

Synthesis of
 β -D-Glc p NAc-(1 \rightarrow 2)-5a-carba- α -D-Man p -
(1 \rightarrow 6)- β -D-Glc p -O(CH₂)₇CH₃:
a reactive acceptor analog
for *N*-acetylglucosaminyltransferase-V

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Abstract

The branching enzyme *N*-acetylglucosaminyltransferase-V (GlcNAcT-V) recognizes the trisaccharide β -D-Glc p NAc-(1 \rightarrow 2)- α -D-Man p -(1 \rightarrow 6)- β -D-Glc p -O(CH₂)₇CH₃ (**1**) as its minimum substrate. We report here the chemical synthesis of β -D-Glc p NAc-(1 \rightarrow 2)-5a-carba- α -D-Man p -(1 \rightarrow 6)- β -D-Glc p -O(CH₂)₇CH₃ (**2**), a carbocyclic analog of **1** where the ring oxygen of the α -D-Man p residue is replaced by a methylene group. Trisaccharide **2** was found to be fully active as an acceptor for GlcNAcT-V, both with the enzyme isolated from hamster kidney and the one cloned from rat kidney. The kinetic parameters K_m and V_{max} for **1** and **2** were functionally equivalent. A preparative glycosylation reaction was performed using **2** as the acceptor with the cloned rat kidney enzyme. A tetrasaccharide formed by the addition of a Glc p NAc residue was the sole product as detected by ¹H NMR spectroscopy and FAB mass spectrometry and was assigned the structure β -D-Glc p NAc-(1 \rightarrow 2)-[β -D-Glc p NAc-(1 \rightarrow 6)]-5a-carba- α -D-Man p -(1 \rightarrow 6)- β -D-Glc p -O(CH₂)₇CH₃ (**13**).

Keywords: *N*-Acetylglucosaminyltransferase-V; Trisaccharide, carbocyclic analog

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1. Introduction

N-Acetylglucosaminyltransferase-V (GlcNAcT-V) is a key glycosyltransferase controlling the branching pattern of cell-surface *N*-linked oligosaccharides [1]. Biosynthetically, this enzyme transfers a GlcNAc residue to large glycoproteins, but substrate recognition appears to be limited to a simple trisaccharide sequence present on all complex *N*-linked sugar chains, namely β -D-Glc pNAc-(1 \rightarrow 2)- α -D-Man p-(1 \rightarrow 6)- β -D-Man p [2]. The design of inhibitors of GlcNAcT-V is a subject of great interest since the activity of this enzyme has been shown to conclusively correlate with the metastatic potential of tumor cells, a phenomenon which may result from the enzyme initiating long polylactosamine chains which become sialylated and fucosylated [3–5].

