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Synthesis of 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-*O*-(6-*O*-methyl-α-D-mannopyranosyl)-(1 → 6)-β-D-glucopyranoside and its 4′,6′-di-*O*-methyl analog. Potential inhibitors of *N*-acetylglucosaminyltransferase V (GnT-V) *

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ABSTRACT

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 bromide and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3-*O*-acetyl-4,6-di-*O*-methyl-α-D-mannopyranosyl bromide were each condensed with 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside, and the products were deprotected to yield, respectively, β-D-Glc*p*NAc-(1 → 2)-6-*O*-Me-α-D-Man*p*-(1 → 6)-β-D-Glc*p* and β-D-Glc*p*NAc-(1 → 2)-4,6-di-*O*-Me-α-D-Man*p*-(1 → 6)-β-D-Glc*p*, as their 4-nitrophenyl glycosides. These trisaccharides are expected to function as inhibitors for *N*-acetylglucosaminyltransferase V.

INTRODUCTION

N-Acetylglucosaminyltransferase V (GnT-V, EC 2.4.1.155) transfers an *N*-acetyl-β-D-glucosamine (β-D-Glc*p*NAc) unit to O-6 of the α-(1 → 6)-linked D-Man*p* residue that forms part of the trimannopyranosyl core of asparagine-linked *N*-glycans[2]. In recent years, this enzyme has been the center of great attention as a potential tumor marker because of its increased activity in cells transformed by tumor viruses[3,4] or oncogenes[5]. Furthermore, work from the laboratory of Dennis et al.[6,7] has suggested that a decrease in intracellular activity of GnT-V and the resulting decrease in specific cell surface structures is correlated with a reduction

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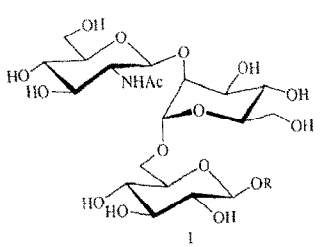
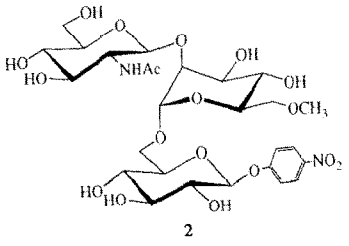


1

R=$(CH_2)_7CH_3$, $C_6H_4NO_2$-4, or CH_2-CH=CH_2



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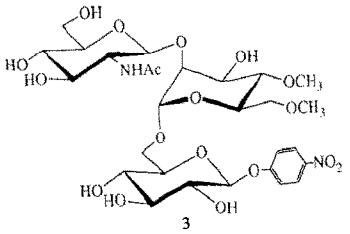


3

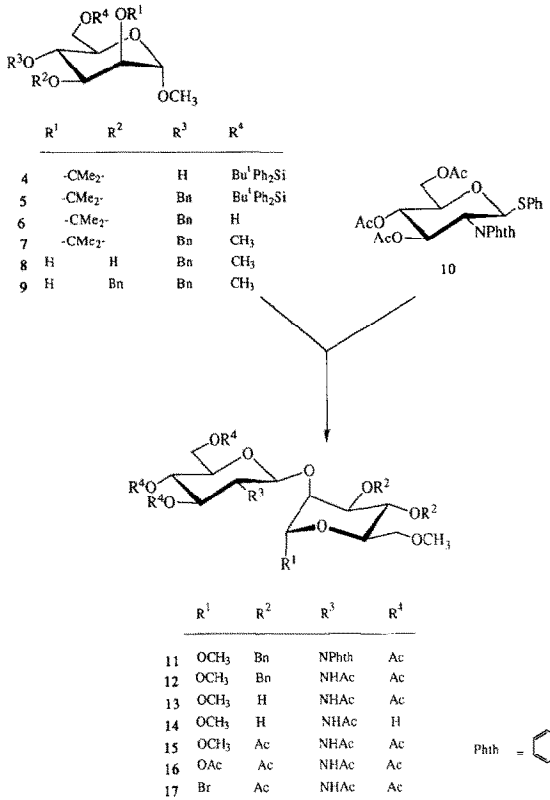
Scheme 1. Structures of the synthetic GnT-V acceptor (**1**) and potential inhibitors (**2** and **3**).

of metastatic potential of human and rodent cells. In addition these authors[8] have shown that, by comparison with nonmalignant tissues, increases in the expression of GnT-V activity and in the resulting cell-surface oligosaccharides are associated with a number of human carcinomas.

For the past few years, our group[9–11] has been actively engaged with the synthesis of acceptor substrates for GnT-V. Now we have focussed our attention on the design, synthesis, and biological evaluation of inhibitors for this particular enzyme[12]. Our strategy for the creation of inhibitors involved defining the specific acceptor substrate for the enzyme, then chemically synthesizing an analog having a masking group on the hydroxyl that would normally serve as the point of attachment for the transferred glycosyl unit, as shown in Scheme 1. On the basis of this rationale, we have envisioned β-D-Glc*p*NAc-(1 → 2)-6-*O*-Me-α-D-Man*p*-(1 → 6)-β-D-Glcp-OC_6H_4(4-NO_2) (**2**) as a potential inhibitor for GnT-V. A similar, presumably equally useful trisaccharide β-D-Glc*p*NAc-(1 → 2)-4,6-di-*O*-Me-α-D-Man*p*-(1 → 6)-β-D-Glc*p*-OC_6H_4(4-NO_2) (**3**) was also synthesized. We preferred the incorporation of a β-D-glucopyranose residue at the reducing terminus in place of the naturally occurring β-D-mannopyranose residue, since the latter is not a prerequisite[13,14] for recognition by GnT-V and its incorporation in the synthesis would be far from simple. Recently, the laboratories of Palcic and Hindsgaul have reported[15,16] the synthesis of a similar trisaccharide, β-D-GlcpNAc-(1 → 2)-6-deoxy-α-D-Man*p*-(1 → 6)-β-D-Glcp-O-$(CH_2)_7CH_3$, and have shown it to be a competitive inhibitor for GnT-V. Compounds **2** and **3** also proved useful[17] in specificity studies of the β-1,4-*N*-acetylglucosaminyltransferase (GnT-VI′) acting on the α-3 and α-6 arms of *N*-linked oligosaccharides.

