Radiosynthesis of [^{11}C]LY2795050 for Preclinical and Clinical PET Imaging using Cu(II)-mediated Cyanation

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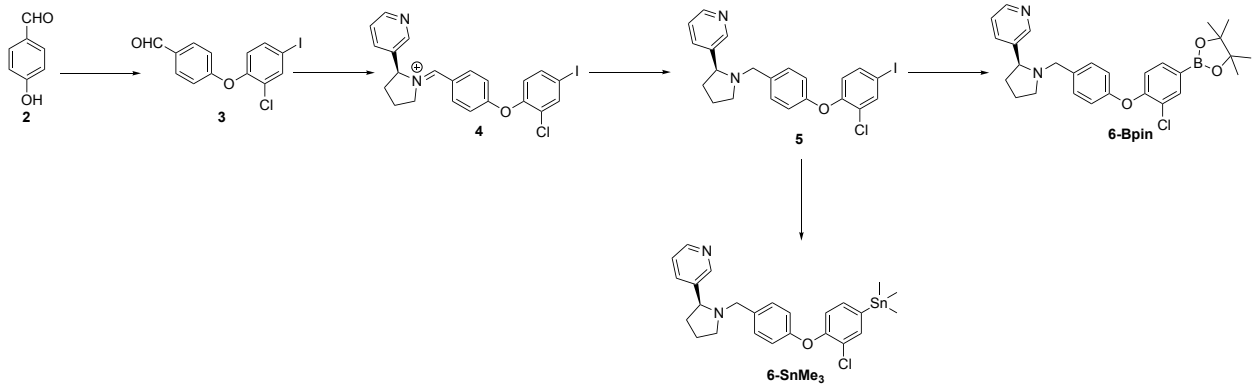
Table of Contents

1. Chemistry and Characterization

1.1 General Considerations

All the chemicals were purchased from commercially available suppliers and used without purification. Automated flash chromatography was performed with Biotage Isolera Prime system. High-performance liquid chromatography (HPLC) was performed using a Shimadzu LC-2010A HT system equipped with a Bioscan B-FC-1000 radiation detector. ^{1}H and ^{13}C NMR spectra: Varian 400 apparatus (400 MHz for ^{1}H NMR and 101 MHz for ^{13}C NMR), in DMSO-d_6 or $CDCl_3$ unless otherwise indicated, δ in ppm rel. to tetramethylsilane (δ = 0), J in Hz. Mass spectra were measured on an Agilent Q-TOF HPLC-MS or VG (Micromass) 70-250-S Magnetic sector mass spectrometer employing the electrospray ionization (ESI) method.

1.2 Synthesis of Precursors 6-Bpin and 6-SnMe$_3$



Scheme S1 Synthesis of **6-Bpin** and **6-SnMe$_3$**

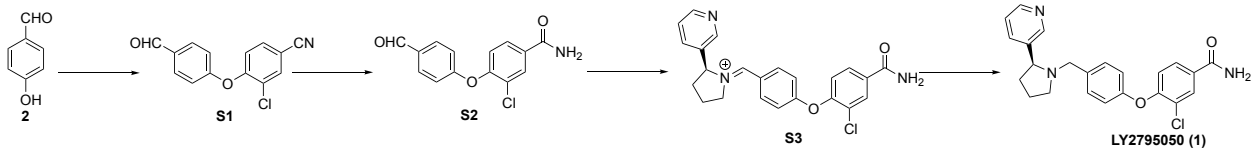
4-(2-Chloro-4-iodophenoxy)benzaldehyde (3): To a solution of 4-hydroxybenzaldehyde **2** (0.50 g, 4.1 mmol) and 2-chloro-1-fluoro-4-iodobenzene (1.1 g, 4.1 mmol) in DMF (10 mL) were added potassium carbonate (1.1 g, 8.2 mmol) and cesium carbonate (0.67 g, 2.1 mmol). The mixture was stirred at 140 °C for 12 h under argon atmosphere. After cooling to the room temperature, the mixture was quenched with saturated ammonium chloride solution and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane, 1:10 to 1:5) to give **3** (0.26 g, 18%) as a yellow oily product. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.94 (s, 1H), 7.86 (d, *J* = 8.8 Hz, 2H), 7.84 (d, *J* = 2.0 Hz, 1H), 7.62 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 1H). HRMS (ESI) found: 380.9151, $C_{13}H_8ICIO_2Na^+$ (M + Na) requires: 380.9150. Characterization data was consistent with previous reports.[1]

(S)-3-(1-(4-(2-chloro-4-iodophenoxy)benzyl)pyrrolidin-2-yl)pyridine (5): To a solution of 4-(2-chloro-4-iodophenoxy)benzaldehyde **3** (0.46 g, 1.3 mmol) and (S)-3-(pyrrolidin-2-yl)pyridine (0.23 g, 1.5 mmol) in 1,2-DCE (15 mL) was stirred at 65 °C for 24 h, whereupon sodium triacetoxyborohydride (0.68 g, 3.2 mmol) and acetic acid (0.18 mL, 3.2 mmol) were added to the reaction. After stirring at 65 °C for 24 h, the mixture was quenched with saturated aqueous sodiumbicarbonate and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (MeOH:DCM, 1:100 to 1:25) to give **5** (0.54 g, 85% over 2 steps) as a yellow oily product. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.67–8.58 (m, 1H), 8.48 (dd, *J* = 4.7, 1.3 Hz, 1H), 7.77 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.45 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.26–7.22 (m, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 8.6 Hz, 1H), 3.72 (d, *J* = 13.1 Hz, 1H), 3.40 (t, *J* = 8.1 Hz, 1H), 3.11 (dd, *J* = 11.0, 6.6 Hz, 2H), 1.96–1.85 (m, 1H), 1.85–1.76 (m, 1H), 1.75–1.65 (m, 1H). HRMS (ESI) found: 491.0384, $C_{22}H_{21}ICIN_2O^+$ (M + H) requires: 491.0382. Characterization data was consistent with previous reports.[1]

(S)-3-(1-(4-(2-chloro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenoxy)benzyl)pyrrolidin-2-yl)pyridine (6-Bpin): To a mixture of (S)-3-(1-(4-(2-chloro-4-iodophenoxy)benzyl)pyrrolidin-2-yl)pyridine **5** (0.54 g, 1.1 mmol), bis(pinacolato)-diboron (0.36 g, 1.4 mmol), $Pd(dppf)Cl_2$ (0.048 g, 0.066 mmol) and potassium acetate (0.32 g, 3.3 mmol) in DMSO (6.0 mL) was reacted at 85 °C under argon atmosphere for 12 h. Then the mixture was diluted with saturated ammonium chloride solution and EtOAc, and the insoluble material was filtered through Celite. The organic layer of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (MeOH:DCM, 1:100 to 1:30) to give **6-Bpin** (0.35 g, 65%) as a brown oily product. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (d, *J* = 1.6 Hz, 1H), 8.50 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.88 (d, *J* = 1.4 Hz, 1H), 7.80 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.60 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.31–7.27 (m, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.92–6.82 (m, 3H), 3.74 (d, *J* = 13.1 Hz, 1H), 3.41 (t, *J* = 8.2 Hz, 1H), 3.16–3.08 (m, 2H), 2.29–2.19 (m, 2H), 1.97–1.87 (m, 1H), 1.86–1.78 (m, 1H), 1.76–1.66 (m, 1H), 1.24 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 160.09, 159.37, 154.93, 152.26, 150.51, 141.40, 134.87 (2C), 128.47 (2C), 125.68, 115.15, 112.92, 112.77, 107.77, 107.61, 105.86, 83.74 (2C), 62.07, 51.09, 49.46, 27.19, 24.89 (4C), 18.66. HRMS (ESI) found: 491.2276, $C_{28}H_{33}BCIN_2O_3^+$ (M + H) requires: 491.2267.

(S)-3-(1-(4-(2-chloro-4-(trimethylstannyl)phenoxy)benzyl)pyrrolidin-2-yl)pyridine (6-SnMe$_3$): To a mixture of (S)-3-(1-(4-(2-chloro-4-iodophenoxy)benzyl)pyrrolidin-2-yl)pyridine **5** (0.11 g, 0.21 mmol), $(Me_3Sn)_2$ (0.084 g, 0.26 mmol), $Pd(PPh_3)_4$ (0.025 g, 0.021 mmol) and lithium chloride (0.014 g, 0.32 mmol) in toluene (15 mL) was reacted at 100 °C under argon atmosphere for 4 h. Then the mixture was diluted with water and EtOAc, and the insoluble material was filtered through Celite. The organic layer of the filtrate was washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and evaporated in vacuo. The residue was purified by column chromatography (MeOH:DCM, 1:100 to 1:30) to give **6-SnMe$_3$** (0.086 g, 76%) as a brown oily product. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.62 (d, J = 1.7 Hz, 1H), 8.50 (dd, J = 4.8, 1.7 Hz, 1H), 7.79 (dt, J = 7.8, 1.9 Hz, 1H), 7.66 (dd, J = 12.0, 1.4 Hz, 1H), 7.58–7.52 (m, 1H), 7.47 (dd, J = 7.6, 2.9 Hz, 1H), 7.20 (d, J = 8.5 Hz, 2H), 6.92–6.85 (m, 3H), 3.73 (d, J = 13.1 Hz, 1H), 3.41 (t, J = 8.1 Hz, 1H), 3.15–3.07 (m, 2H), 2.25 (d, J = 8.8 Hz, 2H), 1.96–1.87 (m, 1H), 1.86–1.79 (m, 1H), 1.75–1.67 (m, 1H), 0.31 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 155.72, 152.75, 149.63, 148.80, 139.39, 138.37, 137.48, 135.00, 134.34, 132.12, 129.90 (2C), 128.51, 123.56, 120.21, 117.84 (2C), 66.93, 57.51, 53.54, 35.28, 22.54, -9.36 (3C). HRMS (ESI) found: 529.1055, $C_{25}H_{30}ClN_2OSn^+$ (M + H) requires: 529.1063.

1.3 Synthesis of standard LY2795050[2]



Scheme S2 Synthesis of standard **LY2795050**

3-Chloro-4-(4-formylphenoxy)benzonitrile (S1): To a solution of 4-hydroxybenzaldehyde **2** (0.32 g, 2.1 mmol) and 3-chloro-4-fluorobenzonitrile (0.25 g, 2.1 mmol) in DMF (6.0 mL) was added potassium carbonate (0.57 g, 4.1 mmol). The mixture was stirred at 100 °C for 2 h under argon atmosphere. After cooling to the room temperature, the solution was poured into ice water and the precipitate was collected to give **S1** (0.48 g, 91%) as a white solid product. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.98 (s, 1H), 7.93 (d, J = 8.5 Hz, 2H), 7.82 (d, J = 1.9 Hz, 1H), 7.58 (dd, J = 8.5, 1.9 Hz, 1H), 7.15–7.06 (m, 3H). HRMS (ESI) found: 258.0317, $C_{14}H_9ClNO_2^+$ (M + H) requires: 258.0316. Characterization data was consistent with previous reports.[2]

3-Chloro-4-(4-formylphenoxy)benzamide (S2): To a mixture of 3-chloro-4-(4-formylphenoxy)benzonitrile **S1** (0.30 g, 1.2 mmol) and potassium (0.080 g, 0.58 mmol) in DMSO (3.0 mL) was added 30% H_2O_2 (0.13 mL, 1.3 mmol) dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. Then the reaction was poured into ice water and the precipitate was collected to give **S2** (0.29 g, 89%) as a white solid product. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.94 (s, 1H), 8.25–8.05 (m, 2H), 8.01–7.85 (m, 3H), 7.58 (s, 1H), 7.36 (d, J = 8.5 Hz, 1H), 7.15 (d, J = 8.5 Hz, 2H). HRMS (ESI) found: 276.0424, $C_{14}H_{11}ClNO_3^+$ (M + H) requires: 276.0422. Characterization data was consistent with previous reports.[2]

