Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 11, pp. 1231-1241, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00096-4

Key Involvement of all Three GlcNAc Hydroxyl Groups in the Recognition of β-D-Glc*p*NAc-(1→2)-α-D-Man*p*-(1→6)-β-D-Glc*p*-OR by *N*-Acetylglucosaminyltransferase-V

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Abstract—*N*-Acetylglucosaminyltransferase-V (GlcNAc T-V) transfers a β-linked GlcNAc residue from UDP-GlcNAc to OH-6' (of the αMan residue) in oligosaccharides terminating in the sequence β-D-Glc*p*NAc-(1 → 2)-α-D-Man*p*-(1 → 6)-β-D-Glc*p*(or Man*p*)-OR (**3**, R = $(CH_2)_7CH_3$). It was previously found that OH-4" (of the GlcNAc residue) in **3** was a critical element for substrate recognition by this enzyme. We show here that OH-3" and OH-6" are also key recognition elements.

Four analogs of trisaccharide **3** where OH-3" and OH-6" were replaced, independently, by NH_2 and NHAc groups, were prepared by multi-step chemical synthesis and kinetically evaluated as substrates for GlcNAc T-V from hamster kidney. These substitutions were selected since they replaced the OH groups with groups probing both hydrogen bonding and steric bulk. The 3"-modified compounds were found to be very poor substrates with K_m values more than 50-fold elevated over that for **3** (26 μM) while the 6"-modified compounds were completely inactive. An intact 3,4,6 triol system in the terminal GlcNAc residue therefore appears to be the key polar group system that is recognized by this enzyme.

Introduction

The glycosyltransferase termed *N*-acetylglucosaminyltransferase-V (GlcNAc T-V, EC 2.4.1.155) transfers a GlcNAc residue from UDP-GlcNAc to complex biantennary asparagine-linked chains such as **1** to generate the additional β-(1 → 6) branch in the product **2**. An increase in the activity of this enzyme has been correlated with the metastatic potential of several cancer cell lines.[1–3] As a result, GlcNAc T-V has become a key target for the development of glycosyltransferase inhibitors. At least 12 different GlcNAc-transferases use UDP-GlcNAc as the donor in mammalian cells[4,5] but these enzymes evidently differ in the recognition of their acceptor oligosaccharide structures. The smallest active acceptor encountered biosynthetically by GlcNAc T-V is a glycopeptide containing the heptasaccharide sequence shown in **1**. Knowledge of exactly how this enzyme binds to **1** is of basic importance in the development of inhibitors for GlcNAc T-V and is the goal of the present research.

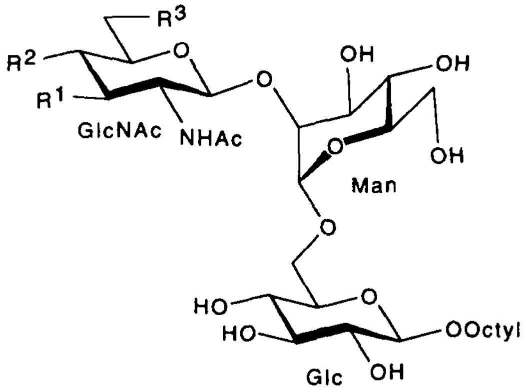
The synthetic trisaccharide derivative **3**[6] was found to be a good model for the more complex heptasaccharide and is much more amenable to chemical modification. This trisaccharide has been shown[7,8] to be an acceptor specific for GlcNAc T-V which converts it to **4**. Recognition of the terminal GlcNAc residue in **3** was examined in a previous study where OH-4" (of the GlcNAc residue) was replaced by H, OMe, F, NH_2 NHAc and inverted to the galacto-configuration.[9] All of these modifications produced compounds which were either extremely poor or non-acceptors. OH-4" in **3** was therefore designated as a key polar group for GlcNAc T-V.[9]

The present paper explores further the recognition of the terminal GlcNAc residue in **3** through the synthesis and enzymatic evaluation of four new analogs where OH-3" and OH-6" were replaced by NH_2 and NHAc groups. As discussed previously,[10] replacement of OH groups by NH_2 groups, to produce **5** and **9**, is sterically conservative provided that the amines are bound in the non-protonated form. If protonated, then the ammonium groups can serve as probes for the presence of negatively charged species in or near the active site. The product amines (or ammonium ions) might in addition still retain the ability to engage in hydrogen bonds with the protein combining site. The replacement of OH groups by acetamido groups, to produce **6** and **10**, is readily accomplished by *N*-acetylation of the free amines. These derivatives are neutral and sterically much more demanding yet might still either donate or accept hydrogen bonds. Together, using the combination of amino and acetamido derivatives, should address whether or not an OH group on an oligosaccharide is intimately involved in recognition.

Results and Discussion

The selection of a versatile intermediate is important to reduce the work required in a multistep synthesis of oligosaccharide analogs. Allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**11**)[11] has been shown to be such a useful intermediate which can easily be converted to suitable building blocks[12–18] for the synthesis of biologically important oligosaccharides containing β-GlcNAc or β-GalNAc residues as well as their analogs. The synthetic strategy used here was to prepare the

Scheme I.



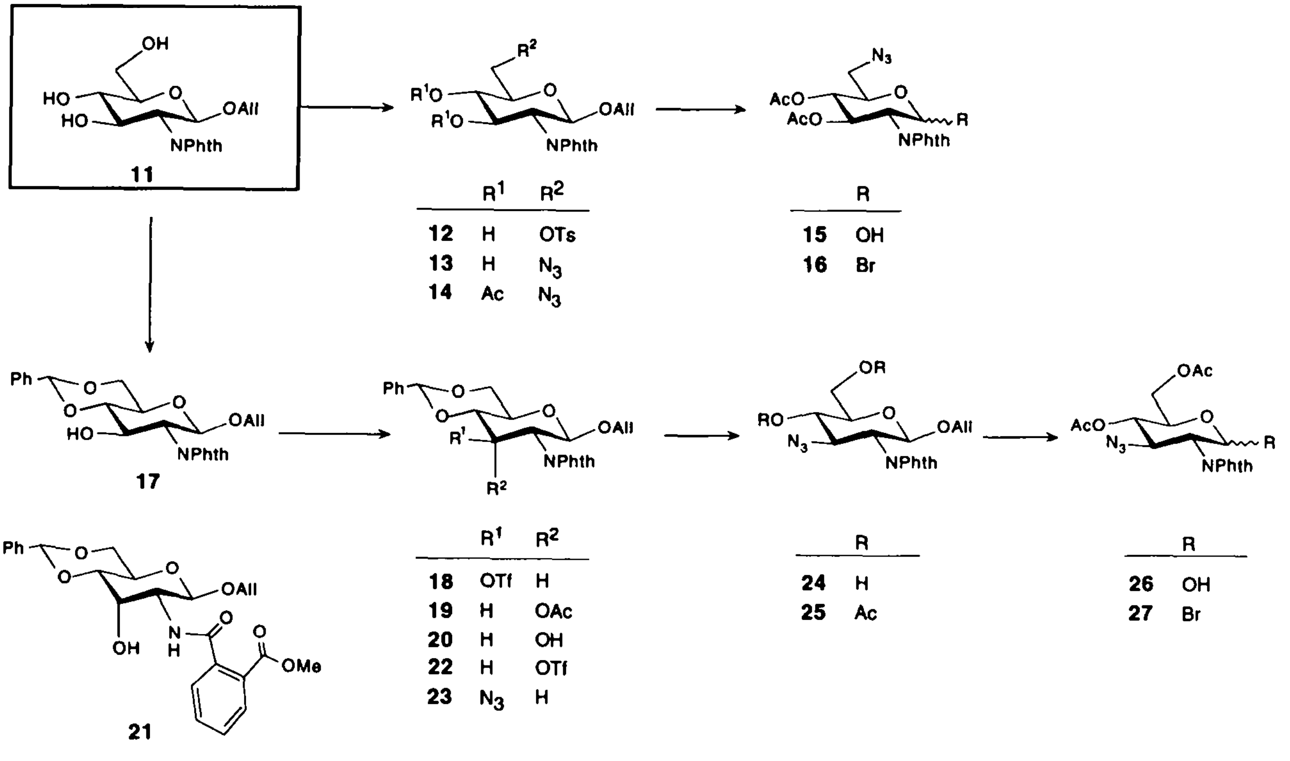
	R^1	R^2	R^3
3	OH	OH	OH
5	NH_2	OH	OH
6	NHAc	OH	OH
7	OH	NH_2	OH
8	OH	NHAc	OH
9	OH	OH	NH_2
10	OH	OH	NHAc

Scheme II.

required 3- and 6-azido glycosyl donors **16** and **27** from **11**. The 6'-*O*-allyl protecting group in the disaccharide-based glycosyl acceptor **28** was incorporated to allow access to the reactive OH group glycosylated by GlcNAc T-V, with the possibility of providing subsequent inhibitors. In the present study, however, only acceptor analogs were prepared and this group was treated as a conventional persistent protecting group.