RESULTS AND DISCUSSION

For the synthesis of the title trisaccharide **2** we employed the known 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside[9] **18** as a glycosyl acceptor and *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 bromide **17** as a glycosyl donor. Bromide **17** was readily prepared from *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-1,3,4-tri-*O*-acetyl-6-*O*-methyl-α-D-mannopyranose **16**. Compound **16** was obtained by condensation of phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[11] **10** with methyl 3,4-di-*O*-benzyl-6-*O*-methyl-α-D-mannopyranoside **9**. The latter was obtained from methyl 6-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-isopropylidene-α-D-mannopyranoside[10] **4** through a succession of chemical steps. Thus, benzylation[18] of compound **4** followed by cleavage of the *tert*-butyldiphenylsilyl ether group at C-6 and subsequent methylation[19] gave syrupy 6-*O*-methyl derivative **7** (77%). Deisopropylidenation of **7** gave **8** (63%) which was converted into the desired **9** by treatment of its stannylene derivative with benzyl bromide[20].

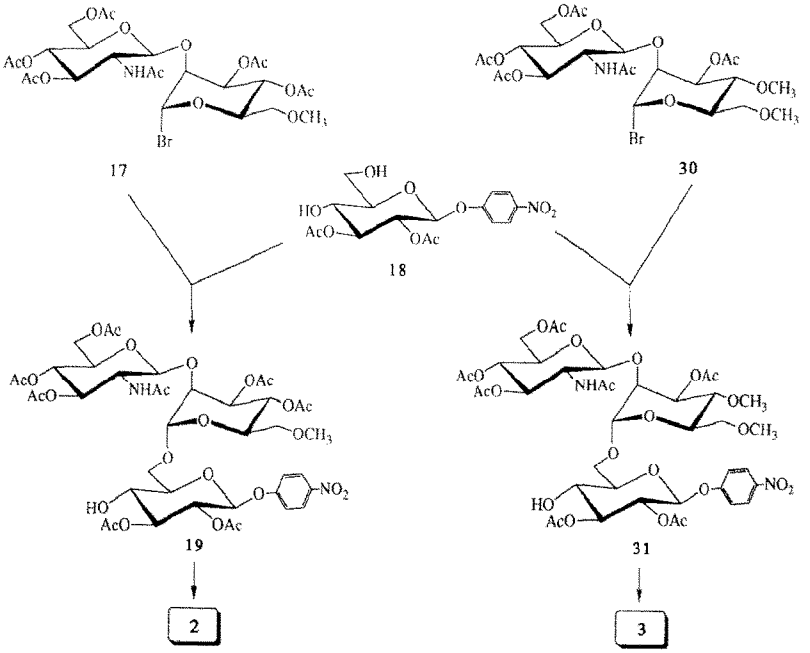


Scheme 2.

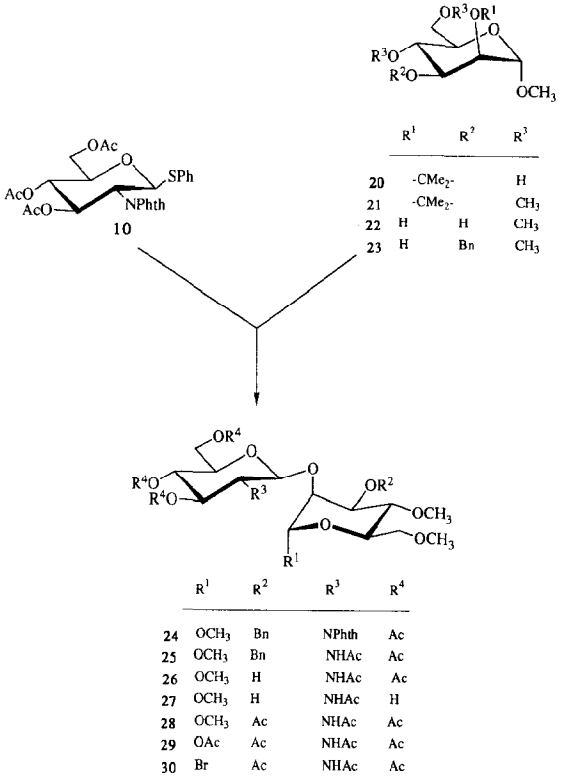
Glycosylation of **9** with thioglycoside **10** promoted by *N*-iodosuccinimide and trifluoromethanesulfonic (triflic) acid[21,22] gave **11** (86%). Treatment of the disaccharide **11** with hydrazine hydrate, followed by acetylation, gave intermediate **12**, which was not characterized. Hydrogenolysis of benzyl groups of **12** gave, after chromatographic purification, **13** (91%) which was acetylated (Py-Ac_2O) to afford the hexacetate **15** (94%). This compound was subjected to acetolysis to furnish **16** (89%). The ^{1}H NMR spectrum of **16** contained a low-field signal at δ 5.98 (1 H, J 2.0 Hz), suggesting that it existed almost exclusively as the α-D anomer. A small portion of **13** was *O*-deacetylated to afford **14** (73%). Treatment of **16** with HBr in glacial acetic acid gave amorphous bromide **17** (87%).

