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Note

Synthesis of 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-(4-*O*-methyl-α-D-mannopyranosyl)-(1→6)-β-D-glucopyranoside. A potential specific acceptor-substrate for *N*-acetylglucosaminyltransferase-V (GnT V)*†

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In an earlier paper in this series[2], we described the synthesis of some oligosaccharides containing the *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-α-D-mannopyranosyl unit. Such compounds were intended as alternative acceptor–substrates for the enzyme UDP-GlcNAc:α-D-mannopyranosyl-(1→6)-*N*-acetyl-β-D-glucosaminyltransferase (GnT-V), which is thought to be responsible for the transfer of a 2-acetamido-2-deoxy-β-D-glucopyranosyl group to O-6 of the (1→6)-linked α-D-mannosyl residue that forms part of the trimannopyranosyl core of asparagine-linked *N*-glycans[3]. Great interest has recently been shown for this enzyme because an increase in (1→6)-branching of *N*-linked glycans is apparently manifested by some virally-transformed cells[4,5], and is also thought to be associated with metastatic potential of certain tumor cells[6]. Also, in previous publications, we argued that the availability of compounds that are capable of acting as acceptor–substrates for an unique and single enzyme, even in the presence of other, related enzymes, would greatly facilitate the identification and quantitative determination of such enzymes[7]. One such compound is the title trisaccharide described herein. Because of lack of a free OH-4, this trisaccharide would be expected to act as an unique acceptor for GnT-V. Moreover, it has been observed[8] that the (1→6)-linked β-D-mannopyranosyl residue is not a prerequisite for recognition by GnT-V. Thus, as has been the case earlier[2], we preferred the incorporation of a β-D-glucopyranose residue at the reducing terminus in place of the

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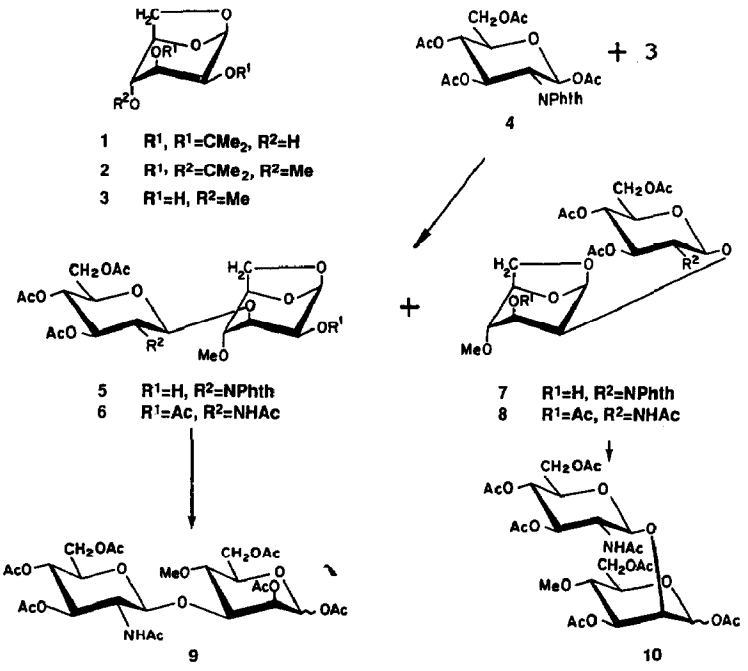
naturally occurring β-D-mannopyranosyl residue, the synthesis of which is hitherto far from simple.

For the synthesis of the title trisaccharide **27**, we employed known 4-nitrophenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside[2] (**25**) as a glycosyl acceptor, and 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-acetyl-4-*O*-methyl-α-D-mannopyranosyl bromide (**24**) as a glycosyl donor. The latter compound was readily prepared from its precursor 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-*O*-acetyl-4-*O*-methyl-D-mannopyranose (**10**). However, for the synthesis of **10**, two routes appeared equally feasible. In the first instance, we attempted the condensation[9] of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**4**) with, 1,6-anhydro-4-*O*-methyl-β-D-mannopyranose (**3**). Compound **3** was obtained in high yield by methylation, according to Kuhn *et al.*[10], of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[11](**1**), followed by cleavage of the acetal group of intermediate **2**. However, the overall yield of **10** *via* this route was discouragingly low, because, in addition to being obtained in low (~9%) yield, the desired disaccharide intermediate **8** was separated with difficulty from its β-(1→3)-linked isomer **6**, which was also isolated in ~7% yield. Acetolysis of **8** in acetic anhydride–acetic acid–sulfuric acid gave **10**, whereas a similar acetolysis of **6** gave the (1→3)-linked isomer **9**. The ^{1}H-n.m.r. spectrum of **10** contained a low-field signal at δ 5.94 (~0.8 H, as compared to the *O*-methyl group protons, $J \sim 1.5$ Hz), suggesting that it was an α,β-anomeric mixture in which the α-D anomer preponderated. A similar anomeric mixture was previously obtained on acetolysis of a related disaccharide[2].

