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Research Article

One-step radiosynthesis of [^{18}F]LBT-999: a selective radioligand for the visualization of the dopamine transporter with PET

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Abstract: LBT-999 (8-((*E*)-4-fluoro-but-2-enyl)-3-beta-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2-beta-carboxylicacid methyl ester) is a recently developed cocaine derivative belonging to a new generation of highly selective dopamine transporter (DAT) ligands (K_D: 9 nM for the DAT and IC_{50} > 1000 nM for the serotonin and norepinephrine transporter). Initial fluorine-18-labelling of LBT-999 was based on the robust and reliable two-step radiochemical pathway often reported for such tropane derivatives, involving first the preparation of (*E*)-1-[^{18}F]fluoro-4-tosyloxybut-2-ene followed by a *N*-alkylation reaction with the appropriate nor-tropane moiety. In the present work, a simple one-step fluorine-18-labelling of LBT-999 is reported, based on a chlorine-for-fluorine nucleophilic aliphatic substitution, facilitating as expected both automation and final high-performance liquid chromatography (HPLC) purification. The process involves: (A) reaction of K[^{18}F]F–Kryptofix®222 with the chlorinated precursor (3.5–4.5 mg) at 165°C for 10 min in DMSO (0.6 mL) followed by (B) C-18 PrepSep cartridge pre-purification and finally (C) semi-preparative HPLC purification on a Waters Symmetry® C-18. Typically, 3.70–5.92 GBq of [^{18}F]LBT-999 (> 95% chemically and radiochemically pure) could be obtained with specific radioactivities ranging from 37 to 111 GBq/μmol within 85–90 min (HPLC purification and Sep-Pak-based formulation included), starting from a 37.0 GBq [^{18}F]fluoride batch (overall radiochemical yields: 10–16%, non-decay-corrected). Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: fluorine-18; LBT-999; dopamine transporter; tropane

Introduction

The cocaine derivative LBT-999 (**1**, 8-((*E*)-4-fluoro-but-2-enyl)-3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester) belongs to a new generation of highly selective dopamine transporter (DAT) ligands (K_D: 9 nM for the DAT and IC_{50} > 1000 nM for the serotonin and norepinephrine transporter[1,2]). As already reported, LBT-999 has been successfully labelled with the positron-emitter carbon-11 (half-life: 20.38 min) at its methyl ester function from the corresponding carboxylic acid precursor, allowing preliminary *in vivo* pharmacological evaluation of this radiotracer by both biodistributions in rodents and brain imaging in non-human primates with positron

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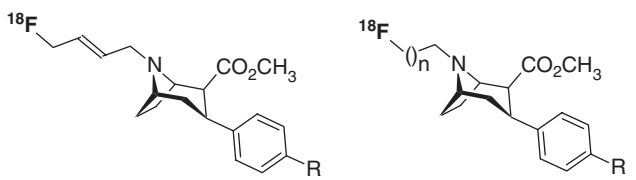
emission tomography (PET).[1–3] The pharmacological characteristics so far obtained demonstrate that LBT-999 is a highly promising candidate for *in vivo* exploration of the DAT in humans.

LBT-999 had been designed with a fluorine atom in its structure (Figure 1), allowing ultimate labelling with fluorine-18, a longer-lived positron-emitter (half-life: 109.8 min) and today one of the most attractive PET isotopes for radiopharmaceutical chemistry.[4–7] The robust and reliable pathway frequently proposed for the labelling of tropane derivatives with fluorine-18 is a two-step radiochemical process, involving first the preparation of an ω-halogeno- or better ω-sulphonyloxyalkyl[^{18}F]fluoride reagent and secondly its coupling to the appropriate nor-tropane moiety. This methodology, which has been applied with success to the preparation of several [^{18}F]fluoroethyl-[8–19] and [^{18}F]fluoropropyl-[8,9,20–26] derivatives like [^{18}F]FECNT and β-[^{18}F]CIT-FP (Figure 1), is based on the well-established radiosyntheses of *n*-bromo-, *n*-tosyloxy- and *n*-mesyloxy-1-[^{18}F]fluoroalkanes ($n = 1$–3) from the corresponding bifunctional alkanes by nucleophilic aliphatic substitution with no-carrier-added [^{18}F]fluoride ion as its activated K[^{18}F]F–Kryptofix®222 complex[27,28] as originally studied by Block *et al.*[29] Based on the radiosynthesis of (*E*)-[^{18}F]FBClNT (Figure 1), to our knowledge the first 4-[^{18}F]fluoro-2-butenyl derivative reported,[30,31] LBT-999 was successfully labelled with fluorine-18 in two radiochemical steps, involving the preparation of (*E*)-1-[^{18}F]fluoro-4-tosyloxy-2-butene[32,33] permitting further evaluation of this radioligand in non-human primates.

