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Automated Radiosynthesis of No-carrier-added [*S-fluoromethyl*-^{18}F]Fluticasone Propionate as a Radiotracer for Lung Deposition Studies with PET

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Abstract. Fluticasone propionate [(*S*)-fluoromethyl-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)-androsta-1,4-diene-17β-carbothioate; FP] is a potent anti-inflammatory steroid with several therapeutic indications, including use as an anti-asthmatic drug when administered as sized particles by inhalation from a pressurised metered-dose inhaler (pMDI). FP was successfully labelled with fluorine-18 ($t_{1/2}$ = 109.6 min; β^+ = 100%) by displacement of tosylate with cyclotron-produced no-carrier-added [^{18}F]fluoride in an (*S*)-tosylmethyl precursor prepared from the known (*S*)-chloromethyl analogue of FP. Radiochemically pure [*S-fluoromethyl*-^{18}F]FP was separated by reverse phase HPLC in 35% radiochemical yield (decay-corrected) within 80 min from the end of radionuclide production (as verified by, radio-HPLC, LC-MS and LC-NMR). The radiosynthesis was automated for the safe production of high radioactivities (20–50 mCi) of [^{18}F]FP in a lead-shielded hot-cell for subsequent incorporation into formulated FP particles within a pMDI and subsequent study of FP deposition in human lung using positron emission tomography (PET).

Keywords: [^{18}F]Fluticasone propionate; fluorine-18; steroid; nucleophilic substitution; no carrier-added.

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Introduction

The semi-synthetic steroid, fluticasone propionate [(*S*)-fluoromethyl-6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)androsta-1,4-diene-17β-carbothioate; FP (1)] behaves as a high affinity agonist at the human glucocorticoid receptor (1) and is a potent anti-inflammatory agent *in vivo* with several therapeutic indications, including use as an anti-asthmatic when administered as sized particles by inhalation from a pressurised metered-dose inhaler (pMDI) (Flixotide™) (2–4). Further information on the deposition of FP particles within the human lung and subsequent drug pharmacokinetics would be valuable for understanding clinical efficacy (3).

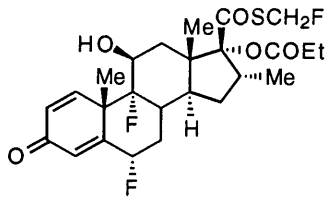

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Figure 1. Structure of fluticasone propionate.

Positron emission tomography (PET) is a quantitative imaging technique, with high sensitivity for the absolute measurement of radioactivity (positron-emitter) concentration *in vivo*, high temporal resolution (a few seconds) and useful spatial resolution (*ca* 4 mm FWHM). PET, in combination with positron-emitting tracers, has now become a powerful technique for the assessment of pharmacokinetics in humans (5). Moreover, modern PET cameras have a long axial field of view, enabling a high proportion of the human thorax to be imaged. PET would therefore be applicable to the study of the fate of inhaled FP particles in human lung, provided that the drug can be labelled isotopically with a positron-emitter, such as fluorine-18 ($t_{1/2}$ = 109.6 min), and used to label FP particles uniformly within a pMDI. Thus, it was of interest to develop a rapid method for labelling FP (1) in high radioactivity and radiochemical purity. The method also would need to be applicable at the no-carrier-added (NCA) level of specific radioactivity to facilitate incorporation of the radiotracer into a pMDI of Flixotide™. It would also have to be amenable to automated control within a shielded enclosure for radiation safety. Here we describe the successful development of such a method.

Experimental and Results

Chemicals

FP (1) and cloticasone propionate (CP) (2; Figure 6) were synthesised as described previously (6). The 17β-carboxy-17α-hydroxy epoxy compound (3) was available from Glaxo Wellcome. Silver(I) *p*-toluenesulphonate, silver(I) trifluoromethanesulphonate, silver(I) oxide and Amberlyst® 15 were purchased from Lancaster Synthesis Ltd. Acetonitrile, dichloromethane, DMF (dimethylformamide), heptane, THF (tetrahydrofuran), isopropanol and ethyl acetate were purchased from BDH Laboratory Supplies Ltd. Dowex® 50, boron tribromide, 4,7,13,16,21,24-hexaoxa-1,10-diazacyclo[8.8.8.]hexacosane (aminopolyether 2.2.2; Kryptofix® 2.2.2; APE 2.2.2) and other chemicals were purchased from Aldrich Chemical Co. Ltd. Silver(I) methanesulphonate was prepared from silver(I) oxide and methanesulphonic acid.

General Methods

Melting points were uncorrected. Elemental analyses were carried out using Carlo Erba 1106 and Perkin-Elmer 240C microanalysers. Merck silica gel Kieselgel 60 (9385) was used throughout for flash column chromatography. ^{1}H-Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC250, Varian XL200 and Varian Unity 400 MHz spectrometers. ^{13}C-NMR spectra were recorded on Bruker AC250 and Varian Unity 400 spectrometers. Infra red (IR) spectra were recorded on a Nicolet 20SXB FT-IR spectrometer. Low and high resolution mass spectrometry (MS) were performed using Finnigan 4600 quadrupole and VG Autospec instruments, respectively. Radioactivity was generally measured in calibrated ionisation chambers (IG12). Measurements were corrected for background radioactivity and decay-corrected to a set time point in each experiement.

HPLC-MS

Separation was performed by HPLC (high performance liquid chromatography) on an Inertsil column (150 x 2.1 mm o.d.) eluted with acetonitrile-water (11:9 v/v) at 0.3 mL/min. FP (1) had a retention time of 8.0 min. The output was analyzed by ESP ionisation with a TSQ-700 mass spectrometer.

HPLC-NMR

Separation was performed using a reverse phase C18 column (250 x 4.6 mm o.d.) eluted with acetonitrile-[$^{2}H_2$]water (11:9 v/v) at 0.3 mL/min. NMR was performed on a Bruker AMX600 spectrometer.

Production of NCA K+-APE 2.2.2-[^{18}F]fluoride

NCA [^{18}F]fluoride (typical specific radioactivity, > 1 Ci/μmol) was produced on a Scanditronix Mk II cyclotron by the ^{18}O(p,n)^{18}F reaction in ^{18}O-enriched water (20–90 atom %) (7). The [^{18}F]fluoride 9(≤ 400 mCi) was recovered from the ^{18}O-enriched water onto an anion exchange column (28 x 3 mm i.d. mm; Dowex AG1X8, 100-200 mesh; Biorad) in the carbonate form, essentially as described previously (8). The [^{18}F]fluoride was eluted with potassium carbonate solution (33.3 mM; 1 mL), and then converted within a glassy carbon vessel (9) into dry K+-APE 2.2.2-[^{18}F]fluoride/carbonate by the addition of APE 2.2.2 (26 mg, 0.069 mmol) and the removal of water by repeated addition and evaporation of acetonitrile at 110°C under nitrogen.