Alternatively, compound **10** was obtained by condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**19**) with methyl 3,6-di-*O*-



benzyl-4-*O*-methyl-α-D-mannopyranoside (**18**), which was, in turn, obtained from methyl α-D-mannopyranoside (**11**) through a succession of fairly good yielding steps. Thus, compound **11** afforded, in fair yield, the 6-*O*-*tert*-butyldiphenylsilyl ether **12** which was converted, in good yield, into its 2,3-*O*-isopropylidene acetal **13**. Methylation[12] of **13**, followed by cleavage of the *tert*-butyldiphenylsilyl ether group at C-6 and subsequent benzylation[14] gave syrupy 6-*O*-benzyl derivative **16** in 78% yield. Hydrolysis of the acetal group gave **17**, which was converted into desired **18** in 58% yield by treatment of its stannylene derivative[15].

Condensation of **18** with bromide **19** and treatment of the crude product with hydrazine hydrate, followed by acetylation, gave after chromatographic purification crystalline methyl 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-4-*O*-methyl-α-D-mannopyranoside (**20**) in 86% yield. Hydrogenolysis of the benzyl groups of **20** and subsequent acetylation afforded, in excellent yield, the hexaacetate **23**, which was subjected to acetolysis to furnish the desired **10**, which was identical (^{1}H-n.m.r., specific rotation, and chromatographic motility) with an authentic sample obtained by acetolysis of **8**. A small portion of **21** was *O*-deacetylated to afford **22**, the ^{13}C-n.m.r. spectrum of which was in agreement with the structure assigned (see Table I). Treatment of a solution of **10** with HBr in glacial acetic acid gave, almost in quantitative yield, amorphous bromide **24**, which as judged by its ^{1}H.n.m.r. (see Experimental section) existed almost exclusively as the α-D anomer. The spectrum of **24** was also reminiscent[2] of its counterpart that was devoid of a methyl group at O-4.

Glycosylation of **25** with bromide **24** in the presence of $Hg(CN)_2$ gave in 64% yield, after column chromatographic purification, the protected trisaccharide derivative **26** as an amorphous solid, the ^{1}H-n.m.r. spectrum of which was in agreement with the structure expected. *O*-Deacetylation of **26** furnished, in 85% yield, 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-(4-*O*-methyl-α-D-mannopyrano-

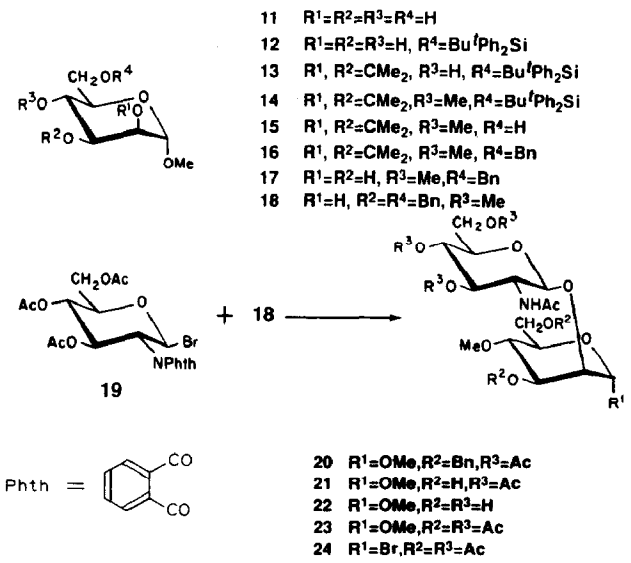


TABLE I

Proposed ^{13}C-n.m.r. assignments for compounds **22**, **27**, and some related compounds[a]

Residue or group	*Compd.*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*OCH_3*	*CH_3CO*
β-D-Glc*p*$OC_6H_4NO_2$-4	[b]	100.20	73.27	77.41	69.73	76.64	60.75		
α-D-Man*p*OMe	[c]	98.73	78.84	70.66	67.40	74.12	61.42	54.14	
β-D-Glc*p*NAc-(1→2)-		101.36	55.80	74.12	70.12	77.20	61.06		23.10
β-D-Glc*p*$OC_6H_4NO_2$-4	[d]	100.31	73.30	76.71	69.69	75.23	66.03		
α-D-Man*p*-(1→6)-		97.57	79.08	70.70	67.26	74.28	61.40		
β-D-Glc*p*NAc-(1→2)-		101.50	55.93	74.05	70.25	77.28	61.16		23.24
4-*O*-Me-α-D-Man*p*-								54.18	
OMe	**22**	98.50	78.92	69.85	77.01	72.64	60.94	59.65	
β-D-Glc*p*NAc-(1→2)-		101.34	55.61	73.88	70.51	77.01	60.94		23.13
β-D-Glc*p*$OC_6H_4NO_2$-4	**27**	99.65	72.96	76.38	69.48	74.96	65.92		
4-*O*-Me-α-D-Man*p*-									
(1→6)-		96.99	79.12	69.77	76.89	72.22	60.85	59.42	
β-D-Glc*p*NAc-(1→2)-		101.24	55.61	73.96	70.34	76.51	60.61		23.09

