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Two-step radiosynthesis of [^{18}F]FE-β-CIT and [^{18}F]PR04.MZ

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The cocaine-derived dopamine reuptake inhibitors FE-β-CIT (8-(2-fluoroethyl)-3-(4-iodophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester) (1) and PR04.MZ(8-(4-fluorobut-2-ynyl)-3-p-tolyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester) (2) were labelled with ^{18}F-fluorine using a two-step route. 2-[^{18}F]Fluoroethyltosylate and 4-[^{18}F]fluorobut-2-yne-1-yl tosylate were used as labelling reagents, respectively. Radiochemically pure (>98%) [^{18}F]FE-β-CIT and [^{18}F]PRD04.MZ (32–86 GBq/μmol) were obtained after a synthesis time of 100 min in about 25% non-decay-corrected overall yield.

Keywords: dopamine transporter; fluorine-18; automated synthesis; tropanes

Introduction

Dopamine transporter (DAT) imaging agents are highly sought after for studies of the availability of this presynaptic binding site in preclinical research and clinical application.[1–4]

Two viable strategies for the synthesis of ^{18}F-labelled radioligands for the presynaptic DAT, direct substitution and ^{18}F-fluoro alkylation, have been reported.[5–11]

In recent years, a variety of ^{18}F-labelled 3-phenyltropane analogues have been reported, and the available labelling strategies were thoroughly elaborated. Although direct labelling superficially appears to be the more elegant route, it suffers from low radiochemical yields, and most approaches to overcome this issue do not translate well into the available automated synthesis modules, for example, due to the use of microwave reactors.[12,13] This is a major issue for clinical application, because routine production of sufficient doses requires starting radioactivities well above the amounts considered to be safe for manual handling.[1–8,12,13]

Radiolabelling of cocaine-analogue radioligands with the positron emitter ^{18}F($t_{1/2}$ = 109.7 min) via nucleophilic fluorination was often complicated by (i) low *N*-alkylation yields, (ii) poor accessibility of sp^2-carbon-bound fluorine, resulting in laborious reaction pathways and (iii) α/β-epimerisation at the C-2 position in the tropane skeleton.[8,12,14–17]

The use of ^{18}F-fluoroalkylating agents has repeatedly been described as a reliable route to furnish the desired radioligands for DAT imaging.[5–8,12] Until recently, automated direct nucleophilic radiofluorination was less popular for this purpose.[10,11] Despite being less straightforward than direct nucleophilic substitution, the ^{18}F-fluoroalkylation route has some exceptional advantages for radiolabelling of cocaine derivatives. The reaction proceeds under milder, less basic conditions, labelling precursors are more readily accessible and the labelled product is often easier to separate from the labelling precursor by preparative HPLC.[5–8,16,18,19]

In addition, 2-[^{18}F]fluoroethyl tosylate has become commercially available, and satellite distribution of other ^{18}F-fluoroalkylating agents can be anticipated, thus enabling one-step labelling processes. Moreover, automated synthesis modules have become available that allow for automation of complex processes including two subsequent HPLC purifications and current good manufacturing practise (cGMP) compliant productions involving the synthesis of ^{18}F-labelled building blocks as intermediates. To the best of our knowledge, ^{18}F-labelling of [^{18}F]FE-β-CIT ([^{18}F]**1**) has not been described yet. This is unfortunate because despite providing useful characteristics such as the opportunity of introducing an ^{123}I-label, and an ^{11}C-label, only ^{18}F will provide a sufficiently long half-life that correlates with the kinetic profile of the ligand to facilitate kinetic modelling in quantitative positron emission tomography studies. Initial direct aliphatic nucleophilic radiofluorination of [^{18}F]PRD04.MZ ([^{18}F]**2**) was]performed under conventional conditions. Unfortunately, only low radiochemical yields of 13 ± 3% were obtained after the reaction times of 60–70 min. For these reasons and given the scope of an ^{18}F-labelled DAT ligand, we have elaborated a suitable two-step method for the radiosynthesis of the ^{18}F-labelled 3-phenyltropanes[^{18}F]FE-β-CIT ([^{18}F]**1**), which has not been described yet, and [^{18}F]PR04.MZ ([^{18}F]**2**).[20]