Alternatively, the [^{18}F]fluoride was obtained as K+-APE 2.2.2-[^{18}F]fluoride/iodide by eluting [^{18}F]fluoride from the anion exchange column with potassium iodide solution (16.6 mM; 1 mL), adding APE 2.2.2 (7 mg, 0.019 mmol) and removing the water by repeated addition and evaporation of acetonitrile at 110°C under nitrogen.

Nitrogen-13 ($t_{1/2}$ = 9.96 min) is co-produced by the ^{16}O(p,α)^{13}N reaction as a significant proportion of the total initial radioactivity. Therefore, fluorine-18 radioactivity was measured after separation from nitrogen-13 (on the ion retardation resin) or after nitrogen-13 radioactivity had been allowed to decay to a relatively insignificant level.

Analytical radio-HPLC

Analytical radio-HPLC was performed on either *i*) a Primesphere C18-HC column (5 μ; 250 x 4.6 mm o.d.) eluted with acetonitrile-water (9:11 v/v) at 2 mL/min (retention time for 1, 30.0 min) (Method 1) or *ii*) a Supelcol Diol column (5 μ, 250 x 4.6 mm o.d.) eluted with heptane-isopropanol (19:1 v/v) at 1.8 mL/min (retention time for 1, 24.3 min) (Method 2). The eluate was monitored for absorbance at 254 nm (Gilson 112 detector; Anachem Ltd) or at 255 nm (LC 235 diode array detector; Perkin Elmer Ltd). Radioactivity was detected by a sodium iodide crystal (20 mm diameter; Bioscan™; Lablogic Ltd) in series with the absorbance detector. Data from the absorbance and radioactivity detectors were acquired simultaneously and analyzed with a Turbochrom™ 3 system (Perkin Elmer Ltd). The analytical methods were calibrated for mass of FP (1) by injections of known amounts.

Synthesis of 6α,9β,11β,16α,17β-9,11-epoxy-6-fluoro-17-hydroxy-16-methyl-3-oxo-androsta-1,4-diene-17-carbothioic acid (4)

To a stirred solution of the 17β-carboxyl-17α-hydroxy compound 3 (6.278 g, 16.68 mmol) in DMF (70 mL) at 20°C, was added 1,1'-carbonyldiimidazole (5.409 g, 33.35 mmol). The mixture was stirred under nitrogen for 30 min giving a clear solution which was then cooled to 10°C. Hydrogen sulphide

was passed through the solution for 15 min and the resulting clear, green solution stirred at 10°C for 90 min. The solution was added over 5 min to stirred 2M-hydrochloric acid (250 mL) at 10°C whereupon an amorphous solid precipitated. This was isolated by filtration, washed with water (200 mL) then dried for 16 h at 60 °C *in vacuo* giving crude product (6.527 g, 99.7%). A portion (130 mg) was recrystallised from acetone-water (1:1, 30 mL) giving the *title compound* (88 mg, 67.7%). ν_{max} (nujol mull)/cm^{-1} 3311 (OH), 1694 (thioacid C=O), 1666 (C3 C=O); δ_H (400 MHz, DMSO-d_6) 6.59 (1H, d, C1 C<u>H</u>=), 6.18 (1H, s, C4 C<u>H</u>=) 6.15 (1H, d, C2 C<u>H</u>=), 5.68 (1H, m, C6 C<u>H</u>) 3.32 (1H, s, C11 C<u>H</u>), 2.87 (1H, m, C16 C<u>H</u>), 1.38 (3H, s, C19 CH_3), 0.86 (3H, s, C18 CH_3); m/z (thermospray +ve) 410 (MNH_4^+), 393 (MH^+).

Synthesis of 6α,9β,11β,16α,17β-9,11-epoxy-6-fluoro-16-methyl-3-oxo-17-propionyloxy-androsta-1,4-diene-17-carbothioic acid (<u>5</u>)

To a stirred solution of <u>4</u> (5.90 g, 15.03 mmol) in acetone (75 mL) at 5°C under nitrogen were added triethylamine (7.115 g, 70.6 mmol) and propionyl chloride (5.56 g, 60.1 mmol). The mixture was stirred at 5°C for 45 min. Diethylamine (5.50 g, 75.15 mmol) was added and the mixture stirred at 20°C for 90 min then poured into stirred 2M-hydrochloric acid (250 mL) at 10°C. After 10 min, further water (200 mL) was added and the amorphous precipitate isolated by filtration, washed with water (200 mL) and dried for 60°C *in vacuo* giving the *title compound* as an unstable solid (6.21 g, 92.2%) which was quickly converted into <u>6</u>. ν_{max} (Nujol mull)/cm^{-1} 1747 (ester C=O), 1700 (thioacid C=O), 1669 (C3 C=O). δ_H (250 MHz, $CDCl_3$) 6.57 (1H, d, C1 C<u>H</u>=), 6.49 (1H, s, C4 C<u>H</u>=) 6.28 (1H, d, C2 C<u>H</u>=), 5.48 (1H, m, C6 C<u>H</u>) 3.38 (1H, s, C11 C<u>H</u>), 2.41 (2H, q, C<u>H</u>$_2$CH$_3$), 1.42 (3H, s, C19 C<u>H</u>$_3$), 1.12 (3H, t, CH$_2$C<u>H</u>$_3$).

Synthesis of 6α,9β,11β,16α,17β-9,11-epoxy-6-fluoro-16-methyl-3-oxo-17-propionyloxy-androsta-1,4-diene-17-carbothioic acid S-fluoromethyl ester (<u>6</u>)

To a stirred solution of <u>5</u> (1.522 g, 3.39 mmol) in DMF (5 mL) was added potassium hydrogen carbonate (0.380 g, 1.11 mmol). The mixture was cooled to −15°C under nitrogen. Bromofluoromethane (0.498 g, 0.25 mL, 4.40 mmol) was added in a single charge and the mixture stirred at −15°C for 40 min then added to a stirred mixture of 2M-hydrochloric acid (50 mL) at −10°C. The mixture was stirred at −10°C for 5 min and the solid product isolated by filtration, washed with water (50 mL) then dried for 3 h at 50°C *in vacuo* giving crude product. This was purified chromatographically on silica (80 g) eluted with dichloromethane: ethyl acetate (10:1). The resulting foam was dissolved in diethyl ether (100 mL) and the solvent removed by evaporation under reduced pressure to give a concentrate (*ca* 20 ml) from which product began to crystallise. Cyclohexane (20

