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**Research Article**

# Synthesis of [$^{18}$F]3-[1-(3-fluoropropyl)-(*S*)-pyrrolidin-2-ylmethoxy]pyridine ([$^{18}$F]NicFP): a potential $\alpha4\beta2$ nicotinic acetylcholine receptor radioligand for PET

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## Summary

Nicotinic acetylcholine receptors are widely distributed throughout the human brain and are believed to play a role in several neurological and psychiatric disorders. In order to identify an effective PET radioligand for *in vivo* assessment of the $\alpha4\beta2$ subtype of nicotinic receptor, we synthesized [$^{18}$F]3-[1-(3-fluoropropyl)-(*S*)-pyrrolidin-2-ylmethoxy]pyridine (NicFP). The *in vitro* $K_D$ of NicFP was determined to be 1.1 nM, and the log *P* value obtained by HPLC analysis of the unlabelled standard was found to be 2.2. The radiosynthesis of [$^{18}$F]NicFP was carried out by a nucleophilic substitution reaction of anhydrous [$^{18}$F]fluoride and the corresponding mesylate precursor. After purification by HPLC, the radiochemical yield was determined to be $11.3 \pm 2.1\%$ and the specific activity was $0.47 \pm 0.18$ Ci/μmol (EOS, $n = 3$). The time of synthesis and purification was $99 \pm 2$ min. The final product was prepared as a sterile saline solution suitable for *in vivo* use. Copyright © 2003 John Wiley & Sons, Ltd.

**Key Words:** $\alpha4\beta2$ nicotinic acetylcholine receptors; PET; radiotracer

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## Introduction

Nicotinic acetylcholine receptors (nAChRs) are a member of the superfamily of ligand-gated ion channels and are believed to play a role in a variety of disease states including Alzheimer's and Parkinson's diseases, schizophrenia and Tourette's syndrome.[1–7] Although neuronal nAChRs consist of pentameric combinations of $\alpha$ and $\beta$ subunits, only a few major subtypes have been identified. Included is the $\alpha4\beta2$ subtype, relatively abundant in the human central nervous system (CNS), and the $\alpha7$ and $\alpha3\beta4$ subtypes found in CNS and peripheral nervous system.

Epibatidine and its derivatives, the first class of compounds examined as potential imaging agents imaging for nAChRs, bind with sub-nanomolar affinity to several receptor subtypes, including $\alpha4\beta2$ and $\alpha3\beta4$. Several epibatidine analogues have been synthesized and labelled with carbon-11, fluorine-18, bromine-76, and iodine-123.[8–11] More recently, a series of 3-pyridyl ether compounds that are more selective for the $\alpha4\beta2$ subtype has been reported. One of the first derivatives, A-85380, has an affinity comparable with epibatidine and has been labelled with iodine-123[12,13] and fluorine-18.[14] Another derivative, A-84543, has been labelled with carbon-11.[15,16] Another reported novel fluorine-18 labelled radioligand, [$^{18}$F]nifrolidine ($K_i$ = 2.88 nM), has demonstrated some promise for the *in vivo* assessment of $\alpha4\beta2$ nicotinic receptors in non-human primates.[17] This paper describes the synthesis and *in vitro* affinity determination ($K_D$) of a new fluorinated $\alpha4\beta2$ PET tracer, 3-[1-(3-fluoropropyl)-(*S*)-pyrrolidin-2-ylmethoxy]pyridine (NicFP, 1). A preliminary report of this effort has been presented.[18]

## Experimental

### *General*

All reagents were purchased from commercial sources and were used without further purification. Proton NMR spectra were recorded on a Brucker 400 MHz FT-NMR spectrometer (Department of Chemistry, Columbia University). Chemical shifts were recorded in ppm ($\delta$) from an internal tetramethylsilane standard in chloroform-$d_3$, and coupling constants ($J$) are reported in Hz. Chromatographic purification of unlabelled compounds was performed with silica gel (Aldrich, 70-230 mesh, ASTM) using the solvent systems indicated in the text.

