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Synthesis of an octasaccharide fragment of high-mannose-type glycans of glycoproteins*,†

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ABSTRACT

O-(α-D-Mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→3)-*O*-[(α-D-mannopyranosyl)-(1→2)-*O*-(α-D-mannopyranosyl)-(1→6)]-*O*-(α-D-mannopyranosyl)-(1→6)-*O*-(β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-2-deoxy-glucopyranose, an octasaccharide fragment of high-mannose type glycan of glycoproteins, was synthesized. Crucial glycosylation of trisaccharide intermediate, benzyl *O*-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside, was successful only with a di-*O*-acetyltetradeca-*O*-benzyl-D-mannopentaosyl chloride. The use of the corresponding hexadeca-*O*-acetyl-D-mannopentaosyl bromide did not give the desired product.

INTRODUCTION

Numerous efforts[2] have been made for chemically synthesizing glycoprotein glycans since it has been recognized that these compounds are of biological significance for intercellular recognition. Moreover, a greater understanding of the chemical properties of these glycans is desirable. Tetradecasaccharide **1**[3,4] is the glycan part of a key isoprenoid sugar intermediate in glycoprotein biosynthesis, and it is transferred onto an asparagine residue of a nascent protein in the endoplasmic reticulum. Eventually, this glycan structure linked to the protein matures into the high-mannose-, complex-, or hybrid-type-glycan through trimming and glycosylation in the endoplasmic reticulum and Golgi apparatus.

In designing a total synthesis of the precursor oligosaccharide **1**, retrosynthetic analysis led us to the three rational segments **2**, **3**, and **4**. We have reported the synthesis of the tri-[5,6], penta-[7], and hexa-saccharide[6,8] corresponding to **2**, **3**, and **4**, respectively, in the protected or nonprotected form. We have also reported[9] the synthesis of the branched mannohexaoside derivative **5**, a partial structure of **1**, where the stereoselective formations of α-D-(1→2), -(1→3), and -(1→6) linkages were elaborated. Through the glycosylation reactions with various mannosyl donors and acceptors, it is now well established that the glycosyl donors, 2-*O*-acyl-D-mannosyl chlorides, gave high yields of

* Dedicated to Professor Serge David on the occasion of his 70th birthday.
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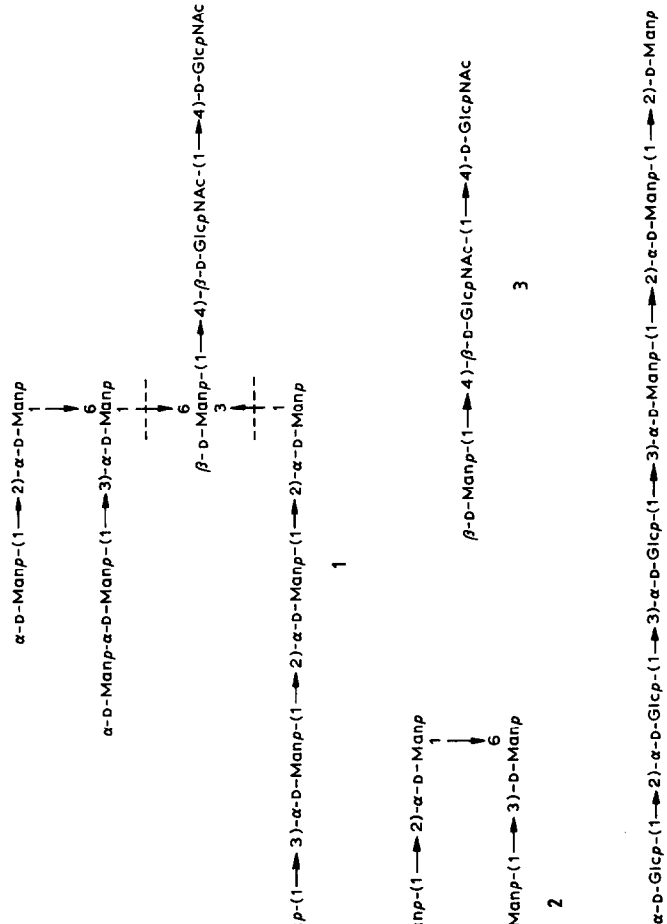
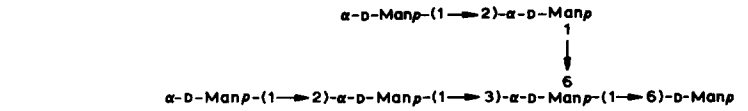

α-D-Manp-(1→2)-α-D-Manp
1
6
α-D-Manp-α-D-Manp-(1→3)-α-D-Manp
1
6
β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcpNAc
3
1
α-D-Glcp-(1→2)-α-D-Glcp-(1→3)-α-D-Glcp-(1→3)-α-D-Manp-(1→2)-α-D-Manp-(1→2)-α-D-Manp
1
α-D-Manp-(1→2)-α-D-Manp
1
6
α-D-Manp-(1→2)-α-D-Manp-(1→3)-D-Manp
2
β-D-Manp-(1→4)-β-D-GlcpNAc-(1→4)-D-GlcpNAc
3
α-D-Glcp-(1→2)-α-D-Glcp-(1→3)-α-D-Glcp-(1→3)-α-D-Manp-(1→2)-α-D-Manp-(1→2)-D-Manp
4

the α anomer in the presence of the promotor, silver trifluoromethanesulfonate, and that HO-6 is more reactive than HO-3 toward a 2-*O*-D-mannosyl-D-mannosyl donor at the branch-point D-mannosyl residue.



