Carbohydrate Research, 216 (1991) 227–235
Elsevier Science Publishers B.V., Amsterdam

Synthesis of 2-deoxy-4-*O*-α- and -β-D-mannopyranosyl-L-*erythro*-pentonic acids*,†

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(Received December 20th, 1990; accepted for publication January 28th, 1991)

ABSTRACT

The title compounds, required for the identification of structural features of the *Bordetella pertussis* endotoxin, have been synthesised by condensation of benzyl 3-*O*-benzyl-2-deoxy-β-L-*erythro*-pentopyranoside with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide and with 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide, respectively, thus affording the fully protected α- and β-linked disaccharides **7** and **12**. Hypoiodite oxidation of the reducing disaccharides **9** and **14**, obtained by conventional deprotection of **7** and **12**, yielded the title compounds.

INTRODUCTION

Endotoxic lipopolysaccharides are major constituents of the outer membrane of Gram-negative bacterial cells. The hydrophilic (side chain + core) and hydrophobic ("Lipid A") domains of these macromolecules (2–20 kDa) are joined by the glycosidic bonds of 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo). In lipopolysaccharides of many Enterobacterial endotoxin preparations, O-5 of one of these Kdo units is substituted by L-*glycero*-α-D-*manno*-heptopyranose. For *S. minnesota* (Family *Enterobacteriaceae*) R-type mutants, the α-anomeric configuration of this heptose has been demonstrated by its cleavage with jack-bean α-D-mannosidase[2], and, for *Proteus mirabilis* R-type mutants[3], by its resistance to treatment with CrO_3 (which destroys β-pyranosides[4,5]). The α configuration has been assigned[6] to the L-rhamnose (6-deoxy-L-mannose) substituent of Kdo[7,8] in lipopolysaccharides of endotoxin preparations of certain *E. coli* K-12 strains because, in the n.m.r. spectrum, $J_{C\text{-}1,H\text{-}1}$ was 174.08 Hz[9]. In lipopolysaccharides that make up the *Bordetella pertussis* (Family *Pseudomonadaceae*) strain 1414, 100, and 134 (ref. 10) endotoxin preparations, the glycose sequence proximal to the hydrophobic domain was shown to be the following hexasaccharide[11,‡].

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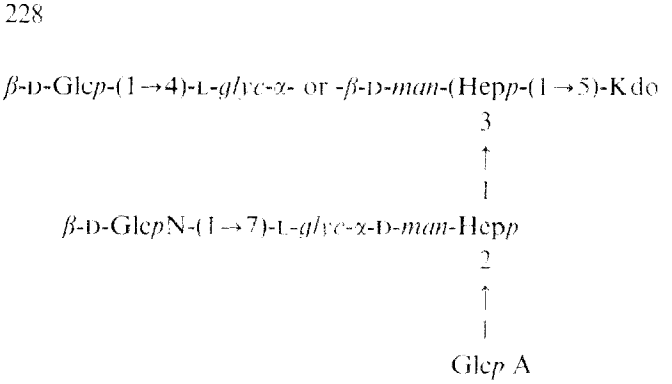
* Dedicated to Professor Grant Buchanan on the occasion of his 65th birthday.

† Chemistry of Bacterial Endotoxins, Part 7. For Part 6, see ref. 1.

‡ The tentative structure, compiled[12] from oligosaccharide fragments obtained by acid hydrolysis, proved to be in error.



However, it was not feasible to determine by n.m.r. spectroscopy whether the L-*glycero*-D-*manno*-heptopyranose linked to Kdo was α or β because of overlapping signals in the $^{13}C-^{1}H$-coupled spectrum. In the ^{1}H-n.m.r. spectrum of the hexasaccharide, δ_{H-1} for one of the heptoses was at 5.08 p.p.m., with the other at 5.54 p.p.m. In the lipopolysaccharides of the *B. pertussis* endotoxin preparations, the heptose residue attached to Kdo is substituted in positions 3 and 4; hence, it affords mannose when treated sequentially with periodate and borohydride. Under the same conditions, the terminal Kdo unit is transformed into 2-deoxy-L-*erythro*-pentonic acid. It follows that one of the title compounds should be present amongst the fragments produced by Smith degradation of the isolated polysaccharide chains. Accordingly, these disaccharides were synthesised. Comparison of the δ_{H-1} values for the disaccharide isolated from the *B. pertussis* endotoxin (δ_{H-1} 5.05) (ref. 13) and for the synthetic compounds described herein [δ_{H-1}: α anomer, 5.06 p.p.m. ($J_{C-1,H-1}$ 171.2 Hz); β anomer, 4.81 p.p.m. ($J_{C-1,H-1}$ 159.5 Hz)] establishes that the natural product is the α anomer.

