is now aligned in an antiparallel fashion with the HC8N7 dipole which overcomes the added Coulombic repulsion between the spectator oxygen and N7 (Figure 6). The e_i values in this case are actually negative for both the spectator carbon and oxygen (-1.39 and -0.84 kcal/mol) as well as for the methylene group α to the nitrogen in the Hoogsteen complex for butyrolactam (-1.23 kcal/mol). Thus, the net interactions with the spectator carbonyl group in the imide are now attractive by 2.2 kcal/mol, whereas they contributed only 0.2 kcal/mol for the succinimide-butyrolactam complex (Figure 2).

In summary, the interesting reversal in the preferences for complexation with imides and lactams can be attributed to predominantly Coulombic secondary interactions. For complexation with another imide or lactam, the repulsion between the spectator oxygen of the imide and the hydrogen-bonding oxygen of the other molecule dominates to make the attraction weaker for the imide than lactam. However, for complexation with adenine derivatives, favorable electrostatic interactions for the HC2N1 and HC8N7 units in the Watson-Crick and Hoogsteen orientations with the spectator carbonyl group enhance the complexation for an imide over a lactam. These analyses and the appropriateness of the intermolecular potential functions upon which they are based are supported by the accord between the present results for relative free energies of binding in chloroform and related experimental data. It is gratifying that the observed differences in binding, which are not large, can be probed with adequate precision by current methods of condensed-phase theory.

Acknowledgment. Gratitude is expressed to Professor Julius Rebek, Jr., for helpful discussions and data prior to publication, to Dr. Julianto Pranata for computational assistance, and to the National Science Foundation for support of this research.

Synthesis and Enzymatic Evaluation of Two Conformationally Restricted Trisaccharide Analogues as Substrates for *N*-Acetylglucosaminyltransferase V

Ingvar Lindh and Ole Hindsgaul*

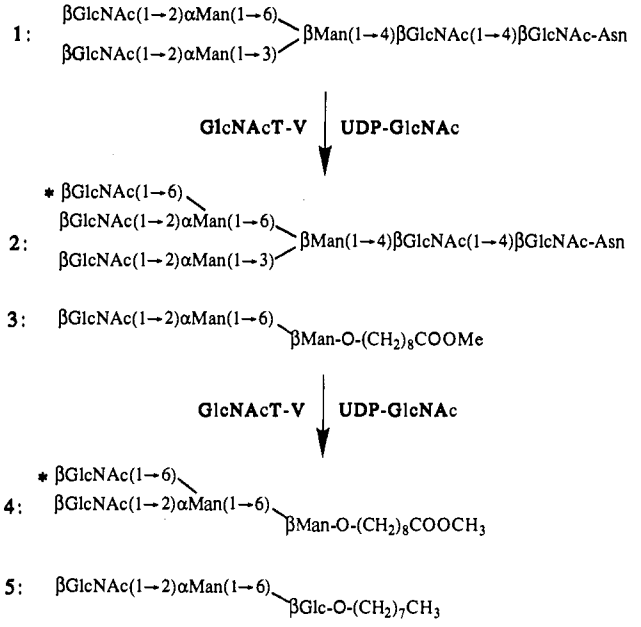
Contribution from the Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada. *Received June 5, 1990*

Abstract: The synthetic trisaccharide βGlcNAc(1→2)αMan(1→6)βGlc-O$(CH_2)_7CH_3$ (**5**) is an acceptor substrate for the enzyme *N*-acetylglucosaminyltransferase V (GlcNAcT-V) which adds an additional βGlcNAc residue to the 6-position of the central αMan unit. Two trisaccharide analogues of **5** have been chemically synthesized where the possibility for rotation about the C5-C6 bond of the βGlc residue has been eliminated by linking O4 and C6 with an ethylene bridge. The resulting fused-ring trisaccharides **8** and **9** represent conformationally restricted models for the "gt" and "gg" conformations, respectively, of asparagine-linked oligosaccharides. Both **5** and its conformationally restricted gg analogue **9** showed similar acceptor properties toward the enzyme from hamster kidney, while the gt trisaccharide **8** was an extremely poor substrate. These results demonstrate that GlcNAcT-V preferentially recognizes the carbohydrate chains of asparagine-linked glycoproteins in their gg conformations.

UDP-GlcNAc:α-mannoside $\beta(1\rightarrow6)$-*N*-acetylglucosaminyltransferase (GlcNAcT-V,EC 2.4.1.155) is a key enzyme involved in the biosynthesis of highly branched asparagine-linked oligosaccharides.[1,2] This enzyme has become the subject of intense investigation following observations that GlcNAcT-V activity increases when cells are transformed by both tumor viruses[3,4] and oncogenes.[5] Specific increases in the activity of this enzyme have also recently been shown to correlate with the metastatic potential of human and rodent tumor cells.[6,7]

Biosynthetically, GlcNAcT-V transfers an *N*-acetyl-D-glucosamine (GlcNAc) residue from uridine 5′-diphospho-GlcNAc (UDP-GlcNAc) to oligosaccharide acceptors having the minimum heptasaccharide sequence **1** (Scheme I), converting it to the octasaccharide **2**.[1,2] We have previously shown that the much simpler synthetic trisaccharide **3**, a partial structure of **1**, is also an effective substrate for the enzyme yielding the expected tetrasaccharide **4**.[8] The enzyme further tolerates the substitution of the βMan residue in **3** by a βGlc residue since trisaccharide **5** was also found to be an excellent acceptor.[9] The aliphatic aglycons in **3** and **5** were incorporated into these structures in order to facilitate the enzyme assay procedures.[10]

Scheme I. Glycosylation Reactions Catalyzed by GlcNAcT-V



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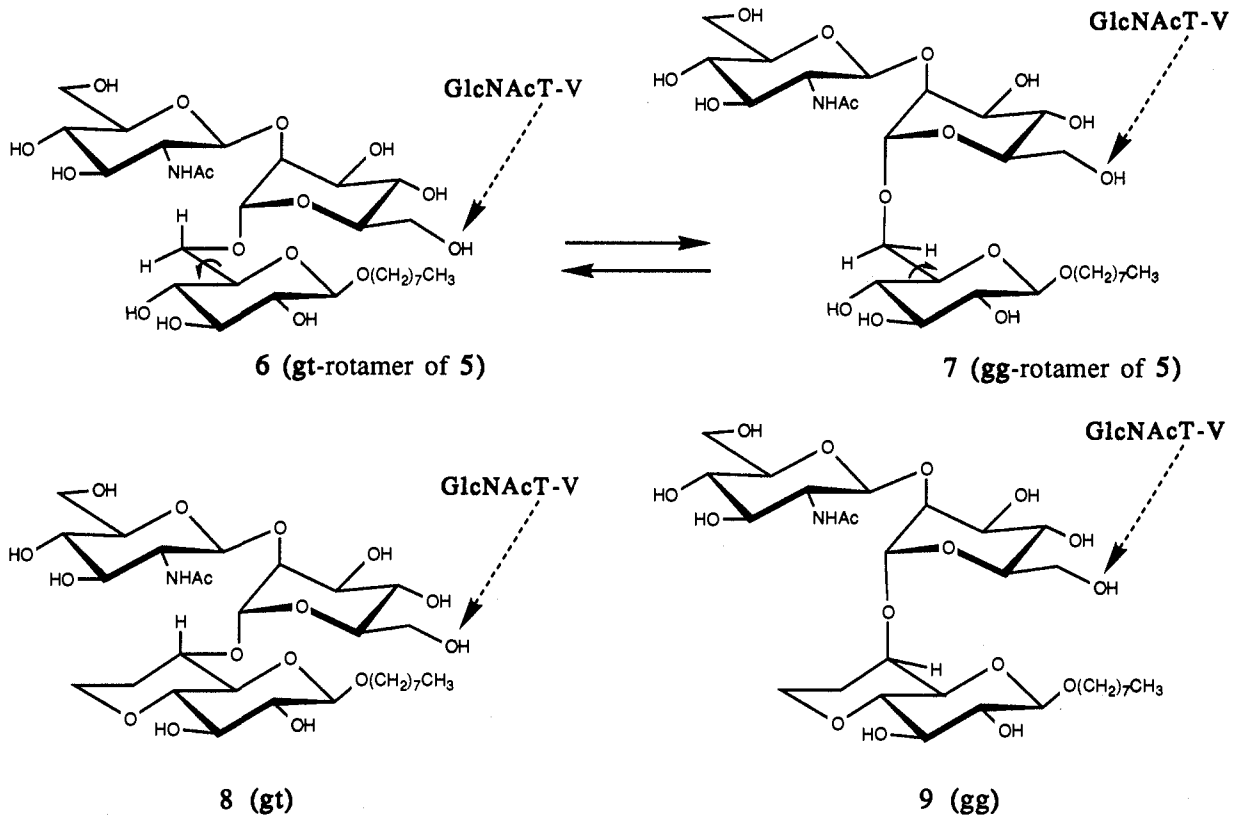
Scheme II



Oligosaccharides such as **5** (and its parent **1**) are known[11-14] to be conformationally labile about the αMan(1→6) linkage and exist, in aqueous solution, as an approximately equimolar mixture of the rapidly interconverting rotamers **6** (the so-called gauche–trans or gt rotamer) and **7** (the gauche–gauche or gg rotamer) (Scheme II). Since GlcNAcT-V requires the entire trisaccharide structure of **5** for good activity,[9] it seemed probable that the enzyme would recogenize this acceptor in only one of these two accessible conformations. We have previously[9] synthesized several analogues of **5** where chemical modifications were made in order to alter the gg ⇌ gt equilibrium (**6** ⇌ **7**), but these analogues, while conformationally biased, could not be used to *unequivocally* establish whether GlcNAcT-V recognized **5** in a specific conformation.

