Supporting Information

An enzymatic route to 5-deoxy-5-[^{18}F]fluoro-D-ribose, a [^{18}F]-fluorinated sugar for PET imaging

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General Information

Materials

Crude extract of 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 solvents were purchased from Sigma-Aldrich Co., UK. All used solvents were HPLC grade or purified and degassed according to standard procedures. Standard samples of 5'-FDA and 5-FDR were chemically synthesised following procedures described earlier.[1] Fluorine-18 production was conducted in a CTI RDS-111 cyclotron (CTI/Siemens) and radioactivity was measured using a Capintec well reader (CRC-15R).

Protein expression and purification

Recombinant fluorinase (EC .5.1.63) was expressed and purified as previously reported.[2] Expression and purification of the *T. vivax* IAG-NH were performed following the protocol described by Versées *et al*.[3] The final concentration of pure protein was determined using a Nanodrop ND1000 spectrophotometer, and SDS-polyacrylamide gel electrophoresis was used to confirm enzyme purity (Figure 1).

Figure 1 SDS-PAGE gel (12% acrylamide) showing purification steps of *T. vivax* IAG-NH. Lanes: 0, molecular markers; 1, purified *T. vivax* IAG-NH; 2, cell-free extract loaded on a NTA column; 3, unbounded fraction; 4, eluted fraction.

High Performance Liquid Chromatography (HPLC)

HPLC analyses were carried out on a Gynkotek HPLC system (P580 pump), a Gynkotek column oven (STHS8S) and a UV detector (UVD340S) coupled in series with a BIOSCAN NaI detector (B-FC-3200). The HPLC system was operated using a Phenomenex Luna C-18 analytical column (250 x 4.6 mm, 5 μm) or a Phenomenex Luna C-18 semipreparative column (250 x 10.0 mm, 5 μm), both equipped with corresponding guard columns. Mobile phase A was water and mobile phase B was ethanol. Product formation was determined by HPLC, monitoring UV and radioactivity simultaneously.

Aliquots of reaction mixtures for HPLC analysis were prepared as follows: 2 μL of enzymatic reaction mixture was added to 48 μL of water. Protein was denatured (100 ºC, 1 min) and removed by centrifugation (13,500 rpm; 2 min). The supernatant was then injected and analysed on a Gynkotec HPLC system using the conditions described below.

HPLC conditions for 5'-[^{18}F]FDA **2** analysis

An aliquot (20 μL) was loaded onto a Phenomenex Luna C-18 analytical column. The following gradient (flow rate of 1 mL/min) was used: 0–13 min, 97.5% to 75% A; 13–14 min, 75% A, 14–15 min 97.5% A. The retention time for 5'-[^{18}F]FDA was 11.5 min.

HPLC conditions for 5-[^{18}F]FDR **5** analysis

An aliquot (20 μL) was loaded onto a Phenomenex Luna C-18 analytical column and eluted with the following gradient (flow rate of 0.75 mL/min): 0–5 min, 100% A; 5–10 min, 100% to 75% A; 10–16 min, 75% A; 16–17 min 75% to 100% A. Under these conditions the retention time for 5-[^{18}F]FDR was 5.3 min and for 5'-[^{18}F]FDA was 14.8 min.

Preparative HPLC conditions for 5-[^{18}F]FDR **5**

An aliquot (100 μL) was loaded onto a Phenomenex Luna C-18 semipreparative column. The following gradient (flow rate of 3 mL/min) was used: 0–6 min, 100% A; 6–11 min, 100% to

75% A; 11–17 min, 75% A; 17–18 min 75% to 100% A. The retention time for 5-[^{18}F]FDR was 5.6 min and 5'-[^{18}F]FDA was 11.5 min.