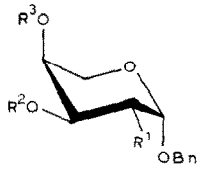
In many lipopolysaccharides, O-3 of the heptose residue attached to O-5 of Kdo is substituted. Consequently, Smith degradation of the isolated polysaccharide chains can be expected to produce one of the title disaccharides. The δ_{H-1} value, easily determined with very small amounts of material, of the mannosyl-pentonic acid present should allow unequivocal identification of the chirality of the anomeric centre of the corresponding heptose residue. At "ambient" temperatures, the signal for H-1′ of the β-D-mannose residue practically coincides with that of HDO, but the two signals are well separated at 303 K.

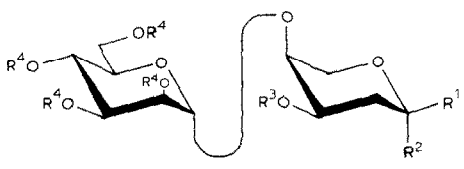
RESULTS AND DISCUSSION

It was considered that benzyl 3-*O*-benzyl-2-deoxy-β-L-*erythro*-pentopyranoside (**5**) would be a suitable aglycon for condensation with an appropriate mannosyl derivative, since, after deprotection of the mannosyl moiety of the resulting fully protected α- and β-linked disaccharides and debenzylation of the deoxypentopyranoside moiety, the reducing disaccharides thus obtained should be easily oxidised to give the title compounds. Consequently, compound **5** was synthesised as follows. Benzyl 4,6-*O*-isopropylidene-β-L-arabinopyranoside (**1**), prepared by a route different from that described[14], was deoxygenated by treatment with tributyltin hydride[15] of its 2-

phenylthiocarbonyl[16] ester **2**. Removal of the isopropylidene group from the resulting 2-deoxypentopyranoside **3** afforded benzyl 2-deoxy-β-L-*erythro*-pentopyranoside (**4**). It was expected that benzylation of the stannylene derivative of **4** under the conditions described by David *et al.*[17] would lead regiospecifically to the equatorial 3-*O*-benzyl derivative, but a mixture (> 90% yield) of the 3- and 4-benzyl ethers **5** and **6** was actually obtained. These compounds were separated by chromatography and identified from the ^{1}H-n.m.r. spectra of their acetylated derivatives. In refluxing toluene, benzylation was relatively rapid, and the ratio of 3- to 4-benzyl ether formed was ~ 1.1:1. Reducing the temperature of the reaction by performing it in boiling benzene slowed it down by a factor of ~ 2, and only increased this ratio to ~ 1.5:1. Condensation of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[18] with the 3-*O*-benzyl derivative **5** in the presence of silver trifluoromethanesulphonate and tetramethylurea[19,20] gave exclusively the fully protected α-disaccharide **7**. No β derivative could be detected in the isolated (50%) compound. Saponification of the benzoyl groups afforded the benzyl 3-*O*-benzyl disaccharide **8** from which the unprotected disaccharide **9** was obtained by hydrogenolysis. Oxidation of **9** by the classical hypoiodite method (*cf.* ref. 21) afforded the α-D-mannosyl acid **10**.

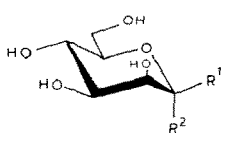
The corresponding β-D-mannosyl acid **15** was prepared using a more recent modification[22] of a relatively old method[23] in which 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide[22] (**11**) was condensed with the 3-*O*-benzyl derivative **5** in

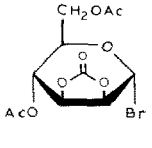


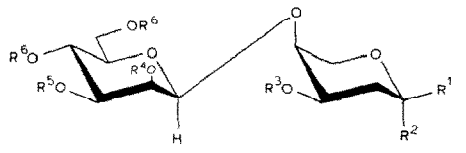


1 $R^1 = OH, R^2, R^3 = -C(Me)_2$
2 $R^1 = -OC(S)OPh, R^2, R^3 = -C(Me)_2$
3 $R^1 = H, R^2, R^3 = -C(Me)_2$
4 $R^1 = R^2 = R^3 = H$
5 $R^1 = R^3 = H, R^2 = Bn$
6 $R^1 = R^2 = H, R^3 = Bn$

