Synthesis of [^{3}H]-Labelled and Unlabelled 2-Deoxy-2-fluoro-*myo*-inositol and 1-Deoxy-1-fluoro-*scyllo*-inositol for Use in Studies of the Phosphoinositide Cycle

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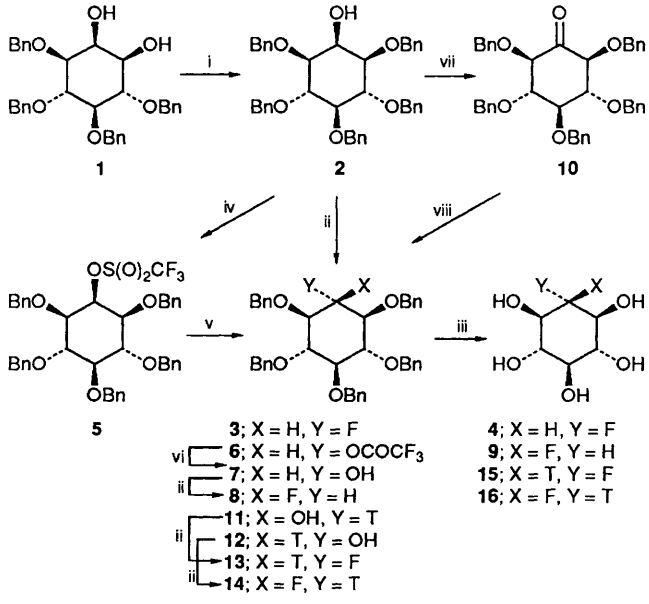
2-Deoxy-2-fluoro-*myo*-inositol and 1-deoxy-1-fluoro-*scyllo*-inositol have been synthesized in their unlabelled and tritiated forms in order to study their potential as inhibitors of the phosphoinositide cycle.

Phospholipase C is a key enzyme of the phosphoinositide cycle and a link in the transmission of some extracellular signals, *e.g.* neurotransmitters, hormones and growth factors, to the cytosol of target cells.[1,2] These molecular signals, when bound to appropriate cell receptors, lead to activation of phospholipase C which hydrolyses the membrane component, phosphatidylinositol, 4,5-bisphosphate [PtdIns(4,5)P_2], to the second messengers inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and the corresponding diacylglycerol (Scheme 1). Since some disease states arise from uncontrolled stimulation of phospholipase C, *e.g.* cell proliferation,[3] we have designed analogues of *myo*-inositol which, if accepted into the phosphoinositide cycle, would lead to analogues of PtdIns(4,5)P_2 which could inhibit the activity of phospholipase C. We reasoned that phospholipase C, a phosphodiesterase, might require the participation of the *cis*-2-hydroxy group on the inositol ring adjacent to the phosphodiester in PtdIns(4,5)P_2 for enzymic activity, by analogy with the mechanism of cleavage of RNA by ribonuclease A.[4] 1-Deoxy-1-fluoro-*scyllo*-inositol **4** and 2-deoxy-2-fluoro-*myo*-inositol **9** were therefore chosen as the target analogues of *myo*-inositol, fluorine being a suitable isostere for a hydroxy group.[5] Since these analogues might also be expected to penetrate the cytoplasmic membrane, if they are accepted into the phosphoinositide cycle they could be capable of controlling the activity of phospholipase C.

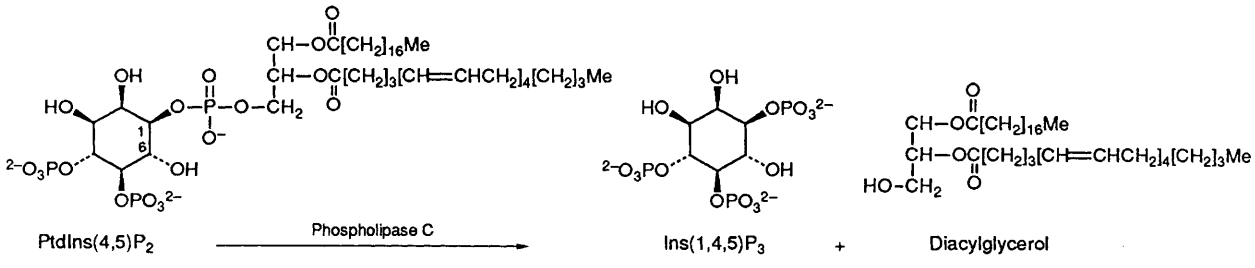
Syntheses of 1-*Deoxy*-1-*fluoro*-scyllo-*inositol* **4** *and* 2-*Deoxy*-2-*fluoro*-myo-*inositol* **9**.—1-Deoxy-1-fluoro-*scyllo*-inositol **4** and 2-deoxy-2-fluoro-*myo*-inositol **9** were synthesized as outlined in Scheme 2. 1,4,5,6-Tetra-*O*-benzyl-*myo*-inositol **1**[6] was converted into 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** (74%) by a modification of the method of Angyal and Tate,[7] in which sodium hydride was used as the activating base. Treatment of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** with (diethylamino)sulphur trifluoride (DAST)[8] led, as expected,[9] to inversion at C-2 to afford 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3**.

