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Synthesis of 2-[^{18}F]Fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine, a Highly Potent Radioligand for *in Vivo* Imaging Central Nicotinic Acetylcholine Receptors

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Summary

This paper reports the synthesis of 2-fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine and its radiolabeling with fluorine-18 ([^{18}F]FK-K_{222}) 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. This fluoro compound is a closely related analog of the high affinity nicotinic ligand A-85380 (3-[2(S)-2-azetidinylmethoxy]pyridine). This compound is the lead compound of a novel 3-pyridyl ether series of new nAChR ligands recently published, and possesses not only subnanomolar affinity, comparable to that of epibatidine, for the $\alpha4\beta2$ subtype, but also a weaker affinity for the other subtypes of nAChRs. 110-140 mCi (4.1-5.2 GBq) of pure 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]fluoro-A-85380) could be obtained in less than 2 hours, with specific radioactivities of 3-5 Ci/μmol (111-185 GBq/μmol) calculated for End of Bombardment (or 1.5-2.5 Ci/μmol (55.5-92.5 GBq/μmol) at End of Synthesis) for a 20 μA, 30 min (36000 μC) irradiation of a 95% enriched [^{18}O]water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F]. Yields (with respect to [^{18}F]fluoride ion) : decay-corrected 49-64% ; non-decay-corrected 25-33%. Total synthesis time from EOB : 105-110 min (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation). Preliminary results in rats showed a substantial uptake of the ligand in the thalamus (1% I.D./g tissue at 30 min) while the cerebellar uptake was 2-fold lower. Thalamic uptake was reduced by 75-85% following a pre-treatment with nicotine, cytisine, epibatidine or fluoro-A-85380. The full pharmacological profile and the potential for eventual clinical applications of this ligand as a tracer for PET experiments are currently under investigation.

Key Words : A-85380, fluorine-18, positron emission tomography, nicotinic receptors

Introduction

The hypothesis that cholinergic dysfunction contributes to cognitive impairments in patients with senile dementia of the Alzheimer type or Parkinson disease[1] has prompted considerable exploration of Positron Emission Tomography (PET) radioligands in order to visualize nicotinic acetylcholine receptors (nAChRs) in human brain. Since a consistent and

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severe loss[2,3] of these receptors is found in these diseases, a radiotracer technique such as PET would find a practical use in the diagnosis of early stage disease as well as in the planning and the monitoring of the treatment of patients.

[^{11}C]Nicotine has been used in several PET centers[4-9]. Problems, such as rapid kinetics, flow dependence of the uptake and rapid metabolism, limit the use of nicotine as a convenient ligand for human brain imaging. More recently, potent specific nicotinic agonists such as ABT-418[10,11] ((S)-3-methyl-5-[1-methyl-2-pyrrolidinyl]isoxazole), *N*-methylcytisine[10,11] and A-84543[12] (3-[(1-methyl-2(S)-pyrrolidinyl)methoxy]pyridine) have been labeled with carbon-11 but *in vivo* pharmacological characterisations showed that these ligands were not suitable. Subsequent efforts have focused on epibatidine ((±)-exo-2-(6-chloro-3-pyridyl)-7-azabicyclo [2.2.1]heptane), a natural compound isolated from the skin of the Ecuadoran poison frog *Epipedobates tricolor*. A fluoro analog ((±)-exo-2-(6-fluoro-3-pyridyl)-7-azabicyclo [2.2.1]heptane) of this substance has been developed and labeled with fluorine-18[13-16]. Due to its high uptake into the brain, specific regiodistribution and high ratio of specific-to-nonspecific binding, this radioligand appears to be ideally suited for PET imaging of nAChRs in the brain. However, the high toxicity[14,17] of both the parent compound and the fluoro derivative may be a considerable limitation for its use as a routine PET tracer in the human brain exploration.

Recent advances in the search for novel nAChR ligands have been made and in the 3-pyridyl ether series developed by Abbott Laboratories, the lead compound A-85380[18,19] (3-[2(S)-2-azetidinylmethoxy]pyridine) not only possesses subnanomolar affinity (52 pM affinity for rat brain [^{3}H]cytisine binding sites), comparable to that of epibatidine (the most potent nAChR ligand reported to date), but also presents high selectivity for the $\alpha4\beta2$ subtype of nAChRs.

We herein report the synthesis of 2-fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (**1**), a fluoro analog of the nicotinic ligand A-85380 and its radiolabeling with fluorine-18 by nucleophilic aromatic nitro-to-fluoro substitution.

