Synthesis of [^{18}F]Norchlorofluoroepibatidine and its N-methyl Derivative: New PET Ligands for Mapping Nicotinic Acetylcholine Receptors

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SUMMARY

Fluorine-18 labeled norchlorofluoroepibatidine (NFEP), a high-affinity nicotinic acetylcholine receptor ligand, was prepared by a one-pot, two-step synthesis: nucleophilic heteroaromatic substitution of a *tert*-Boc protected precursor (7-*tert*-butyloxycarbonyl-exo-2-(2'-N,N,N-trimethylammonium-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane iodide) using no-carrier-added [^{18}F]fluoride followed by deprotection with trifluoroacetic acid. Subsequent reductive N-methylation with formaldehyde and sodium cyanoborohydride afforded fluorine-18 labeled N-methyl-norchlorofluoroepibatidine (N-methyl-NFEP). The unusually high radiochemical yield for the first step (70%) and the quantitative conversions in the deprotection and N-methylation steps afforded overall radiochemical yields of 55-65% (decay corrected based on starting [^{18}F]fluoride) for [^{18}F]NFEP (synthesis time 65 min) and 45-55% for [^{18}F]N-methyl-NFEP (synthesis time 75 min), with a specific activity of 2-9 Ci/μmole (EOB).

Key words: nicotinic acetylcholine receptors, epibatidine, fluorine-18, positron emission tomography.

INTRODUCTION

Epibatidine (EPB, exo-2-(2'-chloro-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane), a compound isolated from the skin of the South American Ecuadoran poison frog, has been shown to exihibit a non-opioid mechanism of action which produced an analgesic effect reported to be 200 times that observed with morphine [1, 2, 3]. The intense interest in epibatidine stems at least in part from the discovery that it is a ligand with one of the highest affinities for neuronal nicotinic acetylcholine receptors (nAChR). The facts that nAChR play a role in various neuropathological and physiological states, including Parkinson's disease, Alzheimer's disease and tobacco dependency, and by far, the lack of a suitable radioligand to map this receptor system *in vivo*, prompted us and other researchers to search for a suitable labeled epibatidine derivative for *in vivo*

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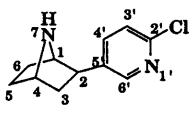
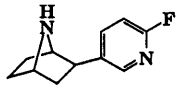
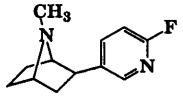
mapping with Positron Emission Tomography (PET). Interestingly, the two stereoisomers of EPB are virtually equipotent. The synthesis for C-11 labeled N-methyl-epibatidine has been reported; however, animal studies were not included [4]. The norchloro analogue (norchloro-epibatidine) has slightly reduced affinity yet higher levels of specific binding *in vivo* for nAChR as compared to epibatidine [5], suggesting that it might be an advantage to prepare F-18 ($t_{1/2}$ = 110 min) labeled norchloro-2-fluoroepibatidine (NFEP) as the target molecule to map nAChR *in vivo* [6, 7, 8]. Our studies demonstrate a high brain uptake of [^{18}F]NFEP in both baboon (12-15%) and mouse, and high specificity of its binding for nAChR *in vivo*. The extraordinarily high thalamus to cerebellum ratio (4.0-6.0 in baboons at the end of 2 hr study), used as the index for specific to nonspecific binding, provides an excellent signal to noise ratio and suggests that [^{18}F]NFEP may be a new scientific tool to investigate the nAChR system and its role in neurodegeneration and addiction [6]. The high pharmacological potency of the parent epibatidine [9, 10, 11] raised the concern as to whether the structurally similar NFEP could be safely administered to humans and whether less potent derivative might increase the safety margin while still maintaining specificity for nAChR. Since N-methylation of epibatidine has been reported to reduce its affinity for central nicotinic binding sites [12], we also synthesized F-18 labeled N-methyl-NFEP in order to carry out comparative studies in baboon. We report here the details of the radiosyntheses for [^{18}F]NFEP (2) and its N-methyl analogue [^{18}F]N-methyl-NFEP (3).



