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New Synthetic Trisaccharide Inhibitors for *N*-Acetylglucosaminyltransferase-V

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Abstract—The trisaccharide octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**5**) is an acceptor substrate for *N*-acetylglucosaminyltransferase-V (EC 2.4.1.155) which adds a β-GlcNAc residue to OH-6 of the central Man-residue. In the present work, 10 analogues of **5**, each missing the potentially reactive OH-6 group, were chemically synthesized. The key intermediate used was octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-amino-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (**6a**), which was synthesized in stepwise fashion by sequential coupling of protected monosaccharide residues. The 6′-amino group in **6a**, was then selectively derivatized by either acylation or alkylation with hydrophobic, hydrophilic, charged, aromatic and potential covalently inactivating groups. The 10 trisaccharide analogues thus produced were evaluated for inhibition against GlcNAcT-V isolated from hamster kidney. All of the compounds were competitive inhibitors with K_i values ranging from 21 to 297 μM. These results indicate that acceptor substrate (or inhibitor)–enzyme complex does not involve critical recognition contacts at the position of transfer.

Introduction

UDP-GlcNAc: α-Mannoside β-(1→6)-*N*-acetylglucosaminyltransferase (GlcNAcT-V, EC 2.4.1.155) is one of the key enzymes involved in the biosynthesis of highly branched asparagine-linked oligosaccharides[1,2]. This enzyme has earned particular interest following observations that its activity increases when cells become transformed.[3–5] Specific increases in the activity of GlcNAcT-V have also been shown to correlate with the metastatic potential of tumor cells.[6,7]

GlcNAcT-V transfers an *N*-acetyl-D-glucosamine (GlcNAc) residue from uridine 5′-diphospho-GlcNAc (UDP-GlcNAc) to glycopeptides bearing the minimum heptasaccharide sequence **1** (Scheme 1), producing the additionally branched structure **2**.[1,2] The much simpler synthetic trisaccharide **3** was shown to also be an effective substrate for the enzyme yielding the expected tetrasaccharide **4**.[8] GlcNAcT-V further tolerates the substitution of the βMan residue in **3** by a βGlc residue since trisaccharide **5** is a fully active substrate.[9] Inclusion of the hydrophobic aglycones in **3** and **5** permitted enzyme-assays to be performed using the 'Sep-Pak' protocol.[10]

Both the 6′-deoxy derivative of **5** ($K_i = 63$ μM) and its 4′-O-methy derivative ($K_i = 14$ μM) were found to be good competitive inhibitors of GlcNAcT-V.[11,12] It was suggested that the large methyl group introduced on O-4′ sterically prevented the formation of product even though both donor and acceptor substrates were bound by the enzyme. We therefore decided to investigate whether trisaccharides of the general structure **6**, modified directly at the position to which GlcNAcT-V transfers, could be used to probe the characteristics of the protein structure near the active site of the enzyme by evaluating them as potential inhibitors. The presence of the 4′-O-methyl group would assure that transfer to position 6 of the central Man residue could not occur in any of the analogues to potentially complicate interpretation of the results, while the 6′-amino group would allow the facile preparation of derivatives using a single precursor primary amine.

Results and discussion

Retrosynthetic analysis suggested that the synthesis of the target molecule could be achieved by the sequential coupling of building blocks **7**–**9** (Scheme 2). Compounds **7** and **9** have already been reported, so a scheme was devised for the preparation of **8**.

D-Mannose was used as the starting material for the synthesis of **8**. Fischer glycosylation of D-mannose with 4-penten-1-ol gave 4-pentenyl α-D-mannopyranoside (**10**).[13] Treatment of **10** with 4-methoxybenzaldehyde dimethylacetal and pyridinium *p*-toluenesulfonate in DMF at 80 °C lead to selective benzylidenation[14] to give 4-pentenyl 4,6-*O*-(4-methoxybenzylidene)-α-D-mannopyranoside (**11**) in 46% yield. Compound **11** was then selectively benzylated, via its 2,3-*O*-dibutylstannylidene derivative,[15] to provide the 3-*O*-benzyl derivative (**12**) in 86% yield. After benzoylation of OH-2, regioselective reductive ring-opening of the 4-methoxybenzylidene acetal in **13** with sodium cyanoborohydride-trifluoroacetic acid in DMF gave the 6-*O*-(4-methoxybenzyl) ether (**14**) in 85% yield.[16]

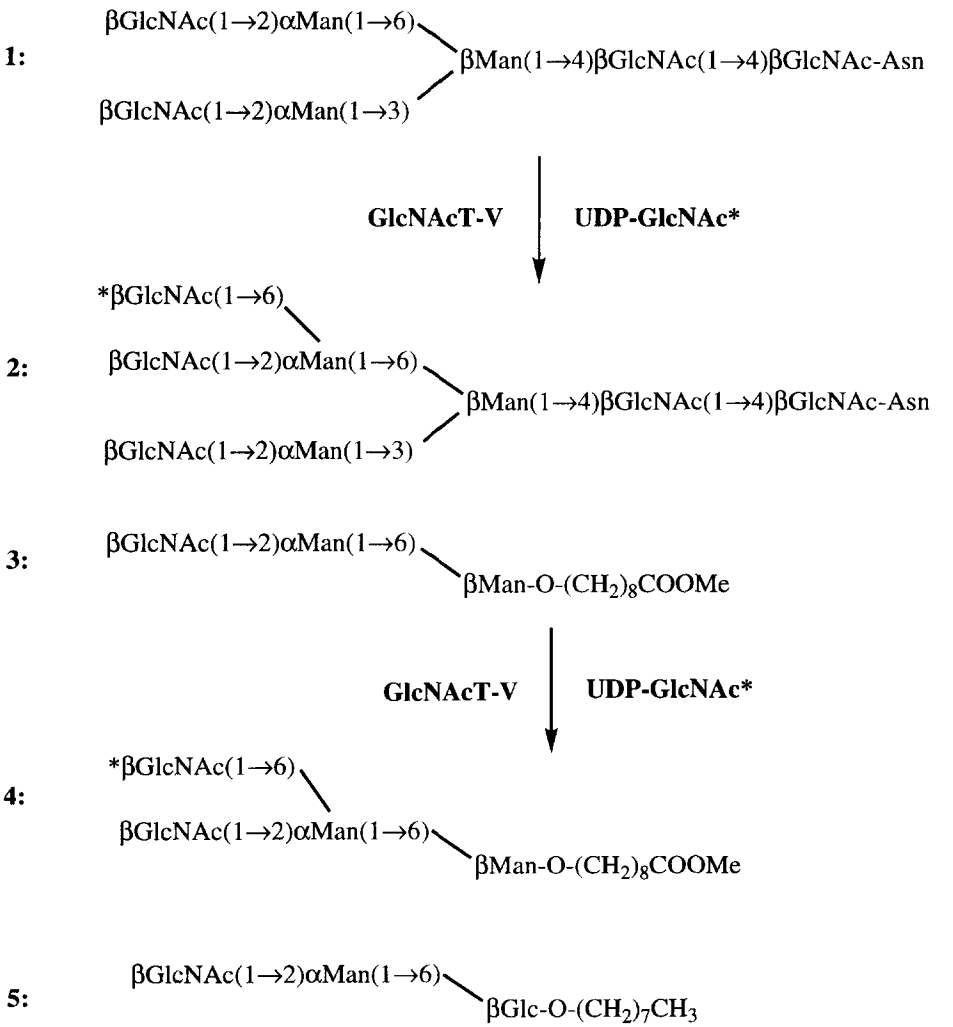
Methylation of OH-4 in **14** in the presence of the benzoyl ester group was achieved using methyl iodide

and sodium hydride in DMF at −15 °C followed by immediate neutralization with acetic acid. Cerium (IV) ammonium nitrate (CAN) was evaluated for the selective deprotection of the *p*-methoxybenzyl group, but failed. 2,4-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in dichloromethane saturated with water[17] afforded the desired product (**16**) in 87% yield. Tosylation of OH-6 in **16**, followed by displacement with sodium azide, gave the key building block **8** (Scheme 3).

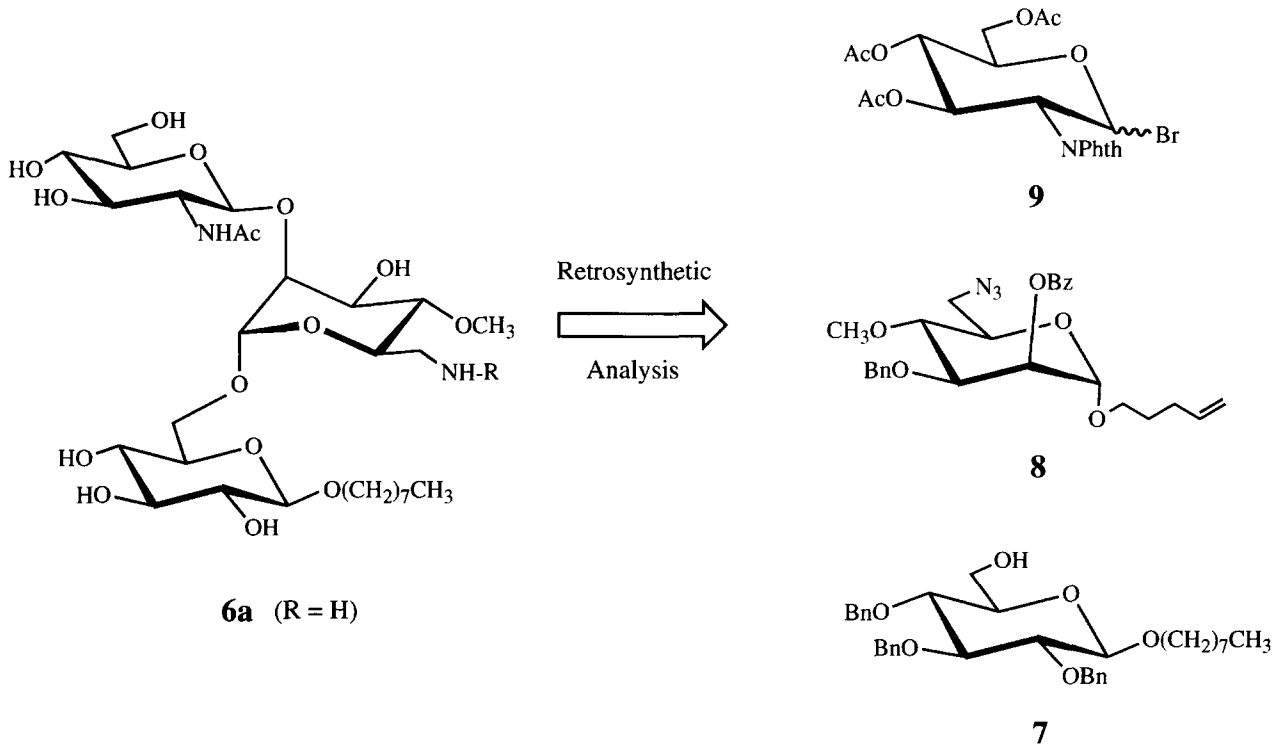
The coupling of **7**[9] and **8** using N-iodosuccinimide (NIS) and silver trifluoromethane sulfonate (AgOTf) as promoters[13] furnished the α-linked disaccharide **18** in 48% yield. The anomeric configuration was confirmed by its ^{13}C NMR spectrum which showed the anomeric carbons at δ103.6 ($J_{C\text{-}1,H\text{-}1}$ = 161.5 Hz) and 97.9 ($J_{C\text{-}1',H\text{-}1'}$ = 170.9 Hz), respectively. One-bond C–H coupling constants of these magnitudes require the presence of the β and α glycosidic linkages, respectively.[18]