Glycosylation of the diol **18** with bromide **17** promoted by silver trifluromethanesulfonate (triflate) and *sym*-collidine, gave the protected trisaccharide derivative **19** (62%). *O*-Deacetylation of **19** furnished the title trisaccharide **2** (86%).

The synthesis of trisaccharide **3** followed a procedure analogous to that described for the preparation of **2**. Thus the reaction of thioglycoside **10** with alcohol **23** (prepared from methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[23] **20** in three steps), promoted again by *N*-iodosuccinimide and triflic acid, gave the β-(1 → 2)-linked disaccharide derivative **24** (76%) which was converted to the glycosyl bromide **30** in five steps, in a manner analogous to that described for conversion of



Scheme 3.



Scheme 4.

11 to **17**. The free disaccharide **27** (78%) was obtained by deprotection of a small portion of **26**.

Condensation of the diol **18** with the glycosyl donor **30** under conditions similar to those described for the reaction of **17** with **18** gave the partially protected trisaccharide **31** (78%), from which the acetyl groups were removed by Zemplén transesterification to afford the desired trisaccharide **3** (71%).

Preliminary evaluation of trisaccharides **2** and **3** shows that they are, as expected, potential inhibitors for GnT-V. These results will be reported in detail elsewhere.

EXPERIMENTAL

General methods.—Unless otherwise indicated, all reactions were carried out at ambient temperatures. Solutions were dried with Na_2SO_4 and concentrated at 40–50°C/2 kPa. TLC was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel 60F-254 (Merck); the compounds were located by UV light and/or by charring with 5% H_2SO_4. Column chromatography was performed on silica gel (Baker Analyzed, 60–200 mesh). The following solvent systems (v/v) were

TABLE I

Selected ^{1}H NMR data for protected and unprotected disaccharides [a]

Compound	Chemical shifts (δ) and coupling constants (Hz)								
	H-1 ($J_{1,2}$) [b]		H-1′ ($J_{1',2'}$) [c]	OCH_3-1	OCH_3-4	OCH_3-6	NAc	OAc	NH ($J_{2,NH}$)
11	4.47		5.51 (8.5)	2.94		3.19		1.87 (3H), 2.04 (6H)	
13	4.66	(1.5)	4.95 (8.5)	3.36		3.40	1.96	2.04 (3H), 2.06 (3H), 2.10 (3H)	6.11 (8.5)
14	4.76		4.55 (8.3)	3.40		3.41	2.05		
15	4.63	(1.5)	5.04 (8.5)	3.36		3.39	1.94	2.00 (6H), 2.02 (6H), 2.08 (3H)	5.92 (8.5)
16	5.98	(2.0)	4.91 (8.5)			3.32	1.97	1.99 (3H), 2.01 (3H), 2.04 (3H), 2.06 (3H), 2.08 (3H), 2.13 (3H)	6.21 (8.5)
24	4.43	(2.0)	5.48 (8.5)	2.98	3.44	3.18		1.87 (3H), 2.04 (6H)	
26	4.61	(1.5)	4.98 (8.5)	3.33	3.55	3.40	1.94	2.02 (3H), 2.04 (3H), 2.10 (3H)	5.95 (8.0)
27	4.74	(< 1)	4.55 (8.4)	3.39	3.51	3.41	2.06		
28	4.57	(2.0)	4.73 (8.5)	3.36	3.47	3.40	1.95	2.03 (3H), 2.04 (3H), 2.08 (3H), 2.10 (3H)	5.64 (8.5)
29	5.94	(2.0)	4.63 (8.5)		3.48	3.37	1.95	2.01 (3H, 2.02 (3H), 2.08 (3H), 2.09 (3H), 2.11 (3H)	5.83 (9.0)

[a] For solutions in $CDCl_3$ at 300 MHz except for compounds **14** and **27**, which were recorded in D_2O at 500 MHz. The reference standards used are listed in the Experimental section. [b] Unprimed locants are used for protons in the reducing-end residue (α-D-Man*p*). [c] Of the nonreducing-end residue (β-D-Glc*p*NAc).

used for chromatography: *A*, 2:1 hexane–$CHCl_3$, *B*, 1:1 hexane–$CHCl_3$, *C*, 3:1 hexane–EtOAc, *D*, 19:1 $CHCl_3$–acetone, *E*, 4:1 $CHCl_3$–acetone, *F*, 1:1 hexane–EtOAc, *G*, 2:1 hexane–EtOAc, *H*, 99:1 $CHCl_3$–MeOH, *I*, 49:1 $CHCl_3$–MeOH, *J*, 19:1 $CHCl_3$–MeOH, *K*, 13:6:1 $CHCl_3$–MeOH–H_2O, *L*, 3:2 $CHCl_3$–acetone, *M*, 2:1 CH_2Cl_2–acetone, *N*, 4:1 CH_2Cl_2–MeOH, *O*, 19:1 CH_2Cl_2–MeOH. Optical rotations were measured at $22 \pm 2°$ with a Perkin-Elmer 241 polarimeter. ^{1}H NMR spectra were recorded either at 90 (Varian EM-390), 300 (Bruker AM-300), or 500 MHz (Bruker AM-500) for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal acetone, δ 2.225). ^{13}C NMR spectra were recorded at 50.3 (Bruker WP-200) or 75.5 MHz (Bruker AM-300) for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (external 1% 1,4-dioxane in D_2O, δ 67.4). Only partial NMR data are reported, but all values were in accord with the proposed structures. The assignments of ^{13}C chemical shifts are tentative. FAB-mass spectra were obtained using an AEI MS-9 instrument with Xe as the bombarding gas and 5:1 1,4-dithiothreitol–1,4-dithioerythritol as a matrix. Elemental analyses were performed by the Robertson Laboratory, 29 Samson Ave., Madison, NJ 08940 (USA).

