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Preparation of [^{3}H]fluoroethyl tosylate and its use in the labelling of the dopamine transporter radioligand [^{3}H]FE-PE2I†

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[^{3}H]Fluoroethyl tosylate, a novel alkylating tritium labelling agent, was synthesized from tritium gas with high specific activity and with 99% radiochemical purity. [^{3}H]Fluoroethyl tosylate was applied in the tritium labelling of the dopamine transporter radioligand [^{3}H]FE-PE2I.

Keywords: [^{3}H]fluoroethyl tosylate; [^{3}H]FE-PE21; dopamine transporter; PET radioligand

Introduction

Fluoroalkyl groups, for example, fluoroethyl and fluoropropyl, allow for straightforward incorporation of fluorine-18 in a molecule.[1] These F-18-labelled reagents are typically incorporated into the target molecule by addition of [^{18}F]fluoroalkyl halides or tosylates to a nucleophile. The requisite [^{18}F]fluoroalkyl halides or tosylates can be synthesized in a one-step radiosynthesis starting from nucleophilic fluorine-18 and are suitable to be produced by automated synthesizer units. Therefore, fluoroalkyl groups are desirable structural motifs in positron emission tomography (PET) radioligands.

For cross-species comparative bridging and pre-clinical validation studies of PET radioligands, compounds labelled with high specific activity tritium may offer an advantage over those labelled with fluorine-18. The long half-life of tritium allows for storage of the compound over an increased period of time, and the low energy particle emission allows for higher resolution *in vitro* imaging than the higher energy positron from F-18 or C-11. A tritium-labelled precursor such as a nonvolatile [^{3}H]fluoroalkyl tosylate would offer a convenient direct route to the corresponding tritiated PET radioligand. The location of the tritium label in the fluoroethyl portion of the molecule allows for better mimicking of the PET tracer *in vivo* as the tritium label and the PET label will be located in the same portion of the molecule and thus produce similar radiolabeled metabolites.

This paper describes the synthesis of [^{3}H]fluoroethyl tosylate and its application in the labelling of the dopamine transporter radioligand [^{3}H]FE-PE2I.[2,3] This was needed for high resolution *in vitro* autoradiographic translational bridging studies prior to clinical PET studies with [^{18}F]FE-PE2I.

Results and discussion

The synthetic route to [^{3}H]fluoroethyl tosylate ([^{3}H]**1**) was envisaged to proceed via reduction using tritium gas of previously described 2-fluorovinyl tosylate (**5**).[4] The potential incorporation of two tritium atoms was expected to provide [^{3}H]fluoroethyl tosylate ([^{3}H]**1**) with high specific activity.

The preparation of the precursor **5** was achieved according to (Scheme 1). Condensation of tosyl chloride with 2,2,2-trifluoroethanol gave 2,2,2-trifluoroethyl tosylate (**3**), and subsequent elimination of **3** in the presence of *n*-BuLi delivered 2,2-difluorovinyl tosylate (**4**) in high yield.[5] Subsequent reduction with $LiAlH_4$ furnished the monofluorinated species **5** in a moderate yield.[4]

A number of hydrogenation catalysts were screened in the reduction of **5** to [^{2}H]**1** under an atmosphere of deuterium gas (Scheme 1, Table 1). The reaction was monitored by the ultraviolet (UV) trace on analytical high-performance liquid chromatography (HPLC), indicating the extent of conversion of the starting material to product. The values reported refer to the relative values of UV-areas of [^{2}H]**1** and **5** are not corrected for differences in response factors. The use of palladium(II) oxide, 5% palladium on carbon in THF or Wilkinson's catalyst in CH_2Cl_2 failed to produce any of the desired product. The reaction employing the iridium-based catalyst [Ir(COD)(PMe_2Ph)(IMes)]PF_6[6] in CH_2Cl_2 resulted in approximately 5% of [^{2}H]**1**, whereas a significantly improved yield of 25% was observed in the presence of the iridium-based Crabtree's catalyst, [Ir(COD)(PCy_3)(py)]PF_6. Employing higher loadings of Crabtree's catalyst of 25 and 50 mol% did not further improve the conversion rate.

