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Synthesis and radiosynthesis of [^{18}F]FPhEP, a novel $\alpha_4\beta_2$-selective, epibatidine-based antagonist for PET imaging of nicotinic acetylcholine receptors

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Abstract—FPhEP (**1**, (+/−)-2-*exo*-(2′-fluoro-3′-phenyl-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane) belongs to a recently described novel series of 3′-phenyl analogues of epibatidine, which not only possess subnanomolar affinity and high selectivity for brain α4β2 neuronal nicotinic acetylcholine receptors (nAChRs), but also were reported as functional antagonists of low toxicity (up to 15 mg/kg in mice). FPhEP (**1**, K_i of 0.24 nM against [^{3}H]epibatidine) as reference as well as the corresponding *N*-Boc-protected chloro- and bromo derivatives (**3a**,**b**) as precursors for labelling with fluorine-18 were synthesized in eight and nine steps, respectively, from commercially available *N*-Boc-pyrrole (overall yields = 17% for **1**, 9% for **3a** and 8% for **3b**). FPhEP (**1**) was labelled with fluorine-18 using the following two-step radiochemical process: (1) no-carrier-added nucleophilic heteroaromatic ortho-radiofluorination from the corresponding *N*-Boc-protected chloro- or bromo derivatives (**3a**,**b**—1 mg) and the activated K[^{18}F]F–Kryptofix$^{®}_{222}$ complex in DMSO using microwave activation at 250 W for 1.5 min, followed by (2) quantitative TFA-induced removal of the *N*-Boc-protective group. Radiochemically pure (>99%) [^{18}F]FPhEP ([^{18}F]-**1**, 2.22–3.33 GBq, 66–137 GBq/μmol) was obtained after semi-preparative HPLC (Symmetry® C18, eluent aq 0.05 M NaH_2PO_4/CH_3CN, 80:20 (v:v)) in 75–80 min starting from a 18.5 GBq aliquot of a cyclotron-produced [^{18}F]fluoride production batch (10–20% nondecay-corrected overall yield). In vitro binding studies on rat whole-brain membranes demonstrated a subnanomolar affinity (K_D 660 pM) of [^{18}F]FPhEP ([^{18}F]-**1**) for nAChRs. In vitro autoradiographic studies also showed a good contrast between nAChR-rich and -poor regions with a low non-specific binding. Comparison of in vivo Positron Emission Tomography (PET) kinetics of [^{18}F]FPhEP ([^{18}F]-**1**) and [^{18}F]F-A-85380 in baboons demonstrated faster brain kinetics of the former compound (with a peak uptake at 20 min post injection only). Taken together, the preliminary data obtained confirm that [^{18}F]FPhEP ([^{18}F]-**1**) has potential for in vivo imaging nAChRs in the brain with PET.

1. Introduction

There is considerable evidence that a variety of functions and disorders of the central nervous system (CNS), including Alzheimer's and Parkinson's diseases, pain, schizophrenia, anxiety, depression, Tourette's syndrome and tobacco dependence, are linked to the neuronal nicotinic acetylcholine receptors (nAChRs).[1] nAChRs are ligand-gated ion channels and most of them are heteromeric pentamers. Several subunits (α2–α10, β2–β4) have been identified so far and different combinations of these subunits define nAChR subtypes. One of the two most abundant subtypes of nAChRs in mammalian brain is the receptor containing α4 and β2 subunits.[1] Positron Emission Tomography (PET) is a radiotracer-based non-invasive medical imaging technique using positron emitters. It is currently the most advanced technology available for studying molecular interactions in vivo and could play a key role in elucidating the involvement of these receptors in various CNS disorders.[2] With the aim of finding a useful PET imaging tool for the study of the nature of these disorders, for their diagnosis or for the monitoring of the effectiveness of their treatment, several α4β2-subtype selective fluorine-containing ligands have been radiolabelled with fluorine-18 (half-life: 109.8 min), one of the most attractive positron-emitting radioisotopes for radiopharmaceutical chemistry.[3–6] These radioligands can be divided into three distinct groups. The first group (Fig. 1) consists of analogues of A-85380 (3-[(*S*)-2-azetidinylmethoxy]-

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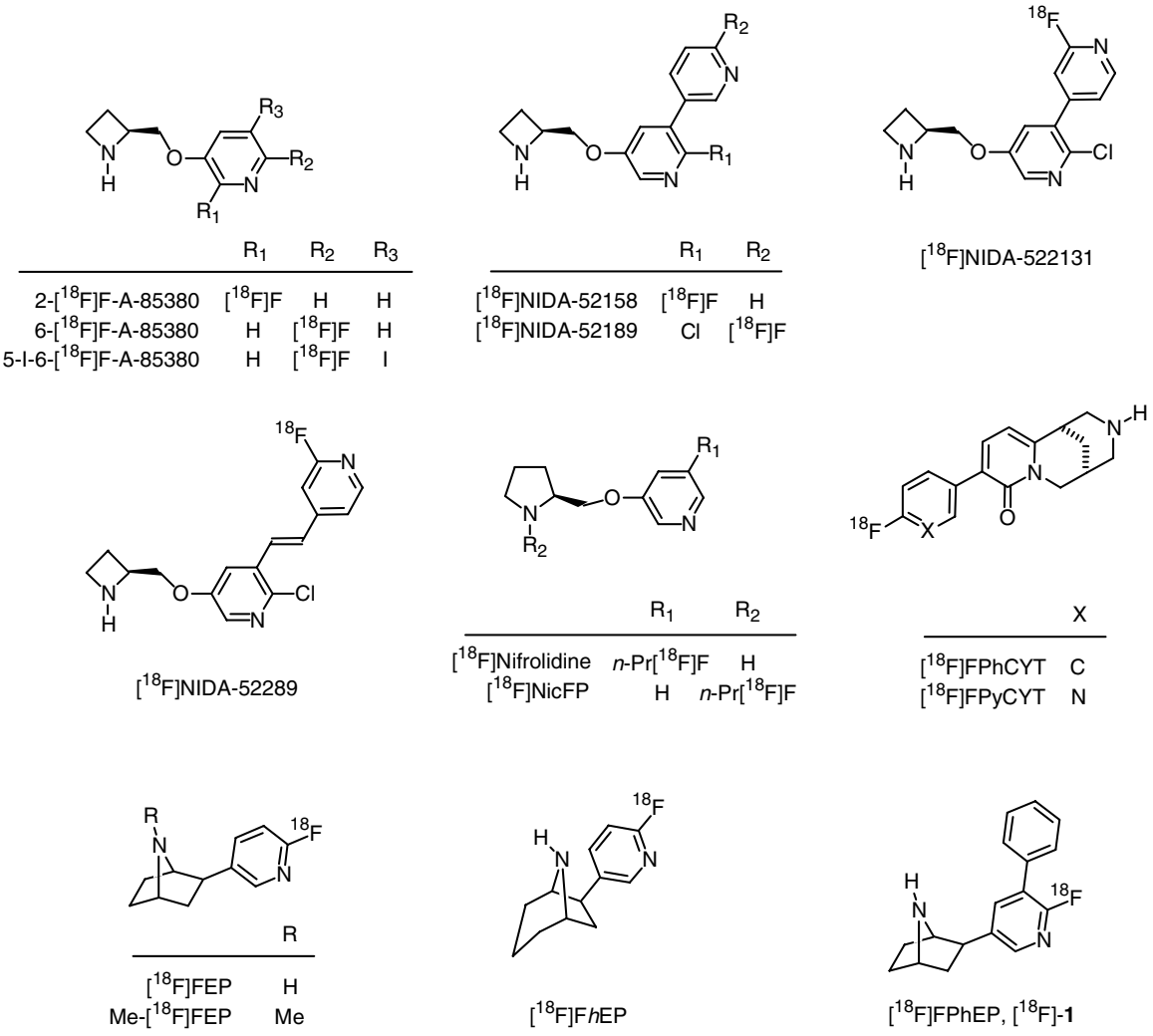


Figure 1. Azetidinyl-, pyrrolidinyl-, (−)-cytisine- and epibatidine-based existing fluorine-18-labelled ligands for nAChR PET imaging including the target tracer of the present work [^{18}F]FPhEP ([^{18}F]-**1**).

