Synthesis and evaluation of [^{11}C]PBD150, a radiolabeled glutaminyl cyclase inhibitor for the potential detection of Alzheimer's disease prior to amyloid β aggregation.

Allen F. Brooks[a], Isaac M. Jackson[a], Xia Shao[a], George W. Kropog[a], Phillip Sherman[a], Carole A. Quesada[a] and Peter J.H. Scott[*a,b]

[a]*Division of Nuclear Medicine, Department of Radiology, The University of Michigan Medical School, 2276 Medical Science I Building, Ann Arbor, Michigan 48109, USA. *Fax:+1(734)615-2557; Tel:+1(734)615-1756; E-mail: pjhscott@umich.edu.*

[b]*The Interdepartmental Program in Medicinal Chemistry, The University of Michigan, 428 Church St., Ann Arbor, Michigan 48109, USA.*

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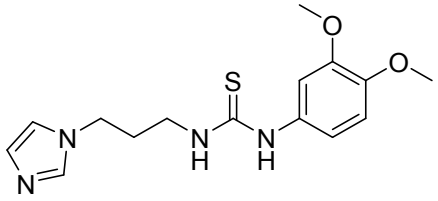
1. CHEMISTRY

1.1 General Considerations

All solvents and reagents were commercially available and used without further purification unless otherwise stated. NMR spectra were recorded with a Varian 400 MHz instrument at room temperature with tetramethylsilane (TMS) as an internal standard. Mass spectra were performed on a Agilent Q-TOF HPLC-MS or VG (Micromass) 70-250-S Magnetic sector mass spectrometer employing the electrospray ionization (ESI) method or electron ionization (EI) 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 with rigorously dried glassware under inert atmosphere.

1.2 Synthesis Procedures

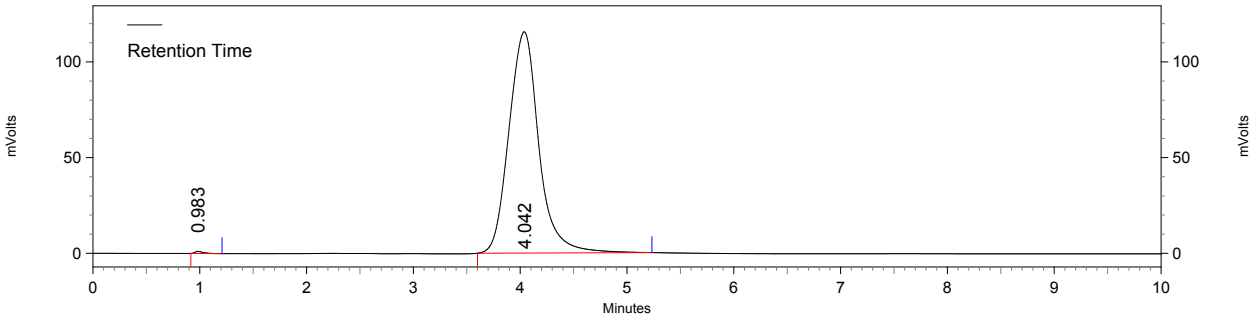


PBD150

PBD150: 4-Isothiocyanato-1,2-dimethoxybenzene (0.200 g, 1.02 mmol) was dissolved in EtOH (2 mL, 0.5 M), and 1-(3-aminopropyl)imidazole (0.12 mL, 1.02 mmol) was added. The solution was heated to and held at reflux for 2 h. The solvent was removed in vacuo and recrystallized from EtOH to provide the product as a white powder (0.22 g, 66.5 % yield, >99% purity by HPLC (Figure S1)); Characterization matched the previous literature report.[1] ^{1}H NMR (400 MHz; d6-DMSO)/δ (ppm): 1.97 (2H, quin, J = 7.0), 3.43 (2H, br), 3.72 (3H, s), 3.74 (3H, s), 3.98 (2H, t, J = 7.0), 6.77 (1H, dd, J = 8.5, 2.2), 6.88 (1H, s), 6.91 (1H, s), 6.95 (1H, d, J = 2.2), 7.18 (1H, s), 7.59 (1H, br), 7.64 (1H, s), 9.36 (1H, s); HRMS: calculated for $[M+H]^+$ (M = $C_{15}H_{20}N_4O_2S$), 321.1380, found 321.1382.

Figure S1: Analytical HPLC trace of PBD150 reference standard

Mobile phase: 20%MeCN, 10 mM NH_4OAc; Column: 150 x 4.6 Luna C18 5µ; Flow rate: 2 mL/min: UV: 254 nm**.**



UV Detector 254 nm

Retention Time	**Area %**
0.983	0.5
4.042	99.5

Figure S2: ^{1}H NMR of PBD150 reference standard

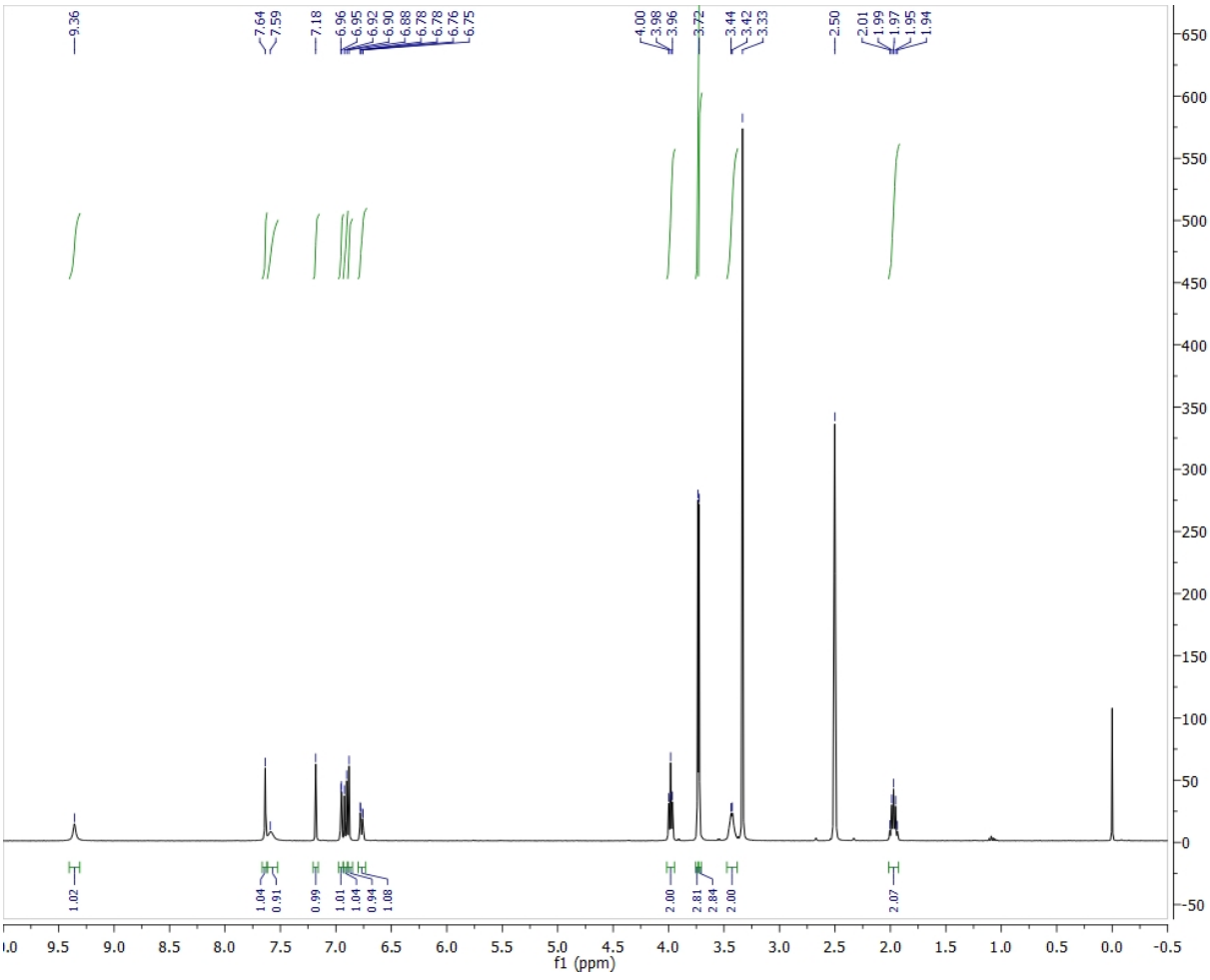
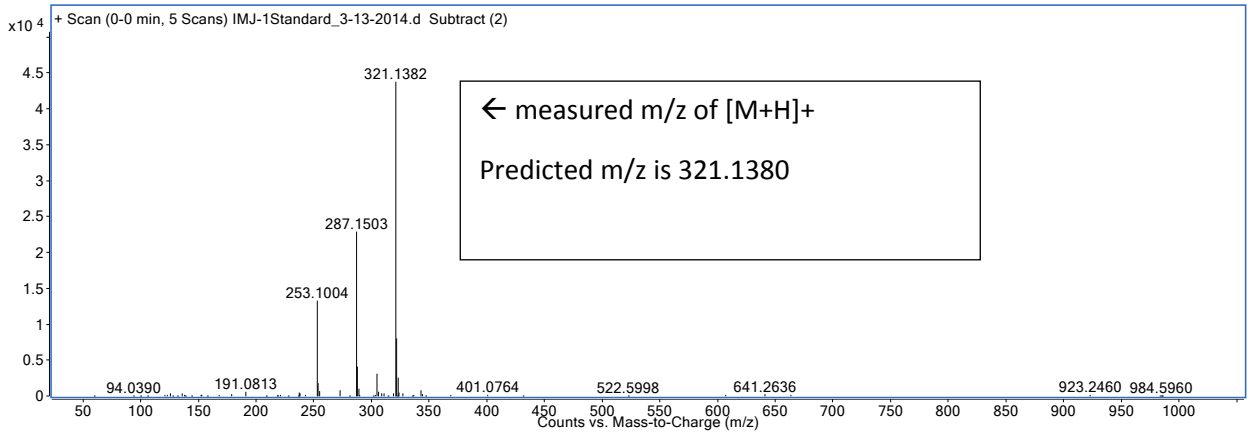
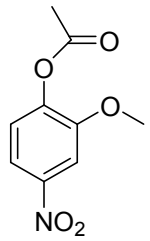


Figure S3: HRMS of PBD150 reference standard





2

2-Methoxy-4-nitrophenyl acetate (2) [2]: 4-Nitroguaiacol (0.5 g, 2.96 mmol) was dissolved in DCM (12 mL, 0.25 M). Pyridine (0.26 mL, 3.26 mmol) was added and the mixture was cooled to 0 °C in a water-ice bath. Acetic anhydride (0.31 mL, 3.26 mmol) was added dropwise and the reaction was warmed to rt and stirred overnight. The reaction mixture was poured into a separatory funnel and washed with an equal volume of water. The organic layer was removed and the aqueous layer was extracted with DCM. The organic layers were combined and washed with water, 2 N HCl, 2 N NaOH and brine then dried over Na_2SO_4. The DCM was removed in vacuo to yield the product as a white powder (0.54 g, 86 % yield); R_f 0.70 (1:1 = hexanes:ethyl acetate); ^{1}H NMR (400 MHz; d6-DMSO)/δ (ppm): 2.32 (3H, s), 3.92 (3H, s), 7.42 (1H, d, *J* = 8.6)7.90 (1H, dd, *J* = 8.6, 2.6) 7.93 (1H, d, *J* = 2.6); ^{13}C NMR (100 MHz; d6-DMSO)/δ (ppm): 20.84, 57.06, 108.34, 116.80, 124.25, 145.03, 146.36, 151.80, 168.41; HRMS: calculated for $[M]^+$ ($M = C_9H_9NO_5$), 211.0481, found 211.0483.