Debenzylation of 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3** was achieved cleanly with dry hydrogen bromide dissolved in dichloromethane from which the product precipitated as it was formed. The yield (48%) was not as high as expected but the method resulted in the formation of analytically pure 1-deoxy-1-fluoro-*scyllo*-inositol **4** and was identical to material prepared by an alternative method.[10]



Scheme 2 *Reagents and conditions:* Bn = CH_2Ph; i, NaH, BnBr (1 mol equiv.), benzene, 100 °C, 1.5 h; ii, THF, Et_3N (3 mol equiv.), DAST, 0–25 °C; iii, CH_2Cl_2, HBr, 25 °C, 2 h; iv, CH_2Cl_2, catalytic pyridine, $(CF_3SO_2)_2O$ (1.5 mol equiv.), −60 to 25 °C, 3 h; v, DMF, $NaOCOCF_3$ (4 mol equiv.), 25 °C, 4 h; vi, MeOH, catalytic Na_2CO_3, 30 °C, 1.5 h; vii, acetone, modified Jones' reagent [$Na_2Cr_2O_7$·$2H_2O$ (2.5 mol equiv.), 3 mol dm^{-3} H_2SO_4, water], 25 °C; viii, THF–MeOH (10:1, v/v), $NaBH_3T$ (5–10 Ci $mmol^{-1}$), 25 °C

Fluorination of a compound using DAST generally proceeds with inversion of configuration at the reaction centre.[9] Hence, to obtain a fluorinated *myo*-inositol derivative, the hydroxy group at the reaction centre has to be of the opposite configuration to that required for the fluorinated derivative. It



Scheme 1 Phospholipase C-catalysed hydrolysis of phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] to the second messengers inositol 1,4,5-trisphosphate [Ins(1,4,5)P_3] and the corresponding diacylglycerol

was therefore necessary to convert 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** into 1,2,3,4,5-penta-*O*-benzyl-*scyllo*-inositol **7** for the synthesis of 2-deoxy-2-fluoro-*myo*-inositol **9**. This involved the esterification of the hydroxy group with trifluoromethanesulphonic (triflic) anhydride[11] to give the triflic derivative **5** of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2**. Sodium trifluoroacetate was employed for the S_N2 displacement of the triflate moiety. This resulted in the formation of the trifluoroacetic ester **6** of 1,2,3,4,5-penta-*O*-benzyl-*scyllo*-inositol. Cleavage of the trifluoroacetate with methanol and a catalytic amount of Na_2CO_3 produced a solid which, after recrystallization from methanol, gave 1,2,3,4,5-penta-*O*-benzyl-*scyllo*-inositol **7** (47% overall yield). Fluorination of the protected *scyllo*-inositol **7** with DAST in the presence of triethylamine gave crystalline 1,3,4,5,6-penta-*O*-benzyl-2-deoxy-2-fluoro-*myo*-inositol **8**. Debenzylation of compound **8** with dry hydrogen bromide gave analytically pure 2-deoxy-2-fluoro-*myo*-inositol **9** (51%).

Synthesis of 1-*Deoxy*-1-*fluoro*-scyllo-[1-^{3}H]*inositol* **15** *and* 2-*Deoxy*-2-*fluoro*-myo-[2-^{3}H]*inositol* **16**.—Preliminary kinetic studies with 1-deoxy-1-fluoro-*scyllo*-inositol **4** and 2-deoxy-2-fluoro-*myo*-inositol **9** showed that both compounds inhibited the incorporation of inositol into the phosphoinositide pathway,[12] but in order to establish if they were also substrates and hence incorporated into the pathway, it was necessary to prepare the compounds in radioactive form.

The initial approach was to establish a method for the stereoselective reduction of 2,3,4,5,6-penta-*O*-benzyl-*scyllo*-inosose (2D-2,4,6/3,5-pentabenzyloxycyclohexanone) **10** to give the desired 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2**, since in preliminary biological testing 1-deoxy-1-fluoro-*scyllo*-inositol **4** showed the most potential as an inhibitor of the PtdIns pathway.[12] The reaction with $NaBH_4$, in the solvent system of tetrahydrofuran (THF)–methanol (10:1, v/v), gave 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** as the major product and 1,2,3,4,5-penta-*O*-benzyl-*scyllo*-inositol **7** as the minor product in the ratio 4:1, respectively (overall yield 65%).

2,3,4,5,6-Penta-*O*-benzyl-*scyllo*-inosose **10** was reduced with sodium borotritiide. Analysis (TLC) showed the existence of mainly 1,3,4,5,6-penta-*O*-benzyl-*myo*-[2-^{3}H]inositol **11** and a minor peak corresponding to 1,2,3,4,5-penta-*O*-benzyl-*scyllo*-[6-^{3}H]inositol **12**. Analysis of this mixture, using a radioscanner for detection, showed the two peaks in the ratio ~4:1.

