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The first synthesis of [^{11}C]J147, a new potential PET agent for imaging of Alzheimer's disease

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ABSTRACT

J147 was synthesized from 2,4-dimethylphenylhydrazine hydrochloride and 3-methoxybenzaldehyde in 2 steps with 71% overall yield. The precursor desmethyl-J147 was synthesized from 3-hydroxybenzaldehyde and 2,4-dimethylphenylhydrazine hydrochloride in 4 steps with 63% overall yield. [^{11}C]J147 was prepared from desmethyl-J147 with [^{11}C]CH_3OTf through O-[^{11}C]methylation and isolated by HPLC combined with solid-phase extraction (SPE) in 35–50% radiochemical yield based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB), with 370–740 GBq/μmol specific activity at EOB.

Alzheimer's disease (AD) is the most common neurodegenerative disorder and almost 30 million people suffer this disease worldwide.[1] A major limitation in finding treatment for AD has been the lack of a reliable early diagnosis for this devastating disease.[2] The search for pathology-specific neuroimaging tools is critical at the present stage, and biomedical imaging technique positron emission tomography (PET) is one of the tools in which it is possible to explore the changes in the brain.[3] The protein aggregations such as amyloid beta plaque (Aβ) deposits or neurofibrillary tangles containing tau-protein are considered to be the popular targets for the development of therapeutic solutions and diagnostic biomarkers like in vivo PET imaging agents for AD.[4] Amyloid cascade hypothesis has resulted in a number of Aβ PET tracers such as [^{11}C]PIB[5] and [^{18}F]Amyvid (formerly known as [^{18}F]AV-45),[6] as indicated in Figure 1, currently in different stages of clinical development and commercialization. However, only a few papers on imaging agents selectively targeting tau aggregates (tau hypothesis) have been published.[7] Recently, a highly selective and specific PET tracer called [^{18}F]-T808 (Fig. 1) for imaging of tau pathologies has been developed by Siemens.[8] In vitro and preclinical in vivo studies suggested [^{18}F]-T808 may be one of new next generation of PET AD probes, a promising candidate progressing to human PET studies for imaging of paired helical filament tau.[8] Currently, the major imaging probe discovery paradigm for AD is based upon high affinity ligands for single disease-specific targets

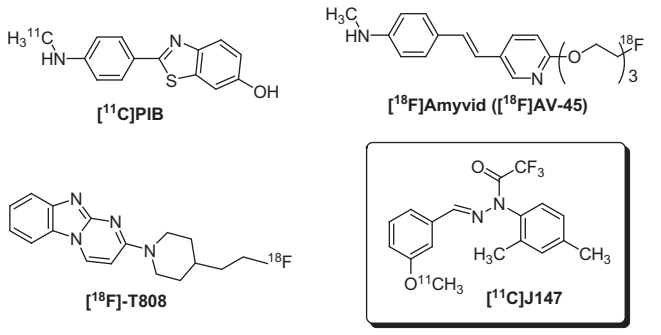

Figure 1. Chemical structures of previously developed [^{11}C]PIB, [^{18}F]Amyvid and [^{18}F]-T808, and newly developed [^{11}C]J147.

such as Aβ or tau. Recently, an alternative drug discovery scheme has been explored and developed, which is based upon efficacy in multiple cell culture models of age-associated pathologies rather than exclusively amyloid metabolism and tau-protein, since age is the greatest risk factor for AD.[9] Using this new approach, a novel, exceptionally potent, orally active, neurotrophic drug called J147 for cognitive enhancement and AD has been identified.[9] We are interested in the development of new PET AD imaging agents, and we apply this new drug discovery approach to our imaging probe discovery practice. In this Letter, we report the synthesis of [^{11}C]J147 (Fig. 1), for the first time.

The reference standard J147 (**2**) was synthesized according to the literature method with modifications.[9] When we applied the