Figure S4: ^{1}H NMR of 2-methoxy-4-nitrophenyl acetate (2)

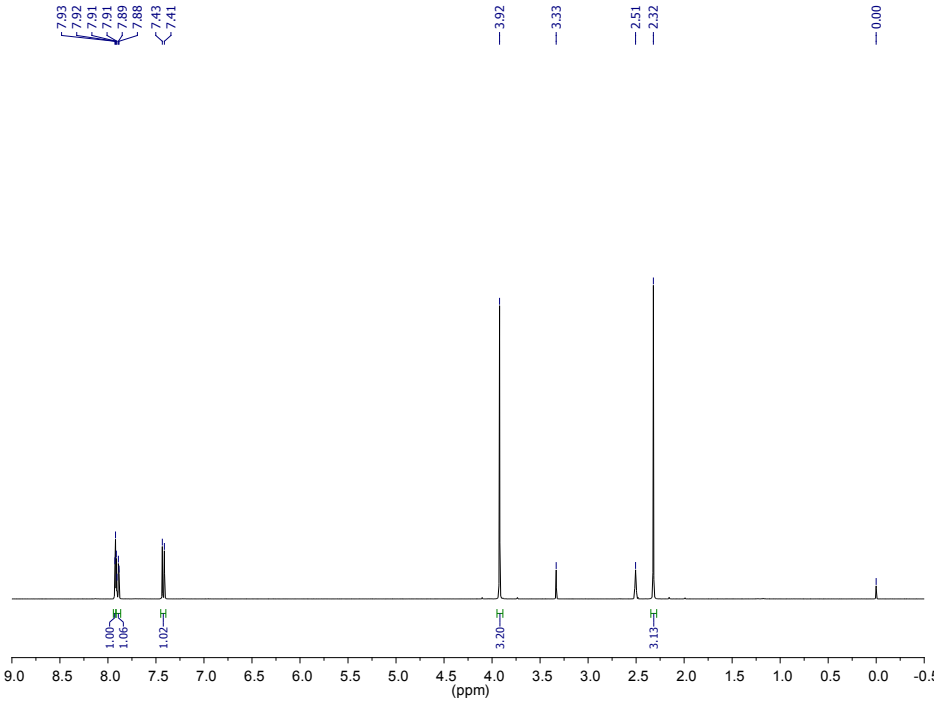


Figure S5: ^{13}C NMR of 2-methoxy-4-nitrophenyl acetate (2)

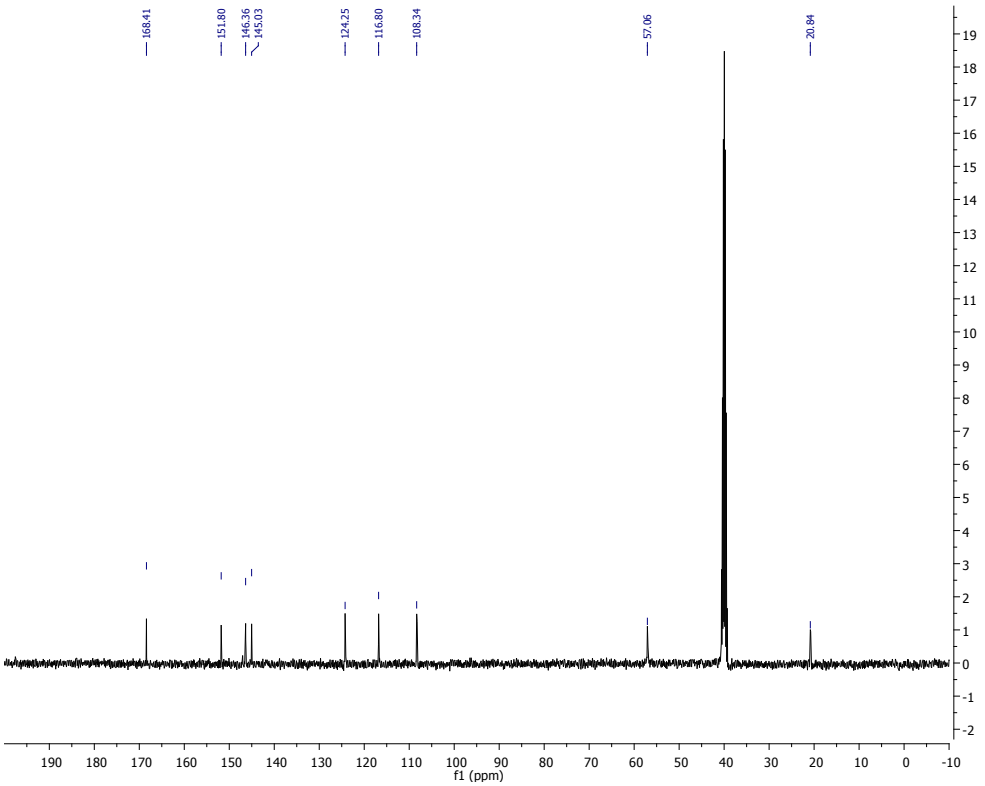
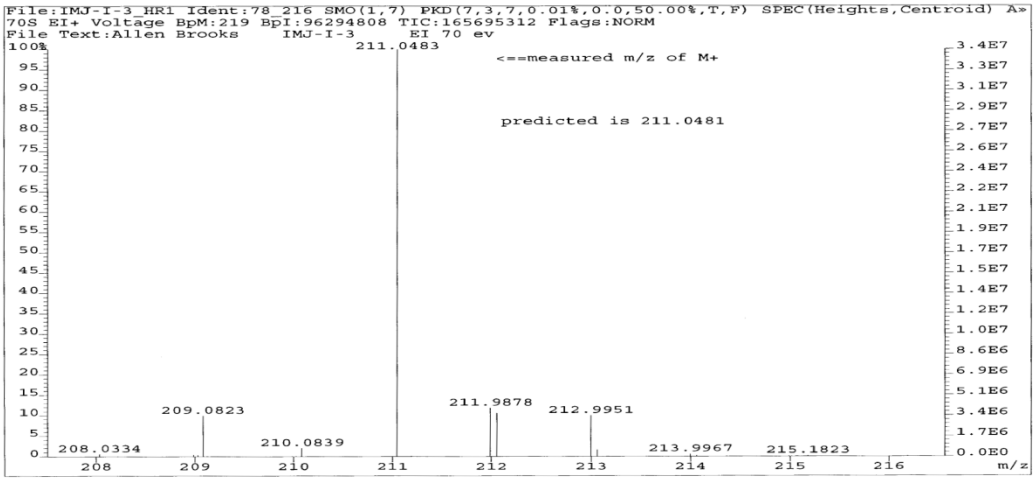
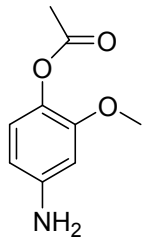


Figure S6: HR-MS of 2-methoxy-4-nitrophenyl acetate (2)





3

4-Amino-2-methoxyphenyl acetate (3) [2]: Nitro **2** (0.20 g, 0.95 mmol) was dissolved in EtOH (3 mL, 0.5 M). To this solution Pd/C (0.020 g, 0.095 mmol) was added and the mixture was sparged with a H_2 balloon (1 atm) overnight at rt. The reaction mixture was filtered through a pad of celite. The filtrate was collected and evaporated in vacuo. The product was purified by silica gel chromatography (hexanes, ethyl acetate gradient). After purification, the product was collected as a yellow oil; (0.139 g, 78 % yield); R_f 0.41 (1:1 = hexanes:ethyl acetate); ^{1}H NMR (400 MHz; d6-DMSO)/δ (ppm): 2.16 (3H, s), 3.65 (3H, s), 5.05 (2H, br, NH_2), 6.08 (1H, dd, *J* = 8.4, 2.4), 6.29 (1H, d, *J* = 2.4), 6.66 (1H, d, *J* =8.4); HRMS: calculated for $[M+H]^+$ (M = $C_9H_{11}NO_3$), 182.0812, found 182.0811.

Figure S7: ^{1}H-NMR of 4-amino-2-methoxyphenyl acetate (3)

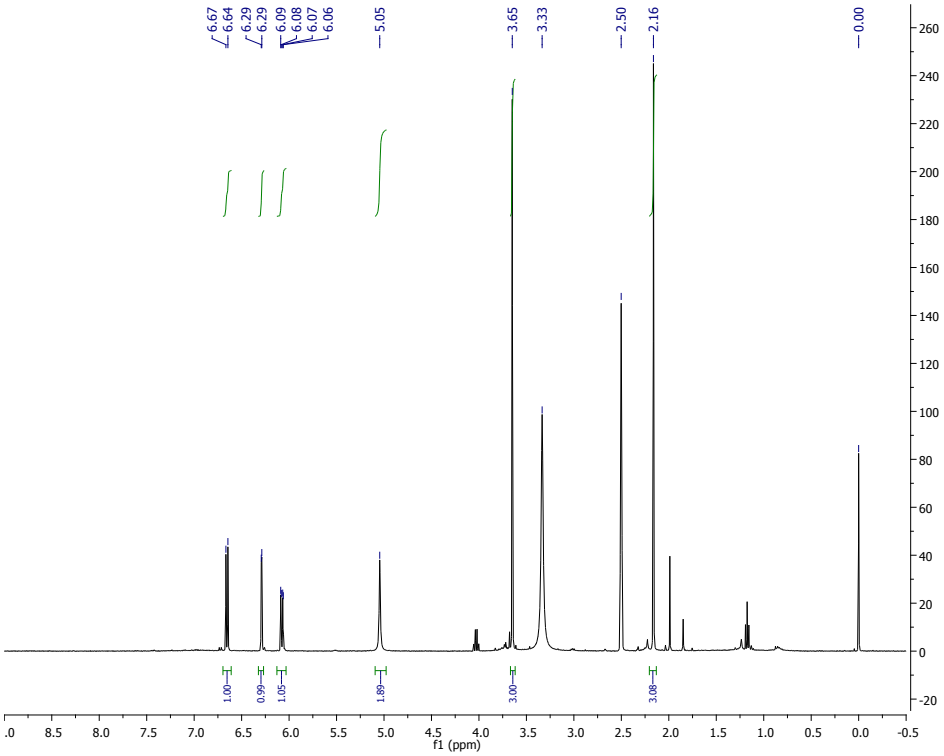
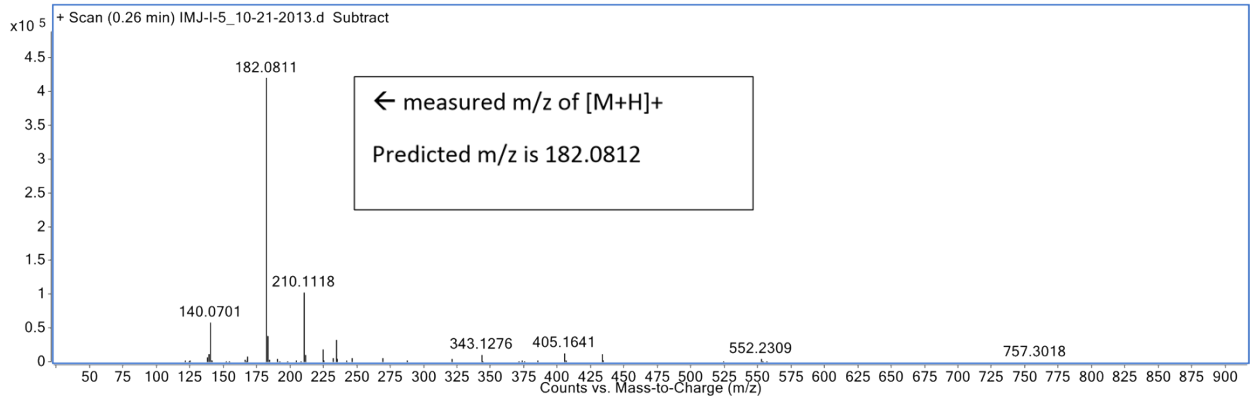
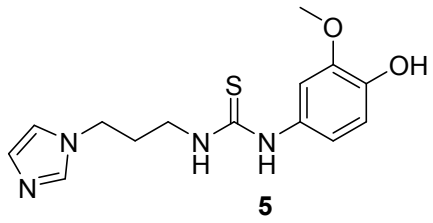