Epibatidine (1, EPB)



Norchlorofluoroepibatidine (2, NFEP)



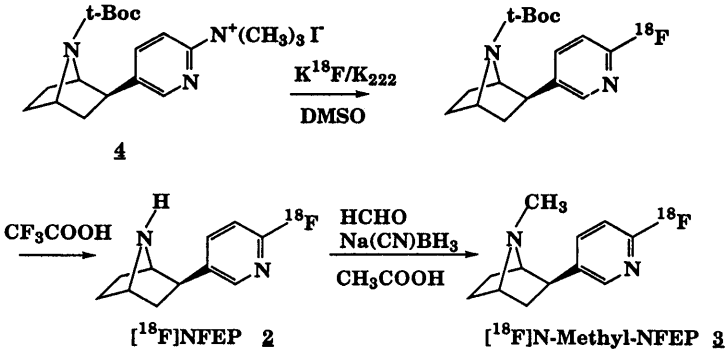
N-Methyl-Norchlorofluoroepibatidine (3, N-Methyl-NFEP)

RESULTS AND DISCUSSION

The one-pot, two-step radiosynthesis (nucleophilic heteroaromatic substitution reaction followed by deprotection) afforded [^{18}F]NFEP with an overall radiochemical yield of 55-65% in a synthesis time of 65 min with a specific activity of 2-9 Ci/μmol (EOB). Subsequent reductive N-methylation with formaldehyde and sodium cyanoborohydride afforded F-18 labeled N-methyl-norchlorofluoroepibatidine ([^{18}F]N-methyl-NFEP) (Scheme 1). The high radiochemical yield for the first step (70%) and the quantitative conversions in the deprotection and N-methylation steps afforded overall radiochemical yields of 45-55% for [^{18}F]N-methyl-NFEP (synthesis time 75 min), with a specific activity of 2-6 Ci/μmole (EOB).

A *tert*-Boc protected epibatidine derivative (4, (±)-7-*tert*-butoxycarbonyl-exo-2-(2'-N,N,N-trimethylammonium-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane iodide) was chosen as the precursor for F-18 labeling. Compound 4 was prepared via a modification of the procedure for the synthesis of epibatidine reported by Clayton and Regan [13], and the details for the synthesis of compound 4 will be published elsewhere [14]. Although it has been previously shown that aromatic nucleophilic substitution reactions can be extended to the F-18 labeling of heterocyclic aromatic rings, the radiochemical yields were low. For example, the F-18 nucleophilic heteroaromatic substitution reaction has been carried out in several substituted pyridine systems as

Scheme 1. Radiosynthesis of [18F]NFEP and [18F]N-methyl-NFEP



well as substituted pyridazine, pyrimidine and pyrazine systems[15]; the yields for the substituted pyridine systems were extremely low (2-15%) in the absence of a second electron withdrawing group. 6-[^{18}F]Fluoropurine and 2-[^{18}F]fluoro-9-β--D-ribofuranosylpurine were prepared in 15-16% yields [16]. 6-[^{18}F]Fluoronicotinic acid diethylamide was prepared in a reasonable yield (40%) from the corresponding 6-chloronicotinic acid [17]; however, a high temperature reaction condition (>200° C) was required. In our study, an unexpectedly high radiochemical yield for F-18 nucleophilic heteroaromatic substitution reaction (70% at EOB) was achieved. This is noteworthy since structurely similar bromo substituted precursors (exo-2-(2'-bromo-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane or its N-protected analogs) afforded only 10-15% radiochemical yield at EOB [7]. The combination of a better leaving group (trimethylammonium group) and a *tert*-Boc protecting group might orient the molecule to a configuration favorable (electronically and sterically) for this substitution reaction. Apart from this advantage, compound **4** has been proven to be a better precursor than the bromo substituted precursors for the synthesis of [^{18}F]NFEP for the following reason. The bromo precursor has an affinity for nAChR similar to EPB; however, *tert*-Boc protected precursor **4** is 12,000 times less potent than EPB based on a binding assay conducted by Dr. Ivy Carroll 's group (unpublished results). Thus, the use of *tert*-Boc protected precursor **4** eliminates the possible contamination of [^{18}F]NFEP with starting material which has high pharmacological potency and may confound the interpretation of PET studies.

