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CHEMICAL SYNTHESIS OF THE HUMAN P^k-ANTIGENIC DETERMINANT*

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ABSTRACT

Condensation of 1,2,3,6-tetra-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-α-D-glucopyranose with 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride in 1,2-dichloroethane in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieve 4 Å gave 1,2,3,6-tetra-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranosyl]-α-D-glucopyranose. Catalytic hydrogenolysis and debenzoylation then gave 4-*O*-(4-*O*-β-D-galactopyranosyl-β-D-galactopyranosyl)-D-glucopyranose, the human blood-group P^k-antigenic determinant. A similar sequence of reaction was performed starting from 1,2,3,6-tetra-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranose.

INTRODUCTION

The human blood-group P system consists[1] of three antigens, P, P_1, and P^k. A trihexosylceramide was identified as the P^k antigen by hemagglutination inhibition[2] studies and by analysis of the glycosphingolipids from P^k erythrocytes[3]. The structure of the trisaccharide moiety of this glycoconjugate is 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-D-glucopyranose (**12**). This trihexosylceramide is also associated with Fabry's disease[4] and urinary-tract infection[5]. P^6 and P_7 determinants have been synthesized. We now report a chemical synthesis of the P^k determinant. Syntheses of methyl 4-*O*-(4-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-β-D-glucopyranoside have been reported by Cox *et al.*[8] and Garegg and Hultberg[9]. The Swedish group used 1,2,3,6,2′,3′,6′-hepta-*O*-benzoyl-α-D-lactose (**6**) as the key alcohol, prepared by selective benzoylation of the known[10] 1,2,3,6,2′,3′-hexa-*O*-benzoyl-α-D-lactose (**4**). On repeating the synthesis[10] of **4**, we realised that the structures proposed by these authors had to be questioned. Since **4** is a potential starting-material for the synthesis of various trisaccharides, we have reinvestigated the synthesis.

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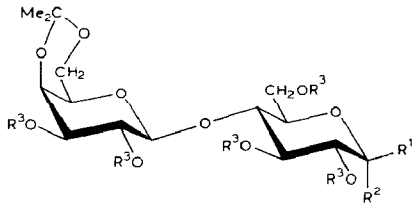
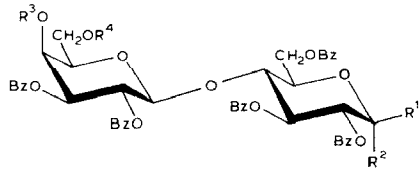
RESULTS AND DISCUSSION

Treatment of 4-*O*-(4,6-*O*-isopropylidene-β-D-galactopyranosyl)-D-glucopyranose[10] (**1**) with benzoyl chloride in pyridine afforded an α,β-mixture of hexabenzoates. Column chromatography on silica gel gave, first, the α-anomer **2** (55%), m.p. 235–236°, $[\alpha]_D$ +208° (chloroform). The α configuration was clear[11] from the n.m.r. signal for H-1 (δ 6.64, $J_{1,2}$ 3.5 Hz). Eluted second was the β-anomer **3** (33%), m.p. 236–237°, $[\alpha]_D$ +106° (chloroform). The β configuration was apparent from the n.m.r. signal for H-1 (δ 6.12, $J_{1,2}$ 7.5 Hz). Baer and Abbas[10] reported that benzoylation of **1** afforded, after four crystallisations from acetone–methanol, a hexabenzoate, m.p. 245–247°, $[\alpha]_D$ +104.8° (chloroform), and assigned the structure **2** on the basis of the spacing (3.5 Hz) of the H-1 doublet and the $[\alpha]_D$ value. Our results suggest that the anomeric configuration and the purity of the hexabenzoate reported by Baer and Abbas are doubtful.



1 R^1, R^2 = H,OH, R^3 = H
2 R^1 = H, R^2 = OBz, R^3 = Bz
3 R^1 = OBz, R^2 = H, R^3 = Bz



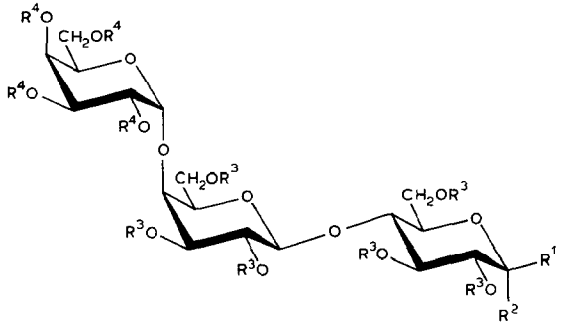
4 R^1 = R^3 = R^4 = H, R^2 = OBz
5 R^1 = OBz, R^2 = R^3 = R^4 = H
6 R^1 = R^3 = H, R^2 = OBz, R^4 = Bz
7 R^1 = OBz, R^2 = R^3 = H, R^4 = Bz

