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Synthesis of [^{11}C]Iressa as a new potential PET cancer imaging agent for epidermal growth factor receptor tyrosine kinase

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Abstract—Iressa (Gefitinib) is an orally active inhibitor of epidermal growth factor receptor tyrosine kinase (EGFR-TK) involved in cell signal transduction processes critical to proliferation, apoptosis, repair, and angiogenesis of cancer cells. [^{11}C]Iressa was first designed and synthesized as a new potential positron emission tomography (PET) cancer imaging agent for EGFR-TK in 30–40% radiochemical yield with 4.0–6.0 Ci/μmol specific activity at end of bombardment (EOB).

The epidermal growth factor receptor tyrosine kinase (EGFR-TK) is an epithelial cell membrane receptor with an intracellular tyrosine kinase component.[1] EGFR-TK was involved in cell signal transduction processes and associated with the proliferation, apoptosis, repair, and angiogenesis of cancer cells.[2–4] More than two-thirds of human cancers including breast cancer and lung cancer derive from epithelial tissues and overexpress EGFR-TK. 4-Anilino-quinazolines, exemplified by the prototype PD 153035, have emerged as extremely potent and selective EGFR-inhibitors. Iressa (Gefitinib) is another orally active EGFR-TK inhibitor for cancer chemotherapy. EGFR-TK inhibitors represent a new class of anticancer drugs. The overexpression of EGFR-TK in cancers provides a promising target for the development of cancer imaging agents for in vivo biomedical imaging technique positron emission tomography (PET). PET is a functional imaging modality that can probe tumor cell physiology. PET and an enzyme-based imaging agent, positron-labeled EGFR-TK inhibitor that inhibits selectively to enzyme EGFR-TK, may prove to be a useful tool for monitoring EGFR-TK levels in tumor tissues and for evaluating the effectiveness of the antitumor drug enzyme inhibitor.

Keywords: Iressa; Carbon-11; Positron emission tomography (PET); Epidermal growth factor receptor tyrosine kinase (EGFR-TK); Cancer imaging.
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Radiolabeled Iressa analogues may enable non-invasive monitoring of EGFR-TK in cancers and cancer response to Iressa therapy using PET imaging technique. To develop new diagnostic PET cancer imaging agents based on potential therapeutic drugs, we have designed and synthesized radiolabeled 4-anilino-quinazolines.[5,6] In our previous work, we have developed carbon-11 and fluorine-18 labeled PD compounds as EGFR-TK ligands as indicated in Figure 1.[5] In this ongoing study, we selected Iressa as a target molecule to synthesize positron radiolabeled Iressa analogues (Fig. 1). The synthesis of [^{18}F]Iressa has appeared in the literature.[1,7] Here, we report the first radiosynthesis of [^{11}C]Iressa.

There is very little synthetic information appearing in the literature. Wishing to study this compound in this laboratory, we decided to make our own material by following the literature methods. Although several patents dealing with the synthesis of Iressa have appeared, there are gaps in synthetic detail among them, and certain key steps gave poor yields or were difficult to repeat in our hands. Consequently, we investigated alternate approaches and modifications that eventually resulted in a high-yielding synthesis of Iressa that was superior to previous work. We have developed an improved synthetic approach for the synthesis of reference standard Iressa (**1**) using a modification of the patent procedure[8] and a new desmethylation methodology for the preparation of desmethylated precursor M523595 (**10**) using a modification of the literature method.[9] In this paper,