pyridine), the lead compound of a series of 3-pyridyl ethers developed by Abbott laboratories.[7–9] They include azetidine-based derivatives, 2-[^{18}F]F-A-85380,[10–29] 6-[^{18}F]F-A-85380,[30–35] 5-iodo-6-[^{18}F]fluoro-A-85380,[36] the more lipophilic [^{18}F]NIDA-52158,[37] [^{18}F]NIDA-52189,[37] [^{18}F]NIDA-522131[38] and [^{18}F]NIDA-52289[39], and pyrrolidine-based [^{18}F]Nifrolidine[40] and [^{18}F]NicFP.[41] A second group includes [^{18}F]FPhCYT[42,43] and [^{18}F]FPyCYT,[44] two derivatives of (−)-cytisine, a natural chiral quinolizidine alkaloid.[45–48] The third group consists of analogues of the alkaloid (−)-epibatidine, a natural product isolated from a South American frog (*Epipedobates tricolor*) and discovered in the 1990s as a non-opioid analgesic agent with a potency in mice 200-fold greater than that of morphine.[49] It was later shown to exert its antinociceptive action via an nAChR mediated mechanism.[50,51] [^{18}F]FEP, a compound in which the chlorine atom of epibatidine has been replaced by a [^{18}F]fluorine-atom,[52–55] showed a brain distribution and in vivo pharmacological characteristics in non-human primates that were exceptionally promising.[56–61] Although the mass of this fluoro-analogue of epibatidine implied in an imaging protocol is far below pharmacologically active doses, its toxicity prohibited its use in humans.[62] Other analogues such as its *N*-methyl derivative ([^{18}F]Me-FEP)[60] and an 8-azabicyclo[3.2.1]octane derivative ([^{18}F]F*h*EP[63–65]) have also been developed. Among all these fluorine-18-labelled derivatives, the agonist 2-[^{18}F]F-A-85380 is the only PET probe for the α4β2 nAChR subtype currently used in humans. It shows high affinity and selectivity for this subtype[10,12,16,26] and has been extensively validated in non-human primate studies.[15,17,21,23,28] It also displays a safe profile—low toxicity,[19,26] no mutagenicity,[19,26] and acceptable effective dose equivalent to the patient in dosimetric studies[20,22,26]—for its use as a PET probe in humans.[27,29] However, this relatively strong-hydrophilic derivative displays rather slow brain kinetics, resulting in long scanning times, and this could be a limitation in routine clinical use. Also, its non-specific binding in regions showing rich n-AChRs densities, such as the thalamus, remains relatively high with 30–40% of the radioligand fixation being nondisplacable. This phenomenon becomes even more important in regions with intermediate and poor n-AChRs densities, such as the cortex, the striatum and the cerebellum.[15]

Recently, a novel series of 3′-phenyl-epibatidine and 3′-phenyl-deschloroepibatidine derivatives was described.[66,67] These compounds not only possess subnanomolar affinity[66,67] and high selectivity[68] for brain α4β2-nAChRs, but also were reported as functional antagonists of low toxicity (up to 15 mg/kg in mice). Within this series, a 2′-fluoro-derivative, namely (+/−)-2-*exo*-(2′-fluoro-3′-phenyl-pyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (**1**, FPhEP), displays a K_i of 0.24 nM against [^{3}H]epibatidine, and offers an opportunity for fluorine-18-labelling by nucleophilic ortho-heteroaromatic substitution.[69–71] We therefore selected it as a potential candidate for imaging the nAChRs with PET.

We herein report the synthesis of FPhEP (**1**) as reference compound as well as the synthesis of both the *N*-Boc-protected chloro- and bromo analogues, **3a** and **3b** (Scheme 1), as precursors for labelling with fluorine-18. We also report the two-step radiosynthesis of [^{18}F]FPhEP ([^{18}F]-**1**) by nucleophilic heteroaromatic ortho-radiofluorination. Finally, preliminary in vitro and in vivo pharmacological evaluation of this new fluorine-18-labelled radioligand, including two selected PET studies, is presented.

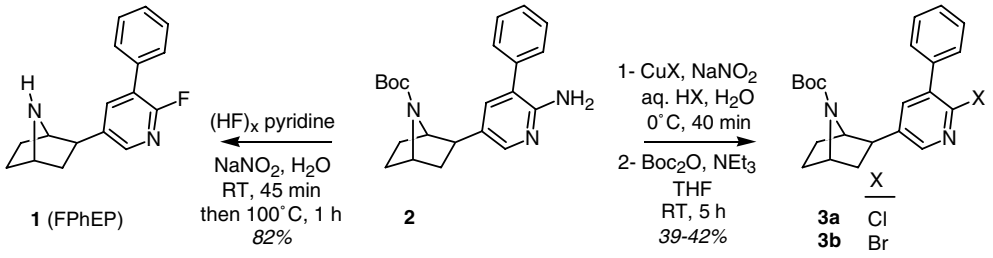
2. Results and discussion

2.1. Chemistry

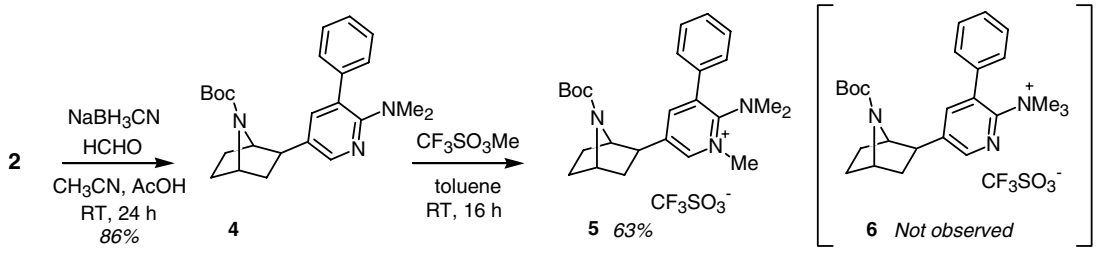
The synthesis of the reference compound FPhEP (**1**) is outlined in Scheme 1. The amino derivative **2** was synthesized according to reported procedures[66,67] and obtained in seven steps and 20% overall yield from commercially available *N*-Boc-pyrrole. It was treated with sodium nitrite in 70% HF/pyridine at room temperature for 45 min, followed by heating at 100 °C for 1 h giving FPhEP (**1**) in 82% yield.

The obvious radiochemical labelling strategy for the preparation of [^{18}F]FPhEP ([^{18}F]-**1**) is nucleophilic heteroaromatic fluorination. These reactions, like the aliphatic nucleophilic radiofluorinations, only require a good leaving group and there is no need for an additional strong electron-withdrawing substituent for activation of the aromatic ring such as in the homoaromatic nucleophilic radiofluorinations. The preferable leaving groups are the trimethylammonium and the nitro functions.[69–71] An attempted synthesis of the *N*-Boc-protected trimethylammonium trifluoromethanesulfonate derivative **6** is outlined in Scheme 2. Methylation of **2** using reductive conditions (formaldehyde and cyanoborohydride in a mixture of acetonitrile and acetic acid at room temperature for 24 h) gave the corresponding 2′-dimethylamino derivative **4** in 86% yield. Treatment of **4** with 1 equiv of methyl trifluoromethanesulfonate in toluene at room temperature for 16 h only gave the non-desired 1′-methylpyridinium derivative **5** in 63% yield. The desired trimethylammonium derivative **6** could not be observed (^{1}H NMR analysis of the crude reaction mixture). Unfortunately, the nitro derivative (structure not shown) also could not be obtained using known conditions (hydrogen peroxide in concentrated sulfuric acid at low temperature)[72] and routinely used in our laboratory for the synthesis of 2-fluoropyridines.[11,69,70]

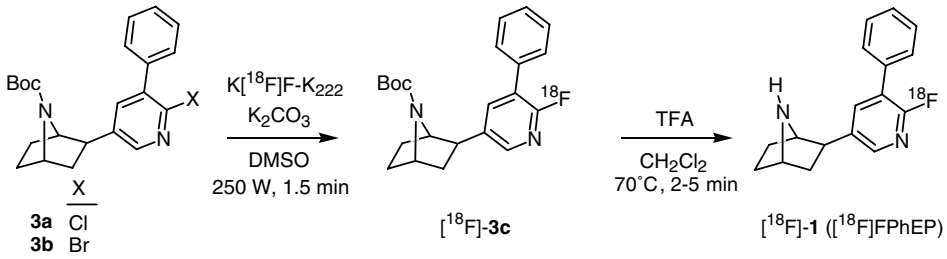
In view of these failures, we turned to the chloro- and bromo derivatives **3a**,**b** as precursors. Halogens are also known to be reasonably efficient leaving groups in this type of reaction.[69–71] Chlorination of **2** (with concomitant loss of *N*-Boc) with sodium nitrite in hydrochloric acid in the presence of copper(I) chloride at 0 °C for 40 min, followed by direct treatment with Boc_2O in THF containing triethylamine at room temperature for 5 h, gave the *N*-Boc-protected 2′-chloro derivative **3a** in 42% yield (Scheme 1). A similar reaction using



Scheme 1.