After treatment of the mixture of **11** and **12** with DAST, the products were isolated and purified by both preparative TLC (PLC) and preparative HPLC. The presence of the desired compounds was confirmed by coelution of 'cold' material with the tritiated material on TLC and by mass spectrometric analyses which identified the presence of a common principal ion for both of the major tritiated components. Hence, the most abundant of the tritiated components, which coeluted exactly with 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3** on TLC, was 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]inositol **13** (96% pure) and the other tritiated derivative was 1,3,4,5,6-penta-*O*-benzyl-2-deoxy-2-fluoro-*myo*-[2-^{3}H]inositol **14** (97% pure).

Debenzylation with dry hydrogen bromide gave the required products 1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]inositol **15** and 2-deoxy-2-fluoro-*myo*-[2-^{3}H]inositol **16**. The purity of the two tritiated species was determined by HPLC and this verified that both 1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]inositol **15** and 2-deoxy-2-fluoro-*myo*-[2-^{3}H]inositol **16** were adequately pure for biological testing (>97% pure).

It has been shown that 2-deoxy-2-fluoro-*myo*-[2-^{3}H]inositol (but not 1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]inositol) is accepted by enzymes of the phosphoinoside pathway to give analogues of intermediates in the cycle.[12]

Experimental

M.p.s were recorded on Köfler block or a Büchi 510 and are quoted uncorrected. Mass spectra were recorded on V.G. Micromass 30F, V.G. Masslab 20-250, V.G. analytical 70-250 SEQ and V.G. ZAB1F spectrometers. Desorption chemical ionisation (DCI, NH_3) electron impact (EI) and fast atom bombardment (FAB–MS) techniques were used. IR spectra were recorded on a Perkin-Elmer 1750 FT–IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini 200 operating at 200 MHz and a Brüker WH300 operating at 300 MHz. Tetramethylsilane was used as the external reference or sodium [2,2,3,3-$^2H_4$]-3-(trimethylsilyl)propionate as an internal reference for samples in D_2O. *J* Values are given in Hz.

^{13}C NMR spectra were recorded on a Varian Gemini 200 operating at 50.4 MHz and a Brüker AM500 operating at 126 MHz. Spectra were referenced internally to solvent carbon resonances or 1,4-dioxane (δ = 67.3 ppm) for samples in D_2O. ^{19}F NMR spectra were recorded on the Brüker AM250 operating at 39.2 MHz. The spectra were referenced externally to $CFCl_3$.

Chemicals were obtained from Aldrich Chemical Company Ltd., Gillingham, Dorset, England or from BDH, Poole, Dorset, England.

Solvents were removed using a rotary film evaporator below 40 °C with a water aspirator for volatile solvents or a rotary oil pump for solvents with boiling points greater than 99 °C. Analytical samples were dried over phosphorus pentoxide at approximately 1 mmHg and 20 °C. TLC was performed on Merck D.C.-Alufolien Kieselgel 60F_{254} 0.25 mm precoated plates, silicagel H_{254} coated to 0.2 mm thickness on 5 × 20 cm glass plates or Merck cellulose F coated to 0.1 mm thickness on 5 × 10 cm plates. Spot detection was by UV light fluorescence, iodine vapour, 10% ammonium molybdate in methanol, radiolabel scanner (Berthold L.V. 2832 automatic tlc linear analyser) or 6% ceric ammonium nitrate in 2 mol dm^{-3} HNO_3. PLC was carried out on silicagel (80% HF_{254}–20% G-Blend 41) coated to a maximum of 1 mm thickness on 20 × 20 cm glass plates.

Flash chromatography was carried out on Merck Kieselgel 60 (230–400 mesh) under a slight positive pressure of air.

HPLC was performed with a Waters 600E multisolvent delivery system, a Waters intelligent sample processor model 712 and a Waters 990 photodiode array detector set at a wavelength range, λ 190–300 nm. Also for analytical HPLC, a Beckman Gold analog interface 406, a 114M solvent delivery module with the analytic microflow head, a Beckman 163 variable wavelength detector, a Beckman 171 radioisotope detector, a liquid scintillator flow cell and Beckman Gold software for integrating; for preparative HPLC, a Gilson model 803 (pump and mixer), a Holochrom UV detector and Applemac software were used.

Synthesis of 1-*Deoxy*-1-*fluoro*-scyllo-*inositol* **4**.—*Preparation of* 1,3,4,5,6-*penta*-O-*benzyl*-myo-*inositol* **2**. This was synthesized using a modified version of the method described by Angyal and Tate.[7] (±)-1,4,5,6-Tetra-*O*-benzyl-*myo*-inositol **1** (5 g, 9.26 mmol)[6] was dissolved in dry benzene (93 cm^3) and treated successively with a solution of benzyl bromide (1.1 cm^3, 9.24 mmol) in benzene (9.9 cm^3) and sodium hydride (60%; 3 g). The mixture was stirred under nitrogen for 1.5 h at 100 °C. Analysis of the reaction mixture (TLC; $CHCl_3$) showed the presence of the major product at R_f 0.23 and trace amounts of hexa-*O*-benzylinositol at R_f 0.72. The reaction mixture was cooled with solid CO_2 before dropwise addition of water to destroy the unchanged sodium hydride. The benzene layer was separated and washed with water (3 × 40 cm^3), dried (K_2CO_3), filtered and evaporated to dryness. The residue was triturated with light petroleum (40–60 °C) to dissolve any hexa-*O*-benzyl-*myo*-