In the present work, a simple one-step fluorine-18-labelling of LBT-999 is reported, based on a chlorine-for-fluorine nucleophilic aliphatic substitution, in order to facilitate both the automation and the final purification process. These results were presented at the XVIIth *International Symposium on Radiopharmaceutical Sciences* (Aachen, Germany, 30 April–4th May 2007),[34] in the same meeting, Riss and Roesch[35] (*Institut für Kernchemie*, Universität Mainz, Germany) presented also a similar one-step radiochemical synthesis of this radioligand (in this case, a tosyloxy-for-fluorine substitution), highlighting the growing interest for [^{18}F]LBT-999.



R	
CH_3 [^{18}F]LBT-999 ([^{18}F]-**1**)	
Cl	(*E*)-[^{18}F]FBClNT

R	n	
Cl	1	[^{18}F]FECNT
I	2	β-[^{18}F]CIT-FP

Figure 1 Chemical structures of [^{18}F]LBT-999 and previously reported [^{18}F]fluorinated tropane-analogues.

Results and discussion

Chemistry

The target compound LBT-999 (**1**) was synthesized in 50% yield from 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**5**)[36] and (*E*)-1-fluoro-4-tosyloxy-2-butene (**6**) according to already reported procedures[1] (Scheme 1B). Its chloro analogue **7** as precursor for labelling with fluorine-18 was synthesized from the same nor-tropane **5** but using (*E*)-1-chloro-4-tosyloxy-2-butene (**4**). Compound **4** was prepared in 20% overall yield (Scheme 1A) from (*E*)-2-butene-1,4-diol (**2**)[37,38] using the following two-step sequence: monochlorination of **2** using 1.1 eq. of triphenylphosphine in refluxing tetrachloromethane (27% yield) followed by tosylation of the remaining alcohol function (using 1.1 eq. of tosyl chloride in dioxane containing triethylbenzylammonium chloride and aqueous potassium hydroxyde, 75% yield). Condensation of **5** with **4** in acetonitrile gave the chloro derivative **7** in 18% yield (Scheme 1B).

Radiochemistry

LBT-999 (**1**) was labelled with fluorine-18 at its 4-fluoro-2-butenyl moiety from the corresponding chloro analogue **7** using the one-step radiochemical process outlined in Scheme 2.

Fluorination with the cyclotron-produced [^{18}F]fluoride as the, no-carrier-added, activated K[^{18}F]F–Kryptofix®222 complex[27,28] was performed in DMSO using 10–13 μmol of precursor at 165°C for 10 min without stirring the contents. After cooling the reaction vessel, 96 to over 99% of the initial radioactivity was still present. The radiochemical yields of fluorine-18 incorporation, calculated from the thin-layer chromatography (TLC)-radiochromatogram and defined as the ratio of radioactivity area of [^{18}F]-**1** over total fluorine-18 radioactivity area, were about 30–40%. At this stage, a C-18 PrepSep cartridge was used to rapidly isolate [^{18}F]-**1** (with a radiochemical purity > 90%, according to radio-HPLC) from the reaction mixture, which represented 30–35% of the total radioactivity amount engaged in the fluorination process whereas most likely unreacted [^{18}F]fluoride, 65–70% of the total radioactivity, was eluted off the cartridge as waste. These

