third GlcNAc-6-sulfotransferase, termed I-GlcNAc6ST by Lee et al.,[23] is restricted in expression to small intestine and colon; its specific oligosaccharide substrate preference has not yet been reported.

These three enzymes, and possibly others that have yet to be identified, have some overlap in tissue expression pattern and substrate specificity. Thus, it is not clear what distinct physiological roles the enzymes play and the extent to which each contributes to L-selectin ligand biosynthesis. Selective inhibitors would be powerful tools for deconvolution of the sulfotransferases' biological functions. Furthermore, they might serve as leads for antiinflammatory therapy. Several avenues for inhibitor design/discovery can be envisioned based on the details of the sulfotransferase reaction, depicted in Figure 2. 3′-Phosphoadenosine-5′-phosphosulfate (PAPS) is utilized as the sulfate donor, from which a sulfuryl group is transferred to the carbohydrate substrate. Kinetic studies[16] and crystallographic data from a distant relative, estrogen sulfotransferase, suggest that the sulfotransferase mechanism proceeds by direct transfer of the sulfuryl group from PAPS to the substrate.[24−26] Inhibitors based on either or both substrates should therefore compete for the active site. Indeed, we have recently identified inhibitors of a bacterial GlcNAc-6-sulfotransferase by screening a kinase-directed library of compounds that resemble the purine ring of both PAPS and ATP.[27] Since all sulfotransferases utilize PAPS as a substrate, one concern with this approach is a potential lack of selectivity for one enzyme from a family of close relatives.

Alternatively, elements of the carbohydrate substrate might be integrated into inhibitor design. Although all three GlcNAc-6-sulfotransferases modify the 6-hydroxyl group of a GlcNAc residue, the preferred structures surrounding this residue within a larger glycoprotein substrate may differ for each enzyme. The details of oligosaccharide recognition have not been investigated for these enzymes and may provide a basis for selectivity. Ultimately, compounds that are effective in cell-based assays will be required for functional deconvolution of the sulfotransferases. Carbohydrate molecules are generally unsuitable for such studies due to their polar nature and consequent poor membrane permeability. This limitation could be overcome with the design of glycomimetics, compounds that retain the critical recognition elements but eliminate unnecessary polar functionality.[28−30] Knowledge of the critical functional groups on an oligosaccharide substrate would therefore serve two purposes: first, to attain inhibitor selectivity among the highly homologous enzymes, and second to design more useful pharmacological tools.

In this report, we identify key substituents on a disaccharide substrate that are required for recognition by HEC-GlcNAc6ST and CHST2. Our approach utilized a panel of compounds distinguished by either the nature or orientation of the substituents on the pyranose rings. We identified a region of the substrate at the reducing terminus that is critical for activity with HEC-GlcNAc6ST but not for CHST2, establishing a basis for the design of specific glycomimetic inhibitors.

Results and Discussion

Enzyme Assays. The two GlcNAc-6-sulfotransferases in this study, HEC-GlcNAc6ST and CHST2, are normally Golgi-resident molecules that are predicted to have a single pass transmembrane domain, a short N-terminal cytosolic tail and a C-terminal catalytic domain in the lumen of the Golgi compartment.[17−20] To facilitate our studies, we desired secreted, soluble enzymes with an expedient means of purification and detection. Toward this end, we subcloned the catalytic domains of the two sulfotransferases, without the transmembrane domain and cytosolic tail, into a vector possessing an N-terminal secretion signal and an additional N-terminal His_6 sequence.

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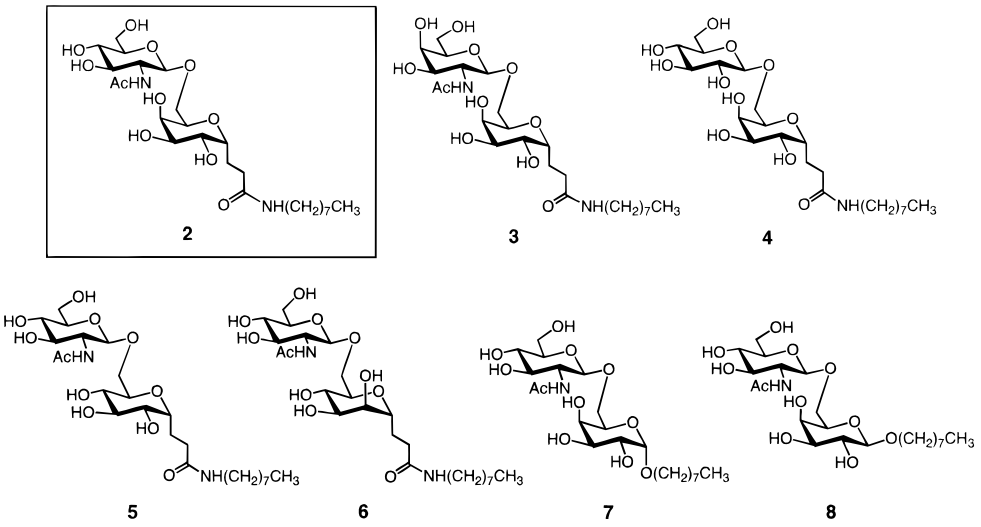


Figure 3. Panel of disaccharide sulfotransferase substrates.

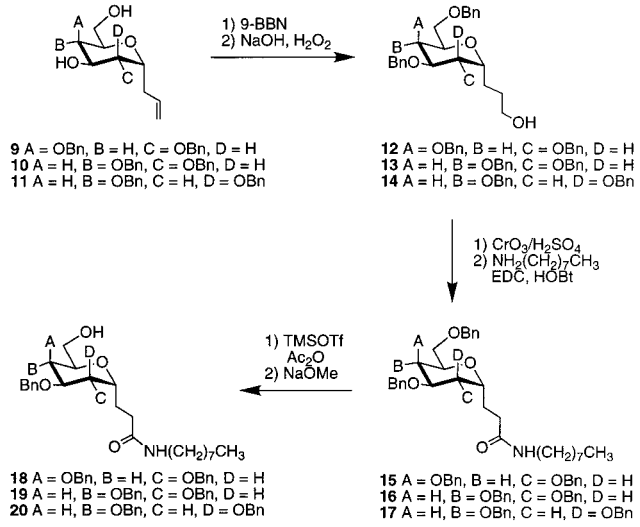
The soluble His_6-tagged constructs were expressed in baculovirus-infected insect cells and purified from the conditioned media on Ni-NTA agarose.

The activities of the enzymes with various oligosaccharide analogues were determined using an in vitro assay similar to that which we have used previously in the study of tissue extracts.[16] The assay utilized the *C*-glycosyl disaccharide GlcNAcβ1,6Galα-*C*-R (**2**, Figure 3), based on the disaccharide within structure 1 outlined in the solid box in Figure 1, as a sulfate acceptor. This substrate was incubated with the enzyme and a ^{35}S-labeled version of the sulfate donor PAPS; the amount of sulfate transfer was determined by separation of the products on a silica gel TLC plate followed by quantitation by phosphorimaging. Initial studies demonstrated that compound **2** is a substrate for the soluble His-tagged versions of both HEC-GlcNAc6ST ($K_m = 0.51 \pm 0.17$ mM) and CHST2 ($K_m = 1.17 \pm 0.21$ mM) with kinetic parameters similar to those of the GlcNAc-6-sulfotransferase activity from lymph node extracts.[16] As observed with enzyme activity from lymph nodes,[16] elaboration of the distal GlcNAc residue of **2** with an additional Gal residue severely diminished substrate activity; its terminal position was required for recognition. In this study, we used compound **2** as the basis for evaluating the effects of structural modifications on substrate activity.

Synthesis of Carbohydrate Analogues. We designed a panel of analogues (**3**−**8**, Figure 3) that would test the importance of various functional groups on the pyranose rings as well as the orientation and nature of the reducing terminal (aglycon) moiety. In all cases, a hydrophobic tail at the reducing end provided a convenient means for purification of the final products using reversed-phase HPLC. Compounds **3**−**6** retain the reducing terminal α-*C*-glycoside of the parent compound **2**. Compounds **3** and **4** bear modifications to the terminal residue, replacing GlcNAc with a GalNAc (**3**) or glucose (**4**) residue. Compounds **5** and **6** substitute the reducing terminal Gal residue of **2** with a glucose or mannose residue, respectively. Finally, compounds **7** and **8** replace the *C*-glycosyl substituent at the reducing end with α- and β-*O*-glycosides, respectively.

Disaccharides **2**−**8** were prepared via glycosidic coupling of a trichloroacetimidate donor with the corresponding selectively protected glycosyl acceptor. The glycosyl acceptors for compounds **2**−**6** were synthesized as shown in Scheme 1. Benzyl-protected *C*-allyl glycosides **9**−**11** were prepared according to Hosomi et al.[31] Hydroboration/oxidation produced alcohols **12**−**14**. Jones' oxidation followed by EDC-mediated coupling with

Scheme 1



octylamine produced **15**−**17**. The 6-*O*-benzyl ether was removed by selective C-6 acetoxylation[32] followed by deacetylation to provide glycosyl acceptors **18**−**20**.

The glycosyl donors for compounds **2**−**6** were prepared as outlined in Scheme 2. Glucosamine hydrochloride (**21**) and galactosamine hydrochloride (**22**) were protected as their peracetylated *N*-*p*-nitrobenzylcarbamate (PNZ) derivatives **23** and **24**. These compounds and peracetylated glucose (**25**) were treated with benzylamine to effect selective removal of the anomeric acetate.[33] The products, **26**−**28**, were converted to the corresponding trichloroacetimidates **29**−**31** using standard procedures.[34] These glycosyl donors were coupled with acceptors **18**−**20** in various combinations to afford disaccharides **32**−**36** (Scheme 3). Finally, a series of protecting group manipulations provided **2**−**6**.

The *O*-glycosyl acceptors **38** and **39** were synthesized as shown in Scheme 4. Treatment of galactose with boron trifluoride etherate and octyl alcohol[35] produced a 2.4:1 mixture

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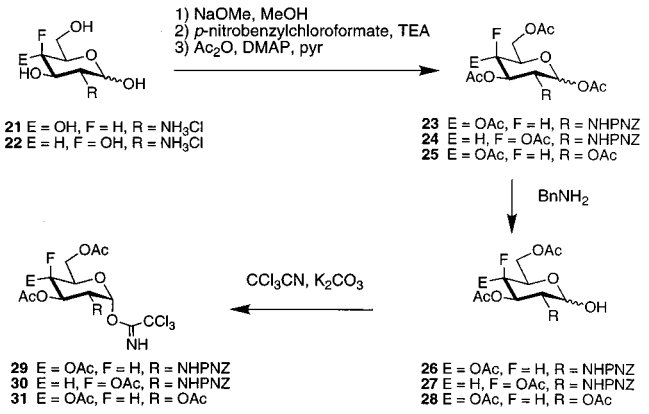
Scheme 2



of the α and *β*-octyl glycosides. The anomers were selectively protected on the 6-position with a TBDMS group and then peracetylated. The silyl-protecting group was removed under mild acidic conditions to furnish glycosyl acceptors **38** and **39** as separable compounds. Reaction of these with glycosyl donor **29** (Scheme 2) followed by protecting group removal steps finally yielded compounds **7** and **8**. All compounds were purified by reversed-phase HPLC before assaying for substrate activity.

Substrate Activities of Carbohydrate Analogues. Compounds **2**−**8** were assayed at a fixed concentration of 1 mM using recombinant His-tagged CHST2 and HEC-GlcNAc6ST. The radioactivity incorporated into the substrates was measured at 2-hour intervals for a total of 8 h. No more than 15% of any substrate was reacted under these conditions. Using these data, relative rates were determined for each substrate. These are expressed as percent activity relative to the basis substrate, compound **2**, in Figure 4 (CHST2) and Figure 5 (HEC-GlcNAc6ST).

The integrity of the GlcNAc residue is essential for both GlcNAc-6-sulfotransferases. Replacement of GlcNAc with a GalNAc residue (**3**) or a glucose residue (**4**) resulted in no significant activity with HEC-GlcNAc6ST and diminished activity with CHST2. Thus, both the orientation of the 4-hydroxyl group and the presence of the 2-*N*-acetamido group are essential for enzymatic sulfation. Since the sulfate transfer reaction takes place at a relatively distant site (*C*-6), this result may reflect decreased binding of the substrates in the enzyme active sites. In the case of glucose substrate **4**, this hypothesis was borne out by competition studies. Addition of 0.5 mM compound **4** to a CHST2 enzyme reaction that included 0.5 mM compound **2** resulted in sulfated products equal to the sum of the products produced in each reaction separately (data not shown). Thus, compound **4** is not inhibiting the sulfotransferase; it is simply not an efficient substrate.

