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p-TRIFLUOROACETAMIDOPHENYL *O*-α-D-MANNOPYRANOSYL-(1→3)-*O*-[α-D-MANNOPYRANOSYL-(1→6)]-β-D-MANNOPYRANOSIDE*

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ABSTRACT

p-Nitrophenyl 2-*O*-benzyl-4,6-*O*-cyclohexylidene-β-D-mannopyranoside (**4**) was condensed with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide. The resulting, protected disaccharide was converted into *p*-nitrophenyl *O*-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-4-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranoside (**8**), which was condensed with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide to give *p*-nitrophenyl *O*-(2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl)-(1→3)-*O*-[2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-4-*O*-benzoyl-2-*O*-benzyl-β-D-mannopyranoside (**9**) in 75% yield. Conversion of the *p*-nitrophenyl group followed by deprotection then yielded the title compound, whose structure was confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

Our laboratory continues the preparation of saccharidic determinants that occur on glycoproteins or glycolipids. Our opinion remains that disaccharidic determinants are most important: they are sufficiently large to generate binding-affinity[2] for antibodies having a ΔG value of 6.4–7.0 kcal.mol^{-1}, quite sufficient for the precipitation interaction needed if these antibodies are to be used for diagnostic studies, or to initiate the complement-mediated lysis of cells. Ordinary, trisaccharidic determinants are too large for this purpose, as the recognition by sera — originally obtained against a trisaccharidic determinant — of a disaccharidic fragment thereof (i.e., a cross reaction), could be of sufficiently high affinity to suggest erroneously the presence of the intact trisaccharide. However, the case of branched, core-like, trisaccharides is a different matter as they may not be as accessible to antibodies as disaccharide ligands. As all *N*-glycosylproteins, be they high mannosyl- or *N*-acetyllactosamine- and/or sialic acid-substituted, contain a trimannosyl branch-point[3], we decided to prepare this antigen and its sera as well. We

*The Preparation of Immunogens Bearing Immunodeterminants Known to Occur on Glycoproteins. Part IV. For Part III, see ref. 1.
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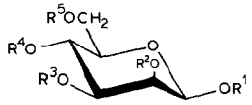
report here the synthesis of the trisaccharide in a form suitable for linkage to a protein carrier, as before. The immunization, harvesting of sera, and characterization of diagnostic antibodies to many of these disaccharides will be reported in the near future.

RESULTS AND DISCUSSION

The starting material, *p*-nitrophenyl β-D-mannopyranoside, was prepared as described by Garegg *et al.*[4]. For the first glycosylation step, an "aglycon" having all positions protected except OH-3 was needed. Initial attempts to prepare the commonly used 4,6-benzylidene acetal (as a first intermediate), did not give satisfactory results. However, Iversen and Bundle[5] recently described the preparation of a 4,6-cyclohexylidene acetal, using 1-ethoxycyclohexene[6], under kinetic and stoichiometric control. Substituting *p*-nitrophenyl β-D-mannopyranoside in this reaction afforded the 4,6-*O*-cyclohexylidene derivative **1** in 52% yield. To protect OH-2 required a somewhat circuitous route. First, partial benzoylation at low temperature (−38°) afforded the 3-benzoate **2** in 59% yield after processing and chromatography. In the next step, a mild benzylation procedure, using benzyltrichloroacetimidate as benzylating agent[7] was used, as it is compatible with both acid- and alkali-sensitive groups. With compound **2** as substrate, the fully protected 2-benzyl ether **3** was obtained in 73% yield. Subsequent debenzoylation with sodium methoxide in methanol then afforded the desired "aglycon" **4**. Direct partial benzylation[8] with benzyl bromide plus silver oxide as promoter (in analogy with the partial benzylation of *p*-nitrophenyl 4,6-benzylidene-α-D-mannopyranoside) would have been much simpler. However, attempts to use this method here failed to give a recognizable product.