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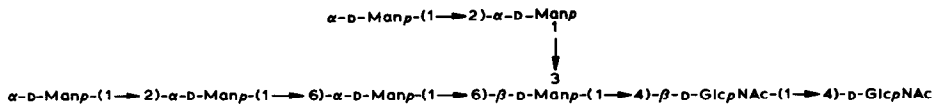

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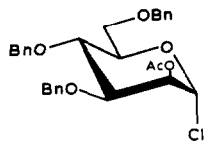
Based on this knowledge, we directed our attention toward the synthesis of larger glycan segments of **1**, and we report herein the first total synthesis of octasaccharide **6** by coupling of segments **2** and **3**. It is to be noted that several elegant syntheses of high-mannose-type glycans have been reported[10,11] independently.

RESULTS AND DISCUSSION

Compounds **7–12** were the necessary monosaccharide synthons and the syntheses of **7** [6,12], **8**[13], **9**[9], **10**[14], and **13**[15] have previously been described. Synthon **11** was readily prepared from the corresponding hemiacetal **12**[14] by treatment with thionyl chloride–*N*,*N*-dimethylformamide[16] in dichloromethane. The trisaccharide synthon **18**, corresponding to segment **2**, was synthesized with the monosaccharide glycosyl donors **10** and **11**, and acceptor **3**. Glycosylation of **13** with **11** in the presence of $AgOSO_2CF_3$ and molecular sieves 4A produced the chitobiose derivative **14** in 62% yield. Acid-catalyzed hydrolysis[17] of **14** gave deacetylated **15** in 82% yield. Coupling of **10** and **15** was promoted by the heterogeneous catalysis procedure of Paulsen and assoc.[18]. Compound **15** was glycosylated by the use of an excess (4 equivs.) of **10** in the presence of silver silicate in 1,2-dichloroethane to give a mixture of **16** (40%) and **19** (36%), which were separated by column chromatography. So far, other coupling conditions could not improve the ratio of β to α anomer of the isomeric products in favor of **16**. The structures of **16** and **19** were readily assigned from the ^{13}C- (ref. 19) and ^1H-n.m.r. spectra. The anomeric carbon signals for **16** appeared at δ 101.5 ($^1J_{C,H}$ 156.3 Hz, C-1″) and 97.0 ($^1J_{C,H}$ 164.8 Hz, C-1,1′), and the anomeric signals for **19** at δ 100.1 ($^1J_{C,H}$ 169.7 Hz, C-1′), 97.1 ($^1J_{C,H}$ 164.8 Hz, C-1 or 1′), and 96.7 ($^1J_{C,H}$ 164.8 Hz, C-1 or 1′).

Deallylation of **16** with $PdCl_2$[20] in AcOH–NaOAc[21] for 1 h at 70° afforded **17** in 58% yield. Conversion of **17** into the acetamido derivative **18** was executed in 90% yield through dephthaloylation[22] with methanolic $BuNH_2$, acetylation, and selective deacetylation.



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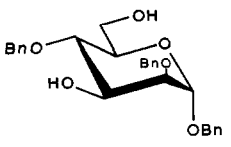

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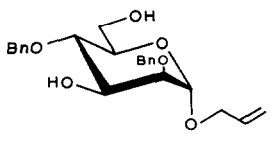

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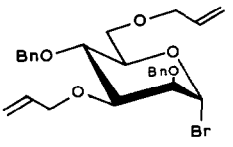

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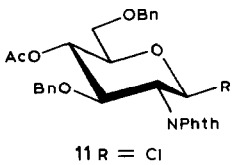
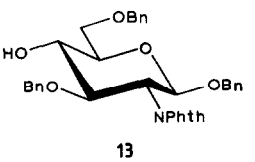


11 R = Cl

12 R = OH



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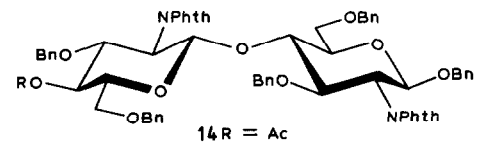
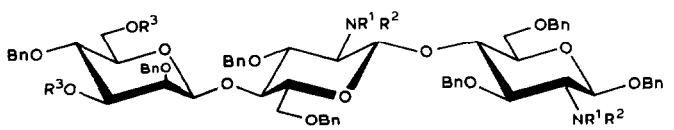


14 R = Ac

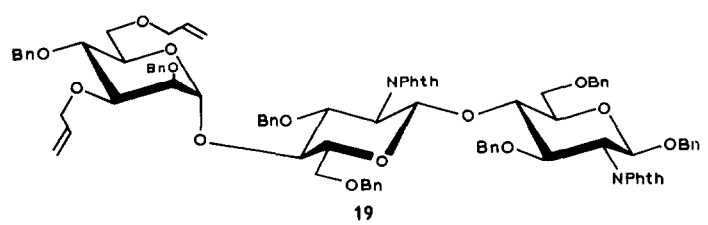
15 R = H



16 R^1, R^2 = Phth; $R^3 = CH_2CH{=}CH_2$

17 R^1, R^2 = Phth; R^3 = H

18 R^1, R^2 = Ac, H; R^3 = H



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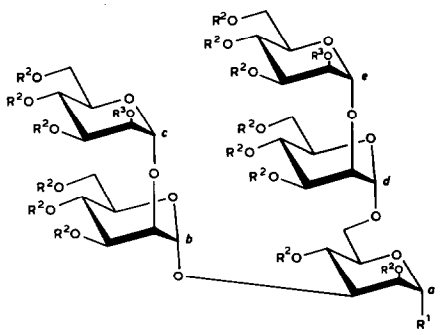
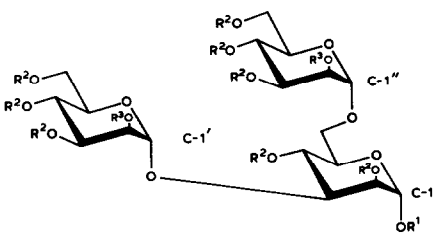
For segment **3**, hexadeca-*O*-acetyl-D-mannopentaosyl bromide **20** and tetradeca-*O*-benzyl derivative **21** were prepared and examined for their efficiency in glycosylation reaction. Glycosylation[23] of **8** by **7** promoted by $AgOSO_2CF_3$ proceeded in the presence of 1,1,3,3-tetramethylurea to give trisaccharide **27**, which was deacetylated with NaOMe in MeOH–oxolane to **23** in 78% yield. Coupling of **7** and **28** under the same conditions afforded **22** in 63% yield. The structure of pentasaccharide **22** was confirmed by ^{13}C-n.m.r. spectroscopy, in which five signals for C-1 in α-D configuration showed at δ