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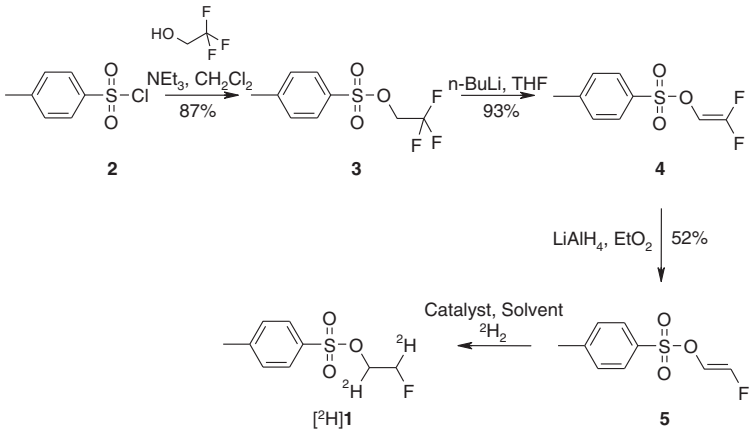


Scheme 1. Synthesis of (*E*)-2-fluorovinyl 4-methylbenzenesulfonate (**5**) and reduction with deuterium to (*E*)-2-fluoroethyl 4-methylbenzenesulfonate ([^{2}H]**1**).

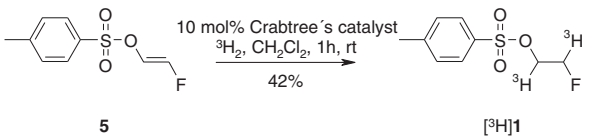
Table 1. Screening of hydrogenation catalysts

Catalyst	Catalyst loading (mol%)	Solvent	% Conversion
PdO	70	THF	0
5%Pd/C	80	THF	0
$Rh(PPh_3)_3Cl$	10	CH_2Cl_2	0
$[Ir(COD)(PMe_2Ph)(IMes)]PF_6$	10	CH_2Cl_2	5
Crabtree's catalyst	10	CH_2Cl_2	25
Crabtree's catalyst	25	CH_2Cl_2	25
Crabtree's catalyst	50	CH_2Cl_2	19

The reaction conditions established of 10 mol% of Crabtree's catalyst were applied to the reduction of **5** with tritium gas on a tritium gas manifold system (Scheme 2). The reaction proceeded without incident, and after purification on normal phase HPLC, the desired product [^{3}H]**1** was obtained with a radiochemical purity of >99% and in a 42% isolated chemical yield. [^{3}H]**1** was stored either in the HPLC eluent (5% ethyl acetate in heptanes) or in toluene. After 6 months storage at −18°C and at a concentration of 240 MBq/mL, the radiochemical purity was still >99%.

Prior to its application in the labelling of FE-PE2I, an aliquot of [^{3}H]**1** was dispensed, and the solvent was removed by a stream of nitrogen gas at ambient temperature. The residue was redissolved in a small volume of CH_3CN, which was the solvent selected for the subsequent labelling reaction.

As depicted in (Scheme 3), treatment of acid precursor **6** with [^{3}H]**1** in MeCN, under microwave heating, furnished the desired [^{3}H]FE-PE2I ([^{3}H]**7**) in an 11% isolated radiochemical yield after normal phase HPLC purification with a specific activity of



Scheme 2. Reduction of (*E*)-2-fluorovinyl 4-methylbenzenesulfonate (**5**) to [^{3}H]2-fluoroethyl 4-methylbenzenesulfonate ([^{3}H]**1**).

1.6 TBq/mmol and a 98% radiochemical purity. It was found that a high reaction temperature was needed for an acceptable yield. However, labelling of the PE2I analogue [^{3}H]FE-β-CIT with [^{3}H]**1** using conventional heating and the corresponding secondary amine as precursor resulted in almost complete conversion, which illustrates a good reactivity of [^{3}H]**1** (data not shown). [^{3}H]**7** was stored in absolute ethanol at −18°C at a concentration of 52 MBq/mL. After 4 months at these storage conditions, the radiochemical purity had decreased to 96%.