mL) was added and remaining diethyl ether removed by evaporation. The precipitate was isolated by filtration, washed with cyclohexane (5 mL) and dried *in vacuo* giving the *title compound* (0.721 g, 44.2%). ν_{max} (nujol mull)/cm^{-1} 1744 (ester C=O), 1710 (thioester C=O), 1666 (C3 C=O) 983 (epoxide). δ_H (400 MHz, $CDCl_3$) 6.53 (1H, d, C1 C<u>H</u>=), 6.47 (1H, s, C4 C<u>H</u>=) 6.27 (1H, d, C2 C<u>H</u>=), 5.85 (2H, m, SC<u>H</u>$_2$F) 5.45 (1H, m, C6 C<u>H</u>F), 3.32 (1H, s, C11 C<u>H</u>OH), 2.67 (1H, m, C16 C<u>H</u>CH$_3$), 2.37 (2H, q, C<u>H</u>$_2$CH$_3$), 1.57 (3H, s, C19 C<u>H</u>$_3$), 1.13 (3H, t, CH$_2$C<u>H</u>$_3$), 1.00 (3H, s, C18 C<u>H</u>$_3$), 0.95 (3H, d, C16 C<u>H</u>$_3$); δ_C (100 MHz, DMSO-d_6) 192.0 (<u>C</u>OS), 184.5 (C3), 172.0 (O<u>C</u>OC$_2$H$_5$), 159.2 (d, C5), 150.2 (d, C1), 127.9 (d, C2), 121.3 (d, C4), 95.4 (C17), 85.5 (d C6), 80.1 (d, <u>C</u>H$_2$F), 64.9 (C9), 62.3 (C11), 48.2 (C14), 47.3 (C13), 42.9 (d, C10), 37.3 (d, C7), 35.3 (C16), 34.2 (C12), 32.5 (d, C8), 30.1 (C15), 26.8 (<u>C</u>H$_2$CH$_3$), 22.2 (C19), 16.5 (C16 <u>C</u>H$_3$), 16.4 (C18), 8.4 (CH$_2$<u>C</u>H$_3$); m/z (thermospray +ve) 503 (MNa^+), 481 (MH^+). (Found: C, 62.7; H, 6.3; F, 7.9; S, 6.7%. $C_{25}H_{30}F_2O_5S$ requires: C, 62.5; H, 6.3; F, 7.9; S, 6.7%).

Synthesis of (S)-iodomethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)androsta-1,4-diene-17β-carbothioate (<u>8</u>)

A stirred mixture of <u>3</u> (1.17 g,; 2.25 mmol) and sodium iodide (6.754 g; 45.06 mmol) in acetone (30 mL) was heated at reflux for 2 h. TLC (silica; chloroform-ethyl acetate, 3:1 v/v) indicated that reaction was complete. Solvent was evaporated off under reduced pressure. Ethyl acetate (200 mL) and water (200 mL) were added to the residue with stirring. The organic phase was isolated, extracted sequentially with water (100 mL) and saturated brine (100 mL) and then dried over exsiccated magnesium sulphate. Solvent was evaporated off under reduced pressure giving the crude <u>8</u> as a pale yellow solid (1.329 g). This was dissolved in acetone (50 mL) at 40°C. Water (10 mL) was added rapidly to the stirred solution whereupon crystallisation proceeded spontaneously. After stirring the mixture for 5 min, more water (40 mL) was added dropwise over 5 min. The mixture was allowed to cool to ambient temperature over 30 min. The crystalline product was then isolated by filtration, washed sequentially with acetone-water (1:1 v/v; 10 mL) and water (10 mL) and then dried for 4 h at 60°C *in vacuo* giving the *title compound* (1.235 g; 90.1%); m.p. 220–223°C; ν_{max} (nujol mull)/cm^{-1} 3369 (OH stretch), 1751 (ester C=O), 1708 (thioester C=O), 1665 (C3 C=O); δ_H (400 MHz, DMSO-d_6) 7.23 (1H, d, C1 C<u>H</u>=), 6.25 (1H, d, C2 C<u>H</u>=), 6.09 (1H, s, C4 C<u>H</u>=), 5.54 (2H, m, SC<u>H</u>$_2$I), 4.19 (1H, m, C11), 2.30 (2H, q, OCOC<u>H</u>$_2$), 1.49 (3H, s, C19 C<u>H</u>$_3$), 0.97 (6H, m, OCOCH$_2$C<u>H</u>$_3$ and C18 C<u>H</u>$_3$), 0.82 (3H, d, C25 C<u>H</u>$_3$); δ_C (100 MHz, DMSO-d_6) 193.7 (C20), 184.1 (C3), 172.0 (O<u>C</u>OC$_2$H$_5$), 162.5 (d, C5), 151.4 (d, C1), 129.9 (d, C2), 119.3 (m, C4), 99.7 (d, C9), 95.5 (C17), 86.6 (dd, C6), 69.8 (dd, C11), 48.3 (C13), 47.7 (dd, C10), 42.7 (C14), 35.5 (C16), 34.9 (C12), 33.4 (C7), 33.3 (C15), 31.6 (m, C8), 26.8 (<u>C</u>H$_2$CH$_3$), 22.5 (C19), 16.7 (C16 <u>C</u>H$_3$),

15.4 ($\underline{C}H_2CH_3$), 8.8 (C24), 6.8 (C21); m/z (Thermospray +ve) m/z 609 (MH^+); $C_{25}H_{31}F_2IO_5S$ requires: C, 49.4; H, 5.1; F, 6.2; I, 20.9; S, 5.3%; Found C, 49.2; H, 5.0; F, 6.3; I, 21.3; S, 5.3%.

Synthesis of (S)-tosylmethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)-androsta-1,4-diene-17β-carbothioate (9a)