In this paper we describe chemical syntheses of trisaccharides **8** and **9** (Scheme II) which contain a fused ring on the βGlc residue thereby providing structurally defined models for the gt and gg rotamers **6** and **7**, respectively, by eliminating the possibility for rotation about the C5–C6 bond. The conformational freedom remaining in these potential enzyme acceptors is therefore limited to changes in the dihedral angles about the less flexible glycosidic linkages (the so-called ϕ and ψ angles)[11-14] and, possibly, minor pyranose ring distortions. These conformationally restricted acceptors are also evaluated as substrates for a partially purified GlcNAcT-V preparation from hamster kidney. The results clearly indicate that the enzyme recognizes its oligosaccharide substrates in the gg conformation.

Chemical Synthesis of Trisaccharides 8 and 9. The preparation of trisaccharides **8** and **9** proceeded by way of the fused ring monosaccharides **22** and **25** to which α-D-*manno*-pyranosyl and 2-acetamido-2-deoxy-β-D-*gluco*-pyranosyl residues were sequentially added, by using the glycosyl donors **26** and **29**, following well-precedented procedures. The synthesis of **22** started with the known D-*gluco* derivative **10**[15] which was oxidized (Scheme III) to aldehyde **11** (44%). The Grignard reaction of **11** with allylmagnesium chloride[16] yielded a 1:1 mixture of 6-*R* (D) and 6-*S* (L) nonopyranosides **12** and **13** in 77% yield, from which **13** could be obtained in pure form following chromatography and crystallization. Ozonolysis of **13**, followed by reduction, gave diol **14** (67%) from which the *p*-methoxybenzyl group was selectively removed to provide triol **15** (80%). Acetylation of **15** then gave triacetate **16** (99%). Acetolysis of the methyl aglycon in **16** had to be carefully controlled since the rate of benzyl ether cleavage was very similar. The use of 63 μM H_2SO_4 in acetic anhydride at −5 °C was found to provide the anomeric acetates **17** in acceptable yield (63%). Reaction of **17** with HBr yielded the α-bromide **18** which, on glycosylation with *n*-octanol using silver zeolite[17] as promoter, provided the β-glycoside **19** (78%) which was de-O-acetylated to triol **20**.

Formation of the 4,8-anhydro ring was accomplished via the 8-*O*-(2,4,6-tri-isopropylbenzenesulfonate) (**21**) (74%) which cyclized on treatment with sodium hydride. The ^{1}H NMR spectrum of the bicyclic product **22**, isolated in 63% yield, was used to establish the stereochemistry of the new chiral center formed in the Grignard reaction used to produce **13**. The observation of a vicinal coupling between H-5 and H-6 of 9.0 Hz, in both **22** and its acetate **23** (Scheme IV), confirmed the antiperiplanar orientation of these two protons. The stereochemistry at C-6 in **22** was next inverted via the 6-*O*-trifluoromethylsulfonyl ester which was treated with tetraethylammonium acetate to furnish the 6-*R* (D) isomer **24** (87%). Compound **24** showed the expected 2.5-Hz coupling between the gauche H-5 and H-6 thereby cor-

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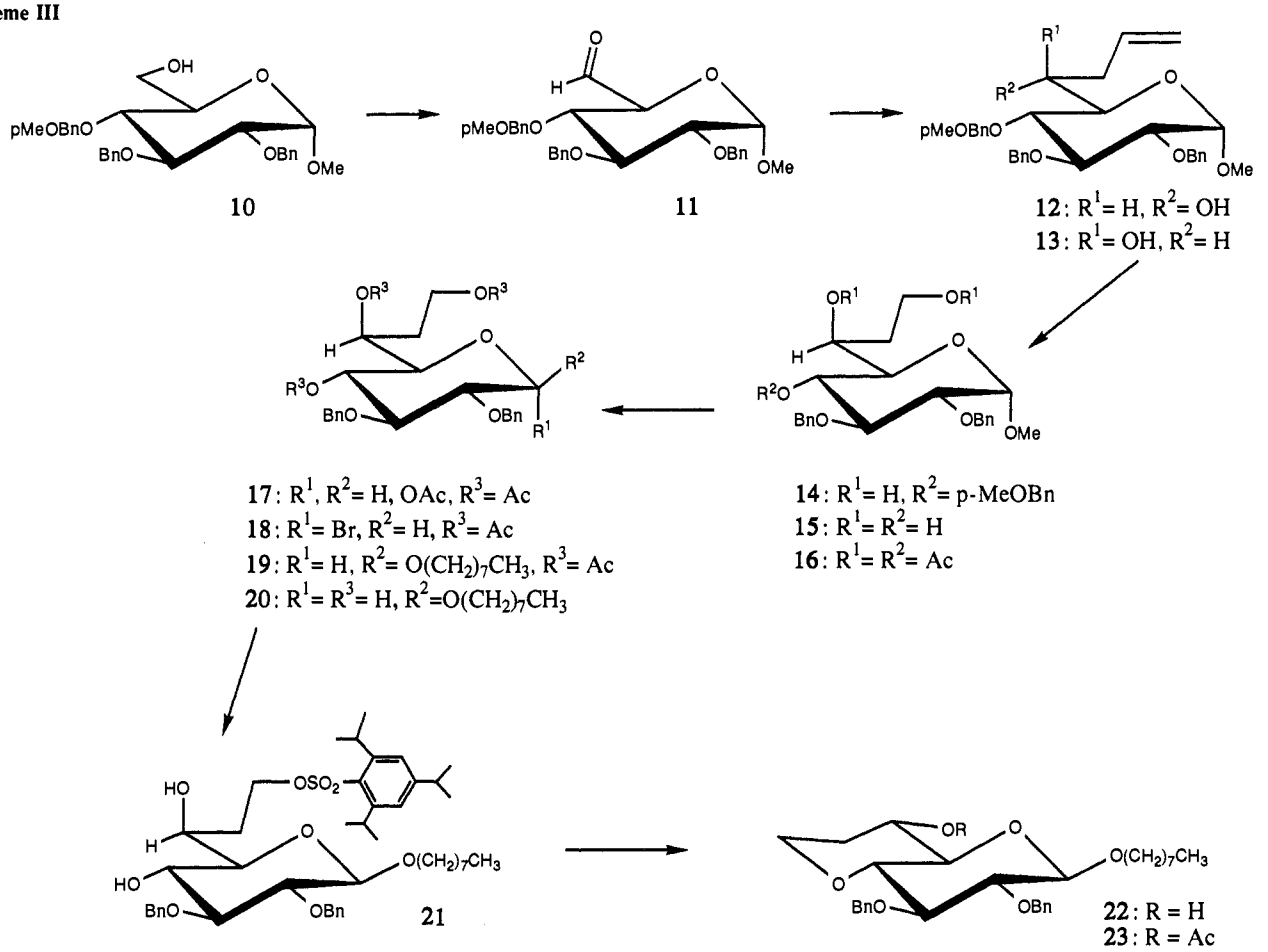
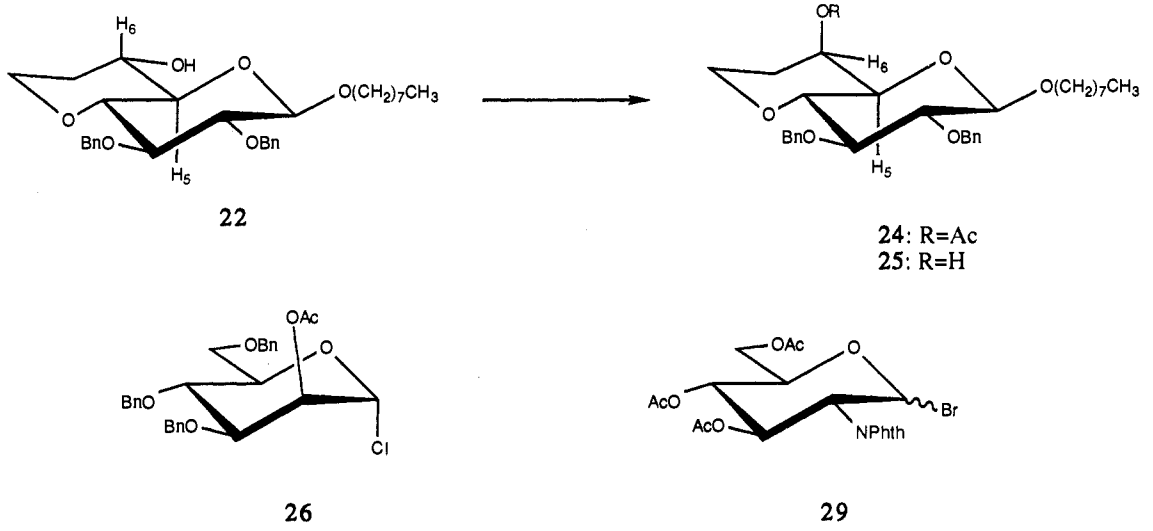
Scheme III



Scheme IV



roborating the structural assignment of **22**. Deacetylation of **24** gave alcohol **25**.