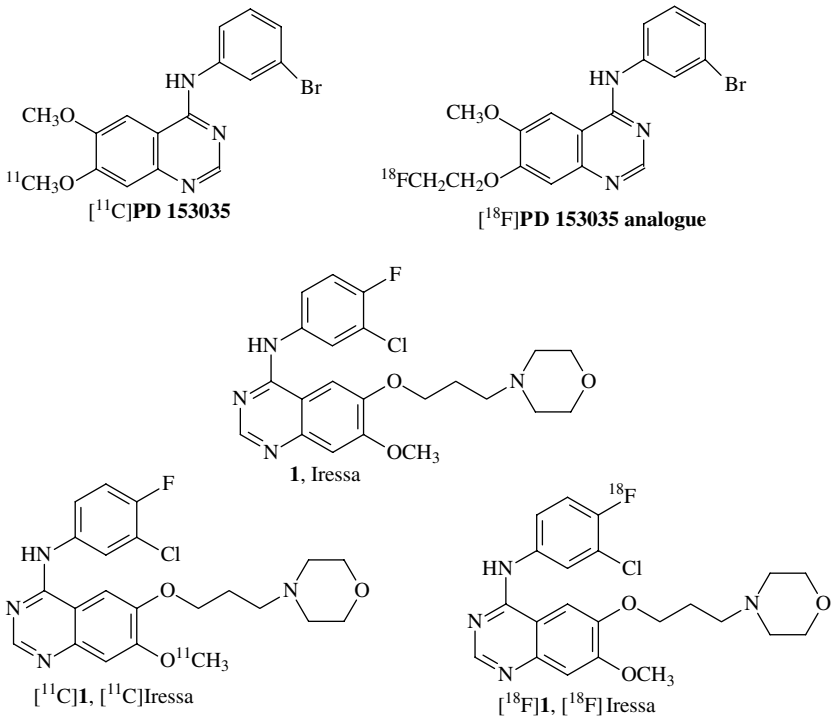
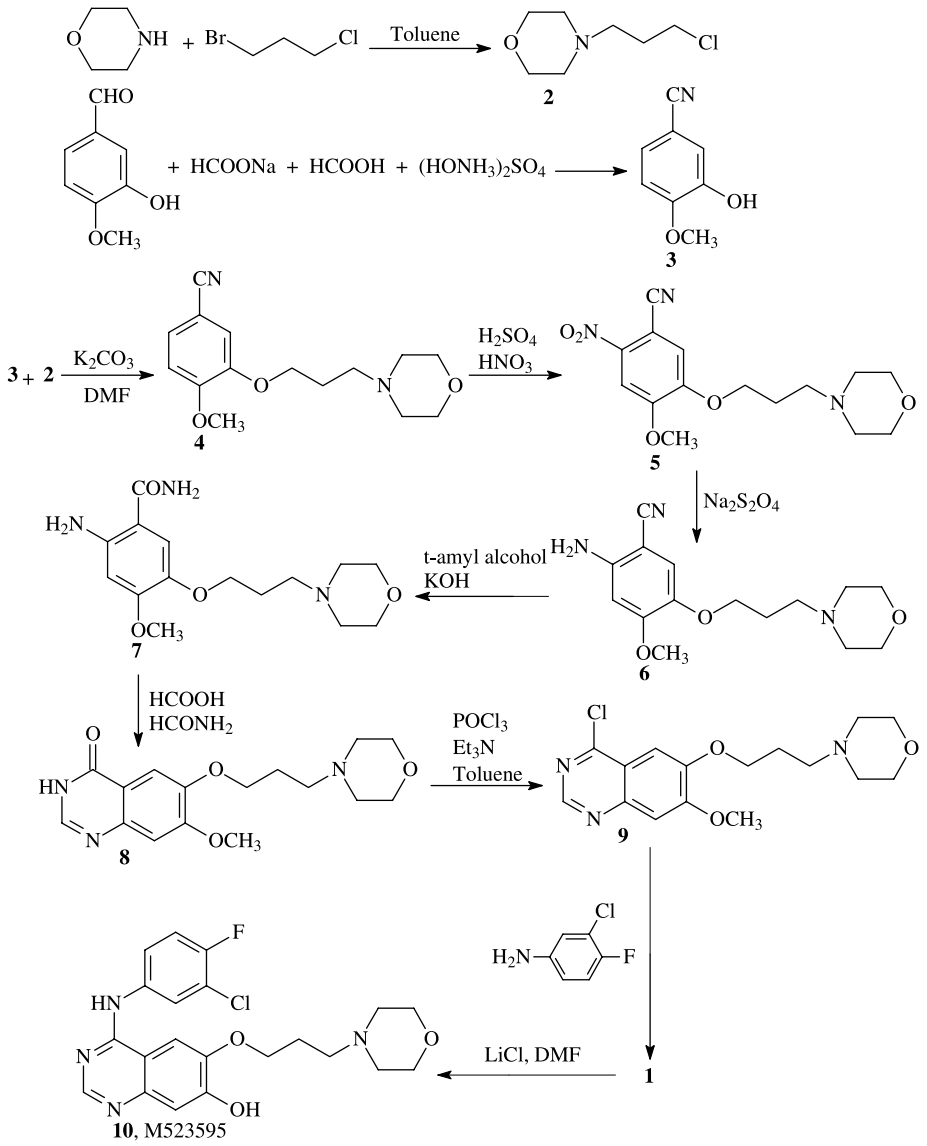