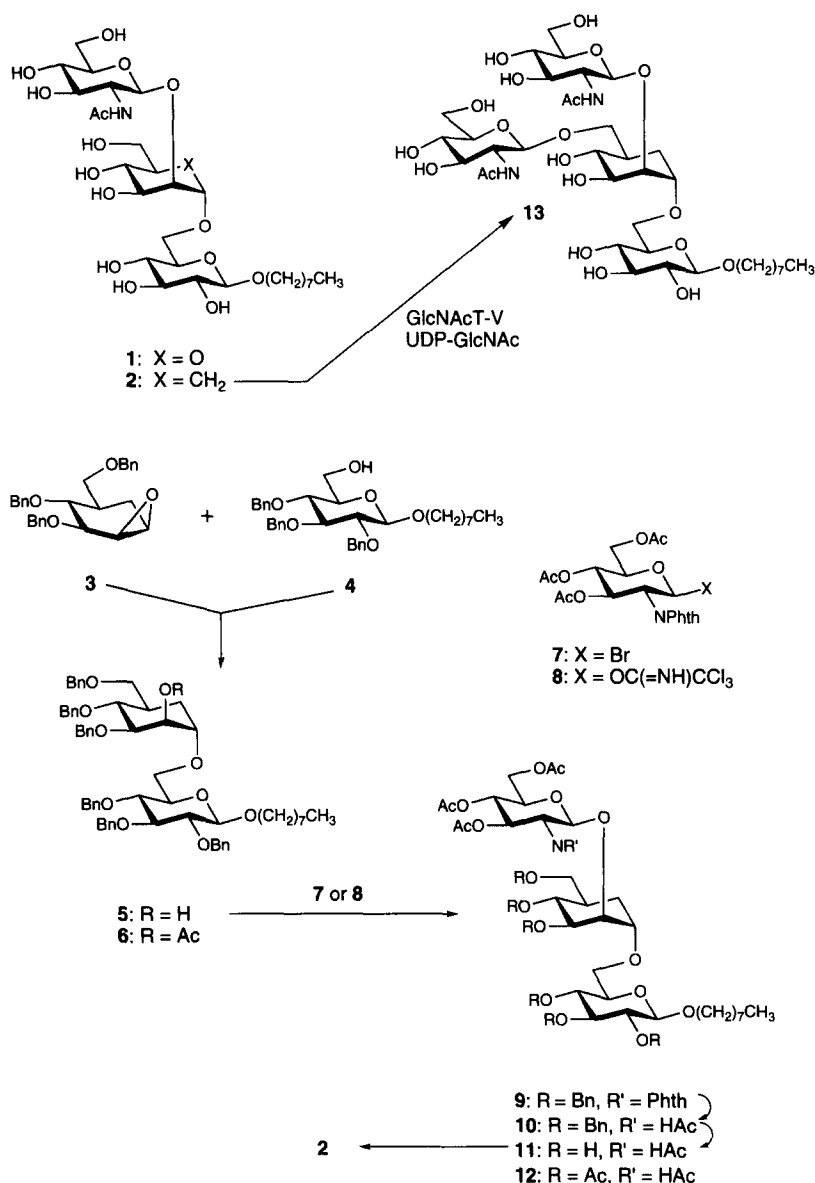
Numerous oligosaccharide analogs have been synthesized and enzymatically evaluated in an effort to assist the rational design of inhibitors for GlcNAcT-V [6–22]. Most of these are based on a *gluco* analog of the natural sequence, namely β -D-Glc pNAc-(1 \rightarrow 2)- α -D-Man p-(1 \rightarrow 6)- β -D-Glc p-O(CH₂)₇CH₃ (**1**), which is much easier to prepare than the corresponding β -D-*manno* compound, yet is equally active [14]. All of the analogs prepared to date have contained pyranose sugar rings where modifications have been made only to the carbon atoms bearing OH groups. The tolerance of the enzyme for non-pyranosyl residues therefore remains unexplored, which is a serious impediment to the design of glycomimetic or conformationally restricted inhibitors [17,22]. In this paper we report the chemical synthesis and enzymatic evaluation of the first non-pyranosidic analog of **1**, where the ring oxygen on the α -D-Man p residue is replaced with a methylene group to yield the carba-sugar, β -D-Glc pNAc-(1 \rightarrow 2)-5a-carba- α -D-Man p-(1 \rightarrow 6)- β -D-Glc p-O(CH₂)₇CH₃ (**2**). The results obtained open up new opportunities for inhibitor design for this important enzyme.

2. Results and discussion

Synthesis of the 5a-carba-trisaccharide (2). — Recently we described a convenient synthesis [23] of ether-linked carba-oligosaccharides of biological interest using 1,2-anhydro-3,4,6-tri-*O*-benzyl-5a-carba- β -D-mannopyranose (**3**) [23,24] as the carba-glycosyl donor. The generality of this procedure is further demonstrated here by the preparation of the target carba-trisaccharide analog **2**.

