Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2220–2224

Synthesis and biodistribution of new radiolabeled high-affinity choline transporter inhibitors [^{11}C]hemicholinium-3 and [^{18}F]hemicholinium-3

Qi-Huang Zheng,[a,*] Mingzhang Gao,[a] Bruce H. Mock,[a] Shuyan Wang,[a] Toshihiko Hara,[a] Rachid Nazih,[a] Michael A. Miller,[a] Tim J. Receveur,[a] John C. Lopshire,[b] William J. Groh,[b] Douglas P. Zipes,[b] Gary D. Hutchins[a] and Timothy R. DeGrado[a]

[a]*Department of Radiology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*
[b]*Krannert Institute of Cardiology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Received 20 December 2006; revised 21 January 2007; accepted 22 January 2007
Available online 4 February 2007

Abstract—The high-affinity choline transporter (CHT1) system is an attractive target for the development of positron emission tomography (PET) biomarkers to probe brain, cardiac, and cancer diseases. An efficient and convenient synthesis of new radiolabeled CHT1 inhibitors [^{11}C]hemicholinium-3 and [^{18}F]hemicholinium-3 by solid-phase extraction (SPE) technique using a cation-exchange CM Sep-Pak cartridge has been well developed. The preliminary evaluation of both tracers through biodistribution studies in 9L-glioma rats has been performed, and the uptakes in the heart and tumor were observed, while very low brain uptake was seen.

The high-affinity choline uptake (CHT1) system located in peripheral and central cholinergic nerve terminals plays a regulating and rate-limiting role in the intraneuronal synthesis of acetylcholine (ACh).[1,2] Three ACh-related proteins are considered specific markers for cholinergic neurons, including choline acetyltransferase (ChAT), the vesicular ACh transporter (VAChT), and the high-affinity choline transporter (CHT1).[3] Synthesis of ACh is catalyzed by ChAT, and ChAT antibodies have been used to map central cholinergic pathways and to identify cholinergic nerves in peripheral tissues.[4–6] VAChT transfers ACh to storage vesicles in nerve terminals or varicosities, and VAChT antibodies have been used to identify cholinergic neurons and nerve fibers in brain and heart.[6–9] The CHT1 protein is highly expressed at cholinergic varicosities and transports choline into neurons for synthesis of ACh, and CHT1 antibodies have been used for immunohistochemical detection in multiple species and tissues.[10–17] Biomedical imaging technique positron emission tomography (PET) studies with carbon-11 and fluorine-18 labeled choline analogues have shown moderate to high choline tracer accumulations in several types of primary and metastatic tumors, which imply choline transporter activity to support accumulation of tracer in the tumors.[18–21] In our previous work,[21] we performed mechanistic studies on the transport of choline in the PC-3 human prostate cancer cell line for clarification of the properties of the choline transporter. Although the uptake of choline analogues in cultured prostate cancer cells was found to be mediated by an intermediate affinity transporter distinct from CHT1, it was found sensitive to inhibition with the CHT1 inhibitor, hemicholinium-3 (HC-3). Results of cellular binding of [^{3}H]HC-3 in PC-3 cells demonstrated the importance of the transporter for choline processing in PC-3 human prostate cancer cells. Favorable tumor cell binding and in vivo biodistribution properties of [^{3}H]HC-3 in a 9L-glioma-bearing rat model encouraged further characterization and development of molecular imaging agents that target the choline transporter in cancer cells.