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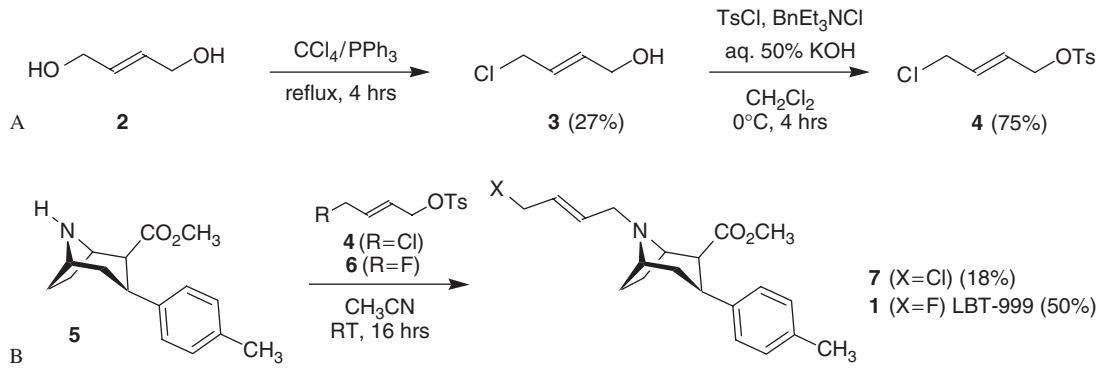
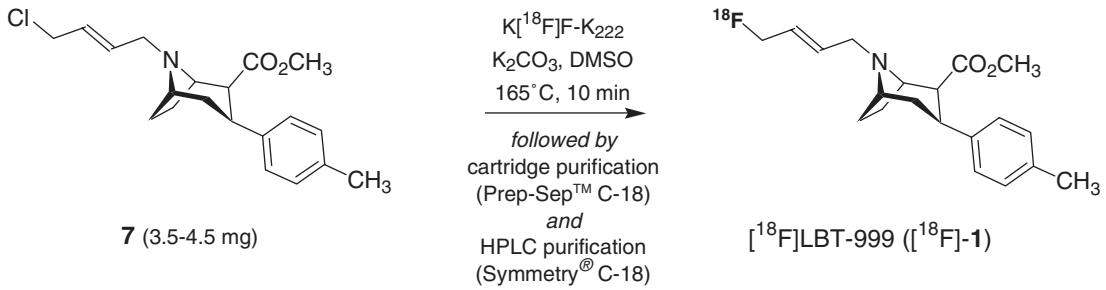


Scheme 1



Scheme 2

values clearly confirmed the radiochemical yields measured by radio-TLC.

HPLC purification of [^{18}F]LBT-999 ([^{18}F]-**1**) was performed on a semi-preparative Symmetry® C18 column (HPLC A, see Experimental), using a mixture of H_2O, CH_3CN and TFA as the eluent. Using these conditions, [^{18}F]-**1** (t_R: 12.5–13.5 min) could be obtained with > 95% chemical and radiochemical purity and was completely separated within a short total purification time (less than 20 min) from the non-labelled chloro-derivative **7** (t_R: 15.5–17.5 min), as well as from an unidentified fluorine-18-labelled side product at t_R: 14.0–15.0 min (up to 10% compared to [^{18}F]-**1**). At least, two chemical structures can be proposed for this side product: one resulting from a *cis–trans* isomerization (leading to the (*Z*)-isomer) and the other resulting from the addition of the fluorine atom to the double bond with concomitant shift of the latter to the terminal position (leading to the 2-fluoro-3-butenyl-isomer). However, no further efforts were made to identify this side product.

Noteworthy, a poor separation (leading to a too low – 90% – chemical purity) was observed with the HPLC system previously used for the purification of [^{18}F]-**1** in the old two-step process (HPLC B, see Experimental, t_R ([^{18}F]-**1**): 9.0–10.0 min).[33]

Introduction of fluorine-18 was also performed using microwave activation (at 100 W for 2 min). Incorporation yields (calculated from the TLC-radiochromatogram or after the C-18 PrepSep cartridge purification) were similar to those described above, with, however, formation in significantly higher ratio of the unidentified fluorine-18-labelled side product (about 40–50% compared to [^{18}F]-**1**). HPLC purification using HPLC A system gave nevertheless > 95% chemically and radiochemically pure [^{18}F]-**1**, still well separated from the side product.

