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α-(2,6)-Sialyltransferase-Catalyzed Sialylations of Conformationally Constrained Oligosaccharides

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Abstract: It is demonstrated that conformationally restricted oligosaccharides can act as acceptors for glycosyltransferases. Correlation of the conformational properties of *N*-acetyl lactosamine (Galβ(1-4)GlcNAc, LacNAc) and several preorganized derivatives with the corresponding apparent kinetic parameters of rat liver α-(2,6)-sialyltransferase-catalyzed sialylations revealed that this enzyme recognizes LacNAc in a low energy conformation. Furthermore, small variations in the conformational properties of the acceptors resulted in large differences in catalytic efficiency. Collectively, our data suggest that preorganization of acceptors in conformations that are favorable for recognition by a transferase may improve catalytic efficiencies.

Introduction

Protein- and lipid-bound oligosaccharides play critical roles in a diverse range of biological processes such as protein folding, cell−cell communication, bacterial adhesion, viral infection, and masking of immunological epitopes.[1,2] They are also important in health science and are involved in the attachment and invasion of pathogens, inflammation, metastasis, and xenotransplantation. Unlike nucleic acid and protein biosynthesis in which the order of attachment of nucleotides and amino acids is read from a template, glycosylation is a nontemplated process. Oligosaccharides are assembled by glycosyltransferases, which transfer monosaccharide residues from nucleoside mono- or diphosphate sugars to growing oligosaccharide chains.[3,4] The level of expression of glycosyltransferases and their donor and acceptor specificities determine the structure of a biosynthesized oligosaccharide. These parameters may differ between cell type and development stage leading to cell-specific glycosylation. The mechanism of this metabolic control is complex, and, in particular, the origin of *acceptor specificity* is not well understood. Several studies have indicated that not only primary structures but also conformational properties of oligosaccharides are important for acceptor specificities. Most oligosaccharides have some degree of flexibility around their glycosidic linkages and, as a result, can adopt several distinct low energy minima on their conformational surface.[5,6] Examination of the structural properties of a wide range of glycoproteins has revealed that glycosidic torsional angles may deviate as much as 20−30° from minimum energy conformations.[7] Furthermore, in the binding site of proteins, not only global but also secondary minima with very different conformations can be complexed.[6] In addition, there is evidence that the conformational properties of a glycosidic linkage can be modulated by the macromolecular structure it is part of.[8] In particular, addition or removal of flanking oligosaccharide residues or attachment of oligosaccharides to proteins and lipids may result in conformational changes. This modulation of properties may have important implications in the control of glycan biosynthesis.

Little is known about the effect of interresidual flexibility of glycosyl acceptors on kinetic parameters of glycosyl transfer.[9] Furthermore, structural studies have provided little information about the conformation of a glycosyl acceptor that is recognized by a glycosyltransferase.[10] To address these issues, we have designed and synthesized several conformationally constrained[11−18] *N*-acetyl lactosamine (LacNAc) derivatives for

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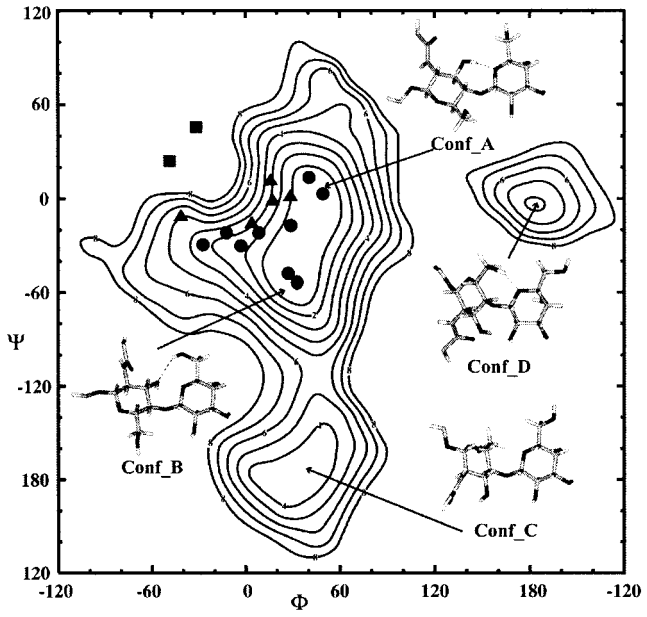

Figure 1. Energy map of LacNAc glycosidic linkage[21] calculated with MM3. Conformations accessible to compounds **3**−**5** have been superimposed as ■ = **3**, ▲ = **4**, ● = **5**.

which apparent kinetic parameters of rat liver α-(2,6)-sialyltransferase-catalyzed sialylations have been determined. These data have been correlated with conformational properties of these acceptors obtained by computer modeling and NMR spectroscopy.

Sialyltransferases are an important class of glycosyltransferases that catalyze the transfer of the *N*-acetyl neuraminic acid (Neu5Ac) moiety of CMP-Neu5Ac to the carbohydrate moiety of glycolipids and proteins. All known sialyltransferases have two conserved regions in their catalytic domain, which are proposed to be important for binding the common substrate CMP-Neu5Ac and catalysis.[19] To date, no NMR or X-ray crystal structures of sialyltransferases have been reported, and information about acceptor specificities has mainly been obtained from studies with chemically modified acceptors. These studies have revealed the C-6′ hydroxyl and acetamido group of LacNAc are essential for sialylation by rat liver α-(2,6)-sialyltransferase.[20] Furthermore, glycosylation of the C-3′ and C-4′ and C-3 hydroxyl lead to inactivation of the substrate, whereas removal of other hydroxyls such as those at C-2′ and C-6 leads to some reduction of acceptor activity.

Previously, the conformational properties of LacNAc have been investigated by molecular mechanics calculations (Figure 1).[21] This disaccharide is highly flexible with a global minimum energy conformation around $\Phi = 50°$ ($\Phi = H_1'-C_1'-O_1'-C_4$) and $\Psi = 0°$ ($\Psi = C_1'-O_1'-C_4-H_4$) (conf A) and a second low minimum around $\Phi = 30°$ and $\Psi = -60°$ (conf B). Regions C and D correspond to remote parts of the main low energy region and should be considered as secondary minima. An analysis of reported X-ray crystal structures of oligosaccharides/protein complexes that contain a LacNAc moiety

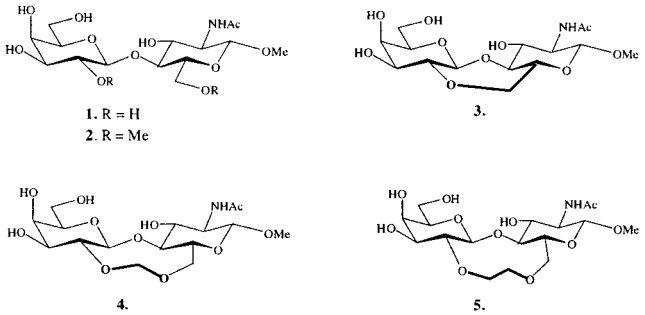

Figure 2. Disaccharide derivatives **1**−**5**.

showed that in the bound state, LacNAc is mainly observed in conf A, some in conf B, and one case in conf D.[7,22]

In conformations A and B, the C-6′ hydroxyl and the acetamido moiety are at the same face of the molecule, and both of these residues serve critical roles in substrate recognition by rat liver α-(2,6)-sialyltransferase.[20] The C-6 and C-2′ hydroxyls, which are not critical for transferase activity, are at the other face of the molecule, and these functionalities were selected for incorporation of tethers (Figure 2). Compound **2** is a reference compound in which the C-2′ and C-6 hydroxyls of LacNAc are methylated. Compound **3** is a 2′,6-anhydro-derivative, whereas **4** and **5** contain methylene and ethylene tethers, respectively.

Results and Discussion

Synthesis. The target compounds **2**, **3**, and **5** were prepared from orthogonally protected LacNAc **8**, which has a *tert*-butyl dimethyl silyl ether at C-6 and an acetyl ester at C-2′ (Scheme 1). This disaccharide could be converted into alcohol **9** which was the precursor of **3** and into diol **10** which was used for the preparation of **2** and **5**. Compound **4** was obtained from the corresponding disaccharide analogue **21** (Scheme 2).

The key disaccharide **8** was obtained in good yield of 69% by coupling the known trichloroacetimidate donor **6**[23] with glycosyl acceptor **7**[24] in dichloromethane at −40 °C using $BF_3 \cdot OEt_2$ as the promoter. Treatment of **8** with HBF_4 in H_2O/acetonitrile resulted in removal of the TBDMS ether to afford **9** in almost quantitative yield. Deacetylation of **9** under standard conditions gave the desired diol **10**.

The reference compound **2** was easily obtained by O-methylation of **10** using methyl iodide, BaO, and $Ba(OH)_2$ in DMF[25] to give **11** followed by debenzylation by catalytic hydrogenation over Pd/C. Other methylation methods such as treatment with methyl triflate in the presence of lutidine or methyl iodide in combination with silver oxide[26] were unsuccessful.

