Synthesis of a PET tau tracer [11C]PBB3 for imaging of Alzheimer's disease

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Abstract—The authentic standard PBB3 and its precursor *N*-desmethyl-PBB3 as well as TBS-protected *N*-desmethyl-PBB3 precursor for radiolabeling were synthesized from 5-bromo-2-nitropyridine, acrolein diethyl acetal, 6-methoxy-2-methylbenzothiazole, and diethylchlorophosphate with overall chemical yield 1% in six steps, 2% in five steps, and 1% in six steps, respectively. [11C]PBB3 was prepared from either desmethyl-PBB3 or TBS-protected desmethyl-PBB3 with [11C]CH$_3$OTf through *N*-[11C]methylation and isolated by HPLC combined with SPE in 20-25% and 15-20% radiochemical yield, respectively, based on [11C]CO$_2$ and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the specific activity at EOB was 370-1110 GBq/μmol with a total synthesis time of ~40-minutes from EOB.

Keywords: [11C]PBB3; Tau tracer; Radiosynthesis; Positron emission tomography (PET); Alzheimer's disease (AD).

Alzheimer's disease (AD) is a dominant public health problem because of increasing life expectancy and no reliable disease-modifying therapies at present.[1-3] Currently, the cause of AD remains unclear and no any effective strategy is approved for preventing, curing and slowing the progress of AD.[4-6] To discover more effective treatments, a reliable diagnostic tool is really needed.[7] Neuroimaging of AD is one of the most active as well as most challenging areas in neuroscience.[8] Advanced biomedical imaging technique positron emission tomography (PET) is a promising modality for AD, and significant advances have occurred in this field of molecular imaging.[9] The development of PET imaging probes for in vivo detection of Alzheimer's brains is critical for early and accurate diagnosis and for the successful discovery of disease-modifying therapies.[10-12] Aggregated β-amyloid plaques (Aβ) and tau protein are two major biomarkers for AD.[13,14] Aβ PET tracers have been well-developed, and the representative radiopharmaceuticals include [11C]PIB[15] and [18F]Amyvid (formerly known as [18F]AV-45),[16] as displayed in Figure 1. The success and limitations of Aβ imaging with [11C]PIB and [18F]Amyvid have spurred efforts worldwide to develop selective PET tau tracers, since Aβ imaging with current tracers still lacks diagnostic accuracy and high degree of correlations between sensitivity/specificity and pathology of AD, and more and more evidences demonstrate that increased tau levels appear to correlate with clinical AD severity.[17] Recently the researchers have been focusing on tau imaging, several highly selective and specific PET tau tracers like [18F]T808 ([18F]AV-680), [18F]T807 ([18F]AV-1451), [11C]PBB3, [18F]THK-5105 and [18F]THK-5117 (Figure 1) have been developed, and promising clinical PET imaging results with these tracers have been reported.[18-24]

