Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1787–1790

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Facile Synthesis of [^{11}C]Edrophonium and its Analogues as New Potential PET Imaging Agents for Heart Acetylcholinesterase

Qi-Huang Zheng,* Xuan Liu, Xiangshu Fei, Ji-Quan Wang, Bruce H. Mock, Barbara E. Glick-Wilson, Michael L. Sullivan and Gary D. Hutchins

Department of Radiology, Indiana University School of Medicine, 975 West Walnut Street, Room 028C, Indianapolis, IN 46202-5121, USA

Received 17 September 2002; accepted 7 February 2003

Abstract—[^{11}C]Edrophonium and its analogues have been synthesized for evaluation as new potential positron emission tomography (PET) imaging agents for heart acetylcholinesterase. The tracers were prepared by *N*-[^{11}C]methylation of precursors using [^{11}C]methyl triflate and isolated by solid-phase extraction (SPE) purification procedure in 50–65% radiochemical yields.

Edrophonium and neostigmine are acetylcholinesterase (AChE) inhibitors used to treat a number of heart conditions in humans due to their high affinities to the enzyme target.[1–7] AChE positive neuron fibers make up a significant portion of the heart's conduction system and sensory nerves, and AChE enzyme-based imaging agents[8] could be useful in studying parasympathetic function in the heart. In vivo biomedical imaging technique positron emission tomography (PET) coupled with appropriate radiopharmaceuticals has become a clinically valuable and accepted diagnostic tool to image heart diseases,[9,10] since it produces powerful images of the human body's biological functions and reveals the mysteries of health and disease. Considerable efforts have been devoted to the development of radiopharmaceuticals for the in vivo imaging of the AChE, however, only a limited number of PET studies have been conducted to image AChE and monitor its response to AChE inhibitors treatment, because of the limited accessibility of radiotracers.[11] Radiotracer development, both for labeled drugs and for labeled tracers, is a key area for advancement of research and clinical applications of PET in the detection and treatment of heart diseases.[12] The short-lived positron emitting radionuclide carbon-11 (half-life $t_{1/2}$ 20.4 min) labeled analogues of edrophonium and neostigmine might be able for PET to image heart AChE in vivo. We have developed [^{11}C]neostigmine (Fig. 1) as a potential PET marker for heart AChE and parasympathetic innervation.[13] As part of our efforts to evaluate potential heart imaging agents,[14,15] we synthesized [^{11}C]edrophonium and its methyl and ethyl analogues.

The reliable routine production, automation and specific radioactivity of carbon-11 heart imaging agents require high yields, short reaction times, lower reaction temperatures and more efficient purification methods. In order to explore novel methodology for the synthesis of carbon-11 heart imaging agents, we have developed a convenient and reliable gas phase production (GPP) method for the most commonly used radiolabeled precursor [^{11}C]methyl triflate[16] from gas phase bromination of [^{11}C]methane (Fig. 2),[17] a fast and efficient solid phase extraction (SPE) method for isolation and purification of ^{11}C-radioligands,[18–21] and a convenient

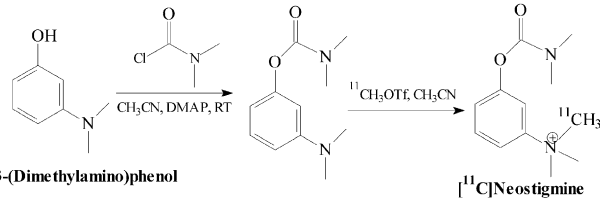


Figure 1. Synthesis of [^{11}C]neostigmine.

$$^{11}CO_2 \xrightarrow[>95\%]{H_2} {}^{11}CH_4 \xrightarrow[70\text{-}75\%]{Br_2} {}^{11}CH_3Br \xrightarrow[\sim 100\%]{AgOTf} {}^{11}CH_3OTf$$

Figure 2. GPP method for [^{11}C]methyl triflate.