7 $R^1 = H, R^2 = OBn, R^3 = Bn, R^4 = Bz$
8 $R^1 = H, R^2 = OBn, R^3 = Bn, R^4 = H$
9 $R^1, R^2 = H, OH, R^3 = R^4 = H$



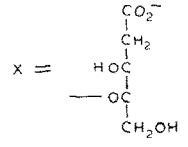




10 $R^1 = H, R^2 = X$
15 $R^1 = X, R^2 = H$

11

12 $R^1 = H, R^2 = OBn, R^3 = Bn, R^4, R^5 = CO, R^6 = Ac$
13 $R^1 = H, R^2 = OBn, R^3 = Bn, R^4 = R^5 = R^6 = H$
14 $R^1, R^2 = H, OH, R^3 = R^4 = R^5 = R^6 = H$



the presence of silver oxide. Removal of the acetyl and carbonyl groups from the fully protected β-disaccharide **12** gave the β analogue **13** (δ_{H-1} 4.65) of **8** (δ_{H-1} 4.97). The unprotected β-disaccharide **14** (δ_{H-1} 4.67) was obtained from **13** in the same way as the α-disaccharide **9** (δ_{H-1} 4.87) was prepared from **8**, and oxidation of **14** to the β-D-mannosyl acid **15** (δ_{H-1} 4.81) was performed by the method used for the conversion of **9** into **10** (δ_{H-1} 5.06).

EXPERIMENTAL

Evaporations were carried out under reduced pressure at 40°. M.p.s were determined on a Kofler hot-plate and are uncorrected. Optical rotations were determined with a Perkin–Elmer model 141 polarimeter. Except where mentioned, all ^{1}H-n.m.r. spectra were recorded on a Bruker A.C. 200 spectrometer, using tetramethylsilane as internal standard. Detailed assignments of the resonances of the fully protected disaccharides **7** and **12** were obtained by COSY and COSY-DMQ techniques, respectively. T.l.c. was performed on silica gel (60 F_{254} on aluminium foil, Merck); all compounds were located by spraying with sulphuric acid and heating on a hot plate. Column chromatography was performed on silica gel (Merck 60, 70–230 mesh).

Benzyl 4,6-O-isopropylidene-β-L-arabinopyranoside (**1**). — 2-Methoxypropene (11.25 mL) was added dropwise to a cooled (10°), stirred mixture of benzyl β-L-arabinopyranoside[14] (13.5 g), 1,4-dioxane (200 mL), anhydrous *p*-toluenesulphonic acid (845 mg), and anhydrous copper sulphate (3 g), in an atmosphere of argon, and the mixture was allowed to attain room temperature. When t.l.c. (1:1 cyclohexane–EtOAc) showed the absence of starting material, the mixture was diluted with ether (1.2 L) and filtered. The solution was washed with cold, saturated aqueous $NaHCO_3$, then with a cold, saturated solution of NaCl, and dried (Na_2SO_4). Solvents were removed, leaving a thick syrup (14.7 g) which was sufficiently pure for the following step. [The syrup obtained from a small-scale preparation (2.4 g of benzyl glycoside) was purified by column chromatography (21 × 4.5 cm) (solvent as for t.l.c.) to give crystalline **1** (2.16 g, 77%), m.p. 57–58° (lit.[14] m.p. 59–59.5°)].

Benzyl 3,4-O-isopropylidene-2-O-phenylthiocarbonyl-β-L-arabinopyranoside (**2**). — Phenyl chlorothionocarbonate (11 mL) was added dropwise to a stirred solution of the above syrup (14.7 g) in freshly distilled (P_2O_5) CH_2Cl_2 (650 mL) and anhydrous pyridine (87 mL) cooled to 0° under argon. The mixture was allowed to attain room temperature and left overnight, when t.l.c. (1:2 EtOAc–cyclohexane) showed the reaction to be complete. The mixture was diluted with CH_2Cl_2 (1 L), washed with cold M HCl, cold, saturated aqueous $NaHCO_3$, ice–water, and saturated aqueous NaCl, and dried (Na_2SO_4). Solvents were removed and the residue was purified by column chromatography (43 × 8.2 cm) (solvent as for t.l.c.). Combined impure fractions were repurified on another column (30 × 3 cm). Solvents were removed to give **2** as a hard oil (18.5 g, 85%), $[\alpha]_D$ +197° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 0.79 and 0.99 (2 s, each 3 H, CH_3), 3.50 (d, 2 H, H-5a,5b), 3.725 (br s, 1 H, $J_{4,3}$ 6, $J_{4,5a} = J_{4,5b}$ = 1–2 Hz, H-4), 3.975 (dd, 2 H, *J* 12.5 Hz, 1 H of CH_2Ph and $J_{3,2}$ 8, $J_{3,4}$ 6 Hz, H-3), 4.20 (d, 1 H, *J* 12.5 Hz, CH_2Ph), 4.65 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 4.85 (dd, 1 H, H-2), 6.45–6.85 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{22}H_{24}O_6S$ (416.5): C, 63.44; H, 5.81. Found: C, 63.33; H, 5.93.

