Synthesis and Nicotinic Acetylcholine Receptor in Vivo Binding Properties of 2-Fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine: A New Positron Emission Tomography Ligand for Nicotinic Receptors

Frédéric Dollé,* Lilian Dolci, Héric Valette, Françoise Hinnen, Françoise Vaufrey, Ilonka Guenther, Chantal Fuseau, Christine Coulon, Michel Bottlaender, and Christian Crouzel

Département de Recherche Médicale, Service Hospitalier Frédéric Joliot, CEA/DSV, 4 Place du Général Leclerc, F-91401 Orsay, France

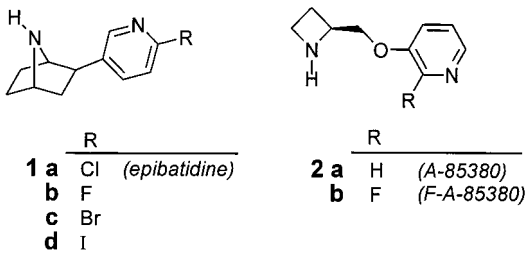
Received February 9, 1999

The lead compound of a new series of 3-pyridyl ethers, the azetidine derivative A-85380 (3-[(*S*)-2-azetidinylmethoxy]pyridine), is a potent and selective ligand for the human α4*β*2 nicotinic acetylcholine receptor (nAChR) subtype. In vitro, the fluoro derivative of A-85380 (2-fluoro-3-[(*S*)-2-azetidinylmethoxy]pyridine or F-A-85380) competitively displaced [^{3}H]cytisine or [^{3}H]-epibatidine with K_i values of 48 and 46 pM, respectively. F-A-85380 has been labeled with the positron emitter fluorine-18 ($t_{1/2}$ (half-life) = 110 min) by no-carrier-added nucleophilic aromatic substitution by K[^{18}F]F-K_{222} complex with (3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]-pyridin-2-yl)trimethylammonium trifluoromethanesulfonate as a highly efficient labeling precursor, followed by TFA removal of the Boc protective group. The total synthesis time was 50−53 min from the end of cyclotron fluorine-18 production (EOB). Radiochemical yields, with respect to initial [^{18}F]fluoride ion radioactivity, were 68−72% (decay-corrected) and 49−52% (non-decay-corrected), and the specific radioactivities at EOB were 4−7 Ci/*μ*mol (148−259 GBq/*μ*mol). In vivo characterization of [^{18}F]F-A-85380 showed promising properties for PET imaging of central nAChRs. This compound does not bind in vivo to α7 nicotinic or $5HT_3$ receptors. Moreover, its cerebral uptake can be modulated by the synaptic concentration of the endogenous ligand acetylcholine. The preliminary PET experiments in baboons with [^{18}F]F-A-85380 show an accumulation of the radiotracer in the brain within 60 min. In the thalamus, a nAChR-rich area, uptake of radioactivity reached a maximum at 60 min (4% I.D./100 mL of tissue). [^{18}F]F-A-85380 appears to be a suitable radioligand for brain imaging nAChRs with PET.

Introduction

The alkaloid (−)-epibatidine (**1a**), a natural product isolated from South American frogs (Chart 1), was discovered in the 1990s as a non-opioid analgesic agent with a potency 200-fold greater than that of morphine in mice[1] and was later shown to exert its antinociceptive action via a neuronal nicotinic acetylcholine receptor (nAChR)-mediated mechanism.[2,3] Extensive pharmacological evaluations have demonstrated that **1a** is a highly potent agonist at several nAChR subtypes, including α4*β*2 and α7, predominant subtypes in the central nervous system, as well as at nAChRs in peripheral autonomic ganglia and skeletal muscle (reviewed in ref 4). Halogen analogues **1b**−**d** (*exo*-2-(2′-halo-5′-pyridyl)-7-azabicyclo[2.2.1]heptane) have been synthesized,[5−10] and their in vivo binding properties have been characterized in mice, rats, and baboons.[11−15] Both their brain distribution and their pharmacological characteristics indicate that for positron emission tomography (PET) as well as for single-photon emission computed tomography (SPECT) radiolabeled forms of epibatidine are exceptionally promising ligands for the study of nAChRs in vivo. However, in rodents, derivative **1a** is toxic at doses only slightly higher than its effective analgesic dose.[16−18] Although the estimated mass of epibatidine derivatives **1b**−**d** to image nAChRs in humans is far below pharmacologically active doses, this toxicity warrants a cautious approach to its potential use in humans.[19]

Chart 1

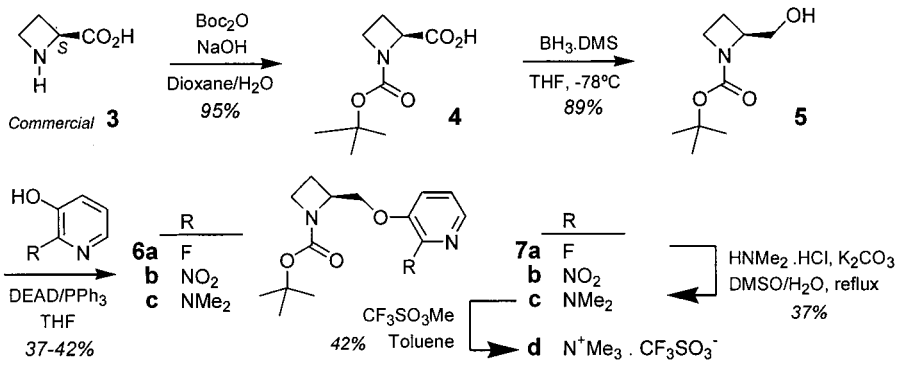


Recently, a new series of 3-pyridyl ether compounds was described.[20] This series not only possesses subnanomolar affinity for brain nAChRs and differentially activates subtypes of neuronal nAChR but also shows a satisfactory safety profile.[21] The lead compound of this series, the azetidine-based derivative A-85380 (**2a**, 3-[(*S*)-2-azetidinylmethoxy]pyridine; Chart 1), is a potent and selective ligand for the human α4*β*2 nAChR subtype (K_i: 50 pM) relative to the human α7 subtype (148 nM).[22] Closely related halo analogues of A-85380 (**2a**) have also been reported as selective nAChR ligands.[23] The novel structural features of these ligands

* Author for correspondence: Dr. Frédéric Dollé. Phone: (33) 1 69 86 77 25 or (33) 1 69 86 77 23. Fax: (33) 1 69 86 78 68. E-mail: dolle@dsvidf.cea.fr.

10.1021/jm9910223 CCC: $18.00
Published on Web 05/29/1999

Scheme 1



retain the high potency of epibatidine (**1a**) and impart a selectivity not observed with epibatidine. In vitro, the fluoro derivative of A-85380 (2-fluoro-3-[(*S*)-2-azetidinylmethoxy]pyridine or F-A-85380, **2b**; Chart 1) competitively displaced [^{3}H]cytisine and [^{3}H]epibatidine with K_i values of 48 pM[23] and 46 pM,[24] respectively. These values are similar to those measured using either the parent compound (50 pM against [^{3}H]cytisine[22] and 17 pM against [^{3}H]epibatidine[24]) or epibatidine (40 pM against [^{3}H]cytisine[22]). The iodo derivative of A-85380, 5-iodo-3-[(*S*)-2-azetidinylmethoxy]pyridine, also displays high affinity for nAChRs (11 pM[24]). The high selectivities for the $\alpha4\beta2$ subtype also avoid the action on ganglionic-type nAChRs which likely mediate untoward effects of nicotinic agonists such as epibatidine and its analogues.[19] Preliminary published results showed that positron and single-photon emitter radiolabeled halogenated derivatives of A-85380 are likely to be suitable ligands for imaging nAChRs in vivo using PET or SPECT.[25−29]

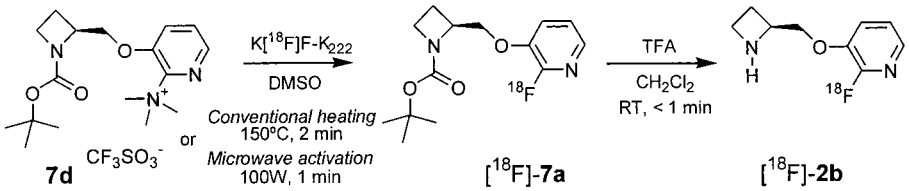
In this report, we present the synthesis of both the closely related fluoro analogue of the nicotinic ligand A-85380, 2-fluoro-3-[(*S*)-2-azetidinylmethoxy]pyridine (F-A-85380, **2b**), and highly efficient precursors for the synthesis of fluorine-18 ($t_{1/2}$ = 110 min)-labeled **2b**, namely, 2-nitro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**7b**) and (3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridin-2-yl)trimethylammonium trifluoromethanesulfonate (**7d**). The radiosynthesis of [^{18}F]-**2b** using a no-carrier-added nucleophilic aromatic substitution with K[^{18}F]F-K_{222} complex is also fully described, and the efficiencies of the labeling precursors used are compared. We also report results on the in vivo binding properties of the ligand F-A-85380 (**2b**) and describe aspects of the brain kinetics of the ligand using PET. Particular attention was also given to clarify some of its pharmacological characteristics, such as rodent interspecies differences (mice versus rats) as well as the possible effects of the anesthesia. Despite similar affinities in vitro, comparison of the in vivo kinetics of halogenated derivatives of epibatidine and A-85380 presents some unexplained differences in mice, such as: a lower binding in the thalamus and a slower clearance from the cerebellum for the fluoro derivative of A-85380, which could both be explained by differences in the lipophilicity of these compounds or nonspecific binding (i.e., binding to other central receptors). Since A-85380 had some affinity for α7 nicotinic receptors (K_i vs [^{125}I]α-bungarotoxin 148 nM[22]), we investigated in vivo if F-A-85380 had some affinity for central α7 nAChRs. Aside from that, since nicotinic agonists (such as nicotine and epibatidine) also competitively antagonize (in the micromolar range) serotonin at $5HT_3$ receptors (which share up to 30% sequence homology with nicotinic receptors[30]), we verified in vivo if the binding of F-A-85380 was modified by administration of a $5HT_3$ antagonist. Moreover, a study was performed to assess if the in vivo uptake of [^{18}F]F-A-85380 was modified by the increase of acetylcholine (the endogenous ligand at nAChRs) concentration. Finally, the cerebral kinetics of [^{18}F]F-A-85380 in awake and isoflurane-anesthetized rats were compared, since volatile halogenated anesthetic agents have been shown to stabilize the slow desensitized conformational state of the $\alpha4\beta2$ nAChRs, an inactive state characterized by high affinity for agonists.[31,32]

