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Synthesis of [^{18}F]SU11248, a new potential PET tracer for imaging cancer tyrosine kinase

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Abstract—*N*-[2-(Diethylamino)ethyl]-5-[(*Z*)-(5-[^{18}F]fluoro-2-oxo-1, 2-dihydro-3*H*-indol-3-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide, a new potential positron emission tomography tracer for imaging cancer tyrosine kinase, has been prepared by the nucleophilic substitution of the nitro-precursor *N*-[2-(diethylamino)ethyl]-5-[(*Z*)-(5-nitro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide with K^{18}F/Kryptofix 2.2.2 followed by a simple chromatography methodology combined solid-phase extraction with high-performance liquid chromatography purification procedures in 15–25% radiochemical yields.

N-[2-(Diethylamino)ethyl]-5-[(*Z*)-(5-fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (SU11248) is a novel tyrosine kinase inhibitor developed by SUGEN, which targets both vascular endothelial growth factor (VEGF) and plated-derived growth factor (PDGF) receptor tyrosine kinases (RTKs) and has been entered to clinical trials as an antitumor drug for the treatment of cancers.[1] The overexpression of the RTKs in tumors indicates that the RTKs are the suitable target for tumor imaging agent development. Positron emission tomography (PET) is a functional biomedical imaging modality that can probe tumor cell physiology. PET and a positron-labeled tyrosine kinase inhibitor that inhibits selectively to RTKs may prove to be a useful tool for monitoring RTK levels in tumor tissues and for evaluating the effectiveness of the antitumor drug. To further develop therapeutic drug SU11248 as a diagnostic agent, we report here the design and synthesis of *N*-[2-(diethylamino)ethyl]-5-[(*Z*)-(5-[^{18}F]fluoro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide ([^{18}F]SU11248) through a simple chromatography-methodology-combined solid-phase extraction (SPE) and high-performance liquid chromatography (HPLC) purification procedures for the first time.

The synthesis of the precursor *N*-[2-(diethylamino)ethyl]-5-[(*Z*)-(5-nitro-2-oxo-1,2-dihydro-3*H*-indol-3-ylidene) methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**9**) and reference standard SU11248 (**1**) was performed using a modification of the literature procedure.[1,2] The synthetic approach is outlined in Scheme 1. Treatment of diketene (**2**) with *N*,*N*-diethylethylenediamine in *tert*-butyl methyl ether furnished *N*-[2-(diethylamino)ethyl]-3-oxobutanamide (**4**) in excellent yield (~100%). The β-ketoamide **4** was prone to decomposition and, therefore, had to be used immediately. Oxime *tert*-butyl 2-(hydroxyimino)-3-oxo-butanoate (**5**) derived from *tert*-butyl acetoacetate (**3**) in quantitative yield was treated with amide **4** in the presence of zinc and acetic acid according to the classic Knorr pyrrole formation conditions, which led to pyrrole *tert*-butyl 4-[[[2-(diethylamino)ethyl]amino]carbonyl]-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (**6**) in 58% yield. The decarboxylation of pyrrole **6** under acidic conditions (H_2SO_4/MeOH) provided the decarboxylated product *N*-[2-(diethylamino)ethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide (**7**) in 87% yield. The α-free pyrrole **7** was treated with chloromethylenedimethylammonium chloride in acetonitrile to form the Vilsmeier–Haack adduct **8** in situ.[2] Addition of 5-fluorooxindole and KOH to the reaction mixture at this stage through Eschenmoser type condensation afforded the reference standard SU11248 (**1**) in 54% yield. Similarly, addition of 5-nitrooxindole and KOH to the reaction mixture afforded the nitro-precursor (**9**) in 61% yield. The overall chemical yields for

