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SYNTHESIS OF A HEXASACCHARIDE UNIT OF A COMPLEX TYPE OF GLYCAN CHAIN OF A GLYCOPROTEIN*

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ABSTRACT

The synthesis of a branching hexasaccharide unit, methyl 3-*O*-[2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-6-*O*-[2,4-di-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside, a core structure of the complex type of glycans of glycoproteins, is described.

INTRODUCTION

Complex types of glycan chains of glycoproteins exhibit, for the glycans, unique branching modes[2] that have not been observed in the case of high-D-mannose types of glycan chains. Although high-D-mannose types of glycans show the 3,6-branching mode at the D-mannopyranosyl residues in the core structure, complex types of glycans exhibit the 2,4- and 2,6-branching modes, in addition to 3,6-branching, at the D-mannopyranosyl residues.

For example, in 1979, Baenziger and Fiete[3] reported that the complete structure of the glycan chain of fetuin, the major glycoprotein in fetal-calf serum, is **1**, which shows both 2,4- and 3,6-branching modes. Svensson *et al.*[4], however, proposed a similar, but isomeric, structure for this glycan. Complex types of glycans of similar structures have recently been reported for such glycoproteins as human α_1-protease inhibitor[5], the membrane glycoprotein of vesicular stomatitis virus[6], α_1-acid glycoprotein of human plasma[7], and the membrane glycoprotein of calf-thymocyte plasma[8].

The unique, branching structure having three antennae, as depicted in **1**, and the presence of similar structures in several glycoproteins of biological importance, have stimulated our efforts directed toward their chemical synthesis. In this context, unambiguous synthesis of di- and tri-saccharides having[9] the sequence [β-D-GlcNAc]$_n$-(1→X)-α-D-Man-(1→OMe, and synthetic routes towards lactosaminyl donors[10,11], were recently reported. As the next step towards the synthesis of **1**, we

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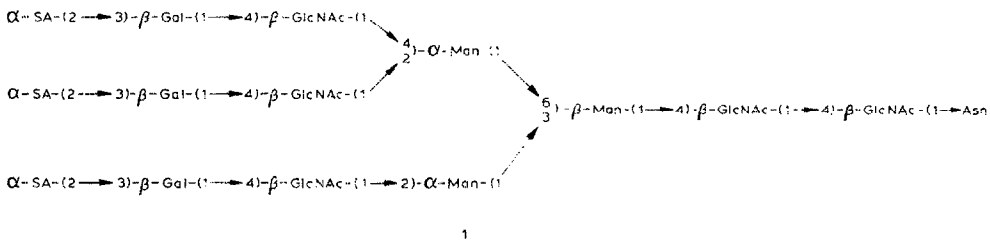
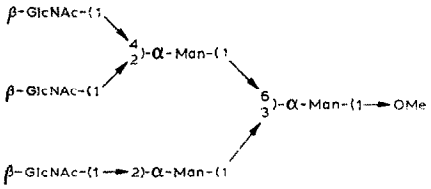


1

SA = sialic acid group



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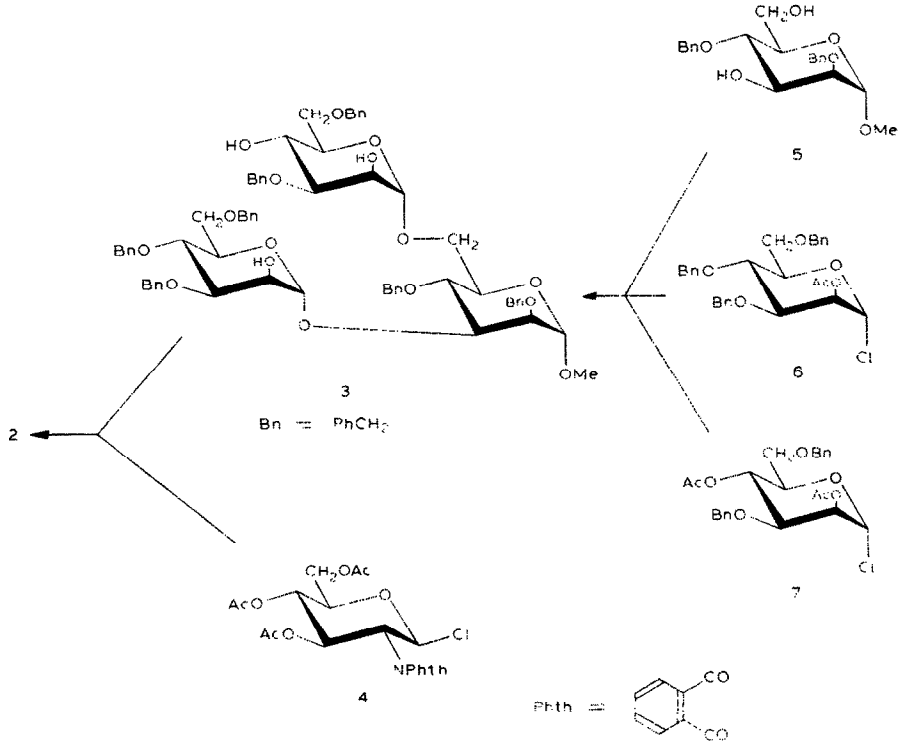
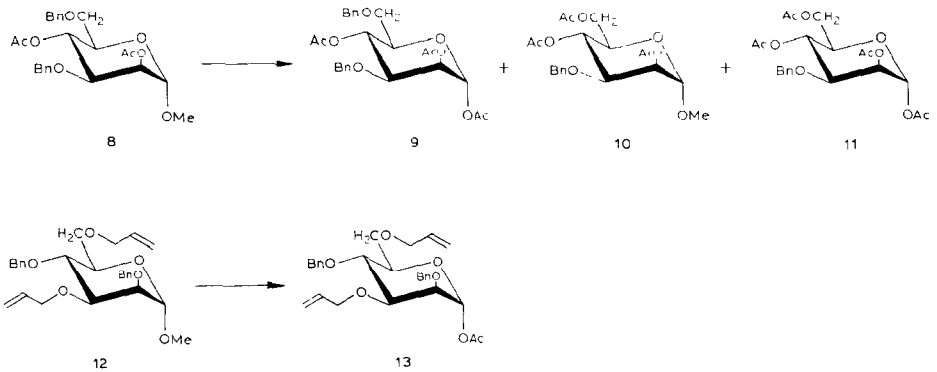