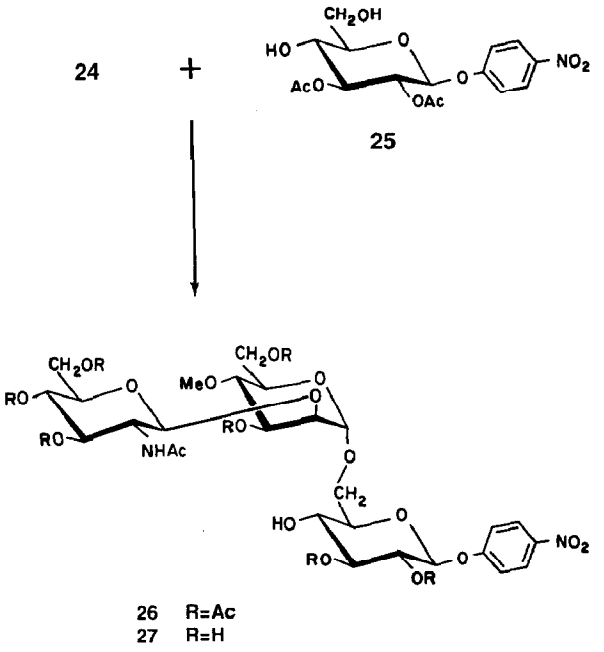
[a] For solutions in $(^2H_3)Me_2SO$ with Me_4Si as the internal standard. Carbonyl and aromatic resonances are not shown. [b] 4-Nitrophenyl β-D-glucopyranoside. [c] Methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside. [d] 4-Nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside. The chemical shifts for the first three compounds are included for comparison purposes; those for compounds *c*[16] and *d*[2] were partially assigned before.

syl)-(1→6)-β-D-glucopyranoside (**27**). The ^{13}C-n.m.r. spectrum of **27** was consistent with the structure assigned (see Table I).

The ^{13}C-n.m.r. signals for disaccharide **22** and trisaccharide **27** were assigned by comparison of the spectra between themselves and with those of their counterparts that are devoid of methyl groups at O-4, namely, methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside, and 4-nitrophenyl *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)-*O*-α-D-mannopyranosyl-(1→6)-β-D-glucopyranoside (see Table I). The ^{13}C-n.m.r. signals for the latter di- and tri-saccharide were partially assigned previously[2,16]. In the ^{13}C-n.m.r. spectra of both **22** and **27**, the carbon atom resonances remained close to those of their respective, nonmethylated counterparts, except for those of carbon atoms affected by alkylation. Thus, in the spectrum of disaccharide **22** and trisaccharide **27**, the signals for C-4 and C-4′ suffered noticeable downfield shifts by comparison to those of their parent compounds, occurring at δ 77.01 and 76.89, respectively. Meanwhile, the signals for C-3 (δ 69.85) and C-5 (δ 72.64), and for C-3′ (δ 69.77) and C-5′ (δ 72.22) of **22** and **27**, were all observed at higher field than those of the respective carbon atoms in the nonmethylated compounds (see Table I) owing to the β shift invariably observed with alkylation[17].

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured at ~27° with a Perkin–Elmer 241 polarimeter.



N.m.r. spectra were recorded at ~25°, ^{1}H-n.m.r. spectra with a Varian EM-390 instrument and ^{13}C-n.m.r. spectra either with a Varian XL-100, a Bruker WP-200, or a Bruker AM-400 instrument, operating at 90, 25.2, 50.3, and 100.6 MHz, respectively; the positions of the peak (δ) are expressed from the tetramethylsilane signal. T.l.c. was conducted on aluminum sheets, precoated with 0.2-mm layers of Silica Gel 60F-25 (E. Merk, Darmstadt, Germany); the compounds were located either by exposure to u.v. light or by spraying the plates with 5% H_2SO_4 in ethanol (or both) and heating. Silica gel used for column chromatography was Baker Analyzed (60–200 mesh). Unless otherwise indicated, the following solvent systems (v/v) were used for chromatography: (*A*) 2:1 Hexane–ethyl acetate; (*B*) 4:1 chloroform–acetone; (*C*) 3:2 chloroform–acetone; (*D*) 1:1 hexane–ethyl acetate; (*E*) 15:1 chloroform–acetone; (*F*) 9:1 chloroform–acetone; (*G*) 19:1 chloroform–methanol; (*H*) 13:6:1 chloroform–methanol–water; and (*I*) 5:4:1 chloroform–methanol–water. Organic solutions were generally dried with anhydrous Na_2SO_4. Pyridine, benzene, and dichloromethane were dried over KOH, Na, and 4A molecular sieves, respectively. Nitromethane was distilled from P_2O_5 immediately before being used. Elemental analyses were performed by Robertson Laboratory, 29 Samson Ave., Madison, New Jersey 08940 (U.S.A.).

*1,6-Anhydro-2,3-*O*-isopropylidene-4-*O*-methyl-β-*D*-mannopyranose* (**2**). — A solution of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[11] (**1**; 10 g), in *N*,*N*-dimethylformamide (250 mL) was stirred for 36 h at room temperature in the presence of BaO (52 g), Ba $(OH)_2 \cdot 8H_2O$ (52 g), and methyl iodide (52 mL). It was then cooled (~0°, bath), diluted with an equal volume of chloroform, and the insoluble material removed by filtration (Celite bed) and thoroughly washed with chloroform. The chloro-

form solution was successively washed with water, aqueous $Na_2S_2O_3$ solution, and water, dried, and concentrated to afford **2** (9.74 g, 91%), syrup, $[\alpha]_D^{27} -33°$ (*c* 2.4, chloroform), R_F 0.26 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.26 (d, 1 H, $J \sim 1.5$ Hz, H-1), 3.46 (s, 3 H OMe), and 1.50 and 1.31 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{10}H_{16}O_5$: C, 55.54; H, 7.46. Found: C, 55.44; H, 7.23.

