Supporting Information

Radioiodinated 9-fluorenone derivatives for imaging of α7-Nicotinic acetylcholine receptors

Hang Gao, Shuxia Wang, Bingchao Qiang, Sixuan Wang and Huabei Zhang*

Key Laboratory of Radiopharmaceuticals of Ministry of Education, College of Chemistry, Beijing Normal University, No. 19 Xinjiekouwai Street, Haidian District, Beijing 100875, China

Content

Materials and methods

Materials and measurements

General information. Reagents and solvents were obtained from commercial sources unless otherwise noted. Thin-layer chromatography (TLC) using silica gel 60 GF254 and a UV lamp at 254nm was used to monitor the reactions. All the NMR spectra were recorded with JEOL JNM ECP600 spectrometer or Bruker-BRUKER AVANCE® III HD 400 spectrometer in $CDCl_3$ solutions at RT. Chemical shifts are given as δ values referring to the internal standard (TMS). MS spectra were recorded on a Waters Quattro Micro® Quadrupole Mass Spectrometer. Shimadzu® LC-20AT system equipped with SPD-20A UV detector (λ = 280 nm) and Bioscan® flow count (3200 NaI/PMT) was used in HPLC analysis and purification. The analytical of radioactive and non-radioactive compounds were performed on a Venusil XBP C18(L) reverse phrase analytical column (Agela Technologies, 5 μm, 150 Å, 4.6 × 250 mm) with elution of acetonitrile/water as the mobile phase at a flow rate of 2 mL/min. The purity of the tested compounds was determined and made sure to be >95%. Radiocounting was counted on a WIZARD^2 2480 automatic γ-counter (PerkinElmer, USA). Kunming mice (female, 18-22 g) and SD mice (female, 180-200 g) were provided by Beijing Xinglong Animal Technology Co, Ltd. All animal experiment protocols were previewed and approved by the Animal Care Committee of Beijing Normal University.

Chemistry

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-bromo-9*H*-fluoren-9-one (3). To a solution of 1,4-diazabicyclo[3.2.2]nonane (109.8 mg, 0.9 mmol) and 2,7-dibromo-9*H*-fluoren-9-one (337.9 mg, 1.0 mmol) in anhydrous toluene (40 mL) added tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$, 45.8 mg, 0.05 mmol, 5% eq), racemic 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP, 62.3 mg, 0.10 mmol, 10% eq), and Cs_2CO_3 (309.9mg, 1.2 mmol) respectively. The mixture was flushed with nitrogen and heated to 85 °C for 24 h. After cooling to room temperature, the mixture was filtered, solvent was removed under vacuum and purified by chromatography on silica gel (DCM/MeOH/Et_3N = 10/1/0.1). The desired purplr fractions containing product were combined and evaporated to remove solvents to give 2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-bromo-9*H*-fluoren-9-one (**3**) a purple solid (197.9 mg, 51.6%) m.p. 176-178 °C. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.61 (d, *J* = 4.0 Hz, 1H), 7.47 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.26 (t, J = 4.0 Hz, 1H), 7.17 (d, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 4.0 Hz, 1H), 6.75 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.07-

4.05 (m, 2H), 3.57 (t, J = 4.0 Hz, 2H), 3.11-3.08 (m, 3H), 3.02-2.95 (m, 2H), 2.14-2.07 (m, 2H), 1.79-1.71 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4 (1C, C_q), 150.4 (1C, C_q), 144.5 (1C, C_q), 137.1 (2C, C_q), 135.9 (1C, C_q), 127.3 (2C, CH), 121.5 (1C, CH), 120.3 (1C, CH), 117.6 (1C, CH), 113.7 (1C, C_q), 109.4 (1C, CH), 57.0 (2C, CH), 46.5 (3C, CH_2), 26.8 (2C, CH_2). IR (KBr) 2926, 2854, 1713, 1597, 1450, 1047, 696. MS (ESI): m/z calcd for $C_{20}H_{19}BrN_2O$, 382.07; found, 383.21 ($M + H^+$).

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-(tributylstannyl)-9*H*-fluoren-9-one (4). A solution of 2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-bromo-9*H*-fluoren-9-one (**3**) (383.3 mg, 1.0 mmol) in anhydrous toluene (30 mL) was added hexabutyldistannane (1160.2 mg, 2.9 mmol) and $Pd(PPh_3)_4$ (115.6 mg, 0.1 mmol, 10% eq) respectively. The mixture was flushed with nitrogen and refluxed at 120 °C for 14 h. The reaction was monitored by TLC (DCM/MeOH/Et_3N = 10/1/0.1) till completed. The mixture was filtered, concentrated under vacuum and purified by chromatography on silica gel (DCM/MeOH/Et_3N = 15/1/0.1). The desired purple fractions were collected, concentrated under vacuum to give 2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-(tributylstannyl)-9*H*-fluoren-9-one (**4**) a purple soild (222.2 mg, 37.4%). ^{1}H NMR (600MHz, $CDCl_3$) δ 7.64 (t, J = 18.0 Hz, 1H), 7.50-7.42 (m, 1H), 7.27 (dd, J = 12.0, 6.0 Hz, 2H), 7.07 (d, J = 6.0 Hz, 1H), 6.75 (dd, J = 12.0, 6.0 Hz, 1H), 4.06 (t, J = 6.0 Hz, 1H), 3.55 (t, J = 6.0 Hz, 2H), 3.13-3.08 (m, 4H), 3.01-2.96 (m, 2H), 2.12-2.07 (m, 2H), 1.75-1.70 (m, 2H), 1.55-1.50 (m, 6H), 1.35-1.29 (m, 6H), 1.08-1.04 (m, 6H), 0.88 (t, J = 12.0 Hz, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.4 (1C, C_q), 149.8 (1C, C_q), 145.2 (1C, C_q), 143.2 (2C, C_q), 135.0 (1C, C_q), 133.3 (1C, CH), 128.9 (1C, CH), 121.5 (1C, CH), 119.3 (1C, CH), 118.9 (1C, CH), 118.3 (1C, C_q), 109.9 (1C, CH), 56.2 (1C, CH_2), 53.2 (1C, CH_2), 51.45 (1C, CH), 46.5 (2C, CH_2), 46.1 (3C, CH_2), 29.1 (2C, CH_2), 27.4 (2C, CH_2), 13.72 (2C, CH_2), 9.75 (2C, CH_2), 8.77 (3C, CH_3). HRMS (ESI): m/z calcd for $C_{32}H_{46}N_2OSn$, 594.26; found, 595.2713 ($M + H^+$).

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-iodo-9*H*-fluoren-9-one (5). A saturated iodine dichloromethane solution (10 mL) was added dropwise to a solution of 2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-(tributylstannyl)-9*H*-fluoren-9-one (**4**) (296.7 mg, 0.05 mmol) in 20 mL DCM at room temperature. The mixture was stirred for 4h and monitored by TLC (DCM/MeOH/Et_3N = 10/1/0.1) till completed. The reaction was quenched by saturated sodium bisulfite solution (10 mL) and the crude was extracted by DCM and purified by chromatography on silica gel (DCM/MeOH/Et_3N = 20/1/0.1). The desired fractions were collected and concentrated under vacuum to give 2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-iodo-9*H*-fluoren-9-one (**5**) a purple solid (125.4 mg, 58.3%), m.p. 211-213 °C. ^{1}H NMR (600MHz, $CDCl_3$) δ 7.83

(d, J = 1.7 Hz, 1H), 7.68 (dd, J = 7.9, 1.7 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 7.07 (d, J = 7.9 Hz, 1H), 7.06 (d, J = 2.9 Hz, 1H), 6.76 (dd, J = 8.4, 2.6 Hz, 1H), 4.06 (q, J = 2.2 Hz, 1H), 3.59 – 3.52 (m, 2H), 3.15 – 3.04 (m, 4H), 2.98 (m, 2H), 2.13 – 2.05 (m, 2H), 1.73 (dd, J = 14.6, 9.6, 4.6 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.5 (1C, C_q), 150.6 (1C, C_q), 145.2 (1C, C_q), 143.2 (2C, C_q), 136.0 (1C, C_q), 135.3 (1C, CH), 133.1 (1C, CH), 121.7 (1C, CH), 120.7 (1C, CH), 117.6 (1C, CH), 109.4 (1C, CH), 90.9 (1C, C_q), 57.1 (1C, CH_2), 51.8 (1C, CH_2), 46.6 (2C, CH_2), 44.7 (1C, CH), 26.9 (2C, CH_2). IR (KBr) 2974, 2924, 1709, 1593, 1446, 1041, 619. HRMS (ESI): m/z calcd for $C_{20}H_{19}IN_2O$, 430.05; found, 431.0620 ($M + H^+$).

9-oxo-9*H*-fluorene-1-carboxylic acid (7). A mixture of CrO_3 (69.0 g, 0.69 mol) in ethanoic acid (40 mL) and water (60 mL) was slowly dropped into a preheated mixture of fluoranthene (20.0 g, 0.1 mol) in ethanoic acid (500 mL). The mixed solution was heated to 100 °C for 120 min and poured into cold water (1500 mL). The precipitated solid was filtered and added to 2 M NaOH (300 mL). The mixture was filtered again and the filtrate was added to a defined amount of concentrated HCl untill the pH reached 3. The precipitated yellow solid was filtered and dried to give 9-oxo-9*H*-fluorene-1-carboxylic acid (**7**) (14.7 g, 66.1%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, J = 7.5 Hz, 1H), 7.71–7.62 (m, 2H), 7.62–7.56 (m, 1H), 7.56–7.50 (m, 1H), 7.47 (d, J = 6.9 Hz, 1H), 7.29 (t, J = 7.2 Hz, 1H). MS (ESI^+): m/z calcd for $C_{14}H_8O_3$, 224.05; found, 225.10 ($M + H^+$).

7-bromo-9-oxo-9*H*-fluorene-1-carboxylic acid (8). Br_2 (10.0 mL, 0.2 mol) was added to a solution of 9-oxo-9*H*-fluorene-1-carboxylic acid (**7**) (15.0 g, 66.9 mmol) in 100 mL of water at room temperature. The mixture was gradually heated to 85°C and stirred for 12 h. The reaction was monitored by TLC, and cooled to R.T. when completed. The mixture was neutralized by the infusion of 10% $NaHSO_3$. The yellow solid in the mixture was filtered and dried, resulting in the title compound 7-bromo-9-oxo-9*H*-fluorene-1-carboxylic acid (**8**) (19.6 g, 96.5%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (dd, J = 7.4, 1.5 Hz, 1H), 7.78 (d, J = 1.7 Hz, 1H), 7.68 – 7.60 (m, 3H), 7.36 (d, J = 7.9 Hz, 1H).

***Tert*-butyl (7-bromo-9-oxo-9*H*-fluoren-1-yl)carbamate (9).** A solution of 7-bromo-9-oxo-9*H*-fluorene-1-carboxylic acid (**8**) (3.0 g, 9.98 mmol) in *t*-BuOH (10 mL) and anhydrous toluene (30 mL) was alkalized with Et_3N (2.0 mL, 14.9 mmol) and added DPPA (3.2 mL, 14.9 mmol). The mixture was heated to 120 °C for 18 h and cooled to room temperature. The toluene was removed under vacuum and the crude was purified by silica gel chromatography (PE: EA = 20:1) to give *Tert*-butyl (7-bromo-9-oxo-9*H*-fluoren-1-yl) carbamate (**9**) as a yellow solid (2.7 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 8.56

Hz, 1H), 7.71 (d, *J* = 1.72 Hz, 1H), 7.59 (dd, *J* = 7.88 Hz, 1.8 Hz, 1H), 7.42 (dd, *J* = 7.44 Hz, 8.36 Hz, 1H), 7.37 (d, *J* = 7.92 Hz, 1H), 7.09 (d, *J* = 7.16 Hz, 1H), 1.55 (s, 9H).

***Tert*-butyl (7-(4-methylpiperazin-1-yl)-9-oxo-9*H*-fluoren-1-yl)carbamate (10).** The typical procedure **a** of Buchwald-Hartwig cross-coupling reaction was followed, starting with **9** (100 mg, 0.3 mmol) and 1-methylpiperazine (40.1 mg, 0.4 mmol). The title compound **10** was obtained after purification as a red solid (58.2 mg, 55.3% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 9.26 (s, 1H), 7.97 (d, *J* = 4.0 Hz, 1H), 7.31-7.24 (m, 2H), 7.12 (s, 1H), 6.91-6.84 (m, 2H), 3.23 (s, 4H), 2.54 (s, 4H), 2.32 (s, 3H), 1.53 (s, 9H). MS (ESI$^+$): m/z calcd for $C_{23}H_{27}N_3O_3$, 393.21; found, 394.1878 (M + H$^+$).

***Tert*-butyl 4-(8-((tert-butoxycarbonyl)amino)-9-oxo-9*H*-fluoren-2-yl)piperazine-1-carboxylate (11).** The typical procedure **a** for Buchwald-Hartwig cross-coupling reaction was followed, starting with **9** (100 mg, 0.3 mmol) and *tert*-butyl piperazine-1-carboxylate (74.5 mg, 0.4 mmol). The title compound **11** was obtained after purification as a red solid (86.1 mg, 67.2%). ^{1}H NMR (400MHz, $CDCl_3$) δ 9.27 (s, 1H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.36-7.32 (m, 2H), 7.17 (d, = 4.0 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 6.92 (dd, *J* = 12.0, 4.0 Hz, 1H), 3.58 (t, *J* = 4.0 Hz, 4H), 3.20 (t, *J* = 4.0 Hz, 4H), 1.53 (s, 9H), 1.48 (s, 9H). MS (ESI$^+$): m/z calcd for $C_{27}H_{33}N_3O_5$, 479.24; found, 480.2203 (M + H$^+$).

1-amino-7-(4-methylpiperazin-1-yl)-9*H*-fluoren-9-one (12). HCl (1 N, 2mL, 2.4 mmol) was added to a solution of *tert*-butyl (7-(4-methylpiperazin-1-yl)-9-oxo-9*H*-fluoren-1-yl)carbamate (**10**) (39.4 mg, 0.1 mmol) in CH_3CN (10 mL) and the resulting mixture was heated to 80 °C for 4 h. The reaction was cooled down, and alkalized to pH = 12 with NaOH (1 N) followed by extraction with DCM. The desired fragments were dried with Na_2SO_4 and filtered and the solvent was removed to give 1-amino-7-(4-methylpiperazin-1-yl)-9*H*-fluoren-9-one (**12**) a red solid (23.6 mg, 80.2%), m.p. 134-136 °C. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.33 (t, *J* = 4.0 Hz, 1H), 7.19 (t, *J* = 4.0 Hz, 1H), 7.13 (t, *J* = 4.0 Hz, 1H), 6.89 (t, *J* = 8.0 Hz, 1H), 6.70 (t, *J* = 4.0 Hz, 1H), 6.38(t, *J* = 4.0 Hz, 1H), 4.34 (s, 2H), 3.32 (t, *J* = 4.0 Hz, 1H), 2.74 (t, *J* = 4.0 Hz, 1H), 2.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.2 (1C, C_q), 175.4 (1C, C_q), 151.7 (1C, C_q), 147.3 (1C, C_q), 145.1 (1C, C_q), 136.4 (1C, CH), 134.8 (1C, C_q), 121.3 (1C, C_q), 119.8 (1C, CH), 116.2 (1C, CH), 115.3 (1C, CH), 111.4 (1C, CH), 109.1 (1C, CH), 54.2 (2C, CH_2), 48.1 (2C, CH_2), 45.1 (1C, CH_3). IR (KBr) 3354, 2930, 1672, 1622, 1496, 1033. HRMS (ESI$^+$): m/z calcd for $C_{18}H_{19}N_3O$, 293.15; found, 294.1604, 295.1651 (M + H$^+$).

1-amino-7-(piperazin-1-yl)-9*H*-fluoren-9-one (13). The typical procedure was followed as step **h**/**k**, starting with **11** (47.9 mg, 0.1 mmol) and red solid was obtained (22.1 mg, 78.9%), m.p. 124-126 °C. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.33 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 4.0 Hz, 1H), 7.14 (dd, J = 8.0, 4.0 Hz, 1H), 6.90 (dd, J = 8.0, 4.0 Hz, 1H), 6.70 (d, J = 4.0 Hz, 1H), 6.37 (d, J = 8.0 Hz, 1H), 5.43 (s, 2H), 3.20 (t, J = 4.0 Hz, 4H), 3.02 (J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 195.3 (1C, C_q), 152.7 (1C, C_q), 147.3 (1C, C_q), 136.6 (1C, C_q), 136.4 (1C, C_q), 134.3 (1C, C_q), 133.3 (1C, CH), 121.2 (1C, C_q), 119.6 (1C, CH), 116.0 (1C, CH), 115.3 (1C, CH), 111.1 (1C, CH), 108.9 (1C, CH), 50.3 (2C, CH_2), 46.1 (2C, CH_2). IR (KBr) 2922, 2852, 1736, 1670, 1618, 1467.IR (KBr) 2926, 2847, 1713, 1597, 1450, 696.

HRMS (ESI$^+$): m/z calcd for $C_{17}H_{17}N_3O$, 279.14; found, 280.1440, 281.1457 (M + H$^+$).

