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SYNTHESIS OF 1-DEOXY-1-FLUORO-L-GLYCEROL AND ITS 3-PHOSPHATE

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ABSTRACT

1-Deoxy-1-fluoro-L-glycerol (**1**) has been stereospecifically synthesised in nine steps *via* crystalline intermediates from D-mannitol. Fluoride-ion displacement of sulphonyloxy groups from C-1 and C-6 of 3,4-*O*-benzylidene-2,5-*O*-methylene-1,6-di-*O*-toluene-*p*-sulphonyl-D-mannitol (**12**), followed, in sequence, by removal of the benzylidene group, periodate oxidation, borohydride reduction, and methanolysis, gave the fluoroglycerol **1** in 9.5% overall yield from D-mannitol. This alternative synthesis shows an improved yield over that previously described and provides confirmation of the optical purity of the product. 1-Deoxy-1-fluoro-L-glycerol 3-phosphate (**4**) was prepared from **1** by selective phosphorylation using dibenzyl phosphorochloridate, hydrogenolysis of the benzyl ester groupings, and characterisation of the product as its dicyclohexylamine salt. A second synthesis of **4** started from 2,2′-*O*-methylenebis(1-deoxy-1-fluoro-L-glycerol) (**16**), an intermediate in the synthesis of **1** from D-mannitol. Phosphorylation of **16** using diphenyl phosphorochloridate, followed by hydrogenolysis of the phenyl ester and methanolysis of the methylene bridge, gave **4**.

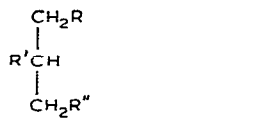
INTRODUCTION

Analogues in which a hydroxyl group of an enzyme substrate has been replaced by fluorine are of potential value both as metabolic inhibitors[1] and as enzyme probes, where the ^{19}F n.m.r. signal may be used to report on the microenvironment of the active site. The effect of such fluorine–hydroxyl substitution on enzyme–ligand interactions has been recently studied in the glycerol kinase–deoxyfluoroglycerol system[2]. 1-Deoxy-1-fluoro-L-glycerol (**1**) used in these studies was stereospecifically synthesised[3] in eight steps and 4% overall yield from D-mannitol. We now report an alternative synthesis of compound **1** from D-mannitol in 9.5% overall yield, *via* a series of highly crystalline intermediates.

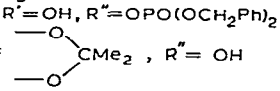
Extension of fluoro-analogue studies to enzymes having L-glycerol 3-phosphate (**3**) as their natural substrate (*e.g.* glycerol phosphate dehydrogenase) depends upon the availability of suitable phosphate analogues. Two synthetic routes to 1-deoxy-1-fluoro-L-glycerol 3-phosphate are described in this work.

RESULTS AND DISCUSSION

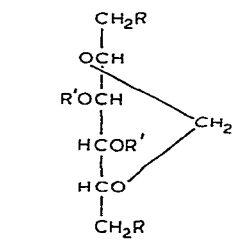
Our earlier synthesis[3] of 1-deoxy-1-fluoro-L-glycerol (**1**) involved successive periodate cleavage and borohydride reduction of 1,2:5,6-di-*O*-isopropylidene-D-mannitol, giving 2,3-*O*-isopropylidene-D-glycerol (**7**). Toluene-*p*-sulphonylation, followed by fluoride-ion displacement of the sulphonyloxy group and acid hydrolysis, gave 1-deoxy-1-fluoro-D-glycerol (**2**). 100% Inversion of configuration at C-2 of **2** was effected by toluene-*p*-sulphonylation, benzoate displacement, and methanolysis, to give the L enantiometer **1**. In this way, C-1 and C-6 of the original D-mannitol molecule became C-3 of both enantiomers of 1-deoxy-1-fluoroglycerol. The presently described route utilises fluoride-ion displacement at C-1 and C-6 of a D-mannitol derivative. Subsequent periodate cleavage of the C-3–C-4 bond then leads to 1-deoxy-1-fluoro-L-glycerol (**1**) in which C-1 is derived from C-1 and C-6 of the D-mannitol. Methods are hence available for selectively labelling **1** in either the C-1 or C-3 position by alternative syntheses starting from commercial D-mannitol-*1*-^{14}C. This could be of particular value in metabolic studies with **1**.