The protected 2′,6 anhydro-derivative **3**[27] was prepared by a three-step procedure starting from **9**. Thus, mesylation of **9** using mesyl chloride (MsCl) in pyridine gave **12**, which was deacetylated using sodium methoxide in methanol to afford **13**. Cyclization of **13** was accomplished using NaH, and although

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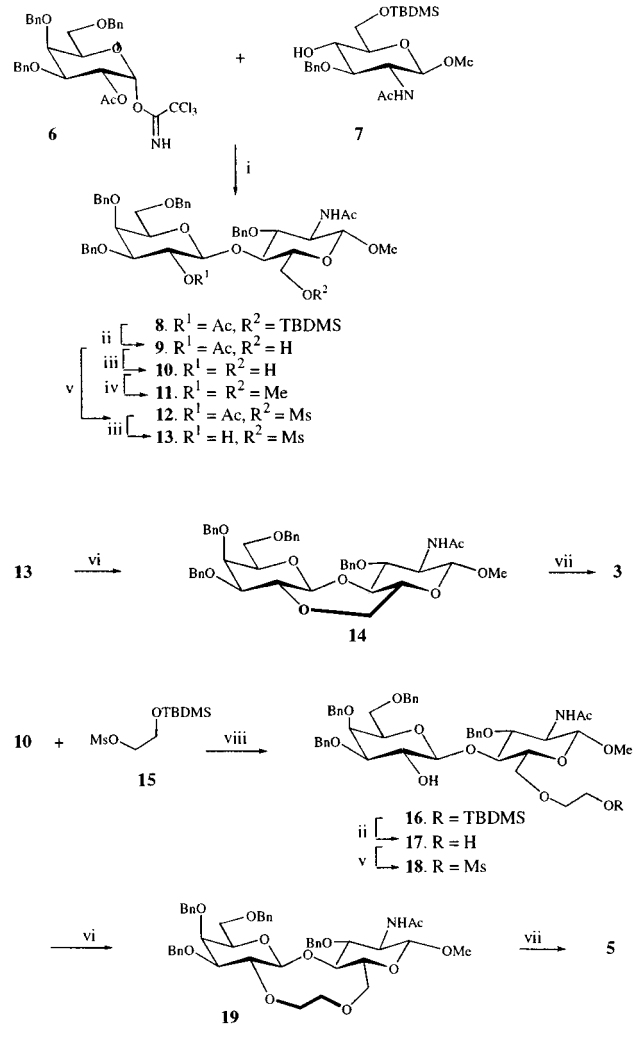
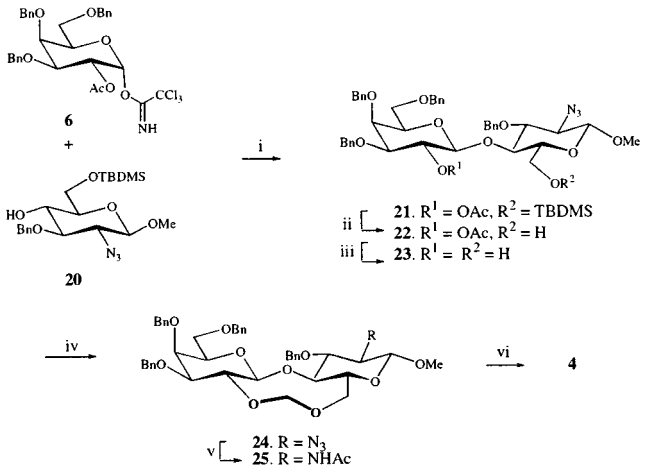
Scheme 1[a]



[a] Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, −40 °C; (ii) 48% HBF_4 in H_2O, MeCN; (iii) NaOMe, MeOH; (iv) MeI, BaO, $Ba(OH)_2$, DMF; (v) MsCl, pyridine; (vi) NaH, DMF; (vii) Pd/C, H_2; (viii) NaH, DMF, 50 °C.

Scheme 2[a]



[a] Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, CH_2Cl_2, −40 °C; (ii) 48% HBF_4 in H_2O, MeCN; (iii) NaOMe, MeOH; (iv) $(PhS)_2CH_2$, NIS, TfOH, CH_2Cl_2; (v) $HS(CH_2)_3SH$ then Ac_2O, pyridine; (vi) Pd/C, H_2.

this reaction involved the formation of a rigid eight-membered ring, compound **14** was obtained in a yield of 70%.

The ethylene-bridged derivative **5** was obtained by a multistep procedure, whereby **10** was first regioselectively condensed with mesylate **15** followed by removal of the TBDMS ether; conversion of the resulting alcohol into a good leaving group allowed macrocyclization. The ethylene linker **15** was prepared by a one-pot two-step procedure,[28] whereby ethylene glycol was monosilylated with sodium hydride and TBDMSCl in DMF followed by sulfonylation by addition of methanesulfonyl chloride and triethylamine. Condensation of **10** with **15** in the presence of NaH gave **16**, and although a relatively large excess of **15** was required to drive the reaction to completion, very little substitution at C-2′ was observed. The TBDMS group of **16** was removed by treatment with HBF_4 in H_2O/acetonitrile to give alcohol **17** which was mesylated using standard conditions to give methyl sulfonate ester **18** in a yield of 47% over three steps. Ethylene-bridged **19** was obtained in a yield of 60% by NaH-mediated cyclization of **18** in DMF. Catalytic hydrogenation over Pd/C of compounds **14** and **19** gave **3** and **5**, respectively.

Attempts to prepare methylene-bridged LacNAc **4** by reaction of diol **10** with $(PhS)_2CH_2$ in the presence of NIS/TMSOTf [12] resulted only in the formation of trace amounts of product. The failure of this reaction was attributed to the presence of the acetamido group, which interfered unfavorably with the reagents. To address this problem, the analogous disaccharide **21** was prepared, which instead of the acetamido has an azido moiety at C-2 (Scheme 2). This disaccharide was obtained in a yield of 80% by a $BF_3{\cdot}OEt_2$-promoted glycosylation of **6** with **20** at −40 °C in dichloromethane. Treatment of **21** with tetrafluoroboric acid in dichloromethane gave **22**, which was deacetylated under standard conditions to afford **23**. In this case, the diol **23** could be cyclized by intramolecular methylene acetal formation by reaction with $(PhS)_2CH_2$ in the presence of NIS/TMSOTf[12] to give **24** in a acceptable yield of 60%. Reduction of the azido group of **24** with propanedithiol[29] followed by N-acetylation with acetic anhydride in pyridine gave **25**. Finally, debenzylation of **25** by catalytic hydrogenation over Pd/C gave the 2′6-methylene acetal **4**.

The presence of the linkers of **3**, **4**, and **5** was confirmed by HMBC experiments which allows the correlation between carbons and protons linked over more than one bond and supported by NOE data. Cross-peaks in the HMBC spectra were observed corresponding to the connectivities between the two sugars other than the glycosidic linkage, that is, H-2′ of galactoside and C-6 of the glucoside of compound **3**, C-7 from the tether of compound **4**, and C-8 from the tether of compound **5**, demonstrating that the disaccharides were connected.

Conformational Analysis. The conformational properties of compounds **1**−**5** were probed by a combination of molecular mechanics simulations and NMR spectroscopy. A systematic conformational search was performed on Ψ and Φ torsional angles of **3**−**5** using the SEARCH procedure of the SYBYL software package (Tripos Inc., St Louis, MO). For the cyclic compounds, conformational families were determined using a clustering procedure. Compound **3** appears to be the most restricted compound with only 14 possible conformers in two conformational families. Compound **4** can adopt 242 conforma-

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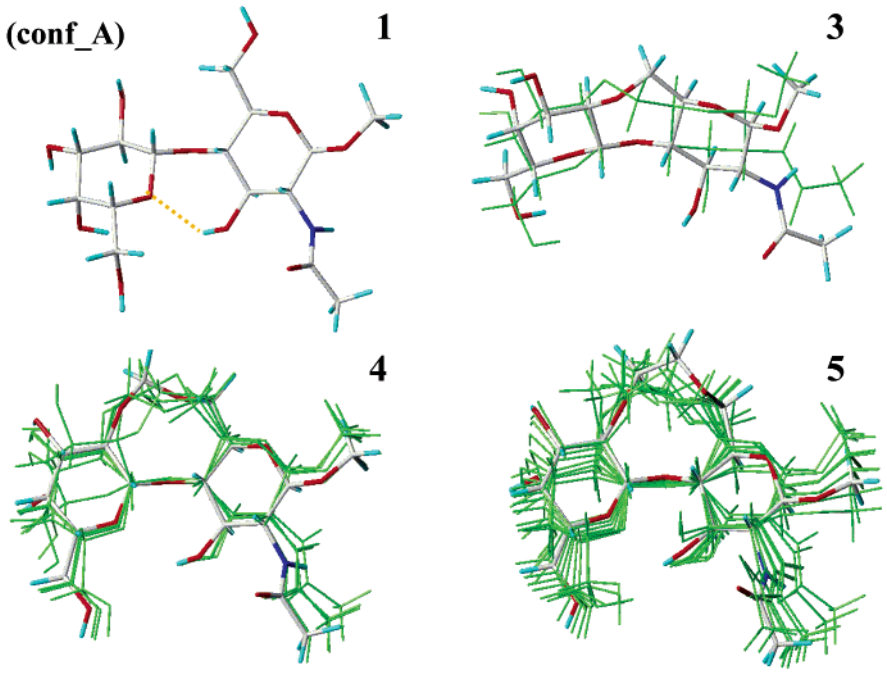

Figure 3. Low energy conformations of compounds **3**−**5**. For each compound, the lowest energy conformation has been colored according to atom types. Conformer A of LacNAc is also displayed for comparison.

tions scattered in seven families, and compound **5** is the most flexible of the cyclic compounds with 2989 conformers in 14 different families. The lowest energy conformer of each family was fully optimized using the MM3 program.[30] Figure 3 displays the obtained low energy conformations, and their complete geometrical characterizations are listed in Supporting Information. To compare the conformational properties of the conformationally constrained compounds with the properties of LacNAc, the low energy conformations of **3**−**5** were superimposed on the energy map of LacNAc (Figure 1). Derivative **3** is highly constrained and can adopt only two conformations, both with similar but unusual conformations at the linkage. Compound **4** can exist in a larger range of conformations; however, the Φ dihedral angle is restricted to values smaller than 30° which are not entirely similar to minimum energy conformations of LacNAc. Compound **5** can attain conformations in the relatively large energy plateau of LacNAc that include syn conformations A and B. However, the anti- (conf C) and gauche−gauche conformer (conf D) cannot be adopted. In all derivatives, the ω torsion angle that is involved in the bridge cannot adopt its lowest energy orientation since such orientation directs the O-6 atom far from the galactose residue and is not compatible with the establishment of the bridge.

NMR Data and Comparison with Molecular Modeling. The ^{1}H and ^{13}C spectra of compounds **1**−**5** were assigned by a combination of one-dimensional proton, and two-dimensional HSQC and HSQC-TOCSY experiments. Assignment of pro-R and pro-S hydroxymethylene protons of the GlcNAc moiety of **1** and **2** was based on relative $^{3}J_{HH}$ coupling constants assuming predominantly gg and gt rotamer populations.[31] The stereospecific assignments for compounds **3**−**5** were based on $^{3}J_{H5,H6}$ and $^{3}J_{C4,H6}$ values and interresidual NOE's assuming tg or eclipsed Gln-H5/H6 rotamer populations.