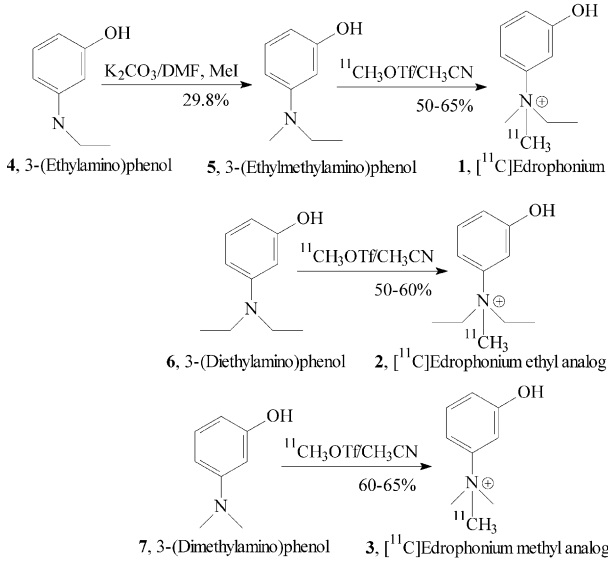
*Corresponding author. Tel.: +1-317-278-4671; fax: +1-317-278-1072; e-mail: qzheng@iupui.edu

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00259-2

labeling and isolation method for the preparation of [^{11}C-methyl]quaternary amines through *N*-[^{11}C]methylation of their precursors,[22,23] in which the similar method for the production of [^{11}C-methyl]quaternary amine [^{11}C]choline was also reported by Pascali et al.[24] We used these new methodologies to label these novel agents.

The synthetic approach for ^{11}C-labeled edrophonium and analogues was shown in Scheme 1. The commercially available starting material 3-(ethylamino)phenol (**4**) was methylated by methyl iodide to give the precursor 3-(ethylmethylamino)phenol (**5**) for radiolabeling.[25,26] The precursor **5** was labeled by [^{11}C]methyl triflate through *N*-[^{11}C]methylation[22–24] and isolated by SPE purification to produce pure target compound [^{11}C]edrophonium, [^{11}C]dimethylethyl-(3-hydroxyphenyl)ammonium (**1**). The commercially available precursor 3-(diethylamino)-phenol (**6**) was N-[^{11}C]methylated and SPE-purified to provide [^{11}C]edrophonium ethyl analogue, [^{11}C]diethylmethyl-(3-hydroxyphenyl)ammonium (**2**). Another commercially available precursor 3-(dimethylamino)-phenol (**7**) was N-[^{11}C]methylated and SPE-purified to provide [^{11}C]edrophonium methyl analogue, [^{11}C]trimethyl-(3-hydroxyphenyl)ammonium (**3**).

A simple technique for convenient labeling and isolation of [^{11}C-methyl]quaternary amines by *N*-[^{11}C]methylation method[22–24] was employed in the radiosynthesis of ^{11}C-labeled edrophonium and analogues. The key part in this technique is a SiO_2 Sep-Pak cartridge containing 0.5–2 g of adsorbent, which was obtained from Waters Corporate Headquarters, Milford, MA. The large polarity difference between the tertiary amine precursor, [^{11}C]methyl triflate and the labeled [^{11}C-methyl]quaternary amine product permitted the use of SPE technique for fast purification of radiotracer from the radiolabeling reaction mixture, and warranted the final product cannot be contaminated with [^{11}C]methyl triflate or derivatives. The reaction mixture was loaded onto the SiO_2 Sep-Pak cartridge by gas pressure. The cartridge was washed with ethanol to conveniently remove non-reacted tertiary amine precursor, reaction solvent, and non-reacted [^{11}C]methyl triflate, which was decomposed by ethanol, therefore, there was no coelution with the product, then the final labeled product [^{11}C-methyl]quaternary amine was eluted with an aqueous solution of 2% acetic acid, which can also contain up to 8% ethanol to enhance recovery of some [^{11}C-methyl]quaternary cations. The presence of acid appears to be crucial for efficient release of the R_3N–$^{11}CH_3^+$ from the silica. Only a minor pH adjustment with 2 M NaOH and 150 mM NaH_2PO_4 mixed solution and sterile filtration are required to make the product ready for injection. To be sure the final product was not contaminated with [^{11}C]methyl triflate or derivatives, a simple experiment was performed in light of the suggestions made by a reviewer. [^{11}C]Methyl triflate was submitted directly, without reacting it with a precursor, to the Sep-Pak procedure, and then the eluates were analyzed on HPLC. The radio-HPLC chromatogram of aqueous eluate unequivocally proved the final product was not contaminated with [^{11}C]methyl triflate or derivatives. The principle of radiolabeling and SPE isolation of [^{11}C-methyl]quaternary amines was illustrated in Figure 3. The schematic diagram of combined *N*-[^{11}C]methylation system apparatus was configured as indicated in Figure 4.