(S)-3-chloro-4-(4-((2-(pyridin-3-yl)pyrrolidin-1-yl)methyl)phenoxy)benzamide (LY2795050; 1): To a solution of 3-chloro-4-(4-formylphenoxy)benzamide **S2** (0.15 g, 0.54 mmol) and (S)-3-(pyrrolidin-2-yl)pyridine (0.097 g, 0.65 mmol) in 1,2-DCE (10 mL) was stirred at room temperature for 24 h, whereupon sodium triacetoxyborohydride (0.29 g, 1.4 mmol) and acetic acid (0.078 mL, 1.4 mmol) were added to the reaction. After stirring at room temperature for 4 h, the mixture was quenched with saturated aqueous sodiumbicarbonate and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography (MeOH:DCM, 1:100 to 1:50) to give **LY2795050** (**1**) (0.17 g, 76%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.63 (d, J = 1.5 Hz, 1H), 8.50 (dd, J = 4.7, 1.3 Hz, 1H), 7.93 (d, J = 2.1 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.61 (dd, J = 8.6, 2.1 Hz, 1H), 7.30–7.26 (m, 2H), 6.94 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.6 Hz, 1H), 6.15–5.50 (m, 2H), 3.75 (d, J = 13.0 Hz, 1H), 3.43 (t, J = 8.2 Hz, 1H), 3.21–3.08 (m, 2H), 2.34–2.18 (m, 2H), 1.98–1.89 (m, 1H), 1.88–1.79 (m, 1H), 1.79–1.70 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 167.88, 156.63, 154.39, 149.38, 148.42, 139.70, 135.92, 135.37, 130.32 (2C), 128.96, 128.80, 127.29, 124.87, 123.84, 119.43 (2C), 119.31, 118.25, 67.13, 57.77, 53.85, 35.43, 22.71. HRMS (ESI) found: 408.1472, $C_{23}H_{23}ClN_3O_2^+$ (M + H) requires: 408.1473. Characterization data was consistent with previous reports.[2]

2. Radiochemistry

2.1 General Considerations

All the chemicals were purchased from commercially available suppliers and used without purification: 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.; Ammonium Acetate and Acetic Acid (glacial) was obtained from Fisher Scientific; and HPLC columns were acquired from Phenomenex. Other synthesis components were obtained as follows: sterile filters were acquired from Millipore; C18 Vac 1cc Sep-Paks were purchased from Waters Corporation; Sep-Paks were flushed with 5 mL of ethanol followed by 10 mL of sterile water prior to use.

2.2 Preparation of [^{11}C]HCN

[^{11}C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ reaction with a GE Medical Systems PETtrace cyclotron using 16.4 MeV proton irradiation (40 min, 60 μA) of nitrogen gas with 0.5% oxygen. [^{11}C]HCN was synthesized from [^{11}C]carbon dioxide by "gas phase" method utilizing a GE PETtrace Carbon-11 Process Panel as previously reported.[3] Briefly, [^{11}C]carbon dioxide from the target was trapped on molecular sieves at room temperature, and then was allowed to release at 350 °C and mixed with hydrogen gas. The conversion of [^{11}C]methane from the gas mixture was performed through a preheated nickel oven at 420 °C, followed by passing it through Ascarite and Sicapent columns to remove water and unreacted [^{11}C]carbon dioxide. The gas together with anhydrous ammonia gas was delivered through a high temperature (950 ºC) platinum oven where the coupling of [^{11}C]methane and ammonia occurred to form [^{11}C]HCN. The non-decay corrected radiochemical yield of [^{11}C]HCN was ~800 mCi starting from 3000 mCi of [^{11}C]carbon dioxide.

2.3 Synthesis Module Modifications

GE TRACERLab FX_M after the modification is shown in **Figure S1**. Solvent reservoirs above V1, V2 and V3 were removed and replaced with Luer lock adapters with needles, which could allow the use of various size V-vials for small volumes of reagents. V17 (helium gas line) was removed from the reactor. V31 was removed from the HPLC pump and connected to the reactor for an additional V-vial to add reagents.

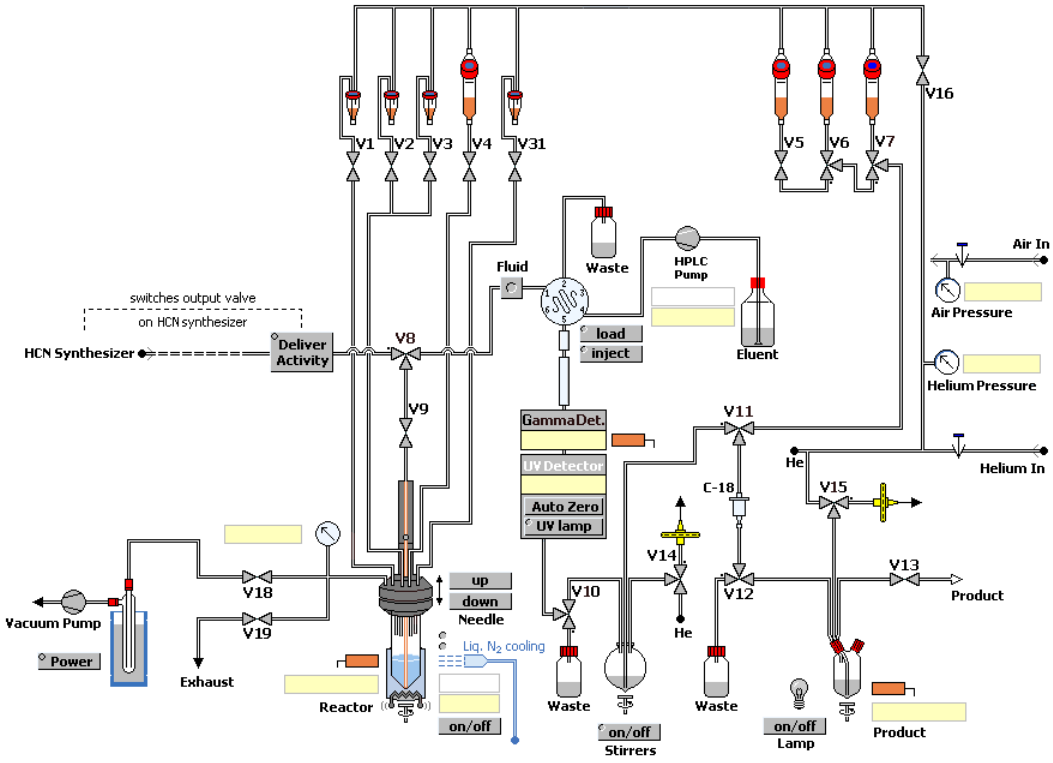
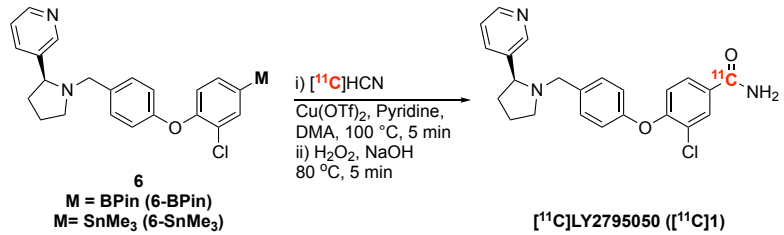


Figure S1 TRACERlab FX_M Synthesis Module Configured for [^{11}C]LY2795050

2.4 Radiosynthesis of [^{11}C]LY2795050 from 6-BPin or 6-SnMe$_3$ (Optimization Screen)



6
M = BPin (6-BPin)
M= SnMe$_3$ (6-SnMe$_3$)

[^{11}C]LY2795050 ([^{11}C]1)

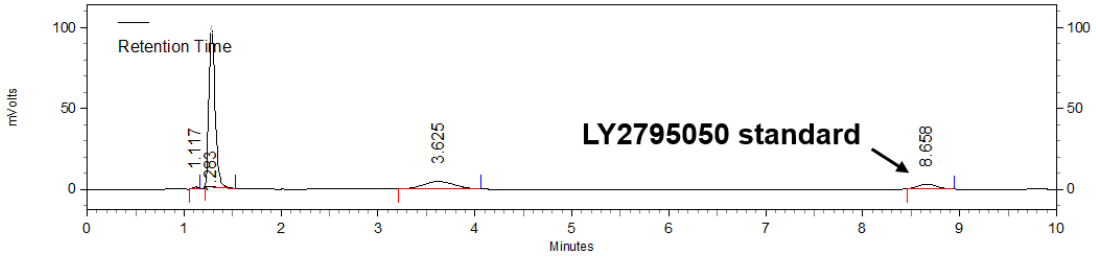
No carrier added [^{11}C]HCN was bubbled into a mixture of pyridine (15 equiv) in DMA (0.25 mL) and H_2O (0.05 mL) directly. $Cu(OTf)_2$ (4 equiv) was added followed by **6-Bpin** or **6-SnMe**$_3$ (1 equiv) and the reaction was heated at at 100 °C for 5 min to generate cyano intermediate **[^{11}C]7**. The reaction mixture was cooled down to 5 °C and hydrolysis to generate [^{11}C]LY2795050 **[^{11}C]1**) was accomplished by treating **[^{11}C]7** with 30% H_2O_2 (0.2 mL) and 5.0 M NaOH (0.2 mL) at 80°C for 5 min. The reaction mixture was quenched with HPLC buffer and analyzed by radio-HPLC (Phenomenex Luna C18(2), 150 x 4.6 mm, 20:80 MeCN:H_2O, 10 mM NH_4OAc, 0.2% acetic acid, pH = 4.5, flow rate = 2.0 mL/min, UV = 254 nm) to determine RCC (Table S1 and Figure S2).

Table S1: Reaction Optimization Studies

Entry	Precursor	Trapping Method	Cu eq.	RCC[a]
1	**6-BPin**	H_2O/pyridine in DMA	4	50%
2	**6-SnMe$_3$**	H_2O/pyridine in DMA	4	31±5%[b]

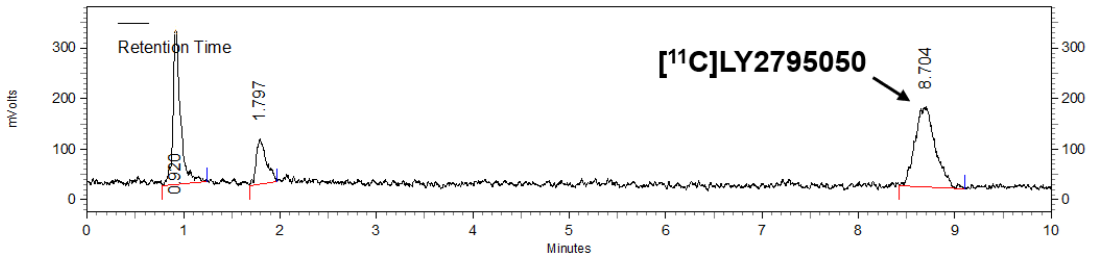
[a]RCC was determined by radio-HPLC. [b]n = 2.

A



UV Detector Ch1-254nm Results

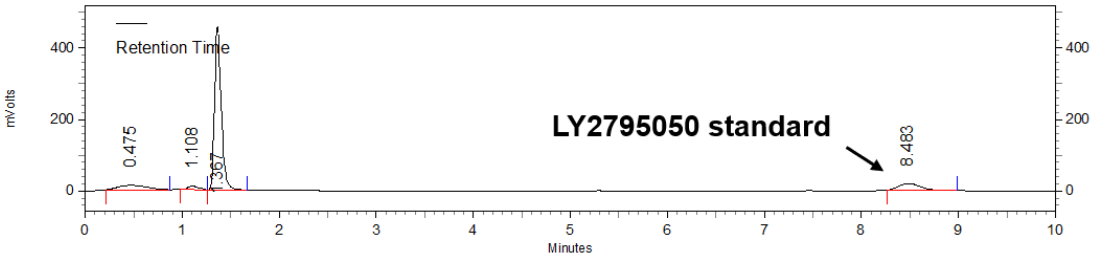
Retention Time	Area	Area %	Height	Width	S/N (ASTM)
1.117	3401	1	1010	0.108	5.00
1.283	435749	75	99629	0.308	493.67
3.625	103230	18	4637	0.850	59.85
8.658	40397	7	3031	0.483	47.95



RAD Results

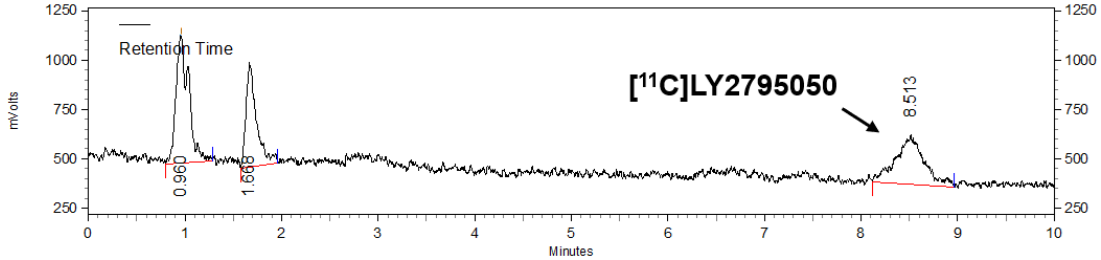
Retention Time	Area	Area %	Width
0.920	1674793	36	0.46
1.797	649825	14	0.28
8.704	2356012	50	0.68