By contrast, when GalNAc substrate **3** (0.5 mM) was added to a CHST2 reaction along with substrate **2** (0.5 mM), a reduction in sulfated products was observed (12% compared to a control reaction lacking compound **3**). Thus, compound **3** might bind unproductively in the enzyme's active site, although further experiments are needed to confirm its mode of inhibition.

Both GlcNAc-6-sulfotransferases are tolerant of conservative modifications to the reducing terminal sugar residue. Replacement of galactose with glucose (**5**) or mannose (**6**) had no significant effect on the efficiency of the reaction for both CHST2 and HEC-GlcNAc6ST. In the case of compound **5**, only a single stereocenter is altered compared to basis substrate **2**. But in case of compound **6**, two stereocenters are altered; one would expect this to effect a significant conformational change on the pyranose ring. Still, the enzymes do not appear to be sensitive to the structural perturbation. Interestingly, the reducing terminal residue is not wholly dispensable. The simple *β*-methyl glycoside of GlcNAc showed no measurable substrate activity with either enzyme under similar conditions (data not shown).

A dramatic distinction between the two enzymes was revealed by modifications to the *C*-glycosyl substituent at the reducing end of substrate **2**. Replacement of the *C*-glycoside with a more native *O*-octyl glycoside, in either the α (**7**) or *β* (**8**) configuration, had no significant effect on the efficiency of CHST2. The α-glycoside, however, appeared to be a slightly better substrate than the *β*-anomer, perhaps reflecting its closer relationship to the native structure **1** (Figure 1).

Surprisingly, the *O*-glycosides showed 10-fold reduced activity with HEC-GlcNAc6ST compared to *C*-glycoside **2**. Again, the α-anomer **7** was slightly superior to the *β*-anomer **8**. To address whether the *O*-glycosides were binding unproductively, we performed competition studies with substrate **2**. Both the α-anomer **7** and the *β*-anomer **8** reduced the observed activity of HEC-GlcNAc6ST with **2** when added at equal concentrations (0.5 mM, data not shown). Thus, the *O*-glycosides appear to disrupt enzyme activity with **2**, although the mechanism of this is still under investigation.

Discussion

A summary of the critical and dispensable functional groups for CHST2 and HEC-GlcNAc6ST is depicted in Figure 6. The results indicate that *C*-glycoside **2** possesses a critical recognition determinant for HEC-GlcNAc6ST activity that is not required by CHST2. The molecular basis of this is an interesting matter for speculation. Several reports have shown *C*- and *O*-glycosidic isosteres to behave similarly in assays of biological recognition.[36−40] Therefore, we think it unlikely that the differential turnover of the *C*-and *O*-linked substrates by the two GlcNAc-6-sulfotransferases is solely the result of the glycosidic linkage. Indeed, one might expect the more native *O*-glycoside to be the superior substrate; this is clearly not the case with HEC-GlcNAc6ST. Both the *C*- and *O*-glycoside substrates possess a C-8 tail, rendering their overall hydrophobic character similar. The only significant difference between the two classes of substrates is the presence of an amide linkage in the *C*-glycosides that is lacking in the *O*-glycosides. The amide can interact with protein components through hydrogen bonding, and a critical fortuitous interaction of this type with HEC-GlcNAc6ST might be required for productive binding that leads to sulfation. It is interesting to note that the amide NH in compound **2** can be superimposed with the amide NH of Ser (or Thr) in structure **1**. An intriguing possibility is that HEC-GlcNAc6ST interacts with that component of the peptide in its native glycoprotein substrate whereas CHST2 is ignorant of the underlying peptide. Also, the peptide-proximal GalNAc residue in **1** was replaced with galactose in **2** for reasons of synthetic accessibility. Interactions of HEC-GlcNAc6ST with the 2-*N*-acetamido group of this residue might be important for productive binding of the native substrate, and the *C*-glycosyl

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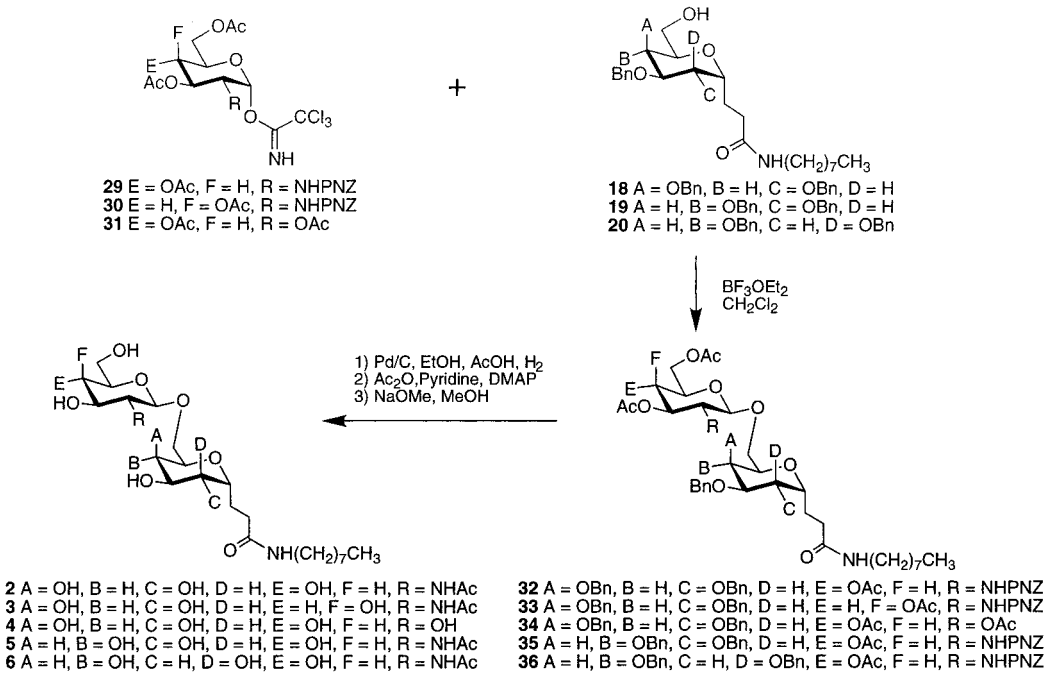
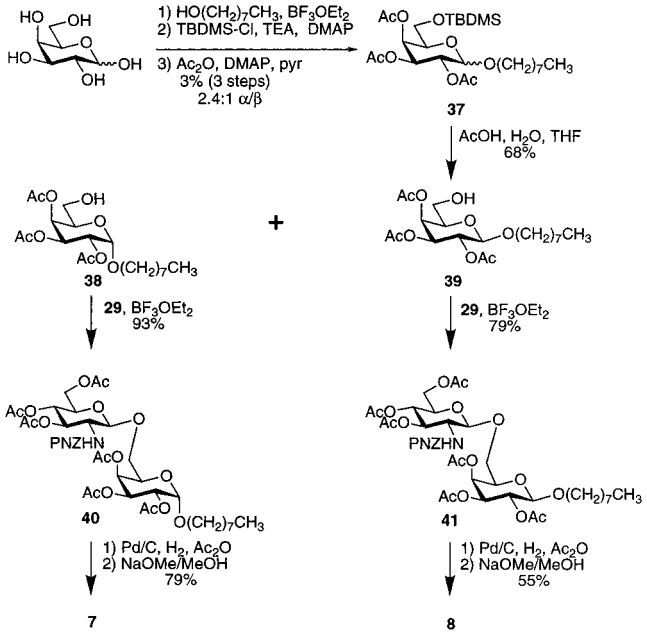
Scheme 3



Scheme 4



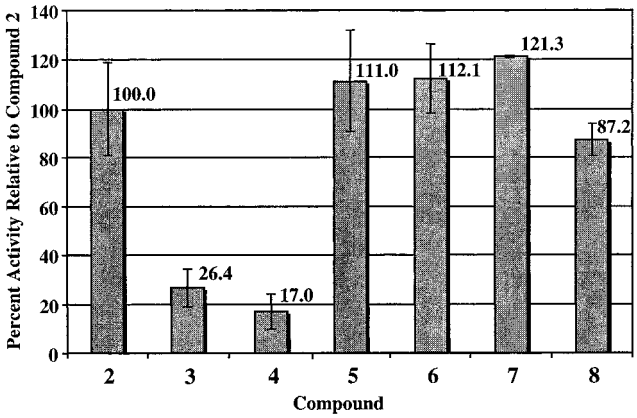


Figure 4. Relative substrate activity of compounds **2**−**8** with CHST2. Values are expressed as percent activity relative to compound **2**. Error bars represent the standard deviation of three replicate experiments.

side chain amide of **2** might fulfill this function in its absence. We are presently addressing these possibilities with an expanded panel of substrates.

The information presented in Figures 4 and 5 provides a basis for the design of glycomimetic inhibitors that can distinguish CHST2 from HEC-GlcNAc6ST. Such compounds should retain the critical recognition elements of GlcNAc, such as the 2-*N*-acetamido and 4-OH groups, while the reducing terminal sugar could be replaced with a variety of carbohydrate or non-carbohydrate moieties, perhaps in library format. Aglycons that lead to unproductive binding might also provide a starting point for inhibitor design. Furthermore, hydrophobic groups could be incorporated into or in place of the reducing terminal sugar to enhance cell permeability without compromising binding activity. Without the proper structural elements for HEC-GlcNAc6ST binding, such compounds should be selective for CHST2. Likewise, one could design a library of compounds that exploits the amide required for productive binding to HEC-GlcNAc6ST. The identification of a discriminating feature using synthetic substrates bodes well for future prospects of selective inhibitor discovery.

Experimental Section

General Methods. All chemical reagents were used as purchased from Sigma and Aldrich. Thin-layer chromatography (TLC) was performed using Analtech Uniplate silica gel plates. Flash chromatography was performed using Merck 60 Å 230−400 mesh silica gel. All reaction solvents were distilled under a nitrogen atmosphere. THF was dried and deoxygenated over benzophenone and Na^0; CH_2Cl_2, pyridine, and CH_3CN were dried over CaH_2; CH_3OH was dried over Mg^0; and toluene was dried over CaH_2.

Biological procedures used doubly distilled and deionized water from a Millipore Milli-Q system. ATP sulfurylase and pyrophosphatase were purchased from Sigma, and APS kinase was purchased from Calbiochem. The $^{35}SO_4^{2-}$ was purchased from American Radiolabeled Chemicals. PEI-cellulose TLC plates were purchased from VWR, and reversed-phase extraction plates (96-well, Oasis) were from Waters.

All ^1H and ^{13}C NMR spectra were recorded using a Bruker AMX-300, AMX-400, or DRX 500 MHz spectrometers as noted. Chemical

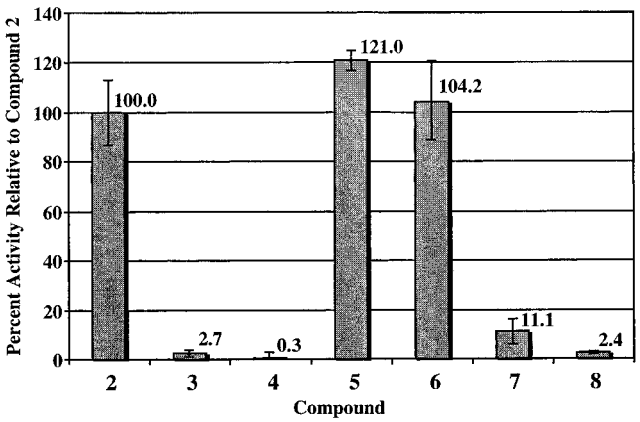


Figure 5. Relative substrate activity of compounds **2**−**8** with HEC-GlcNAc6ST. Values are expressed as percent activity relative to compound **2**. Error bars represent the standard deviation of three replicate experiments.

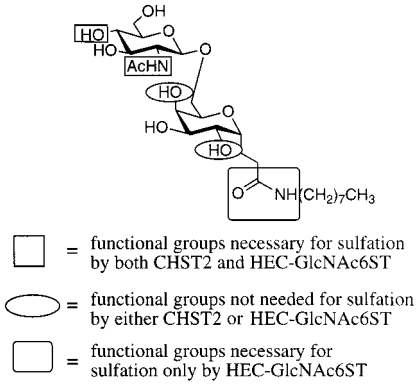


Figure 6. Disaccharide substrate with important functional groups illustrated.

shifts are reported in δ relative to tetramethylsilane. Coupling constants (J) are reported in Hz. Fast atom bombardment (FAB+) spectra and elemental analyses were obtained at the U. C. Berkeley Mass Spectral and Microanalytical Laboratories, respectively. Infrared spectra were recorded on a Perkin-Elmer series 1600 Fourier transform infrared spectrometer.