The synthesis of the P^k trisaccharide was then carried out on both **2** and **3**. Removal of the acetal group from **2** by treatment[10] with aqueous 90% trifluoroacetic acid afforded the diol **4**, m.p. 241–243°, $[\alpha]_D$ +147° (chloroform). Likewise, **3** gave the diol **5**, m.p. 223–225°, $[\alpha]_D$ +68° (chloroform). The anomeric configurations of **4** and **5** were indicated by the $J_{1,2}$ values for the H-1 doublets (**4**, 3.5 Hz; **5**, 7.5 Hz). The physical properties of **4** reported by Baer and Abbas[10] are at variance with our data and, indeed, are close to those for **5**. Compound **4** has been used in synthesis by Garegg and Hultberg[9], but no comment on the structure was made.

Selective esterification of **4** with benzoyl cyanide[12] in dichloromethane–pyridine gave the crystalline 6′-benzoate **6** (87%), which has also been prepared by another route[9]*. Likewise, selective esterification of **5** gave the crystalline 6′-benzoate **7** (88%). Condensation of **6** with freshly prepared 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[13] in 1,2-dichloroethane in the presence of 2,4,6-trimethylpyridine, silver triflate, and molecular sieve 4Å gave, after column

*Water was reported[9] as the solvent used for determination of the optical rotation This is erroneous; chloroform was the solvent used (P. J. Garegg, personal communication).

chromatography on silica gel, the pure but amorphous trisaccharide **8** (89%). A similar condensation in toluene was described by Garegg and Hultberg[9], but the product was not characterised. The α configuration of the new interglycosidic linkage in **8** was clear[11] from the ^{13}C-n.m.r. signal for C-1″ (101.51 p.p.m.). Catalytic hydrogenolysis of **8** gave the known[9] trisaccharide heptabenzoate **10**.



8 $R^1 = H, R^2 = OBz, R^3 = Bz, R^4 = Bn$
9 $R^1 = OBz, R^2 = H, R^3 = Bz, R^4 = Bn$
10 $R^1 = R^4 = H, R^2 = OBz, R^3 = Bz$
11 $R^1 = OBz, R^2 = R^4 = H, R^3 = Bz$
12 $R^1, R^2 = H, OH, R^3 = R^4 = H$

In a similar sequence of reactions, **7** was converted into the pure, amorphous trisaccharide **9** (83%) and thence into **11**.

Debenzoylation of **10** and **11** yielded the target compound **12** (80%) as an analytically pure, amorphous solid, the 300-MHz ^{1}H-n.m.r. spectrum of which was in full agreement with the structure assigned.

EXPERIMENTAL

General methods. — Melting points were determined in capillary tubes with a Büchi apparatus and are uncorrected. Optical rotations were measured at 20–22° with a Perkin–Elmer Model 141 polarimeter. ^{1}H-N.m.r. spectra were recorded with Perkin–Elmer R-32 (90 MHz) and Bruker AM-300 (300 MHz) instruments. ^{13}C-N.m.r. spectra were recorded at 22.63 MHz with a Bruker WH-90 instrument. Purity of products was determined by t.l.c. on Silica Gel 60F 154 (Merck) with detection by charring with sulphuric acid. Column chromatography was performed on Silica Gel 60 (Merck, 63–200 μm) which was used without pre-treatment. Elemental analyses were performed by the Service Central de Micro-Analyse du Centre National de la Recherche Scientifique (Vernaison, France).

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-4,6-*O*-isopropylidene-β-*D*-galactopyranosyl)-α-* (**2**) *and -β-*D*-glucopyranose* (**3**). — To a cooled solution of purified[10] **1** (500 mg) in pyridine (12 mL) was added, dropwise, benzoyl chloride (2

mL). The mixture was stirred for 1 h at room temperature and then for 3 h at 60°, and crushed ice (5 g) was added after cooling. The mixture was stirred for 1 h, diluted with dichloromethane (100 mL), washed with aqueous 10% potassium hydrogensulphate, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of silica gel (50 g) with dichloromethane–ethyl acetate (30:1) to give **2** (725 mg, 55%), m.p. 235–236° (from ethyl acetate–hexane), $[\alpha]_D$ +208° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 8.10–7.0 (m, 30 H, 6 Ph), 6.64 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.25 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9 Hz, H-3), 5.72 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2'), 5.43 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9 Hz, H-2), 5.03 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.5 Hz, H-3'), 4.84 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'), 3.47 (m, 2 H-6'a,6'b), 2.86 (m, 1 H, H-5'), 1.25 and 1.15 (2 s, 6 H, CMe_2).

