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Note

Synthesis of 1-*O*-β-D-ribofuranosyl-D-ribitol 5-(disodium phosphate)

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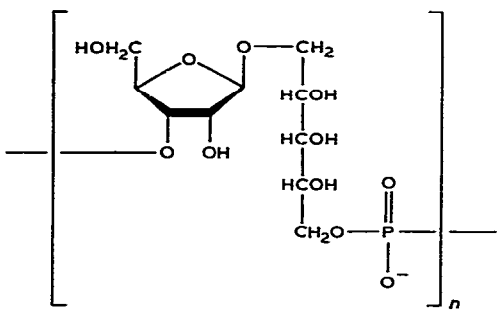
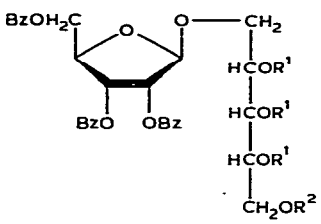
The bacterium *Haemophilus influenzae* type b, which causes meningitis in children, elaborates an antigen composed[1,2] of 1-*O*-β-D-ribofuranosyl-D-ribitol units joined by phosphodiester linkages from O-5 of the ribitol residue to O-3 of the ribosyl residue in the next ribofuranosylribitol unit (**1**).

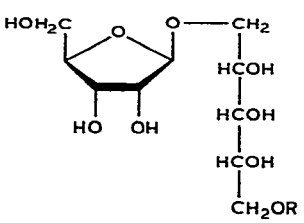
We now report a synthesis of the ribofuranosylribitol phosphate monomer 7



1



2 $R^1 = R^2 =$ Bzl
3 $R^1 = R^2 =$ H
4 $R^1 =$ Bz, $R^2 =$ Tr
5 $R^1 =$ Bz, $R^2 = P(O)(OPh)_2$



6 $R = P(O)(O^-NHEt_3^+)_2$
7 $R = P(O)(O^-Na^+)_2$

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expressed in **1**. This was required for immunological studies aimed at improved diagnosis of infections caused by this organism.

Condensation of 3,5-di-*O*-benzoyl-α-D-ribofuranose 1,2-(methyl orthobenzoate) with 2,3,4,5-tetra-*O*-benzyl-D-ribitol, as previously described[3], afforded **2**, from which the benzyl groups were removed by catalytic hydrogenolysis. The product **3** was tritylated at O-5 of the ribitol residue and then benzoylated. Hydrogenolytic detritylation of the product **4** was followed by phosphorylation with diphenyl phosphorochloridate to give **5**. Hydrogenolysis of **5** followed by debenzoylation with sodium methoxide in methanol gave the monophosphate **6**, which was isolated as its bis(triethylammonium) salt and then converted into the disodium salt **7**. The total yield in the synthesis of **7** from 2,3,4,5-tetra-*O*-benzyl-D-ribitol was 43%.

EXPERIMENTAL

General. — Melting points are corrected. Concentrations were performed at diminished pressure at a bath temperature below 40°. Optical rotations were measured with a Perkin–Elmer 241 instrument. ^{13}C- and ^{1}H-n.m.r. spectra (at 25.05 and 99.55 MHz, respectively) were recorded with a Jeol JNM FX 100 instrument. N.m.r. spectra, recorded in p.p.m. downfield from internal tetramethylsilane for $CDCl_3$ solutions and from external tetramethylsilane for D_2O solutions, were invariably in accordance with the postulated structures. T.l.c. was performed with precoated silica gel plates (F_{250}, Merck) and the spots were detected either in u.v. light or by charring with 8% aqueous H_2SO_4. Column separations were performed on silica gel 60 (0.040–0.063 mm, Merck).

2,3,4,5-Tetra-O-benzyl-1-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-D-ribitol (**2**). — Compound **2** was obtained by condensing 3,5-di-*O*-benzoyl-α-D-ribofuranose 1,2-(methyl orthobenzoate)[4,5] with 2,3,4,5-tetra-*O*-benzyl-D-ribitol[6] as described before[3]. The product, which was not fully characterized before[3], had $[\alpha]_D^{22}$ +6° (*c* 1, chloroform).

Anal. Calc. for $C_{59}H_{56}O_{12}$: C, 74.0; H, 5.90. Found: C, 73.8; H, 5.91.

1-O-(2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl)-D-ribitol (**3**). — A solution of **2** (3.54 g, 3.70 mmol) in acetic acid (80 ml) was hydrogenated over 10% palladium-on-charcoal, to give chromatographically pure **3** (1.97 g, 89%), $[\alpha]_D^{22}$ +16° (*c* 1, chloroform).