*Benzyl 2-deoxy-3,4-O-isopropylidene-β-*L-erythro-*pentopyranoside* (**3**). — Tributyltin hydride (10.8 mL) was added to a solution (obtained by sonication) of 2,2-azobis-(isobutyronitrile) (AIBN) (1.1 g) in anhydrous toluene (70 mL), and the mixture was added in an atmosphere of argon to a solution of syrupy **2** (5 g) in toluene (250 mL) which was stirred and heated in an oil bath at 120°. When t.l.c. (1:2 EtOAc–cyclohexane) showed the reaction to be complete (~ 1 h), the solution was cooled and solvents were removed. The residue was extracted with hot CH_3CN (3 × 150 mL). The combined, cooled extracts were washed with hexane (4 × 150 mL), and the hexane washings were extracted with CH_3CN (50 mL). The combined CH_3CN extracts were concentrated to dryness, to give **3** as an oil (3.9 g). A sample, purified by column chromatography (solvent as for t.l.c.), had $[\alpha]_D$ +111° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.36 and 1.525 (2 s, each 3 H, 2 CH_3), 1.875 (ddd, 1 H, $J_{2a,1}$ 5, $J_{2a,3}$ 6, $J_{2a,2b}$ 14 Hz, H-2a), 2.175 (ddd, 1 H, $J_{2b,1} = J_{2b,3}$ = 5, $J_{2a,2b}$ 14 Hz, H-2b), 3.76 (dd, 1 H, $J_{5a,4}$ 2, $J_{5a,5b}$ 12 Hz, H-5a), 3.925 (dd, 1 H, $J_{5b,4}$ 2.5 Hz, H-5b), 4.16 (m, 1 H, H-4), 4.47 (m, 1 H, H-3), 4.50 and 4.79 (2 d, each 1 H, *J* 12 Hz, C*H*$_2$Ph), 4.99 (dd, 1 H, H-1), 7.14–7.37 (m, 5 H, Ph).

Anal. Calc. for $C_{15}H_{20}O_4$ (264.325): C, 68.16; H, 7.63. Found: C, 68.05; H, 7.45.

*Benzyl 2-deoxy-β-*L-erythro-*pentopyranoside* (**4**). — The preceding syrup (**3**, ~ 3.9 g) was dissolved in a 0.5M solution of trifluoroacetic acid in 8:2 CH_3OH–H_2O, and the mixture was kept at room temperature. When t.l.c. (1:1 EtOAc–cyclohexane, then EtOAc) showed hydrolysis to be complete, the mixture was poured onto a stirred excess of dry Amberlite IR-45 (HO^-) resin. When the mixture was neutral, the resin was filtered off and washed with 8:2 CH_3OH–H_2O. The filtrate was concentrated to dryness and the residue was purified by column chromatography (40 × 3 cm) (EtOAc). Crystalline **4** (1.35 g) (EtOAc) had m.p. 104–105°, $[\alpha]_D$ +171° (*c* 1, chloroform); lit.[24] for the enantiomer, m.p. 106–107°, $[\alpha]_D$ −174° (chloroform).

Anal. Calc. for $C_{12}H_{16}O_4$ (224.26): C, 64.27; H, 7.19. Found: C, 64.22; H, 7.26.