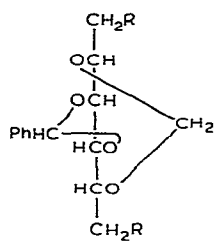
1 R = F, R′ = R″ = OH
2 R = R′ = OH, R″ = F
3 R = R′ = OH, R″ = OPO_3^{2-}
4 R = F, R′ = OH, R″ = OPO_3^{2-}
5 R = OPO_3^{2-}, R′ = OH, R″ = F
6 R = F, R′ = OH, R″ = $OPO(OCH_2Ph)_2$



7 R,R′ = CMe_2, R″ = OH



8 R = OTs, R′ = H
9 R = OTs, R′ = Bz
10 R = F, R′ = H
11 R = OH, R′ = H

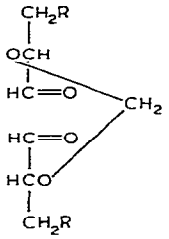


12 R = OTs
13 R = F

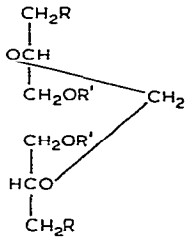
The previous synthesis[3] of **2** provided evidence that racemisation had not occurred under the basic conditions of fluoride-ion displacement and that enantiomers **1** and **2** were optically pure. The alternative synthetic route involves isolation, in high yields, of a series of pure, crystalline compounds having a minimum of two asymmetric centres. The possibility that racemisation occurs in the course of the synthesis is accordingly low, and the identical properties of compound **1** prepared by either route strongly support its optical purity.

2,5-*O*-Methylene-1,6-di-*O*-toluene-*p*-sulphonyl-D-mannitol (**8**) readily undergoes intramolecular displacement of the sulphonyloxy groups by HO-3 and HO-4, giving 1,4:3,6-dianhydro-2,5-*O*-methylene-D-mannitol[4]. Fluoride-ion displacement of the 1,6-sulphonyloxy groups must accordingly be carried out on a suitably blocked derivative of **8**. The 3,4-dibenzoate **9** was too labile under the exchange conditions employed, and treatment of **9** with tetrabutylammonium fluoride in acetonitrile yielded a complex product mixture. Use of the benzylidene acetal was more successful, and fluoride-ion displacement on 3,4-*O*-benzylidene-2,5-*O*-methylene-1,6-di-*O*-

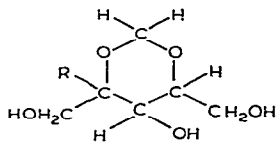
toluene-*p*-sulphonyl-D-mannitol (**12**) afforded crystalline 3,4-*O*-benzylidene-1,6-dideoxy-1,6-difluoro-2,5-*O*-methylene-D-mannitol (**13**) in high yield. Hydrogenolysis of **13** removed the benzylidene group, giving 1,6-dideoxy-1,6-difluoro-2,5-*O*-methylene-D-mannitol (**10**). Periodate oxidation of the diol **10** gave the dialdehyde **14** which was not isolated but immediately reduced, using buffered borohydride, to give crystalline 2,2′-*O*-methylenebis(1-deoxy-1-fluoro-L-glycerol) (**16**) in 73% yield from **10**. Ness *et al.*[5] described the periodate oxidation of 2,5-*O*-methylene-D-mannitol (**11**) to give a syrupy dialdehyde **15** which was hydrogenated over Raney nickel to give 2,2′-*O*-methylenebisglycerol (**17**). When the above workers subjected the dialdehyde **15** to basic conditions, a cyclisation occurred to give 4-formyl-5-hydroxy-4,6-di(hydroxymethyl)-1,3-dioxane (**21**) which on catalytic hydrogenation gave crystalline 5-hydroxy-4,4,6-tri(hydroxymethyl)-1,3-dioxane (**22**). The cyclic product **21** probably arises *via* intramolecular aldol-condensation between a carbanion at C-2 of one glyceraldehyde moiety of the dialdehyde **15** and the carbonyl group of the second glyceraldehyde. Application of the periodate–borohydride conditions to 2,5-*O*-methylene-D-mannitol (**11**) yielded only the cyclic product **22**. It is probable that the base-catalysed cyclisation of the dialdehyde **15** is more facile than that of the difluoride **14** because of greater product stabilisation in the former case. Depending on the configuration assumed on ring closure, the cyclic product **21** can undergo formation of a pyranoid ring which is not possible with **14**. The detailed structures of compounds **21**, **22**, and their fluorinated analogues are being further investigated and will be reported elsewhere.