Homologation of the bicyclic alcohols **22** and **25** to trisaccharides **8** and **9** (Scheme V) was accomplished by sequential reactions with glycosyl donors **26** and **29**, followed by deprotection. Briefly, condensation of **22** with **26**,[18] promoter by silver triflate and tetramethylurea, gave disaccharide **27** (86%) which was deacetylated to alcohol **28**. Glycosylation of **28** with **29**,[19] promoted by silver triflate and 2,6-lutidine, then provided the fully protected trisaccharide **30** (86%). Treatment of **30** with hydrazine, followed by acetylation, gave the acetamido trisaccharide **31** (84%) which was sequentially hydrogenated and deacetylated to furnish the target 6-*S* (L) trisaccharide **8** (77%). The corresponding series of reactions were carried out on the 6-*R* (D) monosaccharide alcohol **25** yielding *O*-acetyl disaccharide **32** (66%), alcohol **33** (99%), phthalimido trisaccharide **34** (62%), and acetamido trisaccharide **35** (80%) finally leading to the fully deprotected

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Scheme V

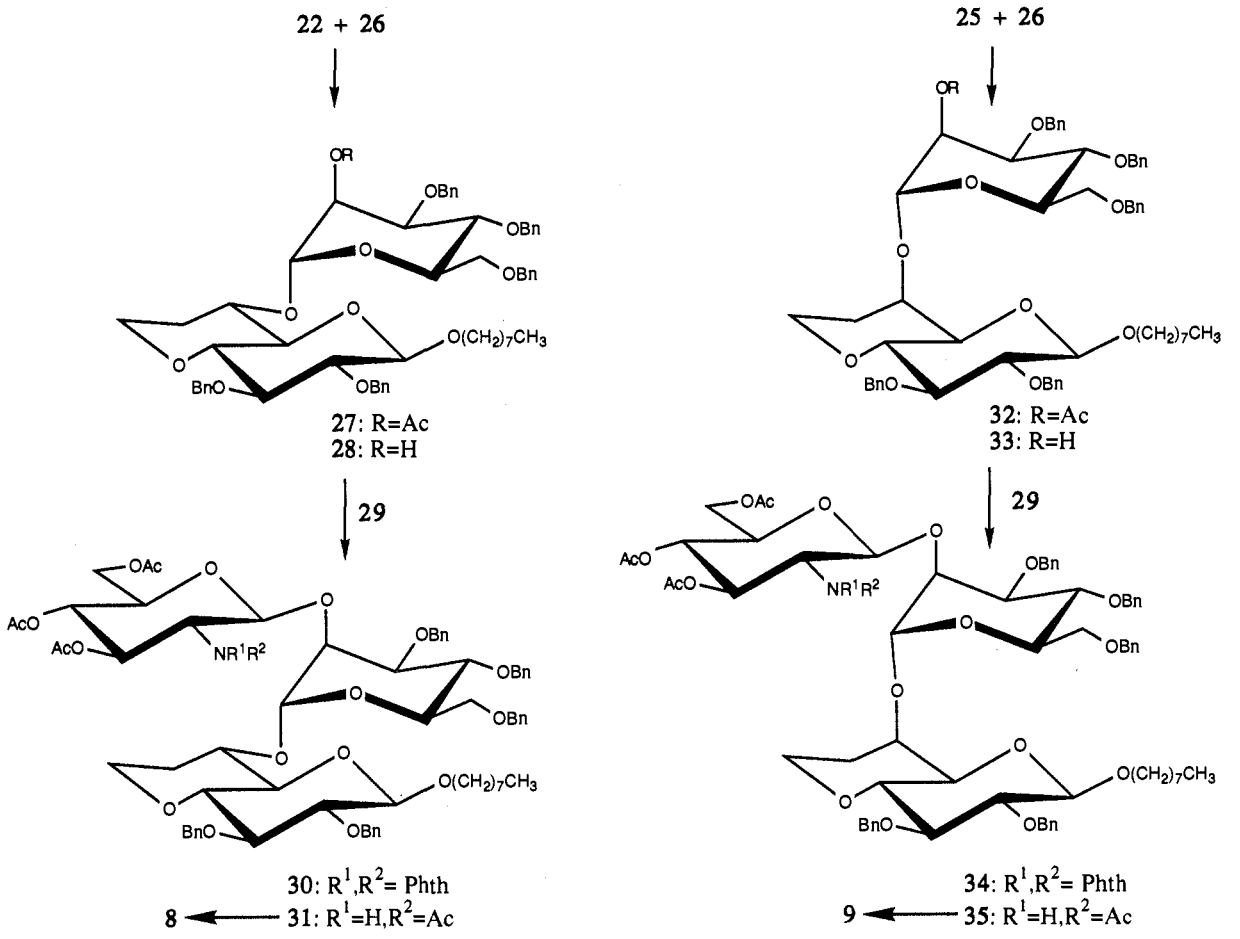

Table I. Selected Chemical Shifts (ppm) and Coupling Constants (Hz) for Key Synthetic Di- and Trisaccharides[a]

nucleus[b]	27	30	31	32	34	35	8	9
H-1 ($J_{1,2}$)	4.353 (8.0)	4.290 (8.0)	4.336 (8.0)	4.375 (7.5)	nd[c]	4.385 (7.5)	4.466 (7.8)	4.468 (8.0)
H-1′ ($J_{1',2'}$)	5.009 (2.0)	nd[c]	4.913 (1.8)	5.217 (2.0)	5.078 (1.8)	5.140 (2.0)	5.038 (1.5)	5.064 (1.8)
H-1″ ($J_{1'',2''}$)		5.533 (8.5)	5.263 (8.5)		5.540 (8.5)	5.050 (8.5)	4.560 (8.2)	4.584 (8.0)
H-2′ ($J_{2',3'}$)	5.338 (3.0)	nd[c]	nd[c]	5.933 (3.0)	nd[c]	nd[c]	4.045 (3.2)	4.206 (3.2)
$OCOCH_3$	2.151	2.052, 2.036, 1.83	2.033, 2.013, 1.997	2.140	2.030 (2×CH_3) 1.875	1.990 (2×CH_3) 1.970		
$NCOCH_3$			1.736			1.760	2.052	2.050
$-CH_2CH_3$	0.865	0.846	0.863	0.860	0.865	0.850	0.861	0.863
other H		5.782 (3″:dd, 9.0, 11.0)	5.573 (NH:d, 7.0)	3.151 (5:dd, 2.5, 9.5)	5.799 (3″:dd, 9.0, 10.5)	5.335 (NH:d, 8.0)	3.983 (6:ddd, 5.0, 9.0, 11.0) 3.285 (5:dd, 9.0, 9.5) 3.109 (4:dd, 9.0, 9.0)	4.281 (6:ddd, 2.0, 2.5, 3.5) 3.060 (2:m)
C-1	103.58	103.50	103.60	103.76	104.55	104.55	103.05	103.47
C-1′	93.57	92.62	92.98	98.75	97.34	98.31	93.97	99.24
C-1″		97.18	97.39		95.85	97.72	100.48	99.99
C-2″		54.55	56.66		54.31	55.72	56.24	56.32
C=O	170.59	170.67, 170.20, 169.44	171.60, 170.62, 169.99, 169.68	169.80	170.69, 170.02, 169.35	180.78, 170.70, 170.17, 169.56	175.60	175.55
CH_2CH_3	14.13	14.11	14.10	14.12	14.10	14.13	14.22	14.22

[a] Spectra for **27–35** were recorded in $CDCl_3$; spectra for **8** and **9** were recorded in D_2O; ^1H NMR at 360 MHz and ^{13}C NMR at 75 MHz. [b] Assignments are tentative, but ^1H connectivities were confirmed by homonuclear decoupling. [c] Not determined due to spectral overlap.

trisaccharide **9** (76%). Selected ^1H and ^{13}C NMR data for the synthetic di- and trisaccharides produced in this study are presented in Table I and fully support their structural assignments.

Evaluation of 5 and Its Conformationally Restricted Analogues 8 and 9 as Acceptor Substrates for GlcNAcT-V. The objective of this work was to establish whether GlcNAcT-V acts preferentially on either the gg or gt rotamers of asparagine-linked oligosaccharides such as **1** and, if so, which rotamer about the C5-C6 bond of the βMan residue in **1** was recognized by the enzyme. The fused ring trisaccharides **8** and **9** were the simplest analogues of these complex oligosaccharides which could be conveniently prepared by chemical synthesis and be expected to serve as models for these two rapidly interconverting rotamers. GlcNAcT-V has not yet been purified to homogeneity, but we have found that the enzyme from hamster kidney binds to a UDP-hexanolamine Sepharose affinity column[20] from which it could be eluted by high ionic strength buffers. This simple procedure resulted in a 140-fold purification and, more importantly, provided an enzyme preparation that was stable for several months at 4 °C. All of the assays for GlcNAcT-V reported here were carried out in the presence of EDTA since GlcNAcT-V is the only

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Table II. Evaluation of Trisaccharides **5**, **8**, **9** as Acceptor Substrates for GlcNAcT-V

acceptor structure	K_m (mM)	V_{max} (pmol/min)	rel[a] V_{max}/K_m
5 (gg ⇌ gt)	0.021 ± 0.002	3.60 ± 0.09	100
8 (gt)	9.8 ± 0.7	0.77 ± 0.2	0.05
9 (gg)	0.029 ± 0.004	11.2 ± 0.4	227

[a] The value for compound **5** is arbitrarily set to 100.

known branching GlcNAc-transferase in the asparagine-linked oligosaccharide pathway which retains activity in the absence of manganese.[1] The hydrophobic aglycons in **5**, **8**, and **9** furthermore allowed simple evaluation of these synthetic trisaccharides as substrates for the enzyme in radioactive assays[10] that measured the rate of transfer of [^{3}H]-GlcNAc from UDP-[^{3}H]-GlcNAc.