Figure 1. Chemical structures of positron labeled PD 153035 and Iressa analogues.

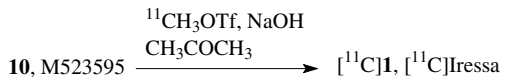
we provide full experimental procedures, yields, and analytical details for this improved Iressa synthesis, as well as new M523595 synthesis. The synthetic approach is outlined in Scheme 1. Commercially available starting material morpholine was reacted with 1-bromo-3-chloropropane to afford *N*-(3-chloropropyl)morpholine (**2**) in 73% yield. Commercially available starting material 3-hydroxy-4-methoxybenzaldehyde was reacted with sodium formate/formic acid and hydroxylamine sulfate to afford 3-hydroxy-4-methoxybenzonitrile (**3**) in 88% yield. The coupling reaction of compounds **2** and **3** gave 4-methoxy-3-(3-morpholinopropoxy)benzonitrile (**4**) in 95% yield. The nitration reaction of compound **4** with HNO_3/H_2SO_4 provided 4-methoxy-5-(3-morpholinopropoxy)-2-nitrobenzonitrile (**5**) in 95% yield. The reduction reaction of compound **5** with $Na_2S_2O_4$ produced 2-amino-4-methoxy-5-(3-morpholinopropoxy)benzonitrile (**6**) in 75% yield. Compound **6** was reacted with *tert*-amyl alcohol to give 2-amino-4-methoxy-5-(3-morpholinopropoxy)benzamide (**7**) in 38% yield. The cyclization reaction of compound **7** with formic acid and formamide provided 7-methoxy-6-(3-morpholinopropoxy)-3,4-dihydroquinazolin-4-one (**8**) in 78% yield. Compound **8** was reacted with a halogenerating agent phosphorus oxychloride to provide 4-chloro-7-methoxy-6-(3-morpholinopropoxy)quinazolin (**9**) in 90% yield. Compound **9** was reacted with 3-chloro-4-fluoroaniline in a displacement reaction to provide reference standard 4-(3′-chloro-4′-fluoroanilino)-7-methoxy-6-(3-morpholinopropoxy)quinazoline (Iressa, **1**) in 91% yield. The desmethylation reaction of compound **1** with LiCl gave desmethylated precursor 4-(3′-chloro-4′-fluoroanilino)-7-hydroxy-6-(3-morpholinopropoxy)quinazoline (M523595, **10**) in 54% yield. The overall yield for Iressa was 11% via nine steps. The overall yield for M523595 was 6% via 10 steps. In comparison with patent and literature procedures, several improvements in the synthetic methodology for Iressa have been made, which include small scale synthetic procedures, higher and reasonable chemical yields, modified synthetic scheme and reaction conditions, and analytical details; and a new desmethylation method using LiCl for M523595 has been reported.