Scheme 1. Synthesis of ^{11}C-labeled edrophonium and analogues (**1**–**3**).

General method for the radiosynthesis of [^{11}C]edrophonium and its ethyl and methyl analogues (1–3)

$^{11}CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in small volume (12.3 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+3% O_2) in a Siemens radionuclide delivery system (RDS-112). The precursor (**5**, **6**, or **7**) (1–2 mg) was dissolved in acetonitrile (250 μL). The mixture was transferred to a small volume, three-neck reaction tube. [^{11}C]methyl triflate was produced by the GPP method[17] from $^{11}CO_2$ as illustrated in Figure 2 and passed into air-cooled reaction tube at −15 to −20 °C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until radioactivity in solution reached a maximum (2–3 min), then reaction tube was isolated and heated at 70–80 °C for 2–3 min. The reaction tube was connected to the Silica Sep-Pak. The product solution was passed onto the Silica Sep-Pak for SPE purification by gas pressure. The reaction tube and Sep-Pak were washed with ethanol (5 mL×2), and the

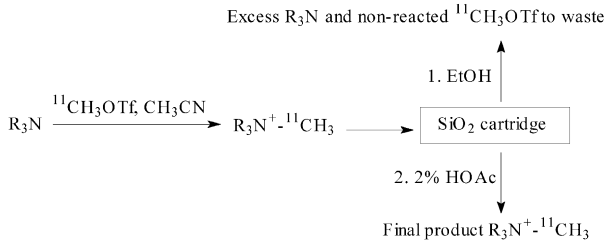


Figure 3. The principle of radiolabeling and SPE isolation of [^{11}C-methyl]quaternary amines.

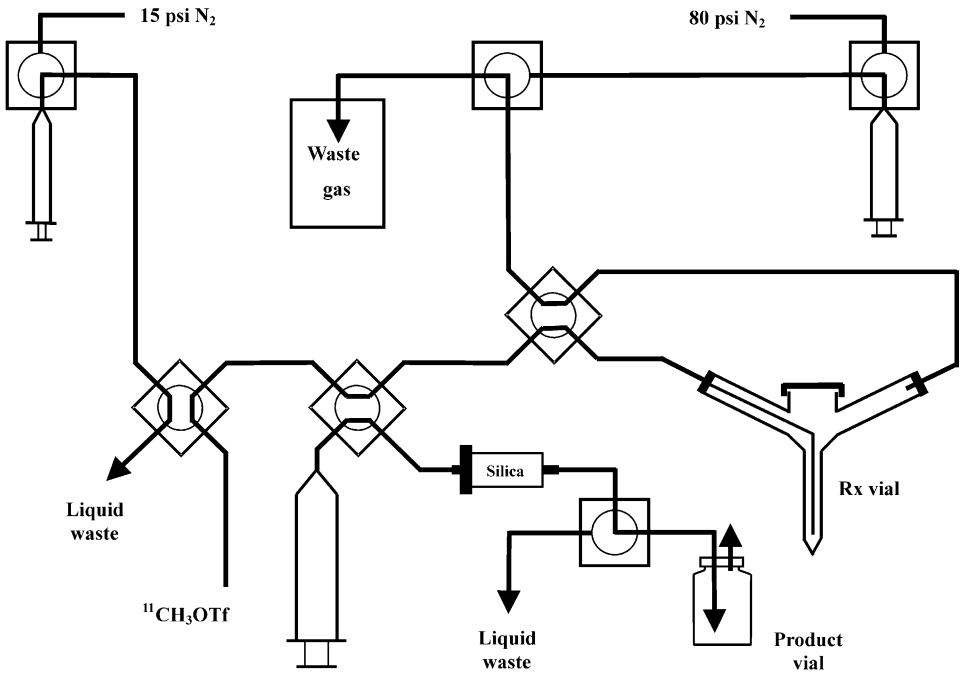


Figure 4. Radiosynthesis and SPE purification system of ^{11}C-labeled edrophonium and analogues.