Table II presents the kinetic data obtained for enzyme assays conducted by using the gg ⇌ gt (**6** ⇌ **7**) conformationally labile trisaccharide acceptor **5** and its two analogues **8** (gt) and **9** (gg) as acceptor substrates in the presence of saturating UDP-GlcNAc. The Michaelis-Menten parameters measured for the flexible **5** with this enzyme preparation were K_m = 0.021 ± 0.002 mM and V_{max} = 3.6 ± 0.09 pmol/min under our standard assay conditions. As seen in Table II, the gt trisaccharide **8** showed extremely low activity with an almost 500-fold elevated K_m (9.8 ± 0.7 mM) and a decreased V_{max} (0.77 ± 0.02 pmol/min). The gg model **9**, however, showed activity very close to that of **5** with a K_m of 0.029 ± 0.004 mM and an increased V_{max} of 11.2 ± 0.4 pmol/min. Comparison of the relative V_{max}/K_m values (Table II), representing the second-order rate constants for the reaction of free enzyme with free substrate for the three compounds, further highlights the specificity of the enzyme for the conformationally restricted gg rotamer **9**.

Conclusion

We consider the data presented in Table II to demonstrate that the gg rotamer of **5**, namely **7**, is the conformation clearly preferred by the enzyme in the kinetically important transfer step catalyzed by GlcNAcT-V. This conclusion can safely be reached since both fused ring analogues **8** and **9** contain the same "artifactual" ethylene bridge between O-4 and C-6, yet only **9** reacts with the enzyme at a rate similar to that of the conformationally unrestricted **5**. The gt structure **8** showed barely detectable activity under normal assay conditions.[10] These results represent the first conclusive experimental evidence that discrete conformations of asparagine-linked oligosaccharides can be distinguished by the enzymes which control their biosynthesis. Knowledge of these acceptor conformations will be of value especially in the design of substrate analogue inhibitors[21] for this class of enzyme.

Experimental Section

General Methods. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at ambient temperatures (22 ± 2 °C). TLC was performed on precoated plates of silica gel (60-F_{254}, Merck) with detection by quenching of fluorescence or by charring after spraying with 5% H_2SO_4 in ethanol. R_f values refer to mobility in TLC. Unless otherwise noted, column chromatography was performed on Silica gel 60 (Merck, 40-63 μm). For gel filtration, Bio-Gel P-2 (200-400 mesh, Bio-Rad Laboratories) was used. ^{1}H NMR spectra were recorded at 360 (Bruker WM-360) or 300 MHz (Bruker AM-300) with either tetramethylsilane (δ = 0 in $CDCl_3$) or in acetone (δ = 2.225 in D_2O) as internal standard at ambient temperature. ^{13}C NMR spectra were recorded at 75.5 MHz (Bruker AM-300) with internal tetramethylsilane (δ = 0 in $CDCl_3$) or external 1,4-dioxane (δ = 67.4 in D_2O) as reference standards. Only partial NMR data are reported. Other spectral features were in accord with the proposed structures. The ^{1}H NMR chemical shifts and coupling constants are reported as though they were first order. Assignments of ^{13}C resonances are tentative. Unless otherwise noted, all reactions were carried out at ambient temperature. In the processing of reaction mixtures, solutions in organic solvents were washed with equal volumes of aqueous solutions. The organic solutions were then dried over sodium sulfate prior to solvent removal on a rotary evaporator under the vacuum of a water aspirator with bath temperature of 40 °C or lower. The microanalyses were carried out by the Analytical Services Laboratory of this department.

The following solvent systems have been designated by letters: A, toluene–ethyl acetate (3:1); B, toluene–ethyl acetate (1:1); C, ethyl acetate; D, hexane–acetone (3:1); E, hexane–acetone (4:1); F, hexane–ethyl acetate (3:1); G, hexane–ethyl acetate (2:1); H, toluene–ethyl acetate (2:1); I, hexane–acetone (3:2); J, dichloromethane–methanol (10:1); K, dichloromethane–methanol–water (40:20:1).

Methyl 2,3-Di-*O*-benzyl-7,8,9-trideoxy-4-*O*-(4-methoxybenzyl)-D-*glycero*-α-D-*gluco*-non-8-enopyranoside (12) and Its L-*glycero* Isomer (13). A solution of dimethyl sulfoxide (780 μL, 11 mmol) in dry dichloromethane (40 mL) was added dropwise to a cooled (−78 °C) solution of oxalyl chloride in dry dichloromethane (40 mL) under argon. The solution was stirred for 15 min at −78 °C, then a solution of **10** (2.7 g, 5.5 mmol) in dichloromethane (50 mL) was added dropwise, and the reaction was stirred for 30 min. Dry triethylamine (3.8 mL, 27 mmol) was added dropwise, and the reaction mixture was warmed to room temperature and quenched with aqueous saturated sodium bicarbonate solution (20 mL). The dichloromethane layer was separated, and the aqueous layer was extracted 3 times with dichloromethane. The combined organic layers were then concentrated in vacuo. The residue was purified by silica gel chromatography by using dichloromethane–methanol (49:1) as eluant. Appropriate fractions were collected and analyzed by NMR spectroscopy which showed the material to consist of ≥90% aldehyde **11** (^{1}H NMR, δ 9.620 ppm; ^{13}C NMR, δ 197.62 ppm). This aldehyde (1.2 g, 44%) was dried over phosphorus pentaoxide under high vaccum for 12 h. Allylmagnesium chloride (2 M in tetrahydrofuran, 4 mL) was diluted with tetrahydrofuran (10 mL) and kept under an argon atmosphere. The aldehyde **11** was then dissolved in tetrahydrofuran (20 mL) and added dropwise over 60 min into the Grignard reagent solution. The reaction was stirred for an additional 15 min by which time TLC (solvent A) showed complete disappearance of **11**. The reaction was quenched by addition of saturated aqueous ammonium chloride. The water layer was extracted with dichloromethane (3 × 50 mL), and the combined organic layers were washed with water (50 mL), dried with sodium sulfate, and then concentrated in vacuo. Purification of **12** and **13** by silica gel chromatography (solvent A) gave a syrup (1.0 g, 2.1 mmol, 77%) consisting of an approximately 1:1 mixture of these two compounds. The *S* (L) isomer **13** crystallized from dichloromethane–hexane: mp 81–82 °C; $[\alpha]_D$ +36.4° (*c* 1.40, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 7.50–6.80 (14 H arom), 5.823 (m, 1 H, CH_2=C*H*-CH_2), 5.130–4.610 (8 H, CH_2= and 3 × CH_2Ar), 4.578 (d, 1 H, *J* = 3.5 Hz, H-1), 3.973 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-3), 3.889 (ddd, 1 H, *J* = 8.5 Hz (D_2O-exchangeable), 5.5, 1.0 Hz, H-6), 3.790 (s, 3 H, CH_3OAr), 3.662 (dd, *J* = 9.5, 9.5 Hz, H-4), 3.518 (dd, 1 H, *J* = 9.5, 1.0 Hz, H-5), 3.495 (dd, 1 H, *J* = 3.5, 9.5 Hz, H-2), 3.351 (s 3 H, OCH_3), 2.416 (m, 1 H, =CH—C*H*H), 2.259 (m, 1 H, =CH—CH*H*), 1.627 (d, 1 H, *J* = 8.5 Hz, 6-OH, D_2O-exchangeable); ^{13}C NMR ($CDCl_3$) δ 98.42 (C-1), 82.14, 79.89, 77.48, 71.62, and 68.10 (C-2, C-3, C-4, C-5, C-6), 75.73, 74.89, and 73.51 (3 × benzylic), 55.34 (OCH_3), 38.66 (C-7). Anal. Calcd for $C_{32}H_{38}O_7$: C, 71.88; H, 7.18. Found: C, 71.67; H, 7.26.

Methyl 2,3-Di-*O*-benzyl-7-deoxy-4-*O*-(4-methoxybenzyl)-L-*glycero*-α-D-*gluco*-octopyranoside (14). Methanol (50 mL) was saturated with ozone at −78 °C, and **13** (1.30 g, 2.43 mmol), dissolved in dichloromethane (50 mL), was added. Ozone was bubbled through the reaction mixture for 20 min, the reaction was warmed to room temperature, and dimethyl sulfide (2.4 mL) was then added. After 30 min sodium borohydride (200 mg) was added, and the reaction was stirred for an additional 30 min. Amberlite IR-120(H) was then added until the solution attained neutrality (pH-paper), the resin was removed by filtration, and solvents were evaporated. The residue was purified by silica gel chromatography to provide **14** (R_f 0.18, solvent B, 880 mg, 1.63 mmol, 67%). Compound **14** was recrystallized from dichloromethane–hexane: mp 133–134 °C; $[\alpha]_D$ +39.5° (*c* 1.2 dichloromethane); R_f 0.75 (solvent C); ^{1}H NMR ($CDCl_3$) δ 7.50–6.80 (14 H, arom), 4.995–4.625 (6 H, 3 × CH_2Ar), 4.577 (d, 1 H, *J* = 3.5 Hz, H-1), 4.055 (br m, 1 H, *J* = 8.0 (D_2O-exchangeable), 9.5, 1.0 Hz, H-6), 3.976 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-3), 3.833 (br, 2 H, H-8 (coupled to OH-8)), 3.790 (s, 3 H, C*H*$_3$OAr), 3.618 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-4), 3.499 (dd, 1 H, *J* = 3.5, 9.5 Hz, H-2), 3.455 (dd, 1 H, *J* = 1.0, 9.5 Hz, H-5), 3.342 (s, 3 H, CH_3), 2.140 (br, 1 H, OH-8), 2.100 (d, 1 H, *J* = 8.0 Hz, OH-6), 1.750 and 1.590 (each m, 1 H, H-7's); ^{13}C NMR ($CDCl_3$) δ 98.36 (C-1), 82.17, 79.88, 77.05, 72.51, 68.83 (C-2, C-3, C-4, C-5, C-6), 75.75, 74.82, and 73.50 (3 × benzylic), 61.60 (C-8), 55.36, and 55.24 (2 × OMe), 35.85 (C-7). Anal. Calcd for $C_{31}H_{38}O_8$: C, 69.11; H, 7.12. Found: C, 69.37; H, 7.16.

