ELSEVIER

Carbohydrate Research 303 (1997) 283–291

CARBOHYDRATE RESEARCH

Synthesis and evaluation of eight aminodeoxy trisaccharide inhibitors for *N*-acetylglucosaminyltransferase-V

Pu-Ping Lu, Ole Hindsgaul *, Hong Li, Monica M. Palcic

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Received 8 April 1997; accepted 28 May 1997

Abstract

N-Acetylglucosaminyltransferase-V is an important enzyme controlling the branching pattern of *N*-linked oligosaccharides. This enzyme recognizes the trisaccharide octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-β-D-glucopyranoside (**5**) as a substrate and adds a β-linked GlcNAc residue to OH-6 of the central α-Man unit. Eight analogs of **5** were chemically synthesized where C-6 of the α-Man residue in **5** was deoxygenated, and structurally diverse modifications were introduced at C-4 of the same residue. The key intermediate prepared for this purpose was octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-4-amino-4,6-dideoxy-α-D-mannopyranosyl-(1 → 6)-β-D-glucopyranoside (**7a**) where the original 4′-amino group was readily derivatized on the unprotected sugar. The eight analogs **7a**–**7h** were evaluated as inhibitors for GlcNAcT-V, both isolated (from hamster kidney) and cloned (from rat kidney). All of the compounds were found to be competitive inhibitors with K_i in the range of 3–106 μM. The conclusion of this work is that recognition of acceptor **5** does not involve contact of the C-6–C-4 end of the α-Man residue with the protein in the E–I (or E–S) complex. © 1997 Published by Elsevier Science Ltd.

Keywords: *N*-Acetylglucosaminyltransferase-V; Enzyme inhibitors; Trisaccharide analogs

1. Introduction

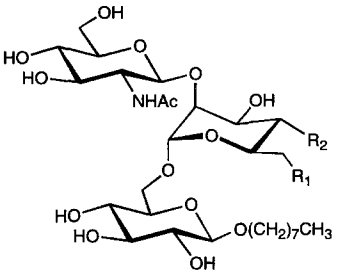
UDP-GlcNAc:α-mannoside β-(1 → 6)-*N*-acetylglucosaminyltransferase V (GlcNAcT-V, EC 2.4.1.155) is an important enzyme controlling the branching pattern of asparagine-linked oligosaccharides [1,2]. A direct correlation between an increase in the activity of this enzyme and the increased metastatic potential of several cancer cell lines has been reported [3–5]. This enzyme has consequently become a target for the development of a glycosyltransferase inhibitor.

Biosynthetically, GlcNAcT-V catalyzes the transfer of an *N*-acetyl-D-glucosamine (GlcNAc) residue from uridine 5′-diphospho-GlcNAc (UDP-GlcNAc) to oligosaccharide acceptors having the minimum heptasaccharide sequence **1** (Scheme 1), converting it to the corresponding octasaccharide **2** [1,2]. Previous work has shown that the much simpler synthetic trisaccharide **3**, which is a partial structure of **1**, is

* Corresponding author.

PII S0008-6215(97)00174-2

also an effective substrate for the enzyme yielding the expected tetrasaccharide **4** [6]. It was also found that trisaccharide **5**, the β-D-gluco analog of **3**, was an excellent acceptor [7]. The aliphatic aglycones in **3** and **5** were incorporated into these structures in order to facilitate the enzyme assay procedures [8].



