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Note

Fluorocarbohydrates
Part XXV[2]. Synthesis of 3-deoxy-3-fluoro-D-glucose 1- and 6-phosphates

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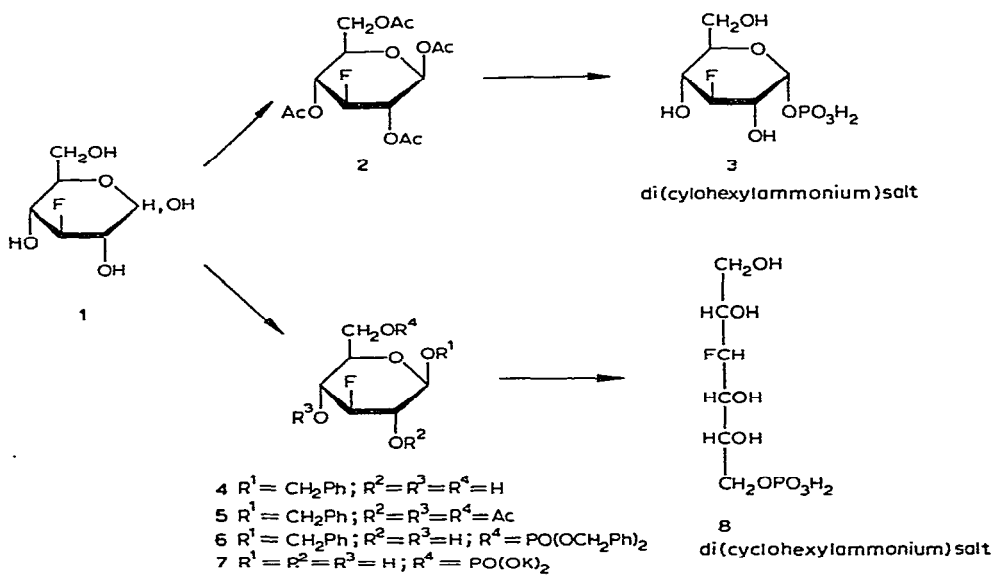
In a preliminary communication[1], we reported the synthesis of 3-deoxy-3-fluoro-α-D-glucose 1-phosphate (3) and 3-deoxy-3-fluoro-D-glucose 6-phosphate (7), and demonstrated that these substrates were competitive inhibitors of UDPG-pyrophosphorylase and phosphoglucomutase, respectively. Previous workers have reported on the chemical synthesis of 6-deoxy-6-fluoro-α-D-galactose 1-phosphate[2] and the enzymic synthesis of 3-deoxy-3-fluoro-D-glucose 6-phosphate[3]. We now report the experimental details of the syntheses of **3** and **7**.



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The synthesis of **3** was based on the method reported for α-D-glucose 1-phosphate[4], and involved treatment of 1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-fluoro-D-glucose (**2**) with anhydrous phosphoric acid and isolation of **3** as the crystalline di(cyclohexylamine) salt. Only the α-D anomer could be detected.

3-Deoxy-3-fluoro-D-glucose 6-phosphate (**7**) was synthesised *via* crystalline benzyl 3-deoxy-3-fluoro-β-D-glucopyranoside (**4**), which was obtained in high yield by treating 3-deoxy-3-fluoro-αβ-D-glucose[5] (**1**) with benzyl alcohol saturated with hydrogen chloride. The structure of **4** was assigned on the basis of a negative specific rotation, the consumption of 2 mol. of periodate, and the formation of a crystalline 2,4,6-triacetate (**5**) that gave the coupling constants expected for the β-D anomer[6]. Treatment of **4** with dibenzyl phosphorochloridate[7] at −40° gave crystalline benzyl 3-deoxy-3-fluoro-β-D-glucopyranoside 6-(dibenzyl phosphate) (**6**) which, after hydrogenolysis, yielded **7**, isolated as a hygroscopic, potassium salt. This salt consumed 2 mol. of periodate and produced 0.84 mol. of formic acid after 72 h, but yielded no formaldehyde. The slow oxidation of **7** was probably due to the formation and slow hydrolysis of a formic ester. These results are consistent with the introduction of the phosphate group at C-6 of 3-deoxy-3-fluoro-D-glucose (**1**). The structure of **7** was further confirmed by reduction with potassium borohydride to give 3-deoxy-3-fluoro-D-glucitol 6-phosphate isolated as the di(cyclohexylammonium) salt (**8**).

