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Acceptor-Substrate Recognition by N-Acetyl-Glucosaminyltransferase-V: Role of the Mannose Residue in βDGlcNAc(1→2)αDMan(1→6)βDGlcOR

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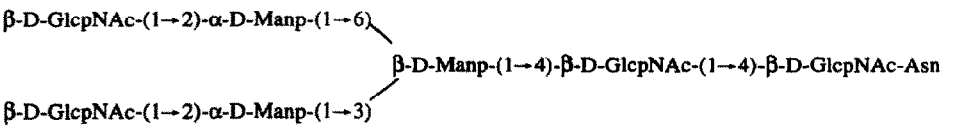
Abstract: βGlcNAc(1→2)αMan(1→6)βGlc-O(CH_2)$_7$$CH_3$ (**4**) is an acceptor specific for N-acetylglucosaminyltransferase-V (GlcNAcT-V), a branching enzyme controlling the biosynthesis of cell-surface Asn-linked oligosaccharides. Three analogs of **4**, where the central mannose residue has been O-methylated at O-3, at O-6, and where the 6-OH group was replaced by fluorine, were chemically synthesized, characterized by NMR-spectroscopy and kinetically evaluated as substrates for GlcNAcT-V. Along with results obtained using previously described derivatives of **4**, the conclusion is drawn that none of the OH groups on the Man residue are critical for recognition by the enzyme. These results should simplify the design of inhibitors for this important tumor-associated enzyme.

INTRODUCTION

N-acetylglucosaminyltransferase-V (GlcNAcT-V) is a key biosynthetic enzyme controlling the branching pattern of cell-surface complex Asn-linked oligosaccharides[1]. This enzyme transfers a GlcNAc residue from UDP-GlcNAc to the biantennary glycopeptide acceptor **1** to create the β(1→6)-branch present in the triantennary structure **2**. The special interest in GlcNAcT-V arises from observations that cell surface β(1→6) branching initiated by this enzyme correlates with the metastatic potential of tumor cells. The corollary is that inhibitors of GlcNAcT-V may show anti-metastatic activity[2].

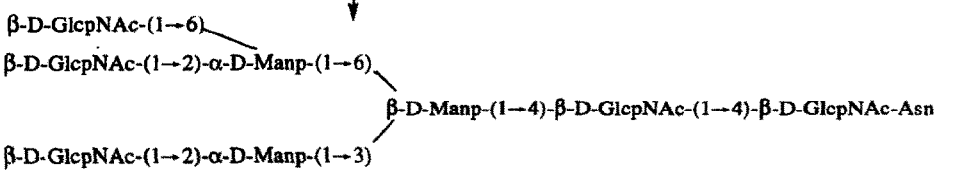
Knowledge of the detailed substrate specificity of GlcNAcT-V is essential for a rational approach to inhibitor design. While the heptasaccharide **1** is the smallest physiological substrate, the simple trisaccharide **3** was recognized early as a good acceptor[3]. Later, its β-*gluco*-analog **4** was found to be equivalent[4]. Derivatives of **4** have been reported with substitutions mainly on the βGlc[4a, 5] and βGlcNAc[6] residues as well

1



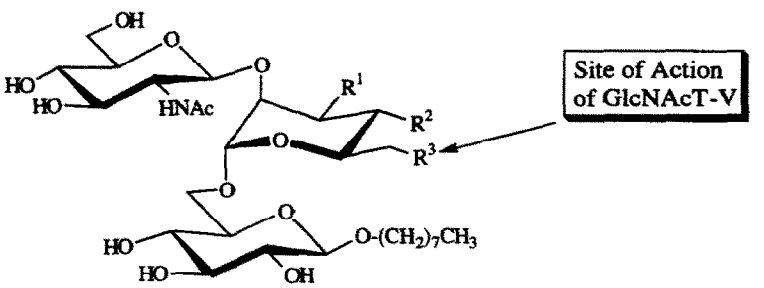
GlcNAcT-V | UDP-GlcpNAc

2



3 β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Manp-O-$(CH_2)_8CO_2CH_3$

4 β-D-GlcpNAc-(1→2)-α-D-Manp-(1→6)-β-D-Glcp-O-$(CH_2)_7CH_3$



	R^1	R^2	R^3	
5	OCH_3	OH	OH	(3'-O-Methyl)
6	OH	OCH_3	OH	(4'-O-Methyl)
7	OH	OH	OCH_3	(6'-O-Methyl)
8	OH	OH	H	(6'-Deoxy)
9	OH	OH	F	(6'-Fluoro)
10	OH	H	OH	(4'-Deoxy)

as some derivatives of the αMan[7] unit. We report here a continuation of these enzyme-specificity studies where the role of the central mannose residue of **4** in substrate recognition by GlcNAcT-V is probed through the synthesis and enzymatic analysis of a series of systematically modified mannose derivatives. Specifically, the syntheses of the new O-methylated trisaccharides **5** and **7**, and the 6-deoxy-6-fluoro derivative **9** are described. A summary of the NMR spectroscopic and enzyme kinetic data obtained with all six manno-derivatives **5**-**10** is also presented.

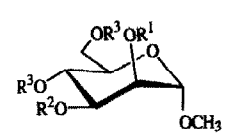
RESULTS AND DISCUSSION

For the synthesis of the title trisaccharide **5** we employed the known octyl 2,3,4-tri-O-benzyl-β–D-glucopyranoside **23**[4a] as a glycosyl acceptor and O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy–β–D-glucopyranosyl)-(1→2)-4,6-di-O-acetyl-3-O-methyl–α–D-mannopyranosyl chloride **22** as a glycosyl donor. Chloride **22** was readily prepared from O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy–β–D-glucopyranosyl)-(1→2)-1,4,6-tri-O-acetyl-3-O-methyl–α–D-mannopyranoside **21**. Compound **21** was obtained by condensation of 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido–β–D-glucopyranosyl bromide[8] **15** with methyl 4,6-di-O-benzyl-3-O-methyl–α–D-mannopyranoside **14**. The latter was obtained from methyl 2,3-O-isopropylidene–α–D-mannopyranoside[9] **11** through a succession of chemical steps. Thus, benzylation of compound **11** followed by cleavage of isopropylidine and subsequent methylation gave syrupy 3-O-methyl derivative **14** (81%).

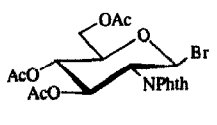
Glycosylation of **14** with bromide **15** promoted by silver trifluromethanesulfonate (triflate) and sym-collidine gave **16** (80%). Treatment of the disaccharide **16** with hydrazine hydrate, followed by acetylation, gave intermediate **17** (92%). Hydrogenolysis of benzyl groups of **17**, gave, after chromatographic purification, **18** (87%) which was acetylated (Py-Ac_2O) to afforded the hexacetate **20** (99%). This compound was subjected to acetolysis to furnish desired **21**. The ^{1}H-n.m.r. spectrum of **21** contained a low field signal at δ 6.04 (1H, *J* 2.0 Hz), suggesting that it existed almost exclusively as the α-D-anomer. A small portion of **18** was O-de-acetylated to afford **19** (91%). Treatment of a solution of **22** in dichloromethane containing acetic anhydride with HCl gave chloride **22** (94%).

Glycosylation of the acceptor **23** with chloride **22** promoted by mercuric cyanide and mercuric bromide gave the α/β mixture of protected trisaccharide derivatives **24** and **25**. These anomers could not be separated by silica gel chromatography but were directly hydrogenolysed (→**26**, **27**) and then acetylated to give the fully acetylated α-(**28**) and β-(**29**). The desired α anomer (18% from **23**) could then be obtained in pure form. This glycosylation reaction in the presence of silver triflate and sym-collidine provided similar α/β mixture of trisaccharides. Zemplén transesterification of **28** furnished **5** (92%). For characterization purposes, β-linked trisaccharide **29** was also deprotected to produce **30**, which was confirmed to be inactive as either acceptor or inhibitor for GlcNAc transferase V.

