Synthesis of [^{11}C]MK-1064 as a new PET radioligand for imaging of orexin-2 receptor

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Abstract—The reference standard MK-1064 {5"-chloro-*N*-((5,6-dimethoxypyridin-2-yl)methyl)-[2,2':5',3"-terpyridine]-3'-carboxamide} was synthesized from methyl 2-chloro-5-iodonicotinate and 5-(chloropyridin-3-yl)boronic acid in 4 steps with 33% overall chemical yield. The precursor desmethyl-MK-1064 {5"-chloro-*N*-((5-hydroxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3"-terpyridine]-3'-carboxamide} for radiolabeling was synthesized from 2-bromopyridin-3-ol and 5"-chloro-[2,2':5',3"-terpyridine]-3'-carboxylic acid in 6 steps with 17% overall chemical yield. The target tracer [^{11}C]MK-1064 {5"-chloro-*N*-((5-[^{11}C]methoxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3"-terpyridine]-3'-carboxamide} was prepared by *O*-[^{11}C]methylation of its corresponding precursor desmethyl-MK-1064 with [^{11}C]CH$_3$OTf under basic condition and isolated by a simplified solid-phase extraction (SPE) method in 50-60% decay corrected radiochemical yields based on [^{11}C]CO$_2$ at end of bombardment (EOB). The overall synthesis time from EOB was 23 min, the radiochemical purity was >99%, and the specific activity at end of synthesis (EOS) was 185-555 GBq/μmol.

The orexin system consists of two neuropeptides: orexin-A (a 33 amino-acid peptide) and orexin-B (a 28 amino-acid peptide); two G-protein coupled receptors: orexin-1 receptor (OX1R) and orexin-2 receptor (OX2R).[1-4] Orexin-A activates both OX1R and OX2R with near equal affinity whereas orexin-B preferentially binds to OX2R. OX1R is selectively expressed in the locus coeruleus. Conversely, OX2R is expressed in the tuberomammillary nucleus.[5] The available experimental evidences strongly support that OX2R plays a key role in motivation, arousal and sleep-wake regulation, and is associated with sleep disorders, irregularities in central vestibular motor control, feeding and gastrointestinal disorders and drug abuse.[6] Although OX1R is thought to regulate sleep-wakefulness and energy homeostasis, particularly food intake, the therapeutic potential of selective antagonism of OX1R is still under evaluation.[6] Orexin receptor is an interesting therapeutic target, and many selective orexin receptor antagonists have been developed.[3,4] However, the *in vivo* selectivity, distribution and involvements of OX1R and OX2R in the pathophysiology of orexin-related disorders are not fully understood.[7] Biomedical imaging technique positron emission tomography (PET) is a useful tool for *in vivo* quantification of various biological processes.[7] OX2R has also become a promising target for molecular imaging of OX2R-mediated diseases and image-guided therapy using PET modality, and several PET radioligands including [^{11}C]BBAC, [^{11}C]BBPC, [^{11}C]EMPA, [^{11}C]CW4, and (1*R*,2*S*)-2-(3-fluorophenyl)-*N*-(5-fluoropyridin-2-yl)-2-(((4-((methoxy-^{11}C)methyl)-2-methylpyrimidin-5-yl)oxy)methyl)cyclopropane-1-carboxamide ([^{11}C]FFMMCC) have been evaluated for imaging of OX2R, as indicated in Figure 1.[7-10] PET scans with [^{11}C]BBAC and [^{11}C]BBPC in a rhesus monkey showed no tracer retention and appropriate brain uptake.[7] [^{11}C]EMPA-PET also showed poor brain uptake in both rats and baboon.[8] [^{11}C]CW4-PET in a baboon showed appropriate early brain uptake, but fast kinetics and high nonspecific binding.[9] PET scan with [^{11}C]FFMMCC showed moderate uptake in rat and monkey brains

under deficiency or blockade of P-glycoprotein, but this radioligand is not selective, and it binds to OX1R and OX2R with the equal affinity (K_i 1 nM for OX1R and OX2R).[10] In view of these radioligands recently developed for PET imaging of OX2R, only a limited success is achieved. Therefore, to investigate the therapeutic effect and receptor occupancy of the OX2R antagonists and to guide the therapy, a new suitable radioligand for OX2R is still needed. Recently, a new selective OX2R antagonist (2-SORA) MK-1064 {5"-chloro-*N*-((5,6-dimethoxypyridin-2-yl)methyl)-[2,2':5',3"-terpyridine]-3'-carboxamide} has been discovered by Merck, it exhibited K_i (nM) and IC_{50} (nM) for OX2R and OX1R, 0.5 and 18, 1584 and 1789, respectively, with selectivity index (SI) K_i/IC_{50} 3168/99, and it is one of the most potent and selective 2-SORA compounds reported to date.[11] Here we report the design, synthesis and radiolabeling of [11C]MK-1064 {5"-chloro-*N*-((5-[11C]methoxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3"-terpyridine]-3'-carboxamide} (Figure 1).
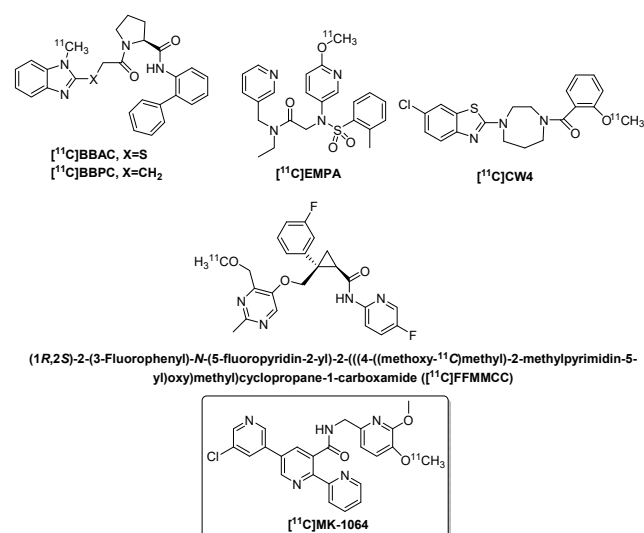
**Figure 1**. PET tracers for orexin-2 receptor.