inositol. The remaining white powder was filtered off and recrystallised from methanol to give purified 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** (4.41 g, 76%), m.p. 125–127 °C (lit.,[7] 128–129 °C) (Found: C, 78.1; H, 6.7. Calc. for $C_{41}H_{42}O_6$: C, 78.07; H, 6.71%); δ_H(200 MHz; $CDCl_3$) 2.5 (1 H, s, OH), 3.40 [2 H, dd, $J_{1(3),2}$ 2.5, $J_{1(3),6(4)}$ 9.7 1- and 3-H]. 3.50 [1 H, t, $J_{5,6(4)}$ 9.6, 5-H], 4.01 [2 H, t, $J_{4(6),5}$ 9.6, $J_{3(1),4(6)}$ 9.6 4- and 6-H], 4.24 [1 H, t, $J_{2,3(1)}$ 2.6, 2-H], 4.84 (10 H, m, CH_2) and 7.3 (25 H, m, Ph); δ_C(50.4 MHz; $CDCl_3$) 67.54 (1 C, s, C-2), 72.81–76.52 (5 C, 3 × s, CH_2), 79.90 (2 C, s, C-1, -3), 81.29 (2 C, s, C-4, -6), 83.26 (1 C, s, C-5) and 127.83–138.96 (30 C, m, Ph); *m/z* (NH_3) 648 [$(M + 18)^+$, 100%].

Preparation of 2,3,4,5,6-*penta*-O-*benzyl*-1-*deoxy*-1-*fluoro*-scyllo-*inositol* **3**. This was accomplished by using a modified version of the method outlined by Posner and Haines.[8] A solution of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** (0.3 g, 0.476 mmol) in dry THF (10 cm^3) containing triethylamine (1.0 cm^3) was cooled to 0 °C before the addition of DAST (100 mm^3, 0.757 mmol) under argon. The reaction mixture was stirred under argon at 25 °C. Analysis [TLC; $CHCl_3$–hexane (93:7, v/v)] showed the presence of product at R_f 0.36, starting material at R_f 0.15 and an oxidised (diethylamino)sulphur derivative at R_f 0.05. The excess of DAST was destroyed with water (1 cm^3) and the solvents were removed under reduced pressure. The product was extracted with dichloromethane (2 × 50 cm^3) and washed successively with water (50 cm^3), saturated brine (50 cm^3) and again with water (50 cm^3). The organic layer was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure to give an orange gum. The crude product was chromatographed [silica gel, 30 × 200 mm; eluent $CHCl_3$–hexane (9:1)] and recrystallized from methanol to give 2,3,4,5,6-*penta*-O-*benzyl*-1-*deoxy*-1-*fluoro*-scyllo-*inositol* **3** (0.193 g, 48%), m.p. 102–103 °C (Found: C, 78.2; H, 6.8. $C_{41}H_{41}FO_5$ requires C, 77.82; H, 6.53%); δ_H(300 MHz; $CDCl_3$) 3.55 (2 H, m, 2- and 6-H), 3.66 [2 H, t, $J_{2(6),3(5)}$ 8.8, $J_{3(5),4}$ 8.8, 3- and 5-H], 3.71 [1 H, t, $J_{4,5(3)}$ 8.9, 4-H], 4.6 [1 H, dt, $J_{1,F}$ 51.2, $J_{1,2(6)}$ 9.0, 1-H], 4.79–4.93 (10 H, m, CH_2) and 7.26–7.40 (25 H, m, Ph); δ_C(126 MHz; $CDCl_3$) 75.37–75.97 (5 C, 3 × s, CH_2), 81.06 [2 C, d, $J_{2(6),F}$ 17.09, C-2, -6], 81.41 [2 C, d, $J_{3(5),F}$ 11.97, C-3, -5], 82.48 (1 C, s, C-4), 96.51 (1 C, d, $J_{1,F}$ 183.31, C-1) and 127.64–138.42 (30 C, m, Ph); δ_F(39.2 MHz; $CDCl_3$ with reference to $CFCl_3$) −195.22 (1 F, dt, $J_{1-H,F}$ 51.27, $J_{2(6)-H,F}$ 12.58); *m/z* (NH_3) 650 [$(M + 18)^+$, 70%].

A minor fraction from the chromatogram was 1,3,4,5,6-penta-*O*-benzyl-2[(diethylamino)sulphinyl]-*myo*-inositol, *m/z* (NH_3) 750 (M^+, 35%).

