Targeting Metal-Aβ Aggregates with Bifunctional Radioligand [^{11}C]L2-b and a Fluorine-18 analogue [^{18}F]FL2-b *(Supporting Information)*

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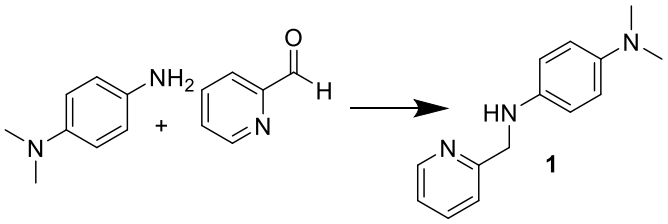
(I) Chemistry and Characterization:

General Considerations:

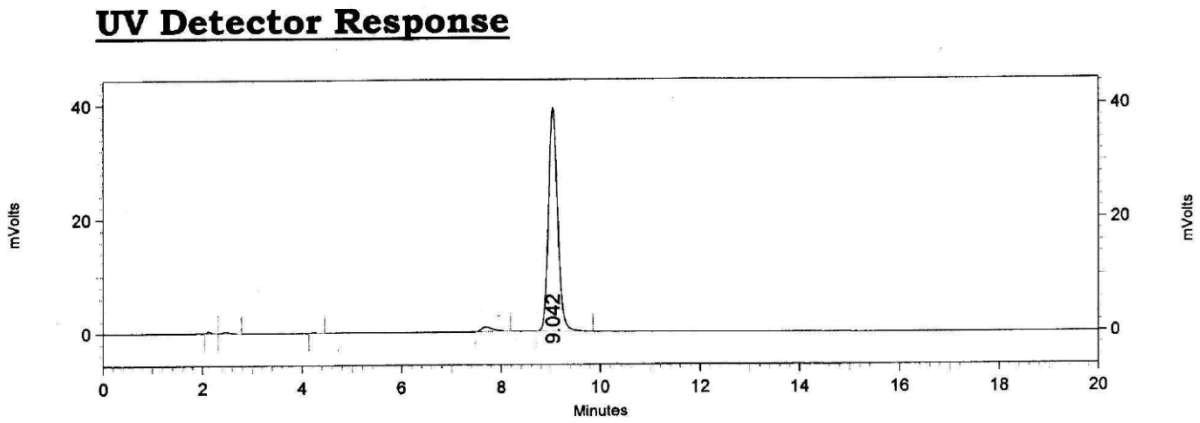
All solvents and reagents were commercially available and used without further purification unless otherwise stated. *N*-methyl-4-nitroaniline, *N*,*N*-dimethyl-p-phenylenediamine, and 2-pyridinecarboxaldehyde were purchased from Sigma Aldrich. 6-Fluoropyridinecarboxaldehyde and 6-chloropyridinecarboxaldehyde were purchased from Oakwood Chemical. NMR spectra were recorded with a Varian 400 MHz instrument at room temperature with tetramethylsilane (TMS) as an internal standard. ^{1}H, ^{13}C, and ^{19}F spectra were recorded at 400 MHz, 100 MHz, and 376 MHz, respectively. Mass spectra were performed on a VG (Micromass) 70-250-S Magnetic sector mass spectrometer or Micromass AutoSpec Ultima Magnetic sector mass spectrometer employing the electrospray ionization (ESI) method. High performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector. All procedures including anhydrous solvents were performed using Schlenk techniques with rigorously dried glassware.



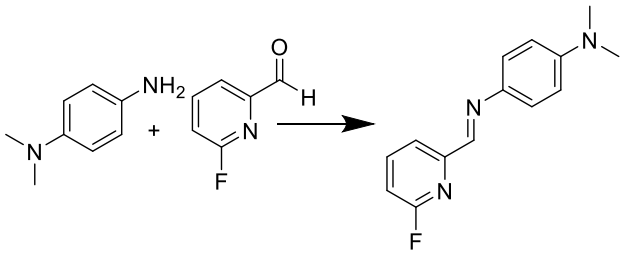
*Synthesis of L-2B; (N^1,N^1-dimethyl-N^4-(pyridin-2-ylmethyl)benzene-1,4-diamine), (**1**):*

The synthesis of **1** was adapted from literature precedent[1] and spectra matched published values. ^{1}H NMR (400 MHz; $CDCl_3$)/δ (ppm): 2.80 (6H, s), 4.40 (2H, s), 6.63 (2H, d, *J* = 8.8Hz), 6.73 (2H, d, *J* = 8.8Hz), 7.12-7.15 (1H, m), 7.32 (1H, d, *J* = 8.0Hz), 7.60 (1H, td, *J* = 7.6 Hz, *J* = 1.6Hz), 8.55 (1H, d, *J* = 4.8Hz); HPLC: 96%, retention time = ~9 min, column: Phenomenex Gemini C18, 250x4.6 mm, mobile phase: 10 mM NH_4HCO_3 in 35% MeCN, pH 10 adjusted with NH_4OH, flow rate: 1.0 mL/min, wavelength: 254 nm.

HPLC of L2-b (1):

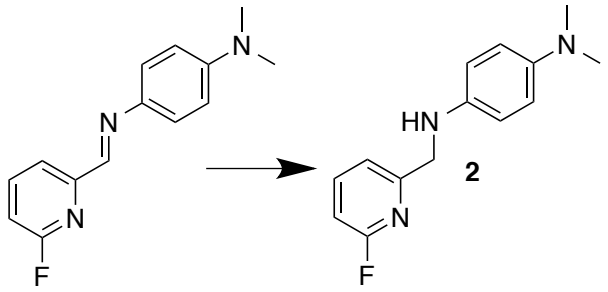


UV Detector Ch1-254nm Results Retention Time	Area	Area %
9.04	503457	96.22



Synthesis of 4-(((6-fluoropyridin-2-yl)methylene)amino)-N,N-dimethylaniline:

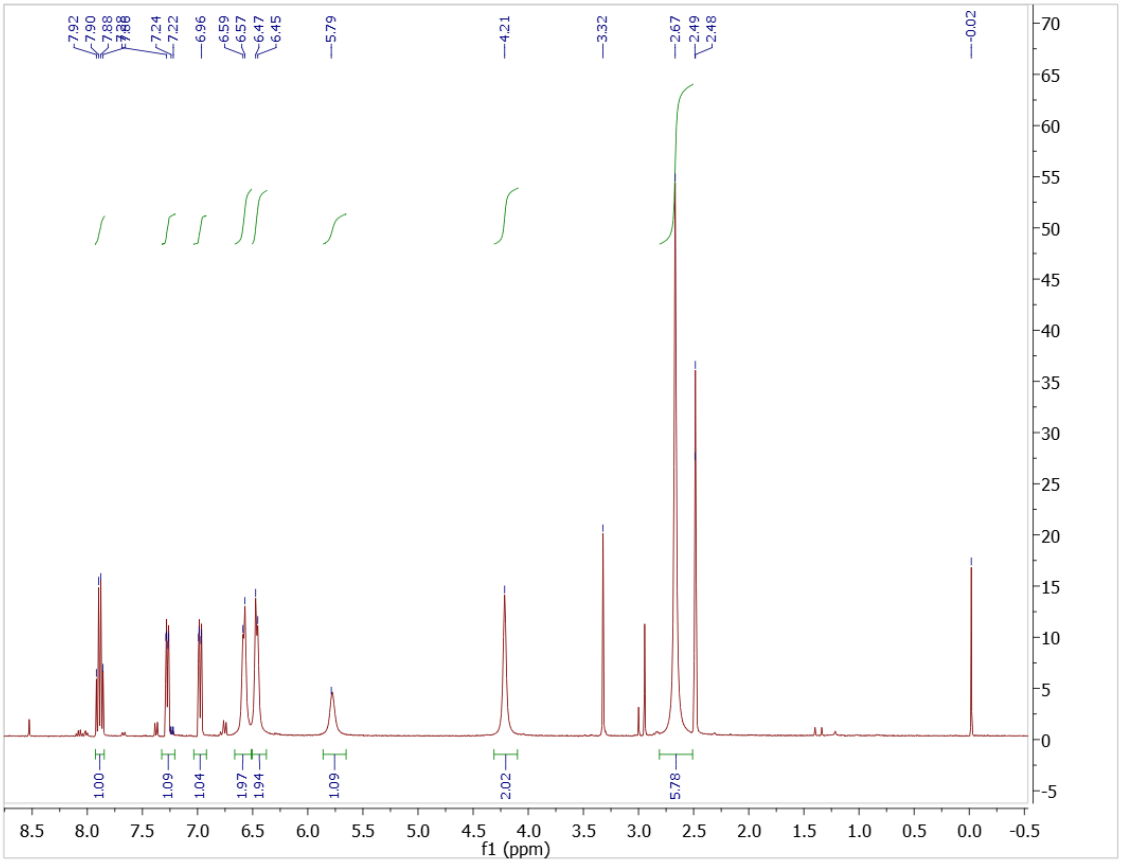
6-Fluoropicolinaldehyde (184 mg, 1.47 mmol) was added to N^1,N^1-dimethylbenzene-1,4-diamine (200 mg, 1.47 mmol) in anhydrous THF (5 mL). To the solution was added sodium sulfate ($NaSO_4$). After 24h of stirring under argon, the solution was filtered and concentrated under vacuum. The crude product was purified via silica gel chromatography (SiO_2, 3:1 = hexanes: ethyl acetate). This yielded the product as a yellow solid (357 mg, quant.); R_f: 0.45 (SiO_2, 3:1 = hexanes:ethyl acetate); mp 102-104 ^oC; ^1H NMR (400 MHz; CD_3OD)/δ (ppm): 2.99 (6H, s), 6.79 (2H, d, J = 8.8 Hz), 7.08-7.11 (1H, m), 7.97-8.05 (2H, composite), 8.51 (1H, s); ^{13}C NMR (100 MHz; CD_3OD)/δ (ppm): 39.30, 109.86, 110.23, 112.37, 118.90, 122.61, 138.50, 142.06, 142.14, 150.73, 152.58; ^{19}F NMR (376.3 MHz, CD_3OD)/δ (ppm): -70.0 (1F, d, J = 6.8Hz).