Synthesis. 3-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl) Propanol (12). General procedure: To a solution of 0.74 g (1.3 mmol) of **10** in 1.3 mL of THF under an atmosphere of N_2, 3.27 mL (1.64 mmol) of 9-BBN was added via syringe. The solution was then heated at reflux for 2.5 h. The reaction mixture was cooled to 0 °C, and 2.5 mL of EtOH, 0.5 mL of 4 M NaOH, and 0.5 mL of H_2O_2 were added. The reaction mixture was stirred under an N_2 atmosphere at 0 °C for 12 h. The solution was then diluted with 20 mL of Et_2O, and the organic layer was washed successively with saturated NH_4Cl (1 × 20 mL), H_2O (2 × 25 mL), and brine (1 × 25 mL) and dried over Na_2SO_4. The crude product was concentrated and purified by silica gel chromatography eluting with 3:2 hexanes/EtOAc to yield 0.69 g (90%) of a pale yellow syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.61−1.64 (m, 3 H), 1.72 (m, 1 H), 2.27 (br s, 1 H), 3.57−3.64 (m, 2 H), 3.65−3.70 (m, 1 H), 3.74 (dd, 1 H, J = 3.0, 7.5), 3.83 (m, 2 H), 3.98 (t, 1 H, J = 3.0), 4.00−4.03 (m, 2 H), 4.48 (d, 1 H, J = 12.0), 4.54 (app d, 2 H, J = 12.0), 4.58 (d, 1 H, J = 11.5), 4.65 (app d, 2 H, J = 12.5), 4.74−4.77 (m, 2 H), 7.28−7.35 (m, 20 H); Lit.:[16] ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.63−1.95 (m, 4 H), 3.18 (br s, 1 H), 3.55−3.67 (m, 3 H), 3.71 (dd, 1 H, J = 2.8, 7.3), 3.75−3.84 (m, 2 H), 3.94−4.01 (m, 3 H), 4.46 (d, 1 H, J = 12.0), 4.52 (d, 1 H, J = 10.3), 4.54 (d, 1 H, J = 11.9), 4.56 (d, 1 H, J = 11.8), 4.64 (app d, 2 H, J = 11.3), 4.67 (d, 1 H, J = 12.2), 4.74 (d, 1 H, J = 13.1), 7.27−7.35 (m, 20 H).

3-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl) propanol (13): 89% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.65 (app sex, 2 H, J = 6.8) 1.79 (m, 2 H), 1.94 (br s, 1 H), 3.55 (m, 2 H), 3.66 (m, 4 H), 3.77 (m, 2 H), 4.05 (m, 1 H), 4.47 (app t, 2 H, J = 12.4) 4.60 (d, 1 H, J = 12.4), 4.61 (d, 1 H, J = 12.0), 4.70 (d, 1 H, J = 11.6), 4.80 (app t, 2 H, J = 10.8), 4.92 (d, 1 H, J = 11.2), 7.10−7.15 (m, 2 H), 7.20−7.31 (m, 18 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 21.0, 29.1, 62.3, 69.1, 71.1, 73.1, 73.5, 74.3, 75.0, 75.4, 78.2, 80.1, 82.4, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.0, 128.3, 128.3, 128.4, 128.4, 137.9, 138.1, 138.2, 138.7; MS (FAB+) m/z 589 (M + Li).

3-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl) propanol (14): 81% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60−1.80 (m, 4 H), 2.40 (br s, 1 H), 3.62−3.70 (m, 3 H), 3.73 (dd, 1 H, J = 5.0, 10.0), 3.75−3.85 (m, 3 H), 3.90−3.95 (m, 1 H), 4.00−4.10 (m, 1 H), 4.54 (d, 1 H, J = 11.5), 4.56−4.61 (m, 6 H), 4.71 (d, 1 H, J = 11.0), 7.2−7.4 (m, 20 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.2, 29.4, 62.2, 69.1, 71.6, 72.2, 72.8, 73.3, 73.6, 74.4, 74.9, 75.2, 76.3, 127.7, 127.8, 127.9, 128.0, 128.0, 128.1, 128.2, 128.3, 128.3, 128.4, 128.4, 128.5, 138.2, 138.2, 138.2, 138.2; MS (FAB+) m/z 589 (M + Li).

3-(2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl) Propanoic Acid (12a). General procedure: A solution of 2.83 g (4.85 mmol) of **12** in 20 mL of acetone was cooled to 0 °C. Over a period of 15 min, 6.0 mL (12 mmol) of 2 M Jones' reagent (prepared using 27 g CrO_3, 100 mL H_2O, 23 mL concentrated H_2SO_4) was added dropwise, after which the reaction flask was warmed to rt and stirred for 1.5 h. Excess Jones' reagent was quenched with 15 mL of *i*-PrOH, and the solution was diluted with 50 mL of ether, washed successively with $NaHCO_3$ (1 × 25 mL), H_2O (2 × 25 mL) and brine (1 × 15 mL), and then dried over Na_2SO_4. Purification of the concentrated product was achieved by silica gel chromatography eluting with 200:1 $CHCl_3$/MeOH to afford 2.69 g (92%) of a clear, colorless syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.95−2.20 (m, 2 H), 2.37−2.44 (m, 1 H), 2.49−2.57 (m, 1 H), 3.65−3.69 (m, 1 H), 3.76−3.83 (m, 2 H), 3.91 (m, 1 H), 4.02−4.06 (m, 3 H), 4.48−4.63 (m, 4 H), 4.67−4.86 (m, 4 H), 7.32−7.36 (m, 20 H); Lit.[16] ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.95 (m, 1 H), 2.03 (m, 1 H), 2.41 (m, 1 H), 2.53 (m, 1 H), 3.68 (dd, 1 H, J = 4.8, 10.5), 3.78 (m, 2 H), 3.91 (app s, 1 H), 4.03 (m, 3 H), 4.50 (d, 1 H, J = 11.9), 4.56 (d, 1 H, J = 11.9), 4.59 (d, 1 H, J = 11.2), 4.62 (d, 1 H, J = 11.4), 4.69 (d, 1 H, J = 11.8), 4.70 (d, 1 H, J = 11.6), 4.75 (d, 1 H, J = 11.9), 4.80 (d, 1 H, J = 11.6), 7.34−7.37 (m, 20 H).

3-(2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl) propanoic acid (13a): 80% yield;^{1}H NMR (300 MHz, $CDCl_3$) δ 2.06 (m, 2 H), 2.52 (m, 2 H), 3.65 (m, 2 H), 3.75 (m, 2 H), 3.88 (m, 2 H), 4.05 (m, 1 H), 4.49 (d, 1 H, J = 10.8), 4.52 (d, 1 H, J = 12.3), 4.64 (d, 1 H, J = 12.3), 4.66 (d, 1 H, J = 11.4), 4.72 (d, 1 H, J = 11.7), 4.83 (d, 1 H, J = 10.8), 4.84 (d, 1 H, J = 10.8), 4.96 (d, 1 H, J = 10.8), 7.13 (m, 2 H), 7.20−7.35 (m, 18 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 20.0, 68.9, 71.2, 73.1, 73.2, 73.5, 75.0, 75.5, 77.9, 79.8, 82.3, 127.6, 127.6, 127.7, 127.9, 128.1, 128.3, 128.4, 128.4, 128.4, 128.5, 137.9, 138.1, 138.6, 178.8; MS (FAB+) m/z 603 (M + Li).

3-(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl) propanoic acid (14a): 83% yield;^{1}H NMR (500 MHz, $CDCl_3$) δ 1.80−1.90 (m, 2 H), 2.30−2.50 (m, 2 H), 3.55−3.60 (m, 1 H), 3.68 (dd, 1 H, J = 3.8, 11.3), 3.70−3.80 (m, 2 H), 3.81−3.89 (m, 2 H), 3.95−4.05 (m, 1 H), 4.51−4.60 (m, 7 H), 4.66 (d, 1 H, J = 11.4), 7.10−7.40 (m, 20 H), 10.35 (br s, 1 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 24.9, 30.2, 54.2, 68.7, 71.3, 71.5, 72.2, 73.3, 73.4, 73.5, 74.6, 75.9, 127.5, 127.7, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.4, 129.0, 129.7, 138.0, 138.0, 138.1, 138.2, 178.4; MS (FAB+) m/z 603 (M + Li).

1-*N*-Octyl-3-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl) Propionamide (15). General procedure: A solution of 1.4 g (2.4 mmol) of **12a**, 0.80 mL (4.8 mmol) of *n*-octylamine, 0.50 g (3.7 mmol) of HOBt, and 0.70 g (3.7 mmol) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) in 20 mL of CH_2Cl_2 was stirred under N_2 at rt. After 12 h, the solution was diluted with 50 mL of $CHCl_3$ and 50 mL of saturated aqueous NH_4Cl. The organic layer was collected and washed with H_2O (2 × 20 mL) and brine (30 mL) and then dried over Na_2SO_4. Purification was achieved by silica gel chromatography eluting with a gradient of 10:1 to 1:1 hexanes/EtOAc to yield 0.99 g (61%) of a clear, pale yellow syrup; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (app t, 3 H, J = 2.8), 1.20−1.36 (m, 12 H), 1.80−2.05 (m, 2 H), 2.23 (m, 2 H), 3.09 (m, 2 H), 3.56 (dd, 1 H, J = 3.2, 10.4), 3.74 (m, 2 H), 3.90−4.10 (m, 4 H), 4.48−4.55 (m, 5 H), 4.60−4.72 (m, 3 H), 6.12 (m, 1 H), 7.28−7.34 (m, 20 H); Lit.[16] ^{1}H

NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3 H, J = 6.7), 1.22−1.34 (m, 12 H), 1.84 (m, 1 H), 1.94 (m, 1 H), 2.20−2.23 (m, 2 H), 3.05 (m, 1 H), 3.12 (m, 1 H), 3.53 (dd, 1 H, J = 3.3, 10.7), 3.68−3.74 (m, 2 H), 3.95−4.04 (m, 4 H), 4.48−4.67 (m, 8 H), 5.94 (br s, 1 H), 7.25−7.35 (m, 20 H).

1-*N*-Octyl-3-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl) propionamide (16): 65% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 3 H, J = 7.2), 1.14 (m, 10 H), 1.35 (m, 2 H), 2.21 (m, 2 H), 3.15 (m, 2 H), 3.44 (m, 1 H) 3.65 (m, 3 H), 3.73 (m, 1 H), 3.79 (dd, 1 H, J = 8.8, 9.2), 4.04 (m, 1 H), 4.45 (d, 1 H, J = 11.2), 4.50 (d, 1 H, J = 13.2), 4.56 (d, 1 H, J = 12.0), 4.65 (m, 2 H), 4.76 (d, 1 H, J = 11.6), 4.81 (d, 1 H, J = 10.8), 4.91 (d, 1 H, J = 11.2), 5.65 (m, 1 H), 7.15 (m, 2 H), 7.20−7.35 (m, 18 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 21.1, 22.6, 26.9, 29.2, 29.3, 29.6, 31.8, 32.1, 39.5, 69.4, 71.1, 72.7, 72.8, 73.4, 74.9, 75.5, 78.2, 79.8, 83.7, 127.6, 127.7, 127.8, 127.9, 127.9, 127.9, 128.1, 128.4, 128.4, 128.4, 137.9, 138.1, 138.1, 138.6, 172.2; MS (FAB+) m/z 714.5 (M + Li).

1-*N*-Octyl-3-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl) propionamide (17): 80% yield;^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, 3 H, J = 7.0), 1.20−1.40 (m, 14 H), 1.80−1.97 (m, 2 H), 2.20−2.35 (m, 2 H) 3.11−3.19 (m, 2 H), 3.55 (m, 1 H), 3.65−3.70 (m, 1 H), 3.75−3.85 (m, 3 H), 3.97−4.02 (m, 3 H), 4.45−4.60 (m, 7 H), 5.86 (m, 1 H), 7.20−7.43 (m, 20 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.6, 25.6, 26.9, 29.2, 29.2, 29.5, 31.8, 39.6, 66.0, 69.1, 71.6, 71.7, 72.2, 73.2, 73.2, 73.8, 75.1, 76.0, 127.7, 127.7, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.3, 128.4, 128.4, 128.5, 138.1, 138.1, 138.1, 138.1, 172.6; MS (FAB+) m/z 714.5 (M + Li).