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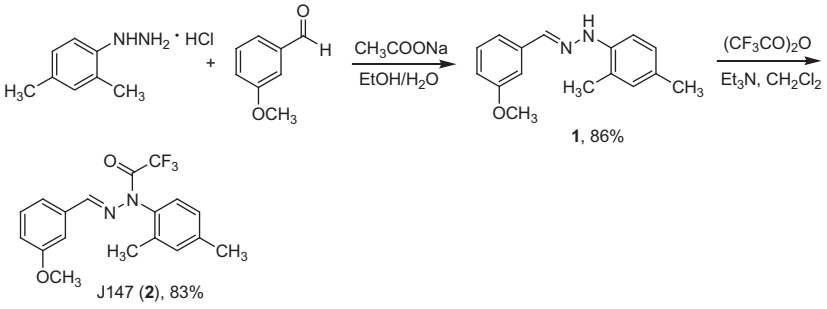
reported reaction conditions to the synthesis of aryl hydrazone **1** from 2,4-dimethylphenylhydrazine hydrochloride and 3-methoxybenzaldehyde in EtOH, the desired compound was not isolated. On the contrary, we obtained a brown unstable reaction mixture which decomposed to tar on standing. Attempts to isolate the aryl hydrazone by prolonging the reaction time, raising reaction temperature and cooling the reaction mixture also proved ineffective, and thus, we turned our attention back to the classic synthetic method.[10] As outlined in Scheme 1, our experiment showed that aryl hydrazone **1** can be successfully prepared by the reaction of 3-methoxybenzaldehyde with 2,4-dimethylphenylhydrazine hydrochloride in the presence of sodium acetate in EtOH aqueous solution. The experiment proceeded expeditiously and delivered very good isolated yield (86%). The isolation procedure simply involved the decantation of the clear solution and solidification the sticky oil, followed by washing with hexanes and drying to obtain high purity aryl hydrazone **1**. Acetylation of **1** with trifluoroacetic anhydride in CH_2Cl_2 in the presence of Et_3N furnished standard **2** in 83% yield.

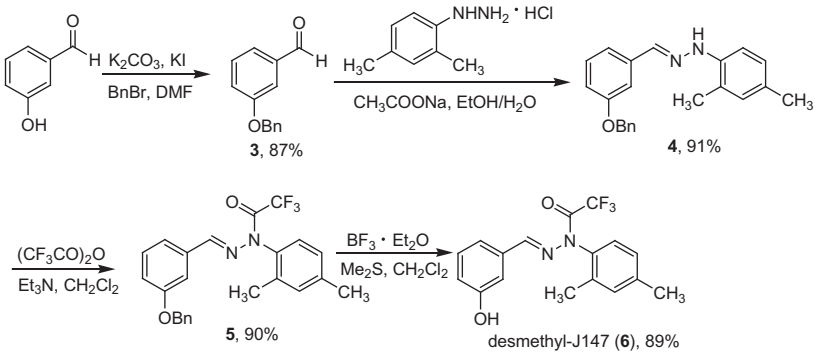
Initial attempts to achieve the precursor desmethyl-J147 (**6**) for ^{11}C-labeling focused on desmethylation of J147. Treatment of **2** with sodium isopropyl thiolate in DMF at reflux resulted with no desired product observed.[11] Stirring **2** with trimethylsilyl chloride (TMSCl) and NaI in acetonitrile at reflux provided only trace amounts of the desired product.[12] At this point we chose to explore an alternative strategy to synthesize the precursor **6**. As shown in Scheme 2, the phenolic hydroxyl group of 3-hydroxybenzaldehyde was protected as benzyl ether by reacting with benzyl bromide in DMF in the presence of K_2CO_3 and KI to afford **3** in 87% yield. Condensation of **3** with 2,4-dimethylphenylhydrazine hydrochloride in the presence of sodium acetate in EtOH aqueous solution gave **4** in 91% yield, which was acetylated with trifluoroacetic anhydride in CH_2Cl_2 in the presence of Et_3N to obtain **5** in 90% yield. Attempt to remove benzyl group by catalytic hydrogenation **5** with 10% Pd/C in EtOAc gave low yield. Prolonging reaction time, changing solvent to THF/MeOH caused a decreased yield with two side products. Eventually, treatment **5** with that $BF_3{\cdot}OEt_2$-Me_2S as a debenzylation agent in CH_2Cl_2 gave precursor **6** in 89% yield.[13]