Experimental

Automated synthesis of ^{18}F-fluorinated labelling agents ([^{18}F]5–6)

All operations were performed in a lead-shielded cell, containing an in-house designed homemade synthesis module. He 5.0 gas was used for all operations at a pressure of 1.4 × 10^5 Pa. [^{18}F]fluoride in $H_2^{18}O$ was extracted from

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the target water using a Waters (GmbH, Eschborn, Germany) light QMA strong anion exchange cartridge. Elution from the resin was performed using a solution of Kryptofix® (Merck KGaA, Darmstadt, Germany) K222 (15 mg, 0.4 μmol) and potassium carbonate (2.1 mg, 15 μmol) in dry MeCN (1 ml). The eluate was concentrated to dryness in 15 min, using a five-step drying procedure. The labelling precursor (15 mg, 35–40 μmol), dissolved in MeCN (1 ml), was added subsequently, and the mixture was stirred at 88°C for 3 min. The reaction mixture was diluted with 50% water in MeCN (1 ml) and transferred into a 2-ml inject loop of a Knaur (Wissenschaftliche Geraetebau Dr. Ing. Herbert Knauer GmbH, Berlin, Germany) semi-preparative HPLC system, equipped with a Lichrospher RP18 (Merck KGaA, Darmstadt, Germany) 5 μm column. A mixture of MeCN and water (55/45) was used as mobile phase at a flow rate of 4.7 ml/min. The product fraction was collected, diluted with water (30 ml) and passed through a Merck Lichrospher EN (Merck KGaA, Darmstadt, Germany) cartridge. After drying of the cartridge in a stream of He, the product was eluted in dimethyl sulfoxide (DMSO) (1 ml) warmed to 65°C, directly into the preheated second reaction vessel, containing precursor. 2-[^{18}F]Fluoroethyl tosylate ([^{18}F]**5**) was obtained in a decay-corrected yield of 60 ± 8% after 55 min; 4-[^{18}F]Fluorobut-2-yne-1-yl tosylate ([^{18}F]**6**) was obtained in a radiochemical yield of 64 ± 4%.

Screening of reaction conditions

Screening reactions[21] were conducted in conical bottom-brown glass Wheaton (Wheaton, Millville, NJ, USA) reaction vessels in triplicate using 2 mg of nortropanes **3** and **4** in DMSO (1 ml) at 80–120°C. Lithium iodide (5 mg, 37 μmol) was added to the vials at 80°C but not at higher temperatures because of the volatility of 2-[^{18}F]fluoroethyl iodide. Samples (100 μl) were withdrawn after 3, 7, 10 and 20 min, quenched in water[26] (400 μl) and analyzed by HPLC.

Exemplified radiosynthesis of [^{18}F]2

After the addition of **6** to the reaction vessel, the reaction mixture was stirred at 120°C for 15 min; after which the reaction was quenched by the addition of the eluent (4 ml) to the reaction vessel. The resultant mixture was drawn into a syringe containing water (5 ml) and passed through a Merck strong cation exchange (SCX) cartridge to remove the DMSO prior to HPLC purification. The trapped radioactivity was eluted with dilute ammonia solution 1 M (1 ml) and purified on a semi-preparative Phenomenex Luna (Phenomenex, Aschaffenburg, Germany) RP18(2) 10 μm 250 × 10 mm HPLC column, using 40% 0.1-M ammonium acetate/60% acetonitrile at pH 4.7 as mobile phase. The product fraction was collected after a total retention time of 13–15 min, diluted with 10 ml of water and passed through a Merck Lichrolut SCX cartridge (200 μg), conditioned with 5 ml of 1-M hydrochloric acid and 20 ml of water. Subsequently, the cartridge was washed with 3 ml of water and the product eluted in a crimp-sealed vial through a sterile filter using phosphate-buffered saline (PBS) (1 ml). Average non-decay-corrected yield was 25% for [^{18}F]**1** and [^{18}F]**2**, respectively.

