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Research Article

Simplified synthesis of *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane ([^{18}F]β-CFT-FP) using [^{18}F]fluoropropyl tosylate as the labelling reagent

Teija Koivula[1,*], Outi Perhola[1], Eeva-Liisa Kämäräinen[2], Tiina Lipponen[1], Jouko Vepsäläinen[3] and Olof Solin[1,4]

[1] *Laboratory of Radiochemistry, Department of Chemistry, University of Helsinki, P.O. Box 55, FI-00014, University of Helsinki, Finland*
[2] *Division of Nuclear Medicine, Helsinki University Central Hospital, FI-00270 Helsinki, Finland*
[3] *Department of Chemistry, University of Kuopio, P.O. Box 1627, FI-70210 Kuopio, Finland*
[4] *Turku PET Centre, P.O. Box 52, FI-20521 Turku, Finland*

Summary

A synthesis method has been developed for the labelling of *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane ([^{18}F]β-CFT-FP), a potential radioligand for visualization of the dopamine transporters by positron emission tomography. The two-step synthesis includes preparation of [^{18}F]fluoropropyl tosylate and its use without purification in the fluoroalkylation of 2β-carbomethoxy-3β-(4-fluorophenyl)nortropane (nor-β-CFT). The final product is purified by HPLC. Optimization of the two synthesis steps resulted in a greater than 30% radiochemical yield of [^{18}F]β-CFT-FP (decay corrected to end of bombardment). The synthesis time including HPLC-purification was approximately 90 min. The radiochemical purity of the final product was higher than 99% and the specific radioactivity at the end of synthesis was typically 20 GBq/μmol. In comparison to alkylation by [^{18}F]fluoropropyl bromide, the procedure described here results in an improved overall radiochemical yield of [^{18}F]β-CFT-FP in a shorter time. Copyright © 2005 John Wiley & Sons, Ltd.

*Correspondence to: Teija Koivula, Laboratory of Radiochemistry, Department of Chemistry, University of Helsinki, P.O. Box 55 (A.I. Virtasen aukio 1), FI-00014, University of Helsinki, Finland.
E-mail: teija.s.koivula@helsinki.fi

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Introduction

The *in vivo* imaging of dopamine transporters (DAT) is important when monitoring neurological function in the brain. Several positron-emitting radioligands labelled with ^{11}C, ^{18}F or ^{76}Br have been developed for the imaging of DAT by positron emission tomography (PET). These include ^{11}C-labelled nomifensine[1] and methylphenidate,[2] as well as the ^{18}F-labelled piperazine derivative named GBR 13119.[3] [^{11}C]Cocaine has also been evaluated as a potential DAT radioligand.[4] However, several of the labelled analogues have proven to possess better physical and binding properties than cocaine itself. Such radioligands are based on the phenyl tropane structure of cocaine: typically compounds such as of 2β-carbomethoxy-3β-(4-fluorophenyl)tropane (CFT or WIN 35,428) and its iodinated derivative β-CIT.[5–11] In fluoromethyl tropanes the phenyl group attached to the tropane ring is fluoromethylated at either the *para* or *ortho* position.[12,13]

Several potential DAT ligands have also been developed by modifying the original tropane structure of cocaine. There is a large number of analogues where the bridging amino group is alkylated either with a fluoroethyl or a fluoropropyl group, e.g. β-CIT-FE and β-CIT-FP [*N*-(3-fluoroethyl or propyl)-2β-carbomethoxy-3β-(4-iodophenyl)nortropane] and also the corresponding chlorinated and methylated compounds FECNT and FPCMT.[14–23] Recent additions among these radioligands include prop-2-enyl- (PE2)[24–26] and fluorobenzyl derivatives.[27] In another class of tropanes the 2β-carboxy group is modified to yield fluoroethyl ester[28,29] or amide congeners.[30] The effect of adding substituent such as a fluoro-2-propoxy group to the 2β-carboxy group has also been evaluated.[31]

