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# Synthesis of 2-[$^{18}$F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine as a radioligand for imaging nicotinic acetylcholine receptors

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**Abstract** Nicotinic acetylcholine receptors (nChRs) are involved in the various pharmacological effects or disease states. In order to study the central nChRs by PET or SPECT, some radioligands have been investigated. In this paper, the procedure for synthesis of 2-[$^{18}$F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (2-[$^{18}$F]-A-85380), a potential PET ligand for in vivo imaging nicotinic acetylcholine receptor was described. 2-[$^{18}$F]-A-85380 was prepared from the precursor, 2-nitro-3-[(1-(tert-butoxycarbonyl)-2-(S)-azetidinyl)methoxy]pyridine(4), which was synthesized with commercial (S)-2-azetid- inecarboxylic acid as starting material. The whole procedure for radiosynthesis and purification was executed in about 1 h and 45–55% of the added fluorine-18 was found in the purified 2-[$^{18}$F]-A-85380, with specific activity of $1.0–2.2\times10^{11}$ Bq/$\mu$mol.

**Keywords** Nicotinic acetylcholine receptors, Precursor, Radioligand, $^{18}$F, Radiosynthesis

**CLC numbers** O615, R817.4 A

## 1 INTRODUCTION

Many studies have revealed that Nicotinic acetylcholine receptors (nChRs) are involved in the various pharmacological effects or disease states, such as tobacco addiction, seizure, analgesia, Alzheimer's Disease and Parkinson's Disease.[1–4] In order to study the central nChRs in living subjects by PET or SPECT, some ligands were synthesized and radiolabelled with $^{18}$F, $^{11}$C or other radioisotopes. Among those potential ligands, [$^{11}$C]-nicotine was the first one used for imaging nChRs in vivo,[5] while epibadine, a natural product isolated from South American frogs, once was the most promising, due to its exceptionally high specificity and selectivity for nChRs in vivo, and has been labeled with $^{18}$F leading to [$^{18}$F]NFEP or [$^{18}$F]N-methyl-NFEP.[6] Unfortunately, toxicity concerns for epibadine and its analogs impede their further use in human beings.[7,8] For

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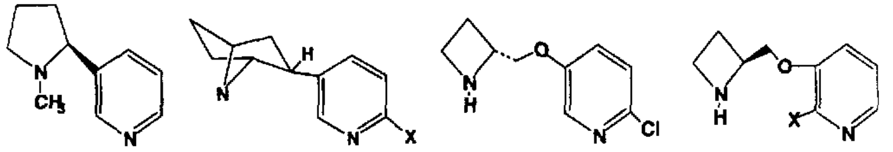
this reason, the later studies concentrated on the synthesis of less toxic epibadine-based tracer. However, some analogs still demonstrated a substantial portion of the parent ligand's toxicity and this remained an impediment to the widespread use of epibadine analogs in human studies.

More recently, a novel series of 3-pyridyl ether with subnanomolar affinity for nChRs were reported,[9–12] including A-85380, A-84543 and ABT-594 (Fig.1). Of which, A-85380 was found to display high affinity toward nChRs, almost as strong as epibadine (the most potent nChRs ligand reported to date), but substantially less toxicity than it.[9] As a result, A-85380-based radioligands were considered as promising PET or SPECT tracers for imaging nChRs in vivo.[13–15] Here we report a modified procedure for the synthesis of 2-[$^{18}$F]fluoro-3-[2(S)-2-azetidinylmethoxy] pyridine (2-[$^{18}$F]-A-85380), a $^{18}$F labeled analog of A-85380 in high yield via a nitro precursor.



**Nicotine**

**X=Cl, Epibatidine**
**X=$^{18}$F, NFEP**

**ABT-594**

**X=H, A-85380**
**X=$^{18}$F, 2-[$^{18}$F]-A-85380**

**Fig.1** Structures for some nChRs ligands

## 2 MATERIALS AND METHODS

All the regents were purchased from Aldrich, Fluka or Sigma Co. and were used without further purification. $^1$H NMR spectra were obtained in $CDCl_3$ on a Bruker 300 MHz NMR spectrometer and were reported in parts per million downfield from tetramethylsilane. Mass spectra were recorded with Finnegan-Mat GC-MS 5100 mass spectrometer using electron impact ionization at 70 eV. TLC analyses were performed on precoated polygram Sil G/UV$_{254}$ plates (Macherey-Nagel Co., Germany). A short wavelength ultraviolet lamp and automatic TLC scanner (Berthold Automatic TLC Linear Analyzer) were used as UV and radioactivity detectors.

### 2.1 K$^{18}$F/Kryptofix$_{222}$

H$^{18}$F was produced by irradiation of $H_2^{18}O$ in a silver target using proton of 17.4 MeV via $^{18}$O(p, n)$^{18}$F nuclear reaction on a JAW cyclotron at Brookhaven National Laboratory. To the aqueous H$^{18}$F solution, potassium carbonate (4 mg) and Kryptofix$_{222}$ (23 mg) was added. The mixture was evaporated to dryness at 120°C under a slow stream of nitrogen, affording K$^{18}$F/Kryptofix$_{222}$ complex as a solid.