Analytical HPLC of [^{18}F]1 and [^{18}F]2

The radiochemical purity and the specific radioactivity of [^{18}F]**1** and [^{18}F]**2** were determined on a Sykam HPLC system (Sykam GmbH, Eresing, Germany), equipped with a Berthold LB501 (Berthold Technologies GmbH & Co. KG, Bad Wildbad Germany) radioactivity detector and a Knauer (Knauer GmbH, Berlin, Germany) ultraviolet (UV) detector. A Phenomenex Luna RP18 5 μm 250 × 4.6 mm analytical HPLC column was used as stationary phase. For [^{18}F]**1**, the mobile phase consisted of 50% 0.01-M phosphoric acid in MeCN; for [^{18}F]**2**, 30% 0.05-M ammonium acetate buffer at pH 4.6 in MeCN was used. An aliquot of 20 μl was withdrawn from the formulated tracer solution and diluted with eluent. Approximately 50 kBq of this solution was injected into the HPLC. Products [^{18}F]**1** and [^{18}F]**2** were eluted after 8–10 and 10–11 min, respectively. Specific activities ranged from 32 to 86 GBq/μmol.

Results and discussion

Chemistry

Reference compounds **1–2** and the corresponding nortropane congeners used as labelling precursors **3–4** were synthesized from natural cocaine as published elsewhere.[20–22,25] 1,2-Bis-(4-toluenesulfonyloxy)ethane (**7**) as a starting material for the synthesis of [^{18}F]**5** is commercially available. The starting material required for the radiosynthesis of [^{18}F]**6**, 1,4-bis-(4-toluenesulfonyloxy)but-2-yne (**8**), was synthesized in a yield of 22% from commercially available but-2-yne-1,4-diol according to a phase-transfer catalytic procedure described for 1,4-bis-(4-toluenesulfonyloxy)but-2-ene.[7]

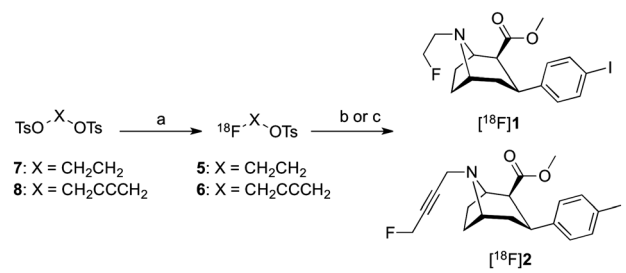
Radiochemistry

The synthesis of both radiotracers was performed in a two-step procedure, by first synthesizing the prosthetic group [^{18}F]**5** or [^{18}F]**6** followed by the ^{18}F-fluoroalkylation of the respective precursor **3** or **4** (Scheme 1).

Preparation of the radiofluorinated alkylating agents was conducted using a homemade synthesis module (Figure 1). The process was based on a report of Block *et al.*[21,24] and modified to our needs. Starting from aqueous [^{18}F]fluoride, potassium carbonate and Kryptofix® K 222, removal of volatiles by azeotropic co-evaporation with MeCN afforded the potassium cryptate complex [$K^+ \subset$ K222]$^{18}F^-$ in a sequence taking roughly 15 min. A solution of the appropriate ditosylate in acetonitrile was added to the residue and heated to reflux for 3 min. The reaction mixture was diluted with HPLC eluent, and the product was purified using semi-preparative reversed phase HPLC.

The product fraction was collected and diluted with H_2O, and the product was separated by solid-phase extraction on a Merck® Lichrolut EN cartridge. This cartridge was dried for 15 min to remove excess moisture from the trapped reagent. This process afforded the labelling reagents [^{18}F]**5** and [^{18}F]**6** in radiochemical yields of about >60% after a total synthesis time of 55 min (Table 1). In our experience, the use of HPLC-purified [^{18}F]**5** and [^{18}F]**6** results in higher yields in the subsequent labelling reactions and reduced formation of non-radioactive by-products that might complicate the purification. We thus resorted to two subsequent HPLC purifications rather than only one after the second step.