The specific activity of [^{3}H]**1** was not determined directly because of weak signal strength on mass spectrometry (MS). However, the specific activity of the carrier free [^{3}H]FE-PE2I should be directly transferrable to [^{3}H]**1** and indicates that, on average, 1.5 tritium atoms were introduced by [^{3}H]**1**. This was reproducible and measured from two different batches of [^{3}H]**1**. Nuclear magnetic resonance (NMR) analysis of [^{3}H]**1** revealed that besides fully labelled, corresponding to two tritiums, and a small amount of nonlabelled [^{3}H]**1**, it was also labelled with one tritium, evenly distributed over the CHT-O and CHT-F groups. This was in accordance with the MS data on the labelled radioligand.

In conclusion, the preparation of the novel radiolabelling agent [^{3}H]fluoroethyl tosylate has been achieved with high specific activity. Its usefulness has been demonstrated by the preparation of the PET radioligand FE-PE2I in its tritiated form. The described method should be extendable to the simple preparation of similar high specific activity alkyl tosylates with tritium gas. Furthermore, as has previously been demonstrated with [^{3}H]methyl nosylate and [^{3}H]methyl tosylate, the stability and nonvolatility allows the solvent to be changed and the reagent to be dispensed in precise quantities.[7]

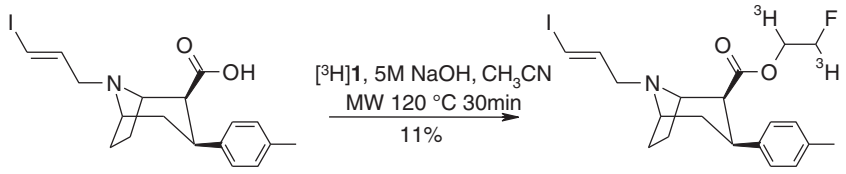
Experimental

General methods

All solvents used were of analytical grade and commercially available. Anhydrous solvents were routinely used for reactions. Reactions were typically run under an inert atmosphere of nitrogen or argon. Tritium gas was handled in a tritium gas manifold system (RC TRITEC AG, Teufen Switzerland). The identities of the final products were established by coelution via HPLC or thin-layer chromatography (TLC) with authentic nonlabelled material.

Preparative chromatography was run on a Gilson 305 system (Agilent Technologies Sweden, Kista, Sweden) with a Gilson UV/VIS-151 and a RAYTEST Ramona detector (Raytest Nordic AB, Höllviken, Sweden).

Gas chromatography mass spectrometry were recorded on a GC/Direct Inlet Probe–MS system supplied by Agilent Technologies,



6 [3H]FE-PE2I, [3H]7

Scheme 3. Tritium labelling of [^{3}H]FE-PE2I ([^{3}H]**7**) with [^{3}H]2-fluoroethyl 4-methylbenzenesulfonate ([^{3}H]**1**). MW, molecular weight.

consisting of a GC 6890N, G1530N, a G2614A Autosampler, G2613A injector and a G2589N mass spectrometer. The mass spectrometer was equipped with an electron impact (EI) ion source. The column used was a DB-5 MS, inner diameter (ID) 0.18 mm × 10 m, 0.18 μm (J&W Scientific). When introduced by GC, a linear temperature gradient was applied starting at 90°C (hold 0.5 min), gradient 40°C/min, ending at 300°C (hold 0.5 min).

^{3}H spectra were recorded on a Bruker DRX600 NMR Spectrometer (Bruker BioSpin Scandinavia AB, Solna, Sweden), operating at 640 MHz for tritium and at 600 MHz for proton, equipped with a 5-mm ^{3}H/^{1}H SEX probe head with *Z*-gradients. ^{1}H decoupled ^{3}H spectra were recorded on samples dissolved in CD_3OD. For ^{3}H-NMR spectra referencing, a ghost reference was used, as calculated by multiplying the frequency of internal tetramethylsilane in a ^{1}H spectrum with the Larmor frequency ratio between ^{3}H and ^{1}H (1.06663975).[8] ^{1}H-NMR spectra were recorded on a Bruker Avance III 500 spectrometer.

