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Synthesis, radiosynthesis and in vivo preliminary evaluation of [^{11}C]LBT-999, a selective radioligand for the visualisation of the dopamine transporter with PET

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Abstract—LBT-999 (8-((*E*)-4-fluoro-but-2-enyl)-3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester), a cocaine derivative belonging to a new generation of highly selective dopamine transporter (DAT) ligands, and its corresponding carboxylic acid derivative, the latter used as precursor for labelling both with tritium and the positron-emitter carbon-11 (half-life: 20.38 min), were synthesized from (*R*)-cocaine. [^{3}H]LBT-999 (>99% radiochemically pure, specific radioactivity of 3.1 TBq/mmol) was prepared from [^{3}H]methyl iodide, allowing its in vitro pharmacological evaluation (K_D: 9 nM for DAT and IC_{50} > 1000 nM for SERT and NET). Routine production batches of 4.5–9.0 GBq of iv injectable solutions of [^{11}C]LBT-999 (with specific radioactivities ranging from 30 to 45 GBq/μmol) were prepared in 25–30 min (HPLC purification and formulation included) using the efficient methylation reagent [^{11}C]methyl triflate. The preliminary in vivo pharmacological evaluation of [^{11}C]LBT-999, using both biodistributions in rats and brain imaging in monkeys with positron emission tomography (PET), clearly illustrates that this ligand is an excellent candidate for quantification with PET of DAT in humans.

1. Introduction

Several affections, including Parkinson's disease, schizophrenia, attention deficit disorder or drug abuse, are related to abnormalities within the brain's dopamine system.[1] In particular, the neuronal dopamine transporter (DAT), a membrane-bound presynaptically located protein, plays a key role in regulating the synaptic concentration of dopamine at nerve terminals and thus dopamine neurotransmission in the brain. Taking into account that DAT density can be considered as a marker of the integrity and number of presynaptic striatal dopamine-producing neurons, considerable efforts have been spent in recent years on the design and development of DAT-selective radioligands as in vivo positron emission tomography (PET) imaging tools for the study of these illnesses and disorders as well as the evaluation of the degree of success of their treatment.

Certain tropane-type derivatives,[2–66] including cocaine,[67,68] represent today the largest class of molecules ever developed for imaging the DAT with PET and therefore labelled with a positron-emitting radioisotope (Table 1). From a chemical point of view, all chemical structures present (a) a 2β (exo) ester group (two exceptions: NS-2214 and β-CPPIT); (b) a 3β (exo) (often para-substituted) phenyl ring (exception: cocaine) and

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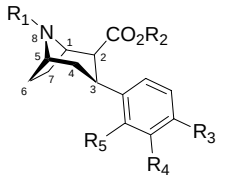
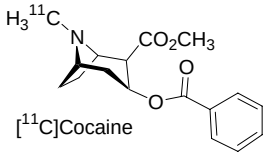
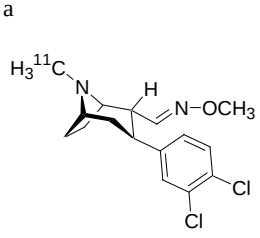
Table 1. Chemical structures of [^{11}C]cocaine and other tropane derivatives showing the positions that have been labelled with the positron-emitters carbon-11, fluorine-18 and bromine-76



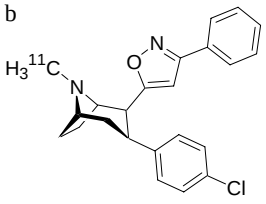


Code	R_1	R_2	R_3	R_4	R_5	Ref.
β-CFT (WIN 35,428)	[^{11}C]CH_3	[^{11}C]CH_3	[^{18}F]F	H	H	2–17
β-CIT (RTI-55)	[^{11}C]CH_3	[^{11}C]CH_3	I	H	H	18–27
β-CCT (RTI-31)	CH_3	[^{11}C]CH_3	Cl	H	H	28–30
β-CMT (RTI-32)	CH_3	[^{11}C]CH_3	CH_3	H	H	28,29
FECNT	CH_2CH_2[^{18}F]F	CH_3	Cl	H	H	31–39
β-CCT-FP (β-FPCT)	$(CH_2)_2CH_2$[^{18}F] F	CH_3	Cl	H	H	31,40
β-CBT	CH_3	CH_3	[^{76}Br]Br	H	H	41
β-FECT (β-FE-CCT)	CH_3	CH_2CH_2[^{18}F]F	Cl	H	H	29,42
β-FETT (β-FE-CMT)	CH_3	CH_2CH_2[^{18}F]F	CH_3	H	H	29,42
β-FIPCT (β-FiP-CCT)	CH_3	$CH(CH_3)CH_2$[^{18}F]F	Cl	H	H	43,44
β-CFT-FE	CH_2CH_2[^{18}F]F	CH_3	F	H	H	45,46
β-CFT-FP	$(CH_2)_2CH_2$[^{18}F] F	CH_3	F	H	H	45
β-CBT-FE	CH_2CH_2F	CH_3	[^{76}Br]Br	H	H	47
β-CIT-FP (FP-CIT)	$(CH_2)_2CH_2$[^{18}F] F	[^{11}C]CH_3	I	H	H	48–52
β-CMT-FP (FP-CMT)	$(CH_2)_2CH_2$[^{18}F] F	CH_3	CH_3	H	H	53
β-CBT-FP (FP-CBT)	$(CH_2)_2CH_2$[^{18}F] F	CH_3	[^{76}Br]Br	H	H	47,54
β-CDCT	CH_3	[^{11}C]CH_3	Cl	Cl	H	30
β-IP-CIT (RTI-121)	[^{11}C]CH_3	$CH(CH_3)_2$	I	H	H	23
NS-2214 (BMS-204756)	[^{11}C]CH_3	See below[a]	Cl	Cl	H	55
β-CIT-FE	CH_2CH_2F	[^{11}C]CH_3	I	H	H	15,56,57
β-CpFMT	CH_3	CH_3	CH_2[^{18}F]F	H	H	58,59
β-CmFMT	CH_3	CH_3	H	CH_2[^{18}F]F	H	58
β-CoFMT (o-FWIN)	CH_3	CH_3	H	H	CH_2[^{18}F]F	59
β-CPPIT	[^{11}C]CH_3	See below[b]	Cl	H	H	60,61
FCT	4-[^{18}F]FBn	CH_2CH_3	Cl	H	H	62
E-IACFT (Altropane)	CH_2CH=CHI	[^{11}C]CH_3	F	H	H	63
MCL301	CH_3	CH_2CH_2[^{18}F]F	I	H	H	64,65
MCL322	CH_3	CH_2CH_2[^{18}F]F	Br	H	H	64,65
FE@CIT	CH_3	CH_2CH_2[^{18}F]F	I	H	H	66