CHT1 is a marker of the functional status of the cholinergic presynaptic terminals and associated with a variety of diseases, such as brain Parkinson's disease, and Alzheimer's disease, coronary heart disease and

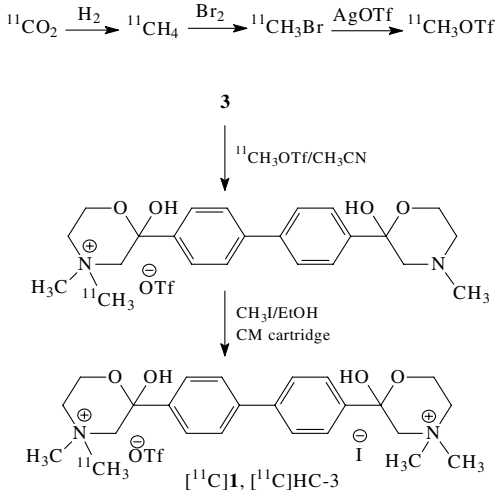
Keywords: High-affinity choline transporter (CHT1); Positron emission tomography (PET); [^{11}C]hemicholinium-3 ([^{11}C]HC-3); [^{18}F]hemicholinium-3 ([^{18}F]HC-3); Biodistribution.
* Corresponding author. Tel.: +1 317 278 4671; fax: +1 317 278 9711; e-mail: qzheng@iupui.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.01.105

cancers.[22,23,21] Thus, choline transporter systems provide attractive targets for the development of PET biomarkers to probe brain, heart, and cancer diseases. HC-3 [2,2-(4,4-biphenylene)bis(2-hydroxy-4,4-dimethylmorpholinium bromide)] and its derivatives represent the most potent known competitive synthetic CHT1 inhibitors.[1] HC-3 is a gold standard for studying the high-affinity choline transport in vitro.[2] Carbon-11 and fluorine-18 labeled HC-3 analogues, [^{11}C]hemicholinium-3 ([^{11}C]HC-3) and [^{18}F]hemicholinium-3 ([^{18}F]HC-3), may serve as in vivo PET agents for the choline transporter. We present here, for the first time, our initial investigation on synthesis and biodistribution of [^{11}C]HC-3 and [^{18}F]HC-3.

Synthesis of HC-3 precursor and reference standard was performed using a modification of the literature method.[24] The synthetic approach is outlined in Scheme 1. The bis-tertiary amine precursor 4,4′-bis-(1-methyl-3-hydroxy-morpholinyl-(3))-biphenyl (**3**) was synthesized from 4,4′-bis-bromoacetyl-biphenyl and 2-(methylamino)ethanol in 80% yield. The reference standard HC-3 (**1**) was synthesized from 4,4′-bis-bromoacetyl-biphenyl and 2-(dimethylamino)ethanol in 92% yield.

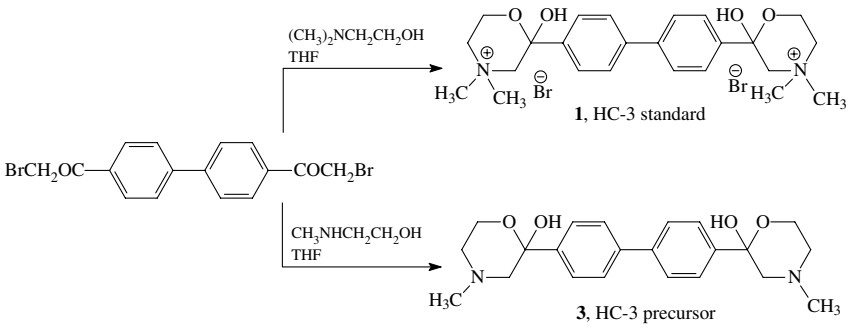
Synthesis of [^{11}C]HC-3 ([^{11}C]**1**) is shown in Scheme 2. The precursor **3** was labeled by a reactive [^{11}C]methylating agent, [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[25] prepared from $^{11}CO_2$, in acetonitrile through the primary N-[^{11}C]methylation and trapped on a cation-exchange CM Sep-Pak cartridge to release the non-reacted bis-tertiary amine precursor with ethanol and to retain the pure ^{11}C-methylated single-side quaternary amine intermediate on the same CM Sep-Pak. The labeled intermediate underwent the secondary N-[^{12}C]methylation by addition of methyl iodide in ethanol to the same cartridge. After 2 min, non-reacted methyl iodide was removed from the cartridge by rinsing with ethanol. The final bis-quaternary amine carbon-11 labeled product [^{11}C]HC-3 was then eluted from the cartridge with saline. The synthesis was performed in an automated multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis.[26] The radiochemical yields were 50–60% based on $^{11}CO_2$ decay corrected to end of bombardment (EOB), and specific activity was in a range of 4.0–6.0 Ci/μmol at EOB. The overall synthesis time was 20–30 min from EOB. The radiochemical purity was >99%. The purification technique we used in the radiosynthesis of [^{11}C]HC-3 is the solid-phase extraction (SPE) method,[23] and the key part in this technique is a CM Sep-Pak cartridge.



