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New synthesis and evaluation of enantiomers of 7-methyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane as stereoselective ligands for PET imaging of nicotinic acetylcholine receptors

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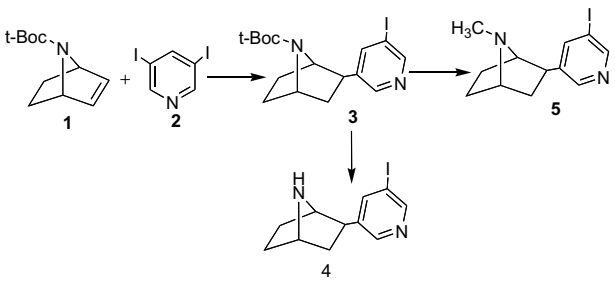
ABSTRACT

A simple and efficient synthesis of nAChR antagonist (±)-7-methyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptane ((±)-NMI-EPB) has been developed. Both enantiomers of (±)-NMI-EPB were separated by semi-preparative chiral HPLC. The enantiomers manifested a substantial difference in their inhibition binding affinities ((+)-NMI-EPB, K_i = 2310, 1680 pM; (−)-NMI-EPB, K_i = 55, 68 pM). The enantiomers were stereoselectively radiolabeled with ^{11}C. In the distribution studies in the rodent brain [^{11}C](−)-NMI-EPB specifically labeled nAChR whereas [^{11}C](+)-NMI-EPB exhibited little specific binding. In the baboon PET study [^{11}C](−)-NMI-EPB did not reach steady-state within 90 min post-injection suggesting that the radioligand may have some limitations for quantitative imaging.

Central nicotinic acetylcholine receptors (nAChR) are involved in tobacco dependence and various conditions and disorders including Alzheimer's disease, Parkinson's disease, schizophrenia, anxiety, depression, Tourette's syndrome, attention-deficit hyperactivity disorder and pain.[1–4] PET imaging of nAChR is useful for studying the role of these binding sites in various disorders. The currently available PET radioligands for human studies (2-[^{18}F]FA and 6-[^{18}F]FA)[5] exhibit drawbacks including slow brain kinetics and low binding potentials. Radiolabeled analogs of high affinity nAChR agonist epibatidine were the first PET radioligands with good imaging properties in animals.[5] Unfortunately, use of these compounds for PET studies in human subjects was too risky due to the toxicity of epibatidine analogs.[6–8] Recently a nAChR antagonist (±)-7-methyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7 azabicyclo[2.2.1]-heptane ((±)-**5**) structurally related to epibatidine was developed[9] and the radiolabeled [^{11}C](±)-**5** was synthesized as a potential PET radioligand-candidate for studies of nAChR in humans with rapid brain kinetics.[10] Since [^{11}C](±)-**5** consists of two enantiomers that may exhibit different binding affinities, it was our hypothesis that separation of a higher affinity radiolabeled enantiomer might yield a PET radioligand with even better imaging properties than those of [^{11}C](±)-**5**. This prompted us to devise a more efficient, simple synthesis of racemic (**5**) with the intention of resolving its enantiomers and evaluating their binding affinities at nAChR to determine if they act in a manner similar to the racemate, or whether one of the isomers behaves differently. Here we describe the new synthesis, radiosynthesis and preliminary in vivo PET imaging properties of ([^{11}C](+)-**5**) and ([^{11}C](−)-**5**) in mice and baboons.

A multi-step synthesis of racemic 7-methyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane **5** has been described previously.[9,11] This procedure was laborious and suffered from low yield. This prompted us to devise a more simple synthesis of unlabeled **5** (Scheme 1). Briefly, palladium catalyzed Heck coupling of compound **1**[9] with the easily available 3,5-diiodopyrdine[12] produced the Boc-protected compound **3** in 86% yield.[13] Deprotection of the Boc group and spontaneously N-methylation occurred in one



Scheme 1. Efficient synthesis of racemic compounds **4** and **5**.

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step with HCOOH and HCHO as reagents.[14] TFA deprotection of compound **3** gave the non-methyl compound **4** quantitatively.