Methyl 4-O-benzyl-6-O-tert-butyldiphenylsilyl-2,3-O-isopropylidene-α-D-mannopyranoside (**5**).—A mixture of **4**[10] (5.5 g, 11.6 mmol), freshly prepared Ag_2O (10

TABLE II

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

Compound	Chemical shifts (δ) [b]										
	C-1	C-1′	C-2	C-2′	C-6	C-6′	OCH_3-1	OCH_3-4	OCH_3-6	$COCH_3$	$COCH_3$
11	96.75 [c]	97.97 [c]	77.91	54.76	71.06	62.36	54.50		58.89	170.65, 170.19, 169.47	20.72, 20.64, 20.48
13	98.94 [d]	99.72 [d]	78.73	55.32	72.33	61.95	54.88		59.29	171.06, 170.74, 170.67 169.45	23.30, 20.68, 20.60
14	98.84	100.23	76.80	55.83	72.95	61.45	56.15		59.23	175.40	23.10
15	98.44	98.44	74.05	55.93	72.08	62.19	55.07		59.33	170.86, 170.65, 170.38 169.75, 169.53	23.32, 20.77, 20.69 20.62
16	90.91	99.23	73.12	54.78	72.04	61.99			59.37	170.95, 170.71, 170.62 169.41, 169.36, 168.61	23.23, 20.99, 20.76 20.71, 20.64, 20.57
24	96.84 [e]	98.05 [e]	77.74	54.83	71.10	62.37	54.51	60.57	58.92	170.67, 170.20, 169.48	20.72, 20.66, 20.49
26	98.90 [f]	99.97 [f]	77.91	54.88	71.81	62.05	55.63	60.66	59.17	170.84, 170.71, 170.63 169.52	23.38, 20.72, 20.65
27	98.69	100.17	77.00	55.84	72.55	61.41	56.17	61.14	59.21	175.50	23.10
28	98.45 [g]	99.35 [g]	74.57	54.98	71.93	62.23	55.18	60.50	59.24	170.73, 170.68, 170.38 169.53	23.41, 21.10, 20.73 20.70, 20.66
29	91.22	100.08	73.96	54.27	71.78	62.09		60.78	59.28	170.00, 170.66, 170.53 169.42, 169.15	23.29, 21.09, 21.05 20.73, 20.69, 20.62

[a] For solutions in $CDCl_3$ at 75.5 MHz except for compounds **14** and **27**, for which the solvent was D_2O. [b] Locants: unprimed, reducing-end residue (α-D-Man*p*); primed, nonreducing-end residue (β-D-Glc*p*NAc). [c,d,e,f,g] Values with the same superscripts may be interchanged.

TABLE III

Selected ^1H and ^{13}C NMR data for protected and unprotected trisaccharides [a]

Nucleus [b]	Chemical shifts (δ) and coupling constants (Hz)			
	19	**2**	**31**	**3**
H-1 ($J_{1,2}$)	n.d. [c]	5.31 (5.4)	n.d. [c]	5.31 (5.5)
H-1′ ($J_{1',2'}$)	4.77 (< 1)	4.85	4.69 (2.0)	n.d. [c]
H-1″ ($J_{1'',2''}$)	5.10 (9.0)	4.51 (8.4)	4.74 (8.5)	4.51 (8.4)
OCH_3-4			3.38	3.40
OCH_3-6	3.25	3.33	3.32	3.31
NAc	1.84	2.00	1.85	2.00
OAc	1.91, 2.02, 2.05, 2.08		2.01, 2.02, 2.17, 2.10	
	2.09, 2.11, 2.13		2.12, 2.13	
C_6H_4-NO_2 (^3J)	8.23 (9.0)	8.30 (9.0)	8.22 (9.0)	8.29 (9.0)
	7.24 (9.0)	7.28 (9.0)	7.08 (9.0)	7.25 (9.0)
C-1	97.68 [d]	100.02	97.86 [c]	99.81
C-1′	97.38 [d]	97.81	97.75 [c]	97.57
C-1″	98.43	100.17	99.65	100.12
C-2′	75.37	76.80	75.70	78.22
C-2″	55.72	56.11	55.18	56.16
C-6	66.55	66.77	66.07	66.96
C-6′	69.55	72.82	72.02	72.41
C-6″	62.10	61.43	62.18	61.43
OCH_3-4			60.17	60.98
OCH_3-6	58.92	59.05	59.00	59.00
$COCH_3$	171.45, 170.97, 170.65	175.44	171.25, 170.83, 170.72	175.47
	170.43, 169.60, 169.45		170.64, 169.51, 169.48	
$COCH_3$	23.14, 20.76, 20.60	23.05	23.30, 21.06, 20.87	23.06
	20.30		20.71, 20.63	
CNO_2	161.79	162.39	161.20	162.35
CO (phenolic)	142.88	143.42	143.11	143.25
Aromatic	125.88, 116.41	127.01, 117.23	126.11, 116.45	127.07, 117.20

[a] Spectra were recorded at 300 MHz (^1H in $CDCl_3$, compounds **19** and **31**) or 500 MHz (^1H in D_2O, compounds **2** and **3**) and 75.5 MHz (^{13}C in $CDCl_3$ for compounds **19** and **31** and in D_2O for **2** and **3**). [b] Locants: unprimed, β-D-Glc*p*; single primed, α-D-Man*p*, double primed, β-D-Glc*p*NAc. [c] Could not be determined due to spectral overlap. [d,e] Values with the same superscripts may be interchanged.

