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The chemical synthesis of β-(1→4)-linked D-mannobiose and D-mannotriose

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A D-cellobiose derivative was converted to D-mannobiose *via simultaneous epimerization* at C-2 and C-2′. Subsequent β-D-glucosylation and epimerization at C-2″ gave D-mannotriose.

Introduction

β-(1⟶4)-Mannans are polysaccharides (both branched and linear) found in wood and plant seeds.[1] Complex branched galactoglucomannan, which contains a backbone built up by β-(1⟶4)-linked D-Man*p* and D-Glc*p* units, is the principal hemicellulose of softwoods. Hardwoods usually contain a linear β-(1⟶4)-glucomannan. Galactomannans occur in the endosperms of a wide range of leguminous seeds. Their backbone consists of β-(1⟶4)-linked D-Man*p* units with single α-D-Gal*p* branches.[2] The principal enzyme for mannan depolymerization is endo-β-(1⟶4)-mannanase (EC 3.2.1.78), which hydrolyses the main chain of β-(1⟶4)-mannans, generating linear or branched oligosaccharides. The main interest in microbial mannanolytic enzymes is related to their possible application in bleaching the softwood pulp, in the hydrolysis of highly viscous seed polysaccharides used in the food industry and in the preparation of oligosaccharides used as food additives. Strains of yeast-like microorganism *Aureobasidium pullulans* were shown to be excellent producers of the endo-β-(1⟶4)-mannanase.[3,4]

There is an interest for biochemical applications of β-(1⟶4)-manno-oligosaccharides. β-(1⟶4)-Mannobiose was identified as the shortest β-mannan fragment triggering the biosynthesis of both endo-β-(1⟶4)-mannanase and β-mannosidase.[3] Nitrophenyl-, naphthyl- and methylumbelliferyl-glycosides of β-(1⟶4)-manno-oligosaccharides are of value as convenient substrates for the assay of the endo-β-mannanase activity.[5,6] The preparation of the β-manno-oligosaccharides normally involves partial hydrolysis of the mannan with crude β-mannanase,[7] resulting in the formation of D-mannose, β-(1⟶4)-mannobiose, β-(1⟶4)-mannotriose and β-(1⟶4)-manno-tetraose. The yields of the oligosaccharides are relatively low and their efficient separation seems to be rather difficult.[8]

The chemical synthesis of β-D-mannopyranosides *via* standard glycosylation approaches is not easy to perform either, since both the neighbouring group participation and the anomeric effect uniformly favour the formation of α-D-mannopyranosides. Previous attempts to the synthesis of the β-mannoside linkage (including use of non-participating groups at C-2 in a mannosyl donor, intermolecular aglycon delivery, 1,2-*O*-dibutylstannylene complexes and enzyme assisted glycosylation techniques) have been well documented and reviewed by Paulsen,[9,10] Barresi and Hindsgaul,[11] Veeneman[12] and, more recently, Gridley and Osborn.[13] Very recently, stereoselective preparation of complex β-D-mannopyranosides was achieved (with an α : β ratio of ~10 : 1) by making use of 4,6-*O*-benzylidene protected α-D-mannopyranosyl sulfoxides[14] and reactive mannopyranosyl derivatives possessing a strong electron-withdrawing (α-toluenesulfonyloxy) group at C-2[15] as glycosyl donors.

In parallel, great effort has been undertaken to develop alternative methods: *e.g.*, epimerization at C-2 in readily available β-D-glucopyranosides. This can be achieved either *via* the oxidation–reduction technique,[16,17] or *via* intermolecular[18–20] or intramolecular[21,22] nucleophilic substitution. Here we describe chemical synthesis of β-(1⟶4)-mannobiose and β-(1⟶4)-mannotriose starting from commercially available and cheap D-cellobiose octa-acetate **1** *via* the epimerization S_N2 reactions.

Results and discussion

Benzyl β-(1⟶4)-mannobioside **10** (Scheme 1) was synthesized from the octa-acetate **1** *via* the preparation of the selectively protected 2,2′-diol **6** followed by simultaneous epimerization at C-2 and C-2′. To this end, compound **1** was first converted (as described in ref. 23) to the benzyl cellobioside **3** (70%) using the Koenigs–Knorr glycosylation reaction in the presence of $Hg(OAc)_2$ (⟶**2**) followed by conventional deacetylation. Subsequent treatment with α,α-dimethoxytoluene and TsOH in DMF[24] provided the 4′,6′-benzylidene acetal **4**, which was then acetylated and characterised as the crystalline penta-acetate **5** (68%).

The diol **6** was prepared from compound **4** by selective benzoylation[25] with benzoyl cyanide (BzCN) and Et_3N in acetonitrile. As it was expected for 4,6-*O*-protected β-D-Glc*p* derivatives, the 3-OH group appeared to be more reactive for the benzoylation than the 2-OH one. It was found that 4 eq. of BzCN was optimal for the reaction, which produced the 3,3′,6-tribenzoate **6** in 33% yield (in addition to a mixture of tetra-*O*-benzoyl derivatives). The same reaction performed with 3 eq. of BzCN gave just 23% of the desired product. The benzoylation of O-3 and O-3′ was unambiguously confirmed by the low-field signals of H-3 (δ 5.37, t, $J_{2,3} = J_{3,4} = 9.4$ Hz) and H-3′ (δ 5.25, t, $J_{2',3'} = J_{3',4'} = 9.5$ Hz) in contrast to up-field signals of H-2 and H-2′ (δ 3.65–3.75) in the ^{1}H NMR spectrum of compound **6** (see Experimental).

Subsequent *simultaneous epimerization* at C-2 and C-2′ was performed *via* triflylation of the diol **6** with triflic anhydride and pyridine in DCM (⟶**7**) followed by the reaction with Bu_4NOBz (intermolecular S_N2) in boiling toluene. That produced the β-(1⟶4)-mannobiose derivative **8** in 76% yield. It should be noted that the same compound could be prepared by the use of the intramolecular nucleophilic substitution technique previously developed in this laboratory.[22] Alcoholysis of the 2,2′-ditriflate **7** with ethanol in the presence of 2,4,6-collidine gave first the corresponding bis-2,3:2′,3′-orthoester **11**, which was hydrolysed with 80% aq. AcOH followed by conventional benzoylation to afford the mannobioside **8** in 48% yield.

