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Synthesis of a Fluorine-18 Labeled Derivative of Epibatidine for In Vivo Nicotinic Acetylcholine Receptor PET Imaging

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Abstract—Epibatidine (*exo*-2-(2′-chloro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane), a natural compound isolated from the skin of the Ecuadorian poison frog *Epipedobates tricolor*, is the most potent nicotinic acetylcholine receptor (nAChR) agonist reported to date. In order to visualize and quantify in vivo these receptors in human brain using Positron Emission Tomography (PET), [^{18}F]norchlorofluoroepibatidine (*exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane), a fluorine-18 ($t_{1/2}$: 110 min) radiolabeled derivative of epibatidine has been designed. The corresponding 2′-bromo-, 2′-iodo- and 2′-nitro *exo*-2-(5′-pyridyl)-7-azabicyclo[2.2.1]heptane analogues as labeling precursors, as well as norchlorofluoroepibatidine as a reference compound have been synthesized by reductive, stereoselective, palladium-catalyzed Heck-type coupling between an *N*-Boc protected azanorbornene and the corresponding halopyridine. [^{18}F]Norchlorofluoroepibatidine has been radiolabeled with fluorine-18 by nucleophilic aromatic substitution from the corresponding Boc-protected halo- and nitro precursors using [^{18}F]FK-K_{222} complex in DMSO by conventional heating (at 150–180 °C for 10 min) or microwave activations (at 100 Watt, for 1 to 2.5 min), followed by TFA-removal of the protective group. Typically, using the microwave activation procedure, 60–80 mCi (2.22–2.96 GBq) of pure [^{18}F]norchlorofluoroepibatidine could be obtained in less than 2 h (110–115 min) from the bromo labeling precursor, with specific radioactivities of 1.5–2.5 Ci/μmol (55.5–92.5 GBq/μmol) calculated for End of Bombardment. The preliminary PET experiments in baboon (*Papio papio*) with [^{18}F]norchlorofluoroepibatidine show a high uptake and a rapid accumulation of the radiotracer into the brain within 30 min. In the thalamus, a nAChR rich area, uptake of radioactivity reached a maximum at 40 min (10% I.D./100 mL tissue). The ratio of radioactivity thalamus/cerebellum (the latter being a nAChR poor area) was 2 at 40 min and increased with time, up to 4.3 at 160 min. Its specific regiodistribution and its high ratio of specific-to-nonspecific binding confirm the ideal profile of [^{18}F]norchlorofluoroepibatidine as a suitable radioligand for PET imaging of nAChRs in the brain.

Introduction

Based on the hypothesis that cholinergic dysfunction contributes to cognitive impairments in patients with senile dementia of the Alzheimer type or Parkinson disease,[1] considerable efforts have been engaged in the design, synthesis, and pharmacological characterization of Positron Emission Tomography (PET) radioligands, in order to visualize and quantify nicotinic acetylcholine receptors (nAChRs) in human brain.

Nicotine (**1**),[2–7] but also ABT-418[8,9] (**2**, (S)-3-methyl-5-[1-methyl-2-pyrrolidinyl]isoxazole), *N*-methylcytisine (**3**)[8,9] and A-84543[10] (**4**, 3-[(1-methyl-2(S)-pyrrolidinyl)methoxy]pyridine) have been labeled with carbon-11 ($t_{1/2}$: 20.4 min) and pharmacologically characterized in vivo (Fig. 1). More recently, a fluoro analogue of the Abbott laboratories 3-pyridyl ether series lead compound A-85380, namely 2-fluoro-3-[2(S)-2-azetidinylmethoxy]-pyridine (F-A-85380, **5**),[11,12] was radiolabeled with fluorine-18 ($t_{1/2}$: 110 min). Its full pharmacological profile and its potential for eventual clinical applications as a tracer for PET experiments are currently under investigation.

Subsequent efforts have also focused on the most potent nAChR agonist reported to date, epibatidine (**6a**, *exo*-2-(2′-chloro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane), a natural compound isolated from the skin of the Ecuadorian poison frog *Epipedobates tricolor*[13,14] (Fig. 2). Both enantiomers have potent antinociceptive activity (200 times more than L-nicotine) and show high affinity (K_i = 54.7 and 55.0 pM) for [^{3}H]nicotine binding site in rat brain.[15] [^{3}H]Epibatidine also shows high uptake into whole brain and a slower clearance compared with [^{3}H]nicotine or [^{3}H]cytisine.[16] Both the brain distribution and the pharmacological characteristics indicate

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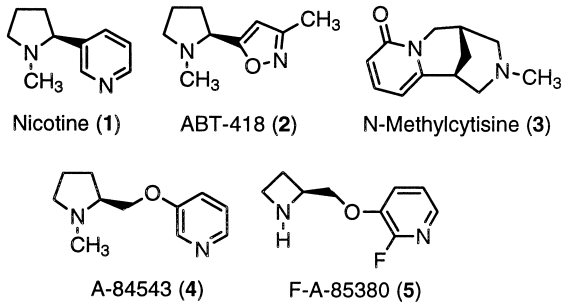


Figure 1. Chemical structure of nicotine (**1**), ABT-418 (**2**), *N*-methylcytisine (**3**), A-84543 (**4**), and F-A-85380 (**5**).

that radiolabeled forms of epibatidine should be exceptionally promising ligands for the study of nicotinic acetylcholine receptors in vivo.

A fluoro analogue (**6b**, exo-2-(2′-fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane) of this substance has been developed and labeled with fluorine-18.[17] Due to its high uptake into the brain, its specific regiodistribution and its high ratio of specific-to-nonspecific binding, this radioligand appears to be ideally suited for PET imaging of nAChRs in the brain.[18] The reported low radiochemical yield prompted us to synthesize three different labeling precursors and to compare the incorporation yield of fluorine-18.

We herein report (1) the synthesis of norchlorofluoroepibatidine (**6b**, *exo*-2-(2′-fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane), (2) its comparative radiolabeling by nucleophilic aromatic fluorination with fluorine-18 from the corresponding 2′-bromo-, 2′-iodo- and 2′-nitro-*exo*-2-(5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane using both conventional heating and microwave activations, and (3) the preliminary PET experiments.

Results and Discussion

Chemistry

Among the different chemical syntheses of epibatidine[19–39] (**6a**), the approach reported by Clayton et al.,[23] involving a reductive palladium-catalyzed Heck-type coupling between an *N*-protected azanorbornene and a iodopyridine, appeared to be not only the shortest synthesis thus far reported, but also the only one which is completely stereoselective, leading exclusively to the desired *exo*-product. This unique convergent approach

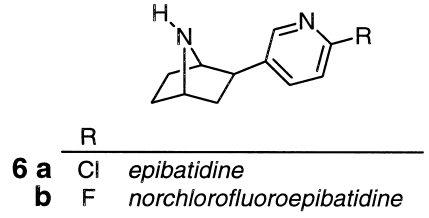


Figure 2. Chemical structure of epibatidine (**6a**) and norchlorofluoroepibatidine (**6b**).

also allows the preparation in the last chemical steps of various derivatives of epibatidine, minimizing therefore the number of intermediate compounds.

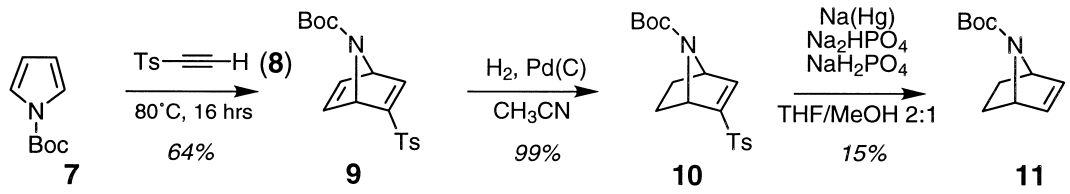
N-Boc-norchlorofluoroepibatidine (**19a**, *exo*-2-(2′-fluoro-5′-pyridyl)-7-*tert*-butoxy carbonyl-7-azabicyclo[2.2.1]-heptane) as well as three labeling precursors, 2′-bromo-, 2′-iodo- and 2′-nitro-*exo*-2-(5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19b**, **19c**, **19d**) were prepared from 7-*tert*-butoxycarbonyl-7-azabicyclo-[2.2.1]hept-2-ene (**11**) and the appropriate substituted halopyridines (**15–18**).

N-Boc-protected azanorbornene **11** was synthesized in 9% overall yield following the route of Altenbach et al. for the known *N*-methoxycarbonyl-protected analogue[40,41] (Scheme 1).