Results and Discussion

Chemistry. The convergent approach reported by Abreo et al.,[20] involving a Mitsunobu coupling between a *N*-protected azetidinemethanol and a hydroxypyridine, is not only the shortest synthesis reported but also the approach which allows the preparation of various derivatives of A-85380 in the last chemical steps, minimizing therefore the number of intermediate compounds.

Protection of the amino function of commercially available (*S*)-2-azetidinecarboxylic acid (**3**) using *tert*-butyl dicarbonate (1.1 equiv) in dioxane/water (2/1, v:v) containing 1 equiv of NaOH gave **4** in nearly quantitative yield (95%) (Scheme 1). The Boc derivative **4** was cleanly reduced by an excess of diborane−dimethyl sulfide complex (5 equiv) in THF to give the azetidinemethanol **5** (89% yield). 2-Fluoro-3-hydroxypyridine (**6a**) was synthesized in 63% yield, from commercially available 2-amino-3-hydroxypyridine, $NaNO_2$, and aqueous fluoboric acid at 0 °C for 1 h using a modified Schiemann reaction[33−35] described for the synthesis of 2-fluoropyridines.[36] Mitsunobu coupling[37,20] of alcohol **5** and 2-fluoro-3-hydroxypyridine (**6a**) using diethyl azodicarboxylate (1.6 equiv) and triphenylphosphine (1.5 equiv) in THF at room temperature gave the ether **7a** in moderate yield (42%). TFA removal of the *tert*-butoxycarbonyl function gave the desired amine **2b** in 96% yield. Concerning derivatives **7b** and **7c**, the key ether-forming step was carried out under similar Mitsunobu conditions as described above for the synthesis of derivative **7a**. Coupling of azetidinemethanol derivative **5** and commercially available 2-nitro-3-hydroxypyridine **6b** or 2-(dimethylamino)-3-hydroxypyridine **6c**

Scheme 2



(synthesized from **6a** in 25% yield using $HNMe_2$·HCl (1.5 equiv), K_2CO_3 (1.5 equiv) in refluxing DMSO/H_2O (3/1 v:v, 110−115 °C) for 24 h) gave the ethers **7b** and **7c** in 40% and 37% yields, respectively. Dimethylamino derivative **7c** was also obtained from **7a** using $HNMe_2$·HCl (1.5 equiv), K_2CO_3 (1.5 equiv) in refluxing DMSO/H_2O (3/1 v:v) in 37% yield. Trimethylammonium derivative **7d** was obtained from **7c** by methylation using methyl trifluoromethanesulfonate (1.45 equiv) in toluene at room temperature (42% yield).

Radiochemistry. In a first approach, radiolabeled 2-[^{18}F]fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine ([^{18}F]-**2b**) was synthesized by (1) nucleophilic aromatic nitro-to-fluoro[29] substitution, followed by (2) TFA removal of the *tert*-butoxycarbonyl protective group. The first radiochemical step consists of the introduction of the fluorine-18 using a no-carrier-added nucleophilic aromatic substitution with K[^{18}F]F-K_{222} (K_{222}: 4,7,13,16,-21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) at the 2-position of the pyridyl ring. The nitro function was 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 K[^{18}F]F-Kryptofix K_{222} complex as the fluorinating reactant, in DMSO as the solvent, by (1) conventional heating (nonstirred sealed reactor, placed in a heating block at 150 °C for 20 min) or (2) microwave activation (100 W for 1 min). After Sep-pak separation, the Boc-protected 2-[^{18}F]fluoropyridine derivative [^{18}F]-**7a** was purified by HPLC. The chemical yields of fluorine incorporation using either procedure were comparable and varied from 70% to 90%. Removal of the *tert*-butoxycarbonyl function in a 10/1 mixture of CH_2Cl_2/TFA at room temperature for 2 min was quantitative. Final HPLC purification gave pure amine [^{18}F]-**2b** in 105−110 min (microwave activation process) or 125−130 min (conventional heating process) synthesis time. Yields were, with respect to initial [^{18}F]fluoride ion radioactivity: decay-corrected, 48−64%; non-decay-corrected, microwave activation and conventional heating process 25−33% and 22−28%, respectively.

Recently, an alternative radiochemical synthesis of [^{18}F]-**2b** was developed, based on the same two-step approach as described above but using a highly efficient nucleophilic aromatic trimethylammonium-to-fluoro substitution (Scheme 2). Radiofluorination of **7d** was investigated as before using either conventional heating (in a nonstirred, nonsealed reactor, placed in a heating block at 145−150 °C for 2 min) or microwave activation (at 100 W for 1 min). The chemical yields of fluorine incorporation using either procedure were comparable and varied from 85% to 95%. Quantitative removal of the *tert*-butoxycarbonyl protective group (in a 50/1 mixture of CH_2Cl_2/TFA at room temperature for 10 s) was performed after Sep-pak separation but without intermediate HPLC purification of the Boc-[^{18}F]fluoropyridine derivative [^{18}F]-**7a**. Final HPLC purification gave pure amine [^{18}F]-**2b** in 43−46 min synthesis time (50−53 min from the end of cyclotron fluorine-18 production (EOB)). Yields were, with respect to initial [^{18}F]fluoride ion radioactivity: decay-corrected, 68−72%; non-decay-corrected, 49−52%.

Direct Boc-deprotection of the non-HPLC-purified 2-[^{18}F]fluoropyridine derivative [^{18}F]-**7a** not only shortened the procedure but also led to simplification to an automated, computer-assisted radiosynthesis without changes in the purity of amine [^{18}F]-**2b**. Irrespective of the radiochemical process (conventional heating or microwave activation) or precursor (nitro or trimethylammonium trifluoromethanesulfonate) employed, no significant differences could be observed in terms of decay-corrected specific radioactivities for [^{18}F]-**2b**. The use of highly efficient precursors such as the nitro derivative **7b** and especially the trimethylammonium trifluoromethanesulfonate derivative **7d**, compared to 2-iodo-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]-pyridine,[38] is undoubtedly the reason for the high radiochemical yields described for the two-step radiosynthesis of [^{18}F]-**2b**.

Typically, starting from a 190 mCi (7.0 GBq) aliquot of a cyclotron [^{18}F]F^- production batch, 95−100 mCi (3.5−3.7 GBq) of pure 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]F-A-85380, [^{18}F]-**2b**) could be obtained in 50−55 min, with specific radioactivities of 4−7 Ci/μmol (148−259 GBq/μmol) calculated for end of bombardment (or 3−5 Ci/μmol (111−185 GBq/μmol) at end of synthesis) for a 20 μA, 30 min (36 000 μC) irradiation of a 95% enriched [^{18}O]water target with a 17 MeV proton beam [^{18}O(p,n)^{18}F].

Formulation of labeled product for i.v. injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) taking up the residue in 5 mL of physiological saline. 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. Administration to animals was always done within 15 min after end of synthesis, in PET as well as mouse/rat biodistribution experiments.

Pharmacology. 1. Biodistribution Studies in Rodents. Preliminary results in rats showed a substantial uptake of [^{18}F]F-A-85380 (i.v. injection of 30 μCi (1.1 MBq)) in the brain. Thalamic (a nAChR-rich area[39]) uptake of radioactivity peaked at 60 min (1.0% I.D./g of tissue). The cerebellum is a nAChR-poor area which contains a density of receptors which is 60% lower than that of thalamus in rats using epibatidine or 85% using cytisine as tritiated ligands.[39] Radioactivity in thalamus and cerebellum decreased rapidly, but the ratio of radioactivity thalamus/cerebellum increased with time

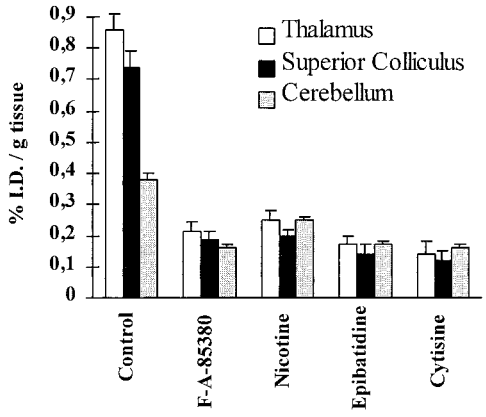


Figure 1. Pretreatment experiments with nicotinic agonists in rats (Sprague−Dawley, $n = 5$ for each experiment, mean ± standard deviation): control, pretreatment with F-A-85380 (0.5 mg/kg i.v. given 50 min before the radiotracer), nicotine (5 mg/kg s.c. given 5 min before the radiotracer), epibatidine (5 μg/kg s.c. given 60 min before the radiotracer), cytisine (5 mg/kg s.c. given 30 min before the radiotracer). Animals received 30 μCi (1.1 MBq) i.v. of [^{18}F]F-A-85380 and were sacrificed 60 min after injection of the tracer.