Glycosylation of **23** with known O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy–β–D-glucopyranosyl)-(1→2)-3,4-di-O-acetyl-6-O-methyl–α–D-mannopyranosyl chloride[10] **31** under Helfrich condition as described above provided the fully protected trisaccharide **32** (64%). Conventional deprotection of **32** proceeded *via* **33** to provide the trisaccharide glycoside **7** (94%).



	R^1	R^2	R^3
11	-CMe_2-		H
12	-CMe_2-		Bn
13	H	H	Bn
14	H	CH_3	Bn



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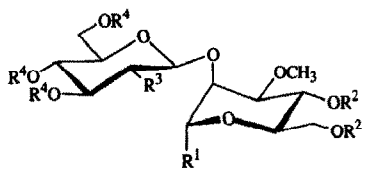
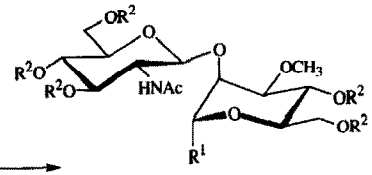
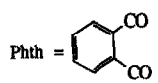


	R^1	R^2	R^3	R^4
16	OCH_3	Bn	NPhth	Ac
17	OCH_3	Bn	HNAc	Ac
18	OCH_3	H	HNAc	Ac

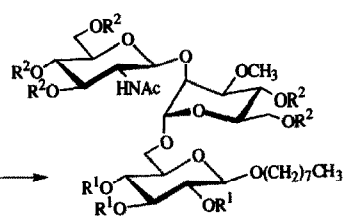


	R^1	R^2
19	OCH_3	H
20	OCH_3	Ac
21	OAc	Ac
22	Cl	Ac



Phth =

22



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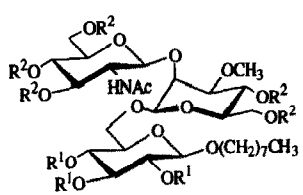
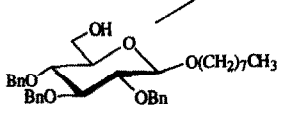
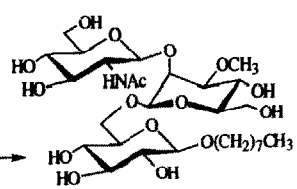




23

	R^1	R^2	
24	Bn	Ac	25
26	H	Ac	27
28	Ac	Ac	29



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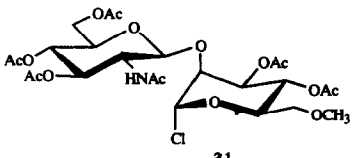
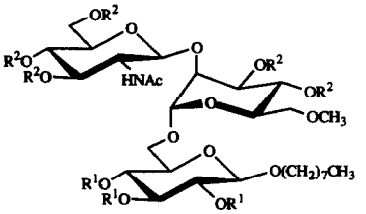
5



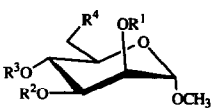
31

23



	R^1	R^2
32	Bn	Ac
33	H	Ac

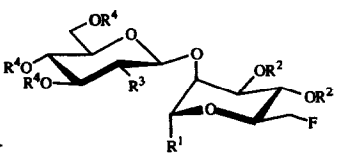
→ 7



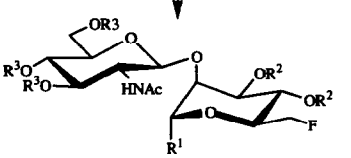
	R^1	R^2	R^3	R^4
34	$-CMe_2-$		Bn	OH
35	$-CMe_2-$		Bn	F
36	H	H	Bn	F
37	Bn	H	Bn	F
38	H	Bn	Bn	F

15

38



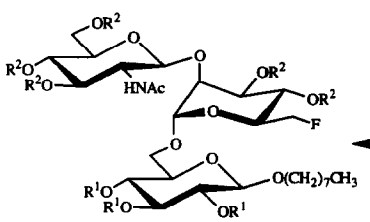
	R^1	R^2	R^3	R^4
39	OCH_3	Bn	NPhth	Ac
40	OCH_3	Bn	HNAc	Ac
41	OCH_3	H	HNAc	Ac



	R^1	R^2
42	OCH_3	H
43	OCH_3	Ac
44	OAc	Ac
45	Cl	Ac

23

45



	R^1	R^2
46	Bn	Ac
47	H	Ac

→ 9

For the synthesis of trisaccharide **9** we followed a strategy similar to that described above which required the preparation of suitably protected acceptor **38**. Thus, fluorination of **34** with DAST gave **35** (44%). Deisopropylidination of **35** gave **36** (93%) which was converted into desired **38** (55%)by treatment of its stannylene derivative with benzyl bromide. The reaction of bromide **15** with alcohol **38** promoted again by silver triflate and *sym*-collidine gave the (1→2)–β–linked disaccharide derivative **39** (98%) which was converted to the glycosyl chloride **45** in five steps, in a manner analogous to that described for conversion of **16** to **22**. The free disaccharide **42** (58%) was obtained by deprotection of a small portion of **41**.

Condensation of the acceptor **23** with the glycosyl donor **45** under conditions similar to those described for reaction of **31** with **23** gave the protected trisaccharide **46** (61%) from which benzyl and acetyl groups were removed sequentially to give trisaccharide **9** (67%). The structures of the synthetic intermediates were confirmed by NMR spectroscopy; the data are collected in Tables 3-7.

The fully-assigned NMR data for deblocked target trisaccharides **3** -**10** are collected in Tables 1 and 2. The similarity of chemical shifts of both protons and carbons, except of course in the vicinity of the chemical modifications, suggest that the overall trisaccharide conformations are similar. The H5-H6 coupling constants of the Glc residue (or the first Man residue in **3**), where observable, suggests that for all compounds both of the so-called "gg" and "gt "rotamers[11] are significantly populated, as has been determined previously through both conformational calculations and detailed NMR-studies[12]. Changes in the ratios of these two rotamers are indicated by the small differences in the coupling constants among the analogs **3** -**10**, but the changes are only modest. Independent evidence obtained using conformationally restricted trisaccharides suggests that only the gg rotamer is bound by the enzyme[13].

Trisaccharides **4** -**10** were evaluated as potential acceptors and inhibitors for GlcNAcT-V using a partially purified preparation from hamster kidney and an established radiochemical assay[7] (Table 8). Compounds **4**[3a], **5**, **6**[7b] and **10**[7b] all retain the 6-OH group to which the enzyme might transfer a GlcNAc residues. The Km values for the 3-O-methyl (**5**) and 4-deoxy trisaccharide (**10**) were slightly higher than for the non-modified acceptor **4** but both remained excellent substrates. The 4-O-methyl derivative also retains the potentially-reactive 6-OH group but it does not react, an observation that has been attributed[7b] to steric-interference of the actual transfer reaction by the O-methyl group. It is a competitive inhibitor with Ki = 14 μM. The remaining derivatives **7**, **8**[7a] and **9** no longer possess the required 6-OH group and therefore cannot react. They are still recognized and bound by the enzyme, however, since they were all found to be competitive inhibitors with Ki values in the range 24-70 μM.

The conclusion of this work is that none of the OH groups of the mannose residue in **4** contribute importantly to acceptor recognition by GlcNAcT-V. This was also found to be the case for the OH groups on the Glc residue[4a, 5]. In contrast, all three OH groups on the terminal GlcNAc residues were found to be essential[6]. Taking these accumulated results into account, our strategy for the design of inhibitors for GlcNAcT-V will shift towards the generation of carbohydrate mimics where only the essential three OH-groups are present.

TABLE 1.

^{1}H Chemical shifts of compounds **3 - 10** obtained at 500 MHz with coupling constants given in parenthesis.