Scheme 2.



Scheme 3.

hydrobromic acid and copper(I) bromide gave the *N*-Boc-protected 2′-bromo derivative, **3b** in 39% yield.

2.2. Radiochemistry

The two-step radiosynthesis of [^{18}F]FPhEP ([^{18}F]-**1**) is outlined in Scheme 3. The chloro- and the bromo derivative (**3a** and **3b**, respectively) were both evaluated for their effectiveness as labelling precursors for the preparation of [^{18}F]FPhEP ([^{18}F]-**1**).

Introduction of the cyclotron-produced fluorine-18 as no-carrier-added naked [^{18}F]fluoride anion was performed in dimethylsulfoxide (DMSO) with the activated K[^{18}F]F–Kryptofix®222 complex[73,74] as the radiofluorinating reactant (Kryptofix®222 (K_{222}): 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) by either conventional heating or microwave activation. The radiochemical yields of fluorine-18 incorporation were calculated from the TLC-radiochromatogram and defined as the ratio of [^{18}F]-**3c** over total fluorine-18 activity. Briefly, a DMSO solution (600 μL) containing 1.0–4.0 mg of the chloro- (**3a**, 2.6–10.3 μmol) or bromo (**3b**, 2.3–9.3 μmol) precursor for labelling was added to 30–60 mCi of the dried K[^{18}F]F–K_{222} complex in a reaction vial (Vacutainer® tube). The unsealed tube was then placed in a heating block at 165 °C for 1–20 min without stirring the contents or in a dedicated microwave oven at 250 W for 30–90 s. Using conventional heating, both precursors (**3a**,**b**) were reactive with incorporation yields increasing with the reaction time up to 20 min. Using 4.0 mg of the chloro precursor (**3a**, 10.3 μmol), yields up to 15–20% were observed. Higher yields in much shorter synthesis time (60–90 s only) were systematically obtained with both precursors when microwave activation was used. In contrast to our previously reported model studies,[69–71] the incorporation yields for the bromo derivative **3b** were only slightly higher than those for the chloro derivative **3a**. Yields ranging from 25% to 40% were observed using 2 mg of the chloro derivative **3a** and microwave activation at 250 W for 90 s. Removal of the *N*-Boc-protective group was performed in 2–5 min in a mixture of trifluoroacetic acid (TFA) and dichloromethane (1:50 (v/v)) at room temperature, quantitatively yielding the amine [^{18}F]-**1** (according to radio-TLC). HPLC purification was performed on a semi-preparative Symmetry® C18 column, using a mixture of aq 0.05 M NaH_2PO_4 and CH_3CN (80:20 (v:v)) as the eluent (HPLC A, see Section 4). This stage was simpler when using the bromo derivative **3b** due to the better separation of [^{18}F]-**1** (t_R: 13.0 min) from the Boc-deprotected bromo derivative (t_R: 17.5 min) than from the corresponding chloro derivative **3a** (t_R: 16.5 min). Also, the use of a more restricted amount of the precursor for labelling (1 mg, for example, instead of 2–4 mg) facilitated the final HPLC purification, offeset however by a lower fluorine-18 incorporation.

Typically, 2.22–3.33 GBq of purified [^{18}F]FPhEP ([^{18}F]-**1**, specific radioactivity: 66–137 GBq/μmol) was obtained in 75–80 min using 1 mg of the bromo precursor **3b** and microwave activation for 90 s (250 W). The starting amount of [^{18}F]fluoride was 18.5 GBq implying a 12–18% nondecay-corrected overall yield.

Formulation of [^{18}F]FPhEP ([^{18}F]-**1**) as iv 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%. The radiotracer preparation was a clear and colourless solution with a measured pH between 5 and 7. As demonstrated by analytical HPLC analysis (HPLC B, See Section 4), the radiotracer preparation was found to be >95% chemically and >99% radiochemically pure and the preparation was shown to be free of non-radioactive precursor and was radiochemically stable for at least 120 min. Administration to animals was performed within 15 min following the end of the synthesis. These results were in compliance with our in-house quality control/assurance specifications.

2.3. Preliminary in vitro pharmacological evaluation

In vitro autoradiographic studies were performed on rat brain slices with [^{18}F]FPhEP ([^{18}F]-**1**) and showed that the highest fixation of radioactivity was present in thalamic nuclei and the lowest in the corpus callosum (Fig. 2, image A). These data are in accordance with the known distribution of nAChRs in the rat brain. Non-specific binding was assessed in adjacent slices in the presence of 300 μM of unlabelled FPhEP (**1**, Fig. 2, image B) or 300 μM of (−)-nicotine (Fig. 2, image C). The results suggest that the non-specific binding associated with this radiotracer is low (image B) and that the specific binding is represented by the fixation to the nAChRs (image C).

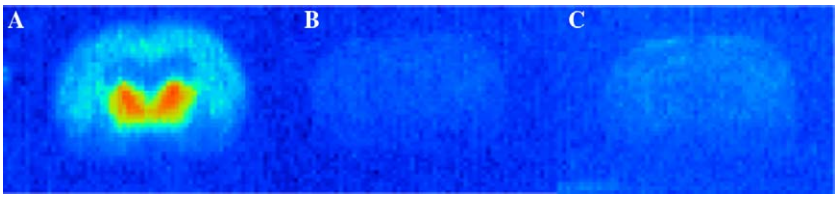


Figure 2. In vitro autoradiography of rat brain slices with [^{18}F]FPhEP ([^{18}F]-**1**). Binding of [^{18}F]-**1** alone (A), in the presence of added unlabelled FPhEP (B) and in the presence of (−)-nicotine (C).

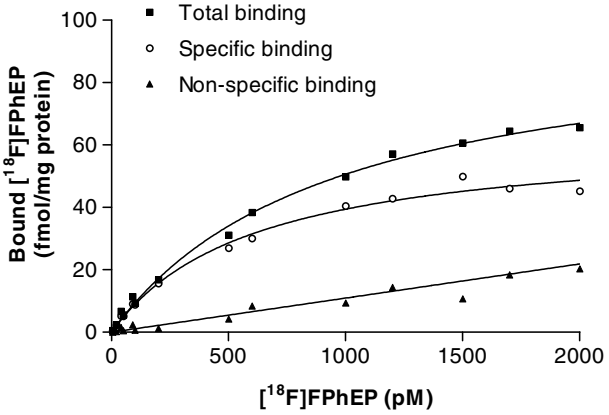


Figure 3. In vitro saturation studies of [^{18}F]FPhEP ([^{18}F]-**1**) on rat whole-brain membranes.

The binding of [^{18}F]FPhEP ([^{18}F]-**1**) was quantified in vitro at 37 °C on rat whole-brain membranes (Fig. 3). Saturation studies showed that the binding of [^{18}F]FPhEP ([^{18}F]-**1**) was saturable and represented a single population of binding sites with a B_{max} of 65 ± 2 fmole/mg of protein and an apparent dissociation constant (K_D) of 660 ± 75 pM (triplicate samples, means ± SD).

Finally, competition assays were performed with 2.5 nM of [^{18}F]-**1** and 14–16 different concentrations of selected competitors, to evaluate the K_i of several nAChR ligands and ligands of other receptors on the [^{18}F]FPhEP ([^{18}F]-**1**) binding. Selectivity of [^{18}F]FPhEP ([^{18}F]-**1**) for the heteromeric α4β2 nAChR subtype was confirmed by the low K_i values with cytisine and nicotine (α4β2-specific; 1.09 and 5.83 nM, respectively), whereas the K_i value of methyllycaconitine (α6/7-specific) was high (1.30 μM). Moreover, the K_i of several non-AChR ligands were all very high, indicating that [^{18}F]FPhEP does not bind to these types of receptors: D1 dopamine receptor (A-69024; >1 μM), D2/3 dopamine receptor (raclopride; >1 μM), 5-HT_2/5-HT_{1C} serotoninergic receptor (ketanserine; 643 nM), $5HT_{1A}$ serotonergic receptor (WAY-100135; 268 nM) and muscarinic acetylcholine receptor (3-quinuclidinyl benzilate; >1 μM). Taken together, all the data obtained clearly demonstrate that [^{18}F]FPhEP ([^{18}F]-**1**) binds to the non-α7 nAChR subtype. These results are also in accordance with the just recently reported binding values of this derivative.[68]