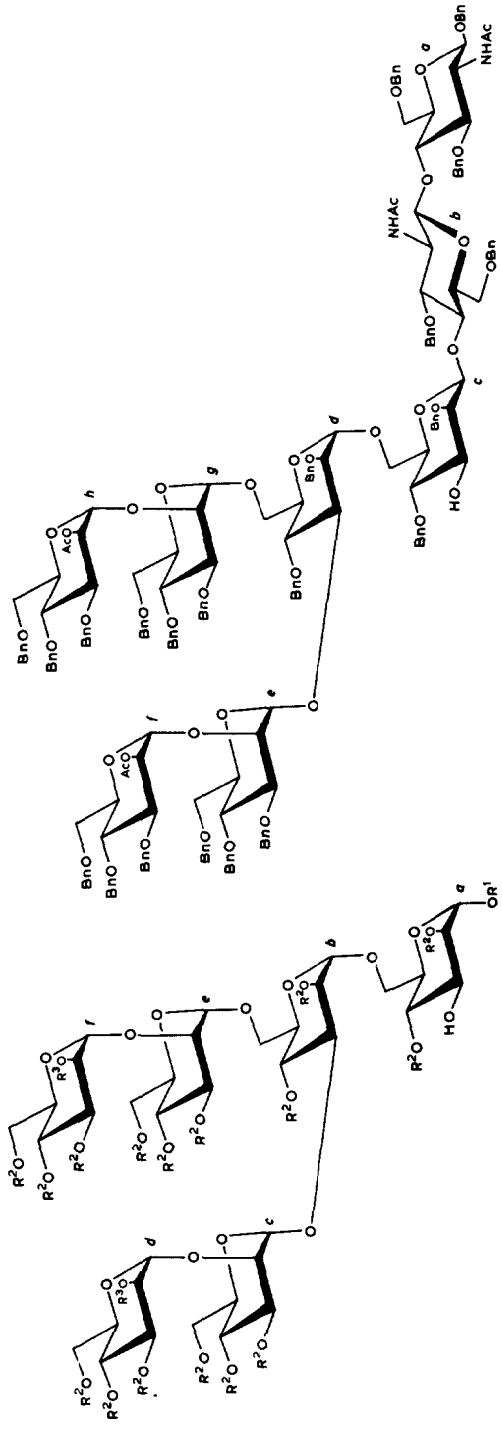
20 R^1 = Br, R^2 = R^3 = Ac
21 R^1 = Cl, R^2 = Bn, R^3 = Ac
22 R^1 = OBn, R^2 = Bn, R^3 = Ac
23 R^1 = OBn, R^2 = Bn, R^3 = H
24 R^1 = OAc, R^2 = R^3 = Ac
25 R^1 = $OCH_2CH{=}CH_2$, R^2 = Bn R^3 = Ac
26 R^1 = OH, R^2 = Bn, R^3 = Ac



27 R^1 = R^2 = Bn, R^3 = Ac
28 R^1 = R^2 = Bn, R^3 = H
29 R^1 = $CH_2CH{=}CH_2$, R^2 = Bn, R^3 = Ac
30 R^1 = $CH_2CH{=}CH_2$, R^2 = Bn, R^3 = H

100.9, 99.6, 99.5, 99.2, and 96.1. Compound **22** was converted into **20**, in 33% overall yield, in four steps (alkaline hydrolysis, catalytic hydrogenolysis, acetylation, and glycosyl bromide formation). Similarly, **9** was glycosylated with **7** and deacetylated to give **30** in 63% yield. Glycosylation of **30** with **7** afforded a 79% yield of **25**, the structure of which was confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy. After deallylation of **25**, the resulting hemiacetal **26** was treated with $SOCl_2$–*N,N*-dimethylformamide in 1,2-dichloroethane to afford **21** in 79% yield.

Model reactions of the branched mannopentaosyl donors **20** and **21** toward the diol acceptor **9** were examined before condensation with the trisaccharide acceptor **18**. Reaction of **20** and **9** in the presence of $AgOSO_2CF_3$ and molecular sieves 4A in 1,2-dichloroethane gave no glycosylation except for an acid-labile product; this product was assumed to be an orthoester-type condensation product. In contrast, **21** treated with **9** in the presence of $HgBr_2$–$Hg(CN)_2$ and molecular sieves 4A in 1,2-dichloroethane gave **31** with the α-D-(1→6)-linkage compound as the preponderant one (36% by h.p.l.c. analysis). A reasonable explanation for the subtle difference in the reaction course caused by the use of glycosyl donors **20** and **21** is not yet available. The structure of **31** was confirmed by conversion through deacetylation and hydrogenation into the known hexasaccharide[9] **32**. The minor products (<2% by h.p.l.c. analysis) could not be isolated. Similarly, pentasaccharide donor **21** could undergo a regio- and stereoselective reaction with diol acceptor **8**, though in low yield. The key glycosylation of **18** with **21** was then carried out under similar conditions. After 6 days at 60° in the presence of $HgBr_2$–$Hg(CN)_2$ as promotor, the reaction gave the coupling product **33**, together with byproducts derived largely from an excess of donor **21**. Fractionation by gel-permeation chromatography and careful purification of the high-molecular-weight fraction by h.p.l.c. made it possible to isolate **33** in 14% yield (19%, based on consumed **18**). The octasaccharide was fully characterized after deprotection by alkaline hydroly-