1-amino-7-bromo-9*H*-fluoren-9-one (14). The typical procedure was followed as step **h**/**k**, starting with **9** (404 mg, 1.08 mmol) in CH_3CN (40 mL) and the resulting mixture was heated to 80 °C for 4 h. The reaction was cooled down, and alkalized to pH = 12 with NaOH (1 N) followed by extraction with EA. The desired fragments were dried with Na_2SO_4 and filtered and the solvent was removed to give 1-amino-7-bromo-9*H*-fluoren-9-one (**7**) (250 mg, 84.5%).^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (d, J = 6.0 Hz, 1H), 7.55 (dd, J = 12.0, 6.0 Hz, 1H), 7.38-7.33 (m, 3H), 7.23 (dd, J = 12.0, 6.0 Hz, 1H), 3.80 (dt, J = 48.0, 12.0 Hz, 4H), 1.79-1.71 (m, 4H), 1.45-1.37 (m, 4H), 1.00-0.95 (m, 6H). MS (ESI$^+$): m/z calcd for $C_{13}H_8BrNO$, 272.98; found, 273.99, 275.98 (M +H$^+$).

7-bromo-1-fluoro-9*H*-fluoren-9-one (15). To a solution of pre-cooled HBF_4 (20 mL) added **14** (1.0 g, 3.7 mmol), the mixture was stirred for 10 min at -5 °C. A mixed solution of $NaNO_2$ (0.3 g, 4.4 mmol) in H_2O (10 mL) was added slowly to the mixture at 5 °C, and stirred for another 30 min. The precipitate was filtered, washed with ethanol and heated to 120 °C in an open bottle for 20 min. The black residue was dissolved in EA, filtered and purified by flash column chromatography (PE: EA = 10: 1) to obtain the title compound as a yellow solid (337.6 mg, 33.4%). 1H NMR (400MHz, $CDCl_3$) δ 7.76 (s, 1H), 7.62-7.58 (m, 1H), 7.49 (dd, J = 12.0, 8.0 Hz, 1H), 7.39 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 6.96 (t, J = 8.0 Hz, 1H). ^{19}F NMR (400MHz, $CDCl_3$) δ -112.41 (1F).

1-fluoro-7-(4-methylpiperazin-1-yl)-9*H*-fluoren-9-one (16). The typical procedure **a** for Buchwald-Hartwig cross-coupling reaction was followed, starting with **15** (82.8 mg, 0.3 mmol) and 1-methylpiperazine (40.1 mg, 0.4 mmol). The title compound **16** was obtained after purification by preparative liquid chromatograph as a purple solid (30.6 mg, 34.6% yield), m.p. 119-121 °C. ^{1}H NMR

(400MHz, $CDCl_3$) δ 7.41 (t, J = 4.0 Hz, 1H), 7.25 (d, J = 4.0 Hz, 1H), 7.18 (s, 1H), 6.99-6.94 (m, 2H), 6.81 (t, J = 8.0 Hz, 1H), 3.34 (t, J = 8.0 Hz, 4H), 2.65 (t, J = 4.0 Hz, 4H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7 (1C, C_q), 152.5 (1C, C_q), 147.3 (1C, C_q), 137.2 (1C, C_q), 135.5 (1C, C_q), 133.8 (1C, C_q), 121.5 (2C, CH), 120.0 (2C, CH), 115.9 (1C, C_q), 115.4 (1C, CH), 111.7 (1C, CH), 54.7 (2C, CH_2), 48.4 (2C, CH_2), 45.9 (1C, CH_3). ^{19}F NMR (400MHz, CDCl3) δ -113.79 (1F). IR (KBr) 2922, 2852, 1710, 1606, 1467, 1236. HRMS (ESI^+): m/z calcd for $C_{18}H_{17}FN_2O$, 296.13; found, 297.1402, 278.1451 (M + H^+).

***Tert*-butyl 4-(8-fluoro-9-oxo-9*H*-fluoren-2-yl)piperazine-1-carboxylate (17).** The typical procedure **a** for Buchwald-Hartwig cross-coupling reaction was followed, starting with **15** (82.8 mg, 0.3 mmol) and 1-methylpiperazine (74.7 mg, 0.4 mmol). The title compound **17** was obtained after purification by preparative liquid chromatograph as a purple solid (46.5 mg, 40.7% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 7.40-7.35 (m, 2H), 7.20 (d, J = 4.0 Hz, 1H), 7.15 (d, J = 4.0 Hz, 1H), 6.92 (dd, J = 8.0, 4.0 Hz, 1H), 6.79 (t, J = 8.0 Hz, 1H), 3.57 (t, J = 4.0 Hz, 4H), 3.20 (t, J = 4.0 Hz, 4H), 1.47 (s, 9H). ^{19}F NMR (400MHz, $CDCl_3$) δ -113.62 (1F). MS (ESI^+): m/z calcd for $C_{22}H_{23}FN_2O_3$, 382.17; found, 383.1550 (M + H^+).

1-fluoro-7-(piperazin-1-yl)-9*H*-fluoren-9-one (18). The typical procedure was followed as step **h**/**k**, starting with **17** (38.2 mg, 0.1 mmol) and purple solid was obtained (22.9 mg, 81.3%), m.p. 101-103 °C. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.42-7.36 (m, 2H), 7.24 (s, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.95 (d, J = 8.0 Hz, 1H), 6.80 (t, J = 8.0 Hz, 1H), 3.23 (t, J = 4.0 Hz, 4H), 3.04 (t, J = 4.0 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8 (1C, C_q), 153.1 (1C, C_q), 147.3 (1C, C_q), 137.1 (1C, C_q), 135.5 (1C, C_q), 133.7 (1C, C_q), 121.5 (1C, CH), 120.1 (1C, C_q), 115.9 (1C, CH), 115.7 (1C, CH), 115.4 (1C, CH), 115.3 (1C, CH), 111.6 (1C, CH), 49.8 (1C, CH_2), 45.9 (1C, CH_2). ^{19}F NMR (400MHz, $CDCl_3$) δ -113.89 (1F). IR (KBr) 2922, 2847, 1712, 1606, 1471, 1234. HRMS (ESI^+): m/z calcd for $C_{17}H_{15}FN_2O$, 282.12; found, 283.1237, 284.1284 (M + H^+).

Radiolabeling

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-[^{125}I]iodo-9*H*-fluoren-9-one (5). To a solution of tributyltin precursors (**4**) (0.2mg, 0.00034 mmol) in Ethanol (200 μL) was added 1 mCi of Na^{125}I in 0.1 N NaOH at R.T., followed by 1 N HCl (50 μL) and hydrogen peroxide (50 μL) at R.T. The reaction mixture was maintained at room temperature for 30 min with intermittent shaking and quenched by saturated sodium bisulfite solution (10 μL) then diluted with a CH_3CN/H_2O mixture (7/3, 200 μL) and applied to a reverse phase HPLC column (Agela Technologies, 5 mm, 150 Å, 4.6 × 250 mm). [^{125}I]**5** elutes at 50.8 min, and was collected and loaded on a Waters Sep-Pak C18 cartridge. The column was washed with an additional water (10 mL), and the product was eluted with methanol (10 mL), then solvent was removed under vacuum. The final product was analyzed by HPLC using a UV detector at 280 nm and a flow count radio detector to determine the radiochemical purity of synthesized compound (Table 1). The total synthesis time was approximately 100 min. The radiochemical yield was 85.4% (decay not corrected), and the radiochemical purity was greater than 98%.

Table 1 HPLC Conditions for [^{125}I]5

Compd	Mobile phase	Flow rate	Product retention time	Precursor retention time
[^{125}I]**5**, prep.	$CH_3CN/H_2O/TFA$ = 28/72/0.1	2 mL/min	50.87 min	>300 min
[^{125}I]**5**, ana.	$CH_3CN/H_2O/TFA$ = 60/40/0.1	2 mL/min	8.31 min	>300 min

***In vitro* binding assay**

Preparation of rat membranes: SD rats (180-200g, female) were decapitated, brains were quickly remove from the body and put on cooling bedplate. The cortexes of the brains (α7-nAChRs highly expressed) were collected, homogenized at 4 °C in 15 vol of cold Tris-HCl buffer (pH = 7.4, 50 nM Tris, 120 nM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$) and centrifuged at 48000 G for 20 min under cooling condition. The supernate was abandoned and the tissues in bottom of the tube were collected, homogenized followed by centrifuged again as described above for 3 times. Subsequently, the bottom tissues were distributed into 15 times the volume of Tris-HCl bufferin the aseptic centrifugal tube and kept at -80 °C.The method of Lowry was used to determine the protein concentration [1].

α7-nAChR bind assay: The protein prepared above was directly used to conducted the saturation binding. The protein were hatched in Tris-HCl buffer for 2h at 37 °C with a gradient concentration of [^{125}I]*α*-bungarotoxin ([^{125}I]***α*-Bgt**, 10 μL) in a total volume of 500 μL. Nonspecific binding was measured by adding *α*-bungarotoxin (*α*-Bgt, 2 μM, 100 μL) with the same condition. The binding assays of each tube was terminated at the end of the time and filtered through polyethyleneimine-pretreated glass fiber filters (Whatman GF/B) by a cell harvester (Mp-48T, Brandel, Gaithersbrug, MD). The glass fiber filters on the cell harvester were washed with pre-cooled buffer for three times and collected. The radioactivity of each sheared filters were measured by an automatic γ-counter. The synthesized compounds as well as reference compound **MLA** were evaluated in the presence of [^{125}I]***α*-Bgt** (0.4 nM). All expertiments were performed independently and carried in triplicate. Cheng-Prusoff equation was used to analyze and calculated the Ki values.

α4β2-nAChR assay: the affinities of studied compounds were conducted with a modified method same as α7 -nAChR. [^{3}H]cytisine (1 nM) was used as the competitive binding ligands, and cytisine (1 μM) was used in nonspecific binding assays. The same methods describe above were used to perform the incubation and filtration, and a liquid scintillation counter was used to measure the resulting samples.

***In vitro* stability studies**

The radioligands (10 μCi) was incubate in fetal bovine serum (BSA, 100 μL, 38g/1000 mL) and saline at 37 °C and R.T., respectively. The specimens were sampled at 1 h, 2 h, 6 h, 12 h, and analyzed by HPLC system. The samples from fetal bovine serum were disposed with acetonitrile (100 μL) and filtered through a Nylon 66 filter (0.22 μm) before the analyses.

Biodistribution in tissues and organs in mice.

The biodistribution of studied compounds were performed in normal female Kunming mice. Each Kunming mouse (18-22g, female, n=3) was injected with radiotracer (0.2 μCi, in 0.1 mL saline, containing 10% Ethanol) *via* the tail vein. The mice were contributed by decapitations at 5, 15, 30, 60, 90 and 120 min post-injection. The organs and tissues of each mouse were collected, weighted and measured by the γ-counter. Aliquots of the injectate were taken as standards and measured together with the organs and tissues. The percent of injected dose per gram of wet tissue (%ID/g tissue) was calculated. The values were expressed as the mean ± SD (n = 3).

Biodistribution studies in brains regions and blockade studies

Biodistribution studies in brain regions of mice were conducted using same method described above. Each mouse was injected 0.2 μCi of radioligand into a lateral tail vein, followed by cervical dislocation. The whole brain of each mouse was carefully removed and dissected on ice, after flushed with saline, the studied regions were collected, weighed, and counted by the γ-counter. Aliquots of the injectate were taken as standards, and the radioactivity content was measured together with the brain tissue samples. The percent of injected dose per gram of tissue (%ID/g tissue) was calculated. The values were expressed as the mean ± SD (n = 3).

Blockade studies

In vivo self-blockade studies were conducted by pre-injection the non-radioactive standards **5**. Compound **5** was dissolved in sterile saline (containing 10 % Ethanol) to make a concentration of 0.125 mg/mL and injected to mice (0.1 mL) at 15 min before the administration of radioligand. At 45 min post-injection of the radiochemical, brain tissues were harvested and interest cerebral regional was collected and counted.

In vivo blocking study of α4β2-nAChR and 5-HT3 receptors were carried out by pre administration of cytisine (0.1 mL, 1 mg/kg) and ondansetron (0.1 mL, 1 mg/kg) *via* subcutaneous injection 15 min before the radiotracer. The radiochemical were dissolved in sterile saline (containing 10 % Ethanol) and made a concentration of 0.25 mg/mL. Control groups were treated with 0.1 mL of the vehicle solution in order to eliminate the interference. At 45 min after the injection of the racers, brain tissues of each mouse were harvested and interest regions were counted by the automatic γ-counter.

***Ex vivo* autoradiography**

After injected of [^{125}I]**5** (60 μCi, 0.1 mL), the Kunming mice were sacrificed at 45 min. The mice of blocking group were pre-treated with SSR180711 (0.1 mL, 0.25 mg/mL) 15 min before the injection of radiotracers. The intact brain was carefully taken out and frozen at preference temperature, the brain was cut into 15-μm-thick sections. Each slices were carefully exposed in a dark room to phosphorus pates for 14 h and scanned by a storage phosphor system. The references used for verifying the brain sections was downloaded from Allen Brain Reference Atlases.

***In vivo* micro-SPECT/CT imaging in Kunming mice**

Kunming mice were anesthetized painlessly and positioned on the bed of micro-SPECT/CT equipment (Triumph SPECT/CT system, TriFoil). After tail vein injected ([^{125}I]**5**, 80 μCi, 0.1 mL saline containing 10% Ethanol), a 60 min dynamic scan was performed successively. In additional, another two points static scans (90 min and 120 min post-injection) were conducted. The blocking group was treated with SSR180711 (0.1 mL, 0.25 mg/mL) *via* tail intravenous 15 min before the injection of radiotracers. The sagittal, coronal and transverse views of mice cerebral were transferred to the corresponding SPECT images and superposed with CT images.

Molecular docking of ligands into α7-nAChRs

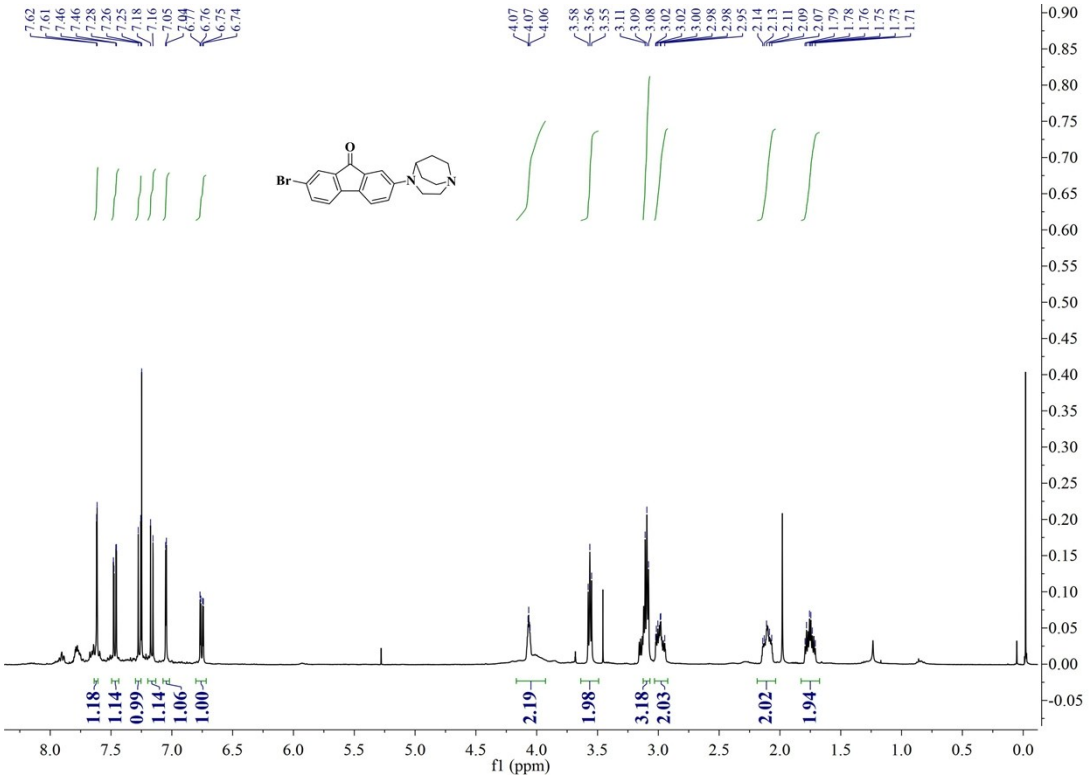
Docking studies were studied in Glide 6.7.[2-4] Protein structures (PDB ID: 3SQ6) were downloaded from Protein Data Bank, and prepared using Maestro protein preparation wizard.[43] Hydrogen-bonds of the complexes were optimized, followed by an energy-minimization of protein-ligand complexes in Maestro until the RMSD reached 0.30 Å. The ligands were prepared in Maestro with OPLS_2005 force field, and the docking grid was generated using the original ligand Epibatidine (EPD) for reference with a maximum size of 20 Å.[6, 7] The OH groups of amino acid chains near the binding site were allowed to rotate. The extra precision docking procedure mode (XP) was implemented and 20 docking complexes were saved for each ligand. The most favourable docking scores (top 5) of each studied ligand were chosen for refinement with molecular dynamic studies.