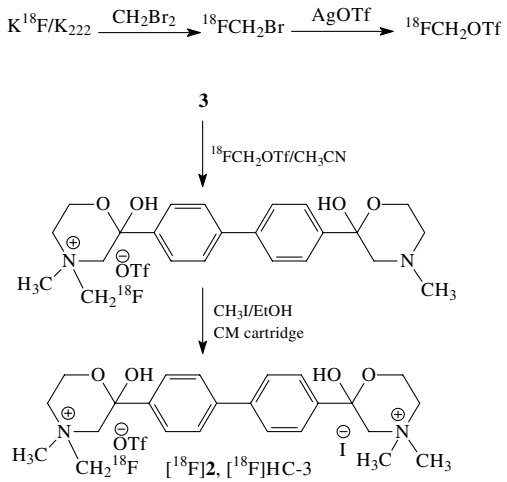
Scheme 2. Synthesis of [^{11}C]HC-3.

Synthesis of [^{18}F]HC-3 ([^{18}F]**2**) is indicated in Scheme 3. Using similar methodology, fluorine-18 labeled target tracer [^{18}F]HC-3 was prepared by primary N-[^{18}F]fluoromethylation of the precursor **3** using a new and reactive [^{18}F]fluoromethylating agent, [^{18}F]fluoromethyl triflate ([^{18}F]FCH_2OTf) prepared starting from K^{18}F/Kryptofix2.2.2 with dibromomethane,[27] followed by secondary N-[^{12}C]methylation using methyl iodide and purified by the SPE technique using a CM Sep-Pak cartridge in an automated multi-purpose ^{18}F-radiosynthesis (FBM) module[20] with 5–10% radiochemical yields based on K^{18}F, 30–40 min overall synthesis time from EOB, >99% radiochemical purity, and >1.0 Ci/μmol specific activity at end of synthesis (EOS).

Both [^{11}C]HC-3 and [^{18}F]HC-3 are radiolabeled bis-quaternary amine salts, therefore, it is not possible for the tracers to cross the blood-brain barrier (BBB). Thus, their application appears limited for in vivo imaging studies of the central nervous system (CNS) diseases in the brain such as Alzheimer's disease, for which a dramatic loss of ACh and its metabolically related enzymes from cortex and hippocampus is a characteristic feature of Alzheimer's disease.[22] Rather, the potential applica-



Scheme 1. Synthesis of HC-3 precursor and reference standard.



Scheme 3. Synthesis of [^{18}F]HC-3.

tion of both tracers would be in heart imaging and cancer imaging.[23,21]