a



b



(c) a methyl group or a longer alkyl chain at the 8-nitrogen atom. From a radiochemical point of view, many of these derivatives have been labelled with the short-lived radioisotope carbon-11 (half-life: 20.38 min) at their *N*-methyl group or methyl ester function using [^{11}C]methyl iodide or [^{11}C]methyl triflate (for example, β-CIT or β-CCT). Also, a relatively large number of these derivatives have been labelled with fluorine-18 (half-life: 109.8 min), today the most widely used positron-emitting radioisotope, by aliphatic nucleophilic fluorination (for example, FE-CNT or β-FE-CCT) or aromatic electrophilic fluorination (β-CFT). Finally, a few of these derivatives have been labelled with bromine-76, a longer-lived positron-emitter (half-life: 16.1 h), by electrophilic bromination (for example, β-CBT). From an imaging point of view, many of these derivatives have been more or less successfully used in pre-clinical and clinical PET studies for the quantification of the DAT in the brain. A constant major drawback characterizing these radioligands is the low selectivity for the DAT, as most of these derivatives have a relatively high affinity for the serotonine transporter (SERT) and/or the norepinephrine transporter (NET) too. Another limitation, especially amplified with carbon-11-labelled radiotracers, is that the uptake of most of these radioligands in the striatum, the dopamine-rich region, increases continuously and

does not reach an equilibrium state, needed for quantification, within the duration of a PET examination. The, in this respect, most suited radioligand so far described seems to be FECNT,[31–39] with the more attractive kinetic characteristics by achieving a peak striatal uptake at 60–75 min post-injection. However, recently, the presence of a polar labelled metabolite, which crosses the blood–brain barrier, was reported, which could possibly compromise the use of this radioligand.[69]

Today, fluorine-18, beyond its adequate physical and nuclear characteristics, appears as the most attractive positron-emitting radioisotope for radiopharmaceutical chemistry and PET imaging, part of this continuous growing interest probably due to the successful use in clinical oncology of [^{18}F]FDG, actually the most widely used PET-radiopharmaceutical.

Briefly, fluorine-18 displays simple decay- and emission properties with a high 97% positron abundance. Compared with the other conventional short-lived positron-emitting radionuclides carbon-11, nitrogen-13 and oxygen-15, fluorine-18 has a relatively low positron energy (maximum 635 keV) and the shortest positron linear range in tissue (2.3 mm), resulting in the highest resolution in PET imaging. Its half-life (109.8 min) is long enough to give access to relatively extended imaging protocols compared to what is possible with carbon-11, therefore facilitating kinetic studies and high-quality plasma metabolite analysis. Moreover, from a chemical point of view, fluorine-18 allows for multi-step synthetic approaches that can be extended over hours. Finally, fluorine-18 can be reliably and routinely produced at the multi-Curie level, using the well-characterized (p,n) nuclear reaction on an oxygen-18-enriched water target on widely implemented biomedical cyclotrons of a relatively low-energy proton beam (e.g., 18 MeV). This distinctive advantage, combined with its favourable half-life, permits the transport and the use of fluorine-18-labelled radiopharmaceuticals (such as the archetype [^{18}F]FDG) at 'satellite' PET units without an on-site cyclotron production facility.