^{18}F-labelling procedures for alkylated tropane analogues are often two-step methods beginning with the production of a labelling reagent, which is then used for the ^{18}F-fluoroalkylation of the nortropane precursor at the bridging amino group of the tropane ring.[16–23] This kind of synthesis methods have been used previously in the production of several ^{18}F-fluoropropylated cocaine analogues, such as [^{18}F]β-CIT-FP and [^{18}F]FPCT.[16–20,23] Labelling in most of these syntheses is via [^{18}F]fluoropropyl bromide.[16,18,19] Separating this labelling reagent from the 1,3-dibromopropane precursor requires a complicated purification using a C-18 cartridge and subsequent distillation. The successful synthesis of [^{18}F]β-CIT-FP without intermediate purification was reported by Kazumata *et al.*[17] In their one-pot method an alternative alkylating agent, [^{18}F]fluoropropyl tosylate, was used.

We have previously prepared the ^{18}F-labelled phenyl tropane analogue *N*-(3-[^{18}F]fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane

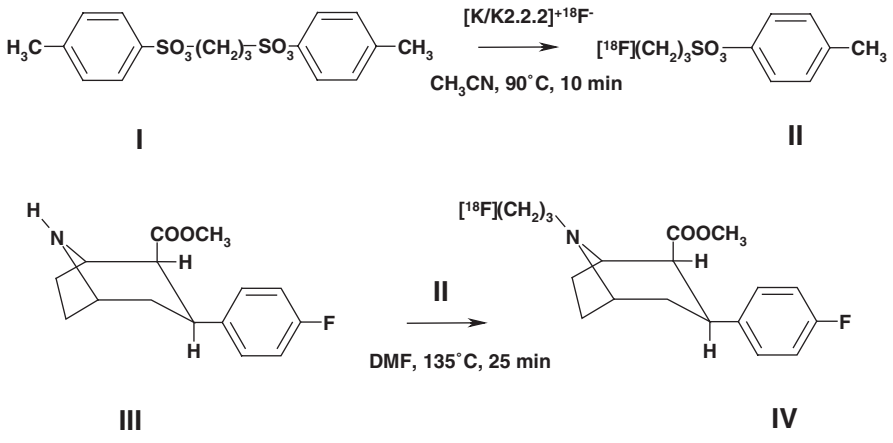

Figure 1. Radiolabelling of [^{18}F]β-CFT-FP (IV) starting from [^{18}F]fluoropropyl tosylate (II) and nor-β-CFT (III)

([^{18}F]β-CFT-FP) by a two-step synthesis using [^{18}F]fluoropropyl bromide or tosylate as the labelling reagent.[19] Labelling with alkyl bromide resulted in a higher radiochemical yield of [^{18}F]β-CFT-FP than labelling with alkyl tosylate. However, because of the protracted and complicated purification step of [^{18}F]fluoropropyl bromide we decided to develop further the approach with [^{18}F]fluoropropyl tosylate as the labelling reagent.

Here we report the development of a rapid two-step synthesis of [^{18}F]β-CFT-FP using [^{18}F]fluoropropyl tosylate as the alkylating agent (Figure 1). Preparation of [^{18}F]fluoropropyl tosylate was studied in various chemical conditions and the reagent was used without separate purification in the labelling of 2β-carbomethoxy-3β-(4-fluorophenyl)nortropane (nor-β-CFT). The ^{18}F-fluoroalkylation reaction was optimized with respect to reaction time and temperature as well as stochiometric conditions. The [^{18}F]β-CFT-FP obtained was then purified by HPLC.