The preliminary biological evaluation of [^{11}C]HC-3 and [^{18}F]HC-3 was carried out through biodistribution studies in a subcutaneous 9L-glioma rat model. We chose against a xenograft mouse model for this study, because a rat model would allow for more practical collection of arterial blood samples and previous work with the PC-3 xenografts showed accumulations of choline radiotracers indicative of poor perfusion of these tumors.[19] Since elevation of choline uptake is found in a wide variety of tumors including gliomas,[28,29] the model serves as a representative model for evaluating choline-based imaging probes. Moreover, this model is also suitable for evaluating in vivo biodistribution properties of brain and heart regions, since our regions-of-interest (ROIs) included normal brain and heart. All animal experiments were performed under a protocol approved by the Indiana University Institutional Animal Care and Use Committee (IACUC). The in vivo biodistribution of [^{11}C]HC-3 was determined in 9L-glioma rats at 5 and 20 min post intravenous (iv) injection of the tracer. Likewise, the in vivo biodistribution of [^{18}F]HC-3 was determined in 9L-glioma rats at 5 and 30 min post iv injection of the tracer. Urine was collected by a syringe from the bladder. The data are listed in Table 1. The data represent the average value in three rats. Biodistribution data of [^{11}C]HC-3 at 5 and 20 min time points and [^{18}F]HC-3 at 5 and 30 min time points in 9L-glioma rats showed the major organs of uptake to be kidney. Heart uptake was observed in different regions including L-atrium, R-atrium, septum, L-ventricle, R-ventricle, and A–V node. Tumor uptake was moderate, and tumor/muscle ratios were 3.0–5.1 for both [^{11}C]HC-3 and [^{18}F]HC-3 at 5–30 min post-injection. In comparison with the uptakes in urine and kidney, the uptake in brain was very low. The minimal penetration of both tracers into brain confirmed the lipophobicity[30] of the bis-quaternary amine tracers, which is consistent with their HPLC retention times. Gliomas are brain tumors derived from glia,[31] and the glioma tumor was implanted in the thigh of the rat, not in the brain. Thus, the tumor uptakes were able to be seen in 9L-glioma rat model in biodistribution study, and both radiotracers had relative good uptake in tumor and good tumor/background ratios. This study does not address the question of whether these tracers can cross the blood-brain barrier when glioma is present. There was little change in the tissue biodistributions from 5 to 30 min post-injection, demonstrating that the disposition of the labeled HC-3 analogues occurs rapidly. Significant fractions of radioactivity were accumulated in urine (~25% at 30 min). Since bone and fat were not the

Table 1. Biodistribution data of [^{11}C]HC-3 and [^{18}F]HC-3 in 9L-glioma rats (% ID/g)

Organ	[^{11}C]HC-3 (n = 3, 5 min)	[^{11}C]HC-3 (n = 3, 20 min)	[^{18}F]HC-3 (n = 3, 5 min)	[^{18}F]HC-3 (n = 3, 30 min)
Blood	1.383 ± 0.267	0.749 ± 0.093	1.467 ± 0.685	0.378 ± 0.198
Plasma	3.648 ± 0.683	1.453 ± 0.383	3.732 ± 0.680	1.272 ± 0.536
Brain	0.060 ± 0.038	0.035 ± 0.008	0.042 ± 0.021	0.030 ± 0.008
Lung	1.081 ± 0.471	0.590 ± 0.074	0.944 ± 0.584	0.533 ± 0.228
Liver	0.848 ± 0.381	1.239 ± 0.369	1.228 ± 0.662	1.536 ± 0.585
Kidney	7.640 ± 2.782	3.656 ± 0.322	8.676 ± 4.732	5.935 ± 5.577
Urine (% dose)	6.381 ± 6.876	24.980 ± 6.583	10.188 ± 12.182	27.623 ± 15.663
Heart regions				
L-Atrium	1.026 ± 0.606	0.457 ± 0.070	0.824 ± 0.324	0.767 ± 0.312
R-Atrium	0.963 ± 0.325	0.398 ± 0.085	2.023 ± 1.848	0.999 ± 0.266
Septum	0.381 ± 0.115	0.223 ± 0.011	0.530 ± 0.390	0.262 ± 0.155
L-Ventricle	0.415 ± 0.162	0.244 ± 0.028	0.438 ± 0.219	0.253 ± 0.134
R-Ventricle	0.401 ± 0.129	0.234 ± 0.009	0.734 ± 0.699	0.243 ± 0.134
A–V node	1.074 ± 0.964	0.478 ± 0.243	5.607 ± 7.484	0.600 ± 0.211
Solar Plexus	0.522 ± 0.258	2.057 ± 1.631	1.186 ± 0.143	1.009 ± 0.693
Ske. Musc.	0.207 ± 0.061	0.159 ± 0.046	0.194 ± 0.087	0.123 ± 0.070
Tumor	0.649 ± 0.176	0.457 ± 0.146	0.952 ± 0.400	0.359 ± 0.165
Tumor/blood	0.466 ± 0.064	0.622 ± 0.219	0.572 ± 0.311	0.978 ± 0.095
Tumor/muscle	3.199 ± 0.522	3.094 ± 1.310	5.085 ± 1.771	3.024 ± 0.393