B



UV Detector Ch1-254nm Results

Retention Time	Area	Area %	Height	Width	S/N (ASTM)
0.475	259698	9	12540	0.667	12.99
1.108	73030	3	9673	0.283	10.02
1.367	2209817	78	456587	0.408	473.01
8.483	288601	10	18731	0.717	42.60

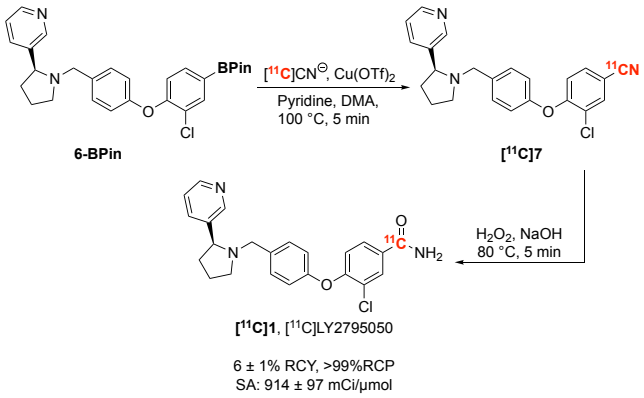


RAD Results

Retention Time	Area	Area %	Width
0.960	5935326	40	0.48
1.668	3980335	27	0.38
8.513	5053863	34	0.85

Figure S2: Radio-HPLC traces for manual synthesis of **[^{11}C]7** from **6-BPin** (A) or **6-SnMe$_3$** (B)

2.5 Automated Radiosynthesis of [^{11}C]LY2795050 from 6-BPin



Pyridine (0.15 mL, 1.0 M DMA stock, 150 μmol, 15 equiv) in 0.1 mL of DMA was mixed in the reactor. No carrier added [^{11}C]HCN was bubbled into the reactor directly. Then $Cu(OTf)_2$ (0.2 mL, 0.2 M DMA stock, 40 μmol, 4 equiv) through V31 and **6-Bpin** (4.9 mg in 0.4 mL DMA, 10 μmol,1 equiv) from vial 1 were added. The reaction was allowed to heat to 100 °C for 5 min. After the temperature was cooled to 5 °C by liquid nitrogen, 30% H_2O_2 (0.2 mL) in vial 2 and NaOH (5M, aq, 0.2 mL) in vial 3 were added to the reactor. The hydrolysis was performed at 80 °C for 5 min, followed by cooling to 25 °C and quenching with acetic acid (0.4 mL, glacial) in vial 4. The reaction mixture was stirred for 2 min and then injected onto a semi-preparative HPLC column for purification (Phenomenex Prodigy C8, 10 μm, 150 x 10 mm, 25:75 MeCN:H_2O, 100 mM NH_4OAc, 1.0% acetic acid, pH = 4.8, flow rate = 5.0 mL/min, UV = 254 nm). The product peak at ~5-7 minutes was collected (see **Figure S3** for a typical semi-preparative HPLC trace) into 55 mL of water and passed through a C-18 sep-pak to remove HPLC solvent. The sep-pak was rinsed with 4 mL of USP water and the product was then eluted with 0.5 mL of ethanol followed by 9.5 mL of USP saline for injection. The final dose was filtered into a dose vial via a 0.22 μm sterile filter, then submitted for quality control testing as outlined below. Total synthesis time was approximately 45 min from end of beam. The non-decay radiochemical yield was 48 ± 10 mCi (6 ± 1%) based on [^{11}C]HCN with radiochemical purity of >99% and specific activity of 914 ± 97 mCi/μmol (n = 3).

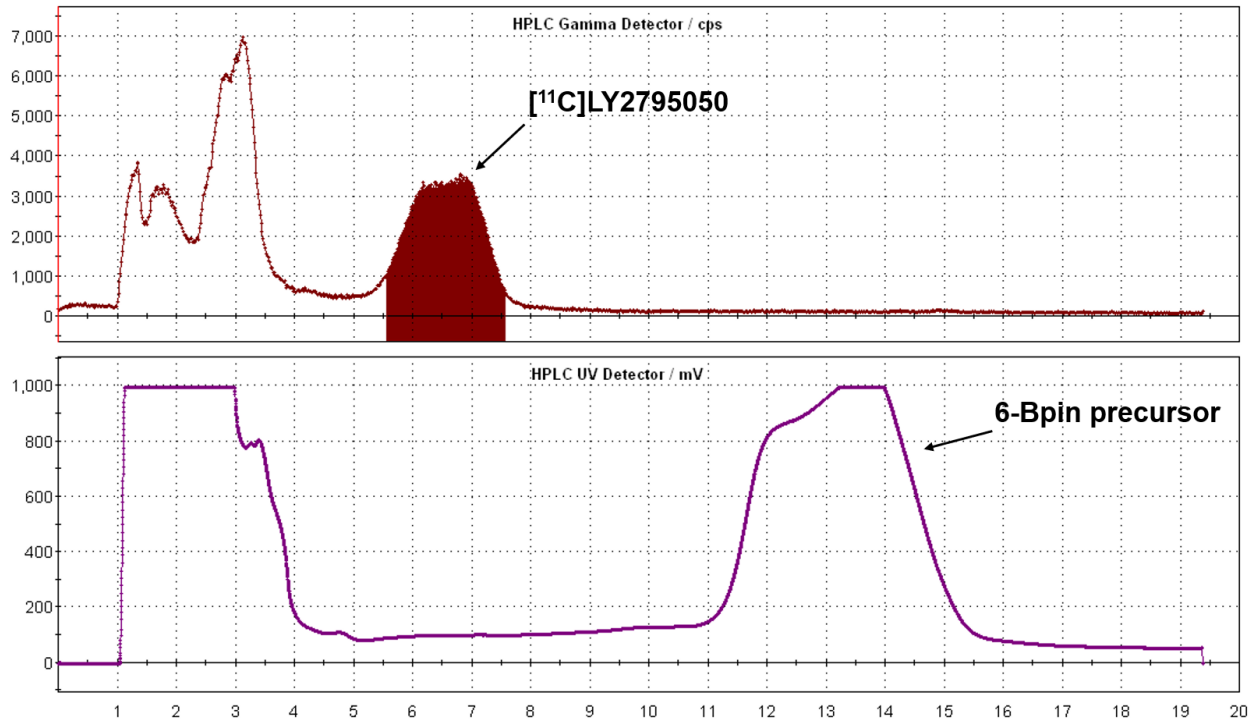


Figure S3 Typical semi-preparative HPLC trace for the automated synthesis of [^{11}C]LY2795050 from **6-BPin**

2.6 Quality control

Quality control of radiopharmaceuticals prepared for clinical use at the University of Michigan PET Center is carried out using guidelines outlined in Chapter 823 of USP and as detailed in the next paragraphs. The key data is summarized in **Table 2** in the main manuscript.

2.6.1 Visual inspection

Doses were visually examined and required to be clear, colorless and free of particulate matter.

2.6.2 Dose pH

The pH of the doses was determined by applying a small amount of the dose to pH-indicator strips and determined by visual comparison to the scale provided. Acceptable doses were between 4.5 and 7.5.

2.6.3 Radiochemical purity, specific activity and radiochemical identity

Radiochemical purity and identity are analyzed using an HPLC equipped with a radioactivity detector and a UV detector. Column: Luna C18 150 × 4.6 mm; mobile phase: 10 mM NH_4OAc in 20% MeCN, pH = 4.5; flow rate: 3.0 mL/min. The retention time of product: ~ 5.3 min (**Figure S4**) Radiochemical purity for doses must be > 90%. LY2795050 (previously prepared) was used as the non-radio-active carbon-12 reference standard. Specific activity was determined based on concentration of cold mass. Radiochemical identity was confirmed and quantified by calculating the relative retention time (RRT = retention time of [^{11}C]LY2795050/[retention time of [^{12}C]LY2795050), and was required to be 0.9–1.10.

2.6.4 Radionuclidic identity

Radionuclidic identity is confirmed by measuring the half-life of [^{11}C]LY2795050 doses and comparing it to the known half-life of carbon-11 (20.8 min). Activities were measured using a Capintec CRC-15 Radioisotope Dose Calibrator and half-life ($t_{1/2}$) was calculated according to equation. Calculated half-life must be 18.4-22.4 min.

$$t_{1/2} = \ln 2(\text{Time difference}/(\ln(\text{ending activity}/\text{starting activity})))$$

2.6.5 Sterile filter integrity test

Sterile filter from dose (with needle still attached) was connected to a nitrogen supply via a regulator. The needle was then submerged in water and the nitrogen pressure gradually increased. If the pressure was raised above the filter acceptance pressure (typically 40 psi) without seeing a stream of bubbles, the filter is considered intact.

2.6.6 Bacterial endotoxins

Endotoxin content in radiopharmaceutical doses is analyzed by a Charles River Laboratories EndoSafe® Portable Testing System and according to the US Pharmacopeia. Doses must contain <175 Endotoxin Units (EU).

2.6.7 Sterility

Culture tubes of fluid thioglycolate media (FTM) and tryptic soy broth (TSB) are inoculated with samples of the radiolabeled product and incubated (along with positive and negative controls) for 14 days. FTM is used to test for anaerobes, aerobes and microaerophiles whilst TSB is used to test for non-fastidious and fastidious microorganisms. Culture tubes are visually inspected on the 3rd, 7th and 14th days of the test period and compared to the positive and negative standards. Positive standards must show growth (turbidity) in the tubes, and dose/negative controls must have no culture growth after 14 days to be indicative of sterility.

2.6.8 ICP-MS

ICP-MS was conducted by EMSL Analytical, Inc. (Cinnaminson, NJ).

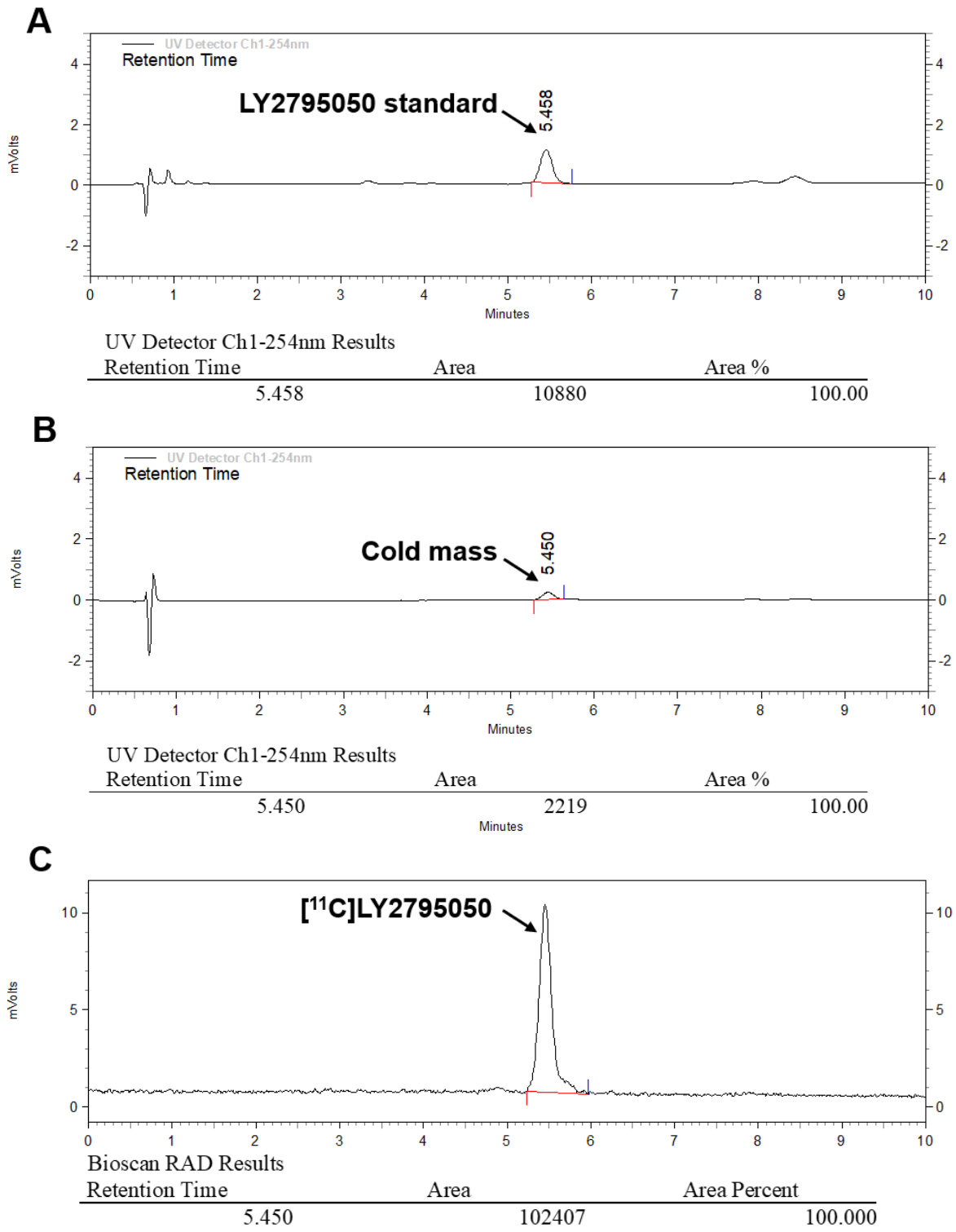


Figure S4 Analytical HPLC trace. (A) UV trace of LY2795050 standard at 254 nm. (B) UV trace of formulated [^{11}C]LY2795050 dose at 254 nm. (C) Radioactivity trace of formulated [^{11}C]LY2795050 dose.