Coupling of **3** with the alkoxide generated from octyl 2,3,4-tri-*O*-benzyl- β -D-glucopyranoside [14] (**4**) by treatment with sodium hydride in *N,N*-dimethylformamide (DMF) in the presence of 15-crown-5 ether for 2 h at 70°C gave a single coupling product **5** (82%), which was also characterized as the acetate **6**. The α -D-*manno* configuration of the 5a-carba-sugar residue in **5** and **6** was supported by the appearance of narrow doublets of doublets due to H-2' at δ 4.17 and 5.57, respectively, in their ¹H NMR spectra (CDCl₃). The anhydro ring had therefore opened by nucleophilic attack at C-1 by the alkoxide to produce exclusively the desired diaxial product.

Introduction of the required 2-acetamido-2-deoxy- β -D-glucopyranose residue at C-2' of **5** was first attempted using 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranose (**7**) as the donor.



pyranosyl bromide (7) [25] as the donor. Condensation of 5 with 7 (4 equiv) was carried out in benzene in the presence of mercury(II) cyanide for 4 h at reflux to afford, after chromatography, 9 (35%). A much better yield was obtained when the trichloroacetimidate 8 [26] was used as the donor. Using BF₃-etherate as the catalyst the coupling smoothly proceeded in dichloromethane in the presence of molecular sieves (4 Å) for 3

Table 1

Kinetic comparison of the normal trisaccharide **1** and the carba-trisaccharide **2** as acceptors for GlcNAcT-V

	Trisaccharide 1 ^a	Relative V_{\max} (%) ^b	Carba- trisaccharide 2	Relative V_{\max} (%) ^b
	K_m		K_m	
GlcNAcT-V (cloned)	27.7 ± 3.5 μ M	79	34.3 ± 4.6 μ M	100
GlcNAcT-V (isolated)	39.7 ± 4.1 μ M	35	31.4 ± 4.5 μ M	40

^a Normal acceptor.^b 100% V_{\max} was 6.1 pmol min⁻¹.

h at 0°C to give **9** in 73% yield. The ¹H NMR spectrum of **9** was in agreement with the assigned carba-trisaccharide structure. The *N*-phthalimido group was removed by treatment with hydrazine hydrate, and the resulting amine was acetylated conventionally to give the *N*-acetyl derivative **10** (57%). Hydrogenolysis of **10** in a mixture of ethanol–ethyl acetate in the presence of 10% Pd/C gave the tri-*O*-acetyl derivative **11** in 81% yield. Under Zemplén conditions, compound **11** was converted into the free crystalline carba-trisaccharide **2** in 91% yield. The structure of **2** was further supported by the ¹H NMR spectrum of its nona-*O*-acetyl derivative **12**.

Evaluation of the 5a-carba-trisaccharide 2 as an acceptor for GlcNAcT-V. — Both the “normal” trisaccharide acceptor sequence **1** and its carba-analog **2** were kinetically evaluated as acceptor substrates for GlcNAcT-V using a well-established radioactive “Sep-Pak assay” [13,19,20]. Both a partially purified enzyme preparation from hamster kidney [19,20] and a recently cloned form of the enzyme from rat kidney [27] were used. The results obtained are summarized in Table 1. For both sources of the enzyme, the K_m values were very similar. Glycosylation of the carba-analog **2** was slightly more rapid. A preparative incubation of **2** with the cloned enzyme in the presence of unlabeled UDP-GlcNAc resulted in its complete conversion to a new structure that was shown to be a carba-tetrasaccharide by ¹H NMR and FAB mass spectrometry. The product was therefore assigned the structure **13**.

This work demonstrates that the ring oxygen of the mannose residue in **1** is not involved in specific recognition by GlcNAcT-V. This finding opens up new opportunities for the design and synthesis of inhibitors for this enzyme where conformational restraints on the mannose hydroxymethyl group can potentially be achieved through tethering to the newly inserted ring carbon of the central mannose residue.