Formulation of [^{18}F]LBT-999 ([^{18}F]-**1**) as an i.v. injectable solution was performed using a home-made Sep-Pak®Plus C18 device. The HPLC-collected fraction containing the radiotracer was diluted with water and the resulting solution was passed through a C18 Sep-Pak® cartridge. The cartridge was then washed with water, partially dried with nitrogen and finally eluted with ethanol followed by physiological saline. The solution was then sterile-filtered and diluted with physiological saline to an ethanol concentration below 10%.

Quality controls of [^{18}F]LBT-999 ([^{18}F]-**1**) were performed on an aliquot of the preparation ready for i.v. injection. The radiotracer preparation was a clear and colourless solution with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis (HPLC C, see Experimental), the radiotracer preparation was found to be > 95% chemically and radiochemically pure (**1**, t_R: 2.25 min). The preparation was also shown to be free of the non-radioactive precursor, the chloro

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derivative **7** (t_R: 3.45 min) as well as the unidentified fluorine-18-labelled side product (t_R: 2.58 min), and was chemically and radiochemically stable for at least 120 min. These results were in compliance with our in-house quality control/assurance specifications.

Typically, using the one-step synthesis described herein, 3.70–5.92 GBq (100–160 mCi) of [^{18}F]LBT-999 ([^{18}F]-**1**) ready to use could be obtained with a specific radioactivity of 37–111 GBq/μmol (1–3 Ci/μmol) within 85–90 min (HPLC purification and Sep-Pak-based formulation included), starting from a 37.0 GBq (1.0 Ci) [^{18}F]fluoride batch (overall non-decay-corrected isolated radiochemical yield: 10–16%).

Experimental

General

Chemicals, flash chromatography and TLC analysis. Chemicals, except (*R*)-cocaine, were purchased from Aldrich-, Fluka- or Sigma France and were used without further purification. Flash chromatographies were conducted on silica gel or alumina gel (0.63–0.200 mm, VWR) columns. TLCs were run on precoated plates of silica gel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by dipping the TLC-plates in a 1% ethanolic ninhydrin solution and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser.

HPLC analysis. [*HPLC A*]: Equipment: system equipped with a Waters 600 pump and Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semi-preparative Symmetry® C18, Waters (300 × 7.8 mm); porosity: 7 μm; eluent H_2O/CH_3CN/TFA: 72:28:0.1 (v/v/v); flow rate: 5 mL/min; temperature: RT; absorbance detection at $\lambda = 220$ nm. [*HPLC B*]: Equipment: system equipped with a Waters 600 pump and Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semi-preparative SunFire™ C18, Waters (250 × 10.0 mm); porosity: 5 μm; eluent H_2O/CH_3CN/TFA: 70:30:0.1 (v/v/v); flow rate: 5 mL/min; temperature: RT; absorbance detection at $\lambda = 220$ nm. [*HPLC C*]: Equipment: Waters Alliance 2690 (or a Waters binary HPLC pump 1525) equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (50 × 4.6 mm); porosity: 5.0 μm; conditions: isocratic elution with solvA/solvB: 55/45 (v/v) [solvent A: H_2O containing Low-UV PIC® B7 reagent (20 mL for 1000 mL); solvent B: H_2O/CH_3CN: 30:70 (v/v) containing Low-UV PIC® B7 reagent (20 mL for 1000 mL)]; flow rate: 2.0 mL/min; temperature: 30°C; absorbance detection at $\lambda = 205$ nm.

Spectroscopies and elemental analyses. NMR spectra were recorded on a Bruker (Wissembourg, France) DPX Avance (200 MHz) apparatus using the hydrogenated residue of the deuterated solvent $CDCl_3$ ($\delta = 7.30$ ppm) or TMS ($\delta = 0$ ppm) as internal standards for ^{1}H-NMR as well as the deuterated solvent $CDCl_3$ ($\delta = 77.0$ ppm) as internal standard for ^{13}C-NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, m for singlet, doublet, triplet and multiplet, respectively). The mass spectra (MS) were measured on a Hewlett-Packard (Issy les Moulineaux, France) 5989A GC/EI-MS spectrometer (EI at 70 eV) and on a Bruker (Wissembourg, France) Esquire-LC spectrometer (ES+). Elemental analyses were performed by the *Service d'Analyses du CNRS* (Vernaison, France) and results were within ±0.4% of theoretical values.