3. Pre-clinical PET Imaging

3.1 General Considerations

Rodent and primate imaging studies were performed at the University of Michigan, in accordance with the standards set by the Institutional Animal Care And Use Committee (IACUC) at the University of Michigan.

3.2 Rodent Imaging Protocol

Rodent imaging studies were done using a male Sprague Dawley rat (230 g, n = 1). The rat was anesthetized (isoflurane), intubated, and positioned in a Concorde MicroPET P4 scanner. Following a transmission scan, the animal was injected i.v. (via intravenous tail vein injection) with [^{11}C]LY2795050 (0.42 mCi) as a bolus over 1 min, and the brain imaged for 60 min (5 × 1 min frames – 2 × 2.5 min frames – 2 × 5 min frames – 4 × 10 min frames). Emission data were corrected for attenuation and scatter, and reconstructed using the 3D maximum a priori (3D MAP) method. By using a summed image, a region of interest (ROI) was drawn over the whole brain on multiple planes, and the volumetric ROI was then applied to the full dynamic data set to generate the associated time-radioactivity curve (**Figure S5**).

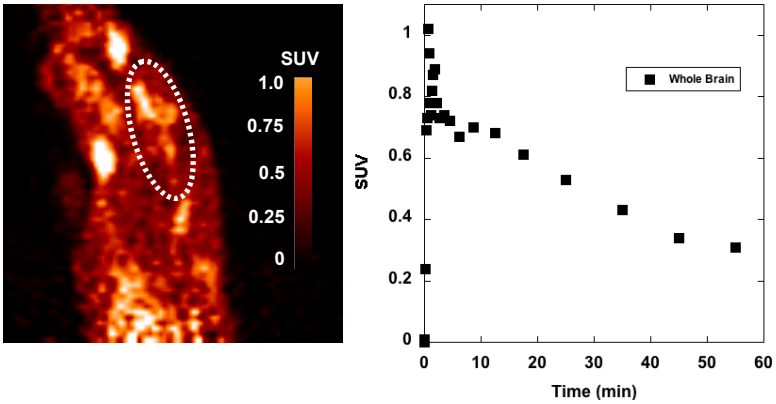


Figure S5 Sagittal rodent PET image of [^{11}C]LY2795050 summed from 0 to 60 min after injection and associated whole brain time-activity curve [dashed line = rodent brain].

3.3 Primate Imaging Protocol

Primate imaging studies were done using a mature female rhesus monkey (n = 1, weight = 8.7 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]LY2795050 (2.8 mCi) or [^{11}C]CFN (4.6 mCi) as a bolus over 1 min, and the brain imaged for 60 min (5 × 2 min frames – 4 × 5 min frames – 3 × 10 min frames). Emission data were corrected for attenuation and scatter, and reconstructed using the 3D MAP method. By using summed images (**Figure 1** in main manuscript), regions-of-interest (ROI) were drawn over brain regions on multiple planes, and the volumetric ROIs were then applied to the full dynamic data sets to generate the associated time-activity curves for [^{11}C]LY2795050 (**Figure S6**) and [^{11}C]carfentanil (**Figure S7**).

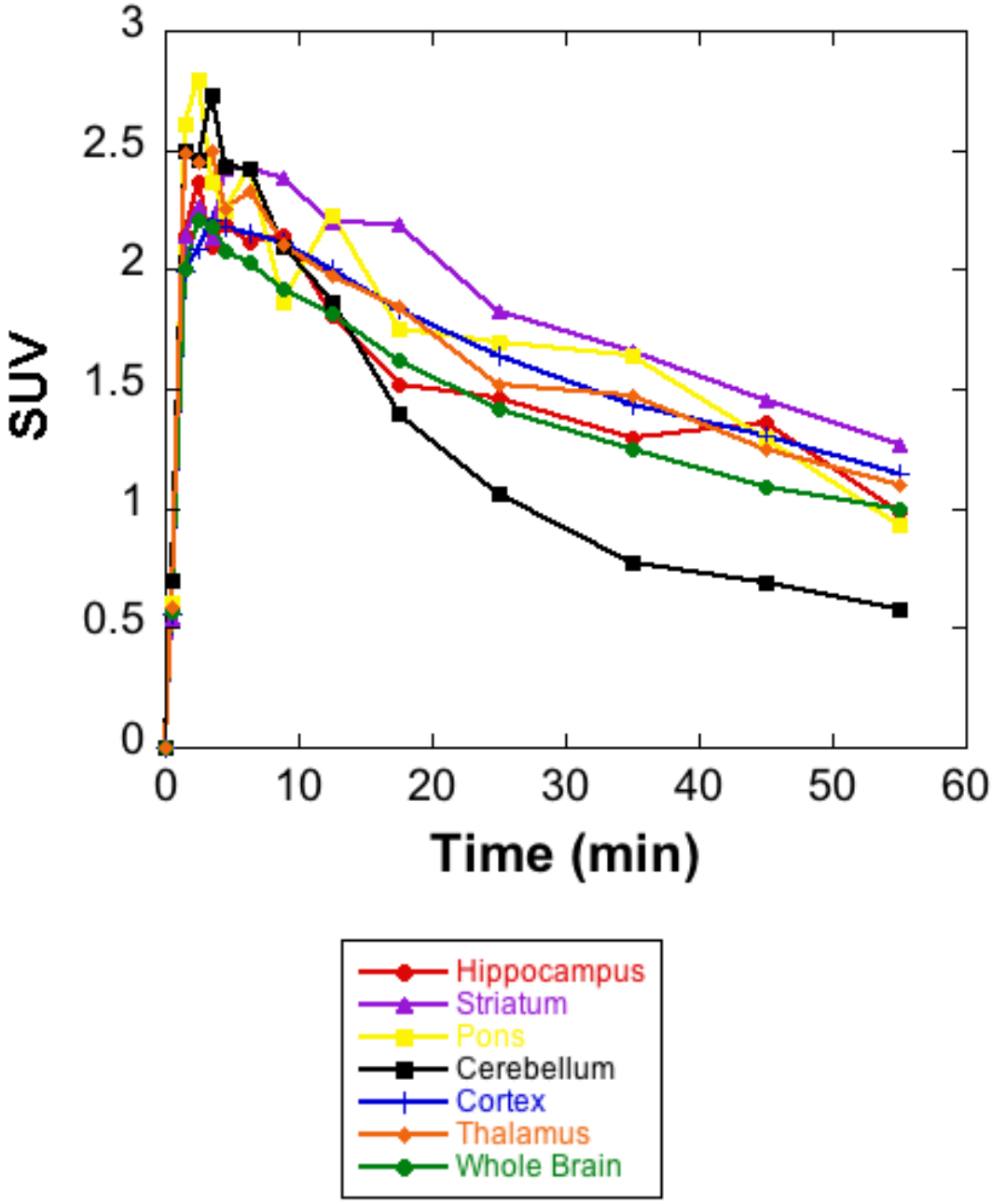


Figure S6 [^{11}C]LY2795050 primate time-activity curves

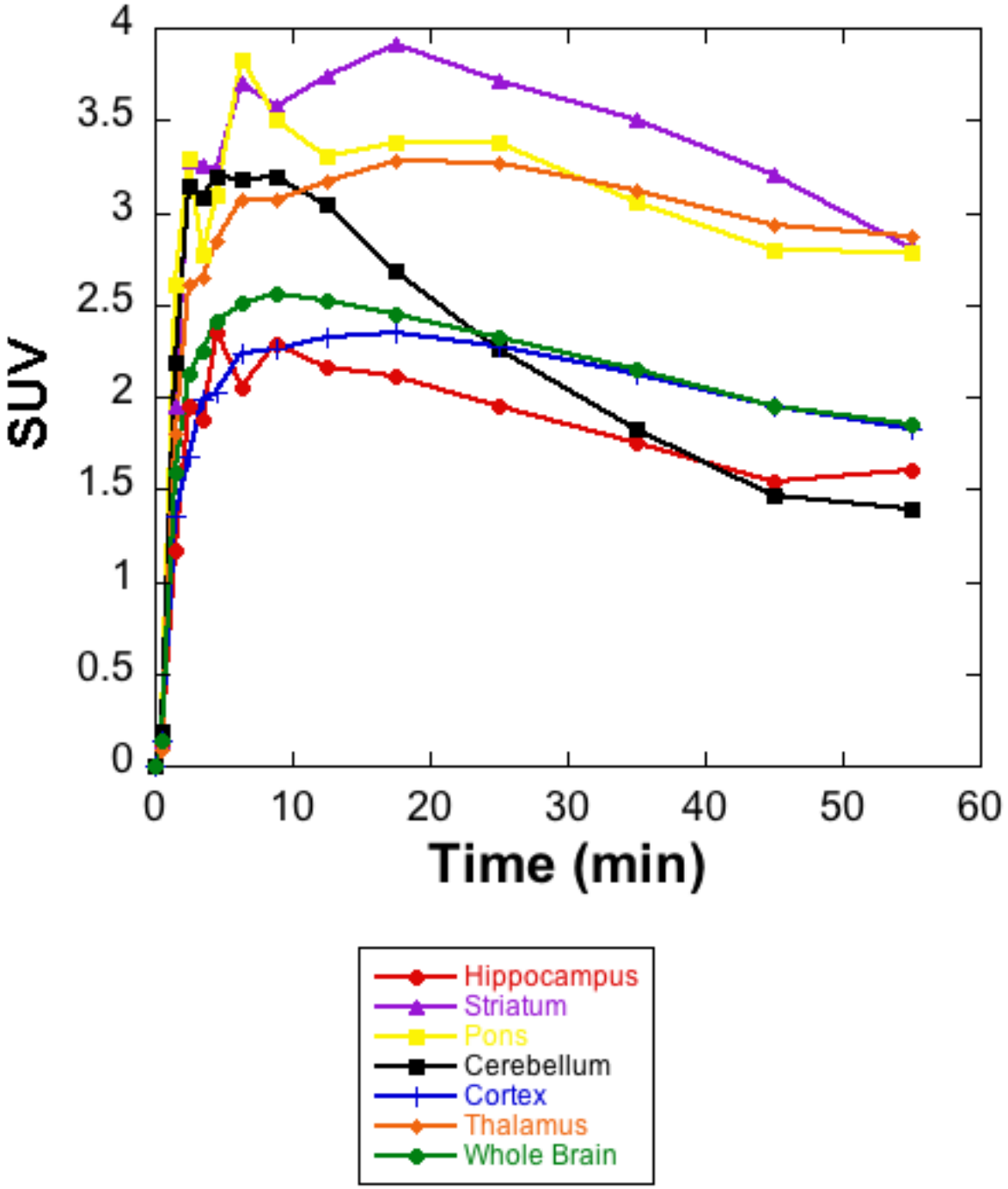


Figure S7 [^{11}C]Carfentanil primate time-activity curves

4. References

[1] Zheng, M. Q.; Nabulsi, N.; Kim, S. J.; Tomasi, G.; Lin, S. F.; Mitch, C.; Quimby, S.; Barth, V.; Rash, K.; Masters, J.; Navarro, A.; Seest, E.; Morris, E. D.; Carson, R. E.; Huang, Y. Synthesis and evaluation of ^{11}C-LY2795050 as a kappa-opioid receptor antagonist radiotracer for PET imaging. *J. Nucl. Med.* **2013**, *54*, 455-463.