1-*N*-Octyl-3-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl) Propionamide (18). General procedure: A solution of 0.32 g (0.46 mmol) of **15** in 2 mL of CH_2Cl_2 and 2 mL of Ac_2O was cooled to −42 °C (CH_3CN/CO_2) under an atmosphere of N_2. Over the course of 1 h, a solution of 0.09 mL (0.05 mmol) of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in 0.3 mL CH_2Cl_2 was added dropwise via syringe, and the solution was stirred for an additional 3 h. The solution was then diluted with 10 mL of $CHCl_3$ and quenched with aqueous $NaHCO_3$. The organic layer was washed successively with H_2O (2 × 25 mL) and brine (1 × 25 mL), and then dried over Na_2SO_4. Concentration afforded a clear, colorless syrup which was carried on to the next reaction without further purification. The crude product was dissolved in 10 mL of a 10 mM solution of NaOMe in MeOH and stirred overnight. The reaction was neutralized using Dowex AG 50w-X2 cation-exchange resin (H^+ form), and the product was concentrated and purified by silica gel chromatography eluting with 1:4 EtOAc/hexanes to give 0.21 g (76%) of a clear, colorless syrup; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (m, 3 H), 1.20−1.40 (m, 12 H), 1.42−1.44 (m, 2 H), 2.10−2.20 (m, 2 H), 3.10−3.20 (m, 2 H), 3.60−3.67 (m, 2 H), 3.74 (m, 1 H), 3.99 (m, 2 H), 4.00−4.10 (m, 2 H), 4.45−4.90 (m, 6 H), 6.28 (app t, 1 H, J = 5.6), 7.27−7.44 (m, 15 H); Lit.[16] ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, 3 H, J = 6.8), 1.28 (m, 10 H), 1.48 (m, 2 H), 1.81 (m, 1 H), 2.04 (m, 1 H), 2.25 (d app t, 1 H, J = 6.1, 13.9), 2.37 (m, 1 H), 3.18 (br s, 1 H), 3.19 (m, 2 H), 3.62 (m, 1 H), 3.68 (m, 1 H), 3.76 (m, 1 H), 3.97 (m, 2 H), 4.04 (d app t, 1 H, J = 2.8, 10.9), 4.14 (m, 1 H), 4.48 (d, 1 H, J = 12.1), 4.65, 1 H, J = 11.9), 4.68 (d, 1 H, J = 12.0), 5.89 (br m, 1 H), 7.19−7.35 (m, 15 H).

1-*N*-Octyl-3-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl) propionamide (19): 82% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.84 (t, 3 H, J = 4.5), 1.25 (m, 10 H), 1.45 (m, 2 H), 1.62 (m, 2 H), 2.08 (m, 2 H), 2.43 (br s, 1 H), 3.18 (m, 2 H), 3.33 (m, 1 H), 3.54 (m, 1 H), 3.62 (m, 2 H), 3.82 (m, 2 H), 3.97 (m, 1 H), 4.58 (d, 1 H, J = 11.0), 4.63 (m, 1 H), 4.75 (d, 1 H, J = 11.0), 4.82 (d, 1 H, J = 11.5), 4.88 (d, 1 H, J = 11.0), 5.95 (m, 1 H), 7.20−7.35 (m, 15 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 22.6, 26.9, 29.2, 29.5, 31.5, 32.1, 34.6, 39.7, 62.3, 72.0, 72.8, 73.1, 74.8, 75.2, 77.2, 78.0, 79.7, 81.8, 127.5, 127.5, 127.7, 127.7, 127.8, 127.9, 128.3, 128.3, 128.4, 138.0, 138.1, 138.5, 172.8; MS (FAB+) m/z 624.5 (M + Li).

1-*N*-Octyl-3-(2,3,4-tri-*O*-benzyl-α-D-mannopyranosyl) propionamide (20): 60% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (t, 3 H, J = 7.0), 1.20−1.30 (m, 10 H), 1.47 (m, 2 H), 1.85 (m, 2 H), 2.24 (m, 1 H), 2.35 (m, 1 H), 2.97 (br s, 1 H), 3.23 (m, 2 H), 3.55 (m, 2 H), 3.68 (m, 2 H), 3.82 (m, 1 H), 3.95 (m, 1 H), 4.08 (m, 1 H), 4.50−4.59 (m, 5 H), 4.70 (d, 1 H, J = 11.5), 5.80 (m, 1 H), 7.20−7.45 (m, 15 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.6, 26.9, 29.2, 29.2, 29.4, 29.5, 31.8, 32.7, 39.7, 61.4, 67.6, 72.8, 72.4, 73.7, 73.7, 74.8, 75.0, 76.5, 127.8, 127.8, 127.9, 128.0, 128.2, 128.2, 128.4, 128.4, 128.4, 138.0, 138.1, 142.6, 172.5; MS (FAB+) m/z 624.5 (M + Li).

2-*N*-*p*-Nitrobenzyloxycarbonyl-1,3,4,6-tetra-*O*-acetyl-D-glucosamine (Mixture of Anomers) (23). General procedure: 5.0 g (23 mmol) of glucosamine·HCl was dissolved in 130 mL of 0.19 M NaOMe in MeOH and was stirred for 20 min. The reaction mixture was cooled to 0 °C, 3.23 mL (23.2 mmol) of TEA and 5.0 g (23 mmol) of *p*-nitrobenzyl chloroformate were added, and the mixture was stirred for 5 h. The thick suspension was concentrated and dissolved in 25 mL of Ac_2O and 50 mL of pyridine. A catalytic amount of *N*,*N*-(dimethylamino)pyridine (DMAP) was added, and the solution was stirred overnight. It was concentrated and coevaporated with toluene (6 × 25 mL). The resulting syrup was dissolved in 40 mL of $CHCl_3$ and washed with saturated $CuSO_4$ (2 × 20 mL), saturated $NaHCO_3$ (2 × 25 mL), and brine (1 × 25 mL). The combined aqueous layers were extracted with 40 mL of $CHCl_3$, and the combined organic layers were dried over Na_2SO_4. The crude product was concentrated and purified by silica gel chromatography eluting with a gradient of 200:1 to 20:1 $CHCl_3$/MeOH to yield 6.3 g (52%) of a yellow syrup comprising a mixture of anomers (1:1 α/β). ^{1}H NMR (500 MHz, $CDCl_3$) 1.88 (s, 3 H), 1.91 (s, 3 H), 1.92 (s, 3 H), 1.95 (s, 3 H), 4.10 (m, 1 H), 4.2 (m, 1 H), 5.0−5.2 (m, 4 H), 5.37 (d, 1 H, J = 9.5) (α anomer H-1), 6.10 (d, 1 H, J = 3.5) (β anomer H-1), 7.35 (d, 2 H, J = 8.5), 8.05 (d, 2 H, J = 9.0); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.2, 20.2, 20.3, 20.5, 52.7, 61.3, 65.2, 69.4, 72.3, 72.3, 90.27, 123.36, 127.6, 127.7, 143.3, 168.5, 168.9, 170.3, 170.7; lit.[41] characteristic peaks: ^{13}C NMR (75 MHz, $CDCl_3$) 55.9, 62.5, 65.5, 69.2, 73.2, 73.2, 92.9.

2-*N*-*p*-Nitrobenzyloxycarbonyl-1,3,4,6-tetra-*O*-acetyl-D-galactosamine (Mixture of Anomers) (24). A mixture of anomers (2.6:1 α/β) (68% yield) was obtained using the previous procedure. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3 H), 2.01 (s, 3 H), 2.16 (s, 3 H), 2.17 (s, 3 H), 4.10 (m, 2 H), 4.25 (m, 1 H), 4.46 (m, 1 H), 4.90 (d, 1 H, J = 9.6), 5.21 (m, 3 H), 5.39 (m, 1 H), 5.72 (d, 1 H, J = 8.4) (α anomer H-1), 6.25 (d, 1 H, J = 3.6) (β anomer H-1), 7.48 (d, 2 H, J = 7.2), 8.19 (d, 2 H, J = 8.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.6, 20.8, 21.0, 21.1, 49.0, 61.2, 65.7, 66.7, 67.7, 68.6, 91.7, 123.8, 128.2, 143.3, 155.4, 169.5, 170.1, 170.4, 170.8; MS (FAB+) m/z 533 (M + Li).

2-*N*-*p*-Nitrobenzyloxycarbonyl-3,4,6-tri-*O*-acetyl-D-glucosamine (Mixture of Anomers) (26). General procedure: Under an atmosphere of N_2, 0.16 mL (1.5 mmol) of distilled benzylamine ($BnNH_2$)was added to a solution of 0.70 g (1.3 mmol) of **24** in 3 mL of THF. The reaction mixture was stirred for 12 h, and an additional 0.02 mL (0.18 mmol) of $BnNH_2$ was added. The solution was stirred another 2 h and then concentrated. The crude product was then purified by silica gel chromatography eluting with 1:1 EtOAc/hexanes to yield 0.61 g (95%) of a gel. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (s, 3 H), 2.05 (s, 3 H), 2.14 (s, 3 H), 3.85 (m, 1 H), 3.97 (m, 1 H), 4.10 (m, 2 H), 4.20 (m, 1 H), 5.05−5.30 (m, 4 H), 7.45 (d, 2 H, J = 8.3), 8.20 (d, 2 H, J = 8.4).

2-*N*-*p*-Nitrobenzyloxycarbonyl-3,4,6-tri-*O*-acetyl-D-galactosamine (mixture of anomers) (27): 74% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (br s, 1 H), 1.93 (s, 3 H), 2.03 (s, 3 H), 2.15 (s, 3 H), 4.11 (m, 2 H), 4.22 (m, 1 H), 4.41 (m, 1 H), 5.10−5.28 (m, 4 H), 5.35 (m, 1 H), 7.48 (d, 2 H, J = 8.7), 8.19 (d, 2 H, J = 8.7); ^{13}C NMR (125 MHz, $CDCl_3$) δ 20.7, 20.7, 22.5, 50.0, 63.4, 65.3, 66.3, 67.6, 68.5, 92.2, 123.8, 128.0, 128.9, 143.8, 149.4, 170.1, 170.4, 170.6.

2,3,4,6-Tetra-*O*-acetyl-D-glucose (28). Distilled $BnNH_2$ (0.85 mL, 7.8 mmol) was added to a solution of 2.65 g (6.79 mmol) of glucose pentaacetate in 5 mL of THF under N_2. The solution was stirred for 12 h, and an additional 0.02 mL (0.18 mmol) of $BnNH_2$ was added. The solution was stirred another 2 h and then concentrated. The crude product was purified by silica gel chromatography eluting with 1:1 EtOAc/hexanes to yield 1.5 g (63%) of a gel. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (s, 3 H), 1.99 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 4.1−4.15 (m, 2 H) 4.15−4.30 (m, 1 H), 4.83 (dd, 1 H, J = 3.6, 10.2), 5.04 (t, 1 H, J = 9.7), 5.2 (d, 1 H, J = 9.5) (β H-1) 5.41 (m, 1 H) (α

(41) Boullanger, P.; Jouineau, M.; Bouammali, B.; Lafont, D.; Descotes, G. *Carbohydr. Res.* **1990**, *202*, 151−164.

H-1), 5.50 (t, 1 H, $J = 9.8$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.7, 62.0, 67.1, 68.5, 69.9, 71.1, 90.0, 169.7, 170.3, 170.3, 171.0; the product was taken on to the next reaction without further purification.

Compound 29. General procedure: A solution of 3.3 g (6.9 mmol) of **26** and 1.04 g (7.56 mmol) of anhydrous K_2CO_3 in 34 mL of CH_2Cl_2 was stirred under an atmosphere of N_2. To this solution was added 6.9 mL (69 mmol) of trichloroacetonitrile by syringe, and the reaction mixture was stirred overnight. The solution was then warmed in a water bath at 30 °C for 1 h. The reaction mixture was then concentrated and purified by silica gel chromatography eluting with a gradient of 1:2 to 1:1 EtOAc/hexanes to yield 2.6 g (61%) of a yellow glass. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 3 H), 2.00 (s, 3 H), 2.01 (s, 3 H), 4.06 (m, 2 H), 4.21 (m, 2 H), 5.1−5.2 (m, 3 H), 5.3 (m, 1 H), 6.35 (d, 1 H, $J = 3.2$), 7.40 (d, 2 H, $J = 8.4$), 8.13 (d, 1 H, $J = 8.8$), 8.77 (s, 1 H); MS (FAB+) *m*/*z* 636 (M + Li); lit. [33] characteristic peaks: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.39 (d, 1 H, $J = 3.6$), 8.75 (s, 1 H).

Compound 30: 55% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.98 (s, 3 H), 2.01 (s, 3 H), 2.17 (s, 3 H), 4.05 (m, 1 H), 4.15 (m, 1 H), 4.36 (m, 1 H), 4.50 (ddd, 1 H, $J = 3.6, 11.6, 13.2$), 4.97 (d, 1 H, $J = 9.6$), 5.18 (m, 2 H), 5.23 (dd, 1 H, $J = 3.2, 11.2$), 5.49 (d, 1 H, $J = 2.4$), 6.43 (d, 1 H, $J = 3.6$), 7.46 (d, 2 H, $J = 8.4$), 8.20 (d, 2 H, $J = 8.4$), 8.78 (s, 1 H).