The synthesis of target tracer [^{11}C]Iressa ([^{11}C]**1**) is shown in Scheme 2. The desmethylated precursor M523595 was reacted with [^{11}C]methyl triflate ($^{11}CH_3OTf$)[10] under basic conditions through *O*-[^{11}C]methylation and purified by semi-preparative HPLC method[11] to give [^{11}C]Iressa in 30–40% radiochemical yields based on [^{11}C]CO_2, 20–30 min overall synthesis time from end of bombardment (EOB), >98% radiochemical purity, and 4.0–6.0 Ci/μmol specific activity at EOB measured by analytical HPLC method.[12] Retention times (t_Rs) in the semi-preparative HPLC system were: t_R **10** = 4.95 min, t_R **1** = 6.78 min, and t_R [^{11}C]**1** = 6.78 min. t_Rs in the analytical HPLC system were: t_R **10** = 2.16 min, t_R **1** = 3.33 min, and t_R [^{11}C]**1** = 3.35 min. The quality control using both semi-preparative and analytical HPLC method demonstrated the success of the labeling and that the labeling did not alternate the potency of the drug, because the HPLC data (retention times) of the labeled compound [^{11}C]Iressa and the drug Iressa are exactly same, and the labeling did not change the chemical structure of the drug. The in vivo biological evaluation of [^{11}C]Iressa is currently underway, and the results will be reported in due course.

Compound **9** is a new compound. The experimental details and characterization data for compounds **1**–**10** and new tracer [^{11}C]**1** are given.[13]



Scheme 1. Synthesis of Iressa and M523595.



Scheme 2. Synthesis of [^{11}C]Iressa.

In summary, an efficient and convenient chemical and radiochemical synthesis of the desmethylated precursor M523595, reference standard Iressa, and target tracer [^{11}C]Iressa has been well developed. The chemistry result provides the foundation for further evaluation of [^{11}C]Iressa as a new potential PET imaging agent for EGFR-TK in cancers.