Compound **11** was transformed into **14**, by tosylation[19] (56 %), displacement using sodium azide (72 %) and acetylation. The introduction of the azido group was evident from an infrared absorption at 2095 cm^{-1} in **13**. The allyl glycoside **14** was then converted to hemiacetal **15** by sequential treatment with [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)iridium (I) hexafluorophosphate[20] in THF followed by iodine in THF-water[21] (64 % in two steps). Treatment of **14** with palladium(II) chloride in acetic acid buffer[22] gave only a poor yield (21 %) of **15**. The hemiacetal **15** was treated with Vilsmeier bromide [*N*,*N*-dimethyl(bromomethylene) ammonium bromide][23] in $CHCl_3$ at 40 °C to produce bromide **16**.

For the production of **27**, **11** was converted to benzylidene derivative **17**.[16] Reaction with triflic anhydride gave triflate **18** which was treated with tetraethylammonium acetate (77 %, two steps) to give



Scheme III.

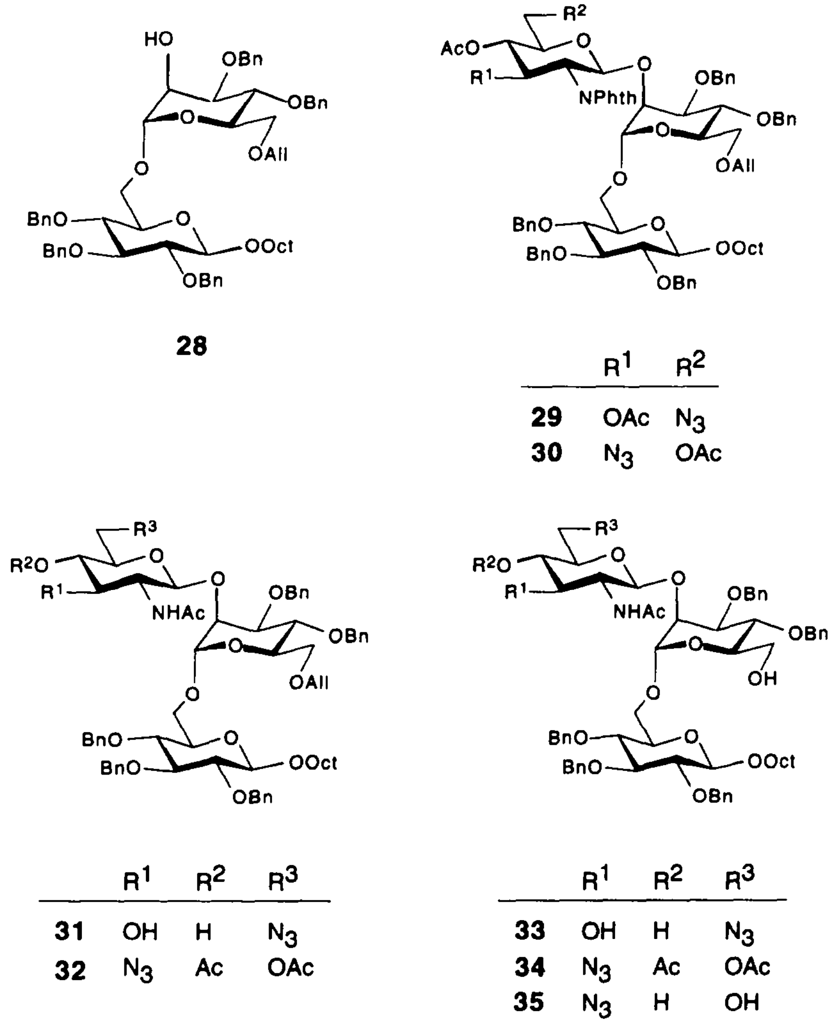
the allosamine derivative **19**. Zemplen deacetylation of **19**, however, yielded a 1:2 mixture of the desired **20** and the phthalimido mono-ester **21** (a similar problem was recently reported; see ref 24). Fortunately, reaction of **21** with triethylamine gave back the desired **20** in quantitative yield. Alternatively, deacetylation of **19** could be carried out using triethylamine in MeOH to produce **20** directly in 92 % yield. Triflation of the alcohol **20**, followed by displacement with sodium azide, yielded the 3-azido glucosamine derivative **23** (56 %). A higher yield could not be obtained due to a competing elimination reaction. The infrared absorption at 2107 cm^{-1} and the ^1H NMR coupling constants ($J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 8.9 Hz) are consistent with the structure assigned to **23**. The benzylidene group in **23** was removed by hydrolysis yielding **24** which was acetylated to give fully protected **25**. De-*O*-allylation of **25** was performed as described for the preparation of **15** in 60 % overall yield. The bromide **27** was prepared as for **16**.

Glycosylation of the disaccharide derivative **28**[9] with bromides **16** and **27** was carried out in the presence of silver triflate and *s*-collidine to yield trisaccharides **29** and **30** in 91 and 83 % yield, respectively. The phthaloyl protecting group in **29** was removed, without affecting the 6'-*O*-allyl group, using 1,2-diaminoethane in *n*-butanol at 90 °C[9] to yield the 2"-amino compound which was then *N*-acetylated to give **31** (65 %, two steps). The allyl protecting group in **31** was removed using palladium(II) chloride in acetic acid to give **33** (55 %). Similarly, compound **30** was converted to the acetamido derivative **32** which was further transformed into **35**.

Hydrogenolysis of **33** and **35** was carried out in the presence of hydrogen chloride (2 eq.) in aldehyde-free ethanol[9] to yield amines **5** and **9** in 94 and 92 % yield, respectively. *N*-Acetylation of amines **5** and **9** then gave the 2", 3"- and 2", 6"-di-acetamido derivatives **6** and **10** in quantitative yield. Selected NMR data for **5**–**10** are collected in Table 1.

Compounds **5**, **6**, **9** and **10** were tested as acceptors for GlcNAc T-V which had been partially purified from hamster kidney according to published procedures.[25] The enzyme assay quantitated the transfer of radiolabelled GlcNAc from UDP-[^3H]-GlcNAc to the acceptors. The octyl aglycone incorporated into the synthetic acceptor analogs allowed a facile separation of the radiolabelled products from unreacted UDP-[^3H]-GlcNAc and its breakdown products since it rendered them sufficiently hydrophobic to be quantitatively adsorbed on to C_{18} cartridges.[26] Elution of the product with MeOH, followed by liquid scintillation counting, then permitted the quantitation of enzymatically-formed product. The results of the kinetic evaluations are reported in Table 2. Results obtained with the previously reported[9] 4"-amino and 4"-acetamido derivatives **7** and **8** are included for comparison.

The K_m for the parent trisaccharide **3** was 26 μM in the series of experiments presented here, and a V_{max} value of 270 pmol/h was obtained with the enzyme preparation under standard conditions. The results in



28

	R^1	R^2
29	OAc	N_3
30	N_3	OAc

	R^1	R^2	R^3
31	OH	H	N_3
32	N_3	Ac	OAc

	R^1	R^2	R^3
33	OH	H	N_3
34	N_3	Ac	OAc
35	N_3	H	OH

Scheme IV.

Table 1. Selected chemical shifts δ (ppm) and coupling constants (Hz) for compounds **5**–**10**[a]

Compounds Substituent	**5** 3-NH_2	**6** 3-NHAc	**9** 6-NH_2	**10** 6-NHAc	**7**[b] 4-NH_2	**8**[b] 4-NHAc
H-1 (J1,2)	4.45 (7.7)	4.47 (7.7)	4.46 (7.7)	4.46 (7.9)	4.46 (7.8)	4.46 (7.8)
H-1' (J1',2')	4.89 (broad s)	4.92 (broad s)	4.90 (broad s)	4.89 (d, 1.1)	4.91 (broad s)	4.89 (broad s)
H-1" (J1",2")	4.57 (8.2)	4.68 (8.1)	4.57 (8.2)	4.54 (8.2)	4.55 (8.2)	4.58 (8.2)
$\mathrm{COC}H_3$	2.09	2.00x2	2.05	2.05, 2.03	2.07	2.06, 2.03
$\mathrm{CH_2C}H_3$	0.86	0.86	0.86	0.86	0.86	0.86
Other *H*s	4.12 (dd, 1.0, 2.0, H-2'), 2.79 (t, 10.0, H-3")	4.15 (broad d, 2.9, H-2')	4.12 (dd, 1.1, 3.1, H-2'), 3.13 (broad dd, ~1.5, 13.5, H-6"a), 2.86 [dd(second order), 7.4, 13.5, H-6"b)	4.09 (dd, 1.1, 3.1, H-2')	2.75 (t, 9.5, 9.5, H-4")	
C-1	103.2	103.1	103.2	103.3	102.3	103.2
C-1'	97.7	97.7	97.6	97.6	96.8	97.7
C-1"	100.7	100.6	100.3	100.4	99.6	100.3
C-2"	55.4	54.5	56.2	56.3	55.7	56.8
$\mathit{C}\mathrm{OCH_3}$	175.7	n.d.	175.7	n.d.	174.8	174.8, 175.6
$\mathrm{CO}\mathit{C}\mathrm{H_3}$	22.9	22.9, 22.8	23.2	23.2, 22.8	22.3	23.2
$\mathrm{CH_2}\mathit{C}\mathrm{H_3}$	14.3	14.3	14.3	14.3	13.4	14.3
Other *C*	56.5 (C-3")	55.5 (C-3")	42.1 (C-6")	40.9 (C-6")	52.8 (C-4")	53.0 (C-4")

[a] The assignments of ^{13}C resonances are tentative.
[b] Data taken from ref 9.