Methyl 2,3-Di-*O*-benzyl-7-deoxy-L-*glycero*-α-D-*gluco*-octopyranoside (15). Ceric ammonium nitrate (1.73 g, 3.16 mmol) was added in portions to a solution of **14** (850 mg, 1.58 mmol) in acetonitrile–water (9:1,

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10 mL). When TLC indicated no remaining starting material (45 min), the reaction was diluted with dichloromethane (100 mL) and washed with aqueous sodium bicarbonate. The aqueous layer was extracted with dichloromethane (2 × 50 mL), and the combined organic layers were washed with water prior to drying and concentration. Solvent removal left a syrup which was purified by silica gel chromatography (solvent C) to provide **15** (532 mg, 1.27 mmol, 80%): $[\alpha]_D$ +40.9° (*c* 1.0 dichloromethane); R_f 0.25 (solvent C); ^{1}H NMR ($CDCl_3$) δ 7.480–7.400 (10 H, arom), 5.013–4.684 (4 H, 2 × CH_2Ar), 4.609 (d, 1 H, *J* = 3.5 Hz, H-1), 4.135–3.462 (7 H, H-2, H-3, H-4, H-5, H-6, H-8's), 3.345 (s, 3 H, OCH_3) 2.836 (d, 1 H, *J* = 2.5 Hz, D_2O-exchangeable, OH-4), 2.677 (d, 1 H, *J* = 7.0 Hz, OH-6), 2.500 (br, 1 H, OH-8, D_2O-exchangeable), 1.933 and 1.643 (each m, 1 H, H-7's); ^{13}C NMR ($CDCl_3$) δ 138.81 and 138.15 (quart. arom), 98.40 (C-1), 81.85, 79.60, 72.87, 69.99, and 66.93 (C-2, C-3, C-4, C-5, C-6), 60.18 (C-8), 55.26 (OCH_3), 35.67 (C-7). Anal. Calcd for $C_{32}H_{30}O_7$: C, 66.00; H, 7.24. Found: C, 65.37; H, 7.09.

Methyl 4,6,8-Tri-*O*-acetyl-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-α-D-*gluco*-octopyranoside (16). Acetic anhydride (0.774 mL, 7.14 mmol) was added dropwise to a stirred solution of triol **15** (500 mg, 1.19 mmol) and *N*,*N*-(dimethylamino)pyridine (2 mg, 16 mmol) in pyridine (5 mL). After 2 h, methanol (2 mL) was added, and stirring was continued for an additional 20 min. Solvent was removed under high vaccum, and the residue was purified by silica gel chromatography (solvent D) to provide **16** (642 mg, 1.18 mmol, 99%) as a syrup which crystallized on standing. Recrystallization from methanol gave needles: mp 87°C; $[\alpha]_D$ +7.6° (*c* 0.50 dichloromethane); R_f 0.40 (solvent D); ^{1}H NMR ($CDCl_3$) δ 7.280–7.460 (10 H, arom), 5.118 (ddd, 1 H, *J* = 2.0, 4.5, 6.5 Hz, H-6), 4.960 (dd, 1 H, *J* = 9.5, 10.0 Hz, H-4), 4.888–4.628 (4 H, 2 × CH_2Ar), 4.604 (d, 1 H, *J* = 3.5 Hz, H-1), 4.058 (m, 2 H, H-8), 3.874 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-3), 3.771 (dd, 1 H, *J* = 10.0, 2.0 Hz, H-5), 3.609 (dd, 1 H, *J* = 9.5, 3.5. Hz, H-2), 3.401 (s, 3 H, OCH_3), 2.069 and 1.900 (each m, 1 H, H-7's), 2.062, 2.030, and 1.889 (each s, 3 H, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.91, 170.52, and 169.68 (C=O), 98.78 (C—1), 79.64, 79.40, 69.68, 68.91, and 66.87 (C-2, C-3, C-4, C-5, C-6), 75.47 and 73.72 (benzylic), 60.85 (C-8), 55.88 (OCH_3), 29.85 (C7), 20.91, 20.88, 20.81 ($COCH_3$). Anal. Calcd for $C_{29}H_{36}O_{10}$: C, 63.95; H, 6.68. Found: C, 63.72; H, 6.50.

1,4,6,8-Tetra-*O*-acetyl-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-α,β-D-*gluco*-octopyranose (17). A freshly prepared solution of sulfuric acid (27 μL) in acetic anhydride (1.5 mL) was added dropwise, with stirring to a solution of **16** (620 mg, 1.14 mmol) in acetic anhydride (6 mL) at −5 °C for 1.5 h. The reaction mixture was then poured into a stirred solution of dichloromethane (150 mL) and saturated aqueous sodium bicarbonate (150 mL) on crushed ice and was stirred for a further 4 h. The aqueous layer was extracted with dichloromethane (50 mL). The combined organic layers were washed with water (50 mL) prior to solvent evaporation. Purification by silica gel chromatography (solvent E) provided **17** (411 mg, 0.72 mmol, 63%, α:β ≈ 9:1) as a syrup: R_f 0.40 (solvent D); ^{1}H NMR ($CDCl_3$) δ 7.360–7.240 (10 H, arom), 6.320 (d, *J* = 3.6 Hz, H-1α), 5.537 (d, *J* = 8.0 Hz, H-1β); ^{13}C NMR ($CDCl_3$) δ 170.85, 170.39, 169.43, and 169.10 (C=O), 94.30 (C-1β), 89.76 (C-1α), 82.09, 80.85, 74.63, 68.40, 66.50 (C-2β, C-3β, C-4β, C-5β, C-6β), 79.12, 78.61, 71.43, 68.28, 66.37 (C-2α, C-3α, C-4α, C-5α, C-6α), 75.32 and 73.48 (benzylic), 60.61 (C-8β), 60.45 (C-8α), 29.49 (C-7α and β), 20.96, 20.82, and 20.72 (3 × CH_3). Anal. Calcd for $C_{30}H_{36}O_{11}$: C, 62.86; H, 6.35. Found: C, 62.76; H, 6.23.

4,6,8-Tri-*O*-acetyl-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-α-D-*gluco*-octopyranosyl Bromide (18). Hydrogen bromide gas was passed for 45 min through a tube (25 × 2 cm) of calcium sulfate into a solution of **17** (388 mg, 0.68 mmol) in dry dichloromethane (20 mL) at O °C. The solution was taken to dryness, and toluene (20 mL) was added and evaporated twice. For characterization purposes, a small quantity of the oily residue was passed through a pad of silica gel (solvent A): $[\alpha]_D$ +174.1° (*c* 2.8 dichloromethane); ^{1}H NMR ($CDCl_3$) δ 7.400–7.24 (10 H arom), 6.360 (d, 1 H, *J* = 4.0 Hz, H-1), 5.165 (ddd, 1 H, *J* = 2.0, 6.5, 8.0 Hz, H-6), 5.061 (dd, 1 H, *J* = 10.0, 9.5 Hz, H-4), 4.890–4.643 (4 H, 2 × CH_2Ar), 4.089 (dd, 1 H, *J* = 2.0, 10.0 Hz, H-5), 4.055 (m, 2 H, H-8), 3.920 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-3), 3.606 (dd, *J* = 4.0, 9.5 Hz, H-2), 2.085–1.800 (11 H, H-7's and 3 × $COCH_3$).

Octyl 4,6,8-Tri-*O*-acetyl-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (19). 1-Octanol (0.3 mL, 1.90 mmol) was added dropwise, under an argon atmosphere, to a stirred solution of **18** (0.926 mmol) and silver zeolite (0.93 g) in dry dichloromethane (10 mL). After stirring at room temperature for 2 h, the reaction was filtered through a pad of Celite which was washed with dichloromethane (30 mL). The filtrate was washed sequentially with saturated aqueous sodium bicarbonate and then water, dried, and concentrated to a syrup which was purified by silica gel chromatography (solvent E) to provide **19** (460 mg, 0.721 mmol, 78%) as a solid. Recrystallization from hexane gave needles: mp 82 °C; R_f 0.36 (solvent F); $[\alpha]_D$ −27.5° (*c* 1.0 dichloromethane); ^{1}H NMR ($CDCl_3$) 7.360–7.200 (10 H arom), 5.104 (ddd, 1 H, *J* = 2.5, 5.5, 8.0 Hz, H-6), 5.055 (dd, 1 H, *J* = 9.5, 9.5 Hz, H-4), 4.936–4.594 (4 H, 2 × CH_2Ar), 4.372 (d, 1 H, *J* = 7.5 Hz, H-1), 4.076 (t, 2 H, *J* = 6.5 Hz, H-8), 3.950 (dt, 1 H, J = 6.0, 9.5 Hz, $OCHHCH_2$), 3.558–3.515 (3 H, H-3, H-2, and $OCHHCH_2$), 3.411 (dd, 1 H, *J* = 2.5, 9.5 Hz, H-5), 2.098–1.260 (14 H, aliphatic), 2.057, 2.044, and 1.878 (each s, 3 H, $COCH_3$), 0.878 (t, 3 H, *J* = 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.92, 170.76, and 169.62 (C=O), 104.32 (C-1), 82.08, 81.78, 73.64, 68.82, and 66.95 (C-2, C-3, C-4, C-5, C-6), 75.17, 75.01 (benzylic), 70.55 (OCH_2CH_2), 60.88 (C-8), 31.87, 29.78, 29.61, 29.45, 29.29, 26.22, and 22.71 (C-7 and aliphatic), 20.97, 20.84 (2×C) and 20.84 ($COCH_3$), 14.13 (CH_3). Anal. Calcd for $C_{32}H_{50}O_{10}$: C, 67.27; H, 7.86. Found: C, 67.21; H, 7.66.