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13. Experimental details and characterization data.
(a) General. All commercial reagents and solvents were used without further purification unless otherwise specified. CH_3OTf was made according to a literature procedure.[10] ^1H 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 ($5 \times 10\ cm^2$). 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. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C_{18} column, 4.6×250 mm; 3:1:3 CH_3CN/MeOH/20 mM, pH 6.7, $KHPO_4^-$ (buffer solution) mobile phase, flow rate 1.5 mL/min, and UV (254 nm) and γ-ray (NaI) flow detectors. Semi-preparative HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 10×250 mm; 3:1:3 CH_3CN/MeOH/20 mM, pH 6.7, $KHPO_4^-$ mobile phase, 5.0 mL/min flow rate, UV (254 nm) and γ-ray (NaI) flow detectors. Sterile vented Millex-GS 0.22 μm filter unit was obtained from Millipore Corporation, Bedford, MA.
(b) Compound **2**. 1-Bromo-3-chloropropane (6 mL, 60.98 mmol) was added slowly to a hot solution of morpholine (12 mL, 137.74 mmol) in toluene (40 mL) at 80 °C. The reaction mixture soon became cloudy, and it was stirred at 80 °C for 24 h. After cooling to room temperature, more toluene (20 mL) was added to dilute the mixture, and then aqueous HCl (18%, 14 mL) was added. The aqueous layer was separated and basified with 50% NaOH solution to pH 11, extracted twice using toluene, and the organic layer was dried over $MgSO_4$ and evaporated to give a liquid residue. The crude product was subject to vacuum distillation to give a colorless liquid **2** (7.33 g, 73%). ^1H NMR (300 MHz, $CDCl_3$): δ 3.71 (t, $J = 4.41$ Hz, 4H, OCH_2CH_2N), 3.61 (t, $J = 6.62$ Hz, 2H, CH_2Cl), 2.48 (t, $J = 7.35$ Hz, 2H, $NCH_2CH_2CH_2Cl$), 2.44 (t, $J = 4.42$ Hz, 4H, NCH_2CH_2O), 1.94 (q, $J = 7.36$ Hz, 2H, $CH_2CH_2CH_2$).
(c) Compound **3**. A mixture of 3-hydroxy-4-methoxybenzaldehyde (10 g, 65.72 mmol), sodium formate (8.94 g, 131.45 mmol), and formic acid (48 mL) was heated to 85 °C. To the above mixture was added hydroxylamine sulfate (6.47 g, 39.42 mmol) in six equal portions at 30 min intervals, and the mixture was heated at 85 °C for 5 h. The reaction was cooled to room temperature and poured to a solution of sodium chloride (40 g) in water (200 mL). The resultant solid was collected by filtration, washed with water, and dried to give an off-white solid **3** (8.62 g, 88%). ^1H NMR (300 MHz, DMSO-d_6): δ 9.78 (s, 1H, OH), 7.24 (dd, $J_1 = 2.21$ Hz, $J_2 = 8.09$ Hz, 1H, Ph-H), 7.00–7.13 (m, 2H, Ph-H), 3.83 (s, 3H, OCH_3).
(d) Compound **4**. A mixture of compound **3** (8 g, 53.64 mmol), K_2CO_3 (13 g, 94.07 mmol), compound **2** (9.54 g, 58.30 mmol), and DMF (50 mL) was heated to 85 °C for 10 h. Solvent was removed under vacuum to leave a residue which was partitioned between *tert*-butyl methyl ether and water. The organic phase was dried over $MgSO_4$ and evaporated to give a very viscous liquid **4** (14.07 g, 95%). ^1H NMR (300 MHz, DMSO-d_6): δ 7.33–7.43 (m, 2H, Ph-H), 7.09 (d, $J = 8.82$ Hz, 1H, Ph-H), 4.03 (t, $J = 5.88$ Hz, 2H, $OCH_2CH_2CH_2N$), 3.82 (s, 3H, OCH_3), 3.55 (t, $J = 5.88$ Hz, 4H, NCH_2CH_2O), 2.10–2.43 (m, 6H, NCH_2), 1.85 (q, $J = 6.62$ Hz, 2H, $CH_2CH_2CH_2$).
(e) Compound **5**. To a 250 mL two-necked flask were added compound **4** (10 g, 36.19 mmol) and HOAc (25 mL). The mixture of H_2SO_4 (70%, 25 mL) and HNO_3 (70%, 5 mL) was cooled to room temperature, then was added slowly to the above solution under ice/water. The mixture was slowly warmed to room temperature and stirred for 50 h. After addition of water (200 mL), the mixture was basified to pH 11 by addition of 50% NaOH aqueous solution. A large amount of yellow solid formed. CH_2Cl_2 was added to the mixture, which dissolved the solid. The aqueous phase was further extracted with CH_2Cl_2. The combined organic phase was washed with water, dried over $MgSO_4$, and evaporated to give a yellow solid. The solid was recrystallized in EtOAc to give a pure yellow solid **5** (11.07 g, 95%), $R_f = 0.44$ (20:1 CH_2Cl_2/MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 7.84 (s, 1H, Ph-H), 7.69 (s, 1H, Ph-H), 4.22 (t, $J = 5.88$ Hz, 2H, $OCH_2CH_2CH_2N$), 3.96 (s, 3H, OCH_3), 3.56 (t, $J = 4.42$ Hz, 4H, NCH_2CH_2O), 2.27–2.44 (m, 6H, NCH_2), 1.90 (q, $J = 6.62$ Hz, 2H, $CH_2CH_2CH_2$).
(f) Compound **6**. To a 250 mL two-necked flask were added compound **5** (5 g, 15.56 mmol), sodium dithionite (8.34 g), and water (80 mL). The mixture was stirred at room temperature for 2 h and the heated to 50 °C overnight. After the mixture was heated to 70 °C, concentrated HCl (37%, 25 mL) was added slowly in a period of 2 h. Heating was continued for another 1 h. The mixture was cooled to room temperature and basified with 50% NaOH aqueous solution to pH 11. The mixture was extracted with CH_2Cl_2 three times to give a residue, which was subject to silica gel chromatography eluted with 30:1 CH_2Cl_2/MeOH to give a very viscous liquid **6** (3.38 g, 75%), $R_f = 0.25$ (30:1 CH_2Cl_2/MeOH). ^1H NMR (300 MHz, DMSO-d_6): δ 6.88 (s, 1H, Ph-H), 6.38 (s, 1H, Ph-H), 5.63 (s, 2H, NH_2), 3.84 (t, $J = 6.62$ Hz, 2H, $OCH_2CH_2CH_2N$), 3.72 (s, 3H, OCH_3), 3.55 (t, $J = 4.41$ Hz, 4H, NCH_2CH_2O), 2.26–2.40 (m, 6H, NCH_2), 1.77 (q, $J = 6.62$ Hz, 2H, $OCH_2CH_2CH_2N$).
(g) Compound **7**. Compound **6** (3.38 g, 11.60 mmol) and *tert*-amyl alcohol (30 mL) were added to a 250 mL

