Synthesis of a Radioiodinated Analog 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

John L. Musachio*[1], Andrew Horti[2], Edythe D. London[1,2,3] and Robert F. Dannals[1]

[1]Divisions of Nuclear Medicine and Radiation Health Sciences
The Johns Hopkins Medical Institutions
600 North Wolfe Street, Nelson Bldg. Rm B1-167
Baltimore, MD 21287 USA

[2]Brain Imaging Section
Intramural Research Program, NIDA
PO Box 5180
Baltimore, MD USA 21224

[3]Department of Pharmacology and Experimental Therapeutics
University of Maryland School of Medicine
655 North Baltimore Street
Baltimore, MD USA 21201

Summary

[^{125}I] or [^{123}I] labeled (±)-*exo*-2-(2-iodo-5-pyridyl)-7-azabicyclo-[2.2.1]heptane (IPH), an analog of the high-affinity nicotinic acetylcholine receptor ligand, epibatidine, was prepared by a nucleophilic non-isotopic exchange reaction. Treatment of the 2-bromo pyridyl precursor with radioiodide and *in situ* generated Cu(I) at high temperature (200 - 220 °C) gave [^{125}I] or [^{123}I]IPH that was purified by reverse phase HPLC. Radiochemical yields ranged from 31 - 50% for the [^{125}I] labeling (average = 37%, n = 7) and 20% for [^{123}I]. Both [^{125}I]IPH and [^{123}I]IPH were of high radiochemical purity (> 96%) and high specific activity (average of 1540 mCi/μmol (57 GBq/μmol) for [^{125}I]IPH and > 1750 mCi/μmol (65 GBq/μmol) for [^{123}I]IPH).

Key Words: epibatidine, ^{123}I, ^{125}I, copper-assisted halogen-exchange, nicotinic receptors, single photon emission computed tomography, brain imaging

* Author for correspondence

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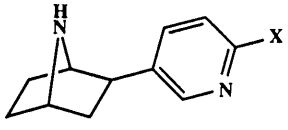
Introduction

Nicotinic acetylcholine receptors (nAChRs) have been implicated in several disease states, including Alzheimer's (1, 2) and Parkinson's diseases (3), and play a role in tobacco addiction (4). Tomographic imaging of central nAChRs has been hampered by the absence of a radiotracer that displays suitable pharmacokinetics and a high degree of specific binding (5). With the discovery of epibatidine, **1**, (*exo*-7-aza-bicyclo-2-(2-chloro-5-pyridyl)-[2.2.1]heptane (Figure 1) and its subsequent pharmacological characterization (6 - 8), a new tool to probe nAChRs has become available. Both [^{3}H]epibatidine and [^{3}H]deschloroepibatidine show exceptionally high specificity and selectivity for nicotinic acetylcholine receptors *in vivo* (5, 9). Prompted by these results, our group has synthesized the [^{18}F] labeled 2-fluoro-pyridyl analog (FPH, **2**) from the bromo precursor (BrPH, **4**) for positron emission tomography (PET) studies of nAChRs (Figure 1) (10). Additionally, syntheses of the N-[^{11}C]methyl and N-2-[^{18}F]fluoroethyl- analogs of epibatidine have been reported (11, 12). Comparable nicotinic acetylcholine receptor ligands labeled with the single photon emitter ^{123}I have only recently been investigated (13). Therefore, we sought to prepare the radioiodinated 2-iodo-pyridyl analog of epibatidine (IPH, **3**, Figure 1). This compound was identified as a suitable candidate for radiolabeling from *in vitro* binding studies; unlabeled IPH revealed subnanomolar affinity (K_i = 0.48 nM) for nAChRs (7). Here we provide details of the synthesis of no-carrier-added [^{125}I] and [^{123}I]-labeled IPH from brominated precursor **4**. To our knowledge, this is the first radioiodinated analog of epibatidine. IPH labeled with [^{125}I] ($t_{1/2}$ = 60 d) has proven useful for *in vitro* binding studies, autoradiography, and *in vivo* biodistribution studies of nAChRs in rodents (14, 15). IPH labeled with [^{123}I] ($t_{1/2}$ = 13.2 h) has been utilized for visualization of nAChRs in primate brain *via* single photon emission computed tomography (14).