			Native	3'-OMe	4'-OMe	6'-OMe	6'-deoxy	6'-F	4'-deoxy
		3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
GlcNAc	H1	4.56 (8.4)	4.55 (8.4)	4.54 (8.4)	4.55 (8.4)	4.54 (8.4)	4.53 (8.3)	4.55 (8.4)	4.57 (8.4)
	H2	3.69 (10.4)	3.69 (10.2)	3.66	3.70 (10.5)	3.68 (10.5)	3.69 (8.4)	3.69	3.71
	H3	3.54 (8.4)	3.53 (9.6)	3.54 (9.8)	3.54 (8.5)	3.53 (8.5)	3.53 (10)	3.53	3.55
	H4	3.44 (9)	3.44	3.47 (9.8)	3.46	3.44	3.45 (9.6)	3.45	3.44 (9.9)
	H5	3.41	3.40	3.39	3.41	3.40	3.40	3.40	3.42
	H6A	3.89 (2.5 12.4)	3.90 (2.2 12.0)	3.89 (2.2 12.3)	3.89 (<3 12.3)	3.89 (2.2 12.6)	3.89 (2.3)	3.89 (2.1 12.4)	3.90
	H6B	3.74 (5.3)	3.74 (5.4)	3.76 (5.3)	3.76 (5.7)	3.74 (5.5)	3.74 (5.7)	3.74	3.74
	NAc	2.04	2.03	2.03	2.05	2.03	2.03	2.02	2.04
Man	H1	4.90 (1.6)	4.88 (1.7)	4.91 (1.5)	4.86 (1)	4.86 (1)	4.83 (1.3)	4.93 (1.2)	4.92 (<1)
	H2	4.10 (3.4)	4.10 (3.4)	4.32	4.08 (3.3)	4.10 (3.4)	4.10 (3.5)	4.14 (3.4)	3.93 (3.0)
	H3	3.83 (9.6)	3.82 (9.6)	3.53	3.88 (9.6)	3.81 (9.8)	3.76 (9.8)	3.84	4.05
	H4	3.49 (9.6)	3.51 (9.6)	3.54 (10)	3.28	3.46	3.33 (9.4)	3.67	1.53 (4.0 12.5)
	H4b								1.66 (11.9)
	H5	3.65	3.62	3.66	3.62	3.76	3.69	3.78 (2.9 27)[a]	3.89
	H6A	3.89 (2.0 11.7)	3.88	3.88 (2 11)	3.85 (<2 10)	3.72 (2.0 11.0)	1.24 (8.3)	4.65 (2.7 47.5)[a]	3.60 (3.2 11.8)
	H6B	3.60 (7.2)	3.63	3.60 (7.5)	3.65 (6.8)	3.54 (7.8)		4.65 (2.7 47.5)[a]	3.52 (6.8)
OCH3				3.43	3.50	3.38			
Glc	H1	4.64 (0.8)	4.44 (8.0)	4.44 (8.0)	4.43 (8.0)	4.43 (8.0)	4.43 (8.0)	4.43 (8.0)	4.43 (8.0)
(or Man)	H2	3.96 (3.1)	3.24 (9.8)	3.24	3.23 (8.9)	3.23 (9.2)	3.23 (9.0)	3.22 (9.3)	3.23 (8.9)
	H3	3.60 (9.6)	3.48	3.46	3.45	3.45	3.45	3.44	3.45 (8.8)
	H4	3.65 (9.5)	3.45	3.45	3.44	3.44	3.45	3.56	3.42 (9.2)
	H5	3.47	3.54	3.57	3.55	3.56	3.55	3.56	3.57
	H6A	3.94 (5.4 11.2)	3.94 (4.8 12.1)	3.93 (5.2 11.2)	3.89 (5 10.8)	3.91 (4.8 11.2)	3.89	3.91 (5.0 11.2)	3.89
	H6B	3.76 (1.9)	3.72 (2.0)	3.75 (1.9)	3.74 (2)	3.72 (<3)	3.73	3.75	3.73
OR	H1A	3.83	3.88	3.87		3.86	3.86	3.85	3.84
	H1B	3.64	3.66	3.67		3.65	3.65	3.65	3.69

[a] The second coupling constant given is between the protons and fluorine

TABLE 2.

^{13}C Chemical shifts of compounds **3** - **10** obtained at 125.77 MHz

			Native	3'-OMe	4'-OMe	6'-OMe	6'-deoxy	6'-F [a]	4'-deoxy
		3	**4**	**5**	**6**	**7**	**8**	**9**	**10**
GlcNAc	C1	100.3	100.5	100.3	100.3	100.2	100.1	99.8	100.3
	C2	56.2	56.4	56.1	56.2	56.1	56.1	56.1	56.2
	C3	74.2	74.3	74.3	74.2	74.2	74.1	74.1	74.2
	C4	70.7	70.9	70.5	70.7	70.7	70.7	70.7	70.8
	C5	76.6	76.8	76.6	76.6	76.6	76.6	76.6	76.6
	C6	61.4	61.6	61.4	61.4	61.4	61.4	61.4	61.5
	NAc	175.6						175.6	
	NAc	23.1	23.0	23.1	23.1	23.1	23.1	22.9	23.1
Man	C1	97.6	97.8	97.6	97.4	97.6	97.6	97.7	98.2
	C2	77.0	77.2	72.7	77.3	76.7	76.8	76.3	75.1
	C3	70.4	70.6	79.4	70.4	70.3	70.2	70.3	65.2
	C4	68.1	68.2	66.8	78.2	68.7	72.9	66.4 (6.3)	30.5
	C5	73.6	73.8	73.6	72.7	71.7	69.5	72.3 (17.3)	70.1
	C6	62.4	62.5	62.4	62.0	72.9	17.6	83.0 (168.4)	65.2
OMe				57.0	61.1	59.1			
Glc	C1	100.8	103.3	103.1	103.1	103.1	103.1	103.1	103.2
(or Man)	C2	71.4	74.1	73.9	73.9	73.9	73.9	73.9	73.9
	C3	74.0	77.0	76.8	76.8	76.8	76.8	76.8	76.8
	C4	67.5	70.4	70.3	70.3	70.3	70.3	70.2	70.5
	C5	75.2	75.1	74.8	74.9	74.9	74.9	74.9	74.9
	C6	66.9	66.7	66.7	66.7	66.7	66.7	66.8	66.6
OR	C1	70.9	71.8	71.6	71.6	71.6	71.6	71.6	71.8

[a] Values in parenthesis are coupling constants between carbon and fluorine.

TABLE 3.

Selected ^{1}H- and ^{13}C-n.m.r. data for protected monosaccharides.[a]

	Chemical shifts (δ) and coupling constants (Hz)						
Nucleus	**12**	**13**	**14**	**35**	**36**	**37**	**38**
H-1 ($J_{1,2}$)	4.95	4.74 (1.5)	4.76 (2.0)	4.93	4.73 (1.25)	4.78 (1.5)	4.77 (1.25)
OCH_3-1	3.38	3.35	3.33	3.36	3.36	3.35	3.32
OCH_3-3	-	-	3.44	-	-		
$(CH_3)_2$	1.36, 1.50	-	-	1.35, 1.49	-	-	-
C-1	98.28	100.64	100.36	98.30	100.74	100.37	98.04
C-6 ($J_{C6,F6}$)	69.33	68.94	69.05	82.31 (172.07)	82.15 (172.82)	82.19 (172.82)	82.28 (182.82)
OCH3-1	54.85	54.98	54.91	54.87	55.10	54.99	54.88
OCH3-3	-	-	57.38	-	-	-	-
$C(CH_3)_2$	26.30, 27.97	-	-	26.21, 27.89		-	-
$C(CH_3)_2$	109.33	-	-	109.44		-	-
CH_2Ph	72.79, 73.47	73.62, 74.64	73.47, 74.93	72.79	74.93	72.04, 75.22	72.93, 74.90
Quat. Arom.	138.32, 138.42	138.05, 138.37	138.32, 138.52	137.98	138.07	137.79, 138.10	137.55, 138.21

[a]Spectra were recorded at 300 MHz (^{1}H in $CDCl_3$) and 75.5 MHz (^{13}C in $CDCl_3$).

TABLE 4.