### 2.2 (S)-1-(tert-butoxycarbonyl)-2-azetidinecarboxylic acid (2)

To an ice-cooled solution of (S)-2-azetidinecarboxylic acid (1) (1.7 g, 16.8 mmol) in 1, 4-dioxane: water (1:1, 50 mL) was added di-tert-butyl dicarbonate (Boc$_2$O, 4.8 g, 21.96 mmol), followed by 4-methyl-morpholine (2.2 mL, 19.5 mmol). After stirred for 3 h at 0°C and for another 16 h with gradual warming to room temperature (RT), the reaction mixture was poured into a cold saturated solution of sodium bicarbonate (50 mL) and washed with ethyl acetate (3×40 mL). The aqueous solution was acidified with HCl solution (3 mol/L) to pH=1 and extracted with ethyl acetate (3×40 mL). The extracts were dried (MgSO$_4$), and concentrated in vacuum to afford the product 2 (3.36 g, 98.8%) as a semisolid. $^1$H-NMR (CDCl$_3$): $\delta$4.81- 4.76 (t, J=15 Hz, 1H), 3.99-3.83 (m, 2H), 2.62-2.38 (m, 2H), 1.48 (s, 9H). MS(DCl/NH$_4$): 202 (M + H$^+$), 219 (M + NH$_4^+$).

### 2.3 (S)-1-(tert-butoxycarbonyl)-2-azetidinemethanol(3)

To a solution of (S)-1-(tert-butoxycarbonyl)-2-azetidinecarboxylic acid (2) (0.936, 4.63 mmol) in THF (15 mL) was added borane/THF complex (1 mmol/L, 24 mL, 23.15 mmol) at 0° under nitrogen. The mixture was allowed to gradually warm to RT and was stirred for 44 h, then 10% aqueous potassium hydrogen sulfate solution (8 mL) was added gradually, and the volatile components were evaporated in vacuum. The remaining slurry was extracted with ethyl acetate (3×30 mL). The organic phase was washed with a saturated solution of sodium hydrogen carbonate (3×20 mL), dried (MgSO$_4$), filtered and concentrated in vacuum, yielding the product 3 (0.86 g, 98.9%) as a colorless oil. $^1$H-NMR (CDCl$_3$): $\delta$ 4.49-4.40 (ddd, J=9.0, 9.0, 3.0 Hz, 1H), 3.95-3.68(m, 4H), 2.23-2.12 (m, 1H), 1.99-1.87 (m, 1H), 1.46 (s, 9H). MS(DCl/NH$_4$): 188 (M+H$^+$).

### 2.4 2-Nitro-3-[(1-(tert-butoxycarbonyl)-2-(S)-azetidinyl)methoxy]pyridine(4)

A solution of triphenylphosphine (1.19 g, 4.52 mmol) and diethylazodicarboxylate (DEAD, 0.63 mL, 4.07 mmol) in THF (15 mL) was stirred for 15 min in an ice-bath under nitrogen. To this solution was added (S)-1-(tert-butoxycarbonyl)-2-azetidinemethanol (3) *(0.85 g, 4.52 mmol) in THF (5 mL), followed by 3-hydroxy-2nitropyridine* (0.45 g, 3.21 mmol) in THF (5 mL). After stirred for 22 h with gradual warming to RT, the reaction mixture was concentrated in vacuum to remove the solvent. The pink residue was chromatographed on a flash silica gel column with elution of ethyl acetate/hexane (1:2 to 2:1) to provide the product 4 (0.82 g, 83.0%) as a yellow solid. $^1$H-NMR (CDCl$_3$): $\delta$8.00 (d, J=6.0Hz, 1H), 7.50-7.60 (ddd, J=9.0, 9.0, 6.0Hz, 2H), 4.53-4.45(m, 2H), 4.26(dd, J=10.6, 2.8 Hz, 1H), 3.78(m, 2H), 2.19 (m, 2H), 1.43 (s, 9H). MS(DCl/NH4): 310 (M +

$H^+$), 327 (M + $NH_4^+$).

### 2.5 (2-[$^{18}$F]-A-85380) (6)

A solution of compound 4 in DMSO (3 mL) was added to dried $K^{18}F$/Kryptofix$_{222}$ The mixture was stirred at 120°C for 10 min, quenched by adding water (3 mL) and extracted with $CH_2Cl_2$ (2×2 mL). The $CH_2Cl_2$ extracts were dried by passing through a $K_2CO_3$ column, providing the BOC-protected and $^{18}$F-substituted intermediate (5).