Trans-glycosidic heteronuclear coupling constants, which are sensitive to orientations around glycosidic linkages, were measured using a quantitative HMBC experiment.[32] The resulting values are listed in Table 1 together with the calculated values for each low energy conformer using the empirical Karplus-type equation proposed by Tvaroska et al.[33] This approach allowed a direct comparison of the experimental coupling constants with the theoretical ones obtained by averaging the populations of conformers using a Boltzmann equation for energy-weighting. For LacNAc **1**, the $^{3}J_{H1'-C4}$ and $^{3}J_{H4-C1'}$ values of 2.9 and 2.5 Hz, respectively, support the mixture of conformers determined by the modeling studies. To obtain a good agreement with experimental data, the computed population of conformer B should be higher. As expected, compound **2** gave similar values to **1** (3.3, 2.6 Hz). Compound **3** provided a small value of $^{3}J_{H1'-C4}$ (1.2 Hz) corresponding to Φ of approximately −60°, and due to spectral overlap, $^{3}J_{H4-C1'}$ could not be measured. Compound **3** provided additional NOE and *J* coupling data that was most consistent with the lowest energy conformer. A $^{3}J_{H5-H6R}$ value of 7.3 Hz, a $^{3}J_{H6S-C2'}$ value of 7 Hz, small $^{3}J_{H6R-C4}$ and $^{3}J_{H6S-C4}$ values (<1.5 Hz), and a large H2′−H6R NOE (2.4 Å) are in agreement with a very unusual eclipsed orientation of the ω torsion angle. In the lowest energy conformer, ω adopts a value of −102° (see Supporting Information) with a small H2′−H6R distance (2.1 Å vs experimentally derived 2.4 Å). Experiments and modeling

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(32) Additional measurements were made using two-dimensional HSQC type experiments such as "Exside" (Krishnamurthy, V. *J. Magn. Reson., Ser. A* **1996**, *121*, 33−41) and HSQCMBC (Williamson, R.; Marquez, B.; Gerwick, W.; Kover, K. *Magn. Reson. Chem.* **2000**, *38*, 265−273). In all the compounds, significant signal overlap and strong coupling restricted their application, and only a few values ($^{3}J_{H1'-C4}$ for compounds **1**, **2**, and **3**) could be obtained which agreed within experimental error with those obtained via the HMBC experiment. The application of other methods that depend on carbon selective pulses (for example, Nishida, T.; Widmalm, G.; Sandor, P. *Magn. Reson. Chem.* **1996**, *34*, 377−382) did not provide useful data.
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Table 1. Comparison of Experimental and Theoretical NMR Values of LacNAc and LacNAc Derivatives[a]

	Φ_H (deg)	$J_{H1'-C4}$ (Hz)	Ψ_H (deg)	$J_{H4-C1'}$ (Hz)	H1′–H4 (Å)	H1′–H6R (Å)	H1′–H6S (Å)
compd **1**							
conf_A	45	2.9	−5	5.5	2.2	2.8	3.2
conf_B	28	4.4	−54	2.1	2.4	4.1	4.5
conf_C	43	3.1	−174	6.7	3.7	5.2	4.1
conf_D	176	6.8	−2.6	5.6	3.6	4.5	5.4
av conf		3.7		3.9	2.3	3.1	3.5
exp		2.9		2.5	2.4	2.9	3.0
compd **2**							
exp		3.3		2.6	2.5	2.9	n.m.
compd **3**							
conf_1	−47	2.7	25	4.6	2.1	4.5	4.2
conf_2	−29	4.3	49	2.6	2.2	2.1	3.7
av conf		3.1		4.2	2.1	2.7	4.1
exp		1.2		n.m.	2.3	4.1	4.0
compd **4**							
conf_1	−0	5.6	−14	5.3	2.0	3.5	4.9
conf_2	−42	3.2	−13	5.3	2.3	5.5	4.2
conf_3	13	5.3	17	5.1	2.1	2.2	3.9
conf_4	24	4.7	0	5.6	2.2	3.7	4.4
conf_5	12	5.4	3	5.6	2.0	4.3	3.8
av conf		5.5		5.3	2.0	3.3	4.8
exp		4.2		5.9	2.1	n.o.	n.o
compd **5**							
conf_1	−5	5.6	−32	4.0	2.15	5.40	4.38
conf_2	−1	5.6	−14	5.3	2.01	3.47	4.94
conf_3	3	5.6	−18	5.1	2.02	5.01	3.83
conf_4	24	4.7	−48	2.7	2.31	4.39	5.41
conf_5	13	5.3	−9	5.5	2.05	2.80	4.41
conf_6	−18	5.1	−22	4.8	2.16	4.06	5.36
conf_7	46	2.9	6	5.5	2.36	3.37	4.12
conf_8	15	5.2	−59	3.4	2.28	4.10	5.34
conf_9	39	3.4	13	5.3	2.25	2.46	3.91
av conf		5.3		4.2	2.13	4.42	4.38
exp		4.5		4.6	2.3	n.o.	n.o.

[a] n.m. not measured, n.o. not observed; exp. *J* values ±15%; exp. NOE values ±0.3 Å.

therefore indicate that the 6-hydroxymethyl−C2′ bridge rotates H6R to eclipse H5, and thus rotates the Φ angle into the unusual −60° value. The proposed conformation moves the C-2 acetamido group away from the face containing the C-6′ hydroxyl. Compound **4** has values of both $^3J_{H1'-C4}$ (4.2 Hz) and $^3J_{H4-C1'}$ (5.9 Hz) significantly larger than those of **1**, consistent with the major cluster of conformers proposed by the modeling studies. Compound **5** also has coupling values larger than that of **1** (4.5, 4.6 Hz), and the $^3J_{H4-C1'}$ is consistent with the predicted high population of Ψ torsional angle close to 0°, and therefore similar to conformer A of compound **1**.

Distance constraints were obtained from relative NOE intensities using the isolated spin-pair approximation. In Table 1, the measured distances are compared to the ones obtained from each low energy conformer. The theoretical averaged distances have been calculated from an $\langle r^{-6}\rangle^{1/6}$ distance using a Boltzmann population. All compounds showed a strong NOE between H1′ and H4, which was expected for compounds restricted to syn conformations. Compounds **1** and **2** showed an NOE between H1′−H6R and H6S, which is indicative of favored gt orientation of the ω torsion angle for the GlcNAc residue in LacNAc. This NOE was very weak for compound **3** and was not observed for compounds **4** and **5**, which is in agreement with the computer modeling data.

Enzyme Kinetics. The apparent kinetic parameters for the α-(2,6)-sialyltransferase-catalyzed reaction of CMP-(^{14}C)-Neu5Ac with acceptors **1**−**5** were determined using a reported assay[34,35] (Table 2). The K_m for **1** was in close agreement with previous data, and its V_{max} was set at 1. Compound **2**, containing methoxy groups at C-6 and C-2′, has a significantly elevated K_m and a somewhat smaller V_{max}, indicating that these hydroxyls are of some importance for the transferase. The anhydro-derivative **3** is not a substrate, whereas the methylene-bridged compound **4** has similar kinetic parameters as compared to that of **2**. Importantly, the ethylene-tethered derivative **5** has the highest catalytic efficiency of all compounds tested, suggesting that it is preorganized in a favorable conformation.

Table 2. Apparent Kinetic Parameters of Rat Liver α-(2,6)-Sialyltranferase-Catalyzed Sialylation of Compounds **1**−**5**[a]

acceptor	K_m (mM)	rel V_{max}	(rel V_{max})/ K_m (mM^{-1})
1	1.7 ± 0.2	1	0.6
2	11.2 ± 0.8	0.5	0.04
3	n.a.	n.a.	n.a
4	11.6 ± 1.9	1.0	0.09
5	1.0 ± 0.1	1.3	1.2

[a] n.a. compound not active up to 2.0 mM of substrate.

A preparative enzymatic reaction was performed to ensure that the sialylation had occurred at C-6 and that the tether of compound **5** was still intact. The structure of the resulting tethered trisaccharide **6** was confirmed by ^{1}H NMR and MS analyses. The spectroscopic data were in agreement with that of the methyl glycoside of the corresponding linear trisaccharide.[36]

The observation that compound **5** is the best acceptor tested indicates that it is preorganized in a conformation that is favorable for the transferase. The low energy conformations of this compound are centered about the A- and B-conformers of LacNAc with A being the most populated one. Thus, these combined findings indicate that the enzyme recognizes LacNAc in one of its main low energy conformations with conf A being the most probable one. Correlating the properties of compounds **4** and **5** reveals that small differences in conformational behavior may result in significant differences in apparent kinetic parameters. In the case of **4**, a small shift outside the main minimum resulted in a much larger K_m, but, importantly, the compound can still act as an acceptor. When larger conformational changes are induced, such as in compound **3**, glycosyl-accepting properties are lost. The similarity of apparent kinetic parameters for compounds **2** and **4** was surprising. Probably, the unfavorable conformation of **4** is compensated by less reduction of flexibility upon binding. The K_m of **5** is approximately 10 times smaller than that of the dimethoxy analogue **2** with a 3-fold increase in V_{max}. Both compounds essentially have the same hydrogen-bonding potential and hydrophobic and hydrophilic surface but differ in conformational properties. Therefore, the significantly higher catalytic efficiency (V_{max}/K_m) of **5** probably results from a more favorable enzyme−substrate association (approximately 1.5−2.0 kcal/mol) due to preorganization of the acceptor in a conformation that is recognized by the enzyme. It is important to note that apart from preorganization of the glycosidic linkages of **2** and **5**, their C-6 and C-2′ substitutions (ethyl bridge vs two methyl groups) are also conformationally different. These

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positions of the disaccharide are thought to be at the periphery of the enzyme-binding site, and it is unlikely that these conformational modifications can account for the observed differences in catalytic efficiency. Thus, we propose that the glycosidic linkage of **5** will lose less conformational flexibility upon binding than **2**, resulting in a more favorable association. This result is highly significant because most of the previously reported studies with conformational constrained oligosaccharides failed to produce more favorable free energies of binding.[11,13,18]

Experimental Section

Molecular Modeling. Nomenclature. Torsion angles at the glycosidic linkage have been defined with reference to the hydrogen atom: $\Phi_H = H_1'-C_1'-O_1'-C_4$ and $\Psi_H = C_1'-O_1'-C_4-H_4$ (see Supporting Information).