The reference standard MK-1064 (**5**) was synthesized according to the reported procedures[11] with modifications, as shown in Scheme 1. Commercially available starting material methyl 2-chloro-5-iodonicotinate was undergone a Suzuki reaction with 5-(chloropyridin-3-yl)boronic acid to obtain compound **1** in 90% yield. Compound **1** was then converted to the intermediate **2** through a Stilling reaction with 2-(tri-n-butylstannyl)pyridine in 53% yield. Compound **2** was hydrolyzed in methanol/water solution of KOH to yield the acid **3** in 98% yield, which subsequently reacted with (5,6-dimethoxypyridin-2-yl)methanamine (**4**) using catalysts 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 1-hydroxy-7-azabenzotriazole (HOAt) under basic

condition with *N*,*N*-diisopropylethylamine (DIPEA) to give MK-1064 in 70% yield.
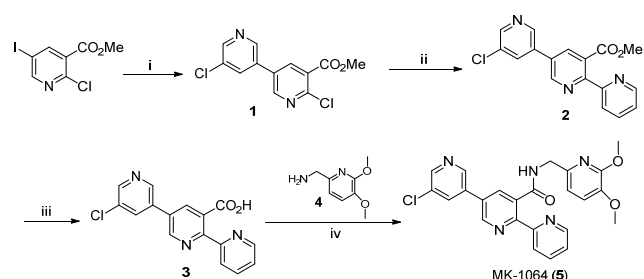
Scheme 1. Synthesis of the reference standard MK-1064 (**5**). Reagents, conditions and yields: (i) 5-(chloropyridin-3-yl)boronic acid, PdCl$_2$(dppf), Cs$_2$CO$_3$, DMF/water, room temperature (RT), 20 h; 90%. (ii) 2-(tri-n-butylstannyl)pyridine, Pd(PPh$_3$)$_4$, CsF, CuI, DMF, 80 ºC, 2 h; 53%. (iii) KOH, MeOH, RT, 2 d; 98%. (iv) (5,6-dimethoxypyridin-2-yl)methanamine (**4**), EDC, HOAt, DMF, DIPEA, RT, 15 h; 70%.

Attempt to desmethlyation of MK-1064 to synthesize the precursor desmethyl-MK-1064 {5"-chloro-*N*-((5-hydroxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3"-terpyridine]-3'-carboxamide, **11**} employing several methods was unsuccessful. Thus a new route for the synthesis of the precursor **11** was designed as depicted in Scheme 2. 2-Bromo-3-hydroxy pyridine was reacted with I$_2$ and K$_2$CO$_3$ in water to afford compound **6** in 96% yield. Compound **6** was then converted to compound **7** protecting phenol hydroxyl group via benzyl bromide in 81% yield. The bromide **7** was alcoholized in DMF by sodium methoxide to afford compound **8** in 85% yield. Compound **8** was reacted with CuCN at 150 ºC for 1.5 h to obtain compound **9** in 73% yield. Compound **9** was undergone a 2-step reaction: deprotection of hydroxyl group and reduction of nitrile group, in one-pot under hydrogen atmosphere using Pd(OH)$_2$/C as catalyst to give the key intermediate **10**, which was converted to the precursor desmethyl-MK-1064 using similar procedure as synthesis of MK-1064 in 35% yield.
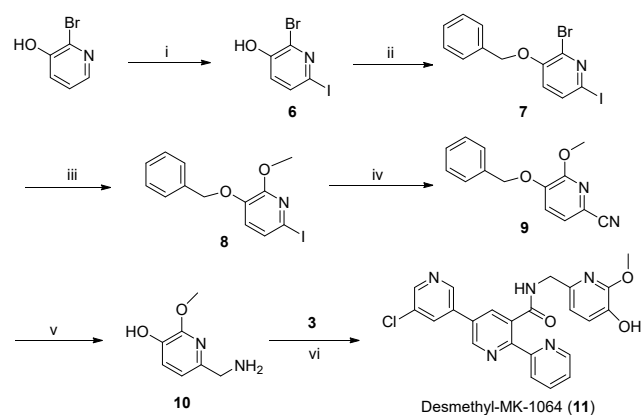
Scheme 2. Synthesis of the precursor desmethyl-MK-1064 (**11**). Reagents, conditions and yields: (i) K$_2$CO$_3$, H$_2$O, I$_2$, 5 h, RT; 96%. (ii) BnBr, K$_2$CO$_3$, CH$_3$CN, 60 ºC, 12 h; 81%. (iii) MeONa, MeOH, DMF, 100 ºC, 1.5 h; 85%. (iv) CuCN, DMF, 150 ºC, 2 h; 73 %. (v) H$_2$ (50 psi), Pd(OH)$_2$/C, MeOH, 8 h. (vi) EDC, HOAt, DMF, DIPEA, RT, 15 h; 35%.