Octyl 2,3-Di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (20). Compound **19** (234 mg, 0.364 mmol) was O-deacetylated by treatment with methanol containing a trace (≈0.01 M) of sodium methoxide. After the reaction was complete as evidenced by TLC (solvent C, R_f 0.41), the mixture was neutralized with IRC 50 (H^+) resin, and the resin was removed by filtration. The solution was taken to dryness, and the solid residue was recrystallized from dichloromethane–hexane to yield **20** (183 mg, 0.354 mmol, 97%): mp 65–66 °C $[\alpha]_D$ −27.3° (*c* 1.37, dichloromethane); ^{1}H NMR ($CDCl_3$) δ 7.380–7.300 (10 H, arom), 4.980–4.680 (4 H, 2 × CH_2Ar), 4.437 (d, 1 H, *J* = 7.5 Hz, H-1), 4.100 (m, br, coupled with OH-6, H-6), 3.910–3.848 (3 H), 3.702 (m, coupled with OH-4, H-4), 3.525 (dt, 1 H, *J* = 6.0, 9.5 Hz, $OCHHCH_2$), 3.451 (dd, 1 H, *J* = 9.0, 9.0 Hz, H-3), 3.397 (dd, 1 H, *J* = 7.5, 9.0, H-2), 3.188 (dd, 1 H, *J* = 2.5, 9.5 Hz, H-5), 2.520 (br, 2 H, D_2O-exchangeable, OH-4 and 6), 2.392 (br, 1 H, D_2O-exchangeable, OH-8), 0.876 (t, 3 H, *J* = 6.5 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 104.02 (C-1), 84.07, 81.81, 76.39, 69.62, and 69.35 (C-2, C-3, C-4, C-5, C-6), 75.31 and 74.72 (benzylic), 70.41 (CH_2CH_2), 60.97 (C-8), 35.39, 31.87, 29.82, 29.44, 29.28, 26.21, and 22.70 (C-7's and aliphatic), 14.13 (CH_3). Anal. Calcd for $C_{30}H_{44}O_7$: C, 69.73; H, 8.60. Found: C, 69.61; H, 8.62.

Octyl 2,3-Di-*O*-benyl-7-deoxy-8-*O*-(2,4,6-triisopropylbenzenesulfonyl)-L-*glycero*-β-D-*gluco*-octopyranoside (21). 2,4,6-Triisopropylbenzenesulfonyl chloride (249 mg, 0.822 mmol) was dissolved in dichloromethane (0.5 ml) and added dropwise at O °C to a solution of **20** (354 mg, 0.685 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (100 mg, 0.822 mmol) stirring in dichloromethane (4 mL) at O °C. After 5 h the reaction was cooled to −18 °C and kept there for 10 h. TLC indicated that unreacted **20** remained, and a stream of argon was passed through the flask to decrease the amount of solvent to about 1 mL, and more 4-(*N*,*N*-dimethylamino)pyridine (25 mg, 0.21 mmol) and 2,4,6-triisopropylbenzenesulfonyl chloride (62 mg, 0.21 mmol) were then added at room temperature. After 60 min methanol (2 mL) was added, and stirring was continued for an additional 30 min. Solvent was removed by evaporation, and the residue was applied to a silica gel column which was eluted with solvent F to provide **21** (400 mg, 0.511 mmol, 74%), as a syrup: R_f 0.30 (solvent F); $[\alpha]_D$ −22.2° (*c* 1.0, dichloromethane); ^{1}H-NMR ($CDCl_3$) δ 7.360–7.180 (12 H, arom), 4.990–4.663 (4 H, 2 × CH_2Ar), 4.420 (d, 1 H, *J* = 7.5 Hz, H-1), 4.228 (t, 2 H, *J* = 6.5, H-8), 4.136 (sept, 2 H, *J* = 7.0 Hz, $ArCH(CH_3)$), 3.982 (br m, 1 H, coupled with OH-6, H-6), 3.883 (dt, 1 H, *J* = 6.5, 9.5 Hz, $OCHHCH_2$), 3.664 (m, 1 H, coupled with OH-4, H-4), 3.518–3.378 (3 H, H-3, H-2, and $OCHHCH_2$), 3.128 (dd, 1 H, *J* = 2.0, 9.5 Hz, H-5), 2.903 (sept, 1 H, *J* = 7.0 Hz, $ArCH(CH_3)$), 2.188 (d, 1 H, *J* = 2.5 Hz, D_2O-exchangeable, OH-4), 1.960 (d, 1 H, *J* = 10.0 Hz, D_2O-exchangeable, OH-6), 0.875 (t, 3 H, *J* = 7.0 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 153.63, 150.81 (2×C), 138.54, 138.36, and 129.42 (quat. arom), 103.99 (C-1), 83.94, 81.76, 76.27, 69.45, and 66.13 (C-2, C-3, C-4, C-5, C-6), 75.22 and 76.65 (benzylic), 70.32 (CH_2CH_2), 66.83 (C-8), 14.14 (CH_3). Anal. Calcd for $C_{45}H_{66}O_6S$: C, 69.01; H, 8.51. Found: C, 69.24; H, 8.49.

Octyl 4,8-Anhydro-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (22). Sodium hydride (50% dispersion in oil, 100 mg) was washed with hexane (4 × 4 mL), *N*,*N*-dimethylformamide (4 mL) was added under argon, and the mixture was cooled to −50 °C. A solution of **21** (537 mg, 0.686 mmol) in *N*,*N*-dimethylformamide (4 mL) was then added dropwise. The reaction mixture was stirred for 3 h, while the temperature was raised slowly to room temperature when methanol (2 mL) was added. The mixture was diluted with dichloromethane (100 mL) and washed with water (3 × 25 mL). The combined water layers were extracted with dichloromethane (2 × 25 mL), and the organic layers were combined, dried, and evaporated. Chromatography on silica gel (solvent F) provided a semicrystalline residue which was recrystallized from hexane to yield **22** (160 mg, 0.321 mmol, 63%): mp 69–70 °C; R_f 0.21 (solvent F) $[\alpha]_D$ +3.9° (*c* 1.0, dichloromethane); ^{1}H NMR (assigned by homonuclear decoupling) ($CDCl_3$) δ 7.370–7.260 (10 H, arom), 4.447 (d, 1 H, *J* = 7.5 Hz, H-1), 4.030 (ddd, 1 H, *J* = 5.0, 1.5, 12.5 Hz, H-8e), 3.950 (dt, 1 H, *J* = 6.5, 9.5 Hz, $OCHHCH_2$), 3.082 (br, on D_2O-exchange becomes dddd, 1 H, *J* = 5.0, 9.0, 11.0, Hz, H-6), 3.583–3.480

(3 H, H-3, H-8a, and OC*H*HCH$_2$), 3.375 (dd, 1 H, J = 7.5, 9.0 Hz, H-2), 3.133 (dd, 1 h, J = 9.0, 9.0 Hz, H-4), 2.989 (dd, 1 H, J = 9.0, 9.0 Hz, H-5), 2.480 (d, 1 H, J = 2.0 Hz, D$_2$O-exchangeable, OH-6), 2.010 (m, 1 H, H-7e), 1.713 (m, 1 H, H-7a) and 0.875 (t, 3 H, J = 6.5 Hz, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 138.32 and 138.49 (quat. arom), 103.98 (C-1), 81.98, 81.90, 79.65, 76.78, and 70.39 (C-2, C-3, C-4, C-5, C-6), 70.70 and 66.07 (C-8 and *C*H$_2$CH$_2$), 32.68, 31.85, 29.88, 29.45, 29.26, 26.17, and 22.69 (C-7 and aliphatic), 14.11 (CH$_3$). Anal. Calcd for $C_{30}H_{42}O_6$: C, 72.24; H, 8.51. Found: C, 72.05; H, 8.37.

Octyl 6-*O*-Acetyl-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (23). Compound **22** (ca. 2 mg) was acetylated in pyridine and acetic anhydride (1:1 v/v, 1 mL) and purified by silica gel chromatography (solvent F) to provide **23** for purposes of later comparison with **24**: ^{1}H NMR (CDCL$_3$) δ 7.390–7.240 (10 H, arom), 4.893–4.715 (4 H, 2×CH$_2$Ar), 4.886 (ddd, 1 H, J = 5.5, 9.0, 11.0 Hz, H-6), 4.328 (d, 1 H, J = 7.5 Hz, H-1), 3.942 (ddd, 1 H, J = 12.0, 1.0, 5.0 Hz, H-8e), 3.799 (dt, 1 H, J = 6.0, 9.0 Hz, OC*H*HCH$_2$), 3.510–3.436 (3 H, H-3, H-8a and OC*H*HCH$_2$), 3.321 (dd, 1 H, J = 7.5, 9.5 Hz, H-2), 3.157 and 3.073 (dd, 1 H each, J = 9.5, 9.5 Hz, H-4 and H-5), 2.050 (m, 1 H, H-7e), 2.002 (s, 3 H, COC*H*$_3$), 0.805 (t, 3 H, J = 6.5 Hz, CH$_3$).