*1,6-Anhydro-4-*O*-methyl-β-*D*-mannopyranose* (**3**). — A solution of **2** (9 g) in chloroform (30 mL) containing trifluoroacetic acid (30 mL) and water (3 mL) was stirred for 4 h at room temperature. It was then concentated, and several portions of toluene were added to, and evaporated from the residue. T.l.c. (*B*) revealed the presence of a major product, slower-migrating than **2**, together with some faster- and some slower-migrating contaminants. The crude product was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–2% methanol in chloroform. Evaporation of the fractions corresponding to the major product gave **3** (4.9 g, 67%), syrup, $[\alpha]_D^{27} -102°$ (*c* 1.1, chloroform), R_F 0.14 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.33 (br. s, 1 H, H-1), and 3.46 (s, 3 H, OMe).

Anal. Calc. for $C_7H_{12}O_5$: C, 47.72; H, 6.87. Found: C, 47.43; H, 6.99.

*3-*O*-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-2-*O*-acetyl-1,6-anhydro-4-*O*-methyl-β-*D*-mannopyranose* (**6**) *and 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-3-*O*-acetyl-1,6-anhydro-4-*O*-methyl-β-*D*-mannopyranose* (**8**). — A mixture of 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranose (**4**; 4.8 g, 10.1 mmol), compound **3** (1.8 g, 10.1 mmol), and 4 A molecular sieves (13.63 g) in dichloromethane (150 mL) was stirred for 1 h at room temperature and then treated with trimethylsilyl trifluoromethanesulfonate ($Me_3SiOSO_2CF_3$; 2.72 mL, 14.07 mmol) and the stirring was continued for an additional 17 h at room temperature. T.l.c. (solvent *B*) then revealed the presence of a product faster-migrating than **3**, a small proportion of **4**, as well as some slower- and some faster-migrating impurities. The mixture was rendered neutral by the dropwise addition of triethylamine, and the solids were filtered off (a bed of Celite), and washed with dichloromehtane. The filtrate and washings were combined and concentrated, and the crude mixture was applied to a column of silica gel. On elution with a solvent gradient consisting of 0–10% acetone in chloroform, evaporation of the fractions containing the product gave a white amorphous sodil, contaminated with some faster-migrating impurities as shown by t.l.c. in *B*, but it was neither purified nor characterized at this stage. The crude product ($\sim$2.88 g, containing **5** and **7**) was boiled for 3 h under N_2 in a mixture of ethanol (45 mL) and hydrazine hydrate (12 mL). The solution was concentrated to dryness to yield a residue, which was dissolved in pyridine (50 mL) and acetic anhydride (25 mL), and stirred overnight at room temperature. The acetic anhydride and pyridine were removed under diminished pressure, and the residue was suspended in chloroform, the solid material filtered off, the filtrate concentrated, and the concentrate applied to a column of silica gel. Elution with a solvent gradient consisting of 0–15% acetone in chloroform gave the (1→3)-linked isomer (see later) as a white solid which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the precipitation of **6** (0.4 g, 7.4%, based on **3**), amorphous, $[\alpha]_D^{27} -36°$ (*c* 1, chloroform), R_F 0.31 (solvent *C*); ^{1}H-n.m.r.

($CDCl_3$): δ 6.0 (d, 1 H, $J \sim 9$ Hz, NH), 5.33 (br. s, 1 H, H-1), 3.46 (s, 3 H, OMe), 2.13 and 1.05 (s, 3 H each, 2 OAc), 1.03 (s, 6 H, 2 OAc), and 1.94 (s, 3 H, NAc).

Anal. Calc. for $C_{23}H_{23}NO_{14}$: C, 50.45; H, 6.07; N, 2.56. Found: C, 50.12; H, 5.69; N, 2.30.

Continued elution of the column gave a fraction (~ 0.4 g) consisting of a mixture of **6** and the slower-migrating product. The last fraction to be eluted afforded the chromatographically homogeneous (1→2)-linked isomer (see later) as a solid which was dissolved in a small volume of dichloromethane. Addition of ether–hexane caused the crystallisation of **8** (0.5 g, 9.2% based on **3**), m.p. 235–236°, $[\alpha]_D^{27} - 34°$ (*c* 0.7 chloroform), R_F 0.22 (solvent *C*); ^{1}H-n.m.r. ($CDCl_3$): δ 6.02 (d, 1 H, $J \sim 9$ Hz, NH), 5.40 (br. s, 1 H, H-1), 3.51 (s, H, OMe), 2.08 and 2.07 (s, 3 H each, 2 OAc), 1.99 (s, 6 H, 2 OAc), and 1.92 (s, 3 H, NAc).

Anal. Calc. for $C_{23}H_{33}NO_{14}$: C, 50.45; H, 6.07; N, 2.56. Found: C, 50.40; H, 5.75; N, 2.40.

3-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-O-acetyl-4-O-methyl-D-mannopyranose (**9**). — Compound **6** (0.2 g) was dissolved in a precooled mixture of 70:30:1 (v/v) acetic anhydride–acetic acid–conc. H_2SO_4 (12 mL), and the mixture was stirred for 15 min at $\sim 0°$ (bath). It was then allowed to gradually warm up to room temperature, and the stirring was continued for an additional 3 h. After neutralization with saturated aqueous sodium acetate, the mixture was diluted with dichloromethane (50 mL), successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated. The concentrate was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–15% acetone in chloroform to afford a solid which was dissolved in a small volume of dichloromethane. Addition of ether–hexane precipitated **9** (0.15 g, 50%), amorphous, $[\alpha]_D^{27} - 6°$ (*c* 0.7, chloroform), R_F 0.43 (solvent *C*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.94 (d, ~ 0.8 H, $J \sim 1.5$ Hz, H-1), 5.60 (d, 1 H, $J \sim 9$ Hz, NH), 3.48 (s, 3 H, OMe), 2.12, 2.09, 2.07, and 2.04 (s, 3 H each, 4 OAc), 2.0 (s, 6 H, 2 OAc), and 1.91 (s, 3 H, NAc).

