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Research Article

Radiosynthesis of [^{18}F]LBT-999, a selective radioligand for the visualization of the dopamine transporter with PET

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Summary

LBT-999 (8-((*E*)-4-fluoro-but-2-enyl)-3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester) is a cocaine derivative belonging to a new generation of highly selective dopamine transporter ligands (K_D:9 nM). LBT-999 was labelled with fluorine-18 at its fluoromethylvinyl moiety using the following two-step radiochemical process: (a) No-carrier-added nucleophilic aliphatic radiofluorination from (*E*)-1, 4-ditosyloxybut-2-ene and the activated K[^{18}F]F-Kryptofix®222 complex in acetonitrile at 70°C for 10 min giving (*E*)-1-[^{18}F]fluoro-4-tosyloxybut-2-ene, followed by (b) condensation of the latter with 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester in *N,N*-dimethylformamide containing potassium iodide for 20 min

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at 90°C and (c) HPLC purification (SunFire™ C18, eluent H_2O/CH_3CN/TFA (72:28:0.1 (v/v/v)). Radiochemically pure (> 95%) [^{18}F]LBT-999 (2.03–2.96 GBq, 37–111 GBq/μmol) was obtained in 95–100 min starting from a 44.4 GBq [^{18}F]fluoride ion production batch (4.6–6.7% non-decay-corrected overall yield).

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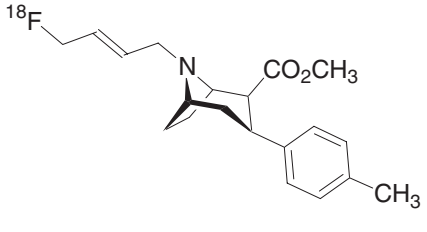
Key Words: Fluorine-18; LBT-999; dopamine transporter; tropane

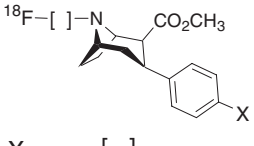
Introduction

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, Figure 1) is a 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 serotonine and norepinephrine transporter).[1] Recently, 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 and the methylation reagent [^{11}C]methyl triflate,[2] permitting preliminary *in vivo* pharmacological evaluation of this radiotracer by both biodistributions in rodents and brain imaging in non-human primates with positron emission tomography (PET).[1,2] Taken together, the results so far obtained demonstrate that LBT-999 is a highly promising candidate for *in vivo* exploration of DAT in humans, using PET.[1,2]

The chemical structure of LBT-999 permits also its labelling at its fluoromethylvinyl moiety with fluorine-18 (half-life: 109.8 min), today one of the most attractive positron-emitting radioisotopes for radiopharmaceutical chemistry.[3–5]



[^{18}F]LBT-999
[^{18}F]-**1**



	X	[ ]	
A	Cl	-CH_2CH_2-	[^{18}F]FECNT
B	F	-CH_2CH_2-	β-[^{18}F]CFT-FE
C	3'-furyl	-CH_2CH_2-	[^{18}F]FE-3FPT
D	I	-$CH_2CH_2CH_2$-	β-[^{18}F]CIT-FP
E	Me	-$CH_2CH_2CH_2$-	β-[^{18}F]CMT-FP
F	Br	-$CH_2CH_2CH_2$-	β-[^{18}F]CBT-FP
G	F	-$CH_2CH_2CH_2$-	β-[^{18}F]CFT-FP
H	Cl	-$CH_2(CH{=}CH)CH_2$-	(E/Z)-[^{18}F]FBCINT

Figure 1.