3. Experimental

General methods. — Melting points were determined with a Mel-Temp capillary melting-point apparatus and uncorrected. Optical rotations were measured with a Jasco DIP-370 polarimeter. ¹H NMR spectra were recorded with a Jeol JNM GSX-270 (270 MHz) instrument or a Varian Unity 500 spectrometer operating at 500 MHz. TLC was

performed on Silica Gel 60 GF (E. Merck, Darmstadt) with detection by charring with H_2SO_4 . Column chromatography was conducted on Wakogel C-300 (300 mesh). Organic solutions were dried over anhydrous Na_2SO_4 and evaporated at $< 50^\circ\text{C}$ under diminished pressure. Sep-Pak C_{18} cartridges were from Millipore–Waters. The fast-atom bombardment mass spectrum (FABMS) was recorded on a Kratos AEI MS-9 instrument with Xe as the bombarding gas and 5:1 1,4-dithiothreitol–1,4-dithioerythritol as the matrix.

Octyl 2,3,4-tri-O-benzyl-6-O-(3,4,6-tri-O-benzyl-5a-carba- α -D-mannopyranosyl)-6-deoxy- β -D-glucopyranoside (5). — To a stirred solution of octyl 2,3,4-tri-O-benzyl- β -D-glucopyranoside [14] (**4**, 325 mg, 0.58 mmol) in DMF (8 mL) maintained was added sodium hydride (297 mg, 8.7 mmol), and the mixture was stirred for 30 min at room temperature. Then, 1,2-anhydro-3,4,6-tri-O-benzyl-5a-carba- β -D-mannopyranose [23,24] (**3**, 600 mg, 1.4 mmol) and 15-crown-5 ether (1.72 mL, 68 mmol) were added, and the mixture was stirred for 2 h at 70°C . After cooling to a room temperature, the mixture was neutralized with acetic acid, diluted with ethyl acetate (80 mL), and the solution was washed thoroughly with water, dried, and evaporated. The residue was chromatographed on a silica gel column (30 g, 1:12 ethyl acetate–toluene, to give **5** (471 mg, 82%) as a syrup: R_f 0.37 (1:8 ethyl acetate–toluene), $[\alpha]_D^{30} + 21^\circ$ (c 1.9, CHCl_3); IR (neat) ν_{\max} 3490 (OH); ^1H NMR data (270 MHz, CDCl_3): δ 7.45–7.15 (m, 30 H, 6 Ph), 4.97–4.31 (m, 13 H, 6 PhCH_2 , H-1), 4.17 (t, 1 H, $J_{1',2'} = J_{2',3'} = 3.1$ Hz, H-2'), 3.95–3.30 (m, 13 H), 2.51 (m, 1 H, OH), 2.15–1.75 (m, 3 H), 1.68–1.50 and 1.40–1.15 (m, 12 H), 0.85 (t, 3 H, $J = 6.9$ Hz, CH_3). Anal. Calcd for $\text{C}_{63}\text{H}_{75}\text{O}_{10}$: C, 76.26; H, 7.62. Found: C, 76.26; H, 7.94.