2.4. PET imaging

PET studies of the brain distribution of [^{18}F]FPhEP ([^{18}F]-**1**) were carried out in primates (*Papio anubis* baboon). Two selected single PET experiments in the same baboon are described here to illustrate this section and the full characterisation of [^{18}F]FPhEP as a PET radiotracer will be published elsewhere. Figure 4A illustrates the uptake of [^{18}F]FPhEP in the baboon brain at 30–60 min, following iv injection of 163 MBq of [^{18}F]FPhEP ([^{18}F]-**1**, 2.1 nmol). Figure 4B (-■-) shows the corresponding time activity curve obtained in the thalamus, known as a α4β2-nAChR-rich region. After injection of [^{18}F]-**1**, the time course of radioactivity showed a rapid accumulation of the radiotracer, reaching a maximal value of 4.86% of injected dose per 100 mL of tissue (% ID/100 mL), 20 min pi. Past 20 min, this value progressively decreased, reaching 1.7 % ID/100 mL at 180 min pi. Figure 4B (-□-) also shows the time activity curve obtained in the same brain region, following iv injection of 2-[^{18}F]F-A-85380 (430 MBq, i.e., 1.2 nmol). The time course of radioactivity showed a rather slow accumulation of the radiotracer, reaching a maximal value of 3.94 % ID/100mL at 80–90 min pi only. The decrease of the radioactivity in this brain region was very slow reaching 3.75% ID/100 mL at 180 min. Thus, [^{18}F]FPhEP ([^{18}F]-**1**)shows faster kinetics with a peak uptake at 20 min but a lower retention during the scanning time.

3. Conclusion

The novel $\alpha_4\beta_2$-selective, nicotinic cholinergic antagonist FPhEP (**1**) has been labelled with fluorine-18 in sufficient yields to allow in vitro and in vivo pharmacological evaluation. In vitro binding studies on rat whole-brain membranes demonstrated a subnanomolar affinity (K_D 660 pM) of [^{18}F]FPhEP ([^{18}F]-**1**) for nAChRs and in vitro autoradiographic studies showed a good contrast between nAChR-rich and -poor regions with a low non-specific binding. Comparison of in vivo PET kinetics of [^{18}F]FPhEP ([^{18}F]-**1**) and [^{18}F]F-A-85380 in a baboon demonstrated the faster brain kinetics of the former compound, with a peak uptake at 20 min post injection. Taken together, the preliminary data obtained confirm that [^{18}F]FPhEP ([^{18}F]-**1**) has potential for in vivo imaging nAChRs in the brain with PET.

4. Experimental

4.1. General

4.1.1. Chemicals, flash chromatographies and TLCs. Chemicals were purchased from Aldrich-, Fluka- or

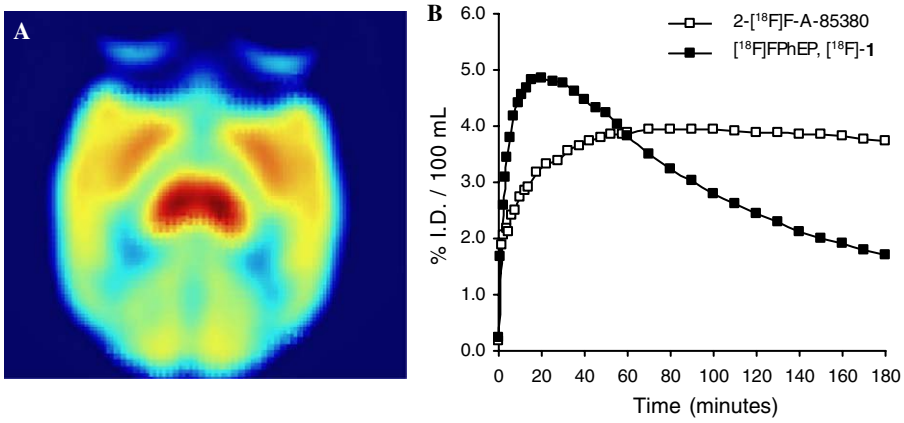


Figure 4. PET imaging of [^{18}F]FPhEP ([^{18}F]-**1**) in a *Papio anubis* baboon. (A) Activity distribution in a slice containing the thalamus at 30–60 min. (B) Time activity curves of [^{18}F]FPhEP and 2-[^{18}F]F-A-85380 (separate experiments).

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 analyser.

4.1.2. HPLCs. [HPLC A]: Equipment: system equipped with a Waters 600 Pump and Waters 600 Controller, a Shimadzu SPD10-AVP UV-multi-wavelength spectrophotometer and a miniature ionisation chamber probe; column: semipreparative, Symmetry® C18, Waters (300 × 7.8 mm); porosity: 7 μm; eluent aq 0.05 M NaH_2PO_4/CH_3CN, 80:20 (v/v); flow rate: 5 mL/min; temperature: RT; absorbance detection at λ = 254 nm. [HPLC B]: Equipment: Waters binary HPLC pump 1525 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 2996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (50 × 4.6 mm); porosity: 3.5 μm; conditions: isocratic elution with solvent A/solvent B: 60:40 (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%) and water (65–75%), pH 3, Waters), 20 mL for 1000 mL; solvent B: H_2O/CH_3CN: 50:50 (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 λ = 280 nm.

4.1.3. Spectroscopies. NMR spectra were recorded on a Bruker AMX (300 MHz) or a Bruker Avance (400 MHz) apparatus, using the hydrogenated residue of the deuterated solvents (CD_2Cl_2, δ = 5.32 ppm; DMSO-d_6, δ = 2.50 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvents (CD_2Cl_2, δ = 53.8 ppm; DMSO-d_6, δ = 39.5 ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, dd, and br for singlet, doublet, triplet, doublet of doublet and broad, respectively). The mass spectra (MS), DCI/NH_4^+, were measured on a Quantum TCQ Discovery spectrometer.

4.1.4. 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 a CGR-MeV 520 cyclotron (17 MeV proton beam) or on an IBA Cyclone-18/9 cyclotron (18 MeV proton beam) and was transferred to the appropriate hot cell.

Target hardware: CGR-MeV 520 cyclotron: home-made, large volume, three-port, keyhole-shaped stainless steel/silver target holder (A complete description of this target hardware and -operation can be found in Ref. 55); IBA Cyclone-18/9 cyclotron: commercial, large volume, 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 at the end of bombardment for a 20 μA, 30 min (10 μA.h) irradiation: CGR-MeV 520 cyclotron: 20.3–24.0 GBq and IBA Cyclone-18/9 cyclotron: 27.7–29.6 GBq.

4.1.5. 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). Microwave activation was performed with a MicroWell 10 oven (2.45 GHz), Labwell AB, Sweden.

4.2. Chemistry

4.2.1. (+/−)-7-*tert*-Butoxycarbonyl-2-*exo*-(2′-amino-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (2). Synthesized according to already reported procedures[66,67] and obtained in seven steps and 20% overall yield from commercially available *N*-Boc-pyrrole. R_f: 0.4 (CH_2Cl_2/MeOH, 95:05 the latter containing 0.5% of aq 28%

ammonia). ^{1}H NMR: (CD_2Cl_2, 298 K): δ: 7.88 (d, J: 1.8 Hz, 1H); 7.47–7.36 (br, 5H); 7.35 (d, J: 1.8 Hz, 1H); 4.68 (br, 2H); 4.29 (br s, 1H); 4.12 (br s, 1H); 2.79 (dd, J: 9.2 & 4.9 Hz, 1H); 1.93 (dd, J: 12.2 & 9.2 Hz, 1H); 1.85–1.38 (br, 5H); 1.36 (s, 9H). ^{13}C NMR: (CD_2Cl_2, 298 K): 155.2 [C]; 154.9 [C]; 145.8 [CH]; 138.8 [C]; 137.1 [CH]; 132.2 [C]; 129.3 [2 CH]; 129.0 [2 CH]; 127.9 [CH]; 121.9 [C]; 79.5 [C]; 62.7 [CH]; 56.2 [CH]; 45.2 [CH]; 40.5 [CH_2]; 30.0 [CH_2]; 29.0 [CH_2]; 28.4 [3 CH_3]. MS $C_{22}H_{27}N_3O_2$: 366 [M+H$^+$].