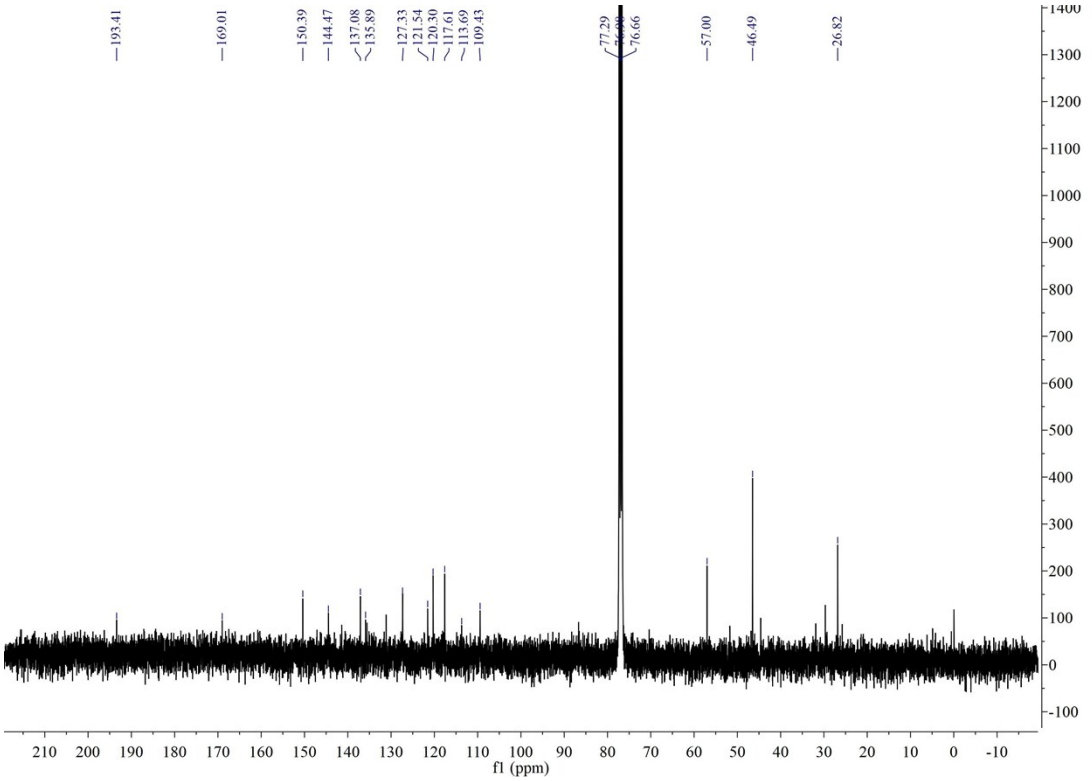
Molecular dynamics simulation

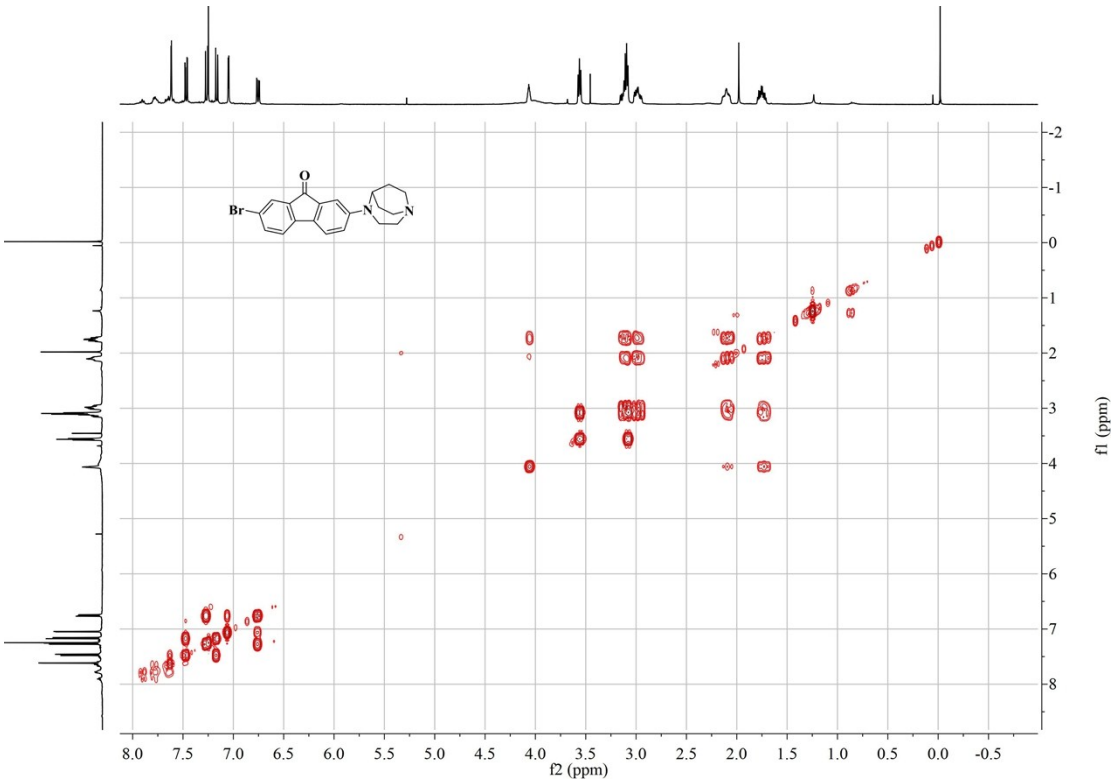
The molecular dynamics (MD) simulations of studied system were carried out in Maestro with Desmond 4.2.[8, 9] The complexes with favourite docking poses were prepared in Maestro. The α7-AChBP/iodo-ASEM and **5** complexes were solvated in 750000 $Å^3$ cubic water boxes. Systems were neutralized by adding reasonable amount of Na^+ and Cl^- ions. The SPC water model was used and a concentration of 0.15 M NaCl was added to simulated physiological environment. Each complex was put in the box, and minimization was conducted. The PRCG method with 3 vectors and 10 minimum steepest descent steps was used to have a gradient threshold less than 25 kcal/mol/Å. The convergence threshold was set to 1.0 kcal/mol/Å while the maximum iterations during the minimization steps were set to 2000. For non-bonded interactions, a cut-off of 9 Å was used. For the long-range electrostatic interaction, the smooth particle mesh Ewald (PME) was used and the Eward tolerance was set to 1e-09. Each studied complexed was minimized and conducted a pore-equilibrate procedure using default relaxation routine before the equilibration and MD simulations. Before the formal simulations, each system was heated to reach 300 K with a time step of 2fs in the NPT ensemble gradually. For the simulations, a multiple time step RESPA integration algorithm was used. The time step for bonder and 'near' nonbonded was 2.0fs while 'far' nonbonded was set to 6.0fs. For each system, 40ns of simulation was implemented in the NPT ensemble was performed using the Nose-Hoover thermostat (T=300 K, relaxation time = 200 ps; P = 1 atm) and Martyna-Tobias-Klein barostat (relaxation time = 500 ps). Energy and atomic coordinate data during the simulations were collected every 1.2 ps and 4.8 ps respectively. Resulting systems and trajectories were checked in Maestro. Root mean square deviations (RMSD) from the initial structures and ligand-protein contacts during the simulation were calculated in Maestro.

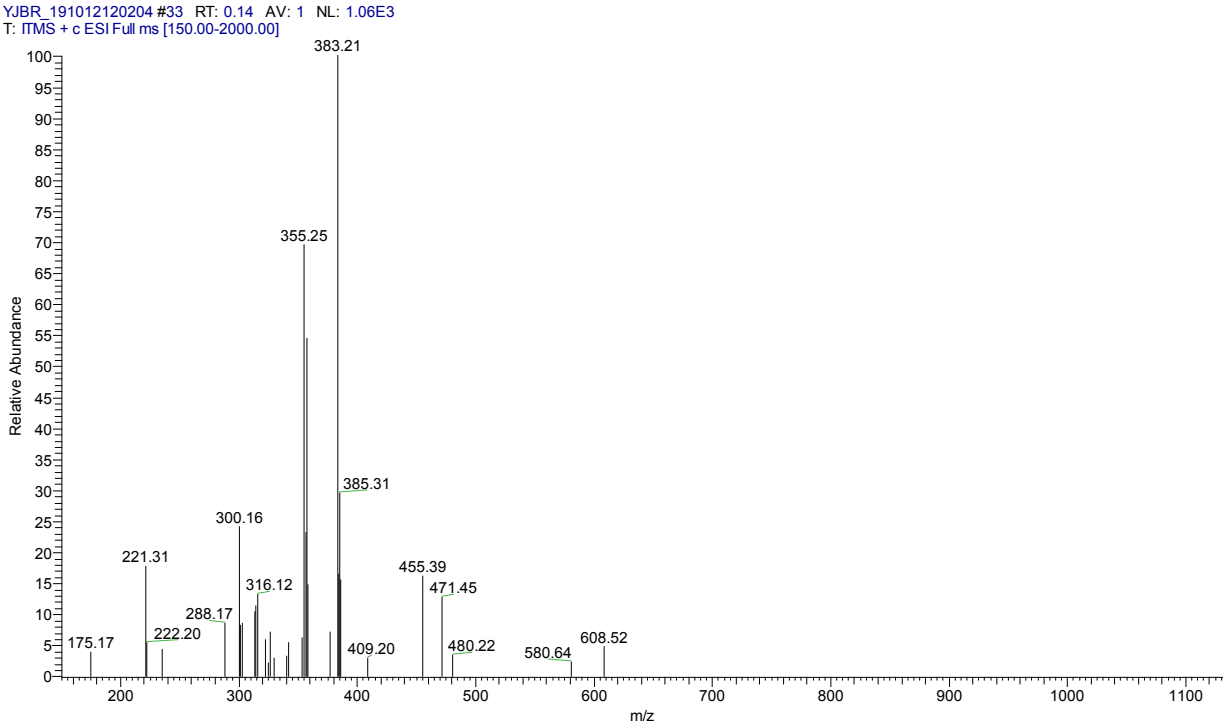
^{1}H NMR, ^{13}C NMR, 1H-1H COSY and MS spectrums of

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-bromo-9H-fluoren-9-one (3)



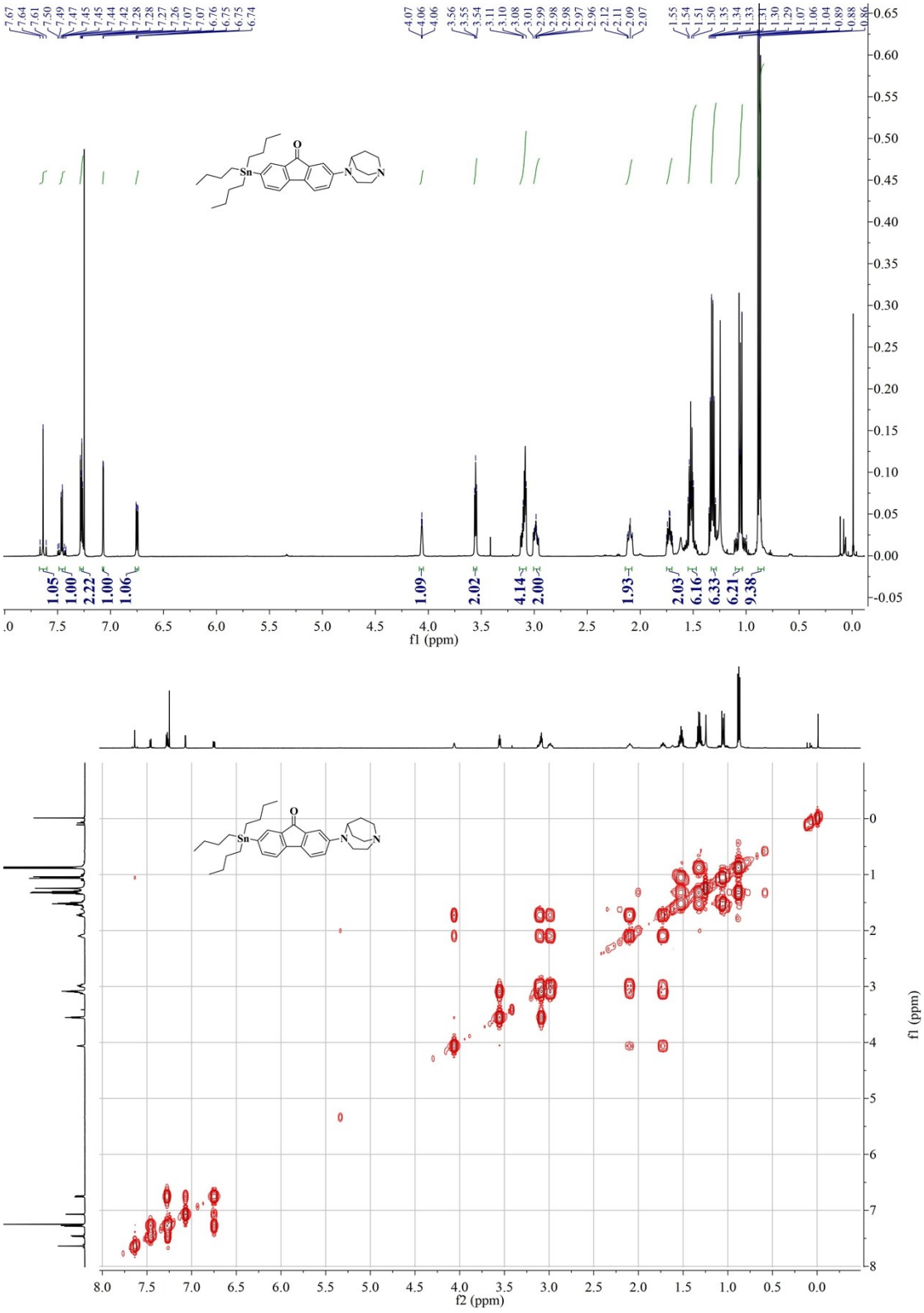


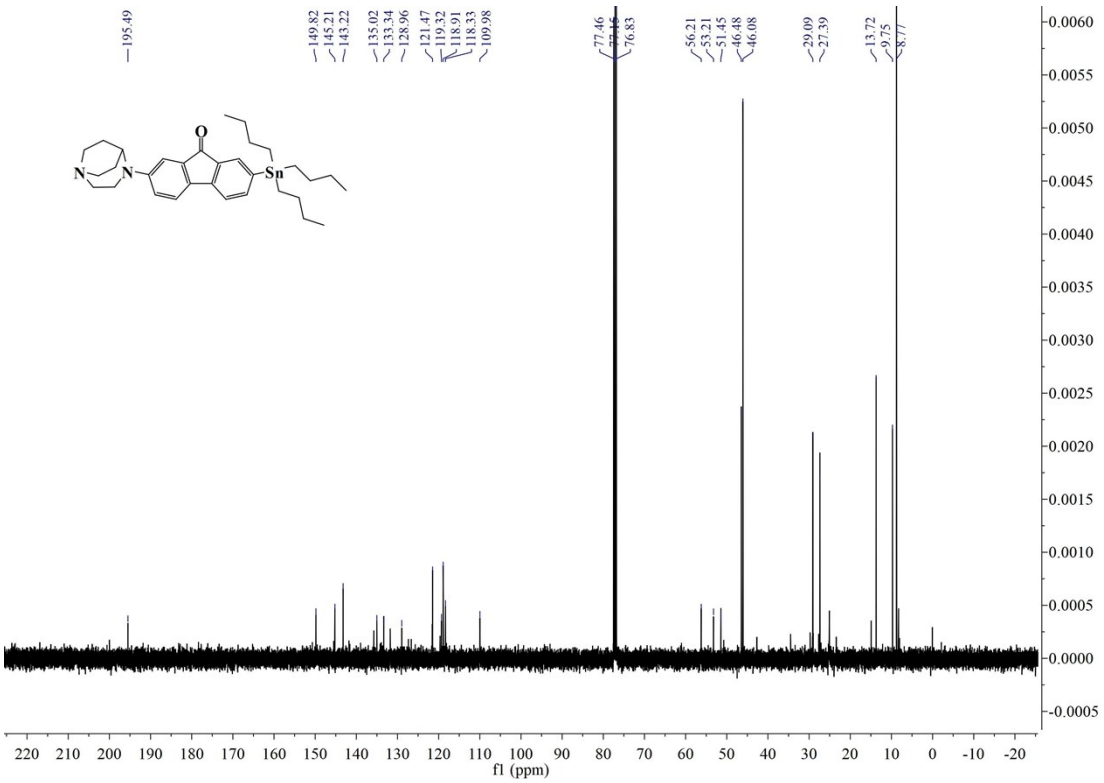

O
Br
N
N
f2 (ppm)
f1 (ppm)


YJBR_191012120204 #33 RT: 0.14 AV: 1 NL: 1.06E3
T: ITMS + c ESI Full ms [150.00-2000.00]
Relative Abundance
m/z
383.21
355.25
385.31
300.16
221.31
455.39
471.45
316.12
288.17
222.20
175.17
409.20
480.22
580.64
608.52

^{1}H NMR, 1H-1H COSY, ^{13}C NMR and MS spectrums of

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-(tributylstannyl)-9*H*-fluoren-9-one (4)