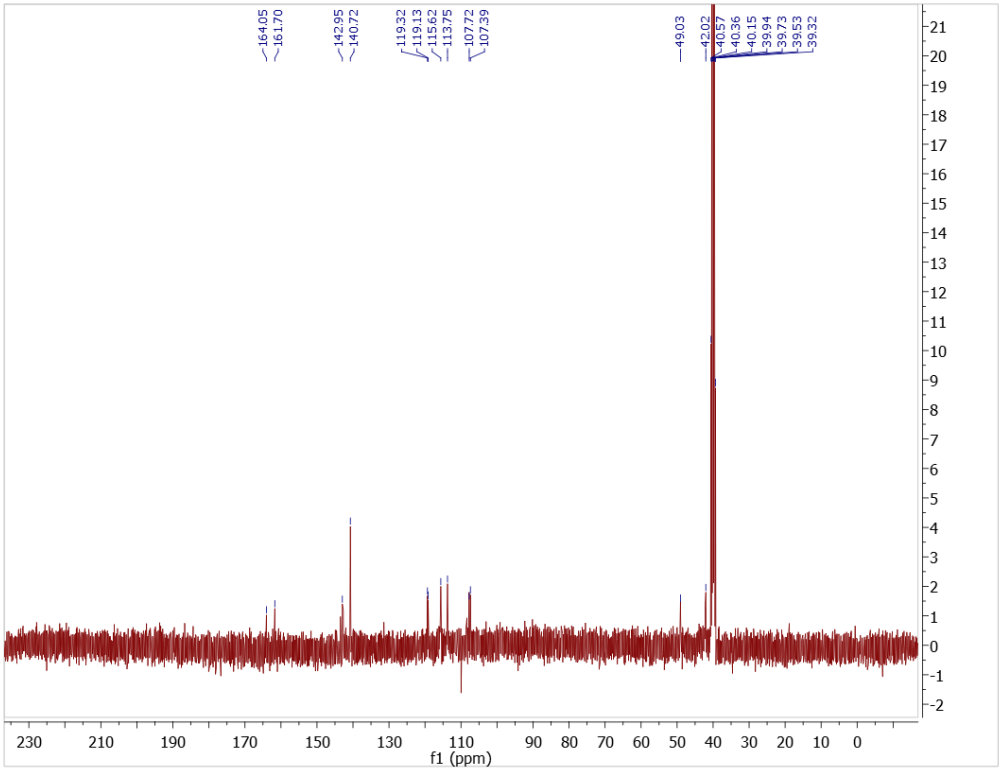
*Synthesis of [^{18}F]FL-2B Reference Standard; (N^1-((6-fluoropyridin-2-yl)methyl)-N^4,N^4-dimethylbenzene-1,4-diamine), (**2**):*

4-(((6-Fluoropyridin-2-yl)methylene)amino)-*N*,*N*-dimethylaniline (130 mg, 0.534 mmol) was dissolved in anhydrous methanol (4 mL) and cooled to 0 ^{o}C in a round-bottom flask. Sodium borohydride ($NaBH_4$, 30.3 mg, 0.801 mmol) was slowly added, and the mixture was stirred at 0^{o} C under argon for 1h. After this time had elapsed, the reaction was quenched with water (10 mL). The product was extracted with diethyl ether, dried over sodium sulfate, and the solvent was removed *in vacuo*. This process provided the product as a yellow oil (86.1 mg, 65.7%); R_f: 0.51 (SiO_2, 1:1=hexanes:ethyl acetate); ^{1}H NMR (400 MHz; DMSO-d_6)/δ (ppm): 2.67 (s, 6H), 4.21 (s, 2H), 5.78 (s, 1H), 6.46 (d, *J* = 8.3 Hz, 2H), 6.58 (d, *J* = 8.3 Hz, 2H), 6.98 (dd, *J* = 8.2, 2.6 Hz, 1H), 7.27 (dd, *J* = 7.5, 2.6 Hz, 1H), 7.89 (q, *J* = 8.1 Hz, 1H); ^{13}C NMR (100 MHz; DMSO-d_6)/δ (ppm): 42.02, 49.03, 107.39, 107.72, 113.75, 115.62, 119.13, 119.32, 140.72, 142.95, 161.70, 164.05; ^{19}F NMR (376.3 MHz, DMSO-d_6)/δ (ppm): -68.7(1F, d, J = 8.0Hz); HPLC: 99%, retention time = 19min, column: Phenomenex Gemini C18, 250x4.6 mm, mobile phase: 10 mM NH_4HCO_3 in 30% MeCN, pH 9.7 adjusted with 3mL/L sat. NH_4OH solution, flow rate: 2.0 mL/min, wavelength: 254 nm; HRMS: calculated for $[M+H]^+$(M = $C_{14}H_{16}FN_3$), 246.1401, found 246.1395.

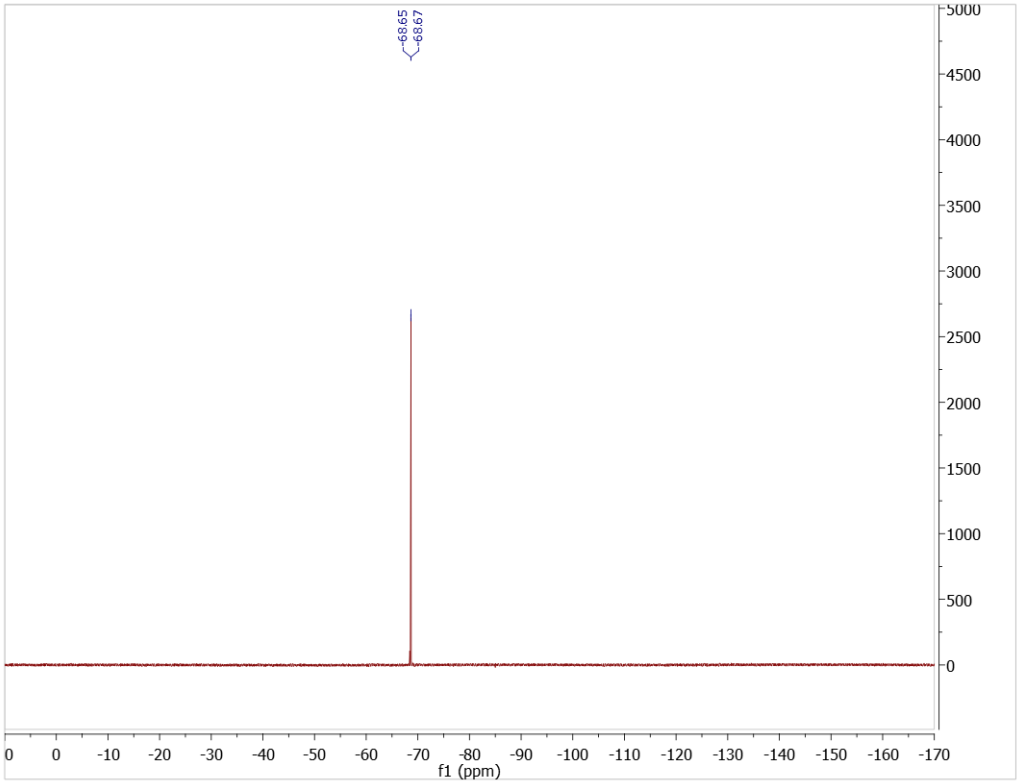
^{1}H NMR (400 MHz) of (2):



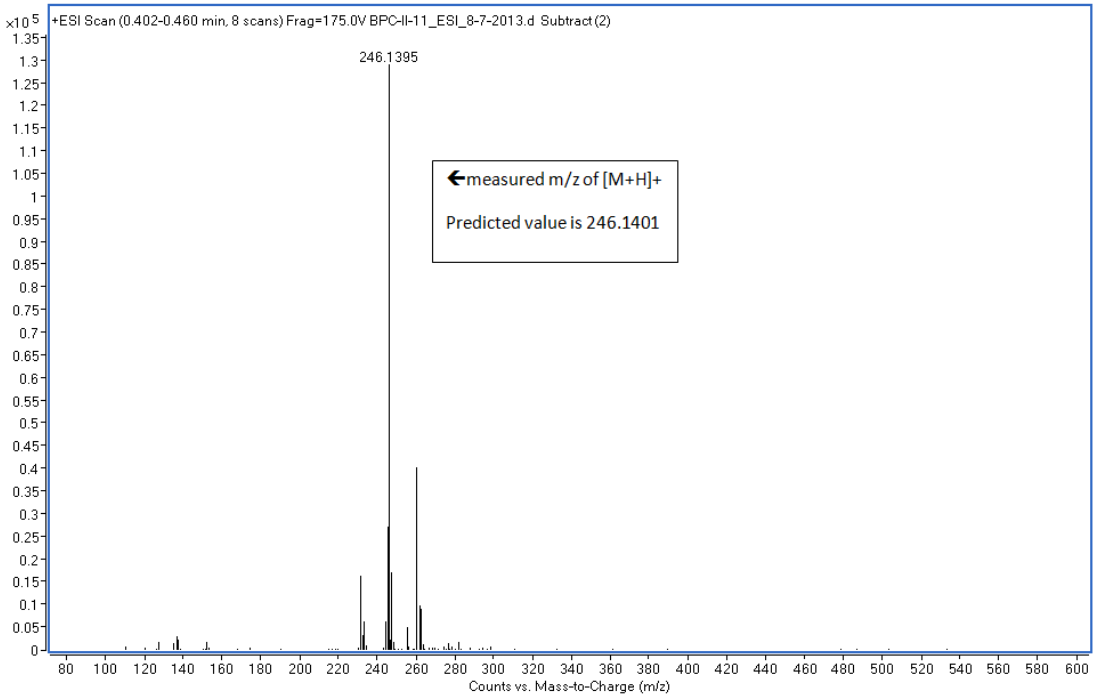
^{13}C NMR (100 MHz) of (2):



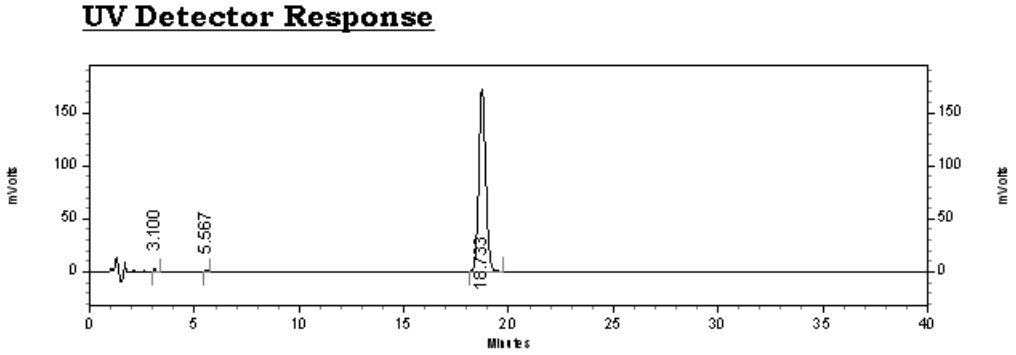
^{19}F NMR (376 MHz) of (2):



HRMS (ESI) of (2):

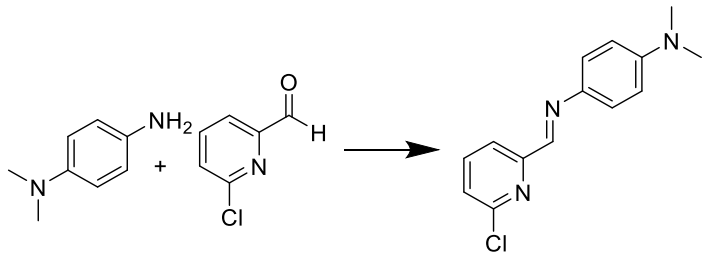


HPLC of (2):



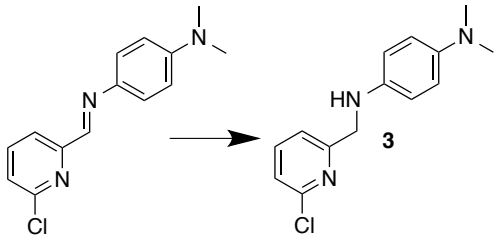
UV Detector
Ch1-254nm Results

Retention Time	Area	Area %
3.10	16070	0.40
5.57	7683	0.19
18.73	3966116	99.40



Synthesis of 4-(((6-chloropyridin-2-yl)methylene)amino)-N,N-dimethylaniline:

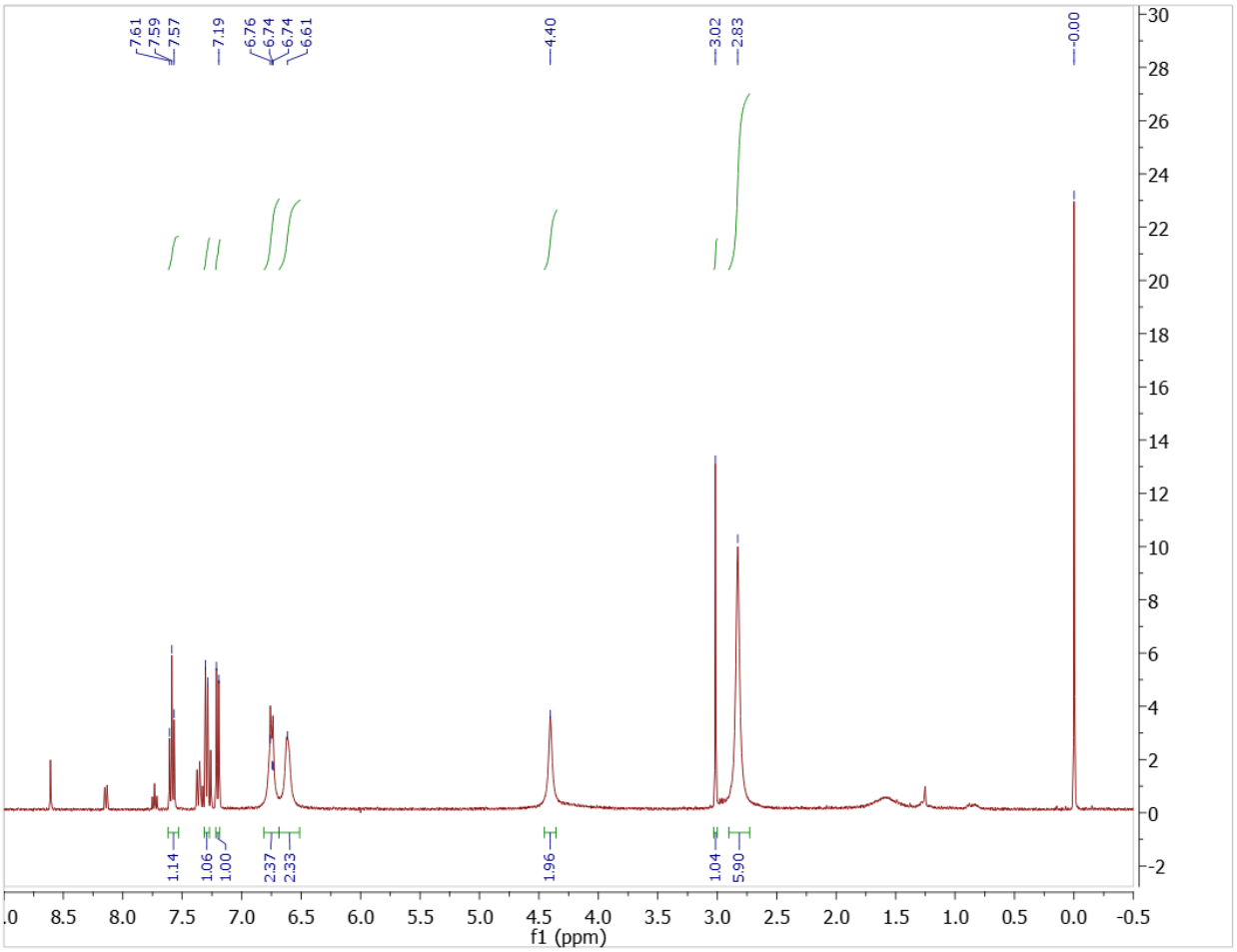
To a round bottom flask was added 6-chloropicolinaldehyde (425 mg, 2.94 mmol) and N^1,N^1-dimethylbenzene-1,4-diamine (400 mg, 2.94 mmol) dissolved in methanol (10 mL). Sodium sulfate was added, and the mixture was stirred for 24 h at room temperature. The mixture was filtered, and the filtrate was concentrated under vacuum. The mixture was purified via silica gel flash chromatography (3:1 = hexanes:ethyl acetate). Product fractions were combined and concentrated under vacuum to provide a yellow solid (49.9 mg, 6.5%); R_f: 0.47 (SiO_2, 3:1=hexanes: ethyl acetate); mp 121-125°C; ^{1}H NMR (400 MHz; $CDCl_3$)/δ (ppm): 3.01 (6H, s), 6.76 (2H, d, J = 8.4), 7.33-7.37 (3H, m), 7.73 (1H, t, J =7.6 Hz), 8.14 (1H, d, J = 7.6 Hz), 8.60 (1H, s); ^{13}C NMR (100 MHz; $CDCl_3$)/δ (ppm): 40.56, 112.52, 119.3, 123.13, 124.69, 139.03, 150.94, 153.32, 156.15.



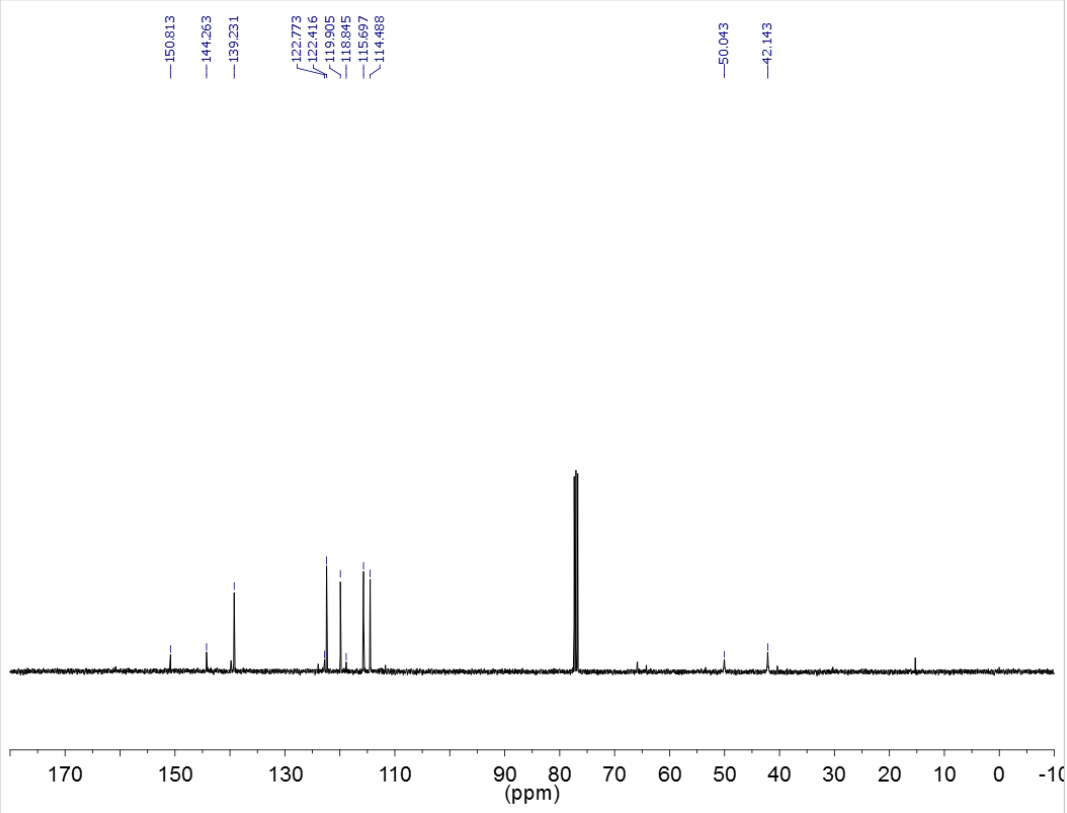
*Synthesis of FL2-b precursor; (N1-((6-chloropyridin-2-yl)methyl)-N4,N4-dimethylbenzene-1,4-diamine) (**3**):*

To a round bottom flask was added 4-(((6-chloropyridin-2-yl)methylene)amino)-*N*,*N*-dimethylaniline (47.0 mg, 0.181 mmol). Anhydrous methanol (3 mL) was added and the solution was cooled to 0 °C. Sodium borohydride ($NaBH_4$, 10.2 mg, 0.27 mmol) was slowly added. The reaction was stirred for 2 h under argon. After this time, the reaction was quenched with water. The product was extracted with ethyl ether, dried over sodium sulfate, filtered, and concentrated *in vacuo*. This process yielded the product as a light-yellow oil (30.9 mg, 65.5%); R_f=0.49 (SiO_2, 1:1=hexanes: ethyl acetate); ^{1}H NMR (400 MHz; $CDCl_3$)/δ (ppm): 2.83 (s, 6H), 3.02 (s, 1H), 4.40 (s, 2H), 6.61 (m, 2H), 6.80 – 6.69 (m, 2H), 7.20 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 7.6 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H); ^{13}C NMR (100 MHz; $CDCl_3$)/δ (ppm): 42.15, 50.05, 114.49, 115.70, 118.85, 122.42, 122.78, 139.23, 144.27, 150.82; HPLC: 98%, retention time = 29min, column: Phenomenex Gemini C18, 250x4.6 mm, mobile phase: 10 mM NH_4HCO_3 in 30% MeCN, pH 9.7 adjusted with 3 mL/L sat. NH_4OH solution, flow rate: 2.0 mL/min, wavelength: 254 nm; HRMS: calculated for $[M+H]^+$(M = $C_{14}H_{16}ClN_3$), 262.1106, found 262.1097.

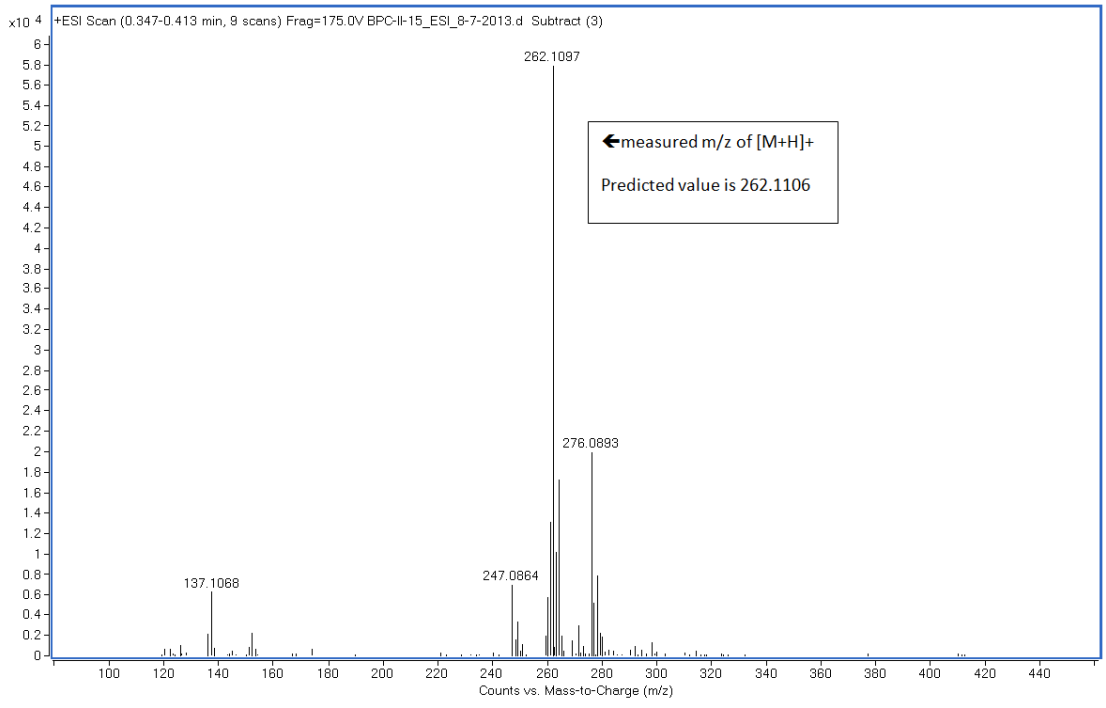
^{1}H NMR (400 MHz) of (3):