14 R = F
15 R = OH



16 R = F, R′ = H
17 R = OH, R′ = H
18 R = F, R′ = Bz
19 R = F, R′ = $OPO(OPh)_2$
20 R = F, R′ = OPO_3^{2-}



21 R = HC=O
22 R = CH_2OH

Treatment of either the methylene acetal **16** or its dibenzoate **18** with methanolic hydrogen chloride gave 1-deoxy-1-fluoro-L-glycerol (**1**) identical with the previously described[3] product. The direct methanolysis of **16** yielded a sample of **1** which was homogeneous by g.l.c. and was obtained in 9.5% overall yield from D-mannitol. This represents a considerable improvement in yield over that obtained by the original method[3].

Selective phosphorylation of **1** with 1 molar equivalent of dibenzyl phosphoro-

chloridate gave a mixture from which the major component, 1-deoxy-1-fluoro-L-glycerol 3-(dibenzyl phosphate) (**6**) was isolated by preparative-layer chromatography. Hydrogenolysis of **6** gave 1-deoxy-1-fluoro-L-glycerol 3-phosphate (**4**), isolated as the crystalline dicyclohexylamine salt in 39% overall yield from **1**. The phosphate **4** was also obtained by treatment of the methylene acetal **16** with diphenyl phosphorochloridate to give the bis(diphenyl phosphate) **19**, followed by hydrogenolysis to the diphosphate **20** and acid hydrolysis to **4**. This latter route gives phosphate **4** in 1.6% overall yield from D-mannitol, compared with 3.3% by selective phosphorylation of **1**.

EXPERIMENTAL

General. — Melting points are uncorrected. Thin-layer chromatography (t.l.c.) was performed on Silica Gel G (Merck) or Cellulose powder CC 41 (Whatman). Detection on silica gel was effected with conc. sulphuric acid. The phosphates were visualised on cellulose by the molybdate spray of Hanes and Isherwood[6]. P.l.c. was performed on glass plates (40 × 20 cm), coated with a layer (1.3 mm) of Silica Gel PF_{254} (Merck). Components were detected as bands of fluorescence or quenching on exposure to u.v. radiation (254 nm). G.l.c. was conducted isothermally on a column (2 m) of Silicone Gum Rubber E-301 (2.5%) on AW-DMCS Chromosorb G (80–100 mesh). The carrier gas was nitrogen, and the chromatograph was a Perkin–Elmer F-11 instrument, fitted with a flame-ionisation detector. Trimethylsilyl derivatives of hydroxy compounds were prepared for g.l.c. by using B.S.A. reagent (Pierce Chemical Company). Optical rotations were determined with a Bellinger and Stanley (Model A) polarimeter (1-dm tube). O.r.d. curves were recorded with a Spectropol 1b (Fica) spectropolarimeter (0.02-dm tube). N.m.r. spectra were measured with a JEOL-JNM-4H-100 n.m.r. spectrometer at 100 MHz, with tetramethylsilane as internal standard. Pyridine and acetonitrile were dried by distillation from phosphorus pentaoxide. Light petroleum refers to the fraction with b.p. 40–60°. Concentrations were performed under diminished pressure with the bath temperature below 40°.