To address local investigator needs for PET tau tracers, we decided to make our own material by following the literature methods. In our previous work, we have reported the synthesis of fluorine-18 labelled tracers [^{18}F]T808 and [^{18}F]T807.[25,26] In this ongoing study, we present the synthesis of a carbon-11 labelled tracer [^{11}C]PBB3. Carbon-11 tracers have a potential advantage of back-to-back same-day studies of pharmacological or behavioral challenges, since it can avoid movement of the study subject from the PET scanner and perform the repeat studies within 2-3 hours to explore the drug effects. The published and patented synthesis of the authentic standard PBB3, its TBS-protected *N*-desmethyl-PBB3 precursor and target tracer [^{11}C]PBB3 lacks synthetic detail, and the key steps gave poor yield and was difficult to reproduce in our hands. Therefore, we investigated alternate approaches and modifications based on the reported literature methods[22,27,28] that eventually resulted in higher-yield synthesis of PBB3, *N*-desmethyl-PBB3, TBS-protected *N*-desmethyl-PBB3 and [^{11}C]PBB3 starting from very beginning materials 5-bromo-2-nitropyridine, acrolein diethyl acetal, 6-methoxy-2-methylbenzothiazole, and diethylchlorophosphate. The synthesis presented here was superior to previous works or addressed more synthetic details to reveal and explain technical artifices. In this letter we provide the complete experiment procedures, yields, analytical details and new findings for the synthesis of PBB3 and [^{11}C]PBB3 from TBS-protected *N*-desmethyl-PBB3 via one-pot-two-step approach, and first present a fully automated one-pot-one-step radiosynthesis of [^{11}C]PBB3 from *N*-desmethyl-PBB3 with relatively high radiochemical yields and shortened radiosynthesis time.
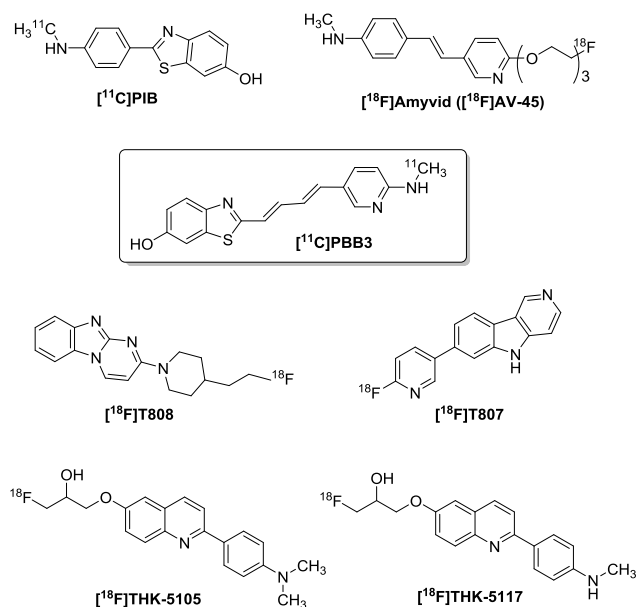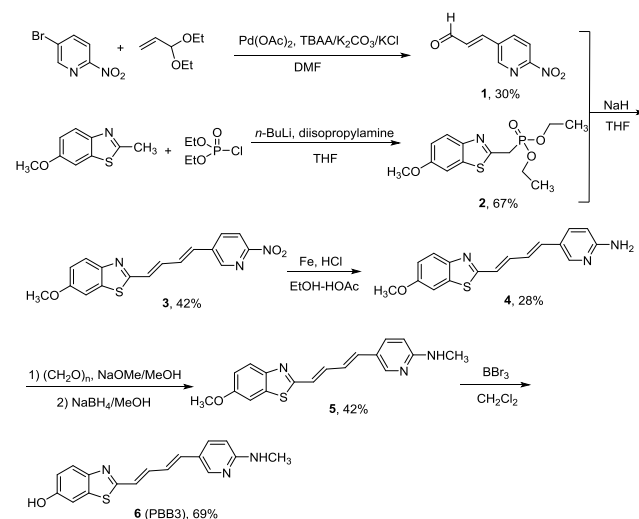
The synthesis of reference standard PBB3 (5-((1*E*,3*E*)-4-(6-methylamino)pyridin-3-yl)buta-1,3-dien-1-yl)benzo[*d*]thiazol-6-ol, **6**) is shown in Scheme 1, according to the patent method[28] with modifications. The core structure diaryldiene **3** was achieved in 42% yield by Wittig-Horner reaction of aldehyde **1** and diethyl phosphonate **2** in the presence of NaH in THF.[29,30] Cinnamaldehyde derivative **1** was prepared from 5-bromo-2-nitropyridine and acrolein diethyl acetal under Cacchi condition (K_2CO_3, tetra-*n*-butyl-ammonium acetate (TBAA), KCl, DMF, 115 °C) with palladium (II) acetate as catalyst in an Ace pressure tube.[31-33] The use of Ace pressure tube improved the yield of compound **1** from 12% to 30%. Coupling diethylchlorophosphate with 6-methoxy-2-methylbenzothiazole in the presence of lithium diisopropylamide (LDA), that was freshly prepared in one-pot, produced diethyl phosphonate derivative **2** in 67% yield.[34,35] The nitro group of compound **3** was reduced with iron in acidic ethanol to yield amine **4** in 28% yield. Reduction with $SnCl_2$ in EtOH or acidic MeOH was less effective. Instead of *N*-monomethylation of compound **4** with CH_3I in the presence of NaH in DMF (14% yield), *N*-monomethyl derivative **5** was obtained by condensation with paraformaldehyde in the presence of NaOMe, followed by reduction with sodium borohydride in MeOH with a moderate yield (42%).[36] Demethylation of compound **5** with BBr_3 in CH_2Cl_2 afforded **6** in 69% yield.

Scheme 1. Synthesis of PBB3 (**6**).

The synthesis of precursors *N*-desmethyl-PBB3 (2-((1*E*,3*E*)-4-(6-aminopyridin-3-yl)buta-1,3-dien-1-yl)benzo[*d*]thiazol-6-ol, **7**) and TBS-protected *N*-desmethyl-PBB3 (5-((1*E*,3*E*)-4-(6-((*tert*-butyldimethylsilyl)oxy)benzo[*d*]thiazol-2-yl)buta-1,3-dien-1-yl)pyridin-2-amine, **8**) is outlined in Scheme 2, according to the patent method[28] with modifications.

Figure 1. Chemical structure of [^{11}C]PIB, [^{18}F]Amyvid ([^{18}F]AV-45), [^{11}C]PBB3, [^{18}F]T808 ([^{18}F]AV-680), [^{18}F]T807 ([^{18}F]AV-1451), [^{18}F]THK-5105 and [^{18}F]THK-5117.

The methoxyl group of compound **4** was desmethylated with BBr$_3$ in CH$_2$Cl$_2$ to provide phenol **7** in 71% yield, and its free hydroxyl group was protected with *tert*-butyldimethylsilyl (TBS) group by reaction with *tert*-butyldimethylsilyl chloride (TBSCl) in the presence of 4-*N,N*-(dimethylamino)pyridine (DMAP) in DMF to afford TSB-protected precursor **8** in 33% yield.
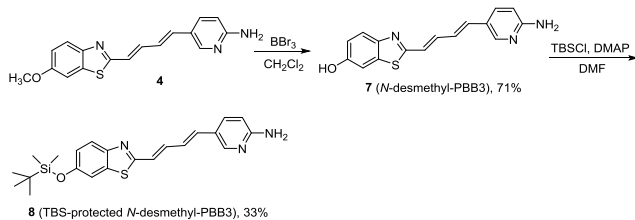
Scheme 2. Synthesis of *N*-desmethyl-PBB3 (**7**) and TBS-protected *N*-desmethyl-PBB3 (**8**).