Scheme 1 (synthetic plan)

designed, as the target, compound **2**, the inner-core hexasaccharide unit of **1**, and developed a synthetic route[12] towards **2**.

A retrosynthetic analysis of the target structure **2** indicated, as a key intermediate, the partially protected mannotriose **3**, which can accept three molecules of the β-D-GlcNAc donor[13] **4**. The key intermediate **3** should, in turn, be retrosynthesized into one α-D-mannopyranosyl acceptor, **5**, and two α-D-mannopyranosyl donors, **6** and **7** (see Scheme 1).

Among the four monosaccharide synthons (**4**, **5**, **6**, and **7**) thus designed, three, namely, synthons **4** (ref. 13), **5** (ref. 14), and **6** (ref. 15), had already been prepared. Therefore, a synthetic route towards the monosaccharide synthon **7** was first studied. Acetolysis of methyl 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-D-mannopyranoside[14a,16] (**8**) afforded the desired triacetate **9** in only 14.4% yield. The other two products, **10** and **11**, were obtained in 36.0 and 7.4% yield, respectively, in addition to a 7.0% recovery of starting material **8**. It may be noted that a parallel experiment on the acetolysis of the 3,6-di-*O*-allyl derivative[14] **12** gave an 80% yield of triacetate **13**. Thus, the relative ease of cleavage of the C–O linkages in acetolysis was found to be in the order 6-*O*-benzyl ≥ 1-*O*-methyl > 6-*O*-allyl. The facile cleavage of the 6-*O*-benzyl group on α-D-Man in acetolysis had been reported by Ponpipom[17] and Schuerch *et al.*[18]. Therefore, in order to synthesize the mannopyranosyl donor **7**, the allyl group[19] was chosen as the protective group for the anomeric oxygen atom, as it can be removed under mild conditions[19,20] without causing cleavage of the 6-*O*-benzyl group.

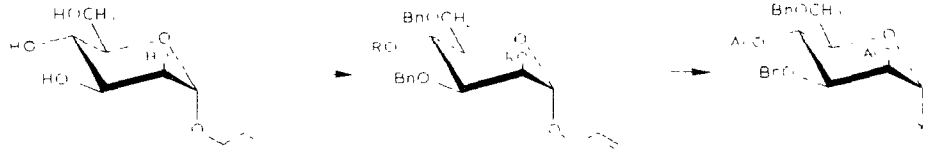


Scheme 2

RESULTS

Allyl α-D-mannopyranoside (**14**) was regioselectively benzylated *via* the partially stannylated intermediate[21], to give the 3,6-di-*O*-benzyl derivative **15** in 54% yield. Acetylation of **15** to **16**, and deallylation of **16** with $PdCl_2$–NaOAc-aq. AcOH, afforded **17** in 64% yield. The hydroxyl derivative **17** was quantitatively converted

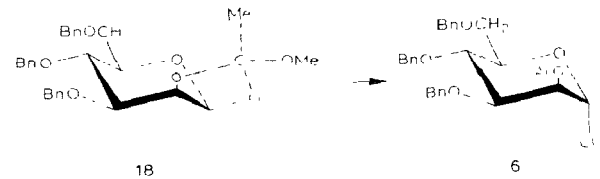
into the chloride **4** by treatment with the complex[22] $[Me_2N^+=CH\text{-}OSOCl]\ Cl^-$, formed *in situ* from $SOCl_2$ and a catalytic amount of *N*,*N*-dimethylformamide (DMF) in 1,2-dichloroethane.