Table 2. Kinetic parameters for GlcNAc T-V acceptor analogs

Compound	GlcNAc T-V		
	K_m (mM)[a]	$V_{max,rel}$[a]	$(V/K)_{relative}$[b]
3	0.026±0.002	100±2	100
5 (3-NH_2)	1.10±0.06	34±1	0.80
6 (3-NHAc)	1.94±0.01	21.7±0.5	0.29
7 (4-NH_2)[c]	6±2	13±2	0.09
8 (4-NHAc)[c]	1.4±0.2	0.95±0.05	0.03
9 (6-NH_2)	not a substrate up to 10 mM		0
10 (6-NHAc)	not a substrate up to 10 mM		0

[a] ± Standard errors.

[b] $V_{max,rel}$ and $(V/K)_{relative}$ were determined relative to compound **3** under the same experimental conditions.

[c] Data taken from ref 9.

Table 2 show clearly that each hydroxyl group on GlcNAc residue is extremely important for recognition by the enzyme; in particular conversion of OH-6" into an amino (**9**) or acetamido group (**10**) yielded compounds for which no acceptor activity could be detected up to 10 mM. For the 3"- and 4"-aminated derivatives **5**–**8**, the K_m values increased between 50- and 300-fold while V_{max} decreased to between 1 and 30 %. It is unlikely that the observed residual activity of **5**–**8** is caused by contaminating parent OH-compound **3** since they should then have the same V_{max} values. Regardless, however, the observed values of K_m and V_{max} would represent an overestimate of the quality of the aminated acceptors if due to contamination. The fundamental result remains that the substitutions reported here at C-3" and C-4' of **3** have an extremely detrimental effect on its ability to act as an acceptor for GlcNAcT-V. All analogs had *V*/*K* values less than 1 % that of the native **3**. The conclusions reached in this study are that an intact triol is required for efficient recognition by GlcNAc T-V. Our efforts at the design of specific acceptor-based inhibitors for this enzyme should therefore incorporate this molecular feature.

Experimental

General methods

TLC was performed on Silica Gel 60-F_{254} (Merck) with detection by quenching of fluorescence, by charring with sulfuric acid, and/or by reaction with ninhydrin. Unless otherwise noted, column chromatography was performed on Silica Gel 60 (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 ± 2°. Melting points were measured with Fisher–Johns melting point apparatus. IR Spectra were recorded with a Nicolet SX-20 FTIR by the spectral services laboratory of the Chemistry Department. ^1H NMR Spectra were recorded at 300 MHz (Bruker AM-300), 360 MHz (Bruker WM-360), or 500 MHz (Varian UNITY 500) on solution in $CDCl_3$ (internal Me_4Si, δ 0), C_6D_6 (internal Me_4Si, δ 0) or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 75, 90 or 125 MHz on the same instruments in $CDCl_3$ (internal Me_4Si, δ 0) or D_2O (internal dioxane, δ 67.4). Only partial NMR data are reported, the other data were in accord with the proposed structures. The assignments of ^{13}C resonances are tentative. Fast atom bombardment-mass spectra (FABMS) were obtained on a Kratos AEIMS9 instrument by the departmental microanalytical laboratory. Elemental analyses were carried out on a CARO ERBA EA1108.

Materials

Allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (**11**),[11] allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**17**)[16] and octyl (6-*O*-allyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (**28**)[9] were prepared by literature methods.

Synthesis of OH-6"modified trisaccharides

*Allyl 2-deoxy-2-phthalimido-6-*O*-*p*-toluenesulfonyl-β-D-glucopyranoside (**12**).* *p*-Toluenesulfonyl chloride (600 mg, 3.15 mmol) was added to a solution of **11** (1 g, 2.86 mmol) in pyridine (100 mL) at 0 °C. The reaction mixture was allowed to come to rt, then stirred for 3 days at 35 °C. Methanol was added to the mixture, and the mixture was stirred for 2 h. Concentration of the solution left a residue which was taken up in dichloromethane. The organic layer was washed with 0.5 N HCl, M $NaHCO_3$, and water, dried(Na_2SO_4), and concentrated to give a syrup which was purified by column chromatography using CH_2Cl_2:MeOH (20:1) to give **12** (790 mg, 56.4 %); R_f 0.15 (5:1 toluene: Me_2CO); $[\alpha]_D$ +9.3° (*c* 1.2 $CHCl_3$); ^1H NMR (360 MHz in $CDCl_3$): δ 7.86–7.34 (m, 8H, aromatic), 5.68 (m, 1H, CH_2=C*H*-CH_2O), 5.19 (d, 1H, $J_{1,2}$ = 8.1 Hz, H-1), 5.10 and 5.03 (2m, each 1H, CH_2=CH-CH_2O), 4.35 (d, 2H, $J_{5,6a} = J_{5,6b}$ = 3.2 Hz, H-6s), 4.29 (dd, 1H, $J_{2,3}$ = 10.3 Hz, $J_{3,4}$ = 8.2 Hz, H-3), 4.18 and 3.95 (2m, 2H, CH_2=CH-CH_2O), 4.08 (dd, 1H, H-2), 3.63 (dt, 1H, $J_{4,5}$ = 8.5 Hz, H-5), 3.54 (t, 1H, H-4), 3.28 and 3.03 (broad signals, 2H, OH × 2), and 2.43 (s, 3H, CH_3-Ph). Anal. calcd for $C_{24}H_{25}NO_9S$: C, 57.25; H, 5.00; N, 2.78; S, 6.37. Found: C, 57.54; H, 5.11; N, 2.85; S, 6.11.

Allyl 6-azido-2-deoxy-2-phthalimido-β-D-glucopyranoside (13). Sodium azide (400 mg, 6.15 mmol) was added to a solution of **12** (540 mg, 1.07 mmol) in *N,N*-dimethylformamide (DMF) (15 mL) and the mixture was stirred at 110 °C for 16 h. Solvent evaporation left a residue which was taken up to dichloromethane. The organic solution was washed with 0.5 N HCl, M $NaHCO_3$, and water, dried (Na_2SO_4), and concentrated. The resulting syrup was purified by column chromatography using CH_2Cl_2:MeOH (10:1) to yield **13** (290 mg, 72.2 %); R_f 0.40 (5:1 toluene:EtOH); $[\alpha]_D$ −19.9° (*c* 1.4 $CHCl_3$); FTIR ($CDCl_3$): 3470 (OH), 2095 (N_3) and 1710 cm^{-1} (CO); ^1H NMR (360 MHz in $CDCl_3$): δ 7.81–7.67 (m, 4H, aromatic), 5.69 (m, 1H, CH_2=C*H*-CH_2O), 5.20 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 5.12 and 5.05 (2m, each 1H, CH_2=CH-CH_2O), 4.24 and 4.02 (m, 2H, CH_2=CH-CH_2O), 4.21 (dd, 1H, $J_{2,3}$ = 10.1, $J_{3,4}$ = 8.3 Hz, H-3), 4.06 (dd, 1H, H-2), 3.92 (broad signal, 2H, OH × 2), 3.57 (dt, 1H, $J_{4,5}$ = 9.1 Hz, $J_{5,6a}$ ~ $J_{5,6b}$ = 4.3 Hz, H-5), 3.45 (d, 2H, H-6s) and 3.42 (t, 1H, H-4); ^{13}C NMR (75.5 MHz): δ 168.53 (CO), 117.78 (CH_2=CH-CH_2O), 97.12 (C-1), 56.58 (C-2), and 51.53 (C-6).