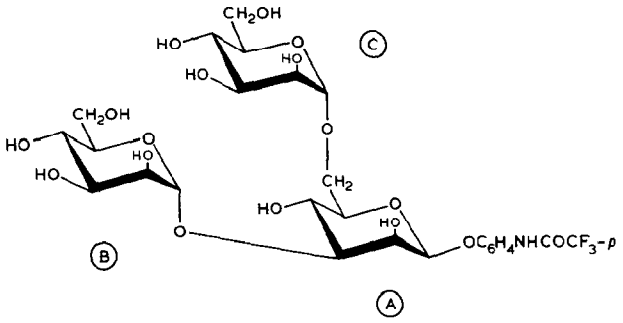
Condensation of **4** with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide, using the silver triflate–collidine complex as promoter[9], then afforded the fully protected disaccharide derivative **5** in 89% yield, after isolation and chromatography. Compound **5** was then converted into a suitable aglycon by the following route: The cyclohexylidene group was removed by mild acid hydrolysis to give the partially protected disaccharide derivative **6**. This product was tritylated, and then benzoylated *in situ*, to give the fully protected intermediate **7**. Compound **7** was then detritylated to give the disaccharide "aglycon" **8** in 70% overall yield from **5**. Condensation of **8** with tetra-*O*-benzoyl-α-D-mannopyranosyl bromide, in exactly the same manner as for the preparation of **5**, then gave the trisaccharide derivative **9** in 75% yield.

In the deprotection sequence, the nitro group in **9** was first converted into a trifluoroacetamido group by catalytic hydrogenation followed by treatment with trifluoroacetic anhydride and pyridine *in situ*; the intermediate **10** was obtained in 89% yield. Compound **10** was next debenzoylated, with sodium methoxide in methanol, to give **11**. Hydrogenation of **11** over palladium-on-charcoal then afforded the final trisaccharide glycoside **12** in 41% yield from **10**.



	R^1	R^2	R^3	R^4	R^5
1	$C_6H_4NO_2$-*p*	H	H	—Cy—	
2	$C_6H_4NO_2$-*p*	H	Bz	—Cy—	
3	$C_6H_4NO_2$-*p*	$PhCH_2$	Bz	—Cy—	
4	$C_6H_4NO_2$-*p*	$PhCH_2$	H	—Cy—	
5	$C_6H_4NO_2$-*p*	$PhCH_2$	αManpBz$_4$	—Cy—	
6	$C_6H_4NO_2$-*p*	$PhCH_2$	αManpBz$_4$	H	H
7	$C_6H_4NO_2$-*p*	$PhCH_2$	αManpBz$_4$	Bz	Ph_3C
8	$C_6H_4NO_2$-*p*	$PhCH_2$	αManpBz$_4$	Bz	H
9	$C_6H_4NO_2$-*p*	$PhCH_2$	αManpBz$_4$	Bz	αManpBz$_4$
10	$C_6H_4NHCOCF_3$-*p*	$PhCH_2$	αManpBz$_4$	Bz	αManpBz$_4$
11	$C_6H_4NHCOCF_3$-*p*	$PhCH_2$	H	H	H

Cy = cyclohexylidene, αManpBz$_4$ = tetra-*O*-benzoyl-α-D-mannopyranosyl



12

The ^{1}H-n.m.r. spectrum **12** showed, *inter alia*, one-proton signals at δ 5.17 ($J_{1,2}$ <1 Hz) and 5.10 ($J_{1,2}$ 1.5 Hz), assigned to H-1 Man B (the 1→3 linked mannopyranosyl group) and H-1 Man A, respectively. The one-proton doublet at 4.32 ($J_{1,2}$ 2.3 Hz) was assigned to H-1 Man C (the 1→6 linked mannopyranosyl group). The ^{13}C-n.m.r. spectrum of **12** showed, *inter alia*, signals at δ 103.96, assigned to C-1 Man B, at 101.33 assigned to C-1 Man C, and at 99.42, assigned to C-1 Man A. A signal was observed at δ 82.37 for C-3 of Man A. Signals were also observed at δ 67.21 for C-6 of Man A, coinciding with C-4 of Man A, and at 62.97 and 62.63 for C-6 of Man B and C-6 of Man C. These data and the specific optical rotation of $[\alpha]_{578}^{23}$ +19°, support the structure assigned to **12**. The corresponding reducing trisaccharide[10] and methyl α-glycoside[11] have been reported.