The data presented here represented the average value in 3 rats. The time points post iv injection of the tracer were 5 and 20 min for [^{11}C]HC-3, and 5 and 30 min for [^{18}F]HC-3, respectively.

tissues of interest, no bone and fat tissues were excised in biodistribution study, and no bone and fat accumulation data were reported.

The experimental details and characterization data for compounds **3** and **1**, new tracers [^{11}C]**1** and [^{18}F]**2**, and biodistribution study are given.[32]

In summary, an efficient and convenient chemical and radiochemical synthesis of the precursors, reference, standards and target tracers has been well developed. The synthetic methodology of [^{11}C]HC-3 and [^{18}F]HC-3 employed bis-tertiary amine precursor, featuring primary N-[^{11}C]methylation with [^{11}C]CH_3OTf and N-[^{18}F]fluoromethylation with [^{18}F]FCH_2OTf in a reaction vessel, purification of ^{11}C-methylated and ^{18}F-fluoromethylated single-side quaternary amine intermediates on a CM Sep-Pak cartridge, followed by secondary N-[^{12}C]methylation with CH_3I and isolated final bis-quaternary amines carbon-11 and fluorine-18 labeled products [^{11}C]HC-3 and [^{18}F]HC-3 by SPE technique on the same cartridge. The radiolabeling reactions and purification are rapid, efficient, and convenient, and resulting radiolabeled products were shown to have moderate to excellent radiochemical yields. Preliminary findings from biodistribution studies of both tracers in 9L-glioma rats indicate that uptakes in the heart and tumor were observed, while very low brain uptake was seen. Further study will be to determine the specificity of binding of HC-3 analogue tracers to choline transporters, and to explore structural modifications to provide tracers for higher lipophilicity needed to cross the blood-brain barrier for the purpose of CNS imaging studies of CHT1.

Acknowledgments

This work was partially supported by the Donald W. Reynolds Foundation, the Susan G. Komen Breast Cancer Foundation BCTR0504022, NIH R01CA108620, and Indiana Genomics Initiative (INGEN) of Indiana University, which is supported in part by Lilly Endowment Inc. The authors would like to thank Barbara E. Glick-Wilson and Michael L. Sullivan for their assistance in production of $^{11}CH_3OTf$ and $K^{18}F$/Kryptofix2.2.2. The referees' criticisms and editor's comments for the revision of the manuscript are greatly appreciated.

