Synthesis of $[{^{18}}\text{F}]$fluoro pivalic-acid: an improved PET imaging probe for the fatty acid synthesis pathway in tumours

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List of abbreviations

HBTU: N,N,N′,N′-Tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate
DBU: 1,8-Diazabicycloundec-7-ene
DIAD: Diisopropyl azodicarboxylate

General

All reagents and solvents were purchased from Sigma-Aldrich, Fluka and used without further purification. Reference compound 3-fluoro-2,2-dimethylpropionic acid (FPIA) was purchased by TC Scientific Inc. Flash column chromatography was carried out on silica gel (Fluka 230-400 mesh, for flash chromatography). Thin layer chromatography was performed on aluminium plates pre-coated with silica (200 µm, 60 F254) which were visualised either by quenching of ultraviolet fluorescence (λmax = 254 nm) or by charring with a KMnO₄ dip. ¹H and ¹³C spectra were obtained on Bruker AV-400, DRX-400 or AV-500 instruments. Chemical shifts (δ) are given in parts per million (ppm) as referenced to the appropriate residual solvent peaks. ¹³C chemical shifts are assigned as s, d, t and q for C, CH, CH₂ and CH₃, respectively. Coupling constant (J) are given in Hertz (Hz). Mass spectra were obtained in positive electrospray ionisation mode on a Micromass LCT Premier equipped with a Waters Atlantis C18 3 µ column 2.1 × 30 mm. Mobile phase (A) water (0.1% formic acid), (B) acetonitrile. HR-MS values are valid up to ±5 ppm. [¹⁸F]Fluoride was acquired from PETNet Solutions (Manchester, UK) or produced with an 11 MeV Siemens Eclipse HP cyclotron -¹⁸O enriched water, >98% ¹⁸O atom, was purchased from Rotem. Semi-preparative HPLC was carried out on a Gilson 121 model and QC HPLC on a QC Agilent 1100 series (quaternary pump with diode array UV detector).
Chemistry

Methyl 2,2-dimethyl-3-[(4-methylbenzenesulfonyl)oxy]propanoate (8)

Methyl 3-hydroxy-2,2-dimethylpropanoate (7, 193 µL, 1.5 mmol) was dissolved in dry pyridine (0.5 mL) and DMAP (9.2 mg, 0.075 mmol) in pyridine (0.5 mL) was added. Tosyl chloride (347 mg, 1.8 mmol) in pyridine (2 mL) was then added and the reaction mixture was stirred at room temperature under nitrogen atmosphere for 3h. The reaction was diluted with CH$_2$Cl$_2$ (30 mL) and water (50 mL). Phases were separated and aqueous layer was extracted with CH$_2$Cl$_2$ (2 x 30 mL). Combined organic layers were washed with 1 M HCl (2 x 50 mL) and brine (50 mL) and dried over Na$_2$SO$_4$. The salt was then filtered off, the reaction mixture concentrated in vacuo and the residue purified by chromatography on silica gel (15% EtOAc/PE). The desired product 8 was isolated as a white solid (270 mg, 70% yield).

8: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.81 (d, $J$ = 8.3 Hz, 2H; Ar), 7.37 (d, $J$ = 8.0 Hz, 2H; Ar), 4.03 (s, 2H; 3-H), 3.63 (s, 3H; OMe), 2.48 (s, 3H; Ph-Me), 1.21 (s, 6H; CH$_3$-2); $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 175.1 (s; CO), 144.9 (s; Ar), 132.7 (s; Ar), 129.8 (d, 2C; Ar), 128.0 (d, 2C; Ar), 75.1 (t; C-3), 52.2 (q; OCH$_3$), 42.8 (s; CMe$_2$), 22.0 (q, 2C; CMe$_2$), 21.7 (q; Ar-Me). MS[ESI,(%)] : 287 [MH$^+$], 309 [MNa$^+$].
Radiochemistry