14 15 R = H 16 R = Ac 17 X = OH

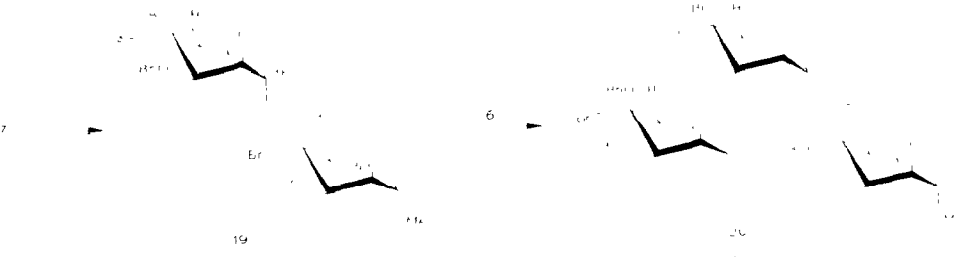
Scheme 3

Having prepared the new α-D-mannopyranosyl donor **7**, regioselective introduction of **7** at the primary hydroxyl group of **5** was now examined. Treatment of **5** with 1.7 molar equivalents of **7** in the presence of $AgOSO_2CF_3$–1,1,3,3-tetramethylurea, according to Hanessian and Banoub[23], afforded the desired D-mannobiosyl derivative **19** in 50% yield. The stereochemistry at C-1b was expected to be α, due to be presence[14] of the 2-*O*-Ac group in the D-mannosyl donor **7**, and this was supported by the ^{13}C-n.m.r. data for the final product (**2**). Introduction of another α-D-mannopyranosyl group, at O-3a of **19**, was achieved, to give the D-mannotriosyl derivative **20** in 62% yield, by using the α-D-mannopyranosyl donor **6**, which was readily available from the orthoester **18** by a reported procedure[14,15]. Subsequent deacetylation of **20** afforded the key intermediate **3**.



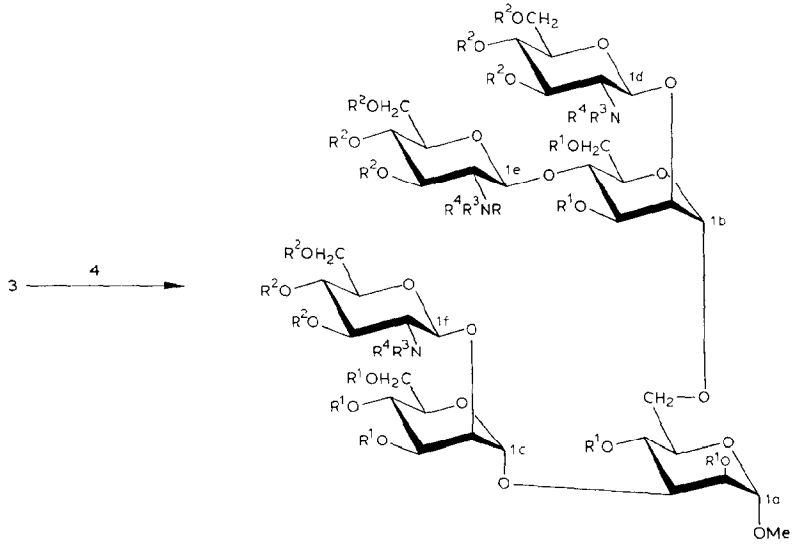
18 6

Scheme 4



19

The transformation of **3** into the target compound **2** was performed by using a method developed by Lemieux *et al.*[24]. The reaction of **3** with 6.25 molar equivalents of the 2-amino-2-deoxy-β-D-glucosyl donor **4** in the presence of $AgOSO_2CF_3$–collidine in 1,2-dichloroethane produced hexasaccharide **21** in 35% yield. Deacylation of **21** with[25] $BuNH_2$ in MeOH, and *N*-acetylation of **21** with Ac_2O–MeOH afforded **22** in 20% yield, and catalytic hydrogenolysis of the benzyl groups of **22** in the presence of 10% Pd–C gave the target hexasaccharide **2**.



Scheme 6

The structure of **2** was proved by the following ^1H- and ^{13}C-n.m.r. data for **2**. The 400-MHz, ^1H-n.m.r. spectra of **2** and the reference D-mannotrioside[14] **23** are shown in Fig. 1. Because we had reported[9] that introduction of a β-D-GlcNAc group on O-2, or O-4, or both, of an α-D-Man residue does not cause any substantial effect on the chemical shift of the anomeric proton of the α-D-Man, a close similarity between the chemical-shift values of H-1a, H-1b, and H-1c in the spectrum of **2** and in that of **23** was expected. Therefore, the assignment of each signal of **2** was made as follows. The signals for H-1a, H-1b, and H-1c were respectively assigned to three doublets (*J* 2 Hz) at δ 4.740, 4.936, and 5.155, in close agreement with the signals for H-1a, H-1b, and H-1c in[26] **23**, which, respectively appeared as three doublets (*J* 2 Hz) at δ 4.738, 4.920, and 5.117. These observations strongly supported the 3,6-branching mode at Man-a in **2**. Signals for H-1d, H-1e, and H-1f appeared at δ 4.605, 4.600, and 4.575 as three doublets, with *J* 8–9 Hz. Although three doublets for the anomeric protons of the GlcNAc groups could not be definitely assigned to any specific group, the observation of the vicinal coupling-constant of 8–9 Hz supported the β-D con-

figuration for each GlcNAc group. We had also observed[9], for the model compounds, that, when an α-D-Man residue carries a β-D-GlcNAc group at O-2, or two such, at O-2 and O-4, the signals for the anomeric carbon atoms of the α-D-Man residues are shielded by 3.2 or 3.8 p.p.m., respectively. As the signals for C-1a, C-1b, and C-1 of **23** had been reported[2,6] to appear at δ 101.8, 100.2, and 103.2, the corresponding