(*E*)-FBClNT (Fig. 1) is a recently reported tropane derivative, structurally closely related to FECNT (Table 1) in which the 8-nitrogen atom is alkylated with an (*E*)-fluorobutenyl group (R_1), offering an opportunity for fluorine-18 labelling.[70,71] Even though this new derivative displays a high in vitro affinity for the DAT (K_i = 2.54 nM) and a high accumulation in rat brain areas dense in DAT, its reported binding to the SERT excludes high selectivity both in vitro and in vivo. Previous structure-activity studies have demonstrated that selectivity for the DAT (over SERT and NET) of tropane-type derivatives was improved with chemical structures presenting both an unsaturated alkyl chain at the 8-nitrogen atom (R_1) and a methyl function at the phenyl ring (R_3 = methyl, instead of the frequently used halogens, see Table 1).[72] Among these compounds, PE2I (Fig. 1) (as well as its bromo analogue PE2Br (structure not shown[73]) were characterized by a moderate in vitro affinity but high selectivity for the DAT.[72,74] In initial in vivo studies, PE2I, labelled with carbon-11 or the SPECT-radioisotope iodine-123, not only accumulated rapidly and selectively in the striatum with a favourable kinetic profile, yielding excellent quality images within 30 min post-injection, but also afforded a high specific- to non-specific-binding ratio within 1 h.[75–83] These results have directed us towards the design of a fluorine-containing structure based on PE2I as the parent molecule (tolyl moiety) and presenting a fluorobutenyl alkyl chain at the 8-nitrogen atom, coded LBT-999 ((*E*)-*N*-(4-fluoro-but-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl) nortropane (**1**), Fig. 1). This new highly DAT-selective analogue can on the one hand be labelled with both the positron-emitter fluorine-18 and carbon-11 for in vivo imaging purposes, and on the other hand be readily accessible to tritium-labelling for in vitro characterization purposes.

This paper presents the synthesis of LBT-999 (**1**) from (*R*)-cocaine as well as the synthesis of the corresponding carboxylic acid derivative as precursor for subsequent tritium/carbon-11 labelling by methylation. Although the ultimate goal of this project is fluorine-18-labelling of this new DAT-ligand, this paper presents a first time radiolabelling of LBT-999 (**1**) with the radiochemically more practical carbon-11. Preliminary in vitro and in vivo pharmacological evaluation, including one selected PET study, is also presented.

2. Discussion

2.1. Chemistry

The target compound LBT-999 (**1**) as well as its precursor acid for tritium- and carbon-11-labelling (**10**) were both synthesized from (*R*)-cocaine (**2**) via the intermediate tropanamine **5**, namely 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (Schemes 1 and 3). Compound **5** was synthesized in 16% overall yield as shown in Scheme 1. Briefly, (*R*)-cocaine (**2**) was converted to ecgonidine methyl ester (**3**) in 84% overall yield using procedures previously described (Scheme 1).[72] 1–4 Addition involving tolylmagnesium bromide at −40 °C afforded preferentially the 2β,3β

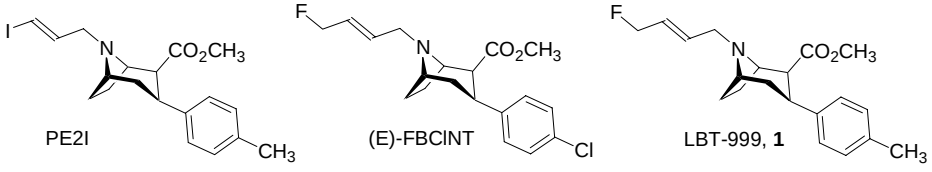
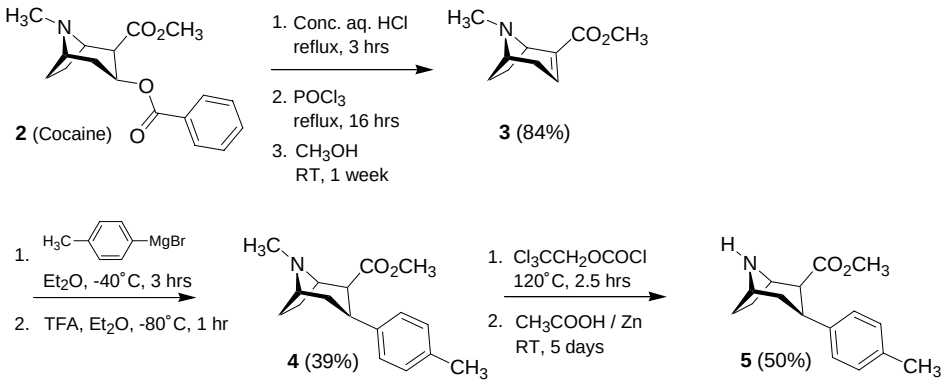
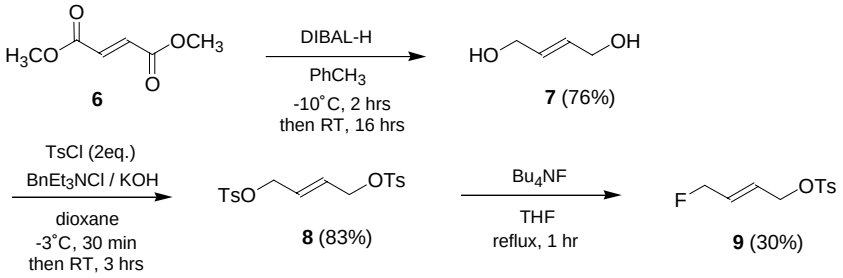


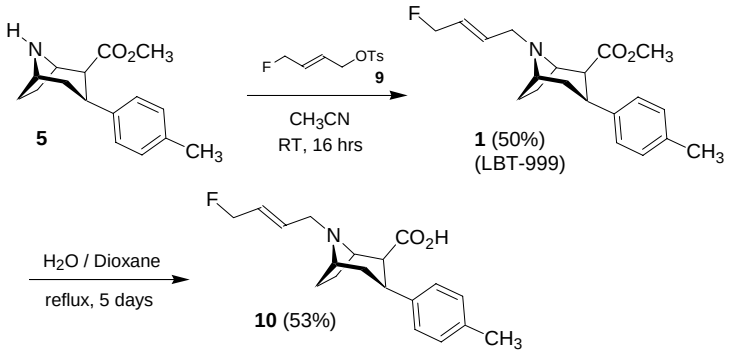
Figure 1. Chemical structures of (*E*)-FBClNT, PE2I and LBT-999 (**1**).