Radioisotope production. No-carrier-added aqueous [^{18}F]fluoride ion was produced via the [^{18}O(p,n)^{18}F] nuclear reaction by irradiation of a 2 mL [^{18}O]water (> 97%-enriched, CortecNet, Paris, France) target on an IBA Cyclone-18/9 cyclotron (18 MeV proton beam) and was transferred to the appropriate hot cell. *Target hardware*: commercial, 2-mL, two-port, stainless steel target holder equipped with a domed-end niobium cylinder insert. *Target to hot cell liquid-transfer system*: 60 m PTFE line (0.8 mm internal diameter; $\frac{1}{16}$ in external diameter), 2.0 bar helium drive pressure, transfer time 3–6 min. Typical production of [^{18}F]fluoride at the end of bombardment for a 20 μA, 30 min (10 μA h) irradiation: 27.7–29.6 GBq (750–800 mCi).

Miscellaneous. Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a 7.5-cm-lead shielded cell using a computer-assisted Zymate-XP robot system (Zymark corporation, USA). Microwave activation was performed with a MicroWell 10 oven (2.45 GHz), Labwell AB, Sweden.

Chemistry

(*E*)-1-Chloro-4-tosyloxy-2-butene (4). *Step 1.* To a CCl_4 solution (100 mL) containing (*E*)-2-butene-1,4-diol[37,38] (**2**, 10.0 g, 113 mmol), triphenylphosphine (33.3 g, 127 mmol, 1.1 eq.) was added . The resulting mixture was vigorously stirred and refluxed for 4 h. The mixture was then cooled to room temperature and diluted with

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CH_2Cl_2 (200 mL). The organic layer was washed with H_2O (2 × 100 mL), dried over $MgSO_4$ and concentrated to dryness under reduced pressure. Purification by flash chromatography (CH_2Cl_2) gave pure (*E*)-1-chloro-4-hydroxy-2-butene (**3**, 3.3 g, 27%). ^{1}H-NMR ($CDCl_3$): δ: 1.69 (b, 1H); 4.06 (dd, $^3J = 4.8$ Hz, $^4J = 1.0$ Hz, 2H); 4.16 (d, $J^3 = 3.6$ Hz, 2H); 5.79–6.01 (m, 2H). ^{13}C-NMR ($CDCl_3$): δ: 44.4; 62.5; 127.0; 133.8. *Step 2.* To a CH_2Cl_2 solution (40 mL) containing (*E*)-1-chloro-4-hydroxy-2-butene (**3**, 2.0 g, 18.8 mmol) and benzyltriethylammonium chloride (220 mg, 0.96 mmol, 0.05 eq.) was added an aq. 50% KOH solution (40 mL). The mixture was cooled to 0°C, then a CH_2Cl_2 solution (40 mL) containing *p*-toluenesulphonyl chloride (3.93 g, 20.6 mmol, 1.1 eq.) was added dropwise while maintaining the temperature below 0°C. The reaction mixture was stirred for another 4 h at 0°C and then diluted with H_2O (40 mL). The aqueous layer was separated and extracted with CH_2Cl_2 (2 × 80 mL). The organic layers were combined, washed with brine (2 × 30 mL), dried over $MgSO_4$ and concentrated to dryness under reduced pressure to give pure (*E*)-1-chloro-4-tosyloxy-2-butene (**4**, 3.65 g, 75%) which was further used without purification. ^{1}H-NMR ($CDCl_3$): δ: 2.45 (s, 3H); 3.99 (d, 2H, $^3J = 5.6$ Hz); 4.55 (d, 2H, $^3J = 5.1$ Hz); 5.82–5.90 (m, 2H); 7.39 (d, 2H, $^3J = 8.2$ Hz); 7.79 (d, 2H, $^3J = 8.2$ Hz). ^{13}C-NMR ($CDCl_3$): *δ*: 21.7; 43.5; 69.4; 126.4; 128.0 (2C); 130.1 (2C); 131.7; 133.2; 145.1.