Synthesis of [^{11}C]PBB3 (5-((1*E*,3*E*)-4-(6-[^{11}C]methylamino)pyridin-3-yl)buta-1,3-dien-1-yl)benzo[*d*]thiazol-6-ol, [^{11}C]**6**) via TBS-protected *N*-desmethyl-PBB3 (**8**) precursor is presented in Scheme 3. The precursor **8** underwent *N*-[^{11}C]methylation[37-40] using the reactive [^{11}C]methylating agent [^{11}C]methyl triflate ([^{11}C]CH$_3$OTf)[41,42] in dimethyl sulfoxide (DMSO) at 100 °C under basic condition (KOH) to give a radiolabeled intermediate TBS-protected [^{11}C]PBB3. Without isolation, this was followed by a quick deprotection reaction using H$_2$O to provide the target tracer [^{11}C]PBB3. The radiolabeling mixture was isolated by semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column, and then concentrated by solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge[43-45] to produce the corresponding pure radiolabeled compound [^{11}C]**6** in 15-20% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO$_2$. This is a 1-pot-2-step radiosynthesis. It is somewhat complicated with low radiochemical yield and long overall reaction time.
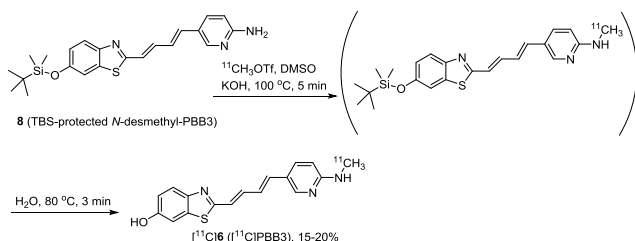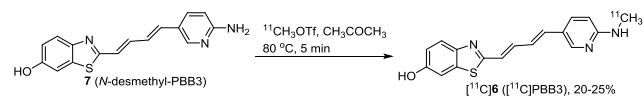

Scheme 3. Synthesis of [^{11}C]PBB3 ([^{11}C]**6**) from TBS-protected *N*-desmethyl-PBB3 (**8**).

The chemical structure of [^{11}C]PBB3 is somewhat similar to the chemical structure of [^{11}C]PIB. The original radiosynthesis of [^{11}C]PIB utilized the hydroxyl group protected precursor for carbon-11 labeling at amino group, followed by deprotection.[46] Later a rapid one-step radiosynthesis of [^{11}C]PIB using unprotected precursor for direct radiolabeling was developed,[47,48] which has become a popular radiosynthesis method for [^{11}C]PIB production in PET radiochemistry facilities. Based on [^{11}C]PIB radiosynthesis methodology, we developed 1-pot-1-step radiosynthesis for [^{11}C]PBB3 for the first time.

Synthesis of [^{11}C]PBB3 via *N*-desmethyl-PBB3 (**7**) precursor is outlined in Scheme 4. The precursor **7** was labeled by [^{11}C]CH$_3$OTf through *N*-[^{11}C]methylation in acetone at 80 °C under neutral condition to directly give the target tracer. The radiolabeling mixture was isolated by RP-HPLC combined with SPE to produce the corresponding pure radiolabeled compound [^{11}C]**6** in 20-25% decay-corrected radiochemical yield. This is a 1-pot-1-step radiosynthesis with higher radiochemical yield and shorter overall synthesis time. No protection of 6-hydroxy group is required, greatly simplifying the synthetic method.

Scheme 4. Synthesis of [^{11}C]PBB3 ([^{11}C]**6**) from *N*-desmethyl-PBB3 (**7**).

Our approach employed more reactive [^{11}C]CH$_3$OTf, instead of commonly used [^{11}C]methyl iodide ([^{11}C]CH$_3$I),[49] in *N*-[^{11}C]methylation of the amino, and thus the radiochemical yield of [^{11}C]**6** was relatively high in both radiosynthesis approaches using protected and unprotected precursors. It is important to note that the strong base KOH at high reaction temperature (forcing conditions) would help the *N*-[^{11}C]methylation of protected precursor **8**, and significantly increase the radiochemical yield of [^{11}C]**6**. However, for the unprotected precursor **7**, it is needed to conduct the *N*-[^{11}C]methylation of the amino under mildly neutral condition, since the unprotected hydroxyl group in the precursor will be [^{11}C]methylated with [^{11}C]CH$_3$OTf first under basic condition at high reaction temperature. Only a relatively small amount of the precursor (0.3-0.5 mg) was used for radiolabeling, instead of more commonly used amount of the precursor (0.9-1.1 mg) in the literature,[27] which improved the chemical purity of the final tracer solution. Furthermore, addition of water to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification, gave better separation of [^{11}C]**6** from its protected or unprotected precursor (**8** or **7**).

The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module.[50-52] This radiosynthesis module facilitated the overall design of the reaction, purification and

reformulation capabilities in a fashion suitable for adaptation to preparation of human doses. In addition, the module is designed to allow in-process measurement of $[^{11}C]$-tracer specific activity (SA, GBq/μmol at EOB) using a radiation detector at the outlet of the HPLC-portion of the system. For the reported syntheses, product SA was in a range of 370-1110 GBq/μmol at EOB. The factors that affect the EOB SA significantly to lead to such a wide range from 370 to 1110 GBq/μmol are mainly from two parts: (1) carrier from the $[^{11}C]$-target, and (2) carrier from the $[^{11}C]$-radiosynthesis unit.[53] We have optimized the $[^{11}C]$ gas irradiation target system and the $[^{11}C]$-radiosynthesis unit to eliminate ^{12}C carrier-added as much as possible and to reach the high end of the SA. To help produce high SA $[^{11}C]CO_2$, we usually do 10-minute target pre-burn for 2-3 times, with the same beam current, prior to the actual production run. These pre-burn warm up the cyclotron target and eliminate significant amount of ^{12}C carrier in the cyclotron's $[^{11}C]$ gas target. We use an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module to produce $[^{11}C]CH_3OTf$, convenient gas phase bromination of $[^{11}C]$methane, and production of $[^{11}C]CH_3OTf$. This 'dry' method, using Br_2 to generate a $[^{11}C]CH_3Br$ intermediate, differs from other 'dry' methods using I_2 and 'wet' methods using $LiAlH_4$ and HI, and seems to help minimize introduction of additional ^{12}C carrier after $[^{11}C]CO_2$ production.[42] To further help produce high SA $[^{11}C]CH_3OTf$, we usually do 2 "test loop" procedures when we set up the module for the actual $[^{11}C]CH_3OTf$ production run. These procedures avoid any leak in the module to introduce additional ^{12}C carrier and eliminate significant amount of original ^{12}C carrier accumulated in the $[^{11}C]CH_3OTf$ production system.