Figure S8: HR-MS of 4-amino-2-methoxyphenyl acetate (3)

Sample IMJ-I-5, by positive ion electrospray:





5

1-(3-(1H-imidazol-1-yl)propyl)-3-(4-hydroxy-3-methoxyphenyl)thiourea: Amine **3** (0.183 g, 1.01 mmol) was dissolved in DCM (10 mL, 0.1 M), and cooled to 0 °C in a water-ice bath. Thiophosgene (0.093 mL, 1.21 mmol) was added dropwise, followed by triethylamine (0.34 mL, 2.42 mmol) added dropwise. The resulting solution was stirred for 1.5 h at rt. The reaction was monitored by TLC and quenched with 10 mL of water once starting material was consumed. The quenched reaction was extracted three times with DCM and the combined organic layers were dried over $MgSO_4$ and evaporated in vacuo to give intermediate **4**; R_f 0.73 (1:1 = hexane:ethyl acetate). Thioisocynate **4** (0.225 g, 1.01 mmol; assumed 100 % conversion) was dissolved in EtOH (2 ml, 0.5 M), and 1-(3-aminopropyl)imidazole (0.241 mL, 2.02 mmol) was added. The solution was heated to and held at reflux for 2 h. The solvent was removed in vacuo and the product was recrystallized from EtOH and purified by silica gel chromatography (DCM, MeOH gradient) to give the product as a white powder; (0.13 g, 41 % yield for two steps); R_f 0.20 (9:1 = DCM:MeOH); ^{1}H NMR (400 MHz; d6-DMSO)/δ (ppm): 1.96 (2H, quin, J = 7.0), 3.43 (2H, br), 3.73 (3H, s), 3.97 (2H, t, J = 7.0), 6.62 (1H, dd, J = 8.4, 2.4), 6.73 (1H, d, J = 8.4), 6.88 (2H, m), 7.18 (1H, s), 7.48 (1H, NH), 7.63 (1H, br), 8.97 (1H, s), 9.27 (1H, OH); ^{13}C NMR (100 MHz; d6-DMSO)/δ (ppm): 30.76, 41.88, 44.27, 55.92, 110.36, 115.70, 117.72, 119.74, 128.75, 130.21, 137.57, 144.73, 147.83,180.86; HRMS: calculated for $[M+H]^+$ ($M = C_{13}H_{16}N_4O_2S$), 307.1223, found 307.1226.

Figure S9: ^{1}H-NMR of 1-(3-(1H-imidazol-1-yl)propyl)-3-(4-hydroxy-3-methoxyphenyl)thiourea (5)

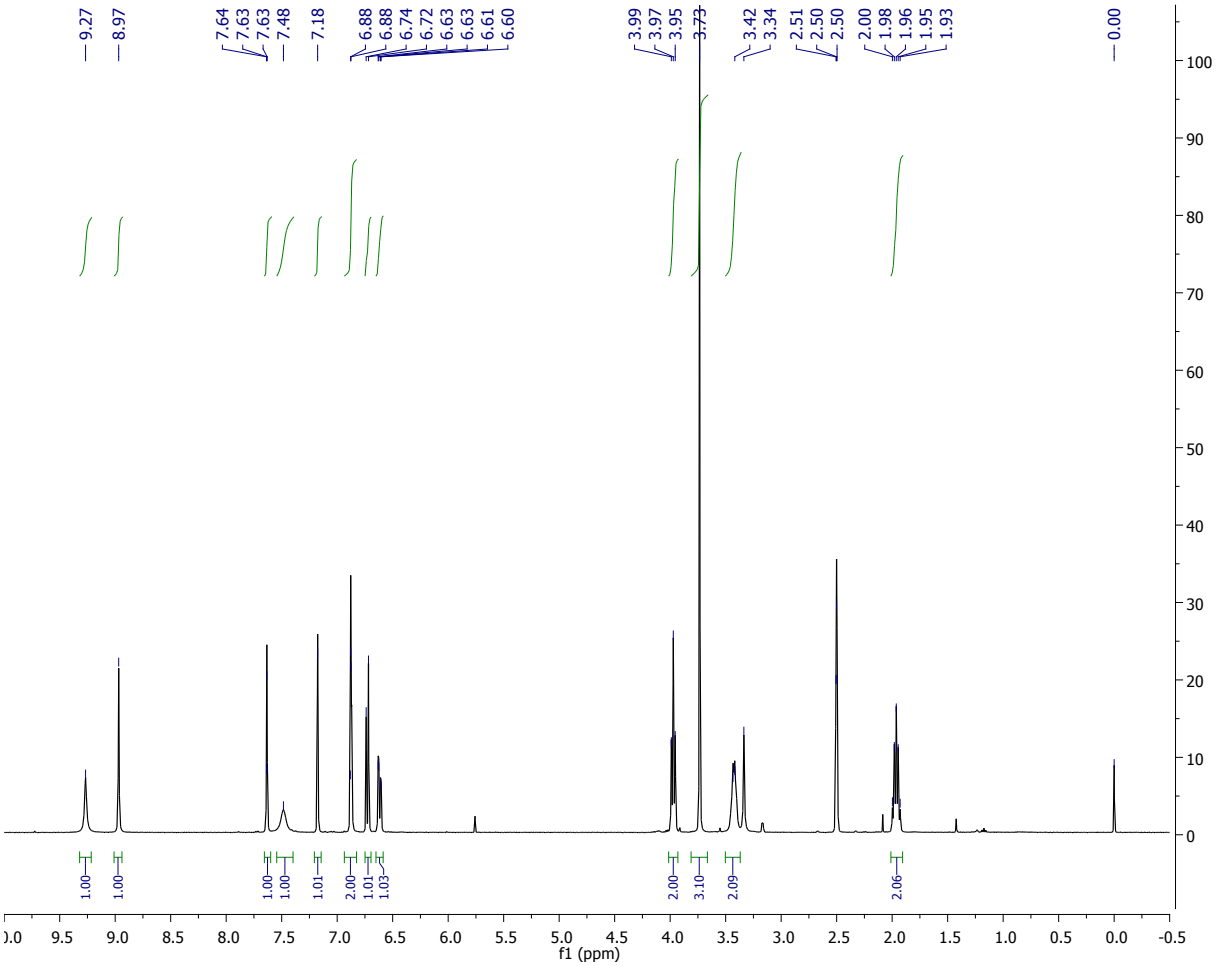


Figure S10: ^{13}C-NMR of 1-(3-(1H-imidazol-1-yl)propyl)-3-(4-hydroxy-3-methoxyphenyl)thiourea (5)