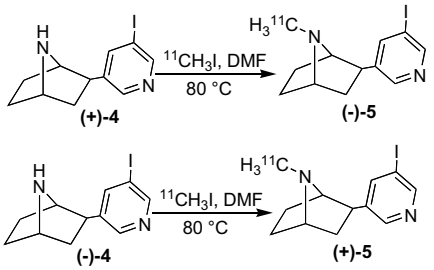
Since **4** and **5** are racemic compounds, the corresponding enantiomers were separated by chiral HPLC and their physical properties are shown in Table 1.

The in vitro inhibition binding assays of (+)-**4**, (−)-**4,** (−)-**5** and (+)-**5** (Table 1) were performed commercially by NovaScreen Biosciences, a Caliper Life Science Co, (Hanover, MD) under conditions similar to those previously published,[15] using rat cortical membranes as a source of nAChRs and [^{3}H]epibatidine as a radioprobe. The in vitro inhibition binding assays of (−)-**5** and (+)-**5** are shown in the Table 1. The *N*-methyl enantiomer (−)-**5** displayed substantially greater binding affinity than that of enantiomer (+)-**5**. In comparison, the K_i value of normethyl precursor (+)-**4** was shown to be essentially identical to its enantiomer (−)-**4**.[9] The more pronounced enantioselectivity of **5** is similar to that of nicotine, that is, exhibiting a 38-fold enantioselectivity of binding affinity with (−)-nicotine versus (+)-nicotine.[16]

It is worth noting that N-methylation of normethyl enantiomers (−)-**4** and (+)-**4** with CH_3I in DMF demonstrated that starting material (+)-**4** stereoselectively yielded *N*-methyl enantiomer (−)-**5**, whereas normethyl compound (−)-**4** produced the corresponding (+)-*N*-methyl enantiomer (+)-**5**. The radiomethylation of (−)-**4** and (+)-**4** with [^{11}C]CH_3I in anhydrous DMF yielded [^{11}C]-(+)-**5** and [^{11}C]-(−)-**5**, correspondingly[17] (Scheme 2). The radioactive products were purified by HPLC and reconstituted in 1 mL ethanol and with 9 mL of 0.9% saline. The final products [^{11}C]-(−)-**5** and [^{11}C]-(+)-**5** were obtained in 18 ± 2% radiochemical yields (n = 3) (non-decay corrected). They were analyzed by analytical HPLC to determine the radiochemical purity and the specific radioactivity. The total radiosynthesis time was 35 min from end of bombardment with an average specific radioactivity of 22,045 ± 1955 mCi/μmol and a radiochemical purity of >98%. The formulated radiotracers were immediately injected into mice or baboons for PET studies.

High affinity radioligand [^{11}C]-(−)-**5** and its low affinity enantiomer [^{11}C]-(+)-**5** were injected in mice and the distribution of radioactivity in the mouse thalamus and cerebellum was studied (Fig. 1). The accumulation of [^{11}C]-(−)-**5** in nAChR-rich thalamus was higher than that of [^{11}C]-(+)-**5** whereas the uptake of both enantiomeric radioligands was comparable in the nAChR-poor cerebellum. As a result, the thalamus/cerebellum ratio was found to be 6 and 1.8 for [^{11}C]-(−)-**5** and [^{11}C]-(+)-**5**, correspondingly. Based on the previously published results with the racemic mixture [^{11}C]-(±)-**5** ((±)-[^{11}C]NMI-EPI)[10] these data suggest that [^{11}C]-(−)-**5** might display a higher binding potential in PET imaging studies than its less active enantiomer [^{11}C]-(+)-**5** or the racemic mixture.

The baboon PET imaging study with active enantiomer [^{11}C]-(−)-**5** demonstrated that the radioligand labels the nAChR-rich regions in the baboon brain whereas the accumulation of radioactivity in the CB-poor cerebellum was lower (Fig. 2). However, PET modeling demonstrated that accumulation of radioactivity in the thalamus did not reach steady-state during 90 min scanning post-injection.[18] The radioactivity accumulation in the thalamus



Scheme 2. Stereoselective synthesis of normethyl enantiomers (−)-**5** and (+)-**5**.

