In vivo Drug Tracking with ^{19}F MRI at Therapeutic Dose

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1. General information

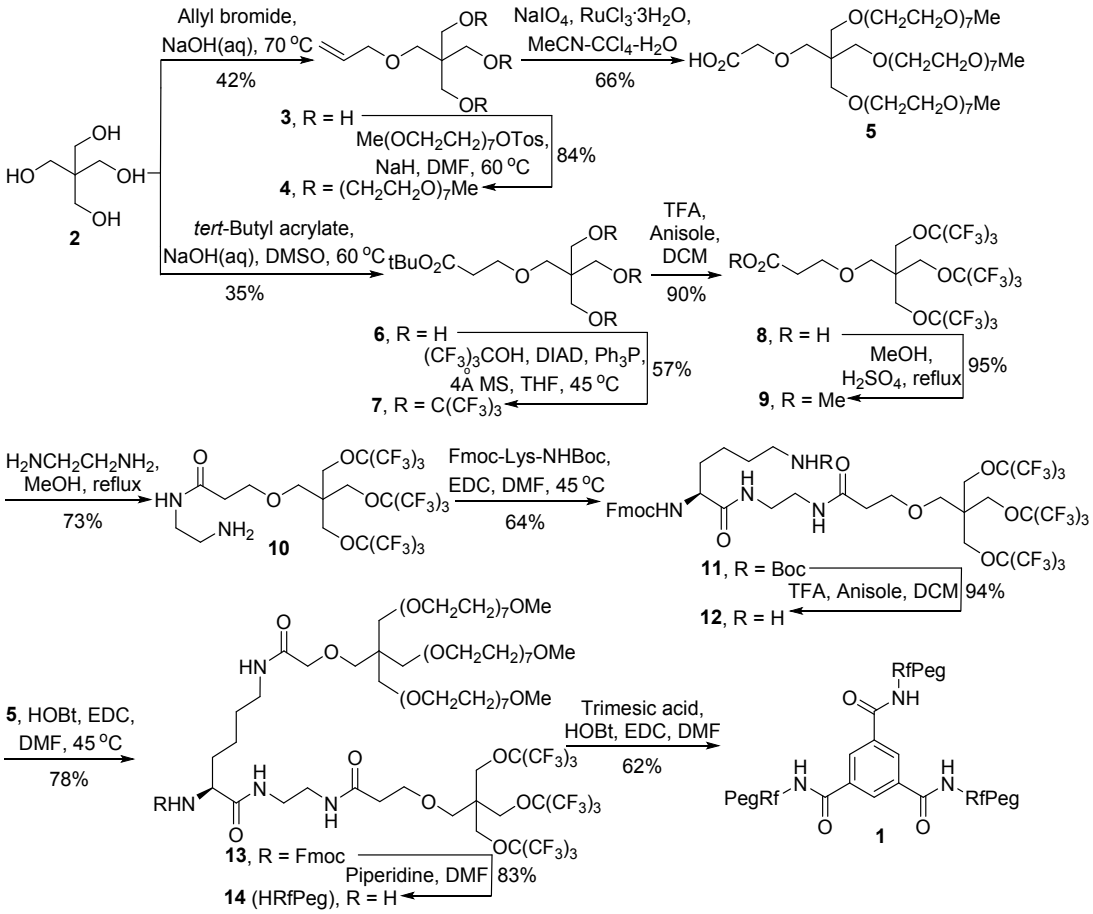
^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer. Chemical shifts are in ppm and coupling constants (*J*) are in Hertz (Hz). ^{1}H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using $CDCl_3$ as solvent. ^{13}C NMR spectra were referenced to solvent carbons (77.16 ppm for $CDCl_3$). ^{19}F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in $CDCl_3$ and 73 mM sodium trifluomethanesulfonate (s, -79.61) in D_2O. The splitting patterns for ^{1}H NMR spectra are denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on an ESI mass spectrometer for compounds below 3,000 Da and a MALDI-TOF spectrometer with α-cyano-4-hydroxylcinnamic acid as matrix using the reflection mode for positive ions for compounds above 3,000 Da.

Unless otherwise indicated, all reagents were obtained from commercial supplier and used without prior purification. All solvents were analytical or HPLC grade. Deionized water was used unless otherwise indicated. DMF, Et_3N, MeOH and THF were dried and freshly distilled prior to use. Flash chromatography was performed on silica gel (200-300 mesh) with petroleum ether (PE)/EtOAc (EA) or CH_2Cl_2/MeOH as eluents.

For amphiphile **1** HPLC analysis: SPD-20A UV detector (254 nm) with a Sunfire C18 column (5 μm, 4.6 x 100 mm), with a gradient elution of 30% methanol in water to 100% methanol over 10 min (flow rate 1.0 ml/min). For DOX HPLC analysis: RF-20A fluorescence detector (E_x = 467 nm, E_m = 550 nm) with a Cosmosil 5C18 column (5 μm, 4.6 x 250 mm) column, with a gradient elution of solvent A (ammonium dihydrogen phosphate buffer, water containing 0.5% v/v acetic acid and 0.01 M of ammonium dihydrogen phosphate, 0.39 ml/min) and solvent B (acetonitrile containing 0.5% v/v acetic acid, 0.21 ml/min) were used as the solvent gradient.

Normal Balb/c nude mice (male, 5-6 week, 22-25g) were bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. Tumor-carrying Balb/c nude mice with tumor volume of 300-800 mm^3 (male, 6-8 week, 23-26g) were bought from Wuhan Cloud-Clone Corp. During the procedures, mice were anesthetized by isoflurane.