Allyl 3,4-di-O-acetyl-6-azido-2,6-dideoxy-2-phthalimido-β-D-glucopyranoside (14). Compound **13** (190 mg, 0.51 mmol) was acetylated with acetic anhydride (2 mL) and pyridine (5 mL) at rt overnight to give **14** (220 mg); R_f 0.16 (2:1 hexane:EtOAc); $[\alpha]_D$ +17.7° (*c* 0.5, $CHCl_3$); ^1H NMR (360 MHz in $CDCl_3$): δ 7.89–7.32 (m, 4H, aromatic), 5.80 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 9.2 Hz, H-3), 5.73 (m, 1H, CH_2=C*H*-CH_2O), 5.46 (d, 1H, $J_{1,2}$ = 8.2 Hz, H-1), 5.17 and 5.10 (2m, each 1H, CH_2=CH-CH_2O), 5.05 (dd, 1H, $J_{4,5}$ = 10.2 Hz, H-4), 4.36 (dd, 1H, H-2), 4.30 and 4.09 (2m, each 1H, CH_2=CH-CH_2O), 3.88 (ddd, 1H, $J_{5,6a}$ = 7.3 Hz, $J_{5,6b}$ = 2.4 Hz, H-5), 3.47 (dd, 1H, $J_{6a,6b}$ = 13.0 Hz, H-6a) 3.23 (dd, 1H, H-6b), and 2.04 and 1.86 (2s, each 3H, CH_3CO); ^{13}C NMR (75.5 MHz): δ 170.16 and 169.66 (CO), 118.12 (CH_2=CH-CH_2O), 96.90 (C-1), 54.67 (C-2), 51.36 (C-6) and 20.68 and 20.47 (CH_3CO). Anal. calcd. for $C_{21}H_{22}N_4O_8$: C, 55.02; H, 4.84; N, 12.22. Found: C, 55.51; H, 4.59; N, 11.91.

3,4-Di-O-acetyl-6-azido-2,6-dideoxy-2-phthalimido-α, β-D-gluco-pyranose (15). Method A: A mixture of palladium(II) chloride (118.4 mg, 0.67 mmol) and sodium acetate (131.5 mg, 1.6 mmol) in acetic acid:water (20:1, 6 mL) was added, under argon at rt, to a solution of compound **14** (201.2 mg, 0.434 mmol). The reaction was stirred at rt for 2 days, then toluene (40 mL) and water (10 mL) were added and the resulting mixture was stirred vigorously for 30 min. After filtration of insoluble materials, the organic phase was separated and the aqueous solution was extracted with toluene 4 times. The organic extracts were combined and concentrated to give a syrup which was purified by column chromatography using toluene:EtOAc (4:1), to yield **15** (40.3 mg, 21.9 %); R_F 0.04 (7:1 toluene: EtOAc); mp 160–163 °C.

Method B: Hydrogen was bubbled into a suspension of [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)-iridium(I) hexafluorophosphate (9.3 mg, 0.011 mmol) in 1 mL of tetrahydrofuran (THF, freshly distilled over $LiAlH_4$) until the red color disappeared and it dissolved. This mixture was added to a solution of **14** (20.1 mg, 0.044 mmol) in THF (1 mL) and the solution was stirred for 2 days at rt under argon. The solvent was evaporated and resulting syrup was purified by flash chromatography using 3:1 hexane:EtOAC to give the 1-propenyl glycoside (17 mg); ^1H NMR (300 MHz in $CDCl_3$): δ 7.90–7.71 (m, 4H, aromatic), 6.16 (m, 1H, CH_3-CH=C*H*-O), 5.79 (dd, 1H, $J_{2,3}$ = 10.4 Hz, $J_{3,4}$ = 8.7 Hz, H-3), 5.62 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 5.08 [(t, 1H, $J_{4,5}$ = 9.6 Hz, H-4) and (dq, 1H, CH_3-C*H*=CH-O)], 4.40 (dd, 1H, H-2), 3.90 (ddd, 1H, H-5), 3.48 (dd, 1H, $J_{5,6a}$ = 7.2 Hz, $J_{6a,6b}$ = 13.4 Hz, H-6a), 3.30 (dd, 1H, $J_{5,6b}$ = 2.6 Hz, H-6b), 2.04 and 1.87 (2s, each 3H, CH_3CO), and 1.47 (dd, 3H, *J* = 1.8 and 6.9 Hz, CH_3-CH=CH-O). The 1-propenyl glycoside was then dissolved in THF:H_2O (4:1, 5 mL), and I_2 (20.7 mg, 0.082 mmol) was added at rt and the mixture was stirred for 5 min. The mixture was then diluted with CH_2Cl_2 (20 mL) and washed with satd $Na_2S_2O_3$ and satd NaCl, dried with Na_2SO_4, and concentrated to dryness. The residue was purified by silica gel chromatography using 5:1 toluene:Me_2CO as eluent to yield **15** (11.7 mg, 63.8 %, two steps); ^1H NMR (360 MHz in $CDCl_3$): δ 7.81–7.70 (m, 4H, aromatic), 6.19 (dd, 0.1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 9.0 Hz, H-3-α), 6.84 (dd, 0.9H, $J_{2,3}$ = 11.6 Hz, $J_{3,4}$ = 8.8 Hz, H-3-β), 5.66 (d, 0.9H, $J_{1,2}$ = 8.2 Hz, H-1-β), 5.43 (d, 0.1H, $J_{1,2}$ = 3.5 Hz, H-1-α), 5.24 (dd, 0.1H, $J_{4,5}$ = 11.0 Hz, H-4-α), 5.21 (dd, 0.9H, $J_{4,5}$ = 9.2 Hz, H-4-β), 4.62 (dd, 0.1H, H-2-α), 4.27 (dd, 0.9H, H-2-β), 4.10 (broad signal, 0.9H, OH-β), 3.90 (dt, 0.9H, $J_{5,6a}$ ~ $J_{5,6b}$ = 4.7 Hz, H-5-β), 3.47 (dd, 0.1H, $J_{5,6a}$ = 2.6 Hz, $J_{6a,6b}$ = 12.8 Hz, H-6a-α), 3.42 (d, 1.8H, H-6s-β), 3.36 (dd, 0.1H, $J_{5,6b}$ = 5.5 Hz, H-6β-α), 2.10 and 1.82 (2s, each 0.3H, CH_3CO-α), and 2.05 and 1.85 (2s, each 2.7H, CH_3CO-β); ^{13}C NMR (75.5 MHz): δ 92.60 (C-1-β), 56.03 (C-2-β), 51.15 (C-6-β), 92.31 (C-1-α), 54.53 (C-2-α), 51.04 (C-6-α).

3,4-Di-O-acetyl-6-azido-2,6-dideoxy-2-phthalimido-α, β-D-gluco-pyranosyl bromide (16). A suspension of *N,N*-dimethyl(bromomethylene)ammonium bromide, prepared from oxalyl bromide (150 μL, ~1.02 mmol) and DMF (80 μL, 1.02 mmol), in chloroform (3 mL) was added to a solution of **15** (85.2 mg, 0.204 mmol) in chloroform (2 mL) at rt. After stirring at 40 °C for 2 h, the reaction mixture was diluted with dichloromethane, and washed with 0.5 % hydrochloric acid, M sodium hydrogen carbonate and water, then dried and concentrated to a pale yellow crystalline solid [R_f 0.26 (2:1 hexane:EtOAc)], which was not further purified but used directly for the next glycosylation reaction; ^1H NMR ($CDCl_3$, 360 MHz): δ 6.60 (d, 0.06H, $J_{1,2}$ = 3.4 Hz, H-1-α) and 6.43 (d, 0.94H, $J_{1,2}$ = 9.2 Hz, H-1-β).

Octyl 6-O-[2-O-(3,4-di-O-acetyl-6-azido-2,6-dideoxy-2-phthalimido-β-D-glucopyranosyl)-6-O-allyl-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (29). To a solution of **28** (385.6 mg, 0.408 mmol) in dry dichloromethane (2 mL), silver trifluoromethanesulfonate (89 mg, 0.347 mmol), *s*-collidine (46 µL, 0.347 mmol), and 4 Å molecular sieves (500 mg) were added. To the resulting mixture was added dropwise a solution of the bromide **16** (~0.2 mmol) in dry dichloromethane (1 mL) at −78 °C. The reaction mixture was allowed to warm to rt and, after 14 h of stirring, excess tetraethylammonium chloride was added and the mixture stirred for another 30 min. The mixture was then diluted with dichloromethane and filtered through Celite. The filtrate was washed sequentially with 0.5 % hydrochloric acid, satd sodium hydrogen carbonate and water, then dried over sodium sulfate, filtered and concentrated. The residual oil was purified by column chromatography using toluene:EtOAc (gradient from 20:1 to 3:1) as eluent to provide **29** (159.4 mg, 91.1 % based on consumed acceptor); R_f 0.33 (8:1 toluene:EtOAc); $[\alpha]_D$ −2.0° (*c* 1.6, CHCl$_3$); ^{1}H NMR (360 MHz in CDCl$_3$): δ 7.85–7.64 (m, 29H, aromatic), 5.81 (dd, 1H, $J_{2'',3''}$ = 10.4 Hz, $J_{3'',4''}$ = 8.9 Hz, H-3"), 5.60 (d , 1H, $J_{1'',2''}$ = 8.5 Hz, H-1"), 5.56 (m, 1H, CH$_2$=C*H*-CH$_2$O), 5.10 (dd, 1H, $J_{4'',5''}$ = 9.4 Hz, H-4"), 4.50 (dd, 1H, H-2"), 4.39 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.28 (broad t, $J_{1',2'} \sim J_{2',3'}$ = 2.4 Hz, H-2'), 4.00 (ddd, 1H, $J_{5'',6''a}$ = 2.3 Hz, $J_{5'',6''b}$ = 7.2 Hz, H-5"), 2.06 and 1.88 (2s, each 3H, C*H*$_3$CO), and 0.87 [t, 3H, C*H*$_3$(CH$_2$)$_7$O]; ^{13}C NMR (75 MHz): δ 170.05 and 169.56 (CO), 116.19 (*C*H$_2$=CH-CH$_2$O), 103.87 (C-1), 97.54 (C-1'), 95.86 (C-1"), 54.46 (C-2"), 51.59 (C-6"), 20.65 and 20.45 (CO*C*H$_3$), 14.09 [*C*H$_3$(CH$_2$)$_7$O].