Compound 31. A solution of 1.5 g (4.3 mmol) of **28** and 0.594 g (4.31 mmol) of anhydrous K_2CO_3 in 21 mL of CH_2Cl_2 was stirred under N_2. To this solution was added 4.31 mL (43.3 mmol) of trichloroacetonitrile by syringe, and the reaction mixture was stirred overnight. The solution was warmed 30 °C for 1 h, and the reaction mixture was then concentrated and purified by silica gel chromatography eluting with 1:1 EtOAc/hexanes to yield 1.48 g (70%) total mass, obtained as 0.756 g of an orange glass (α anomer) and 0.724 g of an orange syrup (β anomer). ^{1}H NMR (500 MHz, $CDCl_3$) (α anomer) δ 2.01 (s, 3 H), 2.03 (s, 3 H), 2.03 (s, 3 H), 2.04 (s, 3 H), 4.12 (dd, 1 H, $J = 2.0, 12.5$), 4.2−4.22 (m, 1 H), 4.27 (dd, 1 H, $J = 4.0, 12.0$), 5.12 (dd, 1 H, $J = 3.5, 10.0$) 5.18 (t, 1 H, $J = 10.0$) 5.56 (t, 1 H, $J = 4.0$), 6.56 (d, 1H, $J = 4.0$), 8.69 (s, 1 H); ^{1}H NMR (500 MHz, $CDCl_3$) (β anomer) δ 2.02 (s, 3 H), 2.03 (s, 3 H), 2.05 (s, 3 H), 2.08 (s, 3 H), 3.89−3.92 (m, 1 H), 4.15 (dd, 1 H, $J = 2.0, 12.5$), 4.31 (dd, 1 H, $J = 4.0, 12.5$), 5.20−5.23 (m, 1 H), 5.26−5.31 (m, 2 H), 5.87 (d, 1 H, $J = 7.0$), 8.71 (s, 1 H); Lit.[42] (α anomer) ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00−2.15 (4 x s, 12 H), 4.15 (dd, 1 H, $J = 2.5, 12.5$), 4.25 (m, 2 H), 5.15 (dd, 1 H, $J = 4.0, 9.5$), 5.20 (t, 1 H, $J = 9.5$), 5.55 (t, 1 H, $J = 9.5$), 6.55 (d, 1 H, $J = 4.0$), 8.70 (br s, 1 H).

Compound 32. General procedure: Glycosyl acceptor **18** (0.156 g, 0.253 mmol) was concentrated in the reaction flask, dried under vacuum over P_2O_5, and placed under an Ar atmosphere. Glycosyl donor **29** (0.47 g, 0.75 mmol) was concentrated and dried over P_2O_5, placed under an argon atmosphere, then dissolved in 4 mL of CH_2Cl_2. This solution was then transferred via syringe to the reaction flask, which was cooled to −42 °C. BF_3OEt_2 (0.016 mL, 0.125 mmol) was added via syringe. The solution was stirred at −42 °C for 6 h and then diluted with $CHCl_3$. The solution was washed with saturated $NaHCO_3$ (1 × 5 mL), and brine (1 × 5 mL), and the aqueous layers were extracted with 5 mL of $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography eluting with 200:1 $CHCl_3$/MeOH to obtain 0.269 g (98%) of a clear syrup. IR (thin film) 3362, 2923, 1746, 1540 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80−0.90 (m, 3 H), 1.20 (m, 12 H), 1.45 (m, 2 H), 1.70−1.94 (m, 2 H), 1.96 (s, 3 H), 2.01 (s, 3 H), 2.03 (s, 3 H), 3.10−3.30 (m, 2 H), 3.60−3.80 (m, 4 H), 3.90−4.05 (m, 4 H), 4.07 (d, 1 H, $J = 12.1$), 4.31 (m, 1 H), 4.50−4.82 (m, 5 H), 5.05−5.30 (m, 4 H), 5.94 (m, 1 H), 6.08 (m, 1 H), 7.28−7.31 (m, 15 H), 7.45 (m, 2 H), 8.16 (d, 2 H, $J = 7.6$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 14.0, 20.6, 20.6, 22.6, 29.2, 29.3, 31.8, 31.8, 32.2, 38.2, 39.7, 39.7, 42.5, 35.0, 46.3, 49.2, 56.2, 62.1, 62.2, 65.0, 65.0, 68.7, 72.5, 73.4, 73.5, 73.6, 123.6, 127.5, 127.8, 127.8, 127.8, 128.0, 128.3, 128.4, 138.4, 138.4, 138.5, 146.7, 155.6, 169.4, 170.4, 170.4, 172.4; HRMS (FAB+) *m*/*z* 1084.5021 (MH^+ $C_{58}H_{74}N_3O_{17}$ requires 1084.5018).

Compound 33: 39% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 3 H, $J = 7.0$), 1.26 (m, 10 H), 1.44 (m, 2 H), 1.69 (m, 2 H), 1.99 (s, 3 H), 2.04 (s, 3 H), 2.14 (s, 3 H), 2.18 (m, 2 H), 3.18 (m, 2 H), 3.71 (m, 1 H), 3.89 (m, 2 H), 3.99 (m, 3 H), 4.11 (m, 2 H), 4.26 (m, 1 H), 4.41 (m, 1 H), 4.48 (m, 1 H), 4.50−4.67 (m, 4 H), 5.10−5.25 (m, 5 H), 5.36 (m, 1 H), 5.40 (m, 1 H), 5.83 (m, 1 H), 6.22 (m, 1 H), 7.10−7.30 (m, 15 H), 7.78 (d, 2 H, $J = 9.0$), 8.20 (d, 2 H, $J = 8.5$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 20.6, 20.6, 20.7, 22.6, 24.4, 26.9, 29.2, 29.3, 29.6, 31.8, 32.5, 39.6, 49.9, 61.2, 61.9, 65.1, 65.3, 65.9, 66.5, 66.6, 67.5, 68.3, 70.4, 70.7, 73.1, 73.2, 102.2, 123.6, 123.7, 127.6, 127.7, 127.8, 127.8, 128.0, 128.4, 128.4, 138.0, 138.3, 143.7, 147.5, 152.4, 155.5, 167.5, 170.2, 170.4, 170.5, 173.0; MS (FAB+) *m*/*z* 1090.7 (M + Li).

Compound 34. General procedure: Glycosyl acceptor **18** (0.058 g, 0.094 mmol) was concentrated in the reaction flask and dried under vacuum over P_2O_5. Glycosyl donor **31** (0.16 g, 0.33 mmol) was concentrated and dried under vacuum over P_2O_5, and dissolved in 1.5 mL of CH_2Cl_2. This solution was transferred via syringe to the reaction flask, which was cooled to −42 °C under N_2, and 0.006 mL (0.047 mmol) of BF_3OEt_2 was added via syringe. The reaction mixture was stirred at −42 °C for 6 h, and then diluted with $CHCl_3$. The solution was then washed with saturated $NaHCO_3$ (1 × 5 mL) and brine (1 × 5 mL), and the aqueous layers were extracted with 5 mL of $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography eluting with 9:1 hexanes/EtOAc yielding 0.035 g (39%) as a clear syrup. IR (thin film) 3390, 2915, 1559 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 3 H, $J = 6.5$), 1.21−1.30 (m, 12 H), 1.24−1.29 (m, 2 H), 2.01 (s, 3 H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.05 (s, 3 H), 2.10−2.20 (m, 2 H), 3.20−3.30 (m, 2 H), 3.60−3.75 (m, 3 H), 3.83 (m, 2 H), 3.90 (m, 1 H), 4.02 (m, 1 H), 4.10−4.20 (m, 1 H), 4.30 (dd, 1 H, $J = 4.2, 8.3$), 4.45 (d, 1 H, $J = 8.0$), 4.54 (d, 1 H, $J = 11.5$), 4.58 (d, 1 H, $J = 11.7$), 4.60−4.70 (m, 2 H), 4.72 (d, 1 H, $J = 12.0$), 4.79 (d, 1 H, $J = 11.8$), 5.00 (app t, 1 H, $J = 8.0$), 5.10 (app t, 1 H, $J = 9.5$), 5.19 (app t, 1 H, $J = 9.5$), 6.20−6.30 (m, 1 H), 7.28−7.33 (m, 15 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 14.1, 20.6, 20.7, 22.6, 22.6, 22.7, 26.9, 29.2, 29.2, 29.2, 29.6, 31.6, 31.7, 39.5, 39.5, 63.4, 68.2, 71.3, 71.7, 72.5, 73.0, 74.3, 74.3, 101.1, 127.6, 127.9, 128.1, 128.3, 128.3, 128.4, 138.2, 138.4, 169.4, 170.0, 170.2, 170.6, 172.5; HRMS (FAB+) *m*/*z* 948.4748 (MH^+ $C_{52}H_{70}N_1O_{15}$ requires 948.4745).

Compound 35: 95% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, 3 H, $J = 6.8$), 1.23 (m, 10 H), 1.45 (m, 2 H), 1.90 (s, 3 H), 1.96 (s, 3 H), 2.06 (s, 3 H), 3.20 (m, 2 H), 3.28 (m, 1 H), 3.52 (m, 1 H), 3.60−3.70 (m, 2 H), 3.76 (app t, 1 H, $J = 8.8$) 3.91 (m, 1 H), 3.99 (m, 1 H), 4.10 (m, 2 H), 4.15−4.25 (m, 2 H), 4.48 (d, 1 H, $J = 11.2$), 4.61 (m, 2 H), 4.73 (d, 1 H, $J = 10.8$), 4.85 (m, 1 H), 4.89 (d, 1 H, $J = 10.8$), 5.00−5.10 (m, 2 H), 5.23 (m, 2 H), 5.32 (m, 2 H), 5.50 (d, 1 H, $J = 8.4$), 6.04 (m, 1 H), 7.27 (m, 15 H), 7.44 (d, 2 H, $J = 8.8$), 8.16 (d, 2 H, $J = 8.8$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 14.1, 20.6, 20.7, 21.0, 22.6, 26.9, 29.2, 29.2, 29.6, 31.7, 39.6, 54.1, 60.4, 62.0, 65.2, 67.4, 68.2, 68.5, 68.7, 71.1, 71.7, 72.1, 72.8, 73.0, 74.7, 77.3, 78.1, 91.7, 123.6, 123.7, 127.7, 127.8, 127.8, 128.0, 128.4, 128.4, 138.0, 138.0, 138.4, 143.5, 143.7, 147.5, 151.2, 155.3, 169.4, 170.5, 170.7, 170.8, 172.5; MS (FAB+) *m*/*z* 1084.7 (M + H).

Compound 36: 91% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 3 H, $J = 7.0$), 1.27 (m, 10 H), 1.45 (m, 2 H), 1.77 (m, 2 H), 1.97 (s, 3 H), 2.02 (m, 6 H), 2.14 (m, 2 H), 3.18 (m, 2 H), 3.57 (m, 2 H), 3.63 (m, 1 H), 3.69 (m, 1 H), 3.85 (m, 2 H), 3.97 (m, 1 H), 4.10 (dd, 1 H, $J = 2.0, 12.5$), 4.24 (m, 1 H), 4.28 (m, 1 H), 4.50−4.70 (m, 6 H), 5.03 (m, 3 H), 5.13 (m, 1 H), 5.24 (m, 2 H), 5.31 (m, 1 H), 7.20−7.35 (m, 15 H), 7.46 (d, 2 H, $J = 8.5$), 8.15 (d, 2 H, $J = 8.5$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 17.1, 18.3, 20.6, 20.7, 22.6, 26.9, 29.2, 29.2, 29.5, 31.8, 39.8, 50.7, 54.1, 56.0, 58.4, 62.0, 64.9, 65.3, 67.4, 68.2, 68.3, 68.6, 71.1, 71.6, 72.5, 73.8, 123.7, 127.7, 127.7, 127.8, 127.8, 127.8, 128.0, 128.0, 128.2, 128.3, 128.4, 128.4, 128.58, 137.8, 137.9, 155.9, 162.3, 169.5, 170.6, 170.7, 171.2; MS (FAB+) *m*/*z* 1090.7 (M + Li).

Compound 32a. General procedure: To a solution of 0.27 g (0.25 mmol) of **32** in 2 mL of EtOH was added 0.016 mL (0.275 mmol) of glacial AcOH and 0.16 g (60 wt %) of 10% Pd/C. H_2 was bubbled through the stirring mixture for 20 min, and the suspension was stirred under H_2 for another 10 h. The mixture was then filtered through Celite and resubmitted to the above reaction conditions using fresh catalyst.

(42) Cook, S. J.; Khan, R.; Brown, J. M. *J. Carbohydr. Chem.* **1984**, *3*, 343−348.