EXPERIMENTAL

Melting points were determined by using an Electrothermal apparatus and are uncorrected. Thin-layer chromatography (t.l.c.) was performed conventionally on either Silica Gel G (Shandon Scientific Co. Ltd.) or CC41 Cellulose (Whatman Biochemicals Ltd.), using *A*, ethyl acetate or *B*, *tert*-pentyl alcohol–water–toluene-*p*-sulphonic acid (60 ml:30 ml:2 g; organic phase). Detection of sugars was by charring with sulphuric acid–ethanol (1:1) at 120° for 5 min, or with ferric chloride–sulphosalicylic acid[8] for CC41 Cellulose plates.

Fluorine analyses were carried out with the fluoride electrode[9], which eliminates interference by phosphate.

3-Deoxy-3-fluoro-α-D-glucose 1-phosphate (**3**). — 1,2,4,6-Tetra-*O*-acetyl-3-deoxy-3-fluoro-D-glucose[5] (**2**) (1.013 g) and anhydrous phosphoric acid (2.129 g) were heated to 50–55°(bath) for 2 h *in vacuo* (1 mmHg). After cooling, the resulting syrup was shaken with ice-cold 2M lithium hydroxide (60 ml) and allowed to stand overnight at 2–4°. Precipitated lithium phosphate was filtered off and the filtrate was passed through a cooled column of Dowex-50(H^+) resin (40 ml). The acidic eluate was collected in a flask containing freshly distilled cyclohexylamine (5.0 ml). Concentration of the solution, followed by addition of acetone to faint turbidity, gave the di(cyclohexylammonium) salt of **3** as needles (970 mg), m.p. 158–162° (dec.), $[\alpha]_D^{22}$ +60.5° (*c* 1.2, water), R_F 0.43 (solvent *B*, cellulose) (Found: C, 45.27; H, 8.58; F, 3.89; N, 6.00; P, 6.53. $C_{18}H_{38}FN_2O_8P \cdot H_2O$ calc.: C, 45.18; H, 8.43; F, 3.98; N, 5.85; P, 6.47%).

Benzyl 3-deoxy-3-fluoro-β-D-glucopyranoside (**4**). — Anhydrous hydrogen chloride was passed for 45 min into a gently shaken mixture of 3-deoxy-3-fluoro-D-glucose (1.85 g) and benzyl alcohol (50 ml) maintained at 0–2°. Nitrogen was then passed into the mixture for 30 min to remove most of the hydrogen chloride, and the solution was diluted with ethyl acetate. After neutralisation with solid sodium hydrogen carbonate, the solution was filtered and concentrated *in vacuo*. The gummy residue was dissolved in boiling dichloromethane and the solution cooled to 0–2°. The crystalline product was collected, washed with a little ice-cold dichloromethane, and air-dried to give **4** (2.40 g), m.p. 95°, $[\alpha]_D^{21}$ −58° (*c* 1.0, methanol), R_F 0.4 (solvent *A*, Silica Gel G) (Found: F, 6.6. $C_{13}H_{17}FO_5$ calc.: 6.9%).

A solution of the glycoside (200 mg) in pyridine (2 ml) and acetic anhydride (0.2 ml) was stored at room temperature for 18 h and then poured into water. Benzyl 2,4,6-tri-*O*-acetyl-3-deoxy-3-fluoro-β-D-glucopyranoside (**5**), separated as a white precipitate and, after crystallisation from aqueous methanol, had m.p. 104°, $[\alpha]_D^{19}$ −62° (*c* 1.0, chloroform), R_F 0.7 (solvent *A*, Silica Gel G) (Found: C, 57.5; H, 5.9; F, 5.0. $C_{19}H_{23}FO_8$ calc.: C, 57.3; H, 5.9; F, 4.8%).