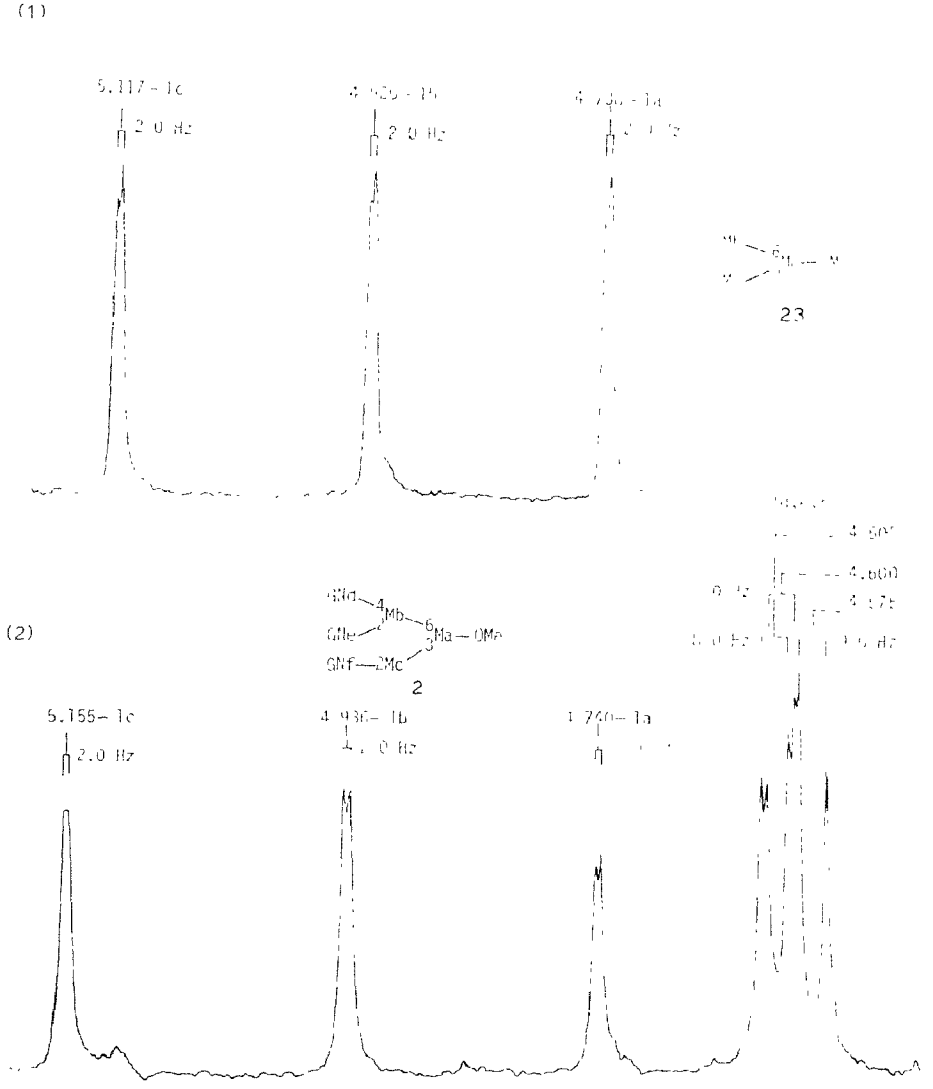


Fig. 1. (1) ^{1}H-N.m.r. spectrum (400 MHz) of methyl D-mannotrioside **23** in D_2O at 57°. (2) ^{1}H-N.m.r. spectrum (400 MHz) of hexasaccharide **2** in D_2O at 75°. [^{1}H-N.m.r. spectra were recorded with a JEOL JNM-FX 400 spectrometer operating at 400.5 MHz. The values of δ_H are expressed in p.p.m. downward from the internal standard, sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. Samples were prepared by "exchanging" them with D_2O, *i.e.*, by dissolving them several times in 99.8% D_2O and evaporating *in vacuo*.]