This was repeated again before the crude reaction mixture filtered, concentrated, and redissolved in 2 mL (20 mmol) of Ac_2O and 4.0 mL (49 mmol) of pyridine. A catalytic amount of DMAP was added, and the reaction mixture was stirred for 12 h. The mixture was concentrated and coevaporated with toluene (7 × 10 mL). The crude product was purified by silica gel chromatography eluting with 5:1 EtOAc/hexanes, and then lyophilized from benzene to yield 0.09 g (45%) of compound **32a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (app t, 3 H, J = 6.5), 1.20−1.25 (m, 12 H), 1.89 (s, 3 H), 1.92 (s, 3 H), 1.97 (s, 3 H), 1.98 (s, 3 H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.07 (s, 3 H), 2.20−2.27 (m, 2 H), 3.10−3.20 (m, 2 H), 3.30−3.40 (m, 2 H), 3.65 (m, 1 H), 3.80 (d, 1 H, J = 9.5), 3.89 (d, 1 H, J = 8.0), 4.00−4.10 (m, 4 H), 4.22−4.25 (dd, 1 H, J = 4.4, 12.3), 4.36 (d, 1 H, J = 8.0), 5.00−5.08 (m, 2 H), 5.10 (m, 1 H) 5.15 (dd, 1 H, J = 3.0, 10.5), 5.24−5.28 (m, 1 H), 5.32 (m, 1 H), 6.09 (d, 1 H, J = 9.5), 7.35 (m, 1 H); Lit.[16] ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.86 (app t, 3 H, J = 8.8), 1.27 (m, 14 H), 1.51 (m, 2 H), 1.4 (s, 3 H), 1.96 (s, 3 H), 2.02 (s, 3 H), 2.04 (s, 3 H), 2.05 (s, 3 H), 2.08 (s, 3 H), 2.11 (s, 3 H), 3.21 (m, 2 H), 3.39 (app t, 1 H, J = 9.8), 3.63 (m, 1 H), 3.86 (app d, 1 H, J = 10.1), 3.92 (d, 1 H, J = 8.1), 4.11 (m, 3 H), 4.28 (dd, 1 H, J = 4.5, 12.4), 4.35 (d, 1 H, J = 8.3), 5.04 (app t, 1 H, J = 9.3), 5.10 (t, 1 H, J = 9.4), 5.14 (dd, 1 H, J = 3.4, 10.6), 5.32 (m, 2 H), 5.60 (d, 1 H, J = 12.0), 7.47 (app t, 1 H).

Compound 33a: 35% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 3 H, J = 5.0), 1.26 (m, 10 H), 1.54 (m, 2 H), 1.95 (m, 1 H), 1.97 (s, 3 H), 1.98 (s, 3 H), 2.03 (s, 3H), 2.05 (s, 3H), 2.06 (s, 3H), 2.13 (s, 3H), 2.16 (s, 3 H), 2.30 (m, 1 H), 3.23 (m, 2 H), 3.41 (dd, 1 H, J = 9.0, 10.0), 3.89 (m, 2 H), 3.95 (app d, 1 H, J = 8.0), 4.10 (m, 1 H), 4.19 (m, 2 H), 4.28 (m, 1 H), 4.38 (d, 1 H, J = 8.0), 5.01 (dd, 1 H, J = 3.0, 11.0), 5.15 (dd, 1 H, J = 3.0, 10.5), 5.31 (m, 1 H), 5.34 (m, 2 H), 5.58 (d, 1 H, J = 9.5), 7.47 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 20.3, 20.6, 20.7, 20.8, 22.6, 23.5, 27.0, 29.2, 29.3, 29.7, 30.9, 31.8, 39.6, 50.6, 61.1, 63.0, 66.4, 67.8, 68.1, 68.6, 69.1, 70.1, 70.7, 72.9, 77.7, 79.1, 101.9, 169.9, 170.1, 170.1, 170.2, 170.4, 170.6, 170.9, 171.0, 172.0; MS (FAB+) m/z 809.4 (M + Li).

Compound 34a: 79% yield; IR (thin film) 3410, 2916, 1743 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 3 H, J = 6.0), 1.20−1.30 (m, 12 H), 1.50−1.52 (m, 2 H), 1.96 (s, 3 H), 2.01 (s, 3 H), 2.02 (s, 3 H), 2.05 (s, 3 H), 2.07 (s, 3 H), 2.10 (s, 3 H), 2.14 (s, 3 H), 2.20−2.21 (m, 2 H), 3.23 (m, 2 H), 3.47 (m, 1 H), 3.70 (m, 1 H), 3.79 (dd, 1 H, J = 2.4, 4.5), 3.89 (dd, 1 H, J = 2.1, 10.0), 4.15−4.20 (m, 1 H), 4.31 (dd, 1 H, J = 4.5, 12.4), 4.45 (d, 1 H, J = 12.0), 5.02 (dd, 1 H, J = 8.0, 9.8), 5.07 (t, 1 H, J = 9.6), 5.15 (dd, 1 H, J = 3.4, 10.6), 5.20 (t, 1 H, J = 9.7), 5.30 (t, 1 H, J = 6.1), 5.34 (m, 1 H) 6.51 (m, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 5.9, 11.7, 13.4, 14.0, 20.0, 20.5, 20.5, 20.6, 20.7, 20.8, 22.6, 26.9, 29.2, 29.2, 29.5, 30.7, 31.7, 39.5, 61.6, 67.7, 68.1, 68.2, 68.5, 68.9, 69.1, 71.2, 71.8, 72.2, 72.7, 169.4, 169.9, 170.1, 170.2, 170.3, 170.6, 171.7, 175.2; HRMS (FAB+) m/z 804.3645 (MH^+ $C_{37}H_{58}N_1O_{18}$ requires 804.3650).

Compound 35a: 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, 3 H, J = 6.4), 1.28 (m, 10 H), 1.55 (m, 2 H), 1.85 (m, 2 H), 1.94 (s, 3 H), 1.96 (s, 3 H), 1.98 (s, 3 H), 2.01 (s, 3 H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.07 (s, 3 H), 2.25 (m, 2 H), 3.25 (m, 2 H), 3.33 (m, 1 H), 3.64 (m, 1 H), 3.75 (m, 1 H), 3.93 (m, 1 H), 4.02 (m, 1 H), 4.10 (dd, 1 H, J = 2.4, 12.4), 4.22 (m, 1 H), 4.28 (dd, 1 H, J = 4.8, 12.4), 4.34 (d, 1 H, J = 8.4), 4,79 (dd, 1 H, J = 9.2, 10.0), 5.05 (m, 3 H), 5.32 (dd, 1 H, J = 9.2, 9.6), 5.91 (d, 1 H, J = 9.6), 7.09 (m, 1 H);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 20.6, 20.6, 20.7, 22.6, 23.2, 27.0, 29.2, 29.2, 29.5, 31.5, 31.8, 39.5, 53.8, 61.7, 68.2, 69.1, 69.5, 70.5, 72.0, 72.4, 72.6, 101.5, 169.2, 169.8, 170.2, 170.5, 170.7, 171.8, 172.5, 173.4; MS (FAB+) m/z 809.5 (M + Li).

Compound 36a: 42% yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, 3 H, J = 6.9), 1.20−1.35 (m, 10 H), 1.55 (m, 2 H), 1.95 (s, 3 H), 1.97 (s, 3 H), 2.02 (s, 3 H), 2.03 (s, 3 H), 2.05 (s, 3 H), 2.07 (s, 3 H), 2.11 (s, 3 H), 2.21 (m, 2 H), 3.18 (m, 2 H), 3.90 (dd, 1 H, J = 6.9, 10.5), 3.65 (m, 1 H), 3.83 (m, 1 H), 3.88 (m, 1 H), 3.95 (m, 1 H), 4.12 (dd, 1 H, J = 2.4, 12.3), 4.20 (m, 2 H), 4.34 (d, 1 H, J = 8.4), 5.05−5.27 (m, 5 H), 5.94 (d, 1 H, J = 9.3), 6.74 (t, 1 H, J = 5.8);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.0, 20.6, 20.6, 20.9, 22.6, 23.1, 23.4, 27.3, 27.4, 29.2, 29.2, 29.5, 31.3, 31.8, 33.9, 34.3, 39.6, 53.7, 61.9, 62.3, 66.9, 68.3, 68.7, 69.1, 70.4, 71.0, 71.9, 72.7, 101.6, 169.2, 169.8, 169.9, 170.2, 170.4, 170.6, 171.1, 171.5; MS (FAB+) m/z 809 (M + Li).

Compound 2. General procedure: Compound **32a** (0.091 g, 0.113 mmol) was dissolved in 3 mL of 0.01 M NaOMe in MeOH and stirred under a N_2 atmosphere for 24 h. The resulting solution was diluted with MeOH and passed through a column of Dowex AG 50w-X2 cation-exchange resin (H^+ form) and concentrated. The product was dissolved in H_2O and lyophilized to yield 61 mg (98%) of a white glass. ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (m, 3 H), 1.30 (m, 12 H), 1.50 (m, 2 H), 1.86−1.94 (m, 2 H), 1.96 (s, 3 H), 2.10−2.20 (m, 1 H), 2.20−2.30 (m, 1 H), 3.10−3.25 (m, 2 H), 3.30−3.38 (m, 3 H), 3.40−3.46 (m, 3 H), 3.60−3.75 (m, 4 H), 3.85−3.90 (m, 3 H), 3.95 (m, 1 H), 4.40 (d, 1 H, J = 8.5); Lit.[16] ^{1}H NMR (500 MHz, D_2O) δ 0.86 (app t, 3 H), 1.27 (m, 12 H), 1.50 (br s, 2 H), 1.93 (m, 3 H), 2.02 (s, 3 H), 2.08 (m, 1 H), 2.18 (m, 1 H), 2.30 (m, 1 H), 3.16 (m, 2 H), 3.44 (m, 2 H), 3.54 (app t, 1 H), 3.66 (app t, 1 H), 3.72 (m, 4 H), 3.77 (app t, 1 H), 3.91 (m, 2 H), 3.97 (m, 4 H), 4.51 (d, 1 H, J = 8.4).

Compound 3: 88% yield; ^{1}H NMR (500 MHz, CD_3OD) δ 0.89 (t, 3 H, J = 7.0), 1.25 (m, 10 H), 1.55 (m, 2 H), 1.99 (s, 3 H), 2.15 (m, 2 H), 2.35 (m, 2 H), 3.15 (m, 2 H), 3.53 (m, 1 H), 3.56 (dd, 1 H, J = 3.5, 11.0), 3.62 (m, 1 H), 3.67 (m, 1 H), 3.75 (m, 3 H), 3.83 (d, 1 H, J = 3.0), 3.88 (m, 3 H), 3.97 (m, 2 H), 4.37 (d, 1 H, J = 8.5); HRMS (FAB+) m/z 551.3170 (MH^+ $C_{25}H_{46}N_2O_{11}$ requires 551.3180).

Compound 4. General procedure: A solution of 0.02 g (0.03 mmol) of **34a** in 1 mL of a 10 mM solution of NaOMe in MeOH was stirred for 24 h. The reaction was passed through a column of Dowex AG 50w-X2 cation-exchange resin (H^+ form), eluting with MeOH. The product was concentrated and redissolved in H_2O and lyophilized to yield 10 mg (78%) of a white glass. IR (thin film) 3423, 2905, 1713 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD), 0.80−0.90 (m, 3 H), 1.20−1.30 (m, 12 H), 1.40−1.50 (m, 2 H), 2.20−2.30 (m, 2 H), 3.10−3.20 (m, 3 H), 3.20−3.40 (m, 5 H), 3.60−3.70 (m, 2 H), 3.80−4.00 (m, 5 H), 4.33 (d, 1 H, J = 7.6), 7.78 (m, 1 H); ^{13} C NMR (100 MHz, CD_3OD) δ 14.6, 23.0, 23.9, 28.2, 30.6, 30.6, 30.7, 33.1, 33.6, 40.7, 40.8, 62.9, 69.7, 70.2, 70.5, 71.9, 72.0, 73.1, 75.2, 75.4, 78.1, 78.3, 176.2; HRMS (FAB+) m/z 510.2921 (MH^+ $C_{23}H_{44}N_1O_{11}$ requires 510.2914).

Compound 5: 95% yield;^{1}H NMR (500 MHz, CD_3OD) δ 0.91 (t, 3 H, J = 7.0), 1.35 (m, 10 H), 1.55 (m, 2 H), 1.86 (m, 2 H), 1.98 (s, 3 H), 2.14 (m, 2 H), 2.35 (m, 2 H), 3.15 (m, 2 H), 3.28 (m, 1 H), 3.33 (m, 2 H), 3.36 (m, 2 H), 3.43 (dd, 1 H, J = 8.5, 10.0), 3.55 (m, 3 H), 3.75 (m, 2 H), 3.82 (m, 1 H), 3.87 (dd, 1 H, J = 2.0, 11.5), 4.15 (d, 1 H, J = 8.5), 4.40 (d, 1 H, J = 8.5);^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.3, 23.9, 28.2, 30.5, 30.6, 30.6, 33.1, 33.2, 40.7, 49.7, 57.4, 62.9, 72.0, 72.3, 72.7, 73.1, 73.6, 75.4, 76.2, 76.9, 78.1, 103.5, 174.0, 176.0; HRMS (FAB+) m/z 551.3169 (MH^+ $C_{25}H_{46}N_2O_{11}$ requires 551.3180).