2. Synthesis and HPLC chromatogram of amphiphile 1



Scheme S1. Synthesis of fluorinated amphiphile **1**

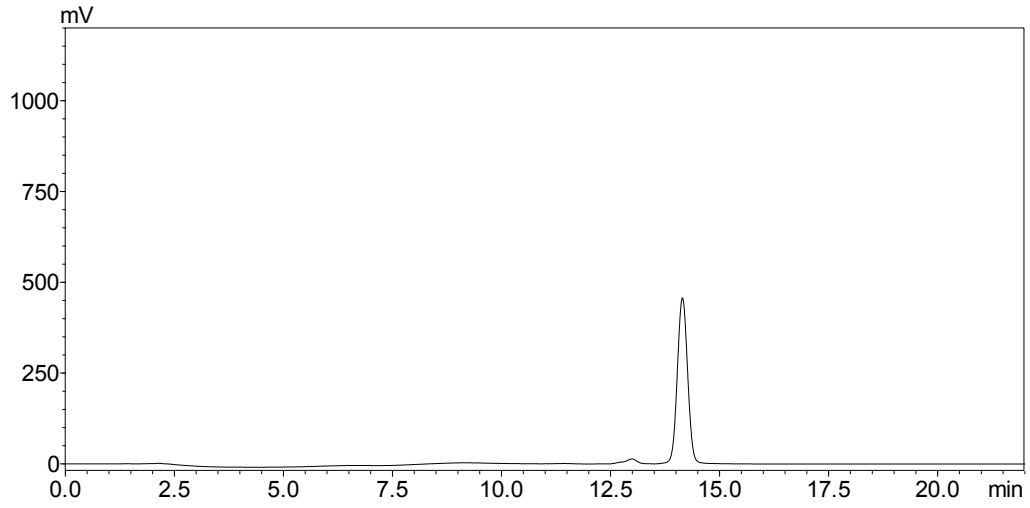


Figure S1. HPLC chromatogram of amphiphile **1**

3. Preparation of fluorinated liposomes

The liposome L1 was prepared with the film dispersion method to encapsulate DOX. A mixture of HSPC/CHOL/amphiphile **1**/DOX (15 mg/5 mg/15 mg/4 mg) was dissolved in 3.0 mL organic solvent (chloroform/methanol = 2/1) and triethylamine (3 equivalent to DOX) was added. The organic solvent was removed by vacuum rotary evaporation to form a dry film on the wall of the flask. PBS (2.0 mL) was added to the flask. The flask was rotated on a rotary evaporation at normal pressure for 2 min and sonicated at 60 ^{o}C for 2 h. Liposome was collected by filtration through a 0.45-μm polycarbonate membrane and a 0.22-μm polycarbonate membrane. The amount of DOX encapsulated in the liposomes was measured by HPLC. Blank liposome L0 was prepared using the same procedures in the absence of DOX addition. The drug-loading content and drug encapsulation efficiency were calculated as below:

$$\text{Drug loading content (\%)} = Wt/Ws \times 100\%$$

$$\text{Drug encapsulation efficiency (\%)} = Wt/Wo \times 100\%$$

Wt: the amount of DOX loaded into nanoparticles; Ws: the amount of nanoparticles after lyophilization; Wo: the initial amount of DOX added.

4. Characterization of fluorinated liposomes

The size and morphology of liposomes L0 and L1 were obtained using TEM. Briefly, liposomes L0 and L1 were diluted to ^{19}F concentration of 20 μM with water and then 5.0 μL of each sample was dropped onto the copper grids and air-dried at 42 °C, respectively. Then the grids were stained with 1% uranyl acetate solution for 30 s before taking images. The size distribution and zeta potential of liposomes were determined by DLS using Zetasizer Nano-ZS.

Table S1. Composition and characterization of liposomes L0 & L1

	liposome compositions	**Liposome characterization**				
	HSPC/CHOL/**1**/DOX (w/w/w/w)	Size (nm)	PDI	Zeta (mV)	Drug loading content	Drug encapsulating efficiency
L0	15:5:15:0	184.7	0.124	-20.3	0	0
L1	15:5:15:4	188.5	0.117	-18.5	10.1%	91%

5. *In vitro* drug release of liposome L1

The release of DOX from liposome L1 was performed using dialysis method with dialysis membrane tubes (molecular weight cutoff: 2000 Da). Briefly, the liposome L1 solution was dispersed in PBS (10 mM) at different pH value (pH 5.0, 6.8, 7.4) and then transferred to dialysis membrane tubes. These tubes were immersed into 200 mL of PBS solution with different pH value and stirred at 37 °C for 24 h. At the time points of 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h, 2.0 mL of the external buffer was collected and replaced by 2.0 mL of fresh buffer. The DOX concentration in the collected buffer was measured using HPLC.

6. Cell culture and *in vitro* anticancer activity assay.

HepG2 cells were cultured in Gibico medium DMEM containing 10% FBS. L02 cells were cultured in Gibico medium 1640 containing 15% FBS. A549 cells were cultured in Gibico medium IMEM containing 10% FBS. All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2.

The cell viability of amphiphile **1** against cells (L02, A549 and HepG2) were evaluated using a microculture tetrazolium (MTT) method. L02 cells, A549 cells and HepG2 cells were seeded in 96-well plates, respectively, and incubated 24 h to adhere. Then the cells were incubated with free amphiphile **1** at different concentrations ranging from 0.15 mM to 0.75 mM for 24 h, followed by replacing the medium with 100 μL MTT (1.0 mg/mL) solution and incubated for another 4 h. Cells treated with normal medium were used as control. Then the medium was replaced with 200 μL DMSO solution and the absorbance values was measured at 490 nm wavelength using a microplate reader. All of the experiments were carried out in three times.

Cell viability (%) was calculated as the formula:

Cell viability (%) = $[(A_{Test}-A_{Blank}) / (A_{Control}-A_{Blank})] \times 100\%$

A_{Test}, $A_{Control}$ and A_{Blank} represented the absorbance of cells with different treatments, untreated cells and blank culture media, respectively.

The antiproliferation activities of anticancer drug DOX, blank liposome L0, and drug-loaded liposome L1 against cancer cells (A549 and HepG2) were evaluated using the same method for amphiphile **1**. A549 cells and HepG2 cells were seeded in 96-well plates, respectively, and incubated 24 h to adhere. Then the cells were incubated with DOX solution and liposome L1 at serial DOX concentrations ranging from 0.001 to 10 μM for 24 h, followed by replacing the medium with 100 μL 1.0 mg/mL MTT solution and incubated for another 4 h. Cells treated with complete medium were used as control. Then the medium was

replaced with 200 μL DMSO solution and the absorbance was measured at 490 nm wavelength using a microplate reader. All of the experiments were carried out in three times.

