Bioorganic & Medicinal Chemistry Letters 25 (2015) 2953–2957

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Fully automated synthesis of [^{18}F]T807, a PET tau tracer for Alzheimer's disease

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ARTICLE INFO

Article history:
Received 24 April 2015
Revised 12 May 2015
Accepted 13 May 2015
Available online 21 May 2015

Keywords:
[^{18}F]T807
Tau tracer
Automation
Positron emission tomography (PET)
Alzheimer's disease (AD)

ABSTRACT

The authentic standard T807 and its nitro-precursor T807P as well as *t*-Boc-protected T807P precursor for radiolabeling were synthesized from (4-bromophenyl)boronic acid, 3-bromo-4-nitropyridine and 3-bromo-6-nitropyridine with overall chemical yield 27% in three steps, 4–7% in three to five steps, and 3–8% in four to five steps, respectively. [^{18}F]T807 was synthesized from T807P by the nucleophilic [^{18}F]fluorination with K[^{18}F]F/Kryptofix 2.2.2 in DMSO at 140 °C followed by reduction with Fe powder/HCOOH through manual synthesis with 5–10% decay corrected radiochemical yield in two steps. [^{18}F]T807 was also synthesized from *t*-Boc-protected T807P by a concurrent [^{18}F]fluorination and deprotection with K[^{18}F]F/Kryptofix 2.2.2 in DMSO at 140 °C and purified by HPLC-SPE method in a home-built automated [^{18}F]radiosynthesis module with 20–30% decay corrected radiochemical yield in one step. The specific activity of [^{18}F]T807 at end of bombardment (EOB) was 37–370 GBq/μmol.

Alzheimer's disease (AD) is an irreversible, progressive brain disease that slowly loses memory and other important mental functions, and nearly 35 million people suffer this disease worldwide.[1–3] The exact causes of AD are not fully understood.[4] Currently there is no prevention or cure, and clinical drug trials have greater than 90% failure rate.[5] The lack of a reliable early diagnosis for AD results in the difficulty to find an efficient treatment for AD, and the lack of effective prognostic biomarkers also hampers the development of therapeutics for AD.[6] Therefore, the development of pathology-specific diagnostic tools has been strongly desired, and advanced molecular imaging technique positron emission tomography (PET) is a promising modality for early detection, disease staging, drug discovery and progression of AD patients.[7] Amyloid and tau are two major interesting targets for the development of therapeutic solutions and diagnostic biomarkers such as in vivo PET probes for AD.[8] Amyloid cascade hypothesis has generated a number of Aβ PET tracers, and the representative radiopharmaceuticals are [^{11}C]PIB[9] and [^{18}F]Amyvid (formerly known as [^{18}F]AV-45),[10] as indicated in Figure 1. Since more and more evidences demonstrate that increased tau levels appear to correlate with clinical AD disease severity, recently the researchers have switched their focus to tau hypothesis, several highly selective and specific PET tau tracers like [^{18}F]T808 ([^{18}F]AV-680), [^{18}F]T807 ([^{18}F]AV-1451) and [^{11}C]PBB3 (Figure 1) have been developed, and promising clinical PET imaging results with these tracers have been reported.[11–15]

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. In our previous work, we have developed a concise and high-yield synthesis of T808 and T808P, and a fully automated radiosynthesis of [^{18}F]T808.[16] In this ongoing study, we select [^{18}F]T807 as another target PET tau tracer. The published and patented synthesis of the authentic standard T807 and target tracer [^{18}F]T807 lacks synthetic detail, and the key steps gave poor yield and was difficult to reproduce in our hands. Therefore, we revisited the reported literature methods[12,13,17,18] and investigated alternate approaches and modifications that eventually resulted in a high-yield synthesis of T807 and [^{18}F]T807 starting from very beginning materials (4-bromophenyl)boronic acid, 3-bromo-4-nitropyridine and 3-bromo-6-nitropyridine that was superior to previous works or addressed more synthetic details to reveal and explain technical artifices. In this letter we report the first synthesis of the nitro-precursor T807P as well as *t*-Boc-protected T807P precursor, provide complete experiment procedures, yields, analytical details and new findings for the synthesis of T807 and