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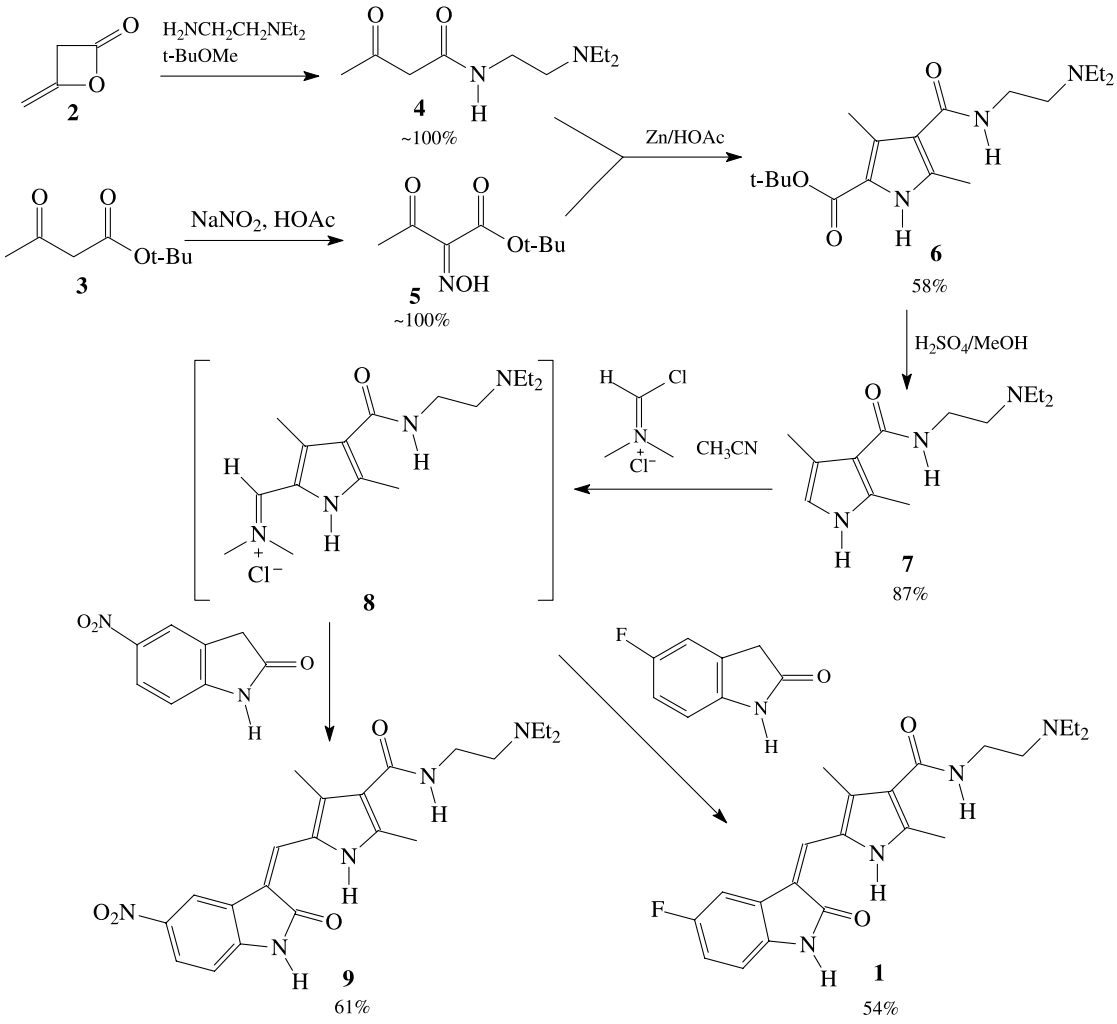
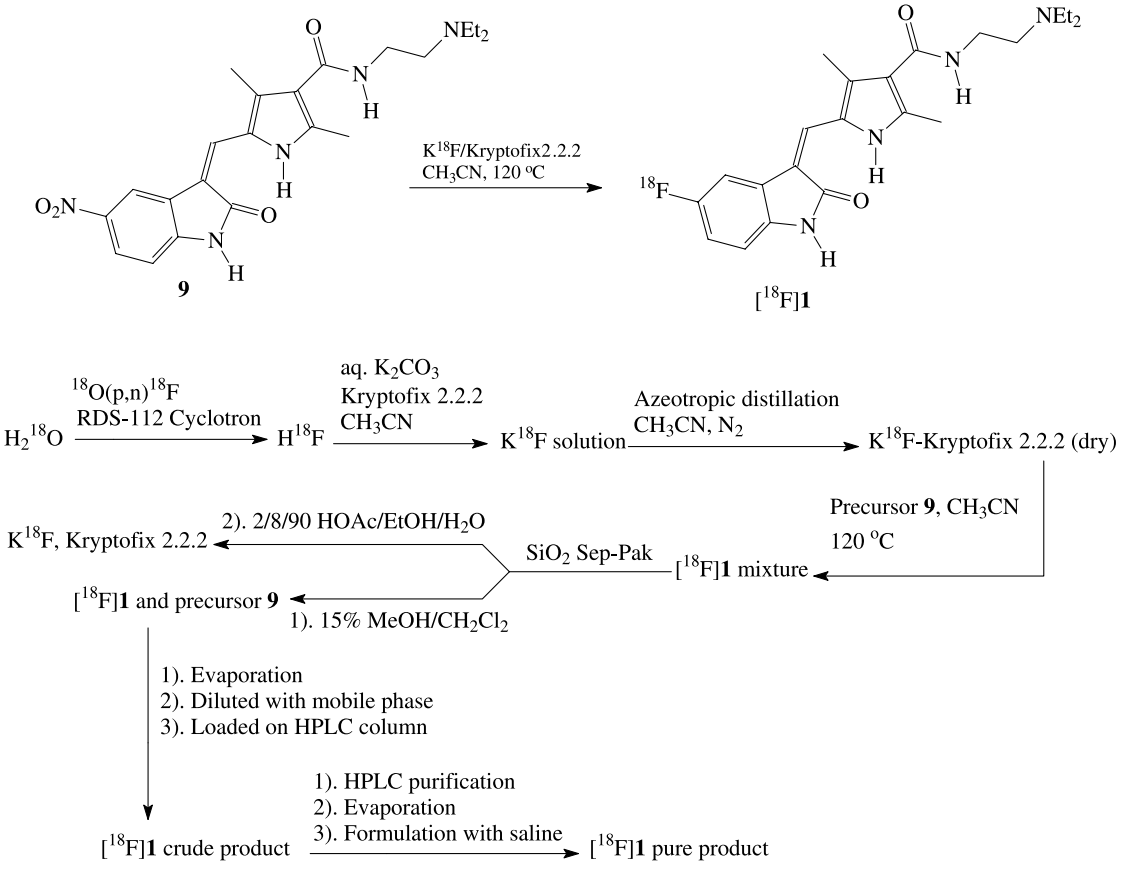