(*E*)-1-Fluoro-4-tosyloxy-2-butene (6). Synthesized from (*E*)-2-butene-1,4-diol (**2**)[37,38] according to Dollé *et al.*[1] ^{1}H-NMR ($CDCl_3$): δ: 2.45 (s, 3H); 4.58–4.62 (m, 2H); 4.85 (dd, 2H, $^2J_{H-F} = 46.4$ Hz, $^3J = 4.8$ Hz); 5.81–5.92 (m, 2H); 7.37 (d, 2H, $^3J = 8.3$ Hz); 7.81 (d, 2H, $^3J = 8.3$ Hz). ^{13}C-NMR (CD_2Cl_2): *δ*: 21.7; 69.9; 82.3 (d, $^1J_{C-F} = 162.7$ Hz); 125.8 (d, $^3J_{C-F} = 12.2$ Hz); 128.2 (2C); 130.3 (2C); 130.8 (d, $^2J_{C-F} = 17.3$ Hz); 133.4; 145.6. MS: *m/e* (%): 225 (3); 224 (7); 155 (32); 92 (37); 91 (100); 89 (19); 72 (62); 65 (56); 39 (52).

3*β*-p-Tolyl-8-aza-bicyclo[3.2.1]octane-2*β*-carboxylic acid methyl ester (5). Synthesized from (*R*)-cocaine hydrochloride according to Emond *et al.*[36] ^{1}H-NMR ($CDCl_3$): δ: 1.60–1.85 (m, 3H); 1.93–2.15 (m, 2H); 2.33 (s, 3H); 2.43 (td, 1H, $^2J = 13.1$ Hz, $^3J = 2.7$ Hz); 2.75 (dd, 1H, $^3J = 5.9$, 1.9 Hz); 2.95 (s, 1H); 3.19 (dt, 1H, $^3J = 13.1$, 5.5 Hz); 3.41 (s, 3H); 3.74 (m, 2H); 7.11 (s, 4H). ^{13}C-NMR ($CDCl_3$): δ: 21.4; 27.6; 29.5; 34.3; 35.7; 51.5; 51.6; 54.1; 56.7; 127.6 (2C); 129.3 (2C); 136.3; 139.6; 174.4. MS: *m/e* (%): 259 (M^+; 22); 228 (3); 200 (5); 170 (5); 141 (10); 115 (14); 84 (70); 83 (93); 69 (80); 68 (100).

8-((*E*)-4-Fluoro-but-2-enyl)-3*β*-p-tolyl-8-aza-bicyclo[3.2.1]octane-2*β*-carboxylic acid methyl ester (1, LBT-999). Synthesized from 3*β*-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2*β*-carboxylic acid methyl ester (**5**) and (*E*)-1-fluoro-4-tosyloxy-2-butene (**6**) according to Dollé *et al.*[1] ^{1}H-NMR ($CDCl_3$): *δ*: 1.57–1.68 (m, 3H); 1.90–2.00 (m, 2H); 2.22 (s, 3H); 2.65 (td, 1H, $^3J = ^2J = 12.4$ Hz, $^3J = 2.6$ Hz); 2.92–3.05 (m, 4H); 3.46 (m, 1H); 3.53 (s, 3H); 3.70 (m, 1H); 4.86 (dd, 2H, $^2J_{H-F} = 47.2$ Hz, $^3J = 4.6$ Hz); 5.80–5.85 (m, 2H); 7.10 (d, 2H, $^3J = 8.0$ Hz); 7.20 (d, 2H, $^3J = 8.0$ Hz). ^{13}C-NMR ($CDCl_3$): *δ*: 20.9; 25.8; 26.0; 33.7; 34.0; 50.8; 52.6; 54.8; 61.3; 62.2; 83.0 (d, $^1J_{C-F} = 161.0$ Hz); 126.1 (d, $^2J_{C-F} = 17.1$ Hz); 127.1 (2C); 128.5 (2C); 134.1 (d, $^3J_{C-F} = 11.6$ Hz); 135.1; 139.8; 171.9. MS: *m/e* (%): 331 (M^+, 24); 272 (13); 258 (6); 180 (7); 154 (46); 141 (97); 140 (100); 122 (66); 108 (60); 68 (33); 53 (35). Anal. Calcd $C_{20}H_{26}FNO_2$: C, 72.48; H, 7.91; N, 4.23; found: C, 72.26; H, 7.94; N, 4.21.