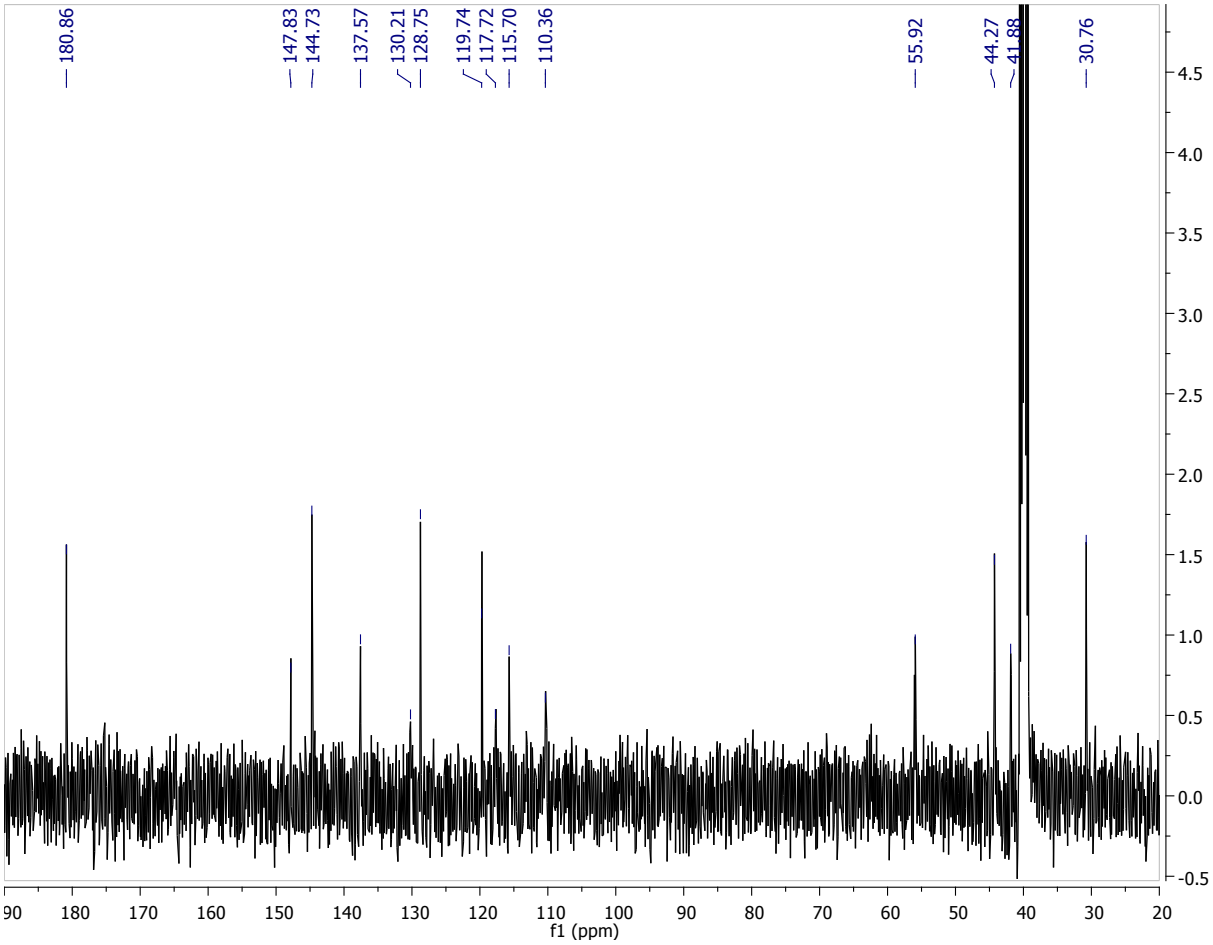
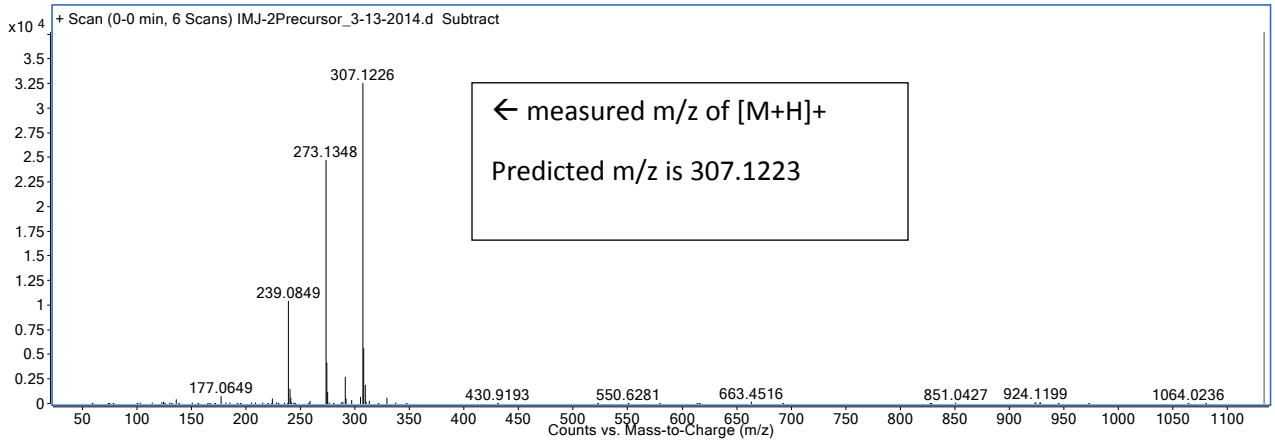
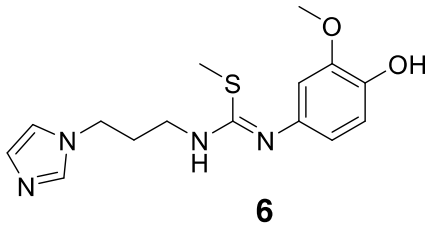


Figure S11: HR-MS of 1-(3-(1H-imidazol-1-yl)propyl)-3-(4-hydroxy-3-methoxyphenyl)thiourea (5)





6

Methyl (Z)-N-(3-(1H-imidazol-1-yl)propyl)-N'-(4-hydroxy-3-methoxyphenyl)carbamimidothioate: Thiourea **5** (0.0500 g, 0.16 mmol) was dissolved in DMF (1.6 mL, 0.1 M) and treated with iodomethane (0.02 mL, 0.33 mmol). The resulting solution was stirred for 90 minutes. The solvent and iodomethane were removed in vacuo and the resulting residue was treated with equal portions of saturated sodium bicarbonate solution and DCM. The organic layer was separated and washed with H_2O and dried over sodium sulfate. The reaction was purified by silica gel chromatography (DCM, MeOH gradient) to give the product (0.0076 g, 11 % yield); R_f 0.09 (9:1 = DCM:MeOH); ^{1}H NMR (400 MHz; d6-DMSO)/δ (ppm): 1.99 (2H, quin, *J* = 6.8), 2.27 (3H, s), 3.19 (2H, br), 3.69 (3H, s), 4.02 (2H, t, *J* = 6.8), 6.12 (1H, dd, *J* = 8.2, 1.8), 6.29 (1H, d, *J* = 1.8), 6.32 (1H, br), 6.59 (1H, d, *J* = 8.2), 6.89 (1H, s), 7.20 (1H, s), 7.65 (1H, s), 8.39 (1H, s); HRMS: calculated for $[M+H]^+$ (M = $C_{15}H_{20}N_4O_2S$), 321.1380, found 321.1379.

Figure S12: ^{1}H-NMR of Methyl (Z)-N-(3-(1H-imidazol-1-yl)propyl)-N'-(4-hydroxy-3-methoxyphenyl) carbamimidothioate: **(6)**

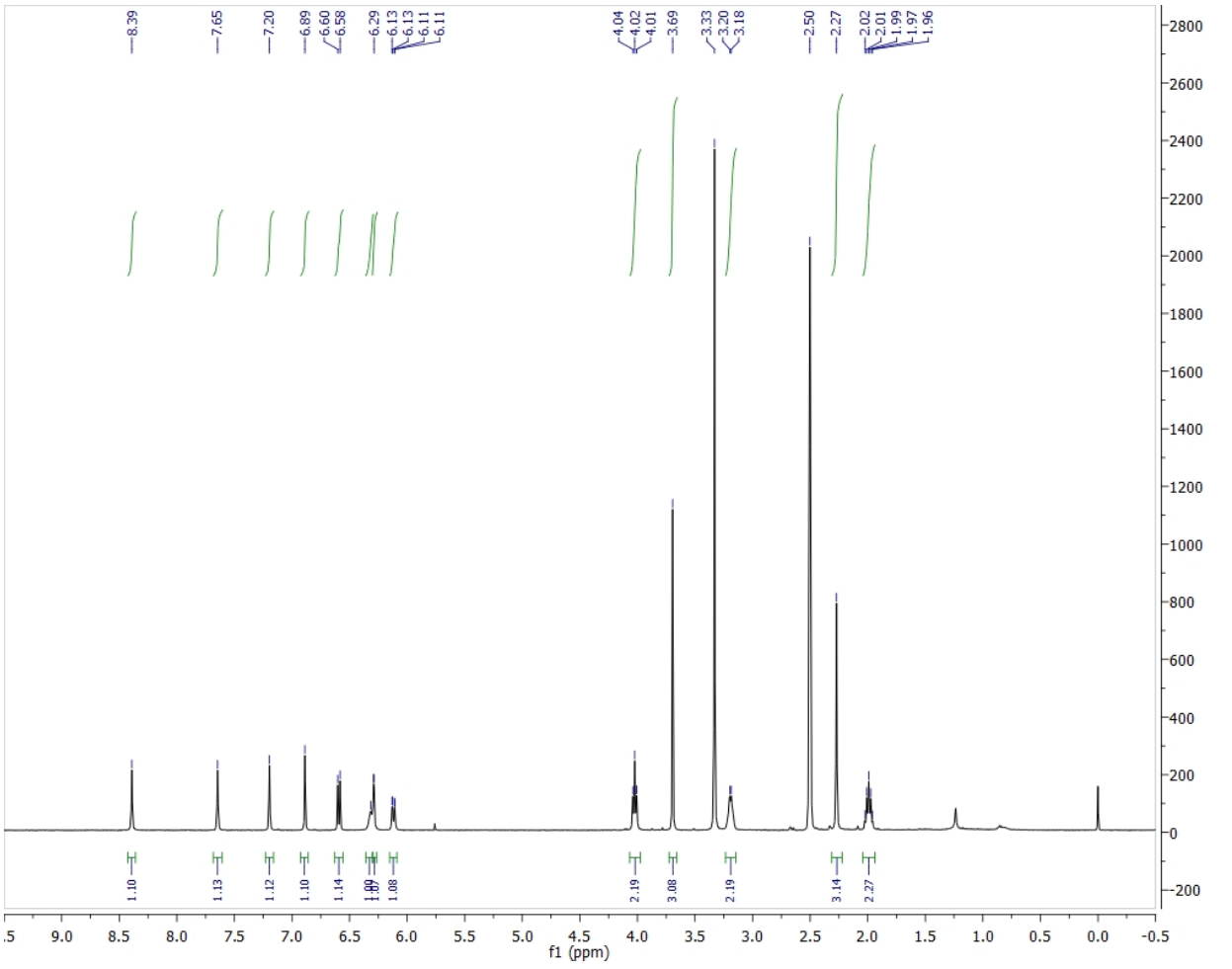
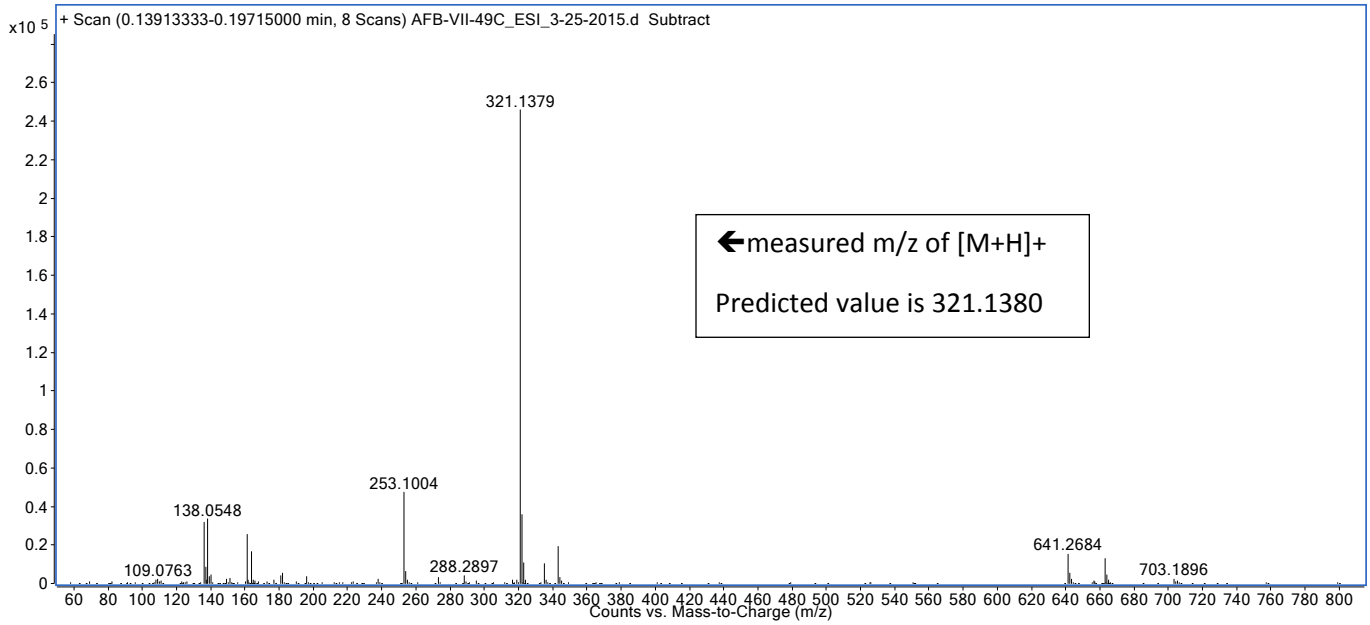