Compound 6: 97% yield;^{1}H NMR (500 MHz, CD_3OD) δ 0.89 (m, 3 H), 1.34 (m, 10 H), 1.83 (m, 1 H), 1.97 (m, 1 H), 1.99 (s, 3 H), 2.25 (m, 1 H), 2.38 (m, 1), 3.17 (m, 2 H), 3.27 (m, 1 H), 3.36 (m, 1 H), 3.44 (dd, 1 H, J = 8.5, 10.0), 3.53 (app t, 1 H, J = 8.0), 3.57 (ddd, 1 H, J = 1.5, 7.6, 9.2), 3.61 (m, 1 H), 3.64 (m, 2 H), 3.66 (m, 2 H), 3.69 (m, 1 H), 3.88 (dd, 1 H, J = 2.5, 12.0), 4.15 (dd, 1 H, J = 2.0, 10.5), 4.41 (d, 1 H, J = 8.5);^{13}C NMR (125 MHz, $CDCl_3$) δ 14.6, 21.4, 23.3, 23.9, 26.0, 28.2, 30.5, 30.6, 30.8, 33.2, 40.6, 57.4, 63.0, 69.9, 70.8, 72.3, 72.8, 73.0, 75.0, 76.2, 77.6, 78.1, 103.4, 173.6, 175.7; HRMS (FAB+) m/z 551.3181 (MH^+ $C_{25}H_{46}N_2O_{11}$ requires 551.3180).

Octyl-2,3,4-tri-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-D-galactopyranoside (Mixture of Anomers) (37). To a stirring mixture of 3.0 g (17 mmol) of galactose in 120 mL CH_3CN under Ar was added 7.87 mL (50.0 mmol) of 1-octanol and 1.05 mL (8.33 mmol) of BF_3OEt_2. This suspension was heated at reflux for 24 h. The mixture was concentrated and filtered through silica gel eluting with 5:1 CH_2Cl_2/MeOH. The crude product was then dissolved and stirred in 5 mL of DMF and treated with catalytic DMAP and 0.21 g (1.4 mmol) of tertbutyldimethylsilyl chloride and 0.21 mL (1.5 mmol) of TEA under Ar. The reaction mixture was stirred at rt for 30 h and then concentrated and purified by silica gel chromatography eluting with 1:1 EtOAc/hexanes. The resultant product was then dissolved in 2 mL of pyridine and stirred. To this solution was added 1 mL (10 mmol) of acetic anhydride and a catalytic amount of DMAP. The reaction mixture was

stirred for 24 h before concentration and coevaporation with toluene (5 × 20 mL). The product was purified by silica gel chromatography eluting with 1:9 EtOAc/hexanes to provide 0.083 g (3.2% over three steps) comprising a mixture of anomers (2.4:1 α/β). ^{1}H NMR (500 MHz, $CHCl_3$) δ −0.01 (s, 3 H), 0.01 (s, 3 H), 0.84 (s, 9 H), 0.87 (m, 3 H), 1.27 (m, 10 H), 1.56 (m, 2 H), 1.95 (s, 3 H), 2.02 (s, 3 H), 2.04 (s, 3 H), 2.10 (s, 3 H), 3.30−3.41 (m, 1 H), 3.55−3.70 (m, 4 H), 3.86 (m, 1 H), 4.02 (dd, 1 H, J = 9.0, 10.0), 4.41 (d, 1 H, J = 6.0), 5.07 (m, 2 H), 5.17 (m, 1 H), 5.34 (dd, 1 H, J = 3.0, 10.0), 5.44 (d, 1 H, J = 3.0), 5.48 (d, 1 H, J = 3.0); ^{13}C NMR (125 MHz, $CDCl_3$) δ −5.8, −5.8, −5.6, 14.0, 18.1, 20.6, 20.7, 20.7, 25.6, 25.7, 25.7, 26.0, 29.2, 29.2, 29.2, 29.2, 29.3, 31.7, 31.7, 60.6, 61.0, 67.1, 68.0, 68.2, 68.3, 68.6, 68.8, 69.3, 70.1, 71.2, 73.4, 95.9, 101.3, 169.4, 169.9, 170.0, 170.1, 170.4; HRMS (FAB+) m/z 555.2964 (MNa+ $C_{26}H_{48}O_9SiNa$ requires 555.2965).

Octyl-2,3,4-tri-*O*-acetyl-α-D-galactopyranoside (38). To a stirring solution of 43.0 mg (0.081 mmol) of **37** in 2 mL of THF/H_2O (3:1) was added 0.5 mL of glacial AcOH. This solution was stirred at rt for 20 h. The reaction was quenched by addition of 5 mL of saturated $NaHCO_3$ and diluted with 10 mL EtOAc. The organic layer was washed with saturated $NaHCO_3$ (1 × 10 mL) and brine (1 × 10 mL). The combined aqueous layers were extracted with CH_2Cl_2, and the combined organic layers were then dried over Na_2SO_4. The crude product was purified by silica gel chromatography eluting with 1:3 EtOAc/hexanes. This yielded 16.0 mg (47%) of a clear glass (α anomer) and 7.0 mg (21% yield) of a second clear glass (β anomer). **α-Anomer (38):** ^{1}H NMR (400 MHz, $CHCl_3$) δ 0.87 (t, 3 H, J = 8.5), 1.27 (m, 10 H), 1.55 (m, 2 H), 2.00 (s, 3 H), 2.07 (s, 3 H), 2.16 (s, 3 H), 3.38 (m, 1 H), 3.48 (m, 1 H), 4.08 (app t, 1 H, J = 8.0), 5.10 (m, 1 H), 5.18 (d, 1 H, J = 4.5), 5.37 (dd, 1 H, J = 4.5, 13.0), 5.43 (app d, 1 H, J = 4.5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 20.4, 20.7, 20.8, 21.3, 22.3, 22.6, 26.0, 29.3, 31.8, 62.0, 67.7, 68.5, 68.6, 68.6, 69.0, 95.6, 169.9, 170.5, 171.1; HRMS (FAB+) m/z 441.2116 (MNa^+ $C_{20}H_{34}O_9Na$ requires 441.2101). **β-Anomer (39):** ^{1}H NMR (500 MHz, $CHCl_3$) δ 0.87 (t, 3 H, J = 6.4), 1.26 (m, 10 H), 1.56 (m, 2 H), 2.00 (s, 3 H), 2.05 (s, 3 H), 2.17 (s, 3 H), 3.48 (m, 2 H), 3.73 (m, 2 H), 3.89 (m, 1 H), 4.48 (d, 1 H, J = 8.0), 5.05 (dd, 1 H, J = 3.6, 10.4), 5.23 (dd, 1 H, J = 8.0, 10.4), 5.36 (app d, 1 H, J = 3.2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 20.6, 20.7, 22.6, 25.8, 29.2, 29.3, 29.3, 31.8, 35.0, 60.7, 68.0, 69.3, 70.4, 70.9, 73.4, 101.4, 166.2, 170.3, 171.2.

Compound 40. The glycosyl acceptor **38** (0.03 g, 0.08 mmol) was concentrated in the reaction flask and dried under vacuum over P_2O_5. Glycosyl donor **29** (0.174 g, 0.213 mmol) was concentrated and dried under vacuum over P_2O_5 and dissolved in 1.5 mL of CH_2Cl_2. This solution was then transferred via syringe to the reaction flask, which was cooled to −42 °C under N_2, and 0.005 mL (0.036 mmol) of BF_3OEt_2 was added via syringe. The reaction mixture was stirred at −42 °C for 5 h and then quenched with saturated $NaHCO_3$ and diluted with $CHCl_3$. The organic layer was then washed with saturated $NaHCO_3$ (1 × 5 mL) and brine (1 × 5 mL), and the aqueous layers were extracted with 5 mL of $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography eluting with a gradient of 1:3 to 1:1 hexanes/EtOAc yielding 65 mg (93%) as a clear glass: ^{1}H NMR (400 MHz, $CHCl_3$) δ 0.86 (t, 3 H, J = 6.8), 1.23 (m, 10 H), 1.52 (m, 2 H), 1.96 (s, 3 H), 2.00 (s, 3 H), 2.03 (s, 3 H), 2.06 (s, 3 H), 2.07 (s, 3 H), 2.11 (s, 3 H), 3.35 (m, 2 H), 3.62 (m, 1 H), 3.65 (m, 2 H), 3.78 (dd, 1 H, J = 6.0, 10.0), 4.10 (m, 2 H), 4.18 (dd, 1 H, J = 6.4, 6.8), 4.25 (dd, 1 H, J = 4.8, 12.4), 4.77 (m, 1 H), 5.04 (m, 3 H), 5.18 (m, 2 H), 5.30 (dd, 1 H, J = 3.6, 10.0), 5.40 (m, 1 H), 5.44 (d, 1 H, J = 2.4), 7.47 (d, 2 H, J = 8.0), 8.21 (d, 2 H, J = 8.4); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 20.3, 20.6, 20.6, 20.7, 22.4, 22.6, 25.1, 26.0, 29.2, 29.2, 29.3, 31.8, 56.4, 61.9, 65.3, 67.0, 67.4, 67.7, 68.3, 68.4, 68.9, 71.7, 74.3, 77.3, 77.6, 95.9, 123.7, 128.2, 144.0, 156.0, 169.3, 169.4, 170.1, 170.2, 170.4, 170.6; HRMS (FAB+) m/z 907.3321 (MNa^+ $C_{40}H_{56}N_2O_{20}Na$ requires 907.3324).

Compound 41. The glycosyl acceptor **39** (0.17 g, 0.04 mmol) was concentrated in the reaction flask and dried under vacuum over P_2O_5. Glycosyl donor **29** (0.083 g, 0.132 mmol) was concentrated and dried under vacuum over P_2O_5, and dissolved in 1.5 mL of CH_2Cl_2. This solution was then transferred via syringe to the reaction flask, which was cooled to −42 °C under N_2, and 0.003 mL (0.023 mmol) of BF_3OEt_2 was added via syringe. The reaction mixture was stirred at −42 °C for 5 h and then quenched with saturated $NaHCO_3$ and diluted with $CHCl_3$. The organic layer was then washed with saturated $NaHCO_3$ (1 × 5 mL) and brine (1 × 5 mL), and the aqueous layers were extracted with 5 mL of $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by silica gel chromatography eluting with a gradient of 1:3 to 1:1 hexanes/EtOAc yielding 0.028 g (79%) as a clear glass: ^{1}H NMR (500 MHz, $CHCl_3$) δ 0.87 (t, 3 H, J = 7.0), 1.25 (m, 10 H), 1.56 (m, 2 H), 1.96 (s, 3 H), 1.97 (s, 3 H), 2.01 (s, 3 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 2.12 (s, 3 H), 3.40 (m, 2 H), 3.67 (m, 2 H), 3.77 (dd, 1 H, J = 5.5, 6.5), 3.86 (m, 2 H), 4.11 (m, 1 H), 4.25 (dd, 1 H, J = 8.0, 12.5), 4.35 (d, 1 H, J = 8.0), 4.79 (m, 1 H), 4.93 (dd, 1 H, J = 3.5, 10.5), 5.02 (app t, 1 H, J = 9.5), 5.05 (m, 1 H), 5.15 (d, 1 H, J = 8.0), 5.19 (m, 2 H), 5.37 (m, 2 H), 7.50 (d, 2 H, J = 8.0), 8.22 (d, 2 H, J = 8.5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 14.2, 20.6, 20.6, 20.7, 22.6, 22.6, 25.8, 29.2, 29.3, 29.4, 31.8, 31.8, 56.4, 61.9, 65.4, 67.0, 67.3, 68.4, 68.9, 68.9, 70.3, 70.3, 71.0, 71.6, 71.9, 101.3, 123.8, 128.1, 147.6, 155.1, 169.4, 169.5, 170.2, 170.2, 170.5, 170.6; HRMS (FAB+) m/z 907.3338 (MNa^+ $C_{40}H_{56}N_2O_{20}$-Na requires 907.3324).