Treatment of **18** with methanolic NaOMe gave **19** with the required free OH-group at C-2 for further coupling. Condensation of **19** and 2-deoxy-2-phthalimido-3,4,6-tri-*O*-acetyl-glucopyranosyl bromide (**9**)[19] using AgOTf as promoter provided the trisaccharide **20** in 72% yield. Removal of the N-phthalimido and O-acetyl groups was achieved using 1,2-diaminoethane in butanol.[20] N-acetylation of the resultant free amine with acetic anhydride in dry methanol gave **21** in 79% overall yield from **20**. Finally, hydrogenolytic cleavage of the benzyl protecting groups and the reduction of the azido group of **21** using 5% palladium-on-charcoal as the catalyst, in 95% ethanol (aldehyde free), furnished the target trisaccharide **6a** in 91% yield. The required analogues of trisaccharide **6a** were prepared conventionally using the reagents indicated in Table 1. All analogues (**6a–j**) were characterized by their ^1H NMR and FAB-MS data.

Compounds **6a–j** were tested as inhibitors of GlcNAcT-V which was partially purified from hamster kidney by modification of published procedures.[12] The activity of the synthetic trisaccharide derivatives were determined using radiochemical 'Sep-Pak assays' as previous described.[10,11] Trisaccharides **6a–j** were all found to be inhibitory. A full kinetic analysis was



Scheme 1. Glycosylation reactions catalysed by GlcNAcT-V.



Scheme 2. Synthetic strategy for the synthesis of **6a**.

carried out only for the tightest binding inhibitor, the N-iodoacyl derivative **6e**, which revealed it to be a competitive inhibitor (Fig. 1). For the remaining compounds, the K_i values were estimated using two to three inhibitor concentrations (see Experimental section). The K_i values ranged from 21–297 μM.

All of the analogues appear to be competitive inhibitors of the acceptor substrate **5** ($K_m = 29$ μM) despite the very large variation in molecular structure attached at the position normally transferred to by the enzyme. This result is very surprising since the OH group to which the enzyme transfers must clearly make contact with groups on the enzyme at some point during the transfer process. The fact that such a large variety of substitutions are tolerated at C-6 of the inhibitory trisaccharides indicates that in forming the E-I complexes, the potentially reactive OH-group does not make important contacts with the enzyme. The data in Table 1 do suggest, however, that positively charged residues near the site of transfer are unfavorable while hydrophobic or anionic groups are much more preferred.

Experimental

General methods

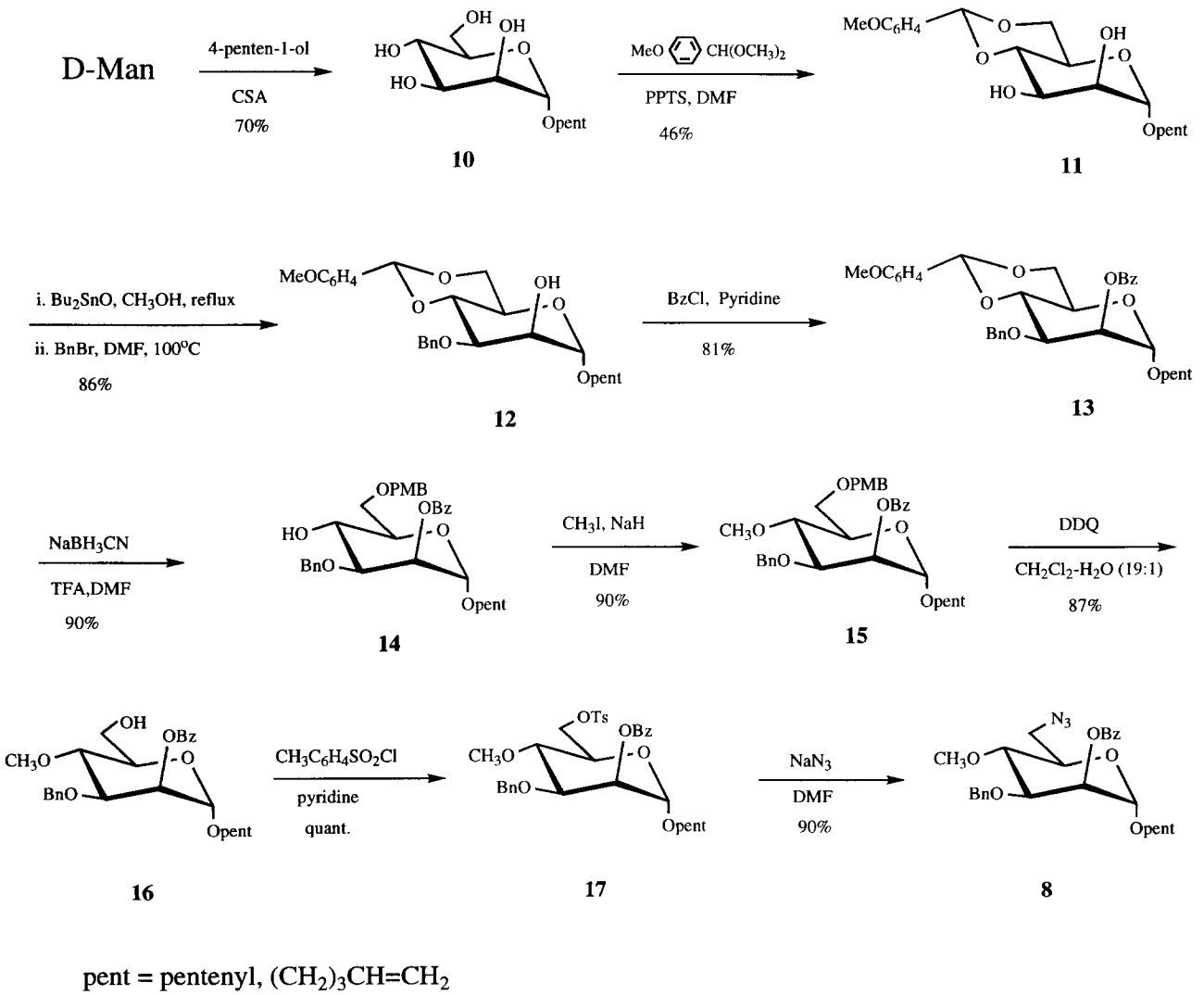
TLC was performed on silica gel 60-F254 (E. Merck) with detection by quenching of fluorescence, by charring with H_2SO_4, and/or by reaction with ninhydrin. Unless otherwise noted, column chromatography was performed on silica gel 60 (E. Merck, 40–63 μm). C-18 Sep-Pak sample preparation cartridges were from Waters Associates. Millex-GV (0.22 mm), filter units were from Millipore. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 22 °C. IR spectra were recorded with a Nicolet SX-20 FT-IR by the spectral services laboratory of the Chemistry Department. ^{1}H NMR spectra were recorded at 360 MHz (Bruker AMR 360), at 400 MHz (Bruker AM 400), or at 500 MHz (Varian UNITY 500) for 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 $CDCl_3$ (internal Me_4Si δ 0) or D_2O (internal 1,4-dioxane, δ 67.4). The assignments of ^{13}C NMR signals are tentative. Fast atom bombardment mass spectra (FAB-MS) were obtained on a Kratos AEIMS9 instrument by using Xe as the bombarding gas and glycerol and HCl as the matrix. Elemental analyses were carried out on a Carlo Erba EA1108.

Protons and carbons of the pentenyl group present in the compounds described in this paper are designated as follows. In ^{1}H NMR, H_a-5 (dddd, $J_{5a,4} = 17.0$ Hz, $J_{5a,5b}$, $J_{5a,3a}$, $J_{5b,3b} = 1.5$ Hz); H_b-5 (dddd, $J_{5b,4} = 10.0$ Hz, $J_{5b,3a}$, $J_{5b,3b} = 1.5$ Hz); H-4 (dddd, $J_{4,3a} = 6.6$ Hz, $J_{4,3b} = 6.5$ Hz); C_3-H (ddddd, $J_{2,3} = 7.0$ Hz); C_2-H (p, $J_{vic} = 7.0$ Hz); C_1-H_a (ddd, $J_{1a,1b}$ 9.5 Hz, $J_{1a,2} = 7.0$ Hz); C1-H_b (ddd, $J_{1b,2} = 7.0$ Hz). 'PMB' refers to signals arising from the *p*-methoxybenzylidene or *p*-methoxybenzyl group.