To a stirred solution of 8 (425 mg; 0.698 mmol) in acetonitrile (80 mL) was added silver(I) *p*-toluenesulphonate (1.036 g, 3.49 mmol). The mixture was stirred at 20°C for 70 min whereupon TLC indicated that reaction was complete. The mixture was filtered through Hyflo Super Cel® to remove silver(I) iodide and the filter cake washed with acetonitrile (20 mL). Solvent was removed from the combined filtrates by evaporation under reduced pressure and the residue was purified chromatographically on silica (Merck 9385, 100 g) eluted with chloroform-ethyl acetate (3:1 v/v). Fractions containing the product were combined and the solvent was evaporated off under reduced pressure. The resulting glass was re-dissolved in diethyl ether and the solution filtered. Solvent was again evaporated off under reduced pressure and the residue dried for 3 h at 20°C *in vacuo* giving the tosylate, 9a (338 mg, 74.2%). TLC (silica; chloroform-ethyl acetate 3:1 v/v) gave one spot (R_f = 0.73). ν_{max} (nujol mull)/cm^{-1} 1745 (ester C=O), 1717 (thioester C=O), 1669 (C3 C=O), 1177 (S=O); δ_H (400 MHz, $CDCl_3$) 7.79 (2H, d, C$\underline{H}$ ortho to SO_3), 7.37 (2H, d, C$\underline{H}$ ortho to tolyl CH_3), 7.12 (1H, d, C1 C$\underline{H}$=), 6.43 (1H, s, C4 C$\underline{H}$=), 6.39 (1H, d, C2 C$\underline{H}$=), 5.60 (1H, d, SC$\underline{H}$O), 5.48 (1H, d, SC$\underline{H}$O), 5.38 (1H, m, C6), 4.41 (1H, d, C11), 3.35 (1H, m, C16) 2.43 (3H, s, ArC$\underline{H}_3$), 2.34 (2H, q, C$\underline{H}_2$CH$_3$), 1.51 (3H, s, C19 C$\underline{H}_3$), 1.10 (3H, t, OCOCH$_2$C$\underline{H}_3$).1.05 (3H, s, C18 C$\underline{H}_3$), 0.88 (3H, d, C16 C$\underline{H}_3$); δ_C (100 MHz, $CDCl_3$) 192.5 (C20), 185.5 (C3), 172.9 (O$\underline{C}$OCH$_2$CH$_3$), 161.1 (d, C5), 150.4 (C1), 145.3 (OSO$_2\underline{C}$), 133.1 (aromatic $\underline{C}CH_3$), 130.3 (aromatic C ortho to CH_3), 129.9 (d, aromatic C meta to CH_3), 128.1 (d, C2), 121.2 (m, C4), 98.7 (d, C9), 96.4 (C17), 86.5 (d, C6), 71.8 (m, C11), 67.3 (S$\underline{C}$H$_2$O), 48.1 (C13), 47.8 (m, C10), 43.2 (C14), 36.3 (C16), 33.8 (m, C12), 33.6 (C7), 32.8 (m, C8 and C15), 27.5 ($\underline{C}H_2CH_3$), 23.0 (C19), 21.7 (tosyl $\underline{C}H_3$), 17.2 (C16 $\underline{C}H_3$), 16.2 (C18), 9.0 (CH$_2\underline{C}H_3$); (Found: m/z (LSIMS +ve) 653.205405 [MH^+]. $C_{32}H_{39}F_2O_8S_2$ requires m/z 653.205444); (Found: C, 58.9; H, 5.8; F, 5.7; S, 9.4% $C_{32}H_{38}F_2O_8S_2$ requires: C, 58.9; H, 5.9; F, 5.8; S, 9.8%).

Synthesis of (S)-mesylmethyl 6α,9α-difluoro-11β-hydroxy-16α-methyl-3-oxo-17α-(propionyloxy)androsta-1,4-diene-17β-carbothioate (9b)

To a stirred solution of 8 (389 mg; 0.639 mmol) in acetonitrile (80 mL) was added silver(I) methanesulphonate (648 mg, 3.19 mmol). The mixture was stirred at 20°C for 70 min whereupon TLC indicated that reaction was complete. The mixture was filtered through Hyflo SuperCel® to remove

silver(I) iodide and the filter cake washed with acetonitrile (20 mL). Solvent was removed from the combined filtrates by evaporation under reduced pressure and the residue triturated with chloroform-ethyl acetate (1:1) whereupon unreacted silver(I) methanesulphonate precipitated. The whole mixture was purified on silica (Merck 9385, 80 g) eluted with chloroform-ethyl acetate (1:1). Fractions containing the product were combined and the solvent was evaporated off under reduced pressure. The residue was dried for 40 h at 20°C *in vacuo* giving a white crystalline solid (9b; 321 mg; 87.2%). This was triturated with diethyl ether (10 mL). The solid was isolated by filtration, washed with diethyl ether (2 mL) and dried for 3 h at 40°C *in vacuo* giving the mesylate, 9b (300 mg; 93.5% recovery). TLC (silica; chloroform-ethyl acetate, 1:1 v/v) gave one spot (R_f = 0.54). TLC (silica; chloroform-ethyl acetate, 1:1 v/v) gave one spot (R_f = 0.54); ν_{max} (nujol mull)/cm^{-1} 3381 (OH stretch), 1746 (ester C=O), 1702 (thioester C=O), 1666 (C3 C=O), 1176 (S=O); d_H (400 MHz, DMSO-d_6) 7.24 (1H, d, C1 C<u>H</u>=), 6.28 (1H, d, C2 C<u>H</u>=), 6.11 (1H, s, C4 C<u>H</u>=), 5.69 (2H, m, SC<u>H</u>$_2$O), 5.56 (1H, m, C6), 4.23 (1H, d, C11), 3.23 (3H, s,$SO_2C\underline{H}_3$ 2.34 (2H, q, OCOC<u>H</u>$_2$), 1.49 (3H, s, C19 C<u>H</u>$_3$), 1.05 (3H, t, $OCOCH_2C\underline{H}_3$).1.04 (3H, s, C18 C<u>H</u>$_3$), 0.88 (3H, d, C16 C<u>H</u>$_3$); δ_C 100MHz, DMSO-d_6) 193.1 (C20), 184.1 (C3), 172.2 ($O\underline{C}OC_2H_5$), 162.6 (d, C5), 151.5 (d, C1), 128.9 (d, C2), 119.2 (m, C4), 99.7 (d, C9), 95.8 (C17), 86.2 (d, C6), 81.8 (SC<u>H</u>$_2$O), 69.9 (m, C11), 48.4 (C13), 47.6 (m, C10), 42.6 (C14), 35.6 (C16), 35.0 (C7), 33.2 (C15), 31.8 (m, C8), 26.7 ($\underline{C}H_2CH_3$), 22.5 (C19), 16.6 (C16 $\underline{C}H_3$), 16.2 (C18), 16.1 ($SO_2\underline{C}H_3$), 8.8 ($CH_2\underline{C}H_3$); m/z (FAB) m/z 577 (MH^+), 599 (MNa^+); (Found: C, 54.4; H, 6.2; F, 6.6; S, 11.3% $C_{26}H_{34}F_2O_8S_2$ requires: C, 54.2; H, 5.9; F, 6.2; S, 11.1%).

Treatment of 9a with potassium fluoride

To a glassy carbon vessel, containing anhydrous potassium fluoride (0.5 mg, 0.01 mmol), APE 2.2.2 (11 mg; 0.030 mmol) and potassium iodide (0.275 mg; 0.00165 mmol), was added a solution of 9a (10 mg; 0.015 mmol) in acetonitrile (0.5 mL). The vessel was capped, pressurised with nitrogen (1.4 bar, 20 p.s.i.) sealed and heated at 100°C for 35 min. The vessel was then allowed to cool and the reaction solution was analyzed by HPLC (Method 1). The main reaction components (1, 8, 9a, 10 and unknown X) were then characterised by retention time, diode-array analysis, mass spectrometry and ^{1}H-NMR.

