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SYNTHESIS OF *p*-TRIFLUOROACETAMIDOPHENYL β-D-GLUCOPYRANOSIDE 4-(D-RIBIT-5-YL PHOSPHATE) CORRESPONDING TO THE *Haemophilus influenzae* TYPE A CAPSULAR ANTIGEN*

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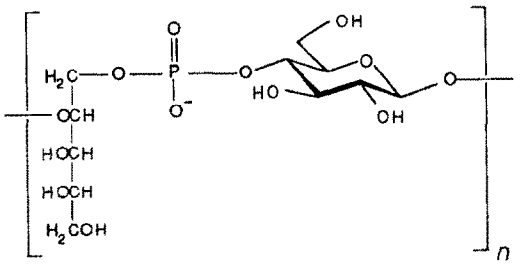
ABSTRACT

The synthesis is described of the title glycoside which corresponds to the *Haemophilus influenzae* type a capsular antigen[1]. The hydrogenphosphonate method[2] was used with 3,3′-(chlorophosphonylidene)bis(2-oxo-1,3-oxazolidene)[3–5] as the condensing agent.

INTRODUCTION

The Gram-negative bacterium *Haemophilus influenzae* type a, which, *inter alia,* causes respiratory disease, has a capsule with the repeating unit **1**.

The *p*-aminophenyl glycoside of the repeating unit of **1** could be attached to a protein *via* a thiourea linkage[6] to give an artificial antigen of potential value for the development of improved diagnostic preparations. We now describe the synthesis of the *p*-trifluoroacetamidophenyl glycoside (**2b**) of the phosphodiester unit of **1**.



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*Dedicated to Professor Bengt Lindberg.

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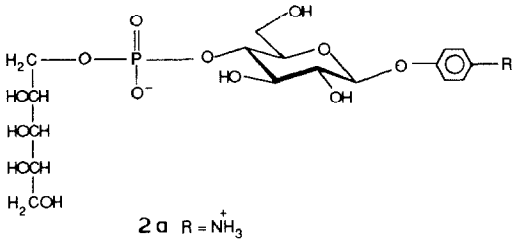
RESULTS AND DISCUSSION

Initially, the phosphotriester method[7,8] was used for the condensation reaction. Thus, 1,2,3,4-tetra-*O*-benzyl-D-ribitol 5-(2,2,2-trichloroethyl 2-chlorophenyl phosphate)[8] was deprotected using zinc in pyridine and acetic acid, and the resulting phosphodiester intermediate was coupled to **5**. Removal of the 2-chlorophenyl group using *N,N,N,N*-tetramethylguanidine and pyridine-2-aldoxime, followed by catalytic hydrogenolysis (Pd/C) produced the phosphodiester **2a** (49% from 1,2,3,4-tetra-*O*-benzyl-D-ribitol 5-(*o*-chlorophenyl phosphate)]. The experimental procedures for this work were the same as those described[8] and, therefore, are not repeated here.

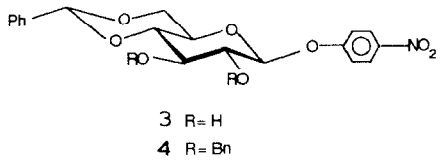
The above deprotection scheme was tedious. An improved route made use of the hydrogenphosphonate method[2] developed in our laboratory.

p-Nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside[9] (**3**) was treated with benzyl bromide and sodium hydride in *N,N*-dimethylformamide to give **4** which, with hydrogen chloride and sodium cyanoborohydride in tetrahydrofuran–ether[10], gave *p*-nitrophenyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**5**, 92%). Reduction of the *p*-nitrophenyl group in **5** with aluminium amalgam[11] and *N*-trifluoroacetylation gave *p*-trifluoroacetamidophenyl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (**6**, 90%).

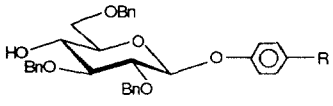
Treatment of 1,2,3,4-tetra-*O*-benzyl-D-ribitol[8] (**7**) with salicyl chlorophosphite[12] in 1,4-dioxane followed by aqueous triethylamine gave 1,2,3,4-tetra-*O*-benzyl-D-ribitol 5-phosphonate as its triethylammonium salt (**8**, 85%). The coupling



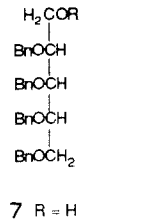
2a R = $\overset{+}{N}H_3$
2b R = NHCOCF$_3$