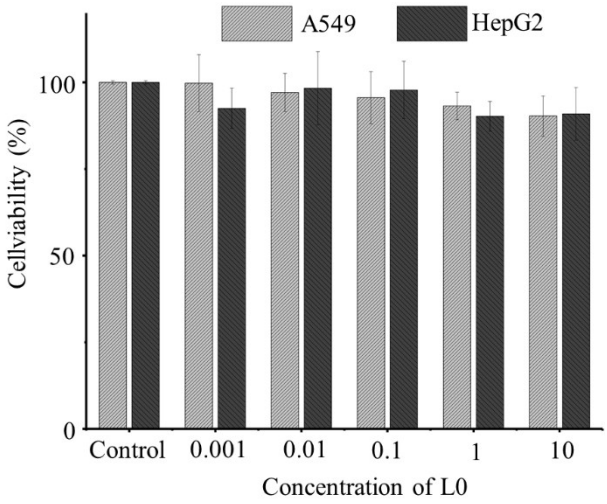


Figure S2. Cytotoxicity assay of L0 on A549 and HepG2 cells (The concentrations of L0 are the same with L1).

7. Internalization of free DOX and DOX-loaded liposome L1

The cellular uptake of DOX and liposome L1 was detected in HepG2 cells using confocal microscope. Briefly, HepG2 cells were seeded into confocal dishes and incubated at 37 °C for 24 h. Then the medium was removed and replaced with medium of DOX solution (5 μg/mL) and liposome L1 (5 μg/mL), respectively. After 2 h incubation, the medium was removed and washed with PBS buffer, followed by Hoechst staining to the nuclei for 5 min, and then to image using confocal microscope.

8. *In Vitro* ^{19}F MRI Experiments

All MRI experiments were performed on a 400 MHz MRI system. The temperature of the magnet room was maintained 25 ^{o}C during the entire MRI experiment.

***In vitro* ^{19}F MRI** of amphiphile **1**: solution of 320 mM ^{19}F was serially diluted 1×, 2×, 4×, 8×, 16×, 32× times by PBS, forming amphiphile **1** solutions with ^{19}F concentrations of 320 mM, 160 mM, 80 mM, 40 mM, 20 mM, 10 mM, respectively. The ^{19}F *in vitro* images were acquired using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32 × 32, FOV = 30 mm × 30 mm, TR = 2000 ms, TE = 5.37 ms, RARE factor = 1, number of average = 4, scan time = 256 s.

***In vitro* ^{19}F MRI** of liposomes L0 and L1: liposomes L0 and L1 of 160 mM ^{19}F was serially diluted 1×, 2×, 4×, 8×, 16×, 32× times by PBS, forming liposomes L0 and L1 solutions with ^{19}F concentrations of 160 mM, 80 mM, 40 mM, 20 mM, 10 mM, 5 mM,

respectively. The ^{19}F *in vitro* images were acquired using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32 × 32, FOV = 30 mm × 30 mm, TR = 3000 ms, TE = 2.993 ms, RARE factor = 8, number of average = 8. Scan time of high concentration samples is 96 s. Scan time of low concentration samples is 384 s.

Table S2. T_1 and T_2 of L0 and L1

	T_1 (ms)	T_2 (ms)
L0	568	15
L1	606	14

9. Mice ^{19}F MRI experiments

Normal mouse 1 was injected with 300 μL amphiphile **1** solution (in saline, ^{19}F dose is 30 mmol/kg) via the tail vein. Normal mouse 2 was injected with 300 μL liposome L1 solution (in PBS, ^{19}F dose is 10 mmol/kg) via the tail vein. ^{1}H MRI and ^{19}F MRI for each mouse were collected at 1 h after the injection with isoflurane as anesthetics (Fig. 5a, 2 upper). ^{1}H MRI: method = RARE, matrix size = 256 × 256, FOV = 80 mm × 40 mm, TR = 1000 ms, TE = 8.15 ms, RARE factor = 4, number of average = 4, scan time = 256 s; ^{19}F MRI: method = FLASH, matrix size = 128 × 64, FOV = 80 mm × 40 mm, TR = 500 ms, TE = 2.26 ms, number of average = 32, scan time = 1024 s.

Tumor mouse 3 was injected with 100 μL amphiphile **1** solution (in saline, ^{19}F dose is 10 mmol/kg) via local injection. Tumor mouse 4 was injected with 100 μL liposome L1 solution (in PBS, ^{19}F dose is 3.3 mmol/kg) via local injection. ^{1}H MRI and ^{19}F MRI for each mouse were collected at 1 h after the injection with isoflurane as anesthetics (Fig. 5b, 2 lower). ^{1}H MRI: method = RARE, matrix size = 256 × 256, FOV = 40 mm × 40 mm, TR = 1500 ms, TE = 7.13 ms, RARE factor = 4, number of average = 4, scan time = 192s. ^{19}F MRI: method = FLASH, matrix size = 64 × 64, FOV = 40 mm × 40 mm, TR = 500 ms, TE = 2.26 ms, number of average = 16, scan time = 512 s

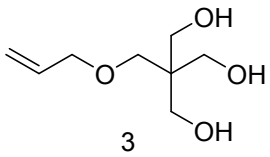
10. *In Vivo* acute toxicity assay

Mice were tail vein injected with solution of amphiphile **1** in saline (1.5 g/kg and 3.0 g/kg, n = 3). Then the mice were observed for a month. During this period, each mouse behaved normally with no obvious acute toxicity.

11. DOX and amphiphile 1 tissue distribution

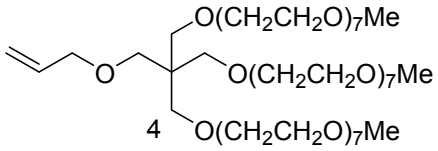
The tumor-carrying mice were injected with 250 μL of L1 at a DOX dose of 5 mg/kg via the tail vein. Distribution of DOX and amphiphile **1** in tumor and kidney were analyzed on groups of 2 mice. At 4 h and 24 h after iv injection, the mice were euthanized and the tumor and kidney were collected. After tissue homogenization, the DOX and amphiphile **1** in tissue samples were extracted with chloroform. Then the concentration of DOX and amphiphile **1** were determined by HPLC and ^{19}F NMR, respectively. The concentration of DOX and amphiphile **1** were expressed as percentage of injected dose per gram tissue (% ID/g tissue).