The 1-pot-1-step radiosynthesis via *N*-desmethyl-PBB3 is a non-specific synthesis route, and 1-pot-2-step radiosynthesis via TBS-protected *N*-desmethyl-PBB3 is a specific synthesis route. The non-specific route required lower reaction temperature, shorter reaction time, and shorter overall synthesis time. Consequently it gave higher radiochemical yield. In addition, it is easier to synthesize the precursor without TBS group than with TBS group. This route is somewhat simpler to implement as it avoids adding a deprotection step. In comparison with these two radiosynthesis approaches, the non-specific route is identified as a more appropriate route for future applications.

Chemical purity and radiochemical purity were determined by analytical HPLC.[54] The chemical purity of the precursors and reference standard was >90%. The radiochemical purity of the target tracer was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target

tracer was >90% determined by reversed-phase HPLC through UV flow detector.

The experimental details and characterization data for compounds **1-8** and for the tracer $[^{11}C]$**6** are given.[55]

In summary, synthetic routes with moderate to high or improved yields have been developed to produce the precursors TBS-protected *N*-desmethyl-PBB3 and *N*-desmethyl-PBB3, the reference standard PBB3, and the target PET radiotracer $[^{11}C]$PBB3. The radiosynthesis employed $[^{11}C]CH_3OTf$ for *N*-$[^{11}C]$methylation at the nitrogen position of the amine precursors, followed by product purification and isolation by a semi-preparative RP HPLC combined with SPE. The desired $[^{11}C]$PBB3 was obtained in high radiochemical yield, radiochemical purity and chemical purity, with a reasonably short overall synthesis time, and high specific activity. This will facilitate studies to evaluate $[^{11}C]$PBB3 as a PET AD tau tracer in animals and humans.

Acknowledgments

References and notes

1. Ariza, M.; Kolb, H. C.; Moechars, D.; Rombouts, F.; Andrés, J. I. *J. Med. Chem.* **2015**, *58*, 4365.
2. Watanabe, H.; Ono, M.; Saji, H. *ScientificWorldJournal* **2015**, *2015*, 124192.
3. James, O. G.; Doraiswamy, P. M.; Borges-Neto, S. *Front. Neurol.* **2015**, *6*, 38.
4. Adlard, P. A.; Tran, B. A.; Finkelstein, D. I.; Desmond, P. M.; Johnston, L. A.; Bush, A. I.; Egan, G. F. *Front. Neurosci.* **2014**, *8*, 327.
5. Jack, C. R. Jr. *Radiology* **2012**, *263*, 344.
6. Zimmer, E. R.; Leuzy, A.; Benedet, A. L.; Breitner, J.; Gauthier, S.; Rosa-Neto, P. *J. Neuroinflammation* **2014**, *11*, 120.
7. Catafau, A. M.; Bullich, S. *Clin. Transl. Imaging* **2015**, *3*, 39.
8. Villemagne, V. L.; Fodero-Tavoletti, M. T.; Masters, C. L.; Rowe, C. C. *Lancet Neurol.* **2015**, *14*, 114.
9. Sabri, O.; Seibyl, J.; Rowe, C.; Barthel, H. *Clin. Transl. Imaging* **2015**, *3*, 13.