Synthesis of [^{11}C]MK-1064 ([^{11}C]**5**) is indicated in Scheme 3. The desmethyl-MK-1064 was labeled by a reactive [^{11}C]methylating agent, [^{11}C]methyl triflate ([^{11}C]CH$_3$OTf)[12,13] prepared from [^{11}C]CO$_2$, through the *O*-[^{11}C]methylation. The labeling reaction was under basic conditions in acetonitrile, and the labeling mixture was isolated by a simplified solid-phase extraction (SPE) method[14-17] to provide target tracers [^{11}C]**5**. The radiochemical yields were 50-60%, decay corrected to end of bombardment (EOB), based on [^{11}C]CO$_2$.
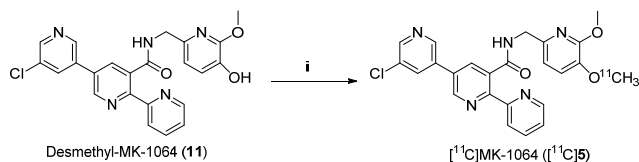
Scheme 3. Synthesis of target tracer [^{11}C]MK-1064 ([^{11}C]**5**). Reagents, conditions and yields: (i) [^{11}C]CH$_3$OTf, CH$_3$CN, 2 N NaOH, 80 °C, 3 min; SPE; 50-60%.

[^{11}C]Methyl iodide ([^{11}C]CH$_3$I) is a most commonly used [^{11}C]methylating agent,[18] but [^{11}C]CH$_3$OTf is more reactive than [^{11}C]CH$_3$I, and thus, the radiochemical yield of ^{11}C]**5** was relatively high. The radiosynthesis including reaction, purification and formulation was performed in an automated self-designed multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity at EOB during synthesis.[19-21] The labeling reaction was conducted using a V-vial method. The purification was performed by a SPE method, because the large polarity difference between the sodium salt of the phenolic hydroxyl precursor (2 N NaOH) and the corresponding labeled *O*-methylated ether product permitted the use of SPE technique for purification of the labeled product from the radiolabeling reaction mixture. A C-18 Plus Sep-Pak cartridge was used in SPE purification technique. It is difficult to directly elute the labeled product from a C-18 Plus Sep-Pak to a vial using small volume of ethanol (1 × 1 mL or 2 × 0.5 mL), due to the back pressure in the C-18 Sep-Pak and dead volume in the transfer tubing. In order to elute most of the labeled product from the C-18 Sep-Pak, we need to increase the volume of the eluent ethanol. Thus, the formulation was completed by Sep-Pak trap/release and rotatory evaporation methods. For the radiotracer produced for animal study, we used ethanol (2 × 2 mL) for elution, and rotatory evaporation was required before reformulation. For the radiotracer produced for human study, we used ethanol (2 × 1 mL), no evaporation required, and a C-18 Sep-Pak was used for direct reformulation.[22-25] We have tried to use a C-18 Light Sep-Pak cartridge instead of a C-18 Plus Sep-Pak cartridge to allow smaller volume (1 mL) of ethanol and to avoid laborious rotary evaporation before formulation. However, there is more serious back pressure in the Light Sep-Pak than in the Plus Sep-Pak, in addition, dead volume in the transfer tubing also affects the elution, and thus it is more difficult to efficiently elute the labeled product from a Light Sep-Pak, which resulted in the low radiochemical yield. The reason is that our home-built fully automated module used many PTFE(polytetrafluoroethylene)/Silicone liners (septa) and Teflon tubing, and these materials cannot afford too high pressure gas (N$_2$) push. The pressure of N$_2$ gas introduced in our module is set at 207 kPa (30 Psi). C-18 Light Sep-Pak cartridge works well in manual or semi-automated radiosynthesis in our lab, because we can easily introduce high pressure gas push during the purification and reformulation process. Overall synthesis time was 23 min from EOB, including approximately 11 min for [^{11}C]CH$_3$OTf production, 5 min for *O*-[^{11}C]methylation reaction, and 7 min for SPE purification, evaporation and reformulation. SPE technique is fast, efficient and convenient and works very well for the *O*-methylated ether tracer purification using the phenolic hydroxyl precursor for radiolabeling.[15,26,27]