washing solution was discarded to a waste bottle. The product was eluted from the Sep-Pak with 90:8:2H_2O:EtOH:HOAc (9.2 mL) and sterile-filtered through a Millex-GS 0.22 μm cellulose acetate membrane (Millipore Corporation, Bedford, MA) and collected into a sterile vial. The pH was adjusted to 5.5–7.0 with 2 M NaOH and 150 mM NaH_2PO_4 mixed solution (0.8 mL). Total radioactivity (200–280 mCi) was assayed and the total volume (10 mL) was noted. The overall synthesis time was 10–15 min. The radiochemical yield of [^{11}C]methyl triflate is 70–75% based on $^{11}CO_2$.[17] The radiochemical yields for target radiotracers **1–3** were 50–65%, based on $^{11}CO_2$, decay corrected to end of bombardment (EOB). Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC method, which employed a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; 3:1:3 CH_3CN: MeOH: 20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, and UV (240 nm) and γ-ray (NaI) flow detectors. Retention times in the analytical HPLC system were: RT**5** = 4.14 min, RT**6** = 5.62 min, RT**7** = 3.35 min; RT**1** = 2.12 min, RT**2** = 2.06 min, RT**3** = 2.06 min. The chemical purities of the precursors **5–7** were >98%. The radiochemical purities of the target radiotracers **1–3** were >99%, and the chemical purities of the target tracers **1–3** were >95%. The average (n = 5–8) specific radioactivity of the target radiotracers **1–3** was >1 Ci/μmol at end of synthesis (EOS).

In summary, the novel methodology based upon the [^{11}C]methyl triflate GPP method and the SPE purification method for the production of ^{11}C-PET radiotracers works very well for the routine synthesis of heart imaging agents ^{11}C-labeled edrophonium and analogues.

Acknowledgements

This work was partially supported by the Indiana 21st Century Research and Technology Fund and the Indiana Genomics Initiative (INGEN) of Indiana University, which is supported in part by Lilly Endowment Inc. The reviewers' criticisms and editor's comments for the revision of the manuscript are greatly appreciated.

References and Notes

1. Katz, R. L. *Anesthesiology* **1967**, *28*, 327.
2. Lockhart, B.; Closier, M.; Howard, K.; Steward, C.; Lestage, P. *Naunyn Schmiedebergs Arch. Pharmacol.* **2001**, *363*, 429.
3. Vinson, P. N.; Justice, J. B. *J. Neurosci. Meth.* **1997**, *73*, 61.
4. Johnson, G.; Moore, S. W. *Mol. Immunol.* **2002**, *39*, 273.
5. Frobert, Y.; Creminon, C.; Cousin, X.; Remy, M. H.; Chatel, J. M.; Bon, S.; Bon, C.; Grassi, J. *Biochim. Biophys. Acta* **1997**, *1339*, 253.
6. Shafferman, A.; Velan, B.; Ordentlich, A.; Kronman, C.; Grosfeld, H.; Leitner, M.; Flashner, Y.; Cohen, S.; Barak, D.; Ariel, N. *EMBO J.* **1992**, *11*, 3561.
7. Hollunger, E. G.; Niklasson, B. H. *Acta Physiol. Scand.* **1981**, *111*, 335.
8. Weissleder, R.; Mahmood, U. *Radiology* **2001**, *219*, 316.
9. Bergmann, S. R. *Semin. Nucl. Med.* **1998**, *28*, 320.
10. Raffel, D. M.; Wieland, D. M. *Nucl. Med. Biol.* **2001**, *28*, 541.
11. Maziere, M. *Pharmac. Ther.* **1995**, *66*, 83.
12. Tewson, T. J.; Krohn, K. A. *Semin. Nucl. Med.* **1998**, *28*, 221.
13. Mulholland, G. K.; Zheng, Q.-H.; Mock, B. H.; Carlson, K. A.; Giger, S.; Vavrek, M. T.; Fain, R.; Hutchins, G. D. *J. Nucl. Med.* **1999**, *40*, 495.
14. Hutchins, G. D.; Chen, T.; Carlson, K. A.; Fain, R. L.;