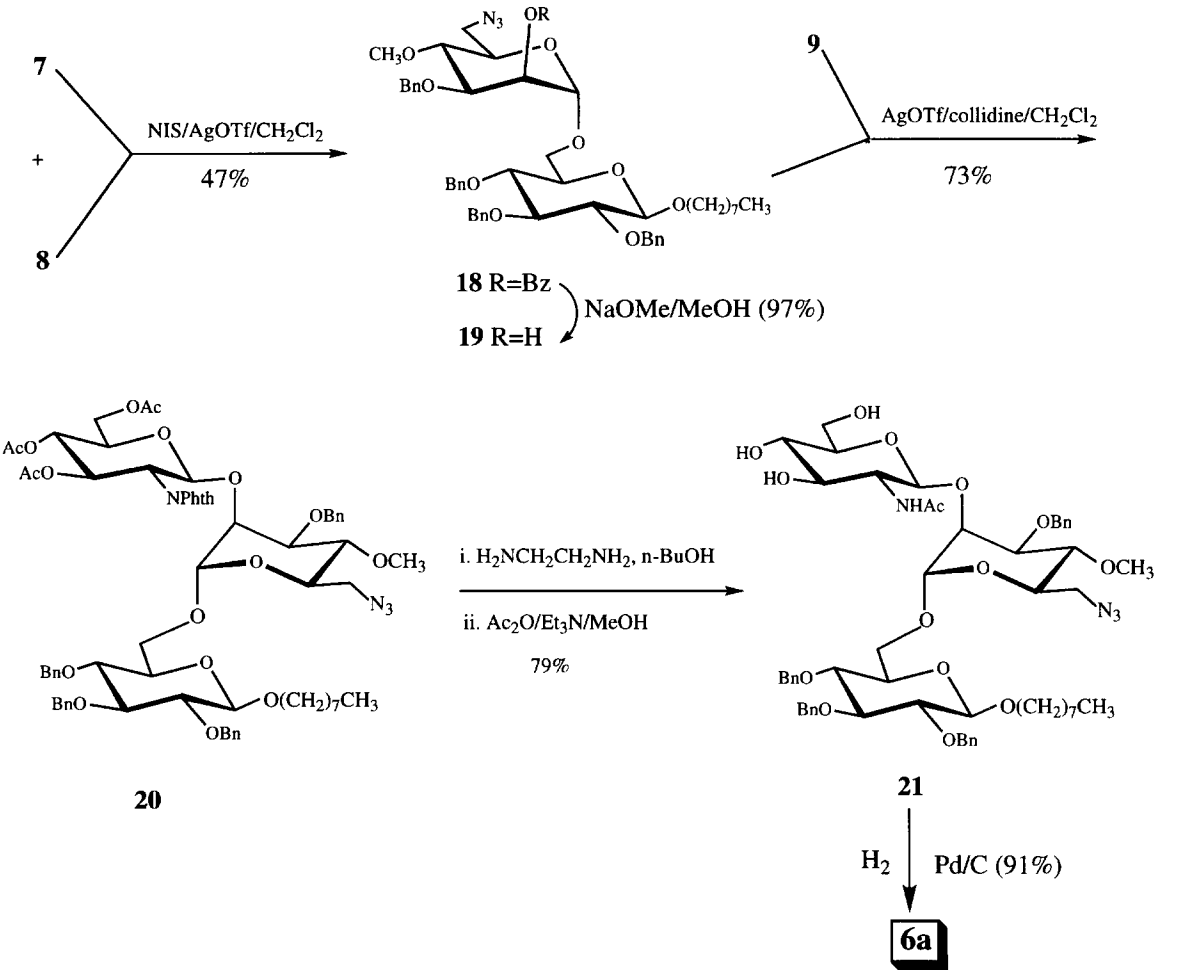
4-Pentenyl 4,6-*O*-(4-methoxybenzylidene)-α-D-mannopyranoside (11). 4-Pentenyl α-D-mannopyranoside (9.89 g, 39.87 mmol) was dissolved in dry dimethylformamide (DMF, 80 ml, 80 °C) and pyridinium

p-toluenesulfonate (100 mg, 0.4 mmol) was added. 4-Methoxybenzaldehyde dimethyl acetal (8.62 g, 47.36 mmol) in DMF (100 ml) was then added dropwise under a stream of argon so as to remove the liberated methanol. After 24 h, TLC indicated complete consumption of starting material. The solvent was evapd in vacuo, and the residue was purified by flash chromatography on silica gel, using first hexane:EtOAc (6:1) to remove by-products, and then hexane-EtOAc (1:2) and EtOAc to elute the product. Compound **11** was obtained as a colorless syrup (6.71 g, 46%); $[\alpha]_D$ +49.8° (*c* 1.92; $CHCl_3$); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.43 (ddd, 2H, J=8.8, 2.1, 2.0 Hz, PMB H-2, H-6), 6.90 (ddd, 2H, J=8.8, 2.1, 2.0 Hz, PMB H-3, H-5), 5.83 (pentenyl H-4), 5.56 (s, 1H, PMBCH), 5.05 (pentenyl H_a-5), 5.02 (pentenyl H_b-5), 4.89 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 4.27 (dd, 1H, $J_{2,3}$=3.5 Hz, H-2), 4.15–4.08 (m, 2H), 3.93 (dd, 1H, $J_{3,4}$=7.5, $J_{4,5}$=7.5 Hz, H-4), 3.90–3.80 (m, 2H), 3.84 (s, 3H, OCH_3), 3.75 (pentenyl H_a-1), 3.47 (pentenyl H_b-1), 2.58 (d, 1H, $J_{3,OH}$=2.2 Hz, OH-3), 2.56 (d, 1H, $J_{2,OH}$=2.9 Hz, OH-2), 2.18 (pentenyl H-3), 1.74 (pentenyl H-2). ^{13}C NMR (75.5 MHz): δ 160.32 (PMB C-4), 137.91 (pentenyl C-4), 129.79 (PMB C-1), 127.62 (PMB C-2), 115.14 (pentenyl C-5), 113.77 (PMB C-3), 102.23 (PMBCH), 100.22 (C-1), 78.96 (C-5), 71.08 (C-2), 68.88 (pentenyl C-1), 68.88 (C-3), 67.29 (C-6), 63.09 (C-4), 55.37 (OCH_3), 30.29, 28.63 (pentenyl C-2/C-3). Anal. calcd for $C_{19}H_{26}O_7$ (366.41): C, 62.28; H, 7.15. Found: C, 61.93; H, 7.04.

4-Pentenyl 3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-α-D-mannopyranoside (12). A suspension of compound **11** (5.90 g, 16.12 mmol) and dibutyltin oxide in methanol (600 ml) was heated at reflux for 3 h. Solvent was removed under diminished pressure to leave a colorless syrup. This dibutylstannylene derivative was dissolved in dry DMF (600 ml), benzyl bromide (2.90 ml, 24.18 mmol) was added and the soln was brought to 100 °C. After 20 min, TLC showed complete disappearance of starting material. The reaction mixture was then concd to dryness under diminished pressure. Chromatography (2:1 hexane: EtOAc) of the residue afforded **12** (6.32 g, 86%) as a colorless oil; $[\alpha]_D$ +31.52° (*c* 1.92; $CHCl_3$); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.44 (d, 2H, J=8.9 Hz, PMB

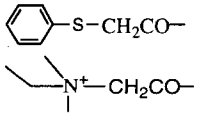
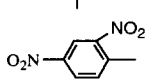
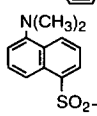


Scheme 3. Synthesis of **8** as key building block.



Scheme 4. Trisaccharide assembly.

Table 1. Evaluation of trisaccharides **6a–j** as inhibitors of GlcNAcT-V

Compound	R	Reagents used for amino derivatization	K_i (μM)
6a	H		297
6b	CH_3CO—	Ac_2O/MeOH	88
6c	C_6H_5CO—	i. C_6H_5COCl/Py ii. $NaOMe_3$/MeOH	36
6d	$Na^+ {}^-OOC$—CH_2CH_2—CO—	i. succinic anhydride/MeOH ii. $NaHCO_3$ aq	31
6e	ICH_2CO—	$(ICH_2CO)_2O$/MeOH	21
6f	CH_2=CH—CO—	CH_2=$CHCOCl$/DMF/$NaHCO_3$	45
6g		i. $(ICH_2CO)_2O$/MeOH ii. C_6H_5SH/$NaHCO_3$ aq	29
6h		i. $(ICH_2CO)_2O$/MeOH ii. $(CH_3)_2NC_2H_5$/MeOH	175
6i		2,4-dinitrofluobenzene/phosphote buffer	32
6j		Dansyl chloride/DMF/$NaHCO_3$ aq	145

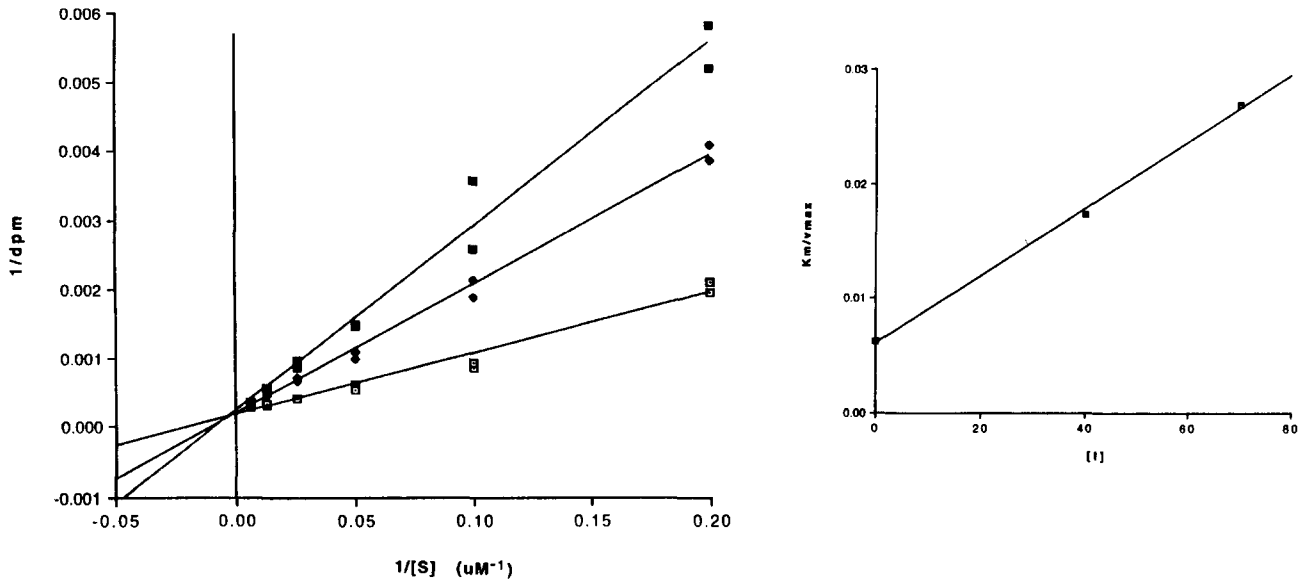


Figure 1. Compound **6e** is a competitive inhibitor for acceptor **5** binding to GlcNAcT-V. Left panel: Inhibitor concentrations were 0 (□), 40 μM (◆) and 70 μM (■); right panel: K_i for compound **6e** was 21 μM by linear regression analysis of K_m/V_{max} vs inhibitor plots.