Octyl 6-O-[2-O-(2-acetamido-6-azido-2,6-dideoxy-β-D-glucopyranosyl)-6-O-allyl-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (31). Compound **29** (153.5 mg, 0.114 mmol) in *n*-butanol (10 mL) was added to ethylenediamine (3 mL) under argon. The solution was stirred at 90 °C for 5 h. Evaporation followed by co-evaporation with toluene twice followed by ethanol twice, gave a yellow syrup, which was dissolved in methanol (5 mL) and acetic anhydride (1 mL) and triethylamine (0.1 mL) were added. After stirring for 14 h at rt, ethanol (20 mL) and water (1 mL) were added and the solution was concentrated to dryness. The residue was purified by column chromatography using a gradient from toluene to toluene:ethanol (20:1) as eluent, to yield **31** (86.9 mg, 64.9 %); $[\alpha]_D$ −19° (*c* 0.7, CHCl$_3$); ^{1}H NMR (360 MHz in 5:1 CDCl$_3$:CD$_3$OD): δ 7.46–7.12 (m, 25H, aromatic), 5.81 (m, 1H, CH$_2$=C*H*-CH$_2$O), 5.22 and 5.15 (2m, each 1H, C*H*$_2$=CH-CH$_2$O), 4.68 (d , 1H, $J_{1'',2''}$ = 8.2 Hz, H-1"), 4.37 (d , 1H, $J_{1',2'}$ = 7.6 Hz, H-1), 4.31 (broad d, H-2'), 1.97 (s, 3H, C*H*$_3$CO), and 0.86 [t, 3H, C*H*$_3$(CH$_2$)$_7$O]; ^{13}C NMR (75 MHz): δ 172.89 (CO), 117.17 (*C*H$_2$=CH-CH$_2$O), 103.82 (C-1), 98.48 (C-1'), 97.26 (C-1"), 59.34 (C-2"), 51.76 (C-6"), 23.52 (CO*C*H$_3$), 14.10 [*C*H$_3$(CH$_2$)$_7$O]. Anal. calcd for C$_{66}$H$_{84}$N$_4$O$_{15}$•2H$_2$O: C, 65.54•H, 7.33; N, 4.63. Found: C, 65.81; H, 7.30; N, 4.71.

Octyl 6-O-[2-O-(2-acetamido-3,4-di-O-acetyl-6-azido-2,6-dideoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (33). To a solution of **31** (65.9 mg, 0.056 mmol), a mixture of palladium(II) chloride (29.8 mg, 0.168 mmol) and sodium acetate (27.6 mg, 0.337 mmol) in acetic acid:water (20:1, 8 mL) was added under argon at rt. The reaction mixture was stirred at rt for 48 h, then dichloromethane (30 mL) and water (10 mL) were added and the resulting mixture was stirred vigorously for 30 min. After filtration of insoluble materials, the organic phase was separated and the aqueous solution was extracted with dichloromethane 4 times. The organic extracts were combined and concentrated to give a syrup which was purified by column chromatography using a gradient of CH$_2$Cl$_2$:MeOH (50:1 to 20:1 on silica gel: NAM-300, Maruishi Kagaku Yakuhin, Gifu, Japan), to yield **33** (35.4 mg, 55.6 %); $[\alpha]_D$ −11.8° (*c* 0.55, CHCl$_3$); ^{1}H NMR (360 MHz in CDCl$_3$): δ 7.40–7.14 (m, 25H, aromatic), 6.66 (broad, 1H, NH), 4.90 (d, partially overlapped with benzyl methylene, $J_{1',2'}$ = 1.9 Hz, H-1'), 4.50 (d , 1H, $J_{1'',2''}$ = 8.2 Hz, H-1"), 4.35 (d , 1H, $J_{1',2'}$ = 7.6 Hz, H-1), 4.20 (broad t, H-2'), 1.90 (s, 3H, C*H*$_3$CO), and 0.86 [t, 3H, C*H*$_3$(CH$_2$)$_7$O]; ^{13}C NMR (75 MHz): δ 173.19 (CO), 103.82 (C-1), 100.00 (C-1'), 97.90 (C-1"), 57.70 (C-2"), 51.79 (C-6"), 23.57 (CO*C*H$_3$), and 14.14 [*C*H$_3$(CH$_2$)$_7$O].

Octyl 6-O-[2-O-(2-acetamido-6-amino-2,6-dideoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (9). Compound **33** (7.1 mg, 6.2 µmol) was dissolved in aldehyde-free ethanol (95 % ethanol distilled from potassium hydroxide and zinc) (4 mL) and 0.01 N hydrochloric acid (1.24 mL, 12.4 µmol) containing 5 % palladium-on-carbon (10 mg) and stirred under hydrogen for 14 h. The catalyst was filtered off and the solution was neutralized by addition of 0.01 N sodium hydroxide and stirring for 30 min. Carbon dioxide was then bubbled through the solution. Solvent evaporation left a glass, which showed a major spot on TLC [R_f 0.09 (60:35:6 CH$_2$Cl$_2$:MeOH:H$_2$O)]. This material was adsorbed on to a Sep-Pak C-18 cartridge in water, the cartridge was washed with water (25 mL) and eluted with methanol (10 mL). The methanol was evaporated and the residue was dissolved in H$_2$O (5 mL), filtered through a Millex GV filter and lyophilized from water, to give **9** (3.8 mg, 92.4 %); NMR data are presented in Table 1; FABMS: m/z 679 (M+Na)$^+$ and 657 (M+H)$^+$.

Octyl 6-O-[2-O-(2,6-di-acetamido-2,6-dideoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (10). To a solution of **9** (1.5 mg, 2.3 µmol) in dry methanol (2 mL), acetic anhydride (140 µL, 15 µmol) and triethylamine (30 µL, 0.22 µmol) were added at rt. The reaction mixture was stirred for 14 h, solvents were evaporated and water then ethanol was added and evaporated. The resulting residue was purified as described for the preparation of **9** to give **10** (1.6 mg, 98 %); R_f 0.52 (30:40:7 CH$_2$Cl$_2$:MeOH:H$_2$O); NMR data are presented in Table 1; FABMS: m/z 721 (M+Na)$^+$.

Synthesis of OH-3'' modified trisaccharides

Allyl 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-allopyranoside (19). To a solution of **17** (2.0 g, 4.22 mmol) in dry dichloromethane (50 mL) and dry pyridine (0.57 mL, 6.33 mmol) was added dropwise a solution of trifluoromethanesulfonic anhydride (1.01 mL, 5.49 mmol) in dry dichloromethane (5 mL) at 0 °C. The reaction mixture was stirred at rt for 1 h, diluted with dichloromethane (100 mL) and washed with 0.5 % hydrochloric acid then water. Evaporation left a foam [R_f 0.65 (8:1 toluene:EtOAc)], presumably the triflate of **22**. This foam was added to 0.1 M tetraethylammonium acetate in dry DMF (50.5 mL, 5.05 mmol) at 0 °C. The resulting mixture was stirred at rt for 14 h. Solvent was evaporated and the residue was suspended in dichloromethane and washed with 0.5 % hydrochloric acid, satd sodium hydrogen carbonate then water. Solvent removal left a syrup which was purified by column chromatography using toluene:EtOAc (10:1) as eluent to give **19** (1.7 g, 77.5 %) as glass; R_f 0.55 (8:1 toluene:EtOAc); [α]$_D$ –44.4° (*c* 2.1 $CHCl_3$); ^{1}H NMR (360 MHz in $CDCl_3$): δ 7.92–7.33 (m, 9H, aromatic), 6.04 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 5.82 (m, 1H, CH_2=CH-CH_2O), 5.74 (t, 1H, $J_{2,3}$ = 2.5 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 5.60 (s, 1H, benzylidene methyne), 5.21 and 5.11 (2m, each 1H, C*H*$_2$=CH-CH_2O), 4.43 (dd, 1H, $J_{5,6a}$ = 5.1, $J_{6a,6b}$ = 10.4 Hz, H-6a), 4.40 (dd, 1H, H-2), 4.35 (m, 1H, one of CH_2=CH-C*H*$_2$O), 4.14 (m, 2H, one of CH_2=CH-C*H*$_2$O and H-5), 3.88 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.85 (t, 1H, $J_{5,6b}$ = 10.4 Hz, H-6b), and 2.06 (s, 3H, CH_3CO).