Compound 40a. To a flask containing **40** (0.065 g, 0.073 mmol) was added 1.0 mL of MeOH and Ac_2O (0.07 mL, 0.74 mmol). This solution was stirred, and to it was added a catalytic amount of 10% Pd/C. H_2 was then bubbled through the stirring mixture for 10 min, and the mixture was allowed to stir under H_2 at rt for 3 h. The mixture was then filtered through Celite and concentrated. The crude product was purified by silica gel chromatography eluting with 1:1 hexanes/EtOAc to yield 0.03 g (55%) of a clear glass. ^{1}H NMR (500 MHz, $CHCl_3$) δ 0.87 (t, 3 H, J = 6.5), 1.30 (m, 10 H), 1.55 (m, 2 H), 1.93 (s, 3 H), 1.97 (s, 3 H), 2.01 (s, 3 H), 2.01 (s, 3 H), 2.06 (s, 3 H), 2.08 (s, 3 H), 2.12 (s, 3 H), 3.34 (m, 1 H), 3.59 (m, 2 H), 3.78 (dd, 1 H,J = 6.5, 10.5), 4.10 (dd, 1 H, J = 2.0, 12.0), 4.16 (app t, 1 H, J = 6.5), 4.25 (dd, 1 H, J = 4.5, 12.0), 4.81 (d, 1 H, J = 8.5), 5.01 (dd, 1 H, J = 9.5, 10.0), 5.10 (m, 2 H), 5.29 (m, 1 H), 5.40 (dd, 1 H, J = 9.5, 10.0), 5.43 (m, 1 H), 5.61 (d, 1 H, J = 8.5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 20.7, 20.7, 20.7, 20.7, 20.8, 22.6, 23.3, 26.0, 29.2, 29.3, 29.3, 30.7, 31.8, 55.3, 62.0, 66.8, 67.0, 67.8, 68.3, 68.4, 68.4, 68.5, 71.7, 71.8, 95.9, 100.0, 169.5, 170.1, 170.3, 170.4, 170.5, 170.6, 170.7; HRMS (FAB+) m/z 770.3210 (MNa^+ $C_{34}H_{53}NO_{17}Na$ requires 770.3211).

Compound 41a. To a flask containing **41** (0.02 g, 0.02 mmol) was added 0.5 mL of MeOH and Ac_2O (0.5 mL, 5 mmol). This solution was stirred, and to it was added a catalytic amount of 10% Pd/C. H_2 was then bubbled through the stirring mixture for 10 min, and the mixture was allowed to stir under H_2 at rt for 12 h. The mixture was then filtered through Celite and concentrated. The crude product was purified by silica gel chromatography eluting with 1:1 hexanes/EtOAc to yield 12 mg (80%) of a clear glass. ^{1}H NMR (400 MHz, $CHCl_3$) δ 0.87 (t, 3 H, J = 6.0), 1.26 (m, 10 H), 1.55 (m, 2 H), 1.95 (s, 3 H), 1.96 (s, 3 H), 2.01 (s, 3 H), 2.02 (s, 3 H), 2.04 (s, 3 H), 2.08 (s, 3 H), 2.12 (s, 3 H), 3.44 (m, 2 H), 3.56 (m, 1 H), 3.75 (m, 2 H), 3.84 (m, 2 H), 4.12 (dd, 1 H, J = 2.6, 10.4), 4.23 (dd, 1 H, J = 4.4, 10.4), 4.43 (d, 1 H, J = 8.0), 4.86 (d, 1 H, J = 8.4), 4.93 (dd, 1 H, J = 3.6, 10.4), 5.02 (dd, 1 H, J = 9.2, 10.0), 5.17 (dd, 1 H, J = 8.0, 10.4), 5.38 (m, 2 H), 5.66 (d, 1 H, J = 8.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.6, 20.6, 20.7, 20.7, 22.6, 22.7, 23.3, 25.8, 29.2, 29.3, 29.4, 31.7, 31.8, 55.3, 61.9, 66.3, 67.3, 68.5, 68.9, 70.2, 71.1, 71.4, 71.8, 76.4, 99.8, 101.2, 169.4, 169.5, 170.2, 170.3, 170.5, 170.7; HRMS (FAB+) m/z 770.3196 (MNa^+ $C_{34}H_{53}NO_{17}Na$ requires 770.3211).

Compound 7. A solution of 0.022 g (0.029 mmol) of **40a** in 1 mL of a 10 mM solution of NaOMe in MeOH was stirred for 24 h. The reaction was passed through a column of Dowex AG 50w-X2 cation-exchange resin (H^+ form), eluting with MeOH. The product was concentrated, redissolved in H_2O, and lyophilized to yield 13.8 mg (96%) of a white glass. ^{1}H NMR (500 MHz, CD_3OD) δ 0.87 (t, 3 H, J = 7.0), 1.27 (m, 10 H), 1.56 (m, 2 H), 1.93 (s, 3 H), 3.27 (m, 3 H), 3.36 (m, 2 H), 3.58−3.65 (m, 2 H), 3.69 (m, 2 H), 3.84 (m, 2 H), 3.86 (d, 1 H, J = 2.0), 3.97 (dd, 1 H, J = 5.5, 10.0), 4.37 (d, 1 H, J = 8.5), 4.72 (d, 1 H, J = 3.0); ^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.2, 23.9, 27.6, 30.6, 30.8, 30.8, 33.2, 57.4, 62.9, 69.2, 70.0, 70.4, 70.7,

71.0, 71.5, 72.3, 76.4, 78.1, 100.4, 103.2, 173.8; HRMS (FAB+) *m/z* 518.2567 (MNa^+ $C_{22}H_{41}NO_{11}Na$ requires 518.2578).

Compound 8. A solution of 0.012 g (0.016 mmol) of **41a** in 1 mL of a 10 mM solution of NaOMe in MeOH was stirred for 24 h. The reaction was passed through a column of Dowex AG 50w-X2 cation-exchange resin (H^+ form), eluting with MeOH. The product was concentrated, redissolved in H_2O, and lyophilized to yield 7.5 mg (95%) of a white glass. ^{1}H NMR (500 MHz, CD_3OD) δ 0.83 (m, 3 H), 1.20−1.35 (m, 10 H), 1.53 (m, 2 H), 1.89 (s, 3 H), 3.23 (m, 3 H), 3.30−3.45 (m, 4 H), 3.50−3.65 (m, 4 H), 3.72−3.85 (m, 2 H), 3.90−3.93 (m, 1 H), 4.11 (d, 1 H, J = 7.2), 4.35 (d, 1 H, J = 8.4); ^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.9, 27.3, 30.6, 30.8, 31.0, 33.2, 42.0, 50.0, 69.5, 70.2, 71.0, 72.3, 72.7, 74.8, 74.9, 75.7, 76.3, 78.1, 103.0, 105.0, 171.5; HRMS (FAB+) *m/z* 518.2579 (MNa^+ $C_{22}H_{41}NO_{11}Na$ requires 518.2578).

Biological Procedures. Subcloning of CHST2 and HEC-GlcNAc6ST. The genes for soluble, truncated versions of CHST2 and HEC-GlcNAc6ST, in which the cytosolic and transmembrane domains were replaced with a His_6 tail, were obtained from the vector pCDNA3.1/HisA (Invitrogen) generated at Roche Bioscience. The genes were subcloned by PCR using the following primers: forward CHST2: GCGGTACCATCCTGGCAATGGCACTCGGGGCACCGGGGGC; reverse CHST2: GCGTCGACGAGACGGGGCTTCCGAAGCAGGGTCTTGC; forward HEC-GlcNAc6ST: GCCGGTACCATCACAACATCAGCTCCCTGTCTATGAAGGC; reverse HEC-GlcNAc6ST: GCGTCGACGTGGATTTGCTCAGGGACAGTCCAGGTAGACA. The PCR products were digested with *Kpn*1 and *Xho*1 and ligated into the baculovirus expression vector pMelBac (Invitrogen).

Growth of Recombinant CHST2. One liter of Sf9 insect cells were grown in Sf-900 II SFM (Gibco, catalog no. 10902) media, infected with the baculovirus expression vector and harvested 3 days after infection. The cells were removed by centrifugation (10 min at 2000*g*), and the supernatant was collected. The sample was stored at −80 °C.

Growth of Recombinant HEC-GlcNAc6ST. Ten liters of Sf9 insect cells were grown in Sf-900 II SFM (Gibco, catalog no. 10902) media, infected with the baculovirus expression vector and harvested 3 days after infection. The cells were removed by centrifugation (10 min at 2000*g*), and the supernatant was collected. The supernatant was concentrated 12-fold, filtered, and washed (20 mM Tris, pH 8.0, 100 mM NaCl, 4 °C). The sample was stored at −80 °C.

Purification of Recombinant CHST2 and HEC-GlcNAc6ST. The supernatants from the above procedures were defrosted and centrifuged (15 min at 23000*g*) to remove any precipitate. The supernatants were then filtered through a Nalgene 0.20 μM filter with a prefilter, and the buffer was exchanged with 20 mM Tris, pH 8.0, 300 mM NaCl, 10 mM imidazole (TNI buffer) at 4 °C. For every 280 mL of concentrated, washed supernatant, a 30-mL nickel-nitrilotriacetic acid affinity resin superflow column (Qiagen catalog no. 30430) was prepared and equilibrated with the TNI start buffer. The crude protein was loaded onto the column, and the column was washed with 5 vols of TNI buffer. The protein was eluted with 2 vols of 20 mM Tris, pH 8.0, 300 mM NaCl, 250 mM imidazole. The fractions were checked for activity using the sulfotransferase assay. The active fractions were concentrated 5-fold by ultrafiltration (Centricon 30; Amicon). The concentrated fractions were then dialyzed into 10% glycerol, 20 mM Tris, pH 8.0, 100 mM NaCl, 0.01% Trition-X-100. The protein concentration was determined by Bradford Assay. The sample was aliquoted and frozen at −80 °C.

Enzymatic Synthesis of ^{35}S-PAPS. Carrier-free ^{35}S-PAPS was synthesized in vitro using a procedure similar to that of Long and co-workers.[43] Briefly, 1 mCi of carrier-free ^{35}S-Na_2SO_4 (American Radiolabeled Chemicals) and 30 mM ATP were incubated with approximately 45 μg of APS kinase (Calbiochem), 0.12 μg ATP sulfurylase (Sigma), and 8.3 μg inorganic pyrophosphatase (Sigma) in 60 μL Buffer A (20 mM Tris-HCl (pH 8.0), 30 mM KCl, 40 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 10% glycerol) for 12 h at 30 °C. Quantification of conversion was accomplished through separation of reaction components by thin-layer chromatography on polyethyleneimine (PEI)-cellulose eluting with 0.9 M LiCl, followed by phosphorimaging analysis (model 445 SI, Molecular Dynamics). Incorporation of the ^{35}S label into ^{35}S-PAPS typically exceeded 90%.

Assays for Sulfotransferase Activity. Standard assay reactions of 50 μL were carried out in 1.5 mL eppendorf tubes at pH 6.5 in Buffer ST (30 mM HEPES, 1% Triton-X-100, 4 mM $Mg(OAc)_2$, 10 mM NaF, 1 mM ATP, 10% glycerol). The reactions were performed with 1 mM disaccharides, 5 μCi ^{35}S-PAPS, 2 μM PAPS, and 2 μL enzyme. Reactions were run for 8 h at room temperature, with 10 μL aliquots taken out every 2 h and diluted with 20 μL of MeOH. These aliquots were then spotted on Whatman 19-lane silica gel TLC plates and eluted with 6:3:2 BuOH:EtOH:H_2O. The plates were dried and exposed by phosphorimaging to determine % incorporation of ^{35}S-sulfate over time. Total radioactivity of product was divided by radioactivity of the whole TLC lane and then multiplied by concentration of PAPS and divided by the % purity of PAPS to yield the final concentration of radiolabeled product. Total substrate turnover did not exceed 15%. Rates were determined by nonlinear regression, and these rates were compared to control substrate **2**. K_m determinations were made by varying concentration of substrate **2** (serial dilutions from 5 mM to 0.04 mM), determining the rates, and using nonlinear regression software (SAS).

Sulfotransferase assays executed during protein purification steps were performed in 96-well microtiter plates using compound **2** (1.25 mM), PAPS (2.5 μM), ATP (1 mM) and 0.05 μCi ^{35}S-PAPS, in 30 mM Hepes pH 6.5, 4 mM $Mg(AcO)_2$, 10 mM NaF, 10% Glycerol, 1% Triton-X-100. Reaction mixtures (20 μL) were incubated overnight at 25 °C. The reactions were stopped with the addition of 100 μL of distilled water. Reactions were then transferred from the 96-well plate to preconditioned Oasis HLB reversed-phase extraction plates (Waters) and washed with distilled water. The radiolabeled substrate was eluted with 200 μL/well MeOH, and the plates were air-dried. The amount of radioactivity incorporated into the substrate was determined by scintillation counting. Reactions were run in duplicate, with and without compound **2**.

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