Furthermore, toxic effects of EPB have been reported due to its high pharmacological potency; our ability to synthesize [^{18}F]NFEP and [^{18}F]N-methyl-NFEP in high yields and high specific activity allows us to carry out tracer studies *in vivo* without hemodynamic effects [18, 19, 20].

In summary, we have developed a highly efficient radiosynthesis of [^{18}F]NFEP and [^{18}F]N-methyl-NFEP. The availability of these F-18 labeled epibatidine analogues at high specific activities makes it possible to examine nAChR *in vivo* with PET and to investigate its role in neurodegeneration and addiction.

EXPERIMENTAL

^{1}H NMR spectra were obtained in $CDCl_3$ on a Bruker 300 MHz NMR spectrometer and reported in ppm downfield from tetramethylsilane. Mass spectra (MS) were recorded with a Finnegan-Mat GC-MS 5100 mass spectrometer using electron impact ionization at 70 eV. Purification and analyses of radioactive mixtures were performed with a Knauer HPLC pump, in-line UV detector (254 nm), and a NaI crystal radioactivity detector. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. The radiochemical purity of the product was determined by radioTLC and by analytical radioHPLC in the presence of unlabeled compound as carrier. Details of semipreparative, analytical HPLC conditions are described in the following experimentals. For the TLC analyses, Macherey-Nagel polygram sil G/UV_{254} plastic-back TLC plates were used. A short wavelength ultraviolet lamp and NaI well counter or automatic TLC scanner (Berthold Automatic TLC Linear Analyzer) were used as UV and radioactivity detectors. Note because of the high toxicity of epibatidine, care should be taken to avoid contact by all routes of exposure.

Synthesis of No-Carrier-Added (NCA) [^{18}F]Norchloro-2-fluoroepibatidine ([^{18}F]NFEP)

The one-pot, two-step radiosynthesis of [^{18}F]norchloro-2-fluoroepibatidine is shown in Scheme 1. A *tert*-Boc protected precursor (**4**, (±)-7-*tert*-butoxycarbonyl-exo-2-(2'-N,N,N-trimethylammonium-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane iodide) was used as the starting material [14]. Nucleophilic aromatic substitution of compound **4** (4 mg) by Kryptofix (23 mg)/K_2CO_3 (4 mg) activated [^{18}F]F^- ion was carried out in DMSO (0.3 mL) at 120°C for 10 min; the detailed procedure for [^{18}F]F^- drying was described in reference 18. Complete cleavage of the protecting group was achieved via acid hydrolysis with trifluoroacetic acid (0.2 mL) in the same reaction vessel refluxed at 120°C for 10 min. The resulting solution was neutralized with concentrated $K_2CO_{3(aq)}$ (100g of K_2CO_3 in 300 mL of H_2O, 0.8 mL) in 2.4 mL of water. The mixture was passed through a C-18 Sep-Pak cartridge which was subsequently eluted with acetonitrile (2 x 2 mL). After evaporation of the solvent, the residue was taken up in a mixture of 0.6 mL of HPLC solvent and 0.6 mL of H_2O and then purified using a semipreparative HPLC (Waters μBondapak C18 column, 7.8 x 300 mm; CH_3CN : 0.025 M phosphate buffer (pH 7.0) = 10 : 90; flow 3.3 mL/min; UV 254 nM). The radioactive product peak detected by radiomonitor was collected (retention time 14 min) and concentrated by evaporation. The area of the UV peak corresponding to the product was compared with a standard calibration curve and was used to determine the specific activity of [^{18}F]NFEP. The retention time for the unprotected precursor was 3.6 min under the same HPLC conditions, thus, there was no concern for possible contamination.