two-necked flask equipped with a condenser. KOH (2.2 g, 39.21 mmol) was added, and the mixture was heated to 82 °C overnight. The reaction was cooled to room temperature and evaporated to give a viscous residue. The residue was transferred to a separation funnel with the aid of CH_2Cl_2 and water, and washed with water. The organic layers were combined and dried over $MgSO_4$. After evaporation to dryness, the residue was dissolved in methanol, and silica gel was added to absorb the solution, then dried under vacuum. The silica gel was transferred to the top of a silica gel column, eluted with 10:1 CH_2Cl_2/MeOH to give white solid **7** (1.35 g, 38%), $R_f = 0.24$ (10:1 CH_2Cl_2/MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.56 (br s, 1H, CONH), 7.11 (s, 1H, Ph-H), 6.81 (br s, 1H, CONH), 6.43 (s, 2H, aniline NH_2), 6.25 (s, 1H, Ph-H), 3.85 (t, $J = 6.61$ Hz, 2H, O$CH_2CH_2CH_2$N), 3.69 (s, 3H, OCH_3), 3.55 (t, $J = 4.41$ Hz, 4H, OCH_2CH_2N), 2.20–2.45 (m, 6H, NCH_2), 1.78 (q, $J = 7.35$ Hz, 2H, $OCH_2CH_2CH_2$N).

(h) Compound **8**. To a 25 mL two-necked flask were added compound **7** (0.5 g, 1.616 mmol), formic acid (0.1 mL, 2.607 mmol), and formamide (5 mL). The mixture was stirred at room temperature overnight. After volatiles were removed at reduced pressure and 90 °C, the resultant mixture was stirred at 100 °C for 7 h. White precipitate formed. After the reaction was cooled to room temperature, the solid was filtered, washed in turn with water, isopropanol, and *tert*-butyl methyl ether, and dried over vacuum to give a white solid **8** (0.4 g, 78%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.06 (s, 1H, NH), 7.97 (s, 1H, Ar-H), 7.42 (s, 1H, Ar-H), 7.12 (s, 1H, Ar-H), 4.09 (t, $J = 6.62$ Hz, 2H, O$CH_2CH_2CH_2$N), 3.89 (s, 3H, OCH_3), 3.57 (t, $J = 4.42$ Hz, 4H, OCH_2CH_2N), 2.20–2.60 (m, 6H, NCH_2), 1.91 (q, $J = 6.62$ Hz, 2H, $OCH_2CH_2CH_2$N).

(i) Compound **9**. To a 25 mL two-necked flask were added compound **8** (0.1 g, 0.313 mmol), $POCl_3$ (0.1 mL, 1.092 mmol), triethyl amine (0.5 mL, 3.587 mmol), and toluene (5 mL). The slurry was stirred at 58 °C for 24 h. The resultant brown slurry was cooled to 0 °C, and water (2 mL) was added slowly. The reaction was then warmed to room temperature and stirred for 1 h. The mixture was transferred to a separation funnel, and more water and toluene were added. The organic layers were combined, dried over $MgSO_4$, and evaporated to dryness. The residue was dissolved in CH_2Cl_2 and transferred to the top of a silica gel column, which was eluted with 30:1 CH_2Cl_2/MeOH to give a white solid **9** (0.095 g, 90%), $R_f = 0.19$ (30:1 CH_2Cl_2/MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.86 (s, 1H, Ar-H), 7.39 (s, 1H, Ar-H), 7.33 (s, 1H, Ar-H), 4.28 (t, $J = 6.62$ Hz, 2H, O$CH_2CH_2CH_2$N), 4.05 (s, 3H, OCH_3), 3.74 (t, $J = 4.41$ Hz, 4H, OCH_2CH_2N), 2.60 (t, $J = 6.62$ Hz, 2H, $OCH_2CH_2CH_2$N), 2.51 (br s, 4H, OCH_2CH_2N), 2.14 (q, $J = 6.62$ Hz, 2H, $OCH_2CH_2CH_2$N). LRMS (EI): 337 (M^+, 14%), 100 (100%). HRMS (EI): calcd for $C_{16}H_{20}ClN_3O_3$: 337.1188, found: 337.1198.