Anal. Calc. for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found: C, 49.76; H, 5.75; N, 1.99.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-1,3,6-tri-O-acetyl-4-O-methyl-D-mannopyranose (**10**). — Compound **8** (0.2 g) was acetolyzed and purified as described for **6** (to give **9**) to give a solid which was dissolved in a small volume of dichloromethane. Addition of ether–hexane precipitated **10** (0.18 g, 70%), amorphous, $[\alpha]_D^{27} - 11°$ (*c* 0.8, chloroform), R_F 0.45 (solvent *C*); ^{1}H-n.m.r. ($CDCl_3$): δ 6.05 (d. 1 H, $J \sim 9$ Hz, NH), 5.92 (d, 0.8 H, $J \sim 1.5$ Hz, H-1), 3.48 (s, 3 H, OMe), 2.13 and 2.10 (s, 3 H each, 2 OAc), 2.05 and 1.99 (s, 6 H each, 4 OAc), and 1.91 (s, 3 H, NAc).

Anal. Calc. for $C_{27}H_{39}NO_{17}$: C, 49.92; H, 6.05; N, 2.16. Found: C, 49.76; H, 5.74; N, 1.81.

Methyl 6-O-tert-butyldiphenylsilyl-α-D-mannopyranoside (**12**). — To a stirred solution of methyl α-D-mannopyranoside (**11**; 5.0 g, 25.8 mmol) and imidazole (2.83 g, 41.6 mmol) in anhydrous *N*,*N*-dimethylformamide (60 mL) was added *tert*-butylchlorodiphenylsilane (9 mL, 34.6 mmol), and stirring was continued for 6 h at room

temperature. The mixture was then poured into ice–water, and the solid extracted with chloroform. The chloroform solution was successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated, and the residue applied to a column of silica gel. Elution with a solvent gradient consisting of 0–15% acetone in chloroform gave **12** (7.97 g, 72%), a thick syrup, $[\alpha]_D^{27} + 23°$ (*c* 1.5, chloroform), R_F 0.34 (solvent *C*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.83–7.23 (m, 10 H, arom.), 4.61 (s, 1 H, H-1), 3.28 (s, 3 H, OMe) and 1.06 (s, 9 H, CMe_3).

Anal. Calc. for $C_{23}H_{32}O_6Si$: C, 63.86; H, 7.46. Found: C, 63.57; H, 7.17.

*Methyl-6-*O*-*tert*-butyldiphenylsilyl 2,3-*O*-isopropylidene-4-*O*-methyl-*α*-*D*-mannopyranoside* (**14**). — To a solution of **12** (7.62 g) in dry acetone (130 mL) were added 2,2-dimethoxypropane (130 mL) and 4-toluenesulfonic acid monohydrate (1.78 g). The mixture was stirred for 24 h at room temperature, made neutral by the addition of triethylamine, and concentrated. The residue was dissolved in chloroform, the solution washed with water, dried, and concentrated. The concentrate was applied to a column of silica gel and eluted with 10–20% ethyl acetate in hexane to afford **13** (7.02 g, 84%), yellow syrup, $[\alpha]_D^{27} + 2°$ (*c* 1.8, chloroform), R_F 0.40 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.78–7.34 (m, 10 H, arom.), 4.88 (s, 1 H, H-1), 3.34 (s, 3 H, OMe), 1.48 and 1.34 (s, 3 H each, CMe_2), and 1.08 (s, 9 H, CMe_2).

A mixture of **13** (1 g), freshly prepared Ag_2O (2 g), and methyl iodide (2 mL) in *N*,*N*-dimethylformamide (15 mL) was stirred for 20 h at room temperature. The solids were removed by filtration (Celite bed) and thoroughly washed with *N*,*N*-dimethylformamide, and the filtrate and washings were combined and concentrated under reduced pressure. The residue was stirred in chloroform (~150 mL), and the precipitated silver salt filtered off and washed with chloroform. The chloroform solution was successively washed with water, aqueous $Na_2S_2O_3$ solution, and water, dried, and concentrated to give a syrup, which showed in t.l.c. (solvent *A*) a major product faster-migrating than **13**, a small proportion of **13**, and some faster-migrating contaminants. The crude mixture was applied to a column of silica gel and eluted with a solvent gradient consisting of 10–20% ethyl acetate in hexane. Evaporation of the fractions corresponding to the major product gave **14** (0.6, 65%), syrup, $[\alpha]_D^{27} + 10°$ (*c* 1.6, chloroform), R_F 0.53 (solvent *A*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.85–7.15 (m, 10 H, arom.), 4.86 (s, 1 H, H-1), 3.43 (s, 3 H, OMe-4), 3.28 (s, 3 H, OMe-1), 1.49 and 1.30 (s, 3 H each, CMe_2), and 1.08 (s, 9 H, CMe_3).