4.2.2. (+/−)-2-*exo*-(2′-Fluoro-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (1, FPhEP). A solution of **2** (183 mg, 0.5 mmol, MW: 365.47) in concentrated 70% hydrofluoric acid/pyridine (1.2 mL) was prepared. Sodium nitrite (138 mg, 2 mmol, 4 equiv, MW: 69.00) in water (0.5 mL) was added at 0 °C and the reaction mixture was stirred for 45 min at room temperature followed by heating at 100 °C for 1 h. The mixture was filtered and the filtrate was poured into 50 mL of aq 14% ammonia and extracted with ethyl acetate. The combined organic extracts were dried with magnesium sulfate and concentrated. The residue was chromatographed on silica gel using CH_2Cl_2/MeOH, the latter containing 0.5% of aq 28% ammonia (98:02–95:05), as eluent to provide **1** (110 mg, 82%) as a yellow oil. R_f: 0.3 (CH_2Cl_2/MeOH, 95:05, the latter containing 0.5% of aq 28% ammonia). t_R (HPLC A): 13.0 min. t_R (HPLC B): 2.28 min. ^{1}H NMR: (CD_2Cl_2, 298 K): δ: 8.07 (d, J: 1.8 Hz, 1H); 8.01 (dd, J: 9.8 & 2.4 Hz, 1H); 7.60–7.50 (br, 2H); 7.50–7.36 (br, 3H); 3.79 (br t, 1H); 3.60 (br s, 1H); 2.85 (dd, J: 9.2 & 4.9 Hz, 1H); 1.90 (dd, J: 12.2 & 9.2 Hz, 1H); 1.69–1.50 (br, 5H). ^{13}C NMR: (CD_2Cl_2, 298 K): 159.4 [d, J: 236 Hz, C]; 145.2 [d, J: 15 Hz, CH]; 141.5 [d, J: 5 Hz, C]; 140.4 [CH]; 134.9 [C]; 129.1 [2 CH]; 128.9 [2 CH]; 128.5 [CH]; 123.2 [d, J: 29 Hz, C]; 63.4 [CH]; 56.8 [CH]; 44.8 [CH]; 40.9 [CH_2]; 31.8 [CH_2]; 30.7 [CH_2]. MS $C_{17}H_{17}FN_2$: 269 [M+H$^+$].

4.2.3. (+/−)-7-*tert*-Butoxycarbonyl-2-*exo*-(2′-chloro-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1] heptane (3a). To a solution of **2** (597 mg, 1.6 mmol, MW: 365.47) in concentrated hydrochloric acid (4 mL) at 0 °C was added sodium nitrite (2.25 g, 32 mmol, 20 equiv, MW: 69.00) in water (2 mL). After 10 min, copper(I) chloride (2.26 g, 22.9 mmol, 14 equiv, MW: 98.99) in hydrochloric acid (2 mL) was added. After 30 min at 0 °C, the mixture was poured into aq 14% ammonia and extracted three times with ethyl acetate. The combined organic layers were dried with magnesium sulfate and concentrated. The residue was treated with di-*tert*-butyldicarbonate (428 mg, 1.96 mmol, 1.2 equiv, MW: 218.25) and NEt_3 (0.27 mL, 1.96 mmol, 1.2 equiv, d: 0.726, MW: 101.19) in THF (4 mL). After 5 h, the solvent was evaporated and the oily residue was chromatographed on silica gel using heptane/EtOAc (90:10–80:20) as eluent to provide **3a** (260 mg, 42%) as a pale yellow solid. R_f: 0.7 (heptane/EtOAc, 50:50). ^{1}H NMR: (CD_2Cl_2, 298 K): δ: 8.26 (d, J: 2.4 Hz, 1H); 7.66 (d, J: 2.4 Hz, 1H); 7.48–7.38 (br, 5H); 4.33 (br t, 1H); 4.21 (br d, 1H); 2.93 (dd, J: 9.2 & 4.9 Hz, 1H); 2.01 (dd, J: 12.2 & 8.5 Hz, 1H); 1.90–1.46 (br, 5H); 1.37 (s, 9H). ^{13}C NMR: (CD_2Cl_2, 298 K): 155.1 [C]; 147.7 [CH]; 147.5 [C]; 141.2 [C]; 138.7 [CH]; 138.2 [C]; 136.7 [C]; 129.7 [2 CH]; 128.6 [2 CH]; 128.5 [CH]; 79.7 [C]; 62.4 [CH]; 56.4 [CH]; 45.2 [CH]; 40.6 [CH_2]; 30.0 [CH_2]; 29.1 [CH_2]; 28.4 [3 CH_3]. MS $C_{22}H_{25}ClN_2O_2$: 387 [M+H$^+$]; 385 [M+H$^+$].

4.2.4. (+/−)-7-*tert*-Butoxycarbonyl-2-*exo*-(2′-bromo-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (3b). To a solution of **2** (130 mg, 0.36 mmol, MW: 365.47) in concentrated hydrobromic acid (2 mL) at 0 °C was added sodium nitrite (490 mg, 7.1 mmol, 20 equiv, MW: 69.00) in water (1 mL). After 10 min copper(I) bromide (492 mg, 5 mmol, 14 equiv, MW: 98.99) in hydrobromic acid (1 mL) was added. After 30 min at 0 °C, the mixture was poured into aq 14% ammonia and extracted three times with ethyl acetate. The combined organic layers were dried with magnesium sulfate and concentrated. The residue was treated with di-*tert*-butyldicarbonate (93 mg, 0.43 mmol, 1.2 equiv, MW: 218.25) and NEt_3 (60 μL, 0.43 mmol, 1.2 equiv, d: 0.726, MW: 101.19) in THF (2 mL). After 5 h, the solvent was evaporated and the oily residue was chromatographed on silica gel using heptane/EtOAc (90:10–80:20) as eluent to provide **3b** (60 mg, 39%) as a brown oil. R_f: 0.7 (heptane/EtOAc, 50:50). ^{1}H NMR: (CD_2Cl_2, 298 K): δ: 8.23 (d, J: 2.4 Hz, 1H); 7.60 (d, J: 2.4 Hz, 1H); 7.46–7.40 (br, 5H); 4.33 (br t, 1H); 4.20 (br s, 1H); 2.91 (dd, J: 8.5 & 4.8 Hz, 1H); 2.01 (dd, J: 12.2 & 9.1 Hz, 1H); 1.90–1.49 (br, 5H); 1.36 (s, 9H). ^{13}C NMR: (CD_2Cl_2, 298 K): δ: 155.1 [C]; 148.1 [CH]; 141.4 [C]; 139.9 [C]; 139.6 [C]; 138.2 [CH]; 129.7 [C]; 128.6 [2 CH]; 128.6 [2 CH]; 128.6 [CH]; 79.8 [C]; 62.3 [CH]; 56.3 [CH]; 45.2 [CH]; 40.6 [CH_2]; 30.0 [CH_2]; 29.1 [CH_2]; 28.3 [3 CH_3]. MS $C_{22}H_{25}BrN_2O_2$: 429 [M+H$^+$]; 431 [M+H$^+$].

4.2.5. (+/−)-7-*tert*-Butoxycarbonyl-2-*exo*-(2′-dimethylamino-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (4). A mixture of **2** (297 mg, 0.81 mmol, MW: 365.47), acetonitrile (22 mL), 37% aq formaldehyde (2.9 mL) and $NaBH_3CN$ (918 mg, 14.6 mmol, 18 equiv, MW: 62.84) was stirred for 3 h at room temperature. Glacial acetic acid was added and stirring was continued. After 24 h, the reaction mixture was decanted into aq 14% ammonia and extracted three times with chloroform. The combined organic extracts were dried with magnesium sulfate and concentrated. The residue was chromatographed on silica gel using heptane/EtOAc (60:40–30:70) as eluent to provide **4** (275 mg, 86%) as a colourless oil. R_f: 0.6 (heptane/EtOAc, 50:50). ^{1}H NMR: (CD_2Cl_2, 298 K): δ: 8.09 (d, J: 2.4 Hz, 1H); 7.53–7.28 (br, 6H); 4.37 (br s, 1H); 4.20 (br s, 1H); 2.85 (dd, J: 8.5 & 4.8 Hz, 1H); 2.73 (s, 6H); 1.97 (dd, J: 12.8 & 9.2 Hz, 1H); 1.90–1.51 (br, 5H); 1.41 (s, 9H). ^{13}C NMR: (CD_2Cl_2, 298 K): 158.8 [C]; 155.3 [C]; 144.3 [CH]; 141.2 [C]; 139.1 [CH]; 129.4 [C]; 129.3 [C]; 128.8 [2 CH]; 128.4 [2 CH]; 127.2 [CH]; 79.5 [C]; 62.5 [CH]; 56.3 [CH]; 45.1 [CH]; 41.6 [2 CH_3]; 40.4 [CH_2]; 30.0 [CH_2]; 29.4 [CH_2]; 28.3 [3 CH_3]. MS $C_{24}H_{31}N_3O_2$: 394 [M+H$^+$].