3,4-O-Benzylidene-2,5-O-methylene-1,6-di-O-toluene-p-sulphonyl-D-mannitol (**12**). — Compound **8**[4] (8 g) was shaken with freshly distilled benzaldehyde (40 ml) and anhydrous zinc chloride (8 g) for 4 h at room temperature. The mixture was poured into stirred, aqueous M potassium carbonate (400 ml), and the resulting suspension was extracted with chloroform (3 × 100 ml). The combined extracts were washed with water, dried ($MgSO_4$), and evaporated to a syrup. Removal of residual benzaldehyde by co-distillation with water afforded a crystalline mass which was recrystallised from ethyl acetate–light petroleum to give **12** (7.4 g, 79%), m.p. 141–142°, $[\alpha]_D^{22}$ +32.5° (*c* 10.0, chloroform); n.m.r. data ($CDCl_3$): δ 2.37 (singlet, 6 protons, 2 tosyl-Me), 4.77 (singlet, 2 protons, –O–CH_2–O–), 5.94 (singlet, 1 proton, benzylic-H).

Anal. Calc. for $C_{28}H_{30}O_{10}S_2$: C, 56.95; H, 5.09; S, 10.85. Found: C, 57.16; H, 5.32; S, 11.02.

3,4-Di-O-benzoyl-2,5-O-methylene-1,6-di-O-toluene-p-sulphonyl-D-mannitol (**9**).

— Conventional esterification of compound **8** (6.5 g) with benzoyl chloride (3.5 g) and pyridine (60 ml) gave the dibenzoate **9** (7.5 g, 81%), m.p. 144–145° (from methanol), $[\alpha]_D^{22}$ −37° (*c* 2.2, chloroform); n.m.r. data ($CDCl_3$): δ 2.41 (singlet, 6 protons, 2 tosyl-Me), 4.76 (singlet, 2 protons, –O–CH_2–O–), 5.35 (multiplet, 2 protons, H-3,4).

Anal. Calc. for $C_{35}H_{34}O_{12}S_2$: C, 59.14; H, 4.79; S, 9.01. Found: C, 58.88; H, 4.72; S, 8.73.

3,4-O-Benzylidene-1,6-dideoxy-1,6-difluoro-2,5-O-methylene-D-mannitol (**13**). — A mixture of compound **12** (15 g) and tetrabutylammonium fluoride (26.5 g) in acetonitrile (200 ml) was boiled for 4 days under reflux, cooled, and partititioned between ether and water. The ether layer was washed with water, dried ($MgSO_4$), and evaporated to give a crystalline residue. Recrystallisation from aqueous methanol gave **13** (5.4 g, 74%), m.p. 86.5–87.5°, $[\alpha]_D^{22}$ +17.5° (*c* 2.0, chloroform); n.m.r. data ($CDCl_3$): δ 4.71 (two multiplets, 4 protons, H-1,1′,6,6′, J_{FH} 47.5 Hz), 5.08 (singlet, 2 protons, –O–CH_2–O–), 6.16 (singlet, 1 proton, benzylic-H).

Anal. Calc. for $C_{14}H_{16}F_2O_4$: C, 58.74; H, 5.59; F, 13.29. Found: C, 58.90; H, 5.81; F, 13.22.

1,6-Dideoxy-1,6-difluoro-2,5-O-methylene-D-mannitol (**10**). — A solution of the benzylidene acetal **13** (2 g) in methanol (35 ml) containing glacial acetic acid (0.5 ml) was shaken with 5% palladium–charcoal (0.75 g) at room temperature under a slight overpressure of hydrogen. On cessation of hydrogen uptake (3 h), the catalyst was removed and the filtrate evaporated. Recrystallisation of the solid residue from ethyl acetate–light petroleum gave **10** (0.7 g, 51%), m.p. 127–128°, $[\alpha]_D^{22}$ −53° (*c* 6.3, water); n.m.r. data (acetone-d_6): δ 4.66 (two multiplets, 4 protons, H-1,1′,6,6′, J_{FH} 47.5 Hz), 4.82 (singlet, 2 protons, –O–CH_2–O–).

Anal. Calc. for $C_7H_{12}F_2O_4$: C, 42.42; H, 6.06; F, 19.15. Found: C, 42.48; H, 5.97; F, 18.88.