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The robust and reliable pathway frequently proposed for the labelling with fluorine-18 of tropane derivatives is a two-step radiochemical process, involving first the preparation of an appropriate halogeno or better sulfonyloxy [^{18}F]reagent. The radiosynthesis 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[6,7] has been extensively studied.[8] This methodology has been applied with success to the preparation of several [^{18}F]fluoroethyl-[9–20] and [^{18}F]fluoropropyl-[9,10,21–26] tropane derivatives (compound A-G, Figure 1), like for example, [^{18}F]FECNT and β-[^{18}F]CIT-FP. More recently, the radiosynthesis of both (*E*)- and (*Z*)-1-[^{18}F]fluoro-4-tosyloxybut-2-ene has also been briefly described and used for the first time for the preparation of (*E*)- and (*Z*)- [^{18}F]FBClNT (compound H, Figure 1).[27,28]

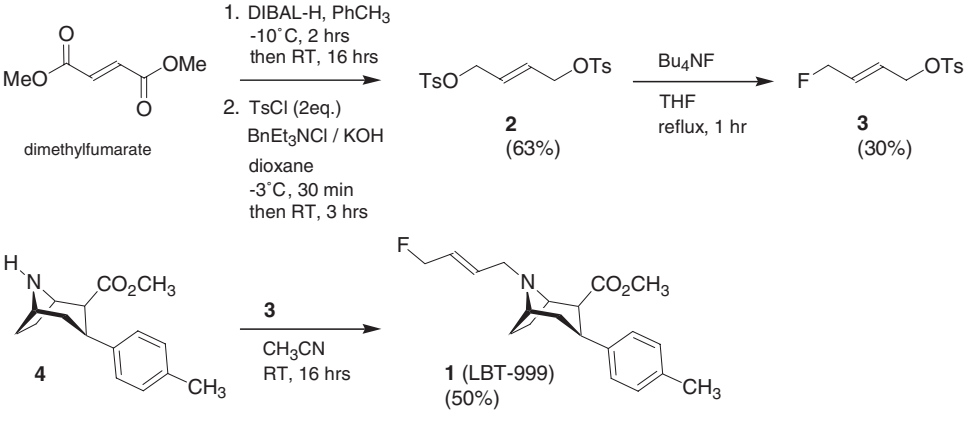
This paper presents the two-step radiochemical synthesis of LBT-999 (**1**) with fluorine-18, involving the preparation of (*E*)-1-[^{18}F]fluoro-4-tosyloxybut-2-ene followed by its condensation with 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester.

Results and discussion

Chemistry

LBT-999 (**1**) was synthesized from 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**4**) and (*E*)-1-fluoro-4-tosyloxybut-2-ene (toluene-4-sulfonic acid (*E*)-4-fluoro-but-2-enyl ester, **3**) using already reported procedures (Scheme 1).[2]

Briefly, (*E*)-1-fluoro-4-tosyloxybut-2-ene (**3**) was prepared in 19% overall yield from dimethyl fumarate (commercially available) using the following



Scheme 1.

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three-step sequence: reduction of both ester functions using DIBAL-H, conversion into their tosylates and finally mono tosyl-to-fluoro substitution using 1.2 eq. of tetrabutylammonium fluoride. Condensation of the nortropane **4** with (*E*)-1-fluoro-4-tosyloxybut-2-ene (**3**) gave LBT-999 (**1**) in 50% yield.

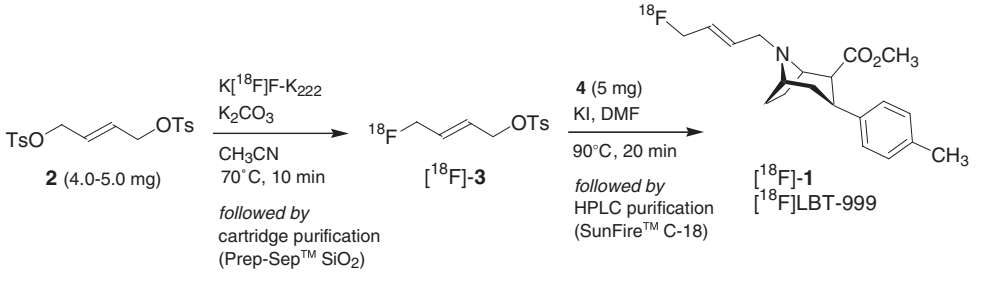
Radiochemistry

LBT-999 (**1**) was labelled with fluorine-18 at its fluoromethylvinyl moiety using the two-step radiochemical process outlined in Scheme 2.