The β-D-*manno*-configuration of both monosaccharide residues followed from the characteristic values of $J_{1,2}$ and $J_{1',2'}$

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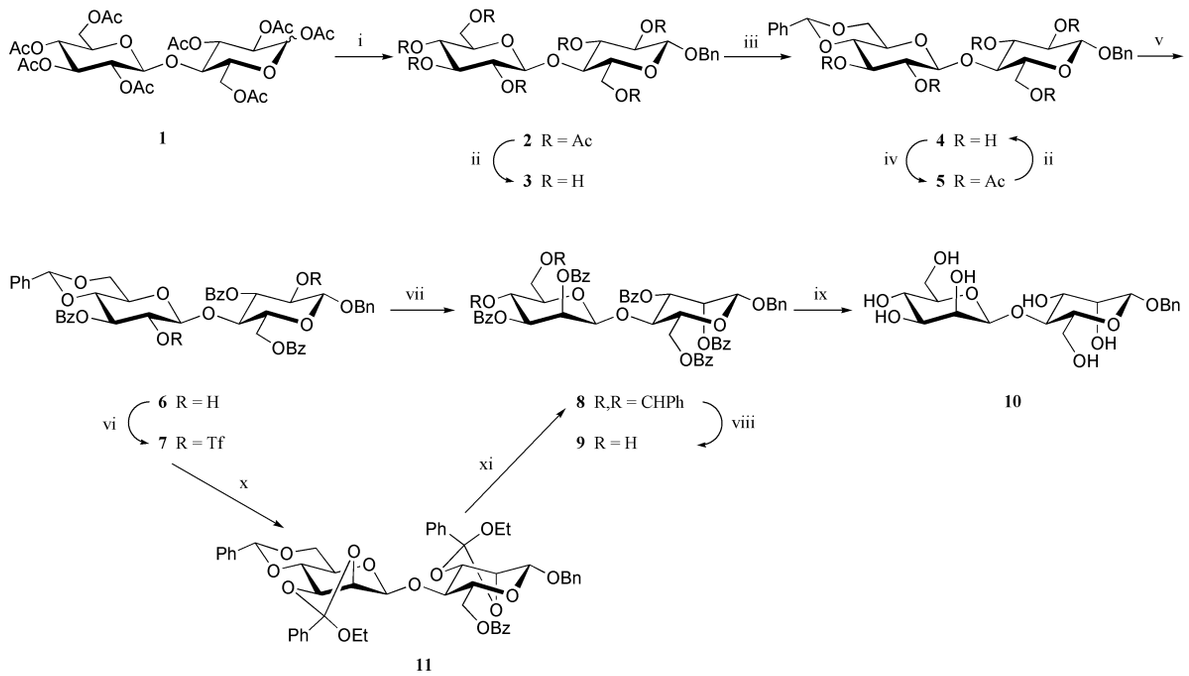
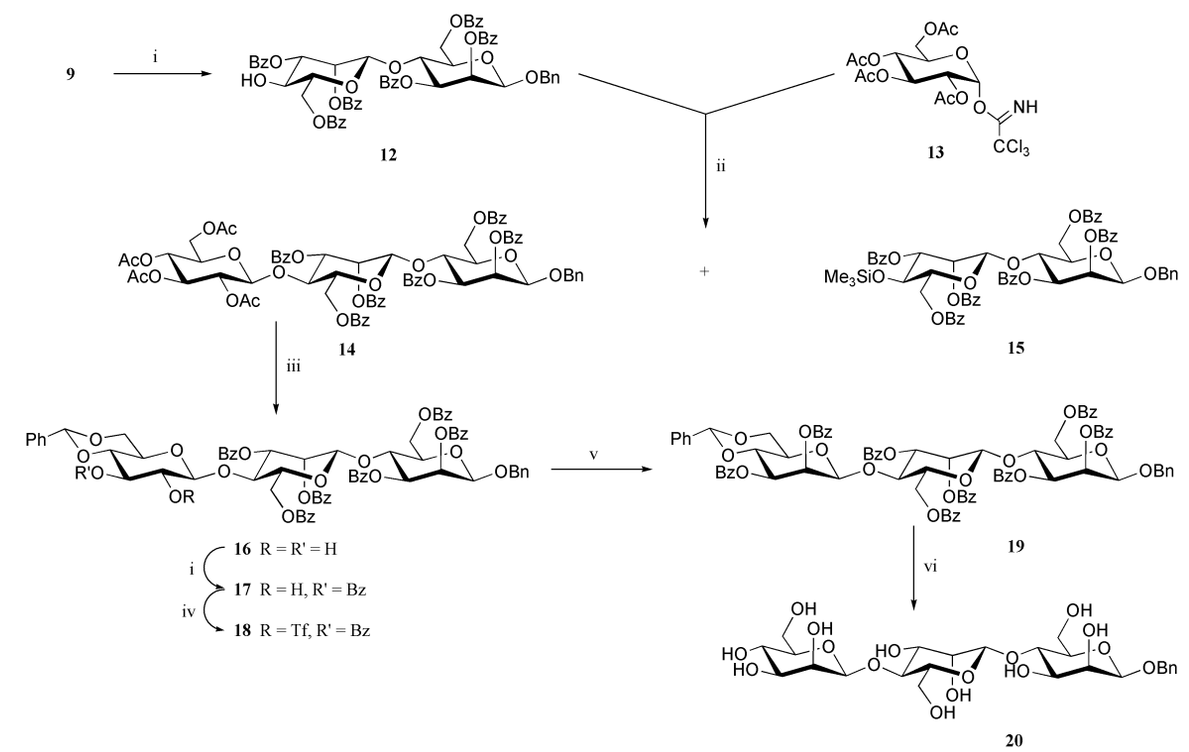


Scheme 1 *Reagents:* i, (a) HBr, AcOH, CH_2Cl_2; (b) BnOH, $Hg(OAc)_2$; ii, NaOMe, MeOH; iii, $PhCH(OMe)_2$, $TsOH{\cdot}H_2O$, DMF; iv, Ac_2O, pyridine; v, BzCN, Et_3N, MeCN; vi, Tf_2O, pyridine, CH_2Cl_2; vii, Bu_4NOBz, toluene; viii, 80% AcOH (70 °C); ix, NaOMe, MeOH, THF; x, EtOH, 2,4,6-collidine, toluene; xi, (a) 80% AcOH (rt); (b) BzCl, pyridine.



Scheme 2 *Reagents:* i, BzCN, Et_3N, MeCN, CH_2Cl_2; ii, TMS triflate, MS 4 Å, CH_2Cl_2; iii, (a) HCl, MeOH; (b) $PhCH(OMe)_2$, $TsOH{\cdot}H_2O$, MeCN; iv, Tf_2O, pyridine, CH_2Cl_2; v, Bu_4NOBz, toluene; vi, (a) 25 : 1 : 350 TFA–H_2O–CH_2Cl_2; (b) NaOMe, MeOH, THF.

(<1 Hz) and $J_{2,3}$ and $J_{2',3'}$ (3.1–3.4 Hz) in the ^{1}H NMR spectrum of compound **8** (see Experimental). For β-D-*gluco*-derivatives the $J_{1,2}$ and $J_{2,3}$ values are about 8.0 and 9.5 Hz, respectively (see Experimental, ^{1}H NMR data for compound **2**). Two-step deprotection of the derivative **8** including acid hydrolysis (⟶**9**) and debenzoylation with methanolic sodium methoxide provided the targeted β-(1⟶4)-mannobioside **10**.

