Electronic Supplementary Information

Fluorinase mediated chemoenzymatic synthesis of [^{18}F]-fluoroacetate

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1. Materials

L-Amino acid oxidase (L-AAO, EC 1.4.3.2, *Crotalus adamanteus*, Type I, 0.3 unit/mg), *S*-adenosyl-L-methionine (SAM) and Standard sodium fluoroacetate (PESTANAL®, analytical standard) were purchased from Sigma-Aldrich Co., UK. A reference sample of 5'-fluorodeoxyadenosine **2** was chemically synthesised following procedures described earlier.[1,2] Fluoride-18 production was conducted in a CTI RDS-111 cyclotron (CTI/Siemens) and radioactivity was measured using a Capintec well reader (CRC-15R).

2. Fluorinase expression and purification

Recombinant fluorinase (EC 2.5.1.63) was expressed and purified according to the known method with slight modifications.[3] In detail the methods used were; *E. coli* BL21 (DE3) was transformed with pET28-flA which was grown in Luria broth (1L) containing kanamycin (0.1 mg mL^{-1}) at 37^{o}C until an absorbance of 0.6 at 600 nm was observed. Over-expression of the fluorinase was induced by adding isopropylthiogalactoside (IPTG) to a final concentration of 0.2 mM and the incubation continued at 30 ^{o}C for 15h. The cells were collected and lysed. After centrifugation, the cell lysate was applied to a column packed with Ni^{2+}-charged His-Bind resin (Novagen). The fluorinase was eluted from the column with Tris-HCl buffer (20 mM, pH 8) containing NaCl (0.4 M) and imidazole (0.4 M). The protein was further purified by size-exclusion fast protein liquid chromatography (FPLC) on a Superdex S-200 (HR 16/60) column (Pharmacia Biotech) and concentrated using a Millipore concentrator. The final concentration of pure protein was 23 mg mL^{-1} (43 mg in total) determined using a Nanodrop ND1000 spectrophotometer. SDS-polyacrylamide gel electrophoresis was used to confirm enzyme purity.

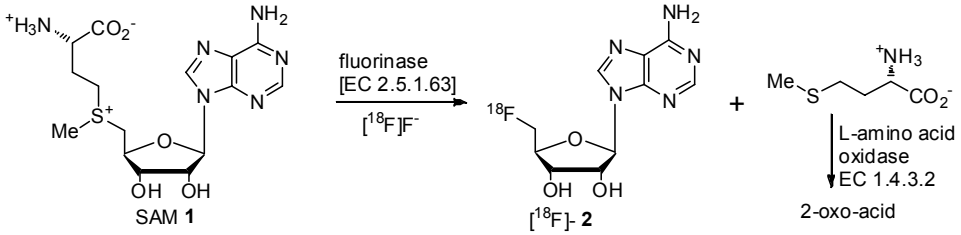
3. Synthesis of "cold" fluoroacetate (optimisation)

An oxidation mixture was prepared by adding CrO_3 (1g) to a solution of concentrated H_2SO_4 (1 mL) in H_2O (2.5 mL). In a typical experiment, 100 µL of the FDA solution (9.5 mg/mL) was added to the oxidation mixture (250 µL) in a sealed 5 mL Wheaton V-vial and heated at 100-160°C for 5 – 50 min. Then the reaction was cooled in a water-ice bath, diluted with water (1-2 mL) and the [^{18}F]-fluoroacetic acid was extracted into diethyl ether (5 × 1 mL). The combined ether extracts were passed through a Sep-Pak Light Si cartridge to remove sulfate and chromate ions and the cartridge was washed with diethyl ether (1 mL). The combined extracts were then washed with aq. Na_2CO_3 (0.5 mL, 8 mg/mL) to secure the product in the aqueous phase as Na [^{18}F]-fluoroacetate and the resulting solution was analysed by HPLC.

HPLC analysis was performed on Dionex HPLC system composed of IP25 isocratic pump, automated injection loop (25 µL), HPLC column (Hamilton PRP-X100, 150 × 3.00 mm), an anion suppressor (Dionex ASRS300, 4 mm) and a conductivity detector connected in series. Elution of all anions was achieved at a 1.5 mL/min flow of aq. Na_2CO_3 (1.5 mM) solution for 10 min and the nature of the ions was confirmed by standard sodium salt solutions: carbonate (R_t = 1.6 min); co-eluting bicarbonate and formate (R_t = 5.0 min and 5.6 min) and fluoroacetate (R_t = 7.7 min).