Octyl 6-O-(2-O-acetyl-3,4,6-tri-O-benzyl-5a-carba- α -D-mannopyranosyl)-2,3,4-tri-O-benzyl-6-deoxy- β -D-glucopyranoside (6). — Compound **5** (40 mg, 0.040 mmol) was treated with acetic anhydride (10 μL) in pyridine (1 mL) for 3 h at room temperature. The mixture was evaporated, and the residue was chromatographed on a silica gel column (1 g, 1:15 ethyl acetate–toluene) to give **6** (38 mg, 92%) as a syrup: R_f 0.58 (1:8 ethyl acetate–toluene), $[\alpha]_D^{26} + 20^\circ$ (c 1.6, CHCl_3); IR (neat) ν_{\max} 1745 (ester); ^1H NMR data (270 MHz, CDCl_3): δ 7.38–7.15 (m, 30 H, 6 Ph), 5.57 (dd, 1 H, $J_{1',2'} = 3.3$, $J_{2',3'} = 2.9$ Hz, H-2'), 4.93 and 4.77 (d, each 1 H, $J_{\text{gem}} = 11.0$ Hz, PhCH_2), 4.87 and 4.53 (d, each 1 H, $J_{\text{gem}} = 11.0$ Hz, PhCH_2), 4.82 and 4.56 (d, each 1 H, $J_{\text{gem}} = 11.0$ Hz, PhCH_2), 4.71 and 4.46 (d, each 1 H, $J_{\text{gem}} = 11.4$ Hz, PhCH_2), 4.45 and 4.42 (d, each 1 H, $J_{\text{gem}} = 11.4$ Hz, PhCH_2), 4.35 (d, 1 H, $J_{1,2} = 7.7$ Hz, H-1), 3.95–3.86 (m, 2 H, H-1' and OCH_2), 3.76 (dd, 1 H, $J_{5,6a} = 4.4$, $J_{\text{gem}} = 10.6$ Hz, H-6a), 3.73–3.59 (m, 5 H, H-3, 6b, 3', 6'a, 6'b), 3.56–3.36 (m, 4 H), 1.90–1.70 (m, 1 H, H-5'a-eq), 1.65–1.45 and 1.40–1.14 (m, 13 H), 0.85 (t, $J = 6.6$ Hz, CH_3). Anal. Calcd for $\text{C}_{65}\text{H}_{77}\text{O}_{11}$: C, 75.48; H, 7.50. Found: C, 75.35; H, 7.12.

Octyl 2,3,4-tri-O-benzyl-6-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-5a-carba- α -D-mannopyranosyl]- β -D-glucopyranoside (9). — (a) To a solution of **5** (86 mg, 0.087 mmol) in benzene (4 mL) were added mercury(II) cyanide (109 mg, 0.43 mmol) and 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl bromide [25] (**7**) (173 mg, 0.35 mmol), and the mixture was stirred for 4 h at reflux temperature. The mixture was filtered through a Celite pad and the filtrate was evaporated. The residual product was chromatographed on a silica gel

column (6 g, 1:12 ethyl acetate–toluene) to give **9** (42.3 mg, 35%) as a syrup: $[\alpha]_D^{23} + 1.5^\circ$ (*c* 1.3, CHCl_3); IR (neat) ν_{\max} 1750 (ester); ^1H NMR (270 MHz, CDCl_3): δ 7.95–7.04 (m, 34 H, 6 Ph, Phth), 5.79 (dd, 1 H, $J_{2'',3''} = 11.0$, $J_{3'',4''} = 9.2$ Hz, H-3''), 5.53 (d, 1 H, $J_{1'',2''} = 8.4$ Hz, H-1''), 5.22 (dd, 1 H, $J_{4'',5''} = 9.9$ Hz, H-4''), 4.95 and 4.90 (d, each 1 H, $J_{\text{gem}} = 11$ Hz, PhCH_2), 4.93 and 4.77 (d, each 1 H, $J_{\text{gem}} = 11.0$ Hz, PhCH_2), 4.81 and 4.70 (d, each 1 H, $J_{\text{gem}} = 10.6$ Hz, PhCH_2), 4.52 (dd, 1 H, H-2''), 4.39 (d, 1 H, $J_{1,2} = 8.4$ Hz, H-1), 4.41–4.30 (m, 4 H, 2 PhCH_2), 4.23 (m, 1 H, H-1'), 4.20–4.01 (m, 3 H, H-2', OCH_2), 3.72–3.08 (m, 13 H), 2.04, 2.02, 1.87 (3 s, each 3 H, 3 Ac), 1.80–1.17 (m, 14 H), 1.06 (m, 1 H, H-5'a-ax), 0.86 (t, 3 H, $J = 6.6$ Hz, CH_3). Anal. Calcd for $\text{C}_{77}\text{H}_{94}\text{NO}_{18}$: C, 69.98; H, 7.17; N, 1.06. Found: C, 70.21; H, 7.02; N, 1.17.