10. Wurtman R. *Metabolism* **2015**, *64(3 Suppl 1)*, S47.
11. Zimmer, E. R.; Leuzy, A.; Gauthier, S.; Rosa-Neto, P. *Can. J. Neurol. Sci.* **2014**, *41*, 547.
12. Ono, M. *Chem. Pharm. Bull.* **2009**, *57*, 1029.
13. Mach, R. H. *J. Nucl. Med.* **2015**, *55*, 1221.
14. Shah, M.; Catafau, A. M. *J. Nucl. Med.* **2014**, *54*, 871.
15. Klunk, W. E.; Engler, H.; Nordberg, A.; Bacskai, B. J.; Wang, Y.; Price, J. C.; Bergström, M.; Hyman, B. T.; Långström, B.; Mathis, C. A. *Neuroimaging Clin. N. Am.* **2003**, *13*, 781.
16. Carpenter, A. P. Jr.; Pontecorvo, M. J.; Hefti, F. F.; Skovronsky, D. M. *Q. J. Nucl. Med. Mol. Imaging* **2009**, *53*, 387.
17. Villemagne, V. L.; Okamura, N. *Alzheimer's Dement.* **2014**, *10*, S254.
18. Zhang, W.; Arteaga, J.; Cashion, D. K.; Chen, G.; Gangadharmath, U.; Gomez, L. F.; Kasi, D.; Lam, C.; Liang, Q.; Liu, C.; Mocharla, V. P.; Mu, F.; Sinha, A.; Szardenings, A. K.; Wang, E.; Walsh, J. C.; Xia, C.; Yu, C.; Zhao, T.; Kolb, H. C. *J. Alzheimer's Dis.* **2012**, *31*, 601.
19. Xia, C. F.; Arteaga, J.; Chen, G.; Gangadharmath, U.; Gomez, L. F.; Kasi, D.; Lam. C.; Liang, Q.; Liu, C.; Mocharla, V. P.; Mu, F.; Sinha, A.; Su, H.; Szardenings, A. K.; Walsh, J. C.; Wang, E.; Yu, C.; Zhang, W.; Zhao, T.; Kolb, H. C. *Alzheimer's Dement.* **2013**, *9*, 666.
20. Chien, D. T.; Bahri, S.; Szardenings, A. K.; Walsh, J. C.; Mu, F.; Su, M. Y.; Shankle, W. R.; Elizarov, A.; Kolb, H. C. *J. Alzheimer's Dis.* **2013**, *34*, 457.
21. Chien, D. T.; Szardenings, A. K.; Bahri, S.; Walsh, J. C.; Mu, F.; Xia, C.; Shankle, W. R.; Lerner, A. J.; Su, M. Y.; Elizarov, A.; Kolb, H. C. *J. Alzheimer's Dis.* **2014**, *38*, 171.
22. Maruyama, M.; Shimada, H.; Suhara, T.; Shinotoh, H.; Ji, B.; Maeda, J.; Zhang, M.-R.; Trojanowski, J. Q.; Lee, V. M.; Ono, M.; Masamoto, K.; Takano, H.; Sahara, N.; Iwata, N.; Okamura, N.; Furumoto, S.; Kudo, Y.; Chang, Q.; Saido, T. C.; Takashima, A.; Lewis, J.; Jang, M. K.; Aoki, I.; Ito, H.; Higuchi, M. *Neuron* **2013**, *79*, 1094.
23. Okamura, N.; Furumoto, S.; Fodero-Tavoletti, M. T.; Mulligan, R. S.; Harada, R.; Yates, P.; Pejoska, S.; Kudo, Y.; Masters, C. L.; Yanai, K.; Rowe, C. C.; Villemagne, V. L. *Brain* **2014**, *137*, 1762.
24. Harada, R.; Okamura, N.; Furumoto, S.; Furukawa, K.; Ishiki, A.; Tomita, N.; Hiraoka, K.; Watanuki, S.; Shidahara, M.; Miyake, M.; Ishikawa, Y.; Matsuda, R.; Inami, A.; Yoshikawa, T.; Tago, T.; Funaki, Y.; Iwata, R.; Tashiro, M.; Yanai, K.; Arai, H.; Kudo, Y. *Eur. J. Nucl. Med. Mol. Imaging* **2015**, *42*, 1052.
25. Gao, M.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 254.
26. Gao, M.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2953.
27. Hashimoto, H.; Kawamura, K.; Igarashi, N.; Takei, M.; Fujishiro, T.; Aihara, Y.; Shiomi, S.; Muto, M.; Ito, T.; Furutsuka, K.; Yamasaki, T.; Yui, J.; Xie, L.; Ono, M.; Hatori, A.; Nemoto, K.; Suhara, T.; Higuchi, M.; Zhang, M. R. *J. Nucl. Med.* **2014**, *55*, 1532.
28. Higuchi, M.; Suhara, T.; Maruyama, M.; Zhang, M.; Shimada, H. JP Patent No. 5422782 B1, 2014.
29. Nielsen, C. B.; Johnsen, M.; Arnbjerg, J.; Pittelkow, M.; McIlroy, S. P.; Ogilby, P. R.; Jørgensen, M. *J. Org. Chem.* **2005**, *70*, 7065.
30. Zhuang, Z. P.; Kung, M. P.; Kung, H. F. *J. Med. Chem.* **2006**, *49*, 2841.
31. Battistuzzi, G.; Cacchi, S.; Fabrizi, G. *Org. Lett.* **2003**, *5*, 777.
32. Alacid, E.; Nájera, C. *Eur. J. Org. Chem.* **2008**, 3102.
33. Nájera, C.; Botella, L. *Tetrahedron* **2005**, *61*, 9688.
34. Hirao, T.; Nakamura, T.; Hagihara, M.; Agawa, T. *J. Org. Chem.* **1985**, *50*, 5860.
35. Lartia, R.; Allain, C.; Bordeau, G. Schmidt, F.; Fiorini-Debuisschert, C.; Charra, F.; Teulade-Fichou, M. *J. Org. Chem.* **2008**, *73*, 1732.
36. Chandra, R.; Oya, S.; Kung, M. P.; Hou, C.; Jin, L. W.; Kung, H. F. *J. Med. Chem.* **2007**, *50*, 2415.
37. Wang, M.; Gao, M.; Steele, B. L.; Glick-Wilson, B. E.; Brown-Proctor, C.; Shekhar, A.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4713.
38. Gao, M.; Shi, Z.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1953.
39. Wang, M.; Gao, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5259.
40. Wang, M.; Gao, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4455.
41. Jewett, D. M. *Int. J. Radiat. Appl. Instrum. A* **1992**, *43*, 1383.
42. Mock, B. H.; Mulholland, G. K.; Vavrek, M. T. *Nucl. Med. Biol.* **1999**, *26*, 467.
43. Gao, M.; Wang, M.; Mock, B. H.; Glick-Wilson, B. E.; Yoder, K. K.; Hutchins, G. D.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2010**, *68*, 1079.
44. Wang, M.; Gao, M.; Miller, K. D.; Zheng, Q.-H. *Steroids* **2011**, *76*, 1331.
45. Wang, M.; Gao, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1569.
46. Mathis, C. A.; Wang, Y.; Holt, D. P.; Huang, G.-F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740.
47. Wilson, A. A.; Garcia, A.; Chestakova, A.; Kung, H.; Houle, S. *J. Labelled Compd. Radiopharm.* **2004**, *47*, 679.
48. Ono, M.; Wilson, A.; Nobrega, J.; Westaway, D.; Verhoeff, P.; Zhuang, Z.-P.; Kung, M.-P.; Kung, H. F. *Nucl. Med. Biol.* **2003**, *30*, 565.
49. Allard, M.; Fouquet, E.; James, D.; Szlosek-Pinaudm, M. *Curr. Med. Chem.* **2008**, *15*, 235.
50. Mock, B. H.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S225.
51. Mock, B. H.; Glick-Wilson, B. E.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S224.

