Synthesis of 3-[(1-[^{11}C]Methyl-2(*S*)-pyrrolidinyl) methoxy]pyridine and 3-[(1-[^{11}C]Methyl-2(*R*)-pyrrolidinyl) methoxy]pyridine: Radioligands for *In Vivo* Studies of Neuronal Nicotinic Acetylcholine Receptors

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Summary

3-[(1-[^{11}C]Methyl-2(*S*)-pyrrolidinyl)methoxy]pyridine, [^{11}C]A-84543, a selective radioligand for neuronal nicotinic acetylcholine receptors (nAChRs) was prepared by *N*-alkylation of *N*-desmethyl A-84543 with [^{11}C]methyl iodide in DMF. The radioligand was purified by semi-preparative reverse-phase HPLC. The average specific radioactivity was 1755 mCi/μmol calculated at end-of-synthesis (EOS). The average time of synthesis, including formulation, was 17 min. The pharmacologically less active (*R*) enantiomer, 3-[(1-[^{11}C]methyl-2(*R*)-pyrrolidinyl) methoxy]pyridine was synthesized in an analogous manner using the appropriate *N*-desmethyl precursor. The specific radioactivity was calculated to be 2368 mCi/μmol (EOS).

Key Words: nicotinic acetylcholine receptors, 3-pyridyl ethers, positron emission tomography, carbon-11

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* Reprints may be obtained from any of the authors.

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Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) are widely distributed throughout the central and peripheral nervous systems, where they modulate a number of physiological functions including neurotransmitter release (1,2). The nAChRs are a family of ligand-gated ion channels formed probably from five subunits of usually two to four homologous types (2,3). In the rat brain at least eight α (α2-α9) subunits and three β (β2-β4) subunits exist with different combinations of subunits resulting in differing receptor pharmacology (4-7). The nAChRs undergo changes with aging (8), but more interestingly a consistent deficit in concentration of nAChRs in tissue from Alzheimer's brain has been reported by postmortem examination (9,10), suggesting that tomographic imaging of nAChRs might be useful in the study of this disorder.

Introduction of non-invasive imaging techniques, such as positron emission tomography (PET), has made possible the study of neuroreceptors in the brains of living human subjects. Such studies have proven useful in the localization and quantification of neuroreceptors and offer insight into the relationship of these receptors in normal and disease states (10-13). Initially carbon-11 labeled forms of nicotine (figure 1), including the racemate and each enantiomer, were used to image nAChRs in the brain of monkeys (14,15) and later in humans (16). However, despite many interesting clinical results obtained using PET and [^{11}C]nicotine, its pharmacological characteristics are far from ideal. High nonspecific binding, very rapid washout from the brain, and rapid metabolism make [^{11}C]nicotine and its enantiomers unsuitable radioligands for quantification of nAChRs *in vivo* by PET (17). A fluorine-18 labeled analog of nicotine has also been described for PET studies (18).

Subsequent efforts in the development of suitable PET radioligands for imaging nAChRs have focused on epibatidine, an azabicycloheptane alkaloid (figure 1). Racemic epibatidine, (±)-exo-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane, has very high affinity for the major brain nAChR subtype (α4β2) (K_i = 0.04 nM), and interestingly resolved epibatidine displayed a lack of enantioselectivity (19), in addition to high affinity for muscle type nAChRs (K_i = 2.7 nM) (20). Racemic [^{3}H]epibatidine has also been shown to label human ganglionic nAChRs (α3β2) (K_d = 0.14 nM) (21), which are believed to be associated with cardiovascular and gastrointestinal side effects. Several epibatidine analogs labeled with carbon-11 and fluorine-18 have been reported (22-24).

More recently, a series of 3-pyridyl ether compounds possessing subnanomolar affinity for brain nAChRs with an ability to differentially activate subtypes of neuronal nAChRs have been reported (25). *In vitro* binding experiments with A-84543, 3-[(1-methyl-2(*S*)-pyrrolidinyl)methoxy]pyridine, revealed subnanomolar affinity for nAChRs (K_i = 0.15 nM) labeled with [^{3}H]cytisine. [^{3}H]Cytisine has been shown to label primarily a receptor subtype in rat brain composed of α4 and β2 subunits (26). The (*R*) enantiomer was 130-fold less potent (K_i = 19.7 nM) at the

[^{3}H]cytisine binding site. In functional assays, A-84543 exhibited 84-fold selectivity to stimulate ion flux at human $\alpha4\beta2$ nAChR subtype compared to human ganglionic nAChRs. Compared to (±)epibatidine, A-84543 was 9000-fold less potent in stimulating ion flux at human ganglionic nAChRs. Based on these results and the need for positron emitting subtype specific radiotracers for nAChRs, we chose to label A-84543 and its pharmacologically less active (*R*) enantiomer with carbon-11 using [^{11}C]methyl iodide. In this paper we report the radiosynthesis, purification, and quality control of these nAChR ligands for PET.