H-2, H-6), 7.39–7.28 (m, 7H, Ar-H), 6.89 (d, 2H, J=8.9 Hz, PMB H-3, H-5), 5.81 (pentenyl H-4), 5.59 (s, 1H, PMBCH), 5.05 (pentenyl H_a-5), 4.98 (pentenyl H_b-5), 4.87 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 4.85, 4.70 (d, each 1H, J_{gem}=12 Hz, $PhCH_2$), 4.25 (dd, 1H, J_{gem} 16.5, $J_{5,6}$=6.0 Hz, H-6), 4.12–4.05 (m, 2H), 3.93 (dd, $J_{3,4}$=9.5, $J_{2,3}$=3.5 Hz, H-3), 3.87–3.78 (m, 3H), 3.71 (pentenyl H_a-1), 3.42 (pentenyl H_b-1), 2.65 (d, 1H, $J_{2,OH}$=1.5 Hz, OH-2), 2.12 (pentenyl H-3), 1.68 (pentenyl H-2); ^{13}C NMR (75.5 MHz): δ 160.05 (PMB C-4), 138.14 (benzyl C-1), 137.92 (pentenyl C-4), 130.15 (PMB C-1), 127.91, 127.83, 127.89 (benzyl methine), 115.09 (pentenyl C-5), 113.61 (PMB C-3), 101.59 (PMBCH), 100.03 (C-1), 78.91 (C-5), 75.83 (C-3), 73.11 benzyl, $PhCH_2$), 70.14 (C-2), 68.90 (pentenyl C-1), 67.21 (C-6), 63.37 (C-2), 55.33 (OCH_3), 30.27, 28.59 (pentenyl C-2/C-3). Anal. calcd for $C_{26}H_{32}O_7$ (456.54): C, 68.40; H, 7.07. Found: C, 67.92; H, 6.99.

4-Pentenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-*O*-(4-methoxybenzylidene)-α-D-mannopyranoside (13). To a soln of **12** (5.92 g, 13.0 mmol) in dry pyridine (50 ml) was added benzoyl chloride (3.84 ml, 33.76 mmol). The reaction mixture was stirred at r.t. overnight. The reaction mixture was poured into ice water (200 ml) and the aq soln was extracted with CH_2Cl_2. The CH_2Cl_2 soln was washed with water, dried ($MgSO_4$), and taken to dryness. Column chromatography (hexane:EtOAc 6:1) afforded **13** (5.92 g, 81.0%) as a colorless oil; $[\alpha]_D$ −35.9° (*c* 1.16; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.11 (dd, 2H, J=8.5, 1.5 Hz, benzoyl, H-2), 7.59 (dddd, 1H, J=8.0, 7.9, 1.5, 1.5 Hz, benzoyl, H-4), 7.50–7.22 (m, 9H, Ar-H), 6.90 (d, 2H, J=8.5 Hz, PMB H-3, H-5), 5.82 (pentenyl H-4), 5.67 (s, 1H, PMBCH), 5.61 (dd, 1H, $J_{2,3}$=2.9, $J_{1,2}$=1.5 Hz, H-2), 5.07 (pentenyl H_a-5), 5.02 (pentenyl H_b-5), 4.95 (d, 1H, $J_{1,2}$=1.5 Hz, H-1), 4.78, 4.72 (d, each 1H, J_{gem}=12.0 Hz, $PhCH_2$), 4.29 (dd, 1H, $J_{3,4}$=9.2 Hz, H-3), 4.21–4.12 (m, 2H), 3.99–3.85 (m, 2H), 3.82 (s, 3H, OMe), 3.75 (pentenyl H_a-1), 3.47 (pentenyl H_b-1), 2.17 (pentenyl H-3), 1.75 (pentenyl H-2); ^{13}C NMR (75.5 MHz): δ 165.83 (C=O), 160.03 (PMB C-4), 138.13 (benzyl, C-1), 137.82 (pentenyl C-4), 133.28 (benzoyl, C-4), 130.04 (PMB C-1), 129.94 (benzoyl C-4), 129.84 (benzoyl C-2, C-5), 128.43, 128.26, 122.58, 122.50, 122.44 (aromatic methine), 115.17 (pentenyl C-5), 113.54 (PMB C-3), 101.64 (PMBCH), 98.82 (C-1), 78.74 (C-5), 74.12 (C-3), 72.02 ($PhCH_2$), 70.46 (C-2), 68.87 (pentenyl C-1), 67.49 (C-6), 63.97 (C-4), 55.29 (OMe), 30.23, 28.54 (pentenyl C-2/C-3). Anal. calcd for $C_{33}H_{36}O_8$ (560.65): C, 70.70; H, 6.47. Found: C, 70.62; H, 6.39.

4-Pentenyl 2-*O*-benzoyl-3-*O*-benzyl-6-(4-methoxybenzyl)-α-D-mannopyranoside (14). A mixture of **13** (5.36 g, 9.56 mmol), sodium cyanoborohydride (6.01 g, 95.7 mmol), crushed molecular sieve 3 Å (10 g) and dry DMF (80 ml) was cooled to 0 °C. Trifluoroacetic acid (7.33 ml, 95.7 mmol) in DMF (60 ml) was then added dropwise. The mixture was stirred at 0 °C for 24 h, then allowed to warm to r.t. and stirred for another 24 h. After filtration of the reaction mixture through celite, the soln was diluted with CH_2Cl_2 and washed with saturated cold aq sodium bicarbonate and ice water. The organic layer was dried ($MgSO_4$) and concd. Chromatography of the residue on silica gel (4:1 hexane:EtOAc) afforded compound **14** (4.55 g, 85%) as a colorless oil; $[\alpha]_D$ −15.1° (*c* 1.97; $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.05 (dd, 2H, J=8.5, 1.5 Hz, benzoyl H-2, H-6), 7.55 (dddd, 1H, J=8.0, 7.9, 1.5, 1.5 Hz, benzoyl H-4), 7.40 (dd, 2H, J=8.5, 7.9 Hz, benzoyl H-3, H-5), 7.32–7.23 (m, 7H, Ar-H), 6.87 (d, 2H, J=8.5 Hz, PMB H-3, H-5), 5.82 (pentenyl H-4), 5.59 (d, 1H, $J_{2,3}$=3.2 Hz, $J_{1,2}$=2.0 Hz, H-2), 5.07 (pentenyl H_a-5), 5.02 (pentenyl H_b-5), 4.98 (d, 1H, H-1), 4.79 (d, 1H, J_{gem}=11.3 Hz, $PMBCH_2$), 4.62 (d, 1H, J_{gem}=11.5 Hz, $PhCH_2$), 4.52 (d, 1H, J_{gem}=11.3 Hz, $PMBCH_2$), 4.51 (d, 1H, J_{gem}=11.5 Hz, $PhCH_2$), 4.13

(dd, 1H, $J_{4,5}$ 10.0, $J_{3,4}$ 9.5 Hz, H-4), 3.91 (dd, 1H, H-3), 3.89–3.72 (pentenyl H_a-1), 3.82 (s, 3H, OMe), 3.50 (pentenyl H_b-1), 2.56 (d, 1H, J=2.0 Hz, OH-4), 2.15 (pentenyl H-3), 1.74 (pentenyl H-2); ^{13}C NMR (75.5 MHz): δ 165.82 (C=O), 159.16 (PMB C-4), 137.96 (pentenyl C-4), 137.75 (benzyl C-1), 133.18 (benzoyl C-4), 129.94, 129.12, 128.46, 128.40, 128.10, 127.88 (aromatic CH), 115.05 (pentenyl C-5), 113.77 (PMB C-3), 98.00 (C-1), 77.72 (C-5), 73.32 ($PMBCH_2$), 71.47 ($PhCH_2$), 71.42 (C-3), 69.73 (pentenyl C-1), 68.55 (C-2), 67.55 (C-4), 67.37 (C-6), 55.29 (OMe), 30.30, 28.62 (pentenyl C-2/C-3). Anal. calcd for $C_{33}H_{38}O_8$ (562.67): C, 70.44; H, 6.81. Found: C, 70.41; H, 6.82.

4-Pentenyl 2-*O*-benzoyl-3-*O*-benzyl-6-(4-methoxybenzyl)-4-*O*-methyl-α-D-mannopyranoside (15). To a soln of **14** (4.36 g, 7.75 mmol) in dry DMF (110 ml) at −18 °C, methyl iodide (730 μl, 11.73 mmol) was added dropwise. Sodium hydride (60% dispersion in oil, 626 mg, 15.64 mmol) was then added. The reaction mixture was stirred at −15 °C for 3 h. Acetic acid was added to quench the reaction (until pH <7). The reaction mixture was concd in vacuo and the residue was extracted with CH_2Cl_2. After solvent evapn, the residue was purified by chromatography (hexane:EtOAc 6:1) yielded **15** (4.04 g, 90%) as a colorless oil; $[\alpha]_D$ −16.7° (*c* 1.26; $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.07 (dd, 2H, J=8.5, 1.5 Hz, benzoyl, H-2), 7.56 (ddd, 1H, J=8.0, 7.9, 1.5, 1.5 Hz, benzoyl, H-4), 7.42–7.20 (m, 9H, Ar-H), 6.89 (d, 2H, J=8.5 Hz, PMB, H-3, H-5), 5.83 (pentenyl H-4), 5.58 (dd, 1H, $J_{2,3}$=3.1, $J_{1,2}$=2.0 Hz, H-2), 5.07 (pentenyl H_a-5), 5.02 (pentenyl H_b-5), 4.96 (d, 1H, H-1), 4.80, 4.54 (d, each 1H, J_{gem}=11.3 Hz, $PMBCH_2$), 4.70, 4.60 (d, each 1H, J_{gem}=11.5 Hz, $PhCH_2$), 4.00 (dd, 1H, $J_{3,4}$=9.5 Hz, H-3), 3.90–3.70 (m, 5H, H-4, H-5, H-6, pentenyl H_a-1), 3.82 (s, 3H, PMBOMe), 3.57 (s, 3H, OMe-4), 3.48 (pentenyl H_b-1), 2.15 (pentenyl H-3), 1.70 (pentenyl H-2); ^{13}C NMR (75.5 MHz): δ 165.83 (C=O), 159.13 (PMB C-4), 138.24 (benzyl C-1), 137.99 (pentenyl C-4), 133.07 (benzoyl C-4), 130.74 (PMB C-1), 130.03 (benzoyl C-1), 129.99, 129.12, 128.38, 128.88, 127.55 (aromatic CH), 78.14 (C-5), 76.19 (C-4), 73.18 ($PMBCH_2$), 71.73 (C-3), 71.56 ($PhCH_2$), 69.26 (C-2), 68.98 (pentenyl C-1), 67.29 (C-6), 61.02 (C_4-OMe), 55.33 (PMB-OMe), 30.30, 28.64 (pentenyl C-2/C-3); Anal. calcd for $C_{34}H_{40}O_8$ (576.69): C, 70.81; H, 6.99. Found: C, 71.11; H, 7.07.