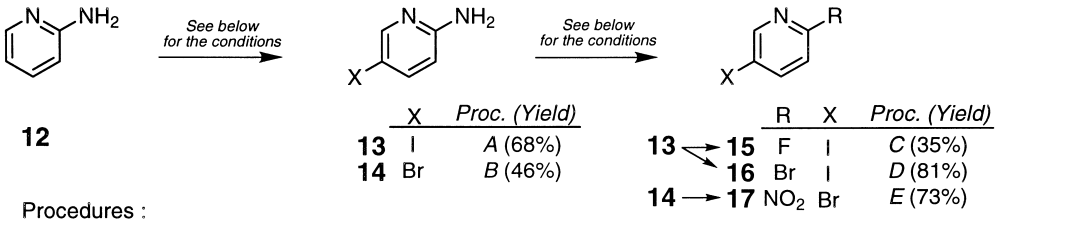
Diels–Alder reaction[23] between commercially available *N*-Boc-pyrrole (**7**) and an efficient acetylene equivalent, *para*-toluenesulfonylacetylene[42] (**8**, synthesized in two steps from the commercially available *bis*-(trimethylsilyl)acetylene in 62% overall yield[43]) at 80 °C for 16 h gave the *N*-Boc protected heterobicyclic compound **9** in 64% yield. Selective careful catalytic hydrogenation (hydrogen and palladium on charcoal (10% Pd content) in acetonitrile[41]) of **9** reduced only the less substituted double bond to give **10** in 99% yield. Overreduction with more than 1.2 equivalent of hydrogen led to *endo*-2-(*p*-tolyl-sulfone)-7-*tert*-butoxycarbonyl-7-azabicyclo-[2.2.1]heptane. Reductive cleavage of the *para*-toluenesulfonyl group of **10** using 6% sodium amalgam[41,44] in methanol–THF gave the *N*-Boc protected 7-azanorbornene **11** in low yield (15%). The use of an excess of the Na(Hg) amalgam led to the reduction of the double bond to give the 7-azanorbornane derivative: 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane. Neither sodium dithionite,[45–48] nor samarium diiodide,[49] both described as a powerful alternative reagent to sodium amalgam for the reductive cleavage of vinylic sulfone, gave the desired *N*-Boc protected 7-azanorbornene **11**. In both cases, the double bond was reduced prior to the desulfonation process as observed by ^{1}H NMR analysis of the crude. No reaction at all was observed when aluminum amalgam[50,51] was used.

Both 2,5-dihalopyridines **15** and **16** as well as 5-bromo-2-nitropyridine (**17**) were prepared from commercially available 2-aminopyridine (**12**) (Scheme 2). 2-Fluoro-5-iodopyridine (**15**) and 2-bromo-5-iodopyridine (**16**) were synthesized in two steps in, respectively, 24 and 55% overall yield. 2-Aminopyridine (**12**) reacted with 0.2 equivalent of periodic acid dihydrate and 0.4 equivalent of iodine in an acetic acid/water/sulfuric acid mixture[52] at 80 °C for 2 h to give 2-amino-5-iodopyridine (**13**). A Schiemann reaction using 50% fluoboric acid and sodium nitrite,[53] gave 2-fluoro-5-iodopyridine (**15**) in 35% yield. Using hydrobromic acid, bromine and sodium nitrite,54 2-bromo-5-iodopyridine (**16**) was obtained in 81% yield.

5-Bromo-2-nitropyridine (**17**) was synthesized in two steps in 34% overall yield. 2-Aminopyridine (**12**) reacted



Scheme 1. Synthesis of 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]hept-2-ene (**11**).



Scheme 2. Synthesis of 2-fluoro-5-iodopyridine (**15**), 2-bromo-5-iodopyridine (**16**), and 5-bromo-2-nitropyridine (**17**).

with bromine in a solution of dichloromethane and acetonitrile at room temperature for 1 h[55] to give desired 2-amino-5-bromopyridine (**14**) as well as 2-amino-3,5-dibromopyridine in 46% and 10% yield, respectively. The oxidation of the amino- to the nitro-function using 10% aqueous hydrogen peroxide and fuming sulfuric acid,[56,57] gave 5-bromo-2-nitropyridine (**17**) in 73% yield. Noteworthy, oxidation of 5-iodo-2-aminopyridine (**13**) under the same conditions as those described above was unsuccessful (90% of starting material was recovered). A similar observation was published for the oxidation of 3-halo-4-aminopyridines: 3-chloro- and 3-bromo derivatives could be converted to the respective 3-chloro- and 3-bromo- 4-nitropyridines, whereas the 3-iodo derivative was not oxidized.[58,59]

As to the reductive palladium-catalyzed Heck-type coupling, reaction of norbornene and halobenzene was described as completely stereoselective; only the *exo*-isomer could be isolated.[60,61] As described for the total synthesis of epibatidine, using the in situ formed $(Ph_3P)_2Pd(OAc)_2$ catalyst, 7-methoxycarbonyl-7-azabicyclo[2.2.1]heptane too was reductively coupled with 2-chloro-5-iodopyridine to give only the *exo*-2-pyridyl-*N*-protected-azanorbornene.[23]

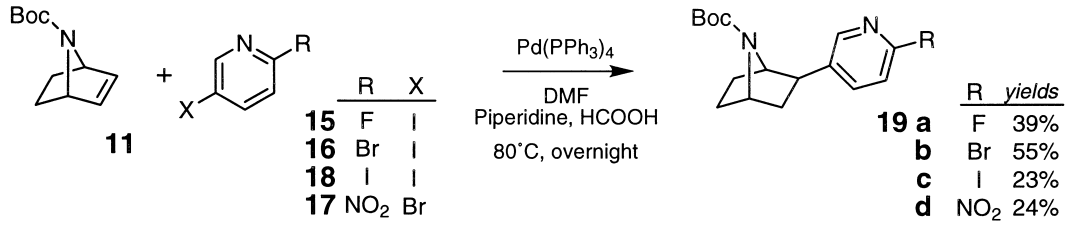
The reductive coupling between 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptene (**11**) and 2-fluoro-5-iodopyridine (**15**), 2-bromo-5-iodopyridine (**16**) or 5-bromo-2-nitropyridine (**17**) using $(Ph_3P)_4Pd$ in DMF containing piperidine and formic acid (at 80 °C, overnight) gave the fluoro-, bromo- and nitro-*exo*-pyridyl-*N*-protected-azanorbornenes **19a**, **19b**, **19d** in 39%, 55% and 24% yield, respectively (Scheme 3). The reductive coupling between 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptene (**11**) and 2,5-diiodopyridine (**18**) using similar conditions gave beside the desired iodo-*exo*-pyridyl-*N*-protected-azanorbornene **19c** (23% yield) an unexpected second *exo*-derivative (11% yield) which could be isolated. Based on its analytical data (^1H and ^{13}C NMR, as well as mass spectrometry), this compound was characterized as *exo*-2-(5′-iodo-2′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane.

The 2-*exo* stereochemistry assigned to **19a–d** was based on analysis of the corresponding ^1H NMR spectra. All spectra showed a doublet of doublet at 2.80–3.10 ppm for the H-2 *exo*-proton with characteristic $J_{2\alpha,3\beta}$, $J_{2\alpha,3\alpha}$ and $J_{2\alpha,1}$ (0.0 Hz) coupling constants[23,33] (Table 1).

TFA removal of the *tert*-butoxycarbonyl function of **19a** in a 4/1 mixture of dichloromethane/TFA at room temperature gave the amine **6b** in 86% yield (Scheme 4).

Radiochemistry

Nucleophilic substitution by means of cyclotron-produced, no-carrier-added [^{18}F]fluoride ion is the method



Scheme 3. Synthesis of epibatidine analogues **19a–d** by reductive palladium-catalyzed Heck-type coupling.

Table 1. Selected ^{1}H NMR data (300 MHz) of the H-2 proton of *N*-protected epibatidine analogues **19a–d**

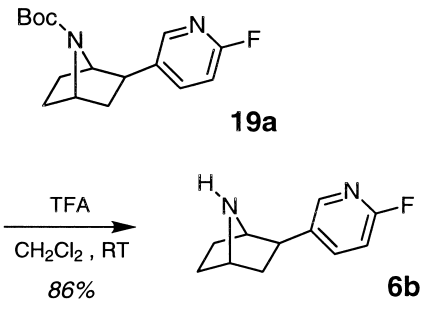
	R	δ[a]	$J_{2\alpha,3\beta}$[b]	$J_{2\alpha,3\alpha}$[b]
19a	F	2.89[dd]	4.2	8.7
19b	Br	2.84[dd]	4.1	8.9
19c	I	2.80[dd]	3.0	9.0
19d	NO_2	3.05[dd]	4.5	8.5

Solvent: CD_2Cl_2
Temperature: 298.0 K
[dd]doublet of doublets
[a]ppm
[b]Hz.

of choice for the synthesis of high specific activity fluorine-18 labeled radioligands for Positron Emission Tomography.

Compared to homoaromatic and aliphatic nucleophilic fluorinations, only few references could be found in the literature describing nucleophilic substitutions with stable [^{19}F]fluoride ion of heteroaromatic derivatives such as 2-substituted pyridines. Usually chloride or bromide were involved as leaving group in these reactions. For example, 2-fluoropyridine was obtained from 2-chloropyridine or 2-bromopyridine and fluoride ion in dimethylsulfone at 200 °C for more than 1 week in 49% and 42% yield, respectively.[53] It was also prepared from 2-nitropyridine in 60% yield by fluorodenitration using fluoride ion in HMPT at 160 °C for 24 h.[62] No example of 2-fluorodeiodination of pyridine derivatives could be found in the literature.