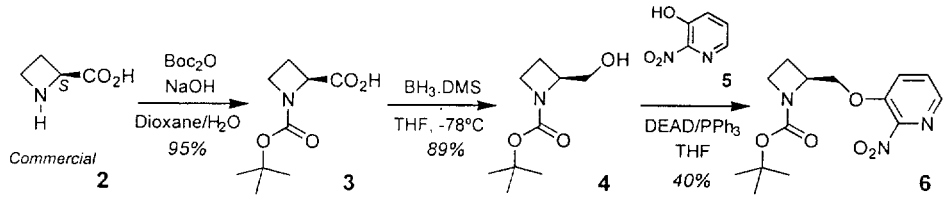
Results and Discussion

Chemistry

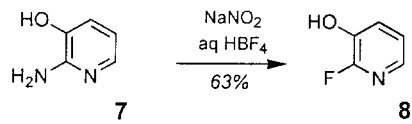
The preparation of the labeling precursor 2-nitro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**6**) and reference compound 2-fluoro-3-[2(S)-2-azetidinylmethoxy] pyridine (**1**) are described in the schemes below.

Protection of the amino function of commercially available (S)-2-azetidinecarboxylic acid (**2**) using *tert*-butyl dicarbonate in dioxane/water containing 1 eq of NaOH, at 0°C for 1 hour and at room temperature for another hour, gave compound **3** in nearly quantitative yield (95%). The boc derivative **3** was cleanly reduced by an excess of diborane in THF at -78°C to

give the azetidinemethanol derivative **4** in 89% yield. Mitsunobu coupling of alcohol **4** and commercially available 3-hydroxy-2-nitropyridine (**5**), using diethylazodicarboxylate and triphenylphosphine in THF at room temperature, gave the ether **6** in moderate yield (40%).

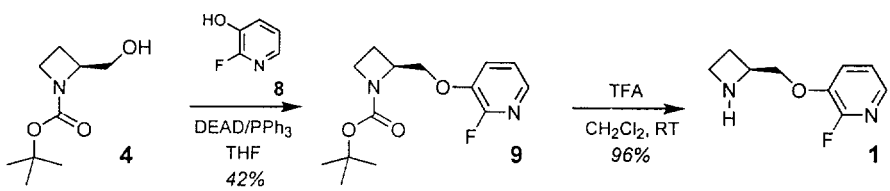


2-Fluoro-3-hydroxypyridine (**8**) was synthesized in 63% yield, from commercially available aminopyridine **7**, $NaNO_2$ and aq. fluoboric acid at 0°C for 1 hour. No analytical data could be found in the literature for this fluoropyridine. Presence of the fluorine atom was confirmed in a first time using usual NMR and mass spectroscopies : The ^1H NMR spectrum showed characteristic fluorine-proton interactions with measured coupling constants of 1.7 & 4.7 Hz (for the td at δ : 7.64), 1.7, 7.7 & 10.8 Hz (for the ddd at δ : 7.42) and 1.3, 4.7 & 7.8 Hz (for the ddd at δ : 7.17) ; COSY experiments also clearly showed correlation peaks between the pyridinyl protons at δ : 7.17 & 7.42 and 7.17 & 7.64. ^{13}C NMR spectrum showed distinctive fluorine-carbon interactions with coupling constants of 233 Hz ($J^1_{F\text{-}C}$, δ : 152.8), 27 Hz ($J^2_{F\text{-}C}$, δ : 140.2), 13 Hz ($J^3_{F\text{-}C}$, δ : 135.6) and 5 Hz ($J^3_{F\text{-}C}$, δ : 126.2). In a second time, from carbon-proton correlation experiments, attribution for both protons and carbons peaks was done : position 6 (C : δ : 135.6 ; H : δ : 7.64) ; position 5 (C : δ : 122.6 ; H : δ : 7.17) ; position 4 (C : δ : 126.2 ; H : δ : 7.42) ; position 3 (C : δ : 140.2) ; position 2 (C : δ : 152.8).

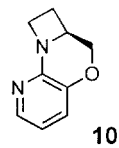


The key ether-forming step was carried out under similar Mitsunobu conditions as described above for the synthesis of derivative **6**. Coupling of azetidinemethanol derivative **4** and 2-fluoro-3-hydroxypyridine (**8**) gave the ether **9** in 42% yield. ^1H NMR (as well as ^{13}C NMR) spectroscopy showed characteristic fluorine-proton (fluorine-carbon) interactions with measured coupling constants similar to those described for the fluoropyridine **8**.

TFA removal of the *tert*-butoxycarbonyl function gave the amine **1** in 96% yield.



It is worth mentioning that fluoro derivative **1**, as its free amine form, cyclisized nearly quantitatively in DMSO at 80°C within 15 minutes to give 1,2,2a,3-tetrahydro-4-oxa-8,8b-diaza-cyclobuta[α]naphtalene (**10**), which is stable for at least 20 hours at this temperature.