Purification and characterization of the radioligand was performed by HPLC methods with a Waters 515 HPLC pump, a Waters PDA UV detector (254 nm), and a Bicron Flow-Scint radiation detector. The column was a reverse-phase column (Phenomenex, ODS, analytical: $4 \times 250$ mm, 5 μm particle size; semi-preparative: $10 \times 250$ mm, 10 μm particle size) and the mobile phases and flow rates used are indicated in the text below. Finally, 3-(2-(*S*)-pyrrolidin-2-ylmethoxy)pyridine (2) was prepared in our laboratory as previously described with modifications as required.[16]

*3-[1-(3-Hydroxypropyl)-(S)-pyrrolidin-2-ylmethoxy]pyridine (3)*. To a solution of 3-(2-(*S*)-pyrrolidin-2-ylmethoxy)pyridine (2) (300 mg, 1.68 mmol) in dichloromethane (20 ml) was added 3-bromo-propanol (235 mg, 0.56 mmol) and potassium carbonate (825 mg). The resulting mixture was stirred at room temperature for 24 h and then deionized water (10 ml) was added. The compound was extracted with dichloromethane ($3 \times 10$ ml), the organic layers were combined, dried over sodium sulphate and evaporated under reduced pressure. The resulting compound was purified by column chromatography (ethyl acetate/ethanol 60:40, v/v) to afford a clear, colorless oil (207 mg, 52%). $^1$H-NMR: 8.38 (s, 1H) 8.25 (m, 1H), 7.25–7.32 (m, 2H), 5.3–5.7 (br s, 1H), 4.24 (m, 1H); 4.05 (m, 1H); 3.82 (m, 2H); 3.40 (m, 1H); 3.16 (m, 1H); 2.87 (m, 1H); 2.44–2.57 (dd, 1H); 1.53–2.23 (m, 7H).

*3-[1-(3-(Methylsulfonyl)-propyl)-(S)-pyrrolidin-2-ylmethoxy]pyridine (4)*. 3-[1-(3-Hydroxypropyl)-(*S*)-pyrrolidin-2-ylmethoxy]pyridine (3) (150 mg, 0.63 mmol) was dissolved in a mixture of triethylamine (2 ml) and dichloromethane (15 ml) and to this was added methansulfonyl chloride (79 mg, 0.69 mmol). The resulting solution was stirred for 3 h at room temperature. The volatile components were removed under reduced pressure, and the product was purified by column chromatography (silica gel; 100% ethyl acetate, $R_f = 0.2$) to provide a clear, colorless oil (98 mg, 50%). $^1$H-NMR: 8.40 (s, 1H) 8.25 (m, 1H), 7.25–7.32 (m, 2H), 4.33–4.42 (m, 2H); 4.05 (m, 1H); 3.87 (dd, 1H); 3.18 (m, 1H); 3.06 (m, 1H); 3.02 (s, 3H); 2.95 (m, 1H); 2.55 (m, 1H); 2.32 (dd, 1H); 1.95–2.13 (m, 2H); 1.67–1.90 (m, 7H). MS *m*/*z*: 315.14.

*3-[1-(3-Fluoropropyl)-(S)-pyrrolidin-2-ylmethoxy]pyridine (NicFP, 1)*. To a solution of 3-(2-(*S*)-pyrrolidin-2-ylmethoxy)pyridine (4) (150 mg, 0.84 mmol) in dichloromethane (10 ml) was added 1-bromo-3-fluoro-

propane (141 mg, 0.84 mmol) and potassium carbonate (415 mg). The resulting mixture was stirred at room temperature for 24 h and then water (10 ml) was added. The product was extracted with dichloromethane (3 × 10 ml), and the organic layers were combined, dried over sodium sulphate and evaporated *in vacuo*. The product was purified by column chromatography (ethyl acetate/ethanol 60:40, v/v) to afford a light-brown oil (41 mg, 20%). $^{1}$H-NMR: 8.40 (s, 1H) 8.25 (m, 1H), 7.25–7.32 (m, 2H), 4.45–4.65 (m, 2H); 3.82–4.02 (m, 2H); 3.20 (m, 1H); 3.08 (m, 1H); 2.92 (m, 1H); 2.56 (m, 1H); 2.32 (dd, 1H); 1.85–2.10 (m, 6H). MS *m*/*z*: 239.16.