5: $R_1 = R_2 = OH$

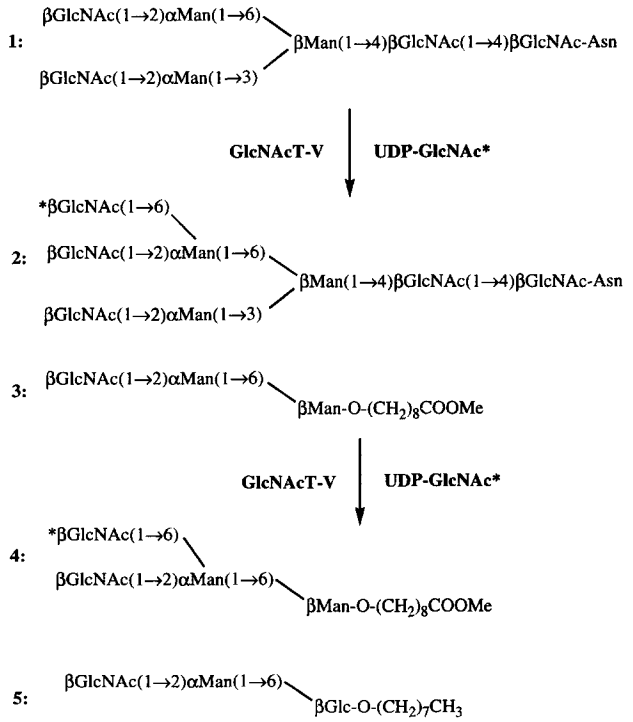
6: $R_1 = H$, $R_2 = OH$

7a: $R_1 = H$, $R_2 = NH_2$

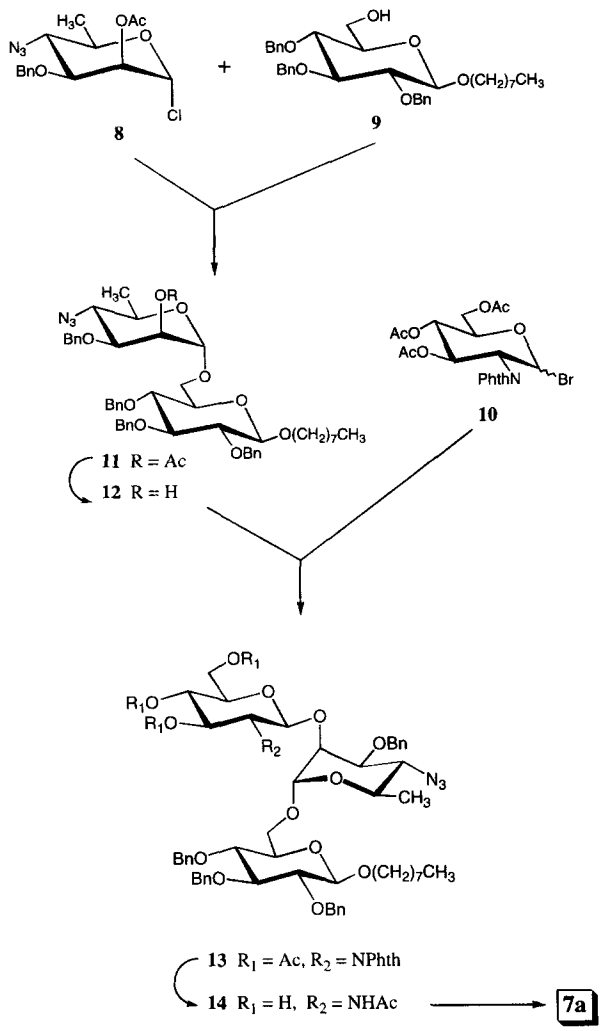
The 6′-deoxy-derivative **6** of trisaccharide **5**, where the reactive 6′-OH had been removed, was the first reported inhibitor specific for a glycosyltransferase and had a K_i value in the range 30–77 μM depending on the source of the enzyme [9,10]. It was later found that substitutions at both C-6 [11] and O-4 [12] of the α-Man residue were tolerated by the enzyme and could lead to enhancements of affinity approaching the μM level. In the present work, we examine the tolerance of GlcNAcT-V to substitution at C-4 of the α-Man residue by preparing a series of structurally diverse derivatives **7a–7h**. In all of these compounds, C-6 of the α-Man residue has been deoxygenated so that the compounds are of necessity only potential inhibitors, not potential acceptors, in order to simplify their evaluation as ligands for GlcNAcT-V. In order to simplify the preparation of the required series of active-site probes, an amino group was installed at C-4 of the α-Man residue. The distinct chemical reactivity of this amino group permitted the facile derivation of a single deprotected trisaccharide **7a** to yield the required target analogs using simple high-yielding synthetic procedures previously described [11].

2. Results and discussion

Compound **8**, the key building block used for the synthesis of trisaccharide **7a**, was synthesized from methyl α-D-mannopyranoside using a published procedure [13]. The coupling of **8** and alcohol **9** [7] using silver trifluoromethanesulfonate (AgOTf) as promotor gave the α-linked disaccharide **11** in 68% yield



Scheme 1. Glycosylation reactions catalyzed by GlcNAcT-V.



Scheme 2. Trisaccharide assembly.

(see Scheme 2). The anomeric configuration was confirmed by its ^{1}H-coupled ^{13}C NMR spectrum which revealed a doublet each for C-1 and C-1′ at δ 103.54 (C-1, $J_{C\text{-}1,H\text{-}1}$ 156.9 Hz) and 97.73 (C-1′, $J_{C\text{-}1',H\text{-}1'}$ 170.9 Hz), respectively. These one-bond C–H coupling constants require the presence of the β and α glycosidic linkages as assigned [14].

Zemplén deacetylation of **11** yielded **12** with OH-2′ free for further coupling. Glycosylation of alcohol **12** with 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranosyl bromide **10** [15] was carried out in the presence of AgOTf and collidine to give trisaccharide **13** in 77% yield. The removal of the *N*-phthalimido and acetyl protecting groups in **14** was achieved using 1,2-diaminoethane-1-butanol [16]. *N*-Acetylation (Ac_2O–MeOH) then gave **14** in 88% overall yield.

The removal of benzyl groups and concomitant reduction of the azido group in **14** were attempted by using hydrogenation (H_2, Pd/C) under numerous conditions but failed. Finally, Birch reduction (Na–l NH_3) was found to give the target trisaccharide **7a** in 81% yield.

The required analogs **7a–7h** were prepared from **7a** using known reagents (Table 1) for selective *N*-derivatization [11], and they were characterized by ^{1}H NMR spectroscopy and FABMS.

Compounds **7a–7h** were evaluated as inhibitors of GlcNAcT-V, both partially purified from hamster kidney [11] and cloned (from rat kidney) [17]. The activity of the synthetic trisaccharide derivatives was determined using an established radioactive 'Sep-Pak' assay technique as previously described [8,12]. The results of the evaluation of trisaccharides **7a–7h** as inhibitors are presented in Table 1. All of the analogs were found to be competitive inhibitors of GlcNAcT-V (Fig. 1). These results demonstrate that partially purified GlcNAcT-V from hamster kidney and cloned rat kidney GlcNAcT-V have similar specificity. The results also demonstrate that GlcNAcT-V tolerates very diverse modifications at C-4′ and still binds the substrate analogs as was previously shown for modifications at C-6′ [11]. Some of the modifications at C-4′ increase the inhibitor activities, though no clear structure–activity relationship is evident. Coupled with the previous study demonstrating a similar wide tolerance for substitution at C-6′ [11], the conclusion is that the α-Man residue to which GlcNAcT-V transfers is not tightly bound by the enzyme prior to transfer of GlcNAc from the donor.

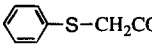
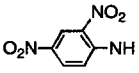
3. Experimental

General methods.—TLC was performed on Silica Gel 60 F_{254} (E. Merck) with detection by quenching of fluorescence, by charring with H_2SO_4, and/or by reaction with ninhydrin. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (E. Merck, 40–63 μm). Beaded Silica Gel 6RS-8060 (Iatrobeads) were from Iatron Laboratories, Inc. (Japan). C_{18} Silica gel (reversed phase) was from Toronto Research Chemicals, Inc. C_{18} Sep-Pak sample preparation cartridges were from Waters Associates. Millex-GV (0.22 μm) filter units were from Millipore. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 °C. ^{1}H NMR spectra were recorded at 300 MHz (Bruker AM 300), 360 MHz (Bruker AMR 360), 400 MHz (Bruker AM 400), or 500 MHz (Varian UNITY 500) on solutions in $CDCl_3$ (internal Me_4Si, δ 0) or D_2O. ^{13}C NMR