4.2.6. (+/−)-5′-(7-*tert*-Butoxycarbonyl-7-azabicyclo[2.2.1]-hept-2-*exo*-yl)-2′-dimethylamino-1′-methyl-3′-phenylpyridinium trifluoromethanesulfonate (5). To **4** (76 mg, 0.19 mmol, MW: 393.52) in toluene (1 mL) was added

methyl trifluoromethanesulfonate (24 μL, 0.21 mmol, 1.1 equiv, MW: 164.10, d: 1.450). The mixture was stirred at room temperature during 16 h and then concentrated to dryness. ^{1}H NMR analysis clearly showed the presence of **5** as the major component (>85%). The residue was chromatographed on silica gel using CH_2Cl_2/MeOH, the latter containing 0.5% of aq 28% ammonia (98:02–95:05), as eluent to provide **5** (64 mg) as a colourless oil. ^{1}H NMR: (CD_2Cl_2, 298 K): δ: 8.52 (s, 1H); 8.09 (s, 1H); 7.52 (br, 3H); 7.28 (br, 2H); 4.36 (br t, 1H); 4.24 (br s, 1H); 4.23 (s, 3H); 3.14 (dd, *J*: 8.5 & 4.8 Hz, 1H); 2.74 (s, 6H); 2.10 (dd, *J*: 12.2 & 8.5 Hz, 1H); 1.92–1.50 (br, 5H); 1.39 (s, 9H).

4.3. Radiochemistry

4.3.1. 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 from the target holder was passed through an anion exchange resin Sep-pak® Light Waters Accell™ Plus QMA cartridge (initially in the chloride form, then washed with aq 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 the last traces of [^{18}O]water. See Ref. 55 for more practical details. The [^{18}F]fluoride ion was then eluted from the resin, using an aq K_2CO_3 solution (1.0 mL of a 4.5 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.

4.4. Preparation of (+/−)-2-*exo*-(2′-[^{18}F]Fluoro-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1]heptane ([^{18}F]-1, [^{18}F]FPhEP)

4.4.1. Incorporation studies with K[^{18}F]F–K_{222}-complex. *(A) General procedure using conventional heating.* DMSO (600 μL) containing the appropriate halogeno derivative **3a**,**b** as precursor for labelling (1.0–4.0 mg) was directly added into the Vacutainer® tube containing the dried K[^{18}F]F–K_{222} complex. The tube (not sealed) was thoroughly vortexed (15 s) and then placed in a heating block (at 165 °C, for 1–20 min) without stirring the contents. The reaction vessel was then cooled using an ice-water bath and the remaining radioactivity was measured, which was 90–95% of the initial radioactivity. The resulting, often dark-coloured 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 the presumed 2-[^{18}F]fluoropyridine [^{18}F]-**3c** over total fluorine-18 radioactivity area (SiO_2-TLC: eluent: EtOAc/heptane, 50:50, R_f: [^{18}F]-**3c**: 0.7 and R_f: [^{18}F]fluoride ion: 0.0).

(B) General procedure using microwave activation. Same as part A, but instead of the heating block a dedicated microwave oven (at 250 W, for 0.5–1.5 min) was used.

4.4.2. Optimized conditions using (+/−)-7-*tert*-butoxycarbonyl-2-*exo*-(2′-bromo-3′-phenylpyridin-5′-yl)-7-azabicyclo[2.2.1]heptane (3b) as precursor for labelling. DMSO (600 μL) containing the bromo derivative **3b** (1.0 mg, 2.3 μmoles) as precursor for labelling was directly added into the Vacutainer® tube containing the dried K[^{18}F]F–K_{222} complex. The tube (not sealed) was thoroughly vortexed (15 s) and then placed in a dedicated microwave oven (at 250 W, for 1.5 min) without stirring the contents. The resulting, dark-coloured reaction mixture was diluted with water (1 mL) and transferred onto a C18 Sep-Pak cartridge (PrepSep™ R-C18, Fisher Scientific, activated beforehand with EtOH (2 mL) and then rinsed with water (10 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 (3–5% of the total radioactivity amount engaged in the fluorination process was lost in the initial tube). The whole 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]-**3c** was eluted from the cartridge with CH_2Cl_2 (3 mL) into a 5 mL reaction vial containing TFA (0.1 mL). Twice 1 mL of CH_2Cl_2 was used to wash the cartridge for maximal transfer of [^{18}F]-**3c** (5–10% of the total radioactivity amount engaged in the fluorination process was left on the cartridge). The incorporation yield was also estimated after the C18 Sep-pak cartridge elution by the CH_2Cl_2-over total eluted radioactivity (DMSO/H_2O + CH_2Cl_2) ratio. The resulting CH_2Cl_2/TFA solution (50:1, v/v) was concentrated to dryness (at 65–75 °C under a gentle nitrogen stream for 4–6 min). The reaction yield (*N*-Boc deprotection) was calculated from the TLC-radiochromatogram (SiO_2-TLC, (A) eluent: EtOAc/heptane, 50:50, R_f: [^{18}F]-**3c**: 0.7 and R_f: [^{18}F]-**1**: 0.0 ; (B) eluent: CH_2Cl_2/MeOH, 95:05, the latter containing 0.5% of aq 28% ammonia, R_f: [^{18}F]-**3c**: 0.9 and R_f: [^{18}F]-**1**: 0.3). The residue was redissolved in CH_2Cl_2 (2 mL) and concentrated again to dryness to minimize TFA presence (at 65–75 °C under a gentle nitrogen stream for 2–3 min). Finally, the above 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 the unlabelled *N*-Boc-deprotected precursor for labelling (BrPhEP: t_R: 17.5 min).

4.4.3. Formulation. 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 of EtOH and then rinsed with 10 mL of water prior to use). The cartridge was washed with 10 mL of water 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 lower the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[75]

4.4.4. Quality control. 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 B), with a sample of authentic **1** (t_R: 2.28 min). Particular attention was paid to the absence of non-radioactive (*N*-Boc-deprotected)-precursors (ClPhEP, t_R: 2.77 min; BrPhEP, t_R: 3.03 min). Chemical and radiochemical stability of the entire preparation was tested by HPLC (HPLC B) at regular 15-min intervals during 120 min. Specific radioactivity of the radiotracer was calculated from three consecutive HPLC (HPLC B) 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. Administration to animals was performed within 15 min following the end of the synthesis.

4.5. In vitro pharmacological evaluation and PET imaging

4.5.1. In vitro autoradiography of rat brain slices with [^{18}F]FPhEP ([^{18}F]-1). Coronal slices (20 μm thick, at bregma—3.30 mm) from frozen brains of Sprague–Dawley rats were thaw-mounted onto gelatin-coated slides. Slices were incubated with 1.2 nM of [^{18}F]FPhEP ([^{18}F]-**1**) for 2 h at 25 °C in 50 mM Tris–HCl buffer (pH 7.0) containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$ and 1 mM $MgCl_2$. Adjacent slices were incubated with 1.2 nM of [^{18}F]FPhEP ([^{18}F]-**1**) in the same buffer together with 300 μM of unlabelled FPhEP (**1**) or with 300 μM of (−)-nicotine in order to evaluate the non-specific binding. Slices were apposed to 3H-Ultrafilm for 2 days at room temperature and the resulting autoradiograms were digitized using a video-camera-based system. The digitized images were analyzed using the Image-Quant® software.

4.5.2. In vitro saturation studies of [^{18}F]FPhEP ([^{18}F]-1) on rat whole-brain membranes. Male Sprague–Dawley rat brain membranes (P2 fraction, 125 μg of protein) were incubated with 0.05–2 nM of [^{18}F]FPhEP ([^{18}F]-**1**) for 2 h at 37 °C in a total volume of 4 mL of 50 mM Tris–HCl buffer (pH 7.0) containing 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$ and 1 mM $MgCl_2$. Non-specific binding was determined in the presence of 300 μM of (−)-nicotine. The binding constants K_D and B_{max} were calculated by non-linear curve fitting using the GraphPad Prism® software. Competition studies used the same experimental procedures (buffer, temperature).

4.5.3. PET imaging of [^{18}F]FPhEP ([^{18}F]-1) in a *Papio anubis* baboon. An adult baboon (14 kg, anaesthetized with 1% isoflurane and 33%/66% O_2/N_2O) was iv injected with 163 MBq of [^{18}F]FPhEP ([^{18}F]-**1**, 2.1 nmol) or 430 MBq of 2-[^{18}F]F-A-85380 (1.2 nmol) and imaged for 180 min on an HR+ Exact Positron Tomograph (CTI PET Systems, Knoxville, TN, USA). For data analysis, an MRI-based region of interest (ROI) was placed on the thalamus. The radioactivity measured in this ROI was corrected for attenuation and fluorine-18-decay and expressed as percent of injected dose per 100 mL of tissue (% ID/100 mL).