Enzymatic preparation of 5-[^{18}F]FDR **5** from SAM **1**

Sequential one-pot synthesis

In a typical radiochemical experiment, the fluorinase enzyme (10 μL, 35.5 mg/mL) was incubated with SAM **1** (10 μL, 20 mM), L-AAO (1 mg) and aqueous [^{18}F]F$^-$ (15 μL, 249 MBq) at 37 ºC for 1 h. An aliquot of the mixture was analysed by radio-HPLC (Figure 2) using the conditions described above. Production of 5'-[^{18}F]FDA **2** was confirmed by comparison with a standard sample of 5'-FDA. The retention time of 5'-FDA in the UV-chromatogram was identical to the retention time of 5'-[^{18}F]FDA **2** in the radioactivity chromatogram. For the stage two of the biotransformation, the reaction mixture was then heated (100 ºC, 1 min) and the denatured L-AAO and fluorinase centrifuged (13,500 rpm; 2 min). The supernatant was not decanted, in order to minimised radiation exposure. A preparation of purified *T. vivax* NH (20 μL, 50 mg/mL) was added to the supernatant. Following the addition of *T. vivax* NH, the reaction mixture was incubated for up to 2 h at 37 ºC, and an aliquot was taken for HPLC analysis every 30 min (radiograms for 30 min, 60 min, 90 min and 120 min incubations are shown in Figures 3–6). Formation of 5-[^{18}F]FDR **5** was observed in 80% radiochemical yield (RCY - decay corrected) after 2-h incubation. The synthesis of 5-[^{18}F]FDR **5** was also confirmed by comparison with a standard sample. In this case, the retention time of 5-FDR in the chromatogram generated by the electrochemical detector was identical to the retention time of 5-[^{18}F]FDR **5** in the radioactivity chromatogram. The isolation of [^{18}F]FDR **5** from the reaction mixture is carried out by HPLC, only for imaging studies with that compound.

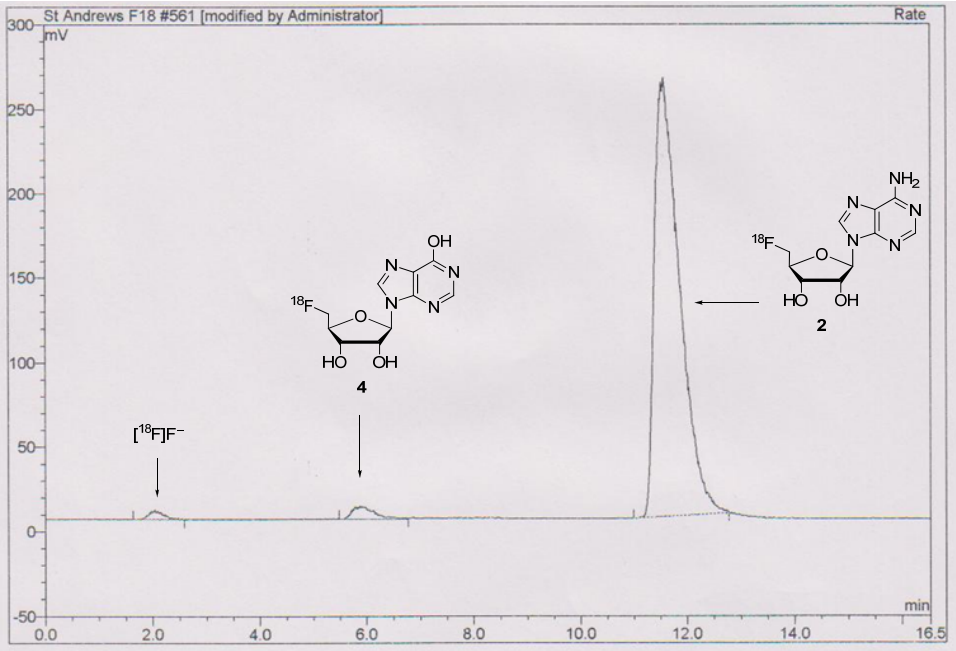

Figure 2 Stage one of the biotransformation: radio-HPLC chromatogram of 5'-[^{18}F]FDA **2** formation from [^{18}F]F^{-} (249 MBq) of fluorinase and L-AAO after 1 h incubation at 37 ºC at pH 7.0. [Note: peak at 5.8 min corresponds to 5'-[^{18}F]FDI **4** due to the presence of deaminase activity in the crude extract of L-AAO. *Tv*NH acts upon both 5'-[^{18}F]FDA **2** and 5'-[^{18}F]FDI **4**]