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http://dx.doi.org/10.1016/j.bmcl.2015.05.035
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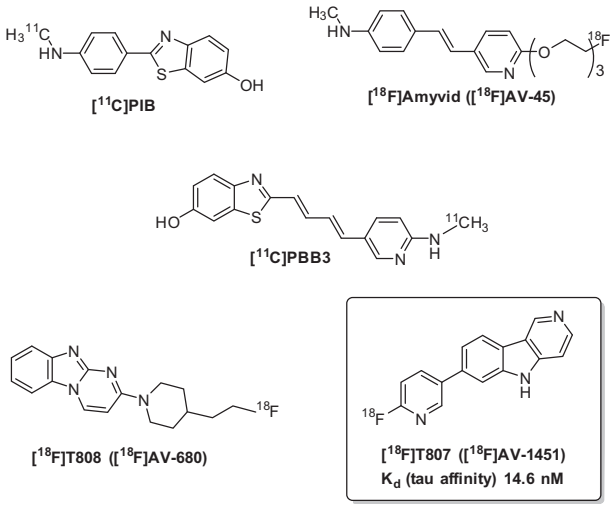


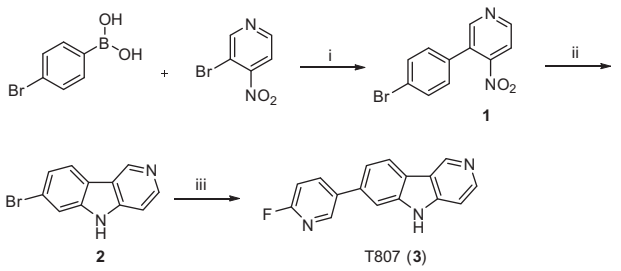
Figure 1. Chemical structure of [^{11}C]PIB, [^{18}F]Amyvid ([^{18}F]AV-45), [^{11}C]PBB3, [^{18}F]T808 ([^{18}F]AV-680), and [^{18}F]T807 ([^{18}F]AV-1451).

[^{18}F]T807, and present a fully automated one-step-one-pot radiosynthesis of [^{18}F]T807 with relatively high radiochemical yields and shortened radiosynthesis time.

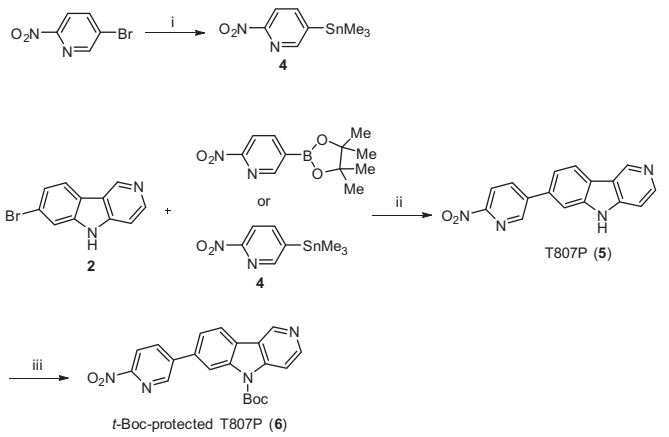
The synthesis of reference standard T807 (7-(6-fluoropyridin-3-yl)-5*H*-pyrido[4,3-*b*]indole, **3**) was shown in Scheme 1, according to the literature method[17] with modifications. (4-Bromophenyl)-boronic acid and 3-bromo-4-nitropyridine were undergone Suzuki coupling reaction in the presence of the catalyst tetrakis(triphenylphosphine)palladium ($Pd(PPh_3)_4$) to produce 3-(4-bromophenyl)-4-nitropyridine (**1**) in 72% yield, which was cyclized using triethyl phosphite (4–8 equiv) at 110 °C to provide 7-bromo-5*H*-pyrido[4,3-*b*]indole (**2**) in 70% yield. The literature yield was 40%.[17] Careful control of reaction temperature and time (3 h) in the cyclization step improved the yield of **2**. Compound **2** was used for further Suzuki coupling reaction with (6-fluoropyridin-3-yl)boronic acid to give T807 in 53% yield.