The trapped radioactive reagent was eluted into a Wheaton reaction vessel, and aliquots of about 50 MBq of [^{18}F]**5** and [^{18}F]**6** were used for the screening of reaction conditions. [^{18}F]**1** was synthesized by the reaction of **3** and [^{18}F]**5** in DMSO, and the time-dependent labelling yield was investigated as a function of the reaction temperature in the absence and in the presence of LiI. To avoid light-induced de-iodination, a brown-glass vessel was used for



Scheme 1. Radiosynthesis of [^{18}F]**1** and [^{18}F]**2**. a: [$K^+ \subset$ K222]$^{18}F^-$, MeCN, 88°C, 3 min; b: **3**, [^{18}F]**5**, dimethyl sulfoxide (DMSO), 120°C, 20 min; and c: **4**, [^{18}F]**6**, DMSO, 120°C, 15 min.

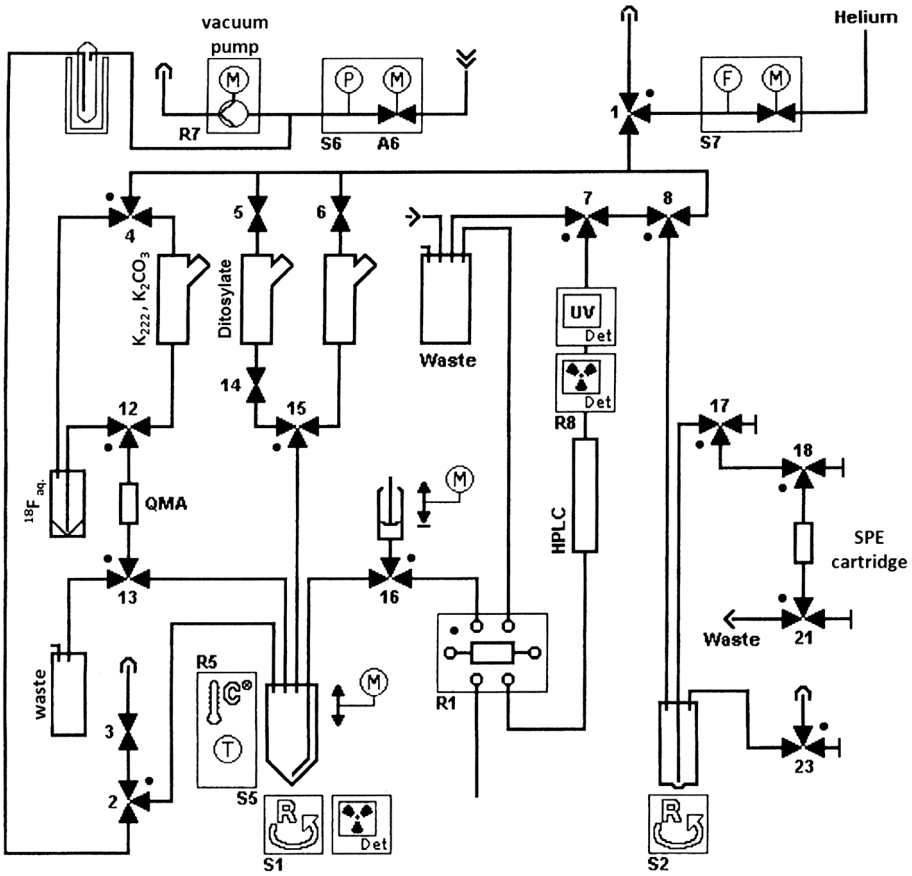


Figure 1. Scheme of the synthesis module. SPE, solid-phase extraction; UV, ultraviolet

Table 1. Labelling conditions, retention times and radiochemical yields

	Conditions	t_R (min)	t_R(QC)/min	RCY (%)
[^{18}F]**1**	DMSO, 20 min, 120°C	11–12	8	64 ± 5
[^{18}F]**2**	DMSO, 15 min, 120°C	13–15	10	67 ± 5.5
[^{18}F]**5**	MeCN, 3 min, 88 °C	8–10	6	60 ± 8
[^{18}F]**6**		10–11	8	64 ± 4

DMSO, dimethyl sulfoxide; QC, quality control; RCY, radiochemical yield.