195.49
149.82
145.21
143.22
135.02
133.34
128.96
121.47
119.32
118.91
118.33
109.98
77.46
76.83
56.21
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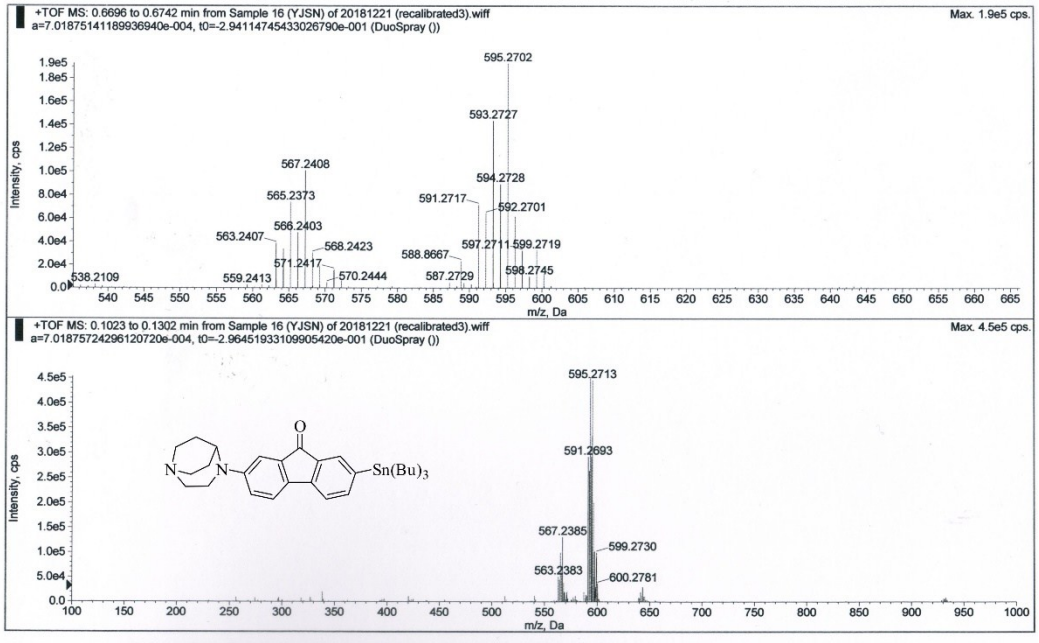
9.75
8.77
O
N
N
Sn
fl (ppm)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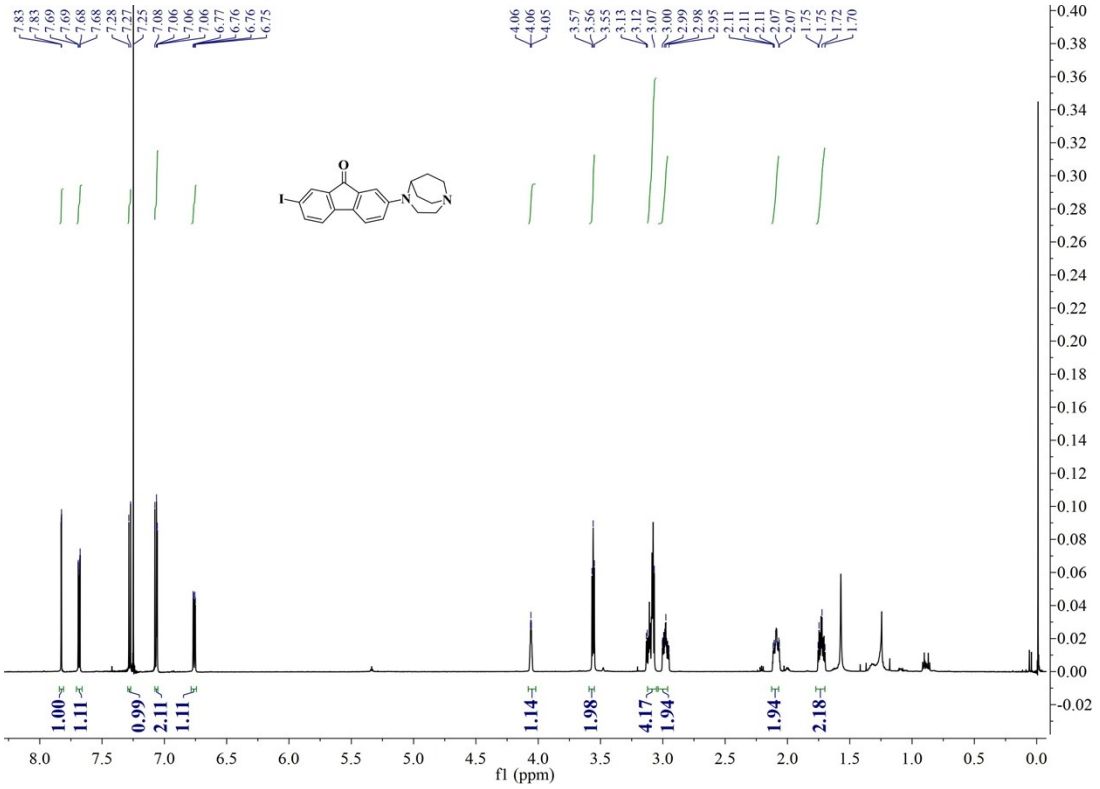
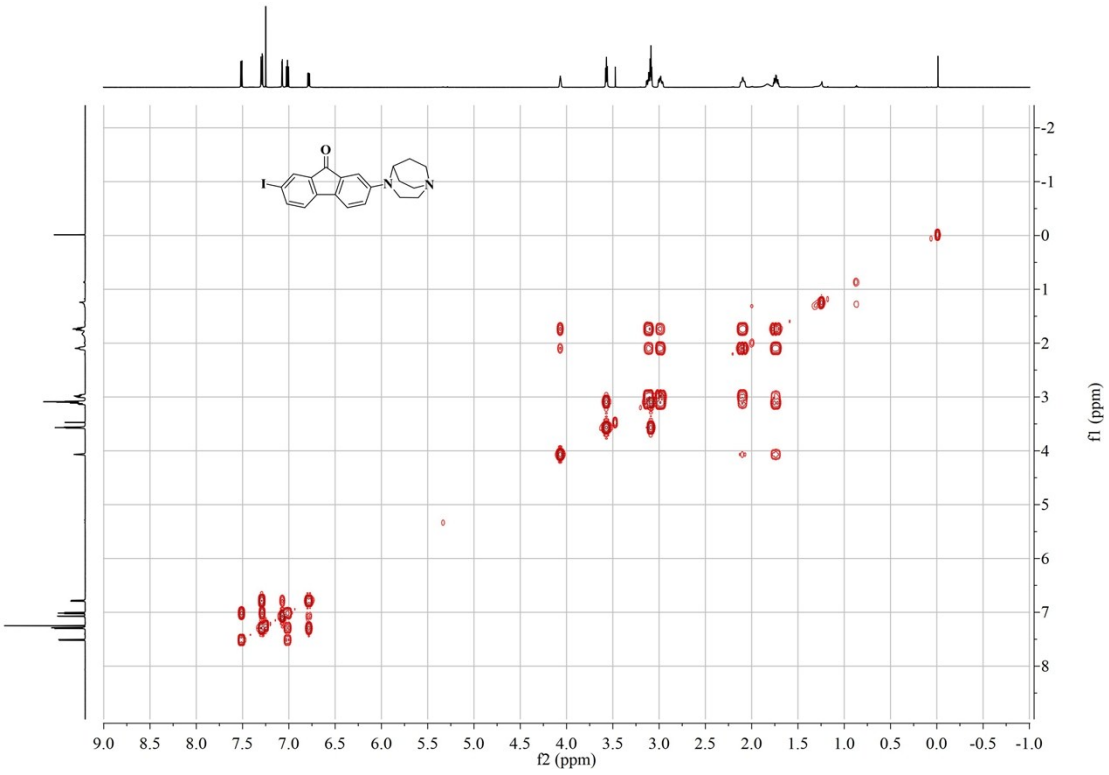
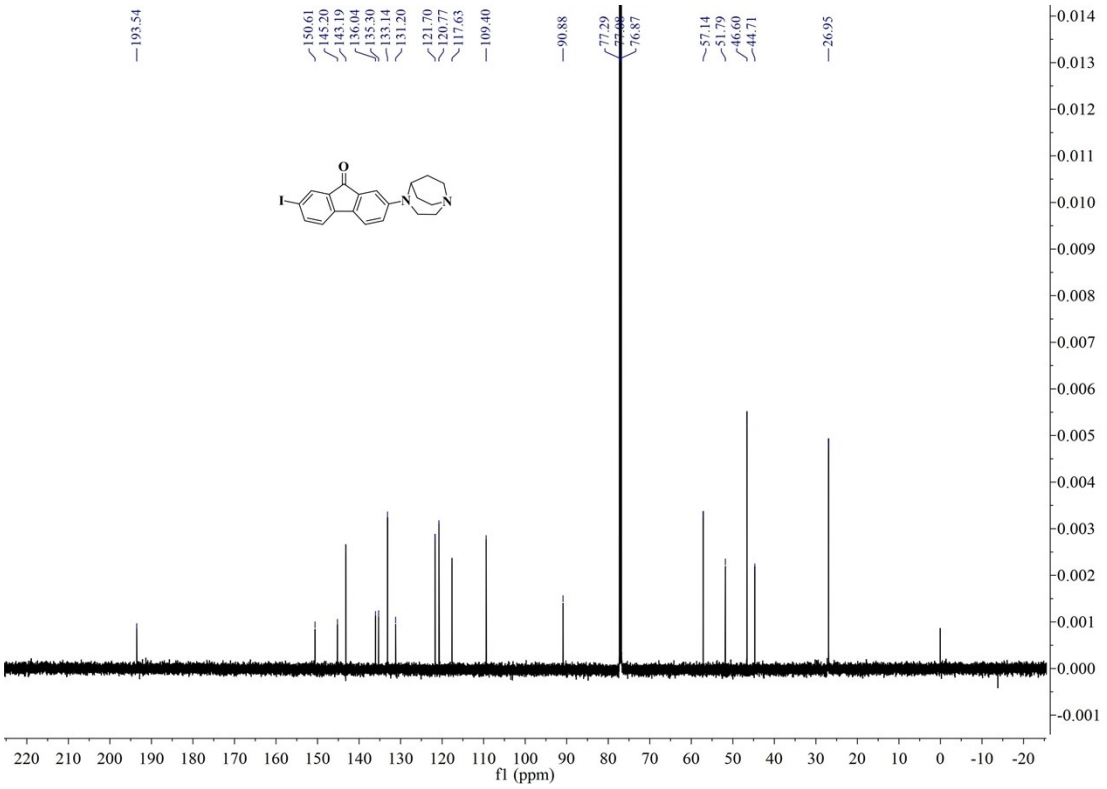
Max. 1.9e5 cps.
Intensity, cps
m/z, Da
595.2702
593.2727
567.2408
594.2728
565.2373
591.2717
592.2701
566.2403
563.2407
568.2423
597.2711
599.2719
588.8667
571.2417
559.2413
570.2444
587.2729
598.2745
538.2109
+TOF MS: 0.1023 to 0.1302 min from Sample 16 (YJSN) of 20181221 (recalibrated3).wiff
a=7.01875724296120720e-004, t0=-2.96451933109905420e-001 (DuoSpray ())
Max. 4.5e5 cps.
Intensity, cps
m/z, Da
595.2713
591.2693
567.2385
599.2730
563.2383
600.2781
O
N
N
Sn(Bu)3

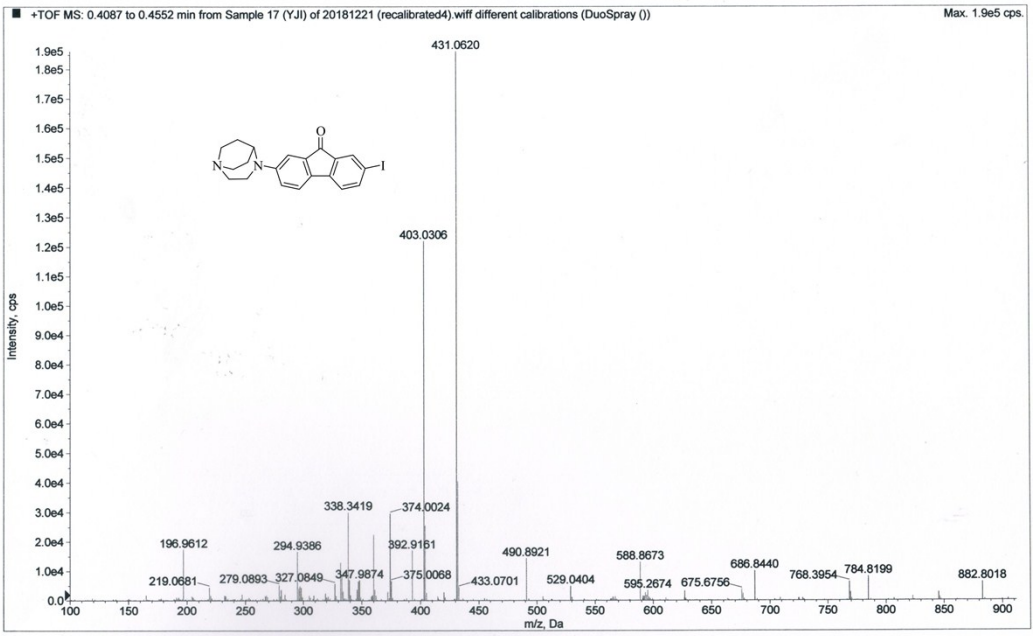
^{1}H NMR, 1H-1H COSY, ^{13}C NMR and MS spectrums of

2-(1,4-diazabicyclo[3.2.2]nonan-4-yl)-7-iodo-9*H*-fluoren-9-one (5)






193.54
150.61
145.20
143.19
136.04
135.30
133.14
131.20
121.70
120.77
117.63
109.40
90.88
77.29
76.87
57.14
51.79
46.60
44.71
26.95
O
I
N
N
f1 (ppm)


+TOF MS: 0.4087 to 0.4552 min from Sample 17 (YJI) of 20181221 (recalibrated4).wiff different calibrations (DuoSpray ())
Max. 1.9e5 cps.
Intensity, cps
m/z, Da
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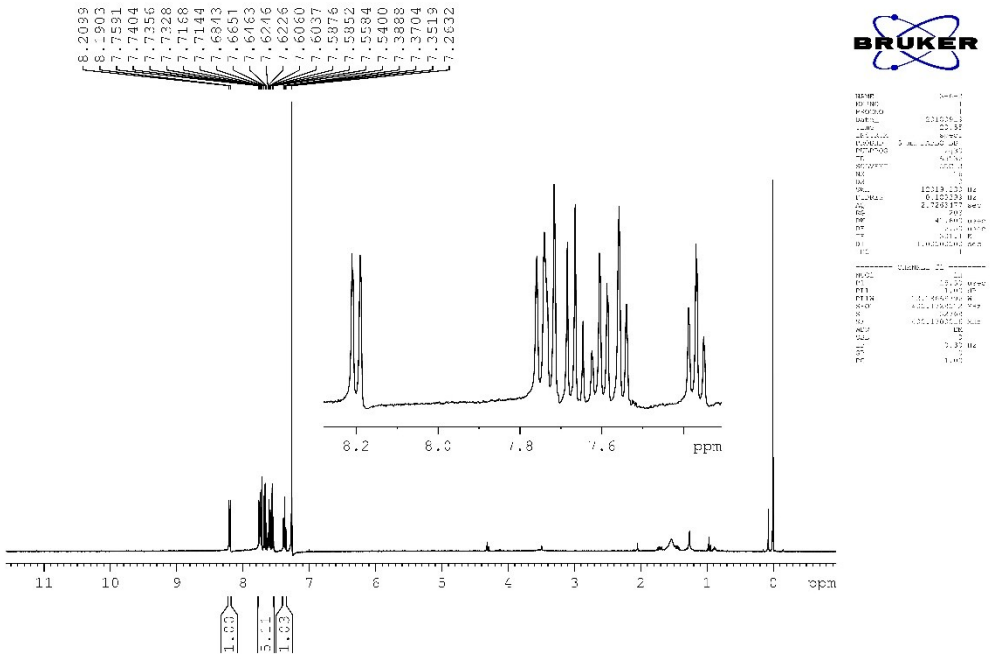
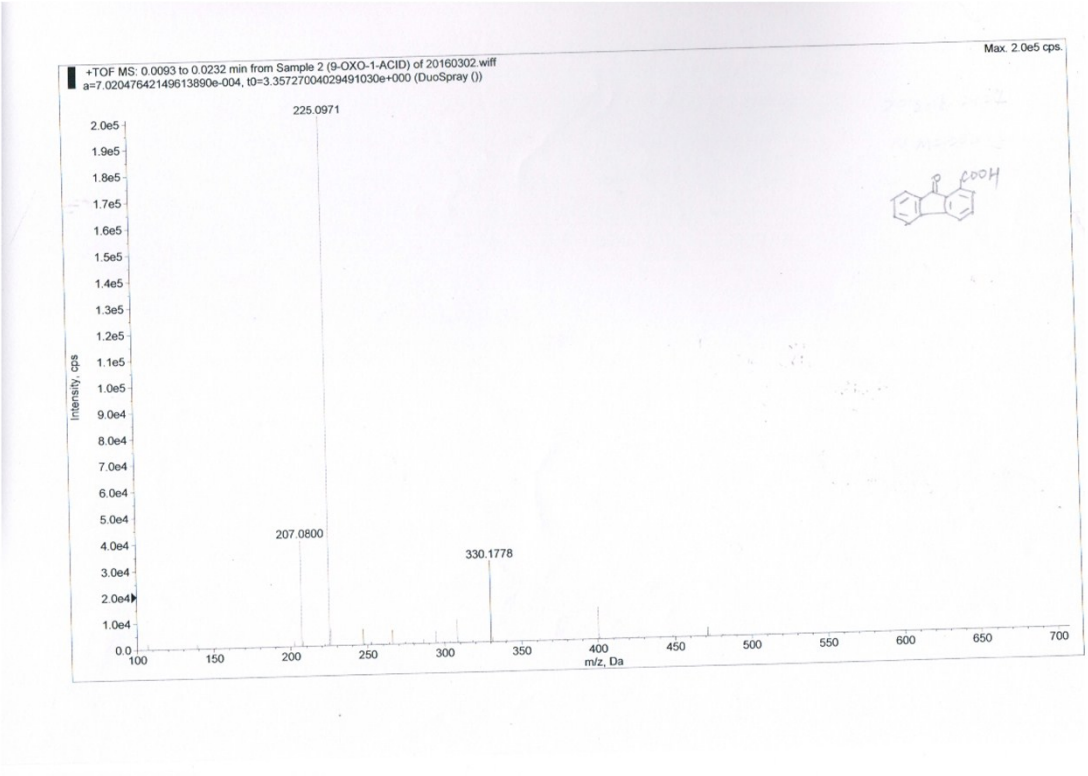
403.0306
374.0024
338.3419
392.9161
294.9386
196.9612
490.8921
588.8673
686.8440
784.8199
882.8018
O
I
N
N