Anal. Calc. for $C_{57}H_{50}O_{17}$: C, 67.98; H, 5.01. Found: C, 68.02; H, 5.03.

Further elution gave **3** (435 mg, 33%), m.p. 236–237° (from ethyl acetate–hexane), $[\alpha]_D$ +106° (*c* 1, chloroform). ^{1}H-N.m.r. data: δ 8.10–7.05 (m, 30 H, 6 Ph), 6.12 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 5.93 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9 Hz, H-3), 5.71 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10 Hz, H-2'), 5.61 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9 Hz, H-2), 5.03 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3.5 Hz, H-3'), 4.76 (d, 1 H, $J_{1,2}$ 8 Hz, H-1'), 3.48 (m, 2 H, H-6'a,6'b), 2.90 (m, 1 H, H-5'), 1.22 and 1.14 (2 s, 6 H, CMe_2).

Anal. Found: C, 68.11; H, 4.96.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-*D*-galactopyranosyl)-α-*D*-glucopyranose* (**4**). — A mixture of **2** (680 mg), trifluoroacetic acid (9 mL), and water (1 mL) was stirred at room temperature for 20 min and then concentrated *in vacuo*, and water (2 × 5 mL) was evaporated from the residue which was crystallised from dichloromethane–ether to give **4** (575 mg, 88%), m.p. 241–243°, $[\alpha]_D$ +147° (*c* 0.5, chloroform); lit.[10] m.p. 225–228° (from methanol–water), $[\alpha]_D$ +83.3° (chloroform). ^{1}H-N.m.r. data (90 MHz, pyridine-d_5–D_2O): δ 8.20–6.90 (m, 30 H, 6 Ph), 6.86 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 5.68 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10 Hz, H-2), 5.40 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3.5 Hz, H-3'), 5.22 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1').

Anal. Calc. for $C_{54}H_{46}O_{17}$: C, 67.07; H, 4.80. Found: C, 66.99; H, 4.74.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3-di-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranose* (**5**). — Compound **3** (600 mg) was treated as described above for **2**. Crystallisation of the product from ethyl acetate–hexane afforded **5** (489 mg, 85%), m.p. 223–225°, $[\alpha]_D$ +68° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, pyridine-d_5–D_2O): δ 8.20–7.0 (m, 30 H, 6 Ph), 6.46 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 5.46 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3 Hz, H-3'), 5.22 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1').

Anal. Calc. for $C_{54}H_{46}O_{17}$: C, 67.07; H, 4.80. Found: C, 67.09; H, 4.90.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-*D*-galactopyranosyl)-α-*D*-glucopyranose* (**6**). — A solution of **4** (578 mg) in dichloromethane (10 mL) and pyridine (1 mL) was stirred overnight at room temperature in the presence of benzoyl cyanide (130 mg). After destruction of the excess of benzoyl cyanide with methanol (5 mL), the mixture was concentrated, and the residue was eluted from a column of silica gel (30 g) with dichloromethane–ethyl acetate (20:1) to give **6**

(560 mg, 87%), m.p. 222–224° (from ethyl acetate–hexane), $[\alpha]_D$ +109° (*c* 1, chloroform); lit.[9] m.p. 196–197° (from ethanol), $[\alpha]_D$ +98° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 7.90–7.30 (m, 35 H, 7 Ph), 6.68 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.13 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9 Hz, H-3), 5.73 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10 Hz, H-2'), 5.54 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9 Hz, H-2), 5.12 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3 Hz, H-3'), 4.84 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 2.38 (1 H, OH).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.41; H, 4.71. Found: C, 68.23; H, 4.71.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranose* (**7**). — Compound **5** (460 mg) was treated as described for **4**. The residue was eluted from a column of silica gel (20 g) with dichloromethane–ethyl acetate (15:1) to give **7** (448 mg, 88%), m.p. 163–165° (from ethyl acetate–hexane), $[\alpha]_D$ +54° (*c* 1, chloroform). ^{1}H-N.m.r. data (90 MHz, $CDCl_3$): δ 8.05–7.05 (m, 35 H, 7 Ph), 6.16 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 5.93 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9 Hz, H-3), 5.86 (dd, 1 H, $J_{1,2}$ 7.5, $J_{2,3}$ 9 Hz, H-2), 5.79 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10 Hz, H-2'), 5.13 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3 Hz, H-3'), 4.78 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 2.52 (1 H, OH).