References and notes

1. Smart, L. A. *J. Med. Chem.* **1983**, *26*, 104.
2. Gilissen, C.; de Groot, T.; Bronfman, F.; van Leuven, F.; Verbruggen, A. M.; Bormans, G. M. *J. Nucl. Med.* **2003**, *44*, 269.
3. Hoover, D. B.; Ganote, C. E.; Ferguson, S. M.; Blakely, R. D.; Parsons, R. L. *Cardiovasc. Res.* **2004**, *62*, 112.
4. Woolf, N. J. *Prog. Neurobiol.* **1991**, *37*, 475.
5. Crick, S. J.; Sheppard, M. N.; Anderson, R. H.; Polak, J. M.; Wharton, J. *J. Anat.* **1996**, *188*, 403.
6. Arvidsson, U.; Riedl, M.; Elde, R.; Meister, B. *J. Comp. Neurol.* **1997**, *378*, 454.
7. Schafer, M. K.; Weihe, E.; Erickson, J. D.; Eiden, L. E. *J. Mol. Neurosci.* **1995**, *6*, 225.
8. Schafer, M. K.; Weihe, E.; Eiden, L. E.; Erickson, J. D. *J. Mol. Neurosci.* **1994**, *5*, 1.
9. Schafer, M. K.; Eiden, L. E.; Weihe, E. *Neuroscience* **1998**, *84*, 361.
10. Suszkiw, J. B.; Pilar, G. *J. Neurochem.* **1976**, *26*, 1133.
11. Kuhar, M. J.; Sethy, V. H.; Roth, R. H.; Aghajanian, G. K. *J. Neurochem.* **1973**, *20*, 581.
12. Apparsundaram, S.; Ferguson, S. M.; Geroge, A. L., Jr.; Blakely, R. D. *Biochem. Biophys. Res. Commun.* **2000**, *276*, 862.
13. Apparsundaram, S.; Ferguson, S. M.; Blakely, R. D. *Biochem. Soc. Trans.* **2001**, *29*, 711.
14. Okuda, T.; Haga, T. *FEBS Lett.* **2000**, *484*, 92.
15. Misawa, H.; Nakata, K.; Matsuura, J.; Nagao, M.; Okuda, T.; Haga, T. *Neuroscience* **2001**, *105*, 87.
16. Kus, L.; Borys, E.; Ping, C. Y.; Ferguson, S. M.; Blakely, R. D.; Emborg, M. E.; Kordower, J. H.; Levey, A. I.; Mufson, E. J. *J. Comp. Neurol.* **2003**, *463*, 341.
17. Ferguson, S. M.; Savchenko, V.; Apparsundaram, S.; Zwick, M.; Wright, J.; Heilman, C. J.; Levey, A. I.; Blakely, R. D. *J. Neurosci.* **2003**, *23*, 9697.
18. Hara, T.; Kosaka, N.; Shinoura, N.; Kondo, T. *J. Nucl. Med.* **1997**, *38*, 842.
19. DeGrado, T. R.; Coleman, R. E.; Wang, S.; Baldwin, S. W.; Orr, M. D.; Robertson, C. N.; Polascik, T. J.; Price, D. T. *Cancer Res.* **2000**, *61*, 110.
20. DeGrado, T. R.; Baldwin, S. W.; Wang, S.; Orr, M. D.; Liao, R. P.; Friedman, H. S.; Reiman, R.; Price, D. T.; Coleman, R. E. *J. Nucl. Med.* **2001**, *42*, 1805.
21. Hara, T.; Bansal, A.; DeGrado, T. R. *Mol. Imaging* **2006**, *5*, 498.
22. Mulholland, K. K.; Wieland, D. M.; Kilbourn, M. R.; Frey, K. A.; Sherman, P. S.; Carey, J. E.; Kuhl, D. E. *Synapse* **1998**, *30*, 263.
23. Gao, M.; Miller, M. A.; DeGrado, T. R.; Mock, B. H.; Lopshire, J. C.; Rosenberger, J. G.; Dusa, C.; Das, M. K.; Groh, W. J.; Zipes, D. P.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem.* **2007**, *15*, 1289.
24. Haarstad, V. B.; Domer, F. R.; Chihal, D. M.; Rege, A. B.; Charles, H. C. *J. Med. Chem.* **1976**, *19*, 760.
25. Mock, B. H.; Mulholland, G. K.; Vavrek, M. T. *Nucl. Med. Biol.* **1999**, *26*, 467.
26. Mock, B. H.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S225.
27. Iwata, R.; Pascali, C.; Bogni, A.; Furumoto, S.; Terasaki, K.; Yanai, K. *Appl. Radiat. Isot.* **2002**, *57*, 347.
28. Gillis, R. J.; Barry, J. A.; Ross, B. D. *Magn. Reson. Med.* **1994**, *32*, 310.
29. Walum, E. *Cell Mol. Neurobiol.* **1981**, *1*, 389.
30. Liu, X.; Wang, J.-Q.; Zheng, Q.-H. *Biomed. Chromatogr.* **2005**, *19*, 379.
31. Barinaga, M. *Science* **1999**, *278*, 1226.
32. Experimental details and characterization data.
(a) General: all commercial reagents and solvents were used without further purification. $^{11}CH_3OTf$ was made according to a literature procedure.[25] $^{18}FCH_2OTf$ was made according to a modification of the literature method.[27] Melting points were determined on a MEL-TEMP II apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 FT NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) are reported in hertz (Hz). The low resolution mass spectra (LRMS) were obtained using an Agilent 1100 series LC/MSD mass spectrometer. Thin layer chromatography