High-performance liquid chromatography radiochemical purity analyses were performed on an Agilent 1100 HPLC-system with a binary pump, autoinjector, DAD and column oven, coupled in series with a Packard Radiomatic Flow Scintillator 525TR (Chemical Instruments AB, Lidingö, Sweden), equipped with a solid scintillator (SolarScint) cell with a volume of 32 μL. The column used was an XBridge 3.5 μm C18 (150 mm × 4.6 mm) (Waters Sverige AB, Sollentuna, Sweden). The column temperature was set to 40°C, and the flow rate to 1.0 mL/min. A linear gradient was applied, starting at 100% A (A: 10 mM NH_4OAc in 5% CH_3CN) and ending at 95% B (B: CH_3CN).

Mass spectra were recorded on a Waters LCMS (Waters Sverige AB, Sollentuna, Sweden) consisting of an Alliance 2795 (LC), Waters 2996 Photodiode Array Detector and a ZMD single quadrupole mass spectrometer. The mass spectrometer was equipped with an electrospray ion source operated in a positive or negative ion mode. The capillary voltage was 3 kV, and cone voltage was 30 V. The mass spectrometer was scanned between *m/z* 100–600 with a scan time of 0.7 s. The column temperature was set to 40°C. The diode array detector was scanned from 200 to 400 nm. The column used was an XBridge 3.5 μm C18 (150 mm × 4.6 mm). The column temperature was set to 40°C, and the flow rate to 1.0 mL/min. A linear gradient was applied, starting at 100% A (A: 10 mM NH_4OAc in 5% CH_3CN) and ending at 95% B (B: CH_3CN).

Liquid scintillation analysis was performed on a PACKARD TRICARB 2900TR (Chemical Instruments AB, Lidingö, Sweden). TLC was performed on Merck TLC-plates (Silica gel 60F_{254}), and UV light (254 nm) visualized the spots. Flash column chromatography was performed on Redisep™ (Teledyne Isco, Lincoln, Nebraska, USA) prepacked columns.

2,2,2-Trifluoroethyltosylate (3)

A solution of 2,2,2-trifluoroethanol (0.73 mL, 10 mmol) and triethylamine (5 mL, 36 mmol) in CH_2Cl_2 (10 mL) was cooled to 0°C. *p*-Toluenesulfonyl chloride (2.3 g, 12 mmol) was added, and the solution was stirred at 0°C for 1 h, then warmed to room temperature and stirred for a further 12 h. The CH_2Cl_2 layer was separated and washed with brine (2 mL), dried over sodium sulfate, filtered, and evaporated. Purification by flash chromatography on silica gel eluting with CH_2Cl_2 yielded 2.21 g of **3** as a colourless solid (8.7 mmol, 87%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm 2.48 (s, 3H) 4.36 (q, *J* = 7.88 Hz, 2H), 7.40 (d, *J* = 8.20 Hz, 2H), 7.83 (d, *J* = 8.20 Hz, 2H). GC–MS *m/z* 254 ([M]$^+$).

2,2-Difluorovinyl tosylate (4)

To a solution of 2,2,2-trifluoroethyl 4-methylbenzenesulfonate (**3**) (2.21 g, 8.7 mmol) in THF (50 mL) at −78°C was added dropwise 1.6 M *n*-butyllithium in hexanes (12.5 mL, 20 mmol). After stirring under a nitrogen atmosphere at −78°C for 1 h, the solution was neutralized with a mixture of THF/H_2O (1:1, 30 mL). Water (~20 mL) was added, and the organic phase was extracted with ethyl acetate (2 × 30 mL), dried over sodium sulfate, filtered, and evaporated. Purification by flash chromatography on silica gel eluting with ethyl acetate/heptane (1:1) yielded 1.9 g of **4** as a colourless liquid (8.1 mmol, 93%). ^{1}H-NMR (500 MHz, MeOD) δ ppm 2.48 (s, 3H), 6.41 (dd, *J* = 14.98, 3.94 Hz, 1H), 7.49 (d, *J* = 7.88 Hz, 2H), 7.84 (d, *J* = 8.51 Hz, 2H). GC–MS *m/z* 234 ([M]$^+$).