[^{18}F]**1**. The results are depicted in Figure 2. In an analogue fashion, [^{18}F]**2** was synthesized from **4** and [^{18}F]**6**. A radiochemical yield of about 64–67% was achieved after a reaction time of 15–20 min at 120°C using DMSO as solvent and 2 mg of the precursor. Following the ^{18}F-fluoroalkylation reaction, both products, [^{18}F]**1** and [^{18}F]**2**, were purified using semi-preparative HPLC.

[^{18}F]**1** was eluted after a reasonable retention time (t_R: 11–12 min), which was necessary to separate it from a polar non-significant by-product, which was hypothesized to correspond to either the saponified acid or the 2α-epimer. For the concentration and isolation of the radiotracer, the product fraction was diluted with water and trapped on a C18 cartridge. The cartridge was washed with water, and the product was eluted with EtOH, followed by 0.9% NaCl solution. This procedure gave [^{18}F]**1** as a colourless, clear solution of neutral pH with a radiochemical purity >98%. No significant UV impurities were detected.

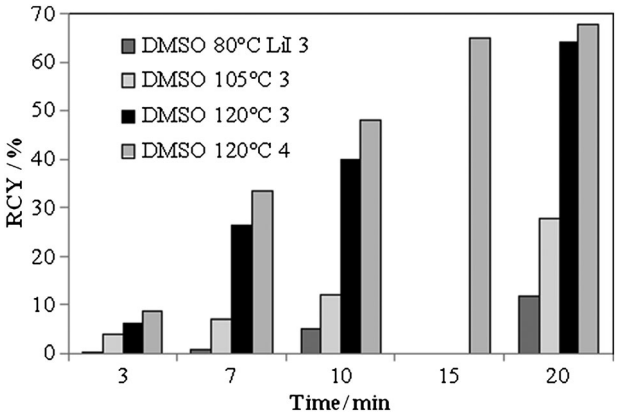


Figure 2. Radiochemical yields (RCYs) of [^{18}F]**1** as a function of reaction temperature and time using 2 mg of the precursor **3**. DMSO, dimethyl sulfoxide.

The HPLC purification of [^{18}F]**2** yielded the product after a retention time of 13–15 min. [^{18}F]**2** was isolated and formulated using a proton-loaded Merck Lichrolut SCX resin. About 95% of the total radioactivity in the diluted product fraction was trapped on the SCX resin. Subsequently, the cartridge was washed with water, and the product was eluted through a sterile filter into a crimp-sealed vial using isotonic PBS. Less than 5% of the total trapped radioactivity remained on the SCX cartridge. The formulation was a colourless, clear solution with a pH value of 7.3. Quality control by analytical HPLC showed a radiochemical purity >98%. No significant UV impurities were detected. In both cases, radio-HPLC conditions were validated by radio-TLC to ensure the absence of co-eluting impurities in the product fraction. The formulations of [^{18}F]**1** and [^{18}F]**2** prepared in this procedure contained either one of both tracers in specific activities of 32–86 GBq/μmol after 100 min starting from [^{18}F]fluoride. In comparison with direct nucleophilic radiofluorination under conventional conditions (13%), formulated [^{18}F]**2** in sterile PBS was obtained in a two-fold higher yield (25%), despite using the slightly more elaborate two-step procedure described herein.[20]

Although [^{18}F]**2** has been produced in even higher yields (32–36%) in a much shorter time (35 min) using a monomodal laboratory microwave reactor, we feel that ease of automation using commercially available synthesis modules combined with conventional heating is probably a more useful route in a radiotracer production context.

Conclusions

[^{18}F]**1** and [^{18}F]**2** have been prepared using a two-step labelling strategy for the first time. This method is compatible with cGMP-compliant synthesis modules and does not require exceptional conditions. The formulated tracers were obtained after a total duration of about 100 min in a non-decay-corrected yield of 25%.

Conflict of Interest

The authors did not report any conflict of interest.

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