Based on the successful synthesis of β-(1⟶4)-mannobiose, we developed its transformation to benzyl β-(1⟶4)-D-mannotrioside **20** (Scheme 2) *via* consecutive β-D-glucosylation (to form a β-D-Glc*p*-β-D-Man*p*-β-D-Man*p* derivative) and epimerization (at C-2″) procedures. The mannobiose glycosyl acceptor **12** was prepared in 92% yield by selective benzoylation[25] of the 4′,6′-diol **9** with BzCN (1 eq.) and Et_3N in acetonitrile-DCM. Its glycosylation[26] with α-D-glucosyl trichloroacetimidate **13**[27,28] in the presence of trimethylsilyl triflate (TMS triflate) and molecular sieves MS 4 Å gave the required trisaccharide **14** (47%) and recovered acceptor **12** (18%). Another compound formed as a by-product was found to be the 4′-trimethylsilyl ether **15** (27%), which was converted to the acceptor **12** on treatment with moist CF_3COOH in chloroform. Any attempts to improve the yield of the trisaccharide **14** using various glycosylation conditions (*e.g.*, $BF_3{\cdot}Et_2O$ instead of TMS triflate or Koenigs–Knorr reaction of acetobromoglucose and

compound **12** in the presence of $Hg(CN)_2$–HgO or $AgSO_3CF_3$) proved to be unsuccessful.

The trisaccharide **14** was converted into the 2″,3″-diol **16** (61%) by deacetylation[29] with HCl in MeOH, followed by treatment of the resulting tetraol with α,α-dimethoxytoluene and TsOH in acetonitrile. Subsequent selective acylation[23] with BzCN (1 eq.) and Et_3N gave the 3″-benzoate **17** (65%). The position of the benzoate was clearly confirmed (as we did it for the diol **6**) by the low-field signal of H-3″ (δ 5.13, t, $J_{2'',3''} = J_{3'',4''}$ = 9.3 Hz) in contrast to the up-field signal of H-2″ (δ 3.36–3.54) in the ^{1}H NMR spectrum (see Experimental). Epimerization at C-2″ was done *via* intermolecular S_N2 reaction: the trisaccharide **17** was first converted (as described above, for the preparation of the ditriflate **7**) to the triflate **18**, which produced the β-D-mannotrioside **19** (88%) on the reaction with Bu_4NOBz in boiling toluene. The β-D-*manno*-configuration of the terminal unsubstituted monosaccharide residue followed from the characteristic values of $J_{1'',2''}$ <1 Hz and $J_{2'',3''}$ = 3.3 Hz in the ^{1}H NMR spectrum of compound **19** (see Experimental). Deprotection of this derivative was performed in conditions similar to the preparation of the mannobioside **10** and provided the β-(1⟶4)-D-mannotrioside **20** in 70% yield.

In conclusion, rather simple and efficient synthesis of β-(1⟶4)-D-mannobiose and -mannotriose was accomplished starting from D-cellobiose octa-acetate by making use of S_N2 epimerization reactions.

Experimental

General procedures

Mps were determined on a Reichert hot-plate apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter; $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra (^{1}H at 300 MHz and ^{13}C{^{1}H} at 75 MHz) were recorded with Bruker AM-300 spectrometer for solutions in $CDCl_3$, unless otherwise indicated. Chemical shifts (δ in ppm) are given relative to those for Me_4Si (for both ^{1}H and ^{13}C); *J*-values are given in Hz. ES mass spectra were recorded with a Micromass Quattro system (Micromass Biotech, UK). TLC was performed on Kieselgel 60 F_{254} (Merck) with *A*, toluene–ethyl acetate (8 : 2); *B*, toluene–ethyl acetate (7 : 3); *C*, toluene–ethyl acetate (1 : 1); *D*, dichloromethane–methanol (9 : 1); and *E*, chloroform–methanol–water (10 : 10 : 3) as developers and detection under UV light or by charring with sulfuric acid–water–ethanol (15 : 85 : 5). Flash-column chromatography (FCC) was performed on Kieselgel 60 (0.040–0.063 mm) (Merck). D-Cellobiose octa-acetate was purchased from Lancaster. Dichloromethane, acetonitrile, pyridine and toluene were freshly distilled from CaH_2. Solutions worked up were concentrated under reduced pressure at <40 °C.

Benzyl β-D-glucopyranosyl-(1⟶4)-β-D-glucopyranoside 3

To a solution of D-cellobiose octa-acetate **1** (5.366 g, 7.91 mmol) in dichloromethane (20 cm^3) and acetic anhydride (0.5 cm^3) was added 45% (w/v) solution of HBr in AcOH (10 cm^3) at 0 °C. The mixture was kept at rt for 1 h, diluted with dichloromethane (300 cm^3) and washed successively with ice-cold water, cold saturated aq. $NaHCO_3$ and water. The dichloromethane solution was dried ($MgSO_4$) and concentrated to give the corresponding hepta-*O*-acetyl-α-D-cellobiosyl bromide, which was immediately dissolved in benzyl alcohol (18 cm^3) containing $Hg(OAc)_2$ (1.75 g, 6.10 mmol). The mixture was heated with stirring at 95 °C for 2 h, and then ethanol (130 cm^3) was poured into the reaction vessel. The resulting crystalline benzyl hepta-*O*-acetyl-β-D-cellobioside **2** was filtered off, washed with ethanol and dried in vacuum [mp 188–190 °C (from ethanol); δ_H 1.91, 1.93, 1.96, 2.01 and 2.08 (15 H, 5 × s, 5 × Ac), 1.94 (6 H, s, 2 × Ac), 3.50 (1 H, ddd, $J_{5',6a'}$ 4.9, $J_{5',6b'}$ 4.3, 5′-H), 3.59 (1 H, ddd, $J_{5,6a}$ 2.2, $J_{5,6b}$ 4.3, 5-H), 3.72 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.5, 4-H), 3.97 (1 H, dd, $J_{6a,6b}$ 12.5, 6-H^a), 4.03 (1 H, dd, $J_{6a',6b'}$ 12.0, 6′-H^a), 4.35 (1 H, dd, 6-H^b), 4.43 (1 H, d, $J_{1',2'}$ 7.9, 1′-H), 4.44 (1 H, d, $J_{1,2}$ 8.0, 1-H), 4.47 (1 H, dd, 6′-H^b), 4.52 and 4.79 (2 H, AB q, *J* 12.4, C*H*$_2$Ph), 4.84 (1 H, dd, 2′-H), 4.91 (1 H, dd, 2-H), 4.99 (1 H, t, $J_{2',3'} = J_{3',4'}$ = 9.5, 3′-H), 5.07 (2 H, 2 coincidental triplets, $J_{2,3} = J_{3,4} = J_{3',4'} = J_{4',5'}$ = 9.5, H-3 and -4′) and 7.17–7.32 (5 H, m, Ph)].