spectra were recorded at 75.5, 100.6 , or 125 MHz, respectively, on the same instruments in $CDCl_3$ ($CDCl_3$, δ 77.0) or D_2O (internal 1,4-dioxane, δ 67.4). In all compounds bearing an octyl aglycone, the signals of the methylene protons of octyl groups were observed at δ 1.20–1.45 (m, 12 H, 6 CH_2) in the ^1H NMR spectra. The assignments of ^{13}C NMR resonances are tentative. FAB mass spectra (FABMS) were obtained on a Kratos AEI-MS9 instrument using glycerol as matrix. Elemental analyses were carried out on a Carlo Erba EA1108 instrument. For the enzyme experiments, cloned rat kidney GlcNAcT-V [17] was a generous gift from Dr. M. Pierce, University of Georgia. Partially purified GlcNAcT-V was isolated from hamster kidney by a published procedure [11]. UDP-[^{3}H]GlcNAc was from American Radiolabeled Chemicals, Inc. (specific activity 40–60 Ci/mmol). UDP-GlcNAc (disodium salt) was from Sigma Chemical Co.

Octyl 2-O-acetyl-4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**11**).—To a mixture of **9** [7] (2.31 g, 4.10 mmol), AgOTf (1.32 g, 5.13 mmol), and 4 Å molecular sieves (4 g) in dry CH_2Cl_2 (10 mL) at −65 °C was added a solution of **8** [13] (1.14 g, 3.36 mmol) in CH_2Cl_2 (5 mL) dropwise under argon. The mixture was allowed to warm to room temperature over 2 h. After stirring for 1 h, tetraethylammonium chloride (580 mg, 3.5 mmol) was added, and the mixture was stirred for another 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, satd $NaHCO_3$, and water, then dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography using 2:1 hexane–EtOAc as eluant to provide **11** (1.97 g, 67.6%): $[\alpha]_D$ +70.2° (*c* 0.6, $CHCl_3$); NMR data ($CDCl_3$): ^1H NMR (360 MHz): δ 7.38–7.20 (m, 20 H, Ar-H), 5.39 (dd, 1 H, $J_{2',3'}$ 3.2, $J_{1',2'}$ 1.9 Hz, H-2'), 4.98 (d, 1 H, J_{gem} 11.0 Hz, $PhCH_2$), 4.97 (d, 1 H, J_{gem} 10.8 Hz, $PhCH_2$), 4.88 (d, 1 H, J_{gem} 11.1 Hz, $PhCH_2$), 4.78 (d, 1 H, H-1'), 4.78 (d, 1 H, J_{gem} 10.8 Hz, $PhCH_2$), 4.73 (d, 1 H, J_{gem} 11.0 Hz, $PHCH_2$), 4.70 (d, 1 H, J_{gem} 11.1 Hz, $PhCH_2$), 4.51 (d, 1 H, J_{gem} 10.7 Hz, $PhCH_2$), 4.48 (d, 1 H, J_{gem} 10.6 Hz, $PhCH_2$), 4.37 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 3.88 (dt, 1 H, J_{gem} 9.4, J_{vic} 6.5 Hz, octyl C_1-H_a), 3.76 (dd, 1 H, $J_{3',4'}$ 9.8 Hz, H-3'), 2.13 (s, 3 H, Ac), 1.25 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.87 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 170.04 (CO), 138.51

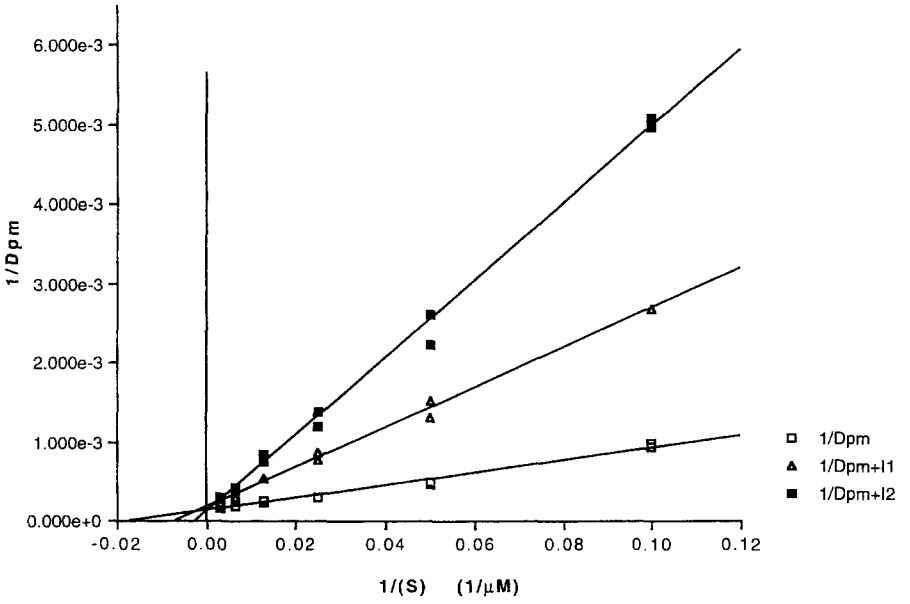
Table 1
Evaluation of trisaccharides **7a–7h** as inhibitors of GlcNAcT-V

Compound	R_2	Reagents Used for Amino Derivatization	*Ki* (μM)	
			a	b
7a	NH_2		106	95
7b	CH_3CONH	Ac_2O–MeOH	20	24
7c	C_6H_5CONH	i. C_6H_5COCl–Py ii. NaOMe–MeOH	10	3
7d	$Na^+{}^-OOCCH_2CH_2CONH$	i. succinic anhydride–MeOH ii. $NaHCO_3$ aq	6	8
7e	ICH_2CONH	$(ICH_2CO)_2O$–MeOH	14	18
7f	$CH_2{=}CHCONH$	$CH_2{=}CHCOCl$–DMF–$NaHCO_3$	13	25
7g		i. $(ICH_2CO)_2O$–MeOH ii. C_6H_5SH–$NaHCO_3$ aq	20	7
7h		1-Fluoro-2,4-dinitrobenzene–Phosphate buffer	60	9

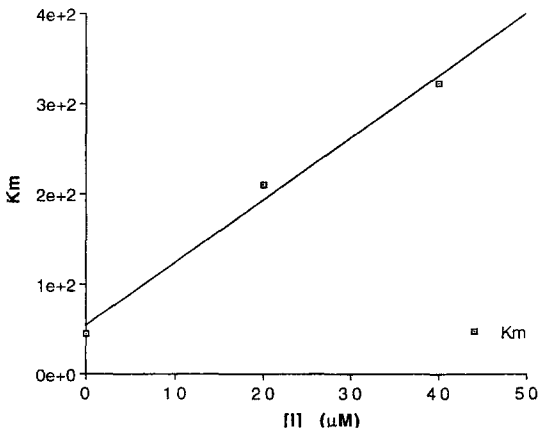
[a] Partially purified GlcNAcT-V from hamster kidney.
[b] Cloned rat kidney GlcNAcT-V.