Radiosynthesis of the target tracer [^{11}C]J147 ([^{11}C]**2**) is indicated in Scheme 3. The phenolic hydroxyl precursor desmethyl-J147 (**6**) was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[14,15] through O-[^{11}C]methylation[16–18] in acetonitrile at 80 °C under basic condition (NaH) and isolated by a semi-preparative high performance liquid chromatography (HPLC) with a Prodigy C-18 column from Phenomenex and a solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge from Waters (a second purification or isolation process)[19–21] to produce the corresponding pure radiolabeled compound [^{11}C]**2** in 35–50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. Addition of $NaHCO_3$ 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]**2** from its phenolic hydroxyl precursor **6**.[19–22] The radiosynthesis was performed in a home-built automated multipurpose ^{11}C-radiosynthesis module, allowing measurement of specific radioactivity during synthesis.[23–25] This ^{11}C-radiosynthesis module includes the overall design of the reaction, purification and reformulation capabilities of the prototype system. In addition, ^{11}C-tracer specific activity (GBq/μmol at EOB) can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system. Briefly, analysis of the chromatographic data utilized PeakSimple software (SRI Instruments, Las Vegas, NV). Immediately following elution of the product peak, the chromatographic data are exported to PeakSimple readable files, and the area of the radioactivity peak is converted to GBq at EOB by comparison to a reference calibration curve previously constructed using the same detector, loop and flow rate. The mass peak from the UV chromatogram (without decay correction) is similarly compared to a standard curve made at the same UV wavelength, mobile phase and flow rate. Simple division of the total EOB radioactivity peak (in GBq) by the total mass peak (in nmoles) gives specific activity at EOB in GBq/μmol. The overall synthesis, purification and reformulation time was 30–40 min from EOB. The specific radioactivity was in a range of 370–740 GBq/μmol at EOB. The specific activity can also be measured by analytical HPLC, which is consistent with the on-the-fly technique to determine the specific activity by semi-preparative HPLC. Chemical purity and radiochemical purity were determined by analytical HPLC.[26] The chemical purity of the precursor **6** and reference standard **2** was >96%. The radiochemical purity of the target tracer [^{11}C]**2** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of [^{11}C]**2** was >93% determined by reverse-phase HPLC through UV flow detector. A C-18 Plus Sep-Pak cartridge was used to significantly improve the chemical purity of the tracer solution.[19–21,26] The chemical purity of the [^{11}C]**2** tracer solution with Sep-Pak purification was usually increased higher 10–20% than that without Sep-Pak purification.[19–21]

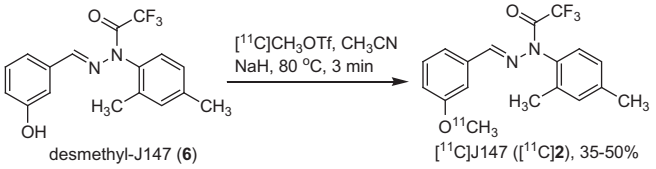
The octanol–water partition coefficient (commonly expressed as Log*P*) is an important physical parameter directly correlated with the biological activities of a wide variety of organic compounds.[27–31] Log*P* provides an assessment of lipophilicity that often correlates with a compound's ability to penetrate the blood brain barrier (BBB). We obtained calculated Log*P* (*C*Log*P*) value of [^{11}C]J147 in comparison with three other AD imaging agents [^{11}C]PIB, [^{18}F]Amyvid and [^{18}F]-T808 from ChemDraw Ultra 9.0 (ChemOffice 2005), and *C*Log*P* values for [^{11}C]J147, [^{11}C]PIB, [^{18}F]Amyvid and [^{18}F]-T808 are 4.55, 3.99, 3.52 and 4.05, respectively. The *C*Log*P* value of [^{11}C]J147 is higher than that of [^{11}C]PIB, [^{18}F]Amyvid and [^{18}F]-T808. The data suggest [^{11}C]J147 has higher lipophilicity to [^{11}C]PIB, [^{18}F]Amyvid and [^{18}F]-T808, more lipophilic than [^{11}C]PIB, [^{18}F]Amyvid and [^{18}F]-T808. In general, there is a suitable range of Log*P* value for favorable BBB penetration.[32] In other words, a compound with higher lipophilicity



Scheme 1. Synthesis of the reference standard J147 (**2**).



Scheme 2. Synthesis of the precursor desmethyl-J147 (**6**).



Scheme 3. Synthesis of the target tracer [^{11}C]J147 ([^{11}C]**2**).

doesn't necessarily pass the BBB easier than a compound with lower lipophilicity if their Log*P* values are in the suitable range. Therefore, we can predict [^{11}C]J147 has suitable ability to pass the BBB. The published biological results of J147[9] further support that [^{11}C]J147 can readily pass the BBB, and it is a promising candidate as potential PET AD imaging agent.