Prior to the above mentioned radiosynthesis of [^{18}F]norchlorofluoroepibatidine ([^{18}F]-**6b**)[17] (14% decay-corrected radiochemical yield by nucleophilic aromatic bromo-to-fluoro substitution in DMSO at 190 °C for 15 min using the activated [^{18}F]FK-K_{222} complex[63]), only two examples of 2-[^{18}F]fluoropyridine derivative syntheses using nucleophilic aromatic [^{18}F]fluorination have been reported: (1) 6-[^{18}F]fluoronicotinic acid diethylamide (**20**)[64] in up to 40% decay-corrected radiochemical yield from the corresponding 2-chloropyridine derivative and [^{18}F]fluoride ion as its cesium salt, in acetamide at 200 °C, (2) 2- and 6-[^{18}F]fluoronicotine (**21** and **22**)[65] in 30 to 40% decay-corrected radiochemical yield in DMSO at 210 °C for 30 min from the corresponding 2- and 6-bromopyridine derivative, respectively, and [^{18}F]fluoride ion again as its cesium salt (Fig. 3).



Scheme 4. Synthesis of norchlorofluoroepibatidine (**6b**).

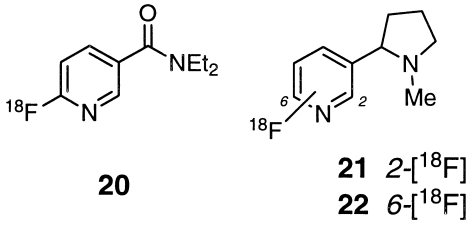


Figure 3. Chemical structure of 6-[^{18}F]fluoronicotinic acid diethylamide (**20**) and 2- and 6-[^{18}F]fluoronicotine (**21** and **22**).

More recently, [^{18}F]F-A-85380 ([^{18}F]-**5**) was synthesized by nucleophilic aromatic nitro-to-fluoro substitution in DMSO by conventional heating at 150 °C for 20 min or by microwave activation at 100 Watt for 1 min (50–60% decay-corrected radiochemical yield).[12] It has also been synthesized in 20% decay-corrected radiochemical yield by nucleophilic aromatic bromo-to-fluoro substitution in DMSO by conventional heating at 150 °C for 20 min.[11]

exo-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane ([^{18}F]-**6b**) was prepared in two steps from the labeling bromo-, iodo- or nitro precursor **19b**, **19c**, **19d**.

The first step consists in the introduction of the fluorine-18 using a no-carrier-added nucleophilic aromatic substitution with [^{18}F]FK-K_{222}, at the alpha position of the pyridyl ring. Beside bromine and iodine, the nitro function was also chosen as leaving group for this substitution, not only for its high potential as leaving group in comparison with a corresponding halo substituent, but also for the expected superior precursor separation from the reaction product. The reaction was performed using the activated [^{18}F]FK-K_{222}-complex[63] as the fluorinating reactant, in DMSO as the solvent, by (1) conventional heating at 150–180 °C for 10–15 min or (2) microwave activation at 100 Watt for 1–2.5 min.

Conventional heating. The incorporation yields for the bromo- (**19b**) and nitro (**19d**) labeling precursors at 180 °C for 10 min, calculated from the TLC-radiochromatogram (and defined as the [^{18}F]fluoropyridine derivative over total fluorine-18 activity area ratio) were 51% and 29%, respectively, with respect to the [^{18}F]fluoride ion. Noteworthy, the iodo substituent (in **19c**), which is usually considered as an excellent leaving group, was almost unreactive in the conditions used (14%) and was not further investigated. Varying the amount of precursor, in the case of the bromo precursor for example, from 14 to 30 μmol, did not change the yield. Longer reaction time did not increase the yield either (51% for the bromo precursor for 15 min, same value for 10 min). At 150 °C and 10 min of reaction, only the nitro precursor was reactive with an incorporation yield superior to the one observed at higher temperature (48%). At 120 °C, only 7% yield was observed for the nitro precursor.

Microwave activations. Using microwave irradiations, good yields of incorporation were observed for the bromo-(**19b**) and the nitro (**19d**) precursors. For the bromo precursor, at 100 W, the incorporation yields increase going from 1 to 2.5 min: 20% at 1 min, 23% at

1.5 min, 54% at 2 min (comparable to those obtained for 10 min at 180 °C of conventional heating) and finally 72% at 2.5 min. Only 9% incorporation yield could be observed for the iodo precursor at 1 min. For the nitro substituent, the incorporation yields at 1 min and 1.5 min were similar, 49% and 45%, respectively, and were comparable to those obtained for 10 min of conventional heating at 150 °C.

Prior to HPLC, the reaction mixture was pre-purified passing it through a C_{18} Sep-pak, the [^{18}F]fluoropyridine derivatives being eluted from the cartridge with CH_2Cl_2. The incorporation yields, determined after the Sep-pak purifications as the ratio of the CH_2Cl_2-eluted radioactivity over the total eluted radioactivity were consistently comparable to the yields estimated by radio-TLC. For example, the incorporation yield for the bromo precursor at 180 °C and 10 min heating was 49% (compared to the 51% radio-TLC yield).

Starting from pure labeling precursor *exo*-2-(2′-bromo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]-heptane (**19b**), two radiolabeled compounds (ratio 1/2.6), representing 85 to 95% of the total radioactivity could be separated on semipreparative SiO_2-HPLC. The minor product was eluted first with a retention time of 7.6 min. The major product, which was eluted with a retention time of 8.9 min, was not separated in these conditions from non-radioactive bromo precursor but co-eluted with an authentic sample of pure *exo*-2-(2′-fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo-[2.2.1]heptane (**19a**).

Starting from pure labeling precursor *exo*-2-(2′-nitro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19d**), four radiolabeled compounds (ratio 1/2.3/1/4.5), representing 85 to 95% of the total radioactivity could be separated on semipreparative SiO_2-HPLC, with retention times of 6.3, 6.9, 7.8 and 8.9 min, respectively. The major product (fourth and last peak) co-eluted with an authentic sample of pure *exo*-2-(2′-fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo-[2.2.1]heptane ([^{18}F]-**19a**) and was easily separated from non-radioactive nitro precursor (>20.0 min).

Removal of the *tert*-butoxycarbonyl function in a 10/1 mixture of CH_2Cl_2/TFA at room temperature for 2 min, followed by HPLC purification gave the amine [^{18}F]-**6b**. The chemical yields of Boc deprotection were quantitative. Direct deprotection of the non-HPLC-purified 2-[^{18}F]fluoropyridine derivative [^{18}F]-**19a** with TFA in CH_2Cl_2 shortened the procedure but led to chemically impure amine [^{18}F]-**6b**. The whole synthesis procedure (included the HPLCs) is fully automated on a computer assisted Zymate robot system (Zymark Corporation, USA). Results (decay-corrected and non decay-corrected yields, synthesis time) are summarized in Scheme 5.

Typically, using the microwave activation procedure, 60–80 mCi (2.22–2.96 GBq) of pure *exo*-2-(2′-[^{18}F]-fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane ([^{18}F]-**6b**) could be obtained in less than 2 h (110–115 min) from the bromo labeling precursor *exo*-2-(2′-bromo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19b**), with specific radioactivities of 1.5–2.5 Ci/μmol (55.5–92.5 GBq/μmol) calculated for End of Bombardment for a 20 μA, 30 min (36000 μC) irradiation of a 95% enriched [^{18}O]water target with a 17 MeV proton beam [^{18}O(p,n)^{18}F]. During the preparation of this manuscript, an alternative radiochemical synthesis of [^{18}F]norchlorofluoroepibatidine appeared in the literature.[66] These authors described a highly efficient one-pot synthesis of [^{18}F]-**6b** starting from a *N*-Boc protected 2-(2′-trimethylammonium-5′-pyridyl)-azabicyclo-[2.2.1]heptane iodide precursor, but using the same two-step approach as described in this manuscript: (1) a no-carrier-added nucleophilic heteroaromatic substitution with [^{18}F]FK-K_{222} followed by (2) a deprotection with TFA.

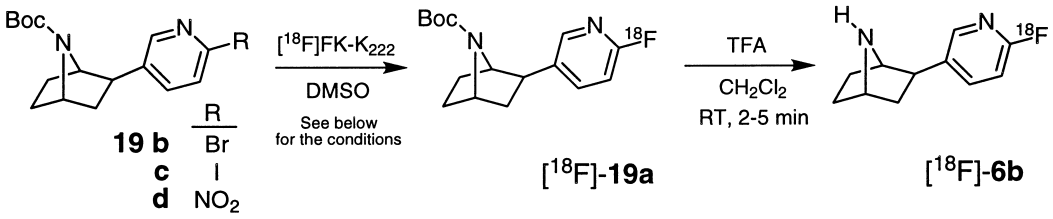
PET imaging

Positron emission tomography (PET) imaging of central nicotinic acetylcholine receptors (nAChRs) is a useful tool to assess the involvement of these receptors in neurodegenerative processes such as Parkinson's and Alzheimer's disease.

PET studies of the brain distribution of [^{18}F]norchlorofluoroepibatidine were carried out in adult *Papio papio* baboon (average weight, 8 kg). A separate MRI examination was performed with a 1.5 Tesla system (GE) in order to provide anatomical images corresponding to PET slices. The PET experiment was performed with a CTI HR + Exact positron tomograph (CTI PET Systems, Knoxville, TN, USA). The baboon's head was positioned in the tomograph using a custom-designed stereotaxic headholder. All the cerebral regions studied (cortex, diencephalon, cerebellum) were contained in axial cross sections parallel to the orbito-meatal anatomical line of reference.