Deprotection of 2,3,4,5,6-*penta*-O-*benzyl*-1-*deoxy*-1-*fluoro*-scyllo-*inositol* **3**. This is a modified version of the protocol described by Trudelle and Spach.[13] 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3** (100 mg, 0.158 mmol) was dissolved in dry CH_2Cl_2 (10 cm^3) and $CaCl_2$-dried hydrogen bromide gas was bubbled through the solution for a few minutes. The reaction was stirred under HBr for 2 h at ambient temperature by which time the deprotected product had precipitated out. The solution was diluted with diethyl ether and the white precipiate was filtered off and washed, first with dichloromethane to remove all the benzyl bromide and then with methanol to remove any polar impurities. The washed precipitate was recrystallized from 90% ethanol to give 1-deoxy-1-fluoro-*scyllo*-inositol **4** (13.9 mg, 48%), m.p. 212 °C (decomp.), (lit.,[14] 250–253 °C) (Found: C, 37.1; H, 6.1. $C_6H_{11}FO_5 \cdot \frac{2}{3}H_2O$ requires C, 37.10; H, 6.40%); δ_H(300 MHz; D_2O) 3.23–3.26 (3 H, m, 3-, 4- and 5-H), 3.52 [2 H, ddd, $J_{2(6),F}$ 13.0, $J_{1,2(6)}$ 9.2, $J_{2(6),3(5)}$ 5.9, 2- and 6-H] and 4.14 [1 H, dt, $J_{1,F}$ 52.1, $J_{1,2(6)}$ 9.3, 1-H]; δ_C(126 MHz; D_2O) 72.54 [2 C, d, $J_{2(6),F}$ 17.6, C-2, -6], 73.24 [2 C, d, $J_{3(5),F}$ 12.2, C-3, -5], 74.09 (1 C, s, C-5) and 95.31 (1 C, d, $J_{1,F}$ 177.8, C-1); δ_F(39.2 MHz; D_2O) −219.6 (1 F, dt, $J_{1-H,F}$ 52.06, $J_{2(6)-H,F}$ 13.06); *m/z* (NH_3) 200 [$(M + 18)^+$, 100%].

Synthesis of 2-*Deoxy*-2-*fluoro*-myo-*inositol* **9**.—*Preparation of* 1,2,3,4,5-*penta*-O-*benzyl*-scyllo-inositol **7**. (a) *Preparation of a triflate derivative of* 1,3,4,5,6-*penta*-O-*benzyl*-myo-*inositol* **2**. This was based on the procedure described by Kimmich and Voelter.[11] A solution of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** (635 mg, 1.008 mmol) in dry CH_2Cl_2 (10 cm^3) containing dry pyridine (400 mm^3) was cooled to −60 °C before the dropwise addition of triflic anhydride (300 mm^3, 1.785 mmol). The reaction mixture was allowed to warm up to ambient temperature and was then kept for 2 h. Analysis (TLC; $CHCl_3$) showed the presence of product at R_f 0.57. The reaction mixture was quenched by the addition of water (4 cm^3), diluted with CH_2Cl_2 (10 cm^3) and washed successively with saturated aq. $NaHCO_3$, water and brine. The organic layer was dried and the solvents were removed under reduced pressure to leave compound **5** as an orange syrup; δ_H(200 MHz; $CDCl_3$) 3.48 [1 H, t, $J_{5,6(4)}$ 9.4, 5-H], 3.51 [2 H, dd, $J_{1(3),2}$ 1.9, $J_{1(3),6(4)}$ 9.3, 1- and 3-H], 3.38 [2 H, t, $J_{4(6),5}$ 9.3, $J_{3(1),4(6)}$ 9.3, 4-and 6-H] and 5.30 [1 H, t, $J_{1(3),2}$ 2.0, 2-H]; δ_F(39.2 MHz; $CDCl_3$ with reference to $CFCl_3$) −75.96 (3 F, s, OSO_2CF_3) and −80.22 [3 F, s, $(CF_3SO_2O)_2O$]; *m/z* (NH_3) 780 [$(M + 18)^+$, 15%].

(b) *Preparation of a trifluoroacetate derivative* **6** *of* 1,2,3,4,5-*penta*-O-*benzyl*-scyllo-*inositol*. The triflate derivative of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** (0.72 g, 0.945 mmol) and sodium trifluoroacetate (0.5 g, 3.676 mmol) were dissolved in dry dimethylformamide (DMF) (6 cm^3) and stirred under nitrogen at ambient temperature. The reaction mixture changed colour from orange-red to red-brown, and after 48 h the reaction mixture was pale yellow. Analysis of this material (TLC; $CHCl_3$) showed the presence of product at R_f 0.47. Water (5 cm^3) was added and the product was extracted in $CHCl_3$ (2 × 25 cm^3), washed with water (2 × 25 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness under reduced pressure to leave the product **6**; δ_H(200 MHz; $CDCl_3$) 3.56–3.74 (6 H, m, inositol ring H_5), 4.60–4.98 (10 H, m, CH_2) and 7.1–7.4 (25 H, m, Ph); *m/z* (NH_3) 744 [$(M + 18)^+$, 80%].