[2] Mitch, C. H.; Quimby, S. J.; Diaz, N.; Pedregal, C.; de la Torre, M. G.; Jimenez, A.; Shi, Q.; Canada, E. J.; Kahl, S. D.; Statnick, M. A.; McKinzie, D. L.; Benesh, D. R.; Rash, K. S.; Barth, V. N. Discovery of aminobenzyloxyarylamides as κ opioid receptor selective antagonists: application to preclinical development of a κ opioid receptor antagonist receptor occupancy tracer. *J. Med. Chem.* **2011**, *54*, 8000-8012.

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5. ^{1}H NMR, ^{13}C NMR and HRMS Spectra for Compounds

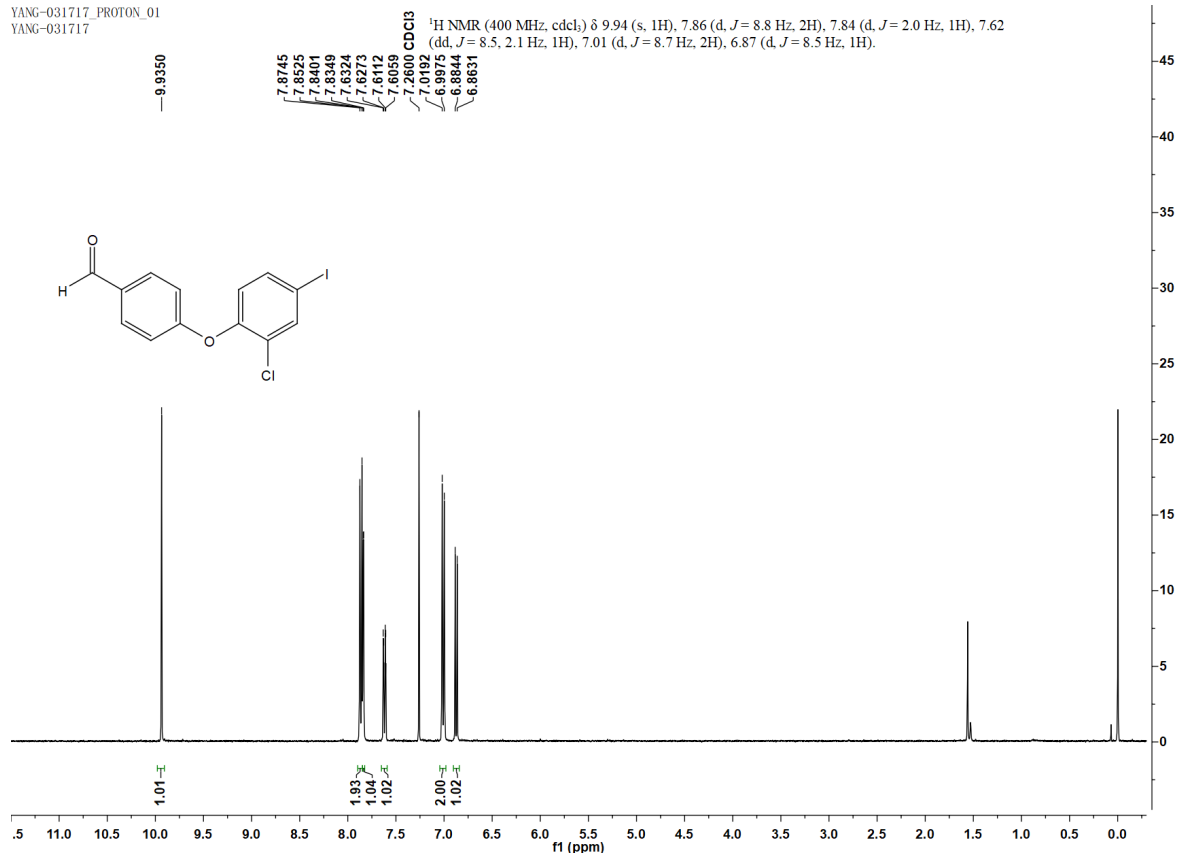


Figure S8 ^{1}H NMR of compound **3**

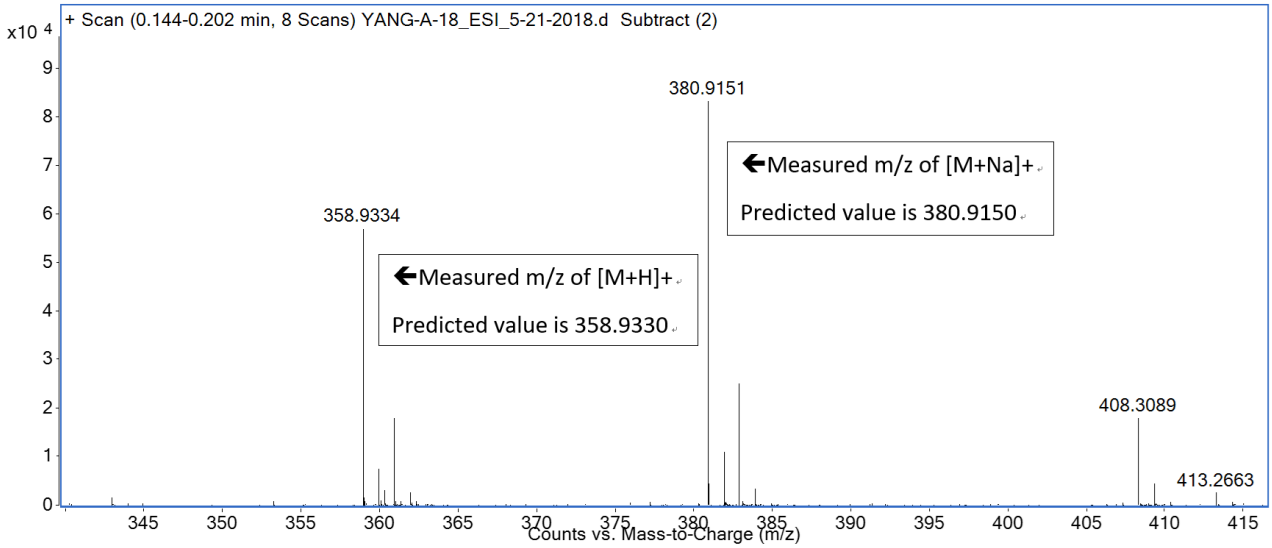
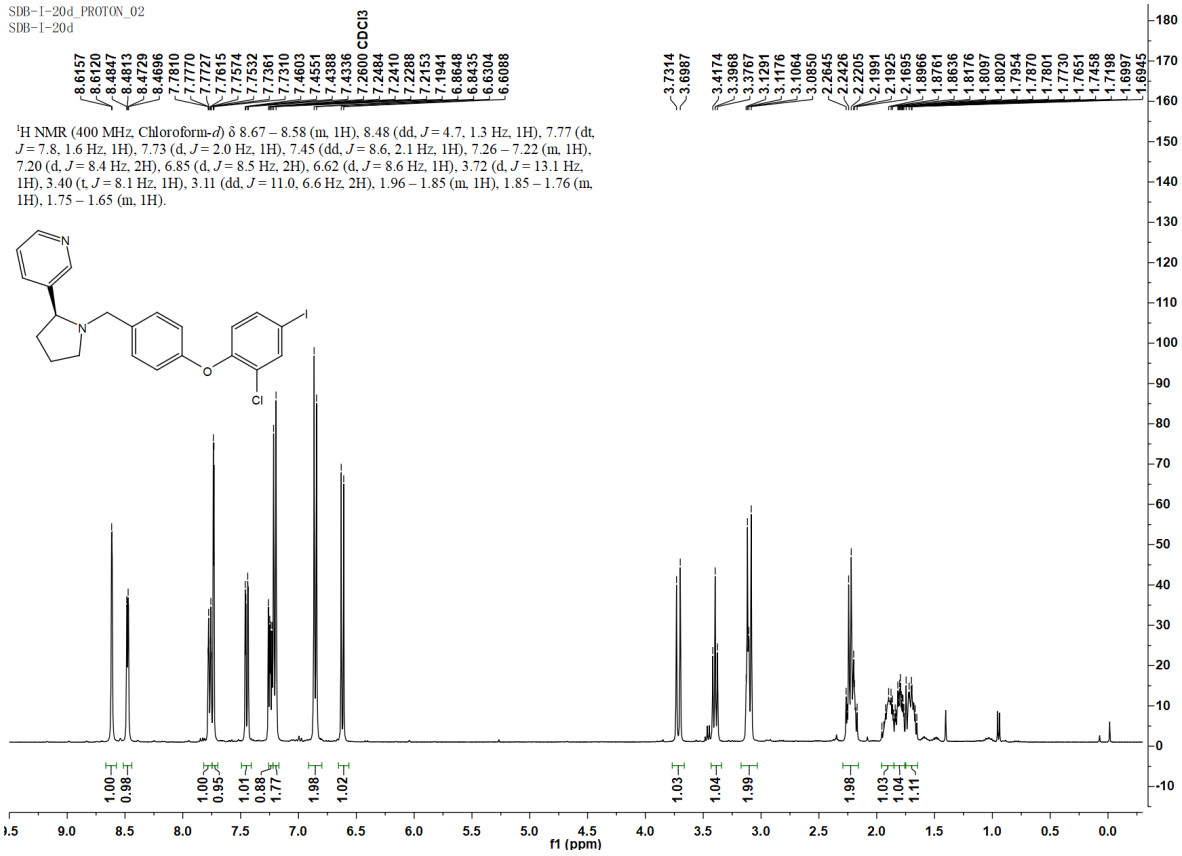


Figure S9 HRMS of compound **3**



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.67 – 8.58 (m, 1H), 8.48 (dd, *J* = 4.7, 1.3 Hz, 1H), 7.77 (dt, *J* = 7.8, 1.6 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.45 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.26 – 7.22 (m, 1H), 7.20 (d, *J* = 8.4 Hz, 2H), 6.85 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 8.6 Hz, 1H), 3.72 (d, *J* = 13.1 Hz, 1H), 3.40 (t, *J* = 8.1 Hz, 1H), 3.11 (dd, *J* = 11.0, 6.6 Hz, 2H), 1.96 – 1.85 (m, 1H), 1.85 – 1.76 (m, 1H), 1.75 – 1.65 (m, 1H).