Figure S13: HR-MS of Methyl (Z)-N-(3-(1H-imidazol-1-yl)propyl)-N'-(4-hydroxy-3-methoxyphenyl) carbamimidothioate: **(6)**

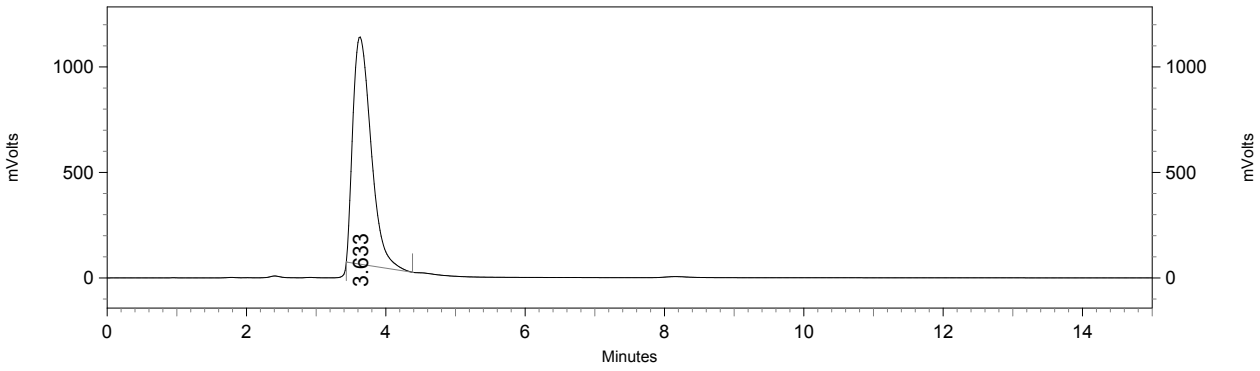


2. HPLC ANALYSIS OF REFERENCE STANDARDS

2.1 HPLC System 1

Column: Phenomenex Luna C18, 150 x 4.6 mm; Mobile phase: 10 mM NH_4OAc in 20% MeCN; Flow rate: 2.0 mL/min; UV wavelength: 254 nm; Column temperature: room temperature.

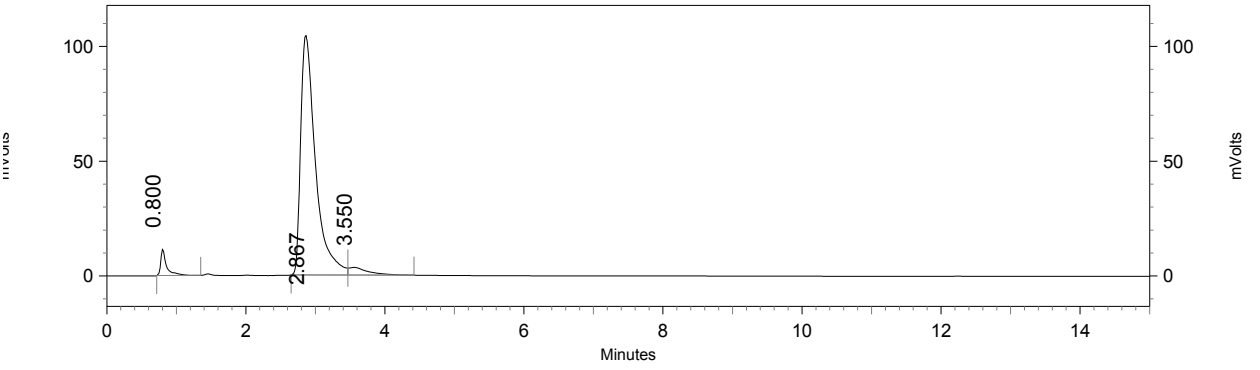
Figure S14: PBD150 Reference Standard Analyzed using System 1



UV Detector Ch1-254nm
Results

Retention Time	Area	Area %
3.63	19554265	100.00

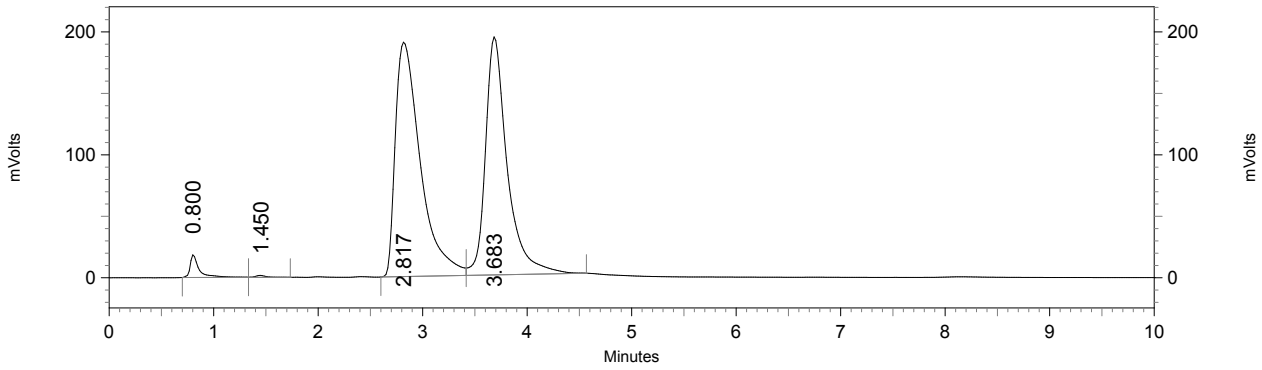
Figure S15: Alternate Methylation Standard 6 Analyzed using HPLC System 1



UV Detector Ch1-254nm
Results

Retention Time	Area	Area %
0.80	67043	3.99
2.87	1546966	92.14
3.55	65011	3.87

Figure S16: Co-Injection of PBD150 and Alternate Methylation Standard 6 Showing Baseline Separation with HPLC System 1



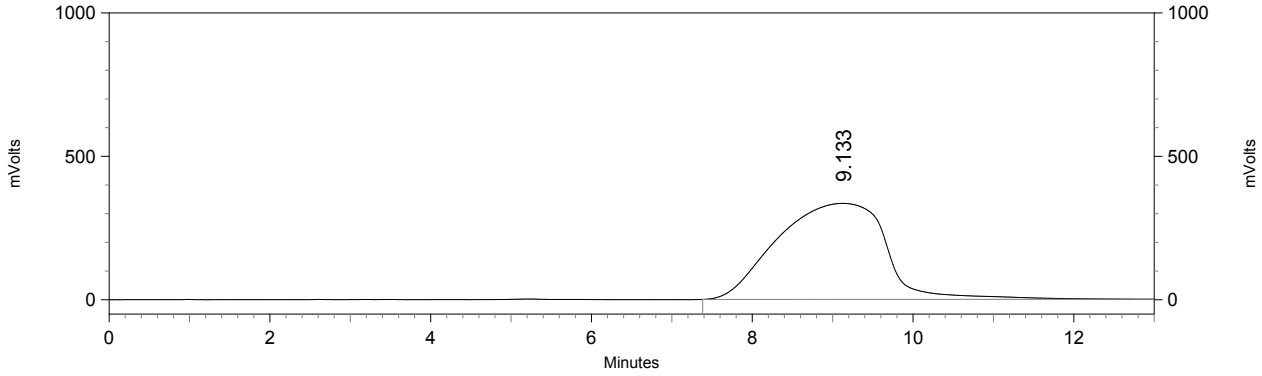
UV Detector Ch1-254nm
Results

Retention Time	Area	Area %
0.80	112370	1.84
1.45	10934	0.18
2.82	3164951	51.94
3.68	2805389	46.04

2.2 HPLC System 2

Column: Phenomenex Luna C18, 150 x 4.6 mm; Mobile phase: 10 mM NH_4OAc in 15% MeCN; Flow rate: 2.0 mL/min; UV wavelength: 254 nm; Column temperature: room temperature.

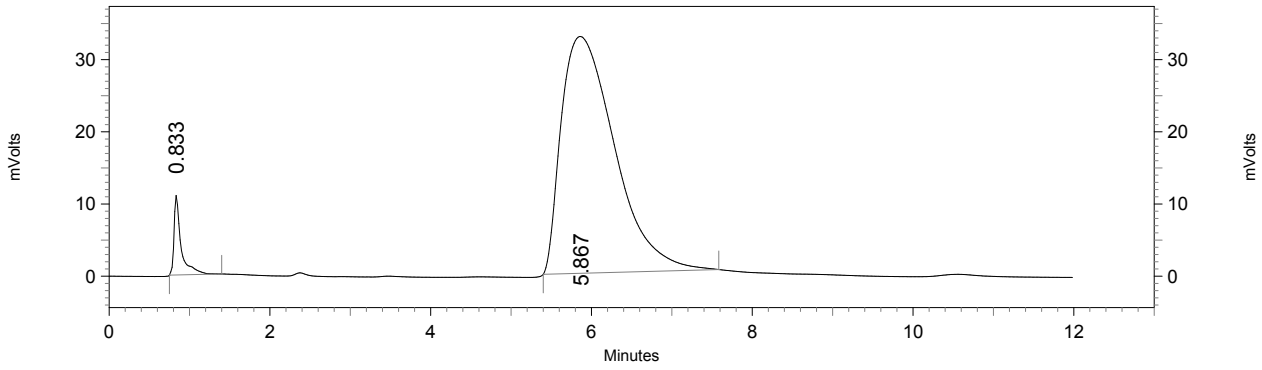
Figure S17: PBD150 Reference Standard Analyzed using HPLC System 2