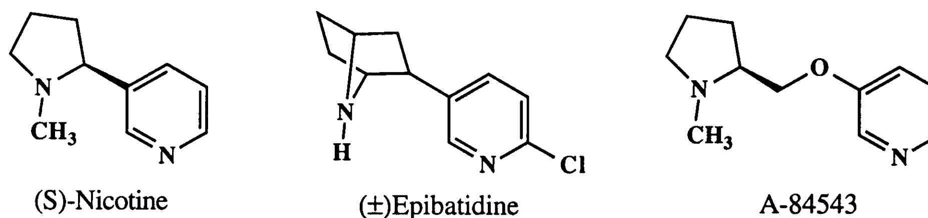


Figure 1. Neuronal Nicotinic Acetylcholine Receptor Agonists

Materials and Methods

3-[(1-Methyl-2(*S*)-pyrrolidinyl)methoxy]pyridine fumarate (A-84543), 3-[(2(*S*)-pyrrolidinyl)methoxy]pyridine fumarate (*N*-desmethyl A-84543), 3-[(1-methyl-2(*R*)-pyrrolidinyl)methoxy] pyridine dihydrochloride, and 3-[(2(*R*)-pyrrolidinyl)methoxy]pyridine dihydrochloride have been previously reported and were synthesized according to literature procedures (25). Chiral starting materials used in these syntheses were (*S*)-2-hydroxymethyl-1-methylpyrrolidine and (*R*) and (*S*)-pyrrolidine-2-carboxylic acid which were purchased from Fluka with purity ≥99%. (*R*) and (*S*)-pyrrolidine-2-carboxylic acid were transformed to (*R*) and (*S*)-1-(*tert*-butoxycarbonyl)-2-pyrrolidinemethanol for which racemization does not occur (25). The key forming ether forming step in all analogs is carried out under Mitsunobu conditions. Optical purity was not determined on synthesised final products. All synthetic intermediates were analysed by ^{1}H NMR. [^{11}C]Carbon dioxide was produced by 16 MeV proton bombardment of a nitrogen gas target using a Scanditronix RNP-16 biomedical cyclotron. Conversion to [^{11}C]methyl iodide has been previously described (27). A dose calibrator (Capintec CRC-12) was used for all radioactivity measurements. High performance liquid chromatographic analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector, and a single 2 inch NaI crystal radioactivity detector. HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax version 1.4).

Synthesis and purification of 3-[(1-[11C]methyl-2(S)-pyrrolidinyl) methoxy]pyridine, [11C]A-84543.

The fumarate salt of *N*-desmethyl A-84543 (2 mg, 6.8 μmol) was dissolved in H_2O (0.2 mL) to which was added NaOH (3 drops of a 6M solution). The mixture was extracted with diethyl ether (1 mL) and the solvent evaporated to give the free base of the *N*-desmethyl compound. The free base was taken up in dimethylformamide (200 μL) and transferred to a small septum-sealed vessel. The vessel was cooled (-78 °C) and [^{11}C]methyl iodide was transferred into the vessel by a stream of nitrogen carrier gas. When the radioactivity in the solution reached a plateau, the stream of nitrogen was stopped, and the vessel was submerged in a 80 °C water bath. After 3 min, 200 μL of HPLC solvent, consisting of 25:15:60 acetonitrile:methanol:water (20 mM NH_4OH), was added to the reaction solution. The mixture was injected onto a Hamilton PRP-1 10μm (305 mm x 7 mm) semi-preparative column and eluted at a flow rate of 5 mL/min. The effluent from the column was monitored with a UV detector and an in-line radioactivity detector. The radioactivity peak corresponding to [^{11}C]A-84543 (t_R = 5.7 minutes, k' = 4.7) was collected in a rotary evaporator and the solvent evaporated to dryness under reduced pressure. The residue was dissolved in sterile, normal saline (7 mL) and filtered through a sterile, Millipore GS 0.22 μm filter, into a sterile pyrogen-free evacuated vial. Sterile aqueous sodium bicarbonate (3 mL, 8.4%) was then added and the radioactivity was measured.

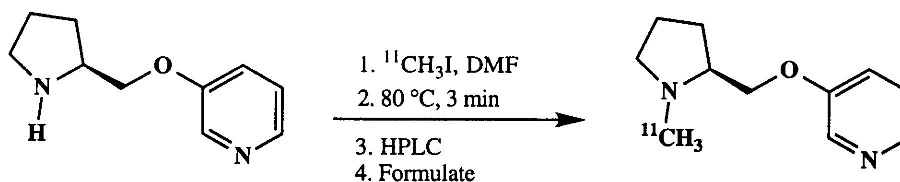
For determination of specific radioactivity and radiochemical purity, an aliquot of the final solution of known volume and radioactivity was injected onto an analytical reverse-phase HPLC column (Hamilton PRP-1 10μm 250 mm x 4.1 mm). A mobile phase of 25:25:50 acetonitrile:methanol:water (20 mM NH_4OH) with a flow rate of 2 mL/min was used to elute the radioligand (t_R = 3.2 minutes, k' = 2.2). The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured by an automated integrating recorder (Hewlett Packard 3390A) and compared to a standard curve relating mass to UV absorbance.

Synthesis and purification of 3-[(1-[11C]methyl-2(R)-pyrrolidinyl) methoxy]pyridine.