8-((*E*)-4-Chloro-but-2-enyl)-3*β*-p-tolyl-8-aza-bicyclo[3.2.1]octane-2*β*-carboxylic acid methyl ester (7). To a 0°C solution of (*E*)-1-chloro-4-tosyloxy-2-butene (**4**, 1.5 g, 5.8 mmol, 1.5 eq.) and triethylamine (800 μL, 5.8 mmol, 1.5 eq.) in acetonitrile (10 mL) was added dropwise 3*β*-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2*β*-carboxylic acid methyl ester (**5**, 1.0 g, 3.86 mmol, 1.0 eq.) dissolved in acetonitrile (20 mL). The resulting reaction mixture was stirred at room temperature overnight and then concentrated to dryness under reduced pressure. The residue was finally purified by flash chromatography on alumina gel (pentane/CH_2Cl_2: 50/50 to 0/100) to give 8-((*E*)-4-chloro-but-2-enyl)-3*β*-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2*β*-carboxylic acid methyl ester (**7**) as a white solid (250 mg, 18%). ^{1}H-NMR ($CDCl_3$): *δ*: 1.58–1.69 (m, 3H); 1.95–2.05 (m, 2H); 2.29 (s, 3H); 2.60 (td, 1H, $^3J = ^2J = 12.6$ Hz, $^3J = 2.6$ Hz); 2.90–2.96 (m, 4H); 3.41 (m, 1H); 3.51 (s, 3H); 3.66 (1H); 4.05 (d, 2H, $^3J = 3.1$ Hz); 5.70–5.75 (m, 2H); 7.08 (d, 2H, $^3J = 8.2$ Hz); 7.16 (d, 2H, $^3J = 8.2$ Hz). ^{13}C-NMR ($CDCl_3$): *δ*: 21.1; 26.0; 26.1; 34.0; 34.2; 44.9; 51.1; 52.9; 54.9; 61.5; 62.4; 127.3 (2C); 127.6; 128.8 (2C); 133.7; 135.3; 140.0; 172.1. MS: ES [MH^+] Calcd: 348.17; found: 348.32.

Radiochemistry

Preparation of the K[^{18}F]F–K_{222} complex. In order to recover and recycle the [^{18}O]water target, 2 mL of aqueous [^{18}F]fluoride from the target holder was passed through an anion-exchange resin (Sep-Pak® Light Waters Accell™ Plus QMA cartridge, chloride form, conditioned by washing with aq. 1 M $NaHCO_3$ (2 mL) and rinsing with water (20 mL) and CH_3CN

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(10 mL)) by helium pressure (1.5–2.0 bar). Helium was blown through the cartridge to maximally extract the last traces of [^{18}O]water. The [^{18}F]fluoride ion was then eluted from the resin, using an aq. K_2CO_3 solution (1.0 mL of a 1.0 mg/mL solution), into a Vacutainer® tube containing Kryptofix®222 (K_{222}: 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, 12.0–15.0 mg). The resulting solution was then gently concentrated to dryness at 145–150°C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F–K_{222} complex as a white semi-solid residue.