X = Cl, epibatidine, **1**
F, **2**
I, **3**
Br, **4**

Figure 1. Epibatidine and 2-halo-pyridyl analogs

Materials and Methods

Chemicals and solvents were used as received. Racemic *exo*-7-(methoxycarbonyl)-2-(2-bromo-5-pyridyl)-7-azabicyclo[2.2.1]heptane, **5**, along with the N-deprotected analog, **4**, were prepared according to the literature method (10). Na[^{125}I]I was purchased from Amersham Corp. (100 mCi/mL (3.7 GBq/mL) in dilute NaOH). Na[^{123}I]I dispensed in 0.1 N NaOH was purchased from Nordion Intl.

(Kanata, Ontario, Canada). Proton NMR spectra were obtained with a Bruker WM-300 (300.13 MHz) instrument. Chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane in $CDCl_3$. High resolution electron impact mass spectroscopy (HREIMS) was performed at the University of Minnesota Mass Spectrometry Service Laboratory. HPLC equipment consisted of a Rheodyne 7125 injector, Water 510 EF and 610 pumps, Waters 486 and 490 UV detectors (254 nm), a flow through NaI(Tl) crystal scintillation detector comprised of EG&G Ortec components, and Rainin Dynamax data acquisition and reprocessing software. Waters C-18 Nova-Pak radial compression cartridges were used for semipreparative (25 mm x 10 cm, 6 μm) and analytical (8 mm x 10 cm , 6 μm) HPLC unless noted otherwise. Radioactivity was measured with a radioisotope dose calibrator (Capintec CRC-7), assuring similar counting geometries and vessel types for each reading. Analytical TLC was conducted on Macherey Nagel silica gel 60 F-254 plates (250 μm) using 90:10:0.4 CH_2Cl_2/MeOH/conc. NH_4OH as developing solvent. Specific radioactivities were determined for aliquots of known radioactivity using analytical HPLC for determination of the mass associated with the UV absorbance peak area for the carrier. The HPLC UV calibration curve was established using nonradioactive standards.

Synthesis of (±)-exo-7-(methoxycarbonyl)-2-(2-iodo-5-pyridyl)-7-azabicyclo[2.2.1] heptane (**6**)

To a 4 mL v-vial purged with argon was added 34 mg (0.11 mmoles) of **5**, prepared as described previously (10). Dry methylsulfoxide was added (700 μL) along with copper(I) iodide (98 mg, 0.51 mmoles, 4.6 equiv) and potassium iodide (86 mg, 0.51 mmoles, 4.6 equiv) . The reaction was heated at 150° C for 4.5 h. The material was then purified by semi-preparative HPLC (6 injections) using a mobile phase of 30/30/40 MeOH/MeCN/H_2O (0.1 M ammonium formate) at a flow rate of 10 mL/min. The peak at t_R = 8.1 min, k' = 2.4 was collected along with residual starting material (t_R = 7.1 min, k' = 2.0). The eluent was concentrated by rotary evaporation, made basic by addition of saturated $NaHCO_3$, and extracted with CH_2Cl_2. The extracts were dried over Na_2SO_4, filtered, and solvent removed under vacuum to give **6** (19 mg, 0.05 mmoles) as a colorless oil in 48% yield. TLC: R_f = 0.82. ^{1}H NMR: ($CDCl_3$, δ) 8.22 (d, 1H, J = 2.5 Hz), 7.63 (d, 1H, J = 8.2 Hz), 7.29 (dd, 1H, J = 8.2, 2.5 Hz), 4.44 (br s, 1H), 4.21 (br s, 1H), 3.67 (s, 3H), 2.84 (dd, 1H, J = 9.0, 4.9 Hz), 2.02 (dd, 1H, J = 12.4, 9.0 Hz), 1.78 - 1.84 (m, 3H), 1.52 - 1.64 (m, 2H). HRMS-EI: *m/z* calcd, 358.01795; found, 358.0187.