Anal. Calc. for $C_{27}H_{38}O_6Si$: C, 66.63; H, 7.87. Found: C, 66.86; H, 7.90.

*Methyl 2,3-*O*-isopropylidene-4-*O*-methyl-α-*D*-mannopyranoside* (**15**). — A solution of **14** (14 g) in anhydrous oxolane (100 mL) was treated with an M solution of tetrabutylammonium fluoride in oxolane (50 mL), and the stirring was continued for 5 h at room temperature. The mixture was concentrated to dryness, and the residue was purified in a column of silica gel with a solvent gradient consisting of 50–75% chloroform in hexane to give **15** (6.5 g, 91%), syrup, $[\alpha]_D^{27} + 51°$ (*c* 1.8, chloroform), R_F 0.30 (solvent *D*); ^{1}H-n.m.r. ($CDCl_3$): δ 4.88 (s, 1 H, H-1), 3.51 (s, 3 H, OMe-4), 3.36 (s, 3 H, OMe-1), and 1.51 and 1.35 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{11}H_{20}O_6$: C, 53.21; H, 8.12. Found: C, 53.24; H, 8.05.

*Methyl 6-*O*-benzyl-2,3-*O*-isopropylidene-4-*O*-methyl-α-*D*-mannopyranoside* (**16**).

— To a stirred mixture of **15** (14.45 g, 58.2 mmol), powdered KOH (8.16 g, 145.5 mmol), and 18-Crown-6 (0.77 g, 2.91 mmol) in oxolane (116 mL) was added benzyl bromide (10.38 mL, 87.3 mmol) dropwise during 15 min. The mixture was stirred for 6 h at room temperature. T.l.c. (solvent *E*) then showed the presence of a product faster-migrating than **15** together with some unreacted **15**. More portions of KOH (4.08 g, 72.75 mmol), 18-Crown-6 (0.39 g, 1.46 mmol), and benzyl bromide (5.19 mL, 43.65 mmol) were added and the mixture was stirred for an additional 16 h at room temperature. It was diluted with dichloromethane (~300 mL) and repeatedly washed with water, dried, and concentrated to give a syrup which showed in t.l.c. (solvent *E*) a major product faster-migrating than **15**, as well as some slower and faster-migrating contaminants. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 0–50% chloroform in hexane. On evaporation, fractions corresponding to the major product gave **16** (15.4 g, 78%), syrup, $[\alpha]_D^{26}+33°$ (*c* 2.1, chloroform), R_F 0.53 (solvent *E*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.33–7.17 (m, 5 H, arom.), 4.88 (s, 1 H, H-1), 4.56 (d, 2 H, $J_{5,6}$ ~ 2 Hz, H-6,6'), 3.47 (s, H, OMe-4), 3.37 (s, 3 H, OMe-1), and 1.52 and 1.33 (s, 3 H each, CMe_2).

Anal. Calc. for $C_{18}H_{26}O_6$: C, 63.88; H, 7.75. Found: C, 64.01; H, 7.46.

Methyl 6-O-benzyl-4-O-methyl-α-D-mannopyranoside (**17**). — The isopropylidene group of **16** (10 g) was removed as described for **2** (to give **3**) to give a solid residue which was dissolved in a little chloroform and applied to a column of silica gel. After elution with a solvent gradient consisting of 50–100% chloroform in hexane, concentration of the fractions corresponding to the major product gave a residue which crystallized from dichloromethane–ether to afford **17** (16 g, 68%), m.p. 87–88°, $[\alpha]_D^{27}+77°$ (*c* 0.8, chloroform), R_F 0.17 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.35–7.23 (m, 5 H, arom.), 3.45 (s, 3 H, OMe-4), and 3.31 (s, 3 H, OMe-1).

Anal. Calc. for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 60.59; H, 7.18.

Methyl 3,6-di-O-benzyl-4-O-methyl-α-D-mannopyranoside (**18**). — A solution of **17** (5.1 g, 17 mmol) and dibutyltin oxide (4.2 g, 17 mmol) in methanol (268 mL) was boiled for 2 h. The solvent was then evaporated to dryness to give a residue, which was dissolved in *N*,*N*-dimethylformamide (54 mL), and benzyl bromide (2.7 mL, 22.9 mmol) was added. The mixture was stirred overnight at 95–100°, and then poured into water and extracted with chloroform. The chloroform solution was washed several times with water, dried, and concentrated to dryness. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 5–15% ethyl acetate in hexane. On evaporation, the fractions corresponding to the product afforded **18** (3.8 g, 58%), syrup, $[\alpha]_D^{27}+49°$ (*c* 1.5, chloroform), R_F 0.37 (solvent *D*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.44–7.16 (m, 10 H, arom.), 3.41 (s, 3 H, OMe-4), and 3.24 (s, 3 H, OMe-1).