31 $R^1 = CH_2CH{=}CH_2, R^2 = Bn, R^3 = Ac$

32 $R^1 = C_3H_7, R^2 = R^3 = H$

33

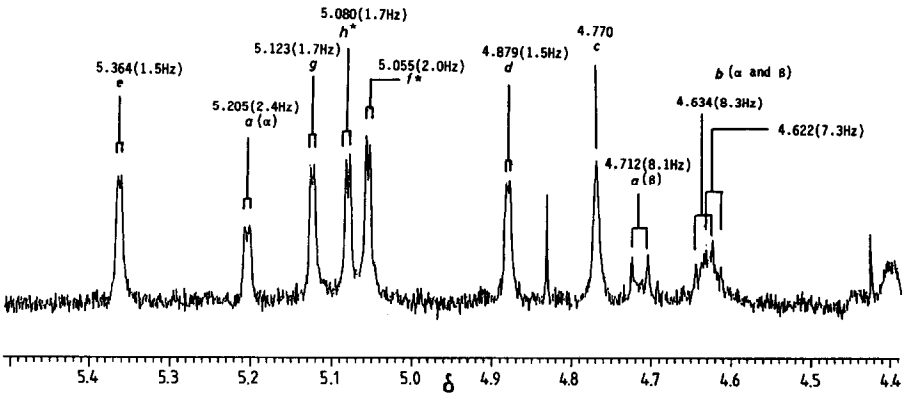


Fig. 1. ^{1}H-N.m.r. spectrum of compound **6**.

sis and hydrogenolysis. The structure of deprotected **6** was assigned on the basis of ^{1}H-n.m.r. spectroscopy. The evidence for the newly formed α-D-(1→6) linkage was attributed to the doublet signal for H-1d at δ 4.879 (J 1.47 Hz), as depicted in Fig. 1, which was in good agreement with the data of related natural glycans[24].

In conclusion, the total synthesis of octasaccharide segment **6** was achieved by a convergent route in a regio- and stereo-selective manner. The slow conversion and low yield in the coupling reaction between the tri- (**18**) and the penta-saccharide (**21**) might be due to the steric nature of the branched glycosyl donor **21**.

EXPERIMENTAL

General methods. — Optical rotations were determined with a Perkin–Elmer Model 241 MC polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on silica gel (Merck 70–230 mesh). Flash-column chromatography was performed on Wako Gel C-300 (200–300 mesh). T.l.c. and h.p.t.l.c. were performed on Silica gel 60 F_{254} (Merck) and products were detected either by u.v. light or by charring with H_2SO_4. H.p.l.c. was performed with a Hitachi 655 L.C. system. 1,2-Dichloroethane used for coupling reactions was distilled and stored with molecular sieves 4A. N.m.r. spectra were recorded with JNM GX 400 [^{1}H(400 MHz)] or FX90Q [^{13}C(22.50 MHz)] spectrometers. Chemical shifts (δ) are expressed downfield from the signal for internal Me_4Si, for solutions in $CDCl_3$, unless noted otherwise, and for solutions in D_2O, downfield from the signal for sodium 4,4-dimethyl-4-sila (2,3-$^2H_4$)-pentanoate.

Benzyl O-(*4-O-acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside* (**14**). — A mixture of **12**[14] (6.4 g, 12 mmol), $SOCl_2$ (10 mL), and *N,N*-dimethylformamide (0.1 mL) in 1,2-dichloroethane (100 mL) was stirred for 2 h at room temperature. Before filtration through Celite, SiO_2 (100 mg) was added to the mixture. The filtrate was concentrated *in vacuo* at 40° to give 4-*O*-acetyl-3,6-di-*O*-benzyl-2-deoxy-2-phthalimi-

do-β-D-glucopyranosyl chloride (**11**), which was used for the next step without further purification; R_F 0.58 (5:1 toluene–EtOAc).

To a mixture of **13**[15] (3.5 g, 6 mmol), $AgOSO_2CF_3$ (9.3 g, 36 mol), and powdered molecular sieves 4A (18 g) in 1,2-dichloroethane (70 mL) was added a solution of **11** (12 mmol) in 1,2-dichloroethane (20 mL) at 0° under Ar. The mixture was stirred for 17 h at 0° to room temperature, diluted with 1,2-dichloroethane (500 mL), filtered through Celite, washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 10:1 toluene–EtOAc gave **14** (4.1 g, 62%), R_F 0.50 (5:1 toluene–EtOAc), $[\alpha]_D$ + 16° (*c* 1.1, $CHCl_3$); ^{13}C-n.m.r.: δ 97.0 ($^1J_{C,H}$ 165 Hz, C-1,1'), 69.3 (C-4'), 56.2, and 55.7 (C-2,2').

Anal. Calc. for $C_{65}H_{60}N_2O_{14}$: C, 71.41; H, 5.53; N, 2.56. Found: C, 71.04; H, 5.53, N, 2.58.

Benzyl O-*(3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**15**). — A mixture of **14** (4.0 g, 3.66 mmol), water (8 mL), and 12M HCl (4 mL) in acetone (200 mL) was refluxed for 4 days at 80°. The mixture was concentrated *in vacuo*, diluted with EtOAc, washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 7:1 toluene–EtOAc gave **15** (3.1 g, 82%), R_F 0.50 (3:1 toluene–EtOAc), $[\alpha]_D$ − 7.8° (*c* 1.1, $CHCl_3$); ^{13}C-n.m.r.: δ 96.9 ($^1J_{C,H}$ 165 Hz, C-1,1'), 70.7 (C-4'), 56.0 and 55.6 (C-2,2').

Anal. Calc. for $C_{63}H_{58}N_2O_{13}$: C, 71.98; H, 5.56; N, 2.67. Found: C, 71.97; H, 5.57; N, 2.61.