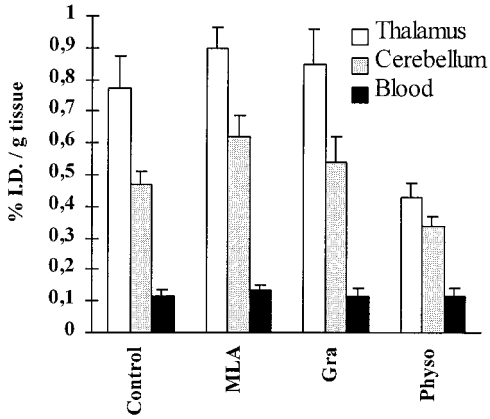


Figure 2. Complementary pretreatment experiments in rats (Sprague−Dawley, $n = 3$ for each experiment, mean ± standard deviation): control, pretreatment with methyllycaconitine (MLA)—a nicotinic α7 antagonist (2 mg/kg i.p. given 20 min before the radiotracer), granisetron (Gra)—a $5HT_3$ receptor antagonist (1 mg/kg s.c. given 30 min before the radiotracer), physostigmine (Physo) (300 μg/kg s.c. given 30 min before the radiotracer). Animals received 30 μCi (1.1 MBq) i.v. of [^{18}F]F-A-85380 and were sacrificed 60 min (methyllycaconitine, granisetron) and 30 min (physostigmine) after injection of the tracer.

(1.8 and 3.5 at 60 and 180 min, respectively, postinjection). The relatively low cerebral uptake prohibits metabolite determination in rat brain. Preliminary results in mice also showed a substantial uptake of [^{18}F]F-A-85380 (i.v. injection of 10 μCi (0.4 MBq)) in the brain. However, the thalamic uptake in rats is 7-fold lower than that measured in mice.[25] This difference between rats and mice is likely due to the difference in the input function and/or in the peripheral volume of distribution: 30 min after injection of the tracer, blood radioactivity was 1.2% I.D./g of tissue in mice, whereas it was only 0.25% I.D./g of tissue in rats. Furthermore, the washout from the cerebellum in mice[25] was faster than in rats. Thalamic, superior colliculus (another nAChR-rich area[39]), and cerebellum uptakes were reduced by 65−80%, 78−85%, and 45−70%, respectively, following a pretreatment with nicotine, epibatidine, cytisine, or F-A-85380 (Figure 1). The parent compound A-85380 had some affinity for α7 nicotinic receptors, but no significant affinity for nicotinic α7 receptors was found in vivo (Figure 2) for the fluoro derivative F-A-85380. Pretreatment with the antagonist methyllycaconitine (2 mg/kg i.p.[40]) did not change cerebral uptake of [^{18}F]F-A-85380 in rats. Aside from that, the cerebral uptake of F-A-85380 was not modified by administration of granisetron (1 mg/kg s.c.[41]), a $5HT_3$ antagonist. A study was also performed to assess the ability of changes in the endogenous ligand concentration to modify the cerebral uptake of [^{18}F]F-A-85380. In microdialysis experiments in rats, a dosage of 300 μg/kg of the acetylcholinesterase inhibitor physostigmine was shown to induced a large increase in acetylcholine.[42] In the same rat species, pretreatment with physostigmine (300 μg/kg s.c.) decreased thalamic uptake of the tracer by 45%.

2. Effects of Anesthesia. Volatile halogenated anesthetic agents have been shown to stabilize the slow desensitized conformational state of the α4β2 nAChRs, an inactive state characterized by high affinity for agonists.[31,32] In rats, the thalamic and cerebellar kinetics of [^{18}F]F-A-85380 were completely modified by isoflurane, the time−activity curve becoming almost flat and the level of uptake being reduced by 50% (Figure 3). This difference between awake and anesthetized rats is likely due to the difference in the input function: 30 min after injection of the tracer, blood radioactivity was 0.25% I.D./g of tissue in awake rats, whereas it was only 0.10% I.D./g of tissue in anesthetized rats. Similar observations can be made when comparing the results already published with another nAChR radioligand, [^{18}F]fluoronorchloroepibatidine in baboons. On one hand, in *Papio anubis* baboons anesthetized with alfaxolone acetate and alfadolone (steroid derivatives),[12] a plateau was observed in the thalamus (1.4% I.D./100 mL of tissue) 40 min after injection of the tracer. These neurosteroids do not affect binding properties of [^{3}H]-nicotine in vitro.[43] On the other hand, in *P. anubis* baboons anesthetized with isoflurane, completely different brain kinetics were observed.[11] Radioactivity (6% I.D./100 mL of tissue) in the thalamus peaked at 5−10 min after injection of the radiotracer. Clearances from the thalamus and the cerebellum were faster. These discrepancies underline the possible effects of the anesthetic agent isoflurane.

3. PET Data. Brain PET images with [^{18}F]F-A-85380 (i.v. injection of 1 mCi (37 MBq or 0.5 nmol)) were performed in isoflurane-anesthetized *Papio papio* baboons. Thalamic uptake of radioactivity peaked at 60−80 min (4.0% I.D./100 mL of tissue). At that time the ratio of radioactivity thalamus/cerebellum was 2 (Figure 4). Injection of unlabeled F-A-85380 (0.3 mg/kg i.v.) at 80 min displaced 60% and 35% of the radioactivity in the thalamus and the cerebellum, respectively (Figure 5). [^{18}F]F-A-85380 shows a relatively slow metabolism in baboons: 60% of the ligand was still present in the plasma 40−80 min postinjection (Figure 4). When compared to the kinetics of [^{18}F]fluoronorchloroepibatidine in the same species (also anesthetized with isoflurane), the main difference is the lower thalamic uptake observed 80 min postinjection: 4% I.D./100 mL of tissue for [^{18}F]F-A-85380, 10% I.D./100 mL of tissue for [^{18}F]fluoronorchloroepibatidine.[8] The cerebellar up-

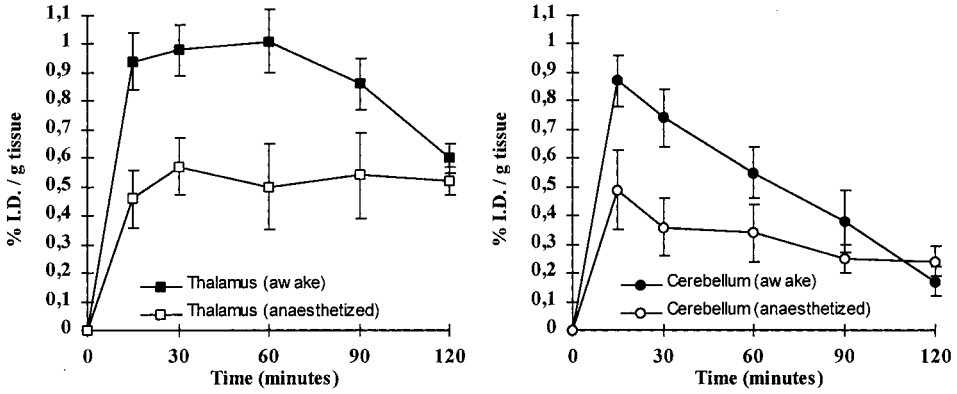


Figure 3. Influence of anesthetic on the thalamic (■,□) and cerebellar (●,○) uptakes of [^{18}F]F-A-85380 in rats (Sprague−Dawley, $n = 3$ for each experiment, mean ± standard deviation): awake (black symbols); anesthetized (white symbols) with isoflurane/N_2O (66%) (with a mimimum alveolar concentration of isoflurane of 1%). Animals received 30 μCi (1.1 MBq) i.v. of [^{18}F]F-A-85380 and were sacrificed at selected times after injection of the tracer.

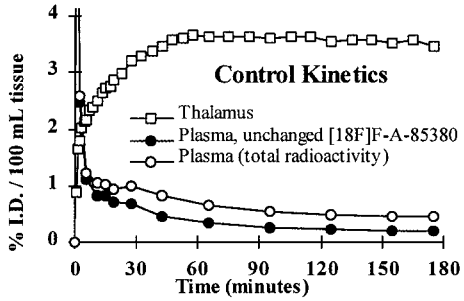


Figure 4. Thalamus PET time activity curve (□) measured in baboon (*Papio papio*) following i.v. injection of 1 mCi (37 MBq or 0.5 nmol) of [^{18}F]F-A-85380: control kinetics (□); plasma, total radioactivity (○), and unchanged [^{18}F]F-A-85380 plasma concentration (●).

take was a little lower for [^{18}F]F-A-85380 (2.3% I.D./100 mL of tissue versus 3% I.D./100 mL of tissue for [^{18}F]fluoronorchloroepibatidine, 80 min postinjection.[8]) The iodo derivative of A-85380[28] is more lipophilic than F-A-85380; [^{123}I]I-A-85380 displayed a higher thalamic uptake in Rhesus monkey anesthetized with neurosteroids (7% I.D./100 mL of tissue, 90 min postinjection) than that observed with [^{18}F]F-A-85380, but the cerebellar uptake was in the same order of value (2% I.D./100 mL of tissue, 90 min postinjection). The main difference between all these epibatidine-like or A-85380 halogenated derivatives is the thalamic uptake level. They all display a relatively high, but partially displaceable, uptake in the cerebellum, despite a low density of nAChRs in this structure.