Results and discussion

Preparation of [^{18}F]fluoropropyl tosylate

The best average radiochemical yield in the preparation of [^{18}F]fluoropropyl tosylate was $61 \pm 4\%$ ($n = 6$) using 10 mg of 1,3-propanediol-di-*p*-tosylate precursor in a volume of 0.5 ml of CH_3CN and a 10 min reaction time. Extending the reaction time or using higher concentration of precursor did not have a significant effect on the radiochemical yield. In fact, contrary to previously reported results,[19] a lower amount of precursor (< 8 mg) lowered the incorporation yield of [^{18}F]fluoropropyl tosylate considerably, see Figure 2. Similar results have also been reported by Block *et al.*[32]

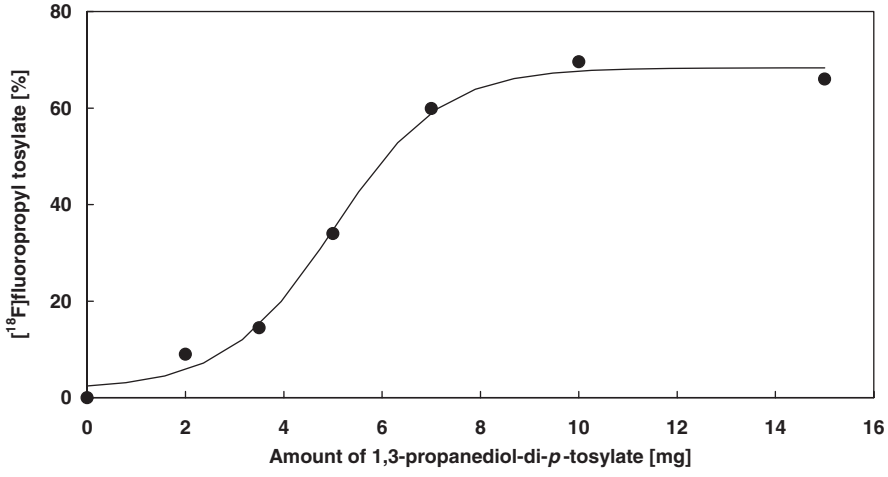

Figure 2. Radiochemical incorporation yield of [^{18}F]fluoropropyl tosylate (by TLC) as a function of amount of 1,3-propanediol-di-*p*-tosylate. The solid line is a sigmoidal fit to the experimental data points

Substitution of the tosylate precursor could proceed using either CH_3CN or DMF as a solvent. However, CH_3CN was chosen considering the final purification and formulation of [^{18}F]β-CFT-FP. A heated ultrasonic bath and a heated oil bath with magnetic stirring were found to promote the labelling reaction equally well.

Commercial C-18 and SiO_2 SPE cartridges have previously been used in the purification of [^{18}F]fluoroalkyl tosylate reagents.[19,21] In our recent experience neither of these cartridges separate [^{18}F]fluoropropyl tosylate from 1,3-propanediol-di-*p*-tosylate. Purification by this means has no effect on the alkylation reaction and it was therefore excluded from the synthesis procedure. This simplifies the method, increases the yield as the handling losses are reduced and also shortens the overall synthesis time.

Preparation of [^{18}F]β-CFT-FP

The most critical parameter in the ^{18}F-fluoroalkylation of nor-β-CFT was the amount of 1,3-propanediol-di-*p*-tosylate remaining after the first reaction step. The unreacted precursor competes with the labelling reagent in the alkylation of nor-β-CFT. An equivalent molar ratio of nor-β-CFT to 1,3-propanediol-di-*p*-tosylate was needed to achieve maximum (90%, decay corrected) incorporation of [^{18}F]fluoropropyl tosylate into [^{18}F]β-CFT-FP, see Figure 3. On a molar basis 10 mg of 1,3-propanediol-di-*p*-tosylate corresponds to approximately 7.5 mg of nor-β-CFT. The optimum reaction condition for the alkylation was a reaction time of 25 min at 135°C. A lower reaction