Selected ^1H chemical shifts and coupling constants for protected and unprotected disaccharides.[a]

	Chemical shifts (δ) and coupling constants (Hz)						
Compound	H-1 ($J_{1,2}$)[b]	H-1' ($J_{1,2}$)[c]	OCH_3-1	OCH_3-3	NAc	OAc	NH ($J_{2,NH}$)
16	4.51 (2.0)	5.49 (8.5)	3.23	3.41	-	1.86 (6H), 2.04 (3H), 2.1 (3H)	-
18	4.64 (1.5)	4.92 (8.25)	3.37	3.43	1.95	2.03 (3H), 2.05 (6H)	-
19	n.d.[d]	4.54 (8.5)	3.40	3.43	2.01	-	-
20	4.68 (1.5)	5.21 (8.5)	3.34	3.34	1.92	1.98 (3H), 2.01 (3H), 2.02 (3H) 2.04 (6H)	5.92 (8.5)
21	6.04 (2.0)	5.12 (8.2)	-	3.37	1.93	1.96 (3H), 1.97(3H), 2.02 (3H) 2.04 (6H), 2.09 (3H)	6.65 (7.0)
22	6.10 (1.5)	5.33 (8.5)	-	3.41	1.95	2.01 (3H), 2.05 (3H), 2.06 (3H) 2.08 (3H), 2.09 (3H)	6.17 (7.5)
40	4.69 (1.5)	5.19 (8.5)	3.33	-	1.91	2.02 (9H)	-
41	4.68 (1.2)	4.98 (8.5)	3.36	-	1.96	2.03 (3H), 2.05 (3H), 2.09 (3H)	-
42	4.59 (1.5)	4.56 (8.0)	3.41	-	2.03	-	-
43	4.66 (2.0)	5.10 (8.5)	3.40	-	1.95	1.98 (3H), 2.02 (3H), 2.03 (3H) 2.05 (3H), 2.07 (3 H)	6.09 (7.5)
45	6.02 (1.5)	5.24 (8.0)	-	-	1.97	2.01 (3H), 2.05 (6H), 2.08 (3H) 2.09 (3H)	6.09 (7.5)

[a]For solutions in $CDCl_3$ at 300 MHz except for compound **19** and **42** which were recorded in D_2O. [b]Unprimed locants are used for protons in the reducing-end residue (α-D-Manp). [c]Of the nonreducing-end residue (β-D-GlcpNAc). [d]Could not be determined due to spectral overlap.

TABLE 5.

Proposed NMR signal assignments for key C atoms of protected and unprotected disaccharides.[a]

	Chemical shifts (δ)[b] and coupling constants (Hz)[c]									
Compound	C-1	C-1'	C-2	C-2'	C-6	C-6'	OCH_3-1	OCH_3-3	$COCH_3$	OCH_3
16	96.68	97.99	80.14	54.40[d]	69.86	62.42	54.83[d]	56.83	169.50, 170.24, 170.68	20.54, 20.70, 20.80
18	98.76	99.26	79.18	55.37[e]	62.60[f]	62.11[f]	55.08[e]	55.97	169.43, 170.57, 170.82 171.32	20.65, 20.72, 23.36
19	98.87	100.36	76.66	55.72	62.45	61.50	56.18	57.11	175.52	23.17
20	97.59	98.35	76.86	55.54	63.18	62.23	54.99	56.59	169.35, 169.67, 170.32 170.46, 170.62, 171.16	20.52, 20.58, 20.65 20.79, 23.08
21	91.11	98.42	76.78	55.13	62.73	62.04	-	56.92	168.17, 169.39, 169.50 170.56, 170.64, 170.83 171.08	20.64, 20.70, 20.76 20.88, 21.00, 23.20
22	90.19	97.62	75.71	55.56	62.29[g]	62.04[g]	-	56.90	169.40, 169.59, 170.49 170.53, 170.63, 171.29	20.61, 20.72, 20.83 23.10
40	97.79	98.36	78.06	56.14	82.30 (172.14)	62.47	55.07	-	169.63, 170.35, 170.65	20.73, 23.31
41	98.75	99.26	77.99	55.00[h]	82.36 (171.31)	61.97	55.33[h]	-	169.47, 170.68, 170.77 171.30	20.60, 20.68, 23.22
42	98.92	99.88	76.68	55.88	83.09 (168.30)	61.45	56.12	-	175.66	22.92
43	98.23[i]	98.46[i]	73.89	56.00	81.86 (173.58)	62.17	55.25	-	169.55, 169.68, 170.36 170.65, 171.13	20.64, 20.70, 20.73 23.23
45	89.21	97.86	76.03	55.94	80.85 (175.84)	62.02	-	-	169.39, 169.50, 170.32 170.47, 170.62, 171.34	20.69, 23.14

[a]For solutions in $CDCl_3$ at 75.5 MHz except for compound **19** and **42** for which solvent was D_2O. [b]Locants: Unprimed, reducing-end residue (α-D-Manp); primed, nonreducing-end residue (β-D-GlcpNAc). [c]$J_{F6,C6}$ (in parentheses). [d,e,f,g,h,i]Values with similar superscripts may be interchanged.

TABLE 6.

Selected ^1H- and ^{13}C-n.m.r. data for trisaccharides.[a]

Nucleus[b]	Chemical shifts (δ) and coupling constants (Hz)				
	28	**29**	**30**	**32**	**46**
H-1 ($J_{1,2}$)	4.47 (8.0)	4.66 (8.0)	4.82 (obsc.)	5.61 (7.8)	4.41 (7.5)
H-1' ($J_{1',2'}$)	4.81 (2.0)	4.42	4.67	4.83 (1.5)	4.83 (1.2)
H-1" ($J_{1",2"}$)	5.25 (8.2)	n.d[c]	4.52 (8.5)	4.40 (8.4)	5.61 (obsc.)
OCH_3-3	3.38	3.38	3.46	-	-
OCH_3-6	-	-	-	3.30	-
NAc	1.90	1.92	2.08	1.93	1.92
CH_2-CH_3	0.85 (6.5)	0.87 (6.5)	0.85 (6.5)	0.87 (6.5)	0.87 (7.0)
C-1 ($J_{C1,H1}$)	101.3 (160.5)	101.29[d]	103.40	103.86	103.85
C-1' ($J_{C1',H1'}$)	97.72 (170.6)	101.16[d]	102.46	97.73	97.57[e]
C-1" ($J_{C1",H1"}$)	97.26 (166.6)	101.10[d]	101.61	98.14	97.74[e]
C-2'	73.06	73.19	77.23	77.74	77.72
C-2"	56.29	54.40	56.44	55.91	56.21
C-6	65.66	67.62	68.10	66.78	66.96
C-6'($J_{F6',C6'}$)	63.16	62.48	62.66	71.86	81.58 (174.86)
C-6"	62.20	62.48	61.51	62.21	62.21
OCH_3-3	57.05	55.99	56.93	-	-
OCH_3-6	-	-	-	59.28	-
CH_2-CH_3	14.06	14.12	14.27	14.11	14.10
OCH_2-CH_2	70.52	70.66	71.98	70.55	70.56

[a]Spectra were recorded at 300 MHz (^1H in $CDCl_3$, compounds **28**, **29**, **32** and **46** and in D_2O for **30**) and 125.7 MHz (^{13}C in $CDCl_3$ for compounds **28** and in D_2O for **30**) or 75.5 MHz (^{13}C in $CDCl_3$ for compounds **29**, **32** and **46**). [b]Locants: Unprimed, β-D-Glcp; single primed, α-D-Manp, double primed, β-D-GlcpNAc. [c]Could not be determined due to spectral overlap. [d,e]Values with similar superscripts may be interchanged.

TABLE 7.

^{19}F Chemical shifts (δ) and coupling constants (Hz) for mono- di- and trisaccharides.[a]

Compound	δ	$J_{F\text{-}6,H6a,6b}$	$J_{F6,H\text{-}5}$
9	-235.36	47.08	26.36
35	-234.42	47.50	25.67
40	-233.76	47.73	27.60
42	-235.02	47.08	26.99
46	-232.46	47.03	24.48

[a]Spectra were recorded at 188.3 MHz (In $CDCl_3$ for compounds **35** and **46** and in D_2OD for **9**) or 376.5 MHz (In $CDCl_3$ for compound **40**).