*Synthesis of [$^{18}$F]3-[1-(3-Fluoropropyl)-(S)-pyrrolidin-2-ylmethoxy]-pyridine ([$^{18}$F]-**1**).* [$^{18}$F]Fluoride was transferred from the cyclotron and trapped on a QMA cartridge (Waters Corp, Woburn MA). Kryptofix 2.2.2 (11 mg) and potassium carbonate (2 mg) in water (1.1 ml) were used to elute the [$^{18}$F]fluoride from the cartridge into a 5 ml reaction vial. Acetonitrile (1 ml) was added and the vial was placed in a heating block (90°C). The acetonitrile/water mixture was evaporated to dryness under a stream of argon. Acetonitrile (1 ml) was added and evaporated once again. After this was repeated for 3 times, the mesylate 4 (1.8–2.0 mg), dissolved in anhydrous acetonitrile (1 ml), was added and the resulting mixture was heated at 90°C for 15 min. To ensure adequate separation by HPLC, excess precursor was consumed by the addition of sodium hydroxide solution (100 µl, 0.1 N), followed by heating for an additional 10 min. The vial was cooled to ambient temperature, and the mixture was diluted with 1 ml mobile phase and injected onto a semi-preparative C-18 Phenomenex Prodigy HPLC column (mobile phase: ammonium acetate buffer (0.1 M, pH = 6.8)/acetonitrile 20:80 (v/v); flow rate = 10 ml/min; retention time = 16–18 min). Quality control analysis by analytical HPLC methods (C-18 Phenomenex Prodigy; mobile phase: ammonium acetate buffer (0.1 M)/acetonitrile 25:75 (v/v); flow rate = 2 ml/min; retention time = 10.5–11.0 min) revealed that the radiolabelled product co-eluted with a fully characterized 1 standard. This HPLC method was also used to determine specific activity and radiochemical and chemical purity of [$^{18}$F]-1.

To obtain preparations of the radiotracer suitable for use *in vivo*, the eluted radioactive peak corresponding to [$^{18}$F]-1 was collected and diluted with 100 ml de-ionized water, passed through a C-18 Sep-Pak® (Waters) and the product was eluted with 1 ml ethanol. A 10 µl sample

of the ethanol solution was analyzed to determine radiochemical and chemical purity, and specific activity.

### *Ligand binding assay*

Male Sprague-Dawley rats ($n = 5$) were decapitated under $CO_2$ anesthesia, and the thalamus of each was removed and homogenized together with a PowerGen 125 tissue homogenizer (Fisher Scientific; 15 s, half-maximum speed) in a 30-fold (w/v) excess of ice-cold Tris–HCl buffer (0.05 M, pH = 7.4). The resulting homogenate was centrifuged at 10,000 *g* for 10 min at 4°C using an Allegra 64R ultracentrifuge (Beckman Coulter). The pellet was collected and resuspended in 30 volumes (w/v) of the same buffer, and was centrifuged a second time. The process was repeated once more. The final pellet was resuspended in ice-cold Tris–HCl buffer (0.05 M, pH = 7.4) to provide a final concentration of 30 mg membrane per 1 ml solution.

Association experiments revealed that [$^{18}$F]NicFP binding had reached equilibrium by 60 min at room temperature. Next, the equilibrium dissociation constant $K_D$ of the compound was measured by *in vitro* receptor binding assays. Briefly, [$^{18}$F]NicFP (2 nM) was incubated for 1.5 h in a total volume of 1.0 ml TRIS acetate buffer (0.05 M, pH = 7.4, 22°C) including NaCl (1 mM) and $CaCl_2$ (1 mM) in the presence of rat thalamic membranes (2 mg/tube). The final concentration of ethanol in these experiments was very low (less than 1/10 000 ml per tube (1 ml total volume per tube)) and would not be expected to interfere with the binding of the tracer. In fact, the specific binding exhibited by NicFP *in vitro* was high (about 80% of the total counts). The effect of different concentrations of test ligand ($10^{-12}$ to $10^{-4}$ M) NicFP was examined, and all assays were performed in triplicate. Non-specific binding was determined by (*S*)-nicotine (10 μM). Experiments were terminated by the addition of 3 ml ice cold buffer, and the unbound tracer was removed by washing twice more (approximately 4 ml/wash) cold buffer using a 48 well cell harvester (Brandel). The data were analyzed by Prism (GraphPad).

### *Lipophilicity estimations*

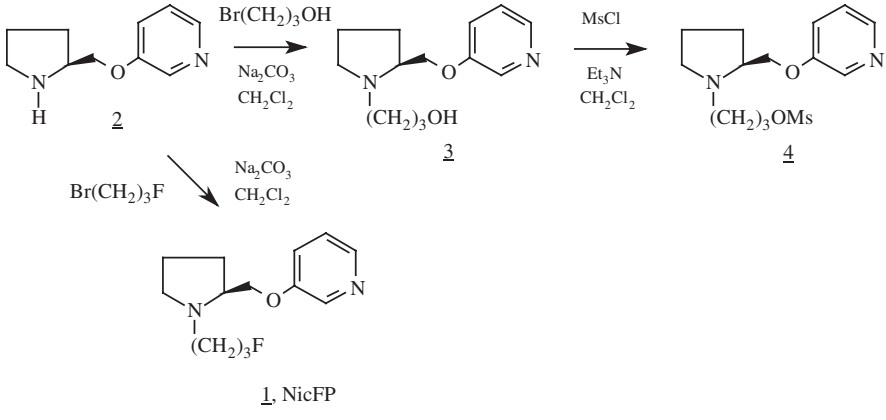
The lipophilicity of 1 was examined by determination of the log *P* value using an HPLC method previously described.[19] Briefly, samples were analyzed using an analytical C-18 column (Phenomenex Prodigy 10 μm,