4-Pentenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-methyl-α-D-manno-pyranoside (16). To a soln of **15** (3.96 g, 6.86 mmol) in CH_2Cl_2:H_2O (19:1, 150 ml) was added a solution of DDQ (1.87 g, 8.24 mmol) in CH_2Cl_2:H_2O (19:1). The resulting mixture was stirred at r.t. under argon for 5 h. The reaction mixture was filtered through celite and the filtrate concd to dryness. The residue was then purified by silica gel flash chromatography, eluting first with CH_2Cl_2 to remove the anisaldehyde, then with CH_2Cl_2:MeOH (99:1) to yield the desired product (2.72 g, yield 87%) as a colorless oil; $[\alpha]_D$ −0.80° (*c* 1.88; $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.05 (dd, 2H, J=8.5, 1.5 Hz, benzoyl H-2, H-6), 7.60 (dddd, 1H, J=8.0, 7.9, 1.5, 1.5 Hz, benzoyl H-4, 7.48 (dd, 2H, J=8.5, 8.0 Hz, benzoyl, H-3, H-5), 7.30 (m, 5H, benzyl Ar-H), 5.82 (pentenyl H-4), 5.55 (dd, 1H, $J_{1,2}$=1.9 Hz, $J_{2,3}$=3.2 Hz, H-2), 5.05 (pentenyl H_a-5), 5.01 (pentenyl H_b-5), 4.90 (d, 1H, H-1), 4.78, 4.60 (d, each 1H, J_{gem}=12.0 Hz, $PhCH_2$), 4.00 (dd, 1H, $J_{3,4}$=8.5 Hz, H-3), 3.90–3.80 (m, 2H, H-5, H-6), 3.75–3.65 (m, 3H), 3.45 (pentenyl H_a-1), 3.61 (s, 3H, OMe), 2.14 (pentenyl H-3), 2.04 (dd, 1H, $J_{6a,OH}$=8.1, $J_{6b,OH}$=5.5 Hz, OH-6), 1.71 (H-2); ^{13}C NMR (75.5 MHz): δ 165.78 (C=O), 138.14 (benzyl C-1), 137.89 (pentenyl C-4), 133.27 (benzoyl C-4), 129.89, 128.50, 128.30, 127.84, 127.59 (aromatic CH), 115.17 (pentenyl C-5), 97.86 (C-1), 77.94 (C-5), 78.28 (C-4), 71.87(C-3), 71.59 ($PhCH_2$), 69.34 (C-2), 67.36 (pentenyl C-1), 62.20 (C-6), 61.15 (C_4-OMe), 30.25, 28.59 (pentenyl C-2/C-3); Anal. calcd for $C_{26}H_{32}O_7$ (456.54): C, 68.40; H, 7.07. Found: C, 68.37; H, 7.06.

4-Pentenyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-methyl-6-*O*-tosyl-α-D-mannopyranoside (17). To a soln of **16** in dry pyridine (60 ml), *p*-toluenesulfonyl chloride (5.515 g, 29.94 mmol) was added. The reaction mixture was stirred at r.t. for 7 h, then poured into cold 5% $NaHCO_3$ (200 ml) and extracted with CH_2Cl_2. The CH_2Cl_2 soln was washed by 0.5% aq. HCl and water, then dried by ($MgSO_4$). Concn afforded the desired product (1.767 g, yield 100%) as a light yellowish oil. ^{1}H NMR ($CDCl_3$): δ 8.05 (dd, 2H, J=8.5, 1.5 Hz, benzoyl H-2, H-6), 7.87 (d, 2H, J=8.0 Hz, tosyl H-2, H-6), 7.59 (dddd, 1H, J=8.0, 7.9, 1.5, 1.5 Hz, benzoyl H-4), 7.48 (dd, 2H, J=8.5, 8.0 Hz, benzoyl H-3, H-5), 7.28 (m, 8H, Ar-H), 5.79 (pentenyl H-4, 5.50 (dd, 1H, $J_{1,2}$=1.9, $J_{2,3}$=3.2 Hz, H-2), 5.05 (pentenyl H_a-5), 4.98 (H_b-5), 4.88 (d, 1H, H-1), 4.75, 4.55 (d, each 1H, J_{gem}=11.5 Hz, $PhCH_2$), 4.39 (dd, 1H, J_{gem}=10.5, $J_{5,6}$ 4.0 Hz, H-6a), 4.28 (dd, J_{gem}=10.5, $J_{5,6b}$=1.9 Hz, H-6b), 3.95 (dd, 1H, $J_{3,4}$=8.5 Hz, H-3), 3.75 (ddd, 1H, $J_{4,5}$=9.5, $J_{5,6}$=4.0, 1.9 Hz, H-5), 3.62 (pentenyl H_a-1), 3.61 (dd, 1H, H-4), 3.51 (s, 3H, OMe), 3.38 (pentenyl H_b-1), 2.09 (pentenyl H-3), 1.68 (pentenyl H-2).

4-Pentenyl 6-azido-2-*O*-benzoyl-3-*O*-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranoside (18). To a soln of **17** (1.767 g, 2.89 mmol) in dry DMF (50 ml) was added sodium azide (1.88 g, 28.94 mmol). The resulting mixture was stirred at r.t. for 12 h, then at 40 °C for 20 h. Concentration left a residue that was extracted with CH_2Cl_2. The CH_2Cl_2 soln was washed with water, dried ($MgSO_4$) and concd. The residue was purified by chromatography, eluting with hexane:ethyl acetate (9:1) to give **18** (1.26 g, yield 90%) as a colorless oil; $[\alpha]_D$ +53.1° (*c* 0.24; $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.08 (dd, 2H, J=8.5, 1.5 Hz, benzoyl H-2, H-6), 7.59 (dddd, 1H J=8.0, 7.9, 1.5, 1.5 Hz, benzoyl H-4, 7.47 (dddd, 2H, J=8.5, 8.0, 1.5, 1.5 Hz, benzoyl H-3, H-5), 7.35–7.21 (m, 5H, Ar-H), 5.82 (pentenyl H-4), 5.58 (dd, 1H, $J_{1,2}$=1.9, $J_{2,3}$=3.2 Hz, H-2), 5.05 (pentenyl H_a-5), 5.00 (pentenyl H_b-5), 4.89 (d, 1H, H-1), 4.78, 4.57 (d, each 1H, J_{gem}=11.5 Hz, $PhCH_2$), 3.98 (dd, 1H, $J_{3,4}$=8.8 Hz, H-3), 3.78 (ddd, 1H, $J_{4,5}$=9.5, $J_{5,6b}$=5.5, $J_{5,6a}$=2.8 Hz, H-5), 3.74 (pentenyl

H_a-1), 3.59 (dd, 1H, H-4), 3.60 (dd, J_{gem} = 13.0 Hz, H-6_a), 3.58 (s, 3H, OMe), 3.49 (pentenyl H_b-1), 3.47 (dd, 1H, J_{gem} = 13.0 Hz, H-6_b), 2.15 (pentenyl H-3), 1.75 (pentenyl H-2); ^{13}C NMR (75.5 MHz): δ 165.78 (C=O), 138.82 (benzyl C-1), 137.89 (pentenyl C-4), 133.25 (benzyl C-4), 129.94, 128.47, 128.32, 127.91, 127.64 (Aromatic CH), 115.12 (pentenyl C-5), 97.80 (C-1), 77.92 (C-5), 76.90 (C-4), 71.54 ($PhCH_2$), 71.46 (C-3), 69.04 (C-2), 67.51 (pentenyl C-1), 61.18 (OMe), 51.53 (C-6), 30.27, 28.59 (pentenyl C-2/C-3); IR ($CHCl_3$): 2099 cm^{-1} (N_3), 1725 cm^{-1} (ester); Anal. calcd for $C_{26}H_{31}N_3O_6$ (481.55): C, 64.85; H, 6.44; N, 8.73. Found: C, 64.67; H, 6.58; N, 8.66.

Octyl 6-azido-2-*O*-benzoyl-3-*O*-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (19). A soln of **18** (482 mg, 1.00 mmol) and **7** (620 mg, 1.10 mmol) in toluene was dried by concentrating in vacuo. The residue was dissolved with anhydrous CH_2Cl_2 and was cooled to 0 °C. *N*-Iodosuccinimide (270 mg, 1.202 mmol) and AgOTf (6.0 mg, 22.5 μmol) were added to the soln. After stirring at r.t. for 20 h, the reaction mixture was dild with CH_2Cl_2, and then washed sequentially with 10% $Na_2S_2O_3$, satd sodium bicarbonate and brine. The organic phase was dried ($MgSO_4$), filtered and concd. The residue was purified by chromatography using hexane:ethyl acetate (9:1) as eluant to provide the title compound as a colorless oil (455 mg, 48%); $[\alpha]_D$ +39.27° (*c* 0.42; $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.08 (dd, 2H, *J* = 8.5, 1.5 Hz, benzoyl H-2, H-6), 7.59 (dddd, 1H, *J* = 8.0, 7.9, 1.5, 1.5 Hz, benzoyl H-4), 7.47 (dddd, 2H, *J* = 8.5, 8.0, 1.5, 1.5 Hz, benzoyl H-3, H-5), 7.38–7.18 (m, 20H, benzyl Ar-H), 5.65 (dd, 1H, $J_{1',2'}$ = 1.9 Hz, $J_{2',3'}$ = 3.2 Hz, H-2'), 4.97 (d, 1H, J_{gem} = 9.5 Hz, $PhCH_2$), 4.96 (d, 2H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.88 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.78 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.75 (d, 1H, H-1'), 4.73 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.55 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.49 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.38 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 3.92 (dd, 1H, $J_{3',4'}$ = 8.8 Hz, H-3'), 3.36 (dd, 1H, J_{gem} = 13.0, $J_{5',6'}$ = 5.0 Hz, H-6'), 3.52 (s, 3H, OMe), 1.62 (p, 2H, J_{vic} = 7.0 Hz, octyl CH_2), 0.84 (t, 3H, J_{vic} = 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 165.59 (benzoyl C=O), 138.62 (benzoyl, C-1), 138.56, 138.07, 137.97 (benzyl C-1), 133.21 (benzoyl C-4), 129.97 (benzoyl C-2, C-6), 128.48, 128.42, 128.33, 128.19, 128.00, 127.94, 127.80, 127.71, 127.66 (aromatic CH), 103.61 ($J_{C\text{-}1,H\text{-}1}$ = 161.5 Hz, C-1), 97.93 ($J_{C\text{-}1',H\text{-}1'}$ = 170.9 Hz, C-1'), 70.17 (octyl C_1), 66.57 (C-6), 61.06 (OCH_3), 51.37 (C-6'), 31.88, 29.85, 29.48, 29.31, 26.21, 22.71 (octyl C_2–C_6), 14.13 (octyl CH_3); Anal. calcd for $C_{56}H_{67}O_{11}N_3$ (958.17): C, 70.20; H, 7.05; N, 4.39. Found: C, 70.30; H, 6.90; N, 4.39.