12. Synthetic procedures of amphiphile 1



3

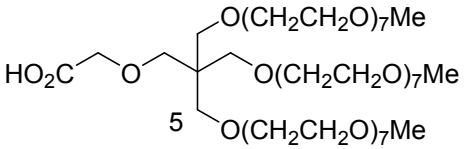
Compound 3. To a stirring solution of pentaerythrotol **2** (68.0 g, 0.5 mol) in NaOH (9.6 g in 200 mL) was added allyl bromide (24.2 g, 0.2 mol) dropwise over 2 h. Then the reaction was heated to 70 ^{o}C for 8 h. The mixture was diluted with water (100 mL) and extracted with EA (150 mL×5). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (PE/EA = 1/1) to give compound **3** as clear oil (14.1 g, 42% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 5.82-5.92 (m, 1H), 5.18-5.28 (m, 2H), 3.97 (d, J = 8.0 Hz, 2H), 3.76 (s, 3H), 3.68 (s, 6H), 3.44 (s, 2H).



4

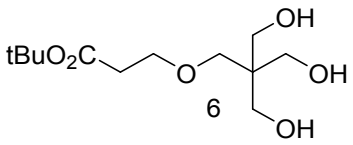
Compound 4. Under an atmosphere of argon, a solution of compound **3** (7.0 g, 40.0 mmol) in DMF (50 mL) was added dropwise into a suspension of NaH (5.8 g, 240.0 mmol) in DMF (100 mL) under ice bath. After stirring for additional 30 min, a solution of $mPEG_7Tos$ (79.0 g, 160.0 mmol) in DMF (100 mL) was added and the resulting mixture was stirred at 60 ^{o}C for 24 h. Then DMF was evaporated under reduced pressure. The crude was purified by column chromatography on silica gel (CH_2Cl_2 /MeOH = 10/1) to give alcohol compound **4** as clear oil (38.4 g, 84% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 5.84-5.89 (m, 1H), 5.24 (d, J = 8.0 Hz, 1H), 5.12 (d, J = 4.0 Hz, 1H), 3.93 (s, 2H), 3.56-3.65 (m, 88H), 3.44 (s, 4H), 3.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.8, 115.5, 71.7, 71.5, 70.5, 70.0-70.1 (m), 69.9, 69.5, 68.7,

58.5, 45.0. HRMS (ESI) calcd for $C_{53}H_{106}NaO_{25}^{+}$ ([M+Na]$^{+}$) 1165.6915, found 1165.6884.



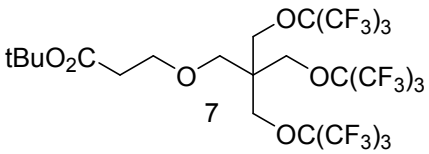
5

Compound 5. To a mixture of $CHCl_3$: CH_3CN : H_2O (1:1:1.5, 210 mL) was added compound **4** (37.7 g, 33.0 mmol), $NaIO_4$ (42.4 g, 198.0 mmol) and ruthenium(III)chloride hydrate (66.0 mg, 0.33 mmol) at 0 °C. The mixture was stirred at room temperature for 3 h. Then the mixture was added water and extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (CH_2Cl_2 /MeOH = 8/1) to give compound **5** as clear oil (25.3 g, 66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.04 (s, 2H), 3.54-3.65 (m, 86H), 3.47 (s, 6H), 3.38 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 71.5, 71.1, 70.6, 70.0-70.2 (m), 69.9, 69.8, 69.7, 68.4, 68.2, 58.6, 44.9. HRMS (ESI) calcd for $C_{52}H_{103}O_{27}^{-}$ ([M-H]$^{-}$) 1159.6692, found 1159.6697.



6

Compound 6. The solution of pentaerythritol **2** (68.1 g, 0.5 mol) in DMSO (100 mL) was heated to 80 °C, then aqueous NaOH (4.0 g in 9 mL H_2O) was added in one portion, *tert*-butyl acrylate (76.9 g, 0.6 mol) was added to the solution dropwise. The mixture was vigorously stirred overnight at 80 °C. After cooling, the solution was extracted with EA. The combined organic phase was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (PE/EA = 1/1) to give compound **6** (46.2 g, 35% yield) as clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.67 (t, J = 8.0 Hz, 2H), 3.65 (s, 6H), 3.52 (s, 2H), 3.10 (s, 3H), 2.50, 2.49(t, J = 8.0 Hz, 2H), 1.46 (s, 9H).



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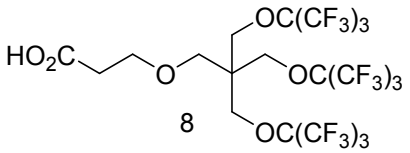
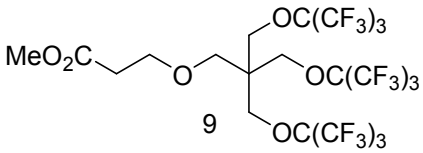
Compound 7. To a stirred suspension of compound **6** (13.2 g, 50.0 mmol), triphenylphosphine (59.0 g, 225.0 mmol), and 4 Å molecular sieves (15.0 g) was added THF (150 mL). Then DIAD (45.5 g, 225.0 mmol) was added dropwise to the mixture at 0 °C.

Afterward, the reaction mixture was stirred for an additional 20 min. Then perfluoro-tert-butanol (53.0 g, 225.0 mmol) was added in one portion, and the resulting mixture was stirred at 45 °C for 48 h in a sealed vessel. The reaction mixture was added water (100 mL) and extracted with EA (100 mL × 3). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, concentrated through rotary evaporation. The residue was subjected to silica gel chromatography (PE/EA = 10/1) to give compound **7** (26.2 g, 57% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.04 (s, 6H), 3.64 (t, *J* = 8.0 Hz, 2H), 3.42 (s, 2H), 2.45 (t, *J* = 8.0 Hz, 2H), 1.44 (s, 9H); ^{19}F NMR (376 MHz, $CDCl_3$) δ −73.64.