52. Wang, M.; Gao, M.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2012**, *70*, 965.

53. Gao, M.; Wang, M.; Green, M. A.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 1965.

54. Zheng, Q.-H.; Mock, B. H. *Biomed. Chromatogr.* **2005**, *19*, 671.

55. *(a). General:* All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH$_3$OTf was prepared according to a literature procedure.[42] ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 MHz NMR Fourier transform spectrometer at 500 and 125 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard tetramethylsilane (TMS, δ 0.0) (^{1}H NMR) and to the solvent signal (^{13}C NMR), and coupling constants (*J*) are reported in hertz (Hz). Liquid chromatography-mass spectra (LC-MS) analysis was performed on an Agilent system, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5 × 10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV254 plates (20 × 20 cm^2). Normal phase flash column 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. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; mobile phase 40% CH$_3$CN/60% 20 mM H$_3$PO$_4$; flow rate 1.0 mL/min; and UV (379 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC was performed using a Gemini ODS (Phenomenex) 5 μm C-18 column, 10 × 250 mm; mobile phase 22% CH$_3$CN/78% 20 mM NH$_4$OH; 8.0 mL/min flow rate; UV (379 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).

(b). (E)-3-(6-Nitropyridin-3-yl)acrylaldehyde (1): A suspension of 5-bromo-2-nitropyridine (1.50 g, 7.4 mmol), acrolein diethyl acetal (3.36 mL, 22.0 mmol), K$_2$CO$_3$ (1.53 g, 11.1 mmol), TBAA (4.50 g, 14.9 mmol), KCl (552 mg, 7.4 mmol), palladium (II) acetate (670 mg, 2.98 mmol), and DMF (30 mL) in an Ace pressure tube under N$_2$ atmosphere was stirred at 115 °C overnight. The reaction mixture was cooled, filtered through Celite, and washed with EtOAC. The combined filtrate was cooled in an ice bath, and an aqueous 2 N HCl (120 mL) was added slowly. After the mixture was stirred at room temperature (RT) for 30 min, it was poured into ice-water and neutralized with saturated aqueous NaHCO$_3$. The mixture was extracted with EtOAc, the combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The above reaction and work up was repeated 10 times. The combined crude product was purified by column chromatography with CH$_2$Cl$_2$/MeOH (from 250:3 to 250:5) as eluent to afford compound **1** as a pale yellow solid (4.01 g, 30%). ^{1}H NMR (CDCl$_3$): δ 9.80 (d, *J* = 7.0 Hz, 1H), 8.79 (d, *J* = 2.0 Hz, 1H), 8.33 (d, *J* = 8.5 Hz, 1H), 8.23 (dd, *J* = 4.0, 8.5 Hz, 1H), 7.58 (d, *J* = 16.0 Hz, 1H), 6.88 (dd, *J* = 7.0, 16.0 Hz, 1H).

(c). Diethyl ((6-methoxybenzo[d]thiazol-2-yl)methyl)phosphonate (2): To a stirred solution of *n*-BuLi (2.5 M in hexanes, 25 mL, 62.5 mmol)) in anhydrous THF (25 mL) was added diisopropylamine (8.76 mL, 62.5 mmol) at -78 °C under N$_2$ atompshere. The mixture was stirred at this temperature for 30 min. To this LDA mixture, a solution of 6-methoxy-2-methylbenzothiazole (5.00 g, 27.9 mmol) in anhydrous THF (40 mL) was added dropwise and the resulting reddish solution was stirred 30 min at -78 °C. Then, a diethylchlorophosphate (4.44 mL, 30.7 mmol) solution in anhydrous THF (20 mL) was added dropwise. After the reaction mixture was stirred 10 min at -78 °C, it was allowed to warm up to RT and stirred at RT for 1 h. The reaction was quenched with aqueous 1 N NH$_4$Cl (100 mL), and the mixture was extracted with CHCl$_3$. The combined organic layer was washed with aqueous 2% Na$_2$CO$_3$, brine and dried over anhydrous Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography with CH$_2$Cl$_2$/MeOH (250:5) as eluent to afford compound **2** as an orange oil (5.91 g, 67%). ^{1}H NMR (CDCl$_3$): δ 7.79 (d, *J* = 9.0 Hz, 1H), 7.22 (d, *J* = 2.5 Hz, 1H), 6.98 (dd, *J* = 2.5, 9.0 Hz, 1H), 4.06 (quin, *J* = 2.0 Hz, 4H), 3.78 (s, 3H), 3.62 (d, *J* = 21.5 Hz, 2H), 1.24 (t, *J* = 2.0 Hz, 6H).

(d). 6-Methoxy-2-((1E,3E)-4-(6-nitropyridin-3-yl)buta-1,3-dien-1-yl)benzo[d]thiazole (3): To a stirred solution of compound **2** (4.41 g, 14.0 mmol) in anhydrous THF (50 mL) was added NaH (60% dispersion in mineral oil, 780 mg, 19.5 mmol) at 0 °C under N$_2$ atmosphere. After the mixture was allowed to warm up to RT and stirred at RT for 30 min, compound **1** (2.30 g, 12.9 mmol) was added. The reaction mixture was stirred at RT overnight, and it was cooled in an ice bath. Saturated aqueous NH$_4$Cl (16 mL) was added and stirred at 0 °C. The sticky solid was decanted, washed with water, and dried *in vacuo*. The crude solid product was suspended in toluene, and washed with toluene and CH$_2$Cl$_2$ to afford compound **3** as an orange solid (1.82 g, 42%). ^{1}H NMR (DMSO-d_6): δ 8.80 (s, 1H), 8.37-8.31 (m,

2H), 7.87 (d, J = 8.0 Hz, 1H), 7.67 (s, 1H), 7.60-7.55 (m, 1H), 7.42-7.37 (m, 1H), 7.17 (d, J = 15.5 Hz, 2H), 7.12 (d, J = 8.0 Hz, 1H), 3.85 (s, 3H).