Anal. Calc. for $C_{21}H_{28}O_6$: C, 68.02; H, 7.26. Found: C, 68.28; H, 7.21.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-benzyl-4-O-methyl-α-D-mannopyranoside (**20**). — A mixture of **18** (3.14 g, 8.08 mmol), silver trifluoromethanesulfonate (2.65 g, 10.31 mmol), 2,4,6-trimethylpyridine (1.20 g, 9.90 mmol), and 4A molecular sieves (6.9 g) in dichloromethane (51 mL), protected from light and moisture, was stirred for 30 min in an atmosphere of N_2. A

solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl bromide (**19**; 5 g, 10.01 mmol) in dichloromethane (25 mL) was then added dropwise with stirring during 20 min, and the stirring was continued for an additional 40 min. More portions of silver trifluoromethanesulfonate (1.33 g, 5.18 mmol) and 2,4,6-trimethylpyridine (0.6 g, 4.95 mmol) were added, followed by the dropwise addition of a solution of bromide **19** (2.8 g, 5.61 mmol) in dichloromethane (17 mL), and the stirring was continued for an additional 20 h. T.l.c. (solvent *F*) then revealed the presence of a major product, faster-migrating than **18**, and some slower- and faster-migrating contaminants (presumably due to decomposition of **19**). The mixture was diluted with an equal volume of dichloromethane, and the solids were filtered off (a bed of Celite) and washed with dichloromethane. The filtrate and washings were combined, successively washed with ice-cold water, cold 3% aqueous HCl, cold saturated $NaHCO_3$, and water, dried, and concentrated to dryness. The foamy solid so obtained (~ 10.8 g) was boiled for 3 h under N_2 in a mixture of ethanol (170 mL) and hydrazine hydrate (35 mL). The solvent was then evaporated to give a residue, which was dissolved in pyridine (190 mL) and acetic anhydride (95 mL), and stirred overnight at room temperature. Pyridine and acetic anhydride were evaporated under diminished pressure, the residue was taken in chloroform, and the solution successively washed with water, aqueous $NaHCO_3$, and water, dried, and concentrated. The residue was applied to a column of silica gel and eluted with a solvent gradient consisting of 25–75% chloroform in hexane. On evaporation, the fractions corresponding to the product gave a solid which crystallized from ethyl acetate to give **20** (5 g, 86% based on **18**), m.p. 190–192°, $[\alpha]_D^{27}$ +25° (*c* 0.9, chloroform), R_f 0.29 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 7.32 (s, 10 H, arom.), 3.50 (s, 3 H, OMe-4), 3.30 (s, 3 H, OMe-1), 2.00 (s, 9 H, 3 OAc), and 1.75 (s, 3 H, NAc).

Anal. Calc. for $C_{36}H_{47}NO_{14}$: C, 60.24; H, 6.60; N, 1.95. Found: C, 59.94; H, 6.64; N, 2.04.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-4-O-methyl-α-D-mannopyranoside (**21**). — A mixture of **20** (6.73 g) and 10% Pd–C (4 g) in glacial acetic acid was shaken under H_2 at ~ 345 kPa for 21 h at room temperature. The suspension was filtered (a bed of Celite), the solid was thoroughly washed with glacial acetic acid, and the filtrate and washings were combined and concentrated under diminished pressure to give a residue which was dissolved in chloroform and applied to a column of silica gel. Elution with a solvent gradient consisting of 1–3% methanol in chloroform, and evaporation of the fractions corresponding to the product gave a solid which was dissolved in dichloromethane. Addition of ether–hexane precipitated **21** (4.1 g, 81%), amorphous, $[\alpha]_D^{27}$ +8° (*c* 0.8, chloroform), R_f 0.13 (solvent *G*); ^{1}H-n.m.r. ($CDCl_3$): δ 3.58 (s, 3 H, OMe-4), 3.36 (s, 3 H, OMe-1), and 2.10–1.99 (cluster of s, 12 H, 3 OAc and NAc).

Anal. Calc. for $C_{22}H_{35}NO_{14}$: C, 49.16; H, 6.56; N, 2.61. Found: C, 48.97; H, 6.31; N, 2.94.

Methyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-4-O-methyl-α-D-mannopyranoside (**22**). — Compound **21** (0.3 g) in 20 mM methanolic sodium methoxide (40 mL) was stirred overnight at room temperature. The base was neutralized with a few

drops of glacial acetic acid, and the solution was de-ionized with Amberlite IR-120 (H^+) cation-exchange resin. The resin was filtered off (Celite bed) and thoroughly washed with methanol, and the filtrate and washings were combined and concentrated to give a material which was dissolved in a little methanol. Addition of ether–hexane caused the precipitation of **22** (0.19 g, 83%), amorphous, $[\alpha]_D^{27}+2°$ (*c* 0.9, water), R_F 0.32 (solvent *H*); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{16}H_{29}O_{11}N\cdot 1.5\ H_2O$: C, 43.83; H, 7.36; N, 3.20. Found: C, 43.66; H, 6.98; N, 2.82.

Methyl 2-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-4-O-methyl-α-D-mannopyranoside (**23**). — A solution of **21** (1.55 g) in 1:2 acetic anhydride–pyridine (60 mL) was stirred overnight at room temperature. Pyridine and acetic anhydride were evaporated under diminished pressure, the last traces being removed by coevaporation with several added portions of toluene, to give a residue which was dissolved in dichloromethane. Addition of ether caused the precipitation of **23** (1.64 g, 91.5%), amorphous, $[\alpha]_D^{27}-96°$ (*c* 0.8, chloroform), R_F 0.15 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 5.92 (d, 1 H, $J\sim 9$ Hz, NH), 3.44 (s, 3 H, OMe-4), 3.33 (s, 3 H, OMe-1), 2.10–2.0 (cluster of s, 15 H, 5 OAc), and 1.90 (s, 3 H, NAc).

Anal. Calc. for $C_{26}H_{39}NO_{16}$: C, 50.24; H, 6.32; N, 2.25. Found: C, 49.95; H, 5.83; N, 1.95.