The synthesis of nitro-precursor T807P (7-(6-nitropyridin-3-yl)-5*H*-pyrido[4,3-*b*]indole, **5**) and *t*-Boc-protected T807P (*tert*-butyl 7-(6-nitropyridin-3-yl)-5*H*-pyrido[4,3-*b*]indole-5-carboxylate, **6**) was outlined in Scheme 2. The Suzuki coupling reaction of the intermediate **2** with 2-nitro-5-pyridineboronic acid pinacol ester gave T807P in only 7% yield, although the reaction conditions including catalyst ligands, solvents and bases have been optimized. The low yield is attributed to the low reactivity of boronic acid ester, since this boronic acid ester contains: (1) strong electron-withdrawing nitro group in pyridinyl ring at its *para*-position; and (2) pyridinyl nitrogen at its *meta*-position resulting in the electron deficiency of pyridinyl ring, and both all decrease the reactivity of the boronic acid ester. Thus alternate reagent and more reactive method need to be developed. To improve the yield of T807P, a Stille cross-coupling reaction was employed to prepare T807P in 26% yield, using 2-nitro-5-(trimethylstannyl)pyridine (**4**) as the replacement of the boronic acid ester to react with the intermediate **2**. The halogen-tin exchange reaction of 5-bromo-2-nitropyridine with hexamethylditin in refluxing dioxane in the presence of the catalyst $Pd(PPh_3)_4$ gave **4** in 55% yield. Another precursor *t*-Boc-protected T807P was prepared from T807P with di-*tert*-butyl-dicarbonate {$(Boc)_2O$} using base catalyst 4-*N*,*N*-dimethylaminopyridine (DMAP) in 85% yield.[19]

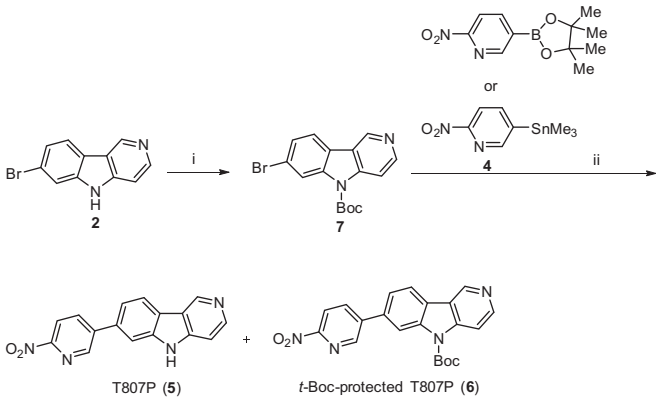
Further studies showed the –NH in compound **2** may result in by-product and lower yield in Suzuki coupling and Stille cross-coupling reactions (Scheme 2). Consequently, alternate synthesis of T807P and *t*-Boc-protected T807P was designed and conducted as shown in Scheme 3. Boc-protected intermediate **7** was synthesized from compound **2** with $(Boc)_2O$ and DMAP in 86% yield. Compound **7** was reacted with 2-nitro-5-pyridineboronic acid pinacol ester or **4** using the same procedure for the preparation of T807P (Scheme 2) to afford two precursors **5** (10–16% yield) and **6** (15–32% yield) at the same time, and the yields of **5** and **6** was changed while reaction time was different. If the reaction time is longer, the yield of **6** will decrease, since the *t*-Boc protecting group is not very stable under high reaction temperature, and can be deprotected under mildly basic conditions.[18,19]



Scheme 1. Synthesis of T807 (**3**). Reagents, conditions and yields: (i) $Pd(PPh_3)_4$, Na_2CO_3, 1,4-dioxane, H_2O, reflux; 72%. (ii) $P(OEt)_3$, 110 °C; 70%. (iii) (6-fluoropyridin-3-yl)boronic acid, $Pd(OAc)_2$, 2-dicyclohexylphosphinobiphenyl, K_2CO_3, dimethoxyethane (DME), H_2O, reflux; 53%.



Scheme 2. Synthesis of T807P (**5**) and *t*-Boc-protected T807P (**6**). Reagents, conditions and yields: (i) $(Me_3Sn)_2$, $Pd(PPh_3)_4$, 1,4-dioxane, reflux; 55%. (ii) $Pd(PPh_3)_4$, Cs_2CO_3, 1,4-dioxane, reflux; 7% (from 2-nitro-5-pyridineboronic acid pinacol ester) and 26% (from **4**). (iii) DMAP, $(Boc)_2O$, CH_2Cl_2; 85%.