Conclusion

In summary, an efficient synthesis of closely related derivatives of the nicotinic ligand A-85380 has been developed. 2-Fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine (**2b**) as reference compound as well as 2-nitro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**7b**) and especially (3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridin-2-yl)trimethylammonium trifluoromethanesulfonate (**7d**) as efficient precursors for the synthesis of [^{18}F]-**2b** were synthesized in good overall yields. [^{18}F]F-A-85380 has been radiolabeled with fluorine-18 by nucleophilic aromatic substitution from the corresponding Boc-protected trimethylammonium trifluoromethanesulfonate or nitro precursor using K[^{18}F]F−K_{222} complex in DMSO by conventional heating (at 150 °C for 2 min) or by microwave activation (at 100 W for 1 min), followed by TFA removal of the protective group (total synthesis time, 50−53 min from the end of cyclotron fluorine-18 production (EOB); yields with respect to starting [^{18}F]fluoride ion, decay-corrected 68−72%, non-decay-corrected 49−52%; specific radioactivities at EOB, 4−7 Ci/μmol (148−259 GBq/μmol)). In vivo characterization of [^{18}F]F-A-85380 showed promising properties for PET imaging of central nAChRs. This compound does not bind in vivo to α7 nicotinic or $5HT_3$ receptors. Moreover, its cerebral uptake can be modulated by the synaptic concentration of the endogeneous ligand acetylcholine. However, both the observed interspecies differences and the impact of anesthesia make it difficult to predict the kinetics of the tracer [^{18}F]F-A-85380 in human brain.

Experimental Section

Chemistry. General. Chemicals were purchased from Aldrich, Fluka, or Sigma (France) and were used without further purification. TLC were run on precoated plates of silica gel 60F254 (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% ninhydrin solution in ethanol or in 1% aqueous $KMnO_4$ 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 (Ar). HPLCs were run on Waters systems equipped with a 510 pump and 440 UV detector or 481 & 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger-Müller counter. HPLC column and conditions: A, column semipreparative SiO_2, Nova-Pak HR silica, Waters (300 × 7.8 mm); porosity 6 μm; temperature room temperature; UV detection at λ 254 nm; B, column semipreparative C-18, Zorbax SB, Hewlett-Packard (250 × 9.4 mm); porosity 5 μm; temperature room temperature; UV detection at λ 254 nm; C, column preparative SiO_2, Zorbax Rx-SIL, Hewlett-Packard (250 × 21.3 mm); porosity 7 μm; temperature room temperature; UV detection at λ 254 nm; D, column semipreparative C-18, μBondapak, Waters (300 × 7.8 mm); porosity 10 μm; temperature room temperature; UV detection at λ 287 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 deuterated solvents (DMSO-d_6, $\delta = 2.50$ ppm; CD_2Cl_2, $\delta = 5.32$ ppm; CD_3OD, $\delta = 4.78$ and 3.30 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvents (DMSO-d_6, $\delta = 39.5$ ppm; CD_2Cl_2, $\delta = 53.8$ ppm; CD_3OD, $\delta = 49.0$ ppm) and/or TMS as internal standards

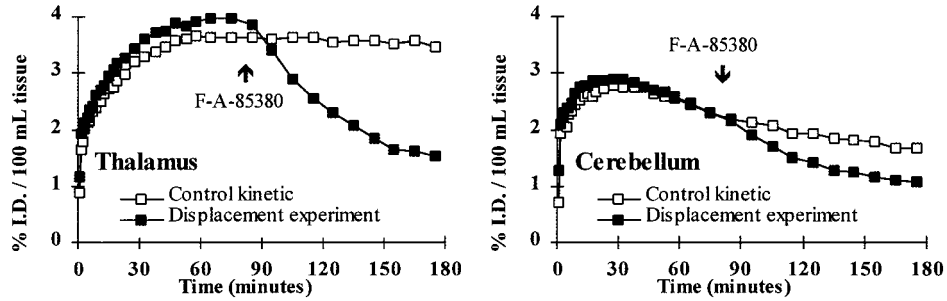


Figure 5. Thalamus and cerebellum PET kinetics measured in baboon (*Papio papio*) following i.v. injection of 1 mCi (37 MBq or 0.5 nmol) of [^{18}F]F-A-85380: control kinetics (□); displacement experiment in the same baboon (■), injection of unlabeled F-A-85380 (0.3 mg/kg, at 80 min) reduced thalamic and cerebellar radioactivities by 60% and 35%, respectively.

for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q, dd, m, b for singlet, doublet, triplet, quadruplet, doublet of doublet, multiplet (or multisharp-peak system) and broad, respectively). The mass spectra (MS), DCI/NH_4^+ were measured on a Nermag R10-10 apparatus. Elemental analyses, performed by the Service Central de Microanalyses du CNRS, 91190 Gif-sur-Yvette, France, were within 0.3% of the theoretical values calculated for C, H, N, and F. Air- or moisture-sensitive reactions were conducted in heat-gun-dried glassware, under an inert atmosphere and with freshly distilled solvents.

2-Fluoro-3-hydroxypyridine (6a). Synthesized from commercially available 2-amino-3-hydroxypyridine according to ref 29. R_f (EtOAc/heptane, 80/20): 0.65. Mp: 131 °C. ^{1}H NMR (DMSO-d_6, 298 K): δ 10.41 (s, 1H); 7.64 (td, J = 1.7 & 4.7 Hz, 1H); 7.42 (ddd, J = 1.7, 7.7 & 10.8 Hz, 1H); 7.17 (ddd, J = 1.3, 4.7 & 7.8 Hz, 1H). ^{13}C NMR (DMSO-d_6, 298 K): δ 152.8 (d, J^1_{F-C} = 233 Hz, C); 140.2 (d, J^2_{F-C} = 27 Hz, C); 135.6 (d, J^3_{F-C} = 13 Hz, CH); 126.2 (d, J^3_{F-C} = 5 Hz, CH); 122.6 (CH). MS (DCI/NH_4^+): C_5H_4FNO 131 [M + NH_4^+]; 114 [M + H^+]. Anal. (C_5H_4FNO) C, H, N.

2-(Dimethylamino)-3-hydroxypyridine (6c). To a solution of 2-fluoro-3-hydroxypyridine 29 (**6a**; 4.0 g, 35.4 mmol) in a mixture of DMSO (300 mL) and water (100 mL) at 10 °C were slowly added 4.35 g of $HNMe_2$·HCl (53.1 mmol, 1.5 equiv) and 7.34 g of K_2CO_3 (53.1 mmol, 1.5 equiv). After stirring at 10 °C for 30 min, the mixture was refluxed (110−115 °C) for 24 h and then diluted with water (500 mL). The product was extracted with Et_2O. The organic layers were combined, washed with water and brine, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was purified by passage through a silica gel column (eluent: heptane/EtOAc, 50/50) to give 1.25 g (25%) of crude **6c** as a solid (mp: 128 °C) which was used without further purification. R_f (EtOAc/heptane, 70/30): 0.40. ^{1}H NMR (CD_2Cl_2, 298 K): δ 7.86 (d, J = 3.0 Hz, 1H); 7.13 (d, J = 6.0 Hz, 1H); 6.91 (dd, J = 3.0 & 6.0 Hz, 1H); 2.75 (s, 6H). COSY experiments also clearly showed correlation peaks between the pyridinyl protons at δ 7.86 & 6.91 and 7.13 & 6.91. ^{1}H NMR (DMSO-d_6, 298 K): δ 9.58 (b, $w_{1/2}$ = 25 Hz, 1H); 7.66 (s, 1H); 7.00 (d, J = 6.0 Hz, 1H); 6.66 (s, 1H); 2.88 (s, 6H). ^{13}C NMR (CD_2Cl_2, 298 K): δ 153.5 (C); 145.9 (C); 139.2 (CH); 121.9 (CH); 120.5 (CH); 42.5 (CH_3). ^{13}C NMR (DMSO-d_6, 298 K): δ 151.7 (C); 143.7 (C); 137.1 (CH); 121.2 (CH); 115.8 (CH); 40.2 (CH_3). MS (DCI/NH_4^+): $C_7H_{10}N_2O$ 139 [M + H^+]. Anal. ($C_7H_{10}N_2O$) C, H, N.

General Procedure for the Mitsunobu Coupling of (*S*)-*N*-(*tert*-Butoxycarbonyl)-2-azetidinemethanol (5) with Various 2-Substituted-3-Hydroxypyridine Derivatives. To a solution of (*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinemethanol (**5**) (1.00 g, 5.3 mmol) in THF (20−30 mL), stirred under argon and cooled to 0 °C, were added 1.4 mL of diethyl azodicarboxylate (DEAD; *d*: 1.106, 8.6 mmol, 1.6 equiv) and 2.1 g of triphenylphosphine (8.0 mmol, 1.5 equiv). After stirring at 0 °C for 10−15 min, the appropriate 3-hydroxypyridine derivative (8.6 mmol, 1.6 equiv) was added. The mixture was stirred at room temperature overnight and then concentrated to dryness. The residue was chromatographed on silica gel. Elution with heptane/EtOAc gave the desired 3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine derivative.