2,2′-O-Methylenebis(1-deoxy-1-fluoro-L-glycerol) (**16**). — Aqueous solutions of diol **10** (3.2 g in 150 ml) and sodium metaperiodate (5 g in 150 ml) were cooled (0°), mixed, and kept overnight in the dark at room temperature. A slight excess of aqueous barium chloride was added, and the mixture was allowed to stand at 0° for 1 h and then filtered to remove precipitated barium salts. The strongly reducing filtrate was added to a solution of potassium borohydride (2 g) in 0.2M disodium hydrogen phosphate buffer (100 ml) and kept at room temperature overnight. The mixture was adjusted to pH 6 with glacial acetic acid and deionised by passage through successive columns of Dowex-50W x8(H^+) and Amberlite IR-45 (HO^-) resins. Evaporation of the eluate gave non-reducing, crystalline **16** (2.5 g, 73%) which was chromatographically homogeneous (R_F 0.6; benzene–methanol, 3:1) and was used for further reactions. Recrystallisation from ethyl acetate–light petroleum gave **16** as long needles, m.p. 31–32°, $[\alpha]_D^{22}$ +10.5° (*c* 5.3, methanol); n.m.r. data ($CDCl_3$): δ 4.58 (quartet, 4 protons, H-1,1′, $J_{1,2} = J_{1',2} = 5$ Hz, $J_{F,1} = J_{F,1'} = 45$ Hz); 4.98 (singlet, 2 protons, –O–CH_2–O–).

Anal. Calc. for $C_7H_{14}F_2O_4$: C, 42.0; H, 7.0; F, 19.0. Found: C, 42.14; H, 7.24; F, 19.37.

2,2'-O-Methylenebis(3-O-benzoyl-1-deoxy-1-fluoro-L-glycerol) (**18**). — Conventional esterification of **16** (1.5 g) with benzoyl chloride (2.75 g) and pyridine (15 ml) gave the dibenzoate **18** (1.6 g, 52%), m.p. 74–75° (from ethanol), $[\alpha]_D^{22}$ +44° (*c* 5.0, chloroform); n.m.r. data ($CDCl_3$): δ 4.63 (quartet, 4 protons, H-1,1', $J_{1,2} = J_{1',2} =$ 4.5 Hz, $J_{F,1} = J_{F,1'} = 47.5$ Hz), 5.04 (singlet, 2 protons, –O–CH_2–O–).

Anal. Calc. for $C_{21}H_{22}F_2O_6$: C, 61.77; H, 5.39; F, 9.31. Found: C, 62.05; H, 5.51; F, 9.23.

1-Deoxy-1-fluoro-L-glycerol (**1**). — (*a*) A solution of dibenzoate **18** (1.1 g) in methanolic hydrogen chloride (50 ml, 5% w/w) was boiled under reflux for 2 days and then evaporated. Hydrogen chloride was removed from the syrupy residue, firstly by co-distillation with methanol and then by stirring a methanolic solution of the residue with Amberlite IR-45 (HO^-) resin. The methanol was removed by evaporation, and a solution of the residue in water was washed with chloroform and evaporated to a syrup. Distillation gave compound **1** (0.26 g, 50%), b.p. 58°/0.5 torr, $[\alpha]_D^{22}$ +8.5° (*c* 4.6, water). G.l.c. (at 90°) of the *O*-trimethylsilyl derivative showed that this sample of **1** had the same retention time as that of **1** prepared by the previously described route[3].

(*b*) A solution of diol **16** (0.53 g) in methanolic hydrogen chloride (50 ml, 5% w/w) was boiled under reflux overnight and then evaporated. Hydrogen chloride was removed from the syrupy residue as in (*a*), and the neutral, methanolic solution was evaporated to give compound **1** (0.46 g, 92%) which was identical in all respects with the compound prepared by method (*a*).

1-Deoxy-1-fluoro-L-glycerol 3-phosphate (**4**) *dicyclohexylamine salt*. — (*a*) Dibenzyl phosphorochloridate (1.5 g) was added dropwise to a stirred solution of fluoroglycerol **1** (0.45 g) in pyridine (4 ml) at −40°. The mixture was kept at −20° overnight and then shaken with water (0.2 ml) to destroy any excess of dibenzyl phosphorochloridate. Chloroform was added, and the solution was washed successively with ice-cold M hydrochloric acid, aqueous sodium hydrogen carbonate, and water, dried ($MgSO_4$), and concentrated. Purification by p.l.c. (benzene–methanol, 5:1) gave chromatographically homogeneous (R_F 0.4), syrupy 1-deoxy-1-fluoro-L-glycerol 3-(dibenzyl phosphate) (**5**, 0.9 g, 54%).