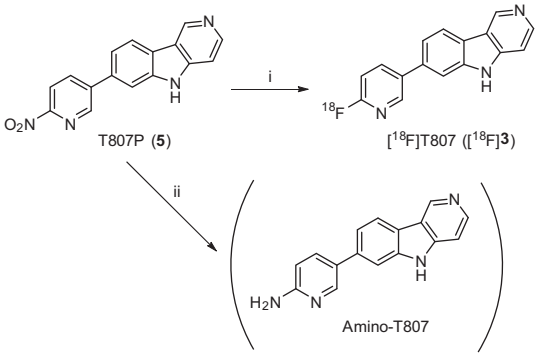


Scheme 3. Alternate synthesis of T807P (**5**) and *t*-Boc-protected T807P (**6**). Reagents, conditions and yields: (i) DMAP, $(Boc)_2O$, CH_2Cl_2; 86%. (ii) $Pd(PPh_3)_4$, Cs_2CO_3, 1,4-dioxane, reflux; **5** (10–16%) and **6** (15–32%).

Synthesis of the target tracer [^{18}F]T807 (7-(6-[^{18}F]fluoropyridin-3-yl)-5*H*-pyrido[4,3-*b*]indole, [^{18}F]**3**) from T807P is shown in Scheme 4. The nitro-precursor T807P was labeled with K[^{18}F]F/Kryptofix 2.2.2 through nucleophilic substitution at 140 °C to form [^{18}F]T807.[12,13,16,18] It is difficult to directly purify labeled product [^{18}F]T807 from unlabeled precursor T807P by a semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) due to their close polarity. Therefore, the reduction of $-NO_2$ to $-NH_2$ is needed after F-18 labeling step. The reaction mixture was treated with Fe powder with formic acid, quenched with 0.1 M $NaHCO_3$, filtered, and purified by HPLC combined with solid-phase extraction (SPE) using a C18 Plus Sep-Pak cartridge to give pure tracer [^{18}F]T807, which was formulated in ethanol/saline. This is a two-step-two-pot radiosynthesis. DMSO is a good reaction solvent for [^{18}F]fluorination at high reaction temperature, since it resulted in higher radiochemical yield.[20–24] However, the solubility of T807P in DMSO is not very high, and thus F-18 labeling yield was low.[18] To reduce excess amount of the unreacted precursor T807P (1 mg) to amino-T807, huge amount of Fe powder (90–100 mg) need to be used, thus it is not easy to perform routine automation, and instead manual synthesis is required. The overall synthesis, purification and formulation time was ~90 min from end of bombardment (EOB), and decay corrected radiochemical yield at EOB based on H[^{18}F]F was 5–10%. Other group[18] has proved it was unable to reach the reported high radiochemical yield (29–47%),[12,13] and our manual procedure reported here was not able to give high radiochemical yield as well.

Synthesis of the target tracer [^{18}F]T807 from *t*-Boc-protected T807P is shown in Scheme 5. *t*-Boc-protected T807P was labeled with K[^{18}F]F/Kryptofix 2.2.2 in DMSO at 140 °C via a concurrent [^{18}F]fluorination and deprotection and purified by RP-HPLC/SPE method to give [^{18}F]T807 in 20–30% decay corrected radiochemical yield. This is a one-step-one-pot radiosynthesis, which was performed in a self-designed and fully automated multi-purpose [^{18}F]-radiosynthesis module.[23,25,26] The solubility of *t*-Boc-protected T807P in DMSO is increased compared to T807P, and thus it improved F-18 labeling yield. *t*-Boc-protected T807P is concurrently deprotected during nucleophilic F-18 substitution with K[^{18}F]F/Kryptofix 2.2.2 at high reaction temperature (140 °C).[18] The overall synthesis, purification and formulation time was 60–70 min from EOB. *t*-Boc-protected T807P has made more easily amenable for routine automation with relatively short radiosynthesis time and much higher radiochemical yield.

Several modifications have been made to improve the chemical purity and specific activity (SA) of the target tracer [^{18}F]T807. 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[20–23] gave better



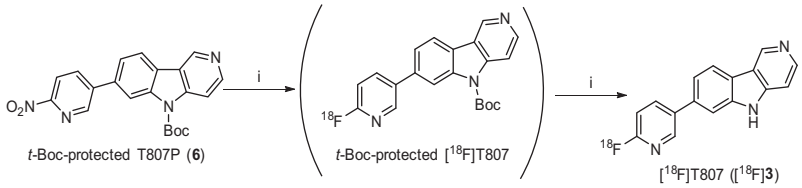
Scheme 4. Synthesis of [^{18}F]T807 ([^{18}F]**3**) from T807P (**5**). Reagents, reaction conditions and yields: (i) K[^{18}F]/K2.2.2, DMSO, 140 °C, 15 min; (ii) Fe powder/HCOOH, 110 °C, 10 min; HPLC-SPE; 5–10%.