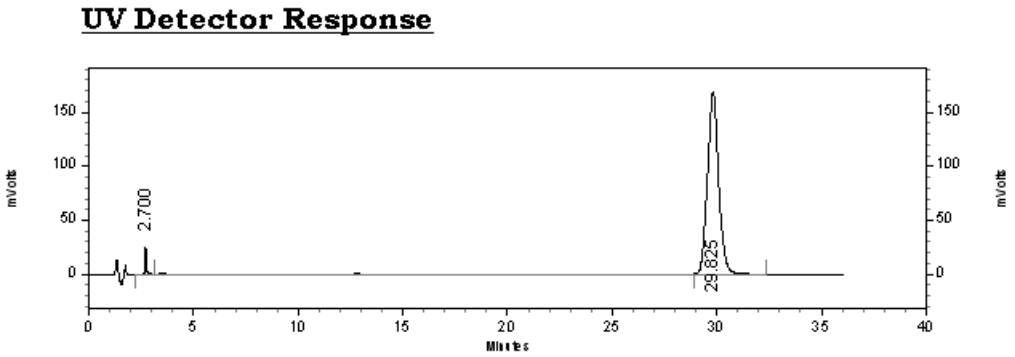
^{13}C NMR (100 MHz) of (3):



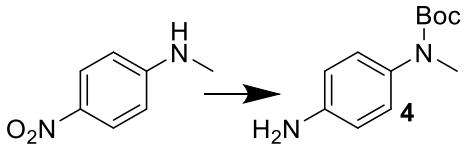
HRMS (ESI) of (3):



HPLC of (3):

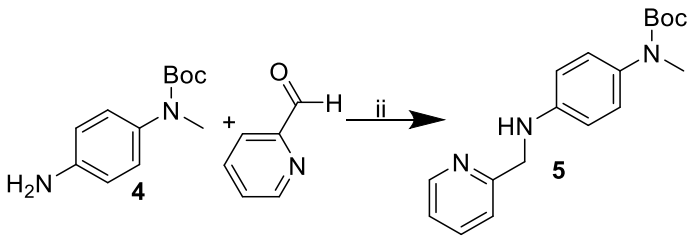


UV Detector Ch1-254nm Results		
Retention Time	Area	Area %
2.70	118591	1.86
29.82	6255436	98.14



*Synthesis of tert-butyl (4-aminophenyl)(methyl)carbamate (**4**)*:

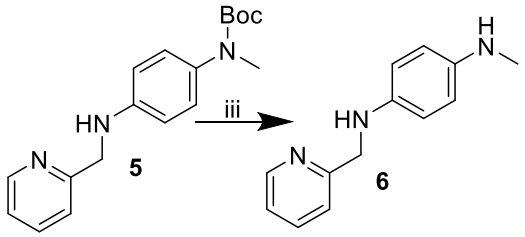
The synthesis of **4** was adapted from literature precedent[1] and spectra matched published values.



*Synthesis of tert-butyl methyl(4-((pyridin-2-ylmethyl)amino)phenyl)carbamate (**5**)*:

Tert-butyl (4-aminophenyl)(methyl)carbamate (**4**) (0.200 g, 0.900 mmol,) was added to 2-pyridinecarboxaldehyde (0.0869 g, 0.811 mmol) in anhydrous THF (6 mL). Sodium sulfate ($NaSO_4$) was added to the mixture and stirred for 24 h. The mixture was filtered and concentrated *in vacuo*. To this product was added anhydrous methanol (6 mL). The solution was cooled to 0 ^oC, and sodium borohydride (0.0614 g, 1.623 mmol) was slowly added. The solution was stirred for 24h under Argon. Water (4 mL) was added to quench the reaction. The resulting solution was extracted with diethyl ether (10 mL) and washed with brine solution (10 mL). The crude product was purified with silica gel chromatography

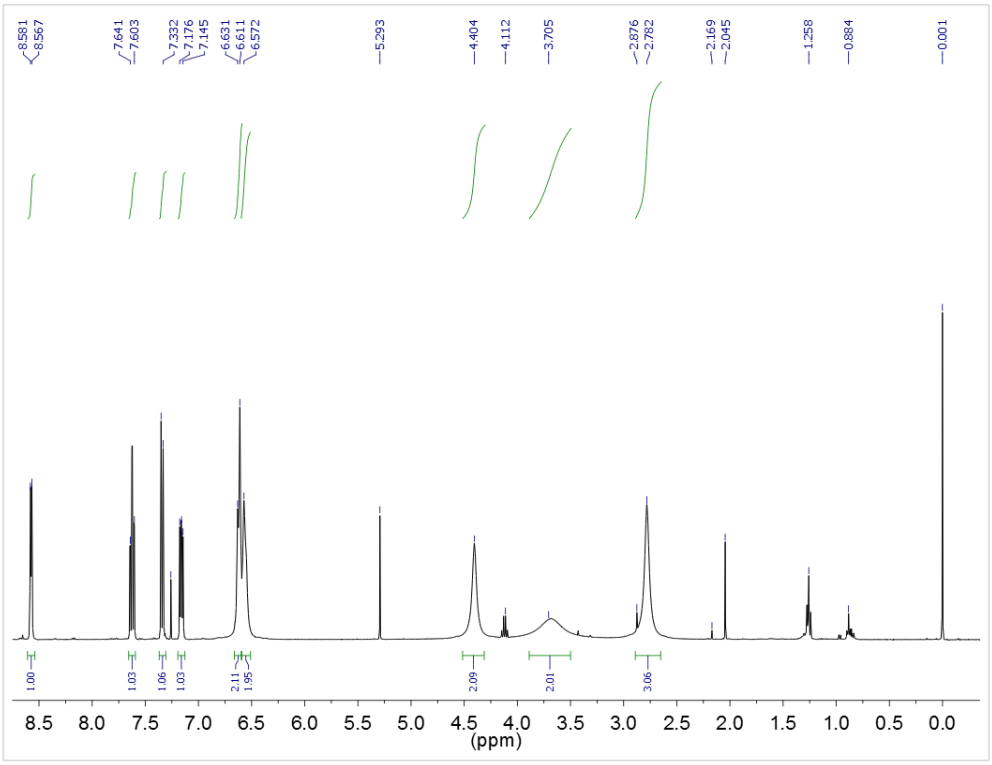
(SiO_2, 1:1 = hexanes: ethyl acetate) which gave the product as a white solid (155 mg, 60.1%); R_f 0.57 (1:1 = hexanes:ethyl acetate); mp 102-104 ^{o} C; ^{1}H NMR (400 MHz; $CDCl_3$)/δ (ppm): 1.42 (9H, s), 3.19 (3H, s), 4.46 (2H, s), 6.61 (2H, d, *J* = 8.4 Hz), 7.00-7.02 (2H, m), 7.19 (1H, dd, *J* = 5.2 Hz, 1.6 Hz), 7.33 (1H, d, *J* = 7.6 Hz), 7.64 (1H, td, *J* = 7.6 Hz, 1.6 Hz), 8.57 (1H, d, *J* = 4.4 Hz); ^{13}C NMR (100 MHz; $CDCl_3$)/δ (ppm): 28.353, 37.704, 49.344, 112.89, 121.69, 122.18, 126.78, 145.65, 149.00, 155.345.



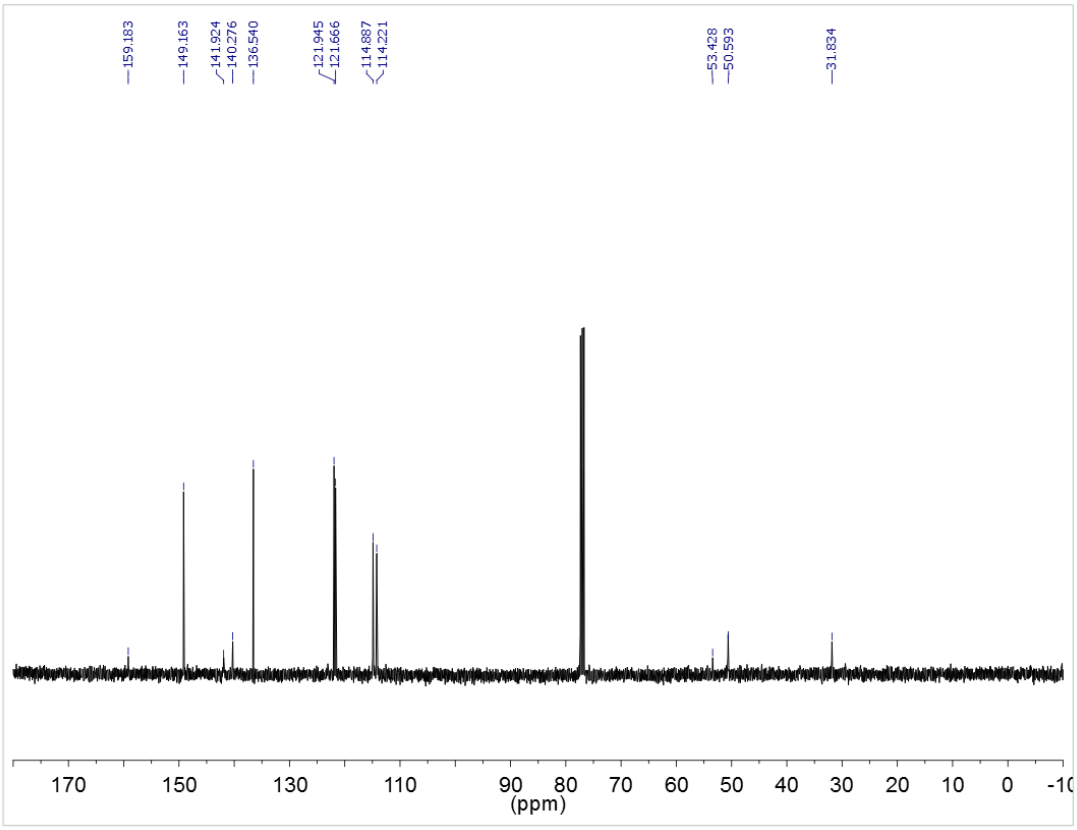
*Synthesis of [^{11}C]L2-b precursor; (N^1-methyl-N^4-(pyridin-2-ylmethyl)benzene-1,4-diamine)(**6**):*

Tert-butyl methyl(4-((pyridin-2-ylmethyl)amino)phenyl)carbamate (45.6 mg, 0.146 mmol) was dissolved in anhydrous methanol (3 mL). The solution was cooled to 0 ^{o}C, and chlorotrimethylsilane (79.1 mg, 0.728 mmol) was slowly added. The reaction mixture was stirred for 24h, during which time it was allowed to warm to room temperature. The solution was quenched with an aliquot of saturated sodium bicarbonate, and the product was extracted with dichloromethane three times. Vacuum drying provided the product as a yellow oil (27.9 mg, 90.2%); R_f: 0.60 (SiO_2, 1:3=hexanes: ethyl acetate); ^{1}H NMR (400 MHz; $CDCl_3$)/δ (ppm):2.87 (3H, s), 3.71 (2H, s), 4.40 (2H, s), 6.57-6.63 (4H,m), 7.16 (1H, t, *J* = 6.2 Hz), 7.34 (1H, d, *J* = 8.0 Hz), 7.62 (1H, td, *J* = 7.6 Hz, 1.6 Hz), 8.57 (1H, m); ^{13}C NMR (100 MHz; $CDCl_3$)/δ (ppm): 31.834, 50.593, 114.221, 114.887, 121.666, 121.945, 136.540, 140.276, 141.942, 149.163, 159.183. HPLC: 99%, retention time = ~5min, column: Phenomenex Gemini C18, 250x4.6 mm, mobile phase: 10 mM NH_4HCO_3 in 35% MeCN, pH 10 adjusted with NH_4OH, flow rate: 1.0 mL/min, wavelength: 254 nm; HRMS: calculated for $[M]^+$(M = $C_{13}H_{15}N_3$), 213.1260, found 213.1253.