Preparation of 8-((*E*)-4-[^{18}F]fluoro-but-2-enyl)-3β-p-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester ([^{18}F]-1, [^{18}F]LBT-999). A. Procedure using conventional heating. DMSO (600 μL) containing 8-((*E*)-4-chloro-but-2-enyl)-3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**7**, 3.5–4.5 mg, 10.0–12.9 μmol) was added into the Vacutainer® tube containing the dried K[^{18}F]F–K_{222} complex. The tube (not sealed) was thoroughly vortexed (30 s) and then placed in a heating block (at 165°C, for 10 min) without stirring the contents. The reaction vessel was then cooled using an ice-water bath, the remaining radioactivity was measured (which should be >96% of the initial radioactivity) and the reaction mixture was analysed by radio-TLC. The reaction yield was calculated from the TLC-radiochromatogram and defined as the ratio of radioactivity area of [^{18}F]LBT-999 ([^{18}F]-**1**) over total fluorine-18 radioactivity area (SiO_2-TLC (EtOAc):R_f:[^{18}F]-**1**:0.5 and R_f:[^{18}F]fluoride ion:0.0). The reaction mixture was diluted with water (1 mL) and transferred onto a C18 cartridge (PrepSep™ R-C18 Extraction Column, Fisher Scientific, activated beforehand with EtOH (2 mL) and then rinsed with water (10 mL)), pre-filled with water (2 mL). The tube was rinsed twice with water (1 mL), which was also transferred and added to the diluted reaction mixture on top of the cartridge (0.5–1.5% of the total radioactivity amount engaged in the fluorination process was lost in the initial tube). An additional portion of water (2 mL) was further added to the diluted reaction mixture on top of the cartridge. The whole mixture was then passed through the cartridge, which was then washed with water (3 mL) and partially dried for 0.5 min by applying a nitrogen stream. [^{18}F]-**1** was eluted from the cartridge with CH_2Cl_2 (3 mL) into an empty 5-mL reaction vial. Elution was repeated twice with 1 mL of CH_2Cl_2 for maximal transfer of [^{18}F]-**1** (only 1–2% was left and lost on the cartridge). The incorporation yield was estimated after the C18 cartridge elution by the CH_2Cl_2 over the total eluted radioactivity (DMSO/H_2O+CH_2Cl_2) ratio. The eluted CH_2Cl_2 solution was concentrated to dryness at 65–75°C under a gentle nitrogen stream for 3–5 min. Finally, the residue was redissolved in the HPLC solvent used for purification (1.0 mL) and the crude was injected onto HPLC (HPLC A). Isocratic elution gave pure [^{18}F]-**1** (t_R: 12.5–13.5 min), well separated from unlabelled **7** (t_R: 15.5–17.5 min) and the unidentified fluorine-18-labelled side product (t_R: 14.0–15.0 min). *B. Procedure using microwave activation*. Same as procedure A, but instead of the heating block a dedicated microwave oven (at 100 W, for 2 min) was used.

Formulation of [^{18}F]LBT-999 ([^{18}F]-1). Formulation of the labelled product for i.v. injection was effected as follows: the HPLC-collected fraction containing the radiotracer was diluted with water (50 mL). The resulting solution was passed through a Sep-Pak®Plus C18 cartridge (Waters, washed with 2 mL of EtOH and then rinsed with 10 mL of water prior to use). The cartridge was washed with water (10 mL) and partially dried by applying a nitrogen stream for 10 s. The radiotracer was eluted with 2 mL of EtOH (less than 10% of the total radioactivity was left on the cartridge) followed by 8 mL of physiological saline and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to take the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[40]

Quality control of [^{18}F]LBT-999 ([^{18}F]-1). The radiotracer preparation was visually inspected for clarity, absence of colour and particulates. An aliquot of the preparation was removed for determination of pH using standard pH paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC (HPLC C), with a sample of authentic **1** (t_R: 2.25 min). Particular attention was paid to the absence of non-radioactive precursor **7** (t_R: 3.45 min). Chemical and radiochemical stability of the entire preparation was tested by HPLC (HPLC C) at regular 15-min intervals during 120 min. Specific radioactivity of the radiotracer was calculated from three consecutive HPLC (HPLC C) analyses (average) and determined as follows: the area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance.

Conclusion

The highly selective dopamine transporter ligand LBT-999 (**1**) has been labelled in one radiochemical step with fluorine-18 and its synthesis represents to our knowledge the first example of a direct nucleophilic

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fluorination with [^{18}F]fluoride leading to a 4-fluoro-2-butenyl-containing PET ligand. The one-step process described herein gives improved overall isolated radiochemical yields and now replaces our former two-step preparation[32,33] of [^{18}F]LBT-999 ([^{18}F]-**1**). Moreover, this process is 10 min shorter and appears to be more reliable (> 20 radiosyntheses performed to date).[39] [^{18}F]LBT-999 is currently under evaluation and its *in vivo* pharmacological profile will be compared to that of [^{11}C]LBT-999.

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