(c) *Preparation of* 1,2,3,4,5-*penta*-O-*benzyl*-scyllo-*inositol* **7**. A solution of compound **6** in methanol (30 cm^3) was treated with trace amounts of Na_2CO_3 and was stirred overnight at ambient temperature. The solvent was removed under reduced pressure and the crude product was extracted in boiling hexane (100 cm^3). The mixture was filtered and the solvent was removed under reduced pressure to leave a yellow solid, which was recrystallized from methanol to give 1,2,3,4,5-*penta*-O-*benzyl*-scyllo-*inositol* **7** [overall yield (for the three steps) 0.297 g, 47%], m.p. 108–109 °C (Found: C, 78.1; H, 6.9. $C_{41}H_{42}O_6$ requires C, 78.07; H, 6.71%); δ_H(200 MHz; $CDCl_3$) 2.63 (1 H, s, OH), 3.56 [2 H, dd, $J_{1,2(6)}$ 8.0, $J_{2(6),3(5)}$ 8.9, 1- and 5-H], 3.66–3.79 (3 H, m, 2-, 3-, 4-and 6-H), 4.99 (10 H, m, CH_2) and 7.37–7.48 (25 H, m, Ph); δ_C(126 MHz; $CDCl_3$) 74.50 (1 C, s, C-6), 75.47–75.86 (5 C, 3 × s, CH_2), 82.50 (2 C, s, C-1, -5), 82.82 (2 C, s, C-2, -4), 83.21 (1 C, s, C-3) and 127.59–138.58 (30 C, m, Ph); *m/z* (NH_3) 648 [$(M + 18)^+$, 40%].

Preparation of 1,3,4,5,6-*penta*-O-*benzyl*-2-*deoxy*-2-*fluoro*-myo-*inositol* **8**. This was prepared by using a modified version of the method described by Posner and Haines.[8] A solution of 1,2,3,4,5-penta-*O*-benzyl-*scyllo*-inositol **7** (0.4 g, 0.635 mmol) in dry THF (13 cm^3) containing dry triethylamine (1.3 cm^3) was cooled to 0 °C before the addition of DAST (140 mm^3, 1.06 mmol) under nitrogen. The reaction mixture was stirred under nitrogen overnight at 25 °C. Analysis of the reaction mixture [TLC; $CHCl_2$–hexane (97:3, v/v)] showed the presence of product at R_f 0.3 and starting material at R_f 0.10. The work-up procedure was equivalent to the protocol described for the preparation of compound **3** and yielded 1,3,4,5,6-*penta*-O-

benzyl-2-*deoxy*-2-*fluoro*-myo-*inositol* **8** (150 mg, 37%), m.p. 159 °C (Found: C, 77.95; H, 6.9. $C_{41}H_{41}FO_5$ requires C, 77.82; H, 6.54%); δ_H(300 MHz; $CDCl_3$) 3.38 [2 H, ddd, $J_{1(3),F}$ 28.8, $J_{1(3),2}$ 1.9, $J_{1(3),6(4)}$ 9.8, 1- and 3-H], 3.5 [1 H, t, $J_{4(6),5}$ 9.4, 5-H], 3.97 [2 H, t, $J_{3(1),4(6)}$ 9.7, $J_{4(6),5}$ 9.7, 4- and 6-H], 4.88 [1 H, dt, $J_{2,F}$ 52.1, $J_{1(3),2}$ 1.9, 2-H], 4.71–4.97 (10 H, m, CH_2) and 7.27–7.36 (25 H, m Ph); δ_C(126 MHz; $CDCl_3$) 72.85–76.76 (5 C, 3 × s, CH_2), 78.53 [2 C, d, $J_{1(3),F}$ 17.5, C-1 and -3], 81.04 [2 C, d, $J_{4(6),F}$ 3.5, C-4 and -6], 83.00 (1 C, s, C-5), 88.12 (1 C, d, $J_{2,F}$ 180.0, C-2) and 127.61–138.60 (30 C, m, Ph); δ_F(39.2 MHz; $CDCl_3$ with reference to $CFCl_3$) −213.62 (1 F, dt, $J_{2-H,F}$ 52.2, $J_{1-H,F}$ 28.7); *m*/*z* (NH_3) 650 [$(M + 18)^+$, 80%].

Preparation of 2-*deoxy*-2-*fluoro*-myo-*inositol* **9**. This was prepared by using a modified version of the protocol described by Trudelle and Spach.[13] In this experiment, a solution of compound **8** (80 mg, 0.127 mmol) in CH_2Cl_2 (8 cm^3) was saturated with $CaCl_2$–dried HBr gas and stirred for 4 h at ambient temperature. The crude product obtained from this reaction was recrystallized from methanol–water (~4:1; v/v) to yield 2-*deoxy*-2-*fluoro*-myo-*inositol* **9** (11.8 mg, 51%), m.p. 223 °C (Found: C, 38.45; H, 6.9. $C_6H_{11}FO_5 \cdot \frac{1}{4}H_2O \cdot CH_3OH$ requires C, 38.45; H, 7.14%); δ_H(300 MHz; D_2O) 3.16 [1 H, t, $J_{5,6(4)}$ 8.9, 5-H], 3.47 [2 H, t, $J_{3(1),4(6)}$ 10.2, $J_{4(6),5}$ 10.2, 4- and 6-H], 3.49 [2 H, ddd, $J_{1(3),F}$ 33.2, $J_{1(3),6(4)}$ 10.0, $J_{1(3),2}$ 1.9, 1- and 3-H] and 4.75 [1 H, dt, $J_{2,F}$ 52.1, $J_{2,3(1)}$ 1.9, 2-H]; δ_C(126 MHz; D_2O) 70.09 [2 C, d, $J_{4(6),F}$ 17.0, C-4, -6], 72.26 (1 C, s, C-5), 73.32 [2 C, d, $J_{1(3),F}$ 30.7, C-1, -3] and 94.07 (1 C, d, $J_{2,F}$ 175.8, C-2); δ_F(39.2 MHz; D_2O with reference to $CFCl_3$) −216.17 [1 F, dt, $J_{2-H,F}$ 51.8, $J_{1-H(3),F}$ 33.8]; *m*/*z* (NH_3) 200 [$(M + 18)^+$, 100%].