Octyl 6-azido-3-*O*-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (20). Compound **19** (427 mg, 0.446 mmol) was treated with methanolic NaOMe (0.05 N, 25 ml) at r.t. for 36 h. Neutralization with Amberlite IR-120 (H^+) resin, removal of the resin by filtration and solvent evapn left a residue that was purified by column chromatography using hexane:EtOAc (4:1) as eluant to provide **20** as a white amorphous powder (365 mg, 96%). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.40–7.22 (m, 20H, Ar-H), 4.98 (d, 1H, J_{gem} = 10.4 Hz, $PhCH_2$), 4.97 (d, 2H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.80 (d, 1H, J_{gem} = 10.6 Hz, $PhCH_2$), 4.75 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.73 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.71 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 4.56 (d, 1H, J_{gem} = 10.6 Hz, $PhCH_2$), 4.38 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.08 (ddd, 1H, $J_{2',3'}$ 3.2 Hz, H-2'), 3.51 (s, 3H, OMe), 2.35 (d, 1H, $J_{2',OH}$ = 2.0 Hz, OH-2', 0.86 (t, 3H, J_{vic} = 7.0 Hz, octyl OCH_3).

Octyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-6-azido-3-*O*-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (21). A mixture of **20** (136 mg, 0.159 mmol), AgOTf (102 mg, 0.398 mmol), molecular sieve 4 Å (350 mg), collidine (19.0 μl, 0.143 mmol) and dry CH_2Cl_2 (2 ml) was cooled to −40 °C. To the resulting mixture was added dropwise a soln of bromide **9** (159 mg, 0.318 mmol) in dry CH_2Cl_2 (1 ml) at −40 °C under argon. The mixture was allowed to warm to r.t. over 1 h. After stirring for 3 h, excess tetraethylammonium chloride was added, and the mixture was stirred for another 30 min. The mixture was then dild with CH_2Cl_2 and filtered through celite. The filtrate was washed sequentially with 0.5% HCl, satd $NaHCO_3$ and water, then dried ($MgSO_4$), and concd. The residue was purified by column chromatography using hexane:EtOAc (2:1) as eluant to provide compound **21** as white crystals (148 mg, 73%). $[\alpha]_D$ +13.2° (*c* 0.38; $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.83 (dd, 2H, *J* = 5.1, 2.9 Hz, phthalimido Ar-H), 7.71 (dd, 2H, *J* = 5.1, 2.9 Hz, phthalimido Ar-H), 7.39–7.12 (m, 20H, Ar-H), 5.83 (dd, 1H, $J_{2'',3''}$ = 10.5 Hz, $J_{3'',4''}$ = 9.5 Hz, H-3''), 5.48 (d, 1H, $J_{1'',2''}$ = 8.3 Hz, H-1''), 5.20 (dd, $J_{4'',5''}$ = 9.8 Hz, H-4''), 4.98 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.95 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.77 (2d, each 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.77 (2d, each 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.72 (2d, each 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.64 (d, 1H, $J_{1',2'}$ = 1.8 Hz, H-1'), 4.50 (d, 1H, J_{gem} = 11.5 Hz, $PhCH_2$), 4.46 (dd, 1H, J_{gem} = 12.8 Hz, $J_{6'',5''}$ = 2.8 Hz, H-6''), 4.45 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.44 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.75 (dd, 1H, $J_{3',4'}$ = 11.0, $J_{2',3'}$ = 3.8 Hz, H-3'), 3.34 (s, 3H, OMe), 2.06, 2.04, 1.89 (3s, each 3H, CH_3CO), 0.88 (t, 3H, J_{vic} = 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 170.71, 170.15, 169.45 (C=O), 138.58, 138.45, 138.05, 137.96 (benzyl C-1), 103.92 (C-1), 97.51 (C-1'), 96.24 (C-1''), 70.73 (octyl C_1), 66.75 (C-6), 62.39 (C-6''), 60.85 (OCH_3), 54.38 (C-2''), 51.41 (C-6'), 31.89, 29.86, 29.54, 29.33, 26.23, 22.70 (octyl C_2-C_6), 20.80, 20.67, 20.53 (CH_3CO), 14.12 (octyl CH_3); Anal. calcd for $C_{69}H_{82}O_{19}N_4$ (1271.44): C, 65.18; H, 6.50; N, 4.41. Found: C, 65.19; H, 6.48; N, 4.40.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-azido-3-*O*-benzyl-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (22). A soln of **21** (140 mg, 0.11 mmol) in butanol (20 ml) was added to ethylenediamine (1 ml). The mixture was stirred at 70 °C for 14 h. Concn was

followed by addition and evaporation twice of toluene followed by methanol. The residue was not characterized but was dissolved in dry methanol (3 ml) to which acetic anhydride (1 ml) and triethylamine (0.1 ml) were added. After stirring 12 h at r.t., water (1 ml) was added, and the soln was concd. The residue was purified on Iatrobeads using CH_2Cl_2:MeOH (10:1) as eluant, to give **22** as a colorless syrup (85 mg, 73%). $[\alpha]_D$ +6.1° (*c* 0.15; $CHCl_3$); ^1H NMR ($CDCl_3$, 360 MHz): δ 7.42–7.16 (m, 20H, Ar-H), 6.28 (br s, 1H, NHAc), 4.96 (2d, each 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.87 (2d, each 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.79 (2d, each 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.72 (d, 1H, $J_{1',2'}$ = 2.0 Hz, H-1'), 4.63 (d, 1H, J_{gem} = 11.0 Hz, $PhCH_2$), 4.55 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1''), 4.43 (d, 1H, J_{gem} = 10.5 Hz, $PhCH_2$), 4.36 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.12 (dd, 1H, $J_{2',3'}$ = 3.5 Hz, H-2'), 3.53 (s, 3H, OMe), 1.95 (s, 3H, NHAc), 0.84 (t, 3H, J_{vic} = 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ 138.49, 138.39, 138.09, 137.64 (benzyl C-1), 128.61, 128.45, 128.31, 127.99, 127.86, 127.77, 127.68 (aromatic CH), 103.90 (C-1), 99.29 (C-1''), 98.03 (C-1'), 75.82, 74.95, 74.89, 71.92 ($PhCH_2$), 70.74 (octyl C_1), 66.49 (C-6), 62.52 (C-6''), 60.85 (CH_3), 58.82 (C-2''), 51.48 (C-6'), 31.87, 29.82, 29.48, 29.30, 26.18, 22.20 (octyl C_2–C_7), 23.64 ($COCH_3$), 14.13 (octyl CH_3); Anal. calcd for $C_{57}H_{76}O_{15}N_4$ (1057.26): C, 64.76; H, 7.25; N, 5.30. Found: C, 64.41; H, 7.45; N, 5.16.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-amino-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6a). Compound **22** (98 mg, 92.7 mmol) was dissolved in 95% EtOH (40 ml) containing 5% palladium on charcoal (300 mg), then 0.01 N HCl (14 ml) was added. The mixture was stirred under one atmosphere of H_2 for 56 h. Removal of the catalyst by filtration followed by evapn left a glass which showed a single spot on TLC. This material was adsorbed on to a Sep-Pak C18 cartridge in water, the cartridge was washed with water (25 ml), and the product eluted with HPLC grade methanol (20 ml). Evapn of the methanol, dissolution of the residue in water, filtration through a Millex filter and lyophilization gave **6a** (56.6 mg, 91%) as a white powder; $[\alpha]_D$ −7.2° (c 0.66, H_2O); ^1H NMR (D_2O, 500 MHz): δ 4.894 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 4.557 (d, 1H, $J_{1'',2''}$ = .5 Hz, H-1''), 4.451 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.099 (dd, 1H, $J_{2',3'}$ = 3.0 Hz, H-2'), 3.922 (dd, 1H, $J_{3',4'}$ = 8.5 Hz, H-3'), 3.867 (ddd, 1H, J_{gem} = 10.0 Hz, J_{vic} = 7.0 Hz, octyl H_a-1), 3.704 (dd, 1H, $J_{2'',3''}$ = 9.6 Hz, H-2''), 3.686 (dd, 1H, $J_{3'',4''}$ = 9.6, $J_{4'',5''}$ = 9.6 Hz, H-4''), 3.678 (ddd, 1H, octyl H_b-1), 3.592 (ddd, 1H, $J_{5'',6''a}$ = 5.6, $J_{5'',6''b}$ = 2.5 Hz, H-5''), 3.548 (dd, 1H, $J_{2'',3''}$ = 9.6 Hz, H-3''), 3.525 (s, 3H, OCH_3), 3.477 (dd, 1H, $J_{2,3}$, $J_{3,4}$ = 8.6 Hz, H-3), 3.430 (dd, 1H, J_{gem} = 11.0, $J_{5,6'a}$ = 3.0 Hz, H-6'a), 3.279 (dd, 1H, $J_{4',5'}$ = 9.6 Hz, H-4'), 3.240 (dd, 1H, $J_{2,3}$ = 8.0 Hz, H-2), 3.080 (dd, 1H, J_{gem} = 11.0, $J_{5',6'b}$ = 8.6 Hz, H-6'b), 2.050 (s, 3H, $COCH_3$), 1.615 (p, 2H, J_{vic} = 7.0 Hz, octyl H-2), 1.38–1.24 (m 12H, octyl CH_2), 0.860 (t, 3H, J_{vic} = 7.0 Hz, octyl CH_3); ^{13}C NMR (75.5 MHz): δ175.43 (C=O), 103.23 (C-1, $J_{C\text{-}1,H\text{-}1}$ = 161.7 Hz), 100.93 (C-1'', $J_{C\text{-}1'',H\text{-}1''}$ = 161.6 Hz), 97.98 (C-1, $J_{C\text{-}1',H\text{-}1'}$ = 172.4 Hz), 79.32 (C-4'), 77.91 (C-2'), 76.76 (C-3), 76.66 (C-5''), 74.88 (C-5), 74.16 (C-3''), 73.97 (C-2), 71.82 (octyl C_1), 70.83 (C-4), 70.43 (C-3'), 70.23 (C-4''), 68.82 (C-5'), 66.99 (C-6), 61.39 (C-6''), 61.29 (OCH_3), 56.27 (C-2''), 41.70 (C-6'), 31.93, 29.62, 29.27, 29.22, 25.87, 22.86 (octyl C_2–C_7), 23.18 (CH_3CO), 14.28 (octyl CH_3); FAB-MS: *m/z* 693 $(M+Na)^+$, 671 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-acetamido-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6b). To a solution of **6a** (1.4 mg, 1.96 μmol) in dry methanol (0.5 ml) was added acetic anhydride (0.5 ml) at room temperature. The reaction mixture was stirred for 14 h, solvents were removed and the resulting residue purified as described for the preparation of **6a** to give **6b** (1.5 mg, 100%). ^1H NMR (D_2O, 400 MHz): δ 4.83 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 4.54 (d, 1H, $J_{1'',2''}$ = 8.5 Hz, H-1''), 4.45 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.07 (dd, 1H, $J_{2',3'}$ = 2.3 Hz, H-2'), 3.51 (s, 3H, OCH_3), 3.26 (dd, 1H, $J_{4',5'}$, $J_{3',4'}$ = 9.6 Hz, H-4'), 3.24 (dd, 1H, $J_{2,3}$ = 8.0 Hz, H-2), 2.06, 2.04 (s, each 3H, NHAc), 0.86 (t, 3H, J_{vic} = 7.0 Hz, octyl CH_3); FAB-MS: *m/z* 735 $(M+Na)^+$, 713 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-benzamido-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6c). To a soln of **6a** (1.3 mg, 1.94 μmol) in dry pyridine (0.5 ml) was added benzoyl chloride (4.5 μl, 38.8 μmol). The reaction mixture was stirred at r.t. for 24 h, and poured into ice water. The aq soln was extracted with CH_2Cl_2. The CH_2Cl_2 soln was washed with 1% HCl, satd $NaHCO_3$ and water, then dried ($MgSO_4$), filtered, and concd. The residue was purified by column chromatography using CH_2Cl_2:MeOH (20:1) as eluant, to provide perbenzoylated product which was treated with 0.05 N NaOMe/MeOH for 24 h. Neutralization with Amberlite IR-120 (H^+) resin, filtration of the resin, and evapn left a residue which was purified as described for the preparation of **6a** to give **6c** (1.0 mg 62%). ^1H NMR (D_2O, 360 MHz): δ 7.78 (dd, 2H, *J* = 8.0, 1.5 Hz, benzoyl H-2, H-6), 7.65 (ddd, 1H, *J* = 8.0, 7.9, 1.5 Hz, benzoyl H-4), 7.57 (dd, 2H, *J* = 8.0, 7.9 Hz, benzoyl H-3, H-5), 4.89 (d, 1H, $J_{1',2'}$ = 1.5 Hz, H-1'), 4.59 (d, 1H, $J_{1'',2''}$ = 8.5 Hz, H-1''), 4.40 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 4.11 (dd, $J_{2',3'}$ = 3.2 Hz, H-2'), 3.60 (s, 3H, OCH_3), 3.17 (dd, 1H, $J_{1,2}$, $J_{2,3}$ = 8.0 Hz, H-2), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic} = 7.0 Hz, octyl CH_3); FAB-MS: *m/z* 797.6 $(M+Na)^+$, 775.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-4-*O*-methyl-6-succinamido-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6d). A mixture of **6a** (1.1 mg, 1.65 μmol) and succinic anhydride (4.4 mg, 43.8 μmol) in dry methanol (0.5 ml) was stirred at r.t. for 3 h and was evapd to dryness. The product was purified by chromatography on Iatrobeads, eluting first with CH_2Cl_2:MeOH (9:1) to remove excess succinic anhydride, then with $CHCl_3$:MeOH:H_2O (65:35:8). The product thus obtained was purified as described for the preparation of **6a** to provide the title compound (1.0 mg, 79%). ^1H NMR (D_2O, 360 MHz): δ 4.88 (d, 1H, $J_{1',2'}$ 1.5 Hz, H-1'), 4.56 (d, 1H, $J_{1'',2''}$ = 8.5 Hz,