The prepared compound **2** was dissolved in MeOH (30 cm^3) and 4.6 mol dm^{-3} methanolic sodium methoxide (0.6 cm^3) was added to the solution. The mixture was kept at rt for 2 h, whereafter it was deionized with Dowex 50W-X4 (H^+) resin, filtered and concentrated to produce the benzyl cellobioside **3** (2.41 g, 70% from the octa-acetate **1**); mp 190–191 °C; $[a]_D^{23}$ −33 (*c* 1, water) [lit.,[21] mp 192.5–193 °C; $[a]_D^{21}$ −37 (*c* 1, water)]; δ_C (D_2O) 60.46 (C-6), 60.96 (C-6′), 69.84 (C-4′), 71.92 (*C*H$_2$Ph), 73.30 (C-2), 73.55 (C-2′), 74.74 (C-3), 75.17 (C-5), 75.89 (C-3′), 76.36 (C-5′), 79.07 (C-4), 101.40 (C-1′), 102.93 (C-1) and 128.87–129.81 and 136.91 (Ph).

Benzyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1⟶4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside 5

A solution of the benzyl glycoside **3** (0.97 g, 2.24 mmol) in *N,N*-dimethylformamide (5 cm^3) was placed in 50–100 cm^3 round-bottomed flask and α,α-dimethoxytoluene (0.64 cm^3, 4.26 mmol) and $TsOH·H_2O$ (32 mg, 0.17 mmol) were added. The flask was then attached to a rotary evaporator, and rotated at ~50 °C under diminished pressure (~40–60 mm Hg) with monitoring the reaction progress by TLC (solvent *D*). After 3 h, a second portion of α,α-dimethoxytoluene (0.35 cm^3, 2.33 mmol), along with more $TsOH·H_2O$ (40 mg, 0.21 mmol), were added to the solution. After a further 2 h, the flask was detached from the evaporator and the mixture was kept at rt for 16 h, before being neutralized with triethylamine (1 cm^3) and concentrated. The residue (crude product **4**) was dissolved in pyridine (6 cm^3) and acetic anhydride (4 cm^3) was added. The solution was kept at rt for 5 h, then diluted with dichloromethane (120 cm^3), washed successively with cold saturated aq. $NaHCO_3$ and water, dried ($MgSO_4$) and concentrated. Toluene was evaporated off from the residue. Crystallisation of the residue from a mixture of ethanol (50 cm^3) and ethyl acetate (5 cm^3) gave the *penta-acetate* **5** (1.113 g, 68% from the benzyl glycoside **3**); mp 211–213 °C (from ethanol–ethyl acetate); $[a]_D^{25}$ −63 (*c* 1, $CHCl_3$); (Found: C, 58.84; H, 5.81. $C_{36}H_{42}O_{16}$ requires C, 59.17; H, 5.79%); δ_H 1.94, 1.95, 1.96, 1.99 and 2.08 (15 H, 5 × s, 5 × Ac), 3.40 (1 H, dt, $J_{5',6b'}$ 4.8, 5′-H), 3.48 (1 H, ddd, $J_{5,6a}$ 4.6, $J_{5,6b}$ 1.8, 5-H), 3.60 (1 H, t, $J_{5',6a'} = J_{6a',6b'}$ = 9.5, 6′-H^a), 3.64 (1 H, t, $J_{3,4} = J_{4,5}$ = 10.1, 4-H), 3.75 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.4, 4′-H), 4.02 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H^a), 4.28 (1 H, dd, 6′-H^b), 4.42 (1 H, d, $J_{1,2}$ 7.9, 1-H), 4.45 (1 H, dd, 6-H^b), 4.51 and 4.80 (2 H, AB q, *J* 11.3, C*H*$_2$Ph), 4.53 (1 H, d, $J_{1',2'}$ 8.1, 1′-H), 4.85 and 4.88 (2 H, 2 × dd, H-2 and -2′), 5.06 (1 H, t, $J_{2,3}$ 10.1, 3-H), 5.18 (1 H, t, $J_{2',3'}$ 9.4, 3′-H), 5.41 (1 H, s, C*H*Ph) and 7.15–7.35 (10 H, m, 2 × Ph).

Benzyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1⟶4)-3,6-di-*O*-benzoyl-β-D-glucopyranoside 6

To a solution of compound **5** (0.95 g, 1.3 mmol) in methanol (20 cm^3) and dichloromethane (6 cm^3) was added 4.6 mol dm^{-3} methanolic sodium methoxide (0.4 cm^3). The mixture was kept at rt for 40 min, whereafter it was deionized with Dowex 50W-X4 (H^+) resin, filtered and concentrated. Acetonitrile (2 × 5 cm^3) was evaporated off from the residue (the expected 4′,6′-*O*-benzylidene derivative **4**), before it was dissolved in the same solvent (10 cm^3) and benzoyl cyanide (0.17 g, 1.3 mmol) and Et_3N (0.25 cm^3) were added to the solution. The mixture was stirred at rt for 30 min, then cooled with an ice-water bath and more benzoyl cyanide (0.511 g, 3.9 mmol) and Et_3N (0.3 cm^3) were added. After 45 min at 0 °C, when TLC

(solvents *B* and *D*) indicated the reaction was complete, the mixture was diluted with methanol (10 cm^3), stirred for a further 30 min at rt and concentrated. The residue was dissolved in dichloromethane (100 cm^3), the solution was washed successively with saturated aq. $NaHCO_3$ and water, and concentrated. FCC [toluene–ethyl acetate, (9 : 1) ⟶ (8 : 2)] of the residue gave the *tribenzoate* **6** (0.356 g, 33% from the pentaacetate **5**) as an amorphous solid; $[\alpha]_D^{23}$ −51.5 (*c* 1, $CHCl_3$); (Found: C, 67.73; H, 5.35. $C_{47}H_{44}O_{14}$ requires C, 67.78; H, 5.32%); δ_H 2.85 (1 H, t, $J_{5',6a'} = J_{6a',6b'}$ = 10.2, 6′-H^a), 3.13 (1 H, ddd, $J_{5',6b'}$ 4.9, 5′-H), 3.30 (1 H, dd, 6′-H^b), 3.48 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.6, 4′-H), 3.59–3.88 (4 H, m, H-2, -2′, -4 and -5), 4.43 (1 H, d, $J_{1,2}$ 7.9, 1-H), 4.48 (1 H, d, $J_{1',2'}$ 7.6, 1′-H), 4.50 (1 H, m, 6-H^a), 4.59 and 4.85 (2 H, AB q, *J* 11.6, CH_2Ph), 4.96 (1 H, dd, $J_{5,6b}$ 1.6, $J_{6a,6b}$ 12.0, 6-H^b), 5.16 (1 H, s, C*H*Ph), 5.25 (1 H, t, $J_{2',3'}$ 9.6, 3′-H), 5.37 (1 H, t, $J_{2,3} = J_{3,4}$ = 9.1, 3-H) and 7.10–8.10 (25 H, m, 5 × Ph).

Benzyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 8

(a) Triflic anhydride (0.116 cm^3, 0.688 mmol) was added dropwise to a cooled (0 °C) stirred solution of compound **6** (0.072 g, 0.086 mmol) in dichloromethane (5 cm^3) containing pyridine (0.139 cm^3, 1.72 mmol). The reaction was monitored by TLC (solvent *A*). After 30 min, the mixture was diluted with dichloromethane, washed successively with ice-cold 0.1 mol dm^{-3} HCl, ice-cold saturated aq. $NaHCO_3$ and water, and dried by filtration through cotton wool. The filtrate was concentrated and toluene was evaporated off from the residue (the expected ditriflate **7**, 0.093 g, 98.5%). A solution of tetrabutylammonium benzoate (0.25 g, 0.688 mmol; dried beforehand by evaporation of anhydrous toluene) in toluene (6 cm^3) was added to the residue and the reaction mixture was stirred under reflux. After 25 min, when TLC (solvent *A*) indicated completion, the mixture was diluted with dichloromethane, washed successively with saturated aq. $NaHCO_3$ and water, dried by filtration through cotton wool and concentrated. FCC (toluene ⟶ toluene–ethyl acetate, 95 : 5) of the residue gave the *mannobioside derivative* **8** (0.067 g, 76% from the tribenzoate **6**) as an amorphous solid; $[\alpha]_D^{23}$ −128 (*c* 1, $CHCl_3$); (Found: C, 70.24; H, 5.15. $C_{61}H_{52}O_{16}$ requires C, 70.38; H, 5.03%); δ_H 3.15 (1 H, dt, $J_{4',5'} = J_{5',6a'}$ = 10.0, 5′-H), 3.24 (1 H, t, $J_{6a',6b'}$ 10.0, 6′-H^a), 3.51 (1 H, dd, $J_{5',6b'}$ 4.3, 6′-H^b), 3.65 (1 H, dt, $J_{5,6a} = J_{5,6b}$ = 2.3, 5-H), 3.94 (1 H, t, $J_{3',4'}$ 10.0, 4′-H), 4.37 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.6, 4-H), 4.57 and 4.77 (2 H, AB q, *J* 12.4, CH_2Ph), 4.62–4.72 (2 H, m, 6-H_2), 4.67 (1 H, br s, 1-H), 4.91 (1 H, d, $J_{1',2'}$ 0.9, 1′-H), 5.29 (1 H, s, C*H*Ph), 5.32 (1 H, dd, 3-H), 5.35 (1 H, dd, 3′-H), 5.68 (1 H, br d, $J_{2,3}$ 3.1, 2-H), 5.86 (1 H, dd, $J_{2',3'}$ 3.4, 2′-H) and 7.00–8.10 (35 H, m, 7 × Ph).

(b) A solution of the ditriflate **7** (0.13 g, 0.119 mmol), ethanol (0.209 cm^3, 3.56 mmol) and 2,4,6-collidine (0.038 cm^3, 0.286 mmol) in toluene (2 cm^3) was stirred under reflux for 1 h 45 min (with TLC control in solvent *A*). The mixture was diluted with dichloromethane, washed successively with saturated aq. $NaHCO_3$ and water, dried by filtration through cotton wool and concentrated. The residue was dissolved in 80% AcOH (5 cm^3) and the mixture was kept at rt for 1 h before being concentrated, and toluene was evaporated off from the residue. This was dissolved in pyridine (2 cm^3) and benzoyl chloride (0.5 cm^3) was added to the solution. After 16 h, the reaction mixture was diluted with dichloromethane, washed successively with saturated aq. $NaHCO_3$ and water, dried by filtration through cotton wool and concentrated. FCC (as above) produced the mannobioside **8** (0.06 g, 47% from the tribenzoate **6**) identical with that obtained in (a).

Benzyl 2,3-di-*O*-benzoyl-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 9

A solution of compound **8** (0.69 g, 0.639 mmol) in 80% acetic acid (30 cm^3) was heated at 70 °C for 2 h, whereafter the mixture was concentrated and toluene was twice evaporated off from the residue. FCC [petroleum ether (40–60 °C)–ethyl acetate, (3 : 2) ⟶ (1 : 1)] then produced the *pentabenzoate* **9** (0.471 g, 76%) as an amorphous solid; $[\alpha]_D^{23}$ −93.5 (*c* 1, $CHCl_3$); (Found: C, 67.90; H, 5.15. $C_{54}H_{48}O_{16}$ requires C, 68.06; H, 5.08%); δ_H 2.69 (1 H, d, $J_{4',OH}$ 4.4, 4′-OH), 3.15 (1 H, dt, $J_{5',6a'} = J_{5',6b'}$ = 3.3, 5′-H), 3.43–3.60 (2 H, m, 6′-H_2), 3.86 (1 H, ddd, $J_{5,6a}$ 4.4, $J_{5,6b}$ 3.1, 5-H), 4.12 (1 H, t, $J_{6',OH}$ 7.1, 6′-OH), 4.17 (1 H, dt, $J_{3',4'} = J_{4',5'}$ = 9.6, 4′-H), 4.50 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.1, 4-H), 4.66 and 4.88 (2 H, AB q, *J* 12.3, CH_2Ph), 4.71 (1 H, dd, $J_{6a,6b}$ 11.8, 6-H^a), 4.78 (1 H, br s, 1-H), 4.87 (1 H, dd, 6-H^b), 5.00 (1 H, br s, 1′-H), 5.20 (1 H, dd, $J_{2,3}$ 3.2, 3-H), 5.51 (1 H, dd, $J_{2',3'}$ 3.3, 3′-H), 5.76 (1 H, br d, 2-H), 5.85 (1 H, br d, 2′-H) and 7.20–8.25 (30 H, m, 6 × Ph).

Benzyl β-D-mannopyranosyl-(1⟶4)-β-D-mannopyranoside 10

To a solution of compound **9** (128 mg, 0.134 mmol) in methanol (4 cm^3) and THF (0.7 cm^3) was added 4.6 mol dm^{-3} methanolic sodium methoxide (0.27 cm^3). The mixture was kept at rt for 14 h, whereafter it was deionized with Dowex 50W-X4 (H^+) resin, filtered and concentrated. Water (3 × 5 cm^3) was evaporated off from the residue to remove methyl benzoate. The disaccharide **10** (53 mg, 91%) was thereby obtained as an amorphous solid; $[\alpha]_D^{20}$ −63 (*c* 1, MeOH); R_f 0.78 (solvent *E*); δ_H (D_2O) (*inter alia*) 3.90 (1 H, br signal, 2-H), 3.95 (1 H, br signal, 2′-H), 4.57 (1 H, br s, 1-H), 4.62 (1 H, br s, 1′-H), 4.62 and 4.80 (2 H, AB q, *J* 11.7, CH_2Ph) and 7.33 (5 H, m, Ph); δ_C (D_2O) 61.63 (C-6), 62.02 (C-6′), 67.70 (C-4′), 70.98 (C-2), 71.52 (C-2′), 71.81 (*C*H_2Ph), 72.66 (C-3), 73.78 (C-3′), 75.90 (C-5), 77.42 (C-5′), 77.84 (C-4), 99.53 (C-1), 101.21 (C-1′) and 129.48–129.78 and 136.92 (Ph); ES-MS(−) data: *m*/*z* 431.51 (100%, $[M - H]^-$) (expected *m*/*z*, 431.15), 467.47 (63%, $[M + {}^{35}Cl]^-$) (expected *m*/*z*, 467.16), 469.53 (25%, $[M + {}^{37}Cl]^-$) (expected *m*/*z*, 469.16), 863.13 (17%, $[2M - H]^-$) (expected *m*/*z*, 863.31), 899.03 (18%, $[2M + {}^{35}Cl]^-$) (expected *m*/*z*, 899.32) and 900.02 (10%, $[2M + {}^{37}Cl]^-$) (expected *m*/*z*, 900.32) ($C_{19}H_{28}O_{11}$ requires *M*, 432.16).