Conformational Analysis of the Cyclic Derivatives. All energy calculations were performed using the TRIPOS force field[37] complemented by carbohydrate energy parameters.[38] First, it was checked that the relaxed (Φ,Ψ) energy map of LacNAc calculated with the TRIPOS force field was similar to the MM3 energy map, previously published.[21] Starting conformations of compounds **3**, **4**, and **5** were built using low energy conformation of LacNAc and editing and optimizing the molecule while checking that no distortion of pyranose rings occurred. The possible conformations of compounds **3**, **4**, and **5** were determined using the SEARCH procedure of SYBYL. The ring closure bond was chosen to be μ6 in compound **3**, ν6 in compound **4**, and ν2 in compound **5**. Tolerance for ring closure was 0.3 Å for bond length and 20° for bond angles. Several conditions were tested on compound **3** and **4**. A search step of 10° with van der Waals cutoff of 0.6 and energy window of 20 kcal/mol was appropriate for the conformational studies. The hydroxylic hydrogen atoms were not taken into account during this rigid search. The energy was evaluated for each conformation, but the electrostatic contribution was not included. These conditions were applied for the three compounds. The resulting conformations were then analyzed by a homemade clustering program to determine conformational families. The lowest energy conformer of each family was then fully optimized with the use of the MM3 force field,[30] using a dielectric constant of 80 to simulate water environment. Some of the families converged to the same conformation. Low energy conformations are displayed, and their characteristics are listed in Supporting Information.

Heteronuclear $^3J_{C-H}$ coupling constants across the glycosidic linkages were calculated using the Karplus-type equation proposed by Tvaroska.[33]

$$^3J_{C-H} = 5.7 \cos^2 \Phi - 0.6 \cos \Phi + 0.5$$

Averaging of NMR parameters $\langle ^3J_{C-H} \rangle$ and $\langle r^{-6} \rangle$ was performed on the population of conformers, taking into account the probability of existence (P_i) of each conformer with energy E_i at a given temperature T as governed by a Boltzmann distribution. This procedure has been detailed in the conformational analysis of sucrose.[39]

NMR Spectroscopy. The disaccharides (2−4 mg) were dissolved in 99.96% D_2O. Data were collected on Varian 500, 600, and 800 MHz spectrometers at 25 °C, except for some data on compound **4**, which were collected at 21 °C. Complete proton and carbon assignments are presented in the Supporting Information. Standard Varian gradient HSQC and HSQC-TOCSY pulse sequences were used for the assignment data.

Heteronuclear Coupling Constants. The three-bond proton-carbon coupling constants ($^3J_{CH}$) were measured according to Zhu et al.[40] A typical data set was 600 × 64 complex points, covering a ^{1}H range of 3.5 ppm and ^{13}C range of 60 ppm. Sufficient transients were collected to obtain a signal-to-noise of ~20:1 for long-range cross-peaks. Data were processed using Gaussian apodization functions and zero-filling in both dimensions. No special noise reduction procedures were used. The cross-peak intensities were measured by integrating the peak volumes in the two-dimensional spectra, or by extracting one-dimensional slices from these cross-peaks. Ratios of the long-range cross-peak and the one-bond reference volumes were used to calculate J values, according to Zhu et al.[40] In cases where neighboring cross-peaks distorted the baseline significantly, making volume integration unreliable, the one-dimensional slices of these volumes were matched, and a scaling factor was extracted. The corrections due to differences in $T1$ and $T2$ for ^{13}C- and ^{12}C-bonded protons canceled within the experimental error for the protons that were measured. The values reported thus have an error of ±15% primarily due to signal-to-noise and uncertainties in integration due to baseline distortions.

NOE Experimental Procedures. For each compound, selective one-dimensional NOESY experiments[41] were used to obtain buildup curves for the anomeric and other resolved protons. For compound **1**, the anomeric protons were overlapped, and therefore a one-dimensional-TOCSY-NOESY sequence was employed.[42] In this experiment, H4′ was selectively irradiated, followed by TOCSY transfer to H1′, which then was selectively irradiated for the subsequent NOESY step. Similarly, H-5 was selectively irradiated prior to the TOCSY transfer to H-1. For each compound, good linearity of the NOE buildup curves was observed up to a mixing time of 300 ms. For compounds **2**, **3**, and **5**, the interproton distances were determined by using the intraring H-1′−H-2′ NOE as an internal reference (3.09 Å).[43] For compounds **1** and **4**, the NOE between H-1′ and H-2′ could not be accurately measured; therefore, H-1−H-5 (2.37 Å)[43] was employed as internal reference.

General Methodology. Chemicals were purchased from Aldrich and Fluka and used without further purification. Molecular sieves were activated at 350 °C for 3 h in vacuo. Dichloromethane was distilled from CaH_2 and stored over 4 Å molecular sieves. All of the reactions were performed under anhydrous conditions and monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was by examination under UV light (254 nm) and by charring with 10% sulfuric acid in methanol. Flash chromatography was performed on silica gel (Merck, mesh 70−230). Extracts were concentrated under reduced pressure at <40 °C (bath). ^{1}H NMR (1-D, 2-D) and ^{13}C NMR spectra were recorded on a Varian Merc300 spectrometer and Varian 500, 600, and 800 MHz spectrometers equipped with Sun workstations. For ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$, chemical shifts (δ) are given in ppm relative to solvent peaks (^{1}H, δ = 7.26; ^{13}C, δ = 77.3) as internal standard. Negative ion matrix assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were recorded using an HP-MALDI instrument using gentisic acid matrix. Optical rotations were measured on a Jasco P-1020 polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^3 g^{-1} at 25 °C, 50 mm cell.

The rat liver α-(2,6)-sialyltransferase was purchased from Sigma. CMP-[^{14}C]Neu5Ac was obtained from Amersham Corp. CTP, CMP-Neu5Ac, and calf alkaline phosphatase were purchased from Calbiochem. ACS liquid scintillation cocktail was obtained from Fisher

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Scientific. All other chemicals were of analytical grade. Compounds **1**−**5** were purified by chromatography on Iatrobeads (Iatron laboratories, 6RS-8060), followed by chromatography on biogel P2 (Biorad).

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (8). Boron trifluoride diethyl etherate (0.9 mL, 7.1 mmol) was added to a stirred suspension of **6** (4.5 g, 7.1 mmol), **7** (2.0 g, 4.7 mmol), and powdered molecular sieves 4 Å (4.0 g) in dry dichloromethane (50 mL). The mixture was left stirring at −40 °C for 3 h. TLC (toluene/ethyl acetate, 1/1, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (1.4 mL, 10.0 mmol) and then filtered over Celite. The filtrate washings were combined and concentrated under reduced pressure. The residue was then diluted in dichloromethane (100 mL), washed successively with a saturated solution of $NaHCO_3$ (20 mL), water (2 × 20 mL), and brine (20 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (gradient hexane/ethyl acetate, 3/1 to 1/1, v/v). The yellow solid was crystallized from diethyl ether/ethanol, and the product was washed with cold diethyl ether to give compound **8** (2.97 g, 69%) as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.60−7.15 (m, 20H, arom), 6.1 (d, 1H, $J_{NH,2}$ 8.8, N*H*), 5.30 (dd, 1H, $J_{2',3'}$ 9.7, H-2′), 4.73 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.46 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.73−4.20 (m, 4H, 2 x OCH_2Ph), 4.40 (d, 1H, $J_{1,2}$ 5.7, H-1), 4.38 (d, 1H, $J_{1',2'}$ 7.9, H-1′), 4.0−3.88 (m, 2H, H-6b, H-6b′), 3.96 (d, 1H, H-4′, $J_{3'-4'}$ 2.74), 3.90 (m, 1H, H-2), 3.72−3.68 (m, 2H, H-5′, H-6a′), 3.61−3.38 (m, 4H, H-3, H-4, H-5, H-6a), 3.50 (m, 1H, H-3′), 3.35 (s, 3H, OCH_3), 2.02 (s, 3H, CH_3C(O)NH), 1.90 (s, 3H, CH_3C(O)O), 0.81 (s, 9H, Si(CH_3)$_3$), −0.03, −0.05 (2s, 6H, Si(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.94, 138.25, 137.74, 137.61, 128.30, 128.27, 128.07, 127.95, 127.78, 127.77, 127.70, 127.62, 127.46, 127.27, 127.19, 101.17, 99.72, 79.99, 75.96, 74.63, 73.47, 73.32, 72.53, 72.42, 72.06, 71.80, 67.79, 62.09, 56.16, 51.01, 25.87, 23.34, 21.16, 18.32, 18.20, −5.21, −5.30. FAB-MS: *m*/*z* 936.5 [M + Na]$^+$. Anal. Calcd for $C_{51}H_{67}NO_{12}Si$: C, 67.01; H, 7.39; N, 1.53; O, 21.00; Si, 3.07. Found: C, 67.18; H, 7.55; N, 1.49. $[\alpha]_D$ = −17.6 (*c* 0.59, $CHCl_3$). mp = 124.9 °C.

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (9). Tetrafluoroboric acid (48% in water, 0.31 mL, 2.33 mmol) was added to a stirred solution of **8** (2.0 g, 2.2 mmol) in acetonitrile (50 mL). The mixture was left stirring at room temperature for 5 min. TLC (toluene/ethyl acetate, 1/1, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (0.33 mL, 2.3 mmol) and concentrated in vacuo. The residue was then dissolved in dichloromethane (100 mL), washed successively with a saturated solution of $NaHCO_3$ (20 mL), water (2 × 20 mL), and brine (20 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (gradient hexane/ethyl acetate, 3/1 to 1/1, v/v) to afford compound **9** (1.7 g, 99%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.82 (d, 1H, $J_{NH,2}$ 8.3, N*H*), 5.26 (dd, 1H, $J_{2',3'}$ 10.1, H-2′), 4.91, 4.50 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.76, 4.60 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.63, 4.46 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.54 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.43 (d, 1H, $J_{1',2'}$ 8.4, H-1′), 4.35, 4.22 (AB q, 2H, J_{AB} 11.9, OCH_2Ph), 3.91 (d, 1H, $J_{3'-4'}$ 2.64, H-4′), 3.88−3.35 (m, 5H, H-4, H-5, H-5′, H-6a′, H6b′), 3.86 (dd, 1H, J_{6a-5} 3.86, J_{6a-6b} 11.6, H-6a), 3.82 (m, 1-H, H-3), 3.80 (dd, 1H, H-6b), 3.70 (m, 1H, H-2), 3.50 (m, 1H, H-3′), 3.41 (s, 3H, OCH_3), 1.97 (s, 3H, CH_3C(O)NH), 1.84 (s, 3H, CH_3C(O)O). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.50, 170.08, 138.76, 138.69, 138.05, 137.98, 128.62, 128.38, 128.23, 128.10, 128.06, 127.99, 127.97, 127.72, 127.62, 127.59, 101.75, 100.81, 80.34, 80.26, 75.86, 75.52, 74.81, 73.70, 73.55, 72.90, 72.51, 72.23, 72.13, 68.30, 61.90, 56.94, 53.85, 23.56, 21.22. FAB-MS: *m*/*z* 821.3 [M + Na]$^+$. Anal. Calcd for $C_{45}H_{53}NO_{12}$: C, 67.57; H, 6.68; N, 1.75; O, 24.00. Found: C, 67.69; H, 6.80; N, 1.88. $[\alpha]_D$ = −9.6 (*c* 0.73, $CHCl_3$).