UV Detector Ch1-254nm
Results

Retention Time	Area	Area %
9.13	31558619	100.00

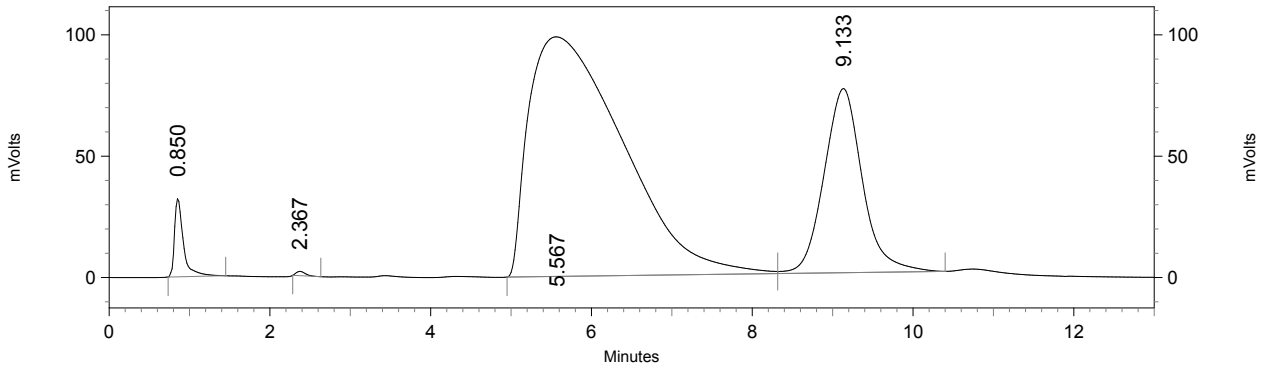
Figure S18: Alternate Methylation Standard 6 Analyzed using HPLC System 2



UV Detector Ch1-254nm
Results

Retention Time	Area	Area %
0.83	67276	4.24
5.87	1517957	95.76

Figure S19: Co-Injection of PBD150 and Alternate Methylation Standard 6 Showing Baseline Separation with HPLC System 2



UV Detector Ch1-254nm
Results

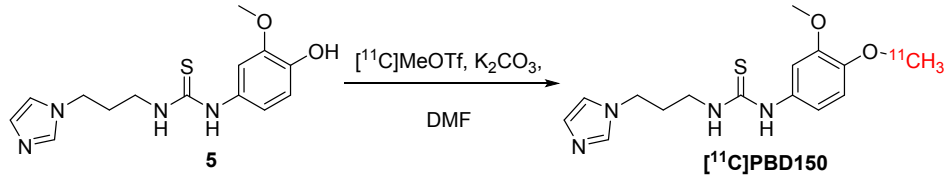
Retention Time	Area	Area %
0.85	248918	2.31
2.37	14048	0.13
5.57	8017838	74.29
9.13	2511471	23.27

3. RADIOCHEMISTRY

3.1 General Radiochemistry 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 Hollister-Stier. Sep-Paks were flushed with 10 mL of ethanol followed by 10 mL of sterile water prior to use.

3.2 Synthesis of [^{11}C]PBD150

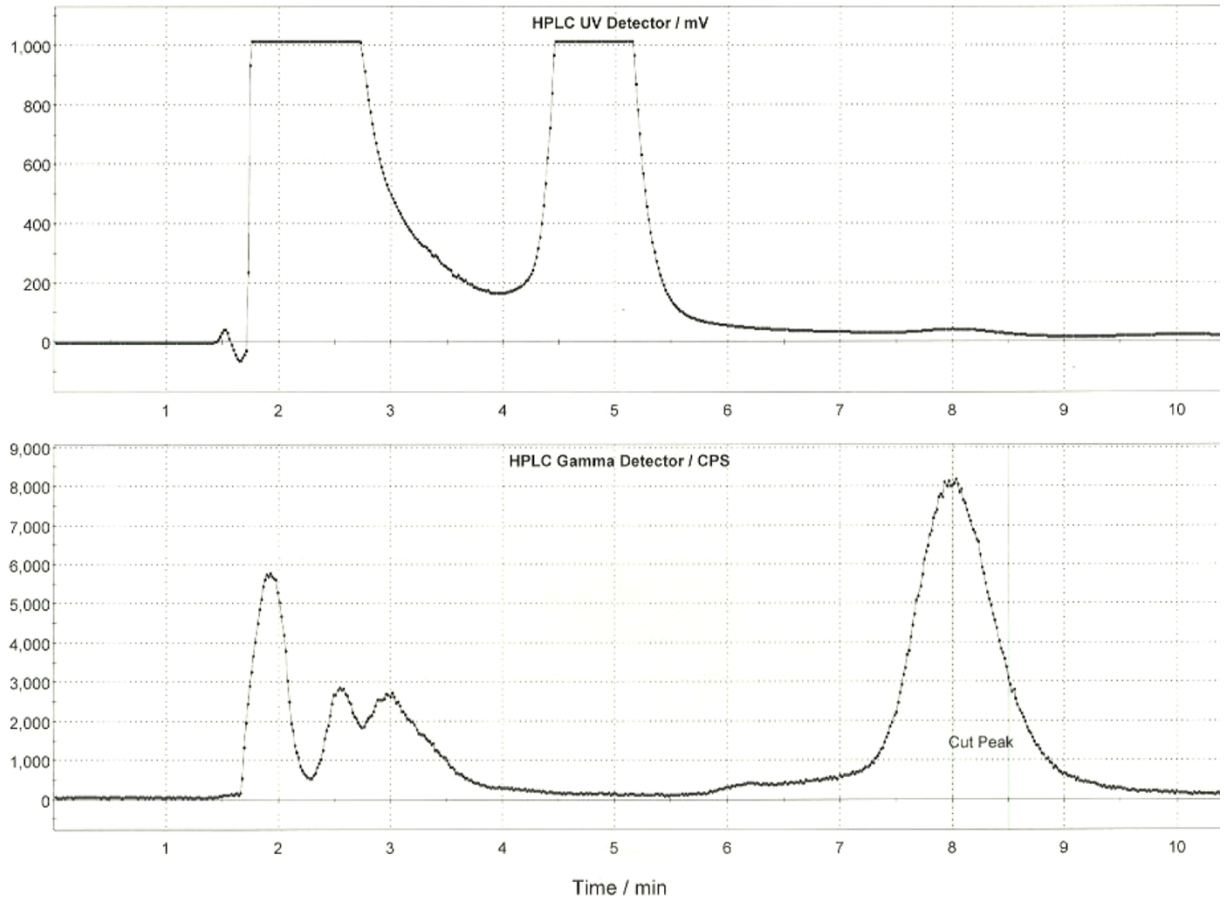


Production of [^{11}C]MeOTf was carried out as described by Shao et al.[3] Briefly, carbon-11 gas target loaded with [^{14}N]N_2 of a General Electric (GE) PETTrace cyclotron was bombarded with a proton beam to generate ~3 Ci of [^{11}C]CO_2. Subsequently, [^{11}C]CO_2 was delivered to the TRACERlab $FX_{C\text{-}Pro}$ 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 through a silver triflate-Graphpac column pre-heated to 190 °C to finally yield ~900 mCi of [^{11}C]CH_3OTf. The resulting [^{11}C]CH_3OTf was delivered to the reactor for the preparation of [^{11}C]PBD150 as outlined below.

The desmethylprecursor **5** (1 mg) was dissolved in 0.1 mL of DMF. To this mixture 10 µL of sat. $K_2CO_{3(aq.)}$ solution was added and the resulting mixture was mixed thoroughly. The mixture was dried over Na_2SO_4 and the solution was transferred to the reactor (careful not include any of the hydrated Na_2SO_4). The reaction solution was sparged with [^{11}C]MeOTf at a flow rate of 15 mL/min for 3-5 min. The reaction mixture was then purified via semi-preparative HPLC (column: Phenomenex Luna C18, 150x10 mm, mobile phase: 10 mMolar Na_2HPO_4, 500 mg/L ascorbic acid in 20 % EtOH, pH 8.0, flow rate: 4 mL/min; see Figure S20 for a typical trace). The product peak was collected at ~8-9 min (collected for 30 seconds at peak of product elution, 2 mL collected) and diluted into 8 mL of saline for a final volume of 10 mL (~4 % *v/v* EtOH). The dose was passed through a 0.22-µm filter into a sterile

dose vial. The synthesis resulted in 66.1 mCi of product collected (7.3% yield from [^{11}C]MeOTf, non-decay corrected, specific activity = 5.7 Ci/μmol, n = 5). The final product was then submitted for quality control testing (see Section 3.3).

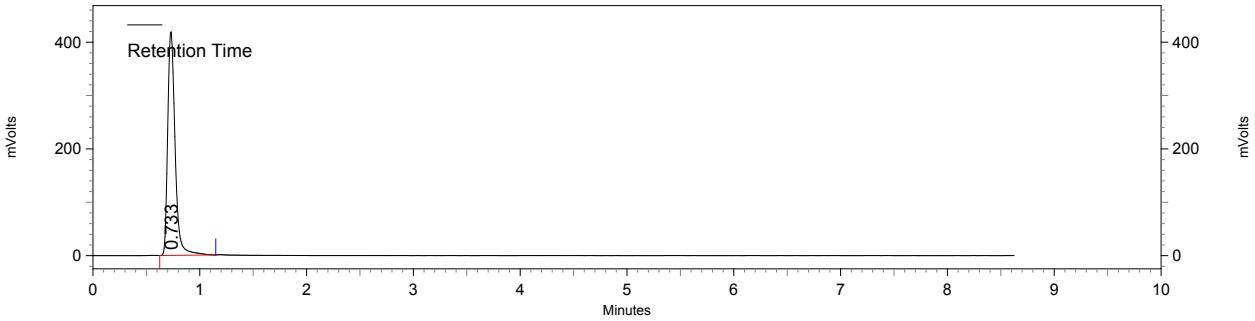
Figure S20: Semi-preparative HPLC trace of [^{11}C]PBD150 Radiosynthesis



3.3 Quality Control of [^{11}C]PBD150

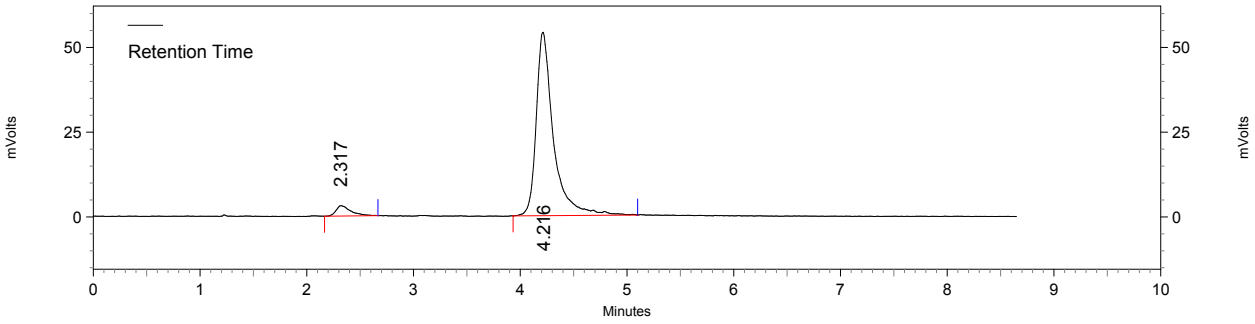
Radiochemical purity of [^{11}C]PBD150 was assessed using Shimadzu LC-2010A HT system equipped with the UV and Rad detectors (column: Phenomenex Luna C18, 150x4.6 mm; mobile phase: 10 mM NH_4OAc in 20% MeCN; flow rate: 2.0 mL/min; wavelength: 254 nm; room temperature; product peak: ~4.2 min; see Figure S21 for a typical trace). The pH of the dose solution was determined (pH = 7.0, n = 5) and radiochemical purity was assessed before use in animal imaging experiments (radiochemical purity ≥95%, n = 5).