4.6 × 250 mm) and a mobile phase of methanol and 0.05 M sodium hydrogen phosphate/sodium dihydrogen phosphate buffer (85:15 v/v, pH = 7.4 at 22°C) at 1.0 ml/min. The lipophilicity was determined by comparison of the retention time of the compound to that of standards having known log *P* values. Relative retention times, RRT, were calculated, and a calibration curve of log *P* vs log RRT was generated. The calibration equations were polynomial with $r^2$ of 0.992. All standard and sample injections were done in triplicate and the results were averaged to provide the final values.

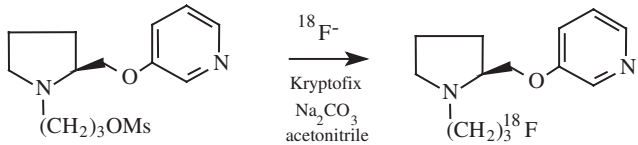
## Results and discussion

The syntheses of 3-[1-(3-fluoropropyl)-(*S*)-pyrrolidin-2-ylmethoxy]-pyridine (NicFP, 1) and the corresponding mesylate 4 are presented in Scheme 1. Alkylation of 2 with either 1-bromo-3-fluoropropane or 3-bromo-1-hydroxypropane provided 3 and 1, respectively, in moderate yield. The mesylate precursor 4 was synthesized by treating 3 with methansulfonyl chloride at room temperature in a mixture of triethylamine and dichoromethane, followed by purification by flash chromatography. Proton NMR spectral data of all compounds (1–4), and mass spectral analysis of 1 and the mesylate precursor 4 were in accordance with the expected molecular structures. The standard 1 was shown to be >95% pure by NMR and HPLC analysis. The mesylate was 95% pure by NMR analysis.




**Scheme 1. Synthesis of 1 (NicFP) and the mesylate precursor 4**




[$^{18}$F]NicFP, 1

**Scheme 2. Synthesis of [$^{18}$F]NicFP**

The corresponding PET tracer [$^{18}$F]-1 was synthesized *via* a nucleophilic substitution reaction between the precursor 4 and [$^{18}$F]fluoride (Scheme 2). The reaction was facilitated by heating the mixture at 90°C for 10 min. The resulting radiolabelled [$^{18}$F]-1 was purified by semi-preparative HPLC methods.

A 10 µl sample of the ethanol solution was analyzed to determine radiotracer purity and specific activity. The time of synthesis and purification was $99 \pm 2$ min. As determined by analytical HPLC methods, a radiochemical yield of $11.3 \pm 2.1\%$ and a specific activity of $0.472 \pm 0.181$ Ci/µmol were realized (EOS, $n = 3$). The chemical purity was >90% as assessed by peak area evaluation of the UV chromatograms. The radiochemical purity was greater than 99%, and up to 67 mCi of product was obtained after HPLC purification. Finally, HPLC based analysis revealed that the lipophilicity of NicFP is moderate ($\log P = 2.2$).

An *in vitro* receptor binding assay was performed to determine the affinity of [$^{18}$F]NicFP to $\alpha 4\beta 2$ nicotinic receptors in rat thalamic membranes, and a $K_D$ value of $1.1 \pm 0.3$ nM ($n = 3$) was measured.

## Conclusion

We report the synthesis, radiosynthesis and *in vitro* ($K_D$) evaluation of the novel $\alpha 4\beta 2$ nAChR PET ligand [$^{18}$F]3-[1-(3-fluoropropyl)-(*S*)-pyrrolidin-2-ylmethoxy]pyridine ([$^{18}$F]NicFP, [$^{18}$F]-1). The radiotracer exhibited good affinity for the nicotinic acetylcholine receptor, and moderate lipophilicity. The corresponding mesylate precursor was labelled with the positron emitting isotope fluorine-18 in a good yield and acceptable specific activity. [$^{18}$F]-1 was subsequently prepared as a formulation suitable for use in *in vivo* studies.

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