Anal. Calc. for $C_{61}H_{50}O_{18}$: C, 68.41; H, 4.71. Found: C, 68.35; H, 4.70.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-α-*D*-glucopyranose* (**8**). — A mixture of **6** (620 mg), freshly prepared 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[13] (500 mg), activated powdered 4 Å molecular sieve (500 mg), and 1,2-dichloroethane (10 mL) was stirred for 15 min at room temperature under dry argon, and then cooled to −20°. 2,4,6-Trimethylpyridine (0.18 mL) and freshly prepared silver triflate (300 mg) were added, and the mixture was stirred for 1 h at −20° and then allowed to attain room temperature overnight. The mixture was diluted with dichloromethane (50 mL), filtered, washed with water, cold 0.1M hydrochloric acid, saturated aqueous sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Elution of the residue from a column of silica gel (100 g) with toluene–ethyl acetate (13:1) gave amorphous **8** (821 mg, 89%), $[\alpha]_D$ +93° (*c* 1, chloroform). N.m.r. data: ^{1}H (300 MHz, $CDCl_3$), δ 8.10–7.0 (m, 55 H, 11 Ph), 6.73 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 6.24 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9 Hz, H-3), 5.79 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10 Hz, H-2'), 5.48 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 9 Hz, H-2), 5.03 (dd, 1 H, $J_{2',3'}$ 10, $J_{3',4'}$ 3 Hz, H-3'), 4.92 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'); ^{13}C, δ 101.84 and 101.51 (C-1' and C-1''), 89.94 (C-1).

Anal. Calc. for $C_{95}H_{84}O_{23}$: C, 71.60; H, 5.31. Found: C, 71.40; H, 5.24.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-galactopyranosyl)-β-*D*-galactopyranosyl]-β-*D*-glucopyranose* (**9**). — A mixture of **7** (300 mg), 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride (235 mg), activated powdered 4 Å molecular sieve (250 mg), and 1,2-dichloroethane (5 mL) was stirred for 15 min at room temperature under dry argon, and then cooled to −20°. 2,4,6-Trimethylpyridine (0.083 mL) and freshly prepared silver triflate (135 mg) were added, and the mixture was stirred for 1 h at −20° and allowed to attain room temperature overnight. The mixture was worked up as described for

the preparation of **8**. Elution of the residue from a column of silica gel (45 g) with toluene–ethyl acetate (13:1) gave amorphous **9** (370 mg, 83%), $[\alpha]_D$ +48° (*c* 1, chloroform). N.m.r. data: ^{1}H (300 MHz, $CDCl_3$), δ 8.10–7.0 (m, 55 H, 11 Ph), 6.13 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.92 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9 Hz, H-3), 5.76 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10 Hz, H-2'), 5.68 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9 Hz, H-2), 5.03 (dd, $J_{2',3'}$ 10, $J_{3',4'}$ 3.5 Hz, H-3'), 4.86 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'); ^{13}C, δ 101.38 (C-1' and C-1''), 92.54 (C-1).

Anal. Calc. for $C_{95}H_{84}O_{23}$: C, 71.60; H, 5.31. Found: C, 71.87; H, 5.19.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranosyl)-α-*D*-glucopyranose* (**10**). — A solution of **8** (235 mg) in acetic acid (10 mL) was hydrogenolysed in the presence of 10% Pd/C (200 mg) for 2 days, filtered, and concentrated. The residue was eluted from a column of silica gel (12 g) with ethyl acetate to give amorphous **10** (138 mg, 76%), $[\alpha]_D$ +124° (*c* 1, chloroform); lit.[9] $[\alpha]_D$ +124° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$ + D_2O): δ 8.10–7.30 (m, 35 H, 7 Ph), 6.76 (d, 1 H, $J_{1,2}$ 4 Hz, H-1), 6.19 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 9 Hz, H-3), 5.87 (dd, 1 H, $J_{1',2'}$ 8, $J_{2',3'}$ 10.5 Hz, H-2'), 5.63 (dd, 1 H, $J_{1,2}$ 4, $J_{2,3}$ 10 Hz, H-2), 5.18 (dd, 1 H, $J_{2',3'}$ 10.5, $J_{3',4'}$ 3.2 Hz, H-3'), 4.95 (d, 1 H, $J_{1'',2''}$ 3.6 Hz, H-1''), 4.93 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1'), 3.35 (dd, 1 H, $J_{5'',6''a}$ 4, $J_{6''a,6''b}$ 12 Hz, H-6''a), 3.26 (dd, 1 H, $J_{5'',6''b}$ 5.6, $J_{6''a,6''b}$ 12 Hz, H-6''b).