Table 8. Kinetic Parameters for **5** - **10**.

Compound	Mannose Modification	K_m (μM)	V_{max} (rel)	K_i (μM)
4	None	26	100	--
5	3-O-methyl	60	47	--
6	4-O-methyl	--	--	14
7	6-O-methyl	--	--	70
8	6-deoxy	--	--	30
9	6-fluoro	--	--	24
10	4-deoxy	74	460	--

EXPERIMENTAL

General Materials and Methods- EDTA, MES, UDP and UDP-GlcNAc were obtained from Sigma. Triton X-100 was from Calbiochem or Sigma. Liquid scintillation cocktail was from ICN (Ecolite(+)) or from Fisher (ScintiverseE). Millex-GV (0.22 μm) filter units were from Millipore. Reverse-phase C18 SepPak cartridges from Waters Associates were pre-equilibrated with 20 mL methanol and 30 mL water before use. UDP-[6-^{3}H(N)]GlcNAc was obtained from American Radiolabelled Chemicals Inc.; in order to reduce background values obtained in radioassays, this material was lyophilized, passed through a C18 SepPak cartridge pre-equilibrated with water, and then re-lyophilized and dissolved in ethanol:water (70:30) for later use. Hamster kidneys were obtained from Pel Freez Biologicals, Rogers, Arkansas. Other materials were of reagent grade.

Optical rotations were measured at 22±2 with a Perkin-Elmer 241 polarimeter. T.l.c. was conducted on glass plates precoated with 250 μm layers of silica gel 60-F_{254} (Whatman); the compounds were located by quenching of fluorescence and/or by charring with 5% sulfuric acid. Column chromatography was performed on silica gel 60 (Merck, 40-63 μm). Iatrobeads refers to a bead silica gel from Iatron Laboratories (Tokyo). The following solvent systems (v/v) were used for chromatography: **A** hexane-ethyl acetate (4:1), **B** hexane-ethyl acetate (3:2), **C** hexane-ethyl acetate (1:1), **D** dichloromethane-methanol (93:7), **E** dichloromethane-methanol (19:1), **F** dichloromethane-methanol (9:1), **G** dichloromethane-methanol (2:1), **H** chloroform-methanol-water (13:6:1), **I** dichloromethane-methanol (97:3), **J** dichloromethane-acetone (4:1), **K** dichloromethane-acetone (5:1), **L** toluene-ethyl acetate-methanol (10:4:0.75), **M** toluene-ethyl acetate-methanol (10:4:1), **N** hexane-ethyl acetate-methanol (70:70:6), **O** ethyl acetate-hexane (2:1), **P** hexane-ethyl acetate (9:1), **Q** dichloromethane-acetone (93:7), **R** hexane-ethyl acetate (3:1), **S** hexane-ethyl acetate (2:1), **T** dichloromethane-methanol (32:1), **U** ethyl acetate-hexane (3:2). ^{1}H NMR spectra were recorded at 300 (Bruker WH-300) or 500 MHz (Varian Unity 500 or Bruker AM-500) on solutions in $CDCl_3$ (internal Me_4Si, δ 0) or D_2O (external Me_4Si, δ 0 or internal acetone, δ 2.225) at ambient temperature. ^{13}C NMR spectra were recorded at 75.5 MHz (Bruker AM-300) or 125.7 MHz (Varian Unity 500 or Bruker AM-500) on solution in $CDCl_3$ (internal Me_4Si, δ 0) or D_2O (1,4-dioxane in D_2O, δ 67.4). ^{19}F NMR spectra were recorded at 188.3 MHz (Bruker WH-200) or 376.5 MHz (Bruker WH-400) on solution in $CDCl_3$ or D_2O (internal $CFCl_3$, δ 0). Only partial NMR data are reported, the other data were in accord with the proposed structures. The chemical shifts and coupling constants (as observed splittings) for ^{1}H resonances are reported as though they were first order. The assignments of ^{13}C chemical shifts are tentative and were assigned based on comparison with published spectral data[7b,10,14]. F.a.b.-mass spectra were obtained using an AEI MS-9 instrument with xenon as the bombarding gas with 1,4-dithiothreitol-1,4-dithioerythritol (5:1) as matrix. Unless otherwise indicated, all reactions were carried out at ambient temperatures, and in the work-up, solutions in organic solvents were washed with equal volumes of aqueous solutions. Organic solutions were generally dried (anhydrous Na_2SO_4) prior to concentration on a rotary evaporator under the vacuum of a water aspirator with bath temperature of 40-50 °C. Elemental analyses were performed by the Analytical Services Laboratory of this department.

Methyl 2,3-O-isopropylidene-4,6-di-O-benzyl-α-D-mannopyranoside (**12**).- To a stirred solution of methyl 2,3-O-isopropylidene-α-D-mannopyranoside[9] (**11**; 3.2 g, 13.66 mmol) in *N,N*-dimethylformamide (80 mL) was added NaH (2.5 g, 80%) portionwise, and stirring was continued for 0.5 h at room temperature

and then benzyl bromide (8 mL, 67.26 mmol) was added, and the stirring was continued for 24 h at the same temperature. After careful addition of methanol to decompose excess NaH, the solvent was evaporated and the residue dissolved in chloroform. The chloroform solution was successively washed with water, aqueous $NaHCO_3$, and water, dried, and concentrated. The crude product was chromatographed (solvent A) to give **12** as a syrup (3.44 g, 61%), $[\alpha]_D$ +33.3 (c 4.8, chloroform), R_F 0.52 (solvent A). *Anal.* Calc. for $C_{24}H_{30}O_6$ (414.50): C, 69.55; H, 7.30. Found: C, 69.86; H, 7.28.

Methyl 4,6-di-O-benzyl–α–D-mannopyranoside (**13**).- A solution of **12** (3.38 g, 8.15 mmol) in chloroform (45 mL) containing trifluroacetic acid (5 mL) and water (0.4 mL) was stirred for 4 h at room temperature. It was then concentrated, and several portions of toluene were added to, and evaporated from the residue. The crude product was purified by chromatography (solvent B→C) to provide **13** as a white solid, (2.63 g, 86%), $[\alpha]_D$ +76.3 (c 1.3, chloroform), {lit.[15] $[\alpha]_D$ +74.7 (c 1, chloroform)}, R_F 0.28 (solvent C). *Anal.* Calc. for $C_{21}H_{26}O_6$ (374.44): C, 67.36; H, 7.00. Found: C, 67.66; H, 7.08.

Methyl 3-O-methyl-4,6-di-O-benzyl–α–D-mannopyranoside (**14**).-A solution of **13** (2.22 g, 5.92 mmol) and dibutyltin oxide (1.47 g, 5.91 mmol) in methanol (35 mL) was boiled for 2 h. The solvent was then evaporated to dryness to give a residue which was dissolved in toluene (35 mL), tetrabutylammonium iodide (2.35 g, 5.93 mmol), and methyl iodide (35 mL, 562 mmol) was added. The mixture was stirred for 16 h at 60 °C, and then poured into water and extracted with dichloromethane. The dichloromethane solution was washed several times with water, dried, and concentrated. The residue was purified by chromatography (solvent B) to provide **14** as a yellow syrup (1.87 g, 81%), $[\alpha]_D$ +82.9 (c 2.9, chloroform), R_F 0.42 (solvent C). *Anal.* Calc. for $C_{22}H_{28}O_6$ (388.46): C, 68.02; H, 7.27. Found: C, 67.62; H, 7.37.