The experimental details and characterization data for compounds **1-6** and for the target tracer [^{11}C]**2** are given.[33]

In summary, a facile synthetic route to PET radioligand [^{11}C]J147 has been developed. This synthetic approach provided J147 and its corresponding normethyl precursor desmethyl-J147 in high overall chemical yields. An automated self-designed multi-purpose [^{11}C]-radiosynthesis module for the synthesis of [^{11}C]J147 has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed O-[^{11}C]methylation radiolabeling on oxygen position of the phenolic hydroxyl precursor. Radiolabeling procedures incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3OTf, which was produced by gas-phase production of [^{11}C]methyl bromide ([^{11}C]CH_3Br) from our laboratory. The target tracer was isolated and purified by a semi-preparative HPLC combined with SPE procedure in high radiochemical yields, short overall synthesis time, and high specific activity. These results facilitate the potential preclinical and clinical PET studies of [^{11}C]J147 as a brain AD imaging agent in animals and humans.

Acknowledgments

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References and notes

1. Någren, K.; Halldin, C.; Rinne, J. O. *Eur. J. Nucl. Med. Mol. Imaging* **2010**, *37*, 1575.
2. Rojo, L. E.; Gaspar, P. A.; Maccioni, R. B. *Curr. Alzheimer Res.* **2011**, *8*, 652.
3. Berti, V.; Pupi, A.; Mosconi, L. *Ann. N.Y. Acad. Sci.* **2011**, *1228*, 81.
4. Mori, T.; Maeda, J.; Shimada, H.; Higuchi, M.; Shinotoh, H.; Ueno, S.; Suhara, T. *Psychogeriatrics* **2012**, *12*, 106.
5. 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.
6. Carpenter, A. P., Jr.; Pontecorvo, M. J.; Hefti, F. F.; Skovronsky, D. M. Q. *J. Nucl. Med. Mol. Imaging* **2009**, *53*, 387.
7. Jensen, J. R.; Cisek, K.; Funk, K. E.; Naphade, S.; Schafer, K. N.; Kuret, J. *J. Alzheimers Dis.* **2011**, *26*, 147.
8. 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. Alzheimers Dis.* **2012**, *31*, 601.
9. Chen, Q.; Prior, M.; Dargusch, R.; Roberts, A.; Riek, R.; Eichmann, C.; Chiruta, C.; Akaishi, T.; Abe, K.; Maher, P.; Schubert, D. *PLoS ONE* **2011**, *6*, e27865.
10. Vogel, A. I.; Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; Longman: London, 1989.
11. Jones, K.; Roset, X.; Rossiter, S.; Whitfield, P. *Org. Biomol. Chem.* **2003**, *1*, 4380.
12. Mathews, W. B.; Zober, T. G.; Ravert, H. T.; Scheffel, U.; Hilton, J.; Sleep, D.; Dannals, R. F.; Szabo, Z. *Nucl. Med. Biol.* **2006**, *33*, 15.
13. Kim, Y. W.; Hackett, J. C.; Brueggemeier, R. W. *J. Med. Chem.* **2004**, *47*, 4032.
14. Jewett, D. M. *Int. J. Radiat. Appl. Instrum. A* **1992**, *43*, 1383.
15. Mock, B. H.; Mulholland, G. K.; Vavrek, M. T. *Nucl. Med. Biol.* **1999**, *26*, 467.
16. Wang, M.; Yoder, K. K.; Gao, M.; Mock, B. H.; Xu, X.-M.; Saykin, A. J.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5636.
17. Gao, M.; Lola, C. M.; Wang, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3222.
18. Gao, M.; Wang, M.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3704.
19. 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.
20. Wang, M.; Gao, M.; Miller, K. D.; Zheng, Q.-H. *Steroids* **2011**, *76*, 1331.
21. Wang, M.; Gao, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1569.
22. Gao, M.; Wang, M.; Hutchins, G. D.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2008**, *66*, 1891.
23. Mock, B. H.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S225.
24. Mock, B. H.; Glick-Wilson, B. E.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S224.
25. Wang, M.; Gao, M.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2012**, *70*, 965.
26. Zheng, Q.-H.; Mock, B. H. *Biomed. Chromatogr.* **2005**, *19*, 671.
27. Haky, J. E.; Young, A. M. *J. Liq. Chromatogr.* **1984**, *7*, 675.
28. Fei, X.; Zheng, Q.-H. *J. Liq. Chromatogr. Related Technol.* **2005**, *28*, 939.
29. Gao, M.; Mock, B. H.; Hutchins, G. D.; Zheng, Q.-H. *Nucl. Med. Biol.* **2005**, *32*, 543.
30. Gao, M.; Wang, M.; Hutchins, G. D.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2529.
31. 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.
32. Mathis, C. A.; Wang, Y.; Holt, D. P.; Huang, G.-F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740.
33. (a). General. All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[15] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, 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). 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 HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; mobile phase 3:1:1 CH_3CN:MeOH:20 mM phosphate buffer solution (pH = 6.7); flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10×250 mm; mobile phase 3:1:1 CH_3CN:MeOH:20 mM phosphate buffer solution (pH = 6.7); flow rate 5.0 mL/min; UV (254 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). *1-(2,4-Dimethylphenyl)-2-(3-methoxybenzylidene)hydrazine (**1**).* To a stirred suspension of 2,4-dimethylphenylhydrazine hydrochloride (951 mg, 5.51 mmol) and sodium acetate (452 mg, 5.51 mmol) in water (5 mL) was added 3-methoxybenzaldehyde (500 mg, 3.67 mmol) in EtOH (3 mL). The reaction mixture was heated to 100 °C and held at this temperature for 20 min with vigorous stirring. The mixture was cooled to 0 °C, the orange sticky oil accumulated at the bottom of the flask. The yellow solution was decanted and washed with cold water. The sticky oil was stored in refrigerator. After the sticky oil solidified, it was washed with hexanes and dried to afford **1** (800 mg, 86%) as a yellow solid, which was stored at −20 °C; mp 58–59 °C. ^{1}H NMR (DMSO-d_6): δ 9.48 (s, 1H), 8.08 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.20–7.19 (m, 2H), 6.92 (d, J = 8.0 Hz, 1H), 6.88–6.86 (m, 2H), 3.80 (s, 3H), 2.19 (s, 6H).