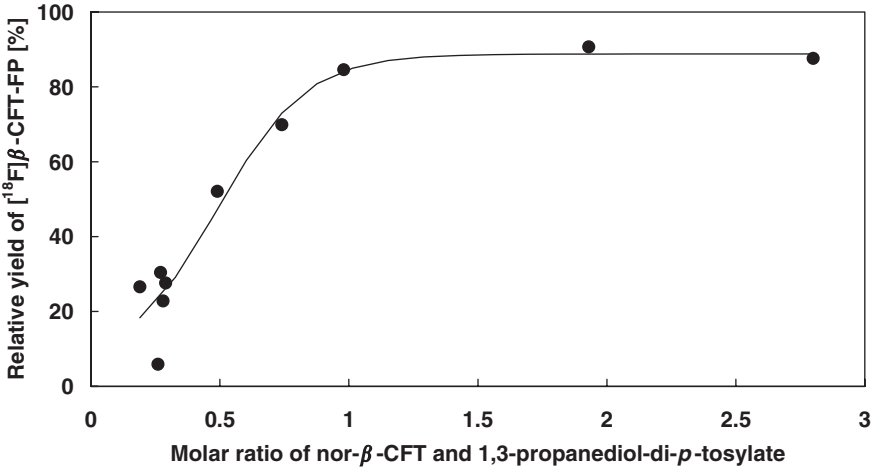

Figure 3. Relative yield of [^{18}F]β-CFT-FP from [^{18}F]fluoropropyl tosylate (decay corrected) as a function of molar ratio of nor-β-CFT and 1,3-propanediol-di-*p*-tosylate. The solid line is a sigmoidal fit to the experimental data points

temperature (90°C) failed to produce [^{18}F]β-CFT-FP. Raising the reaction temperature (150°C) or prolonging reaction time over 25 min did not improve the yield. The effect of a base (Cs_2CO_3) on the labelling reaction could not be demonstrated.

[^{18}F]β-CFT-FP was purified by HPLC. Under described conditions [^{18}F]β-CFT-FP eluted at 13–15 min and its inactive precursor nor-β-CFT at 8–10 min. The radiochemical yield of the separated [^{18}F]β-CFT-FP was 18 $\pm$ 2% (with decay correction to end of bombardment >30%) and the radiochemical purity exceeded 99%.

The overall radiochemical yield of formulated [^{18}F]β-CFT-FP at the end of synthesis (EOS) was approximately 10%. The total synthesis including formulation lasted about 105 min. The specific radioactivity of [^{18}F]β-CFT-FP at EOS was 15–30 GBq/μmol (400–800 Ci/mmol). The stability of the product was confirmed for up to 8 h after EOS, at which time the radiochemical purity of [^{18}F]β-CFT-FP was >97%.

Experimental

Materials

1,3-Propanediol-di-*p*-tosylate was purchased from Aldrich. The inactive precursor 2β-carbomethoxy-3β-(4-fluorophenyl)nortropane (nor-β-CFT) and the reference standard for the product *N*-(3-fluoropropyl)-2β-carbomethoxy-3β-(4-fluorophenyl)nortropane (β-CFT-FP) were synthesised as described

earlier.[19] Other reagents and chemicals were obtained from commercial sources. HPLC-solvents were of HPLC gradient grade and other chemicals were of analytical grade.

Production of [^{18}F]fluoride

[^{18}F]fluoride was produced at the Laboratory of Radiochemistry, University of Helsinki with an IBA Cyclone 10/5 cyclotron using 10 MeV protons in the ^{18}O(p,n)^{18}F nuclear reaction on ^{18}O-enriched water. The irradiated target water was transferred through teflon tubing from the target chamber to the laboratory.

Preparation of [^{18}F]fluoropropyl tosylate

No-carrier-added (n.c.a.) aqueous [^{18}F]fluoride was added to a solution of Kryptofix-2.2.2 (15 mg, 40 μmol) and K_2CO_3 (3.0 mg, 22 μmol dissolved in 100 μl of H_2O) in dry acetonitrile (800 μl). The reaction mixture was dried by azeotropic distillation (115°C) with three 1 ml portions of CH_3CN under a N_2-stream. 1,3-propanediol-di-*p*-tosylate (purity 98%) in 500 μl of dry CH_3CN was added to the dried residue. This solution was then heated in a closed vial at 90°C. The crude reaction mixture was subsequently cooled to room temperature.