2-Fluoro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (7a). The procedure described above was used with 2-fluoro-3-hydroxypyridine (**6a**; 0.97 g, 8.6 mmol) to give 630 mg (42%) of **7a** as a yellow oil after flash chromatography (eluent: heptane/EtOAc, 80/20 to 60/40), which may or may not solidify (mp: 48 °C). For analytical purposes, an aliquot (100 mg) was repurified on HPLC to give pure **7a** as a colorless oil [HPLC A; eluent: heptane/EtOAc, 60/40; flow rate: 6.0 mL/min; retention time: 7.5−9.0 min]. R_f (EtOAc/heptane, 50/50): 0.45. t_R (HPLC A; eluent: heptane/EtOAc, 60/40; flow rate: 6.0 mL/min): 8.5 min. ^{1}H NMR (CD_2Cl_2, 298 K): δ 7.72 (td, J = 1.5 & 4.8 Hz, 1H); 7.40 (ddd, J = 1.6, 7.6 & 8.5 Hz, 1H); 7.12 (dd, J = 4.8 & 7.5 Hz, 1H); 4.48 (m (6 peaks), 1H); 4.37 (bq, $w_{1/2}$: 19 Hz, 1H); 4.16 (dd, J = 2.7 & 10.2 Hz, 1H); 3.85 (t, J = 7.8 Hz, 2H); 2.32 (m (8 peaks), 2H); 1.39 (s, 9H). ^{13}C NMR (CD_2Cl_2, 298 K): δ 156.4 (C); 154.2 (d, J^1_{F-C} = 235 Hz, C); 142.7 (d, J^2_{F-C} = 26 Hz, C); 137.7 (d, J^3_{F-C} = 13 Hz, CH); 123.6 (d, J_{F-C} = 4 Hz, CH); 122.3 (d, J_{F-C} = 4 Hz, CH); 79.7 (C); 70.0 (CH_2); 60.5 (CH); 47.5 (CH_2); 28.5 (CH_3); 19.3 (CH_2). MS (DCI/NH_4^+): $C_{14}H_{19}FN_2O_3$ 300 [M + NH_4^+]; 283 [M + H^+]. Anal. ($C_{14}H_{19}FN_2O_3$) C, H, N.

2-Nitro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (7b). The procedure described above was used with 3-hydroxy-2-nitropyridine[29] (**6b**; 1.20 g, 8.6 mmol) to give 650 mg (40%) of **7b** as a yellow powder (mp: 73 °C) after flash chromatography (eluent: heptane/EtOAc, 75/25 to 60/40). R_f (EtOAc/heptane, 50/50): 0.25. ^{1}H NMR (CD_2Cl_2, 298 K): δ 8.06 (d, J = 6.0 Hz, 1H); 7.64 (d, J = 9.0 Hz, 1H); 7.55 (dd, J = 6.0 & 9.0 Hz, 1H); 4.60 (b, $w_{1/2}$ = 25 Hz, 1H); 4.50 (m (8 peaks), 1H); 4.19 (dd, J = 1.5 & 9.0 Hz, 1H); 3.81 (t, J = 9.0 Hz, 2H); 2.32 (q, J = 9.0 Hz, 2H); 1.37 (s, 9H). ^{13}C NMR (CD_2Cl_2, 298 K): δ 156.3 (C); 149.5 (C); 147.5 (C); 139.7 (CH); 129.0 (CH); 124.4 (CH); 79.8 (C); 69.8 (CH_2); 60.3 (CH); 47.9 (CH_2); 28.5 (CH_3); 19.0 (CH_2). MS (DCI/NH_4^+): $C_{14}H_{19}N_3O_5$ 327 [M + NH_4^+]; 310 [M + H^+]. Anal. ($C_{14}H_{19}N_3O_5$) C, H, N.

2-(Dimethylamino)-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (7c). The procedure described above was used with 2-(dimethylamino)-3-hydroxypyridine (**6c**; 1.19 g, 8.6 mmol) to give 600 mg (37%) of **7c** as a yellow oil after flash chromatography (eluent: heptane/EtOAc, 70/30 to 55/45). R_f (EtOAc/heptane, 70/30): 0.30. ^{1}H NMR (CD_2Cl_2, 298 K): δ 7.79 (d, J = 4.8 Hz, 1H); 7.05 (d, J = 7.8 Hz, 1H); 6.71 (dd, J = 4.8 & 7.8 Hz, 1H); 4.49 (b, $w_{1/2}$ = 20 Hz, 1H); 4.24 (b, $w_{1/2}$ = 25 Hz, 1H); 4.02 (dd, J = 2.4 & 8.0 Hz, 1H); 3.85 (t, J = 7.8 Hz, 2H); 2.99 (s, 6H); 2.33 (dd, J = 7.2 & 14.7 Hz, 2H); 1.38 (s, 9H). ^{13}C NMR (CD_2Cl_2, 298 K): δ 156.1 (C); 153.4 (C); 145.8 (C); 139.2 (CH); 119.0 (CH); 115.4 (CH); 79.4 (C); 69.0 (CH_2); 61.8 (CH); 47.2 (CH_2); 40.9 (CH_3); 28.5 (CH_3); 19.5 (CH_2). MS (DCI/NH_4^+): $C_{16}H_{25}N_3O_3$ 308 [M + H^+].

2-Fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine (2b). To a solution of 2-fluoro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**7a**) (0.4 g, 1.4 mmol) in CH_2Cl_2 (20 mL), cooled to 0 °C, was added 4 mL of TFA. The mixture was stirred at 0 °C for 1 h and then concentrated to dryness to give 405 mg of **2b** as its TFA salt (96%). For analytical purposes, an aliquot (200 mg) was purified on HPLC [HPLC B; eluent: acetonitrile/water/TFA, 10/90/0.15; flow rate: 6.0 mL/min; retention time: 4.5−5.5 min]. The fractions containing the desired amine were combined, basified with 1 N aqueous

NaOH, and extracted with CH_2Cl_2. The organic layers were combined, dried with Na_2SO_4, and concentrated (at 30 °C) to dryness to give **2b** as a colorless oil. This oil was diluted with diethyl ether, and a hydrochloric acid solution in diethyl ether was added dropwise. After concentration, the light-yellow residue was triturated/concentrated with two portions of diethyl ether to give **2b** as its HCl salt (mp: 126 °C). R_f ($CHCl_3$/MeOH/NH_4OH, 90/10/1): 0.15. t_R (HPLC B; eluent: acetonitrile/water/TFA, 10/90/0.15; flow rate: 6.0 mL/min): 5.0 min. t_R (HPLC B; eluent: acetonitrile/water/TFA, 5/95/0.15; flow rate: 6.0 mL/min): 9.5 min. ^{1}H NMR (HCl salt, CD_3OD, 298 K): δ 7.79 (d, J = 4.5 Hz, 1H); 7.68 (t, J = 9.6 Hz, 1H); 7.30 (dd, J = 5.1 & 8.1 Hz, 1H); 4.85 (m, partly masked by deuterated solvent, 1H); 4.45 (d, J = 6 Hz, 2H); 4.08 (m (6 peaks), 2H); 2.68 (m (10 peaks), 2H). COSY experiments were fully in accordance with the proposed structure and undoubtedly confirmed the presence of a hidden peak at δ 4.85. ^{1}H NMR (TFA salt, CD_2Cl_2, 298 K): δ 7.75 (d, J = 4.5 Hz, 1H); 7.38 (t, J = 8.7 Hz, 1H); 7.15 (dd, J = 5.1 & 7.8 Hz, 1H); 4.90 (b, $w_{1/2}$ = 24 Hz, 2H); 4.36 (bs, $w_{1/2}$ = 9 Hz, 2H); 4.13 (b, $w_{1/2}$ = 19 Hz, 2H); 2.69 (bt, $w_{1/2}$ = 24 Hz, 2H). ^{13}C NMR (free amine, CD_2Cl_2, 298 K): δ 154.1 (d, J^1_{F-C} = 235 Hz, C); 141.9 (d, J^2_{F-C} = 26 Hz, C); 138.0 (d, J^3_{F-C} = 11 Hz, CH); 123.9 (d, J_{F-C} < 2 Hz, CH); 122.4 (d, J_{F-C} < 2 Hz, CH); 72.0 (CH_2); 57.5 (CH); 43.9 (CH_2); 23.1 (CH_2). ^{13}C NMR (HCl salt, CD_3OD, 298 K): δ 154.9 (d, J^1_{F-C} = 236 Hz, C); 142.7 (d, J^2_{F-C} = 25 Hz, C); 139.2 (d, J^3_{F-C} = 13 Hz, CH); 125.2 (CH); 123.7 (CH); 68.9 (CH_2); 60.4 (CH); 44.9 (CH_2); 21.8 (CH_2). ^{13}C NMR (TFA salt, CD_2Cl_2, 298 K): δ 161.4 (q, J^2_{F-C} = 38 Hz, C, CF_3CO_2H); 154.0 (d, J^1_{F-C} = 237 Hz, C); 141.5 (d, J^2_{F-C} = 25 Hz, C); 138.6 (d, J^3_{F-C} = 12 Hz, CH); 124.5 (d, J_{F-C} < 2 Hz, CH); 122.8 (d, J_{F-C} < 2 Hz, CH); 116.3 (q, J^1_{F-C} = 290 Hz, C, CF_3CO_2H); 68.0 (CH_2); 59.6 (CH); 44.4 (CH_2); 21.2 (CH_2). MS (HCl salt, DCI/NH_4^+): $C_9H_{11}FN_2O$ 221 [M·HCl + H^+]; 219 [M·HCl + H^+]; 183 [M + H^+]. **2b**, hydrochloride: Anal. ($C_9H_{11}FN_2O$·1.1HCl·0.07Et_2O) C, H, N. **2b**, TFA salt: Anal. ($C_9H_{11}FN_2O$·1.6TFA·0.15CH_2Cl_2·0.5H_2O) C, H, N, F.