(c). *N-(2,4-Dimethylphenyl)-2,2,2-trifluoro-N′-(3-methoxybenzylidene)acetohydrazide (J147, **2**).* To a stirred solution of **1** (500 mg, 1.97 mmol) and Et_3N (0.33 mL, 2.36 mmol) in CH_2Cl_2 (5 mL) was added trifluoroacetic anhydride (0.33 mL, 2.36 mmol) dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 1.5 h. After the mixture was concentrated, the residue was purified by column chromatography with acetone/hexanes (1:10) to afford compound **2** (571 mg, 83%) as a pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.26–7.23 (m, 4H), 7.19 (d, J = 8.0 Hz, 1H), 7.12 (d, J = 7.5 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.92 (dd, J = 8.5, 2.0 Hz, 1H), 3.79 (s, 3H), 2.39 (s, 3H), 2.08 (s, 3H). HRMS (ESI-TOF, *m/z*): calcd for $C_{18}H_{18}N_2O_2F_3$ ([M+H]$^+$) 351.1320; found 351.1311.

(d). *3-(Benzyloxy)benzaldehyde (**3**).* A suspension of 3-hydroxybenzaldehyde (2.0 g, 16.4 mmol), K_2CO_3 (3.39 g, 24.6 mmol) and KI (0.07 g) in DMF (10 mL) was stirred and heated to 65 °C under nitrogen atmosphere. Benzyl bromide (2.53 mL, 21.3 mmol) was added dropwise. After the reaction mixture was stirred at 65 °C overnight, the mixture was cooled to room temperature. Water was added and the mixture was extracted with Et_2O. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was triturated with cooled hexanes to give **3** (3.02 g, 87%) as a white solid; mp 42–43 °C. ^{1}H NMR ($CDCl_3$): δ 9.97 (s, 1H), 7.48–7.43 (m, 5H), 7.41–7.38 (m, 2H), 7.35–7.32 (m, 1H), 7.26–7.24 (m, 1H), 5.12 (s, 2H).

(e). *1-(3-(Benzyloxy)benzylidene)-2-(2,4-dimethylphenyl)hydrazine (**4**).* To a stirred suspension of 2,4-dimethylphenylhydrazine hydrochloride (2.68 g, 15.54 mmol) and sodium acetate (1.27 g, 15.54 mmol) in water (20 mL) was added **3** (3.0 g, 14.1 mmol) portionwise, followed by EtOH (10 mL). The reaction mixture was heated to 75 °C and held at this temperature for 10 min with vigorous stirring until yellow solid precipitated. The mixture was cooled to 0 °C and filtered. The solid was washed with cold water, hexanes and dried to afford **4** (4.25 g, 91%) as a yellow solid, which was stored at −20 °C; mp 70–71 °C. ^{1}H NMR (DMSO-d_6): δ 9.48 (s, 1H), 8.05 (s, 1H), 7.48 (d, J = 7.5 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.35–7.28 (m, 4H), 7.19 (d, J = 7.5 Hz, 1H), 6.95–6.92 (m, 2H), 6.86 (s, 1H), 5.15 (s, 2H), 2.19 (s, 3H), 2.18 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 158.7, 140.9, 137.5, 137.1, 137.0, 130.9, 129.7, 128.4, 127.8, 127.7, 127.4, 127.1, 120.7, 118.6, 114.5, 112.4, 111.1, 69.2, 20.2, 17.4. HRMS (ESI-TOF, *m/z*): calcd for $C_{22}H_{23}N_2O$ ([M+H]$^+$) 331.1800; found 331.1810.