*Benzyl 3-*O- *and 4-*O-*benzyl-2-deoxy-β-*L-erythro-*pentopyranosides* (**5** *and* **6**). — (*a*) A mixture of **4** (0.9 g, 4.02 mmol) and dibutyltin oxide (1 g, 4.02 mmol) in anhydrous toluene (120 mL) was boiled under reflux for 16 h with continuous removal of water. The resulting solution was concentrated (to ~ 100 mL), tetrabutylammonium bromide (1.295 g, 4.02 mmol) and benzyl bromide (1 mL, 8.44 mmol, 2.1 mol. equiv.) were added, and the mixture was boiled under reflux for 4 h, after which time t.l.c. (1:1 EtOAc–cyclohexane) showed the reaction to be complete. The solution was cooled and concentrated to dryness, and the residue was submitted to column chromatography (33 × 2 cm) (solvents as for t.l.c.). The 4-*O*-benzyl derivative **6** (0.59 g, 47%), which was eluted first, had m.p. 108–109°, $[\alpha]_D$ +141° (*c* 1, chloroform). ^{1}H-N.m.r. data: ($CDCl_3$): δ 1.9 (m, 2 H, H-2a,2b), 2.21 (d, 1 H, *J* 8 Hz, HO-3), 3.57 (m, 1 H, H-4), 3.78 (m, 2 H, H-5a,5b), 4.08 (m, 1 H, H-3), 4.57 (m, 4 H, 2 C*H*$_2$Ph), 4.935 (t, 1 H, H-1), 7.27 and 7.30 (2 m, 10 H, 2 Ph).

Anal. Calc. for $C_{19}H_{22}O_4$ (314.385): C, 72.59; H, 7.05. Found: C, 72.45; H, 6.91.

^{1}H-N.m.r. data ($CDCl_3$) of acetylated (acetic anhydride–pyridine, 40°, 6 h) **6**: δ

1.80 (m, 1 H, H-2a), 1.96 (s, 3 H, Ac), 2.2 (m, 1 H, H-2b), 3.64 (m, 1 H, H-4), 3.73 (m, 2 H, H-5a,5b), 4.52 (m, 4 H, 2 CH_2Ph), 4.96 (t, 1 H, H-1), 5.22 (m, 1 H, H-3), 7.25 (m, 10 H, 2 Ph).

Eluted second was the 3-*O*-benzyl derivative **5** (0.66 g, 52%), m.p. 74–75°, $[\alpha]_D$ +124° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.96 (m, 2 H, H-2a,2b), 2.34 (s, 1 H, OH), 3.78 (m, 2 H, H-5a,5b), 3.88 (m, 2 H, H-3,4), 4.53 (m, 4 H, 2 CH_2Ph), 4.96 (t, 1 H, H-1), 7.26 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{19}H_{22}O_4$ (314.385): C, 72.59; H, 7.05. Found: C, 72.57; H, 6.98.

^{1}H-N.m.r. data ($CDCl_3$) of acetylated **5**: δ 1.9–2.1 (m, 2 H, H-2a,2b), 2.08 (s, 3 H, Ac), 3.74 (m, 2 H, H-5a,5b), 4.04 (m, 1 H, H-3), 4.50 (m, 4 H, 2 CH_2Ph), 4.99 (s, 1 H, H-1), 5.26 (s, 1 H, H-4), 7.23 and 7.26 (2 s, 10 H, 2 Ph). Ratio **5**:**6** ~ 1.1:1.

(*b*) The reaction described in (*a*) was repeated with the benzyl glycoside **4** (1.33 g) and the corresponding molar proportions of reactants, but the solvent used was anhydrous benzene. The time needed for complete reaction was ~ 9 h, and **6** (0.644 g, 34.6%) and **5** (1.09 g, 58.6%) were then isolated as in (*a*). Ratio **5**:**6** ~ 1.5:1.