EXPERIMENTAL

General methods. — These were essentially the same as those described previously[12].

p-*Nitrophenyl 4,6-O-cyclohexylidene-β-*D*-mannopyranoside* (**1**). — A solution of *p*-nitrophenyl β-D-mannopyranoside (3.62 g, 12 mmol) in DMF (55 mL) was treated with 1-ethoxycyclohexene (3.16 mL, 24 mmol) and a catalytic amount of *p*-toluenesulfonic acid (40 mg) at room temperature. After 2 h, the mixture was made neutral by addition of triethylamine (2 mL), diluted with toluene–ethyl acetate (2:1, 350 mL) and washed with water (3 × 200 mL), dried (Na_2SO_4), and evaporated. T.l.c. (toluene–EtOAc 1:2) of the residue showed the presence of a major product at $R_F \approx 0.33$ and several minor faster- or slower-moving components. Chromatography on a column of silica gel (same solvent) gave 2.38 g (52%) of the title compound, which crystallized on standing, m.p. 155–160°, $[\alpha]_{578}^{23}$ −93° (*c* 3.18, $CHCl_3$); ^{1}H-n.m.r. ($CDCl_3$ + 30% CD_3OD, 220 MHz): δ 8.18 and 7.13 (both, 2 H each, $J_{H,H}$ 10 Hz, AB-spectrum, *p*-NO_2-C_6H_4 group), 5.28 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.22 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 3.5 Hz, H-2), 4.08 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 4.00–3.82 (m, 2 H, 2 × H-6), 3.77 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 7.5 Hz, H-3), and 3.42 (ddd, 1 H, $J_{4,5}$ 9.5, $J_{5,6}$ 9.5, $J_{5,6'}$ 6 Hz, H-5); ^{13}C-n.m.r. ($CDCl_3$ + 30% CD_3OD, 25.05 MHz): δ 100.64 (cyclohexylidene acetal C), 98.26 (C-1), 71.01 (C-3 and C-4), 69.99 (C-2), 68.22 (C-5), and 61.41 (C-6).

p-*Nitrophenyl 3-O-benzoyl-4,6-O-cyclohexylidene-β-*D*-mannopyranoside* (**2**). — A solution of compound **1** (1.91 g, 5 mmol) in pyridine (50 mL) was cooled to −38° and a solution of benzoyl chloride (0.55 mL, 5 mmol) in pyridine was added dropwise, with stirring, during 20 min. Stirring was continued overnight, during which period the mixture was allowed to slowly attain room temperature. T.l.c. (toluene–EtOAc 4:1) showed a major product at R_F 0.41 and also some faster (presumably the dibenzoate) and slower-moving components; only traces of the starting material remained. The mixture was diluted with toluene (200 mL) and washed with water (2 × 100 mL), saturated aqueous $NaHCO_3$ (100 mL), and water again (100 mL), dried (Na_2SO_4) and evaporated. Chromatography on a column of silica gel (same solvent as before), gave 1.44 g (59%) of the title compound. Crystallization from 2-propanol gave an analytical sample having m.p. 177–178°, $[\alpha]_{578}^{23}$ −74° (*c* 1.41, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CDCl_3$): δ 8.25–6.91 (m, 9 H, aromatic H), 5.37 (s, 1 H, $J_{1,2}$ <1 Hz, H-1), 5.23 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, H-3), 4.61 (d, 1 H, $J_{1,2}$ <1, $J_{3,4}$ 3 Hz, H-2), 4.43 (dd, 1 H, $J_{3,4}$ 10, $J_{4,5}$ 9 Hz, H-4), 4.14–3.86 (m, 2 H, 2 × H-6), 3.58 (ddd, 1 H, $J_{4,5} = J_{5,6}$ 9, $J_{5,6'}$ 7 Hz, H-5), and 2.36–1.14 (m, 10 H, cyclohexylidene H); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 100.40 (cyclohexylidene acetal C), 97.67 (C-1), 72.96 (C-3), 69.35 (C-4), 68.77 (C-2), 66.97 (C-5), and 61.36 (C-6).