Specific activity is an important parameter for a brain radiotracer. If a reverse-phase (RP) high performance liquid chromatography (HPLC) is used as purification method, then on line determination of specific activity at EOB is accurate. If a SPE cartridge is used as purification method, then the on-the-fly technique to determine specific activity at EOB is not applied. The specific activity for the ^{11}C-tracers produced in our PET chemistry facility usually ranges from 370 to 1110 GBq/μmol at EOB according to our previous works. The specific activity of [^{11}C]MK-1064 was 185-555 GBq/μmol at the end of synthesis (EOS) determined by analytical HPLC[28,29] and calculated, which are in agreement with the "on line" determined values. To achieve the highest specific activity, several procedures were performed.[30,31] The ^{11}C gas target we used is the Siemens RDS-111 Eclipse cyclotron ^{11}C gas target. The technical trick to produce high specific activity [^{11}C]CO$_2$ is we will usually do 2-3 times pre-burn with the same beam current and short time like 10 min before production run. This pre-burn will warm up the cyclotron and eliminate significant amount of ^{12}C carrier-added in the cyclotron ^{11}C gas target. We use an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module to produce [^{11}C]CH$_3$OTf, convenient gas phase bromination of [^{11}C]methane, and production of [^{11}C]CH$_3$OTf. This is a 'dry' method using Br$_2$ to generate a [^{11}C]CH$_3$Br intermediate. Comparing with other 'dry' methods using I$_2$ and 'wet' methods using LiAlH$_4$ and HI, our 'dry' method seems to help minimize introduction of additional ^{12}C carrier after [^{11}C]CO$_2$ production.[13] To further help produce high specific activity [^{11}C]CH$_3$OTf, we usually do 2

'test loop' procedures when we set up the module for the actual [11C]CH$_3$OTf production run, and 1 actual [11C]CH$_3$OTf production run before we do [11C]methylation labeling reaction. 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 [11C]CH$_3$OTf production system. Therefore, the specific activity of our ^{11}C-tracers is significantly improved.

Chemical purity and radiochemical purity were determined by analytical HPLC.[28,29] The chemical purity of the precursor and reference standard was >95%. 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 octanol-water partition coefficient (commonly expressed as Log P) is an important physical parameter directly correlated with the biological activities of a wide variety of organic compounds.[32-35] Log P provides an assessment of lipophilicity that often correlates with a compound's ability to penetrate the blood brain barrier (BBB). We obtained Log P and calculated Log P (CLog P) values of PET tracers for OX2R (Figure 1) from ChemBioDraw Ultra 14.0 (ChemOffice) as listed in Table 1. Log P and CLog P data of [11C]MK-1064 are similar to those of [11C]FFMMCC, and in the optimal range of $LogD_{7.4}$ (2.0-3.5) reported for an optimum central nervous system (CNS) penetration of drug molecules.[36] These data suggest the [11C]MK-1064 has appropriate lipophilicity for brain uptake.

Table 1. Log P and Clog P values of PET tracers for OX2R.

Compound	Log P	Clog P
[11C]BBAC	4.21	4.40
[11C]BBPC	3.79	3.06
[11C]EMPA	2.24	3.43
[11C]CW4	4.98	4.11
[11C]FFMMCC	3.65	3.29
[11C]MK-1064	3.2	3.15

The experimental details and characterization data for compounds **1-3**, **5-11** and for the tracer [11C]**5** are given.[37]

In summary, a simple and moderate-to-high-yield synthetic route to synthesize MK-1064, desmethyl-MK-1064 and [11C]MK-1064 has been developed. An automated self-designed multi-purpose [11C]-radiosynthesis module for the synthesis of [11C]MK-1064 has been built. The target tracer was easily prepared by O-[11C]methylation of its corresponding

desmethylated precursor using a reactive [11C]methylating agent, [11C]CH$_3$OTf, and isolated by a simplified SPE purification procedure in high radiochemical yields, short overall synthesis time, and high specific radioactivities. These methods are efficient and convenient. It is anticipated that the approaches for the design, synthesis and automation of new tracer, authentic standard and radiolabeling precursor, and improvements to increase radiochemical yield and specific activity of the tracer described here can be applied with advantages to the synthesis of other ^{11}C-radiotracers for PET imaging. These chemistry results combined with the reported *in vitro* and *in vivo* biological data[11] of MK-1064 encourage further *in vivo* biological evaluation of [11C]MK-1064 as a new candidate PET radioligand for imaging of OX2R in brain diseases.