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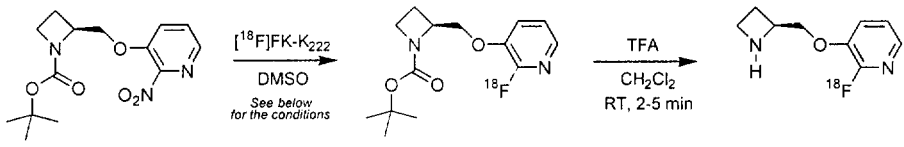
Radiochemistry

2-[^{18}F]Fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]-**1**) was prepared in 2 steps from the labeling nitro precursor **6**.



6 [^{18}F]-**9** [^{18}F]-**1**

Conditions	*Yields* (with respect to [^{18}F]fluoride ion)	
Conventional heating 150°C, 20 min	55-70% [a] ; 32-40% [b] Synthesis time : 85-90 min [c]	48-63% [a] ; 22-28% [b] Synthesis time : 125-130 min [c]
or		
Microwaves 100W, 1 min	57-71% [a] ; 37-46% [b] Synthesis time : 65-70 min [c]	49-64% [a] ; 25-33% [b] Synthesis time : 105-110 min [c]

[a] Decay-corrected ; [b] Non decay-corrected ; [c] from EOB

The first step consists of the introduction of the fluorine-18 using a nucleophilic aromatic substitution, performed at the alpha position of the pyridinyl ring. The nitro function was chosen as substituent 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[20] as the fluorinating reactant, in DMSO as the solvent, by (1) conventional heating at 150°C for 20 minutes or (2) microwave activation at 100 Watt for 1 minute. After Sep-pak separation, the Boc-protected 2-[^{18}F]fluoropyridine derivative [^{18}F]-**9** was purified by HPLC. Removal of the *tert*-butoxycarbonyl function in a 10/1 mixture of CH_2Cl_2/TFA at room temperature for 2 minutes, followed by HPLC purification gave the amine [^{18}F]-**1**. The average time gain using the microwave activation procedure over the conventional heating procedure was 20 minutes. The chemical yields of fluorine incorporation using the one or the other procedure were comparable and varied from 70% to 90%. The chemical yields of Boc deprotection were quantitative. Direct deprotection of the non-HPLC-purified 2-[^{18}F]fluoropyridine derivative [^{18}F]-**9** with TFA in CH_2Cl_2 shortened the procedure but led to chemically impure amine [^{18}F]-**1**. The whole synthesis procedure (included the HPLCs) is fully automated on a computer assisted Zymate robot system (Zymark corporation, USA). Decay-corrected and non-decay-corrected yields (with respect to [^{18}F]fluoride ion) as well as synthesis times are given in the scheme.

Typically, using the microwave activation procedure, 110-140 mCi (4.1-5.2 GBq) of pure 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]-**1**) could be obtained in less than 2 hours, with specific radioactivities of 3-5 Ci/μmol (111-185 GBq/μmol) calculated for End of Bombardment (or 1.5-2.5 Ci/μmol (55.5-92.5 GBq/μmol) at End of Synthesis) for a 20 μA, 30 minutes (36000 μC) irradiation of a 95% enriched [^{18}O]water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F].

Preliminary in vivo *studies in rats*

Following *intravenous* injection of 30 μCi of 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy] pyridine ([^{18}F]-**1**), thalamus uptake peaked at 30 minutes (1% of the injected dose per gram of tissue), remained on a plateau for 60 minutes and then slowly decreased (0.7% of the injected dose per gram of tissue at 180 minutes). Ratio of thalamus/cerebellum radioactivity was 2 at 60 minutes. Pre-treatment with nicotine (5 mg/kg), cytisine (5 mg/kg), epibatidine (5 μg/kg) or fluoro-A-85380 (5 μg/kg) reduced both thalamic and cerebellar uptake by 75-85%.