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (10). Sodium methoxide (5.4 mg, 0.1 mmol) was added to a stirred solution of **9** (450 mg, 0.56 mmol) in methanol (20 mL). The mixture was left stirring at room temperature for 48 h. TLC (hexane/acetone, 1/1, v/v) indicated completion of the reaction. The mixture was neutralized with Dowex 50H$^+$ resin until pH = 7, filtered, and concentrated in vacuo. The residue was purified by flash silica gel chromatography (gradient hexane/acetone, 3/1 to 1/1, v/v) to afford compound **10** (418 mg, 98%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.42 (d, 1H, $J_{NH,2}$ 7.9, N*H*), 4.92, 4.59 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.80, 4.48 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.59, 4.23 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.50, 4.18 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.6 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.52 (d, 1H, $J_{1',2'}$ 8.3, H-1′), 4.04 (t, 1H, $J_{3,4}$ 9.2, H-3), 3.95 (dd, 1H, J_{6a-5} < 2, J_{6a-6b} 12.1, H-6a), 3.90 (m, 1H, H-4), 3.85 (m, 1H, $J_{2',3'}$ 9.7, H-2′), 3.82 (dd, 1H, $J_{3'-4'}$ 2.6, H-4′), 3.81 (dd, 1H, H-6b), 3.5 (m, 2H, H-6a′, H6b′), 3.40 (s, 3H, OCH_3), 3.30 (dd, 1H, $J_{3',4'}$ 2.6, H-3′), 3.29 (m, 1H, H-2), 3.25 (m, 1H, H-5), 3.22 (dt, 1H, $J_{5'-6a'}$ 7.9, $J_{5'-6b'}$ 7.9, H-5′), 1.79 (s, 3H, CH_3C(O)NH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.90, 139.13, 138.78, 138.13, 137.93, 128.68, 128.54, 128.43, 128.33, 128.03, 127.99, 127.92, 127.82, 127.65, 127.52, 104.03, 101.41, 82.15, 79.86, 76.803, 75.29, 74.71, 74.11, 73.71, 73.58, 72.87, 72.50, 72.14, 71.00, 68.36, 56.96, 56.83, 23.63. MALDI-TOF: *m*/*z* 780.4 [M + Na]$^+$. Anal. Calcd for $C_{43}H_{51}NO_{11}$: C, 68.15; H, 6.78; N, 1.85; O, 23.22. Found: C, 68.28; H, 6.91; N, 1.90. $[\alpha]_D$ = 5.6 (*c* 0.43, $CHCl_3$).

Methyl 2-Acetamido-2-deoxy-6-*O*-methyl-4-*O*-(2-*O*-methyl-β-D-galactopyranosyl)-β-D-glucopyranoside (2). Methyl iodide (0.86 mL, 9.23 mmol), anhydrous barium oxide (778 mg, 5.0 mmol), and barium hydroxide octahydrate (750 mg, 2.5 mmol) were added to a stirred solution of **10** (318 mg, 0.42 mmol) in dry *N*,*N*-dimethylformamide (3 mL). The mixture was left stirring at room temperature for 24 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The mixture was diluted with ethyl acetate (20 mL), washed successively with a saturated solution of $NaHCO_3$ (10 mL), water (5 × 10 mL), and brine (10 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash chromatography (gradient hexane/acetone, 3/1 to 1/1, v/v) to afford compound **11** (198 mg, 60%). 10% palladium on charcoal (125.0 mg) was added to a solution of **11** (100 mg, 0.13 mmol) in ethanol (10 mL). The mixture was vigorously stirred under an atmosphere of hydrogen for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated the completion of the reaction. After filtration on Celite and concentration, the crude material was purified by chromatography (Iatrobeads, chloroform/methanol/water, 74/24/2, v/v/v) to afford compound **2** (52 mg, 94%) as an amorphous white solid. MALDI-TOF: *m*/*z* 448.3 [M + Na]$^+$. $[\alpha]_D$ = −20.6 (*c* 0.30, H_2O). For ^{1}H NMR and ^{13}C NMR data, see Supporting Information.

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-6-*O*-methanesulfonyl-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (12). Methanesulfonyl chloride (70 μL, 0.9 mmol) was added to a cooled (−20 °C) and stirred solution of **9** (479 mg, 0.6 mmol) in dry pyridine (20 mL). After stirring for 6 h at −20 °C, TLC (toluene/ethyl acetate, 1/1, v/v) indicated completion of the reaction. After coevaporation with toluene, the reaction mixture was diluted with dichloromethane (100 mL), washed successively with a saturated solution of $NaHCO_3$ (20 mL), water (2 × 20 mL), and brine (20 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (hexane/ethyl acetate, gradient 3/1, 3/2 followed by 1/1, v/v) to afford compound **12** (463 mg, 88%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 6.40 (d, 1H, $J_{NH,2}$ 9.7, N*H*), 5.34 (dd, 1H, $J_{1',2'}$ 7.9 $J_{2'3'}$ 10.1, H-2′), 4.94, 4.51 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.74, 4.44 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.69, 4.54 (AB q, 2H, J_{AB} 11.9 OCH_2Ph), 4.63 (dd, 1H, $J_{6b,5}$ 4.0, H-6b), 4.60, 4.40 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.55 (d, 1H, $J_{1,2}$ 5.7, H-1), 4.47 (dd, 1H, $J_{6a,5}$ 5.3, $J_{6a,6b}$

10.1, H-6a), 4.39 (d, 1H, $J_{1',2'}$ 7.9, H-1′), 4.23 (dd, 1H, $J_{2,3}$ 8.4, H-2), 3.99−3.79 (m, 1H, H-4), 3.97 (d, 1H, $J_{3'-4'}$ 2.6, H-4′), 3.93 (m, 1H, H-5), 3.82 (m, 1H, H-3), 3.61−3.46 (m, 3H, H-5′, H-6a′, H-6b′), 3.53 (dd, 1H, $J_{2'-3'}$ 10.1, H-3′), 3.44 (s, 3H, OCH_3), 2.82 (s, 3H, CH_3SO$_2$), 2.07 (s, 3H, CH_3C(O)O), 1.99 (s, 3H, CH_3C(O)NH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.78, 170.51, 138.47, 138.10, 137.99, 128.84, 128.70, 128.58, 128.55, 128.48, 128.28, 128.15, 128.12, 127.95, 127.73, 101.77, 100.19, 80.00, 77.66, 77.43, 77.23, 76.81, 75.50, 74.93, 74.08, 73.78, 72.89, 72.63, 72.43, 71.90, 69.42, 68.39, 56.92, 48.41, 37.26, 29.92, 23.34, 21.27. MALDI-TOF: *m*/*z* 900.5 [M + Na]$^+$. Anal. Calcd for $C_{46}H_{55}NO_{14}$: C, 62.93; H, 6.31; N, 1.60; O, 25.51; S, 3.65. Found: C, 63.05; H, 6.36; N, 1.75. $[\alpha]_D = -24.8$ (*c* 0.13, $CHCl_3$).

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-6-*O*-methanesulfonyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (13). Sodium methoxide (5.4 mg, 0.1 mmol) was added to a solution of **12** (447 mg, 0.51 mmol) in methanol (10 mL). After stirring at room temperature for 48 h, TLC (hexane/acetone, 1/1, v/v) indicated completion of the reaction; the mixture was then neutralized with Dowex 50H$^+$ until pH = 7.0, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (gradient hexane/acetone, 3/1 to 1/1, v/v) to afford compound **13** (418 mg, 98%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.88 (d, 1H, $J_{NH,2}$ 7.7, N*H*), 4.86, 4.72 (AB q, 2H, J_{AB} 11.5, OCH_2Ph), 4.81, 4.54 (AB q, 2H, J_{AB} 11.3, OCH_2Ph), 4.72, 4.57 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.60, 4.40 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.62 (d, 1H, $J_{1,2}$ 7.4, H-1), 4.60 (m, 1H, H-6b), 4.47 (m, 1H, H-6a), 4.44 (d, 1H, $J_{1',2'}$ 7.7, H-1′), 4.00 (dd, 1H, $J_{3,4}$ 8.5, H-3), 3.99 (dd, 1H, $J_{3',4'}$ 3.8, H-3′), 3.96 (dd, 1H, $J_{2',3'}$ 9.6, H-2′), 3.86 (m, 2H, H-4, H-5), 3.80 (m, 1H, H-4′), 3.64 (m, 1H, H-2), 3.60 (dd, 1H, $J_{6a'-6b'}$ 11.8, H-6b′), 3.57 (dd, 1H, $J_{6a'-5}$ 2.2, H-6a′), 3.48−3.30 (m, 1H, H-5′), 3.46 (s, 3H, OCH_3), 2.96 (s, 3H, CH_3SO$_2$), 1.85 (s, 3H, CH_3C(O)NH). MALDI-TOF: *m*/*z* 858.0 [M + Na]$^+$. Analytical data are in agreement with Lemieux et al.[25]