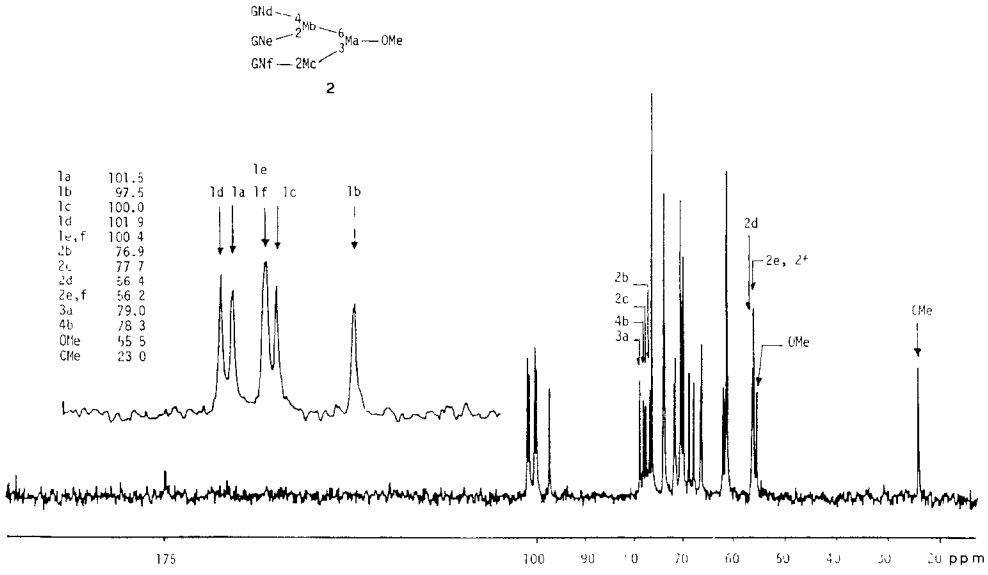


Fig. 2. ^{13}C-N.m.r. spectrum of hexasaccharide **2** in D_2O at 80°. [The ^{13}C-N.m.r. spectrum was recorded with a JEOL JNM-FX 100FT spectrometer operating at 25.05 MHz. The values of δ_C are expressed in p.p.m. downward from tetramethylsilane, referenced indirectly with an internal standard of 1,4-dioxane (δ_C 66.9).]

signals for C-1a, C-1b, and C-1c in **2** would be expected to appear at δ 101.8, 96.4 (100.2 — 3.8), and 100.0 (103.2 — 3.2). In fact, three signals, with $^1J_{CH}$ 170 Hz, were observed, at δ 101.5, 100.0, and 97.5, in the spectrum of **2** (see Fig. 2), and were readily assigned to the signals for C-1a, C-1c, and C-1b, respectively. The remaining two signals, observed at δ 101.9 and 100.4 in the ratio of 1 : 2, with $^1J_{CH}$ 156 Hz, were assigned as follows. As it had been reported[9] that the anomeric carbon atoms of β-D-GlcNAc groups linked to O-2 or O-3 of α-D-Man are relatively shielded, and appear at δ 99.6–100.3, whereas those of β-D-GlcNAc groups linked to O-4 or O-6 of α-D-Man appear at δ 101.8–102.0, the signals at δ 101.9 and 100.4 were readily assigned to C-1d, and C-1e and C-1f, respectively. The ^{13}C- and ^{1}H-n.m.r. data for the anomeric carbon atoms and protons of **2** were reasonably assigned, based on our previous observations, and were found to be compatible with the target structure **2**.

In conclusion, a regio- and stereo-controlled, synthetic sequence leading to the hexasaccharide unit **2**, a core structure of the complex type of glycans of glycoproteins, has now been developed. In close connection with our approach to the synthesis of **2**, it may be noted that an approach to complex types of glycans having two antennae was recently reported by Arnarp and Lönngren[11].

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-

point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241MC polarimeter for solutions in $CHCl_3$ at 25 °, unless noted otherwise. Column chromatography was performed on columns of Silica Gel Merck (70–230 mesh; E. Merck, Darmstadt, Germany). Thin-layer chromatography was conducted on precoated plates (layer thickness, 0.25 mm; E. Merck, Darmstadt, Germany) of Silica Gel 60 F_{254}. Flash chromatography was performed on columns of Silica Gel C-300 (200–300 mesh; Wako-Pure Chemical Industries, Osaka, Japan). I.r. spectra were recorded with an EPI-G2 Hitachi spectrophotometer, as KBr disks for the crystalline samples, and as neat films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with a Varian HA-100 n.m.r. spectrometer, and ^{13}C-n.m.r. spectra with a JNM-FX 100FT n.m.r. spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in p.p.m. downward from the internal standard, tetramethylsilane, for solutions in $CDCl_3$, unless noted otherwise.

Acetolysis of methyl 2,4-di-O-acetyl-3,6-di-O-benzyl-α-D-mannopyranoside (**8**). — To a solution of **8** (307.7 mg), $[\alpha]_D$ +56.8° (*c* 0.48, MeOH), in Ac_2O (3 mL) was added, dropwise, 2% H_2SO_4–AcOH (3 mL) at 0–5 °. After stirring for 30 min at 0–5 °, NaOAc (0.3 g) was added, and the mixture was evaporated *in vacuo*. The residue was partitioned between EtOAc and H_2O, and the organic layer was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was flash-chromatographed on SiO_2 C-300 (30 g) with 7:1 toluene–EtOAc, to give the following fractions.

(*i*) A 1:1 mixture (42.8 mg) of **8** (R_F 0.47 in 5:1 toluene–EtOAc) and **9** [(**8** + **9**): δ_H 6.10 (d, *J* 2 Hz, H-1 of **9**) and 3.38 (s, OMe of **8**) in the ratio of 1:3].

(*ii*) **9** (25.6 mg), R_F 0.41, $[\alpha]_D$ +9.1° (*c* 0.96); δ_H 6.09 (d, 1 H, *J* 2 Hz), 5.32 (q, 1 H, *J* 2.4 Hz, H-2), 5.29 (t, 1 H, *J* 10 Hz, H-4), 2.14 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), and 1.92 (s, 3 H, Ac).

Anal. Calc. for $C_{26}H_{30}O_9$: C, 64.18; H, 6.22. Found: C, 64.10; H, 6.26.