1, 43% of absorbance response: λ_{max} 236 nm; m/z 501 [MH^+]; δ_H 7.32 (1H, C2 C<u>H</u>=), 6.34 (1H, d, C1 C<u>H</u>=), 6.28 (1H, C4 C<u>H</u>=), 5.86 (2H, d, SCH_2F, J ^{19}F-^{1}H 50Hz), 5.51 (1H, dm, C<u>H</u>F, J ^{19}F-^{1}H 49Hz), 4.28 (1H, dm, C<u>H</u>OH), 3.33 (1H, m, C16, C<u>H</u>CH_3), 2.35 (2H, m, C19, C<u>H</u>$_2CH_3$), 1.48 (3H, s, C10 CH3), 1.04 (3H, t, $CH_2C\underline{H}_3$), 1.01 (3H, s, C13 C<u>H</u>$_3$), 0.89 (3H, d, C16 C<u>H</u>$_3$).

8, 21% of absorbance response: λ_{max} 239 nm; m/z 609 [MH^+]; δ_H 7.32 (1H, C2 C<u>H</u>=), 6.34 (1H, d, C1 C<u>H</u>=), 6.28 (1H, C4 C<u>H</u>=), 5.51 (1H, dm, C<u>H</u>F, J ^{19}F-^{1}H 49Hz), 4.47 (2H, s, SCH_2I), 4.27 (1H, dm,

CHOH), 3.27 (1H, m, C16, CHCH$_3$), 2.36-2.27 (2H, m, C19, CH$_2$CH$_3$), 1.47 (3H, s, C10 CH3), 1.03 (3H, t, CH$_2$CH$_3$), 1.02 (3H, s, C13 CH$_3$), 0.89 (3H, d, C16 CH$_3$).

9a, 4% of absorbance response; λ_{max} 237 nm; m/z 653 [MH+]; δ_H 7.77 (2H, d, C21, aromatic), 7.45 (2H, d, C22, aromatic), 7.31 (1H, dd, C2, CH=), 6.34 (1H, d, C1 CH=), 6.30 (1H, m, C16 , CHCH$_3$), 6.28 (1H, d, C4 CH=), 5.51 (1H, dm, CHF, J ^{19}F-^{1}H 49Hz), 5.51 (2H, s, SCH$_2$OTs), 4.29 (1H, dm, CHOH), 3.20 (1H, m, C16, CHCH$_3$), 2.35-2.25 (2H, m, C19, CH$_2$CH$_3$), 1.48 (3H, s, C10 CH$_3$), 1.04 (3H, t, CH$_2$CH$_3$), 1.03 (3H, t, Ph-CH$_3$) 0.90 (3H, s, C13 CH$_3$), 0.86 (3H, d, C16 CH$_3$).

10, 24% of absorbance response: λ_{max} 240 nm; m/z 949 [MH+].

Unknown X, 8% of absorbance response: λ_{max} 238 nm; m/z 541 [MH+]; δ_H 7.33 (1H, d, C2 CH=), 6.35 (1H, d, C1 CH=), 6.29 (1H, s, C4 CH=), 5.50 (1H, dm, C6 CHF, J ^{19}F-^{1}H 49Hz), 5.46, 5.43 (2H, d, SCH$_2$X), 4.28 (1H, dm, CHOH), 3.29 (1H, m, C16, CHCH$_3$), 2.35 (2H, m, CH$_2$CH$_3$), 1.48 (3H, s, C10 CH$_3$), 1.02 (3H, t CH$_2$CH$_3$), 0.99 (3H, s, C13 CH$_3$), 0.89 (3H, d, C16 CH$_3$).

Syntheses of [S-fluoromethyl-^{18}F]FP (11) by treatment of 8, 9a, or 9b with NCA K+-APE 2.2.2-[^{18}F]fluoride/iodide

A solution of 8, 9a or 9b (15 mg; *ca* 15 μmol) in anhydrous acetonitrile (500 μL) was added to a glassy-carbon vessel containing dry K+-APE 2.2.2-[^{18}F]fluoride/iodide (*ca* 3 mCi). The vessel was sealed, pressurised to 20 p.s.i. with nitrogen and heated for 30 min at 100°C. The vessel was then allowed to cool and the product was analyzed by radio-HPLC (Method 1; e.g. Figure 2).

Reactions with 9a (10 mg; 0.015 mmol) were also carried at 125°C for 35 min and analysed similarly. Radiochemical yields of 11 were calculated from the HPLC analyses (Table 1).

Table 1. Radiochemical yield [^{18}F]FP (11) in reactions of precursors with NCA K+-APE 2.2.2[^{18}F]fluoride/iodide.

Precursor	Yield of [^{18}F]FP (11) (%)
Iodide (8)	4–5 (n = 4)
Tosylate (9a)	15–35 (n = 9)
Mesylate (9b)	15–18 (n = 5)

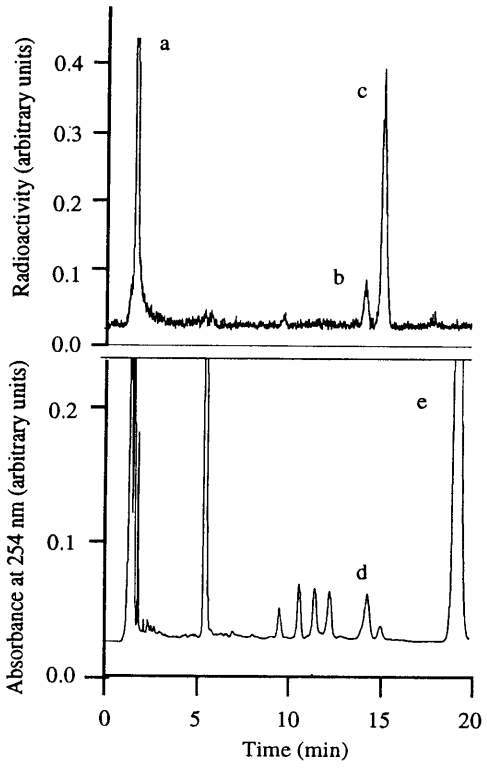


Figure 2. Chromatogram from the reverse phase HPLC analysis (Method 1) of the reaction mixture from the treatment of 9a with K+-APE 2.2.2-[^{18}F]fluoride/iodide. Key: a - [^{18}F]fluoride-; b - radioactive unknown; c-[*S-fluoromethyl*-^{18}F]FP (11); d - FP (1), e - 9a .

Automated Preparation of [S-fluoromethyl-^{18}F]FP (11) from tosylate (9a)

An automated apparatus, housed in a lead-shielded hot-cell, was constructed for the safe preparation of high radioactivities of pure [*S-fluoromethyl*-^{18}F]FP (11) from tosylate (9a) and cyclotron-produced [^{18}F]fluoride (≤ 400 mCi) (Figure 3).