(benzyl C_1), 138.47 (benzyl C_1), 137.98 (benzyl C_1), 137.18 (benzyl C_1), 103.54 (C-1, J_{CH} 156.9 Hz), 97.73 (C-1′, J_{CH} 170.9 Hz), 75.68, 74.88, 74.77, 71.43 ($PhCH_2$), 70.11 (octyl C_1), 66.35 (C-6), 63.96 (C-4′), 31.81, 29.78, 29.43, 29.25, 26.18, 22.63 (octyl C_2-C_7), 20.94 ($CO\mathit{CH_3}$), 18.46 (C-6′), 14.05 (octyl CH_3). Anal. Calcd for $C_{50}H_{63}N_3O_{10}$ (866.07): C, 69.34; H, 7.33; N, 4.85. Found: C, 69.62; H, 7.41; N, 4.73.

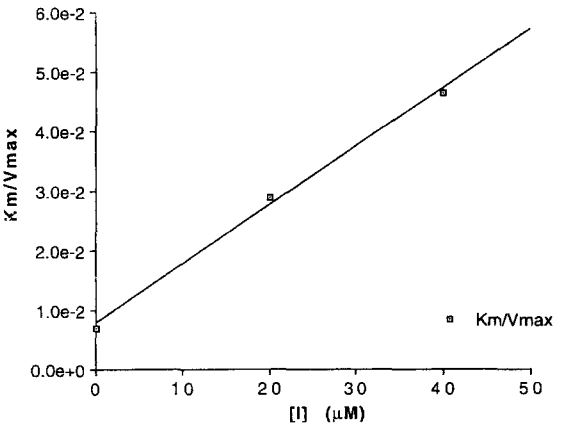
Octyl 4-azido-3-O-benzyl-4,6-dideoxy-α-D-mannopyranosyl-(1 → 6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**12**).—Compound **11** (1.95 g, 2.25 mmol) was treated with methanolic NaOMe (0.05 N, 100 mL) at room temperature for 2 h. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration, and concentration left **12** as a syrup (1.81 g, 98%): ^{1}H NMR ($CDCl_3$, 360 MHz): δ 7.42–7.18 (m, 20 H, Ar), 4.968 (d, 1 H, J_{gem} 10.5 Hz, $PhCH_2$), 4.966 (d, 1 H, J_{gem} 10.6 Hz, $PhCH_2$), 4.90 (d, 1 H, J_{gem} 10.5 Hz, $PhCH_2$), 4.88 (d, 1 H, $J_{1',2'}$ 1.2 Hz, H-1′), 4.79 (d, 1 H, J_{gem} 10.6 Hz, $PhCH_2$), 4.73 (d, 1 H, J_{gem} 10.5 Hz, $PhCH_2$), 4.68



A: Primary plot



B: Secondary plot K_m vs [*I*]



C: Secondary plot K_m/V_{max} vs [*I*]

Fig. 1. Kinetic evaluation of **7d** as a competitive inhibitor of cloned GlcNAcT-V. Plot A demonstrates that **7d** is competitive with the acceptor, with inhibitor concentration 0 (□), 20 μM (△) and 40 μM (■). The K_i for **7d** was 8 μM, determined by linear regression analysis of plots B and C. This figure is typical of the behavior observed for analogs **7a–7h** tested with both cloned and isolated enzymes.

(s, 2 H, $PhCH_2$), 4.51 (d, 1 H, J_{gem} 10.5 Hz, $PhCH_2$), 4.37 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.03 (ddd, 1 H, $J_{2',3'}$ 4.5 Hz, H-2'), 3.89 (dt, 1 H, J_{gem} 9.5, J_{vic} 6.2 Hz, octyl C_1-H_a), 3.78 (dd, 1 H, $J_{3',4'}$ 11.0 Hz, H-3'), 2.34 (d, 1 H, $J_{2',OH}$ 1.8 Hz, $C_{2'}$-OH), 1.24 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.88 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3).

*Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl-(1 → 2)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**13**).—A mixture of **12** (788 mg, 0.96 mmol), AgOTf (491 mg, 1.91 mmol), 4 Å molecular sieves (1.5 g), collidine (101 μL, 0.77 mmol) and dry CH_2Cl_2 (12 mL) was cooled to −78 °C. To the resulting mixture was added dropwise a solution of bromide **10** [15] (715 mg, 1.43 mmol) in dry CH_2Cl_2 (6 mL) under argon. The mixture was allowed to warm to room temperature over 1 h. After addition of tetraethylammonium chloride (250 mg, 1.5 mmol), the mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, satd $NaHCO_3$, and water, then dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography using 2:1 hexane–EtOAc as eluant to provide **13** (910 mg, 77%): $[\alpha]_D$ +31.8° (*c* 0.4, $CHCl_3$); NMR data ($CDCl_3$): ^1H (360 MHz), δ 7.83 (dd, 2 H, *J* 5.4, 3.0 Hz, phthalimido C_3-H, C_6-H), 7.69 (dd, 2 H, *J* 5.4, 3.0 Hz, phthalimido C_4-H, C_5-H), 7.41–7.13 (m, 20 H, Ar), 5.87 (dd, 1 H, $J_{2'',3''}$ 10.9, $J_{3'',4''}$ 9.2 Hz, H-3''), 5.43 (d, 1 H, $J_{1'',2''}$ 8.5 Hz, H-1''), 5.20 (dd, 1 H, $J_{4'',5''}$ 9.9 Hz, H-4''), 4.98 (d, 1 H, J_{gem} 10.9 Hz, $PhCH_2$), 4.96 (d, 1 H, J_{gem} 10.8 Hz, $PhCH_2$), 4.78 (d, 1 H, J_{gem} 11.1 Hz, $PhCH_2$), 4.76 (d, 1 H, J_{gem} 10.5 Hz, $PhCH_2$), 4.74 (d, 1 H, J_{gem} 11.6 Hz, $PhCH_2$), 4.73 (d, 1 H, J_{gem} 10.6 Hz, $PhCH_2$), 4.55 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.53 (d, 1 H, J_{gem} 11.6 Hz, $PhCH_2$), 4.46 (dd, 1 H, H-2''), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.31 (d, 1 H, J_{gem} 11.1 Hz, $PhCH_2$), 4.26 (dd, 1 H, J_{gem} 12.3, $J_{5'',6''}$ 2.4 Hz, H-6''), 4.13 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 4.06 (dt, 1 H, J_{gem} 9.5, J_{vic} 6.3 Hz, octyl C_1-H_a), 3.95 (ddd, 1 H, $J_{4'',5''}$ 10.2, $J_{5'',6''a}$ 4.7, $J_{5'',6''b}$ 2.5 Hz, H-5''), 3.20 (dd, 1 H, $J_{4',5'}$ 9.9, $J_{3',4'}$ 9.8 Hz, H-4'), 3.10 (dq, 1 H, $J_{5',6'}$ 6.0, $J_{4',5'}$ 9.8 Hz, H-5'), 2.08, 2.05, 1.89 (s, each 3 H, 3 Ac), 0.87 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3), 0.55 (d, 3 H, 3 H-6'); ^{13}C (75.5 MHz), δ 170.70, 170.14, 169.39 (C=O), 138.49, 138.37, 138.03, 137.39 (benzyl C_1), 128.44, 128.39, 128.33, 128.04, 127.94, 127.71, 127.53 (aromatic methine), 103.85 (C-1), 97.07 (C-1'), 96.07 (C-1''), 75.76, 74.74, 74.73, 70.68 (Ph*CH*$_2$), 70.37 (octyl C_1), 66.57 (C-6), 63.35 (C-4'), 62.22 (C-6''), 54.33 (C-2''), 31.85, 29.83 29.49, 29.28, 26.20, 22.66 (octyl C_2-C_7), 20.76, 20.63, 20.51 (3 *CH*$_3$CO), 17.91 (C-6'), 14.07 (octyl CH_3). Anal. Calcd for $C_{68}H_{80}N_4O_{18}$ (1241.41): C, 65.79; H, 6.50; N, 4.51. Found: C, 65.87; H, 6.64; N, 4.55.

*Octyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-4-azido-3-*O*-benzyl-4,6-dideoxy-α-*D*-mannopyranosyl-(1 → 6)-2,3,4-tri-*O*-benzyl-β-*D*-glucopyranoside* (**14**).—A solution of **13** (840 mg, 0.68 mmol) in butanol (120 mL) was added to 1,2-ethylenediamine (24 mL). The mixture was stirred at 80 °C for 12 h. Concentration was followed by addition and evaporation twice of toluene. The residue was not characterized but was dissolved in dry MeOH (10 mL) to which Ac_2O (10 mL) was added. After stirring at room temperature overnight, water (10 mL) was added, and the solution was concentrated. The residue was purified by column chromatography on Iatrobeads using 9:1 CH_2Cl_2–MeOH as eluant, to provide **14** (610 mg, 88%) as a colorless syrup: $[\alpha]_D$ +24.6° (*c* 1.25, $CHCl_3$); NMR data ($CDCl_3$): ^1H (300 MHz), δ 7.40–7.10 (m, 20 H, Ar-H), 4.85 (d, 1 H, $J_{1',2'}$ 1.9 Hz, H-1'), 4.53 (d, 1 H, $J_{1'',2''}$ 8.2 Hz, H-1''), 4.36 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.11 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 1.91 (s, 3 H, Ac), 1.18 (d, 3 H, $J_{5',6'}$ 6.5 Hz, 3 H-6'), 0.87 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C (75.5 MHz), δ 172.36 (C=O), 138.46, 138.38, 138.06, 136.97 (benzyl C_1), 103.83 (C-1), 99.09 (C-1''), 97.65 (C-1'), 70.67 (octyl C_1), 66.44 (C-6), 64.06 (C-4'), 62.40 (C-6''), 58.41 (C-2''), 31.86, 29.81, 29.47, 29.30, 26.20, 22.69 (octyl C_2-C_7), 23.58 (*CH*$_3$CO), 18.73 (C-6'), 14.11 (octyl CH_3). Anal. Calcd for $C_{56}H_{74}N_4O_{14}$ (1027.23): C, 65.48; H, 7.26; N, 5.45. Found: C, 65.27; H, 7.22; N, 5.45.

*Octyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-4-amino-4,6-dideoxy-α-*D*-mannopyranosyl-(1 → 6)-β-*D*-glucopyranoside* (**7a**).—To a mixture of 1 NH_3 (80 mL) and *tert*-butanol (0.3 mL) was added a solution of **14** (193 mg, 0.285 mmol) in THF (5 mL) at −78 °C. Small pieces of sodium were then added until the mixture remained blue, and stirring was continued for 4 h. Ammonium chloride (solid) was added until the blue color disappeared. After evaporation of NH_3, the residue was dissolved in water. The aq solution was loaded onto a C_{18} reversed-phase silica gel column (12 g). The column was eluted with water (3 × 30 mL), followed by MeOH (100 mL). The methanolic eluate was evaporated to dryness, and the residue was purified by chromatography on Iatrobeads using 65:35:8 CH_2Cl_2–MeOH–H_2O as eluant to provide the crude