^{1}H NMR and MS spectrums of

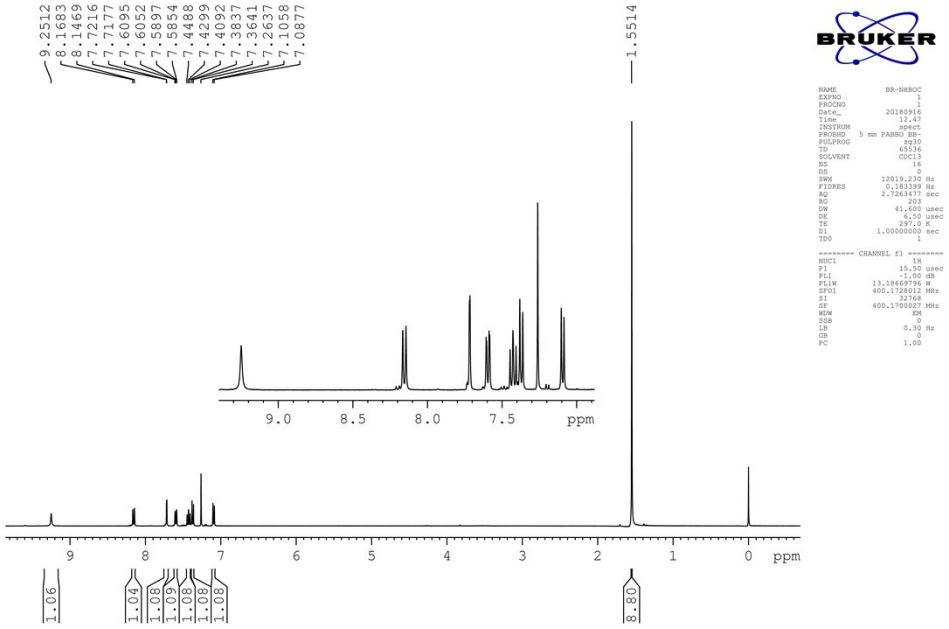
9-oxo-9*H*-fluorene-1-carboxylic acid (7)





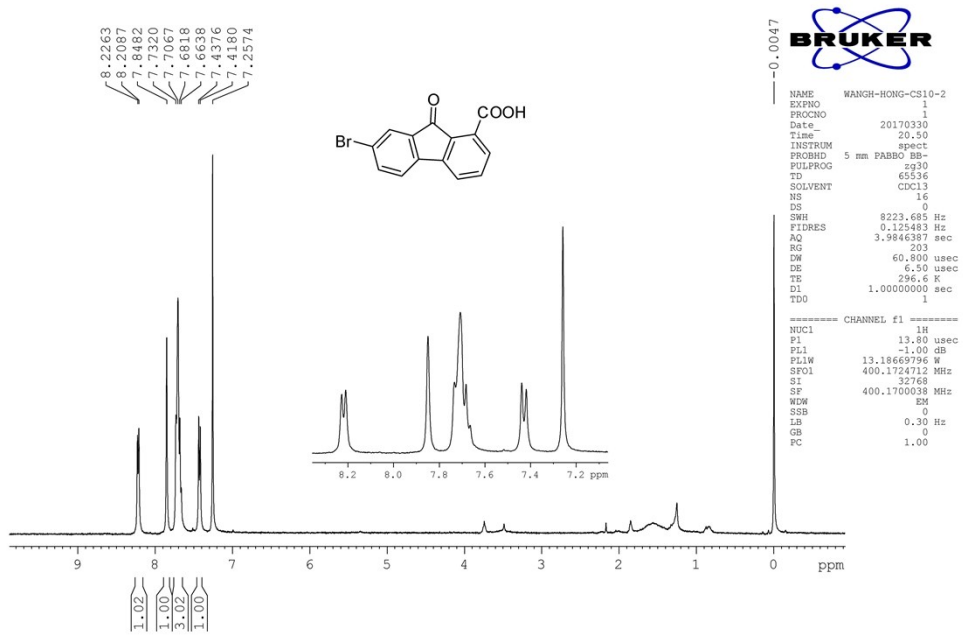
^{1}H NMR spectrum of

7-bromo-9-oxo-9*H*-fluorene-1-carboxylic acid (8)



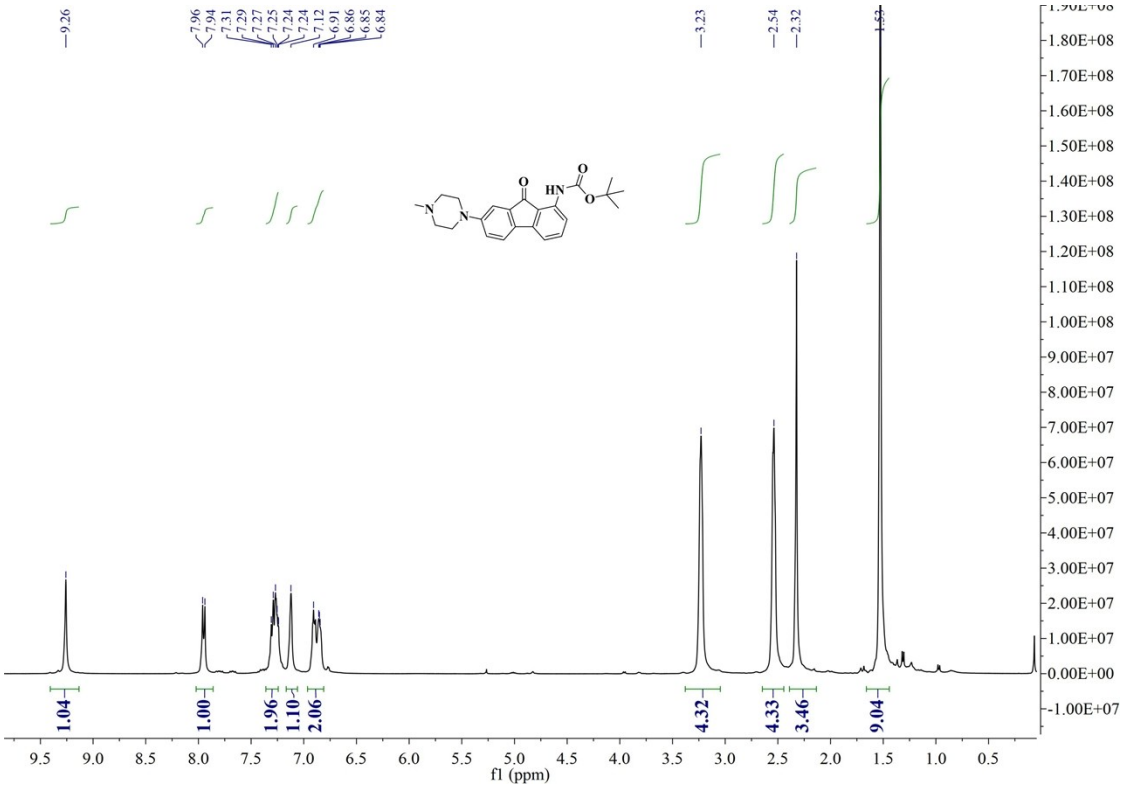
^{1}H NMR spectrum of

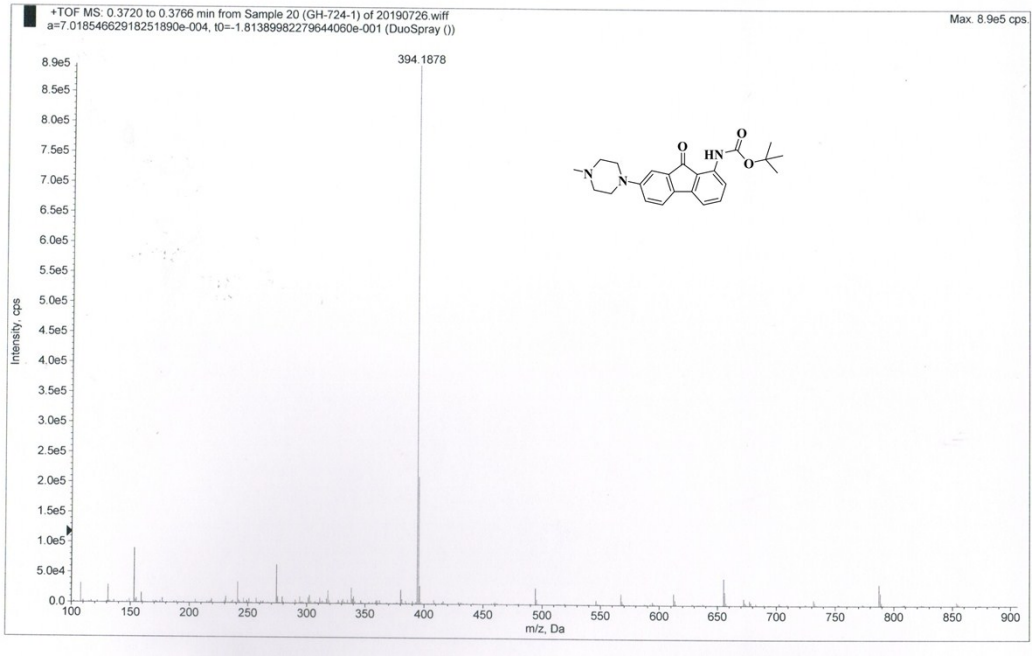
tert-butyl (7-bromo-9-oxo-9*H*-fluoren-1-yl)carbamate (9)



^{1}H NMR and MS spectrums of

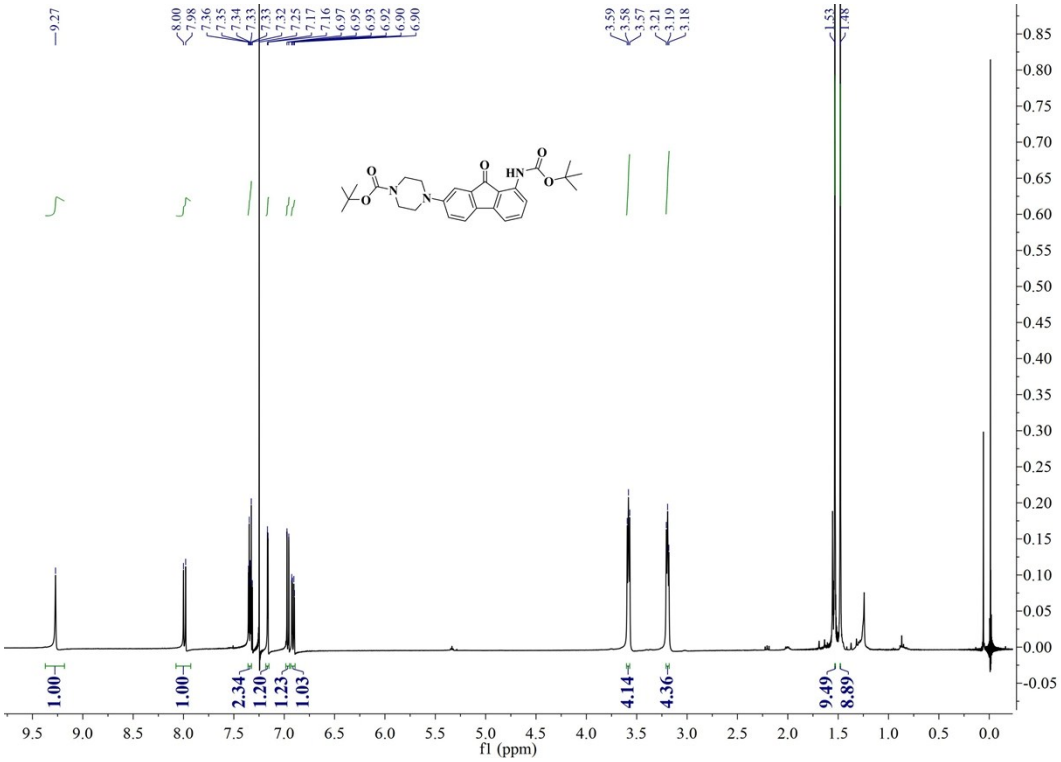
***tert*-butyl (7-(4-methylpiperazin-1-yl)-9-oxo-9*H*-fluoren-1-yl)carbamate (10)**

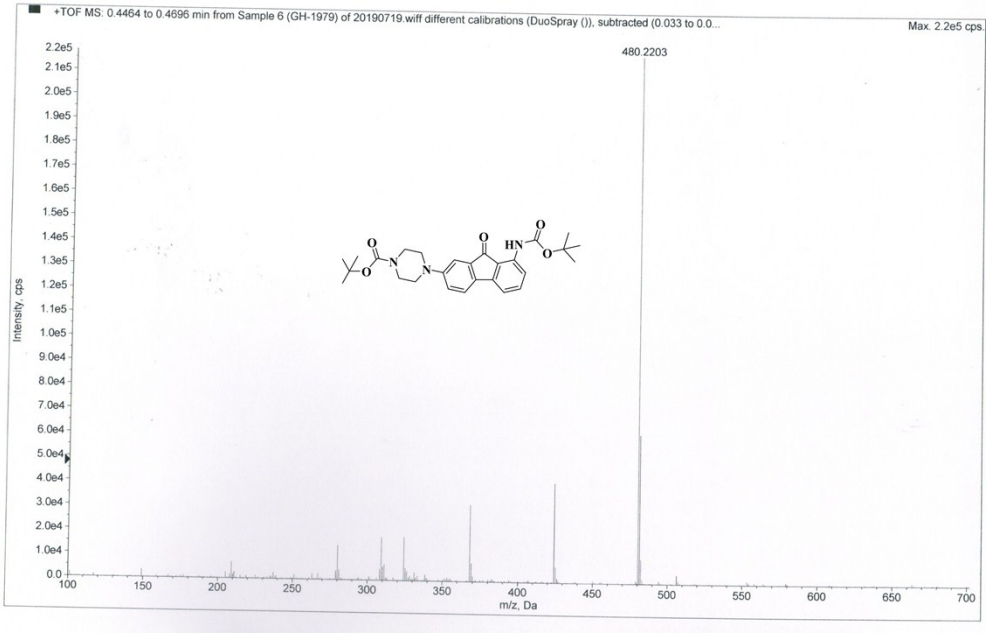




^{1}H NMR and MS spectrums of

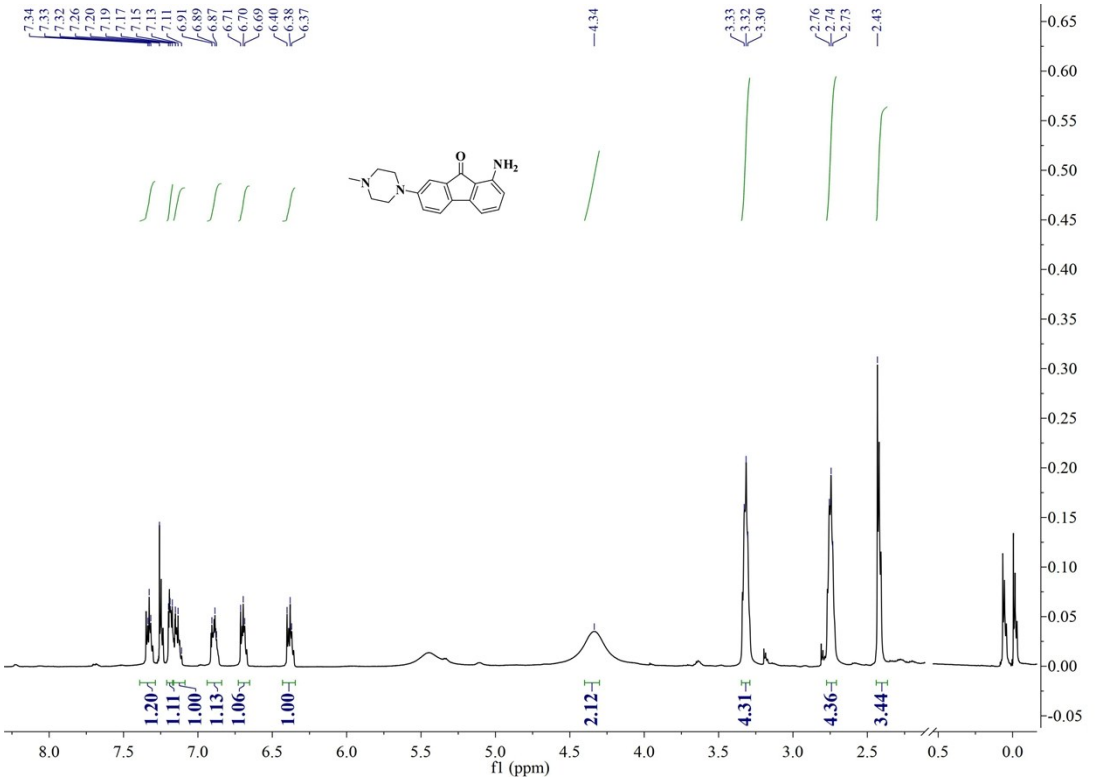
***tert*-butyl 4-(8-((*tert*-butoxycarbonyl)amino)-9-oxo-9*H*-fluoren-2-yl)piperazine-1-carboxylate (11)**