All stages of the preparation, except for injection of the reaction products onto HPLC, were remotely-controlled and timed with an external programmable logic controller (Toshiba). (11) was separated by HPLC on a Primesphere C18-HC column (5 μ; 250 x 10 mm o.d.) eluted with acetonitrile-water (13:12 v/v) at 4.5 mL/min with eluate monitored for absorbance at 254 nm (Gilson 112 detector; Anachem Ltd) and for radioactivity with a plastic scintillant PM-tube detector linked to a scaler-ratemeter (Mini-Instruments Ltd). The radioactive fraction having the same retention time as authentic

FP (1) (24 min) was collected in a volume of 15–20 mL. A radioactive byproduct eluted at 22.2 min. The whole preparation required 80 min from the end of radionuclide production.

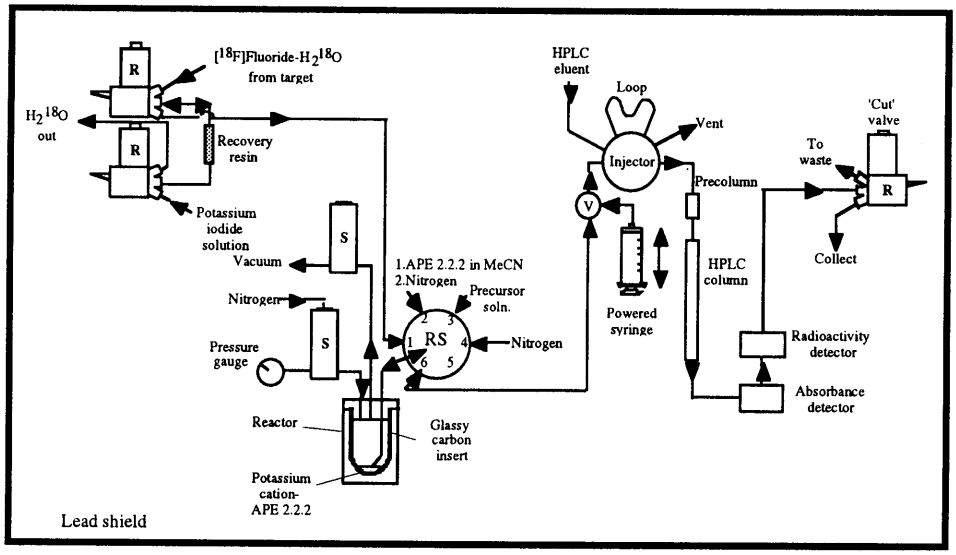


Figure 3. Diagram of the apparatus constructed for the shielded and remotely-controlled radiosynthesis of high radioactivities of [*S-fluoromethyl*-^{18}F]FP (11). **R**, **S**, **RS** and **V** denote pneumatically controlled slider valves (Rheodyne), solenoid operated valves (Rheodyne), pneumatically-controlled 6-way rotary valve (Rheodyne) and a two-way solenoid valve, respectively.

Routine analysis of preparations of [S-fluoromethyl-^{18}F]FP (11)

The isolated radioactive product was routinely analysed by reverse phase HPLC (Method 1) to measure radiochemical purity, chemical purity and specific radioactivity (Figure 4). Radiochemical purity exceeded 99% (n = 8). In some analyses, a U.V. absorbance peak was detected with the same retention time as FP (1). Generally, this peak represented ≤ 3 μg of FP (1) in the whole preparation. In some preparations the FP was below the detection limit. On this basis, the specific radioactivity of preparations of 11 ranged between 150 mCi and greater than 4 Ci per μmol at the end of synthesis from irradiations producing 60 mCi of [^{18}F]fluoride. Only a very low levels of one nonradioactive impurity was detected in the HPLC analysis. Preparations were radiochemically stable.

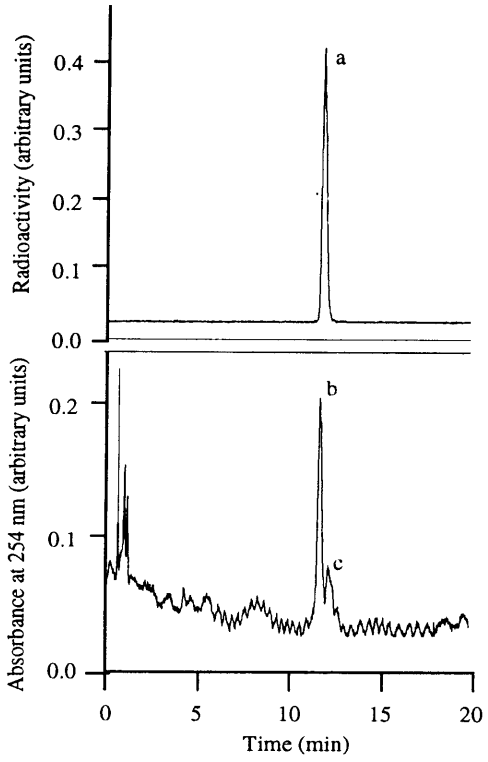


Figure 4. Chromatogram from reverse phase HPLC analysis of [*S-fluoromethyl* ^{18}F]FP (11). Key: a.- [^{18}F]FP (11); b - FP (1); c - unknown. (See Experimental for elution conditions).

Discussion

Only two positron-emitting radioisotopes could realistically be considered for labelling FP (1) without structural modification, carbon-11 ($t_{1/2}$ = 20.3 min) or fluorine-18 ($t_{1/2}$ = 109.6 min). We chose to label FP (1) with fluorine-18 to allow more time for radiosynthesis, formulation of the radiotracer within a pMDI and data acquisition in PET studies. Fluorine-18 is readily produced as [^{18}F]fluoride in high radioactivity (1–4 Ci) at a NCA level of specific radioactivity by the proton irradiation of ^{18}O-enriched water (7). Moreover, there are now efficient procedures for reclaiming the ^{18}O-enriched water and for converting the [^{18}F]fluoride into salts that are powerfully nucleophilic radiofluoridation reagents in organic solvents (8). One of the most widely used of these reagents is the [^{18}F]fluoride salt of potassium cation-aminopolyether 2.2.2 (K^+-APE 2.2.2) (10). Therefore, a labelling procedure was sought based on the use of this reagent.