Synthesis of (±)-exo-2-(2-iodo-5-pyridyl)-7-azabicyclo[2.2.1]heptane (**3**, IPH)

To a 4 mL vial was added **6** (19 mg, 53 μmol) and 1 mL of 45% hydrobromic acid in acetic acid (Lancaster Synthesis Inc., Windham, NH). The vial was kept at room temperature for 22 h. Approximately 2 mL of 6 M NaOH was added to bring the pH to 4. The crude reaction was purified by HPLC (15 injections) using an

analytical column and a mobile phase of 10/10/80 MeOH/MeCN/H_2O (0.1 M ammonium formate) at a flow rate of 3 mL/min. The N-deprotected product (t_R = 3.2 min, k' = 2.6) was collected. Extractive work-up, as described for **6**, gave **3** (15 mg, 50 μmol, 94% yield) as an oil that solidified upon standing. TLC: R_f = 0.43. ^1H NMR: ($CDCl_3$, δ) 8.26 (d, 1H, J = 2.4 Hz), 7.62 (d, 1H, J = 8.2 Hz), 7.46 (dd, 1H, J = 8.2, 2.5 Hz), 3.79 (br s, 1H), 3.55 (br s, 1H), 2.71 (dd, 1H, J = 8.8, 4.8 Hz), 1.90 (dd, 1 H, J = 12.1, 9.0 Hz), 1.56 - 1.62 (m, 5H). HRMS-EI: *m/z* calcd, 300.0125; found, 300.0136.

*Synthesis and purification of (±)-exo-2-(2-[^{125}I]iodo-5-pyridyl)-7-aza-bicyclo[2.2.1]heptane, [^{125}I]**3**.*

As an example of a typical experiment: 10 μL (1045 μCi, 38.6 MBq) of [I-125] NaI, 25 μL of 0.1 M $Na_2S_2O_5$(aq) (2.5 μmol), 100 μg (0.4 μmol) of **4** dissolved in 100 μL of glacial HOAc, and 10 μL of 2.6 mM $CuSO_4$•$5H_2O$ (0.026 μmol) were added to a 1.5 mL glass Mini-Aktor reaction vessel (Alltech Associates Inc., Deerfield, IL) . The vessel was sealed with a teflon cap and placed in a 200° C sand-bath. After heating for 20 min, the reaction was cooled at room temperature for 5 min., and the contents of the vessel injected onto an analytical cartridge eluted with 7.5/7.5/85 MeOH/MeCN/0.1 M ammonium formate at 4 mL/min. [^{125}I]**3** was collected (t_R = 4.9 min, k' = 6.2) and resolved from starting **4** (t_R = 3.2 min, k' = 3.7). The HPLC solvent was concentrated to less than 300 μL *via* rotary evaporation at 40 °C to give 378 μCi (14 MBq) of [^{125}I]**3** (36% radiochemical yield, 98% radiochemical purity). For specific activity determination, the concentrated solution of [^{125}I]**3** of known activity (412 μCi, 15.2 MBq, in a plastic syringe) was reinjected on the analytical cartridge. A specific radioactivity of 2060 mCi/μmol (76 GBq/μmol) was determined by comparison of the area of the carrier peak to that of a standard sample of **3**. [^{125}I]**3** was collected from the analytical column and the solvent was evaporated. This twice purified radioligand was formulated in aqueous ammonium formate for *in vitro* studies and in 0.9% sterile saline for *in vivo* studies.

*Synthesis and purification of (±)-exo-2-(2-[^{123}I]iodo-5-pyridyl)-7-aza-bicyclo[2.2.1]heptane, [^{123}I]**3***

To a v-vial containing 28.6 mCi (1 GBq) of Na[^{123}I]I, originally dispensed in 0.12 mL of 0.1 M NaOH and then evaporated to dryness, was added 200 μL of water. The radioiodide solution was withdrawn in a 1 mL plastic syringe and passed through a cation exchange resin (25 mm Novo-Clean IC-H disk, Alltech Associates Inc., Deerfield, IL). The resin was washed with an additional 400 μL of water. The effluent was collected in a 1.5 mL glass Mini-Aktor reaction vessel fitted with a rubber septum. The radioiodide solution was then concentrated in a chemical hood to less than 30 μL by heating at 90° C under an argon stream with a charcoal filter vent.