Scheme 1. Synthesis of SU11248 (**1**) and nitro-precursor (**9**).

the reference standard **1** and nitro-precursor **9** in five steps are moderate.

The nitro-precursor **9** was labeled by a conventional nucleophilic substitution[3–7] with $K^{18}F$/Kryptofix 2.2.2 in acetonitrile at 120 °C for 15–20 min to provide target tracer [^{18}F]SU11248 ([^{18}F]**1**). The synthetic approach is indicated in Scheme 2. The radiolabeling reaction is a key step in the radiosynthesis of final compound. In this reaction, Kryptofix 2.2.2 was used as a phase transfer catalyst, which catalyzed the fluorination of nitro-precursor. A novel chromatography-methodology-combined SPE with HPLC techniques was employed in the isolation and purification of the target tracer from radiolabeling reaction mixture. A flow chart for the isolation procedure is shown in Scheme 2. The radiolabeling mixture was passed through a Silica Sep-Pak to remove Kryptofix 2.2.2 and nonreacted $K^{18}F$. The large polarity difference between nitro-precursor, labeled product, Kryptofix 2.2.2, and nonreacted $K^{18}F$ permitted the use of a simple SPE technique[8–11] for fast isolation of nitro-precursor and labeled product from the radiolabeling reaction mixture. The key part in this technique is a SiO_2 Sep-Pak type cartridge that contains ~0.5–2 g of adsorbent. The Sep-Pak was eluted with 15% MeOH/CH_2Cl_2 and the solvent was evaporated under high vacuum to give the mixture residue of the precursor and product, and then eluted with 2/8/90 HOAC/EtOH/H_2O to desalt $K^{18}F$ and remove the Kryptofix 2.2.2, so that the SiO_2 Sep-Pak cartridge can be repeatedly used in the tracer production. The existence of the catalyst Kryptofix 2.2.2 and nonreacted $K^{18}F$ would affect the purification of labeled product from its mixture with precursor and the quality control of the tracer production;[12] therefore, they needed to be removed before [^{18}F]**1** was separated from the mixture. The SPE technique reduces the tasks and complexity prior to HPLC purification of the radiolabeling mixture. Then, the mixture containing precursor and product was diluted with HPLC mobile phase and loaded on the HPLC column and purified by semipreparative HPLC method. The retention time (RT) difference between target tracer [^{18}F]**1** and the precursor **9** in the preparative HPLC system we used was ~1.7 min, which warranted the efficient purification of the target tracer from unlabeled precursor. The nitro-precursor has similar structure with the final fluoro-tracer, they may have similar biological activity, and the precursor would affect the PET studies of the tracer. Therefore, the precursor needed to be removed before [^{18}F]**1** was formulated in saline as final



Scheme 2. Synthesis, isolation, and purification of [^{18}F]SU11248 ([^{18}F]**1**).

pure product for further study. The radiochemical yield of [^{18}F]**1** was 15–25% at the end of bombardment (EOB).

The application of combined chromatography methodology using both SPE and HPLC techniques simplified the isolation and purification procedures of the radiosynthesis of [^{18}F]SU11248, so that it will be amenable for automation.[5] The optimization through SPE and HPLC methods for the tracer [^{18}F]SU11248 production provides a general and useful methodology, which could be used to purify all fluorine-18 PET radiotracers prepared by nucleophilic substitution of the precursors radiolabeling with K^{18}F/Kryptofix 2.2.2.

The experimental details are given for the new compound nitro-precursor **9** and tracer [^{18}F]**1**, and only characterization data are given for other known compounds **4**–**7** and **1**.[13]

In conclusion, a novel and general chromatography-methodology-combined SPE with HPLC techniques has been well developed, and the detailed isolation and purification procedures used in the radiosynthesis of [^{18}F]SU11248 have been described. The synthetic procedures that provide nitro-precursor and reference standard SU11248 also have been developed in this regard. These methods are efficient and convenient, and work very well for the routine production of a variety of other fluorine-18 PET tracers used in our PET imaging center. These chemistry results provide the foundation for further evaluation of the tracer [^{18}F]SU11248 as a new potential PET tumor imaging agent for tyrosine kinase in vivo.