The synthesis of the (*R*) enantiomer, 3-[(1-[^{11}C]methyl-2(*R*)-pyrrolidinyl) methoxy]pyridine, was carried out using the appropriate *N*-desmethyl precursor, 3-[(2(*R*)-pyrrolidinyl)methoxy]pyridine dihydrochloride. The experimental conditions were identical as to those described above. During the purification, the radioactivity peak corresponding to 3-[(1-[^{11}C]methyl-2(*R*)-pyrrolidinyl)methoxy]pyridine eluted from the semi-preparative HPLC column at a retention time of 5.7 min (k' = 4.7). On the analytical HPLC column the retention time was 3.2 min (k' = 2.2).

Results and Discussion

The synthesis of 3-[(1-[^{11}C]methyl-2(*S*)-pyrrolidinyl)methoxy]pyridine, [^{11}C]A-84543, and its pharmacologically less active (*R*) enantiomer, 3-[(1-[^{11}C]methyl-2(*R*)-pyrrolidinyl)methoxy]pyridine, involved the *N*-alkylation of the appropriate (*S*) and (*R*) *N*-desmethyl precursors with [^{11}C]methyl iodide. The synthesis of [^{11}C]A-84543 is outlined in scheme 1.



Scheme 1. Radiosynthesis of [^{11}C]A-84543

Reverse-phase semi-preparative HPLC was used to purify [^{11}C]A-84543 (Figure 2). *N*-desmethyl A-84543 eluted at 3.3 minutes ($k' = 2.3$) while [^{11}C]A-84543 eluted at 5.7 minutes ($k' = 4.7$). The radioactivity peak corresponding to the product was collected remotely and the HPLC solvent removed via rotary evaporation under vacuum. [^{11}C]A-84543 was formulated in sterile normal saline, microfiltered into a sterile, evacuated dose-vial, and diluted with sterile sodium bicarbonate. The (*R*) enantiomer was prepared in an analogous manner. Using this procedure, both radioligands proved to be sterile and pyrogen free.

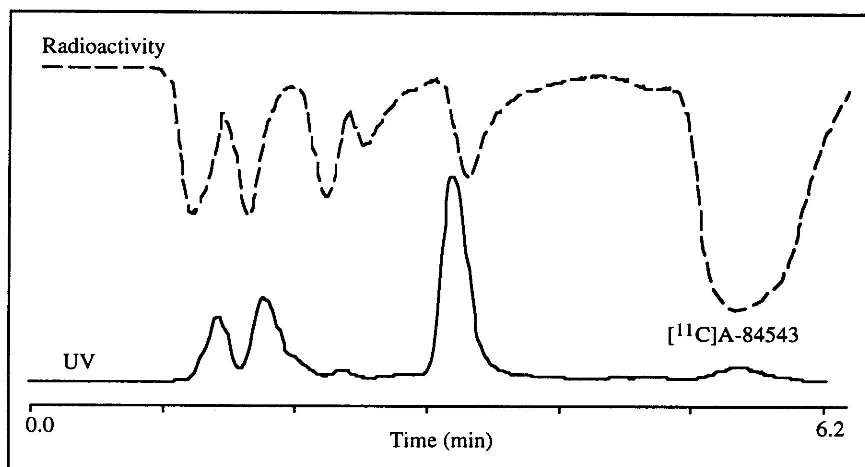


Figure 2. Preparative chromatogram of [^{11}C]A-84543

The average time of synthesis of [^{11}C]A-84543 from end of bombardment was 17 minutes with a non-decay corrected radiochemical yield of 27% based on [^{11}C]methyl iodide. To determine specific radioactivity and final radiochemical purity of the radioligand, a known aliquot of radioactivity was injected onto an analytical reverse-phase HPLC column. Comparison of the carrier peak associated with the radioactivity to that of a standard sample of A-84543 enabled calculation of the specific radioactivity. The resulting chromatogram showed [^{11}C]A-84543 to be of high radiochemical (>99%) and chemical purity determined at 254 nm. The radioactive product co-eluted with an authentic sample of A-84543 confirming its identity, while the specific radioactivity calculated at the end of synthesis was 1755 mCi/μmol. The (*R*) enantiomer, 3-[(1-[^{11}C]methyl-2(*R*)-pyrrolidinyl) methoxy]pyridine, was prepared in 17 minutes from end of bombardment with a non-decay corrected radiochemical yield of 9% and specific radioactivity of 2368 mCi/μmol. The radiochemical purity was >99% and high chemical purity determined at 254 nm .

Conclusions

3-[(1-[^{11}C]Methyl-2(*S*)-pyrrolidinyl)methoxy]pyridine, [^{11}C]A-84543, and 3-[(1-[^{11}C]methyl-2(*R*)-pyrrolidinyl)methoxy]pyridine were both prepared at high specific radioactivity by *N*-alkylation of the corresponding *N*-desmethyl precursors with [^{11}C]methyl iodide. The radiosyntheses produced radiochemically pure radioligands in good radiochemical yield. A sufficient amount of each radioligand can be prepared to allow for *in vivo* studies of neuronal nAChRs in the brain with PET.

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