Allyl 4,6-O-benzylidene-2-deoxy-2-phthalimido-β-D-allopyranoside (20) and allyl 4,6-O-benzylidene-2-deoxy-2-(2-methoxycarbonylbenzamido)-β-D-allopyranoside (21). Method A: (1) Sodium methoxide (50 mg) was added to a solution of compound **19** (1.5 g, 3.13 mmol) in dry methanol (60 mL) and dichloromethane (50 mL), and stirred for 6 h at rt. After treatment with Amberlite IR-120(H$^+$), the resin was removed by filtration, washed with methanol and concentrated to dryness. Compound **21** (400 mg, 22.2 %) [R_f 0.42 (7:1 toluene:EtOH)] was precipitated by adding dichloromethane (50 mL) to the residue. The mother liquor was further purified by column chromatography using toluene:EtOH (7:1) as eluent to give **20** (420 mg, 30.7 %) and **21** (530 mg, 41.1 %; total 63.3 %) [R_f 0.37 (7:1 tolueneEtOH)]. (2) Addition of triethylamine (2 mL) to a suspension of **21** (920 mg) in dichloromethane (60 mL) and stirring at rt for 3 h resulted in complete conversion to **20** which was obtained in quantitative yield after solvent evaporation.

Method B: Compound **19** (2.5 g, 5.21 mmol) was treated with triethylamine (5 mL) in dry methanol (100 mL) at rt for 2 days. Evaporation followed by crystallization from ether gave **20** (2.1 g, 92.1 %).

20: [α]$_D$ –82° (*c* 1.5 chloroform); mp 127–129 °C; ^{1}H NMR (360 MHz in $CDCl_3$): δ 7.88–7.34 (m, 9H, aromatic), 6.01 (d, 1H, $J_{1,2}$ = 8.6 Hz, H-1), 5.80 (m, 1H, CH_2=C*H*-CH_2O), 5.60 (s, 1H, benzylidene methyne), 5.18 and 5.09 (2m, each 1H, C*H*$_2$=CH-CH_2O), 4.43 (m, 2H, H-3 and H-6a), 4.32 (m, 2H, one of CH_2=CH-C*H*$_2$O and H-2), 4.21 (dt, $J_{4,5}$ = $J_{5,6b}$ = 9.5 Hz, $J_{5,6a}$ = 4.9 Hz, H-5), 4.13 (m, 1H, one of CH_2=CH-C*H*$_2$O), 3.85 (t, 1H, $J_{6a,6b}$ = 10.5 Hz, H-6b), 3.79 (dd, 1H, $J_{3,4}$ = 2.4 Hz, H-4) and 2.98 (broad s, 1H, OH); ^{13}C NMR (75.5 MHz): δ 117.38 (*C*H$_2$=CH-CH_2O), 101.72 (C-1*), 96.30 (*C*HPh*), and 56.32 (C-2). Anal. calcd for $C_{24}H_{23}NO_7$: C, 65.90; H, 5.30; N, 3.20. Found: C, 65.73; H, 5.27; N, 3.26.

21: [α]$_D$ –43.7° (*c* 0.65 $CHCl_3$); mp 217–219 °C; ^{1}H NMR (360 MHz in $CDCl_3$): δ 7.94–7.34 (m, 9H, aromatic), 6.15 (d, 1H, $J_{2,NH}$ = 8.3 Hz, NH), 5.91 (m, 1H, CH_2=C*H*-CH_2O), 5.63 (s, 1H, benzylidene methyne), 5.33 and 5.19 (2m, each 1H, C*H*$_2$=CH-CH_2O), 4.83 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 4.68 (broad dt, 1H, H-3), 4.40 (m, 2H, H-6a and one of CH_2=CH-C*H*$_2$O), 4.29 (dt, 1H, $J_{2,3}$ = 2.7 Hz, H-2), 4.11 (m, 1H, one of CH_2=CH-C*H*$_2$O), 4.06 (dt, $J_{4,5}$ = $J_{5,6b}$ = 9.3 Hz, $J_{5,6a}$ = 4.8 Hz, H-5), 3.86 (s, 3H, CH_3O), 3.83 (t, 1H, $J_{6a,6b}$ = 10.1 Hz, H-6b), 3.75 (dd, 1H, $J_{3,4}$ = 2.8 Hz, H-4), and 2.91 (d, 1H, $J_{3,OH}$ = 1.7 Hz, OH). Anal. calcd for $C_{25}H_{27}NO_8 \cdot {}^1/_2H_2O$: C, 62.75; H, 5.90; N, 2.93. Found: C, 62.80; H, 5.72; N, 2.98.

Allyl 3-azido-4,6-O-benzylidene-2,3-dideoxy-2-phthalimido-β-D-glucopyranoside (23). To a solution of **20** (352.7 mg, 0.81 mmol) in dry dichloromethane (5 mL) and dry pyridine (131 μL, 1.22 mmol) was added dropwise a solution of trifluoromethanesulfonic anhydride (204 μL, 1.22 mmol) in dry dichloromethane (1 mL) at 0 °C. The reaction mixture was stirred at rt for 1 h, diluted with dichloromethane (30 mL) and washed with 0.5 % hydrochloric acid followed by water. Evaporation left a foam [R_f 0.64 (8:1 toluene:EtOAc)], presumably the triflate of **22**. The foam was dissolved in dry DMF (3 mL) and then sodium azide (263.3 mg, 4.05 mmol) dissolved in DMF (3 mL) was added at –10 °C. The resulting mixture was stirred at –10 °C for 20 h. Solvent was evaporated and the residue was suspended in dichloromethane and washed with 0.5 % hydrochloric acid, satd sodium hydrogen carbonate then water. Solvent removal left a syrup which was purified by column chromatography using toluene:EtOAc (gradient from 30:1 to 8:1) as eluent to give **23** (210.5 mg, 56.5 %) as a syrup; R_f 0.66 (8:1 toluene:EtOAc); mp 103–105°; [α]$_D$ –39.5° (*c* 0.97, $CHCl_3$); FTIR ($CDCl_3$): 2107 (N_3) and 1715 cm^{-1} (CO); ^{1}H NMR (360 MHz in $CDCl_3$): δ 7.92–7.36 (m, 9H, aromatic), 6.68 (m, 1H, CH_2=C*H*-CH_2O), 6.65 (s, 1H, benzylidene methyne), 6.28 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1), 6.13 and 6.02 (2m, each 1H, C*H*$_2$=CH-CH_2O), 5.64 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 8.9 Hz, H-3), 5.42 (dd, 1H, $J_{5,6a}$ = 4.9 Hz, $J_{6a,6b}$ = 10.9 Hz, H-6a), 5.25 and 5.01 (2m, each 1H,

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*Assignments are tentative.

CH$_2$=CH-C*H*$_2$O), 5.14 (dd, 1H, H-2), 4.87 (t, 1H, $J_{5,6b}$ = 9.5 Hz, H-6b), 4.76 (t, 1H, $J_{4,5}$ = 8.5 Hz, H-4), and 4.70 (dt, H-5). Anal. calcd for $C_{24}H_{22}N_4O_6$: C, 62.33; H, 4.79; N, 12.12. Found: C, 62.18; H, 4.93; N, 11.90.