g), and benzyl bromide (10 mL) in DMF (80 mL) was stirred for 48 h at 45°C. The solids were filtered off (Celite bed), washed with DMF, and the combined filtrate was concentrated. The residue was stirred in $CHCl_3$ (200 mL), and the precipitated silver salts were filtered off and washed with $CHCl_3$. The $CHCl_3$ solution was successively washed with water, aq $NaHCO_3$, and water, dried, and concentrated. The crude product was chromatographed (Solvent $A \rightarrow B$) to give **5** as a syrup (4.20 g, 64.1%); $[\alpha]_D$ +9.6° (*c* 1.7, $CHCl_3$); R_f 0.7 (solvent *B*); ^1H NMR (90 MHz, $CDCl_3$): δ 7.80–7.23 (m, 15 H, arom.), 4.90 (s, 1 H, H-1), 3.33 (s, 3 H, OMe), 1.50, 1.35 (2 s, 6 H, CMe_2), and 1.07 (s, 9 H, CMe_3). *Anal.* Calcd for $C_{33}H_{42}O_6Si$: C, 70.43; H, 7.52. Found: C, 70.69; H, 7.58.

Methyl 4-O-benzyl-2,3-O-isopropylidene-α-D-mannopyranoside (**6**).—To a solution of **5** (4.52 g, 8.03 mmol) in anhyd oxolane (50 mL) was added tetrabutylammo-

nium fluoride in oxolane (18 mL), and the mixture was stirred for 6 h at room temperature. After concentration the residue was chromatographed (solvent *C*), providing **6** as a syrup (2.13 g, 82%); $[\alpha]_D + 54.3°$ (*c* 1.2, $CHCl_3$); R_f 0.3 (solvent *C*); ^1H NMR (90 MHz, $CDCl_3$): δ 7.33 (br s, 5 H, arom.), 4.89 (s, 1 H, H-1), 3.31 (s, 3 H, OMe), 1.44, and 1.33 (2 s, 6 H, CMe_2). *Anal.* Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 63.03; H, 7.49.

Methyl 4-O-benzyl-2,3-O-isopropylidene-6-O-methyl-α-D-mannopyranoside (**7**).—A solution of **6** (2.1 g, 6.5 mmol) in DMF (40 mL) was stirred for 16 h at room temperature in the presence of BaO (8 g), $Ba(OH)_2 \cdot 8\ H_2O$ (8 g), and MeI (11 mL). The mixture was cooled (0°C) and diluted with an equal volume of $CHCl_3$, and the insolubles were filtered off (Celite bed) and washed with $CHCl_3$. The $CHCl_3$ solution was successively washed with water, aq $Na_2S_2O_3$, and water, dried, and concentrated to afford **7** as a syrup (1.69 g, 77%); $[\alpha]_D + 44.8°$ (*c* 1.1, $CHCl_3$); R_f 0.3 (solvent *C*); ^1H NMR (90 MHz, $CDCl_3$): δ 7.25 (br s, 5 H, arom.), 3.34 (s, 6 H, OMe-6 and OMe-1), 1.46, and 1.33 (2 s, 6 H, CMe_2). *Anal.* Calcd for $C_{18}H_{26}O_6$: C, 63.89; H, 7.74. Found: C, 64.19; H, 7.77.

Methyl 4-O-benzyl-6-O-methyl-α-D-mannopyranoside (**8**).—A solution of **7** (3.79 g, 11.2 mmol) in $CHCl_3$ (13 mL) containing trifluoroacetic acid (13 mL) and water (1.3 mL) was stirred for 1 h at room temperature. After concentration, and successive additions and evaporations of toluene, the crude product was chromatographed ($CHCl_3 \rightarrow$ solvent *D*) to provide **8** as a syrup (2.11 g, 63%); $[\alpha]_D + 75°$ (*c* 1.1, $CHCl_3$); R_f 0.2 (solvent *E*); ^1H NMR (300 MHz, $CDCl_3$): δ 7.40–7.24 (m, 5 H, arom.), 4.71 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.36 (s, 3 H, OMe-6), and 3.33 (s, 3 H, OMe-1); ^{13}C (75.5 MHz): δ 138.42 (arom. *ipso*), 100.77 (C-1), 74.62 ($PhCH_2$), 71.31 (C-6), 59.10 (OCH_3-6), and 54.81 (OCH_3-1). *Anal.* Calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 60.46; H, 7.37.

Methyl 3,4-di-O-benzyl-6-O-methyl-α-D-mannopyranoside (**9**).—A solution of **8** (0.9 g, 3.02 mmol) and dibutyltin oxide (0.75 g, 3.01 mmol) in MeOH (40 mL) was boiled for 1 h. The solvent was then evaporated, the residue was dissolved in DMF (10 mL), and benzyl bromide (0.5 mL, 4.21 mmol) was added. The mixture was stirred for 5 h at 110°C, then poured into water. The aqueous mixture was extracted with $CHCl_3$, and the $CHCl_3$ solution was washed several times with water, dried, and concentrated. The residue was chromatographed (solvent *C*) to provide **9** as a syrup (0.49 g, 42%); $[\alpha]_D + 36.1°$ (*c* 1.1, $CHCl_3$); R_f 0.6 (solvent *F*); ^1H NMR (300 MHz, $CDCl_3$): δ 7.37–7.25 (m, 10 H, arom.), 4.76 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 3.38 (s, 3 H, OMe-6), and 3.34 (s, 3 H, OMe-1); ^{13}C (75.5 MHz): δ 138.39 and 137.94 (arom., *ipso*), 100.34 (C-1), 75.01 and 71.82 ($PhCH_2$), 71.36 (C-6), 59.16 (OCH_3-6), and 54.76 (OCH_3-1). *Anal.* Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.18; H,7.19.