separation of labeled product [^{18}F]T807 from its corresponding precursor *t*-Boc-protected T807P and unreacted [^{18}F]fluoride. The amounts of the precursor *t*-Boc-protected T807P used were ~1 mg. Small amount of the precursor was used for radiolabeling instead of large amount of the precursor (2.5 mg) reported in the literature,[11] 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]T807 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.[16] The SA was 37–370 GBq/μmol at EOB. 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 (Siemens RDS-111 Eclipse) and home-built automated [^{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]FEDAA1106, etc.[20–23] 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.[27] The SA of the title tracer [^{18}F]T807 was 37–370 GBq/μmol at EOB, which was similar to the values previously reported by our lab.[20–23] No-carrier-added [^{18}F]fluoride ion in [^{18}O]water was trapped without a QMA cartridge. This way[20–23,28] significantly increased the SA of the prepared F-18 labeled product. As indicated in the literature,[28] 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]T807 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.[20–23] The reason was that there was a low-level contamination of QMA anionic resins with fluoride ion.[28]

Furthermore, in order to make more product radioactivity, we also modified the published semi-preparative HPLC conditions[12,13,18] including column, mobile phase and flow rate to shorten the retention time of [^{18}F]T807. Although this modification helped to shorten the overall production time, it potentially could bring the organic solvent residue issue (CH_3CN from mobile phase) since CH_3CN residue has limit restriction for clinical usage. In addition, to our F-18 labeling experiences on F-18 tracers,[16,20–23] 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 (0.1–0.3 μg/mL) contaminating the tracer solution. [^{18}F]T807 was also in the same case. Therefore, we used a C-18 Plus Sep-Pak cartridge for the purposes to further remove the organic solvent residue from HPLC mobile phase, the precursor and most of possible non-radiolabeled undesired side-products. The product fraction (5–10 mL) was diluted in 30 mL water, passed through the C18 cartridge, and washed with 4 × 5 mL water. Thus, the CH_3CN residue in the final product (EtOH/saline solution) is lower than limit, which can be detected by gas chromatography (GC) in quality control (QC) analysis. A C-18 Plus Sep-Pak cartridge instead of rotatory evaporation was used to significantly improve the chemical purity of the tracer solution.[16,20–23] In this study, the Sep-Pak purification can further increase the chemical purity more than 10%.

Chemical purity and radiochemical purity were determined by analytical HPLC.[29] The chemical purity of T807, T807P and *t*-Boc-protected T807P was >93% determined by HPLC through UV flow detector. The radiochemical purity of the target tracer [^{18}F]T807 was >98% determined by radio-HPLC through γ-ray (PIN diode) flow detector.

The experimental details and characterization data for compounds **1**–**7** and for the tracer [^{18}F]**3** are given.[30]



Scheme 5. Synthesis of [^{18}F]T807 ([^{18}F]**3**) from *t*-Boc-protected T807P (**6**). Reagents, reaction conditions and yields: (i) K[^{18}F]/K2.2.2, DMSO, 140 °C, 15 min; HPLC-SPE; 20–30%.

In summary, T807 was synthesized by the patent method with modifications and improved yields. Synthesis of the precursors T807P and *t*-Boc-protected T807P is first reported. A two-step-two-pot manual radiosynthesis of [^{18}F]T807 from T807P has described for comparison. An improved, concise and automated one-step-one-pot radiosynthesis of [^{18}F]T807 from *t*-Boc-protected T807P has been developed, which were superior to previous works. The radiosynthesis employed a concurrent [^{18}F]fluorination and deprotection. 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, radiochemical purity and chemical purity. New and improved results in the synthetic methodology, radiolabeling, preparative separation and analytical details for T807, T807P, *t*-Boc-protected T807P and [^{18}F]T807 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]T807 as a PET AD tau tracer in animals and humans.

Acknowledgments

This work was partially supported by the United States 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 the United States National Science Foundation (NSF) Major Research Instrumentation Program (MRI) grant CHE-0619254.