(*iii*) Compound **10** (34.0 mg), R_F 0.26, $[\alpha]_D$ −1.0° (*c* 1.47); δ_H 5.36 (q, 1 H, *J* 2.4 Hz, H-2), 5.24 (t, 1 H, *J* 10 Hz, H-4), 4.74 (d, 1 H, *J* 2 Hz, H-1), 3.38 (s, 3 H, OMe), 2.16 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), and 2.02 (s, 3 H, Ac).

Anal. Calc. for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39. Found: C, 58.59; H, 6.41.

(*iv*) A 3:1 mixture (86.8 mg) of **10** and **11** (R_F 0.22); (**10** + **11**): δ_H 6.08 (d, *J* 2 Hz, H-1 of **11**), 4.72 (d, *J* 2 Hz, H-1 of **10**, partially overlapped with CH_2Ph signals), and 3.36 (s, OMe of **10**). The combined yields of **9**, **10**, and **11** were 14.4, 36.0, and 7.4%, respectively. A 7.0% recovery of **8** was also observed.

3,6-Di-O-allyl-2,4-di-O-benzyl-α-D-mannopyranosyl acetate (**13**). — To a solution of **12** (12.6 g) in Ac_2O (100 mL) was added, dropwise, 2% H_2SO_4–AcOH (100 mL) at 0–5 °. After stirring the mixture for 10 min at 5 °, NaOAc (8.0 g) was added, to neutralize the H_2SO_4. The mixture was evaporated *in vacuo*, and the residue was partitioned between EtOAc and H_2O. The organic layer was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated, to give an oil which was chromatographed on SiO_2 (700 g) with 10:1 toluene–EtOAc, to give **13** (10.6 g, 79.1%); R_F 0.54 in 5:1 toluene–EtOAc; $[\alpha]_D$ +32.2° (*c* 1.06); δ_H 7.5–7.3

(m, 10 H, aromatic), 6.24 (d, 1 H, *J* 2 Hz, H-1), 6.16–5.76 (m, 2 H, 2 -C*H*=CH_2), 5.44–5.10 (m, 4 H, 2 -CH=CH_2), 4.98 and 4.63 (ABq, 2 H, *J* 9 Hz, CH_2Ph), 4.80 (s, 2 H, CH_2Ph), and 2.04 (s, 3 H, Ac).

Anal. Calc. for $C_{28}H_{34}O_7$: C, 69.69; H, 7.10. Found: C, 69.73; H, 7.07.

*Allyl 3,6-di-*O*-benzyl-α-*D*-mannopyranoside* (**15**). — A mixture of **14** (20 g, 91 mmol) and $(Bu_3Sn)_2O$ (77.5 g, 130 mmol) in toluene (1.000 L) was boiled and stirred under reflux for 3 h with continuous, azeotropic removal of water, cooled, and evaporated *in vacuo*. A solution of the residue in benzyl bromide (200 mL) was stirred under Ar for 2 days at 90°, cooled, evaporated *in vacuo*, a solution of the residue in EtOAc mixed with aq. KF, and the mixture stirred for 30 min. The precipitate of Bu_3SnF was filtered off, the filtrate extracted with EtOAc, and the extract washed with water, dried ($MgSO_4$), and evaporated *in vacuo* to an oil which was chromatographed on SiO_2 (1 kg) with 1:1 toluene–EtOAc, to give **15** (19.5 g, 53.6%); R_F 0.44 in 1:1 toluene–EtOAc; $[\alpha]_D$ +30.8° (*c* 0.39); δ_H 7.30 (s, 5 H, aromatic), 7.28 (s, 5 H, aromatic), 6.1–5.65 (m, 1 H, -C*H*=CH_2), 5.4–5.05 (m, 2 H, CH=CH_2), and 4.88 (d, 1 H, *J* 1 Hz, H-1).

Anal. Calc. for $C_{23}H_{28}O_6$: C, 68.98; H, 7.05. Found: C, 68.96; H, 7.07.

*Allyl 2,4-di-*O*-acetyl-3,6-di-*O*-benzyl-α-*D*-mannopyranoside* (**16**). — To a solution of **15** (1.3 g, 3.2 mmol) in pyridine (15 mL) was added Ac_2O (7 mL) at 0–5°, and the mixture was stirred for 16 h at 20°. The usual processing, and chromatography on SiO_2 with 3:1 toluene–EtOAc afforded a quantitative yield of **16**; R_F 0.59 in 3:1 toluene–EtOAc; $[\alpha]_D$ +11.1° (*c* 0.45); δ_H 7.28 (s, 5 H, aromatic), 7.26 (s, 5 H, aromatic), 6.0–5.64 (m, 1 H, -C*H*=CH_2), 4.86 (bs, 1 H, H-1), 2.11 (s, 3 H, Ac), and 1.90 (s, 3 H, Ac).

Anal. Calc. for $C_{27}H_{32}O_8$: C, 66.92; H, 6.66. Found: C, 66.94; H, 6.66.