References and notes

1. Boss, C.; Roch, C. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2875.
2. Lebold, T. P.; Bonaventure, P.; Shireman, B. T. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 4761.
3. Bingham, M. J.; Cai, J.; Deehan, M. R. *Curr. Opin. Drug Discov. Devel.* **2006**, *9*, 551.
4. Gotter, A. L.; Roecker, A. J.; Hargreaves, R.; Coleman, P. J.; Winrow, C. J.; Renger, J. J. *Prog. Brain Res.* **2012**, *198*, 163.
5. Callander, G. E.; Olorunda, M.; Monna, D.; Schuepbach, E.; Langenegger, D.; Betschart, C.; Hintermann, S.; Behnke, D.; Cotesta, S.; Fendt, M.; Laue, G.; Ofner, S.; Briard, E.; Gee, C. E.; Jacobson, L. H.; Hoyer, D. *Front. Neurosci.* **2013**, *7*, 230.
6. Merlo Pich, E.; Melotto, S. *Front. Neurosci.* **2014**, *8*, 26.
7. Liu, F.; Majo, V. J.; Prabhakaran, J.; Castrillion, J.; Mann, J. J.; Martinez, D.; Kumar, J. S. D. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2172.
8. Wang, C.; Moseley, C. K.; Carlin, S. M.; Wilson, C. M.; Neelamegam, R.; Hooker, J. M. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3389.

9. Wang, C.; Wilson, C. M.; Moseley, C. K.; Carlin, S. M.; Hsu, S.; Arabasz, G.; Schroeder, F. A.; Sander, C. Y.; Hooker, J. M. *Nucl. Med. Biol.* **2013**, *40*, 1000.

10. Oi, N.; Suzuki, M.; Terauchi, T.; Tokunaga, M.; Nakatani, Y.; Yamamoto, N.; Fukumura, T.; Zhang, M.-R.; Suhara, T.; Higuchi, M. *J. Med. Chem.* **2013**, *56*, 6371.

11. Roecker, A. J.; Mercer, S. P.; Schreier, J. D.; Cox, C. D.; Fraley, M. E.; Steen, J. T.; Lemaire, W.; Bruno, J. G.; Harrell, C. M.; Garson, S. L.; Gotter, A. L.; Fox, S. V.; Stevens, J.; Tannenbaum, P. L.; Prueksaritanont, T.; Cabalu, T. D.; Cui, D.; Stellabott, J.; Hartman, G. D.; Young, S. D.; Winrow, C. J.; Renger, J. J.; Coleman, P. J. *ChemMedChem.* **2014**, *9*, 311.

12. Jewett, D. M. *Int. J. Radiat. Appl. Instrum. A* **1992**, *43*, 1383.

13. Mock, B. H.; Mulholland, G. K.; Vavrek, M. T. *Nucl. Med. Biol.* **1999**, *26*, 467.

14. Zheng, Q.-H.; Mulholland, G. K. *Nucl. Med. Biol.* **1996**, *23*, 981.

15. Gao, M.; Wang, M.; Hutchins, G. D.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2008**, *66*, 1891.

16. Gao, M.; Wang, M.; Miller, K. D.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2012**, *70*, 1558.

17. Gao, M.; Gao, A. C.; Wang, M.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2014**, *90*, 181.

18. Allard, M.; Fouquet, E.; James, D.; Szlosek-Pinaudm, M. *Curr. Med. Chem.* **2008**, *15*, 235.

19. Mock, B. H.; Zheng, Q.-H.; DeGrado, T. R. *J. Label. Compd. Radiopharm.* **2005**, *48*, S225.

20. Mock, B. H.; Glick-Wilson, B. E.; Zheng, Q.-H.; DeGrado, T. R. *J. Label. Compd. Radiopharm.* **2005**, *48*, S224.

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

22. Gao, M.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 254.

23. 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.

24. Wang, M.; Gao, M.; Miller, K. D.; Zheng, Q.-H. *Steroids* **2011**, *76*, 1331.

25. Wang, M.; Gao, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1569.

26. Gao, M.; Gao, A.; Wang, M.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2014**, *91*, 71.

27. Gao, M.; Gao, A.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 5581.

28. Gao, M.; Yang, Q.; Wang, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2013**, *74*, 61.

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

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

31. Gao, M.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1371.

32. Haky, J. E.; Young, A. M. *J. Liq. Chromatogr.* **1984**, *7*, 675.

33. Fei, X.; Zheng, Q.-H. *J. Liq. Chromatogr. & Rel. Technol.* **2005**, *28*, 939.

34. Gao, M.; Mock, B. H.; Hutchins, G. D.; Zheng, Q.-H. *Nucl. Med. Biol.* **2005**, *32*, 543.

35. Gao, M.; Wang, M.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2529.

36. Waterhouse, R. N. *Mol. Imaging Biol.* **2003,** *5*, 376.

37. *(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.[13] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded at 500 MHz on a Bruker Avance II 500 MHz 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*) were 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. The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic spectrometer. 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. 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 43% CH$_3$CN/57% H$_2$O; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-GS 0.22 μm filter units were obtained from Millipore Corporation (Bedford, MA).