Benzyl O-*(3,6-di*-O-*allyl-2,4-di*-O-benzyl-β-D-*mannopyranosyl)-(1→4)*-O-*(3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**16**). — To a stirred mixture of **15** (2.9 g, 2.8 mmol), Ag silicate (12 g), and dried molecular sieves 4A powder (10 g) in 1,2-dichloethane (100 mL), at 0° under Ar, was added a solution of **10** (5.63 g, 11.2 mmol) in 1,2-dichloroethane (20 mL). The mixture was stirred for 16 h at 0–18°, diluted with CH_2Cl_2, filtered, and the filtrate washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 10:1 toluene–EtOAc gave **16** (1.62 g, 40%) and **19** (1.45 g, 36%).

Compound **16**. R_F 0.47 (5:1 toluene–EtOAc), $[\alpha]_D$ − 2.1° (*c* 1.0, $CHCl_3$); ^{13}C-n.m.r. δ 101.5 ($^1J_{C,H}$ 156 Hz, C-1'') and 97.0 ($^1J_{C,H}$ 165 Hz, C-1,1').

Anal. Calc. for $C_{89}H_{88}N_2O_{18}$: C, 72.54; H, 6.02; N, 1.90. Found: C, 72.61; H, 6.15; N, 1.75.

Compound **19**. R_F 0.53 (5:1 toluene–EtOAc), $[\alpha]_D$ + 15.6° (*c* 1.2, $CHCl_3$); ^{13}C-n.m.r. δ 100.1 ($^1J_{C,H}$ 170 Hz, C-1''), 97.1 and 96.7 ($^1J_{C,H}$ 165 Hz, C-1,1').

Anal. Calc for $C_{89}H_{88}N_2O_{18}$: C, 72.54; H, 6.02; N, 1.90. Found: C, 72.61; H, 6.15; N, 1.75.

Benzyl O-*(2,4-di*-O-*benzyl-β*-D-*mannopyranosyl)-(1→4)*-O-*(3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranosyl)-(1→4)-3,6-di*-O-*benzyl-2-deoxy-2-phthalimido-β*-D-*glucopyranoside* (**17**). — A mixture of **16** (1.37 g, 0.93 mmol), $PdCl_2$ (0.36 g, 2.0 mmol), and AcONa (0.37 g 4.5 mmol) in 95% AcOH (30 mL) was stirred for 1 h at 70°,

concentrated *in vacuo*, diluted with EtOAc, and filtered through Celite. The filtrate was washed with aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue on gel silica with 4:1 toluene–EtOAc afforded **17** (0.75 g, 58%), R_F 0.55 (2:1 toluene–EtOAc), $[\alpha]_D$ −4.5° (*c* 1.4, $CHCl_3$).

Anal. Calc. for $C_{83}H_{80}N_2O_{18}$: C, 71.53; H, 5.79; N, 2.01. Found: C, 71.87; H, 5.91; N, 1.88.

Benzyl O-*(2,4-di*-O-*benzyl-β*-D-*mannopyranosyl)-(1→4)*-O-*(2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranosyl)-(1→4)-2-acetamido-3,6-di*-O-*benzyl-2-deoxy-β*-D-*glucopyranoside* (**18**). — A mixture of **17** (201 mg, 0.144 mmol), $BuNH_2$ (5 mL), and MeOH (5 mL) was heated under reflux for 6 d and concentrated *in vacuo* to give a residue, which was stirred in 1:1 pyridine–Ac_2O (10 mL) for 24 h at room temperature. The mixture was concentrated and chromatographed on silica gel (7:1 CH_2Cl_2–acetone) to afford a crude product, which was *O*-deacetylated with a catalytic amount of NaOMe in MeOH. The base was neutralized with Amberlite CG-50 cation-exchange resin and the mixture concentrated *in vacuo*. Column chromatography of the residue on silica gel with 7:1 CH_2Cl_2–acetone gave **18** (111 mg, 65%), R_F 0.47 (3:1 CH_2Cl_2-acetone), $[\alpha]_D$ −38.5° (*c* 0.8, $CHCl_3$); ^{1}H-n.m.r.: δ 1.92 (s, 3 H, NAc) and 1.68 (s, 3 H, NAc).

Anal. Calc. for $C_{71}H_{80}N_2O_{16}\cdot H_2O$: C, 69.02; H, 6.69; N, 2.27. Found: C, 69.08; H, 6.62; N, 2.26.

Benzyl O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1→3)*-O-*[(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1→6)]-2,4-di*-O-*benzyl-α*-D-*mannopyranoside* (**28**). — To a mixture of **8**[13] (2.0 g, 4.4 mmol), $AgOSO_2CF_3$ (5.5 g, 21 mmol), and 1,1,3,3,-tetramethylurea (5 mL) in 1,2-dichloroethane (15 mL) was added dropwise at 0° a solution of **7**[6,12] (6.8 g, 13.3 mmol) in 1,2-dichloroethane (10 mL). After stirring for 48 h at room temperature, the mixture was filtered, and the filtrate washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 10:1 toluene–oxolane gave a fraction containing **27** (6.9 g), which was stirred with M methanolic NaOMe (0.25 mL) in oxolane (30 mL) for 16 h at room temperature. The base was neutralized with Amberlist A-15 cation-exchange resin, the suspension filtered, and the filtrate concentrated *in vacuo*. Column chromatography of the crude product on silica gel with 10:1 toluene–oxolane gave **28** (4.6 g, 78%), R_F 0.40 (5:1 toluene–oxolane), $[\alpha]_D$ +60° (*c* 0.6, $CHCl_3$); ^{13}C-n.m.r.: δ 101.6 ($^1J_{C,H}$ 172.1 Hz, C-1'), 100.0 ($^1J_{C,H}$ 168.5 Hz, C-1''), and 96.3 ($^1J_{C,H}$ 172.1 Hz, C-1).

Anal. Calc. for $C_{81}H_{88}O_{16}$: C, 73.94; H, 6.61. Found: C, 73.67; H, 6.65.