Methyl O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1 → 2)-3,4-di-O-benzyl-6-O-methyl-α-D-mannopyranoside (**11**).—Phenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[11] **10** (2.64 g, 4.99 mmol) and alcohol **9** (1.57 g, 4.04 mmol) were dissolved in dry CH_2Cl_2 (26 mL), pulverized,

activated 4A molecular sieves (1.9 g) and *N*-iodosuccinimide (2.54 g, 11.3 mmol) were added, and the mixture was stirred in the dark for 30 min under Ar. After cooling (0°C; bath), a solution of trifluoromethanesulfonic acid (0.1 mL) in CH_2Cl_2 (37 mL) was added dropwise, and the stirring was continued for 2 h. Dichloromethane (200 mL) was added, and the solids were filtered off (Celite bed) and washed with CH_2Cl_2. The combined filtrate was successively washed with water, aq $NaHCO_3$, aq $Na_2S_2O_3$, and water. Evaporation of the solvent and purification of the residue by chromatography (solvent $C \rightarrow G \rightarrow F$) gave amorphous **11** (2.8 g, 86%); $[\alpha]_D$ −8.2° (*c* 1.3, $CHCl_3$); R_f 0.1 (solvent *F*). *Anal.* Calcd for $C_{42}H_{47}NO_{15}$: C, 62.60; H, 5.88; N, 1.74. Found: C, 62.22; H, 5.86; N, 1.86.

Methyl O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)-6*-O-*methyl-α*-D-*mannopyranoside* (**13**).—A mixture of **11** (2.7 g) and hydrazine hydrate (11 mL) in EtOH (37 mL) was refluxed for 3 h. The mixture was concentrated, the residue was dissolved in pyridine (50 mL), and Ac_2O (25 mL) was added. After being stirred overnight at room temperature the mixture was cooled to 0°C, and the excess Ac_2O was decomposed by dropwise addition of MeOH. After concentration, a solution of the residue in $CHCl_3$ (200 mL) was successively washed with water, aq $NaHCO_3$, and water. Evaporation of the solvent and chromatography ($CHCl_3 \rightarrow$ solvent *E*) gave **12** (2.0 g), which was sufficiently pure for the next step.

A mixture of **12** (2.0 g) and 10% Pd–C (2.0 g) in glacial acetic acid (30 mL) was shaken under H_2 at 345 kPa for 2 days at room temperature. The suspension was filtered (Celite bed), the solid was thoroughly washed with MeOH, and the filtrate and washings were combined and concentrated. The residual syrup was chromatographed (solvent $H \rightarrow I$) to yield amorphous **13** (1.36 g, 91%); $[\alpha]_D$ +8.3° (*c* 1.2, $CHCl_3$); R_f 0.1 (solvent *J*). *Anal.* Calcd for $C_{22}H_{35}NO_{14}$: C, 49.16; H, 6.56; N, 2.61. Found: C, 48.92; H, 6.49; N, 2.73.

Methyl O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1 → 2)-6*-O-*methyl-α*-D-*mannopyranoside* (**14**).—A solution of **13** (0.18 g) in 20 mM methanolic NaOMe (25 mL) was stirred overnight at room temperature. The base was neutralized with a few drops of glacial acetic acid, and the solution was deionized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (Celite bed) and thoroughly washed with MeOH. The combined filtrate was concentrated, and a solution of the residue in water was lyophilized to give amorphous **14** (0.1 g, 73%); $[\alpha]_D$ −10.2° (*c* 0.5, H_2O); R_f 0.3 (solvent *K*). FABMS: *m/z* 412 [11.5%, $(M + 1)^+$] and 434 [100%, $(M + Na)^+$]. *Anal.* Calcd for $C_{16}H_{29}NO_{11}$: C, 46.71; H, 7.11; N, 3.41. Found: C, 46.35; H, 7.11; N, 3.05.

Methyl 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-*mannopyranoside* (**15**).—A solution of **13** (1.11 g) in 1:2 Ac_2O–pyridine (45 mL) was stirred overnight at room temperature. Methanol was added dropwise at 0°C, the solution was concentrated, and the residue was subjected to additions and evaporations of toluene to give amorphous **15** (1.2 g

93%); $[\alpha]_D$ −6.2° (*c* 1.2, $CHCl_3$); R_f 0.3 (solvent *J*). *Anal.* Calcd for $C_{26}H_{39}NO_{16}$: C, 50.24; H, 6.32; N, 2.25. Found: C, 50.02; H, 6.19; N, 1.98.

O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-1,3,4-tri-O-acetyl-6-O-methyl-α-D-mannopyranose (**16**).—A solution of compound **15** (1.17 g) in Ac_2O (30 mL) containing 1% (v/v) of concd. H_2SO_4 was stirred for 17 h at room temperature. The mixture was diluted with CH_2Cl_2 (200 mL), successively washed with water, satd $NaHCO_3$, and water, dried, and concentrated. The residue was dissolved in a small amount of EtOAc, and the solution diluted with ether to cause the precipitation of amorphous **16** (1.09 g, 89%); $[\alpha]_D$ −7.6° (*c* 1.1, $CHCl_3$); R_f 0.3 (solvent *L*). *Anal.* Calcd for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found: C, 49.82; H, 6.01; N, 2.07.

4-Nitrophenyl 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-di-O-acetyl-β-D-glucopyranoside (**19**).—To a cold (0°C, bath), stirred solution of **16** (0.97 g) in CH_2Cl_2 (12 mL) was added a 31% solution of HBr in glacial acetic acid (12 mL), and stirring was continued for 12 h at 0°C. The mixture was then poured into ice–water and the product was extracted into CH_2Cl_2. The extract was successively washed with cold water, cold satd $NaHCO_3$, and cold water, dried, and concentrated to give *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 bromide (**17**) as an amorphous solid (0.87 g, 87%); $[\alpha]_D$ +54.3° (*c* 0.9, $CHCl_3$), R_f 0.2 (solvent *E*); ^1H NMR (90 MHz, $CDCl_3$): δ 7.28 (d, 1 H, *J* 9 Hz, N*H*), 6.45 (s, 1 H, H-1), 3.26 (s, 3 H, OMe-6), and 2.10–1.98 (cluster of s, 18 H, 5 OAc and 1 NAc).