(b) To a solution of **5** (151 mg, 0.152 mmol) in CH_2Cl_2 (3 mL) maintained under argon were added 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl trichloroacetimidate [26] (**8**) (369 mg, 0.637 mmol), $\text{BF}_3 \cdot \text{OEt}$ (18.8 mL, 0.15 mmol), and molecular sieves MS-4A (150 mg). The mixture was stirred for 3 h at 0°C , diluted with chloroform (40 mL), thoroughly washed with water, dried, and evaporated. The residue was chromatographed similarly to give **9** (158 mg, 73%) as a syrup. The product was identical with that obtained under (A), above.

Octyl 2,3,4-tri-O-benzyl-6-O-[3,4,6-tri-O-benzyl-2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-5a-carba- α -D-mannopyranosyl]- β -D-glucopyranoside (10). — A mixture of **9** (42.3 mg, 0.030 mmol) and hydrazine hydrate (1 mL) was heated for 2 h at 80°C , and then evaporated. The residue was treated with acetic anhydride (34 μL , 0.36 mmol) and pyridine (1 mL) for 5 h at room temperature, and evaporated. The residual product was chromatographed on a silica gel column (5 g, 1:2 ethyl acetate–toluene) to give **10** (23 mg, 57%) as a syrup: $[\alpha]_D^{29} + 9.4^\circ$ (*c* 0.50, CHCl_3); IR (neat) ν_{\max} 1750 (ester), 1650 (amide); ^1H NMR (270 MHz, CDCl_3): δ 7.40–7.15 (m, 30 H, 6 Ph), 5.75 (m, 1 H, NH), 5.53 (t, 1 H, $J_{2'',3''} = J_{3'',4''} = 10.6$ Hz, H-3''). 5.12–4.10 (m, 18 H), 3.98 (m, 1 H, OCH_2), 3.90–3.32 (m, 14 H), 2.01, 2.00, 1.99 (3 s, 12 H, 4 Ac), 1.80–1.50 (m, 3 H, H-5', 5'a-ax, 5''a-eq), 1.45–1.19 (m, 12 H), 0.87 (t, 3 H, $J = 6.6$ Hz, CH_3). Anal. Calcd for $\text{C}_{77}\text{H}_{94}\text{NO}_{18}$: C, 70.72; H, 6.72; N, 0.99. Found: C, 71.03; H, 6.79; N, 1.27.

Octyl 6-O-[2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-5a-carba- α -D-mannopyranosyl]- β -D-glucopyranoside (11). — A solution of **10** (17.0 mg, 0.013 mmol) in 1:1 ethanol–ethyl acetate (1 mL) was hydrogenolyzed in the presence of 10% Pd/C at atmospheric pressure for 12 h at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated to give **11** (8.1 mg, 81%) as a syrup: $[\alpha]_D^{24} - 17.4^\circ$ (*c* 0.41, MeOH); IR (neat) ν_{\max} 3400 (OH), 1750 (ester), 1650 (amide); ^1H NMR (270 MHz, D_2O): δ 5.27 (dd, 1 H, $J_{2'',3''} = 10.3$ Hz, H-3''), 5.07 (t, 1 H, $J_{3'',4''} = J_{4'',5''} = 9.5$ Hz, H-4''), 4.82 (d, 1 H, $J_{1'',2''} = 8.1$ Hz, H-1''), 4.45–4.39 (m, 1 H, H-1'), 4.41 (d, 1 H, $J_{1,2} = 8.1$ Hz, H-1), 4.20 (dd, 1 H, $J_{5'',6''} = 2.2$, $J_{\text{gem}} = 12.5$ Hz, H-6''a), 4.15 (t, 1 H, $J_{1',2'} = J_{2',3'} = 3.3$ Hz, H-2'), 4.00 (m, 1 H, H-5''), 3.94–3.83 (m, 2 H, H-2'', 6''b), 3.83–3.38 (m, 11 H), 3.24 (t, 1 H, $J_{1,2} = J_{2,3} = 8.1$ Hz, H-2), 2.12, 2.08, 2.05, 1.97 (4 s, each 3 H, 4 Ac), 1.92–1.20 (m, 15 H), 0.85 (t, 3 H, $J = 6.6$ Hz, CH_3).