(*E*)-2-Fluorovinyl 4-methylbenzenesulfonate (5)

To a solution of 2,2-difluorovinyl 4-methylbenzenesulfonate (**4**) (1.9 g, 8.1 mmol) in Et_2O (50 mL) cooled to −10°C was added $LiAlH_4$ (0.31 g, 8.1 mmol) in one portion. The solution was warmed to room temperature and stirred for 12 h. After cooling to 0°C, the mixture was neutralized with an aqueous solution of NaOH (1.0 mL, 0.1 M), then filtered through a plug of silica, and evaporated. Purification by flash chromatography on silica gel eluting with a gradient of heptane to heptane/CH_2Cl_2 (5:1) yielded 0.91 g of **5** as a colourless oil (8.1 mmol, 52%). ^{1}H-NMR (500 MHz, MeOD) δ ppm 2.48 (s, 3H), 6.91–7.23 (m, 2H), 7.48 (d, *J* = 8.20 Hz, 2H), 7.80 (d, *J* = 8.20 Hz, 2H). GC–MS *m/z* 216 ([M]$^+$).

General procedure for the D_2 reduction of (*E*)-2-fluorovinyl 4-methylbenzenesulfonate (5)

A solution of substrate and catalyst in solvent was cooled to −78°C in a dry ice/acetone slurry bath. The system was evacuated three times, filled with nitrogen in the first two instances and with deuterium gas in the third instance. The flask was warmed to room temperature and stirred for 1 h. A small sample of the crude residue was dissolved in MeOH and analysed by HPLC.

[^{3}H]2-Fluoroethyl 4-methylbenzenesulfonate ([^{3}H]1)

A solution of (*E*)-2-fluorovinyl 4-methylbenzenesulfonate (**5**) (1.6 mg, 7.4 μmol) and Crabtree's catalyst (0.6 mg, 0.7 μmol) in CH_2Cl_2 (300 μL) was stirred under a tritium atmosphere (926 mbar) at room temperature for 1 h. The solvent was evaporated, and the residue was taken up in Et_2O (~1 mL) prior to filtration through a short plug of silica. The crude material was purified by preparative HPLC on a Kromasil column (KR60-10CN, 4.6 × 250 mm) eluting with 5% EtOAc in heptane at 2 mL/min. 5040 MBq of [^{3}H]**1** with a radiochemical purity of 99% was obtained. ^{3}H-NMR (250 MBq/mL, 600 MHz, MeOD) δ ppm 4.19–4.25 (m, 1T, CHT-O), 4.47–4.56 (m, 1T, CHT-F).

[^{3}H]FE-PE2I, [^{3}H]*N*-(3-iodoprop-2*E*-enyl)-2β-carbofluoroethoxy-3β-(4-methylphenyl)nortropane ([^{3}H]7)

To a solution of [^{3}H]**1** (925 MBq) in MeCN (300 μL) was added a solution of *N*-(3-iodoprop-2*E*-enyl)-2β-carboxy-3β-(4-methylphenyl)nortropane (**6**) (0.1 mg, 2.4 μmol) in MeCN (200 μL) and 5 M NaOH (4 μL, 20 μmol).

The solution was heated under microwave irradiation at 120°C for 0.5 h. TLC analysis (10% MeOH/CH_2Cl_2) showed almost complete consumption of [^{3}H]**1**. The crude reaction mixture was partitioned between CH_2Cl_2 and water, and the aqueous layer re-extracted with a further portion of CH_2Cl_2. The solvent was evaporated, and the resulting material was purified by preparative HPLC on a Kromasil column (C8, 5 μm, 10 × 250 mm) eluting with 75% MeCN modified with 0.1% NEt_3 at 3 mL/min. Fractions containing the product were combined, concentrated, and dissolved in absolute ethanol to give 104 MBq of [^{3}H]**7** with a specific activity of 1.6 TBq/mmol and a 98% radiochemical purity. The product was stored in absolute ethanol at a concentration of 52 MBq/mL. LC/MS [$(M+H)^+$]: 458 (8.2%), 459 (1.8%), 460 (55.0%), 461 (12.8%), 462 (100%), 463 (22.3%), 464 (2.8%).

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Conflict of Interest

The authors did not report any conflict of interest.

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