A solution of the glycosyl bromide **17** (0.86 g, 1.28 mmol) in CH_2Cl_2 (30 mL) was added at 0°C to a stirred mixture of 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside[9] (**18**, 0.47 g, 1.22 mmol), *sym*-collidine (0.22 mL, 1.65 mmol), silver trifluoromethanesulfonate (0.47 g, 1.83 mmol), and pulverized 4A molecular sieves (1.3 g) in CH_2Cl_2 (20 mL). After 6 h, CH_2Cl_2 (200 mL) was added, the mixture was filtered (Celite bed), the solids were washed with CH_2Cl_2 (100 mL), and the combined filtrate was concentrated. Chromatography (solvent *M*) gave unreacted **18** (0.15 g), followed by amorphous **19** (0.5 g 62%, based on **18** consumed); $[\alpha]_D$ −11.3° (*c* 1.1, $CHCl_3$); R_f 0.4 (solvent *M*). *Anal.* Calcd for $C_{41}H_{54}N_2O_{25}$: C, 50.51; H, 5.58; N, 2.87. Found: C, 50.69; H, 5.70; N, 2.71.

4-Nitrophenyl O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-O-(6-O-methyl-α-D-mannopyranosyl)-(1 → 6)-β-D-glucopyranoside (**2**).—Compound **19** (0.1 g) was *O*-deacetylated in 10 mM methanolic NaOMe (11 mL) exactly as described for the preparation of **14** to give, after freeze-drying, amorphous **2** (0.06 g, 86%); $[\alpha]_D$ −55.8° (*c* 0.5, H_2O); R_f 0.3 (solvent *K*); FABMS: *m/z* 681 [2.5%, $(M + 1)^+$] and 703 [1.6%, $(M + Na)^+$]. *Anal.* Calcd for $C_{27}H_{40}N_2O_{18}$: C, 47.65; H, 5.92; N, 4.12. Found: C, 47.68; H, 6.12; N, 3.94.

Methyl 2,3-O-isopropylidene-4,6-di-O-methyl-α-D-mannopyranoside (**21**).—Methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[23] (**20**, 1.56 g, 6.66 mmol) was methylated as described for the preparation of **7** to give **21** as a syrup (1.6 g, 92%); $[\alpha]_D$

+38.9° (*c* 3.7, $CHCl_3$); {lit.[24] $[\alpha]_D^{20}$ + 40° (*c* 1, $CHCl_3$)}; R_f 0.3 (solvent *G*); ^1H NMR (90 MHz, $CDCl_3$): δ 4.86 (s, 1 H, H-1), 3.50 (s, 3 H, OMe-4), 3.40 (s, 3 H, OMe-6), 3.36 (s, 3 H, OMe-1), 1.52, and 1.33 (2 s, 6 H, CMe_2). *Anal.* Calcd for $C_{12}H_{22}O_6$: C, 54.95; H, 8.45. Found: C, 55.06; H, 8.52.

*Methyl 4,6-di-*O*-methyl-α-*D*-mannopyranoside* (**22**).—Deacetonation of **21** (8.16 g) as described for the preparation of **8**, gave after chromatography (solvent *E*), compound **22** as a syrup (5.5 g, 80%); $[\alpha]_D$ +79.2° (*c* 1.1, $CHCl_3$); {lit.[24] $[\alpha]_D^{20}$ + 99° (*c* 2, CH_3OH)}; R_f 0.1 (solvent *E*); ^1H NMR (90 MHz, $CDCl_3$): δ 4.52 (s, 1 H, H-1), 3.55 (s, 3 H, OMe-4), 3.43 (s, 3 H, OMe-6), 3.35 (s, 3 H, OMe-1); ^{13}C (50.3 MHz): δ 101.12 (C-1), 60.53 (OMe-4), 59.18 (OMe-6), and 54.97 (OMe-1). *Anal.* Calcd for $C_9H_{18}O_6$: C, 48.64; H, 8.16. Found: C, 48.59; H, 8.32.

*Methyl 3-*O*-benzyl-4,6-di-*O*-methyl-α-*D*-mannopyranoside* (**23**).—Benzylation of **22** (4.32 g, 19.0 mmol) as described for the preparation of **9** gave, after chromatogaphy (solvent *C*), compound **23** as a syrup (3.9 g, 66%); $[\alpha]_D$ +45.7° (*c* 1.3, $CHCl_3$); R_f 0.2 (solvent *F*); ^1H NMR (90 MHz, $CDCl_3$): δ 7.43–7.07 (m, 5 H, arom.), 4.51 (s, 1 H, H-1), 3.48 (s, 3 H, OMe-4), 3.35 (s, 3 H, OMe-6), 3.28 (s, 3 H, OMe-1); ^{13}C (50.3 MHz): δ 100.51 (C-1), 60.79 (OMe-4), 59.29 (OMe-6), and 54.99 (OMe-1). *Anal.* Calcd for $C_{16}H_{24}O_6$: C, 61.52; H, 7.74. Found: C, 61.59; H, 7.68.