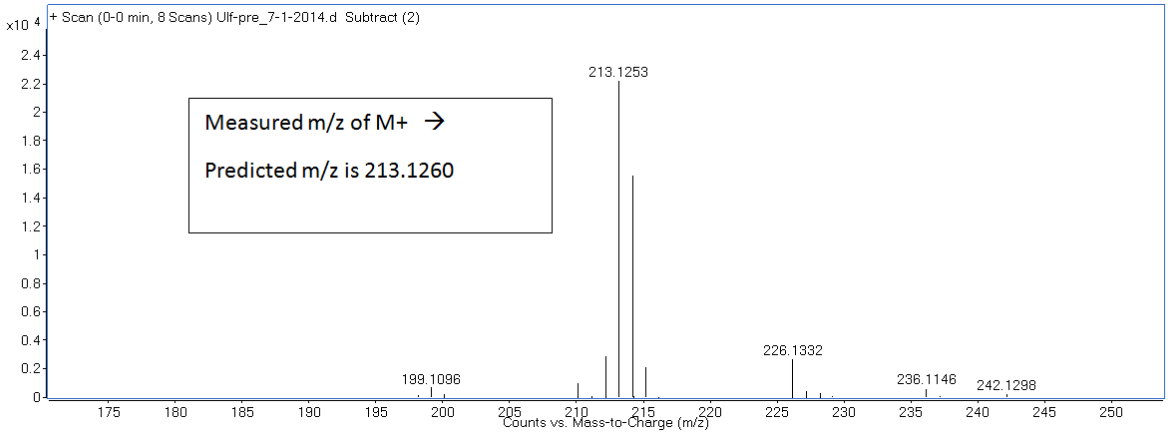
^{1}H NMR (400 MHz) of (6):



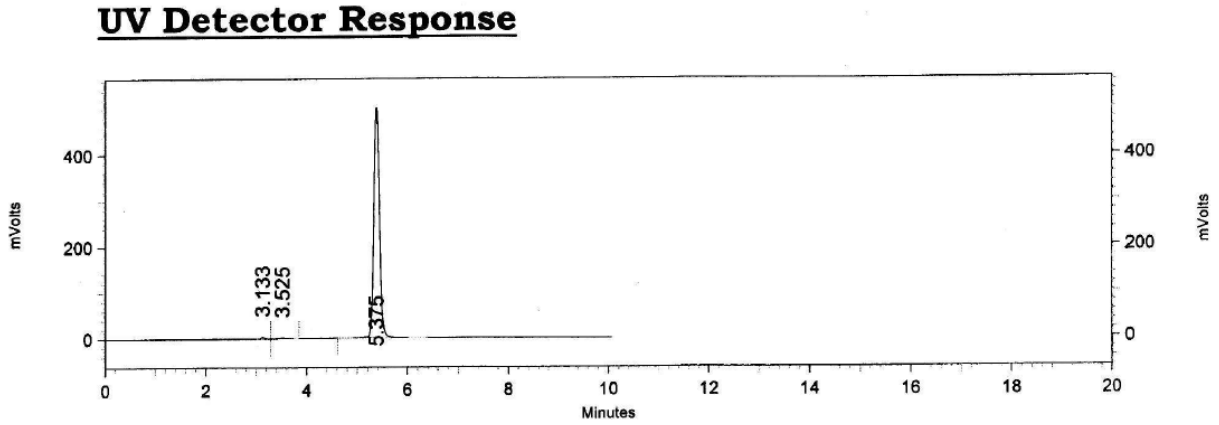
^{13}C NMR (100MHz) of (6):



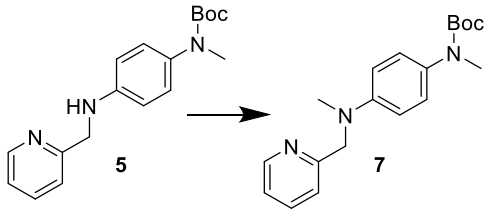
HRMS (ESI) of (6):



HPLC of (6):

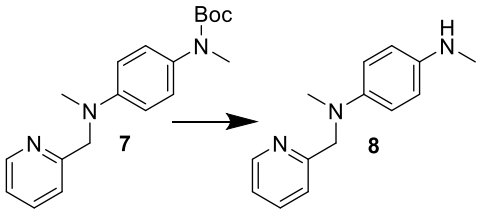


UV Detector Ch1-254nm Results Retention Time	Area	Area %
5.38	3926798	99.14



Synthesis of tert-butyl methyl(4-(methyl(pyridin-2-ylmethyl)amino)phenyl)carbamate (7):

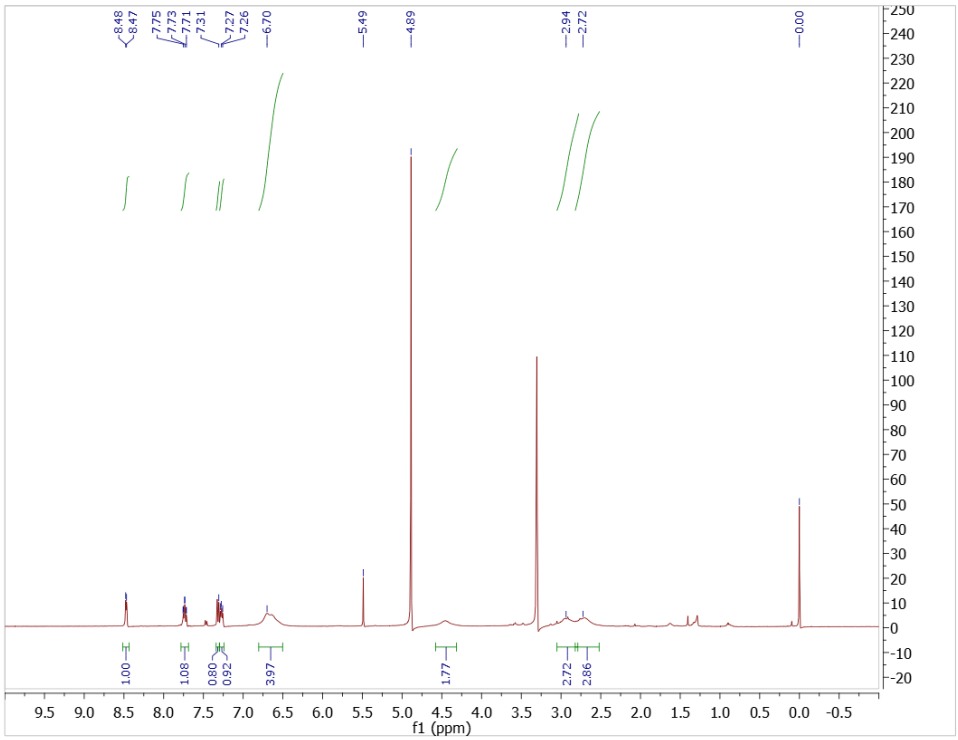
Tert-butyl methyl(4-((pyridin-2-ylmethyl)amino)phenyl)carbamate (300 mg, 0.957 mmol) was dissolved in anhydrous methanol (10 mL). Formaldehyde (288mg, 9.58 mmol; 37% solution in water) was added. The solution was cooled to 0 ^{o}C while stirring for 20 min. After this time period, sodium borohydride (362 mg, 9.58 mmol) was slowly added to the mixture. The solution was stirred at 0 ^{o}C for one hour. The mixture was allowed to warm to room temperature, and the pH was adjusted to 7 by slowly adding hydrochloric acid (1 M). The crude product was extracted with ethyl acetate three times and purified using silica gel flash chromatography (SiO_2, 1:1 = hexanes: ethyl acetate). This yielded the product as an off white solid (13.6 mg, 4.3%); R_f: 0.30 (SiO_2, 1:1=hexanes: ethyl acetate). ^{1}H NMR (400 MHz; $CDCl_3$)/δ (ppm): 1.40 (9H, s), 3.11 (3H, s), 3.13 (3H, s), 4.63 (2H, s), 6.68 (2H, d, *J* = 9.0 Hz), 7.01 (2H, d, *J* = 8.8 Hz), 7.23-7.30 (2H, m), 7.74 (1H, td, *J* = 7.7 Hz, *J* = 1.6 Hz), 8.50 (1H, m); ^{13}C NMR (100 MHz; $CDCl_3$)/δ (ppm): 27.20, 38.33, 57.83, 112.10, 112.39, 121.27, 122.21, 126.57, 126.63, 133.12, 137.37, 137.40, 147.49, 148.52, 159.08.



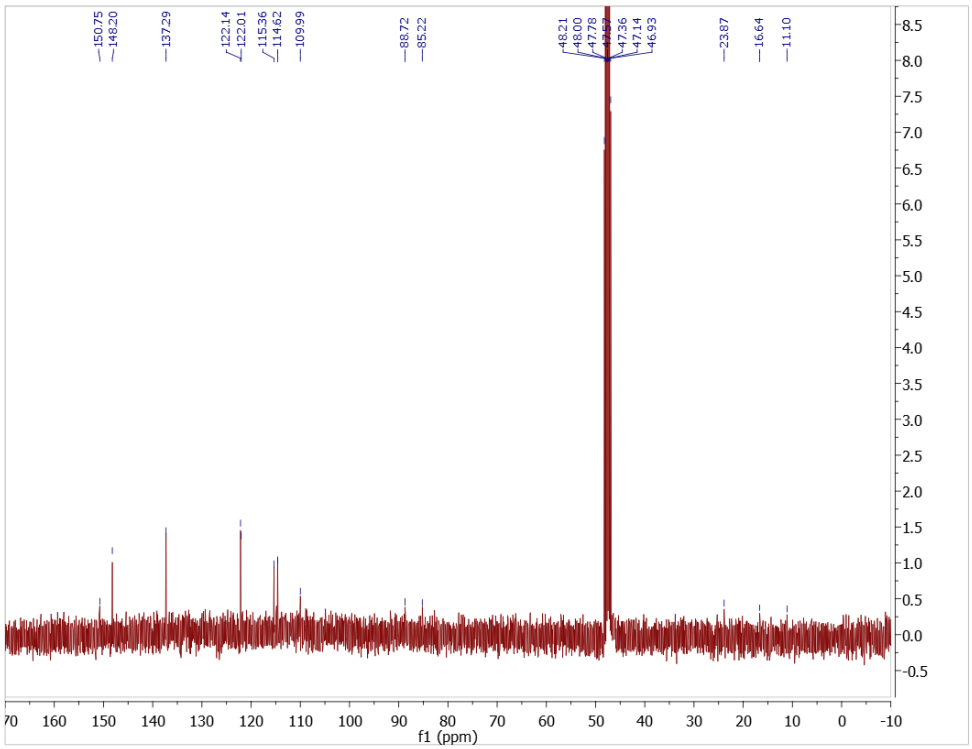
*Synthesis of Alternate Methylation Reference Standard; (N^1,N^4-dimethyl-N1-(pyridin-2-ylmethyl)benzene-1,4-diamine)(**8**):*

To an oven-dried round bottom flask was added tert-butyl methyl(4-(methyl(pyridin-2-ylmethyl)amino)phenyl)carbamate (10.0 mg, 0.0305 mmol) dissolved in anhydrous methanol (2mL). The solution was cooled to 0 ^{o}C, and trimethylsilyl chloride (16.6 mg, 0.153 mmol) was slowly added to the mixture. After the solution stirred for 12h, the reaction was quenched with saturated aqueous $NaHCO_3$ (5 mL). The product was extracted three times with dichloromethane (10 mL), dried over sodium sulfate, and the solvent was removed *in vacuo*. This process yielded the product as a yellow oil (3.9 mg, 60.0%); R_f 0.12 (1:1 = hexanes:ethyl acetate); ^{1}H NMR (400 MHz; CD_3OD)/δ (ppm): 2.70 (3H, s), 2.95 (3H, s), 4.46 (2H, s), 6.70 (4H, m), 7.27 (1H, t, *J*=4.8 Hz), 7.32 (1H, d, *J* =7.6 Hz), 7.74 (1H, td, *J* = 7.6 Hz, *J* = 1.6 Hz), 8.47 (1H, d, *J* = 4.4 Hz); ^{13}C NMR (100 MHz; CD_3OD)/δ (ppm): 23.87, 109.99, 114.62, 114.48, 115.36, 122.01, 122.14, 137.29, 148.20, 150.75 HPLC: retention time = 8 min, column: Phenomenex Gemini C18, 250x4.6 mm, mobile phase: 35% MeCN, pH 10 adjusted with triethylamine, flow rate: 1.0 mL/min, wavelength: 254 nm; HRMS: calculated for $[M+H]^+$(M = $C_{14}H_{17}N_3$), 228.1495, found 228.1495.

