Bioorganic & Medicinal Chemistry Letters 24 (2014) 254–257

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Concise and high-yield synthesis of T808 and T808P for radiosynthesis of [^{18}F]-T808, a PET tau tracer for Alzheimer's disease

Mingzhang Gao, Min Wang, Qi-Huang Zheng *

Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16th Street, Room 202, Indianapolis, IN 46202, USA

ARTICLE INFO

Article history:
Received 30 October 2013
Revised 6 November 2013
Accepted 11 November 2013
Available online 20 November 2013

Keywords:
[^{18}F]-T808
Tau tracer
Automation
Positron emission tomography (PET)
Alzheimer's disease (AD)

ABSTRACT

The authentic standard T808 and its corresponding mesylate precursor T808P were synthesized in six steps using ethyl vinyl ether and trichlorocetyl chloride as starting materials. The overall chemical yields of T808 and T808P were 35% and 52%, respectively. [^{18}F]-T808 was synthesized from T808P by the nucleophilic substitution with K[^{18}F]F/Kryptofix 2.2.2 and isolated by HPLC combined with solid-phase extraction (SPE) purification in 35–45% radiochemical yield with 37–370 GBq/μmol specific activity at end of bombardment (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, couple highly selective and specific fluorine-18 PET tracers called [^{18}F]-T808 and [^{18}F]-T807 (Fig. 1) for imaging of tau pathologies have been developed by Siemens.[8,9] Subsequently, early clinical PET imaging results with tau radioligands [^{18}F]-T807 and [^{18}F]-T808 have been reported.[10,11] Meanwhile, a promising carbon-11 PET tracer called [^{11}C]PBB3 (Fig. 1) for imaging of tau pathology in a tauopathy mouse model and in Alzheimer patients compared to normal controls has been published by other group as well.[12]

The importance of PET tau tracers is well recognized, and broader research investigation to fully explore and validate the utility of tau tracer-PET is important. However, the limited commercial availability, complicated and/or patented synthetic procedure, and high costs of starting materials and precursor can present an obstacle to more widespread evaluation of these intriguing agents. In an attempt to study these compounds in our PET center, we decided to make our own material by following the literature methods. We selected [^{18}F]-T808 as a start of the PET tau tracer project. The published and patented synthesis of the authentic standard T808 and its corresponding mesylate precursor T808P, and target tracer [^{18}F]-T808 has gaps in synthetic detail, and the key step coupling reaction required small-scale microwave-assisted synthesis, gave poor yield and was difficult to reproduce in our hands. Therefore, we revisited the reported literature methods[8,11,13,14] and investigated alternate approaches and modifications that eventually resulted in a concise and high-yield synthesis of T808, T808P and [^{18}F]-T808 starting from very beginning materials ethyl vinyl ether and trichloroacetyl chloride that was superior to previous works or addressed more synthetic details to reveal and explain technical tricks. In this Letter, we provide complete experiment procedures, yields, analytical details and new findings for this synthetic route of T808 and T808P, and present a fully automated radiosynthesis of [^{18}F]-T808 with relatively high radiochemical yields and shortened radiosynthesis time.

The synthesis of T808 (**8**) and T808P (**9**) is shown in Scheme 1, according to the literature method[13,14] with modifications. Ethyl vinyl ether was reacted with trichloroacetyl chloride at 0 °C for 5 h and then stirred at room temperature (RT) for 12 h to produce

* Corresponding author. Tel.: +1 317 278 4671.
E-mail address: qzheng@iupui.edu (Q.-H. Zheng).

0960-894X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.11.025

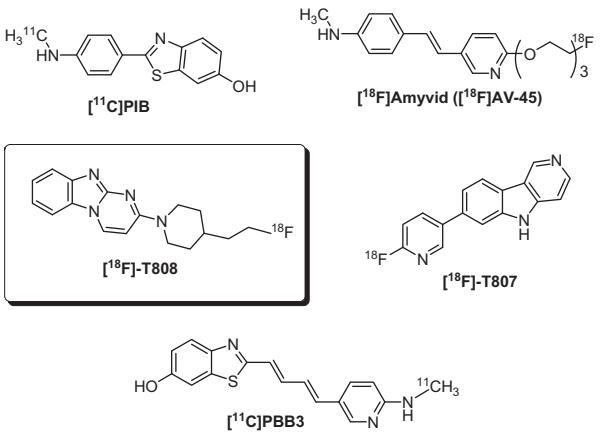
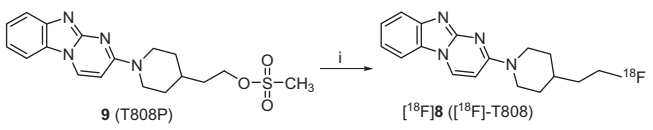