The baboon was **iv** injected with 0.73 mCi (27 MBq) of [^{18}F]norchlorofluoroepibatidine and imaged for 160 min (33 images: 6×1 min, 7×2 min, 8×5 min, 10×10 min). Arterial blood samples were withdrawn from a femoral artery at designated times. For PET data analysis, regions of interest were delineated on images on which anatomical structures (frontal cortex, thalamus, cerebellum) can be clearly identified. The concentration of radioactivity in each region of interest was determined during each sequential scan and expressed as percent of the injected dose per 100 mL (% I.D./100 mL) of tissue.

Brain images show evident rapid accumulation of the radiotracer within 30 min (Fig. 4). In the thalamus, a nAChR rich area, uptake of radioactivity reached a maximum at 40 min (10% I.D./100 mL tissue). The ratio of radioactivity thalamus/cerebellum (the latter being a nAChR poor area) was 2 at that time. Time activity curves in these two structures were strikingly different. While the radioactivity in the thalamus plateaued from 40 min until the end of the experiment (apparent $t_{1/2}$ about 20 h), a rather rapid washout ($t_{1/2} = 60$ min) of the radioactivity was observed in the cerebellum. Therefore,



Conditions	Yields (with respect to [^{18}F]fluoride ion)	
Conventional heating[#]		
19b (R: Br)	25–35%[a]; 15–22%[b]	15–25%[a]; 7–12%[b]
19d (R: NO_2)	12–17%[a]; 7–10%[b]	7–12%[a]; 3–6%[b]
Synthesis time	75–80 min[c]	115–120 min[c]
Microwave activations[£]		
19b (R: Br)	35–45%[a]; 22–28%[b]	21–32%[a]; 10–16%[b]
19d (R: NO_2)	12–17%[a]; 7–10%[b]	7–12%[a]; 3–6%[b]
Synthesis time	70–75 min[c]	110–115 min[c]

[#]R: -Br (180 °C, 10 min); -NO_2 (150 °C, 10 min).

[£]R: -Br (100 W, 2.5 min); -NO_2 (100 W, 1.0 min).

[a]Decay-corrected.

[b]Non decay-corrected.

[c]From EOB (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation).

Scheme 5. Radiosynthesis of *N*-Boc protected [^{18}F]norchlorofluoroepibatidine ([^{18}F]-**19a**) and [^{18}F]norchlorofluoroepibatidine ([^{18}F]-**6b**); Conditions and yields.

the ratio thalamus/cerebellum increased with time, up to 4.3 at 160 min. Clearance from the blood was rapid.

Our results on the brain kinetics of [^{18}F]norchlorofluoroepibatidine in isoflurane anesthetized *Papio papio* corroborate those reported by Villemagne et al.[67] in *Papio anubis* anesthetized with alfaxolone acetate and alfadolone (steroid derivatives) and to a lesser extent, those reported by Ding et al.[68] in the same species, *Papio anubis*, anesthetized with isoflurane. The higher uptake observed in both our study and the one reported by Ding et al.[68] (compared to the one reported by Villemagne et al.[67]) could be due to the use of isoflurane as anesthetic agent. Volatile halogenated anesthetic agents have been shown to stabilize the slow desensitized conformational state of the nAChRs, an inactive state characterized by high affinity for agonists.[69]

Conclusion

Epibatidine (**6a**, *exo*-2-(2′-chloro-5′-pyridyl)-7-azabicyclo-[2.2.1]heptane), a natural compound isolated from the skin of the Ecuadorian poison frog *Epipedobates tricolor*, is the most potent nicotinic acetylcholine receptor (nAChR) agonist reported to date. In order to visualize and quantify in vivo these receptors in human brain using Positron Emission Tomography (PET), [^{18}F]norchlorofluoroepibatidine ([^{18}F]-**6b**, *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-azabicyclo[2.2.1] heptane), a fluorine-18 ($t_{1/2}$: 110 min) radiolabeled derivative of epibatidine (**6a**) has been designed.

The corresponding 2′-bromo-, 2′-iodo- and 2′-nitro *exo*-2-(5′-pyridyl)-7-azabicyclo[2.2.1]heptane analogues (**19b–d**) as labeling precursors, as well as norchlorofluoroepibatidine (**6b**) as a reference compound have been synthesized by reductive, stereoselective, palladium-catalyzed Heck-type coupling between an *N*-Boc protected azanorbornene and the corresponding halopyridine. [^{18}F]Norchlorofluoroepibatidine ([^{18}F]-**6b**) has been radiolabeled with fluorine-18 by nucleophilic aromatic substitution from the corresponding Boc-protected halo- and nitro precursors using [^{18}F]FK-K_{222} complex in DMSO by conventional heating (at 150–180 °C for 10 min) or microwave activations (at 100 W, for 1 to 2.5 min), followed by TFA-removal of the protective group. Typically, using the microwave activation procedure, 60–80 mCi (2.22–2.96 GBq) of pure [^{18}F]-**6b** could be obtained in less than 2 h (110–115 min) from the bromo labeling precursor *exo*-2-(2′-bromo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19b**), with specific radioactivities of 1.5–2.5 Ci/μmol (55.5–92.5 GBq/μmol) calculated for End of Bombardment.

The preliminary PET experiments in baboon (*Papio papio*) with [^{18}F]norchlorofluoroepibatidine ([^{18}F]-**6b**) show a high uptake and a rapid accumulation of the

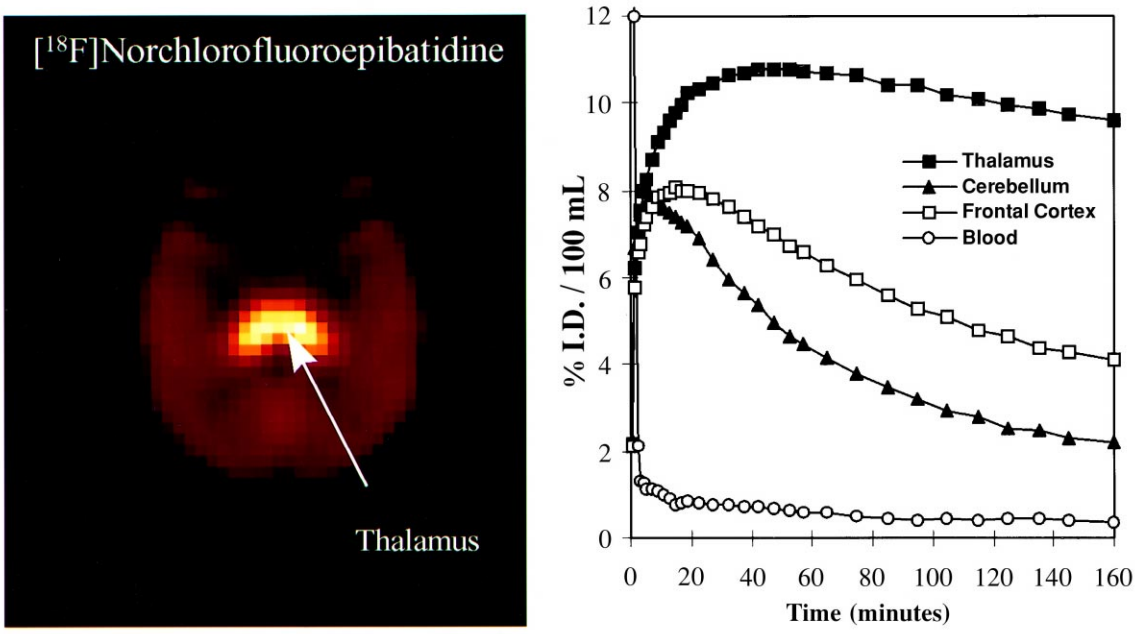


Figure 4. Baboon (*Papio papio*) brain PET images and time–activity curves for selected brain areas and blood following iv injection of [^{18}F]norchlorofluoroepibatidine ([^{18}F]-**6b**).

radiotracer into the brain within 30 min. In the thalamus, a nAChR rich area, uptake of radioactivity reached a maximum at 40 min (10% I.D./100 mL tissue). The ratio of radioactivity thalamus/cerebellum (the latter being a nAChR poor area) was 2 at that time. Time activity curves in these two structures were strikingly different. While the radioactivity in the thalamus plateaued from 40 min until the end of the experiment (apparent $t_{1/2}$ about 20 h), a rather rapid washout ($t_{1/2}$ = 60 min) of the radioactivity was observed in the cerebellum. Therefore, the ratio thalamus/cerebellum increased with time, up to 4.3 at 160 min. Clearance from the blood was rapid. Its specific regiodistribution and its high ratio of specific-to-nonspecific binding confirm the ideal profile of [^{18}F]norchlorofluoroepibatidine as a suitable radioligand for PET imaging of nAChRs in the brain.

Experimental

General

Chemicals were purchased from Aldrich, Fluka, or Sigma France, unless otherwise stated, and were used without further purification. TLC were run on precoated plates of silica gel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV lamp and/or (2) by iodine staining and/or (3) by dipping the TLC plates in a 1% aqueous $KMnO_4$ solution and subsequent heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyzer. Flash chromatography was conducted on silica gel 63–200 μm (Merck) at 0.3 bar (compressed air) unless otherwise stated. HPLCs were run on Waters systems equipped with a 510 pump and either of the following UV detectors: models 440 (single wavelength), 481 or 486 (multi-wavelength); the effluent was also monitored for radioactivity with a Geiger–Müller counter; the HPLC column and conditions were for **A** [Column, semipreparative SiO_2 Lichrosorb Merck (250×10 mm); porosity, 7 μm; temperature, rt; UV detection at λ, 254 nm]; and for **B** [Column, semipreparative C-18 μBondapak Waters (300×7.8 mm); porosity, 10 μm; temperature, rt; UV detection at λ, 254 nm].