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30. *(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 23% CH_3CN/77% 20 mM H_3PO_4; 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 25% CH_3CN/75% 20 mM H_3PO_4; 5.0 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). 3-(4-Bromophenyl)-4-nitropyridine (**1**):* To a solution of 1,4-dioxane (60 mL) and water (10 mL) was added 3-bromo-4-nitropyridine (1.02 g, 5.0 mmol), (4-bromophenyl)boronic acid (1.10 g, 5.5 mmol), $Pd(PPh_3)_4$ (0.23 g, 0.2 mmol), and Na_2CO_3 (1.33 g, 12.5 mmol). Then the reaction was allowed to stir at reflux temperature (110 °C) for 18 h. The reaction was cooled down, poured into water (40 mL) and extracted with EtOAc (80 mL × 3). The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated in reduced pressure. The residue was purified by silica gel column chromatography using an eluent (10:90 EtOAc/hexanes) to afford a yellow

solid product **1** (1.0 g, 72%). R_f = 0.50 (1:2 EtOAc/hexanes), mp 135–137 °C. ^{1}H NMR ($CDCl_3$): δ 7.21 (d, J = 8.0 Hz, 2H, Ph-H), 7.61 (d, J = 8.0 Hz, 2H, Ph-H), 7.68 (d, J = 5.2 Hz, 1H, Py-H), 8.78 (s, 1H, Py-H), 8.84 (d, J = 5.2 Hz, 1H, Py-H). ^{1}H NMR (DMSO-d_6): δ 7.41 (dt, J = 2.2, 9.0 Hz, 2H, Ph-H), 7.71 (dt, J = 2.2, 9.0 Hz, 2H, Ph-H), 8.03 (d, J = 5.5 Hz, 1H, Py-H), 8.87 (s, 1H, Py-H), 8.93 (d, J = 5.5 Hz, 1H, Py-H). MS (ESI): 279 ([M+H]$^+$, 100%).

*(c). 7-Bromo-5H-pyrido[4,3-b]indole (**2**):* A solution of compound **1** (0.84 g, 3.0 mmol) in 30 mL of triethyl phosphate was heated at 110 °C for 3 h under N_2. The reaction was monitored with TLC for every 1 h. After the starting material was disappeared, the reaction mixture was cooled down, and volatiles were removed in vacuo. The residue was purified by silica gel chromatography using an eluent (2:98 MeOH/CH_2Cl_2) to give a light brown color solid product **2** (0.52 g, 70%). R_f = 0.48 (1:12 MeOH/CH_2Cl_2), mp 258–260 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 7.42 (dd, J = 2.0, 8.5 Hz, 1H, Ar-H), 7.49 (dd, J = 1.0, 5.5 Hz, 1H, Ar-H), 7.76 (d, J = 2.0 Hz, 1H, Ar-H), 8.1 (d, J = 8.5 Hz, 1H, Ar-H), 8.4 (d, J = 5.5 Hz, 1H, Ar-H), 9.36 (s, 1H, Ar-H), 11.82 (s, 1H, NH). MS (ESI): 247 ([M+H]$^+$, 100%).

*(d). 7-(6-Fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole (**3**, T807):* Palladium(II) acetate (18 mg, 0.08 mmol) was added to a solution containing compound **2** (198 mg, 0.8 mmol), (6-fluoropyridin-3-yl)boronic acid (158 mg, 1.12 mmol), dicyclohexylphosphinobiphenyl (30.8 mg, 0.088 mmol) and K_2CO_3 (332 mg, 2.4 mmol) in DME (50 mL) and water (6 mL). The reaction mixture was refluxed for 48 h, cooled down to room temperature (rt), diluted with water and extracted with ethyl acetate (50 mL × 3). The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated in reduced pressure. The residue was purified by silica gel chromatography using an eluent (2:98 MeOH/CH_2Cl_2) to obtain a white solid product **3** (112 mg, 53%). R_f = 0.33 (1:12 MeOH/CH_2Cl_2), mp >300 °C. ^{1}H NMR (acetone-d_6): δ 7.20 (ddd, J = 0.5, 3.5, 8.5 Hz, 1H, Ar-H), 7.50 (dd, J = 0.8, 6.0 Hz, 1H, Ar-H), 7.61 (dd, J = 1.5, 8.0 Hz, 1H, Ar-H), 7.89 (d, J = 1.0 Hz, 1H, Ar-H), 8.30–8.33 (m, 1H, Ar-H), 8.35 (d, J = 8.0 Hz, 1H, Ar-H), 8.47 (d, J = 5.0 Hz, 1H, Ar-H), 8.58 (dd, J = 0.5, 2.0 Hz, 1H, Ar-H), 9.39 (s, 1H, Ar-H), 10.95 (br s, 1H, NH). MS (ESI): 264 ([M+H]$^+$, 100%). MS (ESI): 262 ([M−H]$^-$, 20%).