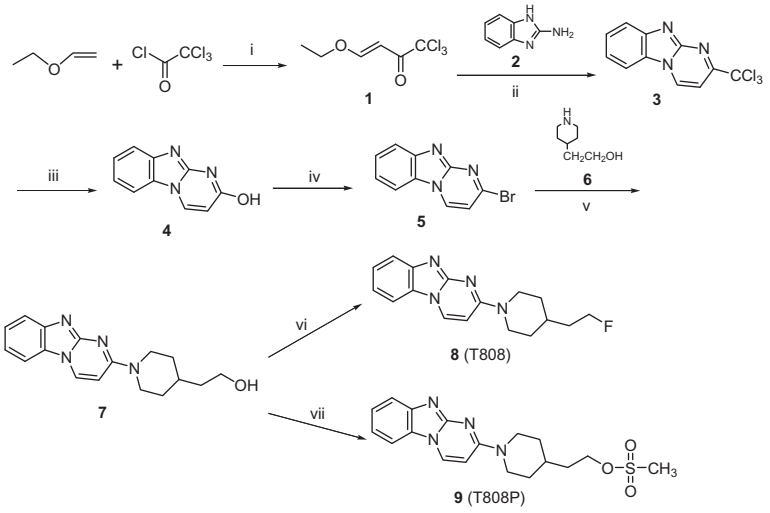
Figure 1. Chemical structure of [^{11}C]PIB, [^{18}F]Amyvid ([^{18}F]AV-45), [^{18}F]-T808, [^{18}F]-T807 and [^{11}C]PBB3.

an intermediate 1,1,1,4-tetrachloro-4-ethoxybutan-2-one, without further isolation, which was quickly converted to compound **1** through dehydrochlorination at heating and distillation in 92% yield.[15] Compound **1** reacted with 1*H*-benzo[*d*]imidazol-2-amine (**2**) to generate compound **3** in 98% yield. Hydrolysis of **3** gave compound **4** in 99% yield. Bromination of **4** with phosphorus oxybromide provided compound **5** in 97% yield. The key step coupling reaction of **5** with 2-(piperidin-4-yl)ethanol (**6**) was conducted in the presence of K_2CO_3 in *N*,*N*-dimethylacetamide (DMA) to afford compound **7** in 80% yield. Several different solvents including DMA, *N*,*N*-dimethylformamide (DMF), dimethylfulfoxide (DMSO), CH_3CN and *n*-BuOH, and catalysts such as K_2CO_3, NaOH and *p*-toluenesulfonic acid (TsOH)[16] were tested for the coupling reaction, and it was found to obtain higher yield when using DMA as a solvent and K_2CO_3 as a base catalyst. The published literature method[13] showed the coupling reaction was performed in a Biotage Initiator microwave reactor (250 W) in small-scale (mg-grade) without adding any base or acid catalyst and with low yield (54%). In comparison with the results reported in the patent,[13] significant improvements in the key step included eliminating the use of microwave reactor, increasing the yield and enlarging the reaction scale from mg-grade to g-grade. Compound **7** reacted with diethylaminosulfur trifluoride (DAST) provided the reference standard **8** in 51% yield. Compound **7** reacted with methanesulfonyl chloride (MsCl) using Et_3N as a base afforded the mesylate precursor **9** in 75% yield. The yield of **9** was increased by optimization of the reaction conditions including decreasing the reaction temperature at 0 °C and extending the reaction time to 1 h.