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-2′,6-anhydro-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (14). Sodium hydride (60% in oil suspension, 19.3 mg, 0.48 mmol) was added to a solution of **13** (134 mg, 0.16 mmol) in dry *N*,*N*-dimethylformamide (20 mL). After stirring at room temperature for 16 h, TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The reaction mixture was quenched with methanol, diluted in ethyl acetate (100 mL), and successively washed with water (2 × 20 mL) and brine (20 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was then purified by flash silica gel chromatography (hexane/acetone, gradient 3/1, 3/2 followed by 1/1, v/v) to afford compound **14** (83 mg, 70%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.40 (d, 1H, $J_{NH,2}$ 7.4, N*H*), 4.96, 4.62 (AB q, 2H, J_{AB} 11.3, OCH_2Ph), 4.93, 4.60 (AB q, 2H, J_{AB} 11.6, OCH_2-Ph), 4.81, 4.70 (AB q, 2H, J_{AB} 12.1, OCH_2Ph), 4.70 (d, 1H, $J_{1,2}$ 7.2, H-1), 4.48, 4.42 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.37 (d, 1H, $J_{1',2'}$ 7.4, H-1′), 4.20 (dd, 1H, $J_{6b,5}$ 6.9, $J_{6b,6a}$ 13.5, H-6b), 4.09 (m, 1H, H-3), 4.02 (dd, 1H, $J_{6a,5}$ 3.3, H-6a), 3.89 (d, 1H, $J_{3',4'}$ 2.5, H-4′), 3.82 (dd, 1H, $J_{3,4}$ 8.5, $J_{4,5}$ 9.6, H-4), 3.75 (m, 1H, H-5), 3.68−3.62 (m, 3H, H6b′, H-6a′, H-5′), 3.61 (m, 1H, H-2′), 3.49 (m, 1H, H-3′), 3.46 (s, 3H, OCH_3), 3.28 (m, 1H, H-2), 1.82 (s, 3H, CH_3C(O)NH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.39, 138.79, 138.63, 137.92, 128.47, 128.23, 127.88, 128.68, 127.59, 127.50, 103.75, 101.03, 85.83, 80.19, 78.83, 78.66, 75.27, 75.08, 74.98, 74.78, 74.31, 73.70, 73.49, 68.74, 57.17, 56.86, 29.95, 23.86. MALDI-TOF: *m*/*z* 762.2 [M + Na]$^+$. Anal. Calcd for $C_{43}H_{49}NO_{10}$: C, 69.81; H, 6.68; N, 1.89; O, 21.63. Found: C, 69.95; H, 6.76; N, 1.92. $[\alpha]_D = -20.8$ (*c* 0.27, CH_2Cl_2).

Methyl 2-Acetamido-2-deoxy-2′,6-anhydro-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside (3). 10% palladium on charcoal (55.0 mg) was added to a solution of **14** (43 mg, 0.058 mmol) in ethanol (3 mL). The mixture was vigorously stirred under an atmosphere of hydrogen for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. After filtration using Celite and concentration of the filtrate, the crude material was purified by column chromatography (Iatrobeads, chloroform/methanol/water, 74/24/2, v/v/v) to afford compound **3** (21 mg, 95%) as a white amorphous solid. MALDI-TOF: *m*/*z* 402.3 [M + Na]$^+$. $[\alpha]_D = -15.6$ (*c* 0.79, D_2O). For ^{1}H NMR and ^{13}C NMR data, see Supporting Information.

1-*O*-*tert*-Butyldimethylsilyl-2-*O*-methanesulfonyloxy-ethane (15). Sodium hydride (60% in oil suspension, 28.2 mg, 0.71 mmol) was added to a solution of ethylene glycol (2.05 g, 33 mmol) in dry *N*,*N*-dimethylformamide (25 mL). After stirring at room temperature for 30 min, *tert*-butyldimethylsilyl chloride (5.0 g, 33 mmol) was added dropwise. The mixture was left stirring at room temperature for 16 h. Triethylamine (6.91 mL, 50 mmol) was then added, followed by slow addition of methanesulfonyl chloride (3.6 mL, 46 mmol). TLC (hexane/ethyl acetate, 3/1, v/v) indicated completion of the reaction. The reaction mixture was quenched with methanol, diluted with ethyl acetate (100 mL), and washed successively with water (5 × 20 mL), brine (20 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was then purified by flash chromatography (gradient hexane/ethyl acetate, 5/1 to 3/2, v/v) to afford compound **15** (5.9 g, 70%) as a white foam. ^{1}H NMR ($CDCl_3$, 300 MHz): 4.58 (dd, 2H, *J* 4.7, 9.1, MsOCH_2), 3.88 (dd, 2H, *J* 4.7, 9.1, TBDMSOCH_2), 3.02 (s, 3H, CH_3-SO$_2$), 0.90 (s, 9H, Si(CH_3)$_3$), 0.09 (2s, 6H, Si(CH_3)$_2$). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 71.22, 61.28, 37.60, 25.96, 25.87, 18.37, −5.27. MALDI-TOF: *m*/*z* 277.1 [M + Na]$^+$. Anal. Calcd for $C_9H_{22}O_4SSi$: C, 42.49; H, 8.72; O, 25.15; S, 12.60; Si, 11.04. Found: C, 42.65; H, 8.76; O, 25.26.

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-6-di-*O*-(2-*O*-methanesulfonyl-ethane)-β-D-glucopyranoside (18). Sodium hydride (60% in oil suspension, 28.2 mg, 0.71 mmol) was added to a solution of **10** (67 mg, 0.09 mmol) in dry *N*,*N*-dimethylformamide (10 mL). After stirring for 30 min at 50 °C, **15** (336 mg, 1.32 mmol) was added dropwise. The mixture was stirred at 50 °C for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The reaction mixture was quenched with methanol, diluted in ethyl acetate (30 mL), washed successively with water (5 × 10 mL), brine (10 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was dissolved in acetonitrile (20 mL), and tetrafluoroboric acid (48% in water, 21.3 μL, 0.16 mmol) was added. The reaction mixture was stirred at room temperature for 5 min after which TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (23.0 μL, 0.16 mmol) and concentrated in vacuo. The residue was then diluted in dichloromethane (10 mL), successively washed with a saturated solution of $NaHCO_3$ (5 mL), water (2 × 5 mL), brine (5 mL), followed by drying over $MgSO_4$. After concentration of the filtrate, the crude mixture was purified by flash chromatography (gradient hexane/acetone, 3/1 to 1/1, v/v). The dried product was then dissolved in pyridine (5 mL), and methanesulfonyl chloride (7.0 μL, 0.09 mmol) was added to the stirred mixture at −20 °C. The reaction mixture was then left stirring at room temperature for 2 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The reaction mixture was quenched with methanol. Toluene was then added to the crude mixture which was concentrated in vacuo. The reaction mixture was diluted with dichloromethane (10 mL) and then washed successively with water (2 × 5 mL), brine (5 mL), followed by drying over $MgSO_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (gradient hexane/acetone, 3/1 to 1/1, v/v) to afford compound **18** (37 mg, 47%) as a white amorphous solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.64 (d, 1H, $J_{NH,2}$ 8.3, N*H*), 4.92, 4.38 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.88, 4.56 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.75, 4.61 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.67 (d, 1H, $J_{1,2}$ 7.4, H-1), 4.50 (m, 2H, H-2, H-5′), 4.36, 4.28 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.33 (d, 1H, $J_{1',2'}$ 8.0, H-1′), 4.1−4.0 (m, 1H, H-4), 4.0−3.88 (m, 1H, H-5), 3.92 (dd, 1H, $J_{3,4}$ 8.4 H-3), 3.84−3.44 (m, 2H, H-6b, H-6a), 3.80 (d, 1H, $J_{3',4'}$ 3.0, H-4′), 3.72 (m, 1H, H-3′), 3.58 (m, 1H, H-6b′), 3.45 (s, 3H, OCH_3), 3.42 (dd, 1H, $J_{2,3}$

6.3, H-2), 3.38 (m, 1H, H-6a′), 2.98 (s, 3H, CH_3SO$_2$), 1.84 (s, 3H, CH_3C(O)NH). ^{13}C NMR (CDCl$_3$, 125 MHz): δ 170.42, 138.87, 138.21, 132.51, 128.70, 128.55, 128.41, 128.55, 128.4, 128.35, 127.96, 127.63, 118.28, 112.42, 103.72, 103.53, 101.46, 100.26, 82.32, 78.85, 74.81, 74.70, 74.07, 73.82, 73.70, 73.07, 72.47, 71.91, 69.88, 69.51, 69.40, 68.69, 57.65, 57.05, 56.20, 37.85, 23.89. MALDI-TOF: *m*/*z* 902.6 [M + Na]$^+$. Anal. Calcd for $C_{46}H_{57}NO_{14}S$: C, 62.78; H, 6.53; N, 1.59; O, 25.45; S, 3.64. Found: C, 62.89; H, 6.68; N, 1.72. $[\alpha]_D = 4.0$ (*c* 0.07, CH_2Cl_2).

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2′,6-di-*O*-(ethane-1,2-diyl)-β-D-glucopyranoside (19). A suspension of sodium hydride (60% in oil suspension, 9.6 mg, 0.24 mmol) in *N*,*N*-dimethylformamide (2 mL) was added over a period of 2 h to a solution of **18** (26.4 mg, 0.03 mmol) in *N*,*N*-dimethylformamide (10 mL). The reaction mixture was then stirred at 50 °C for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. The reaction mixture was quenched with methanol, and then diluted in ethyl acetate (30 mL), successively washed with water (5 × 10 mL), brine (10 mL), followed by drying over MgSO$_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (hexane/acetone, gradient 3/1, 3/2 followed by 1/1, v/v) to afford compound **19** (14.1 mg, 60%) as an amorphous white solid. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.48 (d, 1H, $J_{NH,2}$ 7.5, N*H*), 5.01, 4.66 (AB q, 2H, J_{AB} 12.3, OCH_2Ph), 4.88, 4.64 (AB q, 2H, J_{AB} 11.9, OCH_2Ph), 4.81 (d, 1H, $J_{1,2}$ 7.8, H-1), 4.80, 4.12 (AB q, 2H, J_{AB} 11.9, OCH_2Ph), 4.77 (d, 1H, $J_{1',2'}$ 7.5, H-1′), 4.70, 4.54 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.29 (dd, 1H, $J_{3,4}$ 8.8, H-3), 4.20 (m, 1H, H-4), 4.16, 3.84 (m, 2H, H6b′, H6a′), 4.10−3.87 (m, 2H, H5′, H6a), 3.93 (m, 1H, H6b), 3.85 (dd, 1H, $J_{3'-4'}$ 2.2, H-4′), 3.60 (dd, 1H, $J_{2',3'}$ 9.4, H-2′), 3.51 (m, 2H, OCH_2-CH_2O), 3.50 (m, 1H, H-5), 3.46 (s, 3H, OCH_3), 3.45 (m, 1H, H-3′), 3.27 (dd, 1H, *J* 5.27, 8.79, OCH$_2$CH_2O), 3.18 (m, 1H, H-2), 2.99 (dd, 1H, OCH$_2$CH_2O), 1.80 (s, 3H, CH_3C(O)NH). ^{13}C NMR (CDCl$_3$, 125 MHz): δ 170.53, 139.46, 138.71, 138.40, 137.45, 128.38, 128.30, 128.05, 127.90, 127.83, 127.68, 127.58, 127.36, 127.12, 126.24, 104.84, 100.65, 83.27, 82.08, 81.10, 78.67, 76.57, 74.46, 74.27, 74.18, 73.43, 73.32, 72.99, 72.42, 70.35, 68.11, 64.78, 58.61, 56.71, 30.90, 23.62. MALDI-TOF: *m*/*z* 806.2 [M + Na]$^+$. $[\alpha]_D = 3.5$ (*c* 0.25, CH_2Cl_2).