Synthesis of No-Carrier-Added (NCA) [^{18}F]N-Methyl-norchloro-2-fluoroepibatidine ([^{18}F]N-methyl-NFEP)

The initial two step radiosynthesis of [^{18}F]N-methyl-NFEP (**3**) is the same as described above for the radiosynthesis of [^{18}F]NFEP (**2**). That is, after C-18 Sep-Pak cartridge was eluted with acetonitrile (4 mL), the solvent volume was reduced to 1 mL by evaporation at 120°C. Formaldehyde (50 μL of 37% HCHO in water) and $Na(CN)BH_3$ (18 mg) was added, followed by

addition of acetic acid (10 μL). The reaction mixture was stirred at room temperature for 5 min. After evaporation of the solvent, the residue was taken up in a mixture of 0.6 mL of H_2O and 0.6 mL of HPLC solvent and purified using a semipreparative HPLC (Waters μBondapak C18 column, 7.8 x 300 mm; CH_3CN : 0.025 M phosphate buffer (pH 7.0) = 20 : 80; flow 2.0 mL/min; UV 254 nM). The radioactive product peak detected by radiomonitor was collected (retention time 15 min) and concentrated by evaporation. The area of the UV peak corresponding to the product was compared with a standard calibration curve and was used to determine the specific activity of [^{18}F]N-methyl-norchloro-2-fluoroepibatidine. The HPLC solvent was removed by rotary evaporation and the residue was taken up in 3 ml of saline (0.9%) and subjected to millipore filtration for sterilization. The area of the UV peak corresponding to the product was compared with a standard calibration curve and was used to determine the specific activity of [^{18}F]N-methyl-NFEP.

Radiochemical Purity Determination

Radiochemical purity of both radiotracers was assayed by radioTLC (silica, cyclohexane : toluene : diethylamine = 75:15:10) and by analytical radioHPLC (Waters μBondapak C18 column, 3.9 x 300 mm; CH_3CN : 0.025 M phosphate buffer (pH 7.0) = 30 : 70; UV 254 nM at a flow rate of 1.0 mL/min) in the presence of unlabeled compound as carrier. The unlabeled norchloro-2-fluoroepibatidine has a R_f value of 0.35 on TLC and a retention time of 5 min on HPLC, and the N-methyl analogue has a R_f value of 0.75 on TLC and a retention time of 10 min on HPLC.

Synthesis of N-methyl-NFEP

A sample of N-methyl-NFEP used as HPLC and TLC standards was prepared according to a modified precedure reported by Bai et al. [21]. To a solution of NFEP·HCl salt (5 mg, 0.022 mmol) and formaldehyde (37% solution in water, 0.1 mL) in acetonitrile (2 mL) was added sodium cyanoborohydride (22 mg, 0.33 mmol). The cloudy reaction mixture was stirred at room temperature for 2 h. After addition of acetic acid (20 μL), the mixture was stirred for another 1h, then treated with K_2CO_3 (1M, 5 mL) and extracted with $CHCl_3$. The organic layers were dried over $MgSO_4$(s) and evaporated. The crude product was purified by column chromatography. Yield: 4.3 mg, 95%. ^{1}H NMR ($CDCl_3$): δ 8.10 (1H, d, J = 2.5 Hz), 8.01 (1H, ddd, J = 8.5, 8.3, 2.5 Hz), 6.83 (1H, dd, J = 8.5, 3.0 Hz), 3.33 (1H, m), 3.14 (1H, d, J = 3.7 Hz), 2.68 (1H, dd, J = 9.2, 5.0 Hz), 2.26 (3H, s), 1.85 (1H, dd, J = 9.2, 11.9 Hz), 1.6-2.0 (5H, m). MS, m/e (rel. intensity): 206 (M^+, 7.7), 110 (4.3), 83 (100), 82 (69).

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