Synthesis of the target tracer [^{18}F]-T808 ([^{18}F]**8**) is indicated in Scheme 2. The mesylate precursor T808P (**9**) was labeled with K[^{18}F]F/Kryptofix 2.2.2 through nucleophilic substitution at 140 °C and isolated by a semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) method with C-18 column and a solid-phase extraction SPE method with a C-18 Plus Sep-Pak cartridge (a second purification or isolation process)[17–19] to produce the corresponding pure radiolabeled compound [^{18}F]**8**[8,11,13,14] in 35–45% radiochemical yield, decay-corrected to EOB, based on H[^{18}F]F. The crude radiolabeling mixture was briefly cooled to 100 °C and treated with 3 N NaOH and heated at 100 °C for 8 min. This base-mediated treatment of the reaction mixture was necessary (1) to prevent co-elution of the precursor with the product by decomposition of unreacated precursor T808P and (2) to prevent co-elution of the unreacted [^{18}F]fluoride with the product due to protonization of the radiolabeled product under acidic conditions, during the semi-preparative HPLC purification. 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 labeled product [^{18}F]-T808 from its corresponding mesylate precursor T808P and unreacted [^{18}F]fluoride.[17–19] The radiosynthesis was performed using a self-designed automated multi-purpose [^{18}F]-radiosynthesis module.[20–22] The overall synthesis, purification and formulation



Scheme 2. Synthesis of [^{18}F]-T808 ([^{18}F]**8**). Reagents, reaction conditions and yields: (i) K[^{18}F]/K2.2.2, DMSO, 140 °C, 8 min; 3 N NaOH, 100 °C, 8 min; HPLC, SPE, 35–45%.



Scheme 1. Synthesis of T808 (**8**) and T808P (**9**). Reagents, reaction conditions and yields: (i) 0 °C to RT, 17 h; 130 °C, 1 h, 92%; (ii) 1*H*-benzo[*d*]imidazol-2-amine (**2**), Et_3N, PhMe, RT to reflux, 2 h, 98%; (iii) NaOH, H_2O, MeCN, RT to reflux, 30 min; HCl, 99%; (iv) O=PBr_3, DMF, $ClCH_2CH_2Cl$, RT to reflux, 3 h; NH_4OH, 97%; (v) 2-(piperidin-4-yl)ethanol (**6**), DMA, K_2CO_3, 130 °C, 6 h, 80%; (vi) Et_2NSF_3, CH_2Cl_2, 0 °C to RT, 1.5 h, 51%; and (vii) MsCl, Et_3N, CH_2Cl_2, 0 °C to RT, 2 h, 75%.

time was 50–60 min from EOB. The specific activity (SA) was 37–370 GBq/μmol at EOB. SA is defined as the radioactivity per unit mass of a radionuclide or a labeled compound. SA (MBq/mg) = $3.13 \times 10^9/A \times t_{1/2}$, where A is the mass number of the radionuclide, and $t_{1/2}$ is the half-life in hours of the radionuclide. For fluorine-18, carrier-free ^{18}F, maximum (theoretical) ^{18}F SA = 63,381 GBq/μmol.[23] Actual SA of ^{18}F-tracers in our PET chemistry facility is depended on two parts: (1) carrier from the ^{18}F target during the production of H[^{18}F]F, the target we use is Siemens RDS-111 Eclipse cyclotron ^{18}F target; and (2) carrier from the ^{18}F radiolabeled precursor during the production of K[^{18}F]F/Kryptofix 2.2.2 by azeotropic distillation in our [^{18}F]-radiosynthesis module. Our study has proved that the maximum in-target SA for our ^{18}F-tracers is ~370 GBq/μmol at EOB produced in our cyclotron and ^{18}F-radiosynthesis unit. The SA for our ^{18}F-tracers usually ranges from 37 to 370 GBq/μmol at EOB according to our previous works for the ^{18}F-tracers produced in this facility, including [^{18}F]Fallypride, [^{18}F]PBR06, [^{18}F]FE-DAA1106, etc.[17–19,22] Theoretically, all ^{18}F-tracers have same SA, and actual SA of ^{18}F-tracers is mainly related to the type of cyclotron and synthetic module.[23] The SA of the title tracer was 37–370 GBq/μmol at EOB, which was similar to the values previously reported by our lab.[17–19,22] No-carrier-added [^{18}F]fluoride ion in [^{18}O]water was trapped without a QMA cartridge. This way[17–19,22,24] significantly increased the SA of the prepared F-18 labeled product. As indicated in the literature,[24] when the cyclotron-produced [^{18}F]fluoride ion was dried without the use of a cartridge, but through cycles of evaporation with added acetonitrile, the SA of the prepared [^{18}F]-T808 was substantially higher, and was similar to that we achieved in the radiosynthesis of other F-18 tracers such as [^{18}F]fallypride and [^{18}F]PBR06 previously reported.[17–19,22] The reason was that there was a low-level contamination of QMA anionic resins with fluoride ion.[24] The amounts of the mesylate precursor used were ~1 mg. Small amount of the precursor was used for radiolabeling instead of large amount of the precursor (3 mg) reported in the literature,[8] which improved the chemical purity of the final tracer solution. A large amount of precursor would increase the radiochemical yield, but decrease the chemical purity of [^{18}F]-T808 tracer solution due to precursor contamination. In addition, a large amount of precursor would also decrease the SA of final labeled product due to potential F-18/F-19 exchange during the radiolabeling. The reaction solvent and temperature were either CH_3CN/120 °C or DMSO/140 °C. Radiolabeling procedure with DMSO at 140 °C resulted in higher radiochemical yield.[17–19,22,25] Furthermore, in order to make more product radioactivity, we also modified the published semi-preparative HPLC conditions including column, mobile phase and flow rate to shorten the retention time of [^{18}F]**8**, since the reported retention time of [^{18}F]-T808 was too long (between 25 and 30 min).[8] To our F-18 labeling experiences on F-18 tracers,[17–19,22] although the HPLC systems we employed have shown good separation of products from precursors, there always was a co-elution of the F-18 labeled product with its corresponding precursor from the HPLC column, very tiny amount of the precursor (0.1–0.4 μg/mL) contaminating the tracer solution. [^{18}F]-T808 was also in the same case. Therefore, we used a C-18 Plus Sep-Pak cartridge for this purpose to further remove the precursor and most of possible nonradiolabeled undesired side-products. A C-18 Plus Sep-Pak cartridge instead of rotatory evaporation was used to significantly improve the chemical purity of the tracer solution.[17–19,22] In this study, the Sep-Pak purification further increased the chemical purity more than 10%.[17–19,22] Chemical purity and radiochemical purity were determined by analytical HPLC.[26] The chemical purity of T808P and T808P was >93% determined by HPLC through UV flow detector. The radiochemical purity of the target tracer [^{18}F]-T808 was >98% determined by radio-HPLC through γ-ray (PIN diode) flow detector.