(b) Methyl 5',6-dichloro-[3,3'-bipyridine]-5-carboxylate (1): To a solution of methyl 2-chloro-5-iodonicotinate (5.95 g, 20.0 mmol) in *N,N*-dimethylformamide (DMF, 82 mL)/water (4.0 mL) was added 5-(chloropyridin-3-yl)boronic acid (3.20 g, 20.3 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (Pd(dppf)Cl$_2$, 1.46 g, 2.0 mmol) and Cs$_2$CO$_3$ (19.6 g, 60.0 mmol). The reaction was stirred at RT for 24 h. Then the reaction mixture was partitioned between EtOAc and water, the organic layer was washed with

water and brine, dried over MgSO$_4$, filtered, and concentrated in vacuo. The residue was purified by trituration with MeOH followed by EtOAc/Et$_2$O to give a yellow solid **1** (5.10 g, 90%). R_f = 0.70 (1:19 MeOH/CH$_2$Cl$_2$), mp 135-137 °C. ^{1}H NMR (CDCl$_3$): δ 4.01 (s, 3H, OCH$_3$), 7.89 (t, J = 2.5 Hz, 1H, Ar-H), 8.34 (d, J = 2.5 Hz, 1H, Ar-H), 8.66 (d, J = 2.5 Hz, 1H, Ar-H), 8.72-8.74 (m, 2H, Ar-H). MS (ESI): 283 ([M+H]$^+$, 100%).

(c) Methyl 5''-chloro-[2,2':5',3''-terpyridine]-3'-carboxylate (2): To a solution of compound **1** (1.66 g, 5.86 mmol) in DMF (30 mL) was added 2-(tri-n-butylstannyl)pyridine (3.02 g, 8.20 mmol), CsF (2.67 g, 17.6 mmol), CuI (223 mg, 1.17 mmol) and Pd(PPh$_3$)$_4$ (677 mg, 0.586 mmol). The mixture was heated to 80 °C for 2 h. The reaction mixture was cooled, diluted with EtOAc (120 mL), and filtered through a pad of Celite. The filtrate was washed with water (2 × 50 mL) and brine (50 mL), dried over MgSO$_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel with eluent (5:95 to 50:50 EtOAc/hexanes) to give a white solid product **2** (1.01 g, 53%). R_f = 0.26 (1:2 EtOAc/hexanes), mp 111-113 °C. ^{1}H NMR (CDCl$_3$): δ 3.84 (s, 3H, OCH$_3$), 7.34 (ddd, J = 1.0, 5.0, 7.5 Hz, 1H, Ar-H), 7.85 (dt, J = 2.0, 8.0 Hz, 1H, Ar-H), 7.94 (t, J = 2.0 Hz, 1H, Ar-H), 8.12 (d, J = 2.0 Hz, 1H, Ar-H), 8.22 (d, J = 8.0 Hz, 1H, Ar-H), 8.63 (d, J = 4.0 Hz, 1H, Ar-H), 8.65 (d, J = 2.0 Hz, 1H, Ar-H), 8.79 (d, J = 2.0 Hz, 1H, Ar-H), 8.94 (d, J = 2.0 Hz, 1H, Ar-H). MS (ESI): 326 ([M+H]$^+$, 100%).

(d) 5''-Chloro-[2,2':5',3''-terpyridine]-3'-carboxylic acid (3): To a solution of compound **2** (0.33 g, 1.0 mmol) in methanol (30 mL)/water (3 mL) was added KOH (0.56 g, 10 mmol). The mixture was stirred at RT for 48 h. The reaction mixture was concentrated in vacuo. The residue was added water and HCl (1 N), and pH value of the mixture was adjusted to 6. The resulting precipitate was filtered, washed with cold water, and dried to afford a white solid product **3** (0.306 g, 98%). R_f = 0.38 (1:1 MeOH/CH$_2$Cl$_2$), mp 185-187 °C. ^{1}H NMR (DMSO-d$_6$): δ 7.47 (t, J = 6.0 Hz, 1H, Ar-H), 7.98 (t, J = 7.5 Hz, 1H, Ar-H), 8.11 (d, J = 7.5 Hz, 1H, Ar-H), 8.43 (s, 1H, Ar-H), 8.50 (s, 1H, Ar-H), 8.60 (d, J = 4.0 Hz, 1H, Ar-H), 8.72 (s, 1H, Ar-H), 9.05 (s, 1H, Ar-H), 9.16 (s, 1H, Ar-H), 13.18 (br s, 1H, OH). MS (ESI): 312 ([M + H]$^+$, 56%); MS (ESI): 310 ([M - H]$^-$, 14%).

(e) 5''-Chloro-N-((5,6-dimethoxypyridin-2-yl)methyl)-[2,2':5',3''-terpyridine]-3'-carboxamide (MK-1064, 5): To a solution of compound **3** (218 mg, 0.70 mmol) in DMF (30 mL) was added (5,6-dimethoxypyridin-2-yl)methanamine (**4**, 124 mg, 0.735 mmol), EDC (268 mg, 1.4 mmol) HOAt (237 mg, 1.4 mmol) and DIPEA (361 mg, 2.8 mmol). The mixture was stirred at RT for 15 h. The reaction mixture was diluted with EtOAc (120 mL), washed with water (2 × 30 mL) and brine (30 mL), dried over MgSO$_4$, filtered, and concentrated in vacuo. The residue was purified by column