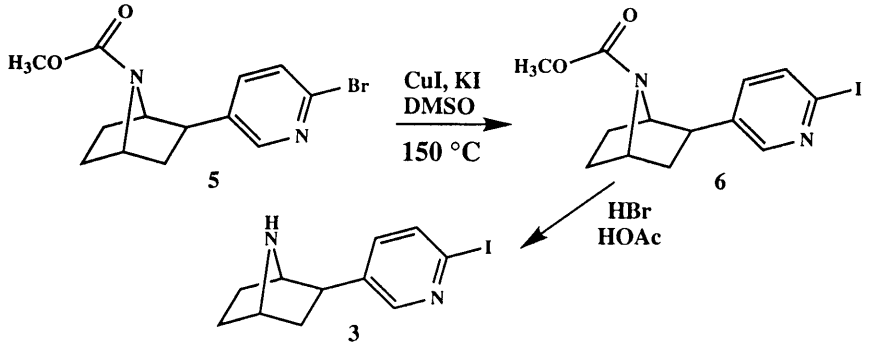
To the reaction vessel was added 25 μL of 0.1 M $Na_2S_2O_5$, 200 μg of **4** dissolved in 100 μL of HOAc, and 10 μL of 2.6 mM $CuSO_4$•$5H_2O$. The vessel was

sealed with a teflon cap and tightly wrapped with teflon tape. The reaction vessel was placed in a 220° C sand-bath, and heated for 40 min before cooling for 4 min at room temperature and 4 min at 0° C. The crude reaction was injected onto an analytical cartridge eluted with 7.5/7.5/85 MeOH/MeCN/0.05 M NaH_2PO_4 at 4 mL/min. After the starting material, **4** (t_R = 2.8 min, k' = 3.1), was eluted from the cartridge, [^{123}I]**3** (t_R = 4.2 min, k' = 5.2) was collected, and the HPLC solvent removed *via* rotary evaporation at 40° C. To remove the last traces of **4**, the radiotracer was repurified on the HPLC column using the above conditions. Rotary evaporation of the radioligand was followed by redissolving the radioactivity in 3 mL of 0.9% sterile saline. The radioactive solution was passed through a 13 mm Gelman Acrodisc sterile filter into a sterile 10 mL dose vial to give 5.8 mCi (0.21 GBq) of [^{123}I]**3** in 20% radiochemical yield (non-decay corrected).

For specific radioactivity and radiochemical purity determination a 100 µL aliquot of the final dose (0.137 mCi, 5.07 MBq) was injected onto the analytical cartridge eluted with 12.5/12.5/75 MeOH/MeCN/0.05 M NaH_2PO_4 at 3 mL/min. [^{123}I]**3** (t_R = 2.9 min, k' = 1.9) was shown to be 97% radiochemically pure with no extraneous UV contaminants observed at the highest sensitivity range of the detector. A minimum specific radioactivity of 1750 mCi/µmol (65 GBq/µmol) was calculated based on the detection limit for authentic IPH (0.078 nmoles).