*(e). 2-Nitro-5-(trimethylstannyl)pyridine (**4**):* A mixture of 5-bromo-2-nitropyridine (0.81 g, 4.0 mmol), hexamethylditin (1.84 g, 5.6 mmol), $Pd(PPh_3)_4$ (169 mg, 0.16 mmol) in 1,4-dioxane (50 mL) was heated to reflux for 6 h. Then the reaction mixture was diluted with water, and extracted with ethyl acetate (60 mL × 3). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by silica gel column chromatography using an eluent (10:90 EtOAc/hexanes) to give a colorless solid product **4** (0.63 g, 55%). R_f = 0.52 (1:5 EtOAc/hexanes), mp 109–110 °C. ^{1}H NMR ($CDCl_3$): δ 0.42 (s, 9H, 3 × CH_3), 8.12–8.18 (m, 2H, Ar-H), 8.65–8.66 (m, 1H, Ar-H). MS (ESI): 289 ([M+H]$^+$, 100%).

*(f). 7-(6-Nitropyridin-3-yl)-5H-pyrido[4,3-b]indole (**5**, T807P):* Method A: A mixture of compound **2** (99 mg, 0.4 mmol), 2-nitro-5-pyridineboronic acid pinacol ester (160 mg, 0.64 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol), and Cs_2CO_3 (391 mg, 1.2 mmol) in 1,4-dioxane (100 mL) was stirred at 100 °C under N_2 atmosphere for 20 h. After cooling to RT, the volatiles were removed under reduced pressure. The residue was purified by silica gel chromatography using an eluent (3:97 MeOH/CH_2Cl_2) to afford a yellow solid product **5** (8 mg, 7%). R_f = 0.32 (1:12 MeOH/CH_2Cl_2), mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 7.52 (d, J = 5.5 Hz, 1H, Ar-H), 7.76 (dd, J = 1.5, 8.0 Hz, 1H, Ar-H), 7.98–8.04 (m, 1H, Ar-H), 8.42–8.47 (m, 3H, Ar-H), 8.61 (dd, J = 2.5, 8.5 Hz, 1H, Ar-H), 9.12 (d, J = 2.0 Hz, 1H, Ar-H), 9.42 (s, 1H, Ar-H), 11.96 (s, 1H, NH). MS (ESI): 291 ([M+H]$^+$, 100%). MS (ESI): 289 ([M−H]$^-$, 40%). Method B (from **4**): A mixture of compound **2** (99 mg, 0.4 mmol), **4** (184 mg, 0.64 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol), and Cs_2CO_3 (391 mg, 1.2 mmol) in 1,4-dioxane (100 mL) was stirred at 100 °C under N_2 atmosphere for 20 h. After cooling to RT, the volatiles were removed under reduced pressure. The residue was purified by silica gel chromatography using an eluent (3:97 MeOH/CH_2Cl_2) to obtain a yellow solid product **5** (30 mg, 26%). Analytical data was identical with Method A.

*(g). tert-Butyl 7-(6-nitropyridin-3-yl)-5H-pyrido[4,3-b]indole-5-carboxylate (**6**, t-Boc-protected T807P):* Di-*tert*-butyl-dicarbonate (($Boc)_2O$, 105 mg, 0.48 mmol) was added to a mixture of DMAP (44 mg, 0.36 mmol) and compound **5** (87 mg, 0.3 mmol) in dichloromethane (50 mL) at rt. The resulting reaction mixture was stirred for 8 h and then was concentrated. The residue was purified by silica gel chromatography using an eluent (2:98 MeOH/CH_2Cl_2) to afford a yellow solid product **6** (100 mg, 85%). R_f = 0.77 (1:12 MeOH/CH_2Cl_2), mp >300 °C. ^{1}H NMR ($CDCl_3$): δ 1.80 (s, 9H, 3 × CH_3), 7.70 (dd, J = 1.5, 8.0 Hz, 1H, Ar-H), 8.14 (d, J = 5.5 Hz, 1H, Ar-H), 8.22 (d, J = 8.5 Hz, 1H, Ar-H), 8.32 (dd, J = 2.5, 8.5 Hz, 1H, Ar-H), 8.39 (dd, J = 0.5, 8.5 Hz, 1H, Ar-H), 8.69 (d, J = 6.0 Hz, 1H, Ar-H), 8.70 (s, 1H, Ar-H), 8.97 (dd, J = 0.5, 2.0 Hz, 1H, Ar-H), 9.34 (s, 1H, Ar-H). MS (ESI): 391 ([M+H]$^+$, 100%). MS (ESI): 413 ([M+Na]$^+$, 20%).