Experimental

General

Chemicals were purchased from Aldrich, Fluka or Sigma France and were used without further purification. TLC were run on pre-coated plates of silicagel 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 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 silicagel 63-200 μm (Merck) at 0.3 bar (Ar). HPLCs were run on Waters systems equipped with a 510 pump, 440 UV detector or 481 & 486 UV-multiwavelength detectors ; the effluent was also monitored for radioactivity with a Geiger-Müller counter ; HPLC Column & Conditions : **A** [column : semipreparative SiO_2 Lichrosorb Merck (250 x 10 mm) ; porosity : 7 μm ; temperature : RT ; UV detection at λ : 254 nm] ; **B** [column : semipreparative C-18 μBondapak Waters (300 x 7.8 mm); porosity : 10 μm ; temperature : RT ; UV detection at λ : 254 nm] ; **C** : [column : semipreparative SiO_2 Prep Nova-Pak® HR Silica, Waters (300 x 7.8 mm); porosity : 6 μm ; temperature : RT ; UV detection at λ : 254 nm]. 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 ; CD_3OD, δ = 4.78 & 3.30 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuteriated solvents (DMSO-d_6, δ = 39.5 ppm ; CD_2Cl_2, δ = 53.8 ppm ; CD_3OD, δ = 49.0 ppm) and/or TMS as internal standards 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 multi sharp-peak system) and broad respectively). The mass spectra (MS), DCI/NH_4^+ were measured on a Nermag R10-10 apparatus. Air- or moisture sensitive reactions were conducted in heat-gun dried glassware, under an inert atmosphere and with freshly distilled solvents. Microwave activations were performed with a MicroWell 10 oven (2.45 GHz), Labwell AB, Sweden.

Complementary data for the preparation and characterisation of compounds **3** and **4** are also available in the literature : See reference 18.

Chemistry

(S)-N-(Tert-butoxycarbonyl)-2-azetidinecarboxylic acid (3)

To a cold (0°C) solution of (S)-2-azetidinecarboxylic acid (**2**) (1.0 g, MW : 101.10, 9.9 mmol) in dioxane (20 mL) and water (10 mL), were successively added 10 mL of 1N aq. NaOH (1.0 eq) and 2.4 g of di-*tert*-butyl dicarbonate (MW : 218.25, 11.0 mmol, 1.1 eq). The mixture was stirred at 0°C for 1 h and at room temperature for another 1 h . The solution was then carefully acidified (pH 2) with 1M aq. $NaHSO_4$, transferred into a separatory funnel and extracted with EtOAc. The organic layers were combined, washed with water, brine and then dried with Na_2SO_4. Concentration gave 1.9 g of pure (S)-*N*-(*tert*-butoxycarbonyl)-2-azetidinecarboxylic acid (**3**) as a white powder (95%).

Rf (EtOAc) : 0.35. ^{1}H NMR (DMSO-d_6, 298.0K) : δ : 4.44 (dd, J : 6.0 & 9.0 Hz, 1H) ; 3.83 (dd, J : 6.0 & 9.0 Hz, 1H) ; 3.75 (b, $w_{1/2}$: 25 Hz, 1H) ; 2.47 (m (6 peaks), 1H) ; 2.00 (m (8 peaks), 1H) ; 1.35 (s, 9H). ^{1}H NMR (DMSO-d_6, 352.0K) : δ : 4.44 (dd, J : 6.0 & 9.0 Hz, 1H) ; 3.83 (dd, J : 6.0 & 9.0 Hz, 1H) ; 3.76 (m (6 peaks), 1H) ; 2.47 (m (6 peaks), 1H) ; 2.03 (m (8 peaks), 1H) ; 1.36 (s, 9H). ^{13}C NMR (DMSO-d_6, 352.0K) : δ : 172.2 [C] ; 154.8 [C] ; 78.7 [C] ; 60.5 [CH] ; 47.2 [CH_2] ; 28.0 [CH_3] ; 19.9 [CH_2]. MS (DCI/NH_4^+) : $C_9H_{15}N_1O_4$: 219 [M + NH_4^+] ; 202 [M + H^+].

(S)-N-(Tert-butoxycarbonyl)-2-azetidinemethanol (4)

To a solution of (S)-*N*-(*tert*-butoxycarbonyl)-2-azetidinecarboxylic acid (**3**) (1.0 g, MW : 201.22, 5.0 mmol) in THF (10 mL), stirred under argon and cooled to -78°C, were carefully added dropwise 2.5 mL of 10M borane dimethylsulfide complex (25 mmol, 5 eq). The mixture was stirred at -78°C for 1 h and at room temperature overnight. The mixture was cooled again to 0°C and the excess of borane was destroyed by careful addition of 3 mL of water. The solution was then transferred into a separatory funnel and extracted with EtOAc. The organic layers were combined, washed with 10% aq. K_2CO_3, water, brine and dried with Na_2SO_4. Concentration gave 830 mg of crude (S)-*N*-(*tert*-butoxycarbonyl)-2-azetidinemethanol (**4**) as a viscous oil (89%) which was used without further purification.