Scheme 1. Preparation of 3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**5**).



Scheme 2. Preparation of toluene-4-sulfonic acid (*E*)-4-fluoro-but-2-enyl ester (**9**).



Scheme 3. Preparation of LBT-999 (**1**) and the corresponding carboxylic acid derivative **10** as precursor for labelling.

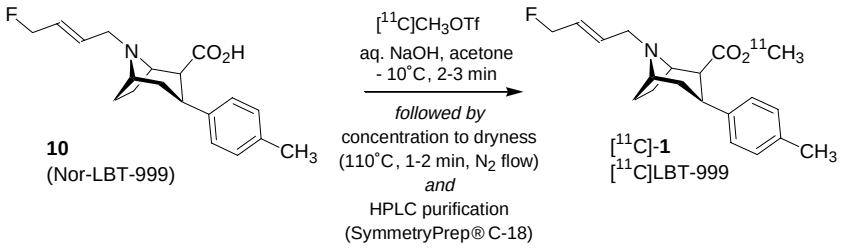
isomer **4**, which was isolated in 39% yield after flash chromatography purification. N-demethylation was performed using the following two-step procedure: (a) treatment of **4** with 2,2,2-trichloroethylchloroformate at 120 °C for 2.5 h, followed by (b) reduction of the intermediate carbamate with zinc powder in acetic acid at room temperature for 5 days, giving the tropanamine **5** in 50% yield.

Toluene-4-sulfonic acid (*E*)-4-fluoro-but-2-enyl ester (**9**, Scheme 2) was prepared in 19% overall yield from dimethylfumarate (**6**) using the following three-step sequence: (a) reduction of both ester functions using DIBAL-H in toluene at −10 °C for 2 h, giving the diol **7** in 76% yield,[84] followed by (b) conversion of the two alcohol functions into their tosylates, using two equivalents of tosyl chloride in dioxane containing triethylbenzylammonium chloride and aqueous potassium hydroxyde, and giving the ditosylate **8** in 83% yield,[85] and finally (c) mono tosyl-to-fluoro substitution using tetrabutylammonium fluoride (1.2 equiv) in refluxing THF, giving the fluorobutenyl tosylate **9** in 30% yield.[71]

Condensation of the tropanamine **5** with toluene-4-sulfonic acid (*E*)-4-fluoro-but-2-enyl ester (**9**) in acetonitrile at room temperature for 16 h gave LBT-999 (**1**) in 50% yield (Scheme 3). Hydrolysis of the methyl ester function was performed in a refluxing 1/1 (v:v) water/dioxane mixture for 5 days and afforded the carboxylic acid derivative **10** in 53% yield.

2.2. Radiochemistry

LBT-999 (**1**) was labelled with carbon-11 at its methyl ester function from the corresponding carboxylic acid derivative **10** and the highly efficient methylation



Scheme 4. Preparation of [^{11}C]LBT-999 ([^{11}C]-**1**).

reagent [^{11}C]methyl triflate. [^{11}C]Methyl triflate was prepared according to a literature procedure from [^{11}C]methyl iodide using silver triflate.[86] [^{11}C]Methyl iodide was prepared from cyclotron-produced [^{11}C]carbon dioxide using the well-known two-step protocol, consisting of the trapping of [^{11}C]CO_2 and conversion into [^{11}C]methanol ($LiAlH_4$) followed by iodination using aqueous HI giving [^{11}C]methyl iodide.[87] On average, about 25 GBq of [^{11}C]CH_3OTf was routinely obtained in 7–8 min after the end of bombardment (EOB) in 70% decay-corrected yield, based on starting [^{11}C]CO_2.

Esterification of carboxylic acid **10** with [^{11}C]methyl triflate (Scheme 4), employing the standard conditions that are now used in our laboratory for the routine radiosynthesis of several radiotracers,[88–91] including [^{11}C]PE2I,[80] gave excellent yields for the preparation of [^{11}C]LBT-999 ([^{11}C]-**1**, Scheme 4). The conditions used were the following: (1) trapping at −10 °C of [^{11}C]methyl triflate in 400 μL acetone containing 0.6–0.8 mg of precursor **10** (1.9–2.5 μmol) and 3 μL of aqueous NaOH (3 N) (about 4 equiv); (2) concentration to dryness of the reaction mixture (at 110 °C, using a nitrogen stream for 1–2 min); (3) taking up the residue in 0.5 mL of the HPLC mobile phase (H_2O/CH_3CN/TFA: 35/65/0.1 (v/v/v)) and (4) purification using a semi-preparative reversed-phase HPLC.

Formulation of LBT-999 ([^{11}C]-**1**) for iv injection was carried out as follows: 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 further diluted with physiological saline.