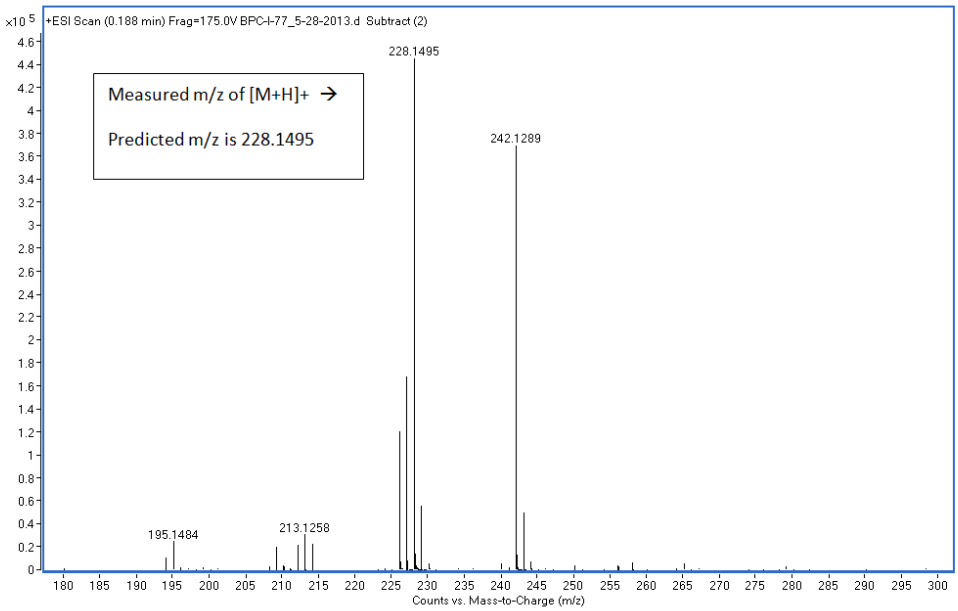
^{1}H NMR (400 MHz) of (8):



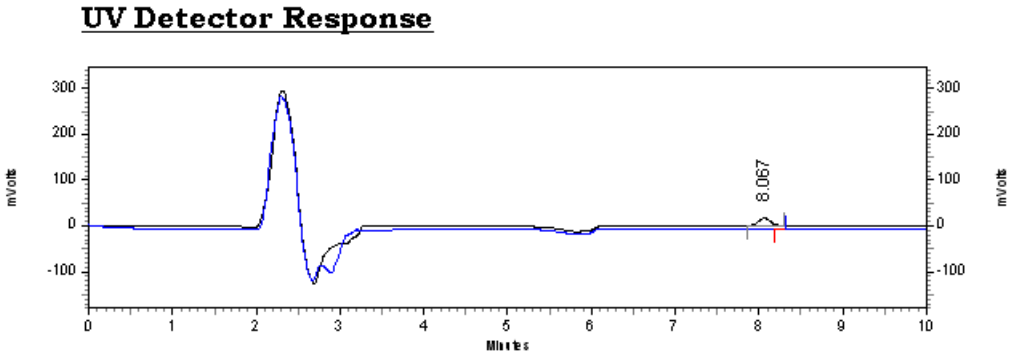
^{13}C NMR (100 MHz) of (8):



HRMS of (8):



HPLC of (8); Blue overlay is blank injection of EtOH:



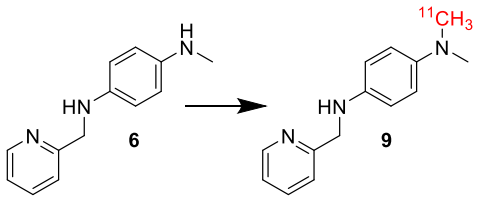
UV Detector
Ch1-254 nm Results

Retention Time	Area	Area %
8.07	165608	100.00

(II) Radiochemistry:

General Considerations

Reagents and solvents were commercially available and used without further purification, unless otherwise noted: sodium chloride, 0.9% USP and sterile water for Injection, USP were purchased from Hospira; Dehydrated Alcohol for Injection, USP was obtained from Akorn Inc.; Ascorbic Acid for Injection, USP was acquired from Bioniche Pharma; Ammonium Bicarbonate was obtained from Fisher Scientific. Shimalite-Nickel was purchased from Shimadzu; iodine was obtained from EMD; phosphorus pentoxide was acquired from Fluka; molecular sieves were purchased from Alltech; and HPLC columns were acquired from Phenomenex. Other synthesis components were obtained as follows: sterile filters were acquired from Millipore; C18-light Sep-Paks and Porapak Q were purchased from Waters Corporation; 10 cc sterile vials were obtained from HollisterStier. Sep-Paks were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use.



*Radiochemical Synthesis of [^{11}C]L2-b (**9**):*

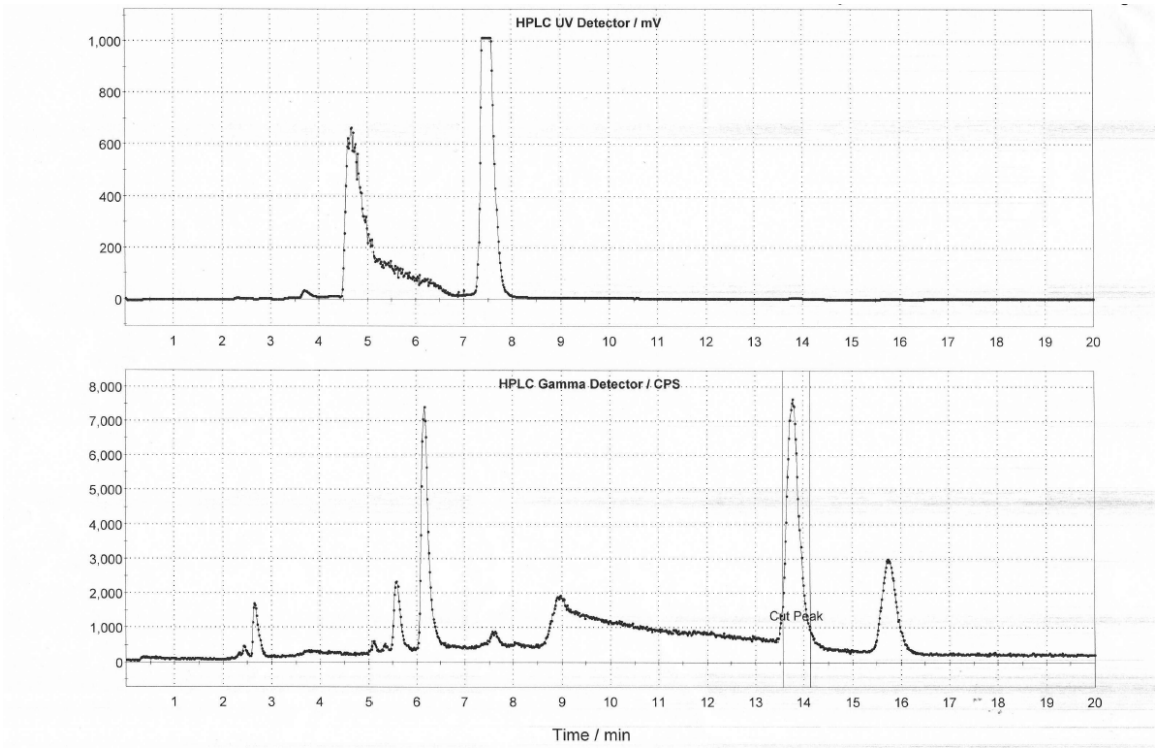
Production of [^{11}C]MeOTf was carried out as described by Shao et al.[3] Briefly, carbon-11 gas target loaded with [^{14}N]N_2 of a General Electric Medical Systems (GEMS) PETTrace cyclotron was bombarded with a proton beam to generate ~3 Ci of [^{11}C]CO_2. Subsequently, [^{11}C]CO_2 was delivered to the TRACERlab $FX_{C\text{-}Pro}$ module and reduced to [^{11}C]CH_4 by heating Shimalite-Nickel column to 350°C for 20 sec. Then, [^{11}C]CH_4 was reacted with iodine at 720°C to produce [^{11}C]CH_3I that was passed via a silver triflate-Graphpac column pre-heated to 190°C to finally yield [^{11}C]CH_3OTf. The resulting [^{11}C]CH_3OTf was delivered to the loop for the preparation of [^{11}C]L2-b as outlined below.

L2-b precursor (1.0 mg) was dissolved in acetonitrile (100 µL), loaded onto the 2 mL steel HPLC loop and conditioned with nitrogen gas for 20 sec at 10 mL/min. [^{11}C]MeOTf was passed through the HPLC loop containing precursor at 15 mL/min for 5 min. The reaction mixture was then purified using semipreparative HPLC (column: Phenomenex Gemini C18, 250x10 mm, mobile phase: 10 mM NH_4HCO_3 in 30% MeCN, pH 10.3, flow rate: 4 mL/min). The product peak (RT ~15 min) was collected into the round-bottomed flask containing 30 mL of water supplemented with 0.1% w/v of ascorbic acid. The solution was then passed through a C-18 extraction disk to remove organic solvent. The disk was washed with 7 mL sterile water/0.1% w/v of ascorbic acid. The product was eluted with 0.5 mL of ethanol followed by 2.0 mL of saline/0.1% w/v of ascorbic acid into the product vial containing 2.5 mL of saline/0.1% w/v of ascorbic acid. The final reformulation was passed through a 0.22-µm filter into a

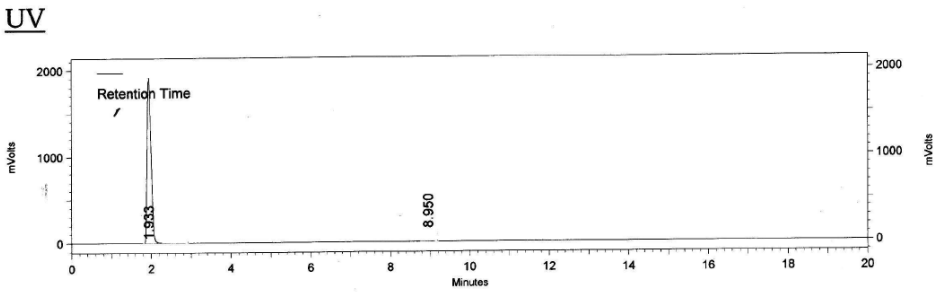
sterile dose vial (yield is ~3.6%, non-decay corrected, mean yield at end of synthesis = 27mCi, clear and colorless, n = 3). The final product was then submitted for a quality control (QC).