Winkle, W.; Vavrek, T.; Mock, B. H.; Zipes, D. P. *J. Nucl. Med.* **1999**, *40*, 846.
15. Olgin, J. E.; Sih, H. J.; Hanish, S.; Jayachandran, J. V.; Wu, J. S.; Zheng, Q.-H.; Winkle, W.; Mulholland, G. K.; Zipes, D. P.; Hutchins, G. *Circulation* **1998**, *98*, 2608.
16. Jewett, D. M. *Appl. Radiat. Isot.* **1992**, *43*, 1383.
17. Mock, B. H.; Mulholland, G. K.; Vavrek, M. T. *Nucl. Med. Biol.* **1999**, *26*, 467.
18. Mulholland, G. K.; Zheng, Q.-H. *Abstracts of Papers of the American Chemical Society* **1996**, *212* 76-NUCL.
19. Zheng, Q.-H.; Mulholland, G. K. *Nucl. Med. Biol.* **1996**, *23*, 981.
20. Fei, X. S.; Zheng, Q-H.; Hutchins, G. D.; Liu, X.; Stone, K. L.; Carlson, K. A.; Mock, B. H.; Winkle, L.; Glick-Wilson, B. E.; Miller, K. D.; Fife, R. S.; Sledge, G. W.; Sun, H. B.; Carr, R. E. *J. Labelled Compd. Radiopharm.* **2002**, *45*, 449.
21. Zheng, Q.-H.; Fei, X. S.; Liu, X.; Wang, J.-Q.; Sun, H. B.; Mock, B. H.; Stone, K. L.; Martinez, T. D.; Miller, K. D.; Sledge, G. W.; Hutchins, G. D. *Nucl. Med. Biol.* **2002**, *29*, 761.
22. Mulholland, G. K.; Zheng, Q.-H.; Mock, B. H.; Vavrek, M. T. *J. Labelled Compd. Radiopharm.* **1999**, *42* (Suppl. 1), S459.
23. Zheng, Q.-H.; Stone, K. L.; Mock, B. H.; Miller, K. D.; Fei, X.; Liu, X.; Wang, J.-Q.; Glick-Wilson, B. E.; Sledge, G. W.; Hutchins, G. D. *Nucl. Med. Biol.* **2002**, *29*, 803.
24. Pascali, C.; Bongi, A.; Iwata, R.; Cambie, M.; Bombardieri, E. *J. Labelled Compd. Radiopharm.* **2000**, *43*, 195.
25. Zheng, Q.-H.; Hutchins, G. D.; Mock, B. H. *J. Labelled Compd. Radiopharm.* **2001**, *44* (Suppl. 1), S410.
26. Experimental procedure: (**A**) General: all commercial reagents and solvents were used without further purification. ^{1}H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal standard TMS (δ 0.0). Chromatographic solvent proportions are expressed on a volume: volume basis. Thin layer chromatography was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. The reaction was performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. (**B**) 3-(Ethylmethylamino)phenol (**5**): a mixture of 3-ethylaminophenol (**4**, 1.37 g, 10 mmol), potassium carbonate (2.07 g, 15 mmol), methyl iodide (1.42 g, 10 mmol), and CH_3CN (50 mL) was stirred at room temperature under nitrogen overnight. The suspension was filtered and the filtrate was taken down to dryness under vacuum. The residue was adsorbed onto a silica gel column and eluted with 1:6 EtOAc:hexane to give the title compound **5** (450 mg, 29.8%) as a yellow oil, R_f = 0.60 (1:3 EtOAc:hexane). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (t, 3H, J_1 = 7.4 Hz, J_2 = 6.6 Hz, $NCH_2\underline{CH_3}$), 2.80 (s, 3H, NCH_3), 3.26–3.33 (dd, 2H, J_1 = 7.4 Hz, J_2 = 6.6 Hz, $N\underline{CH_2}CH_3$), 5.70 (brs, 1H, OH), 6.17–6.19 (m, 2H, ArH), 6.30–6.32 (m, 1H, ArH), 7.02–7.07 (m, 1H, ArH).