A solution of **5** (0.9 g) in ethanol (50 ml) was shaken with 10% palladium–charcoal (0.1 g) under a slight overpressure of hydrogen. On cessation of hydrogen uptake (0.5 h), the catalyst was removed and the filtrate was concentrated to give syrupy **4** (0.45 g, ~100%) which was chromatographically homogeneous by t.l.c. (R_F 0.69; cellulose; propan-1-ol–ammonia–water, 5:4:1). Addition of cyclohexylamine to an ice-cold, ethanolic solution of the acid gave the crystalline dicyclohexylamine salt (0.70 g, 73%), m.p. 171–173°, identical in all respects, except optical rotation, with the racemic compound. It showed a positive, plain o.r.d. curve: $[\alpha]^{25}$ values +13.2° (400 nm), +16.5° (350 nm), +21.5° (320 nm), +26° (300 nm), +50° (250 nm), +170° (200 nm) (*c* 0.5, water).

(*b*) Diphenyl phosphorochloridate (1.1 g) in pyridine (6 ml) was added dropwise to a stirred solution of 2,2'-*O*-methylenebis(1-deoxy-1-fluoro-L-glycerol) (**16**, 0.32 g)

in cooled (0°) pyridine (10 ml) and allowed to stand at 0° overnight. The reaction mixture was worked up as in (*a*) to give a syrup which was purified by p.l.c. (ethyl acetate–light petroleum, 1:1) to give bis(diphenyl phosphate) **19**, which was chromatographically homogeneous by t.l.c. (R_F 0.4; silica gel; ethyl acetate–light petroleum, 1:1).

A solution of compound **19** (0.65 g) in methanol (50 ml) was shaken with Adams' catalyst (0.8 g) at room temperature under a slight overpressure of hydrogen. On cessation of hydrogen uptake (3 h), the catalyst was removed and the filtrate was concentrated to give the syrupy diphosphate **20** (0.36 g), which was chromatographically homogeneous by t.l.c. (cellulose; propan-1-ol–ammonia–water, 5:4:1).

A solution of **20** (0.36 g) in M hydrochloric acid (10 ml) was boiled under reflux overnight and then concentrated to a syrup, and hydrogen chloride was removed from the residue by repeated addition and evaporation of benzene. The resulting, syrupy **4** was characterised as the crystalline dicyclohexylamine salt (0.2 g, 17% from **16**) which was identical in all respects with that prepared by method (*a*).

*1-Deoxy-1-fluoro-*D*-glycerol 3-phosphate* (**5**) *dicyclohexylamine salt.* — 1-Deoxy-1-fluoro-D-glycerol[3] (**2**) was treated as described in (*a*) above for the L enantiomer. The crystalline product **5** had m.p. 171–173° and was identical in all respects, except optical rotation, with the racemic compound. It showed a negative, plain o.r.d. curve of identical magnitude to that obtained for the L enantiomer. Ghangas and Fondy[7] gave m.p. 162–166°, [α] values −5.2° (400 nm), −7.0° (350 nm), and −9.0° (320 nm), for an analytically impure sample of the dicyclohexylamine salt prepared by a similar route.

*1-Deoxy-1-fluoro-*DL*-glycerol 3-phosphate dicyclohexylamine salt.* — 1-Deoxy-1-fluoro-DL-glycerol[8] was treated exactly as for the optically pure enantiomers. The crystalline dicyclohexylamine salt had m.p. 171–173° (lit.[7] 159–163°) and R_F 0.69 (cellulose; propan-1-ol–ammonia–water, 5:4:1).

Anal. Calc. for $C_{15}H_{34}FN_2O_5P$: C, 48.39; H, 9.14; F, 5.11; N, 7.53; P, 8.33. Found: C, 48.29; H, 9.27; F, 5.20; N, 7.32; P, 8.50. Fluorine was determined by the method of Woodward *et al.*[9].

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