Benzyl 2,3,6-tri-*O*-benzoyl-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 12

(a) A solution of benzoyl cyanide (0.065 g, 0.494 mmol) in dichloromethane (0.5 cm^3) was added to a cooled (−40 °C) stirred solution of the 4′,6′-diol **9** (0.471 g, 0.494 mmol) in acetonitrile (3 cm^3) and dichloromethane (1 cm^3) containing Et_3N (0.103 cm^3, 0.741 mmol). After 30 min (during this period the temperature was allowed to rise to −10 °C), when TLC (solvent *A*) indicated the reaction was complete, the solution was diluted with methanol (10 cm^3) and cooling was discontinued. After another 20 min, the mixture was concentrated and a further portion of methanol (10 cm^3) was evaporated off from the residue. FCC [toluene–ethyl acetate, (95 : 5) ⟶ (8 : 2)] of the residue gave the *hexabenzoate* **12** (0.482 g, 92%) as an amorphous solid; $[\alpha]_D^{23}$ −104 (*c* 1, $CHCl_3$); (Found: C, 69.34; H, 5.02. $C_{61}H_{52}O_{17}$ requires C, 69.31; H, 4.96%); δ_H 3.18 (1 H, dt, $J_{5',6a'} = J_{5',6b'}$ = 2.2, 5′-H), 3.71 (1 H, dt, $J_{5,6a} = J_{5,6b}$ = 3.2, 5-H), 3.76 (1 H, dd, $J_{6a',6b'}$ 12.2, 6′-H^a), 3.90 (1 H, t, $J_{3',4'} = J_{4',5'}$ = 9.8, 4′-H), 4.38 (1 H, t, $J_{3,4} = J_{4,5}$ = 9.6, 4-H), 4.42 (1 H, dd, 6′-H^b), 4.55 and 4.75 (2 H, AB q, *J* 12.2, CH_2Ph), 4.65 (1 H, br s, 1-H), 4.68 (1 H, m, 6-H^a), 4.75 (1 H, m, 6-H^b), 4.89 (1 H, br s, 1′-H), 5.14 (1 H, dd, $J_{2',3'}$ 3.0, 3′-H), 5.43 (1 H, $J_{2,3}$ 3.4, 3-H), 5.68 (1 H, br d, 2-H), 5.75 (1 H, br d, 2′-H) and 7.00–8.15 (35 H, m, 7 × Ph).

(b) 99% Aq. CF_3COOH (1 cm^3) was added to a solution of compound **15** (105 mg) in chloroform (9 cm^3). The resulting solution was kept at rt for 15 min, concentrated and toluene (3 × 7 cm^3) was evaporated off from the residue to give pure compound **12** (quantitatively) identical with that obtained above.

Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 14

A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **13**[27,28] (0.235 g, 0.477 mmol), the disaccharide derivative **12** (0.360 g, 0.341 mmol) and freshly activated molecular sieves 4 Å (powder, 1 g) in dry dichloromethane (5 cm^3) was stirred under argon for 30 min. TMS triflate (0.02 cm^3, 0.113 mmol) was then added and stirring was continued for a further 2 h. The reaction was quenched by addition of *N,N*-diisopropylethylamine (3 drops), the solids were filtered off using a Celite pad, and the solvent was removed under reduced pressure. FCC [toluene–ethyl acetate, (9 : 1) ⟶ (8 : 2)] of the residue produced the *trisaccharide derivative* **14** (0.224 g, 47%) as an amorphous solid; $[a]_D^{23}$ −108 (*c* 1, $CHCl_3$); (Found: C, 64.44; H, 5.02. $C_{75}H_{70}O_{26}$ requires C, 64.93; H, 5.09%); δ_H 1.69 and 1.70 (6 H, 2 × s, 2 × Ac), 1.88 (6 H, s, 2 × Ac), 3.13 (1 H, br d, 5′-H), 3.40–3.50 (2 H, m, 5″-H and 6″-H^a), 3.71 (1 H, dd, $J_{5'',6b''}$ 4.2, $J_{6a'',6b''}$ 12.0, 6″-H^b), 3.79 (1 H, br dd, 5-H), 4.04–4.18 (2 H, m, 6′-H_2), 4.25 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.2$, 4′-H), 4.46 (1 H, t, $J_{3,4} = J_{4,5} = 9.4$, 4-H), 4.49 (1 H, d, $J_{1'',2''}$ 7.8, 1″-H), 4.62 and 4.83 (2 H, AB q, *J* 12.2, CH_2Ph), 4.65 (1 H, dd, $J_{5,6a}$ 3.5, $J_{6a,6b}$ 11.9, 6-H^a), 4.72 (1 H, br s, 1-H), 4.73–4.87 (3 H, m, 2″-, 3″-H and 6-H^b), 4.93 (1 H, t, $J_{3'',4''} = J_{4'',5''} = 9.3$, 4″-H), 5.00 (1 H, br s, 1′-H), 5.43 (1 H, dd, 3′-H), 5.53 (1 H, dd, 3-H), 5.75 (1 H, br d, $J_{2,3}$ 2.7, 2-H), 5.85 (1 H, br d, $J_{2',3'}$ 2.5, 2′-H) and 7.15–8.23 (35 H, m, 7 × Ph). Also isolated were the recovered disaccharide **12** (0.065 g, 18%) and the 4′-trimethylsilyl ether **15** (0.105 g, 27%), δ_H 0.01 (9 H, s, Me_3Si), 3.55 (1 H, br dd, 5′-H), 3.97 (1 H, br dd, $J_{5,6a}$ 3.7, 5-H), 4.18 (1 H, br d, $J_{6a',6b'}$ 11.3, 6′-H^a), 4.39 (1 H, $J_{5',6b'}$ 2.4, 6′-H^b), 4.49 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.4$, 4′-H), 4.65 (1 H, t, $J_{3,4} = J_{4',5'} = 9.3$, 4-H), 4.78 and 5.00 (2 H, AB q, *J* 12.2, CH_2Ph), 4.83 (1 H, dd, $J_{6a,6b}$ 13.3, 6-H^a), 4.89 (1 H, br s, 1-H), 4.97 (1 H, m, 6-H^b), 5.16 (1 H, br s, 1′-H), 5.42 (1 H, dd, 3′-H), 5.68 (1 H, dd, 3-H), 5.92 (1 H, br d, $J_{2,3}$ 2.8, 2-H), 5.98 (1 H, br d, $J_{2',3'}$ 2.4, 2′-H) and 7.30–8.40 (35 H, m, 7 × Ph).