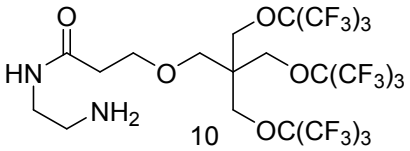


Compound 8. To a stirring solution of compound **7** (25.7 g, 28.0 mmol) and anisole (4.5 g, 42.0 mmol) in CH_2Cl_2 (200 mL) was added trifluoroacetic acid (63.9 g, 560.0 mmol) and the reaction mixture was stirred at room temperature for 4 h. Then, the mixture was concentrated under vacuum. The residue was purified by column chromatography on silica gel (PE/EA = 3/1) to give compound **8** as white solid (21.7 g, 90% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 4.14 (s, 6H), 3.70 (t, *J* = 8.0 Hz, 2H), 3.47 (s, 2H), 2.54 (t, *J* = 8.0 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.17; ^{13}C NMR (100 MHz, CD_3OD) δ 174.9, 121.6 (q, *J* = 291.0 Hz), 80.2-81.4 (m), 68.2, 67.1, 47.4, 35.6. HRMS (ESI) calcd for $C_{20}H_{12}F_{27}O_6^-$([M-H]$^-$) 861.0208, found 861.0209.



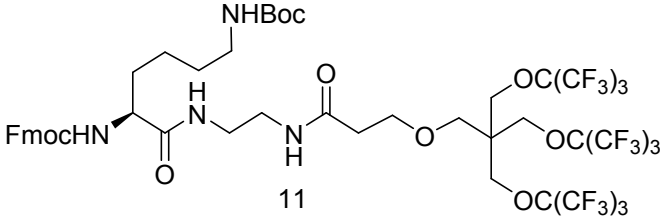
Compound 9. To a stirring solution of compound **8** (21.5 g, 25.0 mmol) in MeOH (100 mL) was added concentrated H_2SO_4 (4.0 mL). After refluxing for 8 h, the mixture was neutralized with saturated sodium bicarbonate solution. The mixture was added water (100 mL) and extracted with EA (100 mL × 3). The combined organic phase was dried over anhydrous Na_2SO_4, concentrated through rotary evaporation. The residue was purified by column chromatography on silica gel (PE/EA = 10/1) to give compound **9** as white solid (20.8 g, 95% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.03 (s, 6H), 3.67-3.70 (m, 5H), 3.41 (s, 2H), 2.55 (t, *J*

= 8.0 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.61; ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 120.3 (q, *J* = 291.0 Hz), 78.9-80.3 (m), 67.00, 66.2, 65.6, 51.7, 46.3, 34.7. HRMS (ESI) calcd for $C_{21}H_{15}F_{27}NaO_6^+$ 899.0330 ([M+Na]$^+$), found 899.0308.



10

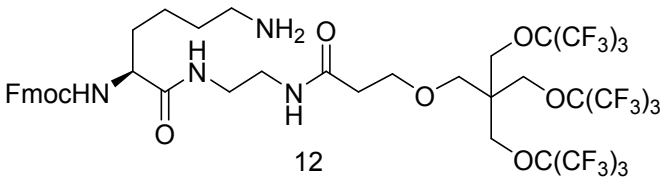
Compound 10. Compound **9** (21.0 g, 24.0 mmol) was dissolved in MeOH (100 mL) and ethylenediamine (30 mL). The mixture was refluxed for 48 h. Then the mixture was evaporated and the residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 10/1) to give compound **10** as clear oil (15.8 g, 73% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 4.15 (s, 6H), 3.71 (t, *J* = 8.0 Hz, 2H), 3.47 (s, 2H), 3.26 (s, 2H), 2.73 (t, *J* = 8.0 Hz, 2H), 2.48 (t, *J* = 8.0 Hz, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.16; ^{13}C NMR (100 MHz, CD_3OD) δ 173.49, 121.7 (q, *J* = 288.0 Hz), 80.31-81.80 (m), 68.91, 67.46, 67.35, 49.85, 47.52, 43.05, 42.12, 37.45; HRMS (ESI) calcd for $C_{22}H_{20}F_{27}N_2O_5^+$ ([M+H]$^+$) 905.0936, found 905.0914.



11

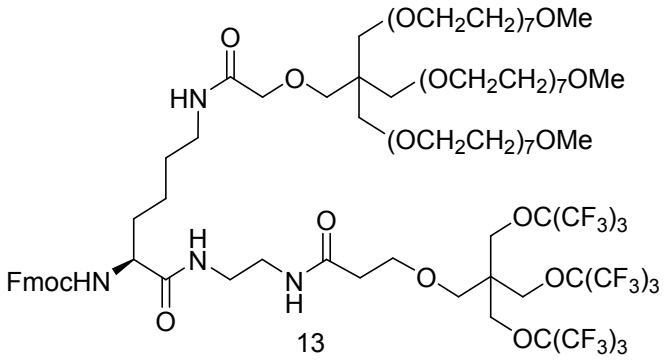
Compound 11. Under an atmosphere of argon, to a stirring solution of HOBt (4.6 g, 33.7 mmol) and Fmoc-Boc-lys-OH (10.5 g, 22.5 mmol) in DMF (100 mL) was added EDC (6.46 g, 33.7 mmol) at 0 °C. After 20 min, compound **10** (13.5 g, 15.0 mmol) in DMF (50 mL) was added in one portion and the reaction mixture was stirred at 45 °C for 12 h. The reaction mixture was washed by brine (200 mL) and extracted with EA (150 mL × 4). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (PE/EA = 2/1) to give compound **11** as white solid (13.0 g, 64% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, *J* = 4.0 Hz, 2H), 7.69 (q, *J* = 4.0 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.33 (t, *J* = 8.0 Hz, 2H), 4.38-4.44 (m, 2H), 4.24 (t, *J* = 8.0 Hz, 2H), 4.12 (s, 6H), 4.0 (q, 4.0 Hz, 1H), 3.65 (t, *J* = 8.0 Hz, 2H), 3.41 (s, 2H), 3.29 (d, *J* = 4.0 Hz, 2H), 3.04 (t, *J* = 8.0 Hz, 2H), 2.42 (t, *J* = 8.0 Hz, 2H), 1.73-1.80 (m, 1H), 1.60-1.68 (m,

1H), 1.28-1.50 (m, 15H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.11; ^{13}C NMR (100 MHz, CD_3OD) δ 175.4, 173.4, 158.6, 145.3, 145.2, 142.6, 128.8, 128.2, 126.2, 121.5 (q, *J* = 290.0 Hz), 80.5-81.2 (m), 79.8, 68.6, 67.9, 67.1, 56.7, 48.4, 48.36, 47.4, 41.0, 40.0, 39.9, 37.3, 32.7, 30.5, 28.9, 24.2; HRMS (ESI) calcd for $C_{48}H_{49}F_{27}N_4O_{10}Na^+$ ([M+Na]$^+$) 1377.2910, found 1377.2897.