Acetolysis of **23**. — A solution of compound **23** (1.4 g) in acetic anhydride (30 mL) containing 1% (v/v) of conc. H_2SO_4 was stirred for 17 h at room temperature. The mixture was then diluted with dichloromethane ($\sim$170 mL), successively washed with water, saturated $NaHCO_3$, and water, dried, and concentrated. The residue was dissolved in a small volume of ethyl acetate, and addition of ether caused the precipitation of **10** (1 g, 68%), amorphous, $[\alpha]_D^{26}-12°$ (*c* 0.55, chloroform), R_F 0.21 (solvent *B*); it had identical chromatographic mobility (solvent *B* and *C*) with an authentic sample of **10** (obtained by acetolysis of **8**); ^{1}H-n.m.r. ($CDCl_3$): δ 6.14 (d, 1 H, $J\sim 9$ Hz, NH), 5.87 (d, $\sim$0.8 H, $J\sim 1.5$ Hz, H-1), 3.45 (s, 3 H, OMe), 2.12 and 2.09 (s, 3 H each, 2 OAc), 2.07 and 1.99 (s, 6 H each, 4 OAc), and 1.89 (s, 3 H, NAc).

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,6-di-O-acetyl-4-O-methyl-α-D-mannopyranosyl bromide (**24**). — To a cold ($\sim$0°, bath), stirred solution of **10** (0.64 g) in dichloromethane (7.7 mL) was added a 31% solution of HBr in glacial acetic acid (7.7 mL), and stirring was continued for 12 h at 0°. The mixture was then poured into ice–water and extracted with dichloromethane, successively washed with cold water, cold saturated $NaHCO_3$, and cold water, dried, and concentrated to give **24** (0.64 g, 97%), amorphous, $[\alpha]_D^{26}+48°$ (*c* 1, chloroform), R_F 0.27 (solvent *B*); ^{1}H-n.m.r. ($CDCl_3$): δ 6.20 (d, $\sim$1 H, $J\sim 1$ Hz, H-1), 2.10 (s, 3 H, OAc), 2.07 (s, 6 H, 2 OAc), 2.02 and 1.99 (s, 3 H each, 2 OAc), and 1.88 (s, 3 H, NAc).

4-Nitrophenyl O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→2)-O-(3,6-di-O-acetyl-4-O-methyl-α-D-mannopyranosyl)-(1→6)-2,3-di-O-acetyl-β-D-glucopyranoside (**26**). — A stirred solution of 4-nitrophenyl 2,3-di-O-acetyl-β-D-glucopyranoside[2] (**25**; 0.22 g, 0.57 mmol), powdered $Hg(CN)_2$ (0.2 g, 0.79 mmol), and powdered 4A molecular sieves (0.6 g) in 1:1 benzene–nitromethane (60 mL) was boiled

until ~25 mL of the solvent had distilled off. After cooling to room temperature, disaccharide bromide **24** (0.63 g, 0.88 mmol) in 1:1 benzene–nitromethane (12 mL) was added, and the stirring was continued for 15 h at ~40°. T.l.c. (solvent *G*) showed the presence of a major product, slightly slower-migrating than **25**, and some slower- and faster-migrating contaminants. The mixture was then cooled and filtered through a bed of Celite, the solids were thoroughly washed with benzene, and the filtrate and washings were combined and diluted with benzene to a total volume of ~200 mL. The solution was successively washed with water, M KI solution, aqueous $NaHCO_3$, and water, dried, and concentrated, and the concentrate applied to a column of silica gel. Elution with a solvent gradient consisting of 0–2% methanol in chloroform and evaporation of the fractions corresponding to the product gave a solid residue which was dissolved in a little dichloromethane. Addition of ether–hexane precipitated **26** (0.37 g, 64%), amorphous, $[\alpha]_D^{27} -13°$ (*c* 0.55, chloroform), R_F 0.29 (solvent *G*); ^{1}H-n.m.r. ($CDCl_3$): δ 8.25 and 7.08 (d, 2 H each, $J \sim 10$ Hz, arom.), 5.88 (d, 1 H, $J \sim 9$ Hz, NH), 3.34 (s, 3 H, OMe), 2.11 (s, 9 H, 3 OAc), 2.05 and 1.99 (s, 6 H each, 4 OAc), and 1.84 (s, 3 H, NAc).

Anal. Calc. for $C_{43}H_{56}N_2O_{26}$: C, 50.78; H, 5.55; N, 2.76. Found: C, 50.74; H, 5.62; N, 2.58.

4-Nitrophenyl O-*(2-acetamido-2-deoxy-β-*D-*glucopyranosyl)-(1→2)*-O-*(4*-O-*methyl-α-*D-*mannopyranosyl)-(1→6)-β-*D-*glucopyranoside* (**27**). — Compound **26** (0.2 g) was *O*-deacetylated in 20mM methanolic sodium methoxide (30 mL) exactly as described for **21** (to give **22**) to give a solid residue, which was dissolved in a small volume of methanol. Addition of ether–hexane caused the precipitation of **27** (0.114 g, 85%), amorphous, $[\alpha]_D^{27} -57°$ (*c* 1.2, water), R_F 0.36 (solvent *I*); ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{27}H_{40}N_2O_{18}$: C, 47.64; H, 5.92; N, 4.12. Found: C, 47.64; H, 6.00; N, 3.87.

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