(TLC) was run using Analtech silica gel GF uniplates ($5 \times 10\ cm^2$). Chromatographic solvent proportions are expressed on a volume:volume basis. Plates were visualized by UV light. All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C_{18} column, 4.6×250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ (buffer solution) mobile phase, flow rate 1.5 mL/min, and UV (270 nm) and γ-ray (NaI) flow detectors. Semi-prep cation-exchange CM Sep-Pak cartridges were obtained from Waters Corporate Headquarters, Milford, MA. Sterile vented Millex-GS 0.22 μm filter unit was obtained from Millipore Corporation, Bedford, MA.

(b) 4,4′-bis-(1-methyl-3-hydroxy-morpholinyl-(3))-biphenyl (**3**). To a solution of 4,4′-bis-bromoacetyl-1-biphenyl (396 mg, 1.0 mmol) dissolved in 50 mL THF was added a solution of 2-(methylamino)ethanol (330 mg, 4.4 mmol) dissolved in 10 mL of THF. The mixture was stirred at room temperature for 3 h and heated to reflux for 2 h. The mixture solution was evaporated under reduced pressure, added with dichloromethane (120 mL), decanted or filtered from the precipitated N-substituted ethanolamine hydrobromide, washed with water and brine. Then the organic solution was extracted with 1N HCl solution, and the pH of HCl solution was adjusted with ammonium hydroxide to 9, aqueous solution was again extracted with dichloromethane, organic solution was dried over magnesium sulfate, filtered, and concentrated to give compound**3** (306 mg, 87%) as a light yellow solid. Mp: 45–46 °C; $R_f = 0.26$ (1:9, MeOH/CH_2Cl_2). ^1H NMR (300 MHz, DMSO-d_6): δ 1.99–2.29 (m, 2H), 2.16 (s, 6H, CH_3), 2.56–2.73 (m, 4H, CH_2), 3.51–3.91 (m, 4H, CH_2), 4.10 (dt, $J = 2.2$, 11.0 Hz, 2H, CH_2), 6.26 (s, 2H, OH), 7.55–7.70 (m, 8H, Ph–H). MS (ESI): 385 ($[M+H]^+$, 30%), 384 (100%).

(c) 2,2-(4,4-biphenylene)bis(2-hydroxy-4,4-dimethylmorpholinium bromide) (HC-3, **1**). 4,4′-bis-bromoacetyl-1-biphenyl (396 mg, 1.0 mmol) and 2-dimethylaminoethanol (178 mg, 2.0 mmol) were added into anhydrous THF (40 mL), stirred at room temperature for 24 h. A white solid was precipitated, filtered, and washed with dry THF to obtain compound **5** (614 mg, 92%). Mp: 178–180 °C. ^1H NMR (300 MHz, DMSO-d_6): δ 3.19 (s, 6H, CH_3), 3.31–3.39 (m, 2H, CH_2), 3.46 (s, 6H, CH_3), 3.60 (d, $J = 5.1$ Hz, 4H, CH_2), 3.66 (d, $J = 13.2$ Hz, 2H, C*H*H), 4.08.

(d) $J = 13.2$ Hz, 2H, CH*H*, 4.51–4.49 (m, 2H, CH_2), 7.39 (d, $J = 1.4$ Hz, 2H, OH), 7.64 (d, $J = 8.1$ Hz, 4H, Ph–H), 7.77 (d, $J = 8.1$ Hz, 4H, Ph–H). MS (ESI): 414 (M^+, 32%), 413 (100%).