H-1″), 4.45 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.08 (dd, 1H, $J_{2',3'}$=3.2 Hz, H-2′), 3.55 (s, 3H, OCH_3), 2.65 (m, 4H, $NHCOCH_2CH_2COOH$), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic}=7.0 Hz, octyl CH_3); FAB-MS: *m/z* 793.6 $(M+Na)^+$, 771.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-6-iodoacetamido-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6e). A mixture of **6a** (8.1 mg, 12.1 μmol) and iodoacetic anhydride (73 mg, 206 μmol) in dry methanol (1 ml) was stirred at r.t. for 24 h and concd. The residue was dissolved in milli-Q water (1 ml) and purified as described for the preparation of **6a**. Lyophilization afforded the desired product (6.3 mg. 62%). ^{1}H NMR (D_2O, 400 MHz): δ 4.81 (d, 1H, $J_{1',2'}$=1.5 Hz, H-1′), 4.54 (d, 1H, $J_{1'',2''}$=8.4 Hz, H-1″), 4.44 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.06 (dd, 1H, $J_{2',3'}$=3.2 Hz, H-2′), 3.52 (s, 3H, OCH_3), 3.28 (dd, 1H, $J_{4',5'}$, $J_{3',4}$=9.7 Hz, H-4′), 3.25 (dd, 1H, $J_{2,3}$=8.3 Hz, H-2), 2.08 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic}=7.0 Hz, octyl CH_3); ^{13}C NMR (100 MHz): δ 175.73 ($NH\underline{C}OCH_3$), 172.69 ($\underline{C}OCH_2I$), 103.10 (C-1), 101.17 (*C-1″*), *97.63* (*C-1′*), *79.11*, *78.16*, *76.63*, *75.03*, *74.18*, 73.92, 71.70 (octyl C_1), 70.64, 70.51, 70.44, 67.00 (C-6), 61.44 (C-6″), 61.36 (OCH_3), 56.29 (C-2″), 41.57 (C-6′), 31.93, 29.65, 29.30, 29.24, 25.93, 22.87 (octyl CH_2), 23.33 ($NHCO\underline{C}H_3$), 14.26 (octyl CH_3), −1.82 (ICH_2); FAB-MS: *m/z* 839.4 $(M+H)^+$, 712.6 $(M-I+H)^+$, 711.6 $(M-I)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-acrylamido-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6f). To a soln of **6a** (1.2 mg, 1.8 μmol) in a mixture of satd $NaHCO_3$ and 95% EtOH (1:1, 1 ml, $NaHCO_3$ precipitate removed) was added acryloyl chloride (1.4 μl, 18 μmol). The mixture was stirred at r.t. for 2 h and concd. The residue was purified as described for the preparation of **6a** to afforded **6f** (1.3 mg, 99%). ^{1}H NMR (D_2O, 360 MHz): δ 6.40 (dd, 1H, J_{trans}=17.0, J_{cis}=10.5 Hz, $COC\underline{H}=CH_2$), 6.23 (d, 1H, *J*=17.0 Hz, acryloyl H_a-3), 5.80 (d, 1H, *J*=10.5 Hz, acryloyl H_b-3), 4.85 (d, 1H, $J_{1',2'}$=1.5 Hz, H-1′), 4.57 (d, 1H, $J_{1'',2''}$=8.4 Hz, H-1″), 4.45 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.08 (dd, 1H, $J_{2',3'}$=3.2 Hz, H-2′), 3.55 (s, 3H, OCH_3), 3.31 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$=9.7 Hz, H-4′), 3.26 (dd, 1H, $J_{2,3}$=8.1 Hz, H-2), 2.09 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic}=7.0 Hz, octyl CH_3), FAB-MS: *m/z* 747.4 $(M+Na)^+$, 725.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-4-*O*-methyl-6-thiophenylacetamido-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6g). To a soln of **6e** (0.78 mg, 0.93 μmol) in 5% $NaHCO_3$ (0.5 ml), a soln of thiophenol (0.19 μl, 1.86 μmol) in 95% EtOH (0.1 ml) was added. The mixture was stirred at r.t. for 1 h and taken to dryness. The residue was purified as described for the prepn of **6a** to give **6g** (0.6 mg, 79%). ^{1}H NMR (D_2O, 360 MHz): δ 7.48–7.30 (m, 5H, Ar-H), 4.79 (d, 1H, $J_{1',2'}$=1.5 Hz, H-1′), 4.52 (d, 1H, $J_{1',2'}$=8.4 Hz, H-1′), 4.41 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.05 (d, 1H, $J_{2',3'}$=3.2 Hz, H-2′), 3.45 (s, 3H, OCH_3), 3.25 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$=9.7 Hz, H-4′), 3.22 (dd, 1H, $J_{2,3}$=8.1 Hz, H-2), 2.04 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic}=7.0 Hz, octyl CH_3); FAB-MS: *m/z* 843.5 $(M+Na)^+$, 821.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-6-(ethyldimethylaminoacetamido)-4-*O*-methyl-α-D-mannopyranosyl]-(1→6)-β-D-glucopyranoside iodide (6h). To a soln of **6e** (0.68 mg, 0.811 μmol) in dry methanol (0.5 ml) was added dimethyl ethylamine (0.2 ml). The reaction mixture was stirred at r.t. for 1 h and taken to dryness. The residue was purified as described for the prepn of **6a** to give **6h** (0.6 mg, 81%). ^{1}H NMR (D_2O, 360 MHz): δ 4.89 (d, 1H, $J_{1',2'}$=1.0 Hz, H-1′), 4.52 (d, 1H, $J_{1'',2''}$=8.4 Hz, H-1″), 4.42 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.16 (dd, $J_{2',3'}$=3.2 Hz, H-2′), 3.36 (s, 3H, OCH_3), 3.25 (s, 6H, $N(C\underline{H}_3)_2C_2H_5$), 2.05 (s, 3H, NHAc), 1.39 (t, 3H, J_{vic}=7.0 Hz, $N(CH_3)_2CH_2C\underline{H}_3$), 0.86 (t, 3H, J_{vic}=7.0 Hz, octyl CH_3); FAB-MS: *m/z* 934.4 $(M+Na)^+$, 785.7 $(M-I+H)^+$, 785.7 $(M-I+H)^+$, 784.7 $(M-I)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-deoxy-6-(2,4-dinitrophenylamino)-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6i). To a soln of **6a** (0.95 mg, 1.42 μmol) in phosphate buffer (0.8 ml) was added 2,4-dinitrofluorobenzene (0.36 μl, 2.84 μmol). The reaction mixture was stirred at r.t. for 4 h and taken to dryness. The residue was purified by prep. TLC which was developed using $CHCl_3$:MeOH:H_2O (65:35:8). The product was further purified as described for the preparation of **6a** to give **6i** (0.7 mg, 59%). ^{1}H NMR (D_2O, 400 MHz): δ 9.18 (dd, 1H, $J_{3,5}$=1.5, $J_{3,6}$=1.0 Hz, DNP H-3), 8.38 (dd, 1H, $J_{5,6}$=9.8 Hz, DNP H-5), 7.26 (dd, 1H, DNP H-6), 4.95 (d, 1H, $J_{1',2'}$=1.0 Hz, H-1′), 4.60 (d, 1H, $J_{1'',2''}$=8.5 Hz, H-1″), 4.38 (d,1H, $J_{1,2}$=8.0 Hz, H-1), 4.14 (dd, 1H, $J_{2',3'}$=3.2 Hz, H-2′), 3.64 (s, 3H, OCH_3), 3.34 (dd, 1H, $J_{3',4'}$, $J_{4',5'}$=9.7 Hz, H-4′), 3.18 (dd, 1H, $J_{2,3}$=8.0 Hz, H-2), 2.01 (s, 3H, NHAc), 0.86 (t, 3H, J_{vic}=7.0 Hz, octyl CH_3); FAB-MS: *m/z* 859.5 $(M+Na)^+$, 837.6 $(M+H)^+$.

Octyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-6-dansylamido-6-deoxy-4-*O*-methyl-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (6j). To a soln of **6a** (1.49 mg, 2.22 μmol) in 0.01 M $NaHCO_3$ (0.5 ml), a soln of dansyl chloride (0.9 mg, 3.33 μmol) in DMF (0.5 ml) was added. The reaction mixture was stirred at r.t. for 4 h and taken to dryness. The residue was purified by prep. TLC which was developed using $CHCl_3$:MeOH (7:3). The product was further purified as described for the prepn of **6a** to give **6j** (1.1 mg. 55%). ^{1}H NMR (D_2O, 400 MHz): δ 8.50 (dd, 1H, $J_{2,3}$=8.0, $J_{2,4}$=1.0 Hz, dansyl H-2), 8.49 (dd, 1H, $J_{3,4}$=8.0 Hz, dansyl H-4), 8.40 (dd, 1H, $J_{7,8}$=7.0, $J_{6,8}$=1.0 Hz, dansyl C_8-H), 7.72 (dd, 1H, dansyl H-3), 7.71 (dd, 1H, $J_{6,7}$=7.0 Hz, dansyl H-7), 7.40, (dd, 1H, dansyl H-6), 4.98 (d, 1H, $J_{1',2'}$=1.5 Hz, H-1′), 4.45 (d, 1H, $J_{1'',2''}$=8.4 Hz, H-1″), 4.34 (d, 1H, $J_{1,2}$=8.0 Hz, H-1), 4.09 (dd, $J_{2',3'}$=3.2 Hz, H-2′), 3.50 (s, 3H, OCH_3), 2.85 (s, 6H, $N(CH_3)_2$), 2.04 (s, 3H, NHAc), 0.86 (t, 3H,

J_{vic} = 7.0 Hz, octyl CH_3); FAB-MS: *m/z* 926.5 $(M+Na)^+$, 904.5 $(M+H)^+$.

Partial purification of GlcNAcT-V

Enzyme was obtained by a modification of published procedures.[11,20] All steps were carried out at 4 °C. Hamster kidney acetone powder prepd from 35 g of kidneys (Keystone Biologicals) was suspended in 150 mL of buffer A (0.1 M sodium acetate, pH 6.0, 0.2 M NaCl, 10 mM EDTA) and homogenized 2 × 30 s in a Waring blender. The suspension was centrifuged at 22,000 *g* for 25 min. The pellet was removed, re-extracted with 250 mL of buffer A, re-homogenized and centrifuged. Homogenization, extraction and centrifugation were repeated on the pellet with 250 mL cold distilled water. GlcNAcT-V was extracted by resuspending the pellet after the aq wash in 200 mL of buffer B (10 mM Tris–HCl buffer, pH 7.6, containing 0.4 M KCl, 10 mM EDTA and 2% Triton X-100) and stirring overnight. The extract was centrifuged at 22,000 *g* for 45 min, the supernatant set aside and the pellet re-extracted for 7 h with 70 mL of buffer B, then centrifuged at 22,000 *g* for 45 min. The supernatant from the second extraction was pooled with the first supernatant and they were dialysed against buffer C overnight (50 mM sodium cacodylate buffer, pH 6.5, containing 10 mM EDTA). Dialysed enzyme was centrifuged 45 min at 22,000 *g* before being loaded onto a UDP-hexanolamine column (1.5 × 6 cm, 7 μmol mL^{-1}) equilibrated with buffer C containing 0.2% Triton X-100. The column was washed with 200 mL buffer C containing 0.2% Triton X-100, 0.1 M NaCl and 20% glycerol, then GlcNAcT-V eluted with 50 mL of buffer C containing 0.2% Triton X-100, 1 M NaCl and 20% glycerol. The eluted enzyme was concentrated in an Amicon ultrafiltration cell (PM-10 membrane) to 5 mL and bovine serum albumin (Sigma) was added to a concentration of 1 mg mL^{-1}. Concentrated enzyme was dialysed into 50 mM sodium cacodylate buffer, pH 6.5, containing 10 mM EDTA, 20% glycerol and 0.1% Triton X-100. This preparation yielded 7 mU of GlcNAcT-V (0.36 mU mg^{-1} protein) where 1 mU is defined as the amount of enzyme forming 1 nmol product min^{-1} at 37 °C, using 1 mM UDP-GlcNAc as a donor and 1 mM synthetic trisaccharide **5** as an acceptor in 25 mM sodium cacodylate buffer, pH 6.5, containing 0.05% Triton X-100, 100% glycerol, 5 mM EDTA and 0.5 mg mL^{-1} BSA.

Evaluation of 6a–j as inhibitors for GlcNAcT-V

GlcNAcT-V was assayed radiochemically using reverse-phase C18 Sep-Pak Plus cartridges to separate radiolabeled hydrophobic product tetrasaccharide from unreacted donor as previously described.[11,12] In a typical assay, acceptor **5**, UDP GlcNAc, UDP-[^{3}H]GlcNAc, and inhibitor were placed in 0.6 mL microcentrifuge tubes and lyophilized to dryness in a speed-vacuum. Buffer, inhibitor and enzyme were added to a total volume of 20 μL, with the following final concns: 3.93 μUnits GlcNAcT-V, 40 μM acceptor, 550 μM UDP GlcNAc, and ~250,000 dpm UDP[^{3}H]GlcNAc. Two to three different concentrations of compounds **6a–j** were utilized ranging from 100 to 1000 μM. Tubes were vortexed, spun briefly in a microcentrifuge and incubated for 1 h at 37 °C. Reactions were quenched with 0.4 mL water, and immediately processed as previously described.[11,12] Each evaluation of compound **6a–j** also contained duplicate tubes of the following: the first contained acceptor **5** at 40 μM final concn. The second contained acceptor **5** at 40 μM plus a known inhibitor (the 6′-deoxy derivative of **5**) at 40 μM final concn. The third contained UDP-GlcNAc enzyme and buffer as a blank control for background counts. The fourth contained compound **6a–j** tested as an acceptor at 1 mM final concn. Data from the first and the second were used to ensure the GlcNAcT-V still showed the same degree of inhibition with the known inhibitor as in previous evaluations.[12] The fourth was included to ensure compounds **6a–j** were not acceptors for the enzyme. The K_is of compounds **6a–j** were obtained by using the formula $\%i = 100[I]/([I]+K_i\{1+[S]/K_m\})$ where $\%i$ is the percent inhibition, $[I]$ is the concn of the inhibitor, $[S]$ is the concn of acceptor **5** (40 μM) and the K_m of acceptor **5** is 26 μM. This equation assumes the inhibition is competitive. Estimates of K_i for two to three different concentrations of inhibitor were comparable within experimental error (±5%) and the averaged results are reported in Table 1. Full kinetic analyses were carried out for compound **6e**. This was found to be a competitive inhibitor since V_{max} was unchanged within experimental error and there was an increase in apparent K_m and K_m/V_{max} for acceptor (Fig. 1). Analysis of secondary plots of K_m/V_{max} (or K_m) vs $[I]$ both gave a K_i of 21 μM for compound **6e** which is identical to the value obtained using the competitive equation above.

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References

1. Cummings, R. D; Trowbridge, I. S. *J. Biol. Chem.* **1982**, *257*, 13421.

2. Schachter, H. *Biochem. Cell. Biol.* **1986**, *64*, 163.

3. Yamshita, K.; Tachibana, Y.; Ohkura, T.; Kobata, A. *J. Biol. Chem.* **1985**, *260*, 3963.

4. Arango, J.; Pierce, M. *J. Cell. Biochem.* **1988**, *37*, 225.

5. Dennis, J. W.; Kosh, E.; Bryce, D.-M.; Breitman, M. L. *Oncogene* **1989**, *4*, 853.

6. Dennis, J. W.; Laferte, S.; Waghorne, C.; Breitman, M. L.; Kerbel, R. S. *Science* **1987**, *236*, 582.

7. Dennis, J. W. *Cancer Surveys* **1988**, *7*, 573.

8. Hindsgaul, O.; Tahir, S. H.; Srivastava, O. P.; Pierce, M. *Carbohydr. Res.* **1988**, *173*, 263.

9. Srivastava, O. P.; Hindsgaul, O.; Shoreibah, M.; Pierce, M. *Carbohydr. Res.*, **1988**, *179*, 137.

10. Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49.

11. Khan, S.H.; Crawley, S.C.; Kanie, O.; Hindsgaul, O. *J. Biol. Chem.* **1993**, *268*, 2468.

12. Hindsgaul, O.; Kaur, K. J.; Srivastava, G.; Blaszczyk-Thurin, M.; Crawley, S. C.; Heerze, L. D.; Placic, M. M. *J. Biol. Chem.* **1991**, *266*, 17858.

13. Fraser-Reid, B.; Udodong, U. E.; Wu, Z.; Ottosson, H.; Merritt, J. R.; Rao, C. S.; Roberts, C.; Madsen, R. *Synlett* **1992**, *268*, 927.

14. Patroni, B.; Stick, R. V.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1988**, *12*, 91.

15. David, S; Hanessian, S. *Tetrahedron* **1985**, *41*, 643.

16. Johansson, R.; Samuelsson, B. *J. Chem. Soc. Perkin Trans.* **1984**, 2371.

17. Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885.

18. Bock, K.; Pederson, C. *J. Chem. Soc., Perkin Trans.* **1974**, 293.

19. Lemieux, R. U.; Takeda, T.; Chung, B. Y. *Am. Chem. Soc. Symp. Ser.* **1976**, *39*, 90.

20. Kanie, O.; Crawley, S. C.; Palcic, M. M.; Hindsgaul, O. *Carbohydr. Res.* **1993**, *243*, 139.

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