Benzyl 3-O-benzyl-2-deoxy-4-O-(2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-β-L-erythro-pentopyranoside (**7**). — A solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide[18] (3.82 g, 5.8 mmol, 2 mol. equiv.) in freshly distilled (P_2O_5) CH_2ClCH_2Cl (20 mL) was added dropwise, in an atmosphere of argon, over a period of 2 h to a mixture of the 3-benzyl derivative **5** (0.91 g, 3 mmol), tetramethylurea (1.04 mL, 8.7 mmol, 3 mol. equiv.), and anhydrous silver trifluoromethanesulphonate (1.47 g, 5.72 mmol, 2 mol. equiv.) in CH_2ClCH_2Cl (15 mL), which was stirred under argon in an oil bath at 40° until t.l.c. (1:2 EtOAc–cyclohexane) showed the reaction to be complete (~ 16 h). Pyridine (2.5 mL) was added to the cooled mixture, which was then diluted with CH_2Cl_2 (30 mL) and filtered through a bed (7 × 9 cm) of silica gel. This was washed with 1:1 EtOAc–cyclohexane (300 mL), and the eluate was concentrated to dryness. The residue was submitted to column chromatography (29 × 3.4 cm) (10:1 toluene–ether). Impure fractions were concentrated and re-chromatographed on the same column. Pure **7** (1.314 g, 50.8%), after being lyophilised from benzene, had $[\alpha]_D$ +51° (*c* 0.5, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.13 (ddd, 1 H, $J_{2a,1}$ 1.5, $J_{2a,3}$ 3.75, $J_{2a,2b}$ 12 Hz, H-2a), 2.33 (ddd, 1 H, $J_{2b,1}$ 3, $J_{2b,3}$ 12 Hz, H-2b), 3.78 and 3.87 (m, 2 H, H-5a,5b), 3.93 (dd, 1 H, H-6'a), 4.11 (m, 1 H, H-3), 4.16 (bs, 1 H, H-4), 4.49 (dd, 1 H, H-6'b), 4.5–4.8 (m, 4 H, 2 CH_2Ph), 4.77 (m, 1 H, H-5'), 5.13 (s, 1 H, H-1), 5.27 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'), 5.66 (dd, 1 H, $J_{2',3'}$ 3 Hz, H-2'), 5.97 (dd, 1 H, $J_{3',4'}$ 10 Hz, H-3'), 6.14 (t, 1 H, $J_{4',5'}$ 10 Hz, H-4'), 7.2–8.1 (m, 30 H, 6 Ph).

Anal. Calc. for $C_{53}H_{48}O_{13}$ (892.967): C, 71.29; H, 5.42. Found: C, 71.15; H, 5.58.

Benzyl 3-O-benzyl-2-deoxy-4-O-α-D-mannopyranosyl-β-L-erythro-pentopyranoside (**8**). — A M solution of NaOMe in anhydrous MeOH (4 mL) was added to a stirred suspension of **7** (890 mg) in MeOH (36 mL). When t.l.c. (8:2 $CHCl_3$–MeOH) showed that transesterification was complete, the solution was neutralised with Amberlite IRN-77 (H^+) resin. The resin was filtered off, the filtrate was concentrated to dryness, and the amorphous disaccharide **8** (422 mg, 88.9%) was triturated with several lots of ether, recovered by centrifugation, and dried. It had $[\alpha]_D$ +139° (*c* 1, chloroform).

^{1}H-N.m.r. data ($CDCl_3$): δ 1.85 (m, 1 H, H-2a); 2.075 (m, 1 H, H-2b), 3.77 (m, 10 H, H-3,4,5a,5b and H-2′,3′,4′,5′,6′a,6′b), 4.45 (m, 4 H, 2 CH_2Ph), 4.92 (s, 1 H, H-1); 4.97 (s, 1 H, H-1′), 7.22 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{25}H_{32}O_9 \cdot H_2O$ (494.539): C, 60.72; H, 6.93. Found: C, 60.61; H, 6.91.

*2-Deoxy-4-*O*-α-*D*-mannopyranosyl-*L*-erythro-pentopyranose* (**9**). — A solution of **8** (409 mg) in MeOH was hydrogenated in the presence of 10% Pd/C until t.l.c. (6.5:4.5:0.4 $CHCl_3$–MeOH–H_2O) showed the removal of the benzyl groups to be complete. The catalyst was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in H_2O, the solution was treated with active charcoal and filtered, and solvents were removed from the filtrate to give the disaccharide **9** (219 mg, 86%), $[\alpha]_D$ +103° (equilibrium) (*c* 1, water). ^{1}H-N.m.r. data (D_2O): δ 1.75 (m, 1 H, H-2a), 2.02 (m, 1 H, H-2b), 3.44–4.22 (m, 10 H, H-3,4,5a,5b and H-2′,3′,4′,5′,6′a,6′b), 4.86 and 5.29 (dd and t, 1 H, H-1α and H-1β), 5.02 (s, 1 H, H-1′).

Anal. Calc. for $C_{11}H_{20}O_9 \cdot H_2O$ (314.297): C, 42.04; H, 7.05. Found: C, 42.04; H, 7.15.