The 9α-fluoro group is considered to be the most metabolically stable of the three fluoro groups in FP (1). Initially we considered this position for labelling. The opening of 9α,11β-epoxides with anhydrous hydrogen fluoride is a well known method for introducing the *trans* 9α-fluoro-11β-hydroxy system into steroids (11). Protonation of the epoxide oxygen atom promotes *trans* nucleophilic attack at the C-9 position by fluoride ion (11). Thus, we considered a similar epoxide ring opening for labelling with fluorine-18. Truly anhydrous NCA hydrogen [^{18}F]fluoride is not an easily accessible nor well characterised reagent. However, we considered that alternative activation of the epoxide ring for opening with [^{18}F]fluoride might be achieved by interaction of the oxygen atom with protic acids other than hydrogen fluoride, including acidic ion exchange resins or Lewis acids. This approach seemed plausible on the basis that simple [^{18}F]fluorohydrins have been prepared previously by treating epoxides with [^{18}F]fluoride under various conditions, albeit in low radiochemical yields (12–16).

The 9α,11β-epoxide (6) was successfully synthesised in three steps from the available 17β-carboxy-17α-hydroxy compound (3) (Figure 5). 6 was treated with K+-APE 2.2.2-fluoride in acetonitrile in the presence of one of a selection of acids (sulphuric acid, Dowex® 50, Amberlyst® or boron tribromide). However, no FP (1) was obtained in these experiments. The reactions were repeated using NCA K+- APE 2.2.2 [^{18}F]fluoride/carbonate in place of potassium fluoride, but no [9-^{18}F]FP (7) was formed. De Groot has recently attempted similar reactions in progestins, also without success at the NCA level of specific radioactivity (17,18). Clearly, the epoxide ring in these steroids is not sufficiently activated for nucleophilic attack by [^{18}F]fluoride under such conditions.

Labelling by nucleophilic substitution at the 6-position was considered. However, this would require the difficult synthesis of an activated precursor while the reaction would lack stereochemical control and could result in elimination rather than substitution, as recently observed in progestin (17,18). Radiofluorination has been achieved in the 6α-position of progesterone via a halofluorination-oxidation strategy with NCA [^{18}F]fluoride, but in only very low radiochemical yield (0.3%, decay-corrected) (19). This approach appeared impractical in view of the high radioactivities (> 20 mCi) that would be required for the preparation of an [^{18}F]FP-loaded pMDI for PET studies.

We finally considered labelling FP (1) in its *S*-fluoromethyl group. In the preparation of FP (1), the fluoromethyl group is introduced by treating the 17β-carbothioic acid with bromofluoromethane or fluoroiodomethane (6). Fluoromethylation of a thioic acid analogue might be feasible for labelling FP (1) with fluorine-18, since [^{18}F]bromofluoromethane has been prepared from NCA [^{18}F]fluoride in up to 62% radiochemical yield (20,21). However, we preferred to develop a single-step labelling procedure, based on nucleophilic displacement of a suitable leaving group, because of its simplicity and potential for greater practical yield.

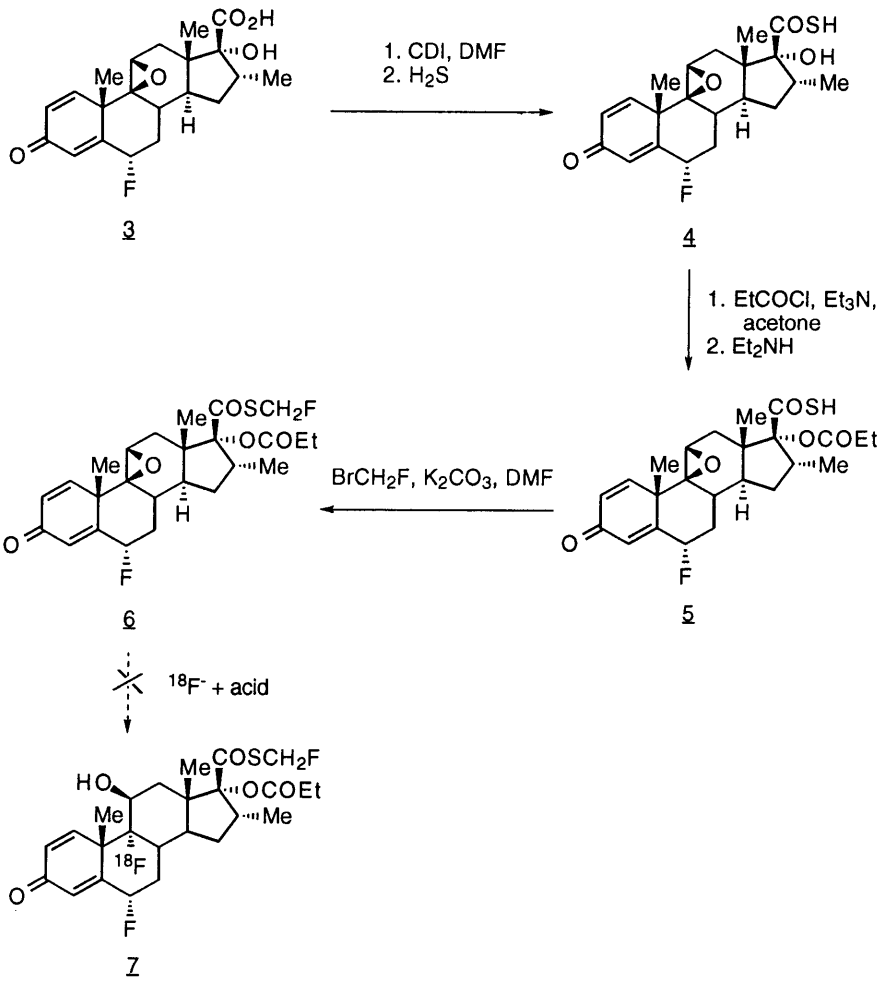
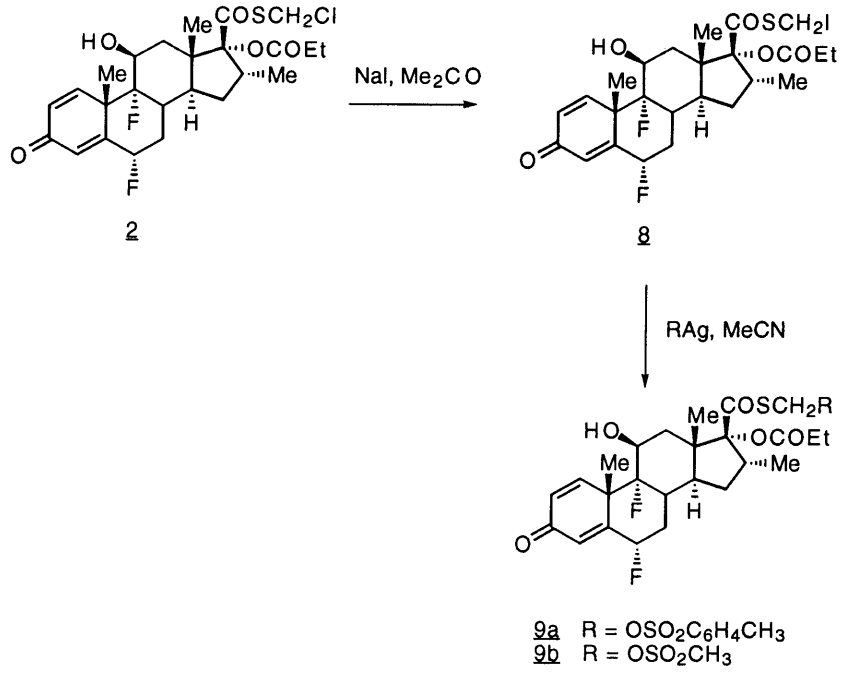


Figure 5. Synthesis of the epoxide (6) for attempted ring opening with NCA [^{18}F]fluoride.