3 R = H
4 R = Bn



5 R = NO_2
6 R = NHCOCF$_3$



7 R = H

8 R = $-\overset{O}{\overset{\|}{P}}(H)-O^{-}\,\overset{+}{H}NEt_3$

TABLE I

N.M.R. DATA FOR SOLUTIONS OF **1** AND **2b** IN D_2O (INTERNAL 1,4-DIOXANE, 64.4 P.P.M.) AT 60°

Compound	*Chemical shifts (p.p.m.)*										
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*
1	102.9	74.1	75.6[a]	74.8[b]	75.8[a]	61.4	63.5	72.6	72.4	80.1[c]	65.7[d]
2b	101.2	73.7	75.8[a]	74.7[e]	76.2[f]	61.3	63.3	73.0	72.7	71.9[g]	67.8[h]

[a]Coupled signals (coupling constants were not determined). [b]$^2J_{P,C}$ 6 Hz, [c]$^3J_{P,C}$ 6.5 Hz, [d]$^2J_{P,C}$ 5 Hz. [e]$^2J_{P,C}$ 6.4 Hz. [f]$^3J_{P,C}$ 4.6 Hz. [g]$^3J_{P,C}$ 7.3 Hz. [h]$^2J_{P,C}$ 5.5 Hz.

of **6** and **8** was performed in pyridine using 3,3'-(chlorophosphonylidene)bis(2-oxo-1,3-oxazolidene). Subsequent oxidation using iodine–water and *O*-debenzylation by hydrogenolysis (Pd/C) gave the title compound **2b** (85%). The ^{13}C-n.m.r. spectrum of **2b** was interpreted with the aid of 2D ^{13}C–^{1}H correlated and ^{1}H–^{1}H correlated (COSY) n.m.r. spectroscopy. Chemical shift data together with the $J_{P,C}$ values are given in Table I and compared to the data given[1] for native capsular antigen from *Haemophilus influenzae* type a.

EXPERIMENTAL

General methods were the same as those described[8]. ^{13}C-N.m.r. shifts are downfield from that of the resonance of internal Me_4Si, and ^{31}P-n.m.r. shifts are downfield from that of the resonance of external 2% H_2PO_3 in D_2O. Only significant shift data are given.

p-*Nitrophenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside* (**4**). — Sodium hydride (50% in mineral oil, 302 mg, 6.3 mmol) was washed with light petroleum and added to a solution of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside[9] (800 mg, 2.1 mmol) in *N,N*-dimethylformamide (20 mL). The mixture was cooled in an ice-bath and benzyl bromide (0.63 mL, 5.3 mmol) was added dropwise. When t.l.c. (toluene–ethyl acetate, 3:1) indicated complete reaction, methanol (1 mL) was added and the temperature was raised to ambient. The mixture was diluted with ether (100 mL), washed with water (3 × 40 mL), dried ($MgSO_4$), and concentrated. Crystallisation of the residue from ether yielded **4** (860 mg, 71%), m.p. 148–149°, $[\alpha]_D^{20}$ −47° (*c* 1, chloroform). ^{13}C-N.m.r. data (25 MHz, $CDCl_3$): δ 66.5, 68.6, 75.2, 75.8, 80.8, 81.2, 81.5, 101.0, 101.4.

Anal. Calc. for $C_{33}H_{31}NO_8$: C, 69.6; H, 5.5; N, 2.5. Found: C, 69.6; H, 5.5; N, 2.5.

p-*Nitrophenyl 2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**5**). — Hydrogen chloride in ether was added dropwise at 0° to a stirred solution of **4** (300 mg, 0.53 mmol) and sodium cyanoborohydride (300 mg, 4.8 mmol) in tetrahydrofuran (7 mL) containing molecular sieves (3Å) until t.l.c. (toluene–ethyl acetate, 4:1) indicated that all **4** was consumed. The mixture was filtered through Celite and

poured into ice–water. The Celite was washed with water and dichloromethane. The aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic solutions were washed with aqueous sodium hydrogencarbonate, dried ($MgSO_4$), and concentrated. Column chromatography (iso-octane–ethyl acetate, 2:1) of the residue on silica gel yielded **5** (280 mg, 92%), m.p. 95° (from ether–light petroleum), $[\alpha]_D^{20}$ −74° (*c* 1, chloroform). ^{13}C-N.m.r. data (25 MHz, $CDCl_3$): δ 69.6, 70.8, 73.6, 74.9, 75.1, 75.4, 81.3, 83.9, 100.8.