Melting points (mp) were measured on a 9200 Electrothermal instrument and are uncorrected. NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents (DMSO-d_6, δ = 2.50 ppm; CD_2Cl_2, δ = 5.32 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuteriated solvent (DMSO-d_6, δ = 39.5 ppm; CD_2Cl_2, δ = 53.8 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, dt, b, m for singlet, doublet, triplet, doublet of doublet, doublet of triplet, broad, and massif, respectively). The mass spectra (MS), DCI/NH_4^+, were measured on a Nermag R10-10 apparatus.

Radiosyntheses were performed in a 5 cm lead-shielded confinement using a computer-assisted Zymate robot system (Zymark Corporation, USA). Microwave activations were performed with a MicroWell 10 oven (2.45 GHz), Labwell AB, Sweden.

Chemistry

Ethynyl *p*-tolyl sulfone (8). In a flame-dried flask fitted with an argon inlet and glass stoppers were stirred 47.08 g (0.25 mol, MW 190.65) of *p*-toluenesulfonyl chloride and 33.18 g (0.25 mol, MW 133.34, 1.0 equiv) of

aluminum chloride in 200 mL of dichloromethane. In another flame-dried flask equipped with an addition funnel was stirred 37.98 g (0.22 mol, MW 170.40) of bis(trimethylsilyl)acetylene in 200 mL of dichloromethane. The latter solution was cooled to 0 °C in an ice-water bath. After 30 min, the solution of the *p*-toluenesulfonyl chloride-aluminium chloride complex was quickly filtered through a glass-wool plug into the addition funnel. The residue was washed with 10 mL of dichloromethane which was also added to the funnel. The complex was added to the cold silylacetylene solution keeping the temperature between 5 and 10 °C. After the addition was finished, the mixture was allowed to room temperature for one day. The mixture was hydrolyzed by pouring it into a slurry of 200 mL of 20% hydrochloric acid and ice. The organic layer was separated. The aqueous layer was extracted twice with 50 mL of dichloromethane. The organic layers were collected and washed three times with 100 mL of water and once with 100 mL of brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated to give a brown solid. Recrystallization in heptane gave 43.88 g (78%) of *p*-tolyl 2-(trimethylsilyl)ethynyl sulfone as creamy crystals: R_f 0.06 (heptane/EtOAc, 90/10); mp 83 °C (lit.[43] 81–82 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 7.85 (d, *J* 8.1 Hz, 2H), 7.37 (d, *J* 8.1 Hz, 2H), 2.42 (s, 3H), 0.21 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 146.5 [C], 139.3 [C], 130.8 [CH], 128.1 [CH], 101.9 [C], 99.3 [C], 22.2 [CH_3], −0.7 [CH_3]; MS $C_{12}H_{16}O_2SSi$, 270 [$M+NH_4^+$].

In a flask fitted with a thermometer and an addition funnel was stirred 27.08 g (0.11 mol, MW 252.41) of *p*-tolyl 2-(trimethylsilyl)ethynyl sulfone in 300 mL of methanol for 30 min. 350 mL of an aqueous solution containing potassium carbonate (6.2×10^{-3} M) and potassium bicarbonate (6.2×10^{-3} M) were placed in the addition funnel. The buffer was added keeping the temperature near 30 °C. After the addition was finished, a white precipitate appeared. The mixture was diluted with 200 mL of water and extracted with four portions of 100 mL of dichloromethane. The organic layers were washed twice with water and once with brine. After drying over anhydrous magnesium sulfate, the solvent was evaporated to give a creamy solid. Recrystallization in heptane/EtOAc gave 14.27 g (79%) of **8** as white crystals: R_f 0.08 (heptane/EtOAc, 90/10); mp 72 °C (lit.[43] 74–75 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 7.85 (d, *J* 7.5 Hz, 2H), 7.39 (d, *J* 7.2 Hz, 2H), 3.69 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 147.1 [C], 138.5 [C], 130.9 [CH], 128.2 [CH], 82.6 [C], 80.9 [C], 22.2 [CH_3]; MS $C_9H_8O_2S$, 198 [$M+NH_4^+$].

***endo*-2-(*p*-Tolyl-sulfone)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]hepta-2,5-diene (9).** In a flask, 9.26 g (0.05 mol, MW 180.22) of **8** and 21.5 mL (0.13 mol, MW 167.21, d 1.00, 2.5 equiv) of **7** were stirred at 80 °C during 16 h. The mixture was then concentrated and was coevaporated twice with EtOAc. The black residue was chromatographed on silica gel. Elution with heptane/EtOAc (95/5 to 70/30) gave 11.35 g (64%) of **9** as a yellow solid: R_f 0.29 (heptane/EtOAc, 60/40); mp 94 °C; ^{1}H NMR (CD_2Cl_2, 295.4 K) δ 7.73 (d, *J* 6.0 Hz, 2H), 7.58 (d, *J* 3.0 Hz, 1H), 7.37 (d, *J* 6.0 Hz, 2H), 6.88 (b, 2H), 5.32 (b, 1H), 5.14 (s, 1H), 2.42 (s, 3H), 1.31 (s, 9H); ^{1}H NMR (DMSO-d_6, 352.0 K) δ 7.72 (d, *J* 8.4 Hz, 2H), 7.70 (s, 1H), 7.44 (d, *J* 8.1 Hz, 2H), 6.95 (s, 2H), 5.33 (s, 1H), 5.13 (s, 1H), 2.41 (s, 3H), 1.26 (s, 9H); ^{13}C NMR (DMSO-d_6, 352.0 K) δ 158.8 [C], 153.5 [C], 153.4 [CH], 145.0 [C], 143.1 [CH], 142.0 [CH], 136.1 [C], 130.4 [CH], 127.9 [CH], 80.8 [C], 68.2 [CH], 66.9 [CH], 27.9 [CH_3], 21.3 [CH_3]; MS $C_{18}H_{21}NO_4S$ 365 [$M+NH_4^+$].

***endo*-2-(*p*-Tolyl-sulfone)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]hept-2-ene (10).** To 5.12 g (14.75 mmol, MW 347.44) of **9** in 200 mL of acetonitrile was added 1.00 g Pd/C (10% Pd content). The reaction vessel was purged first with argon and then carefully with hydrogen. 400 mL (17.78 mmol, 1.2 equiv*) of hydrogen was led into the reaction mixture with vigorous stirring at room temperature. After the addition was complete, the catalyst was removed by filtration over Celite® and the solvent was evaporated in vacuo. 5.09 g (99%) of **10** was obtained as a white solid: R_f 0.51 (heptane/EtOAc, 1/1); mp 141–143 °C; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 7.78 (d, *J* 9.0 Hz, 2H), 7.38 (d, *J* 9.0 Hz, 2H), 7.02 (s, 1H), 4.79 (s, 1H), 4.74 (d, *J* 3.0 Hz, 1H), 2.43 (s, 3H), 1.97 (bt, 2H), 1.26 (b, 2H), 1.20 (s, 9H); ^{1}H NMR (DMSO-d_6, 333.0 K) δ 7.78 (d, *J* 9.0 Hz, 2H), 7.47 (d, *J* 9.0 Hz, 2H), 7.15 (d, *J* 2.1 Hz, 1H), 4.74 (s, 1H), 4.70 (d, *J* 1.2 Hz, 1H), 2.42 (s, 3H), 1.90 (bt, 2H), 1.19 (s, 9H), 1.11 (b, 2H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.0 [C], 149.2 [C], 145.3 [C], 144.1 [CH], 137.3 [C], 130.4 [CH], 128.2 [CH], 80.7 [C], 62.2 [CH], 61.2 [CH], 27.9 [CH_3], 25.4 [CH_2], 24.4 [CH_2], 21.7 [CH_3]; ^{13}C NMR (DMSO-d_6, 333.0 K) δ 154.1 [C], 148.0 [C], 144.7 [C], 144.6 [CH], 136.7 [C], 130.2 [CH], 127.5 [CH], 79.9 [C], 61.7 [CH], 60.5 [CH], 27.5 [CH_3], 24.6 [CH_2], 23.6 [CH_2], 21.0 [CH_3]; MS $C_{18}H_{23}NO_4S$, 367 [$M+NH_4^+$].