The experimental details and characterization data for compounds **1**, **3**–**5**, **7**–**9** and for the tracer [^{18}F]**8** are given.[27]

In summary, a concise and high-yield synthetic route to T808, T808P and [^{18}F]-T808 has been developed. This synthetic approach provided T808 and its mesylate precursor T808P in high overall chemical yields. An automated self-designed multi-purpose [^{18}F]-radiosynthesis module for the synthesis of [^{18}F]-T808 has been built. The radiosynthesis employed nucleophilic substitution of the mesylate precursor with K[^{18}F]F/Kryptofix 2.2.2. The target tracer was isolated and purified by a semi-preparative HPLC combined with SPE procedure in relatively high radiochemical yields, shortened overall synthesis time, and high specific activity and radiochemical purity. New and improved results in the synthetic methodology, radiolabeling, preparative separation and analytical details for T808, T808P and [^{18}F]-T808 have been presented. These methods are efficient and convenient. It is anticipated that the approaches for the design, synthesis and automation of new tracer and radiolabeling precursor, and improvements to increase radiochemical yield, chemical purity and specific activity of the tracer described here can be applied with advantage to the synthesis of other ^{18}F-radiotracers for PET imaging. These results facilitate the potential preclinical and clinical PET studies of [^{18}F]-T808 as a PET AD tau tracer in animals and humans.

Acknowledgments

This work was partially supported by Indiana State Department of Health (ISDH) Indiana Spinal Cord & Brain Injury Fund (ISDH EDS-A70-2-079612). ^{1}H NMR spectra were recorded on a Bruker Avance II 500 MHz NMR spectrometer in the Department of Chemistry and Chemical Biology at Indiana University Purdue University Indianapolis (IUPUI), which is supported by a NSF-MRI Grant CHE-0619254.

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. Alzheimer's 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. Alzheimer's Dis.* **2012**, *31*, 601.
9. 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.
10. 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.
11. 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.
12. 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.
13. Szardenings, A. K.; Zhang, W.; Kolb, H. C.; Cashion, D. K.; Chen, G.; Kasi, D.; Liu, C.; Sinha, A.; Wang, E.; Yu, C.; Gangadharmath, U. B.; Walsh, J. C. US Patent No. 0182812 A1, 2011.
14. Cashion, D. K.; Chen, G.; Kasi, D.; Kolb, H. C.; Liu, C.; Sinha, A.; Szardenings, A. K.; Wang, E.; Yu, C.; Zhang, W.; Gangadharmath, U. B.; Walsh, J. C. WO Patent No. 119565 A1, 2011.