Benzyl 4,6-*O*-benzylidene-β-D-glucopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 16

A solution of HCl in methanol [prepared at 0 °C from acetyl chloride (0.4 cm^3) and methanol (10 cm^3)] was added to a solution of compound **14** (0.21 g, 0.151 mmol) in dichloromethane (3 cm^3). The resulting solution was kept at room temperature for 45 h [TLC (solvent *D*) then showed the formation of one major product], concentrated, and toluene (3 × 7 cm^3) was evaporated off from the residue. The residue was dissolved in acetonitrile (5 cm^3), α,α-dimethoxytoluene (0.045 cm^3, 0.302 mmol) and TsOH·H_2O (20 mg, 0.105 mmol) were added, and the mixture was stirred at rt with monitoring the reaction progress by TLC (solvents *C* and *D*). After 3 h, a second portion of α,α-dimethoxytoluene (0.023 cm^3, 0.151 mmol) was added. After a further 2 h, the reaction was quenched by addition of Et_3N (0.5 cm^3) and the mixture was concentrated. FCC (solvent *C*) of the residue gave the derivative **16** (0.12 g, 61% from compound **14**) as an amorphous solid; $[a]_D^{23}$ −102 (*c* 1, $CHCl_3$); δ_H 2.70 (2 H, m, 2 × OH), 2.90 (1 H, t, $J_{5'',6a''} = J_{6a'',6b''} = 10.2$, 6″-H^a), 3.10–3.28 (3 H, m, 4″-, 5′- and 5″-H), 3.35–3.48 (3 H, m, 2″-, 3″-H and 6″-H^b), 3.70 (1 H, br dd, $J_{5,6a}$ 4.0, 5-H), 3.96 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.5$, 4′-H), 4.06 (1 H, br d, $J_{6a',6b'}$ 11.7, 6′-H^a), 4.18 (1 H, d, $J_{1'',2''}$ 7.5, 1″-H), 4.36 (1 H, dd, $J_{5',6b'}$ 3.4, 6′-H^b), 4.38 (1 H, t, $J_{3,4} = J_{4,5} = 9.2$, 4-H), 4.54 and 4.75 (2 H, AB q, *J* 12.3, CH_2Ph), 4.60 (1 H, dd, $J_{6a,6b}$ 12.3, H-6^a), 4.64 (1 H, br s, 1-H), 4.70 (1 H, br d, H-6^b), 4.90 (1 H, br s, 1′-H), 5.13 (1 H, s, C*H*Ph), 5.36 (1 H, dd, 3′-H), 5.43 (1 H, dd, 3-H), 5.67 (1 H, br d, $J_{2,3}$ 2.8, 2-H), 5.72 (1 H, br d, $J_{2',3'}$ 2.8, 2′-H) and 7.05–8.12 (40 H, m, 8 × Ph); δ_C 62.90 and 63.22 (C-6 and -6′), 66.45 (C-5″), 68.16 (C-6″), 70.42 (2 C, C-2 and *C*H_2Ph), 70.63 (C-2′), 72.10 and 72.35 (C-3 and -3′), 73.51 (C-3″), 73.73 (C-2″), 74.24 and 74.28 (C-5 and -5′), 74.65 (C-4), 75.06 (C-4′), 80.16 (C-4″), 96.94 (C-1) 98.91 (C-1′), 101.90 (*C*HPh), 104.33 (C-1″), 128.56–136.81 (Ph) and 165.16–167.04 (C=0); ES-MS(+) data: *m*/*z* 1329.96 (100%, $[M + Na]^+$) (expected *m*/*z*, 1329.40; $C_{74}H_{66}O_{22}$ requires *M*, 1306.40).

Benzyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 17

A solution of benzoyl cyanide (0.011 g, 0.0849 mmol) in dichloromethane (0.3 cm^3) was added to a cooled (−45 °C) and stirred solution of the 2″,3″-diol **16** (0.111 g, 0.0849 mmol) in acetonitrile (3 cm^3) and dichloromethane (0.5 cm^3) containing Et_3N (0.018 cm^3, 0.127 mmol). The temperature was allowed to rise slowly to −10 °C. After 1 h 10 min, when TLC (solvents *A* and *C*) indicated the reaction was complete, the solution was diluted with methanol (3 cm^3) and cooling was discontinued. After another 20 min, the mixture was concentrated and a further portion of methanol (3 cm^3) was evaporated off from the residue. FCC [toluene–ethyl acetate, (95 : 5) ⟶ (9 : 1)] of the residue produced the *trisaccharide heptabenzoate* **17** (0.078 g, 65%) as an amorphous solid; $[a]_D^{23}$ −102 (*c* 1, $CHCl_3$); δ_H 2.88 (1 H, d, $J_{2'',OH}$ 4.2, 2″-OH), 2.93 (1 H, t, $J_{5'',6a''} = J_{6a'',6b''} = 9.9$, 6″-H^a), 3.17 (1 H, dd, $J_{5'',6b''}$ 3.3, 6″-H^b), 3.36–3.54 (4 H, m, 2″-, 4″-, 5′- and 5″-H), 3.71 (1 H, ddd, $J_{5,6a}$ 3.7, 5-H), 3.97 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.5$, 4′-H), 4.12 (1 H, br d, $J_{6a',6b'}$ 11.3, 6′-H^a), 4.29 (1 H, dd, $J_{5',6b'}$ 3.5, 6′-H^b), 4.33 (1 H, d, $J_{1'',2''}$ 7.2, 1″-H), 4.39 (1 H, t, $J_{3,4} = J_{4,5} = 9.4$, 4-H), 4.54–4.75 (2 H, AB q, *J* 12.4, CH_2Ph), 4.58 (1 H, dd, $J_{6a,6b}$ 12.2, 6-H^a), 4.64 (1 H, br s, 1-H), 4.73 (1 H, dd, $J_{5,6b}$ 1.9, 6-H^b), 4.89 (1 H, br s, 1′-H), 5.12 (1 H, s, C*H*Ph), 5.13 (1 H, t, $J_{2'',3''} = J_{3'',4''} = 9.3$, 3″-H), 5.37 (1 H, dd, 3′-H), 5.43 (1 H, dd, 3-H), 5.67 (1 H, br d, $J_{2,3}$ 3.3, 2-H), 5.71 (1 H, br d, $J_{2',3'}$ 3.2, 2′-H) and 7.05–8.10 (45 H, m, 9 × Ph); ES-MS(+) data: *m*/*z* 1434.02 (100%, $[M + Na]^+$) (expected *m*/*z*, 1433.43; $C_{81}H_{70}O_{23}$ requires *M*, 1410.43);

Benzyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranosyl-(1⟶4)-2,3,6-tri-*O*-benzoyl-β-D-mannopyranoside 19