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References and notes

1. Karlin, A. *Nat. Rev. Neurosci.* **2002**, *3*, 102.
2. Valk, P. E.; Bailey, D. L.; Townsend, D. W.; Maisey, M. N. In *Positron Emission Tomography—Basic Science and Clinical Practice*; Valk, P. E., Bailey, D. L., Townsend, D. W., Maisey, M. N., Eds.; Springer: London—Berlin—Heidelberg—New York—Hong-Kong—Milan—Paris—Tokyo, 2003; pp 1–884.
3. Kilbourn, M. R.; Welch, M. J. *Appl. Rad. Isot.* **1986**, *37*, 677.
4. Kilbourn, M. R. In *Fluorine-18 Labeling of Radiopharmaceuticals, Nuclear Science Series*; National Academy: Washington, DC, 1990.
5. Lasne, M.-C; Perrio, C.; Rouden, J.; Barré, L.; Roeda, D.; Dollé, F.; Crouzel, C.. In *Chemistry of beta+-emitting Compounds based on Fluorine-18, Topics in Current Chemistry*; Krause, W., Ed.; Springer: Berlin, Heidelberg, 2002; Vol. 222, pp 201–258.
6. Welch, M. J.; Redvanly, C. S. In *Handbook of Radiopharmaceuticals—Radiochemistry and Applications*; Welch, M. J., Redvanly, C. S., Eds.; Wiley-Interscience: New York—Chichester—Brisbane—Toronto, 2003; pp 1–848.
7. Abreo, M. A.; Lin, N.-H.; Garvey, D. S.; Gunn, D. E.; Hettinger, A.-M.; Wasicak, J. T.; Pavlik, P. A.; Martin, Y. C.; Donnelly-Roberts, D. L.; Anderson, D. J.; Sullivan, J. P.; Williams, M.; Arneric, S. P.; Holladay, M. W. *J. Med. Chem.* **1996**, *39*, 817.
8. Sullivan, J. P.; Donnelly-Roberts, D. L.; Briggs, C. A.; Anderson, D. J.; Gopalakrishnan, M.; Piattoni-Kaplan, M.; Campbell, J. E.; McKenna, D. G.; Molinary, E.; Hettinger, A.-M.; Garvey, D. S.; Wasicak, J. T.; Holladay, M. W.; Williams, M.; Arneric, S. P. *Neuropharmacology* **1996**, *35*, 725.
9. Holladay, M. W.; Wasicak, J. T.; Lin, N.-H.; He, Y.; Ryther, K. B.; Bannon, A. W.; Buckley, M. J.; Kim, D. J. B.; Decker, M. W.; Anderson, D. J.; Campbell, J. E.; Kuntzweiller, T. A.; Donnelly-Roberts, D. L.; Piattoni-Kaplan, M.; Briggs, C. A.; Williams, M.; Arneric, S. P. *J. Med. Chem.* **1998**, *41*, 407.
10. Koren, A. O.; Horti, A. G.; Mukhin, A. G.; Gündisch, D.; Kimes, A. S.; Dannals, R. F.; London, E. D. *J. Med. Chem.* **1998**, *41*, 3690.
11. Dollé, F.; Valette, H.; Bottlaender, M.; Hinnen, F.; Vaufrey, F.; Guenther, I.; Crouzel, C. *J. Labelled Compd. Radiopharm.* **1998**, *41*, 451.
12. Horti, A. G.; Scheffel, U.; Koren, A. O.; Ravert, H. T.; Mathews, W. B.; Musachio, J. L.; Finley, P. A.; London, E. D.; Dannals, R. F. *Nucl. Med. Biol.* **1998**, *25*, 599.
13. Horti, A.; Koren, A. O.; Ravert, H. T.; Musachio, J. L.; Mathews, W. B.; London, E. D.; Dannals, R. F. *J. Labelled Compd. Radiopharm.* **1998**, *41*, 309.
14. Dollé, F.; Dolci, L.; Valette, H.; Hinnen, F.; Vaufrey, F.; Guenther, I.; Fuseau, C.; Coulon, C.; Bottlaender, M.; Crouzel, C. *J. Med. Chem.* **1999**, *42*, 2251.