Methyl 2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-2′,6-di-*O*-(ethane-1,2-diyl)-β-D-glucopyranoside (5). 10% palladium on charcoal (14.0 mg) was added to a solution of **19** (9.0 mg, 0.012 mmol) in ethanol (3 mL). The mixture was vigorously stirred under an atmosphere of hydrogen for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. After filtration using Celite and concentration, the crude material was purified by chromatography (Iatrobeads, chloroform/methanol/water, 74/24/2, v/v/v) to afford compound **5** (4.9 mg, 96%) as an amorphous white solid. MALDI-TOF: *m*/*z* 446.0 [M + Na]$^+$. $[\alpha]_D = -14.6$ (*c* 0.56, D_2O). For ^{1}H NMR and ^{13}C NMR data, see Supporting Information.

Methyl 2-Azido-2-deoxy-3-*O*-benzyl-4-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (22). Boron trifluoride diethyl etherate (87.5 μL, 0.7 mmol) was added to a stirred solution of **6** (438.2 mg, 0.70 mmol), **20** (194.8 mg, 0.50 mmol), and powdered molecular sieves 4 Å (200 mg) in dichloromethane (20 mL). The mixture was stirred at −40 °C for 3 h. TLC (hexane/ethyl acetate, 7/3, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (97.6 μL, 0.70 mmol) and filtered over Celite. The filtrate washings were combined and concentrated in vacuo. The residue was then diluted in dichloromethane (50 mL) and successively washed with a saturated solution of NaHCO$_3$ (10 mL), water (2 × 10 mL), and brine (10 mL), followed by drying over MgSO$_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (gradient hexane/ethyl acetate, 9.5/0.5 to 8/2, v/v) to give compound **21** (359 mg, 80%). Tetrafluoroboric acid (48% in water, 129 μL, 0.70 mmol) was added to a stirred solution of **21** (359 mg, 0.40 mmol) in acetonitrile (50 mL). The mixture was stirred at room temperature for 5 min. TLC (hexane/ethyl acetate, 7/3, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (0.325 mL, 2.3 mmol) and concentrated in vacuo. The residue was then diluted in dichloromethane (50 mL) and successively washed with a saturated solution of NaHCO$_3$ (10 mL), water (2 × 10 mL), and brine (10 mL), followed by drying over MgSO$_4$. After evaporation of the solvent, the residue was purified by flash silica gel chromatography (gradient hexane/ethyl acetate, 9.5/0.5 to 8/2, v/v) to give compound **22** (297.67 mg, 95%) as an amorphous white solid. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.42 (dd, 1H, $J_{2'3'}$ 9.9, H-2′), 5.06, 4.74 (AB q, 2H, J_{AB} 10.7, OCH_2Ph), 5.00, 4.56 (AB q, 2H, J_{AB} 11.5, OCH_2Ph), 4.69, 4.51 (AB q, 2H, J_{AB} 12.4, OCH_2-Ph), 4.61 (d, 1H, $J_{1',2'}$ 8.0, H-1′), 4.33, 4.23 (AB q, 2H, J_{AB} 11.8, OCH_2-Ph), 4.18 (d, 1H, $J_{1,2}$ 8.0, H-1), 3.99 (d, 1H, $J_{3'-4'}$ 2.5, H-4′), 3.87 (dd, 1H, $J_{6b,5}$ 2.5, $J_{6b,6a}$ 12.1, H-6b), 3.76 (dd, 1H, $J_{6a,5}$ 3.3, H-6a), 3.62−3.44 (m, 3H, H-3, H-4, H5′), 3.54 (s, 3H, OCH_3), 3.52 (m, 1H, H-6b′), 3.51 (dd, 1H, H-3′), 3.33 (m, 1H, H-6a′), 3.32 (m, 1H, H-2), 3.30 (m, 1H, H-5), 2.10 (s, 3H, CH_3C(O)O). ^{13}C NMR (CDCl$_3$, 125 MHz): δ 169.61, 138.83, 138.55, 138.14, 138.10, 128.63, 128.58, 128.39, 128.25, 128.17, 128.02, 127.94, 127.70, 127.58, 127.51, 103.10, 101.36, 81.38, 80.68, 76.28, 75.52, 75.49, 74.99, 73.79, 73.73, 73.02, 72.37, 72.16, 68.28, 66.25, 60.90, 57.60, 21.37. MALDI-TOF: *m*/*z* 806.3 [M + Na]$^+$. Anal. Calcd for $C_{43}H_{49}N_3O_{11}$: C, 65.89; H, 6.30; N, 5.36; O, 22.45. Found: C, 65.96; H, 6.43; N, 5.41. $[\alpha]_D = -17.3$ (*c* 0.40, CH_2Cl_2).

Methyl 2-Azido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-β-D-glucopyranoside (23). Sodium methoxide (5.4 mg, 0.1 mmol) was added to a solution of **22** (223 mg, 0.29 mmol) in dry methanol (30 mL). The mixture was stirred at room temperature for 48 h. TLC (hexane/ethyl acetate, 7/3, v/v) indicated completion of the reaction. The mixture was neutralized with Dowex 50H$^+$ resin, filtered, and the filtrate was concentrated in vacuo. The residue was purified by flash silica gel chromatography (gradient hexane/acetone, 9/1 to 7/3, v/v) to afford compound **23** (210 mg, 98%) as an amorphous white solid. ^{1}H NMR (CDCl$_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 4.97, 4.82 (AB q, 2H, J_{AB} 11.0, OCH_2Ph), 4.83, 4.55 (AB q, 2H, J_{AB} 11.5, OCH_2Ph), 4.68, 4.53 (AB q, 2H, J_{AB} 11.9, OCH_2Ph), 4.61 (d, 1H, $J_{1',2'}$ 7.5, H-1′), 4.37 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.29, 4.21 (AB q, 2H, J_{AB} 11.4, OCH_2Ph), 4.20 (dd, 1H, $J_{6b,5}$ 2.7, $J_{6b,6a}$ 12.6, H-6b), 3.93 (m, 1H, H-5′), 3.91 (d, 1H, $J_{3'-4'}$ 1.9, H-4′), 3.90 (m, 1H, H-2′), 3.84 (dd, 1H, $J_{6a,5}$ 2.2, H-6a), 3.56 (m, 1H, H-6b′), 3.55 (s, 3H, OCH_3), 3.49−3.20 (m, 3H, H-3, H-4, H-5), 3.36 (m, 1H, H-2), 3.35 (m, 1H, H-3′), 3.27 (dd, 1H, $J_{6a',5'}$ 2.7, $J_{6b',6a'}$ 12.4, H-6a′). ^{13}C NMR (CDCl$_3$, 125 MHz): δ 138.85−128.58 (arom), 104.08, 103.33, 82.21, 75.38, 75.03, 74.80, 76.75, 75.52, 75.16, 73.67, 73.50, 72.35, 72.17, 72.01, 68.21, 66.60, 66.31, 61.22, 57.32. MALDI-TOF: *m*/*z* 764.3 [M + Na]$^+$. Anal. Calcd for $C_{41}H_{47}N_3O_{10}$: C, 66.38; H, 6.39; N, 5.66; O, 21.57. Found: C, 66.48; H, 6.47; N, 5.78. $[\alpha]_D = -12.4$ (*c* 0.35, CH_2Cl_2).

Methyl 2-Azido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2′,6-di-*O*-(methane-1,2-diyl)-β-D-glucopyranoside (24). Formaldehyde diphenylmercaptal (58.0 mg, 0.25 mmol) and powdered molecular sieves 4 Å (50 mg) were added to a stirred solution of **23** (156 mg, 0.21 mmol) in a mixture of acetonitrile and dichloromethane (34 mL, 7/3, v/v). The mixture was stirred at room temperature for 1 h, then cooled to −35 °C, and a solution of *N*-iodosuccinimide (234 mg, 1.04 mmol) and trifluoromethanesulfonic acid (5.5 μL, 0.063 mmol) in dry acetonitrile (3 mL) was added to the reaction mixture. The mixture was left stirring at −35 °C for 2 h. TLC (hexane/ethyl acetate, 3/1, v/v) indicated completion of the reaction. The mixture was neutralized with triethylamine (50 μL) and filtered over Celite. The filtrate and washings were combined and concentrated. The residue was then diluted in dichloromethane (30 mL), successively washed with a saturated solution of $Na_2S_2O_3$ (10 mL), a saturated solution of NaHCO$_3$, water (2 × 10 mL), brine (10 mL), followed by drying over MgSO$_4$. After evaporation of the solvents, the residue was purified by flash silica gel chromatography (gradient hexane/acetone, 9/0.5 to 8/2, v/v) to afford compound **24** (95 mg, 60%) as an amorphous