Typically, starting from 50 to 60 GBq of a [^{11}C]CO_2 production batch, 4.5–9.0 GBq of [^{11}C]-**1** was obtained within 25–30 min of radiosynthesis, including HPLC purification and formulation. The total decay-corrected radiochemical yield of [^{11}C]-**1**, based on starting [^{11}C]CO_2, was 19–45% (n = 10). No attempts were made to further optimize these reactions, the yields obtained being sufficient for our purposes. The specific radioactivity measured at the end of the radiosynthesis ranged from 30 to 45 GBq/μmol).

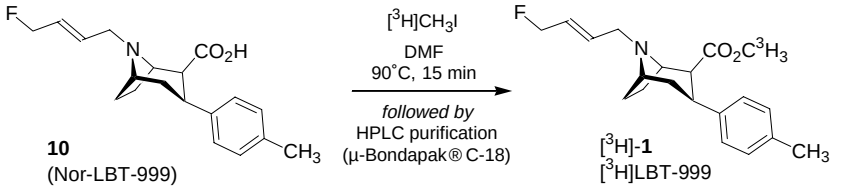
Concerning quality controls, the radiopharmaceutical preparation was a clear and colourless solution and its pH was between 5 and 7. As demonstrated by the HPLC analysis, the radiopharmaceutical preparation was found to be >99% chemically and radiochemically pure and the preparation was shown to be free of non-radioactive precursor (derivative **10**) and was radiochemically stable for at least 60 min. Administration to animals was performed within 10 min following the end of the synthesis. These results were in compliance with our in-house quality control/assurance specifications.

LBT-999 (**1**) was labelled with tritium at its methyl ester function from the corresponding carboxylic acid derivative **10** and commercially available [^{3}H]methyl iodide (Scheme 5).

Esterification of carboxylic acid **10** with [^{3}H]methyl iodide (Scheme 5) employed the following standard conditions: (1) heating at 90 °C for 15 min a DMF/toluene solution (300 μL/150 μL) containing [^{3}H]methyl iodide and 0.7 mg of precursor **10** (2.2 μmol); (2) purification using semi-preparative HPLC. [^{3}H]LBT-999 ([^{3}H]-**1**, >99% radiochemically pure) was obtained with a specific radioactivity of 3.1 TBq/mmol.

2.3. PET imaging and preliminary in vitro/in vivo pharmacological evaluation

In vitro binding experiments on rat striatal membranes showed that [^{3}H]LBT-999 bound to a single site with a K_d of 9 nM (B_{max}: 17 pmol/mg protein) and displayed a very high selectivity for the DAT (IC_{50} versus GBR 12909—another DAT-selective inhibitor—and PE2I: 2.4 and 18 nM, respectively; IC_{50} versus paroxetine,



Scheme 5. Preparation of [^{3}H]LBT-999 ([^{3}H]-**1**).

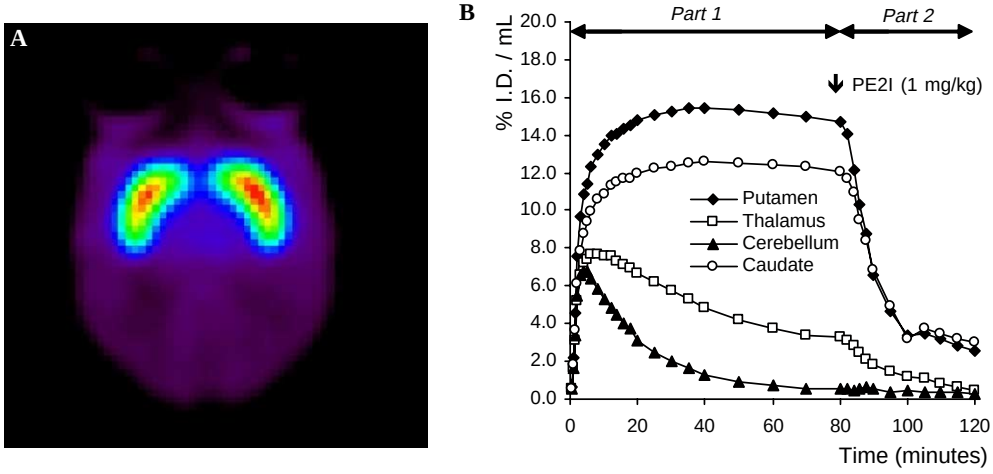


Figure 2. PET imaging of [^{11}C]LBT-999 ([^{11}C]-1) in a Papio anubis baboon. An adult baboon (10 kg, anaesthetized with 1% isoflurane and 33%/66% O_2/N_2O) was iv injected with 160 MBq of [^{11}C]LBT-999 ([^{11}C]-1) and imaged for 80 min on an HR+ Exact positron tomograph (CTI PET Systems, Knoxville, TN, USA). At 80 min, PE2I (1 mg/kg) was iv injected and PET data were acquired for another 40 min. For data analysis, MRI-based regions of interest (ROI) were placed on the putamen, the caudate, the thalamus and the cerebellum. The radioactivity measured in each ROI was corrected for attenuation and carbon-11-decay and expressed as percent of injected dose per 100 mL of tissue (% ID/100 mL).

citalopram, MADAM, nisoxetine and desipramine >1 μM). Ex vivo cerebral biodistribution in rats performed with [^{11}C]LBT-999 showed a striatum/cerebellum radioactivity ratio of 18 and 25 at 30 and 60 min post-injection, respectively. Moreover, the radiotracer accumulation was strongly prevented by a pre-injection of GBR 12909 (a DAT-selective inhibitor), whereas paroxetine (a SERT-selective inhibitor) and nisoxetine (a NET-selective inhibitor) had no effect.