15. Valette, H.; Bottlaender, M.; Dollé, F.; Guenther, I.; Fuseau, C.; Coulon, C.; Ottaviani, M.; Crouzel, C. *J. Nucl. Med.* **1999**, *40*, 1374.
16. Valette, H.; Bottlaender, M.; Dollé, F.; Guenther, I.; Coulon, C.; Hinnen, F.; Fuseau, C.; Ottaviani, M.; Crouzel, C. *Life Sci.* **1999**, *64*, PL93.
17. Chefer, S. I.; Horti, A. G.; Koren, A. O.; Gündisch, D.; Links, J. M.; Kurian, V.; Dannals, R. F.; Mukhin, A. G.; London, E. D. *NeuroReport* **1999**, *10*, 2715.
18. Liu, N.; Ding, Y.-S.; Wang, T.; Garza, V.; Fowler, J. S. *Nucl. Sci. Tech.* **2002**, *13*, 92.
19. Valette, H.; Dollé, F.; Bottlaender, M.; Marzin, D. *Nucl. Med. Biol.* **2002**, *29*, 849.
20. Bottlaender, M.; Valette, H.; Roumenov, D.; Dollé, F.; Coulon, C.; Ottaviani, M.; Hinnen, F.; Ricard, M. *J. Nucl. Med.* **2003**, *44*, 596.
21. Valette, H.; Bottlaender, M.; Dollé, F.; Coulon, C.; Ottaviani, M.; Syrota, A. *J. Neurochem.* **2003**, *84*, 105.
22. Kimes, A. S.; Horti, A. G.; London, E. D.; Chefer, S. I.; Contoreggi, C.; Ernst, M.; Friello, P.; Koren, A. O.; Kurian, V.; Matochik, J. A.; Pavlova, O.; Vaupel, D. B.; Mukhin, A. G. *FASEB* **2003**, *17*, 1331.
23. Chefer, S. I.; London, E. D.; Koren, A. O.; Pavlova, O. A.; Kurian, V.; Kimes, A. S.; Horti, A. G.; Mukhin, A. G. *Synapse* **2003**, *48*, 25.
24. Schmaljohann, J.; Minnerop, M.; Karwath, P.; Gündisch, D.; Falkai, P.; Guhlke, S.; Wullner, U. *Appl. Rad. Isot.* **2004**, *61*, 1235.
25. Schmaljohann, J.; Gündisch, D.; Minnerop, M.; Joe, A.; Bucerius, J.; Dittmar, C.; Jessen, F.; Guhlke, S.; Wullner, U. *Appl. Rad. Isot.* **2005**, *63*, 433.
26. Vaupel, D. B.; Tella, S. R.; Huso, D. L.; Wagner, V. O.; Mukhin, A. G.; Chefer, S. I.; Horti, A. G.; London, E. D.; Koren, A. O.; Kimes, A. S. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 355.
27. Mitkovski, S.; Villemagne, V. L.; Novakovic, K. E.; O'Keefe, G.; Tochon-Danguy, H.; Mulligan, R. S.; Dickinson, K. L.; Saunder, T.; Grégoire, M.-C.; Bottlaender, M.; Dollé, F.; Rowe, C. C. *Nucl. Med. Biol.* **2005**, *32*, 585.
28. Valette, H.; Bottlaender, M.; Dollé, F.; Coulon, C.; Ottaviani, M.; Syrota, A. *Synapse* **2005**, *56*, 217.
29. Gallezot, J.-D.; Bottlaender, M.; Grégoire, M.-C.; Roumenov, D.; Deverre, J.-R.; Coulon, C.; Ottaviani, M.; Dollé, F.; Syrota, A.; Valette, H. *J. Nucl. Med.* **2005**, *46*, 240.
30. Ding, Y.-S.; Liu, N.; Wang, T.; Marecek, J.; Garza, V.; Ojima, I.; Fowler, J. S. *Nucl. Med. Biol.* **2000**, *27*, 381.
31. Horti, A. G.; Chefer, S. I.; Mukhin, A. G.; Koren, A. O.; Gündisch, D.; Links, J. M.; Kurian, V.; Dannals, R. F.; London, E. D. *Life Sci.* **2000**, *67*, 463.
32. Koren, A. O.; Horti, A. G.; Mukhin, A. G.; Gündisch, D.; Dannals, R. F.; London, E. D. *J. Labelled Compd. Radiopharm.* **2000**, *43*, 413.
33. Scheffel, U.; Horti, A. G.; Koren, A. O.; Ravert, H. T.; Banta, J. P.; Finley, P. A.; London, E. D.; Dannals, R. F. *Nucl. Med. Biol.* **2000**, *27*, 51.
34. Ding, Y.-S.; Fowler, J. S.; Logan, J.; Wang, G.-J.; Telang, F.; Garza, V.; Biegon, A.; Pareto, D.; Rooney, W.; Shea, C.; Alexoff, D.; Volkow, N. D.; Vocci, F. *Synapse* **2004**, *53*, 184.
35. Gündisch, D.; Koren, A. O.; Horti, A. G.; Pavlova, O. A.; Kimes, A. S.; Mukhin, A. G.; London, E. D. *Synapse* **2005**, *55*, 89.
36. Koren, A. O.; Schefer, S. I.; Mukhin, A. G.; Pavlova, O. A.; Horti, A. G.; Vaupel, D. B.; London, E. D.; Kimes, A. S. *J. Labelled Compd. Radiopharm.* **2001**, *44*, S257.
37. Zhang, Y.; Pavlova, O. A.; Chefer, S. I.; Hall, A. W.; Kurian, V.; Brown, L. L.; Kimes, A. S.; Mukhin, A. G.; Horti, A. G. *J. Med. Chem.* **2004**, *47*, 2453.
38. Zhang, Y.; Horti, A. G. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 947.
39. Zhang, Y.; Hall, A. W.; Horti, A. G. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 385.
40. Chattopadhyay, S.; Xue, B.; Collins, D.; Pichika, R.; Bagnera, R.; Leslie, F. M.; Christian, B. T.; Shi, B.; Narayanan, T. K.; Potkin, S. G.; Mukherjee, J. *J. Nucl. Med.* **2005**, *46*, 130.
41. Dumont, F.; Sultana, A.; Balter, A.; Waterhouse, R. N. *J. Labelled Compd. Radiopharm.* **2003**, *46*, 1261.
42. Marrière, E.; Rouden, J.; Tadino, V.; Lasne, M.-C. *Org. Lett.* **2000**, *2*, 1121.
43. Bisson, W.; Mu, L.; Rouden, J.; Buck, A.; Schubiger, B. A.; Westera, G. 32nd Annual Meeting of the Society of Neuroscience, November 2–7, 2002, Orlando, USA.
44. Roger, G.; Lagnel, B.; Rouden, J.; Besret, L.; Valette, H.; Demphel, S.; Gopisetti, J.; Coulon, C.; Ottaviani, M.; Wrenn, L. A.; Letchworth, S. R.; Bohme, G. A.; Benavides, J.; Lasne, M.-C.; Bottlaender, M.; Dollé, F. *Bioorg. Med. Chem.* **2003**, *11*, 5333.
45. Hall, M.; Zerbz, L.; Leonard, S.; Freedman, R. *Brain Res.* **1993**, *600*, 127.
46. Schmeller, T.; Sauerwein, M.; Sporer, F.; Wink, M.; Müller, W. E. *J. Nat. Prod.* **1994**, *57*, 1316.
47. Anderson, D. J.; Arneric, S. P. *Eur. J. Pharmacol.* **1994**, *253*, 261.
48. Glennon, R. A.; Dukat, M. *Med. Chem. Res.* **1996**, 465.
49. Spande, T. F.; Garaffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 3475.
50. Qian, C.; Li, T.; Shen, T. Y.; Libertine-Garahan, L.; Eckman, J.; Biftu, T.; Ip, S. *Eur. J. Pharmacol.* **1993**, *250*, R13.
51. Badio, B.; Daly, J. W. *Mol. Pharmacol.* **1994**, *45*, 563–569.
52. Horti, A.; Ravert, H. T.; London, E. D.; Dannals, R. F. *J. Labelled Compd. Radiopharm.* **1996**, *38*, 355.
53. Liang, F.; Navarro, H. A.; Abraham, P.; Kotian, P.; Ding, Y.-S.; Fowler, J.; Volkow, N.; Kuhar, M. J.; Carroll, F. I. *J. Med. Chem.* **1997**, *40*, 2293.
54. Ding, Y.-S.; Liang, F.; Fowler, J. S.; Kuhar, M. J.; Carroll, F. I. *J. Labelled Compd. Radiopharm.* **1997**, *39*, 827.
55. Dolci, L.; Dollé, F.; Valette, H.; Vaufrey, F.; Fuseau, C.; Bottlaender, M.; Crouzel, C. *Bioorg. Med. Chem.* **1999**, *7*, 467.
56. Ding, Y.-S.; Gatley, S. J.; Fowler, J. S.; Volkow, N. D.; Aggarwal, D.; Logan, J.; Dewey, S. L.; Liang, F.; Carroll, F. I.; Kuhar, M. J. *Synapse* **1996**, *24*, 403.
57. Horti, A.; Scheffel, U.; Stathis, M.; Finley, P.; Ravert, H. T.; London, E. D.; Dannals, R. F. *J. Nucl. Med.* **1997**, *38*, 1260.
58. Villemagne, V. L.; Horti, A.; Scheffel, U.; Ravert, H. T.; Finley, P.; Clough, D. J.; London, E. D.; Wagner, H. N.; Dannals, R. F. *J. Nucl. Med.* **1997**, *38*, 1737.
59. Gatley, J. S.; Ding, Y.-S.; Brady, D.; Gifford, A. N.; Dewey, S. L.; Carroll, F. I.; Fowler, J. S.; Volkow, N. D. *Nucl. Med. Biol.* **1998**, *25*, 449.
60. Ding, Y. S.; Molina, P. E.; Fowler, J. S.; Logan, J.; Volkow, N. D.; Kuhar, M. J.; Carroll, F. I. *Nucl. Med. Biol.* **1999**, *26*, 139.
61. Ding, Y.-S.; Logan, J.; Bermel, R.; Garza, V.; Rice, O.; Fowler, J. S.; Volkow, N. D. *J. Neurochem.* **2000**, *74*, 1514.
62. Molina, E. P.; Ding, Y.-S.; Carroll, F. I.; Liang, F.; Volkow, N. D.; Pappas, N.; Kuhar, M.; Abumrad, N.; Gatley, J. S.; Fowler, J. S. *Nucl. Med. Biol.* **1997**, *24*, 743.

63. Patt, J. T.; Deuther-Conrad, W.; Wohlfarth, K.; Feuerbach, D.; Brust, P.; Steinbach, J. *J. Labelled Compd. Radiopharm.* **2003**, *46*, S168.
64. Deuther-Conrad, W.; Patt, J. T.; Feuerbach, D.; Wegner, F.; Brust, P.; Steinbach, J. *Farmaco* **2004**, *59*, 785.
65. Patt, J. T.; Deuther-Conrad, W.; Brust, P.; Patt, M.; Sabri, O.; Steinbach, J. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S90.
66. Carroll, F. I.; Lee, J. R.; Navarro, H. A.; Brieaddy, L. E.; Abraham, P.; Damaj, M. I.; Martin, B. R. *J. Med. Chem.* **2001**, *44*, 4039.
67. Carroll, F. I.; Ware, R.; Brieaddy, L. E.; Navarro, H. A.; Damaj, M. I.; Martin, B. R. *J. Med. Chem.* **2004**, *47*, 4588.
68. Huang, Y.; Zhu, Z.; Xiao, Y.; Laruelle, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4385.
69. Dolci, L.; Dollé, F.; Jubeau, S.; Vaufrey, F.; Crouzel, C. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 975.
70. Karramkam, M.; Hinnen, F.; Vaufrey, F.; Dollé, F. *J. Labelled Compd. Radiopharm.* **2003**, *46*, 979.
71. Dollé, F. *Curr. Pharm. Des.* **2005**, *11*, 3221.
72. Wiley, R. H.; Hartman, J. L. *J. Am. Chem. Soc.* **1951**, *73*, 494.
73. Coenen, H. H.; Klatte, B.; Knoechel, A.; Schueller, M.; Stocklin, G. *J. Labelled Compd. Radiopharm.* **1986**, *23*, 455.
74. Hamacher, K.; Coenen, H. H.; Stöcklin, G. *J. Nucl. Med.* **1986**, *27*, 235.
75. Lemaire, C.; Plenevaux, A.; Aerts, J.; Del Fiore, G.; Brihaye, C.; Le Bars, D.; Comar, D.; Lux, A. *J. Labelled Compd. Radiopharm.* **1999**, *42*, 63.