*2,4-Di-*O*-acetyl-3,6-di-*O*-benzyl-*D*-mannopyranose* (**17**). — A mixture of **16** (500 mg, 1.03 mmol), NaOAc (200 mg, 2.4 mmol), and $PdCl_2$ (200 mg, 1.1 mmol) in AcOH (2 mL)–H_2O (0.1 mL) was stirred for 14 h at 20°, and evaporated *in vacuo* below 30°. A solution of the residue in EtOAc was successively washed with aq. $NaHCO_3$ and H_2O, dried ($MgSO_4$), and evaporated *in vacuo*. Chromatography of the residue on SiO_2 (30 g) with 4:1 toluene–EtOAc afforded **17** (294 mg, 64%); R_F 0.30 in 3:1 toluene–EtOAc; $[\alpha]_D$ −18.9° (*c* 0.37), unchanged after 16 h; δ_H ($CDCl_3$–D_2O): 7.25 (s, 5 H, aromatic), 7.24 (s, 5 H, aromatic), 5.31 (dd, 1 H, $J_{1,2}$ 2, $J_{2,3}$ 3.5 Hz, H-2), 5.14 (d, 1 H, *J* 2 Hz, H-1), 5.10 (t, 1 H, *J* 10 Hz, H-4), 3.90 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10 Hz, H-3), 2.08 (s, 3 H, Ac), and 1.88 (s, 3 H, Ac).

Anal. Calc. for $C_{24}H_{28}O_8$: C, 64.85; H, 6.35. Found: C, 64.36; H, 6.46.

*2,4-Di-*O*-acetyl-3,6-di-*O*-benzyl-α-*D*-mannopyranosyl chloride* (**7**). — To a solution of **17** (2.0 g, 4.5 mmol) in $Cl(CH_2)_2Cl$ (28 mL) were added $SOCl_2$ (0.66 mL, 9 mmol) and DMF (0.1 mL) at 0–5°. The mixture was stirred for 14 h at 15–20°, filtered through a bed of SiO_2 (2.5 g), and the filtrate co-evaporated with toluene (three times), to give **7** (quantitative yield). An analytical sample of **7** was obtained by chromatography on SiO_2 with 3:1 toluene–EtOAc (R_F 0.52); $[\alpha]_D$ +40.0° (*c* 0.27); δ_H 7.26 (s, 10 H, aromatic), 6.02 (d, 1 H, *J* 1 Hz, H-1), 5.41 (dd, 1 H, $J_{1,2}$ 2,

$J_{2,3}$ 4 Hz, H-2), 5.30 (t, 1 H, J 10 Hz, H-4), 4.12 (dd, 1 H, $J_{2,3}$ 4, $J_{3,4}$ 10 Hz, H-3), 2.10 (s, 3 H, Ac), and 1.89 (s, 3 H, Ac).

Anal. Calc. for $C_{24}H_{27}ClO_7$: C, 62.27; H, 5.88; Cl, 7.66. Found: C, 61.69; H, 5.82; Cl, 7.72.

Methyl 2,4-di-O-benzyl-6-O-(2,4-di-O-acetyl-3,6-di-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**19**). — To a mixture of **5** (1.65 g, 4.4 mmol), $AgOSO_2CF_3$ (3.44 g, 13.4 mmol), and 1,1,3,3-tetramethylurea (4.2 mL, 35 mmol) in $Cl(CH_2)_2Cl$ (20 mL) was added, dropwise, a solution of **7** (2.00 g, 4.3 mmol) in $Cl(CH_2)_2Cl$ (5 mL) at 0–5°. After stirring for 5 h at 20°, a solution of **7** (1.5 g, 3.2 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added dropwise at 0–5°, the mixture stirred for 16 h at 20°, diluted with $Cl(CH_2)_2Cl$ (50 mL), filtered, and the filtrate processed, to give an oil which was chromatographed on SiO_2 (200 g) with 4:1 toluene–EtOAc, to give **19** (1.75 g, 49.5%); R_F 0.38 in 3:1 toluene–EtOAc; $[\alpha]_D$ +29.7° (*c* 0.58); δ_H 7.32–7.20 (m, 20 H, aromatic), 5.44 (bs, 1 H, H-2b), 5.26 (t, 1 H, J 10 Hz, H-4b), 4.94 (bs, 1 H, H-1a or H-1b), 3.24 (s, 3 H, OMe), 2.10 (s, 3 H, Ac), and 1.86 (s, 3 H, Ac).

Anal. Calc. for $C_{49}H_{52}O_{13}$: C, 67.49; H, 6.54. Found: C, 67.41; H, 6.55.

Methyl 3-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-2,4-di-O-benzyl-6-O-(2,4-di-O-acetyl-3,6-di-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**20**). — To a mixture of **19** (1.40 g, 1.75 mmol), $AgOSO_2CF_3$ (0.93 g, 3.6 mmol), and tetramethylurea (1.5 mL, 12.6 mmol) was added dropwise a solution of **6** (1.25 g, 2.45 mmol) in $Cl(CH_2)_2Cl$ (5 mL) during 40 min at 0–5°. The mixture was stirred for 3 days at 20°, diluted with $Cl(CH_2)_2Cl$ (100 mL), filtered (Celite), and the filtrate processed as usual. Chromatography on SiO_2 (150 g) with 4:1 toluene–EtOAc afforded **20** (1.38 g, 61.9%); R_F 0.49 in 3:1 toluene–EtOAc; $[\alpha]_D$ +35.2° (*c* 0.29); δ_H 7.4–7.2 (m, 35 H, aromatic), 5.54 and 5.46 (dd, 2 H, J 2.4 Hz, H-2b,2c), 5.21 (d, 1 H, J 2 Hz, H-1), 5.20 (t, 1 H, J 9 Hz, H-4b), 4.96 (d, 1 H, J 2 Hz, H-1), 3.20 (s, 3 H, OMe), 2.14 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), and 1.88 (s, 3 H, Ac).