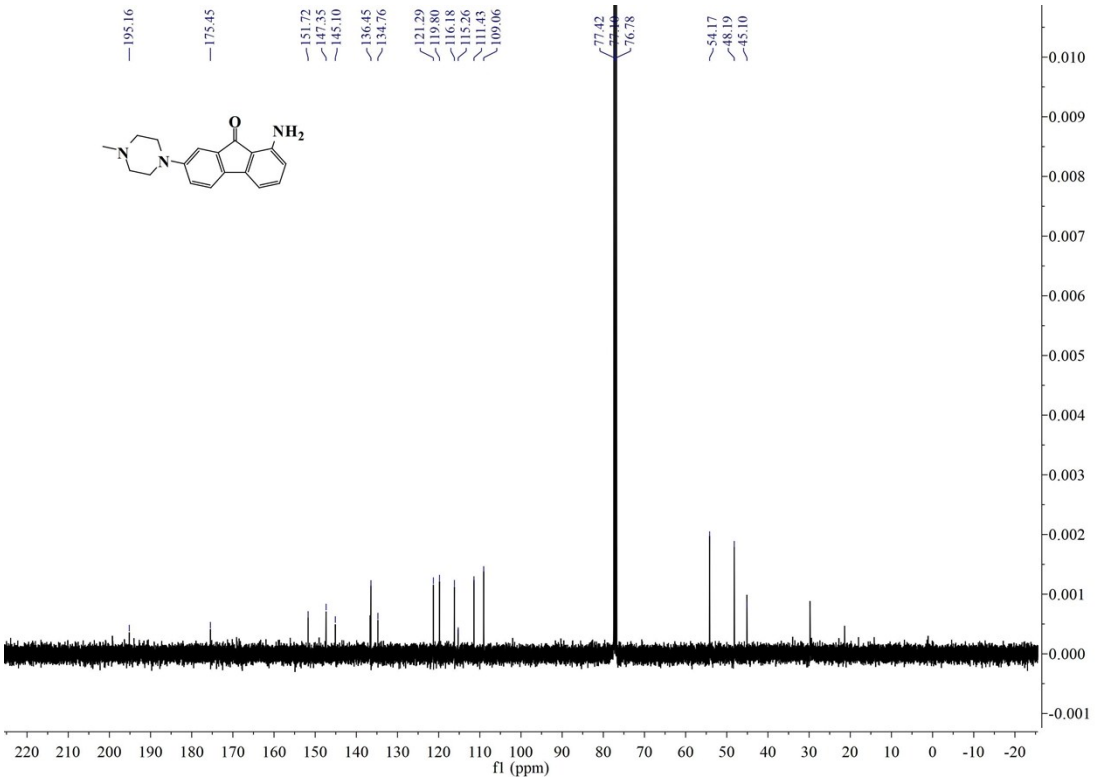


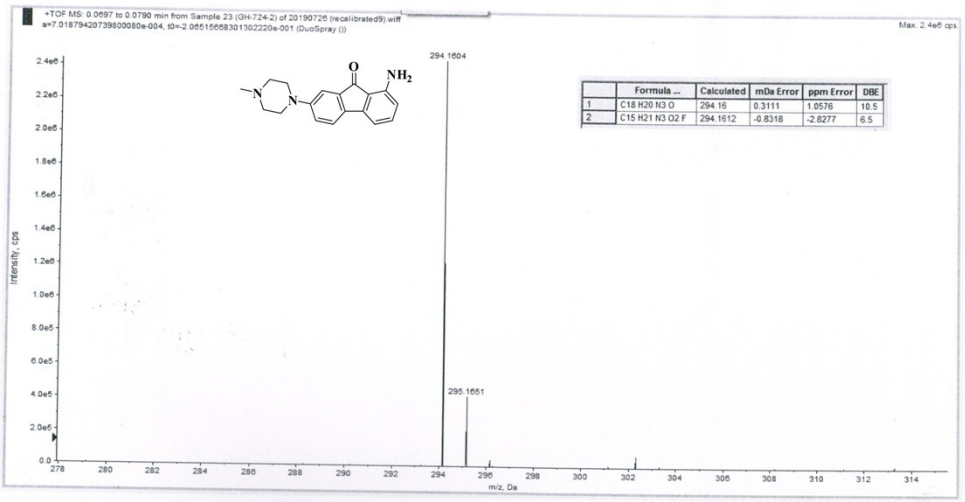


^{1}H NMR, ^{13}C NMR and MS spectrums of

1-amino-7-(4-methylpiperazin-1-yl)-9*H*-fluoren-9-one (12)

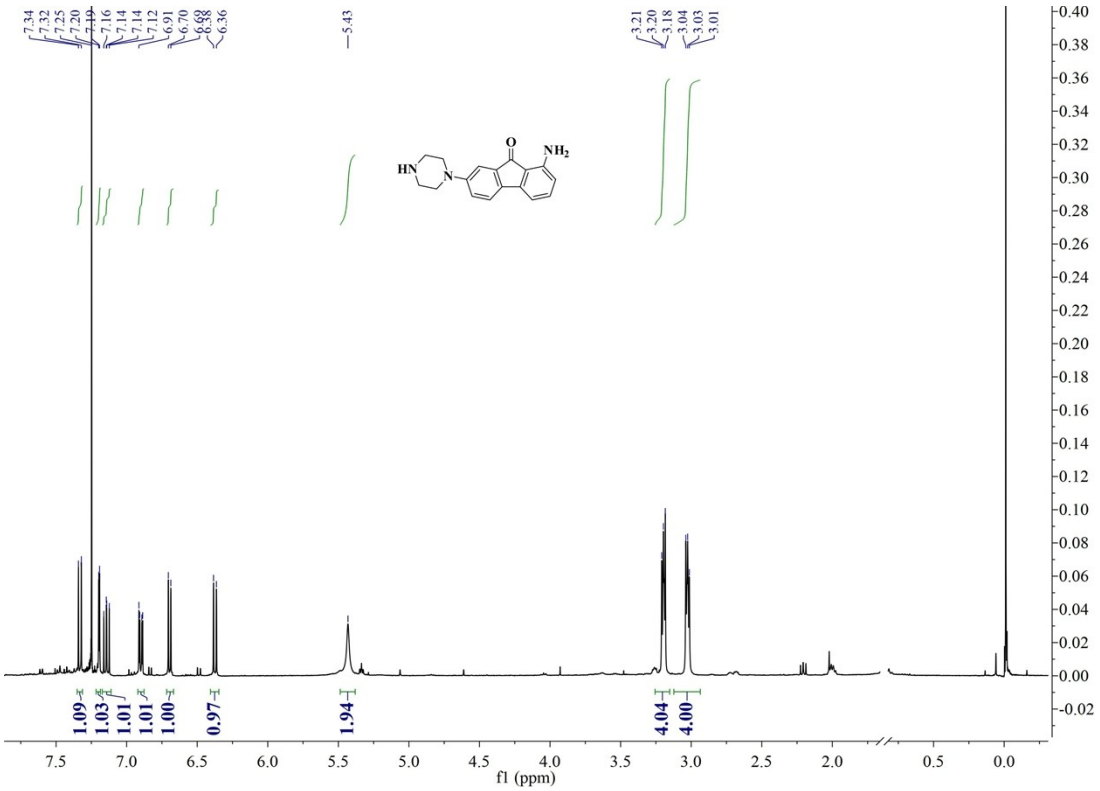


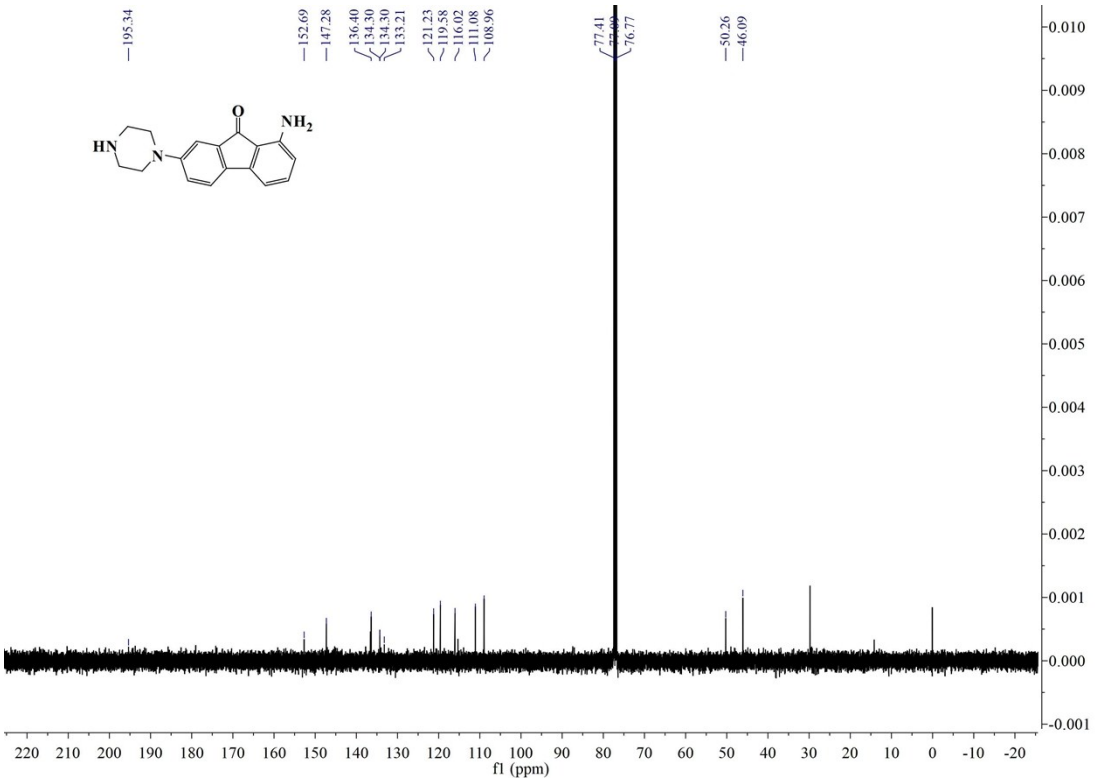




^{1}H NMR, ^{13}C NMR and MS spectrums of

1-amino-7-(piperazin-1-yl)-9*H*-fluoren-9-one (13)




195.34
152.69
147.28
136.40
134.30
134.30
133.21
121.23
119.58
116.02
111.08
108.96
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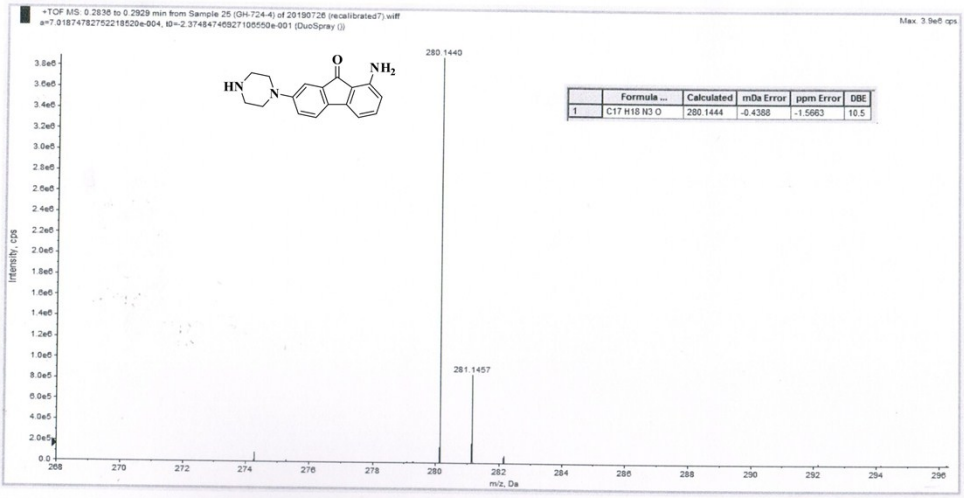
50.26
46.09
O
NH2
HN
N
f1 (ppm)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Intensity, cps
m/z, Da
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281.1457
O
NH2
HN
N
Formula ...
Calculated
mDa Error
ppm Error
DBE
1
C17 H18 N3 O
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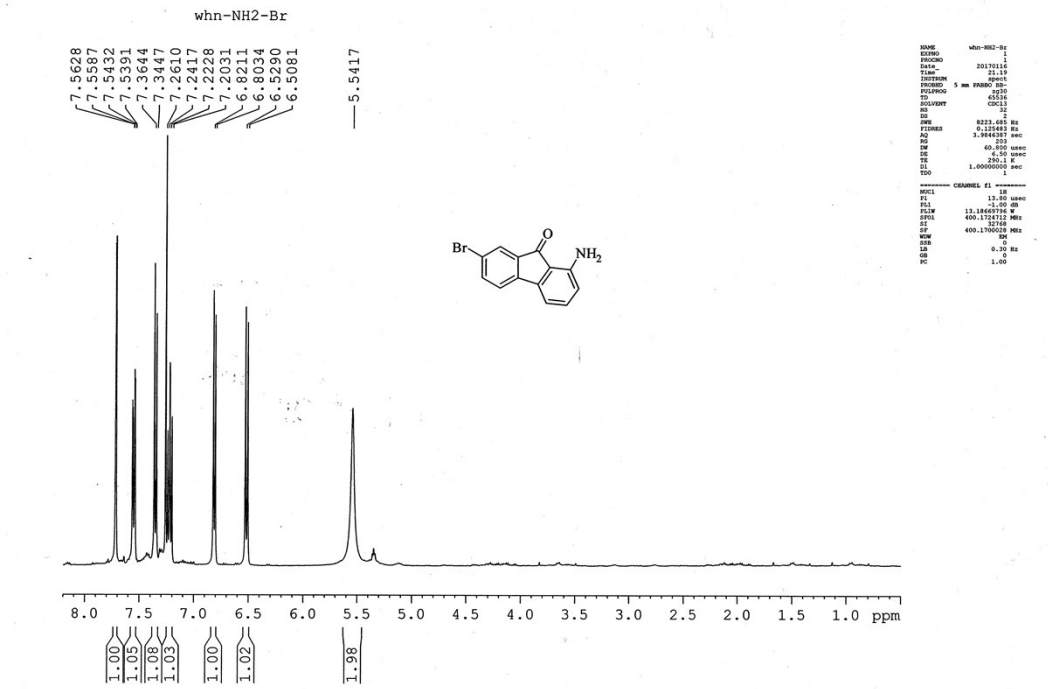
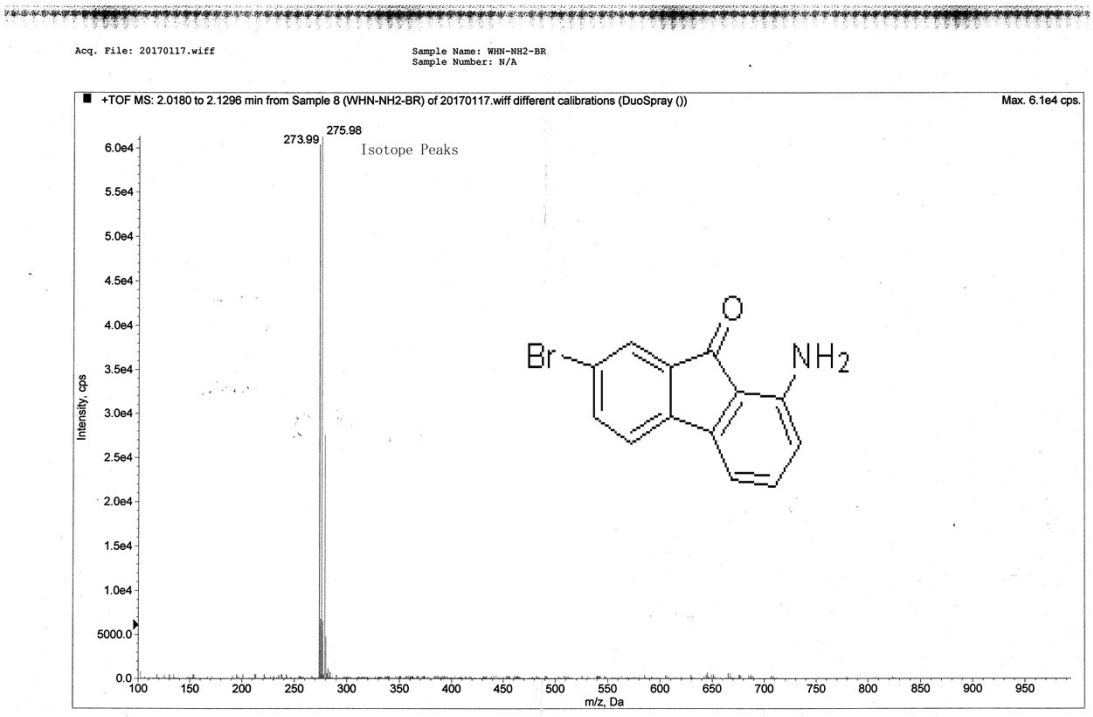
-1.5663
10.5