white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.10, 4.71 (AB q, 2H, J_{AB} 7.4, OCH_2O), 4.98, 4.86 (AB q, 2H, J_{AB} 10.7, OCH_2Ph), 4.90, 4.56 (AB q, 2H, J_{AB} 11.3, OCH_2Ph), 4.69, 4.64 (AB q, 2H, J_{AB} 12.4, OCH_2Ph), 4.68 (d, 1H, $J_{1',2'}$ 7.4, H-1′), 4.38, 4.30 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.18 (d, 1H, $J_{1,2}$ 7.7, H-1), 4.08 (dd, 1H, $J_{6b',5'}$ 2.8, $J_{6b',6a'}$ 12.1, H-6b′), 4.02 (m, 1H, H-5′), 3.98 (dd, 1H, $J_{6a',5'}$ 4.7, H-6a′), 3.88 (d, 1H, $J_{3'-4'}$ 2.8, H-4′), 3.78 (dd, 1H, $J_{2'3'}$9.6, H-2′), 3.60 (dd, 1H, $J_{6b,6a}$ 8.5, H-6b), 3.49 (m, 1H, H-3), 3.46 (m, 1H, H-5), 3.44 (t, 1H, $J_{3,4}$˜$J_{4,5}$ 9.1, H-4), 3.41 (dd, 1H, H-3′), 3.38 (m, 1H, H-2), 3.32 (m, 1H, $J_{6a,5}$ 4.9, $J_{6a,6b}$ 8.5 H-6a), 3.54 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 138.83, 138.68, 138.46, 137.97, 128.66, 128.64, 128.53, 128.37, 128.18, 128.13, 128.05, 127.96, 127.86, 127.80, 127.70, 127.62, 104.54, 103.25, 100.29, 84.51, 83.33, 81.41, 75.76, 74.79, 74.14, 73.73, 73.26, 73.06, 72.81, 70.71, 68.41, 66.31, 57.32. MALDI-TOF: *m*/*z* 776.3 $[M + Na]^+$. Anal. Calcd for $C_{42}H_{47}N_3O_{10}$: C, 66.92; H, 6.28; N, 5.57; O, 21.22. Found: C, 66.96; H, 6.43; N, 5.66. $[\alpha]_D = -57.7$ (*c* 0.53, CH_2Cl_2).

Methyl 2-Acetamido-2-deoxy-3-*O*-benzyl-4-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-2′,6-di-*O*-(methane-1,2-diyl)-β-D-glucopyranoside (25). 1,3-Propanedithiol (43.5 μL, 0.43 mmol) and triethylamine (42 μL, 0.43 mmol) were added to a stirred solution of **24** (83 mg, 0.11 mmol) in a mixture of pyridine and water (10 mL, 4/1, v/v). The mixture was stirred at room temperature for 6 h. TLC (hexane/acetone, 7/3, v/v) indicated completion of the reaction. The mixture was coevaporated with toluene, concentrated in vacuo, and then chromatographed over silica gel (gradient DCM/methanol, 1/0 to 9/1, v/v) to afford compound **25** (73 mg, 89%). Acetic anhydride (5 mL) was added to a solution of the above crude product (70 mg, 0.096 mmol) in pyridine (10 mL). The mixture was stirred at room temperature for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. Toluene was added to the mixture which was concentrated in vacuo. The residue was purified by flash chromatography (gradient hexane/acetone, 3/1 to 1/1, v/v) to afford compound **25** (72.4 mg, 98%) as an amorphous white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40−7.10 (m, 20H, arom), 5.36 (d, 1H, $J_{NH,2}$ 8.0, N*H*), 5.07, 4.69 (AB q, 2H, J_{AB} 7.4, OCH_2O), 4.98, 4.64 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.86, 4.54 (AB q, 2H, J_{AB} 11.5, OCH_2Ph), 4.68, 4.60 (AB q, 2H, J_{AB} 12.1, OCH_2Ph), 4.70 (d, 1H, $J_{1,2}$ 7.2, H-1), 4.68 (d, 1H, $J_{1',2'}$ 7.4, H-1′), 4.32, 4.25 (AB q, 2H, J_{AB} 11.8, OCH_2Ph), 4.12−3.92 (m, 1H, H-5′), 4.07 (dd, 1H, $J_{2,3}$ 7.8, H-3), 4.04 (dd, 1H, $J_{6b',5}$ 2.7, $J_{6b',6a'}$ 12.3, H-6b′), 3.96 (m, 1H, 3.3, H-6a′), 3.86 (d, 1H, $J_{3',4'}$ 2.2, H-4′), 3.76 (dd, 1H, $J_{2,'3'}$ 9.6, H-2′), 3.60−3.40 (m, 1H, H-5), 3.56 (m, 1H, H-6b), 3.43 (s, 3H, OCH_3), 3.33 (dd, 1H, $J_{6b,5}$ 2.7, H-6a), 3.42 (m, 1H, H-4), 3.41 (dd, 1H, H-3′), 3.28 (m, 1H, H-2), 1.80 (s, 3H, CH_3C(O)NH). ^{13}C NMR ($CDCl_3$, 125 MHz): 170.6, 139.81, 139.23, 138.81, 138.49, 138.06, 137.95, 128.63, 128.59, 128.57, 128.35, 128.17, 128.03, 127.96, 127.91, 127.84, 127.74, 127.66, 127.61, 104.84, 101.26, 100.06, 84.32, 81.53, 80.94, 75.31, 74.72, 74.15, 73.68, 73.13, 73.00, 72.87, 70.90, 68.64, 57.55, 56.90, 23.84. MALDI-TOF: *m*/*z* 792.4 $[M + Na]^+$. Anal. Calcd for $C_{44}H_{51}NO_{11}$: C, 68.64; H, 6.68; N, 1.82; O, 22.86. Found: C, 68.78; H, 6.67; N, 1.90. $[\alpha]_D = -19.2$ (*c* 0.68, CH_2Cl_2).

Methyl 2-Acetamido-2-deoxy-4-*O*-(β-D-galactopyranosyl)-2′,6-di-*O*-(methane-1,2-diyl)-β-D-glucopyranoside (4). 10% palladium on charcoal (74.0 mg) was added to a solution of **25** (60.0 mg, 0.078 mmol) in ethanol (3 mL). The mixture was vigorously stirred under an atmosphere of hydrogen for 16 h. TLC (chloroform/methanol, 9/1, v/v) indicated completion of the reaction. After filtration using Celite and concentration, the crude material was purified by chromatography (Iatrobeads, chloroform/methanol/water, 74/24/2, v/v/v) to afford compound **4** (28.7 mg, 90%) as a white amorphous solid. MALDI-TOF: *m*/*z* 432.2 $[M + Na]^+$. $[\alpha]_D = -11.0$ (*c* 0.57, D_2O). For ^{1}H NMR and ^{13}C NMR data, see Supporting Information.

Enzyme Studies. Reported methods[20,34,35,44] were employed for assaying sialyltransferase activity. For studies of the relative rates, incubation mixtures contained CMP[^{14}C]NeuAc (9 nmol, 6180 cpm/nmol) and substrate (120 nmol), bovine serum albumin (1 mg/ml), 0.2 mU of enzyme in sodium cacodylate (50 mM, pH 6.5) containing 0.1% Triton ×100 in a total volume of 60 μL were incubated at 37 °C for a period of 30 min. The radiolabeled product was isolated using a procedure modified by Horenstein et al.[35] based on Paulson's ion-exchange chromatography on a Dowex 1 × 8−200 (PO_4^{2-}, 100−200 mesh) Pasteur pipet column.[34] Columns (5 cm high) were eluted twice with 1 mM PO_4^{2-} (4 mL) buffer to ensure that no radiolabeled product was left on the column.

Kinetic Studies. Apparent kinetic parameters of the α-2,6-ST for synthetic acceptors were determined under the above standard conditions using a saturating concentration of CMP-[^{14}C]Neu5Ac.[43] Assays were performed in duplicate using 50 μU of enzyme. The concentration of oligosaccharide acceptor was varied around the K_m value (six different concentrations, ranging from 0.5 to 3.6 mM for acceptors **1** and **5**, and 0.5−6.0 mM for acceptors **2** and **4**), whereas the concentration of CMP-[^{14}C]Neu5Ac was kept constant at 200 μM (1655 cpm/nmol). The time of incubation at 37 °C was varied to 15 min to limit the CMP-[^{14}C]-Neu5Ac consumption to 10−15% to ensure initial rate conditions. The kinetic parameters V_{max} and K_m were determined using the GraphPad computer program obtained from Prism.

Preparative Sialylation. Incubations were carried out in 150 μL of sodium cacodylate buffer (25 mM, pH 7.2) containing 0.5% Triton ×100 and bovine serum albumin (1 mg/mL). Acceptor **4** (1.0 mg), CMP-Neu5Ac (0.9 mg), 3 U of alkaline phosphatase, and 6.9 mU of sialyltranferase are incubated at 37 °C for 36 h. Every 12 h, 0.9 mg of CMP-Neu5Ac was added to the incubation mixture to compensate for any hydrolysis of the donor. The reaction was monitored by TLC on silica gel plates using $CHCl_3$−MeOH−H_2O (60:35:6, v/v). The acceptor Galβ1 → 4GlcNAcβ-OMe has an R_f of 0.63, and the product NeuAcα2 → 6Galβ1→4GlcNAcβ-OMe has an R_f of 0.33. At the completion of the reaction, the sample was purified on Iatrobeads followed by biogel P2. ^{1}H NMR (D_2O, 500 MHz, DOH set at 4.76): δ 4.55 (d, 1H, $J_{1'2'}$ = 7.8 Hz, H-1′), 4.43 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 3.48 (s, 3H, OCH_3), 3.34 (m, 1H, H-2′), 2.67 (dd, 1H, $J_{3e'',3a''}$ = 12.7, $J_{3e'',4''}$ = 4.9 Hz, H-3e″), 2.04, 2.02 (2s, 6H, 2 CH_3C(O) NH), 1.68 (t, 1H, $J_{3a'',4''}$ = 11.7 Hz, H-3a″). MALDI-TOF: *m*/*z* = 737.3 $[M^+ + Na]$.

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Supporting Information Available: Synthesis of monosaccharide **20**, enzyme kinetics plots, ^{1}H NMR and ^{13}C NMR spectra of compounds **1**−**5**, NOE experiments figure of compounds **1**, **3**, and **5**, showing NOEs from Gal-H-1, region of quantitative HMBC for compound **4**, nomenclature, and detailed geometrical description of all optimized conformers of **1**, **3**, **4**, and **5** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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