7-*tert*-Butoxycarbonyl-7-azabicyclo[2.2.1]hept-2-ene (11). To 5.07 g (14.51 mmol, MW 349.45) of **10** in 30 mL of a methanol/dry THF (1/2) solution was added 7.20 g (60.01 mmol, MW 119.98) of NaH_2PO_4 and 7.80 g (54.94 mmol, MW 141.96) of Na_2HPO_4. The reaction vessel was then cooled to −70 °C with an ethanol/liquid nitrogen bath. 6% Na(Hg)† (8.90 g)‡ was added in small portions and at the end of the addition the mixture was allowed to return to rt. After one night, the solution was

* Overreduction with more hydrogen led to *endo*-2-(*p*-tolyl-sulfone)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane: R_f 0.51 (heptane/EtOAc, 1/1); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 7.78 (d, *J* 6.0 Hz, 2H), 7.38 (d, *J* 6.0 Hz, 2H), 4.30 (t, *J* 4.5 Hz, 1H), 4.26 (t, *J* 4.5 Hz, 1H), 3.55 (m, 1H), 2.48 (b, 1H), 2.43 (s, 3H), 1.99–1.66 (b, 5H), 1.39 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.0 [C], 145.3 [C], 137.8 [C], 130.1 [CH], 128.1 [CH], 80.4 [C], 64.9 [CH], 58.4 [CH], 58.1 [CH], 32.5 [CH_2], 29.5 [CH_2], 28.3 [CH_3], 25.0 [CH_2], 21.7 [CH_3]; MS $C_{18}H_{25}NO_4S$ 352 [$M+H^+$], 369 [$M+NH_4^+$].

† Prepared from Na (0.60 g) and Hg (9.50 g) according to the procedure described in Vogel's Textbook of Practical Organic Chemistry, 5th ed.; Longman Scientific & Technical, John Wiley & Sons: New York.

‡ Overreduction with more Na(Hg) led to 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane: R_f 0.53 (heptane/EtOAc, 1/1); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 4.12 (s, 2H), 1.71 (d, *J* 8.8 Hz, 4H), 1.41 (s, 9H), 1.38 (d, *J* 8.5 Hz, 4H); ^{1}H NMR (DMSO-d_6, 318.0 K) δ 4.04 (s, 2H), 1.62 (d, *J* 4.5 Hz, 4H), 1.38 (s, 9H), 1.02 (d, *J* 7.5 Hz, 4H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 156.1 [C], 79.3 [C], 56.6 [CH], 29.9 [CH_2], 28.4 [CH_3], 24.0 [CH_2].

filtered over Celite® and concentrated to dryness. The yellow residue was chromatographed on silica gel. Elution with heptane/EtOAc (95/5) gave 416 mg (15%) of **11** as a pale oil: R_f 0.53 (heptane/EtOAc, 1/1); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 6.20 (s, 2H), 4.60 (s, 2H), 1.80 (d, *J* 8.9 Hz, 2H), 1.38 (s, 9H), 1.08 (d, *J* 7.7 Hz, 2H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.4 [C], 134.9 [CH], 79.6 [C], 60.0 [CH], 28.3 [CH_3], 23.9 [CH_2]; MS $C_{11}H_{17}NO_2$ 196 [$M+H^+$].

2-Amino-5-iodopyridine (13). A solution of 5.71 g (60.67 mmol, MW 94.12) of 2-aminopyridine (**12**), 5.95 g (23.44 mmol, MW 253.81) of iodine and 3.31 g (14.64 mmol, MW 225.94) of periodic acid dihydrate in acetic acid (40 mL), water (15 mL) and concentrated sulfuric acid (1 mL) was heated at 80 °C during 2 h. The mixture was then added to a solution of $Na_2S_2O_3$ to neutralize the iodine. The solution was extracted with dichloromethane. The organic layers were combined, washed with water and brine, dried over anhydrous magnesium sulfate and concentrated to dryness. The residue was chromatographed on silica gel. Elution with heptane/EtOAc (50/50 to 10/90) gave 9.12 g (68%) of **13** as a yellow solid: R_f 0.16 (heptane/EtOAc, 1/1); mp 125–126 °C (lit.[52] 132–133 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.15 (d, *J* 1.9 Hz, 1H), 7.62 (dd, *J* 2.3 & 8.7 Hz, 1H), 6.36 (d, *J* 8.7 Hz, 1H), 4.90 (b, 2H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 158.1 [C], 153.6 [CH], 145.8 [CH], 111.4 [CH], 77.4 [C]; MS $C_5H_5IN_2$ 221 [$M+H^+$], 238 [$M+NH_4^+$].

2-Amino-5-bromopyridine (14). To a solution of **12** (2.00 g, 21.25 mmol, MW 94.12) in 120 mL of dichloromethane and 60 mL of acetonitrile was added dropwise a 1.07 M solution of bromine in dichloromethane until all the starting material had been consumed according to TLC (approximately 20 mL). Then 20.0 g (238.1 mmol, MW 84.01) of $NaHCO_3$ was added and the solution was stirred for 1 h. The mixture was then filtered and concentrated to dryness. The brown residue was chromatographed on neutral alumina (Merck, 70–230 mesh). Elution with CH_2Cl_2/CH_3CN (85/15) gave 535 mg (10%) of 2-amino-3,5-dibromopyridine and 1.69 g (46%) of **14** as yellow solids: R_f 0.49 (CH_2Cl_2/CH_3CN, 85/15); mp 136 °C (lit.[55] 135–138 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.06 (d, *J* 2.1 Hz, 1H), 7.48 (dd, *J* 2.4 & 8.7 Hz, 1H), 6.45 (d, *J* 8.6 Hz, 1H), 4.50 (b, 2H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 157.8 [C], 149.0 [CH], 140.3 [CH], 110.4 [CH], 108.3 [C]; MS $C_5H_5BrN_2$ 173, 175 [$M+H^+$].

2-Amino-3,5-dibromopyridine:[55] R_f 0.75 (CH_2Cl_2/CH_3CN, 85/15); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.02 (d, *J* 2.1 Hz, 1H), 7.77 (d, *J* 2.1 Hz, 1H), 5.24 (b, 2H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.0 [C], 147.9 [CH], 142.2 [CH], 107.1 [C], 104.8 [C].

2-Fluoro-5-iodopyridine (15). To a solution of 2.70 g (12.62 mmol, MW 220.01) of **13** in fluoboric acid 48% (50 mL) was added 7.00 g (101.46 mmol, MW 68.99) of $NaNO_2$ at 0 °C. The mixture was stirred at rt for 2 h and then neutralized with KOH. The product was extracted with EtOAc. The organic layers were combined, washed with water and brine, dried over anhydrous magnesium sulfate and concentrated to dryness. The residue was chromatographed on silica gel. Elution with heptane/EtOAc (80/20) gave 982 mg (35%) of **15** as a salmon-colored solid: R_f 0.57 (heptane/EtOAc, 1/1); mp 33 °C; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.41 (s, 1H), 8.06 (dt, *J* 2.4 & 7.2 Hz, 1H), 6.81 (dd, *J* 2.9 & 8.5 Hz, 1H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 163.6 [C, J_{F-C} 232.5 Hz], 154.1 [CH, J_{F-C} 15.0 Hz], 149.5 [CH, J_{F-C} 7.5 Hz], 112.2 [CH, J_{F-C} 37.5 Hz], 87.8 [C]; MS C_5H_3FIN 224 [$M+H^+$].

2-Bromo-5-iodopyridine (16). To a solution of 6.75 g (30.68 mmol, MW 220.01) of **13** in 48% aqueous hydrobromic acid (48 mL) was added 5.0 mL of bromine at 0 °C and 24.2 mL of an aqueous solution of $NaNO_2$ (3.25 M). The mixture was stirred at rt for 1 h and then neutralized by adding 145 mL of 3 M aq NaOH. The unreacted bromine was quenched with $Na_2S_2O_3$. The product was extracted with EtOAc. The organic layers were combined, washed with water and brine, dried over anhydrous magnesium sulfate and concentrated to dryness. The residue was chromatographed on silica gel. Elution with heptane/EtOAc (100/0 to 80/20) gave 7.11 g (81%) of **16** as white solid: R_f 0.58 (heptane/EtOAc, 1/1); mp 117–119 °C (lit.[52] 122–126 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.57 (s, 1H), 7.84 (dd, *J* 2.4 & 8.3 Hz, 1H), 7.29 (d, *J* 8.3 Hz, 1H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 156.5 [CH], 147.0 [CH], 141.7 [C], 130.3 [CH], 92.2 [C]; MS C_5H_3BrIN 284, 286 [$M+H^+$], 301, 303 [$M+NH_4^+$].

5-Bromo-2-nitropyridine (17). To a solution of 8 mL of aqueous 10% hydrogen peroxide and 12 mL of concentrated sulfuric acid was added dropwise a solution of 1.17 g (6.78 mmol, MW 173.02) of **14** in 16 mL of concentrated sulfuric acid. During the addition, the solution was cooled to 0 °C in an ice-water bath. After the addition was complete, the solution was allowed to rt during 5 h. The mixture was slowly poured into an ice–water mixture. 877 mg of **17** as a yellow precipitate were filtered off. The filtrate was basified with KOH and then extracted with EtOAc. The organic layers were combined, washed with water and brine, dried over anhydrous magnesium sulfate and concentrated to dryness to give an additional 131 mg of **17** as a yellow powder: Total yield 1.01 g (73%); R_f 0.52 (heptane/EtOAc, 1/1); mp 144–145 °C (lit.[56,57] 148–150 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.70 (s, 1H), 8.21 (d, *J* 8.4 Hz, 1H), 8.18 (d, *J* 8.0 Hz, 1H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.8 [C], 150.5 [CH], 142.9 [CH], 127.4 [C], 119.8 [CH]; MS $C_5H_3BrN_2O_2$ 203, 205 [$M+H^+$].