^{1}H NMR and MS spectrums of

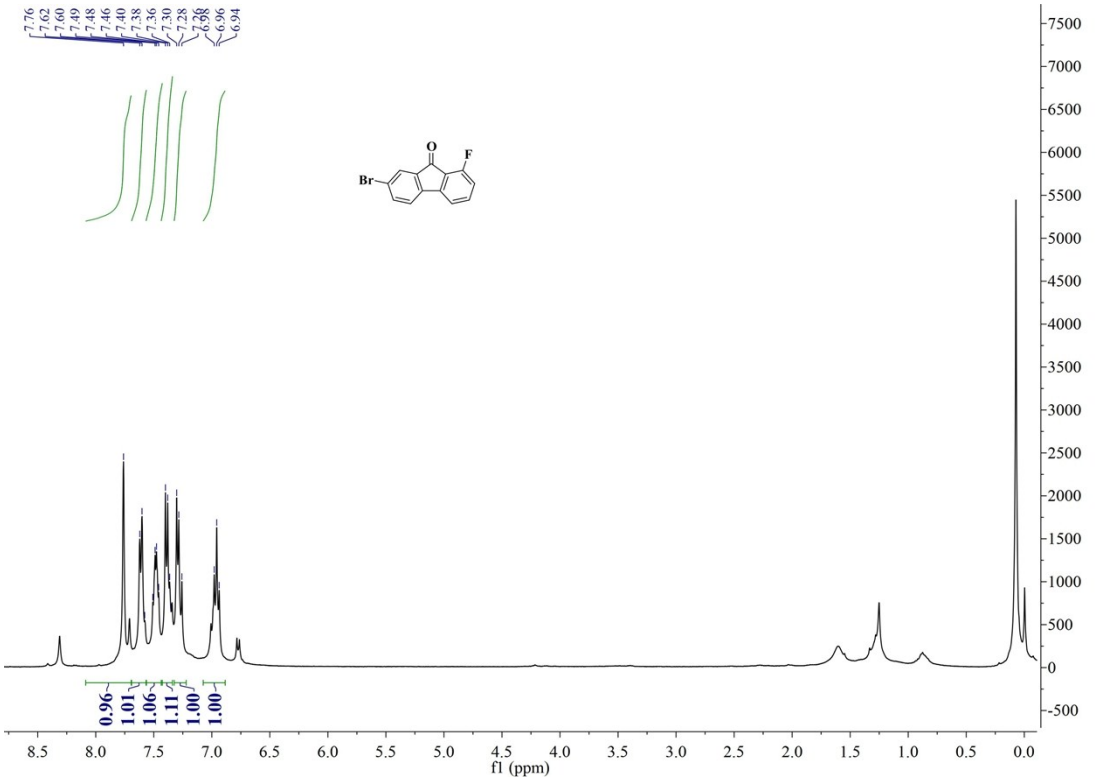
1-amino-7-bromo-9*H*-fluoren-9-one (14)

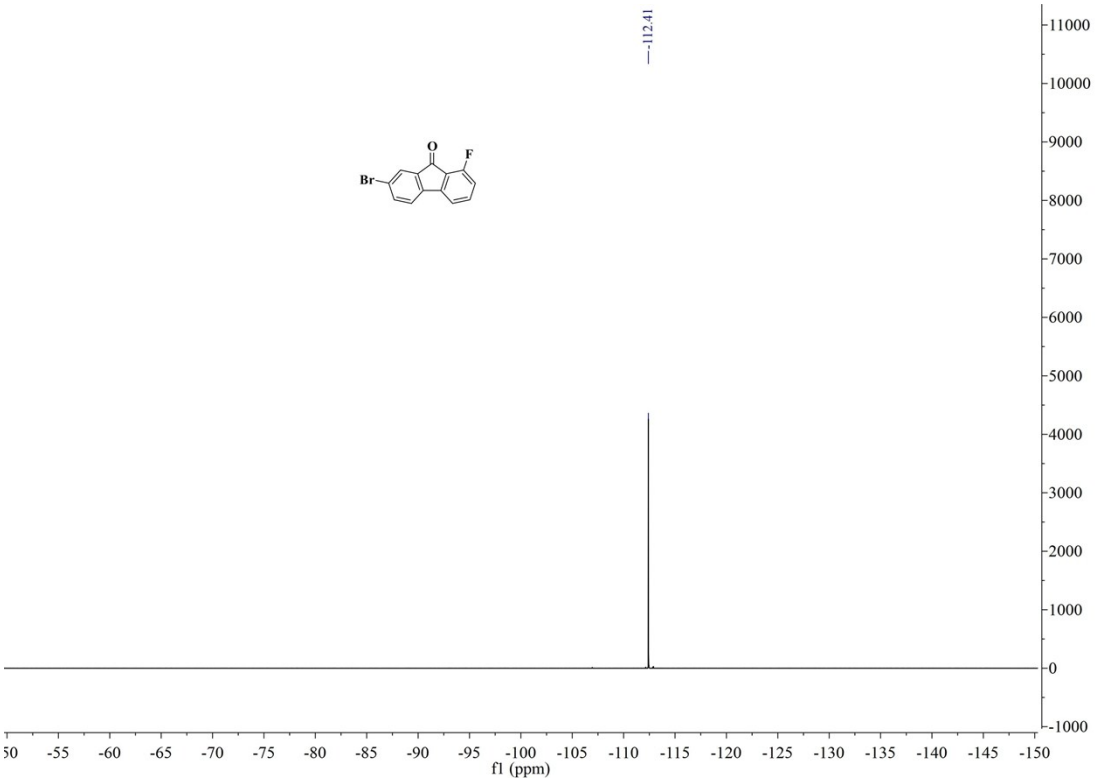




^{1}H NMR and ^{19}F NMR spectrums of

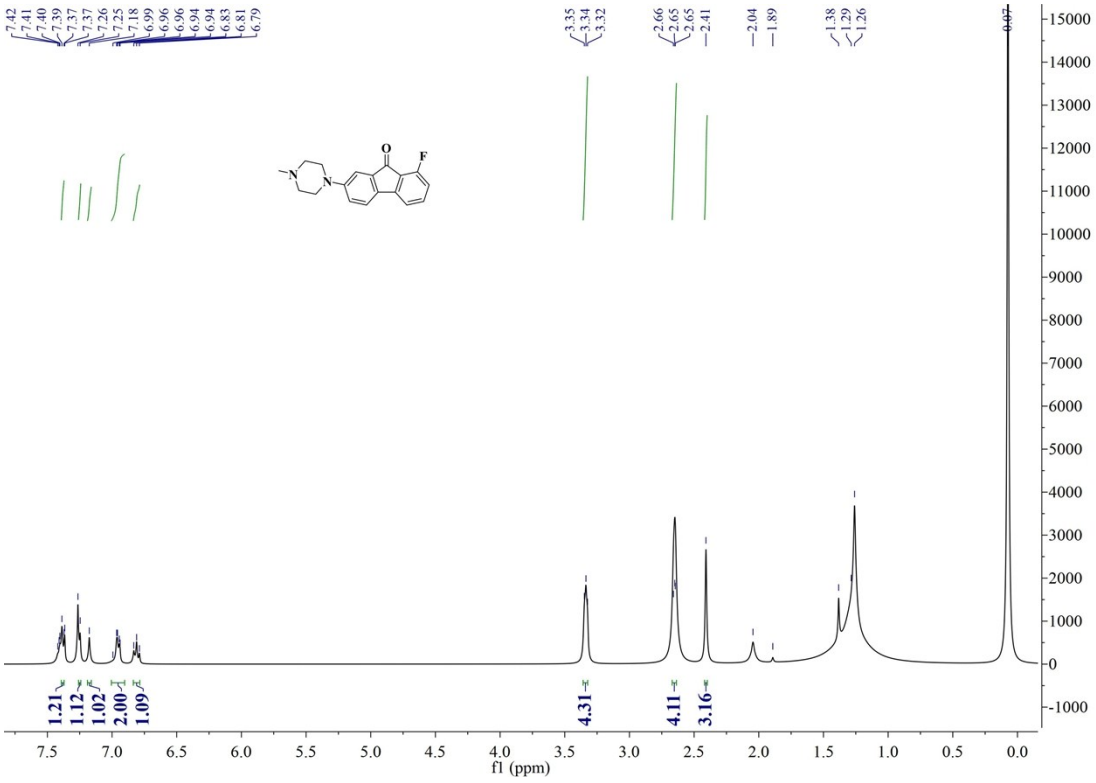
7-bromo-1-fluoro-9*H*-fluoren-9-one (15)

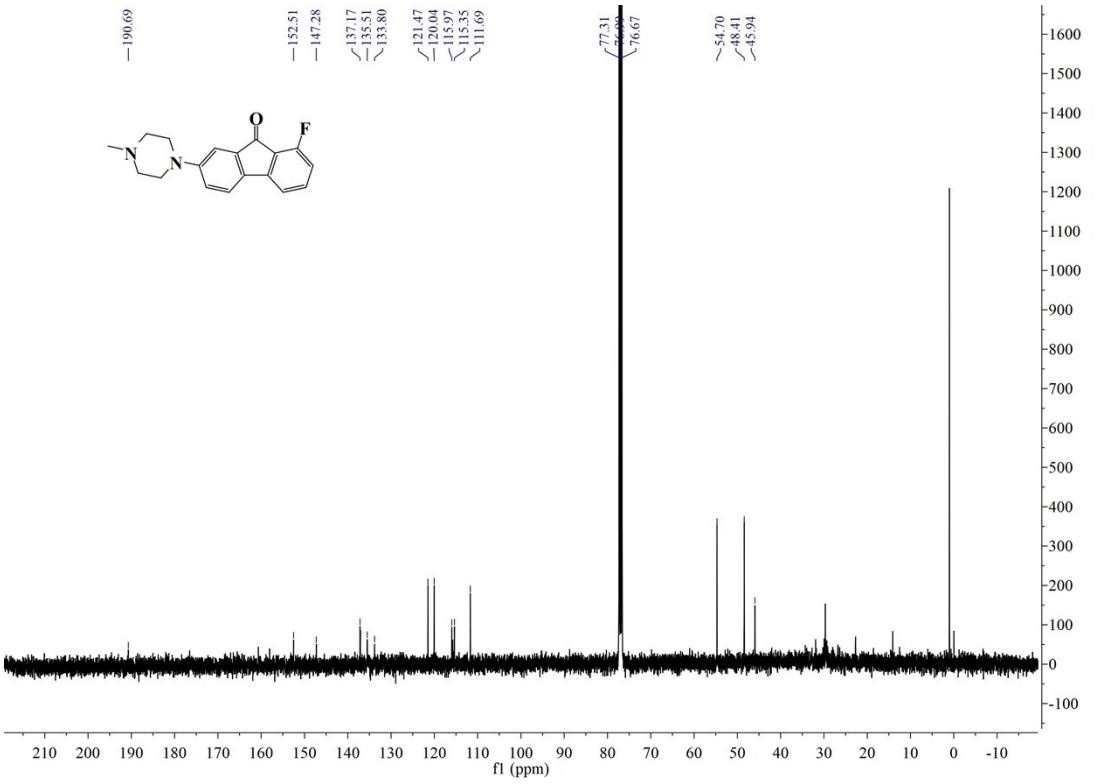


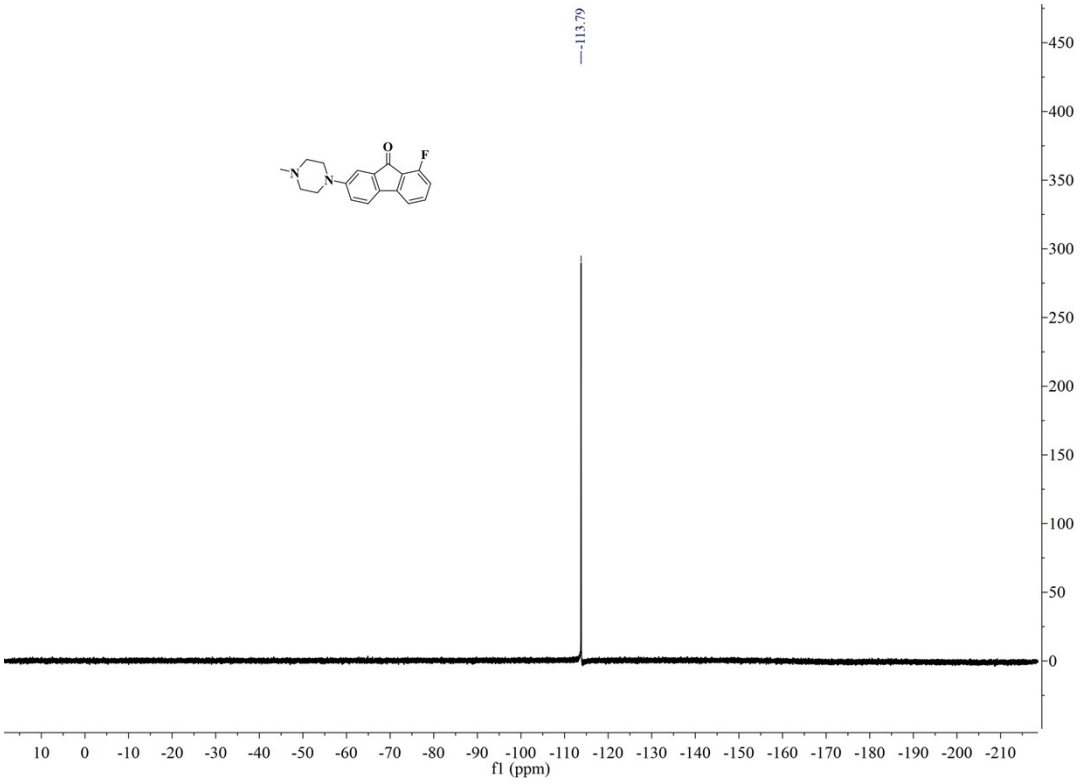


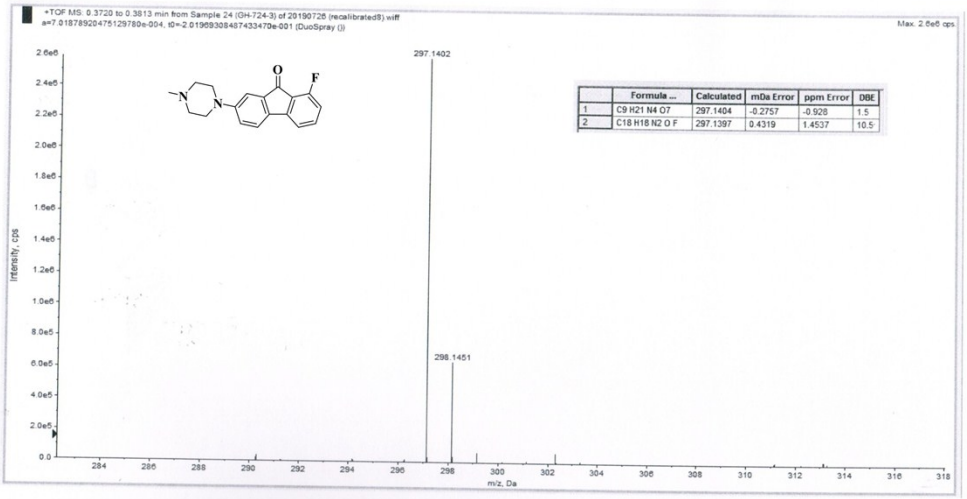
^{1}H NMR, ^{13}C NMR, ^{19}F NMR and MS spectrums of

1-fluoro-7-(4-methylpiperazin-1-yl)-9*H*-fluoren-9-one (16)