Figure S21: Analytical HPLC trace of [^{11}C]PBD150



UV Detector 254 nm

Retention Time	Area	Area %
0.733	2077952	100
4.192	2220	0



Rad

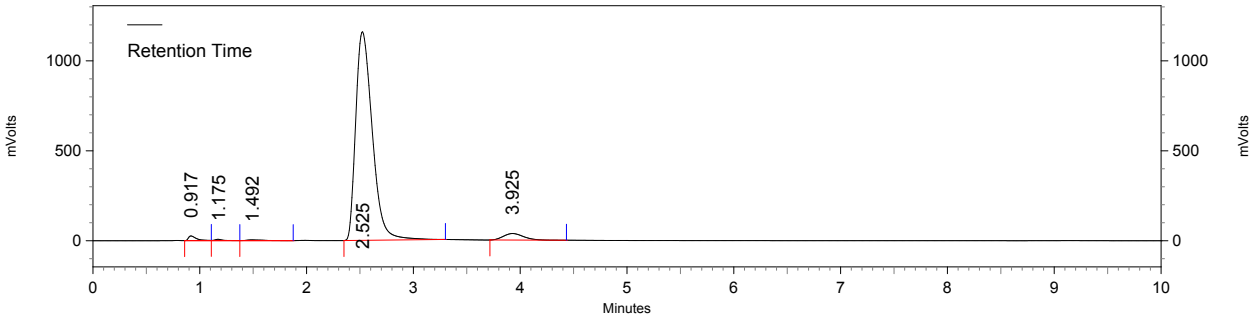
Retention Time	Area	Area %
2.317	29579	5
4.216	544907	95

3.4 Notes on Failed Radiosyntheses of [^{11}C]PBD150

The procedure for synthesizing [^{11}C]PBD150 outlined in Section 3.2 was followed except 1 mg of NaH was employed as the base. Following ^{11}C-methylation, the crude reaction mixture was analyzed by HPLC (Figure S22).

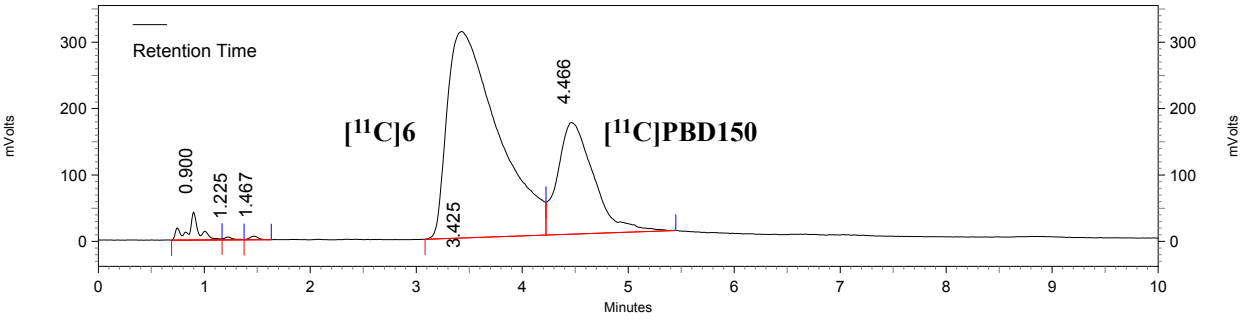
Figure S22: HPLC Analysis of Crude Reaction Mixture when using NaH as Base

UV



UV Detector Ch1-254nm Results Retention Time	Area	Area %	Height	Width	S/N (ASTM)
0.917	132335	1.00	26071	0.250	26.34
1.175	34529	0.26	7331	0.267	7.41
1.492	38784	0.29	3898	0.500	3.94
2.525	12495660	94.83	1159488	0.950	1171.49
3.925	475556	3.61	36218	0.717	36.59

RAD



RAD Results Retention Time	Area	Area %	Width
0.900	307031	2.09	0.47
1.225	23741	0.16	0.21
1.467	30939	0.21	0.26
3.425	10350629	70.46	1.14
4.466	3978094	27.08	1.22

4. SMALL ANIMAL PRE-CLINICAL PET IMAGING

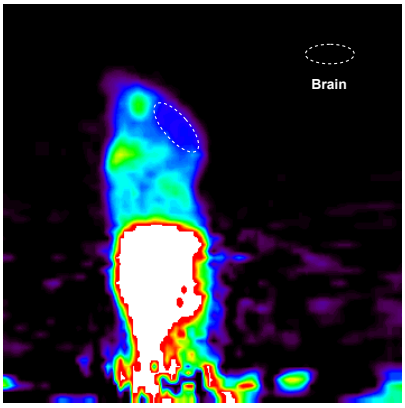
4.1 General Considerations

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. Doses of [^{11}C]PBD150 were used within 5 – 10 mins of recording specific activity at EOS.

4.2 Baseline Imaging Study in Mouse

The mouse imaging study was performed in a female CD-1 mouse (n = 1, weight = 21 g). The rat was anesthetized (isoflurane), and positioned in a Concorde MicroPET P4 scanner. Following a transmission scan, the animal was injected *i.v.* (tail vein) with [^{11}C]PBD150 (1.09 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 were corrected for attenuation and scatter, and then reconstructed using the 3D maximum a priori (3D MAP) algorithm (Figure S23). By using a summed image, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves which confirmed no brain uptake (data not shown).

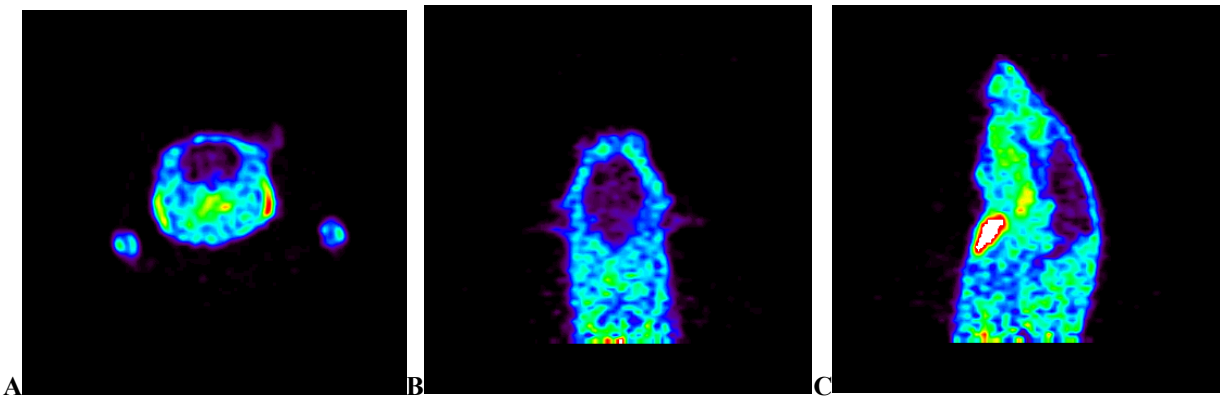
Figure S23: Summed (0 – 60 min) Sagittal PET Image from [^{11}C]PBD150 Baseline PET Imaging Study in Mouse



4.3 Baseline Imaging Study in Rat

Baseline rat imaging studies were performed with female Sprague-Dawley rats (n = 2, weight = 308g, 346g). Rats were anesthetized (isoflurane), and positioned in a Concorde MicroPET P4 scanner. Following a transmission scan, the animals were injected *i.v.* (tail vein) with [^{11}C]PBD150 (1.01 ± 0.09 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 were corrected for attenuation and scatter, and then reconstructed using the 3D maximum a priori (3D MAP) algorithm (Figure S24). By using a summed image, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves (Figure S27).

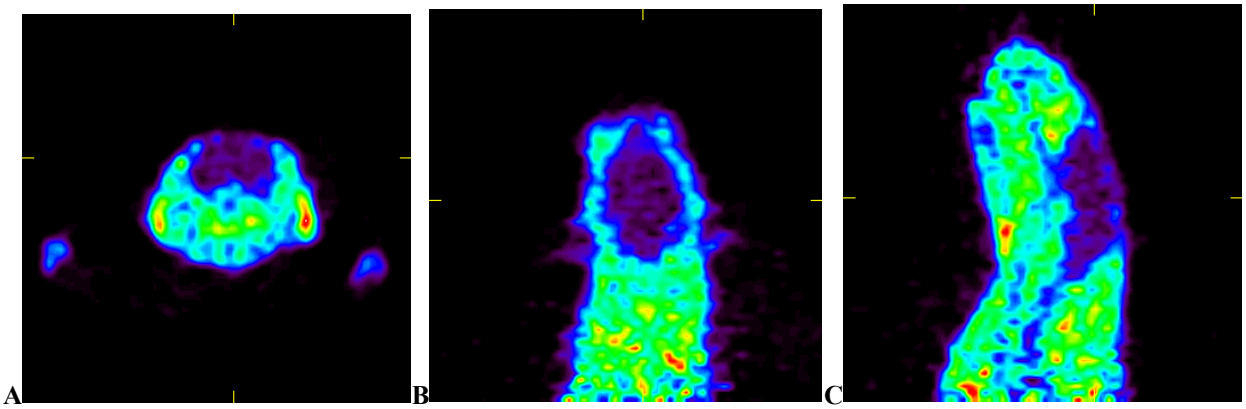
Figure S24: Summed (0 – 60 min) Transverse (A) Coronal (B) and Sagittal (C) PET Images from [^{11}C]PBD150 Baseline PET Imaging Study in Rat



4.4 Cyclosporin Blocking Study in Rat

The imaging experiment described in Section 4.3 was repeated using a female Sprague Dawley rat (n = 1, animal weight = 294 g) pre-treated with cyclosporin A (manufactured for Bedford Labs, Bedford, OH. Lot # 1856175, each ml contains cyclosporin USP 50 mg; polyoxyethyated castor oil 650 mg; and absolute alcohol 33.2% (v/v)) per the procedure reported by Blanckaert et al.[4] Each rat was injected i.v. with 50mg/kg (approx. 0.3 mL of stock was diluted to 1.0 mL with saline) over a 3 minute infusion, one hr. prior to administration of the [^{11}C]PBD150 tracer dose (0.94 mCi). The brain was then scanned for 60 min as described in Section 4.3. Data were corrected for attenuation and scatter, and reconstructed using the 3D MAP method (Figure S25). Using a summed image, ROIs were drawn and time-radioactivity curves generated as described in Section 4.3 (Figure S27).