Figure S10 ^{1}H NMR of compound **5**

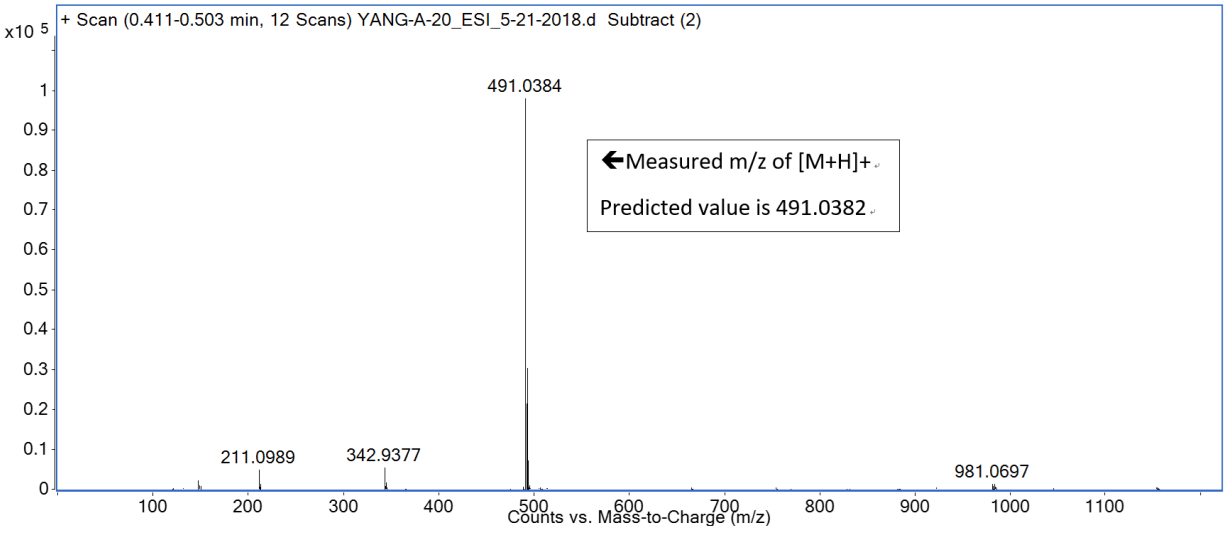
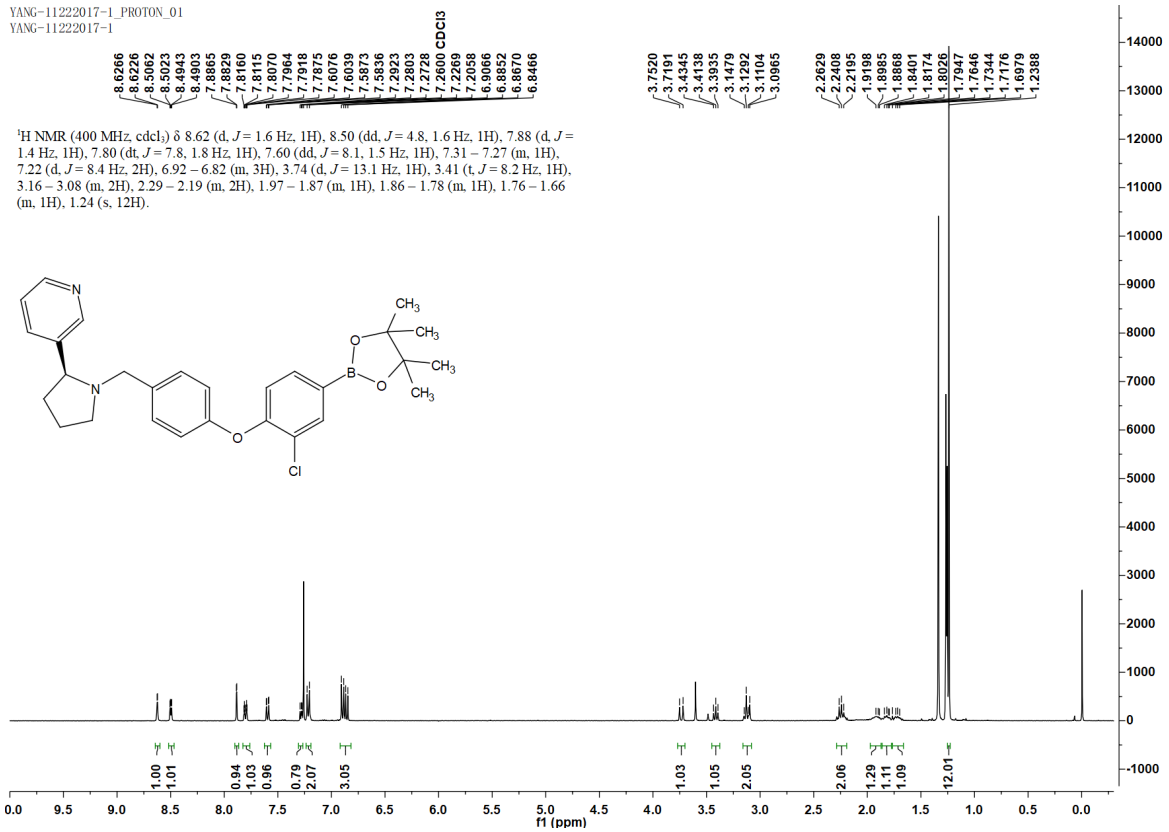


Figure S11 HRMS of compound **5**



^{1}H NMR (400 MHz, cdcl$_3$) δ 8.62 (d, *J* = 1.6 Hz, 1H), 8.50 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.88 (d, *J* = 1.4 Hz, 1H), 7.80 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.60 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.31 – 7.27 (m, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.92 – 6.82 (m, 3H), 3.74 (d, *J* = 13.1 Hz, 1H), 3.41 (t, *J* = 8.2 Hz, 1H), 3.16 – 3.08 (m, 2H), 2.29 – 2.19 (m, 2H), 1.97 – 1.87 (m, 1H), 1.86 – 1.78 (m, 1H), 1.76 – 1.66 (m, 1H), 1.24 (s, 12H).