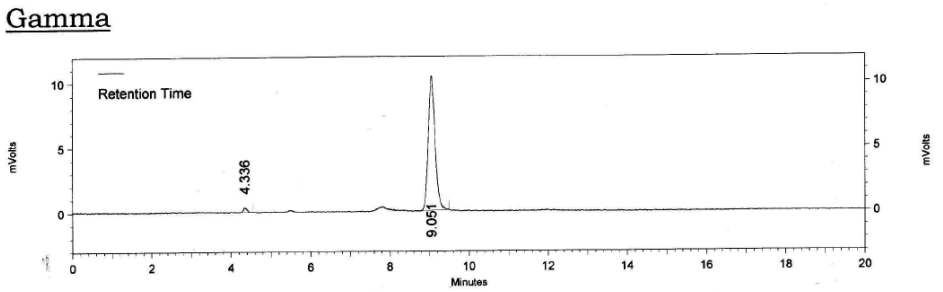
Quality control of [^{11}C]L2-b: Radiochemical purity of [^{11}C]L2-b was assessed using Shimadzu LC-2010A HT system equipped with the UV and Rad detectors (column: Phenomenex Gemini C18, 250x4.6 mm; mobile phase: 10 mM NH_4HCO_3 in 35% MeCN, pH 10.3; flow rate: 1.0 mL/min; wavelength: 254 nm; room temperature; product peak: ~9.0 min). Autoradiolysis was assessed by injecting [^{11}C]L2-b onto QC column taken directly from the original dose vial. Two injections were performed 30 min apart. No autoradiolysis was detected with ascorbate formulation

Typical Semi-preparative HPLC trace for [^{11}C]L2-b synthesis:



Typical Analytical HPLC trace for [^{11}C]L2-b quality control:



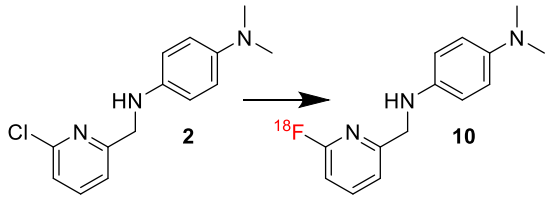


UV Detector
Ch1-254nm Results

Retention Time	Area	Area %	Height	Width	S/N (ASTM)
1.933	13407532	100	1914636	1.150	43898.36
8.950	589	0	54	0.458	7.01

RAD Results

Retention Time	Area	Area %	Width
4.336	2135	2	0.29
9.051	123326	98	0.68



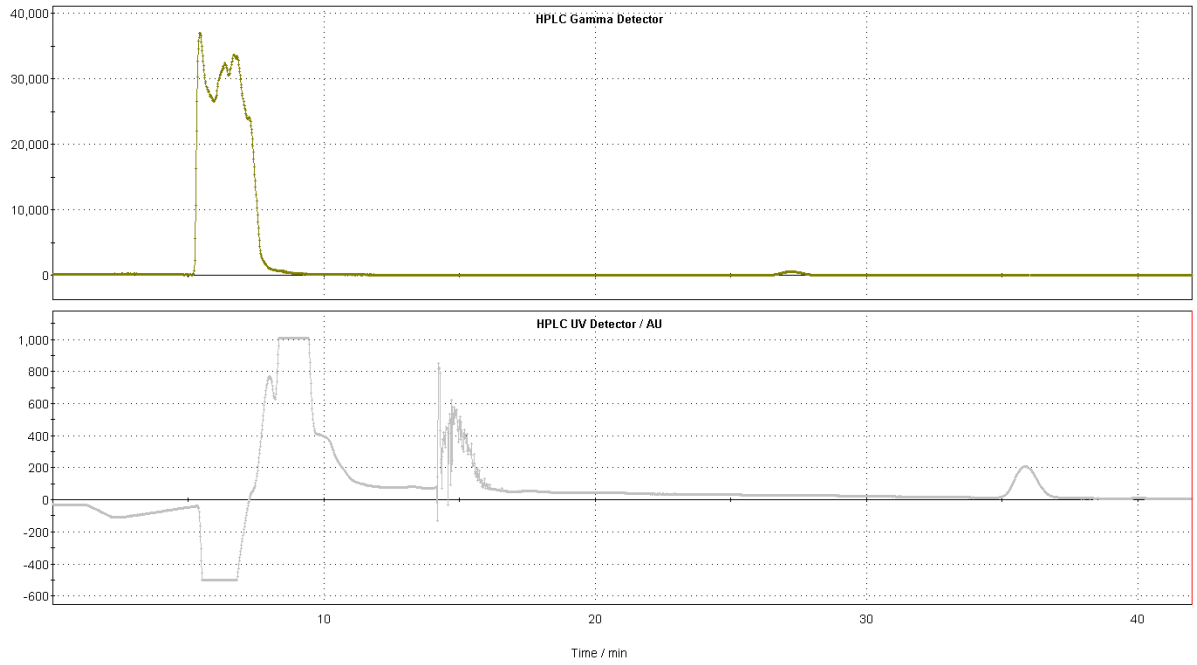
*Radiochemical Synthesis of [^{18}F]FL2-b (**10**):*

Potassium [^{18}F]fluoride was prepared using a TRACERLab FX_{FN} automated radiochemistry synthesis module (General Electric, GE). [^{18}F]Fluoride was produced via the $^{18}O(p,n)^{18}F$ nuclear reaction using a 16 MeV GE PETTrace cyclotron (40 μA beam for 30 min generated 1500 mCi of [^{18}F]fluoride). The [^{18}F]fluoride was delivered to the synthesis module (in a 1.5 mL bolus of [^{18}O]water) and trapped on a QMA-light Sep-Pak to remove [^{18}O]water. [^{18}F]Fluoride was eluted into the reaction vessel using aqueous potassium carbonate (3.5 mg in 0.5 mL of water). A solution of 2.2.2. cryptand (15 mg in 1 mL of acetonitrile) was added to the reaction vessel, and the resulting solution was dried azeotropically to give dry potassium [^{18}F]fluoride 2.2.2. cryptand. Evaporation was achieved by heating the reaction vessel to 100 °C and drawing full vacuum for 4 min. After this time, the reaction vessel was subjected to an argon stream and simultaneous vacuum draw for an additional 4 min. A solution of vacuum dried FL2-b precursor(1.0mg) dissolved in 0.5mL anhydrous DMSO was added to the reactor, and the mixture was stirred at 130°C for 30 min. The reactor was cooled to 55°C, and 2mL semi-preparative solvent was added to the crude reaction mixture. The resulting solution was purified using HPLC (column: Phenomenex

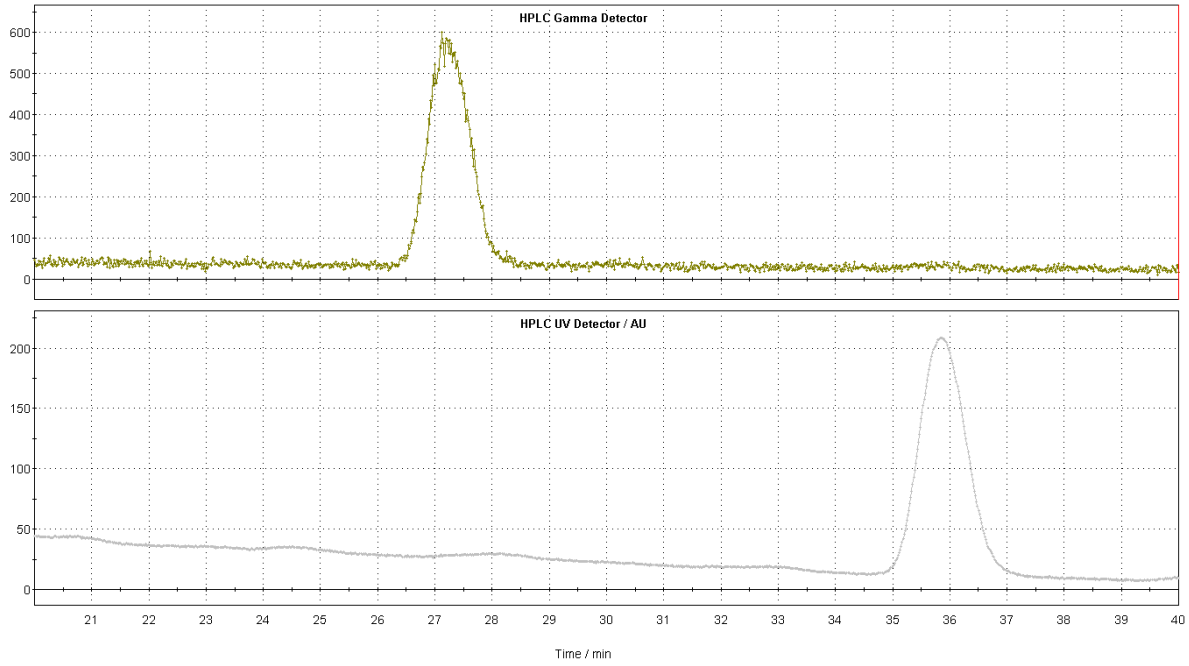
Gemini C18, 250x10 mm, mobile phase: 10 mM NH_4HCO_3 in 40% MeCN supplemented with 100mg/L L-ascorbic acid, pH ~9.7 adjusted with 3mL/L sat. NH_4OH, flow rate: 2.5 mL/min) The product peak (~27 min retention time) was collected and diluted into a round-bottom flask containing 50mL water supplemented with 50µL ascorbic acid solution (500mg/mL in water). The solution was then passed through a C-18 extraction disk to remove organic solvent. The disk was washed with 5 mL sterile water supplemented with 10µL ascorbic acid solution (500mg/mL in water). The product was eluted with 0.5 mL of ethanol followed by 4.5 mL of sterile water supplemented with 10µL ascorbic acid solution (500mg/mL in water). The final formulation was passed through a 0.2µM needle filter into a sterile dose vial [0.14% non-decay corrected radiochemical yield, mean yield at end of synthesis = 2.49 mCi, >99% radiochemical purity, specific activity = 970 Ci/mmol, clear and colorless, n = 3].