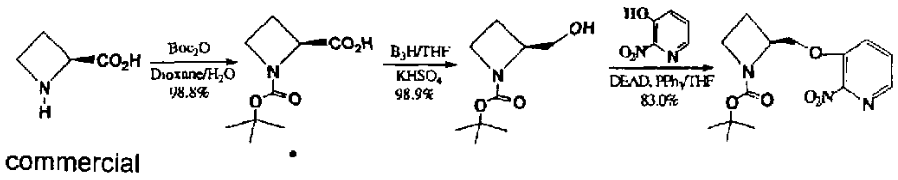
Complete cleavage of the protecting group was achieved via acid hydrolysis with $CF_3COOH$ (0.2 mL) at 120°C for 10 min. The resulting solution was neutralized with concentrated $K_2CO_3$(aq) (100 g of $K_2CO_3$ in 300 mL of $H_2O$, 0.5 mL) in water (2.4 mL). The mixture was passed through a C-18 sep-Pak cartridge, which was subsequently eluted with $CH_3CN$ (2×2 mL). After evaporation of the solvent, the residue was purified using a semipreparative HPLC (Waters $\mu$ Bondapak C18 column, 7.8×300 mm: $CH_3CN$:$H_2O$:TFA = 100:900:1.5; flow rate: 3.0 mL/min; UV254nm, retention time: 9.2 min), affording 2-[$^{18}$F]-A-85380(6) with an overall radiochemical yield of 45-55% and specific activity 1.0-2.2×$10^{11}$Bq/$\mu$mol at EOB in a radiosynthesis time of about 1 h.

The BOC-protected intermediate and no BOC-protected product could be detected by radio TLC (silica, ethyl acetate : hexane = 40:60; $R_f$(5)=0.55 and $R_f$(2-[$^{18}$F]-A-85380) = 0.0). Also, the radiochemical purity of 2-[$^{18}$F]-A-85380 was assayed by radioTLC (silica, 10 mL of the HPLC solvent and 2 drops acetic acid, $R_f$(2-[$^{18}$F]-A-85380) = 0.40).

## 3 RESULTS AND DISCUSSION

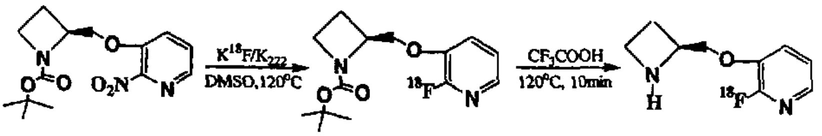
The key to radiotracers for nChRs was syntheses of labeling precursors. In this paper, we described a synthetic strategy for a labeling precursor (4) with 2-nitro as a leaving group and carried out its radiosynthesis to 2-[$^{18}$F]-A-85380. Nitro function was chosen as the leaving group due to its easy substitution by fluorine and available separation of the product from the reaction mixture. The synthetic scheme for the precursor 4 is described in Fig.2.

The synthesis of the labeling precursor started from commercially available (S)-2-azetid- inecarboxylic acid (1). Its amino function should be protected before further steps. In this experiment, it was performed using di-tert-butyl dicarbonate in 1, 4-dioxane-water containing 4-methyl-morpholine, at 0°C for 3 h and at room temperature for another hours, in an almost quantitative yield (98.8%). The boc derivative (2) was clearly reduced by an excess of borane/THF complex at 0°C under nitrogen to give (S)-1-(tert-butoxycarbonyl)-2-azetidinemethanol (3) in 98.9% yield, without the reaction temperature as low as -78°C but in a higher yield as previously reported procedure.[15] The crucial ether-forming step of (S)-1-(tert-butoxycarbonyl)-2-azetidinemethanol (3) and commercially available 3-hydroxy-2nitropyridine was carried out under Mitsunobu coupling condition, using DEAD and triphenylphosphine in THF at room temperature, gave the labeling precursor (4) in good yield (83.0%), much higher than the reported result (40.0%).[15]



1 2 3 4

Fig.2 The synthesis of the labeling precursor



4 5 2-[$^{18}$F]-A-85380

Fig.3 The radiosynthesis of 2-[$^{18}$F]-A-85380

2-[$^{18}$F]fluoro-3-[2(S)-2-azetidinylmethoxy]pyridine (2-[$^{18}$F]-A-85380) was prepared in 2 steps from the t-Boc protected precursor (4) (Fig.3). The first step is the nuleophilic aromatic substitution of compound (4) with fluorine-18, which was performed by Kryptofix$_{222}$/$K_2CO_3$ activated [$^{18}$F]$F^-$ in DMSO at 120°C for 10 min. The yields of F-18 substitution varied from 50%–65% with respect to initial [$^{18}$F]$F^-$ radioactivity. Complete cleavage of the t-Boc protecting group was achieved via acid hydrolysis with trifluoroacetic acid. Final HPLC purification afforded pure 2-[$^{18}$F]-A-85380 in a synthesis time of 55–60 min with specific activity of $(1.0–2.2)\times10^{11}$ Bq/$\mu$mol and an overall radiochemical yield of 45%-55% at the end of bombardment (EOB), better than the reported results with an overall radiochemical yield of 25%–33% (non-decay-corrected) in a synthesis time of 105–110 min.[15] The product was found to be more than 97% radiochemically pure. Also, this provides a shortened and simplified procedure since no intermediate HPLC purification was needed.

In summary, we report a modified and simplified procedure for the synthesis of 2-[$^{18}$F]fluoro-3-[2(S)-2-azetidinylmethoxy] pyridine (2-[$^{18}$F]-A-85380) in high yield via a nitro precursor, which was prepared from commercial (S)-2-azetidinecarboxylic acid as starting material.

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