Benzyl O-*(2*-O-*acetyl-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1→3)*-O-*[(2*-O-*acetyl-3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1→2)*-O-*(3,4,6-tri*-O-*benzyl-α*-D-*mannopyranosyl)-(1→6)]-2,4-di*-O-*benzyl-α*-D-*mannopyranoside* (**22**). — A solution of **7**[6,12] (5.5 g, 10.8 mmol) in 1,2-dichloroethane (50 mL) was added to a mixture of **28** (4.6 g, 3.5 mmol), $AgOSO_2CF_3$ (4.0 g, 15.6 mmol) and 1,1,3,3-tetramethylurea (4 mL) in 1,2-dichloroethane (50 mL) at 0° under Ar. The mixture was stirred for 48 h at room temperature, then filtered, and the filtrate washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 40:1 toluene–oxolane

gave **22** (5.0 g, 63%), R_F 0.70 (5:1 toluene–oxolane), $[\alpha]_D$ + 38° (*c* 0.3, $CHCl_3$); ^{13}C-n.m.r.: δ 100.9 ($^1J_{C,H}$ 173 Hz, C-1^b), 99.6, 99.5 ($^1J_{C,H}$ 172 Hz, C-1^c,1^e), 99.2 ($^1J_{C,H}$ 170 Hz, C-1^d), and 96.1 ($^1J_{C,H}$ 170 Hz, C-1^a).

Anal. Calc. for $C_{139}H_{146}O_{28}$: C, 73.71; H, 6.51. Found: C, 73.34; H, 6.58.

Benzyl O-*(3,4,6-tri-*O-*benzyl-*α-D-*mannopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*benzyl-*α-D-*mannopyranosyl)-(1→3)-*O-*[(3,4,6-tri-*O-*benzyl-*α-D-*mannopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*benzyl-*α-D-*mannopyranosyl)-(1→6)]-2,4-di-*O-*benzyl-*α-D-*mannopyranoside* (**23**). — A mixture of **22** (5.0 g, 2.2 mmol) and M methanolic NaOMe (3 mL) in oxolane (50 mL) was stirred at room temperature, the base neutralized withAmberlist A-15 cation-exchange resin, and the filtrate concentrated *in vacuo*. Column chromatography of the residue on silica gel with 5:1 toluene–EtOAc gave **23** (3.7 g, 72%), R_F 0.49 (4:1 toluene–EtOAc), $[\alpha]_D$ + 50.5° (*c* 1.0, $CHCl_3$); ^{13}C-n.m.r.: δ 101.2 (C-1^b, 1^c, 1^e), 99.2 (C-1^d), and 95.8 (C-1^a).

Anal. Calc. for $C_{135}H_{142}O_{26}$: C, 74.35; H, 6.58. Found: C, 74.46; H, 6.58.

O-*(2,3,4,6-Tetra-*O-*acetyl-*α-D-*mannopyranosyl)-(1→2)-*O-*(3.4.6-tri-*O-*acetyl-*α-D-*mannopyranosyl)-(1→3)-*O-*[(2,3,4,6-tetra-*O-*acetyl-*α-D-*mannopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*acetyl-*α-D-*mannopyranosyl)-(1→6)]-1,2,4-tri-*O-*acetyl-D-*mannopyranose* (**24**). — The deacetylated compound **23** (1.0 g,0.46 mmol) was dissolved in MeOH (2 mL), stirred with 10% Pd–C (1.0 g) and HCO_2H (4 mL) for 48 h at room temperature under Ar, the suspension filtered through Celite, and the filtrate concentrated *in vacuo*. A mixture of the crude product and Ac_2O (10 mL) in pyridine was stirred for 16 h at room temperature, and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 10:1 toluene–oxolane gave **24** (554 mg, 65%), R_F 0.53 (1:1 toluene–oxolane), $[\alpha]_D$ + 31.5° (*c* 0.4, $CHCl_3$).

Anal. Calc. for $C_{64}H_{86}O_{43}$: C, 49.80; H, 5.67. Found: C, 49.60; H, 5.65.

Conversion of **24** *into* O-*(2,3,4,6-tetra* O-*acetyl-*α-D-*mannopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*acetyl-*α-D-*mannopyranosyl)-(1→3)-*O-*[(2,3,4,6-tetra-*O-*acetyl-*α-D-*mannopyranosyl)-(1→2)-*O-*(3,4,6-tri-*O-*acetyl-*α-D-*mannopyranosyl)-(1→6)]-2,4-di-*O-*acetyl-*α-D-*mannopyranosyl bromide* (**20**). — A mixture of **24** (70 mg, 45 μmol) and 30% HBr–AcOH (0.5 mL) in 1,2-dichloroethane (1 mL) was stirred for 2 h at room temperature, diluted with $CHCl_3$, washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo* to give **20** (49 mg, 70%), R_F 0.57 (1:1 toluene–oxolane); ^{1}H-n.m.r.: δ 6.33 (s, 1 H, H-1^a) and 2.18–1.85 (48 H, 16 CH_3CO). This compound was not characterized further.

Allyl O-*(3,4,6-tri-*O-*benzyl-*α-D-*mannopyranosyl)-(1→3)-*O-*[(3,4,6-tri-*O-*benzyl-*α-D-*mannopyranosyl)-(1→6)]-2,4-di-*O-*benzyl-*α-D-*mannopyranoside* (**30**). — Reaction of **9**[9] (1.0 g, 2.25 mmol) with **7**[6,12] (3.6 g, 7.05 mmol) in the presence of AgOSO_2CF_3 (2.25 g, 8.75 mmol) and 1,1,3,3-tetramethylurea (2.5 mL) in dichloroethane (40 mL) took place for 16 h at 0–15°. The mixture was worked up as described for the preparation of **28**. Column chromatography of the crude product on silica gel with 10:1 toluene–oxolane gave a fraction containing **29** (35 g) which was treated with M methanolic NaOMe in oxolane, and purified by column chromatography on silica gel with 10:1 toluene–oxolane to give **30** (2.0 g, 63%), R_F 0.41 (5:1 toluene–oxolane), $[\alpha]_D$ + 55° (*c*

0.25, $CHCl_3$); n.m.r.: (^1H) δ 5.23 (s, 1 H, H-1'), 5.07 (d, 1 H, J 1.7 Hz, H-1''), and 4.81 (d, 1 H, J 1.7 Hz, H-1); (^{13}C), δ 101.6 ($^1J_{C,H}$ 173 Hz, C-1'), 99.8 ($^1J_{C,H}$ 168 Hz, C-1''), and 96.4 ($^1J_{C,H}$ 171 Hz, C-1).