15. Tietze, L. F.; Voss, E.; Hartfiel, U. *Org. Synth. Coll.* **1993**, *8*, 254. Vol. 69, p.238 (1990).
16. Ducray, R.; Jones, C. D.; Jung, F. H.; Simpson, I.; Curwen, J.; Pass, M. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4702.
17. 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.
18. Wang, M.; Gao, M.; Miller, K. D.; Zheng, Q.-H. *Steroids* **2011**, *76*, 1331.
19. Wang, M.; Gao, M.; Miller, K. D.; Sledge, G. W.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1569.
20. Mock, B. H.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S225.
21. Mock, B. H.; Glick-Wilson, B. E.; Zheng, Q.-H.; DeGrado, T. R. *J. Labelled Compd. Radiopharm.* **2005**, *48*, S224.
22. Wang, M.; Gao, M.; Zheng, Q.-H. *Appl. Radiat. Isot.* **2012**, *70*, 965.
23. Lapi, S. E.; Welch, M. J. *Nucl. Med. Biol.* **2013**, *40*, 314.
24. Lu, S.; Giamis, A. M.; Pike, V. W. *Curr. Radiopharm.* **2009**, *2*, 49.
25. Kuhnast, B.; Hinnen, F.; Dolle, F. *J. Labelled Compd. Radiopharm.* **2009**, *52*, S286.
26. Zheng, Q.-H.; Mock, B. H. *Biomed. Chromatogr.* **2005**, *19*, 671.

27. (a). *General:* All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific, and used without further purification. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded 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. 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 20% EtOH/80% H_2O; flow rate 1.0 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10 × 250 mm; mobile phase 50% CH_3CN/50% H_2O; 4.0 mL/min flow rate; 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). *(E)-1,1,1-Trichloro-4-ethoxybut-3-en-2-one (**1**):* A 250 mL round-bottomed flask was charged with trichloroacetyl chloride (35.3 g, 194 mmol). Under nitrogen the flask was cooled with an ice bath to 0 °C and ethyl vinyl ether (41.9 g, 582 mmol) was added dropwise. The mixture was kept on stirring at 0 °C for 5 h and then at RT for 12 h. The dropping funnel was replaced by a short Vigreux column and excess ethyl vinyl ether was removed at 25 °C under reduced pressure (20 mmHg). The bath temperature was raised to 130 °C under reduced pressure (20 mmHg) to start elimination of gas (hydrogen chloride), which was accompanied by formation of a deep black color solution and required 1 h or so until no gas came out. Distillation of the residue under reduced pressure afforded **1** (38.8 g, 92%) as a yellowish oil, bp 75–77 °C/2 mmHg (lit.[15] bp 116–118 °C/13 mmHg). ^{1}H NMR ($CDCl_3$): δ 1.41 (t, J = 7.0 Hz, 3H, CH_3), 4.10 (q, J = 7.0 Hz, 2H, OCH_2), 6.15 (d, J = 12.5 Hz, 1H, 3-H), 7.86 (d, J = 12.5 Hz, 1H, 4-H).

(c). *2-(Trichloromethyl)benzo[4,5]imidazo[1,2-a]pyrimidine (**3**).* Compound **1** (13.47 g, 62.0 mmol) was added to a mixture of compound **2** (7.99 g, 60.0 mmol) and triethylamine (6.26 g, 62.0 mmol) in toluene (250 mL). The reaction mixture was heated to reflux for 2 h, concentrated in vacuo, filtered, and dried in air to give **3** (16.8 g, 98%) as a yellow solid, R_f = 0.31 (2% MeOH/CH_2Cl_2), mp 226 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 7.51 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.62 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.76 (d, J = 7.0 Hz, 1H, Ar-H), 7.94 (d, J = 8.0 Hz, 1H, Ph-H), 8.42 (d, J = 8.0 Hz, 1H, Ph-H), 9.78 (d, J = 7.0 Hz, 1H, Ar-H). MS (ESI$^+$): 286 ([M+H]$^+$, 100%).