Results and Discussion

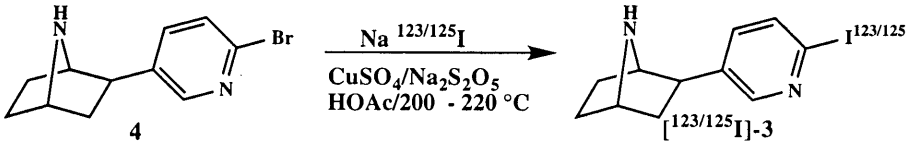
The synthesis of an authentic sample of IPH, **3** (16), involved treatment of the known N-protected BrPH, **5** (10), with CuI and KI in DMSO at 150 °C followed by N-deprotection (Scheme 1). This procedure was based on a published method (17) for the preparation of halogenated pyridines except that DMSO was substituted for carcinogenic hexamethylphosphoramide. Reverse-phase HPLC was used to isolate the intermediate **6** in 48% yield and recover unreacted **5** (typically 15% recovery). Initial attempts to resolve unreacted **5** from **6** by normal phase TLC proved unsuccessful. Removal of the methoxycarbonyl group of **6** using HBr in acetic acid produced **3** in 94% yield (Scheme1) that was again purified by HPLC. In all likelihood, however, hundreds of milligrams of **3** could be purified by normal phase



Scheme 1. Synthesis of authentic IPH, **3**

column chromatography as **3** is almost the exclusive component of the reaction mixture and can be easily visualized at 254 nm on UV absorbing TLC plates.

With the unlabeled IPH standard in hand, various routes to prepare radioiodinated **3** were explored. Owing to the ease of radioiododestannylation reactions (18), we attempted to prepare the 2-trialkylstannyl-pyridyl analog of epibatidine from **5**. Using the method of Rocca as a guide (19), **5** was treated with n-butyllithium at -75° C followed by addition of Me_3SnCl; however, evidence for the formation of the stannylated pyridine was not found. Alternatively, a Cu(I) assisted non-isotopic exchange radiolabeling was explored as this method has already proven useful for preparation of several radioiodinated tracers (20 - 22). Moreover, this procedure appeared promising as 2-halo-pyridines are noted to undergo nucleophilic aromatic substitution (23). Treatment of **4** (100 μg), contained in a Mini-Aktor pressure vessel with Na[^{125}I]I, copper sulfate, and sodium metabisulfite in glacial acetic acid at 200° C, provided [^{125}I]**3**, which was purified by HPLC (Scheme 2). Isolated radiochemical yields of 31 - 50 % using [I-125] were obtained after heating for 20 min. Prolonged heating (e.g., 1 h) and varying the concentration of precursor (50 μg - 200 μg) did not improve radiochemical yields.



Scheme 2. Radiosynthesis of I-125/I-123 labeled IPH, **3**

Purification of radioiodinated **3** was achieved by a two-pass reverse-phase HPLC method. A C-18 Nova-Pak analytical radial compression cartridge (8 mm x 10 cm) was employed using a ternary mobile phase. The use of semi-preparative columns and longer retention times (> 15 min) for the purification of [^{125}I]**3** resulted in excessively broad peaks. Figure 2 depicts the UV and radiometric traces for the purification of [^{125}I]**3**. The radioligand was collected in a small volume (*ca.* 6 mL) and the solvent concentrated at 40° C under reduced pressure. Attempts to isolate the radiotracer by solid-phase extraction using a C-18 cartridge proved unsuccessful as greater than 50% of the radioactivity broke through the stationary phase due to the hydrophilicity of [^{125}I]**3**. After first-pass HPLC purification approximately 99% of the bromo precursor is removed; the remaining 1% (*ca.* 1 - 2 μg) was removed in a second purification that also served for specific activity determination. The total time required for two-pass purification of radioiodinated IPH (approximately 40 min) is compatible with the short-lived ^{123}I. For *in vivo* rodent studies, [^{125}I]**3** was formulated in 0.9% sterile saline while for *in vitro* binding studies [^{125}I]**3** was dissolved in aqueous ammonium formate (0.4 - 1.6 M). [^{125}I]**3** in aqueous ammonium formate is quite stable being of approximately 96% radiochemical purity as determined by HPLC after storage for 21 days at - 20 °C. Specific radioactivities for [^{125}I]**3** ranged

from 520 - 2050 mCi/μmol (19 GBq - 76 GBq/μmol) with an average of 1540 mCi/μmol (57 GBq/μmol). Under several HPLC conditions [^{125}I]**3** co-eluted with the unlabeled standard.