product. The crude product was absorbed onto a C_{18} Sep-Pak cartridges (10 mg/cartridge) in water, and the cartridges were washed with water (50 mL) and eluted with HPLC-grade MeOH (50 mL). Concentration of the eluate, dissolution of the residue in water, filtration through a Millex filter, and lyophilization gave the target product **7a** (98 mg, 81%) as a white powder: $[\alpha]_D$ −22.3° (*c* 0.3, H_2O); NMR data (D_2O): ^1H (500 MHz), δ 4.94 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.62 (d, 1 H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.48 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.18 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 4.02 (dd, 1 H, $J_{3',4'}$ 10.0 Hz, H-3'), 3.99 (dd, 1 H, J_{gem} 11.0, $J_{5,6a}$ 4.0 Hz, H-6a), 3.94 (dd, 1 H, J_{gem} 12.0, $J_{5'',6''a}$ 2.2 Hz, H-6''a), 3.91 (dt, 1 H, J_{gem} 12.0, J_{vic} 6.2 Hz, octyl C_1-H_a), 3.79 (dd, 1 H, $J_{5'',6''b}$ 5.4 Hz, H-6''b), 3.78 (dd, 1 H, $J_{5,6b}$ 6.0 Hz, H-6b), 3.75 (dd, 1 H, $J_{2'',3''}$ 10.0 Hz, H-2''), 3.70 (dt, 1 H, octyl C_1-H_b), 3.59 (m, 1 H, H-5), 3.21 (dd, 1 H, $J_{2,3}$ 9.5 Hz, H-2), 3.11 (dd, 1 H, $J_{3',4'}$, 10.0 Hz, H-4'), 2.07 (s, 3 H, NH*Ac*), 1.64 (p, 2 H, J_{vic} 7.0 Hz, octyl CH_2), 1.36 (d, 3 H, $J_{5',6'}$ 6.2 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C (100.6 MHz), δ 175.52 (C=O), 103.24 (C-1, J_{CH} 161.5 Hz), 100.24 (C-1'', J_{CH} 162.0 Hz), 97.96 (C-1', J_{CH} 171.6 Hz), 76.80 (C-3), 76.69 (C-5''), 75.77 (C-2'), 74.85 (C-5), 74.09 (C-3''), 73.92 (C-2), 71.62 (octyl C_1), 70.66 (C-4''), 70.15 (C-4), 67.72 (C-3'), 67.22 (C-5'), 66.71 (C-6), 61.33 (C-6''), 56.13 (C-2''), 55.12 (C-4'), 31.93, 29.58, 29.28, 29.20, 25.87, 22.84 (octyl C_2-C_7), 23.10 ($CO\mathit{CH_3}$), 17.85 (C-6'), 14.26 (octyl CH_3); FABMS: *m/z* 663.52 $[M + Na]^+$, 641.42 $[M + H]^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-4-acetamido-4,6-dideoxy-α-D-mannopyranosyl-(1 → 6)-β-D-glucopyranoside (**7b**).—To a solution of **7a** (0.9 mg, 1.40 μmol) in dry MeOH (0.5 mL) was added Ac_2O (0.5 mL). The mixture was stirred at room temperature overnight, solvents were removed, and the resulting residue was absorbed onto a Sep-Pak C_{18} cartridge in water, the cartridge was washed with water (10 mL), followed by HPLC-grade MeOH (10 mL). Concentration of the eluate, dissolution of the residue in water, filtration through a Millex filter, and lyophilization afforded **7b** (0.96 mg, quant.): ^1H NMR (D_2O, 360 MHz): δ 4.92 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.64 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.49 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.16 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.28 (dd, 1 H, $J_{2,3}$ 8.0 Hz, H-2), 2.10 (s, 3 H, NH*Ac*), 2.05 (s, 3 H, NH*Ac*), 1.20 (d, 3 H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); FABMS: *m/z* 705.87 $[M + Na]^+$, 683.85 $[M + H]^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-4-benzamido-4,6-dideoxy-α-D-mannopyranosyl-(1 → 6)-β-D-glucopyranoside (**7c**).—To a solution of **7a** (2.0 mg, 3.12 μmol) in pyridine was added benzoyl chloride (28 μL, 220 μmol). The mixture was stirred at room temperature for 8 h and poured into ice water. The aq solution was extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed with 0.5 M HCl, satd aq $NaHCO_3$, and water, then dried over ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography using 1:1 hexane–EtOAc as eluant to provide perbenzoylated product (not characterized), which was then treated with 0.05 N NaOMe–MeOH for 8 h. Neutralization with Amberlite IR-120 (H^+) resin, removal of resin by filtration, and concentration left a residue that was purified as described for the purification of **7b** to give **7c** (0.9 mg, 39%) as a white powder: ^1H NMR (D_2O, 360 MHz): δ 7.85 (dd, 2 H, *J* 8.0, 1.5 Hz, benzoyl C_2-H, C_6-H), 7.68 (ddd, 1 H, *J* 8.0, 8.0, 1.5 Hz, benzoyl C_4-H), 7.58 (dd, 2 H, *J* 8.0, 8.0 Hz, benzoyl C_3-H, C_5-H), 4.98 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.68 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.48 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.20 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 2.13 (s, 3 H, NH*Ac*), 1.27 (d, 3 H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); FABMS: *m/z* 767.66 $[M + Na]^+$, 745.48 $[M + H]^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-4,6-dideoxy-4-succinamido-α-D-mannopyranosyl-(1 → 6)-β-D-glucopyranoside (**7d**).—A mixture of **7a** (2.5 mg, 3.9 μmol) and succinic anhydride (20 mg, 200 μmol) in dry MeOH (3 mL) was refluxed for 4 h and concentrated to dryness. The residue was purified by chromatography on Iatrobeads eluting first with 9:1 CH_2Cl_2–MeOH to remove excess succinic anhydride, then with 65:35:8 CH_2Cl_2–MeOH–H_2O to obtain the crude product that was purified as described for the preparation of **7b** to provide the title compound (1.7 mg, 59%) as a white powder: ^1H NMR (D_2O, 400 MHz): δ 4.88 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.61 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.46 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.12 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.91 (dd, 1 H, $J_{3',4'}$ 10.5 Hz, H-3'), 3.26 (dd, 1 H, $J_{2,3}$ 8.5 Hz, H-2), 2.61 and 2.56 (m, each 2 H, $CO\mathit{CH_2CH_2}COOH$), 2.08 (s, 3 H, NH*Ac*), 1.17 (d, 3 H, $H_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); FABMS: *m/z* 763.64 $[M + Na]^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1 → 2)-4,6-dideoxy-4-iodoacetamido-α-D-mannopyranosyl-(1 → 6)-β-D-glucopyranoside (**7e**).—A mixture of **7a** (10 mg, 15.6 μmol) and iodoacetic anhydride (165 mg, 468 μmol) in dry MeOH (1.5 mL) was stirred at room temperature for 24 h and