Octyl 6-O-[2-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-5a-carba- α -D-mannopyranosyl]- β -D-glucopyranoside (2). — Compound **11** (8.1 mg, 0.010 mmol) was

treated with N methanolic sodium methoxide (1 mL) for 0.5 h at room temperature. The mixture was neutralized with Amberlite 120 (H^+) resin and then concentrated to give **2** (6.2 mg, 91%) as a crystalline solid: mp 140–142°C, R_f 0.62 (3:1 acetonitrile– H_2O), $[\alpha]_D^{24}$ 4.6° (c 0.31, MeOH); IR (neat) ν_{max} 3430 (OH), 1650 (amide); 1H NMR data (270 MHz, D_2O): δ 4.53 (d, 1 H, $J_{1'',2''} = 8.4$ Hz, H-1''), 4.42 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1), 4.13 (m, 1 H, H-1'), 3.95–3.33 (m, 18 H), 3.24 (t, 1 H, $J_{2,3} = 8.3$ Hz, H-2), 2.04 (s, 1 H, Ac), 1.96 (m, 1 H, H-5'), 1.80–1.20 (m, 14 H), 0.85 (t, 3 H, $J = 7.0$ Hz, CH_3).

Octyl 2,3,4-tri-O-acetyl-6-O-[2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranosyl)-3,4,6-tri-O-acetyl-5a-carba- α -D-mannopyranosyl]- β -D-glucopyranoside (12). — Compound **2** (6.2 mg, 0.095 mmol) was acetylated with acetic anhydride (20 μ L) in pyridine (0.2 mL) overnight at room temperature. The mixture was evaporated, and the residue was chromatographed on a silica gel (1 g, 1:4 acetone–toluene) to give **12** (8.6 mg, 88%) as a syrup: R_f 0.17 (1:4 acetone–toluene), $[\alpha]_D^{30} + 49^\circ$ (c 1.02, $CHCl_3$); IR (neat) ν_{max} 1750 (ester), 1650 (amide); 1H NMR data (270 MHz, $CDCl_3$): δ 5.56 (d, 1 H, $J_{1'',2''} = 8.1$ Hz, H-1''), 5.40 (dd, 1 H, $J_{2'',3''} = 10.6$, $J_{3'',4''} = 9.2$ Hz, H-3''), 5.25–4.83 (m, 7 H), 4.47 (d, 1 H, $J_{1,2} = 8.1$ Hz, H-1), 4.34–4.25 (m, 2 H, H-1',2'), 4.12–3.43 (m, 10 H), 2.09, 2.08, 2.06, 2.04, 2.03, 2.02, 2.01, 1.99, and 1.90 (9 s, 3, 3, 3, 3, 3, 6, 3, 3, 3 H, 10 Ac), 1.80 (m, 1 H, H-5'), 1.70–1.20 (m, 14 H), 0.87 (t, 3 H, $J = 6.6$ Hz, CH_3). Anal. Calcd for $C_{47}H_{70}NO_{24}$: C, 54.64; H, 6.83; N, 1.36. Found: C, 54.33; H, 7.17; N, 1.52.