12

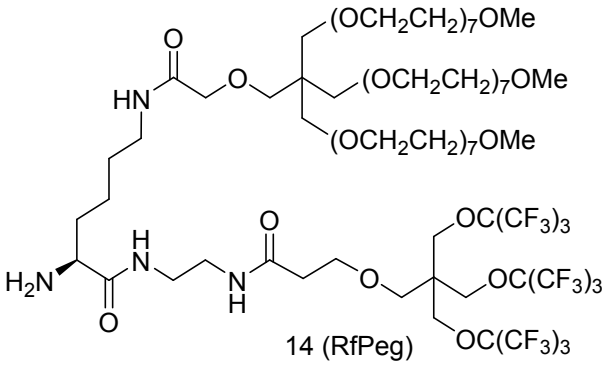
Compound 12. To a stirring solution of compound **11** (12.9 g, 9.5 mmol) and anisole (1.5 g, 14.3 mmol) in CH_2Cl_2 (100 mL) was added trifluoroacetic acid (21.7 g, 190.0 mmol) and the reaction mixture was stirred at room temperature for 4 h. Then, the mixture was concentrated under vacuum. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 8/1) to give compound **12** as white solid (11.2 g, 94% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (d, *J* = 4.0 Hz, 2H), 7.56 (t, *J* = 4.0 Hz, 2H), 7.29 (t, *J* = 8.0 Hz, 2H), 7.21 (t, *J* = 8.0 Hz, 2H), 4.27-4.35 (m, 2H), 4.12 (t, *J* = 8.0 Hz, 1H), 4.00 (s, 6H), 3.92 (q, *J*=4.0 Hz, 1H), 3.53 (t, *J* = 8.0 Hz, 2H), 3.29 (s, 2H), 3.18 (s, 4H), 1.67–1.75 (m, 1H), 1.49-1.60 (m, 3H), 1.27–1.37 (m, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -71.15; ^{13}C NMR (100 MHz, CD_3OD) δ 175.1, 173.5, 158.6, 145.3, 145.2, 142.6, 128.8, 128.1, 126.2, 121.6 (q, *J* = 291.0 Hz), 121.1, 80.4-81.7 (m), 68.7, 67.9, 67.2, 56.4, 48.4, 40.5, 40.1, 39.8, 37.4, 32.4, 28.1, 23.9; HRMS (ESI) calcd for $C_{43}H_{42}F_{27}N_4O_8^+$ ([M+H]$^+$) 1255.2566, found 1255.2542.



13

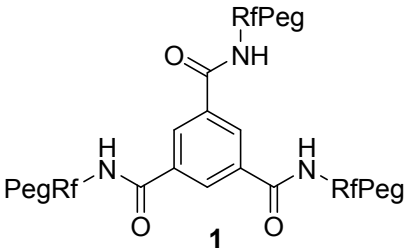
Compound 13. Under an atmosphere of argon, to a stirring solution of HOBt (1.9 g, 14.4 mmol) and compound **5** (11.1 g, 9.6 mmol) in DMF (50 mL) was added EDC (2.8 g, 14.4 mmol) at 0 °C. After 20 min, compound **12** (10.0 g, 8.0 mmol) was added in one portion at

room temperature and the reaction mixture was stirred at 45 °C for 12 h. Then the reaction mixture was washed by brine (200 mL) and extracted with CH_2Cl_2 (150 mL, four times). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 10/1) to give compound **13** as colorless oil (15.0 g, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 4.0 Hz, 2H), 7.63 (d, *J* = 4.0 Hz, 2H), 7.40 (t, *J* = 8.0 Hz, 2H), 7.31 (t, *J* = 8.0 Hz, 2H), 4.35-4.45 (m, 2H), 4.21 (t, *J* = 8.0 Hz, 2H), 4.04 (s, 6H), 3.89 (s, 2H), 3.53-3.65 (m, 90H), 3.44 (d, *J* = 4.0 Hz, 6H), 3.37-3.39 (m, 13H), 2.90 (s, 2H), 2.40 (d, *J* = 4.0 Hz, 2H), 1.26–1.69 (m, 6H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.61; ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 170.9, 143.8, 141.4, 127.8, 127.1, 125.1, 120.2 (q, *J* = 291.0 Hz), 120.0, 79.0-79.8 (m), 72.0, 71.0, 70.5-76.0 (m), 70.2, 70.0, 59.0, 47.2, 46.2, 45.3, 36.5; HRMS (ESI) calcd for $C_{95}H_{143}F_{27}N_4Na_2O_{34}^{2+}$ $([M/2+Na])^+$ 1221.4469, found 1221.4430.



14 (RfPeg)

Compound 14. To a solution of compound **13** (7.2 g, 3.0 mmol) in DMF (30 mL) was added piperidine (6 mL). The mixture was stirred at room temperature for 4 h. After the DMF was removed under reduced pressure. The residue was purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 5/1) to give compound **14** (RfPeg) as colorless oil (5.4 g, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.20 (d, *J* = 8.0 Hz, 2H), 4.18-4.31 (m, 1H), 4.05 (s, 6H), 3.90 (s, 4H), 3.56-3.66 (m, 85H), 3.38-3.45(m, 22H), 2.88 (s, 4H), 2.45 (t, *J* = 8.0 Hz, 2H), 1.41–1.85 (m, 6H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.58; ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 170.6, 170.2, 119.9 (q, *J*=291.0 Hz), 78.5-79.7 (m), 71.7, 71.1, 70.5, 70.2-70.4 (m), 70.0, 69.8, 67.4, 65.9, 65.3, 58.7, 54.6, 45.9, 45.1, 40.0, 38.9, 38.1, 36.2, 33.7, 29.4, 22.6; HRMS (ESI) calcd for $C_{80}H_{135}F_{27}N_4O_{32}^{2+}$ $([M/2+H])^+$ 1088.4309, found 1088.4315.