2,3,4-Tri-O-benzoyl-1-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-5-O-triphenylmethyl-D-ribitol (**4**). — A solution of **3** (1.69 g, 2.83 mmol) in dry pyridine (25 ml) was treated with triphenylmethyl chloride (1.02 g, 3.65 mmol) at room temperature for 24 h. The reaction was monitored by t.l.c. (water-saturated ethyl acetate). Chloroform (15 ml) was added and the mixture was cooled in ice–water. Benzoyl chloride (1.58 ml, 13.6 mmol) was added dropwise to the stirred solution at 0°. After storage at room temperature overnight, when the reaction was complete (t.l.c.; toluene–ethyl acetate, 8:1), ice and, after 15 min, chloroform (150 ml) were added. The mixture was extracted with water, aqueous sodium hydrogencarbonate, and then water,

dried (Na_2SO_4), filtered, and concentrated to a syrup which was purified by means of column chromatography on silica gel (toluene–ethyl acetate, 8:1), to give **5** (3.15 g, 96%), $[\alpha]_D^{22}$ +8° (*c* 1, chloroform).

2,3,4-Tri-O-benzoyl-1-O-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-D-ribitol 5-(diphenyl phosphate) (**5**). — A solution of **4** (2.00 g, 1.73 mmol) in acetic acid (75 ml) was hydrogenated over 10% palladium-on-charcoal, and then filtered through Celite. The Celite was washed with acetic acid, and the combined filtrates were concentrated to dryness. Diphenyl phosphorochloridate (0.94 ml, 4.51 mmol) was added dropwise to a solution of the residue in chloroform (20 ml) and pyridine (20 ml) at 0°. The mixture was kept at 5° overnight; t.l.c. (toluene–ethyl acetate, 4:1) then indicated completion of reaction, and ice was added. After 15 min, chloroform (100 ml) was added, and the mixture was extracted with water, aqueous sodium hydrogencarbonate, and then water, dried (Na_2SO_4), filtered, and concentrated to an amorphous product that was purified by column chromatography on silica gel (toluene–ethyl acetate, 4:1), to give **6** (1.48 g, 75%), $[\alpha]_D^{22}$ +14° (*c* 1, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 105.7 (C-1) and 66.7 (d, $J_{C,P}$ 5.5 Hz, C-5).

Anal. Calc. for $C_{64}H_{53}O_{18}P$: C, 67.4; H, 4.68. Found: C, 67.3; H, 4.71.

1-O-β-D-Ribofuranosyl-D-ribitol 5-[bis(triethylammonium) phosphate] (**6**). — A solution of **5** (1.48 g, 1.30 mmol) in acetic acid (100 ml) was hydrogenated over Adams' catalyst, and then filtered through Celite. The Celite was washed with acetic acid, and the combined filtrates were concentrated to near dryness and then distilled with toluene to remove any remaining acetic acid. Immediately after concentration to dryness, the product was treated with sodium methoxide (from 0.15 g of sodium) in methanol (75 ml) at 5° (refrigerator) overnight and then at room temperature for 4 h. The solution was treated with Dowex-50(H^+) resin, filtered, and then treated with an excess of triethylamine and concentrated to dryness. A solution of the residue in water (50 ml) was washed with diethyl ether (2 × 25 ml), concentrated to ~15 ml, filtered through cotton wool, concentrated, and dried *in vacuo*, to yield syrupy **6** (0.715 g, 95%), $[\alpha]_D^{22}$ −14° (*c* 1, water).

1-O-β-D-Ribofuranosyl-D-ribitol 5-(disodium phosphate) (**7**). — An aqueous solution of **6** was passed through a column of Dowex-50(Na^+) resin and then lyophilized, and the residue was purified on a column of Sephadex G-25 which was eluted with water containing 1% of pyridine. The conversion into the sodium salt was essentially quantitative. The product **7** had $[\alpha]_D^{22}$ −27° (*c* 0.55, water). ^{13}C-N.m.r. data (D_2O): δ 107.5 (C-1′), 83.3 (C-4′), 75.0 (C-2′), 72.2 (C-3′), 71.9 (d, $J_{C,P}$ 6.5 Hz, C-4), 71.2 (C-2 or C-3), 70.9 (C-3 or C-2), 69.1 (C-1), 65.8 (d, $J_{C,P}$ 4.3 Hz, C-5), and 63.2 (C-5′); the assignments are based on published values for ribofuranosides[7] and ribitol derivatives[8], and on the spectrum obtained for 5-*O*-β-D-ribofuranosyl-D-ribitol[3].

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