Anal. Calc. for $C_{25}H_{27}NO_9$: C, 61.84; H, 5.61; N, 2.89. Found: C, 61.75; H, 5.41; N, 2.78.

p-*Nitrophenyl 3-O-benzoyl-2-O-benzyl-4,6-O-cyclohexylidene-β-*D*-mannopyranoside* (**3**). — To a stirred solution of **2** (1.44 g, 2.97 mmol) in cyclohexane–dichloromethane (2:1, 30 mL), benzyl trichloroacetimidate (1.14 mL, 9 mmol) and a catalytic amount (3 drops, ~0.15 mL) of triflic acid were added. After stirring overnight at room temperature, examination by t.l.c. (8:1 toluene–EtOAc) showed

a major product having R_F 0.50 and only a small amount of unreacted (slower moving) starting material. The mixture was made neutral by addition of triethylamine (0.5 mL) and evaporated. The residue was purified by chromatography on a column of silica gel (same solvents as before) to give 1.26 g (73%) of **2**, which crystallized on standing. Recrystallization from methanol gave an analytical sample having m.p. 145–148° and $[\alpha]_{578}^{23}$ −83° (*c* 0.91, $CHCl_3$); ^{1}H-n.m.r. (220 MHz, $CDCl_3$): δ 8.27–6.91 (m, 14 H, aromatic H), 5.34 (s, 1 H, $J_{1,2}$ <1 Hz, H-1), 5.16 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, H-3), 4.93 and 4.80 (both d, 1 H each, $J_{H,H}$ 12 Hz, AB-spectrum benzyl-CH_2), 4.39 (d and dd, 1 H each, coincident signals, d has $J_{1,2}$ <1, $J_{2,3}$ 3 Hz, H-2, dd has $J_{3,4}$ 10, $J_{4,5}$ 9 Hz, H-4), 3.98 (m, 2 H, 2 × H-6), 3.52 (ddd, 1 H, $J_{4,5} = J_{5,6}$ = 9, $J_{5,6'}$ 7 Hz, H-5), and 2.27–1.20 (m, 10 H cyclohexylidene H); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 100.30 (acetal C, cyclohexylidene group), 98.50 (C-1), 75.69 and 75.49 (benzyl-CH_2 and C-2), 72.96 (C-3), 68.90 (C-4), 67.36 (C-5), and 61.31 (C-6).

Anal. Calc. for $C_{32}H_{33}NO_9$: C, 66.81; H, 5.78; N, 2.43. Found: C, 66.61; H, 5.97; N, 2.17.

p-*Nitrophenyl 2-O-benzyl-4,6-O-cyclohexylidene-β-*D*-mannopyranoside* (**4**). — A solution of **3** (1.26 g, 2.2 mmol) in methanol (50 mL) containing sodium methoxide (1 mL of a 0.2M solution in methanol) was boiled under reflux for 30 min. Examination by t.l.c. (2:1 toluene–EtOAc) showed no remaining starting material and a new, major product at R_F 0.46. After cooling, sodium ions were removed by treatment with Amberlite IR-120 (H^+) resin, and the solution was evaporated. Purification of the residue on a column of silica gel (same solvents as above) gave 0.22 g (69%) of the chromatographically pure **4**; $[\alpha]_{378}^{23}$ −102° (*c* 2.48, $CHCl_3$); ^{1}H-n.m.r. (220 MHz, $CDCl_3$): δ 8.18 and 7.00 (both d, 2 H each, $J_{H,H}$ 10 Hz, AB spectrum, *p*-$NO_2C_6H_4$ group), 7.55–7.02 (m, 5 H, benzyl aromatic H), 5.23 (d, 1 H, $J_{1,2}$ ~1 Hz, H-1), 5.13 and 4.65 (both d, 1 H each, $J_{H,H}$ 11.5 Hz, AB spectrum, benzyl-CH_2), 4.13 (dd, 1 H, $J_{1,2}$ ~1, $J_{2,3}$ 4 Hz, H-2), 4.07–3.82 (m, 4 H, H-3, H-4 and 2 × H-6), 3.36 (ddd, 1 H, $J_{4,5} = J_{5,6}$ 9.5, $J_{5,6'}$ 6.5 Hz, H-5), and 2.11–1.09 (m, 10 H, cyclohexylidene H); ^{13}C-n.m.r. (25.05 MHz, $CDCl_3$): δ 100.40 (acetal C, cyclohexylidene group), 98.84 (C-1), 77.93 (C-2), 76.08 (benzyl-CH_2), 71.40 (C-3), 70.47 (C-4), 68.72 (C-5), and 61.12 (C-6).