Figure S12 ^{1}H NMR of compound **6-Bpin**

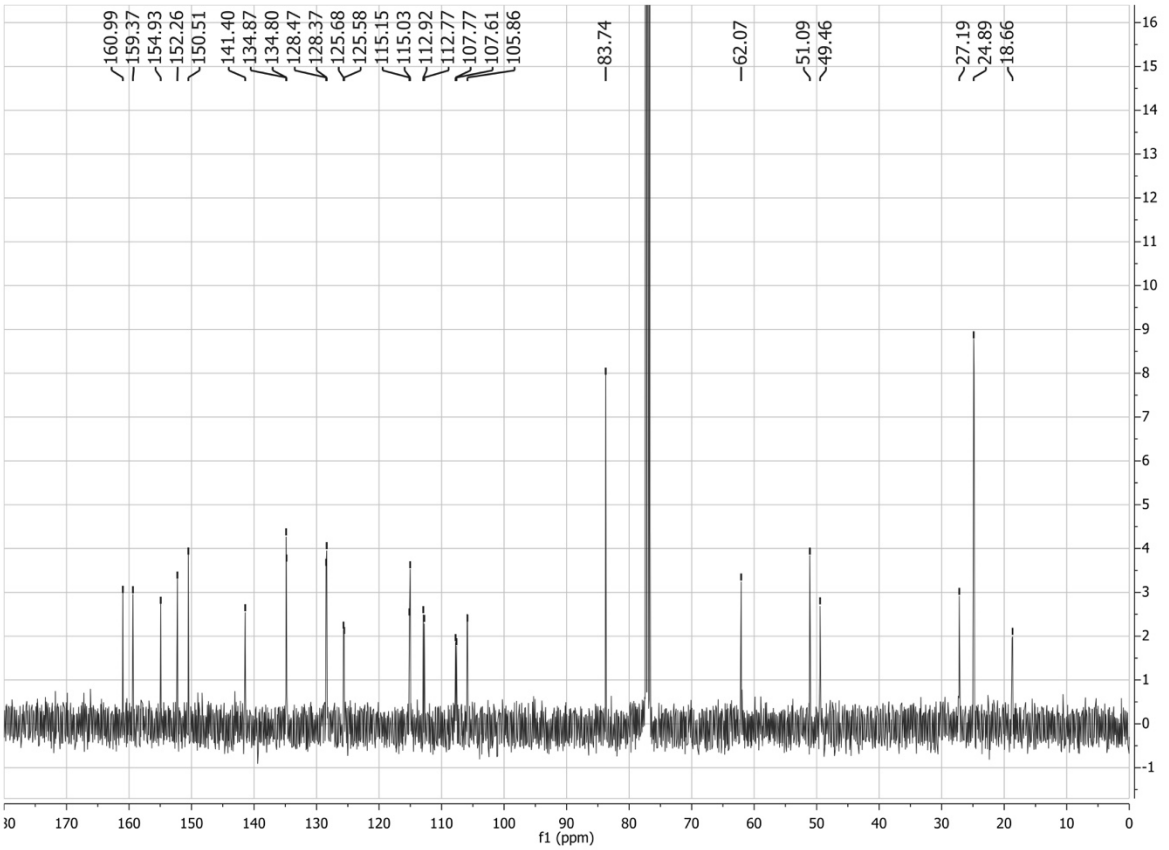


Figure S13 ^{13}C NMR of compound **6-Bpin**

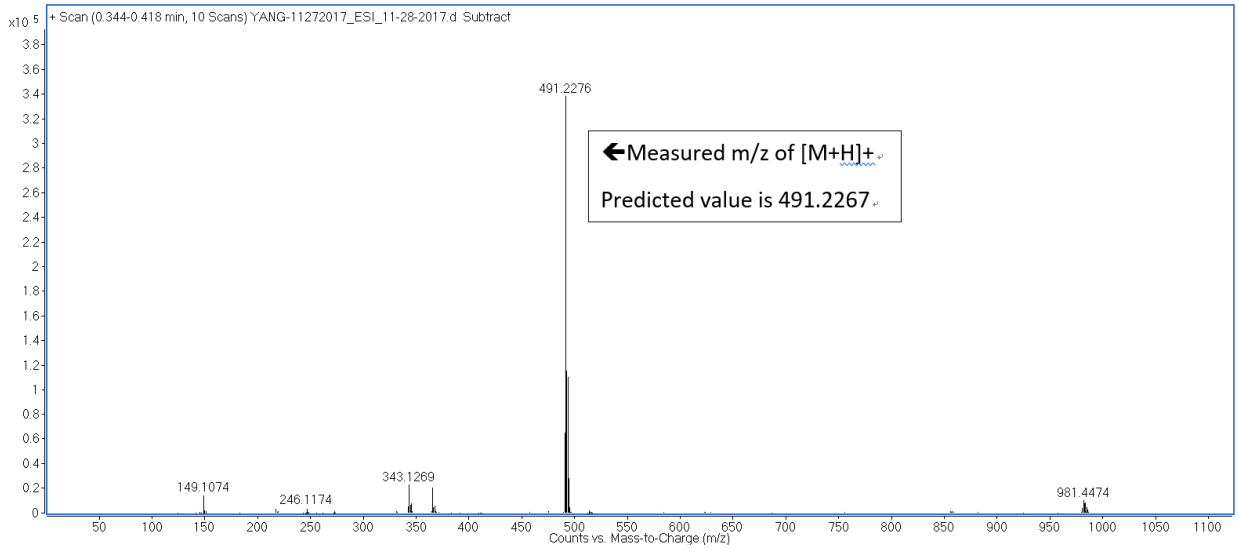


Figure S14 HRMS of compound **6-Bpin**

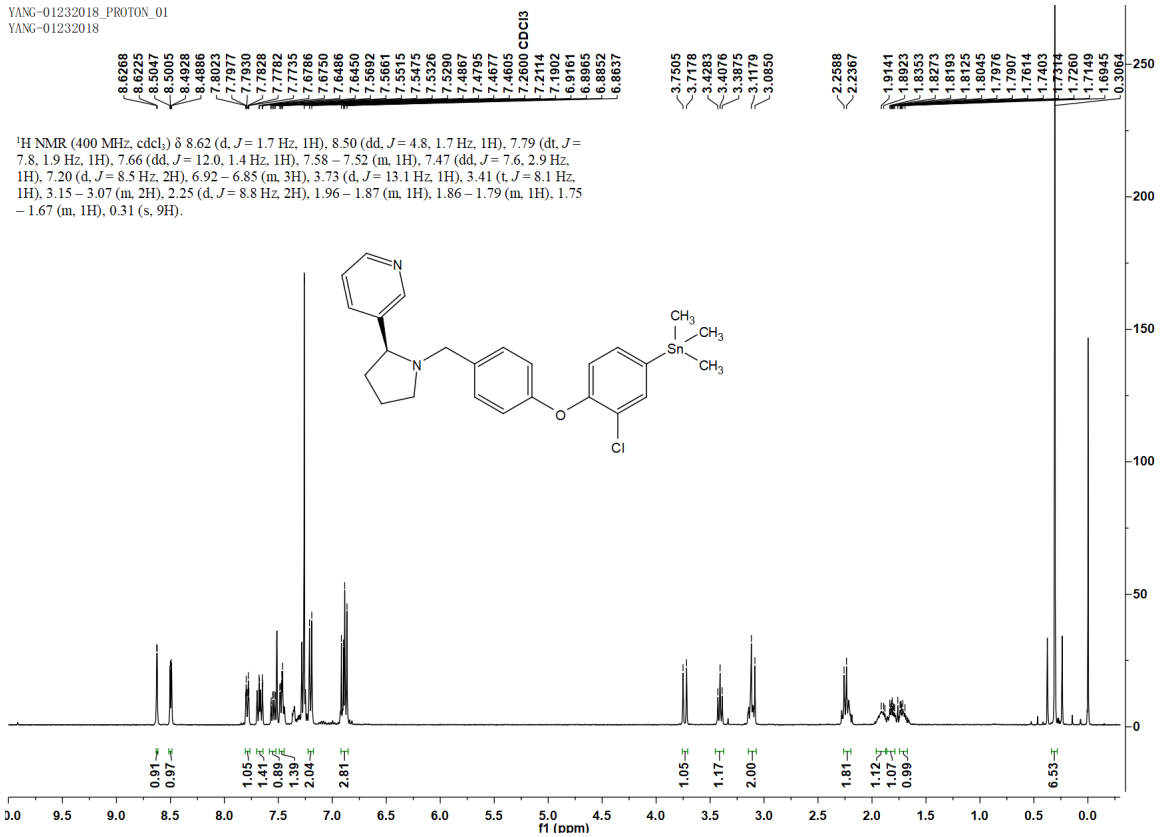


Figure S15 ^{1}H NMR of compound **6-SnMe$_3$**

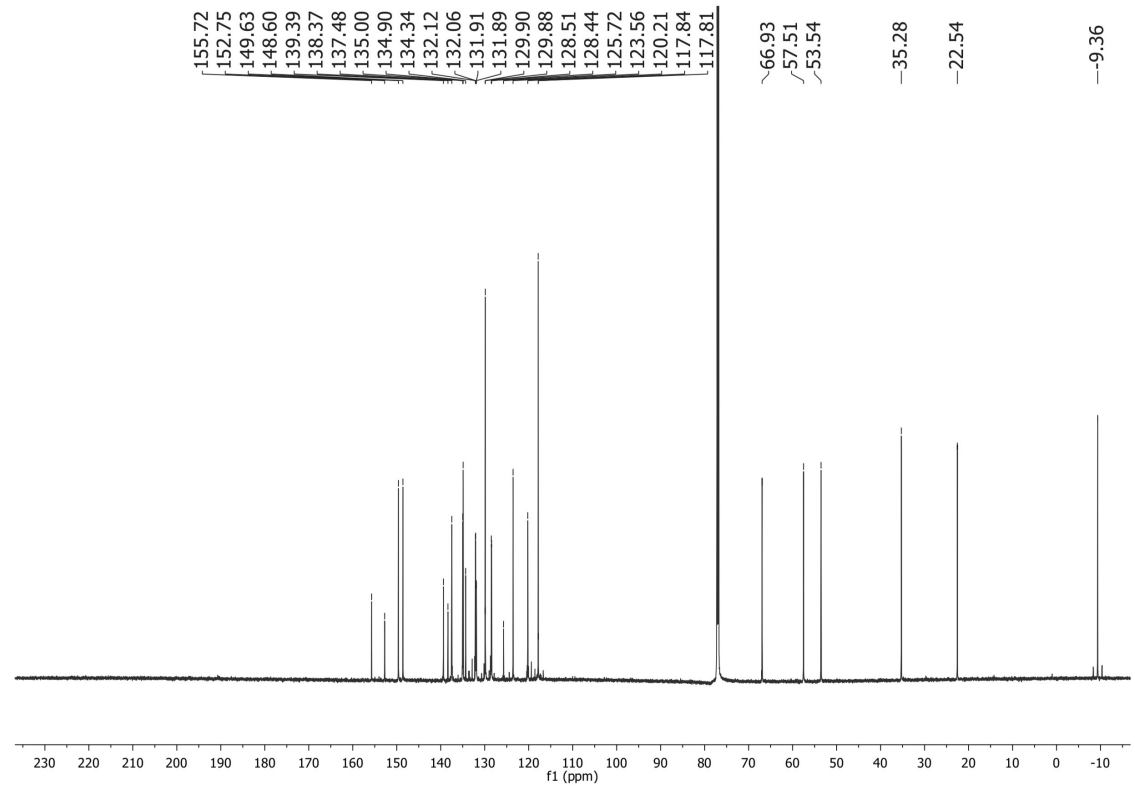


Figure S16 ^{13}C NMR of compound **6-SnMe$_3$**

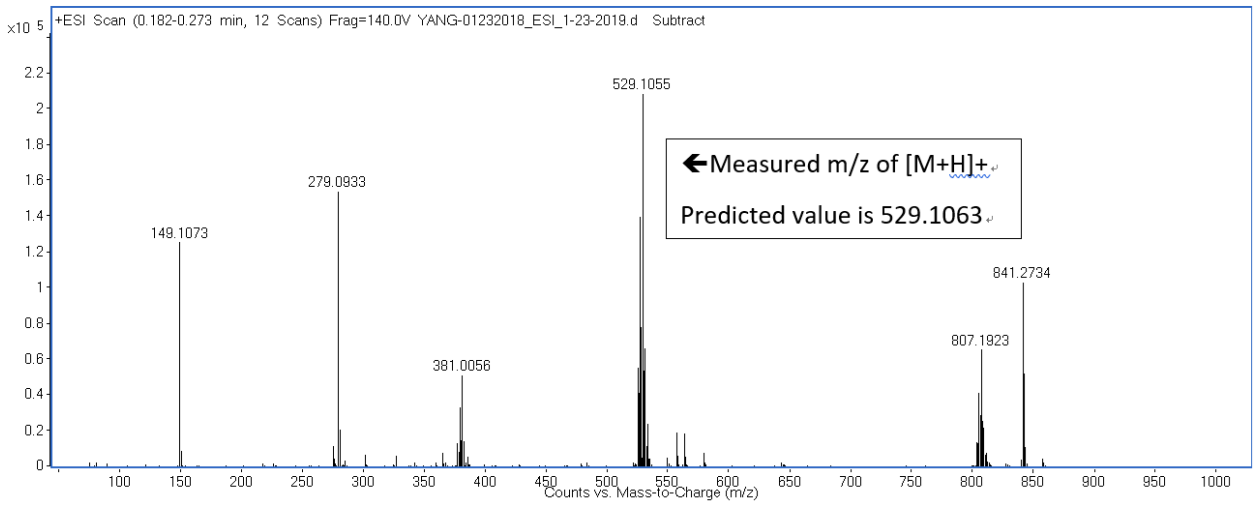


Figure S17 HRMS of compound **6-SnMe$_3$**

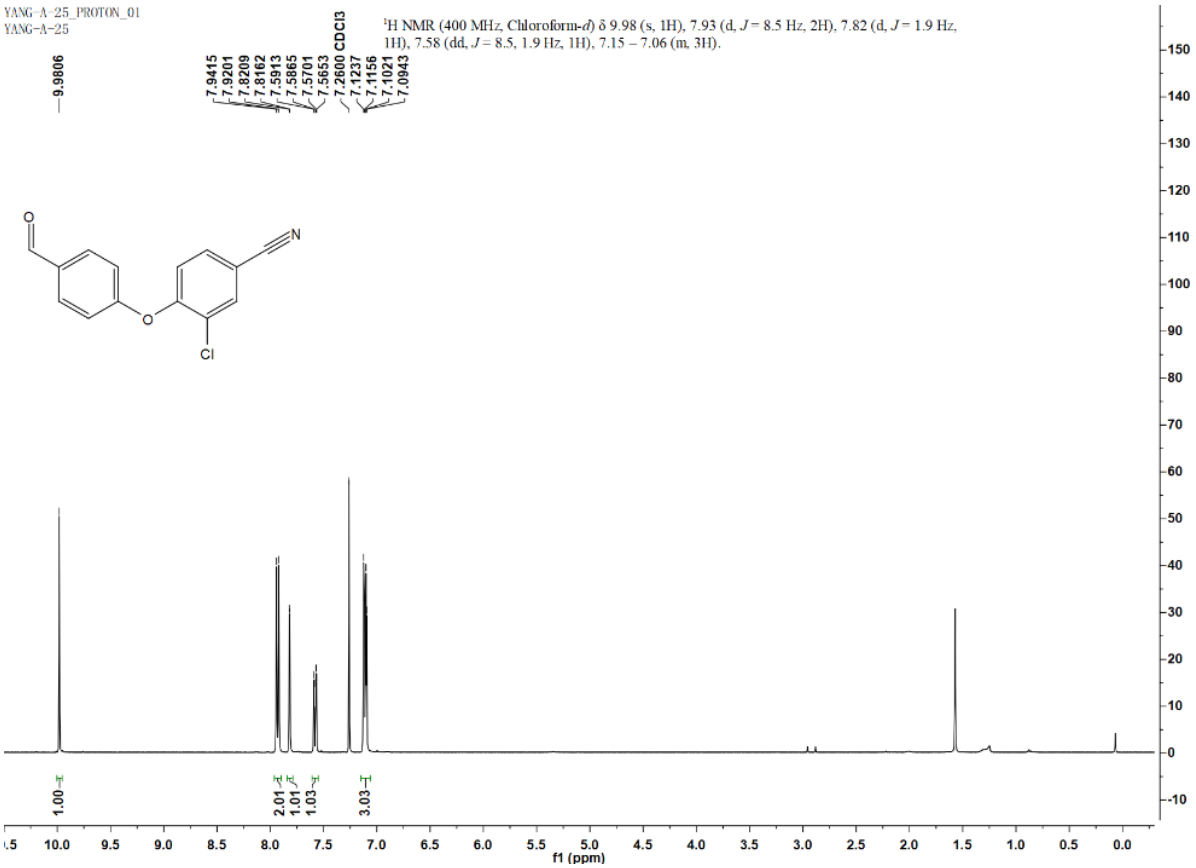


Figure S18 ^{1}H NMR of compound **S1**

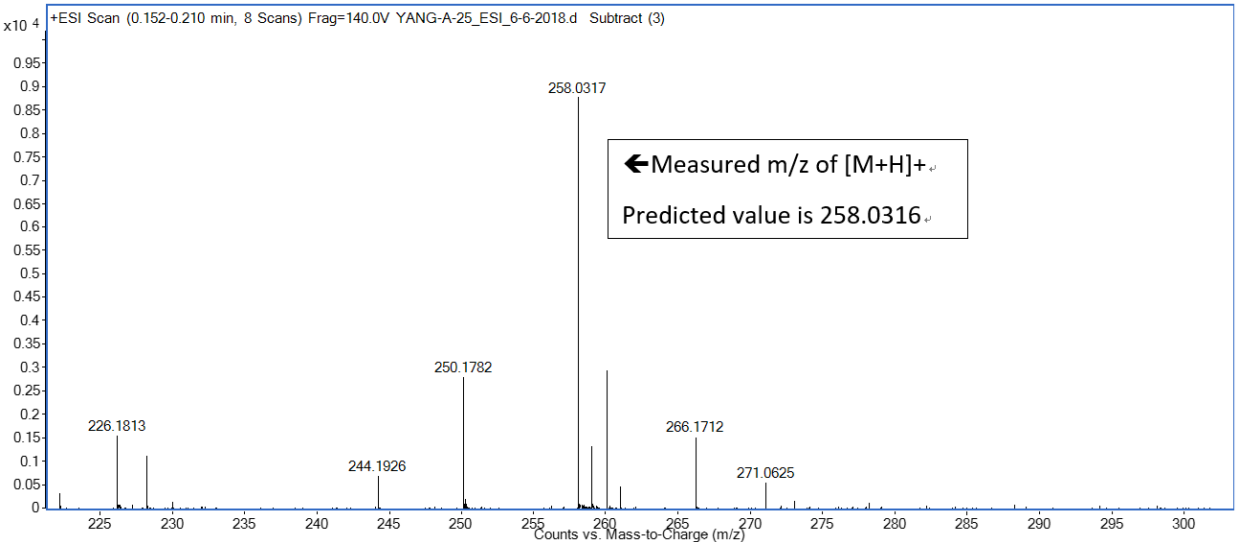


Figure S19 HRMS of compound **S1**

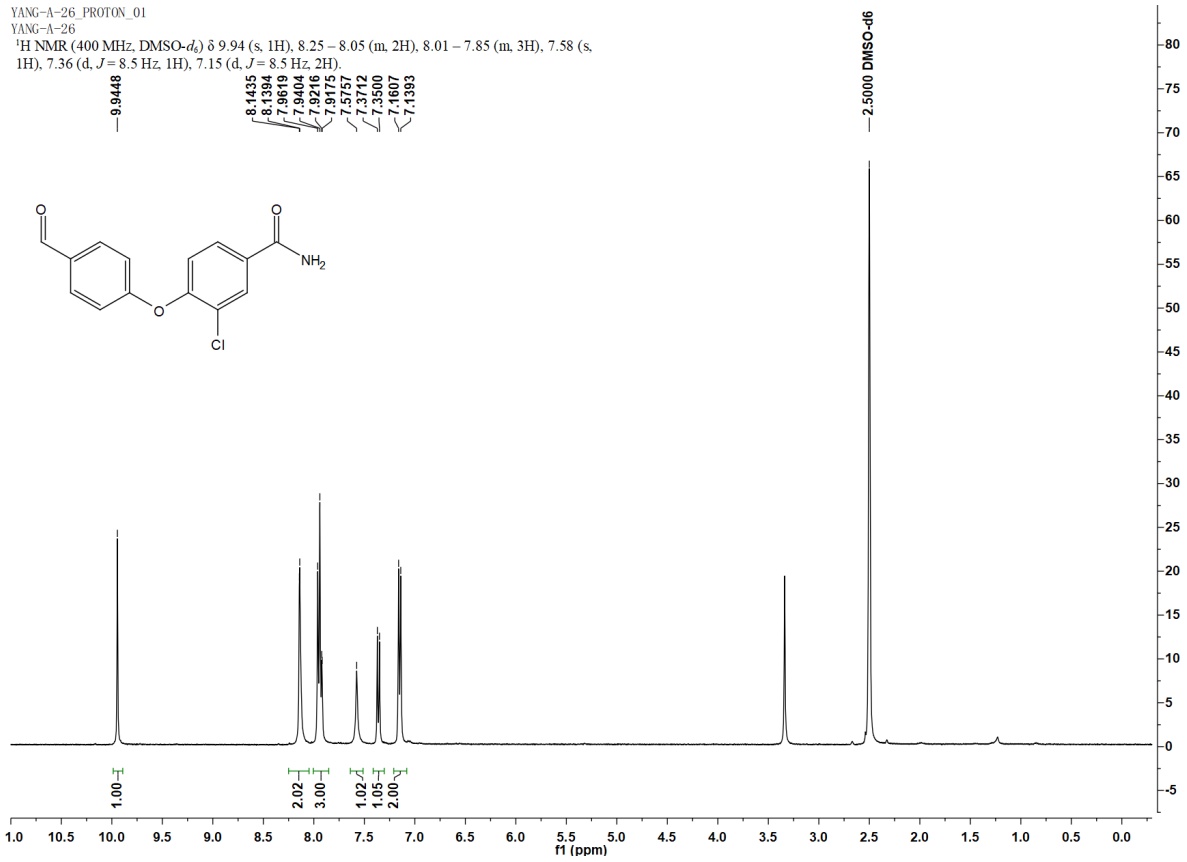


Figure S20 ¹H NMR of compound **S2**

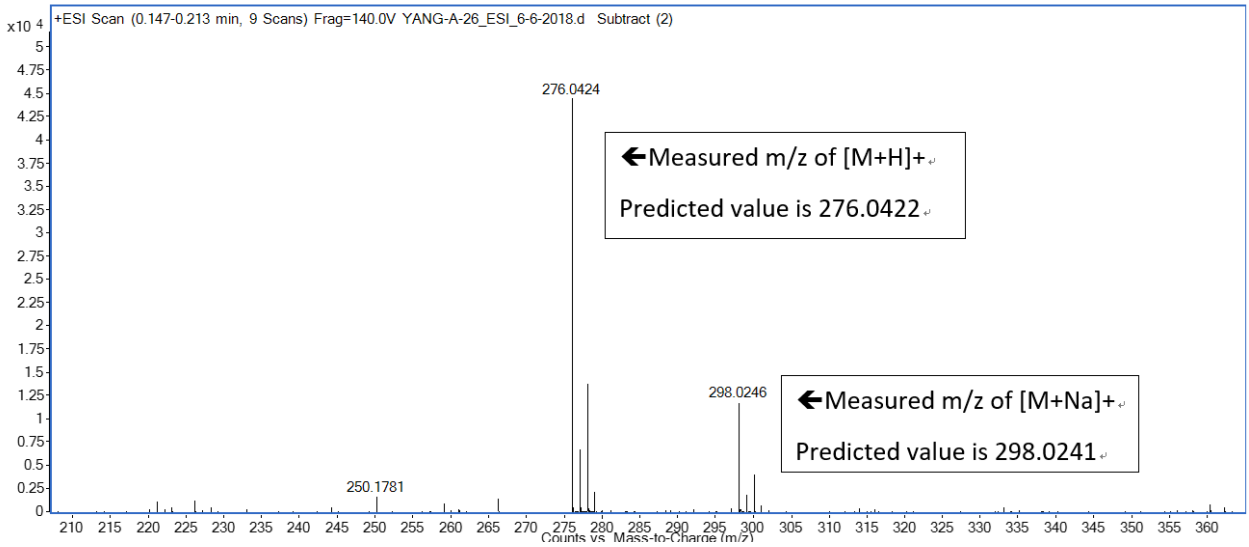
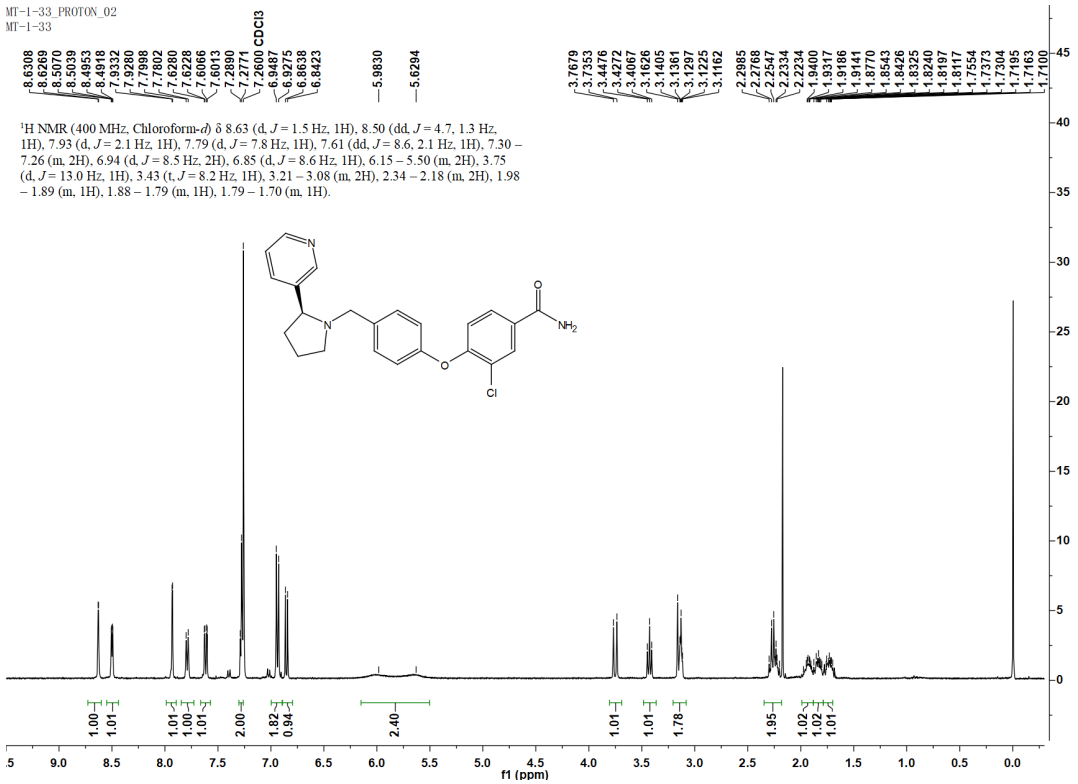
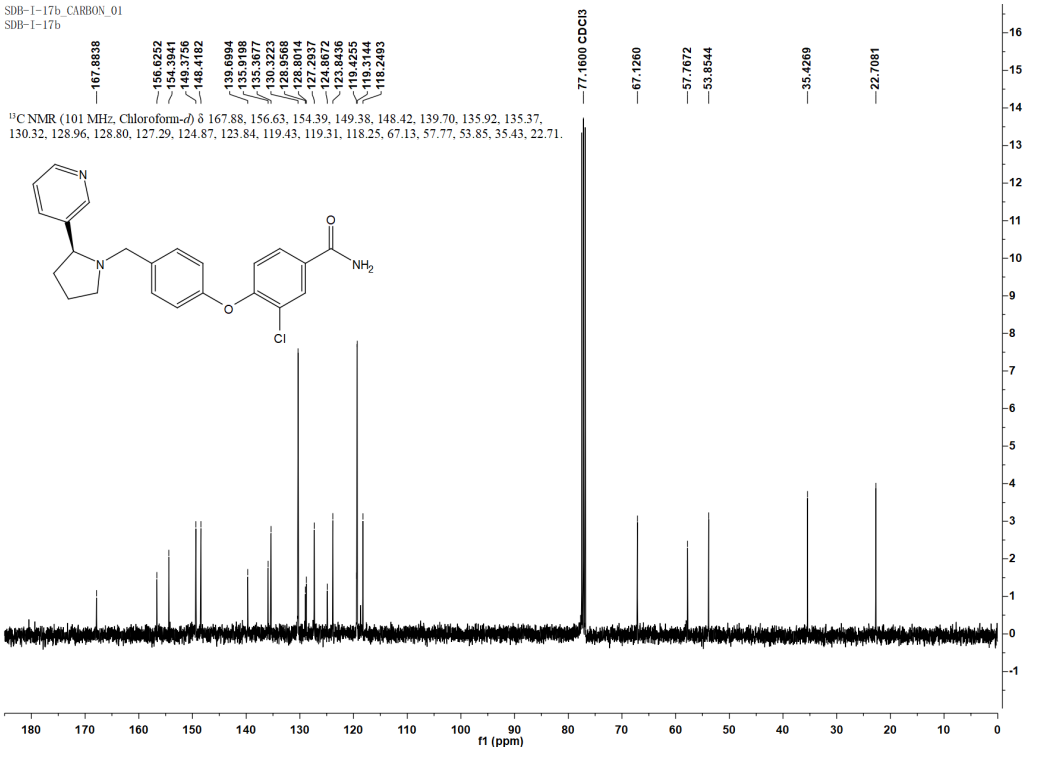


Figure S21 HRMS of compound **S2**