Rf (EtOAc/heptane : 50/50) : 0.40. ^{1}H NMR (CD_2Cl_2, 310.0K) : δ : 4.35 (bq, $w_{1/2}$: 23 Hz, 1H) ; 3.83 (q, J : 9.0 Hz, 2H) ; 3.80-3.55 (m (8 peaks), 2H) ; 2.15 (m (12 peaks), 1H) ; 1.93 (b, $w_{1/2}$: 27 Hz, 1H) ; 1.43 (s, 9H). ^{13}C NMR (CD_2Cl_2, 310.0K) : δ : 154.6 [C] ; 80.3 [C] ; 67.1 [CH_2] ; 64.2 [CH] ; 47.1 [CH_2] ; 28.5 [CH_3] ; 18.4 [CH_2]. MS (DCI/NH_4^+) : $C_9H_{17}N_1O_3$: 188 [M + H^+].

2-Nitro-3-[2(S)-(N-(tert-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (6)

To a solution of (S)-*N*-(*tert*-butoxycarbonyl)-2-azetidinemethanol (**4**) (0.50 g, MW : 187.24, 2.7 mmol) in THF (20 mL), stirred under argon and cooled to 0°C, were added 0.7 mL of DEAD (diethylazodicarboxylate, MW : 174.16, d : 1.106, 4.3 mmol, 1.6 eq) and 1.05 g of triphenylphosphine (MW : 262.29, 4.0 mmol, 1.5 eq). After stirring at 0°C for 10 min, 3-hydroxy-2-nitropyridine (**5**, 0.60 g, MW : 140.10, 4.3 mmol, 1.6 eq) 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 (75/25 to 60/40) gave 380 mg of 2-nitro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**6**) as a yellow powder (40%).

Rf (EtOAc/heptane : 50/50) : 0.25. ^{1}H NMR (CD_2Cl_2, 298.0K) : 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, 298.0K) : 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^+].

2-Fluoro-3-hydroxypyridine (8)

To a solution of 2-amino-3-hydroxypyridine (**7**) (2.0 g, MW : 110.12, 18.2 mmol) in aq. 48 wt. % fluoboric acid (75 mL), cooled to 0°C, were carefully added 10.0 g of $NaNO_2$ (MW : 69.00, 145.0 mmol). The mixture was stirred at 0°C for 1 h, then slowly basified with 1N aq. NaOH, transferred into a separatory funnel and extracted with EtOAc. The organic layers were combined, washed with water, brine and dried with Na_2SO_4. Concentration gave 1.3 g of crude 2-fluoro-3-hydroxypyridine (**8**) as a yellow-brown solid residue (63%).

Rf (EtOAc/heptane : 80/20) : 0.65. ^{1}H NMR (DMSO-d_6, 298.0K) : 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, 298.0K) : δ : 152.8 [d, $J^1_{F\text{-}C}$: 233 Hz, C] ; 140.2 [d, $J^2_{F\text{-}C}$: 27 Hz, C] ; 135.6 [d, $J^3_{F\text{-}C}$: 13 Hz, CH] ; 126.2 [d, $J^3_{F\text{-}C}$: 5 Hz, CH] ; 122.6 [CH]. MS (DCI/NH_4^+) : $C_5H_4F_1N_1O_1$: 131 [M + NH_4^+] ; 114 [M + H^+].

2-Fluoro-3-[2(S)-(N-(tert-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (9)

To a solution of (S)-*N*-(*tert*-butoxycarbonyl)-2-azetidinemethanol (**4**) (0.50 g, MW : 187.24, 2.7 mmol) in THF (20 mL), stirred under argon and cooled to 0°C, were added 0.7 mL of DEAD (diethylazodicarboxylate, MW : 174.16, d : 1.106, 4.4 mmol, 1.6 eq) and 1.05 g of triphenylphosphine (MW : 262.29, 4.0 mmol, 1.5 eq). After stirring at 0°C for 10 min, 2-fluoro-3-hydroxypyridine (**8**, 0.35 g, MW : 113.09, 3.1 mmol, 1.15 eq) 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 (80/20 to 60/40) gave 317 mg of 2-fluoro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**9**) as a yellow oil (42%). For analytical purposes, an aliquot (100 mg) was repurified on HPLC to give pure 2-fluoro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**9**) as a colorless oil [HPLC A ; eluant : heptane/EtOAc : 60/40 ; flow rate : 6.0 mL/min ; retention time : 7.5 to 9.0 min].