Anal. Calc. for $C_{33}H_{33}NO_8$: C, 69.3; H, 5.8; N, 2.5. Found: C, 69.5; H, 5.9; N, 2.6.

p-*Trifluoroacetamidophenyl 2,3,6-tri-*O*-benzyl-β-*D*-glucopyranoside* (**6**). — A long strip of aluminium foil (300 mg) was rolled up and dipped successively in ether, ethanol, aqueous 2% mercury(II) chloride for 10 s, ethanol, and ether, and then immersed in a stirred solution of **5** (190 mg, 0.3 mmol) in tetrahydrofuran–water, 10:1 (11 mL). After 30 min, when t.l.c. (toluene–ethyl acetate, 3:1) indicated complete reaction, the mixture was filtered through Celite which was then washed with tetrahydrofuran (10 mL). The combined filtrate and washings were concentrated and pyridine was evaporated from the residue to remove traces of water. A solution of the residue in pyridine (2 mL) was then added to a solution of trifluoroacetic anhydride (113 μL, 0.8 mmol) in pyridine (0.5 mL) at 0°. The mixture was stirred for 30 min, water (0.5 mL) was added at room temperature, and the mixture was concentrated. Column chromatography (toluene–ethyl acetate, 4:1) of the residue on silica gel yielded **6** (175 mg, 90%), m.p. 149–150° (from ether–light petroleum), $[\alpha]_D^{20}$ −44° (*c* 1, dichloromethane). ^{13}C-N.m.r. data (25 MHz, $CDCl_3$): δ 69.8, 70.9, 73.6, 74.6, 75.0, 75.3, 81.4, 84.0, 101.6.

Anal. Calc. for $C_{35}H_{34}F_3NO_7$: C, 65.9; H, 5.4; N, 2.2. Found: C, 65.8; H, 5.5; N, 2.1.

*1,2,3,4-Tetra-*O*-benzyl-*D*-ribitol 5-(triethylammonium phosphonate)* (**8**). — 1,2,3,4-Tetra-*O*-benzyl-D-ribitol (**7**; 1 g, 2.0 mmol) was rendered anhydrous by the evaporation of pyridine therefrom. To a solution of the residue in 1,4-dioxane–pyridine (3:1, 8 mL) was added a solution of salicyl chlorophosphite[2] (510 mg, 2.5 mmol) in 1,4-dioxane (1 mL). The mixture was stirred for 10–15 min at room temperature. T.l.c. (toluene–ethyl acetate, 9:1) then indicated that all **7** had been consumed. Triethylamine (2 mL) and water (1 mL) were added, stirring was continued for 15 min, the mixture was partitioned between dichloromethane and water, and the organic layer was washed twice with water and concentrated. Column chromatography (dichloromethane–methanol, 99:1 → 95:5) of the residue on silica gel gave **8** (1.2 g, 85%), $[\alpha]_D^{20}$ −13° (*c* 1, dichloromethane). N.m.r. data: ^{13}C (25 MHz, $CDCl_3$), δ 8.4, 45.4 (triethylammonium), 62.7 ($J_{C,P}$ 3.7 Hz, C-5), 70.3 (C-1); ^{31}P (161.7 MHz, pyridine), δ 4.8 ($J_{P,H}$ 632.4 Hz).

p-*Trifluoroamidophenyl β-*D*-glucopyranoside 4-(*D*-ribit-5-yl phosphate)* (**2b**). — Compounds **8** (135 mg, 0.2 mmol) and **6** (125 mg, 0.2 mmol) were rendered anhydrous by evaporation of added pyridine therefrom. A solution of the residue in pyridine (1 mL) and 3,3′-(chlorophosphonylidene)bis(2-oxo-1,3-oxazolidene)

(127 mg, 0.5 mmol) was added. After 15 min, t.l.c. (chloroform–methanol, 9:1) revealed a new spot. A solution of iodine (100 mg, 0.4 mmol) in pyridine–water (95:5, 2 mL) was added, and the mixture was stirred for 5 min and then partitioned between aqueous sodium hydrogensulfite and chloroform. The organic layer was concentrated and column chromatography (dichloromethane–methanol, 100:0 → 80:20) of the residue on a short column of silica gel gave protected diester (222 mg, 90%) which was hydrogenolyzed over 10% Pd/C (250 mg) at 400 kPa in ethyl acetate (30 mL), ethanol (6 mL), and water (6 mL) for 12 h. The mixture was filtered through a short column of silica gel and then concentrated. Column chromatography (with water) of the residue on Bio-Gel P-2 followed by lyophilization yielded **2b** (102 mg, 94%), as a white powder, $[\alpha]_D^{20}$ −34° (*c* 1, water). N.m.r. data: ^{31}P (161.7 MHz, H_2O), δ 0.66; ^1H (270 MHz, D_2O, internal TSP), δ 5.16 ($J_{1,2}$ 7.9 Hz, H-1). The ^{13}C-n.m.r. data are given in Table I.

Anal. Calc. for $C_{19}H_{30}F_3N_2O_{14}P \cdot 1.5\ H_2O$: C, 36.5; H, 5.3; N, 4.5. Found: C, 36.4; H, 4.9; N, 4.5.

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