Allyl 3-azido-2,3-dideoxy-2-phthalimido-β-D-glucopyranoside (24). Compound **23** (800 mg, 1.73 mmol) was treated with acetic acid (16 mL) and water (4 mL) at 45 °C for 14 h. Evaporation of solvents followed by co-evaporation with toluene gave a syrup which was purified by column chromatography using toluene:EtOAc (1:1) as eluent to yield **24** (560 mg, 86.5 %); R_f 0.41 (10:1 CH$_2$Cl$_2$:MeOH); $[\alpha]_D$ −1.9° (*c* 1.2 CHCl$_3$); mp 115–118 °C (softened ~85 °C); ^{1}H NMR (360 MHz in CDCl$_3$): δ 7.94–7.75 (m, 4H, aromatic), 5.70 (m, 1H, CH$_2$=C*H*-CH$_2$O), 5.27 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 5.14 and 5.07 (2m, each 1H, C*H*$_2$=CH-CH$_2$O), 4.40 (dd, 1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 9.1 Hz, H-3), 4.26 and 4.05 (2m, each 1H, CH$_2$=CH-C*H*$_2$O), 4.11 (dd, 1H, H-2), 4.01–3.89 (m, 2H, H-6s), 3.83 (dt, 1H, $J_{4,5}$ = 9.5 Hz, $J_{4,OH}$ = 4.4 Hz, H-4), 3.62 (dt, 1H, $J_{5,6a}$ ~ $J_{5,6b}$ = 3.7 Hz, H-5), 3.00 and 2.00 (2 broad signals, each 1H, OH). Anal. calcd for $C_{17}H_{18}N_4O_6$: C, 54.54; H, 4.84; N, 14.97. Found: C, 54.42; H, 4.80; N, 14.81.

*Allyl 4,6-di-*O*-acetyl-3-azido-2,3-dideoxy-2-phthalimido-β-D-glucopyranoside (25).* Compound **24** (560 mg, 1.5 mmol) was acetylated with acetic anhydride (5 mL) and pyridine (10 mL) at rt for 14 h. Methanol (2 mL) was slowly added to the mixture which was then extracted with dichloromethane. The extract was washed with 0.5 N hydrochloric acid and satd sodium bicarbonate, dried (Na$_2$SO$_4$), filtered and concentrated to give **25** (640 mg); R_f 0.41 (2:1 hexane:EtOAc); $[\alpha]_D$ +35° (*c* 1.5; CHCl$_3$); ^{1}H NMR (360 MHz in CDCl$_3$): δ 7.94–7.74 (m, 4H, aromatic), 5.70 (m, 1H, CH$_2$=C*H*-CH$_2$O), 5.24 (d, 1H, $J_{1,2}$ = 8.5 Hz, H-1), 5.14 and 5.07 (2m, each 1H, C*H*$_2$=CH-CH$_2$O), 5.13 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 4.38 (dd, 1H, $J_{2,3}$ = 11.5 Hz, H-3), 4.30 (dd, 1H, $J_{5,6a}$ = 4.4 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.27 and 4.24 (2m, each 1H, CH$_2$=CH-C*H*$_2$O), 4.19 (dd, 1H, H-2), 4.18 (dd, 1H, $J_{5,6b}$ = 2.8 Hz, H-6b), 3.82 (ddd, 1H, H-5), 2.12 and 2.11 (2s, each 3H, CH$_3$CO).

*4,6-Di-*O*-acetyl-3-azido-2,3-dideoxy-2-phthalimido-α, β-D-glucopyranose (26).* Method A: To a solution of **25** (569.8 mg, 1.24 mmol), a mixture of palladium(II) chloride (580.4 mg, 3.72 mmol) and sodium acetate (610 mg, 7.44 mmol) in acetic acid:water (20:1, 20 mL) was added under argon at rt. The reaction mixture was stirred at rt for 2 days, then toluene (100 mL) and water (50 mL) were added and the resulting mixture was stirred vigorously for 30 min. After filtration of insoluble materials, the organic phase was separated and the aqueous solution was extracted with toluene four times. The organic extracts were combined and concentrated to give a syrup which was dissolved in toluene (30 mL) to give crystalline **26** (197 mg). The mother liquor was concentrated and purified by column chromatography using toluene:EtOAc (4:1) to yield **26** (total yield 300 mg, 57.7 %), R_f 0.06 (7:1 toluene:EtOAc); mp 175–177 °C.

Method B: Hydrogen was bubbled into a suspension of [bis(methyldiphenylphosphine)](1,5-cyclooctadiene)-iridium(I) hexafluorophosphate (54.5 mg, 0.064 mmol) in 10 mL of THF (freshly distilled over LiAlH$_4$) until the red color disappeared and it dissolved in THF. This mixture was added to a solution of **25** (761.8 mg, 1.66 mmol) in THF (20 mL) and the solution was stirred for 3 days at rt under argon. The solvent was evaporated and the resulting syrup was purified by flash chromatography using 3:1 hexane:EtOAc to give the 1-propenyl glycoside (563.8 mg); ^{1}H NMR (300 MHz in CDCl$_3$): δ 7.93–7.75 (m, 4H, aromatic), 6.12 (m, 1H, CH$_3$-CH=C*H*-O), 5.42 (d, 1H, $J_{1,2}$ = 8.3 Hz, H-1), 5.15 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4), 5.04 (dq, 1H, CH$_3$-C*H*=CH-O), 4.48 (dd, 1H, $J_{2,3}$ = 11.4 Hz, H-3), 4.31 (dd, 1H, $J_{5,6a}$ = 4.6 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a), 4.23 (dd, 1H, H-2), 4.18 (dd, 1H, $J_{5,6b}$ = 2.2 Hz, H-6b), 3.88 (ddd, 1H, H-5), 2.13 and 2.11 (2s, each 3H, CH$_3$CO), and 1.47 (dd, 3H, *J* = 1.4 and 6.9 Hz, C*H*$_3$-CH=CH-O). The isomerized product was then dissolved in THF:H$_2$O (4:1, 60 mL), and I$_2$ (684 mg, 2.7 mmol) was added at rt and stirred for 5 min. The mixture was diluted with CH$_2$Cl$_2$ (200 mL) and washed with satd Na$_2$S$_2$O$_3$ and satd. NaCl, dried with Na$_2$SO$_4$ and concentrated to dryness. The residue was purified by silica gel chromatography using 5:1 toluene:Me$_2$CO as eluent to yield **26** (423.7 mg, 60.9 %, two steps); ^{1}H NMR (360 MHz in CDCl$_3$): δ 7.94–7.76 (m, 4H, aromatic), 5.41 (d, 0.7H, $J_{1,2}$ = 8.5 Hz, H-1-β), 5.38 (d, 0.3H, $J_{1,2}$ = 3.8 Hz, H-1-α), 5.12 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4-β and m, H-4-α), 4.61 (dd, 0.7H, $J_{2,3}$ = 11.6 Hz, H-3-β), 4.39–4.27 (m, 1.2H, H-2, H-3, H-5, H-6a-α), 4.26 (dd, 0.7H, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6a-β), 4.19 (dd, 0.7H, $J_{5,6b}$ = 2.3 Hz, H-6b-β), 4.15 (dd, 0.3H, $J_{6a,6b}$ = 12.2 Hz, $J_{5,6b}$ = 2.2 Hz, H-6b-α), 4.07 (dd, 0.7H, H-2-β), 4.01 (broad signal, 0.3H, OH-α), 3.88 (ddd, 0.7H, H-5-β), and 3.16 (broad signal, 0.7H, OH-β), 2.15 and 2.11 (2s, each 0.9H, CH$_3$CO-α), and 2.13 and 2.12 (2s, each 2.1H, CH$_3$CO-β). Anal. calcd for $C_{18}H_{18}N_4O_8$: C, 51.68; H, 4.33; N, 13.39. Found: C, 51.86; H, 4.31; N, 13.30.

*4,6-Di-*O*-acetyl-3-azido-2,3-dideoxy-2-phthalimido-α, β-D-glucopyranosyl bromide (27).* A suspension of *N,N*-dimethyl(bromomethylene)ammonium bromide, prepared from oxalyl bromide (69.3 µL, 0.51 mmol) and DMF (39.9 µL, 0.51 mmol), in chloroform (2 mL) was added to a solution of **26** (43 mg, 0.103 mmol) in chloroform (2 mL) at rt. After stirring at 40 °C for 4 h, the reaction mixture was diluted with dichloromethane, and washed with 0.5 % hydrochloric acid, satd. sodium hydrogen carbonate and water, then dried and concentrated to pale yellow solid [R_f 0.38 and 0.26 (2:1 hexane:EtOAc)] which was not further purified and used for the next glycosylation reaction; ^{1}H NMR (CDCl$_3$, 360 MHz), δ 6.56 (d, 0.57H, $J_{1,2}$ = 3.6 Hz, H-1-α) and 6.26 [m (second order), 0.43H, $J_{1,2}$ = 9.0 Hz, H-1-β].