Syntheses of 1-*Deoxy*-1-*fluoro*-scyllo-[1-^{3}H]*inositol* **15** *and* 2-*Deoxy*-2-*fluoro*-*myo*-[2-^{3}H]*inositol* **16**.—*Preparation of* 2,3,4,5,6-*penta*-O-*benzyl*-scyllo-*inosose* **10**. This was synthesized by an adaptation of the method described by Liotta *et al.*[15] A solution of 1,3,4,5,6-penta-*O*-benzyl-*myo*-inositol **2** (0.6 g, 0.95 mmol) in acetone (5 cm^3) was cooled to 0 °C before the dropwise addition of modified Jones' reagent [sodium dichromate dihydrate (0.7 g, 2.35 mmol), water (1 cm^3) and H_2SO_4 (0.45 cm^3)]. The reaction mixture was allowed to warm up to ambient temperature and was stirred until the red-orange reagent gradually turned green. Analysis (TLC; $CHCl_3$) showed the presence of the product at R_f 0.58. Water (20 cm^3) was added to the reaction mixture to solubilise the Cr^{3+} salts whilst the product was extracted in dichloromethane (2 × 20 cm^3). The dichloromethane layer was washed with saturated aq. $NaHCO_3$ (20 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness under reduced pressure to give a residue. The crude product was recrystallized from ethyl acetate to give 2,3,4,5-penta-*O*-benzyl-*scyllo*-inosose **10** (0.445 g, 74%), m.p. 160 °C (lit.,[7] 163–164 °C) (Found: C, 78.2; H, 6.4. Calc. for $C_{41}H_{40}O_6$: C, 78.32; H, 6.41%); ν_{max}($CHCl_3$)/cm^{-1} 1745 (CO); δ_H(300 MHz; $CDCl_3$) 3.63 [2 H, t, $J_{3(5),4}$ 9.4, $J_{2(6),4(5)}$ 9.4, 3- and 5-H], 3.88 [1 H, t, $J_{4,5(3)}$ 9.2, 4-H], 4.16 [2 H, d, $J_{2(6),3(5)}$ 9.7, 2- and 6-H], 4.54–4.94 (10 H, m, CH_2) and 7.35 (25 H, m, Ph); *m*/*z* (NH_3) 646 [$(M + 18)^+$, 30%].

Reduction of 2,3,4,5,6-*penta*-O-*benzyl*-scyllo-*inosose* **10**. The method described by Varma and Kabalica was used.[16] 2,3,4,5,6-Penta-*O*-benzyl-*scyllo*-inosose **10** (50 mg, 0.080 mmol) was dissolved in THF–methanol (10:1, v/v; 5 cm^3) and $NaBH_4$ (7 mg) was added in portions with constant stirring of the reaction mixture. The mixture was kept for 20 min at ambient temperature. Analysis (TLC; $CHCl_3$) showed the presence of a major peak at R_f 0.23 and a minor peak at R_f 0.17. The reaction was quenched with ice-cold brine (1 cm^3) and the product was extracted with dichloromethane (3 × 2 cm^3), then these fractions were washed with brine (3 × 2 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness. Analysis of the crude material (^{1}H NMR) showed that the main product was the penta-*O*-benzyl-*myo*-inositol **5** and the minor product was penta-*O*-benzyl-*scyllo*-inositol **7** in the ratio of ~4:1, respectively, in 65% overall yield.

Preparation of 1,3,4,5,6-*penta*-O-*benzyl*-myo-[2-^{3}H]*inositol* **11**. 2,3,4,5,6-Penta-*O*-benzyl-*scyllo*-inosose **10** (100 mg, 0.159 mmol) was dissolved in THF–methanol (10:1, v/v; 3 cm^3 from 5 cm^3) and the remainder of the solution (in three washes) was used to form a suspension with $NaBH_3T$ (5–10 Ci $mmol^{-1}$; 100 mCi). This suspension was carefully pipetted dropwise into the stirred solution. The mixture was stirred for 4 h before the addition of $NaBH_4$ (10 mg) and was then stirred overnight. Analysis (TLC) showed a major peak corresponding to the title compound **11** and minor peaks corresponding to starting material and 2,3,4,5,6-penta-*O*-benzyl-*scyllo*-[1-^{3}H]inositol **12**. The unchanged $NaBH_4$ was destroyed with ice-cold brine (1 cm^3) and the solvents were removed under reduced pressure. The residue was extracted with dichloromethane (2 × 5 cm^3) and washed with ice-cold brine (3 × 5 cm^3). The extract was dried ($MgSO_4$), filtered and evaporated to dryness. The residue was used in the next preparation.