p-*Nitrophenyl* O-*(2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-(1→3)-2-*O*-benzyl-4,6-*O*-cyclohexylidene-β-*D*-mannopyranoside* (**5**). — A solution of the aglycon **4** (0.59 g, 1.25 mmol) and tetra-*O*-benzyl-α-D-mannopyranosyl bromide (0.92 g, 1.40 mmol) in 1:1 toluene–nitromethane (10 mL) was stirred at −25° under dry argon. To this mixture, silver triflate (0.36 g, 1.40 mmol) and 2,4,6-collidine (140 μL, 1.06 mmol) in the same solvent (5 mL) were added dropwise during 5 min. After stirring for an additional 15 min, t.l.c. (8:1 toluene–EtOAc) showed the presence of a new major product at R_F 0.45 and no remaining starting materials. The mixture was made neutral by addition of triethylamine (1 mL), diluted with toluene (100 mL), and filtered through Celite, washed subsequently with water (2 × 50 mL), aq. 0.5M $Na_2S_2O_3$ (2 × 25 mL), and water again (50 mL). After

drying (Na_2SO_4) and evaporation, the residue was purified by chromatography on a column of silica gel (same solvents as before); yield 1.17 g (89%) of the title compound as a chromatographically homogeneous, armophous solid, $[\alpha]_{578}$ −55.3° (*c* 1.42, $CHCl_3$); ^{1}H-n.m.r. data (220 MHz, $CHcl_3$): δ 8.27–6.91 (m, 29 H, aromatic H), 6.05 (narrow m, 2 H, H-3 and H-4, both Man B), 5.91 (broad s, 1 H, H-2B), 5.63 (d, 1 H, $J_{1,2}$ ~1 Hz, H-1A), 5.18 (s, 2 H, benzyl-CH_2), 4.89 (s, 1 H, $J_{1,2}$ <1 Hz, H-1B), 4.70 (dd, 1 H, $J_{5,6}$ 2.5, $J_{6,6a}$ 12 Hz, H-6B), 4.42 (dd, 1 H, $J_{5,6a}$ 5, $J_{6,6a}$ 12 Hz, H-6a B), 4.36 (dd, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4 A), 4.31–4.20 (m, 1 H, H-5B), 4.09 (dd, $J_{1,2}$ ~1, $J_{2,3}$ 3.5 Hz, H-2A), 4.06–3.84 (m, 3 H, H-3A and 2 × H-6A), 3.31 (ddd, 1 H, $J_{4,5} = J_{5,6}$ 9.5, $J_{5,6a}$ 5 Hz, H-5A), and 1.75–1.18 (m, 10 H, cyclohexylidene H); ^{13}C-n.m.r. (25.05 MHz): δ 100.50 (acetal C, cyclohexylidene group), 98.74 (2 × C-1, A and B), 75.89, 75.54 and 75.10 (benzyl-CH_2, C-2A and C-3A), 70.52 and 70.13 (C-3B and C-5B), 69.50 (C-2B and C-4A), 68.82 (C-5A), 67.50 (C-4B), 63.87 (C-6B), and 61.21 (C-6A).