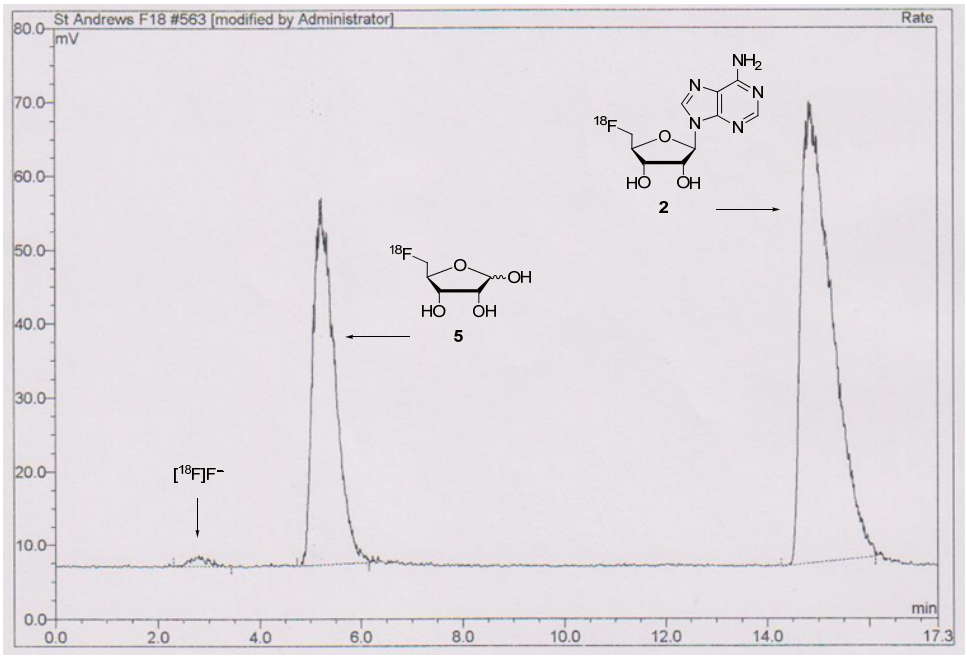

Figure 3 Stage two of the biotransformation: radio-HPLC chromatogram of 5-[^{18}F]FDR **5** formation after 30 min incubation with *Tv*NH at 37 ºC.

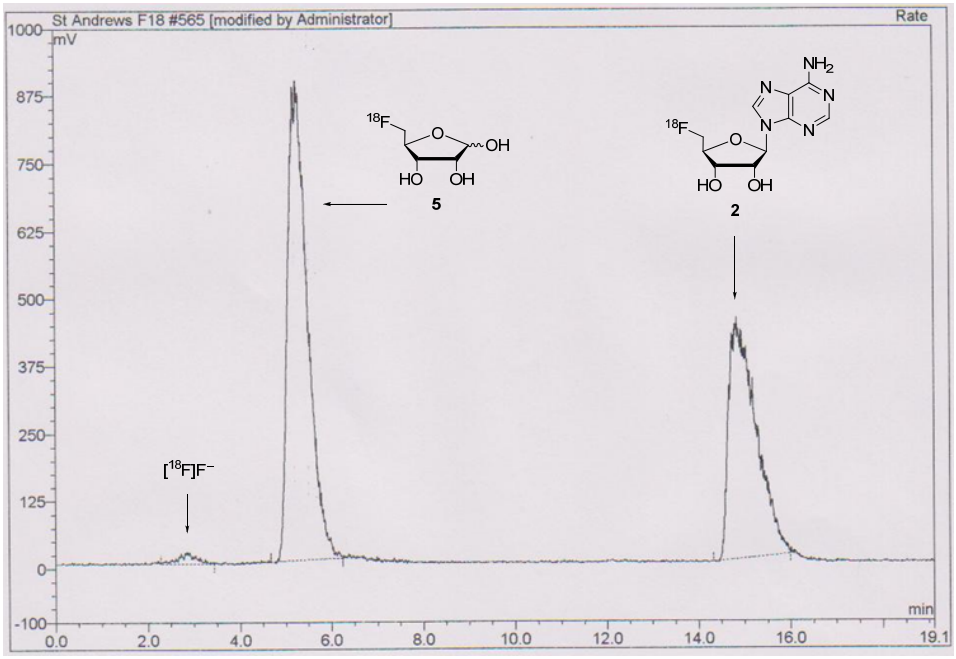

Figure 4 Stage two of the biotransformation: radio-HPLC chromatogram of 5-[^{18}F]FDR **5** formation after 60 min incubation with *Tv*NH at 37 ºC.