2-(Dimethylamino)-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (7c). From 2-fluoro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**7a**). To a solution of **7a** (0.60 g, 2.1 mmol) in a mixture of DMSO (30 mL) and water (10 mL) at 10 °C were added 260 mg of $HNMe_2$·HCl (3.15 mmol, 1.5 equiv) and 430 mg of K_2CO_3 (3.15 mmol, 1.5 equiv). After stirring at 10 °C for 10 min, the mixture was refluxed for 24 h and then diluted with water (50 mL). The product was extracted with Et_2O. The organic layers were combined, washed with water and brine, dried over anhydrous sodium sulfate, and concentrated to dryness. The residue was chromatographed on silica gel. Elution with heptane/EtOAc (60/40) gave 235 mg (37%) of **7c** as a yellow oil. For analytical purposes, an aliquot (100 mg) was repurified on preparative HPLC to give pure **7c** as a light-yellow oil [HPLC C; eluent: heptane/EtOAc, 60/40; flow rate: 40.0 mL/min; retention time: 8.0−9.0 min]. Analytical data, see above.

(3-[2(*S*)-*N*-(*tert*-Butoxycarbonyl)-2-azetidinylmethoxy]-pyridin-2-yl)trimethylammonium Trifluoromethanesulfonate (7d). To a solution of 200 mg of 2-(dimethylamino)-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]-pyridine (**7c**; 0.65 mmol) in 2 mL of toluene was added 0.11 mL of methyl trifluoromethanesulfonate (*d*: 1.45, 0.95 mmol, 1.45 equiv). The solution was stirred at room temperature for 1 h under an argon atmosphere and then diluted with 100 mL of water. The product was extracted with CH_2Cl_2. The organic layers were combined, washed with brine, dried over anhydrous sodium sulfate, and concentrated to dryness. Finally, the residue was triturated/concentrated with two portions of diethyl ether to give 120 mg (42%) of **7d** as a white powder. Mp: 115 °C. ^{1}H NMR (CD_2Cl_2, 298 K): δ 8.12 (d, J = 3.3 Hz, 1H); 7.75 (d, J = 8.1 Hz, 1H); 6.63 (b, $w_{1/2}$ = 15 Hz, 1H); 4.67 (b, $w_{1/2}$ = 20 Hz, 1H); 4.44 (bs, $w_{1/2}$ = 15 Hz, 2H); 4.00−3.75 (m (6 peaks), 2H); 3.73 (s, 9H); 2.45 (b, $w_{1/2}$ = 25 Hz, 1H); 2.20 (b, $w_{1/2}$ = 25 Hz, 1H); 1.40 (s, 9H). ^{13}C NMR (CD_2Cl_2, 298 K): δ 157.1 (C); 147.7 (C); 143.0 (C); 139.5 (CH); 129.1 (CH); 125.0 (CH); 121.4 (q, J = 320 Hz, CF_3); 80.3 (C); 71.8 (CH_2); 59.8 (CH); 54.7 (CH_3); 47.9 (CH_2); 28.4 (CH_3); 19.3 (CH_2). Anal. ($C_{18}H_{28}F_3N_3O_6S$·0.5H_2O) C, H, N.

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

Production of Aqueous [^{18}F]F^-. No-carrier-added aqueous [^{18}F]fluoride ion was produced on a CGR-MeV 520 cyclotron by irradiation of a 2 mL water target using a 20 MeV proton beam on 95% enriched [^{18}O]water [^{18}O(p,n)^{18}F] and was transferred to the appropriate hot cell. Typical production: 700−800 mCi (25.9−29.6 GBq) of [^{18}F]F^- at the end of bombardment for a 20 μA, 30 min (36 000 μC) irradiation. A complete description of the target hardware and operation can be found in ref 8.

Preparation of the K[^{18}F]F-K_{222} Complex. To recover and recycle the [^{18}O]water target, 2 mL of aqueous [^{18}F]fluoride from the target was passed through an anion-exchange resin (AG1 × 8, Bio-Rad, 100−200 mesh); see ref 8 for more practical details. The [^{18}F]fluoride ion was then eluted from the resin using 1.0 mL of a 4.5 mg/mL aqueous K_2CO_3 solution. After addition of 11.0−15.0 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 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 semisolid residue.

Preparation of 2-[^{18}F]Fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine ([^{18}F]-2b). From 2-nitro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**7b**): see ref 29. From (3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridin-2-yl)trimethylammonium trifluoromethanesulfonate (**7d**): freshly distilled DMSO (600 μL) containing 2.0−3.5 mg (6.5−11.3 μmol) of the labeling precursor **7d** was directly added into the tube containing the dried K[^{18}F]F-K_{222} complex. The tube (not sealed) was heated in a heating block without stirring at 145−150 °C during 2 min or placed in a microwave oven (microwaves, 100 W, were applied to the system for 1 min). The resulting yellow-brown reaction mixture was then cooled using a water bath, diluted with 3 mL of water, and passed through a C18 Sep-pak cartridge (3−7% of the total radioactivity amount engaged in the fluorination process was lost in the initial tube). The cartridge was washed with 3.0 mL of water (carrying off 5−15% of the total radioactivity amount engaged in the fluorination process) and partially dried for 0.5 min by applying a nitrogen stream. The Boc-protected 2-[^{18}F]fluoropyridine derivative [^{18}F]-**7a** was eluted from the cartridge (4−7% of the total radioactivity amount engaged in the fluorination process was lost on the cartridge) with CH_2Cl_2 (3 mL followed by two successive rinses of 1.0 mL) into a 5 mL reaction vial containing 0.1 mL of TFA.

Yields, defined as the 2-[^{18}F]fluoropyridine derivative over total fluorine-18 activity area ratio, were calculated from the TLC radiochromatogram (SiO_2 TLC, eluent: heptane/EtOAc, 50/50; R_f: [^{18}F]-**7a** 0.50 and [^{18}F]F^- 0.0) and confirmed after the Sep-pak elution by the CH_2Cl_2 over total radioactivity (DMSO/H_2O + CH_2Cl_2) eluted counting ratio. The yield of substitution varied from 85% to 95% with respect to initial [^{18}F]fluoride ion radioactivity. As demonstrated by HPLC analysis of aliquots, radiosynthesized Boc-protected 2-[^{18}F]-fluoropyridine derivative [^{18}F]-**7a** coeluted [HPLC A; eluent: heptane/EtOAc, 60/40; flow rate: 5.0 mL/min; retention time: 4.5−5.0 min] with an authentic sample of 2-fluoro-3-[2(*S*)-*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**7a**).

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 yield of deprotection was quantitative: No Boc-protected 2-[^{18}F]fluoropyridine derivative [^{18}F]-**7a** could be detected by radiochromatography (SiO_2 TLC, eluent: heptane/EtOAc, 50/50; R_f: [^{18}F]-**7a** 0.5 and [^{18}F]-**2b** 0.0). The above residue was redissolved in 2 mL of CH_2Cl_2 and concentrated again to dryness to minimize TFA presence (at 65−75 °C under a gentle nitrogen stream for 4−6 min). Finally, the residue was dissolved in 1−2 mL of the HPLC solvent used for

purification, and the crude was injected onto HPLC. Isocratic elution [HPLC B; eluent: acetonitrile/water/TFA, 10/90/0.15; flow rate: 6.0 mL/min) gave pure labeled 2-[^{18}F]fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine ([^{18}F]-**2b**). t_R: 5.5−6.0 min. Yield of [^{18}F]-**2b** (with respect to initial [^{18}F]fluoride ion radioactivity): decay-corrected 68−72%; non-decay-corrected 49−52%. Total synthesis time from EOB: 50−53 min (this includes the recovery of the [^{18}F]fluoride ion from the target and the K[^{18}F]F-K_{222} complex preparation).

Typically, starting from a 190 mCi (7.0 GBq) aliquot of a cyclotron [^{18}F]F^- production batch, 95−100 mCi (3.5−3.7 GBq) of pure 2-[^{18}F]fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine ([^{18}F]F-A-85380, [^{18}F]-**2b**) could be obtained in 50−55 min, with specific radioactivities of 4−7 Ci/μmol (148−259 GBq/μmol) calculated for end of bombardment (or 3−5 Ci/μmol (111−185 GBq/μmol) at end of synthesis) for a 20 μA, 30 min (36 000 μC) irradiation of a 95% enriched [^{18}O]water target with a 17 MeV proton beam [^{18}O(p,n)^{18}F]. The whole synthesis procedure (included the HPLCs) is fully automated on a computer-assisted Zymate robot system (Zymark Corp.).