chromatography on silica gel with eluent (0:100 to 3:97 MeOH/CH$_2$Cl$_2$) to give a white solid product **5** (226 mg, 70%). R_f = 0.56 (1:9 MeOH/CH$_2$Cl$_2$), mp 170-172 °C. ^{1}H NMR (DMSO-d$_6$): δ 3.79 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$), 4.37 (d, J = 6.0 Hz, 2H, CH$_2$), 7.16 (d, J = 8.0 Hz, 1H, Ar-H), 7.33 (d, J = 8.0 Hz, 1H, Ar-H), 7.42 (ddd, J = 1.0, 4.5, 7.5 Hz, 1H, Ar-H), 7.94 (dt, J = 1.5, 7.5 Hz, 1H, Ar-H), 8.07 (d, J = 7.5 Hz, 1H, Ar-H), 8.36 (d, J = 2.0 Hz, 1H, Ar-H), 8.50 (t, J = 2.0 Hz, 1H, Ar-H), 8.53 (d, J = 4.0 Hz, 1H, Ar-H), 8.72 (d, J = 2.0 Hz, 1H, Ar-H), 8.88 (t, J = 6.0 Hz, 1H, NH), 9.06 (d, J = 2.0 Hz, 1H, Ar-H), 9.16 (d, J = 2.0 Hz, 1H, Ar-H). MS (ESI): 462 ([M+H]$^+$, 100%).

(f) 2-Bromo-6-iodopyridin-3-ol (6): To a solution of 2-bromo-3-hydroxy pyridine (20.0 g, 115 mmol) in water (250 mL) was added K$_2$CO$_3$ (31.8 g, 230 mmol) and I$_2$ (29.2 g, 115 mmol). The mixture was stirred at RT for 5 h, then cooled to 0 °C and treated with concentrated HCl until solids precipitated from solution (pH ~6). The solids were isolated by filtration and dried to give a brown solid **6** (33.1 g, 96%). R_f = 0.46 (1:49 MeOH/CH$_2$Cl$_2$), mp 152-154 °C. ^{1}H NMR (DMSO-d$_6$): δ 7.07 (d, J = 8.0 Hz, 1H, Ar-H), 7.59 (d, J = 8.0 Hz, 1H, Ar-H), 11.31 (br s, 1H, OH). MS (ESI): 298 ([M-H]$^-$, 100%).

(g) 3-(Benzyloxy)-2-bromo-6-iodopyridine (7): To a solution of compound **6** (12.0 g, 40.0 mmol) in CH$_3$CN (150 mL) was added K$_2$CO$_3$ (11.6 g, 80.0 mmol) and benzyl bromide (8.21 g, 48.0 mmol). The mixture was stirred at 60 °C for 12 h, then cooled to RT, filtered, washed with CH$_3$CN, and concentrated. The residue was recrystallized from EtOAc/hexanes to give a pale brown solid **7** (12.6 g, 81%). R_f = 0.82 (1:2 EtOAc/hexanes), mp 82-84 °C. ^{1}H NMR (CDCl$_3$): δ 5.16 (s, 2H, CH$_2$), 6.82 (d, J = 8.5 Hz, 1H, Ar-H), 7.32-7.36 (m, 1H, Ar-H), 7.37-7.43 (m, 4H, Ar-H), 7.51 (d, J = 8.5 Hz, 1H, Ar-H). MS (ESI): 390 ([M+H]$^+$, 100%).

(h) 3-(Benzyloxy)-6-iodo-2-methoxypyridine (8): To a solution of compound **7** (6.50 g, 16.6 mmol) in DMF (60 mL) was added MeONa (5.76 g, 26.6 mmol, 25% in methanol). The mixture was heated to 100 °C and stirred for 1.5 h, then cooled to RT, and partitioned between EtOAc and water. The organic phase was washed with water and brine, dried over MgSO$_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel with eluent (5:95 to 20:80 EtOAc/hexanes) to give a white solid product **8** (4.81 g, 85%). R_f = 0.70 (1:5 EtOAc/hexanes), mp 48-50 °C. ^{1}H NMR (CDCl$_3$): δ 3.99 (s, 3H, OCH$_3$), 5.10 (s, 2H, CH$_2$), 6.71 (d, J = 8.0 Hz, 1H, Ar-H), 7.13 (d, J = 8.0 Hz, 1H, Ar-H), 7.31-7.33 (m, 1H, Ar-H), 7.34-7.39 (m, 4H, Ar-H). MS (ESI): 342 ([M+H]$^+$, 100%).