A commercial standard of sodium fluoroacetate (PESTANAL®, analytical standard, purity 99.3%) was used as a reference for the calibration of fluoroacetate. An eight point calibration curve (R^2 = 0.997) correlating the area under the curve (AUC) to the FAc concentration (over the 1-134 nmol range) was generated. Yields were thus calculated and plotted against time for all of the oxidation temperatures explored (see Fig 1).

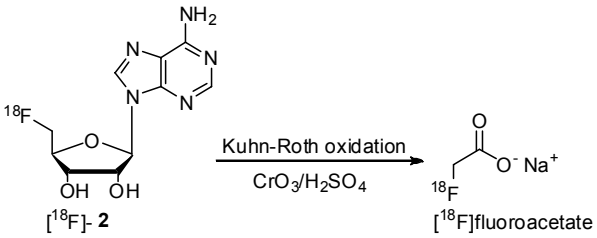
4. Synthesis of [^{18}F]- 2



In a typical experiment, the fluorinase (30-40 µL, 23 mg/mL) was added to a mixture of SAM **1** (15 µL, 20 mM), L-AAO (1 mg), [^{18}F]-F^- (20-30 µL, 130-160 MBq) and incubated at 37°C with magnetic stirring for 1h. Then water (50 µL) was added and the enzymes denatured by heating on a boiling water bath for 3 min, followed by centrifugation of the precipitated protein (14.500 rpm, 4 min). This gave the supernatant (~80-100 µL) containing [^{18}F]-FDA which was used directly in the next step. For quality control, 2 µL of the reaction mixture were diluted with water (50 µL), processed as described above, and a 20 µL aliquot was injected into a Gyncotek HPLC system (manual injection loop (100 µL), dual head pump, column (Phenomenex Luna C-18(2), 250 × 4.60 mm, 5 micron) and UV (UVD340S)) with a NaI single crystal radioactive detector connected in series. [^{18}F]-F^- (R_t = 3.4 min, confirmed by [^{18}F]-F^-standard) and [^{18}F]-FDA **2** (R_t = 10.0 min, confirmed by a cold standard) was eluted at a 1 mL/min gradient flow of a ethanol in water (2.5% to 25% in 13 min). Depending

on the fluorinase and the [^{18}F]-F$^-$ concentration in the reaction media, [^{18}F]-F$^-$ incorporation levels were 90-99%**.**

5. Synthesis of [^{18}F]-fluoroacetate



[^{18}F]- **2** [^{18}F]fluoroacetate

An oxidation mixture was prepared by adding CrO_3 (1g) to a solution of concentrated H_2SO_4 (1 mL) in H_2O (2.5 mL). The [^{18}F]-**2** solution (80-100 μL), obtained as described above, was added to the oxidation mixture (250 μL) in a sealed 5 mL Wheaton V-vial at room temperature. The vessel was heated at 140^{o}C for 20 min, then cooled for 1-2 min in a water-ice bath. Water (1-2 mL) was added and the [^{18}F]-fluoroacetic acid was extracted into diethyl ether (5 × 1 mL). The combined ether extracts were passed through a Sep-Pak Light Si cartridge to remove sulfate and chromate ions and the cartridge was washed with diethyl ether (1 mL). The combined extracts were then washed with aq. Na_2CO_3 (0.5 mL, 8 mg/mL) to secure the product in the aqueous phase as Na [^{18}F]-fluoroacetate and the level of radiolabelling assessed by HPLC.

HPLC analysis was performed as previously described on a Dionex HPLC system with a NaI single crystal radioactivity detector connected to the column effluent. Two radioactive compounds were detected and identified as [^{18}F]-F$^-$ (R_t = 3.1 min) and [^{18}F]-FAc$^-$ (R_t = 6.8 min)(*n.b.* as the radioactive detector sits before the conductivity detector, retention times are reduced compare to those of the unlabelled standards)

For the calculation of decay-corrected RCY, all radioactive fractions were collected and their radioactive content recorded in the Capintec well reader (CRC-15PET).

References

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2. T. D. Ashton and P. J. Scammells, *Bioorg. Med. Chem. Lett*., 2005, **15**, 3361.
3. C. Dong, F. Huang, H. Deng, C. Schaffrath, J. B. Spencer, D. O´Hagan and J. H. Naismith, *Nature*, 2004, **427**, 561.