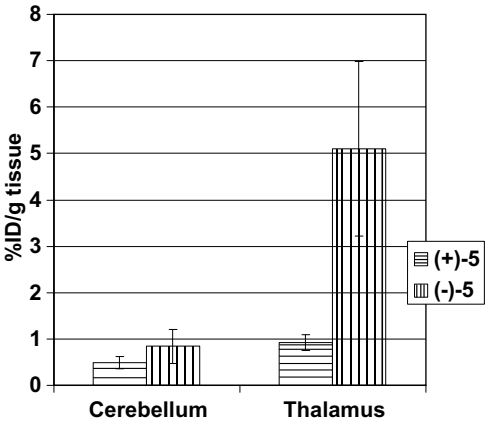


Figure 1. Accumulation of [^{11}C]-(−)-**5** and [^{11}C]-(+)-**5** radioactivity in CD1 mouse brain, 60 min post-injection. Data are mean %ID/g tissue ± SD (n = 3).

(Fig. 2) had not reached the peak and the Th/CB ratio was still rising. This brain kinetics of [^{11}C]-(−)-**5** were different in comparison with [^{11}C]-(±)-**5** ((±)-[^{11}C]NMI-EPI) that reached the peak in all brain regions at 5–10 min post-injection and the thalamus/cerebellum ratio of the enantiomer had reached the plateau at about 90 min post-injection.[10] This finding suggests that [^{11}C]-(−)-**5** ([^{11}C](−)-NMI-EPB) requires more than 90 min of scanning for reliable quantification of nAChR in baboon brain and may not be suitable for quantification of nAChRs in the baboon brain because the scanning time for the most ^{11}C-PET radioligand is limited to 90–120 min due to the short half-life ($t_{1/2}$ = 20 min) of ^{11}C. If radiolabeled with [^{123}I] the enantiomer (−)-**5** ((−)-NMI-EPB) may be of interest as potential SPECT radioligand for imaging of nAChR in human subjects.

In summary, our work has resulted in the simple and efficient synthesis of (±)-7-methyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]-heptane ((±)-NMI-EPB). A substantial increase of yield and shorter synthesis steps are the crucial advantages of this method. Most importantly, we discovered that the enantiomers of (±)-NMI-EPB displayed substantial difference in their binding affinities at nAChRs. The in vivo enantioselectivity of the radiola-

Table 1
Chiral HPLC separation of enantiomers of **4** and **5,** and comparison of their nAChR inhibition binding affinity (K_i) with epibatidine

	Eluent	Retention time (t_R, min)	ee* (%)	$[\alpha]_D22$ ($CHCl_3$)	Physical properties	K_i (pM)
(−)-**4**	A	14.0	>98	−8.85 c = 0.78	Colorless oil	45 ± 7[2]
(+)-**4**	A	12.3	>98	9.53 c = 0.75	Colorless oil	48 ± 7[2]
(−)-**5**	B	6.8	>98	−14.4 c = 0.8	Colorless oil	2310; 1680
(+)-**5**	B	7.8	>98	15.5 c = 0.79	Colorless oil	55, 68
Epibatidine	—	—	—	—	—	46; 56

A: hexane/*i*-PrOH/Et_3N 30:470:1.5. B: hexane/*i*-PrOH/Et_3N 15:485:2.
* ee, enantiomeric excess.

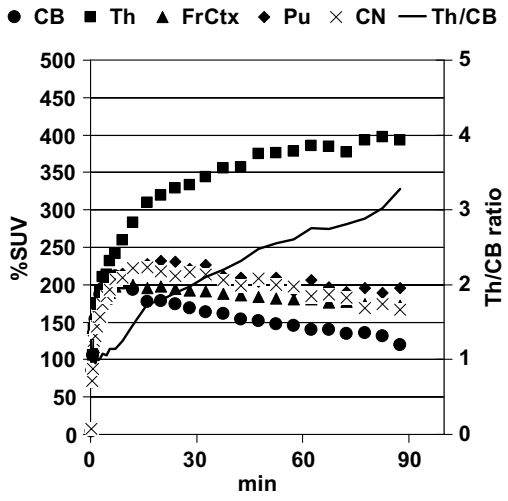


Figure 2. Regional time-uptake curves of [^{11}C]-(−)-**5** in the baboon brain. Symbols: CB, cerebellum; Th, thalamus; FrCtx, frontal cortex; Pu, putamen; CN, caudate nucleus; Th/CB, thalamus/cerebellum ratio.

beled [^{11}C](-)-NMI-EPB and [^{11}C](+)-NMI-EPB were also observed in the distribution studies in the rodent brain with PET. In baboon PET study, [^{11}C](−)-NMI-EPB did not reach steady-state within 90 min post-injection suggesting that it may be not suitable for quantification of nAChRs. Further studies on radiolabeling the enantiomer (−)-**5** ((−)-NMI-EPB with [^{123}I] hold promise as potential SPECT radioligand for imaging of nAChR in human subjects and are in progress.