Benzyl 3-deoxy-3-fluoro-β-D-glucopyranoside 6-(dibenzyl phosphate) (**6**). — To a cooled (−40°) solution of benzyl 3-deoxy-3-fluoro-β-D-glucopyranoside (**4**) (1.0 g) in anhydrous pyridine (20 ml), dibenzyl phosphorochloridate (freshly prepared from 2.0 g of dibenzyl phosphate[8]) was added portionwise during 30 min, followed by ice (10 g), and stirring was continued for a further 30 min. The solution was evaporated, and a solution of the residue in chloroform (100 ml) was washed successively with 2M hydrochloric acid (100 ml), saturated, aqueous sodium hydrogen carbonate (100 ml), and water (100 ml), and dried ($MgSO_4$). The filtered solution was evaporated to dryness *in vacuo* to a syrup which crystallised on standing. Recrystallisation from benzene–cyclohexane gave **6** (1.3 g), m.p. 117–120°, $[\alpha]_D^{22}$ −4.2° (*c* 2.7, chloroform), R_F 0.55 (solvent *A*, Silica Gel G) (Found: C, 60.92; H, 5.66; F, 3.50; P, 5.45. $C_{27}H_{30}FO_8P$ calc.: C, 60.90; H, 5.68; F, 3.57; P, 5.82%).

3-Deoxy-3-fluoro-D-glucose 6-phosphate (**7**). — To a solution of benzyl 3-deoxy-3-fluoro-D-glucopyranoside 6-(dibenzyl phosphate) (**6**, 1.25 g) in ethanol (50 ml) and water (50 ml), 10% palladium-on-charcoal (400 mg) was added and the mixture was hydrogenated at 1 atmosphere until the uptake of hydrogen ceased (45 min). After filtration, the solution was concentrated *in vacuo* to 30 ml and treated with 10% aqueous potassium hydroxide to pH 7.5. The resulting solution was lyophilized to yield the di-potassium salt of **7** (729 mg), R_F 0.35 (solvent *B*, cellulose) (Found: F, 5.50. $C_6H_{10}FK_2O_8P$ calc.: F, 5.62%). No satisfactory C and H analyses could be obtained for this very hygroscopic salt which consumed 2.0 mol. of periodate and produced 0.84 mol. of formic acid after 72 h.

3-Deoxy-3-fluoro-D-glucitol 6-phosphate (**8**). — To a solution of **7** (dipotassium salt, 500 mg) in water (5.0 ml) was added potassium borohydride (150 mg) in water (5.0 ml) during 5 min. Stirring was continued a further 10 min, followed by the slow addition of Dowex-50 (H^+) resin until the evolution of gas ceased. The solution was diluted with water (30 ml) and stirred for 30 min with a further amount (5.0 ml) of

Dowex-50 (H^+) resin. After filtration, the solution was evaporated to dryness *in vacuo* and methanol was distilled several times from the syrupy residue to remove boric acid. A solution of the residue in water (2.0 ml) was neutralised with freshly distilled cyclohexylamine to pH 10.0 and evaporated to dryness *in vacuo*. A solution of the resulting syrup in water (2.0 ml) was triturated with acetone to incipient turbidity and allowed to stand several hours at 0–2°. The crystals were collected, washed with a little acetone, air-dried, and recrystallised from aqueous acetone to give **8** as the di(cyclohexylamine) salt (420 mg), m.p. 162–165°, $[\alpha]_D^{22}$ −30.5° (*c* 1.1, water), R_F 0.37 (solvent *B*, cellulose) (Found: C, 44.6; H, 8.8; F, 3.9; N, 5.7; P, 6.5. $C_{18}H_{40}FN_2O_8P \cdot H_2O$ calc.: C, 44.9; H, 8.8; F, 4.05; N, 5.8; P, 6.6%).

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