The radiochemical yield of [^{18}F]fluoropropyl tosylate was investigated by varying the following parameters: 2–15 mg (5–38 μmol) of precursor, and 10 or 20 min reaction time. The reaction mixture was heated either in an ultrasonic bath or oil bath (reaction vial equipped with magnetic stirring). DMF was also tested as a solvent with 10 mg of 1,3-propanediol-di-*p*-tosylate.

Preparation of [^{18}F]β-CFT-FP

Three hundred microliters of dry DMF solution containing a variable amount of nor-β-CFT precursor (2–20 mg, 7–69 μmol) with or without base, Cs_2CO_3, was added into the cooled labelling reagent mixture. The reaction vial was sealed and heated at 135°C varying the reaction time from 10 to 50 min. Significantly lower (90°C) and higher (150°C) reaction temperatures with 25 min heating time were also tested. The solution was subsequently cooled to room temperature and 700 μl of HPLC-mobile phase was added.

Crude [^{18}F]β-CFT-FP was purified by semi-preparative reversed-phase HPLC on a Waters μBondapak® C_{18} column (7.8 × 300 mm, 10 μm) using 0.01 M H_3PO_4/CH_3CN (80:20; v/v) as an eluent with flow rate of 4 ml/min. The chromatographic system consisted of Merck-Hitachi L-7100 HPLC pump, Rheodyne 7010 injector with 2 ml loop and LKB Bromma 2151 UV-detector ($\lambda = 254$ nm) in series with NaI(Tl) crystal for radioactivity detection.

The collected product fraction was concentrated *in vacuo* and the residue was dissolved in 2 ml of sterile saline.

Analysis of radiolabelled products

Radiochemical purity and stability of [^{18}F]β-CFT-FP was analyzed by TLC and reversed-phase HPLC. The identity of the product was confirmed by comparing radiochromatograms of [^{18}F]β-CFT-FP with the UV-chromatograms of non-labelled reference material. Analytical HPLC was also used to determine the specific radioactivity of [^{18}F]β-CFT-FP. A standard curve was generated to calculate the mass of the final solution.

The analytical HPLC apparatus was comprised of a PC-controlled system with Waters Millennium®^{32} software, Waters 600E pump, Rheodyne 7125 injector with 100 µl loop, Waters photodiode array detector (PDA 996, range 190–400 nm) and NaI(Tl) crystal for radioactivity detection. A Waters µBondapak® C_{18} column (3.9 × 300 mm, 10 µm) was eluted at a flow rate of 2 ml/min using a mixture of 0.01 M H_3PO_4 (A) and CH_3CN (B) either in isocratic (A/B: 80:20) or gradient mode. The gradient program consisted of four steps: 0–7 min isocratic (A/B: 80:20); 7–17 min linear shift to A/B: 30:70; 17–20 min isocratic (A/B: 30:70); 20–25 min return to initial conditions (A/B: 80:20). Using these conditions ($\lambda = 254$ nm), nor-β-CFT, [^{18}F]β-CFT-FP and [^{18}F]fluoropropyl tosylate eluted at approximately 4, 6, and 14 min, respectively.

TLC was carried out using silica gel plates (60 F_{254}, Merck) and hexane/ether/TEA as the mobile phase. The eluent composition was 6.5:3.5:0.1 for the labelling reagent ($R_f = 0.43$) and 6.5:3.5:1 for the crude and final products ($R_f = 0.73$). UV visualization was accomplished with UV-lamp at 254 nm. Radioactivity was detected with a Raytest MiniGita TLC-scanner (raytest GmbH, Straubenhardt, Germany).

Conclusion

[^{18}F]β-CFT-FP can be produced efficaciously from nor-β-CFT using [^{18}F]fluoropropyl tosylate as the fluoroalkylating agent. The synthesis procedure is faster, simpler and results in a higher radiochemical yield of [^{18}F]β-CFT-FP when compared to the earlier two-step method using [^{18}F]fluoropropyl bromide.[19] Optimized chromatographic purification of the final product yields [^{18}F]β-CFT-FP with good radiochemical and chemical purities as well as reasonable specific radioactivity.

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