Octyl 6-*O*-Acetyl-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-D-*glycero*-β-D-*gluco*-octopyranoside (24). Trifluoromethanesulfonic anhydride (102 mg, 0.36 mmol) in dichloromethane (300 μL) was added dropwise at −10 °C to a solution of **22** (75 mg, 0.15 mmol) and pyridine (150 μL) in dichloromethane (3 mL) stirring at the same temperature under argon. After 20 min, TLC showed a single product (R_f 0.74 in solvent F), most likely the trifluoromethanesulfonyl ester of **22**. The reaction was diluted with dichloromethane (30 mL) and washed with ice cold 5% aqueous hydrochloric acid and twice with ice cold water. The organic layer was taken to dryness without external heating, and toluene (3 × 5 mL) was added and evaporated from the residue which was then dissolved in 0.5 M tetraethylammonium acetate in *N*,*N*-dimethylformamide (2 mL). The reaction was stirred for 2 h, solvent was removed under vacuum, and the residue was dissolved in dichloromethane (50 mL) and washed twice with water, dried, and concentrated to syrup which was purified by chromatography on silica gel (solvent F) to provide **24** (71 mg, 0.13 mmol, 87%) as a syrup, R_f 0.53 (solvent F), $[\alpha]_D$ −23.1° (c 1.57 dichloromethane); ^{1}H NMR (assigned by homonuclear decoupling) (CDCl$_3$) δ 7.390–7.240 (10 H, arom), 5.360 (ddd, J = 2.0, 2.0, 2.5 Hz, H-6), 4.893–4.715 (4 H, 2×CH$_2$Ar), 4.412 (d, 1 H, J = 8.0 Hz, H-1), 3.899 (dt, 1 H, J = 6.0, 9.5 Hz, OC*H*HCH$_2$) 3.832 (ddd, 1 H, J = 3.0, 3.0 Hz, 12.0 Hz, H-8e), 3.707 (2H, H-4 and H-8a), 3.526 (dd, 1 H, J = 9.0, 9.0 Hz, H-3), 3.496 (dt, 1 H, J = 6.0, 9.5 Hz, OC*H*HCH$_2$) 3.377 (dd, 1 H, J = 8.0, 9.0 Hz, H-2), 3.204 (dd, 1 H, J = 2.5, 9.0 Hz, H-5), 2.108 (s, 3 H, COC*H*$_3$), 1.860–1.240 (14 H, aliphatic, H-7's), 0.860 (s, 3 H, J = 6.5 Hz, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 169.84 (C=O), 103.39 (C-l), 81.80, 81.25, 75.34, 70.67, and 66.53 (C-2, C-3, C-4, C-5, C-6), 74.62 (2×benzylic), 69.76 (*C*H$_2$CH$_2$), 61.77 (C-8), 31.40, 29.86, 29.23, 28.91, 28.77, 25.64, and 22.17 (C-7 and aliphatic), 20.79 (COC*H*$_3$), 13.60 (CH$_3$). Anal. Calcd for $C_{33}H_{44}O_7$: C, 71.07; H, 8.22. Found: C, 71.05; H, 8.25.

Octyl 4,8-Anhydro-2,3-di-*O*-benzyl-7-deoxy-D-*glycero*-β-D-*gluco*-octopyranoside (25). Compound **24** (71 mg, 0.13 mmol) was O-deacetylated as described for the preparation of **20**. After neutralization with IRC 50 (H$^+$) resin, the resin was removed by filtration, and solvent was evaporated to give **25** (64 mg, 0.13 mmol). An analytical sample was prepared by passing a portion of this material through a pad of silica gel (solvent G): $[\alpha]_D$ −10.7° (c 1.40 dichloromethane); ^{1}H NMR (assigned by homonuclear decoupling) (CDCl$_3$) δ 7.385–7.250 (10 H, arom), 4.895–4.728 (4 H, 2×CH$_2$Ar), 4.492 (d, 1 H, J = 7.5 Hz, H-l), 4.220 (br ddd, J = 2.5, 2.5, 3.0 Hz, H-6, sharper after D$_2$O-exchange), 3.916 (dt, 1 H, J = 6.5, 9.5 Hz, OC*H*HCH$_2$), 3.803 (m, 2 H, H-8's), 3.682 (dd, 1 H, J = 9.5, 9.5 Hz, H-4), 3.546 (2 H, H-3, OC*H*HCH$_2$), 3.346 (dd, 1 H, J = 7.5, 9.0 Hz, H-2), 3.146 (dd, 1 H, J = 2.5, 9.5 Hz, H-5), 2.246 (br s, 1 H, D$_2$O-exchangeable, OH-6), 1.855 (m, 2 H, H-7's), 0.872 (t, 3 H, J = 6.5 Hz, CH$_3$); ^{13}C NMR (CDCl$_3$) δ 104.09 (C-1), 82.20, 81.92, 74.88, 72.77, and 64.83 (C-2, C-3, C-4, C-5, C-6), 75.28 and 75.02 (benzylic), 70.60 (*C*H$_2$CH$_2$), 62.16 (C-8), 31.87, 31.40, 29.83, 29.45, 29.29, and 26.18 (C-7 and aliphatic), 14.11 (CH$_3$). Anal. Calc for $C_{33}H_{42}O_6$: C, 72.24; H, 8.51. Found: C, 72.05; H, 8.53.

Octyl 6-*O*-(2-*O*-Acetyl-3,4,6-tri*O*-benzyl-α-D-*manno*-pyranosyl)-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-gluco-octopyranoside (27). A solution of **26** (0.189 mmol) in dry dichloromethane (1.5 mL) was added to a solution of **22** (63 mg, 0.126 mmol), tetramethylurea (30 μL, 0.252 mmol), and silver trifluoromethanesulfonate (49 mg, 0.189 mmol) in the same solvent (1 mL). The reaction was stirred for 30 min after which time TLC showed complete disappearance of **22**. The reaction was then diluted with dichloromethane (10 mL), silver trifluoromethanesulfonate (30 mg) and 2,6-lutidine (30 μL) were added to destroy excess **26**, and, after stirring for 20 min, tetraethylammonium bromide (40 mg) was added to precipitate excess silver. Solids were removed by filtration through a pad of Celite which was washed with dichloromethane (25 mL). The filtrate was washed with aqueous saturated sodium bicarbonate (30 mL) and water (2 × 25 mL) and dried over sodium sulfate before concentration and purification by silica gel chromatography (solvent F). Disaccharide **27** (106 mg, 0.109 mmol, 86%) was obtained as a syrup, $[\alpha]_D$ +72.0° (c 2.9 dichloromethane); R_f 0.20 (solvent F); NMR data are presented in table **I**. Anal. Calcd for $C_{59}H_{72}O_{12}$: C, 72.80; H, 7.47. Found: C, 72.79; H, 7.27.

Octyl 6-*O*-[2-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-*gluco*-pyranosyl)-3,4,6-tri-*O*-benzyl-α-D-*manno*-pyranosyl]-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (30). Treatment of acetate **27** (103 mg, 0.106 mmol, R_f 0.57 in solvent G) with methanolic sodium methoxide for 1 h at room temperature resulted in its complete conversion into alcohol **28** (R_f 0.20, solvent G) which was not further characterized. Neutralization of the mixture with IRC-50 (H$^+$) resin, subsequent removal of the resin by filtration, evaporation, and passage through a pad of silica gel (solvent F) left **28** (68 mg, 0.073 mmol) as an oil which was dried under high vacuum overnight. This oil was dissolved in dry dichloromethane (1 mL), silver trifluoromethanesulfonate (188 mg, 0.73 mmol), 2,6-lutidine (85 μL, 0.73 mmol), and molecular sieves (4A) were added, and the mixture was cooled to −50 °C. A solution of bromide **29** (39 mg, 80 μmol) in dry dichloromethane (1 mL) was then added dropwise, and, after 15 min, the reaction mixture was warmed to room temperature over 1 h, by which time TLC indicated the presence of only ≈25% of **28**. The mixture was cooled to −50 °C, more **29** (39 mg) in dry dichloromethane (1 mL) was then added dropwise and, after 15 min, the reaction was warmed to room temperature and kept there for 1 h. The mixture was then diluted with dichloromethane (50 mL) and filtered through a pad of Celite. The filtrate was washed sequentially with ice-cold 5% HCl, saturated aqueous sodium bicarbonate, and finally with water. Solvent was evaporated, and the residue was purified by silica gel chromatography (solvent H) to provide trisaccharide **30** (85 mg, 63 μmol, 86%) as a syrup: $[\alpha]_D$ +72.0° (c 2.9 dichloromethane): R_f 0.35 (solvent H); NMR data are presented in Table **I**. Anal. Calcd for $C_{77}H_{89}NO_{20}$: C, 68.57; H, 6.65; N, 1.04. Found: C, 68.16; H, 6.42; N, 0.95.

Octyl 6-*O*-[2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*gluco*-pyranosyl)-3,4,6-tri-*O*-benzyl-α-D-*manno*-pyranosyl]-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (31). A mixture of **30** (78 mg, 63 μmol) and hydrazine hydrate (305 μL, 6.3 mmol) in dry methanol (4 mL) was refluxed for 10 h. The reaction was taken to dryness, the residue was dissolved in pyridine (2 mL), and acetic anhydride (1 mL) was added. After stirring for 5 h ethanol (4 mL) was added to destroy the excess acetic anhydride. Solvent was evaporated, and a solution of the residue in dichloromethane (25 mL) was washed with 5% HCl, saturated aqueous sodium bicarbonate, and water. Evaporation of solvent and purification of the residue on silica gel (solvent D) gave trisaccharide **31** (67 mg, 53 μmol, 84%) as a wax: $[\alpha]_D$ +18.6° (c 0.82, dichloromethane); R_f 0.45 (solvent I); NMR data are presented in Table **I**. Anal. Calcd for $C_{71}H_{89}NO_{19}$: C, 67.64; H, 7.13; N, 1.11. Found: C, 67.40; H, 6.90; N, 1.23.