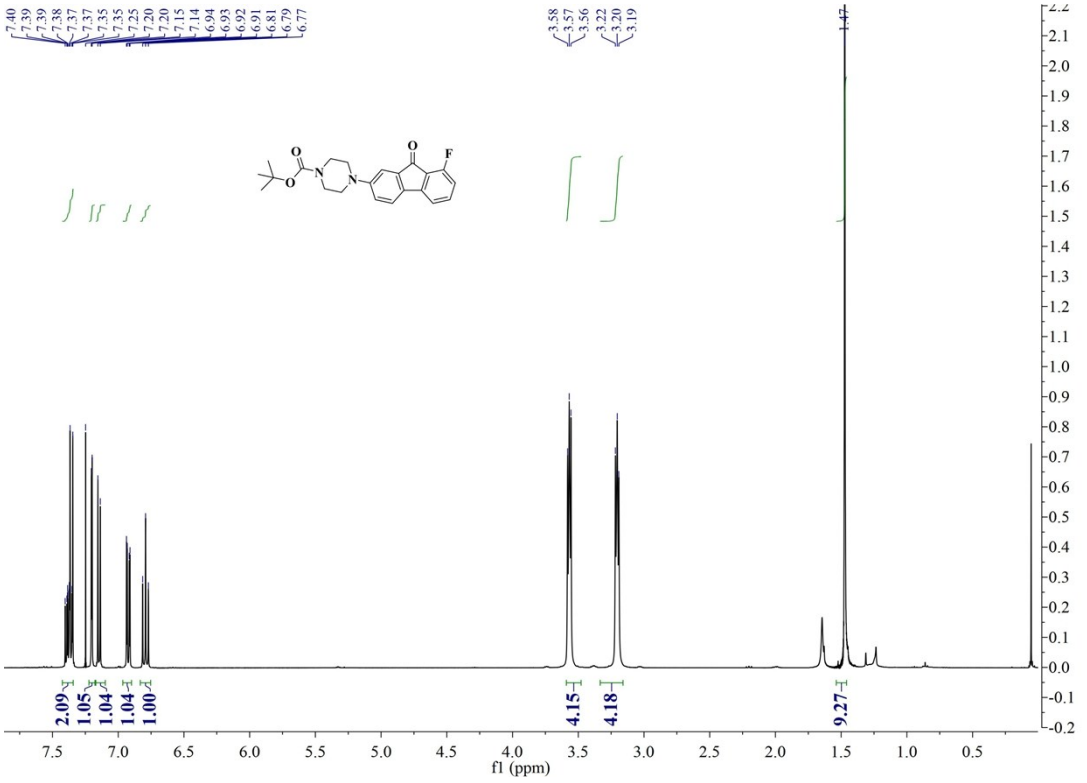


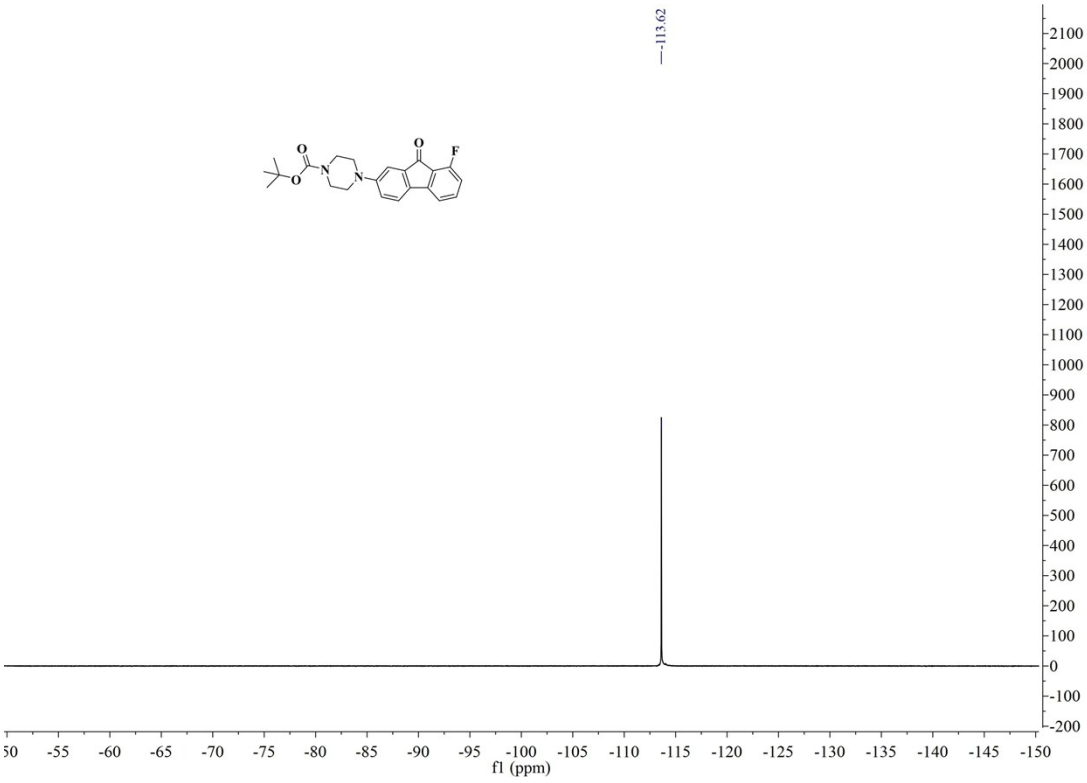

-113.79
O
F
N
N
450
400
350
300
250
200
150
100
50
0
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

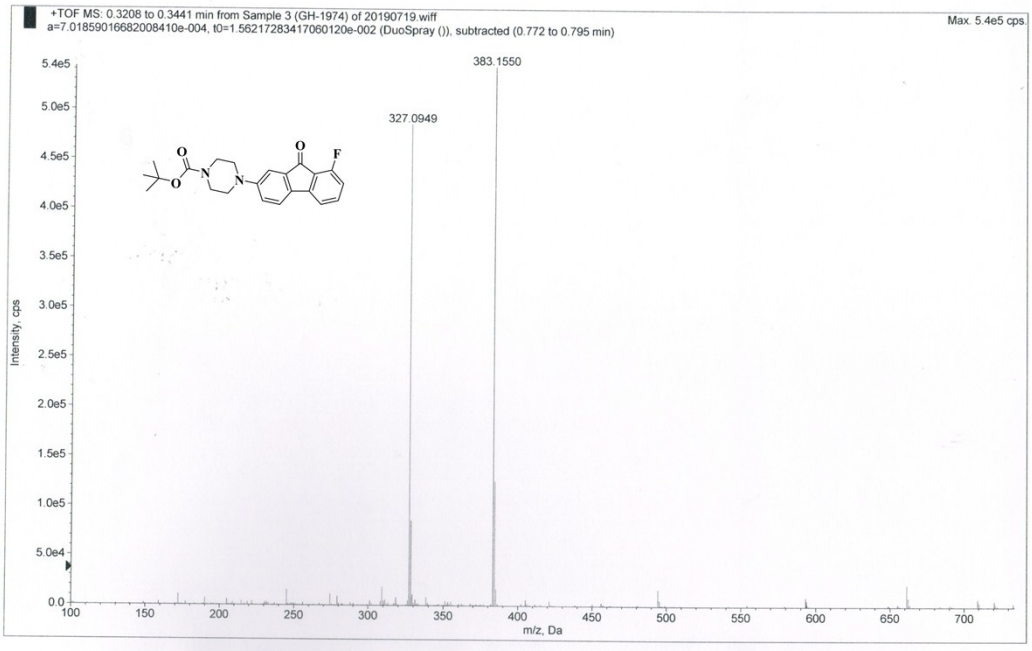

+TOF MS: 0.3720 to 0.3813 min from Sample 24 (GH-724-3) of 20190726 (recalibrated8).wiff
a=7.01878920475129780e-004, t0=-2.01969308487433479e-001 (DuoSpray ()
Max. 2.6e6 cps.
297.1402
298.1451
Intensity, cps
m/z, Da
O
F
N
N
Formula ... | Calculated | mDa Error | ppm Error | DBE
1 | C9 H21 N4 O7 | 297.1404 | -0.2757 | -0.928 | 1.5
2 | C18 H18 N2 O F | 297.1397 | 0.4319 | 1.4537 | 10.5

^{1}H NMR, ^{19}F NMR and MS spectrums of

tert-butyl 4-(8-fluoro-9-oxo-9*H*-fluoren-2-yl)piperazine-1-carboxylate (17)

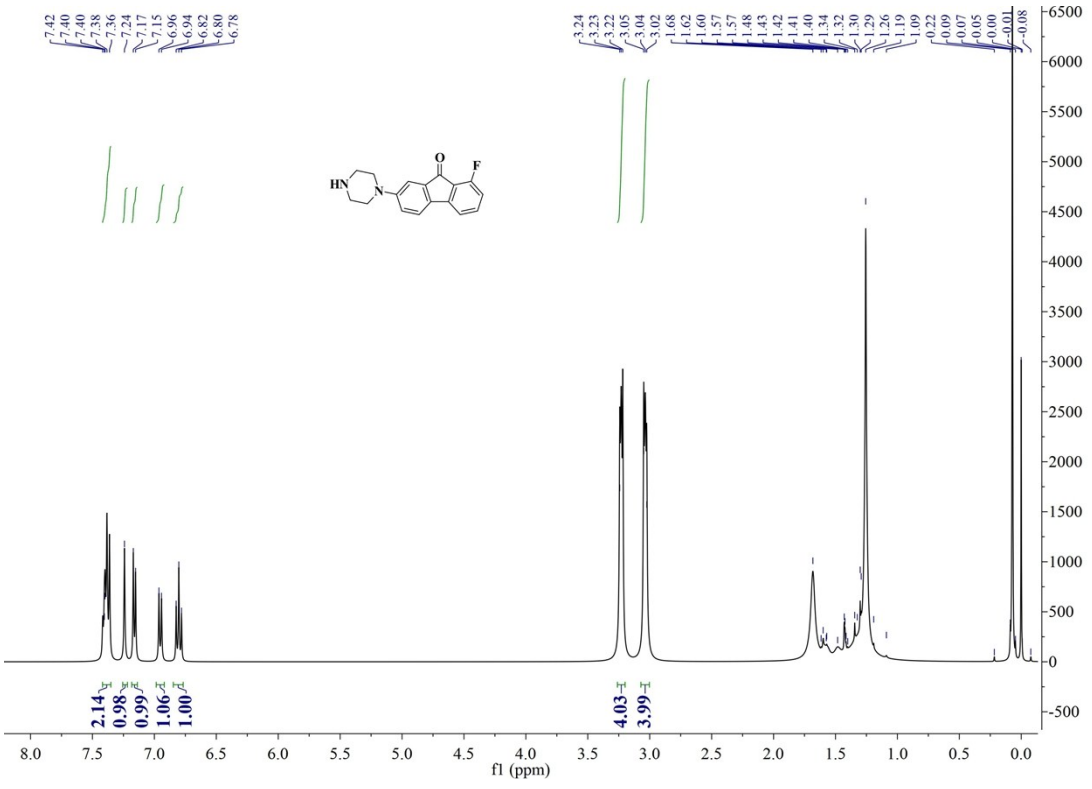


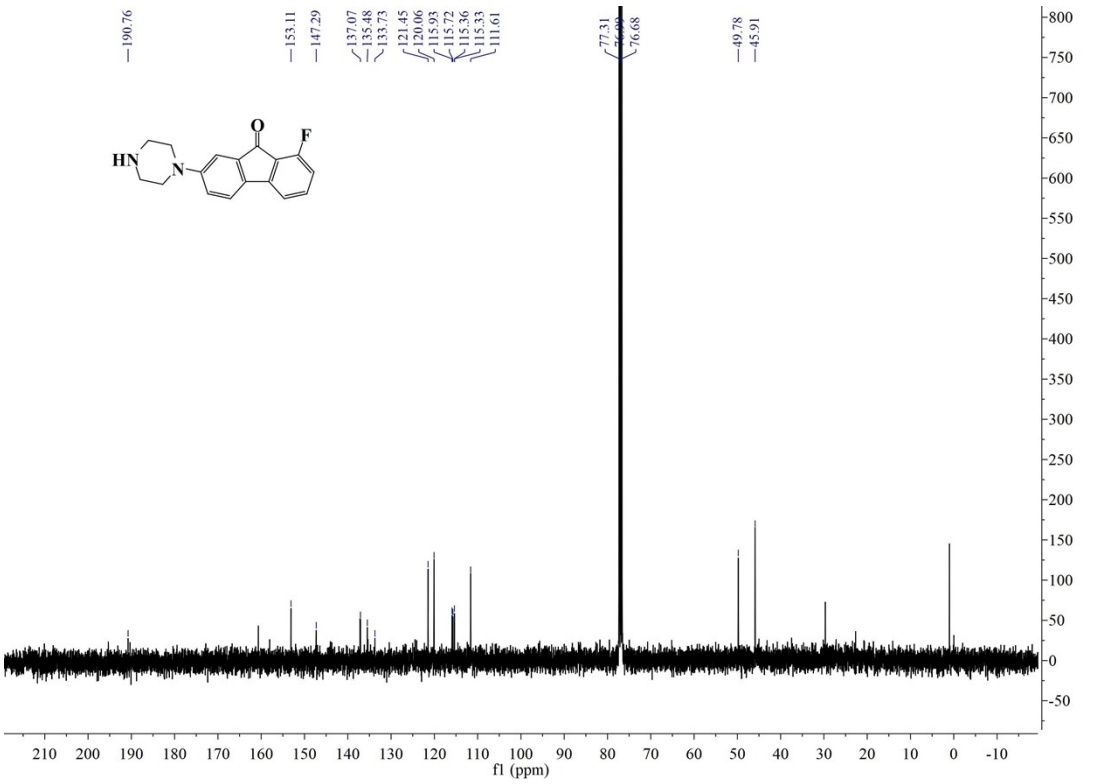


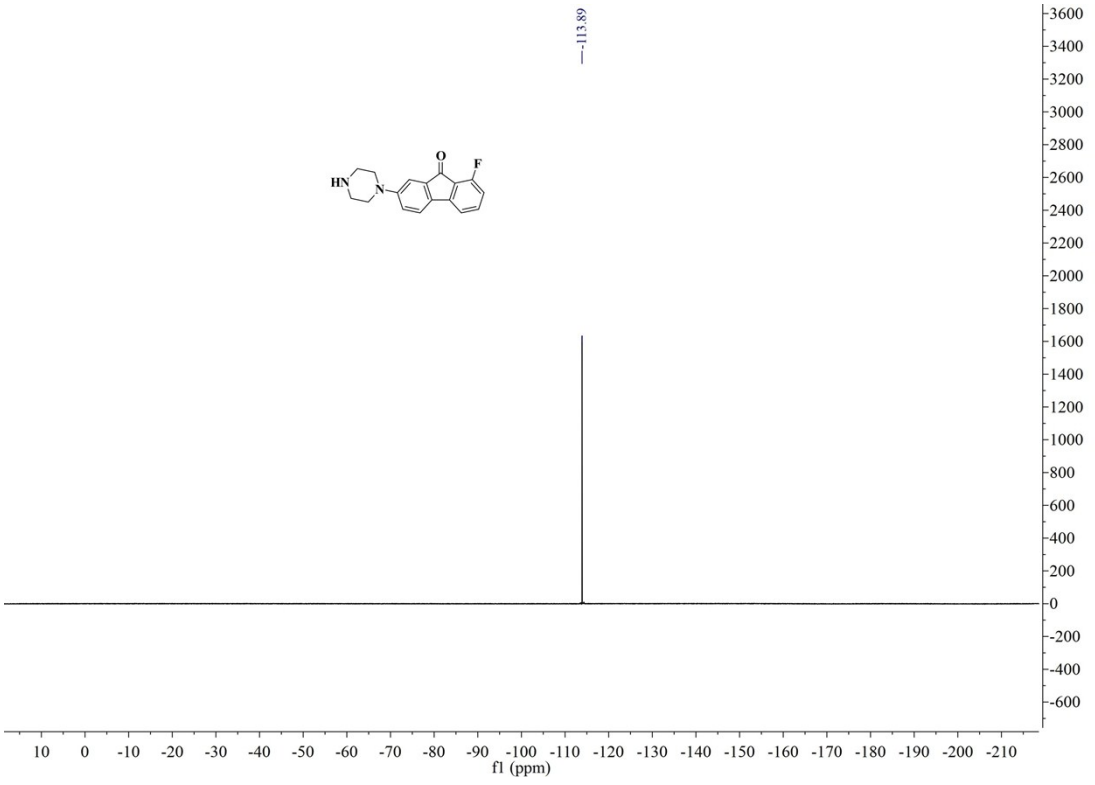


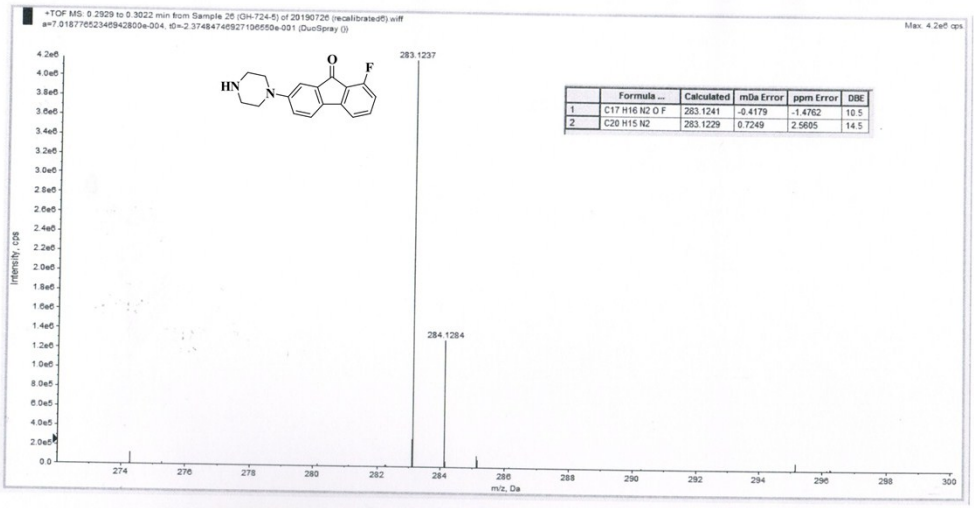
^{1}H NMR, ^{13}C NMR, ^{19}F NMR and MS spectrums of

1-fluoro-7-(piperazin-1-yl)-9*H*-fluoren-9-one (18)




190.76
153.11
147.29
137.07
135.48
133.73
121.45
120.06
115.93
115.72
115.36
115.33
111.61
77.31
76.68
49.78
45.91
O
F
HN
N
fl (ppm)


113.89
O
F
HN
N
fl (ppm)


+TOF MS: 0.2929 to 0.3022 min from Sample 26 (GH-724-5) of 20190726 (recalibrated6).wiff
a=7.018776523469428 00e-004, t0=-2.374847469271066 59e-001 (DuoSpray ())
Max. 4.2e6 cps
Intensity, cps
m/z, Da
283.1237
284.1284
HN
N
O
F
Formula ... | Calculated | mDa Error | ppm Error | DBE
1 | C17 H16 N2 O F | 283.1241 | -0.4179 | -1.4762 | 10.5
2 | C20 H15 N2 | 283.1229 | 0.7249 | 2.5605 | 14.5

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