Quality control of [^{18}F]FL2-b: Radiochemical purity of [^{18}F]FL2-b was assessed using Shimadzu LC-2010A HT system equipped with the UV and Rad detectors (column: Phenomenex Gemini C18, 250x4.6 mm; mobile phase: 10 mM NH_4HCO_3 in 30% MeCN, pH 10 adjusted with 3mL/L sat. NH_4OH solution; flow rate: 2.0 mL/min; wavelength: 254 nm; room temperature; product peak: ~19 min)

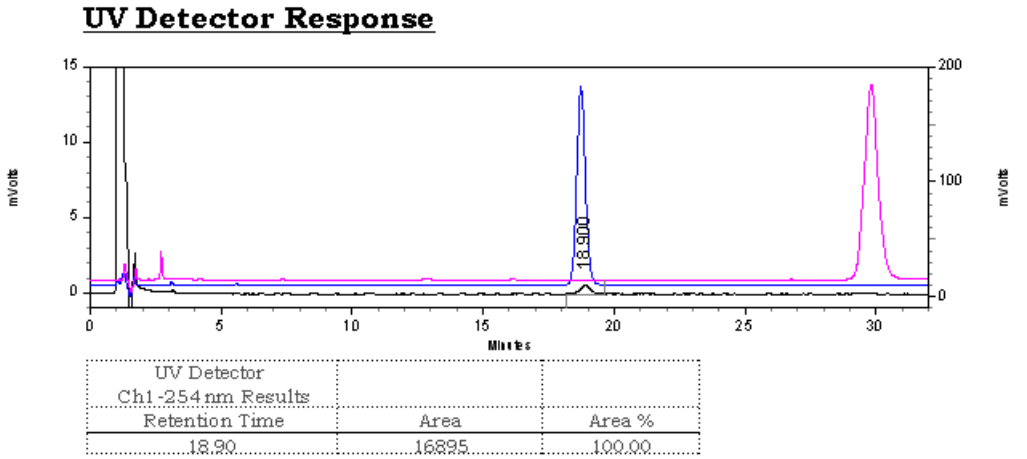
Typical Semi-preparative HPLC trace for [^{18}F]FL2-b(9) synthesis:



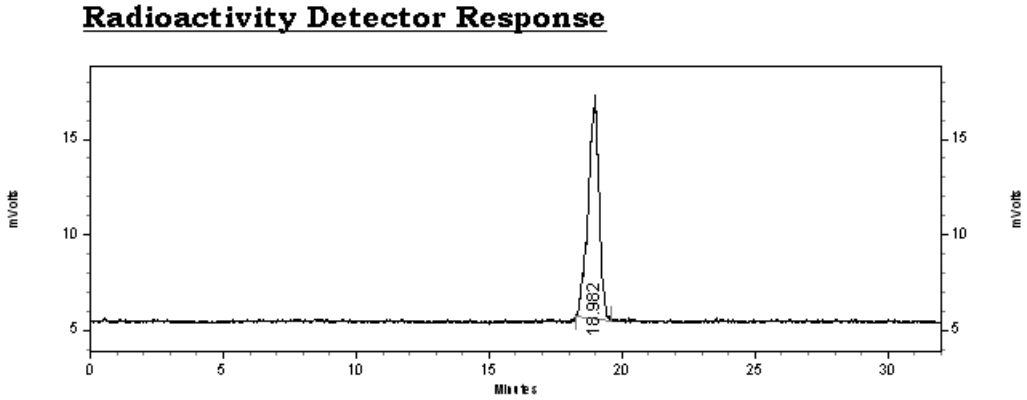
Typical Semi-preparative HPLC trace for [^{18}F]FL2-b(9) synthesis (zoomed into product/precursor peaks):



Typical Analytical HPLC trace for [^{18}F]FL2-b(black; left y-axis) quality control. Blue overlay is FL2-b reference standard(right y- axis; offset +10mVolts); pink overlay is FL2-b precursor(right y-axis; offset +15 mVolts):



UV Detector Ch1-254nm Results		
Retention Time	Area	Area %
18.90	16895	100.00



RAD Results		
Retention Time	Area	Area %
18.98	324408	100.00

(III) Autoradiography:

Autoradiography studies: Frozen blocks (1x1 inch) of amygdala from the postmortem brain of AD patient and normal control were used for the autoradiography binding studies. Tissue was obtained from the University of Michigan Alzheimer's Disease Center. Frozen blocks were sliced into 20 μm sections using a Hacker Instruments cryostat set to -15ºC. Tissue was thaw-mounted on the 1x3 inch polylysine-subbed glass slides. Sections used for autoradiography experiments were incubated for 5 min with phosphate buffer saline (PBS) – ethylenediaminetetraacetic acid (EDTA) buffer pH 7.4. To determine total binding, brain sections were transferred to a solution of various [^{11}C]L2-b, [^{18}F]FL2-b or [^{11}C]PiB concentrations (see Figure 2 in main manuscript) in PBS-EDTA (pH 7.4) and incubated for 30 min. Similarly, the non-specific binding and displacement experiments were conducted by incubating adjacent tissue sections in the same concentration of [^{11}C]L2-b, [^{18}F]FL2-b or [^{11}C]PiB, but supplementing them with 1 μM of "cold" L2-b, PiB, or AV-45 in PBS-EDTA (pH 7.4) for 30 min at room temperature. Subsequently, all tissue sections were washed with PBS-EDTA (pH 7.4) for 2 min (x2) and rinsed with water for 5 sec to remove unbound radioactivity. Finally, all slides were dried under the continuous airflow for 5 min before being exposed to high-resolution plate for 15 min. The exposed plate was then scanned using Typhoon 7000 phosphoimager. Image analysis was performed using software ImageQuant (Molecular Dynamics).

Binding Study: To quantify the amount of bound radiotracer to the brain sections, calibration standards were made for each autoradiography experiment. Each standard was prepared as series of 5 μL drops of known [^{11}C]L2-b, [^{18}F]FL2-b or [^{11}C]PiB concentration pipetted onto TLC plate and exposed simultaneously with the brain sections. Then, the standard curve (fmol of substance vs total counts) was generated to be further used to calculate fmol of radiotracer bound in the area of interest on the brain tissue. The phosphoimager presents the signal for the region of interest as counts per pixel. Therefore, we used a known pixel size (25 μm) to calculate counts per square millimeter. The final calculation involved conversion of counts per square millimeter to femtomoles per square millimeter in the regions of interest. Data analysis including determination of Kd and Bmax values was performed with GraphPad Prism (Version 6.0b) using nonlinear regression.

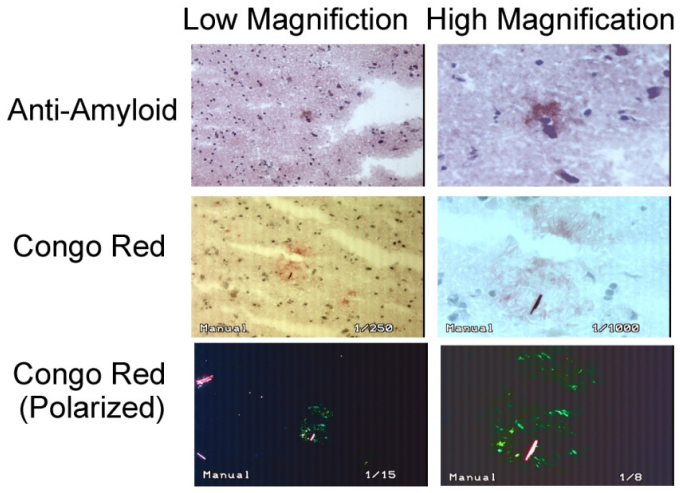
(IV) Immunohistochemistry:

Amyloid burden was established in AD brain tissue using Congo Red and Anti-beta-Amyloid (see Figure below).

Amyloid Antibody: Immunohistochemistry was performed according to Vectastain Elite ABC Kit instructions. Briefly, 20 µm tissue sections used for autoradiography were formalin-fixed for 5 days and used directly for immunohistochemistry. Fixed sections were washed in 70% ethanol for 30 min followed by incubation with 1% sodium dodecylsulfate (SDS) for 15 min at rt. Next, tissue sections were briefly washed with PBS pH 7.4 and then endogenous peroxidase activity was quenched with 0.3% H2O2 in 70% methanol for 15 min at room temperature. These sections were washed with PBS-T solution for 1 min (x3) and blocked with PBS-TBA for 30 min at room temperature. Then, the tissue slides were incubated for 24 hours at 4^{o} C with primary antibody (Millipore anti-beta-amyloid 1-42) diluted 1:1000 in PBS-TBA. After 24 hours, these sections were washed with PBS-T for 5 min (x3) followed by the biotinylated secondary antibody application for 30 min at room temperature (diluted in PBS-T per Vectastain instructions). Once secondary antibody incubation was complete, all sections were washed in PBS-T for 5 min (x3). The ABC solution (diluted in PBS-T per Vectastain instructions) was applied for 30 min at room temperature. Then, a 5 min rinse (x3) was performed before transferring sections to the DAB solution (tablet by Sigma) for 10 min at room temperature. Finally, the tissue sections were rinsed in water pH 4.0 and counterstained with Giemsa before coverslipping them with Permount.

Congo Red: AD patient blocks were sectioned at 10µm, mounted on poly-lysine subbed slides and fixed with 4% formaldehyde overnight. Sections were rinsed with many changes of 70% ethanol (to remove the formaldehyde) and then placed in Congo Red working solution for 10 minutes. Slides were rinsed in distilled water and then placed in Mayer's hematoxylin for 10 minutes, differentiated in running tap water, dehydrated through alcohols and coverslipped. Sections were viewed with white and polarized light.

Representative amyloid burden identified by immunohistochemistry and congo red staining



(V) MicroPET:

General Considerations:

All animal studies were performed in accordance with the standards set by the University Committee on Use and Care of Animals (UCUCA) at the University of Michigan.

Primate Imaging:

Primate imaging studies were done using a mature female rhesus monkey (n = 2, weight = 6.3 kg). The monkey was anesthetized (isoflurane), intubated, and positioned in a Concorde MicroPET P4 scanner. Following a transmission scan, the animal was injected i.v. with [^{11}C]L2-b (4.3 and 4.7 mCi) as a bolus over 1 min, and the brain imaged for 60 min (5 x 2 min frames – 4 x 5 min frames – 3 x 10 min frames). Image data was reconstructed using the 3D MAP algorithm. Using a summed image, regions of interest were drawn over the brain, cortex, and cerebellum. From this, time-radioactivity curves were calculated.

SUV Data From Scans:

Time	Brain			Cortex			Cerebellum		
(seconds)	Scan 1	Scan 2	Average	Scan 1	Scan 2	Average	Scan 1	Scan 2	Average
30	0.511532	0.634212	0.572872	0.452283	0.5266535	0.489468	0.423134	0.812397	0.617765
90	2.21171	1.743459	1.977584	1.886824	1.7442211	1.815522	1.66371	2.584114	2.123912
150	2.249388	1.836736	2.043062	1.939014	1.8007517	1.869883	1.813614	2.401608	2.107611
210	2.103721	1.714533	1.909127	2.066361	1.7838862	1.925124	1.803647	2.183685	1.993666
270	1.723965	1.594791	1.659378	1.905269	1.9347157	1.919992	1.694404	1.971032	1.832718
375	1.517996	1.445532	1.481764	1.717114	1.5770876	1.647101	1.561684	1.680829	1.621256
525	1.173578	1.28767	1.230624	1.586537	1.5053217	1.545929	1.375498	1.215526	1.295512
750	0.971332	1.134568	1.05295	1.250012	1.28466	1.267336	1.112676	1.080532	1.096604
1050	0.845761	1.265487	1.055624	1.034822	1.2473174	1.14107	0.935055	1.252569	1.093812
1500.5	0.758199	0.808368	0.783283	0.813754	0.8925544	0.853154	0.782737	0.743009	0.762873
2101.5	0.696685	0.718775	0.70773	0.794514	0.7300518	0.762283	0.730657	0.689131	0.709894
2702.5	0.624224	0.689836	0.65703	0.823847	0.7507128	0.78728	0.687124	0.728313	0.707718
3301.4	0.629079	0.60789	0.618485	0.787	0.6728648	0.729932	0.656092	0.709541	0.682816

References:

(1) Choi, J.-S.; Braymer, J. J.; Nanga, R. P. R.; Ramamoorthy, A.; Lim, M. H., Design of small molecules that target metal-Aβ species and regulate metal-induced Aβ aggregation and neurotoxicity. *Proc. Nat. Acad. Sci. U.S.A.* **2010,** *107*, 21990-21995.

(2) Yan, S.; Wang, L.; Frye, L. L.; Chen, W.; Loury, D. J., Purinone compounds as kinase inhibitors. WO Patent, **2013**, WO2013116382 A1.

(3) Shao, X.; Hoareau, R.; Runkle, A. C.; Tluczek, L. J. M.; Hockley, B. G.; Henderson, B. D.; Scott, P. J. H. Highlighting the versatility of the Tracerlab synthesis modules. Part 2: fully automated production of [^{11}C]-labeled radiopharmaceuticals using a Tracerlab $FX_{C\text{-}Pro}$. *J. Labelled Comp. Radiopharm.* **2011**, *54*, 819-838.