Octyl 6-*O*-[2-*O*-(2-Acetamido-2-deoxy-β-D-*gluco*-pyranosyl)-α-D-*manno*-pyranosyl]-4,8-anhydro-7-deoxy-L-*glycero*-β-D-*gluco*-octopyranoside (8). Compound **31** (59 mg, 47 μmol) was dissolved in 95% ethanol (15 mL) containing 5% palladium on carbon (60 mg) and stirred under an atmosphere of hydrogen for 16 h, by which time TLC showed complete disappearance of **31** to give a major new product (R_f 0.52, solvent J) which was devoid of UV absorption. Removal of the catalyst by filtration, evaporation of the solvent, and a rapid purification on short silica gel column (solvent J) provided a glassy material (31 mg) after drying for 12 h on high vacuum over phosphorus pentaoxide. The residue was dissolved in dry methanol containing a trace of sodium methoxide and stirred until all the material was converted into a single compound: R_f 0.55 (solvent K). After neutralization with IRC 50 (H$^+$), removal of resin, and evaporation, the residue was dissolved in water (5 mL) and loaded onto a Millipore C-18 Sep-Pak cartridge which was washed with water (3 × 5 mL). Trisaccharide **8** was then eluted from the cartridge with methanol (15 mL). The methanol was removed by evaporation, and the residue was obtained as an amorphous powder after lyophilization (24.8 mg, 36.3 μmol, 77%). A portion of **8** (12 mg) was further passed through a column of Bio-gel P-2 (200–400 mesh) with aqueous 10% ethanol as eluent, and the carbohydrate-containing fractions were combined and concentrated, and then lyophilized to provide **8** as an amorphous powder (12 mg), $[\alpha]_D$ −2.1° (c 0.42 water); NMR data are presented in Table **I**.

Octyl 6-*O*-(2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-*manno*-pyranosyl)-4,8-anhydro-2,3-*O*-benzyl-7-deoxy-D-glycero-β-D-*gluco*-octopyranoside (32). A solution of **26** (110 mg, 0.196 mmol) was added to a solution of **25** (64 mg, 0.13 mmol), tetramethylurea (31 μL, 0.26 mmol), and

silver trifluoromethanesulfonate (50 mg, 0.19 mmol) in the same solvent (1 mL). The reaction was stirred for 2.5 h by which time TLC showed complete disappearance of **25**. The reaction mixture was then processed as described for the preparation of **27**. Purification by silica gel chromatography (solvent F) gave disaccharide **32** (84 mg, 86 μmol, 66%) which was as a syrup: $[\alpha]_D$ +15.6° (*c* 0.85, dichloromethane), R_f 0.58 (solvent F); NMR data are presented in Table I. Anal. Calcd for $C_{59}H_{72}O_{12}$: C, 72.80; H, 7.47. Found: C, 72.08; H, 7.15.

Octyl 6-*O*-[2-*O*-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-*gluco*-pyranosyl)-3,4,6-tri-*O*-benzyl-α-D-*manno*-pyranosyl]-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-D-*glycero*-β-D-*gluco*-octopyranoside (34). Treatment of acetate **32** (50 mg, 51 μmol; R_f 0.58 in solvent F) with methanolic sodium methoxide for 12 h at room temperature resulted in its complete conversion into alcohol **33** (R_f 0.40, solvent F) which was not further characterized. Neutralization of the mixture with IRC-50 (H^+) resin, subsequent removal of the resin by filtration, and evaporation left alcohol **33** (37 mg, 51 μmol) as an oil which was dried under high vacuum over phosphorus pentaoxide for 15 h. Reaction of **33** (37 mg, 51 μmol) with **29** as described for the preparation of **30** provided, after purification on silica gel (solvent G), trisaccharide **34** (43 mg, 32 μmol, 62%) as a syrup: $[\alpha]_D$ −19.6° (*c* 1.2 dichloromethane); R_f 0.40 (solvent G); NMR data are presented in Table I. Anal. Calcd for $C_{77}H_{89}NO_{20}$: C, 68.56; H, 6.28; N, 1.04. Found: C, 68.35; H, 6.34; N, 1.07.

Octyl 6-*O*-[2-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-*gluco*-pyranosyl)--3,4,6-tri-*O*-benzyl-a-D-*manno*-pyranosyl]-4,8-anhydro-2,3-di-*O*-benzyl-7-deoxy-D-glycero-β-D-*gluco*-octopyranoside (35). A mixture of **34** (41 mg, 30 μmol) and hydrazine hydrate (147 μL, 3.04 mmol) in dry methanol (2 mL) was refluxed for 7 h and taken to dryness. The residue was then dissolved in pyridine (2 mL), and acetic anhydride (1 mL) was added. After stirring for 5 h, excess acetic anhydride was decomposed with ethanol (4 mL), solvent was evaporated, and a solution of the residue in dichloromethane (25 mL) was washed with 5% HCl, saturated aqueous sodium bicarbonate, and water. Evaporation of solvent and purification by silica gel chromatography (solvent D) provided trisaccharide **35** (30 mg, 24 μmol, 80%) as a syrup: $[\alpha]_D$ +1.8° (*c* 0.7 dichloromethane); R_f 0.35 (solvent D); NMR data are presented in Table I. Anal. Calcd for $C_{71}H_{89}NO_{19}$: C, 67.64; H, 7.13; N, 1.11. Found: C, 67.27; H, 6.90; N, 1.11.

Octyl 6-*O*-[2-*O*-(2-Acetamido-2-deoxy-β-D-*gluco*-pyranosyl)-α-D-*manno*-pyranosyl]-4,8-anhydro-7-deoxy-D-*glycero*-β-D-*gluco*-octopyranoside (9). The benzyl- and acetyl-protecting groups in **35** (21 mg, 17 μmol) were removed as described for the preparation of **8** to give **9** as an amorphous white powder (8.7 mg, 13 μmol; R_f 0.70 in solvent K). A portion of **9** was passed through a column of Bio-gel P-2 (200–400 mesh) with aqueous 10% ethanol as eluent, and appropriate fractions were combined, concentrated, and then lyophilized to provide an amorphous powder: $[\alpha]_D$ −7.3° (*c* 0.21 water); NMR data are presented in Table I.

Partial Purification of GlcNAcT-V. Uridine 5′-diphospho-*N*-acetylglucosamine:α-mannoside β(1→6)-*N*-acetylglucosaminyltransferase (GlcNAcT-V) was obtained by a modification of a procedure reported for glycosyltransferase extraction from rabbit liver.[21] Hamster kidney acetone powder (17.25 g of mature hamster kidneys, Pel Freeze Biologicals) was prepared as described, but 2-[*N*-morpholino]ethanesulfonic acid (50 mM Mes, pH 6.5) was substituted for cacodylic acid in all buffers and $MnCl_2$ was omitted from all buffers. Also protease inhibitors (0.05 mg/mL aprotinin, 0.5 mg/mL soybean trypsin inhibitor, and 0.1 mM phenylmethylsulfonyl fluoride) were incorporated throughout the extraction procedure.

GlcNAcT-V was partially purified by affinity chromatography on UDP-hexanolamine Sepharose (13 μmol ligand/mL gel, 1 × 3 cm). The crude detergent-solubilized extract[22] was diluted 4-fold with 50 mM Mes, pH 6.5, and 10 mM EDTA (buffer A) to a final volume of 200 mL. This was applied to the column at a flow rate of 15 mL/h. The column was washed with 30 mL of buffer A containing 0.25% (w/v) Triton X-100 (buffer B), and then enzyme activity was eluted with buffer **B** containing 3 M NaCl and 5 mM UDP. The eluate was exhaustively dialyzed against buffer B to obtain a 140-fold purified GlcNAcT-V fraction (15 mL, 0.9 mU, 90% recovery). This extract was stable for at least 2 months at 4 °C in the prsence of 1 mg/mL bovine serum albumin (BSA). One mU is defined here as the amount of enzyme transferring one nanomole of GlcNAc/min to **5** (1.4 mM) in the presence of UDP-GlcNAc (1.14 mM) in 50 μL at 37 °C. Protein concentrations (data not shown) were estimated with the Bio-Rad Protein assay using BSA for the standard curve.

Evaluation of 5, 8, and 9 as Acceptors for GlcNAcT-V. GlcNAcT-V activity was assayed as previously described in detail.[10,21] Briefly, the following assay components were dried under reduced pressure in 1.5 mL plastic microfuge tubes: 0.46 μCi UDP-[^{3}H]-GlcNAc, 420 nmol unlabeled UDP-GlcNAc, and 0.025–1.0 μmol of compound **8** or 0.50–50 nmol of either compounds **5** or **9**. The K_m for UDP-GlcNAc is 1.1 mM.[21] Fifty microliters of the GlcNAcT-V solution described above was then added to the dried assay components, and the tubes were vortexed briefly and then incubated at 37 °C between 2 and 4 h. Product formation was linear with time over this range. The incubation mixtures, at five concentrations of acceptor, were then diluted with water (1 mL) and applied to Sep-Pak C18 cartridges[10] preequilibrated with water. The cartridges were washed with water to remove UDP-[^{3}H]-GlcNAc until constant radioactivity (near background) was obtained. Labeled reaction products were then eluted in 2 × 5 mL of methanol and counted as DPM in ACS cocktail in a Beckman LS 3801 liquid scintillation counter. The kinetic parameters V_{max} and K_m were obtained by using a computer program based on the method of Wilkinson.[23] The results are reported in Table II.

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