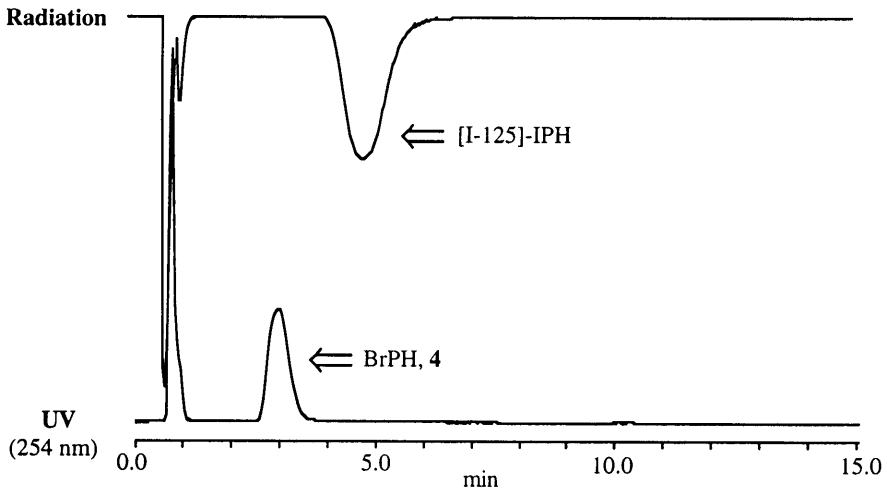


Figure 2. HPLC purification of [^{125}I]IPH from bromo precursor, **4**.

Utilizing a small aliquot of [^{123}I]NaI (0.26 mCi/9.6 MBq, 2 μL of 0.1 M NaOH) and the identical conditions for the ^{125}I radiolabeling, [^{123}I]**3** was obtained in an isolated radiochemical yield of 58%. However, when the ^{123}I radiolabeling was scaled-up using greater amounts of activity (3.5 - 7.0 mCi, 130 - 260 MBq) and subsequently larger volumes of dispensing base (20 - 45 μL), radiochemical yields dropped precipitously (< 10%). The deleterious effects of the 0.1 M NaOH dispensing solution have been noted previously for ^{123}I radiolabelings (24). Quenching the dispensing base with an equivalent amount of 0.74 M phosphoric acid or evaporating the 0.1 M NaOH solution prior to radiolabeling was ineffective in improving isolated yields of [^{123}I]**3**. Lambrecht reported the use of a cation exchange membrane to circumvent the problem of large volumes of NaOH for ^{123}I radioiodinations (25). These membranes have the dual effect of reducing pH (i.e. acidifying) while removing cations. It is believed that sodium ion concentration affects radiochemical yield for some exchange labelings (26), and our results here support this notion. Interestingly, other radiotracers prepared by copper exchange labeling (21, 22) do not seem to be as sensitive to the amount of dispensing NaOH. When mCi levels of [^{123}I]NaI were employed for the synthesis of [^{123}I]**3**, the [^{123}I]NaI was first passed through a Novo-Clean IC-H cation exchange disk, and the aqueous radioiodide solution was evaporated (90° C, Ar stream with charcoal vent). The dried [^{123}I]iodide was then treated with **4** in glacial acetic acid, sodium metabisulfite, and copper sulfate at 220° C for 40 min. Two-pass HPLC purification gave [^{123}I]**3** that was formulated in sterile saline and passed through a sterile filter (20% radiochemical yield, non-decay corrected). The radioligand was of 97% radiochemical purity with a

specific radioactivity of at least 1750 mCi/μmol (65 GBq/μmol) based on the detection limit of the HPLC method. The precursor, **4**, was not detected in the final formulation. After 23 h at room temperature, [^{123}I]**3** was still of 89% radiochemical purity as measured by HPLC.

Conclusions

An analog of epibatidine, IPH, having radioiodine in the 2 position of the pyridine ring was prepared by Cu(I) assisted non-isotopic exchange labeling. The ^{125}I and ^{123}I labeled products were obtained in acceptable yields with high specific radioactivities. Radiolabeled IPH shows promise for *in vitro* and *in vivo* studies of central nAChRs.

Acknowledgments

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