Methyl O-(2-phthalimido-3,4,6-tri-O-acetyl-2-deoxy–β–D-glucopyranosyl)-(1→2)-3-O-methyl-4,6-di-O-benzyl–α–D-mannopyranoside (**16**).-To a solution of alcohol **14** (1.82 g, 4.69 mmol) was added silver trifluoromethanesulfonate (6.0 g, 23.35 mmol), *sym* collidine (3.1 mL, 23.35 mmol), and pulverized activated molecular sieves (4Å, 7.8 g). To the resulting mixture cooled to -50 °C was added dropwise, a solution of bromide **15** (3.5 g, 7.02 mmol) in dichloromethane (25 mL). The above mixture protected from light was stirred at -50 °C for 15 min in an atmosphere of argon and then allowed to warm to room temperature over a period of 2 h, by which time t.l.c. (solvent C) showed the complete disappearance of **14** and **15** and the formation of a new slower migrating product. It was then diluted with dichloromethane (200 mL), and the solids were filtered off (Celite) and washed with dichloromethane. The filtrate and washings were combined, successively washed with cold water, ice cold 5% HCl, saturated aqueous $NaHCO_3$, and water. Evaporation of the solvent and purification of the residue by chromatography (solvent B) gave **16** (3.0 g, 80%), as an amorphous, $[\alpha]_D$ 5.1 (c 1, chloroform), R_F 0.53 (solvent C). *Anal.* Calc. for $C_{42}H_{47}NO_{15}$ (805.84): C, 62.60; H, 5.88; N, 1.74. Found: C, 62.30; H, 5.69; N, 1.72.

Methyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy–β–D-glucopyranosyl)-(1→2)-3-O-methyl–α–D-mannopyranoside (**18**).-A mixture of **16** (2.20 g, 2.73 mmol) and 85% hydrazine-hydrate (21 mL) was heated to reflux in ethanol (85 mL) for 3 h. The reaction mixture was then taken to dryness and the residue was dissolved in pyridine (100 mL) and acetic anhydride (50 mL) was added. After stirring overnight at room temperature, the solvent was evaporated, and the solution of the residue in chloroform (200 mL) was successively washed with water, aqueous $NaHCO_3$, and water. Evaporation of the solvent and purification of the residue by chromatography (solvent D) gave **17** (1.8 g, 92%), as an amorphous solid, R_F 0.29 (solvent D), which was sufficiently pure for the next step.

A mixture of **17** (1.8 g, 2.51 mmol) and 10% Pd-C (1.8 g) in 95% ethanol (70 mL) and glacial acetic acid (5 mL) was stirred under one atmosphere H_2 for 18 h at room temperature. The suspension was filtered (Celite), the solid was thoroughly washed with methanol, and the filtrate and washings were combined and concentrated. The residual syrup was purified by chromatography (solvent E→F) to yield **18** as a white solid (1.17 g, 87%), $[\alpha]_D$ -13 (c 0.6, chloroform), R_F 0.47 (solvent F). *Anal.* Calc. for $C_{22}H_{35}NO_{14}$ (537.52): C, 49.16; H, 6.56; N, 2.61. Found: C, 49.13, H, 6.69, N, 2.93.

Methyl O-*(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-3-O-methyl-α-D-mannopyranoside* (**19**).-Compound **18** (80.5 mg, 0.15 mmol) in 0.1 M methanolic sodium methoxide (6 mL) was stirred overnight at room temperature. The base was neutralized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (Celite) and thoroughly washed with methanol, and the filtrate and washings were combined and concentrated to give a material which was chromatographed (solvent G) to give a solid residue. This material was dissolved in water and filtered through a 0.22 μm Millex filter and then lyophilized to give **19** (55.8 mg, 91%), as a white powder, R_F 0.37 (solvent H). FAB-MS: *m/z* 412 [3.3%, $(M+1)^+$] and 434 [100%, $(M+Na)^+$].

Methyl O-*(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-3-O-methyl-4,6-di-O-acetyl-α-D-mannopyranoside* (**20**).- A solution of **18** (1.05 g, 1.95 mmol) in 1:2 acetic anhydride-pyridine (30 mL) was stirred overnight at room temperature, the solvent was evaporated under high vacuum, and toluene was evaporated from the residue to give a material which was passed through a short column of silica gel (solvent I) to provide **20** as a white solid (1.2 g, 99%), $[\alpha]_D$ +8 (c 1.4, chloroform), R_F 0.13 (solvent I). *Anal.* Calc. for $C_{26}H_{39}NO_{16}$ (621.60): C, 50.24; H, 6.32; N, 2.25. Found: C, 50.00; H, 6.38; N, 2.29.

O-*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-1,4,6-tri-O-acetyl-3-O-methyl-α-D-mannopyranose* (**21**).-A solution of compound **20** (1.1 g, 1.77 mmol) in acetic anhydride (30 mL) containing 1% (v/v) of conc. H_2SO_4 was stirred for 12 h at room temperature. The mixture was then diluted with dichloromethane (300 mL), successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated. The residue was chromatographed (solvent I) to give **21** as a white solid (0.61g, 53%), $[\alpha]_D$ +7 (c 2.2, chloroform), R_F 0.52 (solvent J). FAB-MS: *m/z* 650 [1.2%, $(M+1)^+$] and 672 [5.6%, $(M+Na)^+$]. *Anal.* Calc. for $C_{27}H_{36}NO_{17}$ (649.61): C, 49.92; H, 6.05; N, 2.16. Found: C, 49.80; H, 6.06; N, 2.15.

O-*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-1,3,4-tri-O-acetyl-3-O-methyl-α-D-mannopyranosyl chloride* (**22**).- Hydrogen chloride gas was passed for 15 min through a tube of Drierite into a solution of **21** (105 mg, 0.16 mmol) in dichloromethane (4 mL) and acetic anhydride (0.5 mL) at O °C. After 0.5 h, the solution was warmed to room temperature where it was kept for 12 h. The solution was then taken to dryness and the by-product acetic acid was removed by co-evaporation with toluene to give a residue which was chromatographed (solvent K) to give **22** as an amorphous solid (95 mg, 94 %), $[\alpha]_D$ +35.5 (c 1.2, chloroform), R_F 0.36 (solvent J). *Anal.* Calc. for $C_{25}H_{36}ClNO_{15}$ (626.02): C, 47.97; H, 5.80; Cl, 5.66; N, 2.24. Found: C, 48.08; H, 5.87; Cl, 5.99; N, 2.24.

Octyl O-*(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(3-O-methyl-4,6-di-O-acetyl-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranoside* (**28**) and *Octyl* O-*(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(3-O-methyl-4,6-di-O-acetyl-β-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-β-D-glucopyranoside* (**29**).-To a stirred solution of octyl 2,3,4-tri-O-benzyl-β-D-glucopyranoside[4a] **23** (26.7 mg, 0.047 mmol) in dry nitromethane (0.5 mL) containing powdered 4Å molecular sieves (75 mg), mercuric cyanide (22 mg, 0.088 mmol) and mercuric bromide (32

mg, 0.088 mmol) was added, dropwise, a solution of chloride **22** (55 mg, 0.088 mmol) in dry nitromethane (1.5 mL) at O °C under dry argon. After stirring at O °C for 30 min. the reaction was slowly allowed to warm to room temperature and stirred for 15 h. The mixture was then diluted with dichloromethane (100 mL) and filtered (bed of Celite), the solids were thoroughly washed with dichloromethane and the filtrate and washings were combined. The solution was successively washed with water, M KI solution, and water, dried and concentrated. The resulting syrup was chromatographed (solvent L) to give first unchanged **23** (8 mg), followed by a mixture of **24** and **25** (20.8 mg) [R_F 0.31 and 0.26 (solvent M)].

A solution of the above mixture in 95% ethanol (2 mL) and glacial acetic acid (0.2 mL) in the presence of 10% Pd-C (30 mg) was stirred under one atmosphere H_2 for 24 h at room temperature. The suspension was processed as described for the preparation of **18**, and the residual syrup (14.7 mg, containing **26** and **27**) was acetylated (2:1 Py-Ac_2O, 3 mL) and processed in the usual manner to give a solid residue which was purified by chromatography Iatrobeads (solvent N) to yield first **28** as a white solid (6 mg, 18% based on reacted **23**), $[\alpha]_D$ +17.3 (c 0.2, chloroform), R_F 0.2 (solvent N) *Anal.* Calc for $C_{45}H_{69}NO_{24}$ (1008.04): C, 53.62; H, 6.90; N, 1.39. Found: C, 53.71; H, 7.08; N, 1.49.