Acknowledgments

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13. 7-*tert*-Butoxycarbonyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**3**). A mixture of **1** (160 mg, 0.82 mmol), 3,5-diiodopyridine **2** (815 mg, 2.46 mmol, 3 equiv), $Pd(PPh_3)_4$ (27 mg, 0.024 mmol), piperidine (203 μL, 2.03 mmol), formic acid (65 μL, 1.7 mmol), and DMF (3 mL) was stirred in a sealed vial at 70 °C for 6 h. The solvent was removed in vacuo and the residue was treated with EtOAc (100 mL). The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography on silica gel with hexane–EtOAc (5:1 to 1:1) to give the product 3 as white solid (285 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 8.66 (d, J = 2.0 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 7.99 (t, 1H), 4.40 (br s, 1H), 4.20 (br s, 1H), 2.80–2.84 (m, 1H), 2.28 s, 3H), 1.78–1.86 (m, 3H), 1.50–1.61 (m, 1H), 1.45 (s, 9H).
14. 7-Methyl-2-*exo*-(3′-iodo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**5**). Compound **3** (128 mg, 0.32 mmol) was dissolved in a mixture of formic acid (0.35 mL) and formalin (0.7 mL), refluxed for 6 h, and cooled to room temperature. The reaction mixture was poured into 5% K_2CO_3 solution in water (20 mL). The aqueous mixture was extracted with $CHCl_3$ (4× 15 mL), the combined extracts were washed with water (20 mL), dried over Na_2SO_4, and the solvent was removed. The residue was chromatographed on silica gel ($CHCl_3$/MeOH 15:1) to give the product as a white solid (76 mg, 75%); ^{1}H NMR (400 MHz, $CDCl_3$/TMS) δ 8.62 (d, J = 2.0 Hz, 1H), 8.47 (d, J = 2.4 Hz, 1H), 8.22 (t, 1H), 3.34 (br s, 1H), 3.17 (br s, 1H), 2.58–2.62 (m, 1H), 2.26 (s, 3H), 1.90–1.95 (m, 2H), 1.81–1.86 (m, 1H), 1.66–1.71 (m, 1H), 1.39–1.44 (m, 2H).
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17. Radiosynthesis of [^{11}C]-(−)-**5** and [^{11}C]-(+)-**5**. Normethyl precursor (+)-**4** or (−)-**4** (1 mg) was dissolved in 200 μL of anhydrous DMF, capped in a small V-vial and cooled to −40 °C. [^{11}C]Methyl iodide was swept by argon flow into the vial. After the radioactivity reached a plateau, the vial was assayed in the dose calibrator and then heated at 80 °C for 5 min. Water (200 μL) was added and the solution was injected onto the semi-preparative HPLC column. The radiolabeled products were purified by HPLC using a Phenomenex Luna C-18 semi-preparative column (10 × 250 mm, 10 μm, 40:60:0.1 v/v/v CH_3CN/0.1 M ammonium acetate/NEt_3, 10 mL/min). The retention time of normethyl precursor (+)-**4** or (−)-**4** was 3.2 min. The product peak ([^{11}C]-(+)-**5** or [^{11}C]-(−)-**5**) having retention time of 8.5 min, was collected in 50 mL of HPLC water. The water solution was transferred through a Waters C-18 Sep-Pak Plus. The product was eluted with 1 mL ethanol into a vial and diluted with 9 mL of 0.9% saline. The final product [^{11}C]-(−)-**5** and [^{11}C]-(+)-**5** were then analyzed by analytical HPLC (Phenomenex Luna C-18 10 μm columns, analytical 4.6 × 250 mm, 40:60 v/v CH_3CN/0.1 M ammonium acetate, 4 mL/min, t_R = 5.0 min) to determine the radiochemical purity (>98%) and the specific radioactivity at the time of synthesis. The total synthesis time was 35 min from EOB with an average radiochemical yield of 18 ± 2% (non-decay corrected) and specific radioactivity was 22,045 ± 1955 mCi/μmol (n = 3). The formulated radiotracers were immediately injected into mice or baboons for PET studies.
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