*2-Deoxy-4-*O*-α-*D*-mannopyranosyl-*L*-erythro-pentonic acid* (**10**). — To a stirred solution of **9** (150 mg, 0.5 mmol) in H_2O (19 mL) were added a solution of 0.05M I_2 in 0.25M KI (2 mL) then, dropwise, 0.1M NaOH (3 mL) until a total of 11 mL of the former and 16.5 mL of the latter had been added, the addition being carried out within 10 min. The mixture was stirred for a further 5 min, cooled in ice, and passed slowly through a column of cold Amberlite IRN-77 (H^+) resin (12 mL) into a stirred slurry of freshly prepared Ag_2CO_3 (0.9 g) in an ice bath. Solids were filtered off and the cold solution was passed slowly through another cold column of the same resin (7 mL). The eluate and washings were neutralised (pH meter) to pH 7 with a saturated solution of $Ba(OH)_2$ and concentrated to a very small volume. The oily residue solidified when triturated with ethanol. The barium salt of **10** (167 mg, 81%) was recovered by centrifugation, washed twice with ethanol, and dried. A decationised sample was pure in t.l.c. (5:3:1:1 1-propanol–conc. NH_4OH–H_2O–AcOH), and had $[\alpha]_D$ +50° (*c* 1, water). ^{1}H-N.m.r. data (250 MHz, D_2O): δ 2.32 (dd, 1 H, $J_{2a,2b}$ 15, $J_{2a,3}$ 4.5 Hz, H-2a), 2.52 (dd, 1 H, $J_{2b,3}$ 9 Hz, H-2b), 3.57–3.88 (m, 8 H, H-4,5a,5b and H-3′,4′,5′,6′a,6′b), 3.97 (m, 1 H, H-2′), 4.17 (m, 1 H, $J_{3,4}$ 9 Hz, H-3), 5.06 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1′; $J_{C\text{-}1',H\text{-}1'}$ 171.19 Hz).

Anal. Calc. for $C_{11}H_{19}Ba_{0.5}O_{10} \cdot 1.5H_2O$ (406.952): C, 32.47; H, 5.45. Found: C, 32.65; H, 5.81.

*Benzyl 3-*O*-benzyl-2-deoxy-4-*O*-(4,6-di-*O*-acetyl-2,3-*O*-carbonyl-β-*D*-mannopyranosyl)-*L*-erythro-pentopyranoside* (**12**). — A solution of the 3-benzyl derivative **5** (0.488 g, 1.554 mmol) in dry, alcohol-free $CHCl_3$ (4 mL) was stirred in the dark for 1 h at room temperature with dry, freshly prepared Ag_2O (1 g) and anhydrous $CaSO_4$ (1.4 g). A solution of 4,6-di-*O*-acetyl-2,3-*O*-carbonyl-α-D-mannopyranosyl bromide[22] (**11**; 1.244 g, 3.5 mmol) in dry, alcohol-free $CHCl_3$ (6 mL) was added dropwise over a period of 3 h. Stirring was continued for a further 0.5 h, when t.l.c. (1:1 EtOAc–cyclohexane) showed that very little starting aglycon remained. The mixture was filtered and the filtrate was concentrated to a thick syrup which was purified on a column (33.5 × 4 cm)

of silica gel (170 g) (same solvent) to give **12** (0.49 g, 53%) as a semi-solid oil, $[\alpha]_D$ +34° (*c* 1.6, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 2.04 and 2.10 (2 s, 6 H, 2 Ac), 2.03 and 2.19 (m, 2 H, H-2a,2b), 3.82 (m, 1 H, H-5'), 3.87–4.07 (m, 4 H, H-3,4,5a,5b), 4.12 (m, 1 H, H-6'a), 4.32 (dd, 1 H, H-6'b), 4.42–4.72 (m, 4 H, 2 C*H*$_2$Ph), 4.77 (m, 2 H, H-2',3'), 5.05 (t, 1 H, $J_{1,2a} = J_{1,2b} = 2.3$ Hz, H-1), 5.425 (d, 1 H, $J_{1',2'}$ 2.3 Hz, H-1'), 5.74 (dd, 1 H, $J_{3',4'}$ 3, $J_{4',5'}$ 6.5 Hz, H-4'), 7.3 (m, 10 H, 2 CH$_2$*Ph*).

Anal. Calc. for $C_{30}H_{34}O_{12}$ (586.593): C, 61.43; H, 5.84. Found: C, 61.29; H, 5.94.