Based on these encouraging results, PET studies of the brain distribution of [^{11}C]LBT-999 ([^{11}C]-**1**) were carried out in primates (Papio anubis baboon). Only one selected single PET experiment, performed on an adult male baboon, is described here to illustrate this section. The full characterization of [^{11}C]LBT-999 as a PET radiotracer will be published elsewhere. Figure 2A illustrates the uptake of [^{11}C]LBT-999 in the baboon brain at 30–60 min, following iv injection of 160 MBq of [^{11}C]LBT-999 ([^{11}C]-**1**). Figure 2B (part 1: 0–80 min) shows the corresponding time–activity curves obtained in selected brain regions (putamen, caudate, thalamus, known to contain DAT, and cerebellum, used as reference area for non-specific binding). After injection of the radiotracer, there was a rapid appearance of radioactivity in the whole of the brain. The time course of radioactivity showed a rapid accumulation (within 20 min) in the caudate nucleus and putamen, reaching a value of 12.6% and 15.4% of injected dose per 100 mL of tissue (% ID/100 mL), respectively, 40 min p.i. At 80 min p.i., these values remained high: 12.0% and 14.7% ID/100 mL, respectively. In the thalamus, radioactivity concentrations were maximal at 8 min p.i.: 7.8% ID/100 mL and then decreased progressively. In the cerebellum, radioactivity concentration peaked at 4 min (5.9% ID/100 mL) and then decreased relatively rapidly. At 40 and 80 min p.i., the uptake in the cerebellum was 1.2 and 0.5 ID/100 mL, respectively. Therefore, ratios of radioactivity caudate nucleus/cerebellum and putamen/cerebellum were 10.2 and 12.5, respectively at 40 min p.i. At 80 min p.i., these ratios further increased to 23.6 and 28.9. These results clearly show that LBT-999 rapidly enters the brain and binds in cerebral regions known to contain dopaminergic terminals. Moreover, early equilibrium states (around 40 min) obtained in caudate and putamen would allow DAT quantifying protocols within a PET examination even using the carbon-11-labelled compound. Figure 2B (part 2: 80–120 min) shows the effect of PE2I (1 mg/kg, iv administered 80 min after the radiotracer injection). A rapid wash-out of the radioactivity in the caudate nucleus, the putamen, and the thalamus was observed. In these structures, the radioactivity decreased by 74%, 81%, and 81%, respectively. In the cerebellum, no displacement of radioactivity was observed. These results confirm the high selectivity profile of LBT-999 in vivo and clearly demonstrate that the high levels of LBT-999 binding in caudate and putamen can be attributed to a DAT selective labelling.

3. Conclusion

LBT-999 (**1**) and the corresponding carboxylic acid derivative (**10**) as precursor for labelling were synthesized from (*R*)-cocaine. The key step in these syntheses was the 1–4 Michael addition of 4-tolylmagnesium bromide to ecgonidine methyl ester, affording, after *N*-demethylation, the needed 2β-3β nortropane isomer. LBT-999 was labelled with tritium at its methylester function using [^{3}H]methyl iodide as well as labelled at the same function using the efficient methylation reagent [^{11}C]methyl triflate, permitting its preliminary in vitro and in vivo pharmacological evaluation. Taken together, the results so far obtained demonstrate that LBT-999 is a highly promising candidate for in vivo exploration and quantification in humans of DAT, using PET. Labelling of LBT-999 with fluorine-18 (half-life: 109.8 min), the most widely used positron-emitting radiohalogen, is currently in progress.

4. Experimental

4.1. General

4.1.1. Chemicals, TLCs, and flash chromatographies. 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 (230–400 Mesh, VWR) columns. TLCs were run on pre-coated plates of silica gel $60F_{254}$ (VWR). 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 followed by heating on a hot plate.

4.1.2. HPLCs. [HPLC 1] Equipment: Beckman (Paris, France) system equipped with a 126P-Solvent module, a 166-NMP pump; column: semipreparative Stability® C-18, CL Cluzeau (250 mm × 10 mm); conditions: eluent: MeOH/H_2O: 50/50 (v/v); flow rate: 2.5 mL/min; temperature: RT; UV detection at λ: 254 nm. [HPLC 2] Equipment: Waters (St-Quentin en-Yvelines, France) systems equipped with a 510 pump, 440 UV detector or Shimadzu SPD-10A UV-multiwavelength detector and a Geiger–Müller detector; column: semipreparative SymmetryPrep® C-18, Waters (300 mm × 7.8 mm); conditions: eluent: $H_2O/CH_3CN/CF_3CO_2H$: 35/65/0.1 (v/v/v); flow rate: 5 mL/min; temperature: RT; UV detection at λ: 220 nm. [HPLC 3] Equipment: Waters (St-Quentin en-Yvelines, France) Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold (Thoiry, France) LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (50 mm × 4.6 mm); conditions: isocratic elution with solvA/solvB: 40/60 (v/v) [solvA: H_2O containing Low-UV PIC® B7 reagent (% 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; solvB: H_2O/CH_3CN: 50/50 (v/v) containing Low-UV PIC® B7 reagent (Waters), 20 mL for 1000 mL]; flow rate: 2.0 mL/min; temperature: 30 °C; UV detection at λ: 220 nm. [HPLC 4] Equipment: System equipped with a Hitachi (Tokio, Japan) L-7100 pump, a Hitachi L-7400 UV detector and a Packard (Paris, France) Radiomatic 150TR radioactivity detector equipped with a tritium-cell (550 μL cell); column: semipreparative μ-Bondapak® C-18, Waters (300 mm × 7.8 mm); conditions: eluent: H_2O/CH_3CN/TEA: 65/35/0.1 (v/v/v); flow rate: 6 mL/min; temperature: RT; UV detection at λ: 254 nm.