p-*Nitrophenyl* O-*(2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl)-(1→3)-2*-O-*benzyl*-α-D-*mannopyranoside* (**6**). — Compound **5** (1.12 g, 1.07 mmol) was dissolved in 90% aqueous acetic acid (100 mL) and heated on a steam bath for 1 h, whereupon t.l.c. (1:1 toluene–EtOAc) showed only one major component, R_F 0.46, and no remaining starting material (fast moving). After cooling and evaporation (residual acetic acid was removed by evaporation of toluene from the residue), the residue was purified by chromatography on a column of silica gel (same solvents as before) to give 0.91 g (88%) of the title component, chromatographically homogeneous, as an amorphous solid, $[\alpha]_{578}^{23}$ −63° (*c* 1.84, $CHCl_3$); ^{1}H-n.m.r. (220 MHz, $CDCl_3$ + 15% CD_3OD) showed *inter alia* δ 8.30–6.95 (m, 29 H, aromatic H), 6.08 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4B), 6.02–5.91 (m, 2 H, H-2B and H-4B), 5.58 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1A), 5.26 and 5.03 (both d, 1 H, each, $J_{H,H}$ 12 Hz, AB-spectrum, benzyl-CH_2), 4.75, 1–4.61 (m, 1 H, H-6B), 4.48–4.33 (m, 2 H, H-6a B and H-5B), 4.20 (2 × dd, 2 H, coinciding signals, one dd has $J_{1,2}$ 1.3, $J_{2,3}$ 3.5 Hz, H-2A, the other dd has $J_{3,4} = J_{4,5}$ 9 Hz, H-4A), 4.07–3.82 (m, 3 H, H-3A and 2 × H-6A), and 3.52 (ddd, $J_{4,5}$ 9, $J_{5,6}$ 5, $J_{6,5a}$ 3 Hz, H-5A); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$): δ 99.62 (C-1B), 98.55 (C-1A), 81.20 (C-3A), 77.40 (C-5A), 76.81 (C-2A), 74.67 (benzyl-CH_2), 70.13 (C-3B and C-5B), 69.45 (C-2B), 66.92 (C-4A and C-4B), 62.92, and 62.19 (2 × C-6).

p-*Nitrophenyl* O-*(2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl)-(1→3)-4*-O-*benzoyl-2*-O-*benzyl*-β-D-*mannopyranoside* (**8**). — A solution of **6** (0.25 g, 0.77 mmol) and chlorotriphenylmethane (0.49 g, 1.75 mmol) in pyridine was stirred at room temperature for 2 days, when t.l.c. (16:1 toluene–EtOAc) showed only traces of starting material (very low R_F) and a new major component at R_F 0.32. Benzoyl chloride (0.5 mL, 4.3 mmol), was added and stirring continued overnight. The mixture now had a major component at R_F 0.41 (t.l.c., same solvents). Water (0.5 mL) was added, and the mixture was stirred for 30 min, to decompose the excess of reagents. After dilution with toluene (100 mL) and washing with, in turn, water (50 mL), sat. aqueous $NaHCO_3$ (2 × 50 mL), and water again (50 mL), the solution