Anal. Calc. for $C_{67}H_{60}O_{23} \cdot H_2O$: C, 64.31; H, 4.99. Found: C, 64.32; H, 5.01.

*1,2,3,6-Tetra-*O*-benzoyl-4-*O*-(2,3,6-tri-*O*-benzoyl-4-*O*-α-*D*-galactopyranosyl-β-*D*-galactopyranosyl)-β-*D*-glucopyranose* (**11**). — A solution of **9** (168 mg) in acetic acid (10 mL) was hydrogenolysed in the presence of 10% Pd/C (150 mg) for 2 days, filtered, and concentrated. The residue was eluted from a column of silica gel (8 g) with ethyl acetate to give amorphous **11** (102 mg, 78%), $[\alpha]_D$ +71° (*c* 1, chloroform). ^{1}H-N.m.r. data (300 MHz, $CDCl_3$ + D_2O): δ 8.10–7.20 (m, 35 H, 7 Ph), 6.20 (d, 1 H, $J_{1,2}$ 8 Hz, H-1), 5.91 (t, 1 H, $J_{2,3}$ and $J_{3,4}$ 9.2 Hz, H-3), 5.75 (dd, 1 H, $J_{1,2}$ 8, $J_{2,3}$ 9.2 Hz, H-2), 5.73 (dd, 1 H, $J_{1',2'}$ 7.5, $J_{2',3'}$ 10.6 Hz, H-2'), 5.17 (dd, 1 H, $J_{2',3'}$ 10.6, $J_{3',4'}$ 3 Hz, H-3'), 4.91 (d, 1 H, $J_{1'',2''}$ 3.1 Hz, H-1''), 4.83 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1'), 4.30 (t, 1 H, $J_{3,4}$ and $J_{4,5}$ 9.2 Hz, H-4), 4.27 (dd, 1 H, $J_{3',4'}$ 3, $J_{4',5'}$ 1 Hz, H-4'), 3.35 (dd, 1 H, $J_{5'',6''a}$ 4.2, $J_{6''a,6''b}$ 12.30 Hz, H-6''a), 3.27 (dd, 1 H, $J_{5'',6''b}$ 5.7, $J_{6''a,6''b}$ 12.30 Hz, H-6''b).

Anal. Calc. for $C_{67}H_{60}O_{23} \cdot H_2O$: C, 64.31; H, 4.99. Found: C, 64.58; H, 5.05.

O-α-D-Galactopyranosyl-(1→4)-O-β-D-galactopyranosyl-(1→4)-D-glucopyranose (**12**). — A solution of **10** (160 mg) in dry methanol (8 mL) was treated with methanolic M sodium methoxide (0.5 mL) for 4 h at 0°, de-ionised with Amberlite IR-120 (H^+) resin, filtered, and concentrated, and water (5 × 5 mL) was evaporated from the residue. The residue was then eluted from a column (20 × 960 mm) of Sephadex G-25 (medium) with water, and the eluate was freeze-dried to give amorphous **12** (59 mg, 90%), $[\alpha]_D$ +101° (*c* 0.5, water). ^{1}H-N.m.r. data (300 MHz, D_2O, internal TSP): δ 5.23 (d, $J_{1,2}$ 3.60 Hz, H-1α), 4.96 (d, 1 H, $J_{1'',2''}$ 3.20

Hz, H-1″), 4.67 (d, $J_{1,2}$ 8.0 Hz, H-1β), 4.52 (d, 1 H, $J_{1',2'}$ 7.20 Hz, H-1′), 4.36 (m, 1 H, $J_{4'',5''}$ 0.8, $J_{5'',6''a}$ and $J_{5'',6''b}$ 4.40 Hz, H-5″), 3.28 (t, $J_{1,2}$ and $J_{2,3}$ 8.0 Hz, H-2β).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39; O, 50.75. Found: C, 42.70; H, 6.40; O, 50.88.

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