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13. Experimental details and characterization data. (a) General: all commercial reagents and solvents were 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 QE 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal standard TMS (δ 0.0). Low-resolution mass spectra were obtained using a Bruker Biflex III MALDI-TOF mass spectrometer, and high-resolution mass measurements were obtained using a Kratos MS80 mass spectrometer, in the Department of Chemistry at Indiana University. Chromatographic solvent proportions are expressed on a volume:volume basis. Thin-layer chromatography was run using Analtech silica gel GF uniplates (5 × 10 cm). Plates were visualized by UV light. Normal phase flash 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-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Sterile Millex-GS 0.22-μm vented filter unit was obtained from Millipore Corporation (Bedford, MA, USA). (b) Compound **4**: compound **2** and *tert*-butyl methyl ether were reacted with *N*,*N*-diethylethylenediamine to give a brown liquid **4** in 100% yield, which was used directly in next step without further purification. (c) Compound **5**: the reaction of compound **3** and acetic acid with a solution of $NaNO_2$ in water gave product oxime **5** in quantitative yield, and the solution was used directly in next step without further purification. (d) Compound **6**: an off-white solid in 58% yield, R_f = 0.70 (46:4:1, CH_2Cl_2/MeOH/NH_4OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.15 (br s, 1H, NH), 6.41 (br s, 1H, NH), 3.45 (q, 2H, J = 5.9 Hz, HNCH_2CH$_2$N), 2.62 (t, 2H, J = 5.9 Hz, HNCH$_2$CH_2N), 2.54 (q, 4H, J = 7.4 Hz, CH_2CH$_3$), 2.49 (s, 3H, ring-CH$_3$), 2.48 (s, 3H, ring-CH$_3$), 1.57 (s, 9H, *t*-Bu), 1.01 (t, 6H, J = 7.4 Hz, CH$_2$CH_3). (e) Compound **7**: a viscous brown oil in 87% yield, R_f = 0.62 (46:4:1, CH_2Cl_2/MeOH/NH_4OH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.53 (br s, 1H, NH), 6.45 (br s, 1H, NH), 6.34 (s, 1H, olefinic H), 3.45 (q, 2H, J = 5.1 Hz, HNCH_2CH$_2$N), 2.61 (t, 2H, J = 5.9 Hz, HNCH$_2$CH_2N), 2.54 (q, 4H, J = 7.4 Hz, CH_2CH$_3$), 2.47 (s, 3H, ring-CH$_3$), 2.24 (s, 3H, ring-CH$_3$), 1.01 (t, 6H, J = 7.4 Hz, CH$_2$CH_3). (f) Compound **1**: an orange solid in 54% yield, R_f = 0.47 (46:4:1, CH_2Cl_2/MeOH/NH_4OH), mp 215–217 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.31 (s, 1H, NH), 8.90 (s, 1H, NH), 7.22 (s, 1H, olefinic H), 7.14 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.2 Hz, phenyl H), 6.85 (td, 1H, J_1 = 8.8 Hz, J_2 = 2.2 Hz, phenyl H), 6.78 (dd, 1H, J_1 = 8.1 Hz, J_2 = 4.4 Hz, phenyl H), 6.72 (t, 1H, J = 4.4 Hz, CONHCH$_2$), 3.54 (q, 2H, J = 5.2 Hz, HNCH_2CH$_2$N), 2.71 (t, 2H, J = 5.9 Hz, HNCH$_2$CH_2N), 2.64 (q, 4H, J = 6.6 Hz, NCH_2CH$_3$), 2.55 (s, 3H, ring-CH$_3$), 2.31 (s, 3H, ring-CH$_3$), 1.05 (t, 6H, J = 7.4 Hz, NCH$_2$CH_3). (g) Compound **9**: chloromethylenedimethylammonium chloride (1.70 g, 13.28 mmol) was added to a 250-mL two-necked flask equipped with an addition funnel and a nitrogen inlet. CH_3CN (20 mL) was added dropwise via the addition funnel to the flask. The pyrrole **7** (2.98 g, 12.56 mmol) was dissolved in CH_3CN (25 mL) and added to the flask through the addition funnel. The amide chloride gradually dissolved and the reaction solution turned dark orange. After 15 min, an orange solid precipitated out of the solution. The reaction was completed in 40 min. Then, to the above flask were added 5-nitrooxindole (2.0 g, 13.24 mmol) and powdered KOH (2.59 g, 56.11 mmol). The mixture was stirred at room temperature for 2 days. An orange precipitate was filtered, washed with water, and CH_3CN to give an orange solid **9** (3.24 g, 61%), mp 265–266 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 13.95 (s, 1H, NH), 11.90 (s, 1H, NH), 9.24 (d, 1H, J = 2.2 Hz, phenyl H), 8.46 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.2 Hz, phenyl H), 8.41 (s, 1H, olefinic H), 7.90 (t, 1H, J = 5.2 Hz, CONHCH$_2$), 7.44 (d, 1H, J = 8.8 Hz, phenyl H), 3.65–3.80 (m, 4H, NHCH_2CH_2N), 2.95 (q, 4H, J = 5.9 Hz, NCH_2CH$_3$), 2.88 (s, 3H, ring-CH$_3$), 2.87 (s, 3H, ring-CH$_3$), 1.39 (t, 6H, J = 7.4 Hz, NCH$_2$CH_3). LRMS (CI, *m*/*z*): 236 (100%), 426 [(M+H)$^+$, 8.2%]. HRMS (CI, *m*/*z*): calcd for $C_{22}H_{28}N_5O_4$: 426.2141, found: 426.2141. (h) Tracer [^{18}F]**1**: no-carrier-added aqueous H^{18}F (0.5 mL) prepared by ^{18}O(p,n)^{18}F nuclear reaction in a RDS-112 cyclotron on an enriched $H_2{}^{18}O$ water (95+%) target was added to a Pyrex vessel that contains K_2CO_3 (4 mg, in 0.2 mL H_2O) and Kryptofix 2.2.2 (10 mg, in 0.5 mL CH_3CN). Azeotropic distillation at 115 °C with HPLC grade CH_3CN (3× 1 mL) under a nitrogen steam efficiently removed water to form anhydrous K^{18}F–Kryptofix 2.2.2 complex. The nitro-precursor **9** (2–3 mg, dissolved in 0.5 mL CH_3CN) was introduced to the K^{18}F–Kryptofix 2.2.2 complex. The radiolabeling reaction was monitored by analytical radio-HPLC method, in which we employed a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3:1:1, CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, UV (240 nm) and γ-ray (NaI) flow detectors. Retention times (RTs) in the analytical HPLC system were: RT**9** = 2.83 min, RT[^{18}F]**1** = 3.66 min, and RTK^{18}F = 1.88 min. The reaction mixture was sealed and heated at 120 °C for 15–20 min and was subsequently allowed to cool down, at which time the crude product was passed through a Silica Sep-Pak cartridge (Waters Corporate Headquarters, Milford, MA, USA) to remove Kryptofix 2.2.2 and unreacted K^{18}F. The Sep-Pak column was eluted with 15% MeOH/CH_2Cl_2 (5.0 mL), and the fractions were passed onto a rotatory evaporator. The solvent was removed by evaporation under high vacuum (0.1–1.0 mmHg) to give a crude product [^{18}F]**1**. The mixture containing precursor and product was purified with semipreparative HPLC method, in which we employed a Prodigy (Phenomenex) 5 μm C-18 column, 10 × 250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 5.0 mL/min flow rate, UV (240 nm), and γ-ray

(NaI) flow detectors. The contents of the mixture residue were diluted with HPLC mobile phase 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$, and injected onto the semipreparative HPLC column. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product [^{18}F]**1** was formulated in saline, sterile-filtered through a sterile vented Millex-GS 0.22-μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis, purification, and formulation time was 60–70 min from EOB. RTs in the semipreparative HPLC system were: RT**9** = 5.03 min and RT[^{18}F]**1** = 6.74 min. The radiochemical yield of [^{18}F]**1** was 15–25%. Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC method. The chemical purities of precursor **9** and standard sample **1** were >96%, the radiochemical purity of target radiotracer [^{18}F]**1** was >99%, and the chemical purity of radiotracer [^{18}F]**1** was ~93%. The specific radioactivities of radiotracer [^{18}F]**1** were 0.8–1.2 Ci/μmol (n = 3–5) at end of synthesis.