(e). 5-((1E,3E)-4-(6-Methoxybenzo[d]thiazol-2-yl)buta-1,3-dien-1-yl)pyridin-2-amine (4): To a stirred mixture of compound **3** (1.47 g, 4.34 mmol), iron powder (1.14 g, 20.4 mmol) and HOAc (28 mL) in EtOH (280 mL) was added aqueous 12 N HCl (6 mL) dropwise. After the reaction mixture was stirred at RT overnight, it was cooled in an ice bath, neutralized with aqueous 6 N NaOH, and filtered to remove the precipitate. MeOH was added to the precipitate, and the mixture was stirred and filtered. The filtrates were combined. After the solvent was evaporated *in vacuo*, the crude product was purified by column chromatography with CHCl$_3$/MeOH (250:10) as eluent to afford compound **4** as a yellow solid (372 mg, 28%). ^{1}H NMR (CDCl$_3$): δ 8.12 (d, J = 2.5 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 7.64 (dd, J = 2.5, 8.5 Hz, 1H), 7.29 (d, J = 2.5 Hz, 1H), 7.20 (dd, J = 10.0, 15.5 Hz, 1H), 7.05 (dd, J = 2.5, 9.0 Hz, 1H), 6.85 (d, J = 15.0 Hz, 1H), 6.80 (dd, J = 10.0, 15.5 Hz, 1H), 6.72 (d, J = 15.5 Hz, 1H), 6.51 (d, J = 9.0 Hz, 1H), 4.63 (s, 2H), 3.88 (s, 3H). LC-MS (ESI, *m/z*): Calcd for C$_{17}$H$_{16}$N$_3$OS ([M+H]$^+$) 310.1; found 310.1.

(f). 5-((1E,3E)-4-(6-Methoxybenzo[d]thiazol-2-yl)buta-1,3-dien-1-yl)-N-methylpyridin-2-amine (5): To a stirred solution of compound **4** (50 mg, 0.16 mmol) in anhydrous MeOH (6 mL) was added NaOMe (81 mg, 1.5 mmol), and followed by paraformaldehyde (48 mg, 1.6 mmol). After the reaction mixture was heated and refluxed for 3 h, it was cooled to 0 °C. Sodium borohydride (121 mg, 3.2 mmol) was added portionwise. The mixture was heated and refluxed for another 1 h, then cooled in an ice bath. Water was added, and the mixture was extracted with CH$_2$Cl$_2$. The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The crude product was purified by preparative TLC plate with CH$_2$Cl$_2$/MeOH (100:5) as eluent to afford compound **5** as a yellow solid (22 mg, 42%). ^{1}H NMR (DMSO-d_6): δ 8.15 (d, J = 2.5 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 7.74 (dd, J = 2.0, 8.5 Hz, 1H), 7.67 (d, J = 2.5 Hz, 1H), 7.32 (dd, J = 10.5, 15.5 Hz, 1H), 7.12 (dd, J = 2.5, 9.0 Hz, 1H), 6.98-6.89 (m, 4H), 6.54 (d, J = 9.0 Hz, 1H), 3.88 (s, 3H), 2.86 (d, J = 4.5 Hz, 3H). LC-MS (ESI, *m/z*): Calcd for C$_{18}$H$_{18}$N$_3$OS ([M+H]$^+$) 324.1; found 324.0.

(g). 5-((1E,3E)-4-(6-Methylamino)pyridin-3-yl)buta-1,3-dien-1-yl)benzo[d]thiazol-6-ol (PBB3, 6): To a stirred suspension of compound **5** (20 mg, 0.062 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) was added BBr$_3$ (1.0 M solution in CH$_2$Cl$_2$, 0.4 mL, 0.4 mmol) dropwise at -78 °C under N$_2$ atmosphere. After the mixture was allowed to warm up to room temperature and stirred at RT overnight, the reaction was quenched with water in an ice bath, and it was neutralized with aqueous 1 N NaOH solution. The precipitate was

collected by filtration, washed with water and dried *in vacuo*. The crude product was purified by preparative TLC plate with CH$_2$Cl$_2$/MeOH (100:5) as eluent to afford compound **6** as a yellow solid (13 mg, 69%). ^{1}H NMR (DMSO-d_6): δ 9.99 (br s, 1H), 8.10 (d, J = 1.5 Hz, 1H), 7.72-7.68 (m, 2H), 7.33 (d, J = 2.0 Hz, 1H), 7.22 (dd, J = 10.5, 15.5 Hz, 1H), 6.98-6.91 (m, 3H), 6.84 (dd, J = 4.5, 15.5 Hz, 2H), 6.49 (d, J = 9.0 Hz, 1H), 2.81 (d, J = 4.5 Hz, 3H). ^{13}C NMR (DMSO-d_6): 162.9, 159.2, 155.8, 148.5, 147.1, 137.6, 135.3, 134.9, 133.4, 123.3, 122.9, 122.8, 120.5, 115.8, 106.6, 30.6, 27.9. LC-MS (ESI, *m/z*): Calcd for C$_{17}$H$_{16}$N$_3$OS ([M+H]$^+$) 310.1; found 310.1.