was dried (Na_2SO_4) and evaporated. The residue was purified by chromatography on a column of silica gel (same solvents as before) to give 0.90 g (89%) on the fully protected intermediate **7**. This derivative (0.74 g, 0.56 mmol) was dissolved in 90% aqueous acetic acid (50 mL) and heated on a steam bath for one h, whereupon t.l.c. (4:1 toluene–EtOAc) showed no remaining starting material (very fast-moving) and one major component at R_F 0.31. After cooling and evaporation followed by chromatography on a column of silica gel (same solvent), 0.54 g (90%) of the title compound was obtained; it crystallized on concentration. Recrystallization from 2-propanol gave an analytical sample having m.p. 222–223° and $[\alpha]_{578}^{21}$ −90.5° (*c* 1.05, $CHCl_3$); ^{1}H-n.m.r. (220 MHz, $CDCl_3$–10% CD_3OD): δ 8.32–7.09 (m, 34 H, aromatic H), 6.02–5.89 (m, 2 H, H-3B and H-4B), 5.73 (dd, 1 H, $J_{3,4} = J_{4,5}$ 7.5 Hz, H-4), 5.67 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3 Hz, H-2B), 5.37 (d, 1 H, $J_{1,3}$ 1 Hz, H-1A), 5.36 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1B), 5.14 and 5.01 (both d, 2 H each, $J_{H,H}$ 12 Hz, AB-spectrum, benzyl-CH_2), 4.59 (dd, 1 H, $J_{5,6}$ 2.5, $J_{6,6a}$ 12 Hz, H-6A), 4.50 (m, 1 H, H-5B), 4.38–4.27 (m, 2 H, H-3A and H-6a B), 4.25 (dd, 1 H, $J_{1,2}$ 1, $J_{2,3}$ 3.5 Hz, H-2A), and 3.98–3.72 (m, 3 H, H-5A and 2 × H-6A); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$ + 10% CD_3OD): δ 99.13 (C-1B), 97.48 (C-1A), 76.96 (C-3A), 76.03 (C-2A), 75.49 (C-2A), 74.23 (benzyl-CH_2), 70.28, 69.79 and 69.65 (C-2B, C-3B, and C-5B), 69.06 (C-4A), 67.21 (C-4B), and 62.82 (C-6B) and 61.65 (C-6A).

Anal. Calc. for $C_{66}H_{51}BO_{18}$: C, 67.09; H, 4.79; N, 1.30. Found: C, 67.47; H, 5.08; N, 1.22.

p-*Nitrophenyl* O-*(2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl)-(1→3)*-O-[*2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl*]-*(1→6)-4*-O-*benzoyl-2*-O-*benzyl*-β-D-*mannopyranoside* (**9**). — The disaccharide "aglycon" **8** (0.51 g; 0.47 mmol) and tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (0.35 g, 0.52 mmol) were dissolved in dry 1:1 toluene–nitromethane (10 mL) and the solution was cooled to −25°. A solution of silver triflate (0.14 g, 0.52 mmol) and *sym*-collidine (56 μL, 0.40 mmol) in the same solvent (5 mL) was added dropwise, with stirring, over a period of 5 min. After stirring for 30 min more at −25°, t.l.c. showed (16:1 toluene–EtOAc) a major product at R_F 0.41. After processing and chromatography (see the preparation of **5** for details), the title compound was obtained as a chromatographically pure, amorphous solid, 0.58 g (75%); $[\alpha]_{578}^{21}$ −49° (*c* 3.25, $CHCl_3$); ^{1}H-n.m.r. (220 MHz, $CDCl_3$): δ 8.50–7.07 (m, 54 H, aromatic H), 6.09 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10 Hz, H-4C), 5.99 (narrow m, 2 H, H-3 and H-4 both Man B), 5.86 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, H-3C), 5.83 (dd, 1 H, $J_{3,4} = J_{4,5}$ 8 Hz, H-4A), 5.76 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3 Hz, H-2C), 5.58 (broad s, 1 H, H-2B), 5.41 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1A), 5.23 (s, 1 H, $J_{1,2}$ <1 Hz, H-1B), 5.15 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1C), 5.20 and 5.08 (both d, 1 H each, $J_{H,H}$ 12.5 Hz, benzyl-CH_2), 4.60 (two dd, 1 H each, coinciding signals, both have $J_{5,6}$ 2, $J_{6,6a}$ 10 Hz, H-6 Man B and Man C), 4.50–4.18 (m, 5 H, H-2A, H-3A, H-6A and 2 × H-6a, Man B and C), 4.10 (ddd, $J_{4,5} = J_{5,6}$ 8, $J_{5,6a}$ 2 Hz, H-5A), and 3.78 (dd, $J_{5,6a}$ 2, $J_{6,6a}$ 11 Hz); ^{13}C-n.m.r. data (25.05 MHz, $CDCl_3$): δ 99.23 (C-1B), 98.25 (C-1C), 97.43 (C-1A), 77.10 (C-3A), 75.45 (C-2A), 74.23 (benzyl-CH_2 and C-5A), 70.18 and 69.94 (C-3B, C-3C, C-5B, and C-5C), 67.26 and 69.06 (C-4A,