Compound **29** (8.3 mg, 25% based on reacted **23**), eluted next, was also obtained as a solid, $[\alpha]_D$ -42.1 (c 0.3, chloroform), R_F 0.12 (solvent N). *Anal.* Calc for $C_{45}H_{69}NO_{24}$ (1008.04): C, 53.62; H, 6.90; N, 1.39. Found: C, 53.79; H, 6.97; N, 1.48.

Octyl O-*(2-acetamido-2-deoxy–β–*D-*glucopyranosyl)-(1→2)-*O-*(3-*O-*methyl–α–*D-*mannopyranosyl)-(1→6)–β–*D-*glucopyranoside* (**5**).- Deacetylation of **28** (4.1 mg, 4.1 μmol), as described for the preparation of **19**, gave a solid residue. A solution of the residue in water (15 mL) was passed through Sep-Pak C-18 cartridge which had been prewashed with 30 mL each of methanol and water. The cartridge was then washed with water (30 mL) and the product was eluted with methanol (30 mL). The methanol was evaporated and a solution of the residue was dissolved in water (5 mL) and filtered through a 0.22 μm Millex filter and then lyophilized to give **5** (2.5 mg, 92%), white powder, R_F 0.39 (solvent H). FAB-MS: *m/z* 694 [40.5%, $(M+1)^+$].

Octyl O-*(2-acetamido-2-deoxy–β–*D-*glucopyranosyl)-(1→2)-*O-*(3-*O-*methyl–β–*D-*mannopyranosyl)-(1→6)–β–*D-*glucopyranoside* (**30**).-Compound **29** (15.7 mg, 15.5 μmol) was O-deacetylated and processed exactly as described for the preparation of **5**, to afford **30** as a white powder (10.3 mg, 99%), R_F 0.42 (solvent H). FAB-MS: *m/z* 672 [3.2%, $(M+1)^+$].

Octyl O-*(2-acetamido-3,4,6-tri-*O-*acetyl-2-deoxy–β–*D-*glucopyranosyl-(1→2)-*O-*(3,4-di-*O-*acetyl-6-*O-*methyl–α–*D-*mannopyranosyl)-(1→6)-2,3,4-tri-*O-*benzyl–β–*D-*glucopyranoside* (**32**).-To a stirred solution of **23** (18.4 mg, 0.05 mmol) in dry nitromethane (0.5 mL) containing powdered 4Å molecular sieves (70 mg), mercuric cyanide (22 mg, 0.073 mmol) and mercuric bromide (36.3 mg, 0.073mmol) was added, dropwise, a solution of chloride **31** (46 mg, 0.073 mmol) in dry nitromethane (1 mL) at O °C under dry argon. After stirring at O °C for 30 min. the reaction was slowly allowed to warm to room temperature and stirred for 15 h. After processing the reaction mixture as described above, the resulting syrup was chromatographed (solvent O) to provide **32** (36.5 mg, 64%), as a white solid, $[\alpha]_D$ +8.7 (c 1.4, chloroform), R_F 0.33 (solvent M). *Anal.* Calc. for $C_{60}H_{81}NO_{21}$ (1152.26): C, 62.54; H, 7.09; N, 1.22. Found: C, 62.75; H, 7.20; N, 1.26.

Octyl O-*(2-acetamido-2-deoxy–β–*D-*glucopyranosyl)-(1→2)-*O-*(6-*O-*methyl–α–*D-*mannopyranosyl)-(1→6)–β–*D-*glucopyranoside* (**7**).-The blocked trisaccharide **32** (31.8 mg, 0.028 mmol) was hydrogenolized as described for the conversion of **17** into **18**, to give **33** [R_F 0.57 (solvent F)] which was O-deacetylated and

final product processed exactly as described for the preparation of **5**, to afford **7** as a white powder (17.5 mg, 94%), R_F 0.35 (solvent H). FAB-MS: *m/z* 672 [2%, $(M+1)^+$] and 694 [9%, $(M+Na)^+$].

Methyl 4-O-benzyl-6-deoxy-6-fluoro-2,3-O-isopropylidene-α-D-mannopyranoside (**35**).- To a cold (-40 °C) solution of **34** (3.34 g, 10.3 mmol) in dichloromethane (30 mL) was slowly added *N,N*-diethylaminosulfur trifluoride (DAST, 4.08 mL, 30.9 mmol). After stirring for 1 h at -40 °C the reaction mixture was allowed to warm to room temperature, and stirring was continued for an additional 1 h. The mixture was cooled to -20 °C, quenched by the addition of methanol (50 mL), and concentrated under reduced pressure. The residue was chromatographed (solvent P) to afford **35** (1.47 g, 44%), as a colorless syrup, $[\alpha]_D$ +52.7 (c 1.6, chloroform), R_F 0.36 (solvent P). *Anal.* Calc. for $C_{17}H_{23}FO_5$ (326.35): C, 62.56; H, 7.10. Found:C, 62.76; H, 7.06.

Methyl 4-O-benzyl-6-deoxy-6-fluoro-α-D-mannopyranoside (**36**).-Deacetonation of **35** (1.44 g, 4.41 mmol) in chloroform (45 mL) containing trifluroacetic acid (6 mL) and water (0.4 mL) as described for the preparation of **13** gave after chromatography (solvent Q) **36** (1.17 g, 93%), as a syrup, $[\alpha]_D$ +47 (c 0.1, chloroform), R_F 0.24 (solvent Q).

Methyl 2,4-di-O-benzyl-6-deoxy-6-fluoro-α-D-mannopyranoside (**37**) and *Methyl 3,4-di-O-benzyl-6-deoxy-6-fluoro-α-D-mannopyranoside* (**38**)- A solution of **36** (1.1 g, 3.84 mmol) and dibutyltin oxide (0.96 g, 3.84 mmol) in methanol (100 mL) was boiled for 1 h. The solvent was then evaporated to dryness to give a residue, which was dissolved in *N,N*-dimethylformamide (15 mL), and benzyl bromide (0.64 mL, 5.38 mmol) was added. The mixture was stirred for 1 h at 100 °C and then poured into water and extracted with dichloromethane. The dichloromethane solution was washed several times with water, dried, and concentrated. The residue was purified by chromatography (solvent A) to provide **37** (0.18 g, 13%), as a syrup, $[\alpha]_D$ +11.3 (c 1.5, chloroform), R_F 0.38 (solvent R). *Anal.* Calc. for $C_{21}H_{25}FO_5$ (376.41): C, 67.00; H, 6.69. Found: C, 67.08; H, 6.75.

Compound **38** (0.77 g, 53%) eluted next, was also obtained as a syrup, $[\alpha]_D$ +41.2 (c 0.2, chloroform), R_F 0.26 (solvent R). *Anal.* Calc. for $C_{21}H_{25}FO_5$ (376.41): C, 67.00; H, 6.69. Found: C, 66.75; H, 6.67.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-benzyl-6-deoxy-6-fluoro-α-D-mannopyranoside (**40**).-To a solution of alcohol **38** (0.67 g, 1.78 mmol) was added silver trifluoromethanesulfonate (2.31 g, 8.99 mmol), *sym* collidine (1.2 mL, 9.05 mmol), and pulverized activated molecular sieves (4Å, 3 g). To the resulting mixture cooled to -50 °C was added dropwise, a solution of bromide **15** (1.33 g, 2.67 mmol) in dichloromethane (10 mL). The above mixture protected from light was stirred at -50 °C for 15 min in an atmosphere of argon and then allowed to warm to room temperature over a period of 2 h, by which time t.l.c. (solvent S) showed the complete disappearance of **15** and **38** and the formation of a new slower migrating product. It was then diluted with dichloromethane (200 mL), and processed as described for the preparation of **16** to give after chromatography (solvent S) amorphous **39** (1.39 g, 98%), $[\alpha]_D$ -15.9 (c 1, chloroform), R_F 0.17.(solvent S). This material was slightly contaminated with some faster migrating impurities, and was utilized without further purification in the next step.