2,5-Diiodopyridine (18). 2,5-Diiodopyridine (**18**) was purchased from Syntheval, France: R_f 0.59 (heptane/EtOAc, 1/1); mp 152 °C (lit.[52] 148–154 °C); ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.57 (d, *J* 3.0 Hz, 1H), 7.62 (dd, *J* 3.0 & 6.0 Hz, 1H), 7.51 (d, *J* 6.0 Hz, 1H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 157.1 [CH], 146.2 [CH], 137.0 [CH], 116.8 [C], 93.5 [C]; MS $C_5H_3I_2N$ 332 [$M+H^+$], 349 [$M+NH_4^+$].

General procedure for the palladium-assisted coupling of 11 with 2-halo-5-iodopyridine or 5-bromo-2-nitropyridine. A solution of **11** (50.0 mg, 0.26 mmol, MW 195.26),

0.41 mmol of 2-halo-5-iodopyridine or 5-bromo-2-nitropyridine, tetrakis(triphenylphosphine)palladium(0) (20 mg, 0.02 mmol, MW 1155.58) in a mixture of DMF (120 μL), piperidine (78 μL) and HCOOH (30 μL) was stirred at 80 °C for one night. EtOAc (10 mL) and water (10 mL) were then added. The organic layer was separated, dried over anhydrous magnesium sulfate and concentrated to dryness. The brown residue was first chromatographed on silica gel. Elution with heptane/EtOAc (99/1 to 91/9) gave the desired *exo*-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane derivative. For analytical purposes, an aliquot was repurified on HPLC to give pure *exo*-2-(2′-halo- or nitro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane as pale-yellow solid [HPLC A].

***exo*-2-(2′-Fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (19a).** The procedure described above was used with **15** (92.0 mg, 0.41 mmol, MW 222.99) to give 28 mg (39%) of the *exo*-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane derivative **19a** after flash chromatography. HPLC purification of an aliquot gave pure **19a**: [HPLC A; eluent, heptane/EtOAc, 75/25; flow rate, 6.0 mL/min; retention time, 7.5–8.0 min]; R_f 0.39 (heptane/EtOAc, 1/1); Rt (HPLC A; eluent: heptane/EtOAc: 75:25; flow rate: 6.0 mL/min): 7.7 min; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.05 (s, 1H), 7.78 (dt, *J* 2.4 & 8.1 Hz, 1H), 6.85 (dd, *J* 3.0 & 5.4 Hz, 1H), 4.33 (bt, 1H), 4.13 (s, 1H), 2.89 (dd, *J* 4.2 & 8.7 Hz, 1H), 1.98 (dd, *J* 9.0 & 15.0 Hz, 1H), 1.45–1.95 (b, 5H), 1.41 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 162.8 [C, J_{F-C} 235.5 Hz], 155.5 [C], 146.5 [CH, J_{F-C} 15.1 Hz], 140.1 [CH, J_{F-C} 7.5 Hz], 139.6 [C], 109.4 [CH, J_{F-C} 37.7 Hz], 79.9 [C], 62.6 [CH], 56.6 [CH], 45.1 [CH], 40.8 [CH_2], 30.0 [CH_2], 29.1 [CH_2], 28.4 [CH_3]; MS $C_{16}H_{21}FN_2O_2$ 293 [$M+H^+$], 310 [$M+NH_4^+$].

***exo*-2-(2′-Bromo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (19b).** The procedure described above was used with **16** (234.0 mg, 0.83 mmol, MW 283.90) to give 94 mg (55%) of the *exo*-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane derivative **19b** after flash chromatography. HPLC purification of an aliquot gave pure **19b**: [HPLC A; eluent heptane/EtOAc, 85/15; flow rate 8.0 mL/min; retention time 9.5–10.0 min]; R_f 0.47 (heptane/EtOAc, 1/1); Rt (HPLC A; eluent: heptane/EtOAc: 85:15; flow rate: 8.0 mL/min): 9.6 min; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.22 (d, *J* 2.4 Hz, 1H), 7.54 (dd, *J* 2.5 Hz, *J* 8.4 Hz, 1H), 7.39 (d, *J* 8.3 Hz, 1H), 4.33 (s, 1H), 4.13 (s, 1H), 2.84 (dd, *J* 4.1 & 8.9 Hz, 1H), 1.97 (dd, *J* 2.7 & 6.1 Hz, 1H), 1.50–1.90 (b, 5H), 1.41 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.4 [C], 149.6 [CH], 141.2 [C], 140.0 [C], 137.6 [CH], 128.1 [CH], 79.8 [C], 62.4 [CH], 56.5 [CH], 45.2 [CH], 40.5 [CH_2], 29.9 [CH_2], 29.0 [CH_2], 28.4 [CH_3]; MS $C_{16}H_{21}BrN_2O_2$ 353, 355 [$M+H^+$], 370, 372 [$M+NH_4^+$].

***exo*-2-(2′-Iodo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (19c).** The procedure described above was used with **18** (1.235 g, 3.73 mmol, MW 330.89) to give 339 mg (34%) of a 2/1 mixture (determined from ^{1}H NMR) of two *exo*-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane derivatives after flash chromatography. HPLC purification of an aliquot gave 17 mg of a pure *exo* side-product (Rt 19.0–20.0 min), identified as *exo*-2-(5′-iodo-2′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane and 30 mg of pure **19c** (Rt 21.0–22.0 min): [HPLC A; eluent, heptane/EtOAc, 90/10; flow rate 9.0 mL/min]; R_f 0.41 (heptane/AcOEt, 1/1); Rt (HPLC A; eluent: heptane/EtOAc: 85:15; flow rate: 8.0 mL/min): 21.5 min; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.22 (d, *J* 3.0 Hz, 1H), 7.62 (d, *J* 9.0 Hz, 1H), 7.32 (dd, *J* 3.0 & 9.0 Hz, 1H), 4.32 (bs, 1H), 4.12 (s, 1H), 2.80 (dd, *J* 3.0 & 9.0 Hz, 1H), 1.97 (dd, *J* 9.0 & 12.0 Hz, 1H), 1.50–1.90 (b, 5H), 1.41 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.0 [C], 150.2 [CH], 141.3 [C], 136.6 [CH], 134.7 [CH], 115.4 [C], 79.5 [C], 62.1 [CH], 56.4 [CH], 45.1 [CH], 40.3 [CH_2], 29.9 [CH_2], 29.2 [CH_2], 28.4 [CH_3]; MS $C_{16}H_{21}IN_2O_2$ 401 [$M+H^+$], 418 [$M+NH_4^+$].

Side-product: *exo*-2-(5′-iodo-2′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1] heptane: R_f 0.41 (heptane/AcOEt, 1/1); Rt (HPLC A; eluent: heptane/EtOAc: 85:15; flow rate: 8.0 mL/min): 19.5 min; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.67 (d, $J < 3.0$ Hz, 1H), 7.92 (dd, *J* 3.0 & 9.0 Hz, 1H), 7.14 (d, *J* 9.0 Hz, 1H), 4.29 (b, 2H), 3.05 (dd, *J* 6.0 & 9.0 Hz, 1H), 2.12 (b, 1H), 1.40–1.90 (b, 5H), 1.35 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 163.4 [C], 155.0 [C], 154.9 [CH], 144.9 [CH], 123.8 [CH], 90.7 [C], 79.5 [C], 61.8 [CH], 56.7 [CH], 50.2 [CH], 37.5 [CH_2], 30.1 [CH_2], 29.3 [CH_2], 28.4 [CH_3]; MS $C_{16}H_{21}IN_2O_2$ 401 [$M+H^+$].

***exo*-2-(2′-Nitro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (19d).** The procedure described above was used with **17** (166 mg, 0.82 mmol, MW 203.00) to give 40 mg (24%) of the *exo*-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane derivative **19d** after flash chromatography. HPLC purification of an aliquot gave pure **19d**: [HPLC A; eluent, heptane/EtOAc, 75/25; flow rate 9.0 mL/min; retention time, 12.0–14.0 min]; R_f 0.16 (heptane/EtOAc, 1/1); Rt (HPLC A; eluent: heptane/EtOAc: 75:25; flow rate: 9.0 mL/min): 12.4 min; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 8.50 (d, *J* 3.0 Hz, 1H), 8.15 (d, *J* 9.0 Hz, 1H), 8.00 (dd, *J* 3.0 & 9.0 Hz, 1H), 4.38 (bs, 1H), 4.22 (s, 1H), 3.05 (dd, *J* 4.5 & 8.5 Hz, 1H), 2.06 (dd, *J* 9.0 & 12.0 Hz, 1H), 1.95–1.55 (b, 5H), 1.43 (s, 9H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 155.5 [C], 148.5 [CH], 138.3 [CH], 132.2 [C], 128.9 [C], 118.2 [CH], 80.1 [C], 62.3 [CH], 56.6 [CH], 45.7 [CH], 40.7 [CH_2], 29.9 [CH_2], 29.0 [CH_2], 28.4 [CH_3]; MS $C_{16}H_{21}N_3O_4$ 320 [$M+H^+$], 337 [$M+NH_4^+$].