C-2B, and C-2C), 67.26 (C-4B and C-4C), 66.38 (C-6A), 62.82 and 62.63 (C-6B and C-6C).

p-*Trifluoroacetamidophenyl O-(α-D-mannopyranosyl)-(1→3)-O-[α-D-mannopyranosyl)-(1→6)]-β-D-mannopyranoside* (**12**). — Compound **9** (0.47 g, 0.28 mmol) was dissolved in ethyl acetate (20 mL) and hydrogenated at room temperature and atmospheric pressure over Adams's catalyst (60 mg). When hydrogen consumption had ceased, trifluoroacetic anhydride (1 mL) and pyridine (2 mL), were added and the tightly stoppered flask was kept for 30 min at 60°. After cooling, methanol (1 mL) was added to decompose the excess of anhydride. The catalyst was removed by decantation and the supernatant solution was diluted with toluene (50 mL), washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated. T.l.c. (8:1 toluene–EtOAc) of the residue showed a major product at R_F 0.36 moving more slowly than the starting material (R_F 0.47). Purification on a column of silica gel (same solvent as before) gave 0.43 g (89%) of the intermediate trifluoroacetamide derivative **10** as an amorphous solid, $[\alpha]_{478}^{21}$ −59° (*c* 2.0, $CHCl_3$).

Compound **10** (0.41 g, 0.24 mmol) was then dissolved in methanol (25 mL), and a 0.2M solution of sodium methoxide in methanol (3 mL) was added. After being kept overnight at room temperature, sodium ions were removed from the mixture by treatment with Amberlite 1R-120 (H^+) resin, and the solution was evaporated. T.l.c. of the residue (40:25:1 $CHCl_3$–MeOH–H_2O) showed only one major component, R_F 0.20. Purification on a column of silica gel (same solvents as before gave 0.14 g (75%) of the debenzoylated derivative **11**, $[\alpha]_{378}^{21}$ +23° (*c* 1.67, CH_3OH). This product (0.14 g) was dissolved in 95% ethanol (10 mL) and hydrogenated at room temperature and atmospheric pressure over 10% palladium-on-charcoal (0.15 g). When hydrogen consumption had ceased (3 days), t.l.c. (3:2:1 EtOAc–2-propanol–H_2O) showed the presence of a major component at R_F 0.40. The catalyst was removed by filtration through Celite and the filtrate was evaporated. Chromatography of the residue on a column of silica gel (same solvents as before) afforded the title compound, 20 mg (55%), as amorphous solid, $[\alpha]_{378}^{21}$ +19° (*c* 1.4, CH_3OH); ^{1}H-n.m.r. (220 MHz, CD_3OH) showed *inter alia:* δ 7.53 and 7.08 (both d, 2 H each, $J_{H,H}$ 10 Hz, AB spectrum, *p*-$CF_3CONHC_6H_4$-group), 5.17 (s, 1 H, $J_{1,2}$ <1 Hz, H-1B), 5.10 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1A), 4.32 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1C), and 4.02 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.3 Hz, H-2A); ^{13}C-n.m.r. (25.05 MHz, CD_3OH): δ 103.96 (C-1B), 101.33 (C-1C), 99.42 (C-1A), 82.37 (C-3A), 76.66 (C-5A), 75.01 (C-5B), 74.28 (C-5C), 72.42 and 72.33 (C-3B and C-3C), 71.94 (C-2A, C-2B and C-2C), 68.77 and 68.88 (C-4B and C-4C), 67.21 (C-4A and C-6A), 62.97, and 62.63 (C-6B and C-6C).

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