*(h). tert-Butyl 7-bromo-5H-pyrido[4,3-b]indole-5-carboxylate (**7**):* The mixture of compound **2** (495 mg, 2.0 mmol), DMAP (293 mg, 2.4 mmol), and ($Boc)_2O$ (698 mg, 3.2 mmol) in CH_2Cl_2 (70 mL) was stirred at rt for 6 h, and then was concentrated to dryness. The solid residue was purified by silica gel chromatography using an eluent (2:98 MeOH/CH_2Cl_2) to give a white solid product **7** (597 mg, 86%). R_f = 0.79 (1:12 MeOH/CH_2Cl_2), mp 156–158 °C. ^{1}H NMR ($CDCl_3$): δ 1.77 (s, 9H, 3 × CH_3), 7.53 (dd, J = 1.5, 8.0 Hz, 1H, Ar-H), 7.89 (d, J = 8.0 Hz, 1H, Ar-H), 8.08 (d, J = 6.0 Hz, 1H, Ar-H), 8.54 (d, J = 0.5 Hz, 1H, Ar-H), 8.64 (d, J = 6.0 Hz, 1H, Ar-H), 9.23 (d, J = 1.0 Hz, 1H, Ar-H). MS (ESI): 347 ([M+H]$^+$, 100%).

*(i). General procedure for preparation of compound **5** and **6**:* These two compounds were synthesized from compound **7** and 2-nitro-5-pyridineboronic acid pinacol ester or compound **4** according to the procedure previously described for the synthesis of compound **5**. From 2-nitro-5-pyridineboronic acid pinacol ester, yield (**5**, 10% and **6**, 15%); from **4**, yield (**5**, 16% and **6**, 32%). Analytical data was identical with that previously described.

*(j). 7-(6-[^{18}F]Fluoropyridin-3-yl)-5H-pyrido[4,3-b]indole ([^{18}F]**3**, [^{18}F]T807):* Method A (from **5**): 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 T807P (**5**, 1 mg) dissolved in DMSO (1.0 mL) was introduced to the reaction vessel and heated at 140 °C for 15 min to affect radiofluorination. After cooling to 60 °C, the reaction mixture was transferred to another 10-mL V-vial containing 90–100 mg Fe powder with 1.0 mL formic acid. The mixture was heated to 110 °C for 10 min, cooled to 60 °C, and then quenched with 0.1 M $NaHCO_3$ (3.0 mL). The mixture was filtered, 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]**3**, 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 **5** = 7.85 min, t_R **3** = 9.88 min, t_R [^{18}F]**3** = 9.91 min. Retention times in the analytical HPLC system were: t_R **5** = 6.56 min, t_R **3** = 7.53 min, t_R [^{18}F]**3** = 7.65 min. The decay corrected radiochemical yields of [^{18}F]**3** were 5–10%. Method B (from **6**): The precursor *t*-Boc-protected T807P (**6**, 1 mg) dissolved in DMSO (1.0 mL) was introduced to the reaction vial containing dry K[^{18}F]F/Kryptofix 2.2.2 complex as previously described and heated at 140 °C for 15 min for [^{18}F]fluorination to afford *t*-Boc-protected [^{18}F]T807, which was concurrently deprotected during [^{18}F]fluorination to form [^{18}F]T807. The contents of the reaction vial were cooled down to ~90 °C 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]**3**, 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 **6** = 13.45 min, t_R **3** = 9.88 min, t_R [^{18}F]**3** = 9.91 min. Retention times in the analytical HPLC system were: t_R **6** = 9.04 min, t_R **3** = 7.53 min, t_R [^{18}F]**3** = 7.65 min. The decay corrected radiochemical yields of [^{18}F]**3** were 20–30%.