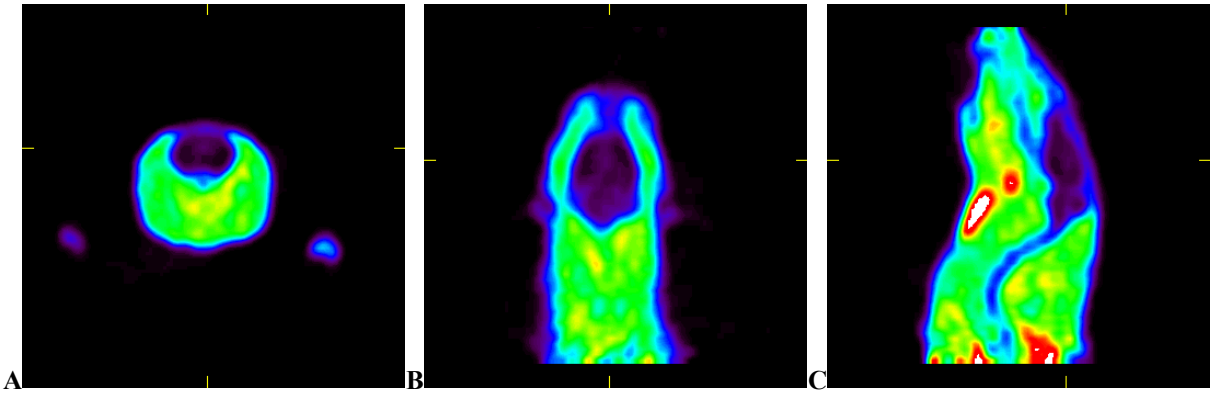
Figure S25: Summed (0 – 60 min) Transverse (A) Coronal (B) and Sagittal (C) PET Images from [^{11}C]PBD150 PET Imaging Study in Rat 60 min Post-administation of 50 mg/kg Cyclosporin A to Block PgP



4.5 Co-administration of PBD150 Therapeutic Dose in Rat

The imaging experiment described in Section 4.3 was repeated using a female Sprague Dawley rat (n = 1, animal weight = 323 g). The radiotracer dose (1.07 mCi) was formulated with unlabelled PBD150 (12 mg corresponding to a dosage of 37 mg/kg) and administered *i.v. via* tail vein injection. The brain was then scanned for 60 min as described in Section 4.3. Data were corrected for attenuation and scatter, and reconstructed using the 3D MAP method (Figure S26). Using a summed image, ROIs were drawn and time-radioactivity curves generated as described in Section 4.3 (Figure S27).

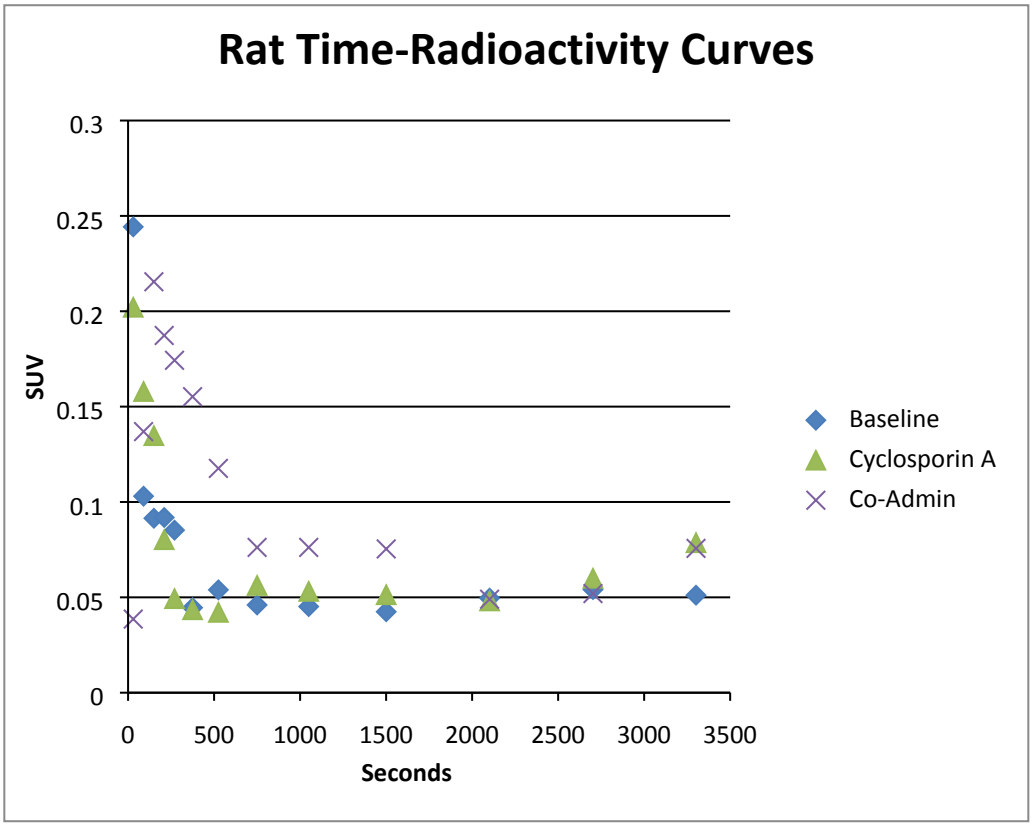
Figure S26: Summed (0 – 60 min) Transverse (A) Coronal (B) and Sagittal (C) PET Images from [^{11}C]PBD150 PET Imaging Study in Rat when Co-administered with 37 mg/kg Unlabeled PBD150.



4.6 Time Radioactivity Curves

Using summed images, regions of interest (ROI) were drawn over the whole brain on multiple planes, and the volumetric ROIs were then applied to the full dynamic data set to generate time–radioactivity curves (Figure S27).

Figure S27: Time-radioactivity Curves for Baseline, Cyclosporin A Pre-treated and PBD150 Co-administered Rat PET Imaging Experiments



5. RAT MICROSOME STABILITY STUDY

Materials

Pooled rat microsomes were stored at -80 °C prior to use.

Study design

The microsome incubation system was prepared according to the **Table S1.**

1. Make a master-mix containing microsome, phosphate buffer and NADPH solution as follows:
    1) Dilute 10μL of microsome (20 mg/mL) with 330 μL 0.1M Phosphate buffer (3.3 mM $MgCl_2$);
    2) Dissolve about 2 mg of NADPH in 120 μL of 0.1M phosphate buffer (3.3 mM $MgCl_2$);
    3) Addition of 40 μL of 10uM test compound to microsome;
    4) Pre-warm the master solution in the 37℃ for 3 min.

2. Add 20μL NADPH to the above master solution to initiate the reaction according to Table S1. The final concentration of test compounds in the reaction system is 1μM.

Table S1. Microsome Incubation System

	Master solution	Stock concentration	Volume	Sampling time (min)
Test compounds	380μL	10 μM	40 μL	0, 5, 10, 15, 30, 45 and 60

3. Aliquot of 40 μL was pipetted from the reaction solution and stopped by the addition of 5 volume of cold acetonitrile containing 10 nM of CE105 as an internal standard at the designated time points. The incubation solution was centrifuged at 3500 g for 15 minutes to precipitate protein. The supernatant was used for LC/MS/MS analysis.

Data Analysis

The natural log peak area ratio (compound peak area/ internal standard peak area) was plotted against time and the gradient of the line determined.

LC-MS/MS Conditions

Chromatographic Conditions:

Column：5 cm x 2.1 mm I.D., packed with 3.5 μm XBridge (Waters)

Mobile Phase A: 0.1% formic acid in purified deionized water

Mobile Phase B: 0.1% formic acid in acetonitrile

Flow Rate: 0.4mL/min; Injection Volume: 3 μL

Run Time: 6.09min

Gradient Program:

Time(min)	%A	%B
0.1	100	0
0.5	100	0
1.5	10	90
3	10	90
3.1	100	0
6.0	100	0
6.10	stop	

MS/MS Conditions

Turbo-Ionspray™ Interface used in the positive ion-mode

MRM-transitions:

Compound	Q1 Mass (m/z)	Q3 Mass (m/z)	DP	EP	CE	CXP
IMJ-I-1	321.2	253.1	48	10	19	10

Metabolic Stability

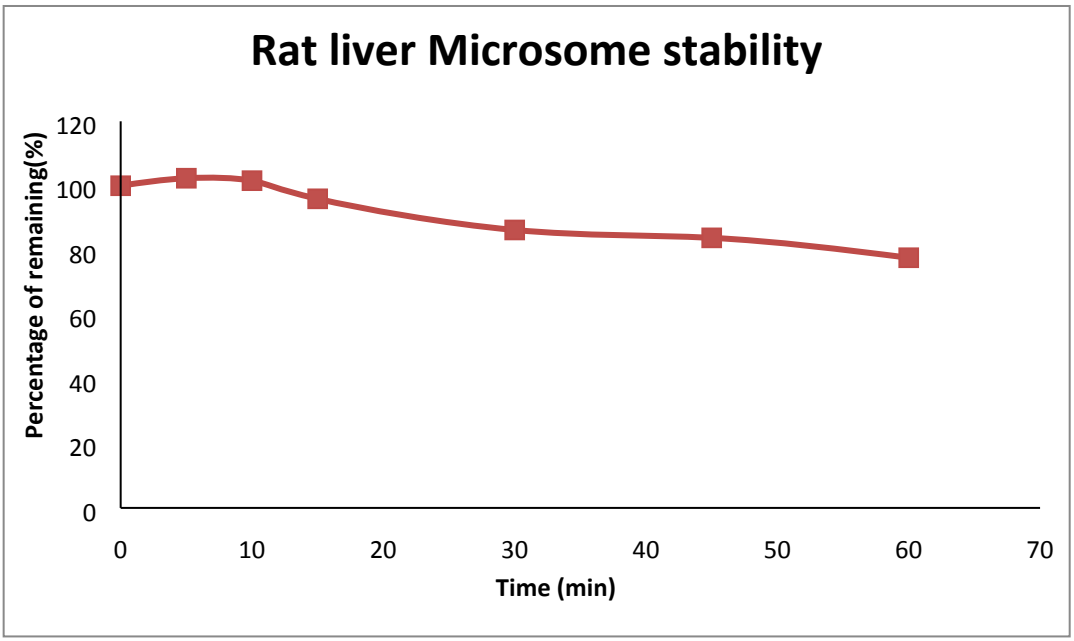
The metabolic stability and biological $t_{1/2}$ of test PBD150 in liver microsome with NADPH is listed in **Table S2,** and plotted in the **Figure S28.**

Table S2. Metabolic Stability and Half Life of PBD150 in Rat Liver Microsomes

	rat
Time (min)	IMJ-I-1
0	100.00
5	102.33

10	101.57
15	95.94
30	86.27
45	83.82
60	77.65
half time (min)	>60

Figure S28. Metabolic Stability of PBD150 in Rat Liver Microsome with NADPH



6. REFERENCES

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3. X. Shao, R. Hoareau, A. C. Runkle, L. J. M. Tluczek, B. G. Hockley, B. D. Henderson and P. J. H. Scott, *J. Labelled Comp. Radiopharm.*, 2011, **54**, 819-838.
4. P. Blanckaert, I. Burvenich, S. Staelens, S. De Bruyne, L. Moerman, L. Wyffels, and F. De Vos, F., *Eur. J. Nucl. Med. Mol. Imaging,* 2009, **36**, 446-453.