***exo*-2-(2′-Fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane (6b).** To 65.0 mg (0.26 mmol, MW 273.29) of **19a** in 2 mL of dichloromethane was added 500 μL of TFA. The solution was stirred for 15 min at rt and concentrated to dryness. The residue was redissolved in 2 mL of CH_2Cl_2 and concentrated again to dryness (twice). HPLC purification gave 35 mg (86%) of pure **6b** as an oily residue: [HPLC B; eluent, aqueous 0.1 M ammonium acetate/MeOH/acetonitrile, 85/7.5/7.5; flow rate 5.0 mL/min; retention time: 6.0–8.5 min]; R_f 0.15 ($CHCl_3$/MeOH/NH_4OH, 90/10/1); Rt (HPLC B; eluent: aqueous 0.1 M ammonium acetate/MeOH/acetonitrile

85:7.5:7.5; flow rate: 5.0 mL/min): 6.8 min; ^{1}H NMR (CD_2Cl_2, 298.0 K) δ 9.34 (b, 1 H), 8.11 (d, $J < 3$ Hz, 1H), 7.88 (dt, J 3.0 & 6.0 Hz, 1H), 6.90 (dd, J 3.0 & 6.0 Hz, 1H), 4.15 (bt, 1H), 4.07 (d, $J < 3.0$ Hz, 1H), 3.10 (dd, J 4.5 & 8.5 Hz, 1H), 2.16 (dd, J 9.0 & 12.0 Hz, 1H), 1.55–2.05 (b, 5H); ^{13}C NMR (CD_2Cl_2, 298.0 K) δ 163.0 [C, J_{F-C} 236.9 Hz], 146.8 [CH, J_{F-C} 15.1 Hz], 140.5 [CH, J_{F-C} 7.5 Hz], 136.4 [C], 109.6 [CH, J_{F-C} 37.7 Hz], 62.7 [CH], 57.9 [CH], 43.8 [CH], 38.3 [CH_2], 29.6 [CH_2], 27.3 [CH_2]; MS $C_{11}H_{13}FN_2$ 193 [$M+H^+$], 210 [$M+NH_4^+$].

Radiochemistry

Production of aqueous [^{18}F]fluoride. No-carrier-added aqueous [^{18}F]fluoride ion was produced on a CGR-MeV 520 cyclotron using the [^{18}O(p,n)^{18}F] reaction and was transferred to the appropriate hot cell.

Target hardware: three-port (vent-, empty-, and fill-port) keyhole-shaped target holder; body, stainless steel, 30 mm internal diameter; cavity depth ca. 3 mm with a water-cooled silver backing; front window: 75 μm He-cooled slightly convex titanium foil; seal: Viton®/Ag O-ring. *Target content*: 2 mL of 95–97% enriched [^{18}O]water. *Target operation:* incident beam: 17 MeV protons (20 μA, 20 mm diameter); typical production: 550–650 mCi (20.3–24.0 GBq) of [^{18}F]F^- at the End Of Bombardment for a 20 μA, 30 min (36000 μC) irradiation. *Target to hot cell liquid-transfer system*: 20 m Teflon line (0.8 mm internal diameter; 1/16 inch external diameter), 1.5–2.0 bar He drive pressure; transfer time 3–5 min).

Preparation of the [^{18}F]-FK-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 were forced through a glass column containing 20–50 mg of an ion exchange resin (AG1X8, Bio-Rad, 100–200 mesh, ionic form: chloride, washed with 10 mL 1 M aq NaOH and then rinsed with 100 mL of water) by He pressure (1.5–2.0 bar). He is blown through the column to extract the last traces of [^{18}O]water. The [^{18}F]fluoride ion was then eluted from the resin using 1.0 mL of a 4.5 mg/mL aq K_2CO_3 solution. After addition of 11.0 to 15.0 mg of Kryptofix® K_{222} (4, 7, 13, 16, 21, 24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane), the resulting solution was then gently concentrated to dryness at 110–120 °C under a nitrogen stream for 10 min to give no-carrier-added [^{18}F]FK-K_{222} complex as a white semi-solid residue.

Preparation of *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane ([^{18}F]-6b). Conventional heating. The [^{18}F]FK-K_{222} complex as a white semisolid residue was dissolved in 200 μL of DMSO (Aldrich, anhydrous, 99.8%, packed under nitrogen in 100 mL Sure/Seal® glass bottle, not distilled) and transferred to a 2 mL reaction vial containing N μmol of the labeling precursor P. The evaporation tube was rinsed twice with 200 μL of DMSO which was then added to the reaction mixture. Resolubilization efficiencies were about 80–95% of the original [^{18}F]fluoride ion. The reaction vial was then tightly sealed with a Teflon cap and heated in a heating block without stirring at a temperature T and during a time t.

$P/(N)$: *exo*-2-(2′-bromo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19b**)/3.7 to 20.4 μmol; *exo*-2-(2′-iodo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19c**)/9.5 to 15.8 μmol; *exo*-2-(2′-nitro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo-[2.2.1]heptane (**19d**)/3.3 to 26.0 μmol.

T: 150 °C or 180 °C; t: 10 to 15 min.

The reaction vial was then cooled using an ice-water bath and the remaining radioactivity was measured. 85% to 95% of the initial activity placed in the vessel was still present. The resulting, often dark-colored reaction mixture was then analyzed by radio-chromatography. The incorporation yields were calculated from the TLC-radiochromatogram and defined as the [^{18}F]fluoropyridine derivative over total fluorine-18 activity area ratio (SiO_2-TLC, eluent: heptane/EtOAc: 30/70, R_f: *exo*-2-(2′-fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19a**): 0.55 and R_f: [^{18}F]fluoride ion: 0.0). The reaction mixture was diluted with 3.0 mL of water and passed through a C_{18} Sep-pak cartridge (Waters). The cartridge was washed with 10.0 mL of water and partially dried for 0.5 min by applying a nitrogen stream. The Boc-protected 2-[^{18}F]fluoropyridine derivatives were eluted from the cartridge with 3.0 mL of CH_2Cl_2 followed by two successive rinses of 1.0 mL (1−10% of the total radioactivity amount engaged in the fluorination process was left on the cartridge). The incorporation yields, now determined after the Sep-pak elution as the ratio of the CH_2Cl_2-eluted radioactivity over the total eluted radioactivity (DMSO/H_2O and CH_2Cl_2) were consistently comparable to the yields estimated by radio-TLC. The mentioned CH_2Cl_2 solution was concentrated to dryness (at 60–80 °C under a gentle nitrogen stream for 4–6 min). The residue was then dissolved in 1–2 mL of the HPLC solvent used for purification and the crude was injected onto HPLC. Isocratic elution [HPLC A; eluent, heptane/EtOAc, 85/15; flow rate 5.0 mL/min] gave pure labeled *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane ([^{18}F]-**19a**), retention time: 8.9 min.

The above HPLC-collected *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane ([^{18}F]-**19a**) was concentrated to dryness (at 60–80 °C under a gentle nitrogen stream for 4–6 min) and the residue was dissolved in 2 mL of CH_2Cl_2/TFA (9/1 v/v). The mixture was allowed to stand without stirring at room temperature for 2 min and was then concentrated to dryness (at 60–80 °C under a gentle nitrogen stream for 4–6 min). The yield of deprotection was quantitative: No Boc-protected 2-[^{18}F]fluoropyridine derivative [^{18}F]-**19a** could be detected by radiochromatography (SiO_2-TLC; eluent heptane/EtOAc, 30/70, R_f: *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane ([^{18}F]-**19a**): 0.55 and *exo*-2-(2′-[^{18}F]-fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane: [^{18}F]-**6b**: 0.0). The above residue was redissolved in 2 mL of

CH_2Cl_2 and concentrated again to dryness to minimize TFA presence (at 60–80 °C under a gentle nitrogen stream for 4–6 min). Finally, the residue was redissolved in 2 mL of the HPLC solvent used for purification and the crude was injected onto HPLC. Isocratic elution [HPLC B; eluent: water/acetonitrile/TFA 90/10/0.15; flow rate: 5.0 mL/min] gave pure labeled *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane ([^{18}F]-**6b**), retention time: 7.7 min.

Microwave heating. The procedure described above was slightly modified: the 2 mL reaction vial was replaced by a Pyrex tube. This tube, not sealed, was placed in the microwave oven. Microwaves (power *W* and during a time *t*) were then applied to the system. The remainder of the synthesis used the same procedure as described above.

P/(*N*): *exo*-2-(2′-bromo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19b**)/14.1 to 31.4 μmol; *exo*-2-(2′-iodo-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptane (**19c**)/14.0 to 15.0 μmol; *exo*-2-(2′-nitro-5′-pyridyl)-7-*tert*-butoxycarbonyl-7-azabicyclo-[2.2.1]heptane (**19d**)/19.6 to 23.0 μmol.

W: 100 Watt; *t*: 1 to 2.5 min.

The whole synthesis procedure (included the HPLCs) is fully automated on a computer assisted Zymate robot system (Zymark corporation, USA).

Formulation and quality control of *exo*-2-(2′-[^{18}F]fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane ([^{18}F]-6b). Formulation of labeled product for **iv** injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) taking up the residue in 5 mL of physiological saline. Animal injections were always done within 15 min after End of Synthesis, in PET-as well as in mouse/rat biodistribution experiments.

The product was found to be >98% chemically and radiochemically pure, as demonstrated by HPLC analysis. It was also shown to be radiochemically stable for at least 180 min in physiological saline.

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