(d). *Benzo[4,5]imidazo[1,2-a]pyrimidin-2-ol (**4**):* NaOH (1 N, 42 mL, 42 mmol) was added to a mixture containing compound **3** (9.16 g, 32.0 mmol) in acetonitrile (160 mL). The reaction was heated to reflux for 30 min, and then the reaction was cooled and concentrated. Added ice to the resulting residue, followed by HCl (1 N, 30 mL) to adjust pH of the solution to 8. Filtered the solid and dried in air to afford **4** (5.87 g, 99%) as a white solid, R_f = 0.16 (10% MeOH/CH_2Cl_2), mp >280 °C (decomposed). ^{1}H NMR (DMSO-d_6): δ 6.09 (d, J = 7.5 Hz, 1H, Ar-H), 7.23 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.29 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.51 (d, J = 7.5 Hz, 1H, Ph-H), 7.89 (d, J = 7.5 Hz, 1H, Ph-H), 8.78 (d, J = 8.0 Hz, 1H, Ar-H), 12.57 (s, OH).

(e). *2-Bromobenzo[4,5]imidazo[1,2-a]pyrimidine (**5**):* Phosphorus oxybromide (17.8 g, 62.0 mmol) was added to suspension of compound **4** (5.74 g, 31.0 mmol) in 1,2-dichloroethane (130 mL) and DMF (1 mL). The reaction mixture was heated to reflux for 3 h. The reaction mixture was concentrated in vacuo, diluted with ice water and quenched with ammonium hydroxide to adjust pH to 8. The resulting residue was filtered and washed with water followed by ethyl ether, and dried under vacuum to obtain **5** (7.46 g, 97%) as a yellow solid, R_f = 0.22 (2% MeOH/CH_2Cl_2), mp 287–289 °C. ^{1}H NMR (DMSO-d_6): δ 6.57 (d, J = 7.5 Hz, 1H, Ar-H), 7.53 (t, J = 7.5 Hz, 1H, Ph-H), 7.49 (t, J = 7.5 Hz, 1H, Ph-H), 7.63 (d, J = 8.0 Hz, 1H, Ph-H), 8.12 (d, J = 8.0 Hz, 1H, Ph-H), 9.15 (d, J = 7.5 Hz, 1H, Ar-H). MS (ESI$^+$): 248 ([M+H]$^+$, 40%).

(f). *2-(1-(Benzo[4,5]imidazo[1,2-a]pyrimidin-2-yl)piperidin-4-yl)ethanol (**7**):* A mixture of compound **5** (1.49 g, 6.0 mmol), compound **6** (970 mg, 7.5 mmol) and K_2CO_3 (1.66 g, 12.0 mmol) in DMA (50 mL) was stirred at 130 °C for 6 h. The reaction mixture was cooled to RT, filtered, and washed with DMA. The combined the organic phase was evaporated in vacuo. The resulting residue was purified by column chromatography (2–15% MeOH/CH_2Cl_2) on silica gel to afford **7** (1.42 g, 80%) as an off white solid, R_f = 0.17 (10% MeOH/CH_2Cl_2), mp 178–180 °C. ^{1}H NMR (DMSO-d_6): δ 1.15–1.17 (m, 2H, CH_2), 1.37–1.41 (m, 2H, CH_2), 1.72–1.80 (m, 3H, CH and CH_2), 2.98–3.00 (m, 2H, CH_2), 3.46–3.49 (m, 2H, CH_2), 4.56 (br s, 1H, OH), 6.87 (d, J = 8.0 Hz, 1H, Ar-H), 7.14 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.26 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.49 (d, J = 8.0 Hz, 1H, Ph-H), 7.93 (d, J = 8.0 Hz, 1H, Ph-H), 8.93 (d, J = 8.0 Hz, 1H, Ar-H). MS (ESI$^+$): 297 ([M+H]$^+$, 100%).