4.1.3. 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 $CDCl_3$ (δ = 7.30 ppm) or TMS (δ = 0 ppm) as internal standards for ^{1}H NMR as well as the deuterated solvent $CDCl_3$ (δ = 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 (ES+). Elemental analyses were performed by the service d'analyses du CNRS (Vernaison, France) and results were within ±0.4% of theoretical values.

4.1.4. Radioisotope availability/production. $[^{11}C]CO_2$ was produced via the $^{14}N[p,\alpha]^{11}C$ nuclear reaction by irradiation of an ultrapure Air Liquide 99.5/0.5 mixture of N_2/O_2 target with an 18 MeV proton beam (at 25 μA) on an IBA Cyclone-18/9 (Louvain-la-Neuve, Belgium) cyclotron (8.5 μA h in about 20 min). At the end of the bombardment, the target contents were transferred to the 5-cm-lead shielded hot cell and passed through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. $[^{11}C]CO_2$ was then separated from the target gas by trapping in an empty stainless-steel coil (150 mm length, 0.51 mm internal diameter), cooled at −186 °C using liquid argon. On average, about 45 GBq (EOB) of $[^{11}C]CO_2$ is routinely obtained in our laboratory for the irradiations described above. $[^3H]CH_3I$ (toluene solution containing copper metal scavenger, 370 MBq/mL, 3.1 TBq/mmol) was purchased from Amersham (Sweden).

4.2. Chemistry

4.2.1. 3β-*p*-Tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methylester (5). Synthesized from (*R*)-cocaine hydrochloride according to Ref. 72. ^{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 Hz, ^3J = 1.9 Hz); 2.95 (s, 1H); 3.19 (dt, 1H, ^3J = 13.1 Hz, ^3J = 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).

4.2.2. Toluene-4-sulfonic acid (*E*)-4-fluoro-but-2-enylester (9). To a solution of (*E*)-but-2-enediol ditosylate (**8**, 0.5 g, 1.26 mmol, synthesized from dimethyl fumarate (**6**, (*E*)-but-2-enedioic acid dimethyl ester) according to Refs. 84,85) dissolved in THF (20 mL) tetrabutylammonium fluoride (1.57 mL, 1 M in THF, 1.57 mmol) was added dropwise and the mixture was heated under reflux for 1 h. The solvent was evaporated, and the resulting solid was purified by flash chromatography (petroleum ether/Et_2O: 8/2). Derivative **9** was obtained as a yellow liquid in 30% yield (91 mg). ^{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).

4.2.3. 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). To a solution of toluene-4-sulfonic acid 4-fluoro-but-2-enyl ester (**9**, 100 mg, 0.41 mmol) in acetonitrile (5 mL) was added dropwise 3β-*p*-tolyl-8-aza-bicy-

clo[3.2.1]octane-2β-carboxylic acid methyl ester (**5**, 100 mg, 0.38 mmol) dissolved in acetonitrile (5 mL). After stirring overnight, the mixture was treated with Et_2O (5 mL), H_2O (15 mL) and HCl 1 M to pH 1. The aqueous phase was separated, basified with Na_2CO_3 and the compound was extracted with Et_2O (5×10 mL). The residue after evaporation of solvent was finally purified by flash chromatography (Et_2O/Et_3N: 99/1) to give **1** (LBT-999) as a white solid (55 mg, 50%). ^{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.

4.2.4. 8-((*E*)-4-Fluoro-but-2-enyl)-3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid (10). Thirty milligrams of 8-((*E*)-4-fluoro-but-2-enyl)-3β-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2β-carboxylic acid methyl ester (**1**, 0.09 mmol) was dissolved in a 1/1 mixture of dioxane and water (5 mL). The resulting solution was refluxed for 5 days. The solvents were removed under vacuum and the residue was purified by semipreparative reversed-phase HPLC (HPLC 1, R_t: 11 min) to give **10** as a white solid (15 mg, 53%). ^{1}H NMR ($CDCl_3$): δ: 1.77–2.14 (m, 3H); 2.19–2.25 (m, 2H); 2.32 (s, 3H); 2.54 (td, 1H, $^3J = {}^2J$ = 13.3 Hz, ^3J = 2.6 Hz); 2.70–2.73 (m, 1H); 3.15–3.27 (m, 3H); 3.68 (s, 2H); 4.92 (dd, 2H, $^2J_{H-F}$ = 46.5 Hz, ^3J = 3.9 Hz); 5.92–5.96 (m, 2H); 7.15 (s, 4H). ^{13}C NMR ($CDCl_3$): δ: 21.0; 25.0; 25.6; 34.6; 35.3; 52.6; 53.5; 59.4; 62.0; 82.1 (d, $^1J_{C-F}$ = 164.5 Hz); 127.3 (2C); 127.9 (d, $^3J_{C-F}$ = 12.0 Hz); 129.3 (2C); 130.3 (d, $^2J_{C-F}$ = 17.1 Hz); 136.7 (2C); 174.4. MS: m/e (%): 317 (M^+, 32); 273 (55); 272 (19); 244 (11); 199 (11); 170 (13); 168 (13); 154 (85); 141 (89); 140 (100); 129 (17); 128 (23); 122 (98); 108 (59); 82 (56); 80 (36); 68 (39); 53 (32).