Preparation of 2,3,4,5,6-*penta*-O-*benzyl*-1-*deoxy*-1-*fluoro*-scyllo-[1-^{3}H]*inositol* **13** *and* 1,3,4,5,6-*penta*-O-*benzyl*-2-*deoxy*-2-*fluoro*-myo[2-^{3}H]*inositol* **14**. The above dried residue was dissolved in dry THF (4 cm^3) containing dry triethylamine (0.1 cm^3) and the solution was cooled to 0 °C under a stream of nitrogen before the addition of DAST (100 mm^3, 0.767 mmol). The reaction mixture was stirred overnight at ambient temperature under nitrogen. Analysis (TLC; $CHCl_3$) showed the presence of product at R_f 0.63. The product was isolated by elution of the mixture through a column of silica gel with CH_2Cl_2–hexane (9:1, v/v). Analysis [TLC; $CHCl_3$–hexane (97:3, v/v)] on silica plates (50 × 200 mm), using a radiolabel scanner, showed the presence of two bands in the ratio of 77:23. A portion of the sample was purified on a preparative silica plate (20 × 20 cm) and run three times in a solvent system of $CHCl_3$–hexane (1:1, v/v) by which time three bands were evident by UV. The least polar band was not radioactive and was unchanged 2,3,4,5,6-penta-*O*-benzyl-*scyllo*-inosose **10**. The other two, radioactive, bands were isolated on a preparative plate and each of the products was extracted from the silica by thorough washing with CH_2Cl_2 (4 × 10 cm^3). The solutions were filtered and evaporated to dryness; a peak with *m*/*z* (EI) 541 ($M^+ - CH_2Ph$, 100%) was obtained for both products. An accumulated scan across the molecular-ion region gave *m*/*z* 632 (M^+) but the peak was too weak to quantify; this was also the case for the unlabelled 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-inositol **3**. Compound **3** coeluted with the major 'hot' material on TLC [$CHCl_3$–hexane (97:3, v/v)]. From the mass spectral and TLC data of the two tritiated products, it was concluded that the less polar and more abundant material was 2,3,4,5,6-penta-*O*-benzyl-1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]-inositol **13**, whilst the more polar of the two was 1,3,4,5,6-penta-*O*-benzyl-2-deoxy-2-fluoro-*myo*-[2-^{3}H]inositol **14**.

A portion of the crude material was also purified by HPLC on a preparative column (Ultrasphere silica column, 5 μ; 4.6 × 250 mm). A gradient was set up using the solvents $CHCl_3$ and hexane. The conditions were: 0–10 min, 70–50% hexane; 10–20 min, 50% hexane; 20–30 min, 50–0% hexane; 30–35 min, 0–70% hexane. The isolated products were tested for their purity on an analytical HPLC column (Ultrasphere silica column, 5μ; 4.6 × 250 mm). Compound **13** was found to be 96% pure while compound **14** was 97% pure.

Preparation of 1-*deoxy*-1-*fluoro*-scyllo-[1-^{3}H]*inositol* **15** *and* 2-*deoxy*-2-*fluoro*-myo-[2-^{3}H]*inositol* **16**. Compounds **13** and **14** were dissolved separately in dry CH_2Cl_2 (2 cm^3) and dry HBr gas was bubbled through the solutions for 2 min. The solution was stirred for 4 h at ambient temperature under HBr

gas. Diethyl ether (1 cm^3) was added to the reaction mixture and the product was extracted with water (2 cm^3). The aq. extract was separated from the organic layer using a pipette. The organic layer was washed three times with water (2 cm^3) to be certain of complete extraction of the product into the aq. layer. The combined aq. layers were then washed with CH_2Cl_2 (3 × 2 cm^3) and evaporated to dryness under reduced pressure. The residue was dissolved in water (2 cm^3) and filtered (pore size 0.44 μm; nylon 66 Rainin filter) into a sealable bottle to which was added ethanol (2 cm^3) to prolong storage of the deprotected tritiated compounds.

For analysis of the tritiated fluoroinositol derivatives, mass spectra (FAB-MS) showed that the appropriate molecular ion was present in both samples. Analysis by TLC [PhOH–water (10:3, v/v)] showed the presence of 1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]inositol **15** at R_f 0.34 and 2-deoxy-2-fluoro-*myo*-[2-^{3}H]inositol **16** at R_f 0.29. The purity of these tritiated fluoroinositol derivatives was tested on an analytical HPLC column [Technicol cyclobond III(α), 4.6 × 250 mm]. A gradient was set up with acetonitrile and water. The conditions were: 20 min, 95–80% acetonitrile; 5 min, 80% acetonitrile; 5 min, 80–70% acetonitrile; 1 min, 70–50% acetonitrile; 10 min, 50% acetonitrile; 1 min, 50–95% acetonitrile. The faster eluting 1-deoxy-1-fluoro-*scyllo*-[1-^{3}H]inositol **15** peaked after 23.3 min, with 97.5% purity, and the more polar 2-deoxy-2-fluoro-*myo*-[2-^{3}H] inositol **16** after 26.5 min, with 98.4% purity.

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