Triflic anhydride (0.036 cm^3, 0.215 mmol) was added dropwise to a cooled (0 °C) stirred solution of compound **17** (0.076 g, 0.054 mmol) in dichloromethane (5 cm^3) containing pyridine (0.044 cm^3, 0.538 mmol). The reaction was monitored by TLC (solvent *A* and *B*). After 1 h 15 min, a second portion of triflic anhydride (0.009 cm^3, 0.054 mmol) was added. After a further 20 min, the mixture was diluted with dichloromethane, washed successively with ice-cold 0.1 mol dm^{-3} HCl, ice-cold saturated aq. $NaHCO_3$ and water, and dried by filtration through cotton wool. The filtrate was concentrated and toluene was evaporated off from the residue (the expected triflate **18**). A solution of tetrabutylammonium benzoate (0.059 g, 0.161 mmol; dried beforehand by evaporation of anhydrous toluene) in toluene (5 cm^3) was added to the residue and the reaction mixture was stirred under reflux. After 50 min, when TLC (solvent *A*) indicated the completion, the mixture was diluted with dichloromethane, washed successively with saturated aq. $NaHCO_3$ and water, dried by filtration through cotton wool and concentrated. FCC (toluene ⟶ toluene–ethyl acetate, 95 : 5) of the residue gave the *mannotrioside derivative* **19** (0.073 g, 88% from the heptabenzoate **17**) as an amorphous solid; $[a]_D^{23}$ −132 (*c* 1, $CHCl_3$); (Found: C, 69.94; H, 5.17. $C_{88}H_{74}O_{24}$ requires C, 69.74; H, 4.92%); δ_H 3.01 (1 H, dt, $J_{5'',6b''}$ 4.5, 5″-H), 3.14 (1 H, t, $J_{5'',6a''} = J_{6a'',6b''} = 9.9$, 6″-H^a), 3.20 (1 H, br dd, $J_{5',6b'}$ 2.6, 5′-H), 3.35 (1 H, dd, 6″-H^b), 3.68 (1 H, ddd, $J_{5,6a}$ 3.6, 5-H), 3.78 (1 H, br d, $J_{6a',6b'}$ 10.9, 6′-H^a), 3.85 (1 H, t, $J_{3'',4''} = J_{4'',5''} = 9.9$, 4″-H), 4.16 (1 H, t, $J_{3',4'} = J_{4',5'} = 9.6$, 4′-H), 4.26 (1 H, dd, 6′-H^b), 4.33 (1 H, t, $J_{3,4} = J_{4,5} = 9.5$, 4-H), 4.52 and 4.73 (2 H, AB q, *J* 12.3, CH_2Ph), 4.57 (1 H, dd, $J_{6a,6b}$ 12.0, 6-H^a), 4.63 (1 H, br s, 1′-H), 4.67 (1 H, br s, 1-H), 4.68 (1 H, dd, $J_{5,6b}$ 1.9, 6-H^b), 4.82 (1 H, br

s, 1″-H), 5.21–5.29 (2 H, m, 3′- and 3″-H), 5.24 (1 H, s, C*H*Ph), 5.40 (1 H, dd, 3-H), 5.66 (1 H, br d, $J_{2,3}$ 3.2, 2-H), 5.69 (1 H, br d, $J_{2',3'}$ 3.1, 2′-H), 5.72 (1 H, br d, $J_{2'',3''}$ 3.3, 2″-H) and 7.10–8.10 (50 H, m, 10 × Ph); δ_C 62.17 and 63.18 (C-6 and -6′), 65.25 (C-5″), 67.38 (C-6″), 68.28 (C-3″), 70.36 (*C*H_2Ph), 70.61 (2 C) and 70.74 (C-2, -2′ and -2″), 71.97 and 72.33 (C-3 and -3′), 73.64 and 73.68 (C-5 and -5′), 73.85 (C-4), 74.56 (C-4′), 76.27 (C-4″), 96.88 (C-1), 99.15 (C-1′), 99.92 (C-1″), 101.97 (*C*HPh), 126.45–137.13 (Ph) and 165.09–167.13 (C=0).

Benzyl β-D-mannopyranosyl-(1⟶4)-β-D-mannopyranosyl-(1⟶4)-β-D-mannopyranoside 20

To a stirred solution of the trisaccharide derivative **19** (70 mg, 0.046 mmol) in dichloromethane (7 cm^3) a mixture of TFA (0.5 cm^3) and water (0.02 cm^3) was added. After stirring at rt for 30 min, the mixture was diluted with dichloromethane, washed successively with saturated aq. $NaHCO_3$ and water, dried by filtration through cotton wool and concentrated. FCC [toluene–EtOAc, (9 : 1) ⟶ (7 : 3)] of the residue gave the corresponding 4″,6″-diol (52 mg, 80%), which was then dissolved in methanol (4 cm^3) and THF (0.7 cm^3) followed by addition of 4.6 mol dm^{-3} methanolic NaOMe (0.27 cm^3). The mixture was kept at rt for 12 h, whereafter it was deionized with Dowex 50W-X4 (H^+) resin, filtered and concentrated. Water (3 × 5 cm^3) was evaporated off from the residue to remove methyl benzoate. The trisaccharide **20** (19 mg, 88% from the 4″,6″-diol, 70% from the protected derivative **19**) was thereby obtained as an amorphous solid; $[a]_D^{20}$ −60 (*c* 1, MeOH); R_f 0.53 (solvent *E*); δ_H (D_2O) (*inter alia*) 3.91 (1 H, d, $J_{1,2}$ 3.1, 2-H), 3.95 (1 H, d, $J_{1',2'}$ 3.0, 2′-H), 4.01 (1 H, br signal, 2″-H), 4.59 (1 H, br s, 1-H), 4.62 (1 H, d, 1″-H), 4.65 (1 H, d, 1′-H), 4.63 and 4.81 (2 H, AB q, *J* 11.9, CH_2Ph) and 7.35 (5 H, m, Ph); δ_C (D_2O) 61.60 (2 C, C-6 and -6′), 62.02 (C-6″), 67.69 (C-4″), 70.94 (C-2′), 71.02 (C-2), 71.49 (C-2″), 71.83 (*C*H_2Ph), 72.48 (C-3′), 72.64 (C-3), 73.75 (C-3″), 75.92 (C-5), 76.03 (C-5′), 77.44 (C-5″), 77.65 (C-4′), 77.79 (C-4), 99.53 (C-1), 101.17 (2 C, C-1′ and -1″) and 129.48–129.77 and 136.90 (Ph); ES-MS(−) data: *m*/*z* 593.35 (100%, $[M - H]^-$) (expected *m*/*z*, 593.21), 629.19 (28%, $[M + {}^{35}Cl]^-$) (expected *m*/*z*, 629.22), 631.24 (16%, $[M + {}^{37}Cl]^-$) (expected *m*/*z*, 631.22), 1187.69 (22%, $[2M - H]^-$) (expected *m*/*z*, 1187.43), 1223.59 (9%, $[2M + {}^{35}Cl]^-$) (expected *m*/*z*, 1223.44) and 1225.70 (6%, $[2M + {}^{37}Cl]^-$) (expected *m*/*z*, 1225.44) ($C_{25}H_{38}O_{16}$ requires *M*, 594.22).

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