Anal. Calc. for $C_{77}H_{87}O_{16}$: C, 73.07; H, 6.70. Found: C, 73.19; H, 6.76.

Allyl O-*(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)-O-[(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-O-benzyl-α-D-mannopyranoside* (**25**). — Reaction of **30** (1.7 g, 1.21 mmol) and **7**[6,12] (1.23 g, 4.79 mmol) in the presence of $AgOSO_2CF_3$ (1.23 g, 4.79 mmol) and 1,1,3,3-tetramethylurea (1.4 ml) in 1,2-dichloroethane (40 mL) took place for 16 h at 0–18°. The mixture was worked up as described for the preparation of **28**. Column chromatography of the crude product on silica gel with 20:1 toluene–oxolane gave **25** (2.35 g, 79%), R_F 0.66 (5:1 toluene–oxolane), $[\alpha]_D$ + 34° (c 0.5, $CHCl_3$); n.m.r.: (^1H), δ 5.19 (d, 1 H, J 1.7 Hz, H-1^b), 5.07 (d, 1 H, J 1.5 Hz, H-1^c or 1^e), 5.01 (d,1 H, J 1.5 Hz, H-1^c or 1^e), 4.92 (d, 1 H, J 2.0 Hz, H-1^d), and 4.80 (s, 1 H, H-1^a); (^{13}C) δ 101.1 ($^1J_{C,H}$ 167 Hz, C-1^b), 99.7 ($^1J_{C,H}$ 169 Hz, C-1^c,1^e), 99.2 ($^1J_{C,H}$ 172 Hz, C-1^d), and 96.2 ($^1J_{C,H}$ 172 Hz, C-1^a).

Anal. Calc. for $C_{135}H_{144}O_{28}$; C, 73.21; H, 6.60. Found: C, 73.22; H, 6.52.

O-*(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)-O-[(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-O-benzyl-D-mannopyranose* (**26**). — A mixture of **25** (2.35 g, 1.06 mmol),$PdCl_2$ (240 mg, 1.36 mmol), and AcONa (240 mg) in 80% AcOH (40 mL) was stirred for 16 h at room temperature, concentrated *in vacuo*, diluted with EtOAc, filtered, and concentrated *in vacuo*. Column chromatography of the residue on silica gel with 10:1 toluene–oxolane gave **26** (1.27 g, 56%), R_F 0.33 (5:1 toluene–oxolane), $[\alpha]_D$ + 33° (c 0.5, $CHCl_3$).

Anal. Calc. for $C_{132}H_{140}O_{28}$; C, 72.90; H, 6.50. Found: C, 73.18; H, 6.86.

O-*(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)-O-[(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→6)]-2,4-di-O-benzyl-D-mannopyranosyl chloride* (**21**). — To a stirred solution of **26** (98 mg, 45 μmol) in 1,2-dichloroethane (2 mL) was added $SOCl_2$ (1 mL), and then *N,N*-dimethylformamide (0.2 mL). Stirring was continued for 30 min at 18°, and silica gel (50 mg) was added to the mixture before filtration and concentration *in vacuo*. Column chromatography of the residue on silica gel with 10:1 toluene–oxolane gave **21** (78 mg, 79%), R_F 0.50 (10:1 toluene–oxolane); ^1H-n.m.r.: δ 6.02 (d, 1 H, J 1.5 Hz, H-1^a).

Anal. Calc. for $C_{132}H_{139}ClO_{27}$; C, 72.28; H, 6.40. Found C, 72.21; H, 6.38.

Allyl O-*(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)-O-[(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→6)]-O-(2,4-di-O-benzyl-α-D-mannopyranosyl)-(1→6)-2,4-di-O-benzyl-α-D-mannopyranoside* (**31**). — A mixture of **21** (120 mg, 54 μmol), **9** (21 mg, 53 μmol), $HgBr_2$ (29 mg, 81 μmol), $Hg(CN)_2$ (20 mg, 79 μmol), and dried molecular sieves 4A powder (200 mg) in 1,2-dichloroethane (1.2 mL) was stirred for 16 h at 60° under Ar. The mixture was diluted

with $CHCl_3$, filtered, and the filtrate washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was chromatographed on silica gel with 5:1 toluene–oxolane to give a fraction containing **31** (110 mg), which was further purified by h.p.l.c. in a column of Nucleosil 50-5 (10 mm diam. × 30 cm) in 3:1 hexane–oxolane to afford **31** (31 mg, 23%), R_F 0.57 (5:1 toluene–oxolane), $[\alpha]_D$ + 31° (*c*, 0.48, $CHCl_3$); ^{1}H-n.m.r.: δ 5.53 (m, 2 H, H-2^d, 2^f), 5.15 (1 H, *J* 1.7 Hz, H-1^c), 5.09 (d, 1 H, *J* 1.5 Hz, H-1^d or 1^f), 5.07 (d, 1 H, *J* 1.5 Hz, H-1^d or 1^f), 5.00 (d, 1 H, *J* 1.6 Hz, H-1^e), 4.95 (d, 1 H, *J* 1.7 Hz, H-1^b), 4.79 (d, 1 H, *J* 1.2 Hz, H-1^a), 2.11 (s, 3 H, CH_3CO), and 2.08 (s, 3 H, CH_3CO).