Formulation of 2-[^{18}F]Fluoro-3-[2(*S*)-2-azetidinylmethoxy]pyridine ([^{18}F]-2b). Formulation of labeled product for i.v. injection was effected as follows: (1) HPLC solvent removal by evaporation; (2) taking up the residue in 5 mL of physiological saline. Administration to animals was always done within 15 min after end of synthesis, in PET as well as mouse/rat biodistribution experiments.

Quality Control. General. HPLC system consisted of two Shimadzu (Kyoto, Japan) LC-10AS pumps, a 2.6 mL mixing chamber, a Valco injector (model C6W; Vici Valco Instruments, TX) with a 1 mL loop, a reverse-phase semipreparative HPLC column (HPLC D) connected to a UV detector (Shimadzu SPD-10A) operated at 287 nm followed by a radioisotope detector (Berthold, Wildbad, Germany; model LB 506, 500 μL cell). A Berthold LB 5035 pump was used to add liquid scintillator (Quickszint Flow 302; Zinsser Analytic, Frankfurt, Germany) to the eluent just before the radioactivity detector. The data acquisition and handling were done on a PC using the software Winflow (version 1.21, JMBS Developments, Grenoble, France). The column (HPLC D) was eluted applying (1) a gradient from 5% acetonitrile (HPLC grade; SDS, Peypin, France) in 0.01 M aqueous phosphoric acid up to 35% in 7.5 min, (2) a gradient from 35% acetonitrile in 0.01 M aqueous phosphoric acid up to 50% in 2.0 min (total run length: 10 min). The flow rate of the eluent as well as the flow rate of the liquid scintillator were maintained at 6 mL/min (Quickszint Flow 302; Zinsser Analytic, Frankfurt, Germany).

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.

In Vivo Kinetic, Distribution, and Blocking Studies in Rodents. All animal use procedures were in accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (decree 87/848) for the care and use of laboratory animals. Biodistribution studies in male Sprague−Dawley rats (weighing 190−200 g) or Swiss mice (weighing 30−40 g) were performed as follows. The animals were sacrificed by decapitation at selected times after [^{18}F]F-A-85380 ([^{18}F]-**2b**) injection in a tail vein. The brain was quickly removed from the skull and dissected on ice. Samples were weighed and placed in small plastic tubes, and their radioactivity was determined, along with that of aliquots of the injected dose, in an automated gamma counter (CG4000, Kontron Instruments, Montigny le Bretonneux, France). Results are expressed as percent injected dose per gram of tissue (% I.D./g of tissue).

Positron Emission Tomography Data Acquisition. PET studies of the brain distribution and kinetics of [^{18}F]F-A-85380 ([^{18}F]-**2b**) were carried out in adult *P. papio* baboons (average weight: 8−10 kg). Animals were anesthetized with ketamine, intubated, and artificially respired with 1−1.5% isoflurane, 67% N_2O, 33% O_2. The PET experiment was performed with a CTI HR+ Exact positron tomograph (CTI PET Systems, Knoxville, TN). The baboon's head was positioned in the tomograph using a custom-designed stereotaxic headholder. 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, corrected for fluorine-18 decay and expressed as percent of the injected dose per 100 mL (% I.D./100 mL) of tissue. Arterial blood samples were withdrawn from a femoral artery at designated times. The blood and plasma fluorine-18 radioactivities were measured in an automated gamma counter (CG4000, Kontron Instruments, Montigny le Bretonneux, France).

Plasma Metabolite Studies. Arterial blood samples were withdrawn from a femoral artery at designated times and were immediately processed. For deproteinization, 0.5 mL of plasma was mixed with 0.7 mL of acetonitrile containing F-A-85380 (0.05 mg/mL) as reference compound. After centrifugation at 2000*g* for 5 min, the supernatant was removed and 1 mL was directly injected into the HPLC system for chromatographic determination of the radioactivity fraction that corresponds to unchanged [^{18}F]F-A-85380 ([^{18}F]-**2b**) (see Quality Control for the HPLC system).

Acknowledgment. The authors wish to thank cyclotron operators Mr. Daniel Gouel and Mr. Christophe Peronne for performing the irradiations, as well as the Section Analyses Instrumentation Spectroscopiques (Henri Virelizier) for MS recording.

References

(1) Spande, T. F.; Garaffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. Epibatidine: A Novel (Chloropyridyl)-azabicycloheptane with Potent Analgesic Activity from an Ecuadoran Poison Frog. *J. Am. Chem. Soc.* **1992**, *114*, 3475−3478.

(2) Qian, C.; Li, T.; Shen, T. Y.; Libertine-Garahan, L.; Eckman, J.; Biftu, T.; Ip, S. Epibatidine is a Nicotinic Analgesic. *Eur. J. Pharmacol.* **1993**, *250*, R13−R14.

(3) Badio, B.; Daly, J. W. Epibatidine, a Potent Analgesic and Nicotinic Agonist. *Mol. Pharmacol.* **1994**, *45*, 563−569.

(4) Sullivan, J. P.; Bannon, A. W. Epibatidine: Pharmacological Properties of a Novel Nicotinic Acetylcholine Receptor Agonist and Analgesic Agent. *CNS Drug Rev.* **1996**, *2*, 21−39.

(5) Horti, A.; Ravert, H. T.; London, E. D.; Dannals, R. F. Synthesis of a Radiotracer for Studying Nicotinic Acetylcholine Receptors: (±)-exo-2-(2-[^{18}F]fluoro-5-pyridyl)-7-azabicyclo[2.2.1]heptane. *J. Labelled Compds. Radiopharm.* **1996**, *38*, 355−365.

(6) Liang, F.; Navarro, H. A.; Abraham, P.; Kotian, P.; Ding, Y.-S.; Fowler, J.; Volkow, N.; Kuhar, M. J.; Carroll, F. I. Synthesis and Nicotinic Acetylcholine Receptor Binding properties of exo-2-(2′-fluoro-5′-pyridyl)-7-azabicyclo[2.2.1]heptane: A New Positron Emission Tomography ligand for Nicotinic Receptors. *J. Med. Chem.* **1997**, *40*, 2293−2295.

(7) Ding, Y.-S.; Liang, F.; Fowler, J. S.; Kuhar, M. J.; Carroll, F. I. Synthesis of [^{18}F]Norchlorofluoroepibatidine and its *N*-methyl Derivative: New PET Ligands for Mapping Nicotinic Acetylcholine Receptors. *J. Labelled Compds. Radiopharm.* **1997**, *39*, 827−832.

(8) Dolci, L.; Dollé, F.; Valette, H.; Vaufrey, F.; Fuseau, C.; Bottlaender, M.; Crouzel, C. Synthesis of a Fluorine-18 Labeled Derivative of Epibatidine for In Vivo Nicotinic Acetylcholine Receptor PET Imaging. *Bioorg. Med. Chem.* **1999**, *7*, 467−479.

(9) Loc'h, C.; Bottlaender, M.; Kassiou, M.; Musachio, J. L.; London, E. D.; Dannals, R. F.; Maziere, B. Evaluation of a Br-76 Analogue of Epibatidine for PET Imaging of Nicotinic Acetylcholine Receptors. *Eur. J. Nucl. Med.* **1997**, *24*, 913 (abstract 39.2).

(10) Musachio, J. L.; Horti, A.; London, E. D.; Dannals, R. F. Synthesis of a Radioiodinated Analogue of Epibatidine: (±)-exo-2-(2-iodo-5-pyridyl)-7-azabicyclo[2.2.1]heptane for in vitro and *in vivo* Studies of Nicotinic Acetylcholine Receptors. *J. Labelled Compds. Radiopharm.* **1997**, *39*, 39−48.

(11) Horti, A.; Scheffel, U.; Stathis, M.; Finley, P.; Ravert, H. T.; London, E. D.; Dannals, R. F. Fluorine-18-FPH for PET Imaging of Nicotinic Acetylcholine Receptors. *J. Nucl. Med.* **1997**, *38*, 1260−1265.

(12) Villemagne, V. L.; Horti, A.; Scheffel, U.; Ravert, H. T.; Finley, P.; Clough, D. J.; London, E. D.; Wagner, H. N.; Dannals, R. F. Imaging Nicotinic Acetylcholine Receptors with Fluorine-18-FPH, an Epibatidine Analogue. *J. Nucl. Med.* **1997**, *38*, 1737−1741.

(13) 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. Mapping Nicotinic Acetylcholine Receptors with PET. *Synapse* **1996**, *24*, 403−407.

(14) Gatley, J. S.; Ding, Y.-S.; Brady, D.; Gifford, A. N.; Dewey S. L.; Carroll, F. I.; Fowler, J. S.; Volkow, N. D. In Vitro and Ex Vivo Autoradiographic Studies of Nicotinic Acetylcholine Receptors Using [^{18}F]Fluoronorchloroepibatidine in Rodent and Human Brain. *Nucl. Med. Biol.* **1998**, *25*, 449−454.

(15) Musachio, J. L.; Villemagne, V. L.; Scheffel, U.; Stathis, M.; Finley, P.; Horti, A.; London, E. D.; Dannals, R. F. [$^{125/123}$I]IPH: a Radioiodinated Analogue of Epibatidine for in vivo Studies of Nicotinic Acetylcholine Receptors. *Synapse* **1997**, *26*, 392−399.