Introduction of the cyclotron-produced fluorine-18 (step 1) as the, no-carrier-added, activated K[^{18}F]F-Kryptofix®222 complex[6,7] was performed at 70°C for 10 min in acetonitrile containing (*E*)-but-2-ene-1,4-diol ditosylate (**2**, about 10–12 μmol) in an unsealed vessel. At this stage, the remaining radioactivity was measured, which was 97% to over 99% of the initial radioactivity. The radiochemical yields of fluorine-18 incorporation, calculated from the TLC-radiochromatogram and defined as the ratio of radioactivity area of [^{18}F]-**3** over total fluorine-18 radioactivity area, were about 30–50%. A SiO_2 Sep-Pak cartridge was then used to rapidly isolate [^{18}F]-**3** (with a radiochemical purity >95%, according to radio-TLC), which represented 30–45% of the total radioactivity amount engaged in the fluorination process whereas 55–70% of the total radioactivity was left on the cartridge, most likely non-reacted [^{18}F]fluoride ion. These values clearly confirmed the radiochemical yields measured by radio-TLC.

The coupling reaction (step 2, Scheme 2) of [^{18}F]-**3** with the nortropane **4** (about 20 μmol) was performed at 90°C for 20 min in *N,N*-dimethylformamide containing potassium iodide in an unsealed vessel. At this stage, the remaining radioactivity was measured again, which was 85–95% of the radioactivity engaged in this second step.

HPLC purification of [^{18}F]LBT-999 ([^{18}F]-**1**) was performed on a semipreparative SunFire™ C18 column (HPLC A). Using these conditions, [^{18}F]-**1** (t_R:8.0–8.5 min) could be obtained within a short total purification time (less than 10 min) with a >95% chemically and radiochemical purity, separated from the non-labelled nortropane **4** (t_R:5.0–5.5 min), as well as



Scheme 2.

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from the fluorine-18-labelled reagent [^{18}F]-**3** (t_R:2.5–3.0 min) and an unidentified [^{18}F]impurity at t_R:6.0–6.5 min. HPLC purification of [^{18}F]-**1** proved to be particularly delicate. For example, the use of a semipreparative Symmetry® C18 column (HPLC B) or a semipreparative Zorbax® SB C-18 column (HPLC C), with the same eluent as described for HPLC A, gave only moderately high radiochemical and chemical purity (85–90%). A too low radiochemical purity (<85%) was also observed using a Zorbax® SB C-18 with different eluents (see HPLC D and HPLC E).

The radiochemical yields for the coupling reaction, defined as the ratio of radioactivity collected for [^{18}F]-**1** after HPLC over the total radioactivity engaged in this chemical step, were about 30–50%. These values correlated well with the ratio of radioactivity collected for uncoupled [^{18}F]-**3** over the total radioactivity (up to 40%). In all runs, the unidentified [^{18}F]impurity (HPLC A, t_R:6.0–6.5 min) represented less than 10% of the total radioactivity engaged in this chemical step. Noteworthy, when the coupling reaction was performed without any potassium iodide, the radiochemical yields of [^{18}F]-**1** were systematically lower to those described above. This observation is in accordance with a recent report, showing that alkali iodide highly efficiently promotes *N*-[^{18}F]fluoroethylations when using the bromo or tosyloxy [^{18}F]fluoroethane reagent.[29] Also, increasing the reaction temperature to over 120°C, did not improve the yields, but led to partial decomposition, which complicated the final HPLC purification.

Formulation of [^{18}F]LBT-999 ([^{18}F]-**1**) as 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 (less than 10% of the total radioactivity engaged in this step was left on the cartridge). The solution was then sterile-filtered and diluted with physiological saline to an ethanol concentration below 10%. The radiotracer preparation was a clear and colorless solution with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis (HPLC F, see experimental), the radiotracer preparation was found to be >95% chemically and radiochemically pure (**1**, t_R:2.28 min). The preparation was shown to be free of non-radioactive precursor nortropane **4** (t_R:1.26 min) and was chemically and radiochemically stable for at least 120 min. Specific radioactivities ranged from 1 to 3 Ci/μmol (37–111 GBq/μmol) at the end of the synthesis.