(i) 5-(Benzyloxy)-6-methoxypicolinonitrile (9): To a solution of compound **8** (4.09 g, 12.0 mmol) in DMF (120 mL) was added copper cyanide (2.15 g, 24.0

mmol). The mixture was heated to 150 °C for 2 h, then cool to RT, diluted with EtOAc (250 mL), and filtered through a pad of Celite. The reaction mixture was washed with water (2 × 50 mL) and brine (50 mL), dried over MgSO$_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel with eluent (5:95 to 30:70 EtOAc/hexanes) to give a white solid product **9** (2.10 g, 73%). R_f = 0.28 (1:5 EtOAc/hexanes), mp 106-108 °C. ^{1}H NMR (DMSO-d$_6$): δ 3.90 (s, 3H, OCH$_3$), 5.22 (s, 2H, CH$_2$), 7.35-7.38 (m, 1H, Ar-H), 7.40-7.43 (m, 2H, Ar-H), 7.45-7.46 (m, 2H, Ar-H), 7.50 (d, J = 8.0 Hz, 1H, Ar-H), 7.64 (d, J = 8.0 Hz, 1H, Ar-H). MS (ESI): 242 ([M+H]$^+$, 100%); MS (ESI): 239 ([M-H]$^-$, 1%).

*(j) 6-(Aminomethyl)-2-methoxypyridin-3-ol (**10**):* To a solution of compound **9** (360 mg, 1.5 mmol) in methanol (70 mL) was added Pearlman's catalyst (Pd(OH)$_2$ on carbon, 120 mg). The mixture was reacted under an atmosphere of hydrogen (50 psi) for 8 h. The reaction contents were filtered through a pad of Celite and the filtrate was collected. The solvent was removed in vacuo to give a bone semi-solid crude product. Because of instability of the product on silica gel column, the product was directly used in next step reaction without further purification. R_f = 0.22 (1:1 MeOH/CH$_2$Cl$_2$). MS (ESI): 155 ([M+H]$^+$, 100%); MS (ESI): 153 ([M-H]$^-$, 5%).

*(k) 5''-Chloro-N-((5-hydroxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3''-terpyridine]-3'-carboxamide (desmethyl-MK-1064, **11**):* Compound **10** was reacted with compound **3** using the similar procedure as synthesis of compound **5** to give a white solid **11**, yield 35%. R_f = 0.45 (1:19 MeOH/CH$_2$Cl$_2$), mp > 251 °C (decomposed). ^{1}H NMR (DMSO-d$_6$): δ 3.30 (s, 3H, OCH$_3$), 4.33 (d, J = 6.0 Hz, 2H, CH$_2$), 7.00 (d, J = 7.5 Hz, 1H, Ar-H), 7.09 (d, J = 7.5 Hz, 1H, Ar-H), 7.41 (dd, J = 5.0, 7.5 Hz, 1H, Ar-H), 7.92 (dt, J = 1.5, 8.0 Hz, 1H, Ar-H), 8.06 (d, J = 8.0 Hz, 1H, Ar-H), 8.34 (d, J = 2.0 Hz, 1H, Ar-H), 8.49 (d, J = 4.0 Hz, 1H, Ar-H), 8.50 (t, J = 2.0 Hz, 1H, Ar-H), 8.72 (d, J = 2.0 Hz, 1H, Ar-H), 8.82 (t, J = 6.0 Hz, 1H, NH), 9.06 (d, J = 2.0 Hz, 1H, Ar-H), 9.14 (d, J = 2.0 Hz, 1H,Ar-H), 9.26 (s, 1H, OH). MS (ESI): 448 ([M+H]$^+$, 100%); MS (ESI): 446 ([M-H]$^-$, 18%). HRMS (ESI): calcd for C$_{23}$H$_{18}$N$_5$O$_3$NaCl 470.0996 ([M+Na]$^+$), found 470.0986.

*(m) 5''-Chloro-N-((5-[^{11}C]methoxy-6-methoxypyridin-2-yl)methyl)-[2,2':5',3''-terpyridine]-3'-carboxamide ([^{11}C]MK-1064, [^{11}C]**5**):* [^{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 50 μA beam current for 15 min on target. The production run produced approximately 25.9 GBq of [^{11}C]CO$_2$ at EOB. Desmethyl-MK-1064 (**11**) (0.1-0.3 mg) was dissolved in CH$_3$CN (300 μL). To this solution was added 2 N NaOH (2 μL). The mixture was transferred to a 5-mL small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH$_3$OTf (13.9 GBq) that was produced by the gas-phase production method[13] within 11 min from [^{11}C]CO$_2$ (25.9 GBq) through [^{11}C]CH$_4$ (21.8 GBq) and [^{11}C]CH$_3$Br (13.9 GBq) 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 80 °C for 3 min. The contents of the reaction vial were diluted with NaHCO$_3$ (0.1 M, 1 mL). The reaction vial was connected to a C-18 Plus Sep-Pak cartridge. The labeled product mixture solution was passed onto the cartridge for SPE purification by gas pressure. The cartridge was washed with H$_2$O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the cartridge with EtOH (2 × 2 mL), and then passed onto a rotatory evaporator. The solvent was removed by evaporation (3 min) under vacuum. The final volume of ethanol after evaporation was ~1 mL. The labeled product [^{11}C]**5** was reformulated with saline (10 mL), sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity (4.7-7.1 GBq) was assayed and the total volume (10-11 mL) was noted for tracer dose dispensing. Retention times in the analytical HPLC system were: t_R **11** = 4.13 min, t_R **5** = 6.56 min, and t_R [^{11}C]**5** = 6.64 min.