(d) [^{11}C]HC-3 ([^{11}C]**1**). Bis-tertiary amine precursor **3** (0.1–0.2 mg) was dissolved in acetonitrile (300 μL). The mixture was placed in a sealed reaction vessel. No carrier-added (high specific activity) [^{11}C]CH_3OTf was passed through the reaction solution, which was cooled at ~ 0 °C, until radioactivity reached a maximum (~3 min), and then the reaction mixture was heated at 80 °C for 2 min. The reaction vessel was connected to a CM Sep-Pak cartridge. The labeled product mixture solution was passed onto the Sep-Pak cartridge to release the non-reacted precursor with ethanol and to retain the pure N-^{11}C-methylated single-side quaternary amine intermediate on the same CM Sep-Pak. Then, the labeled intermediate underwent the secondary N-[^{12}C]methylation by addition of CH_3I in ethanol to the same cartridge. After 2 min, the Sep-Pak cartridge was washed with ethanol (5 mL) and water (2 mL) to remove non-reacted CH_3I, and the washing solution was discarded to a waste bottle. The final product [^{11}C]**1** was eluted from the CM Sep-Pak with saline (2–4 mL) and sterile-filtered through a 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and the total volume was noted. The overall synthesis time was 20–30 min from EOB. The radiochemical yields decay corrected to EOB, from $^{11}CO_2$, were 50–60%, the radiochemical purity was >99%, and the chemical purity of the target tracer was >95% measured by analytical HPLC. Retention times in the analytical HPLC system were: t_R**3** = 3.45 min, t_R**1** = 2.36 min, t_R[^{11}C]**1** = 2.36 min.

(e) [^{18}F]HC-3 ([^{18}F]**2**). Precursor **3** (0.6–1.0 mg) was dissolved in acetone (0.6 mL), and the mixture was placed in a sealed reaction vessel. No carrier-added (high specific activity) [^{18}F]fluoromethyl bromide prepared by a literature method [20] was passed through a silver triflate column at 20 °C to form [^{18}F]FCH_2OTf. [^{18}F]FCH_2OTf was trapped in the reaction solution, and then the reaction mixture was heated at 40 °C for 10 min. The reaction vessel was connected to a CM Sep-Pak cartridge. The labeled product mixture solution was passed onto the Sep-Pak cartridge to release the non-reacted precursor with ethanol and to retain the pure N-^{18}F-fluoromethylated single-side quaternary amine intermediate on the same CM Sep-Pak. Then, the labeled intermediate underwent the secondary N-[^{12}C]methylation by addition of CH_3I in ethanol to the same cartridge. After 2 min, the Sep-Pak cartridge was washed with ethanol (5 mL) and water (2 mL) to remove non-reacted CH_3I, and the washing solution was discarded to a waste bottle. The final product [^{18}F]**2** was eluted from the CM Sep-Pak with saline (2–4 mL) and sterile-filtered through a 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and the total volume was noted. The overall synthesis time was 30–40 min from EOB. The radiochemical yields decay corrected to EOB, from $K^{18}F$, were 5–10%, the radiochemical purity was >99%, and the chemical purity of the target tracer was >95% measured by analytical HPLC. Retention times in the analytical HPLC system were: t_R**3** = 3.45 min, t_R [^{18}F]**2** = 2.42 min.

(f) Biodistribution study. The 9L-glioma rats (200–300 g) were injected intravenously with sub-pharmacologic doses (1–3 mCi) of [^{11}C]HC-3 and/or [^{18}F]HC-3 via the tail vein while under conscious restraint. At 5 and 20 min post-injection of [^{11}C]HC-3 or at 5 and 30 min post-injection of [^{18}F]HC-3, rats were sacrificed by decapitation under halothane anesthesia, their tissues quickly excised, weighed, and the decay-corrected radioactive content measured using a Wallac 1470 gamma counter. The results are expressed as percentage of injected dose (%ID) for urine and as percentage of injected dose per mass of tissue (%ID/g) for all other tissues. For calculation of total blood activity, blood mass was assumed to be 7% of the body mass.