(g). *2-(4-(2-Fluoroethyl)piperidin-1-yl)benzo[4,5]imidazo[1,2-a]pyrimidine (**8**, T808):* To a solution of compound **7** (237 mg, 0.80 mmol) in CH_2Cl_2 (30 mL) was slowly added DAST (774 mg, 4.8 mmol) at 0 °C. The reaction mixture was continued to stir at 0 °C for 1 h, and then at RT for 30 min. The solvent was evaporated and the resulting residue was purified by column chromatography (1–4% MeOH/CH_2Cl_2) on silica gel to obtain **8** (122 mg, 51%) as a white solid, R_f = 0.43 (10% MeOH/CH_2Cl_2), mp 176–178 °C. ^{1}H NMR ($CDCl_3$): δ 1.25–1.33 (m, 2H, CH_2), 1.63–1.72 (m, 2H, CH_2), 1.82–1.89 (m, 3H, CH and CH_2), 3.01 (t, J = 12.3 Hz, 2H, CH_2), 4.49 (t, J = 6.0 Hz, 2H, CH_2), 4.58 (t, J = 6.0 Hz, 2H, CH_2), 6.42 (d, J = 8.0 Hz, 1H, Ar-H), 7.20 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.35 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.55 (d, J = 8.0 Hz, 1H, Ph-H), 7.71 (d, J = 8.0 Hz, 1H, Ph-H), 8.23 (d, J = 8.0 Hz, 1H, Ar-H). MS (ESI$^+$): 299 ([M+H]$^+$, 100%).

(h). *2-(1-(Benzo[4,5]imidazo[1,2-a]pyrimidin-2-yl)piperidin-4-yl)ethyl methanesulfonate (**9**, T808P):* A solution of methanesulfonyl chloride (149 mg, 1.3 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a mixture of compound **7** (296 mg, 1.0 mmol) and Et_3N (303 mg, 3.0 mmol) in CH_2Cl_2 (40 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then at RT for 1 h. The reaction mixture was evaporated in vacuo, and the resulting residue was purified by column chromatography (1–4% MeOH/CH_2Cl_2) on silica gel to afford **9** (281 mg, 75%) as a white solid, R_f = 0.40 (10% MeOH/CH_2Cl_2), mp 237–239 °C. ^{1}H NMR ($CDCl_3$): δ 1.25–1.30 (m, 2H, CH_2), 1.71–1.75 (m, 2H, CH_2), 1.86–1.88 (m, 5H, CH and 2× CH_2), 3.01–3.05 (m, 5H, CH_2 and CH_3), 6.44 (d, J = 8.0 Hz, 1H, Ar-H), 7.17 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.35 (dt, J = 1.0, 8.0 Hz, 1H, Ph-H), 7.56 (d, J = 8.0 Hz, 1H, Ph-H), 7.71 (d, J = 8.0 Hz, 1H, Ph-H), 8.26 (d, J = 8.0 Hz, 1H, Ar-H). MS (ESI$^+$): 375 ([M+H]$^+$, 100%).

(i). *2-(4-(2-[^{18}F]Fluoroethyl)piperidin-1-yl)benzo[4,5]imidazo[1,2-a]pyrimidine ([^{18}F]**8**, [^{18}F]-T808):* No-carrier-added (NCA) aqueous H[^{18}F]F was produced by ^{18}O(p,n)^{18}F nuclear reaction using a Siemens Eclipse RDS-111 cyclotron by irradiation of $H_2{}^{18}O$ (2.5 mL). H[^{18}F]F (7.4-18.5 GBq) in [^{18}O]water plus 0.1 mL K_2CO_3 solution (1.7 mg) and Kryptofix 2.2.2 (10 mg) in 1.0 mL CH_3CN with additional 1 mL CH_3CN were placed in the fluorination reaction vial (10-mL V-vial) and repeated azeotropic distillation (17 min) was performed at 110 °C to remove water and to form the anhydrous K[^{18}F]F-Kryptofix 2.2.2 complex. The precursor T808P (**9**, 1 mg) dissolved in DMSO (1.0 mL) was introduced to the reaction vessel and heated at 140 °C for 8 min to affect radiofluorination. After cooling to 100 °C, the crude reaction mixture was treated with 3 N NaOH (1 mL). The reaction was heated at 100 °C for 8 min. The contents of the reaction vial were cooled down and diluted with 0.1 M $NaHCO_3$ (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) to release [^{18}F]**8**, followed by saline (10 mL). 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 **8** = 11.52 min, t_R **9** = 18.87 min, t_R [^{18}F]**8** = 11.52 min. Retention times in the analytical HPLC system were: t_R **8** = 5.64 min, t_R **9** = 9.02 min, t_R [^{18}F]**8** = 5.64 min. The decay corrected radiochemical yields of [^{18}F]**8** were 35–45%.