Rf (EtOAc/heptane : 50/50) : 0.45. Rt (HPLC A ; eluant : heptane/EtOAc : 60/40 ; flow rate : 6.0 mL/min) : 8.5 min. ^{1}H NMR (CD_2Cl_2, 298.0K) : 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, 298.0K) : δ : 156.4 [C] ; 154.2 [d, $J^1_{F\text{-}C}$: 235 Hz, C] ; 142.7 [d, $J^2_{F\text{-}C}$: 26 Hz, C] ; 137.7 [d, $J^3_{F\text{-}C}$: 13 Hz, CH] ; 123.6 [d, $J_{F\text{-}C}$: 4 Hz, CH] ; 122.3 [d, $J_{F\text{-}C}$: 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^+) : $C_{14}H_{19}F_1N_2O_3$: 300 [M + NH_4^+] ; 283 [M + H^+].

2-Fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (1)

To a solution of 2-fluoro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**9**) (0.4 g, MW : 282.31, 1.4 mmol) in CH_2Cl_2 (20 mL), cooled to 0°C, were 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 pure 2-fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (**1**) as its TFA salt (96%). For analytical purposes, an aliquot (200 mg) was repurified on HPLC [HPLC B ; eluant : acetonitrile/water/TFA : 10/90/0.15 ; flow rate : 6.0 mL/min ; retention time : 4.5 to 5.5 min]. The fraction containing the desired amine were combined, basified with 1N aq. 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 **1** 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 2-fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (**1**) as its HCl salt.

Rf ($CHCl_3$/MeOH/NH_4OH : 90/10/1) : 0.15. Rt (HPLC B ; eluant : acetonitrile/water/TFA : 10/90/0.15 ; flow rate : 6.0 mL/min) : 5.0 min. Rt (HPLC B ; eluant : acetonitrile/water/TFA : 5/95/0.15 ; flow rate : 6.0 mL/min) : 9.5 min. ^{1}H NMR (HCl salt, CD_3OD, 298.0K) : 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 solvant, 1H) ; 4.45 (d, J : 6 Hz, 2H) ; 4.08 (m (6 peaks), 2H) ; 2.68 (m (10 peaks), 2H). ^{1}H NMR (TFA salt, CD_2Cl_2, 298.0K) : 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, 298.0K) : δ : 154.1 [d, $J^1_{F\text{-}C}$: 235 Hz, C] ; 141.9 [d, $J^2_{F\text{-}C}$: 26 Hz, C] ; 138.0 [d, $J^3_{F\text{-}C}$: 11 Hz, CH] ; 123.9 [d, $J_{F\text{-}C}$ < 2 Hz, CH] ; 122.4 [d, $J_{F\text{-}C}$ < 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, 298.0K) : δ : 154.9 [d, $J^1_{F\text{-}C}$: 236 Hz, C] ; 142.7 [d, $J^2_{F\text{-}C}$: 25 Hz, C] ; 139.2 [d, $J^3_{F\text{-}C}$: 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, 298.0K) : δ : 161.4 [q, $J^2_{F\text{-}C}$: 38 Hz, C, $CF_3\underline{C}O_2H$] ; 154.0 [d, $J^1_{F\text{-}C}$: 237 Hz, C] ; 141.5 [d, $J^2_{F\text{-}C}$: 25 Hz, C] ; 138.6 [d, $J^3_{F\text{-}C}$: 12 Hz, CH] ; 124.5 [d, $J_{F\text{-}C}$ < 2 Hz, CH] ; 122.8 [d, $J_{F\text{-}C}$ < 2 Hz, CH] ; 116.3 [q, $J^1_{F\text{-}C}$: 290 Hz, C, $\underline{C}F_3CO_2H$] ; 68.0 [CH_2] ; 59.6 [CH] ; 44.4 [CH_2] ; 21.2 [CH_2]. MS (HCl salt, DCI/NH_4^+) : $C_9H_{11}F_1N_2O_1$: 221 [M.HCl + H^+] ; 219 [M.HCl + H^+] ; 183 [M + H^+].

* COSY experiments were fully in accordance with the proposed structure and undoubtedly confirmed the presence of a hidden peak at δ : 4.85.

*1,2,2a,3-Tetrahydro-4-oxa-8,8b-diaza-cyclobuta[α]naphtalene (**10**)*

2-Fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (**1**) (50 mg, MW : 182.20, 0.27 mmol) was quantitatively converted to 1,2,2a,3-tetrahydro-4-oxa-8,8b-diaza-cyclobuta[α]naphtalene (**10**) in DMSO-d_6 (0.3 mL) at 80°C (sealed tube) during NMR experiments (30-60 min).