Methyl O-*(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-*D*-glucopyranosyl)-(1 → 2)-3-*O*-benzyl-4,6-di-*O*-methyl-α-*D*-mannopyranoside* (**24**).—A reaction of alcohol **23** (1.72 g, 5.51 mmol) with the thioglycoside[11] **10** (3.68 g, 6.96 mmol) as described for the preparation of **11** gave, after chromatography (solvent *C* → *G*), disaccharide **24** as an amorphous solid (3.06 g, 76%); $[\alpha]_D$ +4.1° (*c* 2.7, $CHCl_3$); R_f 0.2 (solvent *F*). *Anal.* Calcd for $C_{36}H_{44}NO_{15}$: C, 59.17; H, 6.07; N, 1.92. Found: C, 59.06; H, 6.11; N, 2.04.

Methyl O-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-4,6-di-*O*-methyl-α-*D*-mannopyranoside* (**26**).—Compound **24** (1.97 g) was treated with hydrazine hydrate and then acetylated as described for the conversion of **11** into **12** to give, after chromatography ($CHCl_3$ → solvent *E*), the amorphous disaccharide **25** (1.37 g); R_f 0.1 (solvent *E*), which was sufficiently pure for the next step.

Hydrogenolysis of **25** (1.31 g), as described for the preparation of **13**, gave, after chromatography ($CHCl_3$ → solvent *H*), the amorphous disaccharide **26** (1.0 g, 89%); $[\alpha]_D$ +17.5° (*c* 2.1, $CHCl_3$), R_f 0.2 (solvent *J*). *Anal.* Calcd for $C_{23}H_{37}NO_{14}$: C, 50.09; H, 6.76; N, 2.54. Found: C, 49.89, H, 6.53, N, 2.39.

Methyl O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-4,6-di-*O*-methyl-α-*D*-mannopyranoside* (**27**).—Deacetylation of **26** (0.2 g) and lyophilization of the resulting product as described for the preparation of **14** gave the amorphous disaccharide **27** (0.12 g, 78%); $[\alpha]_D$ −7.6° (*c* 1.1, H_2O); R_f 0.3 (solvent *N*); FABMS: *m/z* 426 [20%, $(M + 1)^+$] and 448 [100%, $(M + Na)^+$]. *Anal.* Calcd for $C_{17}H_{31}NO_{11}$: C, 47.99; H, 7.35; N, 3.29. Found: C, 47.62; H, 7.72; N, 2.96.

Methyl O-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-3-*O*-acetyl-4,6-di-*O*-methyl-α-*D*-mannopyranoside* (**28**).—Compound **26** (0.36 g) was

acetylated as described for the preparation of **15** to give the amorphous disaccharide **28** (0.38 g, 98%); $[\alpha]_D$ −14.3° (*c* 0.9, $CHCl_3$); R_f 0.3 (solvent *J*). *Anal.* Calcd for $C_{25}H_{39}NO_{15}$: C, 50.59; H, 6.62; N, 2.36. Found: C, 50.43; H, 6.59; N, 2.22.

*O-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-1,3-di-*O*-acetyl-4,6-di-*O*-methyl-α-*D*-mannopyranose* (**29**).—Acetolysis of **28** (0.45 g) as described for the preparation of **16** gave the amorphous disaccharide **29** (0.38 g, 81%); $[\alpha]_D$ −11.1° (*c* 1.1, $CHCl_3$), R_f 0.2 (solvent *E*). *Anal.* Calcd for $C_{26}H_{39}NO_{16}$: C, 50.24; H, 6.32; N, 2.25. Found: C, 50.20; H, 6.19; N, 2.17.

4-Nitrophenyl O-*(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-*O*-(3-*O*-acetyl-4,6-di-*O*-methyl-α-*D*-mannopyranosyl)-(1 → 6)-2,3-di-*O*-acetyl-β-*D*-glucopyranoside* (**31**).—Reaction of **29** (0.3 g) with HBr as described for the preparation of **17** gave *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1 → 2)-3-*O*-acetyl-4,6-di-*O*-methyl-α-D-mannopyranosyl bromide **30** as an amorphous solid (0.28 g, 90%); $[\alpha]_D$ +13.6° (*c* 0.5, $CHCl_3$); ^1H NMR (90 MHz, $CDCl_3$): δ 8.03 (d, 1 H, *J* 9 Hz, N*H*), 6.36 (d, 1 H, $J < 1$ Hz, H-1), 3.46 (s, 3 H, OMe-4), 3.35 (s, 3 H, OMe-6), 2.07 (s, 9 H, 3 OAc), and 1.98 (s, 6 H, OAc and NAc).

A reaction of the alcohol[9] **18** (0.13 g, 0.34 mmol) with glycosyl donor **30** (0.27 g, 0.42 mmol) as described for the preparation of **19** gave, after chromatography (solvent *J*), the amorphous trisaccharide **31** (0.25 g, 78%); $[\alpha]_D$ −13.9° (*c* 1.1, $CHCl_3$); R_f 0.2 (solvent *O*). *Anal.* Calcd for $C_{40}H_{54}N_2O_{24}$: C, 50.74; H, 5.75; N, 2.96. Found: C, 51.04; H, 5.59; N, 2.68.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-(1 → 2)-*O*-(4,6-di-*O*-methyl-α-*D*-mannopyranosyl)-(1 → 6)-β-*D*-glucopyranoside* (**3**).—Deacetylation of **31** (0.27 g) as described for the preparation of **2** gave, after chromatography (solvent *K*), the amorphous trisaccharide **3** (0.14 g, 71%); $[\alpha]_D$ −61.5° (*c* 0.6, H_2O); R_f 0.5 (solvent *K*). FABMS: *m/z* 717 [17%, $(M + Na)^+$]. *Anal.* Calcd for $C_{28}H_{42}N_2O_{18}$: C, 48.41; H, 6.09; N, 4.03. Found: C, 48.33; H, 6.19; N, 3.89.

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