Method A. Aqueous [^18]F fluoride was trapped into a QMA cartridge and eluted into a 2 mL Wheaton vial with K$_2$CO$_3$ (200 µL of a 12mg/mL stock solution) and K222 (800µL of a 18mg/mL stock solution). The fluoride was dried at 120 °C and an azeotrope of MeCN (1mL) used to aid drying. Precursor 8 (8 mg) in DMF (300 µL) was added and the reaction mixture was heated at 105 °C for 10 min and then cooled down to 30 °C using compressed air. The reaction mixture was quenched with water (700 µL) and labelled intermediate [^18]F]9 isolated by semipreparative HPLC [Phenomenex Gemini 5µ C18 110A (100 X 10 mm, 5 micron)] column, isocratic 30 % EtOH/water method, flow rate 3 mL/min, retention time (rt) = 9 min]. NaOH (1M, 200 µL) was added and the mixture heated at 60 °C for 5 min, cooled down to room temperature and neutralized with HCl (1M). Ethanol was removed at 45 °C under vacuum and PBS was added to reach neutral pH and the right concentration for injection.

Method B. KHCO$_3$ (200 µL of a 12mg/mL stock solution) was used instead of K$_2$CO$_3$ and the labelling carried out as previously described.

Method C. Aqueous [^18]F fluoride was dried in the presence of TBAHCO$_3$ (1.5 M, 22 µL) and the labelling carried out as previously described.
The HPLC analysis was carried out using an Agilent Eclipse XDB-C18, 5 μm, 4.6 × 150 mm column and isocratic 40% MeOH/water (flow rate: 1 mL/min) as HPLC method. A) Reaction mixture; B) Purified \(^{18}\text{F}\)FPIA after semipreparative HPLC; C) formulated \(^{18}\text{F}\)FPIA.
QC analysis

Method A. HPLC column: Agilent Eclipse XDB-C8 5 μm 4.6 × 150 mm

HPLC method: isocratic 20% MeOH/AMF 50mM buffer (pH=4), flow rate: 1 mL/min.

A) Radio-chromatogram; B) UV (220 nM) chromatogram of the formulated compound; C) UV (220 nM) chromatogram of the formulated compound spiked with cold FPIA.
Method B.  
HPLC column: Agilent Eclipse XDB-C8 5 μm 4.6 × 150 mm 

HPLC method: isocratic 20% MeOH/H₃PO₄ 0.005 M buffer, flow rate: 1 mL/min.

A) Radio-chromatogram;  
B) UV (220 nM) chromatogram of the formulated compound;  
C) UV (220 nM) chromatogram of the formulated compound spiked with cold FPIA.
**Biology**

All animal experiments were performed by licensed investigators in accordance with the United Kingdom Home Office Guidance on the Operation of the Animal (Scientific Procedures) Act 1986 and within published guidelines for the welfare and use of animals in cancer research. Female BALB/c mice (aged 6 - 8 weeks; Charles River, Wilmington, MA, USA) were used. Dynamic $^{18}$F-FPIA imaging scans were carried out on a dedicated small animal PET scanner (Siemens Inveon PET module, Siemens Medical Solutions USA, Inc., Malvern, PA, USA) following a bolus i.v. injection of ~3.7 MBq of the radiotracer. Dynamic scans were acquired in list mode format over 60 min. The acquired data were then sorted into 0.5 mm sinogram bins and 19 time frames for image reconstruction (4 × 15 s, 4 × 60 s, and 11 × 300 s), which was done by iterative reconstruction (2D-OSEM). The Siemens Inveon Research Workplace software was used for visualisation of radiotracer uptake. For image visualization, iterative reconstruction was performed (3D-OSEM). For tumour experiments, $2\times10^6$ EMT6 murine breast adenocarcinoma cells were injected subcutaneously on the back of mice. Animals were used when the xenografts reached approximately 150 mm$^3$. Tumour dimensions were measured continuously using a caliper and tumour volumes were calculated by the equation: volume = $(\pi/6) \times a \times b \times c$, in which a, b, and c represent 3 orthogonal axes of the tumour.