*Octyl 6-*O*-[2-*O*-(4,6-di-*O*-acetyl-3-azido-2,3-dideoxy-2-phthalimido-β-D-glucopyranosyl)-6-*O*-allyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl]-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (30).* To a solution of **28** (189 mg, 0.20

mmol) in dry dichloromethane (1 mL), silver trifluoromethanesulfonate (33.4 mg, 0.13 mmol), *s*-collidine (19.8 µL, 0.15 mmol), and 4 Å molecular sieves (300 mg) were added. To the resulting mixture was added dropwise a solution of the bromide **27** (~0.1 mmol) in dry dichloromethane (1 mL) at –40 °C. The reaction mixture was allowed to warm to rt and, after 14 h of stirring, excess tetraethylammonium chloride was added; stirring was continued for another 30 min. The mixture was then diluted with dichloromethane and filtered through Celite. The filtrate was washed sequentially with 0.5 % hydrochloric acid, satd sodium hydrogen carbonate and water, and then dried over sodium sulfate, filtered, and concentrated. The residual oil was purified by column chromatography using toluene:EtOAc (gradient from 20:1 to 4:1) as eluent to provide **30** (60 mg, 82.7 % based on consumed acceptor); R_f 0.26 (8:1 toluene:EtOAc); $[\alpha]_D$ +3.6° (*c* 1.6, $CHCl_3$); ^1H-NMR (360 MHz in $CDCl_3$): δ 7.88–7.10 (m, 29H, aromatic), 5.59 (m, 1H, $CH_2{=}C\mathit{H}{-}CH_2O$), 5.39 (d , 1H, $J_{1'',2''}$ = 8.2 Hz, H-1''), 5.18 (t, 1H, $J_{3'',4''} \sim J_{4'',5''}$ = 9.5 Hz, H-4''), 4.64 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1'), 4.20 (broad t, $J_{2',3'}$ = 2.1 Hz, H-2'), 2.14 and 2.05 (2s, each 3H, $C\mathit{H}_3CO$), and 0.86 [t, 3H, $C\mathit{H}_3(CH_2)_7O$]; ^{13}C NMR (75 MHz): δ 170.75 and 169.32 (CO), 116.33 ($\mathit{C}H_2{=}CH{-}CH_2O$), 103.96 (C-1), 97.82 (C-1'*), 96.53 (C-1''*), 60.99 (C-3''), 54.00 (C-2''), 20.85 and 20.69 ($COCH_3$), 14.13 [$\mathit{C}H_3(CH_2)_7O$].

Octyl 6-O-[2-O-(2-acetamido-4,6-di-O-acetyl-3-azido-2,3-dideoxy-β-D-glucopyranosyl)-6-O-allyl-3,4-di-O-benzyl-α-D-mannopyranosyl]-2,3,4-tri-O-benzyl-β-D-glucopyranoside (32). Ethylenediamine (1 mL) was added to a solution of compound **30** (30.2 mg, 0.022 mmol) in *n*-butanol (4 mL) under argon. The solution was stirred at 90 °C for 5 h. Evaporation followed by co-evaporation with toluene twice and ethanol twice gave a yellow syrup, which was dissolved in pyridine (3 mL) and acetic anhydride (1 mL). After 14 h at rt, ethanol (10 mL) was added and the solution was concentrated to dryness. The residue was purified by column chromatography using a gradient from toluene to toluene:EtOH (20:1) as eluent, to yield **32** (19.8 mg, 69.4 %, two steps), R_f 0.46 (7:1 toluene:EtOH); ^1H NMR (360 MHz in $CDCl_3$): δ 7.40–7.15 (m, 25H, aromatic), 6.06 (d, 1H, $J_{2'',NH}$ = 6.8 Hz, NH), 5.85 (m, 1H, $CH_2{=}C\mathit{H}{-}CH_2O$), 5.24 and 5.16 (2m, each 1H, $C\mathit{H}_2{=}CH{-}CH_2O$), 5.19 (d , 1H, $J_{1'',2''}$ = 8.5 Hz, H-1''), 4.38 (d , 1H, $J_{1',2'}$ = 7.6 Hz, H-1'), 4.27 (dd, 1H, $J_{5'',6''a}$ = 5.0 Hz, $J_{6''a,6''b}$ = 12.0 Hz, H-6''a), 4.21 (broad t, $J_{2',3'}$ = 1.9 Hz, H-2'), 4.16 (dd, 1H, $J_{5'',6''b}$ = 2.1 Hz, H-6''b), 2.12, 2.05 and 1.92 (3s, each 3H, $C\mathit{H}_3CO$), and 0.86 [t, 3H, $C\mathit{H}_3(CH_2)_7O$]; ^{13}C NMR (75 MHz): δ 172.35, 170.71 and 169.53 (CO), 117.13 ($\mathit{C}H_2{=}CH{-}CH_2O$), 103.94 (C-1), 98.06 (C-1'), 97.04 (C-1''), 61.18 (C-3''), 57.48 (C-2''), 23.47, 20.84 and 20.78 ($CO\mathit{C}H_3$), 14.13 [$\mathit{C}H_3(CH_2)_7O$].

Octyl 6-O-[2-O-(2-acetamido-3-amino-2,3-dideoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (5). To a solution of compound **32** (16.7 mg, 0.013 mmol), a mixture of palladium(II) chloride (7 mg, 0.039 mmol) and sodium acetate (6.5 mg, 0.079 mmol) in acetic acid:water (20:1, 3 mL), which was previously degassed and flushed with argon, was added under argon at rt. The reaction mixture was stirred at rt for 48 h, then dichloromethane (10 mL) and water (5 mL) were added and the resulting mixture was stirred vigorously for 30 min. After filtration of insoluble materials, the organic phase was separated and the aqueous solution was extracted with dichloromethane four times. The organic extracts were combined and concentrated to give a syrup which was purified by column chromatography using gradient toluene:Me_2CO (5:1) to yield **34** (5.5 mg, 34.3 %) [R_f 0.21 (3:1 toluene:Me_2CO)], which was then treated with sodium methoxide (5 mg, dispersion in mineral oil) in dry methanol (3 mL) at rt for 1 h. The reaction mixture was diluted with dichloromethane (20 mL), and washed with 0.5 % HCl and water, dried (Na_2SO_4) and concentrated to dryness. The resulting residue was purified by column chromatography using toluene:EtOH (10:1) as eluent to give **35** (5 mg, 98 %) [R_f 0.28 (7:1 toluene:EtOH)]. Compound **35** (5 mg, 4.4 µmol) was dissolved in aldehyde free ethanol (95 % ethanol was distilled from potassium hydroxide and zinc) (7 mL) and 0.01 N hydrochloric acid (0.9 mL, 9 µmol) containing 5 % palladium-on-carbon (10 mg) and stirred under hydrogen for 4 h. The catalyst was removed by filtration and the solution was neutralized with 0.01 N sodium hydroxide for 30 min, then carbon dioxide was bubbled through the solution. Solvent evaporation left a glass, which showed a major spot on TLC [R_f 0.04 (60:35:6 CH_2Cl_2:MeOH:H_2O)]. The resulting residue was purified as described for the preparation of **9**, to give **5** (2.7 mg, 94 %); NMR data are presented in Table 1; FABMS: m/z 679 $(M+Na)^+$ and 657 $(M+H)^+$.

Octyl 6-O-[2-O-(2,3-di-acetamido-2,3-dideoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-β-D-glucopyranoside (6). To a solution of **5** (0.8 mg, 1.2 µmol) in dry methanol (1 mL), acetic anhydride (70 µL, 7.5 µmol) and triethylamine (15 µL, 0.11 µmol) were added at rt. The reaction mixture was stirred for 14 h, solvents were removed and water then ethanol were added and evaporated. The resulting residue was purified as described for the preparation of **9**, to give **6** (0.9 mg, quantitative yield); R_f 0.54 (30:40:7 CH_2Cl_2:MeOH:H_2O); NMR data are presented in Table 1; FABMS: m/z 721 $(M+Na)^+$.

Enzymatic evaluation of 5, 6, 9 and 10 as GlcNAc T-V acceptors. Acceptor analogs **5**, **6**, **9** and **10** (0.04–10 nmol) were incubated for 3 h with GlcNAc T-V (4.5 µU) and UDP-GlcNAc (11 nmol, 60,000 dpm/nmol) in 50 mM sodium cacodylate, pH 6.5, with 20 % glycerol, 10 mM EDTA, 1 mg/mL BSA and 0.1 % Triton X-100 (final volume 10 µL). Reactions were quenched with water, and mixtures were transferred onto C_{18} Sep Pak

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*Assignments are tentative.

cartridges. Unreacted radiolabelled donor was removed by washing with water until background counts were obtained. Radiolabelled product was eluted with MeOH (2 × 3 mL) and quantitated by liquid scintillation counting.[26] Michaelis constants (K_m) shown in Table 2 were obtained by fitting rate data to the Michaelis-Menten equation[27] using unweighted nonlinear regression analysis (SigmaPlot 4.0).

Acknowledgments

This work was supported by National Sciences and Engineering Council of Canada through research grants to both M. M. P. and O. H. and a Postgraduate Scholarship to S. C. C. The Alberta Heritage Foundation for Medical Research also supported this work through a Post-Doctoral Fellowship (O. K).

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(*Received in U.S.A.* 15 *February* 1994; *accepted* 30 *May* 1994)