[1]H NMR (400 MHz, Chloroform-*d*) δ 8.63 (d, *J* = 1.5 Hz, 1H), 8.50 (dd, *J* = 4.7, 1.3 Hz, 1H), 7.93 (d, *J* = 2.1 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.61 (dd, *J* = 8.6, 2.1 Hz, 1H), 7.30 – 7.26 (m, 2H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 6.15 – 5.50 (m, 2H), 3.75 (d, *J* = 13.0 Hz, 1H), 3.43 (t, *J* = 8.2 Hz, 1H), 3.21 – 3.08 (m, 2H), 2.34 – 2.18 (m, 2H), 1.98 – 1.89 (m, 1H), 1.88 – 1.79 (m, 1H), 1.79 – 1.70 (m, 1H).

Figure S22 [1]H NMR of compound **LY2795050 (1)**



[13]C NMR (101 MHz, Chloroform-*d*) δ 167.88, 156.63, 154.39, 149.38, 148.42, 139.70, 135.92, 135.37, 130.32, 128.96, 128.80, 127.29, 124.87, 123.84, 119.43, 119.31, 118.25, 67.13, 57.77, 53.85, 35.43, 22.71.

Figure S23 [13]C NMR of compound **LY2795050 (1)**

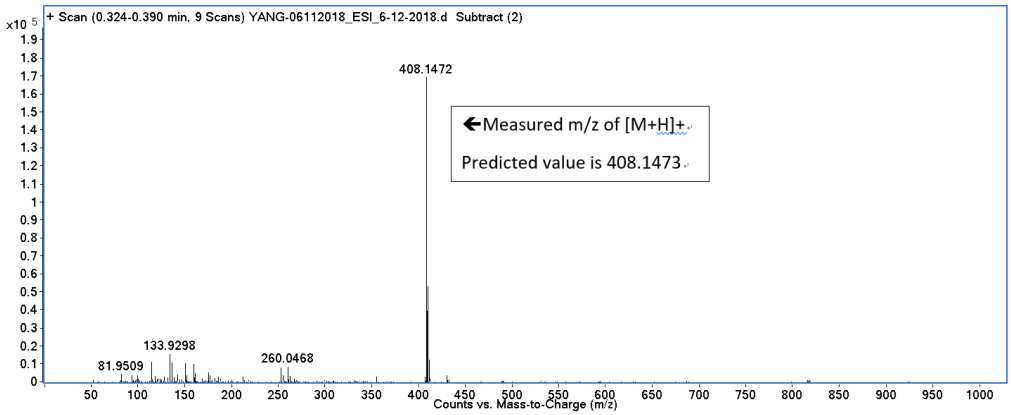


Figure S24 HRMS of compound **LY2795050 (1)**