Anal. Calc. for $C_{155}H_{166}O_{33}$: C, 72.79; H, 6.56. Found: C, 72.92; H, 6.89.

O-α-D-*Mannopyranosyl-(1→2)*-O-α-D-*mannopyranosyl-(1→3)*-O-*[*α-D-*mannopyranosyl-(1→2)*-O-α-D-*mannopyranosyl-(1→6)]*-O-α-D-*mannopyranosyl-(1→6)*-O-*β*-D-*mannopyranosyl-(1→4)*-O-*(2-acetamido-2-deoxy-β*-D-*glucopyranosyl)-(1→4)-2-acetamido-2-deoxy glucopyranose* **(6).** — *Conversion of* **31** *into* **32.** A mixture of **31** (29 mg, 13 μmol) and M methanolic NaOMe (20 μL) in oxolane (20 mL) was stirred for 16 h at room temperature, the base neutralized with Amberlist A-15 cation-exchange resin, the suspension filtered through Celite, and the filtrate concentrated *in vacuo*. The residue was dissolved in 5:1 MeOH–HCO_2H (2.4 mL), stirred with 10% Pd–C (20 mg) for 30 min at 50° under Ar, the suspension filtered through Celite, and the filtrate concentrated *in vacuo*. Gel filtration chromatography (Toyopearl HW-40, H_2O) of the residue gave propyl *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→3)-*O*-[α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→6)]-*O*-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**32**) (11 mg, 94%), R_F 0.26 (2:1:1 BuOH–EtOH–water), $[\alpha]_D$ + 69° (*c* 0.2, water); ^{1}H-n.m.r. (D_2O, 60°): δ 5.352 (d, 1 H, *J* 2 Hz, H-1^c), 5.133 (d, 1 H, *J* 2 Hz, H-1^e), 5.063 (d, 1 H, *J* 2 Hz, H-1^d or H-1^f), 5.055 (d, 1 H, *J* 2 Hz, H-1^d or H-1^f), 4.879 (d, 1 H, *J* 2 Hz, H-1^a), and 4.863 (d, 1 H, *J* 2 Hz, H-1^a).

Condensation of **18** *with* **21.** A mixture of **21** (70 mg, 31 μmol), **18** (40 mg, 31 μmol), $HgBr_2$ (17 mg, 47 μmol), $Hg(CN)_2$ (12 mg, 47 μmol), and dried molecular sieves 4A powder (250 mg) in 1,2-dichloroethane (1.3 mL) was stirred for 72 h at 60° under Ar. Then **7**[6,12] (70 mg), $HgBr_2$ (17 mg), and $Hg(CN)_2$ (12 mg) were added to the mixture and stirring was continued for further 72 h at 60°. The mixture was filtered, the filtrate washed with water and aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated *in vacuo*. Gel permeation chromatography of the residue with Toyopearl HW-40F (150 mL) in 1:1 $CHCl_3$–MeOH gave a fraction (45 mg) containing **33**. This fraction was further purified by h.p.l.c. in a column of Nucleosil 50-5 (10 mm diam. × 30 cm) in 3:2 hexane–oxolane to afford benzyl *O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→3)-*O*-[(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)]-*O*-(2,4-di-*O*-benzyl-α-D-mannopyranosyl)-(1→6)-*O*-(2,4-di-*O*-benzyl-β-D-mannopyranosyl)-(1→4)-*O*-(2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**33**; 15 mg, 14%), R_F 0.69 (1:1 toluene–oxolane), $[\alpha]_D$ + 1.4° (*c* 0.7, $CHCl_3$); ^{1}H-n.m.r. δ 5.509 (m, 2 H, H-2^f, 2^h), 5.083 (d, 1 H, *J* 1.7 Hz, H-1^f or 1^h), 5.035 (d, 1 H, *J* 1.5 Hz, H-1^f or H-1^h),

2.110 (s, 3 H, CH_3CO), 2.067 (s, 3 H, CH_3CO), 2.053 (s, 3 H, CH_3CO), and 1.923 (s, 3 H, CH_3CO).

Similarly, **18** (9 mg) was recovered from the lower-molecular-weight fraction.

Deprotection of **33** *into* **6**. Compound **33** (5 mg) was deacetylated with a few drops of M methanolic NaOMe in oxolane (0.25 mL), then the base was neutralized with Amberlist CG-50 cation-exchange resin. The mixture was filtered and concentrated *in vacuo*. The crude product was dissolved in 4:1 MeOH–HCO_2H (1 mL), stirred with 10% Pd–C for 3 h at 50° under Ar, filtered, and concentrated *in vacuo*. Gel filtration chromatography (Toyopearl HW-40, water) of the residue gave **6** (1.9 mg), R_F 0.61 (1:1:8 water–AcOH–MeOH), $[\alpha]_D$ + 31° (*c* 0.1, water); ^{1}H-n.m.r. (D_2O, 60°): δ 5.365 (d, 1 H, *J* 1.5 Hz, H-1^c), 5.205 (d, 0.5 H, 2.4 Hz, H-1$^a\alpha$), 5.124 (d, 1 H, *J* 1.7 Hz, H-1^g), 5.080 (d, 1 H, *J* 1.7 Hz, H-1^f or 1^h), 5.055 (d, 1 H, *J* 1.9 Hz, H-1^f or 1^h), 4.880 (d, 1 H, *J* 1.5 Hz, H-1^d), 4.770 (bs, 1 H, H-1^e), 4.713 (d, 0.5 H, *J* 8.1 Hz, H-1$^a\beta$), 4.634 (d, 0.5 H, *J* 8.3 Hz, H-1^b), 4.622 (d, 0.5 H, *J* 7.3 Hz, H-1^b), 2.078 (s, 3 H, CH_3CO), and 2.049 (s, 3 H, CH_3CO).

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