Compound **39** (1.34 g) was treated with 85% hydrazine-hydrate (8.5 mL) then acetylated, as described for the conversion of **16** to **17**, to give after chromatography (solvent S→D) the disaccharide **40** as a white solid (0.95 g, 80%), $[\alpha]_D$ -2.3 (c 1.1, chloroform), R_F 0.63 (solvent D). *Anal.* Calc. for $C_{35}H_{44}FNO_{13}$ (705.51): C, 59.56; H, 6.28; N, 1.99. Found: C, 59.42; H, 6.39; N, 2.25.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-6-deoxy-6-fluoro-α-D-mannopyranoside (**41**).-Hydrogenolysis of **40** (0.93 g, 1.32 mmol) as described for the preparation of **18**, gave, after chromatography (solvent T→E) amorphous **41** (0.68 g, 98%), $[\alpha]_D$ +9.6 (c 1.5, chloroform), R_F 0.25 (solvent E).

Methyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-6-deoxy-6-fluoro-α-D-mannopyranoside (**42**).- Deacetylation of **41** (28 mg, 0.053 mmol) in 0.1 M methanolic sodium methoxide (2.2 mL) and processing of the resulting product as described for the preparation of **19**, gave **42** as a white powder (12.4 mg, 58%), R_F 0.39 (solvent H). FAB-MS: *m/z* 400 [5%, $(M+1)^+$] and 422 [100%, $(M+Na)^+$].

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-acetyl-6-deoxy-6-fluoro-α-D-mannopyranoside (**43**).- Compound **41** (0.64 g, 1.22 mmol) was acetylated in 1:2 acetic anhydride-pyridine (30 mL) as described for the preparation of **20**, to give **43** (0.7 g, 94%), as an amorphous powder, $[\alpha]_D$ -0.6 (c 1.2, chloroform), R_F 0.4 (solvent E). *Anal.* Calc. for $C_{25}H_{36}FNO_{15}$ (609.55): C, 49.26; H, 5.95; N, 2.30. Found: C, 49.03; H, 5.93; N, 2.34.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,4-tri-O-acetyl-6-deoxy-6-fluoro-α-D-mannopyranoside (**44**).- A solution of compound **43** (0.4 g, 0.66 mmol) in acetic anhydride (10 mL) containing 1% (v/v) of conc. H_2SO_4 was acetolysed as described for the preparation of **21** to give a solid residue. The residue was dissolved in a small amount of ethyl acetate, and the addition of ether caused the precipitation of **44** as an amorphous powder (0.36 g, 85%), $[\alpha]_D$ -0.3 (c 1.6, chloroform), R_F 0.41 (solvent J). *Anal.* Calc. for $C_{26}H_{36}FNO_{16}$ (637.56): C, 48.98; H, 5.69; N, 2.20. Found: C, 48.98; H, 5.88; N, 2.21.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4-di-O-acetyl-6-deoxy-6-fluoro-α-D-mannopyranosyl chloride (**45**).-Reaction of a solution of **44** (108.5 mg, 0.17 mmol) in dichloromethane (4.5 mL) with hydrogen chloride as described for the preparation of **22** gave a residue which was chromatographed (solvent U) to give amorphous **45** (90 mg, 86%), $[\alpha]_D$ +45.5 (c 0.8, chloroform), R_F 0.24 (solvent U).; *Anal.* Calc. for $C_{24}H_{33}ClFNO_{14}$ (613.96): C, 46.95; H, 5.45; Cl, 5.77; N, 2.28. Found: C, 46.96; H, 5.32; Cl, 5.79; N, 2.28.

Octyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl-(1→2)-O-(3,4-di-O-acetyl-6-deoxy-6-fluoro-α-D-mannopyranosyl)-(1→6)-2,3,4-tri-O-benzyl-β-D-glucopyranoside (**46**).-To a stirred solution of **23** (28.5 mg, 0.051 mmol) in dry nitromethane (0.5 mL) containing powdered 4Å molecular sieves (86 mg), mercuric cyanide (14.5 mg, 0.057 mmol) and mercuric bromide (22.5 mg, 0.062 mmol) was added, dropwise, a solution of chloride **45** (33.3 mg, 0.051 mmol) in dry nitromethane (1.5 mL) at O °C under dry argon. After stirring at O °C for 30 min. the reaction was slowly allowed to warm to room temperature and stirred for 15 h. The mixture was then processed in the usual manner and the resulting syrup was chromatographed (solvent C) to give first unchanged **23** (6.5 mg) followed by **46** (27.1 mg, 61%, based on reacted **23**), as a white solid, $[\alpha]_D$ +15.3 (c 1.4, chloroform), R_F 0.43 (solvent C). FAB-MS: *m/z* 1140 [0.3%, $(M+1)^+$] and 1162 [0.2%, $(M+Na)^+$]. *Anal.* Calc. for $C_{59}H_{78}FNO_{20}$ (1140.22): C, 62.15; H, 6.90; N, 1.23. Found: C, 61.47; H, 6.78; N, 1.27.

Octyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(6-deoxy-6-fluoro-α-D-mannopyranosyl)-(1→6)-β-D-glucopyranoside (**9**).-The blocked trisaccharide **46** (25 mg, 0.022 mmol) was hydrogenolysed as described for the preparation of **18**, to give **47** [R_F 0.63 (solvent F)], which was then O-deacetylated and the final product processed exactly as described for the conversion of **28** into **5**, to afford **9** as a white powder (9.67 mg, 67%), R_F 0.47 (solvent H). FAB-MS: *m/z* 682 [68%, $(M+1)^+$].

Enzyme Preparation-*N*-acetylglucosaminyltransferase V was partially purified as described previously[7]. Briefly, dialyzed extract from 24 g of hamster kidney was applied to a 3.4 mL bed-volume UDP-hexanolamine Sepharose column (7 μmol/mL). This column was washed with 50 mL buffer (50 mM MES pH 6.5, containing 10 mM EDTA, 0.25% Triton X-100 and 20% glycerol), and then with the same buffer containing 0.1M NaCl. GlcNAcT-V was eluted with 20 mL each of buffer containing 0.1 M NaCl +5 mM UDP and then buffer containing 0.25 M NaCl and 5 mM UDP. Eluates were concentrated to less than 1.5 mL by ultrafiltration and dialyzed into 50mM sodium cacodylate buffer pH 6.5 containing 10 mM EDTA, 20% glycerol and 0.1% Triton X-100. Precipitate formed during ultrafiltration and dialysis was removed by centrifugation. This preparation yielded 6 mU of GlcNAcT-V (1 mU/mg protein), where 1 mU is defined as 1 nmol/min using 1.1 mM UDP-GlcNAc and 400 μM synthetic trisaccharide acceptor **4** in 50 mM sodium cacodylate pH 6.5 with 20 % glycerol, 10 mM EDTA and 0.1 % Triton X-100 at 37 °C.

GlcNAcT-V Enzyme Kinetics-GlcNAcT-V was assayed radiochemically using reverse-phase SepPak C-18 cartridges to separate labelled hydrophobic product tetrasaccharide from unreacted radiolabelled sugar-nucleotide donor UDP-GlcNAc as described[7]. Kinetic studies with acceptor analogs contained 2 μU of enzyme, and 11 nmol UDP-GlcNAc (30,000 dpm/nmol); substrates were lyophilized in 600 μL plastic microfuge tubes, and enzyme and buffer were added to give a final volume of 10 μL. The tube containing substrates and enzyme was vortexed, microfuged briefly and incubated at 37 °C for up to 2 hours. The reaction was quenched using 0.4 mL water and the reaction mixture transferred with water onto a pre-equilibrated SepPak C18 cartridge. Unreacted radiolabelled donor was removed by washing with 100 mL water, and labelled product was eluted slowly with 2 X 4 mL MeOH and collected for liquid scintillation counting. Kinetic constants were obtained by fitting rate data to the appropriate equations[7] using unweighted nonlinear regression (SigmaPlot 4.1, Macintosh version). Methods used for the data analysis and determination of the mode of inhibition have been previously described in detail[7].

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