concentrated. The residue was dissolved in water (1 mL) and stirred at room temperature for 30 min to decompose excess iodoacetic anhydride. The product was then isolated on a C_{18} Sep-Pak cartridge as described for the preparation of **7b**. Lyophilization after Millex filtration afforded **7e** (9 mg, 71.3%) as a white powder: ^{1}H NMR (D_2O, 400 MHz): δ 4.93 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.44 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.50 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.17 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-2'), 2.10 (s, 3 H, NH*Ac*), 1.66 (p, 2 H, *J* 7.0 Hz, octyl CH_2), 1.24 (d, 3 H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); ^{13}C NMR (100.6 MHz): δ 175.51 (C=O), 173.19 (ICH_2CO), 103.15 (C-1), 100.06 (C-1''), 97.72 (C-1'), 71.60 (octyl C_1), 67.01 (C-6), 61.45 (C-6''), 56.23 (C-2''), 54.69 (C-4'), 31.94, 26.97, 29.31, 29.23, 25.98, 21.86 (octyl C_2-C_7), 23.12 (CH_3CO), 17.99 (C-6'), 14.26 (octyl CH_3), −1.48 (ICH_2); FABMS: *m/z* 831.57 $[M + Na]^+$, 809.64 $[M + H]^+$.

*Octyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-4-acrylamido-4,6-dideoxy-α-*D*-mannopyranosyl-(1 → 6)-β-*D*-glucopyranoside* (**7f**).—To a solution of **7a** (2.3 mg, 3.59 μmol) in a mixture of aq $NaHCO_3$ (0.1 N, 0.5 mL) and DMF (0.5 mL) was added acryloyl chloride (2.9 μL, 35.9 μmol). The resulting mixture was stirred at room temperature for 12 h and concentrated. The residue was purified by chromatography on Iatrobeads using 65:35:4 CH_2Cl_2–MeOH–H_2O as eluant to give the crude product. The crude product was further purified as described for the preparation of **7b** to give **7f** (1.30 mg, 52%) as a white powder: ^{1}H NMR (D_2O, 500 MHz): δ 6.27 (dd, 1 H, J_{trans} 17.0, J_{cis} 9.5 Hz, $COCH{=}CH_2$), 6.22 (dd, 1 H, J_{trans} 17.0, J_{gem} 2.5 Hz, acryloyl C_3-H_a), 5.80 (dd, 1 H, J_{cis} 9.5, J_{gem} 2.5 Hz, acryloyl C_3-H_b), 4.91 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.62 (d, 1 H, $J_{1'',2''}$ 8.5 Hz, H-1''), 4.46 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.14 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.96 (dd, 1 H, $J_{3',4'}$ 10.5 Hz, H-3'), 3.26 (dd, 1 H, $J_{2,3}$ 9.0 Hz, H-2), 2.07 (s, 3 H, NH*Ac*), 1.18 (d, 3 H, $J_{5',6'}$ 6.0 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); FABMS: *m/z* 717.24 $[M + Na]^+$, 695.35 $[M + H]^+$.

*Octyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-4,6-dideoxy-4-(1-thiophenylacetamido)-α-*D*-mannopyranosyl-(1 → 6)-β-*D*-glucopyranoside* (**7g**).—To a solution of **7e** (1.5 mg, 1.85 μmol) in 0.1 N $NaHCO_3$ (0.5 mL) was added thiophenol (0.77 μL, 11.3 μmol). The mixture was stirred at room temperature for 24 h and evaporated to dryness. The residue was purified as described for the preparation of **7b** to give **7g** (1.4 mg, 96%) as a white powder: ^{1}H NMR (D_2O, 400 MHz): δ 7.52–7.25 (m 5 H, Ar-H), 4.91 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.62 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.48 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.13 (dd, 1 H, $J_{2',3'}$ 3.0 Hz, H-2'), 2.08 (s, 3 H, NH*Ac*), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); FABMS: *m/z* 813.76 $[M + Na]^+$.

*Octyl 2-acetamido-2-deoxy-β-*D*-glucopyranosyl-(1 → 2)-4,6-dideoxy-4-(2,4-dinitrophenylamino)-α-*D*-mannopyranosyl-(1 → 6)-β-*D*-glucopyranoside* (**7h**).—To a solution of **7a** (2.3 mg, 3.59 μmol) in phosphate buffer (0.5 mL) was added 1-fluoro-2,4-dinitrobenzene (1.35 μL, 43.2 μmol). The mixture was stirred at room temperature for 24 h, then at 80 °C for an additional 1 h until the complete consumption of the starting material was noted by TLC. After concentration, the residue was purified by chromatography on Iatrobeads eluting first with 9:1 CH_2Cl_2–MeOH to remove unreacted 1-fluoro-2,4-dinitrobenzene, then with 65:35:8 CH_2Cl_2–MeOH–H_2O to yield the crude product that was purified as described for the preparation of **7b** to give **7h** (2.8 mg, 97%) as a yellow powder: ^{1}H NMR (D_2O, 300 MHz): δ 9.14 (brs, 1 H, Ph C_3-H), 8.35 (d, 1 H, *J* 9.0 Hz, Ph C_5-H), 7.40 (d, 1 H, *J* 9.0 Hz, Ph C_6-H), 5.19 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.67 (d, 1 H, $J_{1'',2''}$ 8.4 Hz, H-1''), 4.45 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.21 (dd, 1 H, $J_{2',3'}$ 3.1 Hz, H-2'), 2.31 (s, 3 H, NH*Ac*), 1.40 (d, 3 H, $J_{5',6'}$, 6.0 Hz, 3 H-6'), 0.86 (t, 3 H, J_{vic} 7.0 Hz, octyl CH_3); FABMS: *m/z* 829.71 $[M + Na]^+$, 807.74 $[M + H]^+$.