(f). *N-(2,4-Dimethylphenyl)-2,2,2-trifluoro-N′-(3-benzyloxybenzylidene)acetohydrazide (**5**).* To a stirred solution of **4** (4.0 g, 12.1 mmol) and Et_3N (2.02 mL, 14.5 mmol) in CH_2Cl_2 (30 mL) was added trifluoroacetic anhydride (2.02 mL, 14.5 mmol) dropwise at 0 °C under nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 1.5 h. After the mixture was concentrated, the residue was purified by column chromatography with acetone/hexanes (from 1:10 to 1:7) to afford **5** (4.64 g, 90%) as a pale yellow solid; mp 112–113 °C. ^{1}H NMR ($CDCl_3$): δ 7.43 (d, J = 7.5 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H),7.34–7.31 (m, 2H), 7.28–7.23 (m, 3H), 7.19 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.04–7.00 (m, 2H), 5.09 (s, 2H), 2.41(s, 3H), 2.07 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 159.2, 144.0, 141.1, 136.8, 136.3, 134.8, 132.7, 129.9, 129.7, 128.9, 128.7, 128.6, 128.2, 127.7, 121.5, 118.2, 112.7, 70.1, 21.4, 17.1. HRMS (ESI-TOF, *m/z*): calcd for $C_{24}H_{22}N_2O_2F_3$ ([M+H]$^+$) 427.1633; found 427.1654.

(g). *N-(2,4-Dimethylphenyl)-2,2,2-trifluoro-N′-(3-hydroxybenzylidene)acetohydrazide (desmethyl-J147, **6**).* To a stirred solution of **5** (200 mg, 0.47 mmol) in Me_2S (3 mL) and CH_2Cl_2 (3 mL) was added $BF_3{\cdot}Et_2O$ dropwise at room temperature. The reaction mixture was stirred at room temperature for 4 h. The mixture was poured into ice-water and extracted with EtOAc. The combined layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by column chromatography with acetone/hexanes (from 1:7 to 1:4) to afford **6** (140 mg, 89%) as a white solid; mp 148–149 °C. ^{1}H NMR ($CDCl_3$): δ 7.23–7.15 (m, 5H), 7.07 (d, J = 7.5 Hz, 1H), 7.03 (d, J = 8.0 Hz, 1H), 6.84 (ddd, J = 8.0, 2.5, 1.0 Hz, 1H), 5.45 (s, 1H), 2.39 (s, 3H), 2.07 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 156.2, 144.3, 141.2, 136.3, 134.8, 132.7, 130.1, 129.5, 128.9, 128.6, 121.2, 118.2, 113.7, 21.4, 17.1. HRMS (ESI-TOF, *m/z*): calcd for $C_{17}H_{15}N_2O_2NaF_3$ ([M+Na]$^+$) 359.0988; found 359.0983.

(h). *N-(2,4-Dimethylphenyl)-2,2,2-trifluoro-N′-(3-[^{11}C]methoxybenzylidene)acetohyrazide ([^{11}C]J147, [^{11}C]**2**).* [^{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 15 min on target. The production run produced approximately 28.5 GBq of [^{11}C]CO_2 at EOB. In a small reaction vial (5 mL), the precursor desmethyl-J147 (**6**) (0.3–0.5 mg) was dissolved in CH_3CN (300 μL). To this solution was added NaH (1 mg). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[15] 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 80 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (0.1 M, 1 mL), and injected onto the semi-preparative 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]**2**. The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity was assayed and total volume was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **6** = 6.67 min, t_R **2** = 12.48 min, t_R [^{11}C]**2** = 12.48 min. Retention times in the analytical HPLC system were: t_R **6** = 4.11 min, t_R **2** = 8.57 min, t_R [^{11}C]**2** = 8.57 min. The decay corrected radiochemical yield of [^{11}C]**2** from [^{11}C]CO_2 was 35–50%.