Anal. Calc. for $C_{74}H_{82}O_{19}$: C, 69.69; H, 6.48. Found: C, 69.42; H, 6.43.

Methyl 2,4-di-O-benzyl-6-O-(3,6-di-O-benzyl-α-D-mannopyranosyl)-3-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**3**). — A solution of **20** (638 mg, 0.5 mmol) in oxolane (10 mL)–MeOH (5 mL)–2M NaOMe (0.2 mL) was stirred for 3 h at 20°. The usual processing gave a quantitative yield of **3**. A small portion of **3** was purified by chromatography on SiO_2, to give an analytical sample: R_F 0.30 in 1:1 toluene–EtOAc; $[\alpha]_D$ +39.5° (*c* 0.39); δ_H 7.4–7.1 (35 H, aromatic), 5.20 (bs, 1 H, H-1), 5.00 (bs, 1 H, H-1), and 3.22 (s, 3 H, OMe).

Anal. Calc. for $C_{68}H_{76}O_{16}$: C, 71.06; H, 6.67. Found: C, 70.70; H, 6.76.

Methyl 2,4-di-O-benzyl-6-O-[3,6-di-O-benzyl-2,4-di-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-3-O-[3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (**21**). — To a mixture of **3** (546 mg, 0.48 mmol), $AgOSO_2CF_3$ (771 mg, 3 mmol), and collidine (0.6 mL) in $Cl(CH_2)_2Cl$ (15 mL) was added, dropwise, a solution of **4** (680 mg, 1.5 mmol) in $Cl(CH_2)_2Cl$ (3 mL) at 0–5°. After stirring for 6 h at 20°, compound **4** (680 mg, 1.5 mmol) in $Cl(CH_2)_2Cl$ (3 mL)

was added, and the mixture was stirred for 16 h at 20°. Processing, and chromatography on SiO_2 (50 g) with 50:3 $CHCl_3$–acetone, afforded **21** (400 mg, 34.7%); R_F 0.70 in 25:2 CH_2Cl_2–acetone; $[\alpha]_D$ +22.4° (*c* 0.38); δ_H 7.8–7.0 (m, 47 H, aromatic), 3.24 (s, 3 H, OMe), and 2.04, 2.00, 1.98, 1.94, 1.92, 1.88, 1.84, 1.82, and 1.78 (9 s, 9 Ac).

Anal. Calc. for $C_{128}H_{133}N_3O_{43}$: C, 64.02; H, 5.58; N, 1.75. Found: C, 64.02; H, 5.56; N, 1.60.

*Methyl 3-*O*-[2-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-3,4,6-tri-*O*-benzyl-α-*D*-mannopyranosyl]-2,4-di-*O*-benzyl-6-*O*-[2,4-di-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-3,6-di-*O*-benzyl-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**22**). — A mixture of **21** (350 mg, 0.15 mmol) and $BuNH_2$ (4.0 mL, 41 mmol) in MeOH (15 mL) was stirred under reflux for 72 h. After addition of further $BuNH_2$ (5.0 mL, 51 mmol), the mixture was boiled under reflux for 6 h, cooled, and evaporated *in vacuo*. To a solution of the residue in MeOH (5 mL) was added, dropwise, Ac_2O (0.3 mL) at 0–5°. After being kept for 16 h at 20°, the mixture was evaporated *in vacuo*, the residue suspended in EtOAc (40 mL), and the suspension stirred vigorously for 1 h, and filtered. (The filtrate did not contain **22**, according to t.l.c. examination.) The solid was chromatographed on SiO_2 (50 g) with 50:24:1 $CHCl_3$–MeOH–H_2O, to give **22** (50 mg, 20%); R_F 0.21 in 80:24:1 $CHCl_3$–MeOH–H_2O; $[\alpha]_D$ +19.4° (*c* 0.18); δ_H (CD_3OD): 7.66–7.0 (m, 35 H, aromatic), 1.95 (s, 6 H, 2 NAc), and 1.86 (s, 3 H, NAc).

Anal. Calc. for $C_{92}H_{115}N_3O_{31}$: C, 62.82; H, 6.59; N, 2.39. Found: C, 62.38; H, 6.70; N, 2.29.

*Methyl 3-*O*-[2-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-6-*O*-[2,4-di-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-α-*D*-mannopyranosyl]-α-*D*-mannopyranoside* (**2**). A mixture of **22** (42 mg) and 10% Pd–C (70 mg) in EtOH (5 mL)–H_2O (0.7 mL) was stirred under H_2 for 8 h at 50–60°. The usual processing gave **2** quantitatively; R_F 0.20 in 10:30:3 $CHCl_3$–MeOH–H_2O; $[\alpha]_D$ +14.7° (*c* 0.34).

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