Rt (HPLC B ; eluant : acetonitrile/water/TFA : 10/90/0.15 ; flow rate : 6.0 mL/min) : 6.5 min. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 7.75 (d, J : 4.2 Hz, 1H) ; 7.04 (dd, J : 1.5 & 7.8 Hz, 1H) ; 6.64 (dd, J : 4.8 & 7.8 Hz, 1H) ; 4.40 (m (9 peaks), 1H) ; 4.32 (m (4 peaks), 1H) ; 4.20 (m (6 peaks), 1H) ; 4.01 (q, J : 9.0 Hz, 1H) ; 3.57 (t, J : 12.0 Hz, 1H) ; 2.62 (m (8 peaks), 1H) ; 2.23 (m (9 peaks), 1H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 150.2 [C] ; 141.0 [C] ; 140.9 [CH] ; 122.4 [CH] ; 114.8 [CH] ; 65.8 [CH_2] ; 59.5 [CH] ; 53.2 [CH_2] ; 20.7 [CH_2]. MS (DCI/NH_4^+) : $C_9H_{10}N_2O_1$: 163 [M + H^+].

Radiochemistry

Production of aqueous [^{18}F]F^-

[^{18}F]F^- was produced on a CGR-MeV 520 cyclotron by irradiation of a 2 mL water target (water-cooled stainless steel target-holder equipped with an 12 μm titanium window He-cooled) using a 20 MeV proton beam on 95% enriched [^{18}O]water [^{18}O(p,n)^{18}F]. On average, about 550-650 mCi (20.3-24.0 GBq) of [^{18}F]F^- is routinely obtained in our laboratory at the End Of Bombardment for a 20 μA, 30 min (36000 μC) irradiation.

Preparation of the [^{18}F]FK-K_{222}-complex

The 2 mL of aqueous [^{18}F]fluoride from the target were passed through a ion exchange resin (AG11A8, Bio-Rad) in order to recover the enriched [^{18}O]-water. The [^{18}F]fluoride ion was eluted from the resin using 1.5 mL of a 3.0 mg/mL aqueous K_2CO_3 solution. After addition of 11.0 to 15.0 mg of Kryptofix® K_{222}, the solution was then gently concentrated to dryness (at 110-120°C under a nitrogen stream for 20 min).

*Preparation of 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]-**1**)*

(1) Conventional heating

The [^{18}F]FK-K_{222}-complex as an oily residue was dissolved in 200 μL of a freshly distilled DMSO and transferred to a 2 mL reaction vial containing 4.0 to 6.0 mg (13.5 to 20.3 μmol) of the labeling precursor 2-nitro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinyl methoxy]pyridine (**6**). The evaporation tube was rinsed twice with 200 μL of DMSO which was then added to the reaction mixture. Resolubilization yields were about 85-95% of the original [^{18}F]fluoride ion. The reaction vial was then tightly closed and heated in an heating block oven without stirring at 150°C during 20 min. The resulting yellow-brown reaction mixture was then cooled using an ice/water bath, diluted with 3 mL of water and passed through a C18 Sep-pak cartridge (Waters). The cartridge was washed with 3.0 mL of water and partially dried for 0.5 min by applying a nitrogen stream. The Boc-protected 2-[^{18}F]fluoropyridine derivative [^{18}F]-**9** was eluted from the cartridge with CH_2Cl_2, 3 mL followed by two successive rinses of 1.0 mL (5-10% of the total radioactivity amount engaged in the fluorination process was left on

the cartridge). The yield of substitution varied from 70% to 90% with respect to [^{18}F]fluoride ion (yields were determined after the Sep-pak elution as the CH_2Cl_2 over DMSO/H_2O radioactivity counting ratio followed by radiochromatography (SiO_2-TLC, eluant : heptane/EtOAc : 50/50, Rf : [^{18}F]-**9** : 0.50 and Rf : [^{18}F]F^- : 0.0). 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 C ; eluant : heptane/EtOAc :60/40 ; flow rate : 5.0 mL/min] gave pure labeled 2-[^{18}F]fluoro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine ([^{18}F]-**9**), retention time : 4.5 to 5.0 min (labeling precursor **6** : Rt : 9.5-12.5 min).

Yield of [^{18}F]-**9** (with respect to [^{18}F]fluoride ion) : 55-70% decay-corrected ; 32-40% non-decay-corrected. Synthesis time from EOB : 85-90 min (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation).

The above HPLC-collected 2-[^{18}F]fluoro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinyl methoxy]pyridine ([^{18}F]-**9**) was concentrated to dryness (at 60-80°C under a gentle nitrogen stream for 5-8 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]-**9** could be detected by radiochromatography (SiO_2-TLC, eluant : heptane/EtOAc : 50/50, Rf : [^{18}F]-**9** : 0.50 and Rf : [^{18}F]-**1** : 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 dissolved in 1-2 mL of the HPLC solvent used for purification and the crude was injected onto HPLC. Isocratic elution [HPLC B ; eluant : 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]-1), retention time : 5.0-6.0 min.