Conclusion

LBT-999 (**1**) has been successfully labelled with fluorine-18. Typically, 55–80 mCi of [^{18}F]-**1** (2.03–2.96 GBq) could be obtained in 95–100 min of

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radiosynthesis (HPLC purification and formulation included), starting from a 1.2 Ci (44.4 GBq) [^{18}F]fluoride ion production batch (overall yields:4.6–6.7% non-decay-corrected and 8.3–12.5% decay-corrected). The potential of [^{18}F]-**1** for *in vivo* exploration of the DAT with PET is currently evaluated in non-human primates.

Experimental

General

Chemicals, Flash chromatography and TLC analysis. Chemicals were purchased from Aldrich-, Fluka- or Sigma France and were used without further purification. Flash chromatographies were conducted on silica gel (0.63–0.200 mm, VWR) columns. TLCs were run on pre-coated 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 analyzer.

HPLC analysis. [HPLC A]: Equipment: system equipped with a Waters 600 Pump and a Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semipreparative SunFire™ C18, Waters (250 × 10.0 mm); porosity: 5 μm; eluent H_2O / CH_3CN / TFA: 72:28:0.1 (v/v/v); flow rate: 5 ml/min; absorbance detection at $\lambda = 220$ nm. t_R ([^{18}F]-**1**): 8.0–8.5 min.

[HPLC B]: Equipment: system equipped with a Waters 600 Pump and a Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semipreparative Symmetry® C18, Waters (300 × 7.8 mm); porosity: 7 μm; eluent H_2O / CH_3CN / TFA: 70:30:0.1 (v/v/v); flow rate: 5 ml/min; absorbance detection at $\lambda = 220$ nm. t_R ([^{18}F]-**1**): 7.0–7.5 min.

[HPLC C]: Equipment: system equipped with a Waters 600 Pump and a Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semipreparative Zorbax® SB C-18, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; eluent H_2O / CH_3CN / TFA: 65:35:0.1 (v/v/v); flow rate: 5 ml/min; absorbance detection at $\lambda = 220$ nm. t_R ([^{18}F]-**1**): 9.0–9.5 min.

[HPLC D]: Equipment: system equipped with a Waters 600 Pump and a Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semipreparative Zorbax® SB C-18, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; eluent

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H_2O / CH_3CN / TEA: 50:50:0.1 (v/v/v); flow rate: 6 ml/min; absorbance detection at $\lambda = 220$ nm. t_R ([^{18}F]-**1**): 10.0–10.5 min.

[HPLC E]: Equipment: system equipped with a Waters 600 Pump and a Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionization chamber probe; column: semipreparative Zorbax® SB C-18, Hewlett Packard (250 × 9.4 mm); porosity: 5 μm; eluent 0.9% aqueous NaCl / EtOH / AcOH: 71:28:1 (v/v/v); flow rate: 7 ml/min; absorbance detection at $\lambda = 220$ nm. t_R ([^{18}F]-**1**): 9.0–9.5 min.

[HPLC F]: 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 (composition (% by weight): methanol (18–22%), heptane sulfonic acid — sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters), 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; UV detection at λ: 205 nm. t_R ([^{18}F]-**1**): 2.28 min.

Spectroscopies and elemental analyses. NMR spectra were recorded on a Bruker DPX Avance (200 MHz) apparatus using the hydrogenated residue of the deuterated solvent $CDCl_3$ ($\delta = 7.30$ ppm) and/or TMS ($\delta = 0.00$ ppm) as internal standards for ^{1}H-NMR as well as the deuterated solvent $CDCl_3$ ($\delta = 77.0$ ppm) and/or TMS 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), ES+, were measured on a Hewlett-Packard 5989A GC/EI-MS spectrometer. Elemental analyses were performed by the service d'Analyses du CNRS (Vernaison, France) and results were within $\pm$ 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; 1/16 in external diameter), 2.0 bar helium drive pressure, transfer time 3–6 min. Typical production of [^{18}F]Fluoride ion at the end of bombardment for a 20 μA, 30 min (10 μA h) irradiation: 750–800 mCi (27.7–29.6 GBq).

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Miscellaneous. Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a 7.5-cm-lead shielded cell using a computer-assisted Zymate robot system (Zymark Corporation, USA).