(16) Sullivan, J. P.; Decker, M. W.; Brioni, J. D.; Donnelly-Robert, D.; Anderson, D. J.; Bannon, A. W.; Kang, C.-H.; Adams, P.; Piattoni-Kaplan, M.; Buckley, M.; Gopalakrishnan, M.; Williams, M.; Arneric, S. P. (±)-Epibatidine Elicits a Diversity of In vitro and In Vivo Effects Mediated by Nicotinic Acetylcholine Receptors. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 624−631.

(17) Bonhaus, D. W.; Bley, K. R.; Broka, C. A.; Fontana, D. J.; Leung, E.; Lewis, R.; Shieh, A.; Wong, E. H. F. Characterization of the Electrophysiological, Biochemical and Behavioral Actions of Epibatidine. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 1199−1203.

(18) Rao, T. S.; Correa, L. D.; Reid, R. T.; Lloyd, G. K. Evaluation of Anti-nociceptive Effects of Neuronal Nicotinic Acetylcholine Receptors (nAChR) Ligands in the Rat Tail-Flick Assay. *Neuropharmacology* **1996**, *35*, 393−405.

(19) 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. Fluoro-norchloroepibatidine: Preclinical Assessment of Acute Toxicity. *Nucl. Med. Biol.* **1997**, *24*, 743−747.

(20) 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. Novel 3-Pyridyl Ethers with Subnanomolar Affinity for Central Neuronal Nicotinic Acetylcholine Receptors. *J. Med. Chem.* **1996**, *39*, 817−825.

(21) 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. Identification and Initial Structure−Activity Relationships of (R)-5-(2-Azetidinylmethoxy)-2-chloropyridine (ABT-594), a Potent, Orally Active, Non-Opiate Analgesic Agent Acting via Neuronal Nicotinic Acetylcholine Receptors. *J. Med. Chem.* **1998**, *41*, 407−412.

(22) 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. A-85380 [3-(2(S)-azetidinylmethoxy)pyridine]: In Vitro Pharmacological Properties of a Novel, High Affinity $\alpha4\beta2$ Nicotinic Acetylcholine Receptors Ligand. *Neuropharmacology* **1996**, *35*, 725−734.

(23) Holladay, M. W.; Bai, H.; Li, Y.; Lin, N. H.; Daanen, J. F.; Ryther, K. B.; Wasicak, J. T.; Kincaid, J. F.; Hettinger, A. M.; Huang, P.; Anderson, D. J.; Bannon, A. W.; Buckley, M. J.; Campbell, J. E.; Donnelly-Roberts, D. L.; Gunther, K. L.; Kim, D. J. B.; Kuntzweiler, T. A.; Sullivan, J. P.; Decker, M. W.; Arneric, S. P. Structure−Activity Studies Related to ABT-594, a Potent non Opioid Analgesic Agent: Effect of Pyridine and Azetidine Ring Substitutions on Nicotinic Acetylcholine Receptor Binding Affinity and Analgesic Activity in Mice. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2797−2802.

(24) Koren, A. O.; Horti, A. G.; Mukhin, A. G.; Gündish, D.; Kimes, A. S.; Dannals, R. F.; London, E. D. 2, 5, and 6-Halo-3(2(S)-azetidinylmethoxy)pyridines: Synthesis, Affinity for Nicotinic Acetylcholine Receptors and Molecular Modeling. *J. Med. Chem.* **1998**, *41*, 3690−3698.

(25) 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. 2-[^{18}F]Fluoro-A85380, an in Vivo Tracer for the Nicotinic Acetylcholine Receptors. *Nucl. Med. Biol.* **1998**, *25*, 599−603.

(26) Musachio, J. L.; Scheffel, U.; Finley, P. A.; Zhan, Y.; Mochizuki, T.; Wagner, H. N., Jr.; Dannals, R. F. 5-[I-125/123]Iodo-3(2(S)-Azetidinylmethoxy)pyridine, a Radioiodinated Analogue of A-85380 for in Vivo Studies of Central Nicotinic Acetylcholine Receptors. *Life Sci.* **1998**, *22*, 351−357.

(27) Vaupel, D. B.; Mukhin, A. S. K.; Horti, A. G.; Koren, A. O.; London, E. D. *In Vivo* Studies with [^{125}I]5-I-A-85380, a Nicotinic Acetylcholine Receptor Radioligand. *Neuroreport* **1998**, *9*, 2311−2317.

(28) Chefer, S. I.; Horti, A. G.; Lee, K. S.; Koren, A. O.; Jones, D. W.; Gorey, J. G.; Links, J. M.; Mukhin, A. G.; Weinberger, D. R.; London, E. D. In vivo Imaging of Brain Nicotinic Acetylcholine Receptors with 5-[^{123}I]Iodo-A-85380 using Single Photon Emisssion Computed Tomography. *Life Sci.* **1998**, *63*, 355−360.

(29) Dollé, F.; Valette, H.; Bottlaender, M.; Hinnen, F.; Vaufrey, F.; Guenther, I.; Crouzel, C. Synthesis of 2-[^{18}F]Fluoro-3-[2(S)-azetidinylmethoxy]pyridine, a Highly Potent Radioligand for in Vivo Imaging Central Nicotinic Acetylcholine Receptors. *J. Labelled Compds. Radiopharm.* **1998**, *41*, 451−463.

(30) Gurley, D. A.; Lanthorn, T. H. Nicotinic Agonists Competitively Antagonize Serotonin at Mouse 5-HT3 Receptors Expressed in Xenopus Oocytes. *Neurosci. Lett.* **1998**, *247*, 107−110.

(31) Violet, J. M.; Downie, D. L.; Nasika, R. C.; Lieb, W. R.; Franks, N. P. Differential Sensitivities of Mammalian Neuronal and Muscle Nicotinic Acetylcholine Receptors to General Anesthetics. *Anesthesiology* **1997**, *86*, 866−874.

(32) Flood, P.; Ramirez-LaTorre, J.; Role, L. $\alpha4\beta2$ Neuronal Nicotinic Acetylcholine Receptors in the Central Nervous System are Inhibited by Isoflurane and Propofol, but $\alpha7$-type Nicotinic Acetylcholine Receptors are Unaffected. *Anesthesiology* **1997**, *86*, 859−865.

(33) March, J. The Schiemann reaction. *Advanced Organic Chemistry*, 4th ed.; John Wiley & Sons: New York, Chichester, Brisbane, Toronto, Singapore, 1992; p 671.

(34) Suschitzky, H. The Balz-Schiemann reaction. In *Advances in Fluorine Chemistry*; Stacey, M., Tatlow, J. C., Sharpe, A. G., Eds.; Butterworth: Washington, DC, 1965; Vol. 4, pp 1−30.

(35) Roe, A. Preparation of aromatic fluorine compounds from diazonium fluoroborates: The Schiemann reaction. In *Organic Reactions*; Adam, R., Ed.; John Wiley & Sons: New York, 1949; Vol. 5, pp 193−228.

(36) Finger, G. C.; Starr, L. D.; Dickerson, D. R.; Gutowsky, H. S.; Hamer, J. Aromatic Fluorine Compounds. XI. Replacement of Chlorine by Fluorine in Halopyridines. *J. Org. Chem.* **1963**, *28*, 1666−1668.

(37) Mitsunobu, O. The use of diethyl azodicarboxylate and triphenylphosphine in synthesis and transformation of natural products. *Synthesis* **1981**, 1−28.

(38) Horti, A.; Koren, A. O.; Ravert, H. T.; Musachio, J. L.; Mathews, W. B.; London, E. D.; Dannals, R. F. Synthesis of a Radiotracer for Studying Nicotinic Acetylcholine Receptors: 2-[^{18}F]Fluoro-3-(2(S)-azetidinylmethoxy)pyridine (2-[^{18}F]A85380). *J. Labelled Compds. Radiopharm.* **1998**, *41*, 309−318.

(39) Perry, D. C.; Kellar, K. J. [^{3}H]Epibatidine Labels Nicotinic Receptors in Rat Brain: an Autoradiographic Study. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1030−1034.

(40) Tani, Y.; Saito, K.; Imoto, M.; Ohno, T. Pharmacological Characterization of Nicotinic Receptor-Mediated Acetylcholine Release in Rat Brain -an in Vivo Microdialysis Study. *Eur. J. Pharmacol.* **1998**, *351*, 181−188.

(41) Pei, Q.; Zetterström, T.; Leslie, R. A.; Grahame-Smith, D. G. 5-HT3 Receptor Antagonists Inhibit Morphine-Induced Stimulation of Mesolimbic Dopamine Release and Function in the Rat. *Eur. J. Pharmacol.* **1993**, *230*, 63−68.

(42) Cuadra, G.; Summers, K.; Giacobini, E. Cholinesterase Inhibitor Effects on Neurotransmitters in Rats Cortex in Vivo. *J. Pharmacol. Exp. Ther.* **1994**, *270*, 277−284.

(43) Bullock, A. E.; Clark, A. L.; Grady, S. R.; Robinson, S. F.; Slobe, B. S.; Marks, M. J.; Collins, A. C. Neurosteroids Modulate Nicotinic Receptor Function in Mouse Striatal and Thalamic Synaptosomes. *J. Neurochem.* **1997**, *68*, 2412−2423.

JM9910223