Yield of [^{18}F]-**1** (with respect to [^{18}F]fluoride ion) : decay-corrected 48-63% ; non-decay-corrected 22-28%. Total synthesis time from EOB : 125-130 min (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation).

(2) Microwave heating

The procedure described above was slightly modified : 600 μL of freshly distilled DMSO containing 4.0 to 6.0 mg (13.5 to 20.3 μmol) of the labeling precursor 2-nitro-3-[2(S)-(*N*-(*tert*-butoxycarbonyl)-2-azetidinylmethoxy]pyridine (**6**) were directly added into the tube containing the dried [^{18}F]FK-K_{222}-complex. The tube (not sealed) was placed in the microwave oven. Microwaves (100 Watt) were applied to the system for 1.0 to 1.2 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. The remainder of the synthesis used the same procedure as described above. The yield of substitution varied from 75% to 90% with respect to [^{18}F]fluoride ion (yields were determined as described above). The average time gain using this procedure was 20 min.

Yield of [^{18}F]-**9** (with respect to [^{18}F]fluoride ion) : 57-71% decay-corrected ; 37-46% non-decay-corrected. Synthesis time from EOB : 65-70 min (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation).

Yield of [^{18}F]-**1** (with respect to [^{18}F]fluoride ion) : decay-corrected 49-64% ; non-decay-corrected 25-33%. Total synthesis time from EOB : 105-110 min (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation).

Typically, using the latter procedure, 110-140 mCi (4.1-5.2 GBq) of pure 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]-**1**) could be obtained in less than 2 h, with specific radioactivities of 3-5 Ci/μmol (111-185 GBq/μmol) calculated for End of Bombardment (or 1.5-2.5 Ci/μmol (55.5-92.5 GBq/μmol) at End of Synthesis) for a 20 μA, 30 min (36000 μC) irradiation of a 95% enriched [^{18}O]water target with a 16 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 corporation, USA).

Formulation and quality control of 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]-1)

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. Animal injections were always done within 15 min after End of Synthesis, in PET- as well as in 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, USA) with a 1 mL loop, a reverse phase semipreparative C-18 μBondapak Waters (Dimensions : 300 x 7.8 mm ; Porosity : 10 μm ; Granulometry : 125Å) 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 (vers. 1.21, JMBS Developments, Grenoble, France). The column was eluted applying (1) a gradient from 5% acetonitrile (HPLC grade ; SDS, Peypin, France) in 0.01M aq. phosphoric acid up to 35% in 7.5 min, (2) a gradient from 35% acetonitrile in 0.01M aq. 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.

Conclusion

This paper reports the synthesis of 2-fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine and its radiolabeling with fluorine-18 ([^{18}F]FK-K_{222}) by nucleophilic aromatic nitro-to-fluoro substitution in DMSO by conventional heating at 150°C for 20 minutes or by microwave activation at 100 Watt for 1 minute. This fluoro compound is a closely related analog of the high affinity nicotinic ligand A-85380 (3-[2(S)-2-azetidinylmethoxy]pyridine). This compound is the lead compound of a novel 3-pyridyl ether series of new nAChR ligands recently published, and possesses not only subnanomolar affinity, comparable to that of epibatidine, for the $\alpha4\beta2$ subtype, but also a weaker affinity for the other subtypes of nAChRs. 110-140 mCi (4.1-5.2 GBq) of pure 2-[^{18}F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine ([^{18}F]fluoro-A-85380) could be obtained in less than 2 hours, with specific radioactivities of 3-5 Ci/μmol (111-185 GBq/μmol) calculated for End of Bombardment (or 1.5-2.5 Ci/μmol (55.5-92.5 GBq/μmol) at End of Synthesis) for a 20 μA, 30 minutes (36000 μC) irradiation of a 95% enriched [^{18}O]water target with a 16 MeV proton beam [^{18}O(p,n)^{18}F]. Yields (with respect to [^{18}F]fluoride ion) : decay-corrected 49-64% ; non-decay-corrected 25-33%. Total synthesis time from EOB : 105-110 minutes (this includes the recovery of the [^{18}F]fluoride ion from the target and the [^{18}F]FK-K_{222}-complex preparation). Preliminary results in rats showed a substantial uptake of the ligand in the thalamus (1% I.D./g tissue at 30 minutes) while the cerebellar uptake was 2-fold lower. Thalamic uptake was reduced by 75-85% following a pre-treatment with nicotine, cytisine, epibatidine or fluoro-A-85380. The full pharmacological profile and the potential for eventual clinical applications of this ligand as a tracer for PET experiments are currently under investigation.

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