Chemistry

*(E)-But-2-ene-1,4-diol ditosylate (**2**)*. Synthesized from dimethyl fumarate ((*E*)-but-2-ene-1,4-dioic acid dimethyl ester) according to Miller *et al*. and Reddy *et al*.[30,31] ^{1}H-NMR ($CDCl_3$): δ: 2.45 (s, 6 H); 4.47 (dd, 4 H, $^3J = 3.0$ Hz, $^4J = 1.4$ Hz); 5.71–5.75 (m, 2 H); 7.36 (d, 4 H, $^3J = 8.3$ Hz); 7.77 (d, 4 H, $^3J = 8.3$ Hz). ^{13}C-NMR ($CDCl_3$): δ: 22.7 (2C); 68.7 (2C); 128.1 (2C); 128.3 (4C); 130.4 (4C); 132.5 (2C); 146.0 (2C).

*Toluene-4-sulfonic acid (E)-4-fluoro-but-2-enyl ester (**3**)*. Synthesized from (*E*)-but-2-ene-1,4-diol ditosylate (**2**) according to Dollé *et al*. and Goodman and Chen.[2,28] ^{1}H-NMR ($CDCl_3$): δ: 2.45 (s, 3 H); 4.58–4.62 (m, 2 H); 4.85 (dd, 2 H, $^2J_{H-F} = 46.4$ Hz, $^3J = 4.8$ Hz); 5.81–5.92 (m, 2 H); 7.37 (d, 2 H, $^3J = 8.3$ Hz); 7.81 (d, 2 H, $^3J = 8.3$ 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).

*3β-p-Tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**4**)*. Synthesized from *(R)*-cocaine hydrochloride according to Emond *et al*.[32] ^{1}H-NMR ($CDCl_3$): δ: 1.60–1.85 (m, 3 H); 1.93–2.15 (m, 2 H); 2.33 (s, 3 H); 2.43 (td, 1 H, $^2J = 13.1$ Hz, $^3J = 2.7$ Hz); 2.75 (dd, 1 H, $^3J = 5.9$ Hz, $^3J = 1.9$ Hz); 2.95 (s, 1 H); 3.19 (dt, 1 H, $^3J = 13.1$ Hz, $^3J = 5.5$ Hz); 3.41 (s, 3 H); 3.74 (m, 2 H); 7.11 (s, 4 H). ^{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)-3β-p-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (1, LBT-999). Synthesized from toluene-4-sulfonic acid (*E*)-4-fluoro-but-2-enyl ester (**3**) and 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**4**) according to Dollé *et al*.[2] ^{1}H-NMR ($CDCl_3$): δ: 1.57–1.68 (m, 3 H); 1.90–2.00 (m, 2 H); 2.22 (s, 3 H); 2.65 (td, 1 H, $^3J = {}^2J = 12.4$ Hz, $^3J = 2.6$ Hz); 2.92–3.05 (m, 4 H); 3.46 (m, 1 H); 3.53 (s, 3 H); 3.70 (m, 1 H); 4.86 (dd, 2 H, $^2J_{H-F} = 47.2$ Hz, $^3J = 4.6$ Hz); 5.80–5.85 (m, 2 H); 7.10 (d, 2 H, $^3J = 8.0$ Hz); 7.20 (d, 2 H, $^3J = 8.0$ 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

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(33); 53 (35). Elemental analyses: Calculated $C_{20}H_{26}FNO_2$: C, 72.48; H, 7.91; N, 4.23. Found: C, 72.26; H, 7.94; N, 4.21.

Radiochemistry

Preparation of the K[^{18}F]F-K_{222}-complex. In order to recover and recycle the [^{18}O]water target, the 2 ml of aqueous [^{18}F]fluoride ion from the target holder were passed through an anion exchange resin (Sep-Pak® Light Waters Accell™ Plus QMA cartridge (chloride form, beforehand washed with aqueous 1 M $NaHCO_3$ (2 ml) and rinsed with water (20 ml) and CH_3CN (10 ml)) by helium pressure (1.5–2.0 bar). Helium was blown through the column to maximally extract [^{18}O]water. The [^{18}F]fluoride ion was then eluted from the resin, using an aqueous 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. This complex was further dried twice by azeotropic removal of water traces by addition of 0.4 ml of CH_3CN followed by concentration to dryness at 145–150°C under a gentle nitrogen stream for 30 s.