*Benzyl 3-O-benzyl-2-deoxy-4-O-β-*D*-mannopyranosyl-β-*L*-erythro-pentopyranoside* (**13**). — Compound **12** (0.74 g, 1.26 mmol) was treated with methanolic sodium methoxide (0.2M, 20 mL) at room temperature for 1 h, when t.l.c. (8:2 $CHCl_3$–MeOH) showed de-esterification to be complete. The cooled solution was treated sequentially with Amberlite IRN-77 (H^+) and IR-45 (HO^-) resins. The syrup (0.577 g, 96%) remaining after removal of solvents had $[\alpha]_D$ +53.5° (*c* 1, chloroform). ^{1}H-N.m.r. data ($CDCl_3$): δ 1.90 (m, 1 H, H-2a); 2.15 (m, 1 H, H-2b), 3.10 (d, 1 H), 3.44 (dd, 1 H), 3.72–4.05 (m, 8 H, H-3,4,5a,5b and H-2',3',4',5',6'a,6'b), 4.34–4.61 (m, 4 H, 2 C*H*$_2$Ph), 4.65 (d, 1 H, H-1'), 4.97 (bs, 1 H, H-1), 7.28 (m, 10 H, 2 Ph).

Anal. Calc. for $C_{25}H_{32}O_9 \cdot 0.5\ H_2O$ (485.53): C, 61.84; H, 6.85. Found: C, 61.96; H, 6.98.

*2-Deoxy-4-O-β-*D*-mannopyranosyl-*L*-erythro-pentopyranose* (**14**). — A solution of **13** (0.577 g) in MeOH was hydrogenated in the presence of 10% Pd–C until t.l.c. (6.5:4.5:0.4 $CHCl_3$–MeOH–H_2O) showed the removal of the benzyl groups to be complete. The catalyst was filtered off and the filtrate was concentrated to dryness. The impure residue [^{1}H-n.m.r. (D_2O): δ 1.66 (m, 1 H, H-2a); 1.88 (m, 1 H, H-2b), 4.67 (s, 1 H, H-1'), 5.15 (bs, part of H-1)] was used directly for the next step and was not further characterised.

*2-Deoxy-4-O-β-*D*-mannopyranosyl-*L*-erythro-pentonic acid* (**15**). — A solution of **14** in H_2O (38 mL) was oxidised as described for the corresponding α anomer, using a total of 22 mL of 0.05M I_2 in 0.25M KI and 33 mL of 0.1M NaOH. The cooled mixture was passed slowly through a column of cold Amberlite IRN-77 (H^+) resin (20 mL) into a stirred slurry of freshly prepared Ag_2CO_3 (2 g). Solids were filtered off and the cold solution was passed slowly through another cold column of the same resin (10 mL). The combined eluate and washings were neutralised (pH 7; pH meter) with a saturated solution of $Ba(OH)_2$, the solvent was removed, and the residue was triturated with ethanol until it solidified. The barium salt of **15** (189 mg) was recovered by centrifugation, washed twice with ethanol, and dried. A decationised sample appeared homogeneous by t.l.c. (5:3:1:1 1-propanol–conc. NH_4OH–H_2O–AcOH); it had $[\alpha]_D$ −27° (*c* 1, water). ^{1}H-N.m.r. data (250 MHz, D_2O): δ 2.38 (dd, 1 H, $J_{2a,2b}$ 15, $J_{2a,3}$ 8 Hz, H-2a), 2.51 (dd, 1 H, $J_{2b,3}$ 5 Hz, H-2b), 3.41 (ddd, 1 H, $J_{5,6'}$ 9, $J_{5,6'a}$ 6 Hz, H-5'), 3.57–3.88 (m, 6 H, H-4,5a,5b and H-3',4',6'a), 3.94 (dd, 1 H, $J_{6'b,5'}$ 2.3, $J_{6'b,6'a}$ 12 Hz, H-6'b), 4.10 (d, 1 H, $J_{2',3'}$ 2.8 Hz, H-2'), 4.16 (m, 1 H, $J_{3,4}$ 8 Hz, H-3), 4.81 (s, 1 H, $J_{1',2'}$ <0.5 Hz, H-1'; $J_{C-1',H-1'}$ 159.5 Hz).

Anal. Calc. for $C_{11}H_{19}Ba_{0.5}O_{10} \cdot 1.5H_2O$ (406.952): C, 32.47; H, 5.45. Found: C, 32.57; H, 5.75.

ACKNOWLEDGMENTS

The authors thank Miss Sophie Mairesse-Lebrun for the microanalyses, and Mrs. Jacqueline Mahuteau for the n.m.r. spectra. Support by the Fondation pour la Recherche Médicale is gratefully acknowledged.

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