Kinetic evaluation of **7a – 7h** *as inhibitors for GlcNAcT-V.*—GlcNAcT-V was assayed radiochemically using reversed-phase C_{18} Sep-Pak cartridges to separate labeled hydrophobic product tetrasaccharides from unreacted radiolabeled sugar-nucleotide donor UDP-GlcNAc as previously described [8,11,12]. Unless otherwise stated, kinetic studies with acceptor analogs contained 2.1 μU cloned GlcNAcT-V or 3.55 μU partially purified hamster kidney GlcNAcT-V, 0.2–6.4 nmol of standard acceptor **5**, and 11 nmol UDP-GlcNAc (containing UDP-[^{3}H]GlcNAc, 200,000 dpm/nmol). In a typical assay, acceptor **5**, UDP-GlcNAc, UDP-[^{3}H]GlcNAc, and inhibitor were placed in 600 μL plastic microcentrifuge tubes and lyophilized to dryness in a speed-vacuum. Enzyme and buffer (50 mM sodium cacodylate, 10 mM EDTA, 20% glycerol, 0.1% Triton X-100, pH 6.5) were added to give a final volume of 20 μL. The tube containing substrates and enzyme was vortexed, microcentrifuged briefly, and incubated at 37 °C for 1 h (cloned enzyme) or 45 min (partially purified enzyme). The reaction was quenched by adding 0.4 mL

of water, and the reaction mixture was loaded onto a pre-equilibrated C_{18} Sep-Pak cartridge. Unreacted radiolabeled donor was removed by washing with 50 mL of water. Labeled product was eluted with 4 mL of MeOH into a scintillation vial, Ecolite scintillation cocktail (10 mL) was then added, and the sample was counted on a Beckman LS-1801 scintillation counter. Kinetic constants were obtained by fitting rate data to the appropriate equations using unweighted nonlinear regression analysis (SigmaPlot 4.1, MacIntosh version). Apparent Michaelis constants ($K_{m\ app}$) and V_{max} were obtained by fitting rate data to the Equation: $u = V_{max}[A]/\{K_{m\ app} + [A]\}$, where u is the reaction rate, and $[A]$ is the concentration of the acceptor. All the analogs (**7a–7h**) were found to be competitive inhibitors since V_{max} values were unchanged within experimental error ($\pm 5\%$) and there were increases in the apparent K_m and K_m/V_{max} values for acceptor (Fig. 1). Inhibition constants (K_i) were determined by analyses of secondary plots of K_m/V_{max} (or K_m) vs. $[I]$ (concentration of inhibitor). The inhibition constants (K_is), obtained for **7a–7h** and evaluated using cloned rat kidney GlcNAcT-V and partially purified GlcNAcT-V from hamster kidney, are listed in Table 1.

Acknowledgements

We thank Ms. C. Compston for assistance with the enzyme assays and Dr. Albin Otter for recording the 500-MHz NMR spectra of compound **7a**. The cloned GlcNAcT-V was a generous gift from Dr. M. Pierce, University of Georgia. This work was supported by grants from the Medical Research Council of Canada (MMP) and the Natural Sciences and Engineering Research Council of Canada (OH). The Alberta Heritage Foundation for Medical Research also supported this work through a scholarship to Pu-Ping Lu.

References

[1] R.D. Cummings and I.S. Trowbridge, *J. Biol. Chem.*, 257 (1982) 13421–13427.
[2] H. Schachter, *Biochem. Cell. Biol.*, 64 (1986) 163–181.
[3] J.W. Dennis, S. Laferte, C. Waghorne, M.L. Breitman, and R.S. Kerbel, *Science*, 236 (1987) 582–585.
[4] J.W. Dennis, *Cancer Surveys*, 7 (1988) 573–594.
[5] J.W. Dennis, in M. Fukuda (Ed.), *Cell Surface Carbohydrates and Cell Development*, CRC press, Boca Raton, FL, 1991, pp 161–194.
[6] O. Hindsgaul, S.H. Tahir, O.P. Srivastava, and M. Pierce, *Carbohydr. Res.*, 173 (1988) 263–272.
[7] O.P. Srivastava, O. Hindsgaul, M. Shoreibah, and M. Pierce, *Carbohydr. Res.*, 179 (1988) 137–161.
[8] M.M. Palcic, L.D. Heerze, M. Pierce, and O. Hindsgaul, *Glycoconjugate J.*, 5 (1988) 49–63.
[9] M.M. Palcic, J. Ripka, K.J. Kaur, M. Shoreibah, O. Hindsgaul, and M. Pierce, *J. Biol. Chem.*, 265 (1990) 6759–6769.
[10] O. Hindsgaul, K.J. Kaur, G. Srivastava, M. Blaszczyk-Thurin, S.C. Crawley, L.D. Heerze, and M.M. Palcic, *J. Biol. Chem.*, 266 (1991) 17858–17862.
[11] P.-P. Lu, O. Hindsgaul, C.A. Compston, and M.M. Palcic, *Bioorg. Med. Chem.*, 4 (1996) 2011–2022.
[12] S.H. Khan, S.C. Crawley, O. Kanie, and O. Hindsgaul, *J. Biol. Chem.* 268 (1993) 2468–2473.
[13] D.R. Bundle, M. Gerken, and T. Peters, *Carbohydr. Res.*, 174 (1988) 239–251.
[14] K. Bock and C. Pedersen, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–295.
[15] R.U. Lemieux, T. Takkeda, and B.Y. Chung, *ACS Symp. Ser.*, 39 (1976) 90–114.
[16] O. Kanie, S.C. Crawley, M.M. Palcic, and O. Hindsgaul, *Carbohydr. Res.*, 243 (1993) 139–164.
[17] M. Shoreibah, G.S. Perng, B. Adler, J. Weinstein, R. Cuddles, D. Wen, J.K. Browne, P. Buckhaults, N. Fregien, and M. Pierce, *J. Biol. Chem.*, 268 (1993) 5381–5385.