The known chloro analogue of FP (1) (cloticasone propionate; CP; 2) (6), served to prepare precursors with leaving groups suitable for nucleophilic displacement by [^{18}F]fluoride. The iodo analogue (8) was first obtained by treating CP (2) with sodium iodide in acetone (Figure 6).

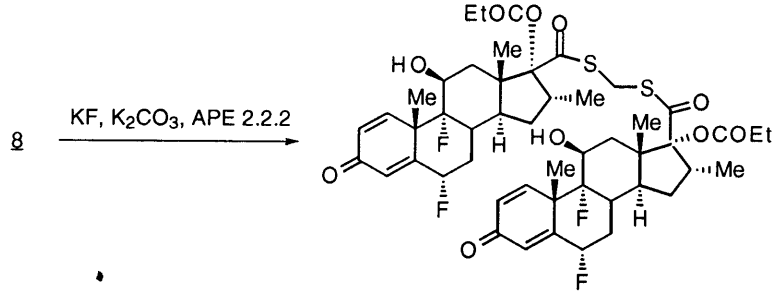
No FP (1) was obtained by treating (8) with potassium fluoride in the presence of APE 2 2.2. In the presence of added potassium carbonate a dimeric product (10) was formed, suggesting that the steroid was susceptible to a base-induced coupling reaction (Figure 7). We therefore considered replacing potassium carbonate in the reaction system with the more weakly basic potassium iodide.



2 8

Figure 6. Synthesis of precursors from CP (2) for nucleophilic substitution with [^{18}F]fluoride.

In practice, the preparation of K+-APE 2.2.2-[^{18}F]fluoride/iodide was easy, since iodide is as effective as carbonate for displacing [^{18}F]fluoride from the anion exchange resin used to recover [^{18}F]fluoride from the proton-irradiated ^{18}O-enriched water. (8) was treated with this reagent at 100°C for 30 min (Figure 8). This gave [*S-fluoromethyl*-^{18}F]FP (11) in radiochemical yields of 4-5% and an



10

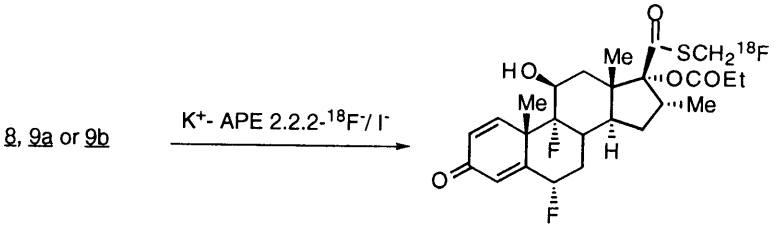
Figure 7. Formation of a dimeric product (10) by treating (8) with K+-APE 2.2.2-fluoride/carbonate.

unknown radioactive compound (radiochemical yield, 19%) (Table 1). In analytical HPLC [*S-fluoromethyl*-^{18}F]FP (11) was identified by its retention time and spectral ultraviolet absorption analysis of its associated carrier with a diode-array detector. These results were encouraging but suggested that a better leaving group than iodide was required for more efficient nucleophilic substitution.

Treatment of (8) with silver(I) mesylate or tosylate in acetonitrile (Figure 6) gave precursors (9a,b) with good leaving groups. However, attempts to prepare the corresponding triflate (9c) from (8) by treatment with silver(I) triflate failed in a variety of solvents.

The tosylate (9a) and mesylate (9b) were each treated with K$^+$-APE 2.2.2-[^{18}F]fluoride/iodide at 100°C for 30 min (Figure 8). The tosylate (9a) gave the highest radiochemical yield of [*S-fluoromethyl*-^{18}F]FP (11) (25%) (Table 1). An unknown radioactive byproduct (X) was formed in 10–15% radiochemical yield (Figure 2). In a separate experiment, the tosylate (9a) was treated with one equivalent of potassium fluoride in the presence of APE 2.2.2. Analysis by liquid chromatography (Figure 2) with inspection of the major peaks by UV spectroscopy, LC-MS and LC-NMR, verified that FP (1) was synthesised under these conditions.

The radiochemical yield of [*S-fluoromethyl*-^{18}F]FP (11) was raised to 35% by performing the radiosynthesis under very dry conditions (*i.e.* with anhydrous acetonitrile) at 125°C for 30 min. At the same time the radiochemical yield of the unknown radioactive byproduct (X) was reduced to less than 5%. In these reactions the main chemical byproduct was (8), formed by displacement of the tosylate group with the free iodide present in the reaction mixture. Therefore, a molar excess of tosylate (9a) over iodide was used in these reactions to maintain an adequate concentration of tosylate. This route was chosen for the routine production of [*S-fluoromethyl*-^{18}F]FP (11). A reverse phase HPLC method was devised for isolating 11 in high radiochemical purity (Figure 2).



11

Figure 8. Radiosynthesis of [*S-fluoromethyl*-^{18}F]FP (11) by nucleophilic substitution with [^{18}F]fluoride.

An automated lead-shielded apparatus was developed to allow high radioactivities (≤ 50 mCi) of pure 11 to be produced from high radioactivities of [^{18}F]fluoride (Figure 3). All operations in the radiosynthesis, except loading of the reaction mixture into a loop injector for HPLC separation, were controlled and timed using an external programmable logic controller (22). The whole preparation takes 80 min and gives 11 in 18–35% radiochemical yield.

Rapid analytical reverse phase HPLC established that isolated 11 had greater than 99% radiochemical purity (Figure 4). The amount of carrier FP (1) varied between preparations, depending on the batch of precusor used for the reaction, but was 3 μg or less, resulting in preparations with specific radioactivities from 150 mCi to greater than 4 Ci per μmol at the end of synthesis (from an irradiation producing 60 mCi of [^{18}F]fluoride).

Thus, an effective method has been developed for labelling FP (1) with fluorine-18 in high radioactivity, chemical purity and radiochemical purity. The radiosynthesis has now been extended to incorporate high radioactivities of 11 into formulated FP particles within an pMDI (23). A full description of this procedure and the future application of this radiotracer to lung deposition studies with PET will be reported elsewhere.

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