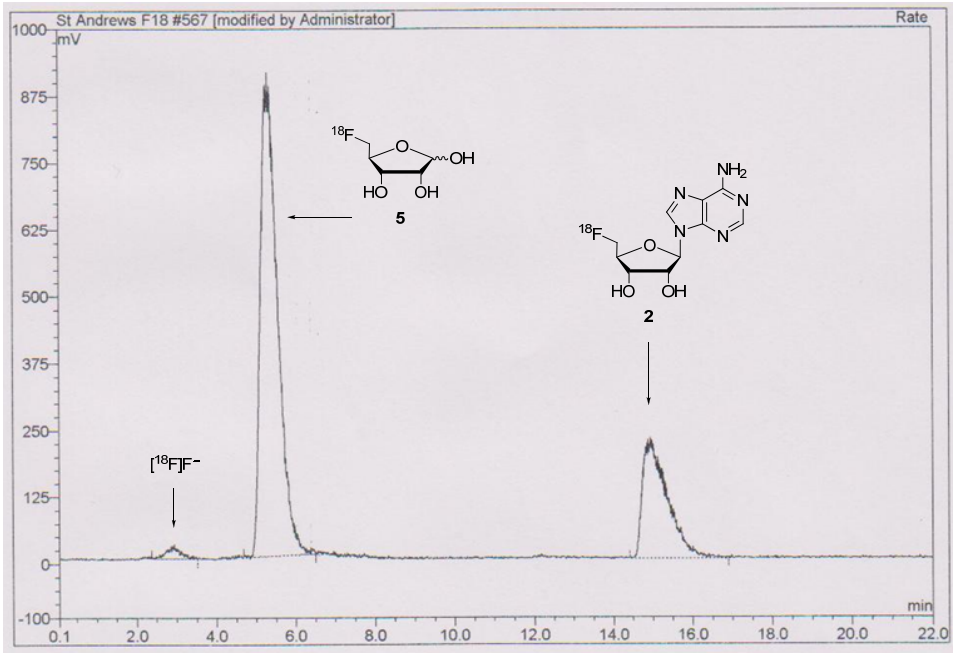

Figure 5 Stage two of the biotransformation: radio-HPLC chromatogram of 5-[^{18}F]FDR formation after 90 min incubation with *Tv*NH at 37 ºC.

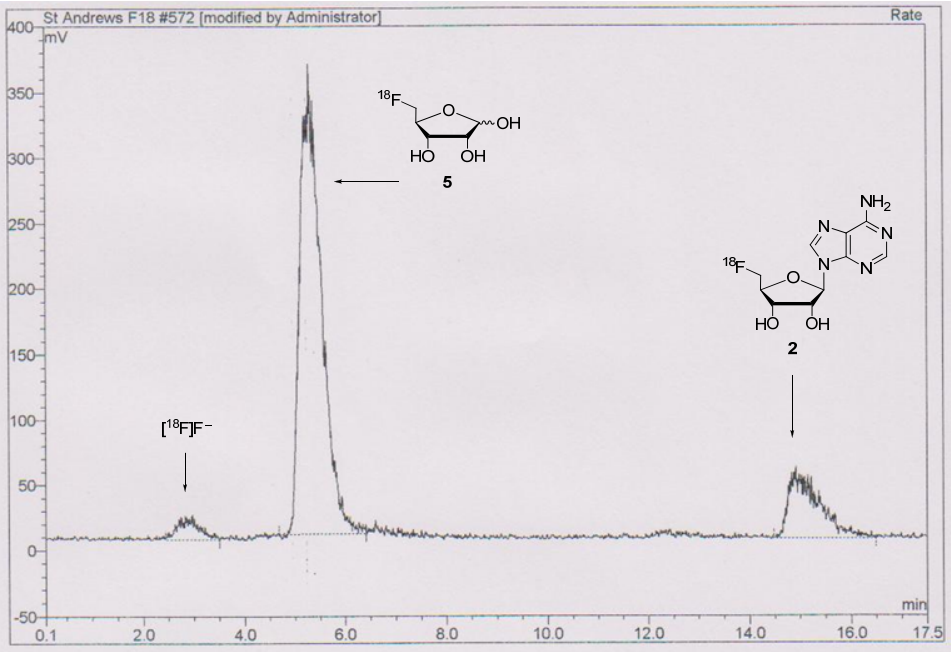

Figure 6 Stage two of the biotransformation: radio-HPLC chromatogram of 5-[^{18}F]FDR formation after 120 min incubation with *Tv*NH at 37 ºC.

References

1. M. Onega, R. P. McGlinchey, H. Deng, J. T. G. Hamilton and D. O´Hagan, *Bioorg. Chem.*, 2007, **35**, 375-385.
2. C. Dong, F. Huang, H. Deng, C. Schaffrath, J. B. Spencer, D. O´Hagan and J. H. Naismith, *Nature*, 2004, **427**, 561–465.
3. W. Versées, K. Decanniere, R. Pellé, J. Depoorter, E. Brosens, D. W. Parkin and J. Steyaert, *J. Mol. Biol.*, 2001, **307**, 1363–1379.