(j) Compound **1**. To a 25 mL two-necked flask were added compound **9** (0.1 g, 0.296 mmol), 3-chloro-4-fluoroaniline (0.18 g, 1.237 mmol), triethyl amine (0.4 mL, 2.152 mmol), and isopropanol (5 mL). The mixture was refluxed for 12 days. After cooling to room temperature, silica gel was added to absorb the solution, then dried under vacuum, and transferred to the top of a silica gel column. The column was eluted with CH_2Cl_2, then 30:1 and 20:1 CH_2Cl_2/MeOH to get unreacted starting material 0.011 g and a white solid **1** (0.12 g, 91%), $R_f = 0.22$ (20:1 CH_2Cl_2/MeOH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.67 (s, 1H, NH), 8.60 (s, 1H, Ar-H), 8.22 (dd, $J_1 = 2.94$ Hz, $J_2 = 6.62$ Hz, 1H, Ar-H), 7.80–7.95 (m, 2H, Ar-H), 7.55 (t, $J = 8.82$ Hz, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 4.29 (t, $J = 5.88$ Hz, 2H, O$CH_2CH_2CH_2$N), 4.04 (s, 3H, OCH_3), 3.68 (t, $J = 4.41$ Hz, 4H, OCH_2CH_2N), 2.40–2.63 (m, 6H, NCH_2), 2.10 (q, $J = 6.61$ Hz, 2H, $OCH_2CH_2CH_2$N).

(k) Compound **10**. Compound **1** (67 mg, 0.15 mmol) and LiCl (32 mg, 0.75 mmol) were added into DMF (10 mL), the reaction mixture was refluxed for 15 h. Then the mixture was cooled to room temperature, water (15 mL) was added. The mixture was extracted by ethyl acetate for three times (3× 50 mL), washed with brine, and dried by $MgSO_4$. The solution was evaporated, residue was purified by column chromatography on silica gel with eluent (5–10% MeOH/CH_2Cl_2) to get compound **10** (35 mg, 54%). $R_f = 0.28$ (10% MeOH/CH_2Cl_2), mp = 133–135 °C. ^{1}H NMR (300 MHz, acetone-d_6): δ 9.01 (s, 1H, NH), 8.53 (s, 1H, Ar-H), 8.25 (dd, $J = 2.57$, 7.77 Hz, 1H, Ph-H), 7.89 (s, 1H, Ph-H), 7.80–7.85 (m, 1H, Ph-H), 7.27(t, $J = 9.18$ Hz, 1H, Ph-H), 7.16 (s, 1H, Ph-H), 4.18 (t, $J = 5.46$ Hz, 2H, OCH_2), 3.67 (t, $J = 4.77$ Hz, 4H, morpholine–OCH_2), 2.60 (t, $J = 6.6$ Hz, 2H, NCH_2), 2.49 (s, 4H, morpholine–NCH_2), 1.98-2.09 (m, 2H, $OCH_2CH_2CH_2$N).

(l) Tracer [^{11}C]**1**. The precursor **10** (0.3–0.5 mg) was dissolved in acetone (300 μL). To this solution was added 3 N NaOH (2–3 μL). The mixture was transferred to a small volume, three-necked reaction tube. $^{11}CH_3OTf$ was passed into the air-cooled reaction tube at −15 to −20 °C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until radioactivity reached a maximum (~3 min), then the reaction tube was heated at 70–80 °C for 3 min. The contents of the reaction tube were diluted with $NaHCO_3$ (1 mL, 0.1 M). The mixture containing precursor and product was purified with semi-preparative HPLC method. The contents of the mixture residue were diluted with HPLC mobile phase 3:1:3 CH_3CN/MeOH/20 mM, pH 6.7 $KHPO_4^-$, and injected onto the semi-preparative HPLC column. The product fraction was collected, the solvent was removed by rotatory evaporation under vacuum, and the final product [^{11}C]**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.