Evaluation of 2 as an acceptor for GlcNAcT-V. — Hamster kidney GlcNAcT-V was purified and assayed as previously described [9]. Purified cloned rat GlcNAcT-V [27] was provided by M. Pierce. The specific activities were 0.057 and 25.6 U mg^{-1} for the hamster and rat enzymes, respectively. Protein concentrations were estimated by a modified Lowary method using a DC-Protein assay kit from Bio-Rad. For kinetic studies reaction mixtures were prepared at 4°C and contained in a total volume of 20 μ L: 1.2–1.4 μ U of enzyme, 10–320 μ M acceptor, 0.55 mM UDP-GlcNAc, 195 000 dpm UDP-[3H]-GlcNAc (60 Ci/mmol from American Radiolabeled Chemical, Inc.), 1 mg mL bovine serum albumin, 0.1% Triton X-100 in 50 mM sodium cacodylate buffer, pH 6.5. Reaction mixtures were incubated for 60 min at 37°C, and reactions were stopped by the addition of water (500 μ L). Radiolabeled product was separated from unreacted donor on Sep-Pak C_{18} reversed-phase cartridges [13] and quantitated by liquid scintillation counting in 10 mL of Ecolite cocktail (ICN). Relative velocities and K_m values were estimated using unweighted non-linear regression analysis of the Michaelis–Menten equation with Sigma Plot 4.0 [13].

Preparative enzymatic synthesis of octyl 6-O-[2,6-di-O-(2-acetamido-2-deoxy- β -D-glucopyranosyl)-5a-carba- α -D-mannopyranosyl]- β -D-glucopyranoside (13). — A preparative reaction was carried out in 360 μ L of 50 mM sodium cacodylate buffer, pH 6.5, containing 2.0 mg (3.4 μ mol) UDP-GlcNAc, 0.5 mg (0.76 μ mol) of acceptor **2**, 0.1% Triton X-100, 20% glycerol, 10 mM EDTA and 1 mU of cloned rat GlcNAcT-V. After 48 h of reaction at ambient temperature, the reaction mixture was diluted with water (10 mL) and applied to two sequentially connected C_{18} reversed-phase cartridges. The cartridges were washed with water (30 mL), and the product was eluted in methanol (10 mL). Methanol was removed by evaporation, and the sample was lyophilized to give 0.54 mg of **13** (83% yield) as a white solid. The 1H NMR spectrum of this crude product

indicated the reaction to be complete. For comparison, ^1H NMR spectra of both **2** and **13** were recorded at 500 MHz (D_2O): **2**: δ 4.53 (d, 1 H, $J_{1'',2''} = 8.4$ Hz, H-1''), 4.43 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.15 (ddd, 1 H, $J_{1',2'} = 3.4$, $J_{2',3'} = 3.4$, $^4J_{2',7'\text{eq}} = 1.2$ Hz, H-2'), 3.25 (dd, 1 H, $J_{2,3} = 9.2$ Hz, H-2), 2.05 (COCH_3), 1.97 (m, 1 H, $J_{1',7'\text{eq}} = 3.7$, $J_{1',7'\text{ax}} = 2.7$, $J_{7'\text{ax}, 7'\text{eq}} = -14$ Hz, H-7'eq), 1.39 (m, H-7ax). **13**: δ 4.57 (d, second order, 1 H, $J_{1'',2''} = 8.3$ Hz, H-1''), 4.51 (d, 1 H, $J_{1'',2''} = 8.4$ Hz, H-1'), 4.43 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.12 (ddd, 1 H, $J_{1',2'} = 3.4$, $J_{2',3'} = 3.4$, $^4J_{2',7'\text{eq}} = 1.2$ Hz, H-2'), 3.25 (dd, 1 H, $J_{2,3} = 9.1$ Hz, H-2), 2.05 ($2 \times \text{COCH}_3$), 1.99 (m, 1 H, H-7'eq), 1.4 (m, H-7ax). FABMS (**13**): 881.0 ($\text{M} + \text{Na}$) $^+$.

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