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). Step 1: CH_3CN (600 µl) containing (*E*)-but-2-ene-1,4-diol ditosylate (**2**, 4.0–5.0 mg, 10.1–12.6 µmol) was directly 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 70°C, for 10 min) without stirring the contents. The reaction vessel was then cooled using an ice-water bath and the remaining radioactivity was measured. The resulting, often colored reaction mixture was then analyzed by radio-TLC. The reaction yield was calculated from the TLC-radiochromatogram and defined as the ratio of radioactivity area of (*E*)-4-[^{18}F]fluoro-but-2-enyl ester ([^{18}F]-**3**) over total fluorine-18 radioactivity area (SiO_2-TLC: R_f: [^{18}F]-**3** : 0.35 (heptane/EtOAc: 80/20), 0.45 (heptane/EtOAc: 70/30), 0.60 (heptane/EtOAc: 50/50) and R_f: [^{18}F]fluoride ion: 0.00 (heptane/EtOAc: 80/20 to 50/50)). The reaction mixture was diluted with Et_2O (1 ml) and transferred onto a SiO_2 Sep-Pak cartridge (PrepSep™ Si Extraction Column, Fisher Scientific, activated beforehand with Et_2O (5 ml)). The tube was rinsed twice with Et_2O (1 ml), which was also transferred and added to the diluted reaction mixture on top of the cartridge (6–10% of the total radioactivity amount engaged in the fluorination process usually stays behind in the initial tube). The whole was then passed through the cartridge and [^{18}F]-**3** was directly eluted into a pre-heated (70°C) 5 ml

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reaction vial. Another 1 ml of Et_2O was used to wash the cartridge for maximal transfer of [^{18}F]-**3** (30–45% of the total radioactivity amount engaged in the fluorination process usually elutes and is >95% pure [^{18}F]-**3**, according to radio-TLC). In addition to the above TLC analysis, the incorporation yield was also calculated after the SiO_2 Sep-Pak cartridge elution by the CH_3CN/Et_2O (containing >95% of [^{18}F]-**3**), over starting fluorine-18 radioactivity ratio. It was confirmed by the SiO_2 Sep-Pak cartridge (most likely non-reacted [^{18}F]fluoride ion), over starting fluorine-18 radioactivity ratio. The resulting CH_3CN/Et_2O solution was concentrated to dryness at 65–75°C under a gentle nitrogen stream for 2–3 min (10–15% of the radioactivity usually is lost most likely due to the volatility of [^{18}F]-**3**).

Step 2: To the above-mentioned residue, were successively added DMF (100 µl) containing KI (2 mg, 12.0 µmol) and DMF (400 µl) containing 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**4**, 5 mg, 19.3 µmol). The vial (not sealed) was placed in a heating block (at 90°C, for 20 min) without stirring the contents. The reaction vessel was then cooled using an ice-water bath and the remaining radioactivity was measured. Finally, the reaction mixture was diluted with 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:8.0–8.5 min), well separated from unlabelled **4** (t_R: 5.0–6.0 min) and starting [^{18}F]-**3** (t_R:2.5–3.0 min).

*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 followed by 8 ml of physiological saline and filtered on a 0.22 µm GS-Millipore filter (vented). Finally, physiological saline was added to lower the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[33]

*Quality control of [^{18}F]LBT-999 ([^{18}F]-**1**).* The radiotracer preparation was visually inspected for clarity, absence of color 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 F), with a sample of authentic **1** (t_R:2.28 min). Particular attention was paid to the absence of non-radioactive precursor (nortropane **4** : t_R:1.26 min). Chemical and radiochemical stability of the entire preparation were tested by HPLC (HPLC F) at regular 15-min intervals during 120 min.

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Specific radioactivity of the radiotracer was calculated from three consecutive HPLC (HPLC F) 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.

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