(h). 2-((1E,3E)-4-(6-Aminopyridin-3-yl)buta-1,3-dien-1-yl)benzo[d]thiazol-6-ol (N-desmethyl-PBB3, 7): To a stirred suspension of compound **4** (166 mg, 0.54 mmol) in anhydrous CH$_2$Cl$_2$ (3 mL) was added BBr$_3$ (1.0 M solution in CH$_2$Cl$_2$, 3 mL, 3.0 mmol) dropwise at -78 °C under N$_2$ atmosphere. After the mixture was allowed to warm up to RT and stirred at RT overnight, the reaction was quenched with water in an ice bath. The precipitate was collected by filtration, and washed with aqueous 5% NaHCO$_3$ and water. The solid was dried *in vacuo* to afford compound **7** as a yellow solid (113 mg, 71%). ^{1}H NMR (DMSO-d_6): δ 9.85 (s, 1H), 8.03 (d, J = 2.0 Hz, 1H), 7.71-7.67 (m, 2H), 7.33 (d, J = 2.5 Hz, 1H), 7.22 (dd, J = 10.5, 15.5 Hz, 1H), 6.96-6.89 (m, 2H), 6.84 (d, J = 15.0 Hz, 2H), 6.48 (d, J = 8.5 Hz, 1H), 6.35 (br s, 2H). LC-MS (ESI, *m/z*): Calcd for C$_{16}$H$_{14}$N$_3$OS ([M+H]$^+$) 296.1; found 296.0.

(j). 5-((1E,3E)-4-(6-((tert-Butyldimethylsilyl)oxy)benzo[d]thiazol-2-yl)buta-1,3-dien-1-yl)pyridin-2-amine (TBS-protected N-desmethyl-PBB3, 8): To a stirred solution of compound **7** (30 mg, 0.10 mmol) and DMAP (37 mg, 0.31 mmol) in DMF (1 mL) was added TBSCl (25 mg, 0.16 mmol). After the reaction mixture was stirred at RT overnight, it was quenched with water. The mixture was extracted with EtOAC. The combined organic layer was washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated *in vacuo*. The crude product was purified by preparative TLC plate with CHCl$_3$/MeOH (100:7) as eluent to afford compound **8** as a yellow solid (14 mg, 33%). ^{1}H NMR (DMSO-d_6): δ 8.04 (d, J = 2.5 Hz, 1H), 7.78 (d, J = 9.0 Hz, 1H), 7.69 (dd, J = 2.5, 8.5 Hz, 1H), 7.52 (d, J = 2.5 Hz, 1H), 7.28 (dd, J = 10.0, 15.0 Hz, 1H), 6.99 (dd, J = 2.5, 8.5 Hz, 1H), 6.96-6.86 (m, 3H), 6.48 (d, J = 9.0 Hz, 1H), 6.37 (br s, 2H), 0.97 (s, 9H), 0.23 (s, 6H). LC-MS (ESI, *m/z*): Calcd for C$_{22}$H$_{28}$N$_3$OSSi ([M+H]$^+$) 410.2; found 410.1.

(k) 5-((1E,3E)-4-(6-[^{11}C]Methylamino)pyridin-3-yl)buta-1,3-dien-1-yl)benzo[d]thiazol-6-ol ([^{11}C]PBB3, [^{11}C]6): Method A (from TBS-protected N-desmethyl-PBB3, **8**). [^{11}C]CO$_2$ was produced by the ^{14}N(p,α)^{11}C nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas

from Praxair, Indianapolis, IN. Typical irradiations used for the development were 55 μA beam current and 30 min on target. The production run produced approximately 45.5 GBq of $[^{11}C]CO_2$ at EOB. In a small reaction vial (5 mL), the precursor **8** (0.3-0.5 mg) was dissolved in DMSO (500 μL). To this solution was added dry KOH (3 mg). No carrier-added (high specific activity) $[^{11}C]CH_3OTf$ that was produced by the gas-phase production method[42] from $[^{11}C]CO_2$ through $[^{11}C]CH_4$ and $[^{11}C]CH_3Br$ with silver triflate (AgOTf) column was passed into the reaction vial at RT, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 100 $^{\circ}C$ for 5 min to form a radiolabeled intermediate TBS-protected $[^{11}C]PBB3$. Then, water (500 μL) was introduced to the reaction vial. The reaction mixture was sealed and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with water (1 mL), and injected onto the semi-preparative RP HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2), followed by 10 mL saline, to release $[^{11}C]$**6**. The eluted product was then sterile-filtered through a sterile vented Millex-FG 0.2 μm filter, and collected into a sterile vial. Total radioactivity (1.7-2.3 GBq) was assayed and total volume (10-11 mL) was noted for tracer dose dispensing, which was diluted with additional saline, since the ethanol concentration in the tracer is required to be <10% in real clinical setting. The overall synthesis, purification and formulation time was ~40 min from EOB. Retention times in the analytical HPLC system were: t_R **8** = 8.83 min, t_R **6** = 6.56 min, and t_R $[^{11}C]$**6** = 6.64 min. Retention times in the semi-preparative HPLC system were: t_R **8** = 15.43 min, t_R **6** = 11.01 min, and t_R $[^{11}C]$**6** = 11.15 min. The radiochemical yield of $[^{11}C]$**6** was 15-20% decay corrected to EOB, based on $[^{11}C]CO_2$. Method B (from *N-desmethyl-PBB3, 7*). The precursor **7** (0.3-0.5 mg) was dissolved in acetone (500 μL) in a 5-mL V-vial. $[^{11}C]CH_3OTf$ was passed into the reaction vial at RT until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 °C for 5 min. The contents of the reaction vial were diluted with water (1 mL), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The purification and formulation procedures were same with Method A. The overall synthesis, purification and formulation time was ~35 min from EOB. Retention times in the analytical HPLC system were: t_R **7** = 4.53 min, t_R **6** = 6.56 min, and t_R $[^{11}C]$**6** = 6.64 min. Retention times in the semi-preparative HPLC system were: t_R **7** = 7.66 min, t_R **6** = 11.01 min, and t_R $[^{11}C]$**6** = 11.15 min. The decay corrected radiochemical yields were 20-25%.