4.3. Carbon-11 radiochemistry

4.3.1. Preparation of [^{11}C]CH_3OTf. [^{11}C]CO_2 was released from the stainless-steel coil trap (see above) by raising its temperature to ambient, swept away by a flow of nitrogen gas (40 mL/min) and trapped at −10 °C (EtOH-ice bath) in a mixture of THF (60 μL) and lithium aluminium hydride/THF (8 μL, 1.0 M). Concentration to dryness (evaporation of solvent at 155 °C using a stream of nitrogen) followed by hydrolysis (100 μL of deionized water) of the formed aluminium complex afforded [^{11}C]CH_3OH, which was distilled, using a flow of nitrogen gas, into 0.8 mL of an aqueous 57% HI solution (heating block at 155 °C). The [^{11}C]CH_3I thus synthesized was continuously swept away by the flow of nitrogen gas, passed through a combined 1/1 (v:v) soda lime/P_2O_5-guard (35 mm length each, 3 mm internal diameter) and converted into [^{11}C]CH_3OTf by passing through a glass column (33 cm length, 5 mm internal diameter), heated at 200 °C and containing silver-triflate-impregnated graphitized carbon (200 mg).

4.3.2. Preparation of [^{11}C]LBT-999 ([^{11}C]-1). [^{11}C]CH_3OTf, carried by a flow of nitrogen gas (40 mL/min), was trapped (bubbling through) at −10 °C (EtOH–ice bath) in a reaction vessel containing 0.6–0.8 mg of the carboxylic acid derivative **10** (1.9–2.5 μmol) dissolved in 400 μL acetone and 3 μL of aq NaOH (3 N) (9 μmol). Trapping of [^{11}C]CH_3OTf was monitored using an ionization-chamber probe. When the reading had reached its maximum (2–3 min), the reaction mixture was concentrated to dryness at 110 °C (heating block), using a nitrogen stream for 1–2 min. The reaction vessel was then cooled (EtOH–ice bath) and the reaction mixture was diluted with 0.5 mL of the HPLC mobile phase and was injected onto the column. HPLC-purification (HPLC 2) gave radiochemically pure [^{11}C]-**1** (R_t: 6.5–7.0 min), well separated from the unlabelled precursor **10** (R_t: 4.0–5.0 min).

4.3.3. Formulation of [^{11}C]LBT-999 ([^{11}C]-1). Formulation of the labelled product for iv 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 EtOH and then rinsed with 10 mL of water prior to use). The cartridge was washed with 7 mL water and partially dried by applying a nitrogen stream for 10 s. The radiotracer was eluted with 2 mL EtOH followed by 8 mL of physiological saline (less than 10% of the total radioactivity was left on the cartridge) 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.[92]

4.3.4. Quality control of [^{11}C]LBT-999 ([^{11}C]-1). The radiotracer preparation was visually inspected for clarity, absence of colour and particulates. An aliquot of the preparation was evaluated for pH using standard pH-paper. Chemical and radiochemical purities were also assessed on this aliquot by HPLC, with a sample of authentic LBT-999 (HPLC 3; R_t: 2.75 min). Finally, specific radioactivity of the radiotracer was calculated from three consecutive HPLC 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.

4.4. Tritium radiochemistry

4.4.1. Preparation of [^{3}H]LBT-999 ([^{3}H]-1). One hundred fifty microliters of a toluene solution of [^{3}H]methyl iodide (370 MBq/ml, 3.1 TBq/mmol) was added to a solution of 0.7 mg of the carboxylic acid derivative **10** (2.20 μmol) in 300 μl of *N*,*N*-dimethylformamide. The resulting solution was heated for 15 min at 90 °C. The reaction mixture was then diluted with 300 μL CH_3CN

and was injected onto the HPLC column. HPLC-purification (HPLC 4) gave radiochemically pure [^{3}H]-**1** (R_t: 5.2 min), well separated from the unlabelled precursor **10** (R_t: 2.5–3.5 min) and non-reacted [^{3}H]methyl iodide (R_t: 2.3 min).

4.4.2. Formulation of [^{3}H]LBT-999 ([^{3}H]-1). Formulation of the labelled product was effected as follows: The HPLC-collected fraction containing the radiotracer was gently concentrated to dryness. The residue was taken up in 100 μL of aq 75% EtOH.

4.4.3. Quality control of [^{3}H]LBT-999 ([^{3}H]-1). Radiochemical purity was assessed on an aliquot by HPLC, with a sample of authentic LBT-999 (HPLC 4, R_t: 5.2 min).

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