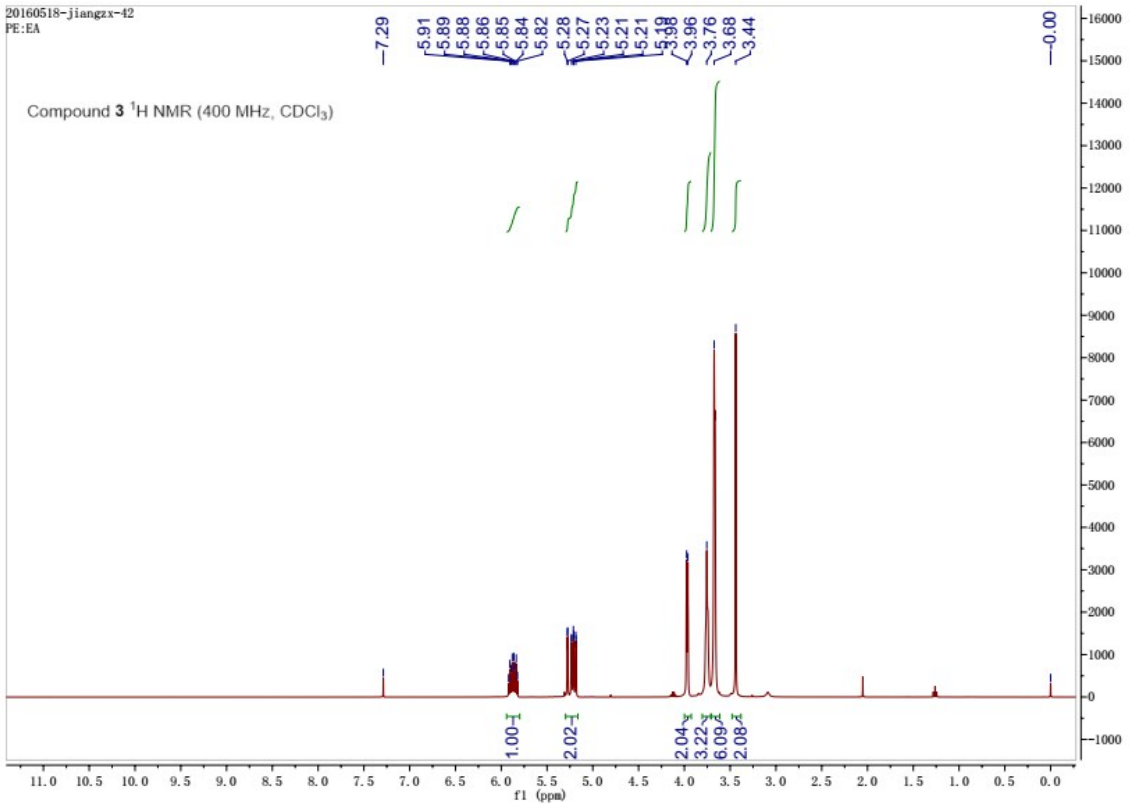


1

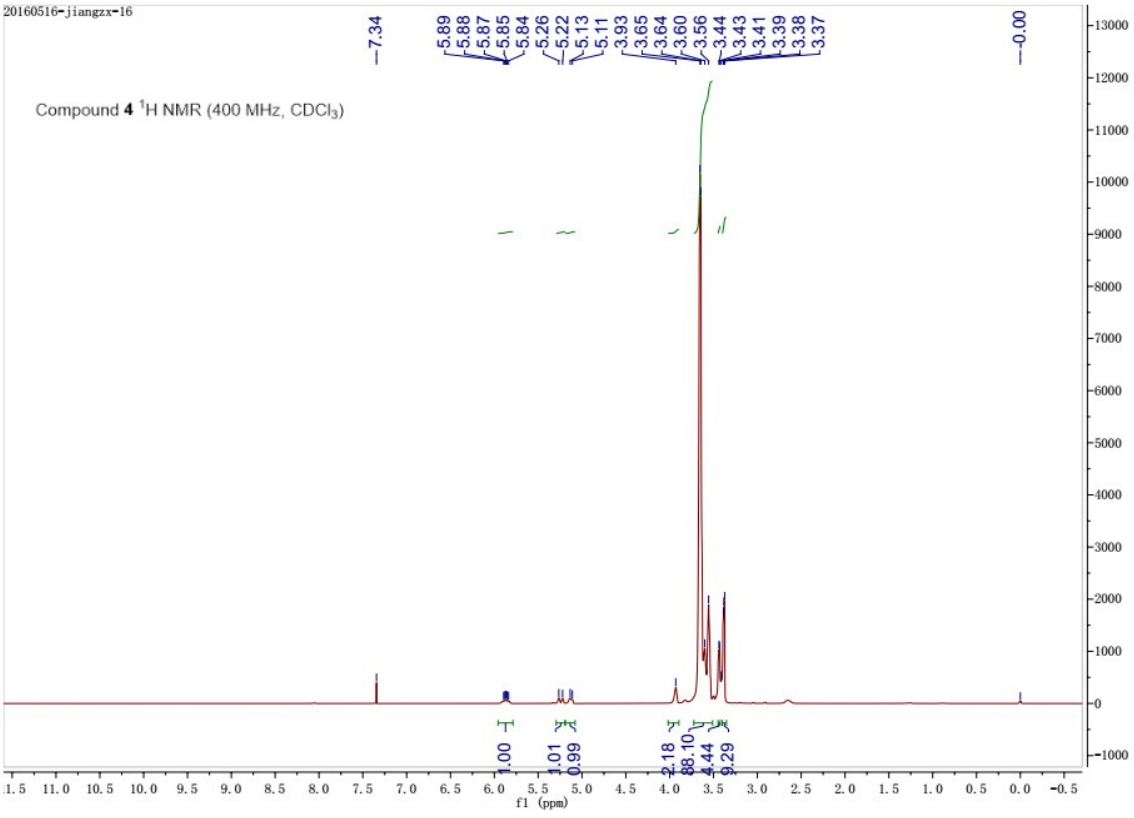
Amphophile 1. Under an atmosphere of argon, to a stirring solution of HOBt (304.0 mg, 2.25 mmol) and trimesic acid (105.0 mg, 0.5 mmol) in DMF (60 mL) was added EDC (431.0 mg, 2.25 mmol) at 0 °C. After 20 min, compound **14** (4.9 g, 2.25 mmol) was added in one portion at room temperature and the reaction mixture was stirred at 45 °C for 24 h. The reaction mixture was washed with brine (200 mL) and extracted with CH_2Cl_2 (150 mL × 3). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by column chromatography on silica gel (CH_2Cl_2/MeOH = 10/1) to give compound **1** as yellowish oil (2.1 g, 62% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 8.60 (s, 3H), 4.51-4.54 (m, 2H), 4.15 (s, 18H), 3.60-3.71 (m, 252H), 3.47-3.56 (m, 57H), 2.47 (t, *J* = 8.0 Hz, 6H), 1.85-1.99 (m, 6H), 1.60-1.64 (m, 6H), 1.44–1.54 (m, 6H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.56; ^{13}C NMR (100 MHz, CD_3OD) δ 174.5, 173.3, 172.7, 168.3, 136.0, 130.8, 127.0, 126.3, 121.5 (q, *J* = 232.0 Hz), 118.8, 111.9, 80.3-81.3 (m), 73.0, 72.2, 71.8, 71.5-71.6 (m), 71.4, 71.3, 70.9, 68.7, 67.3, 59.1, 55.7, 47.4, 46.6, 40.1, 39.9, 39.6, 37.3, 32.7, 30.5, 24.5; MS (MALDI-TOF) calcd for $C_{249}H_{399}F_{81}N_{12}NaO_{99}^+$ $[M+Na]^+$ 6703.5, found 6702.3.

13. ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, MS and HRMS Spectra of Compounds

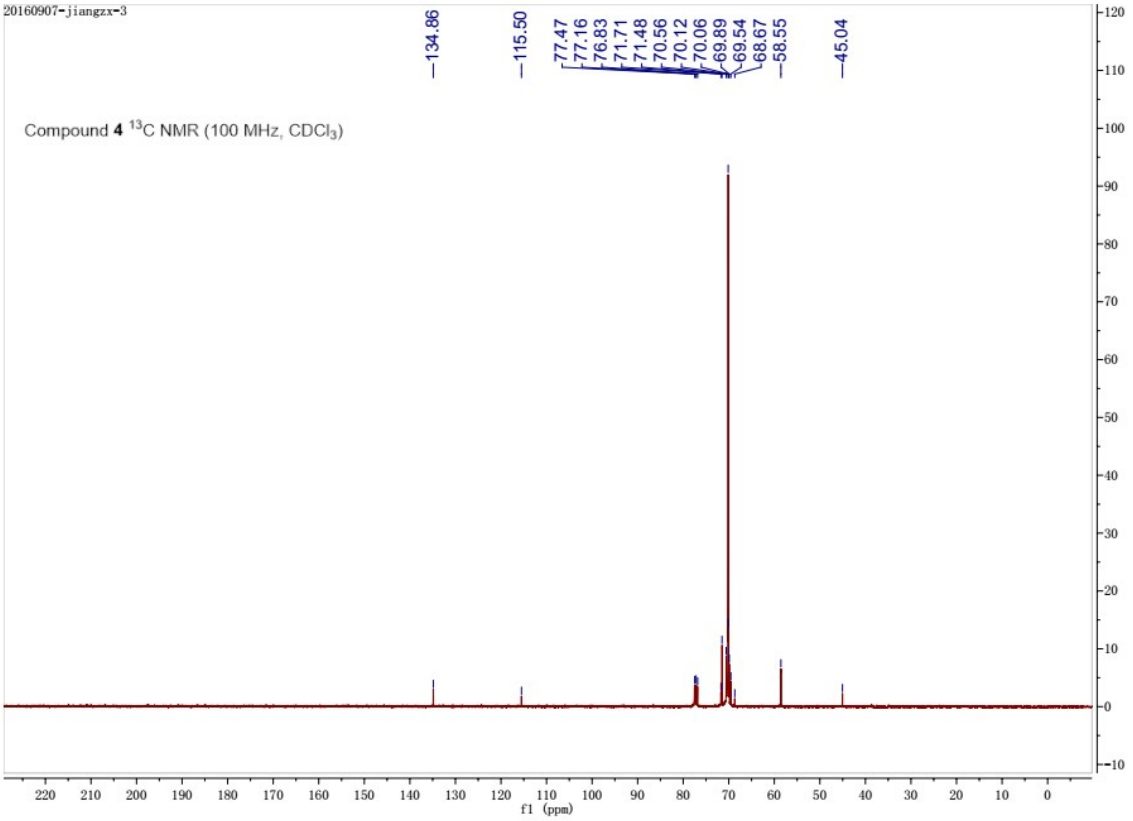
^{1}H NMR spectra of Compound **3** (400 MHz, $CDCl_3$)



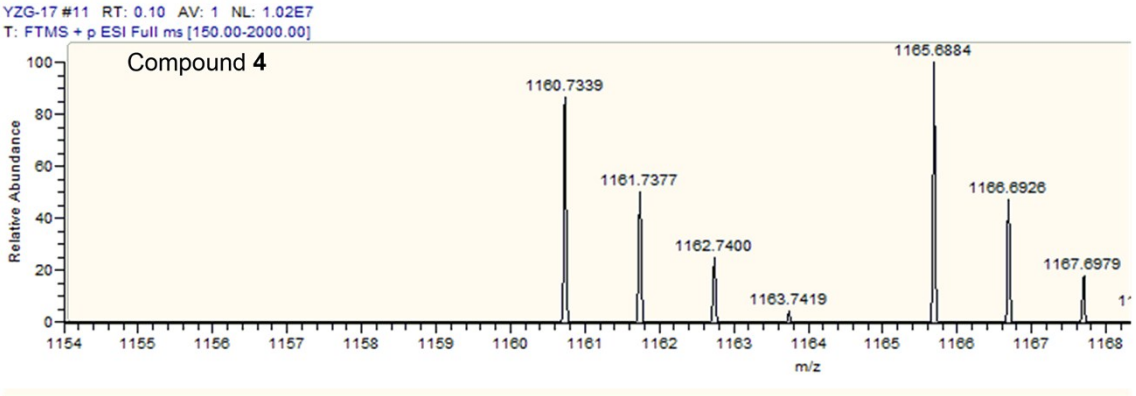
^{1}H NMR spectra of Compound **4** (400 MHz, $CDCl_3$)



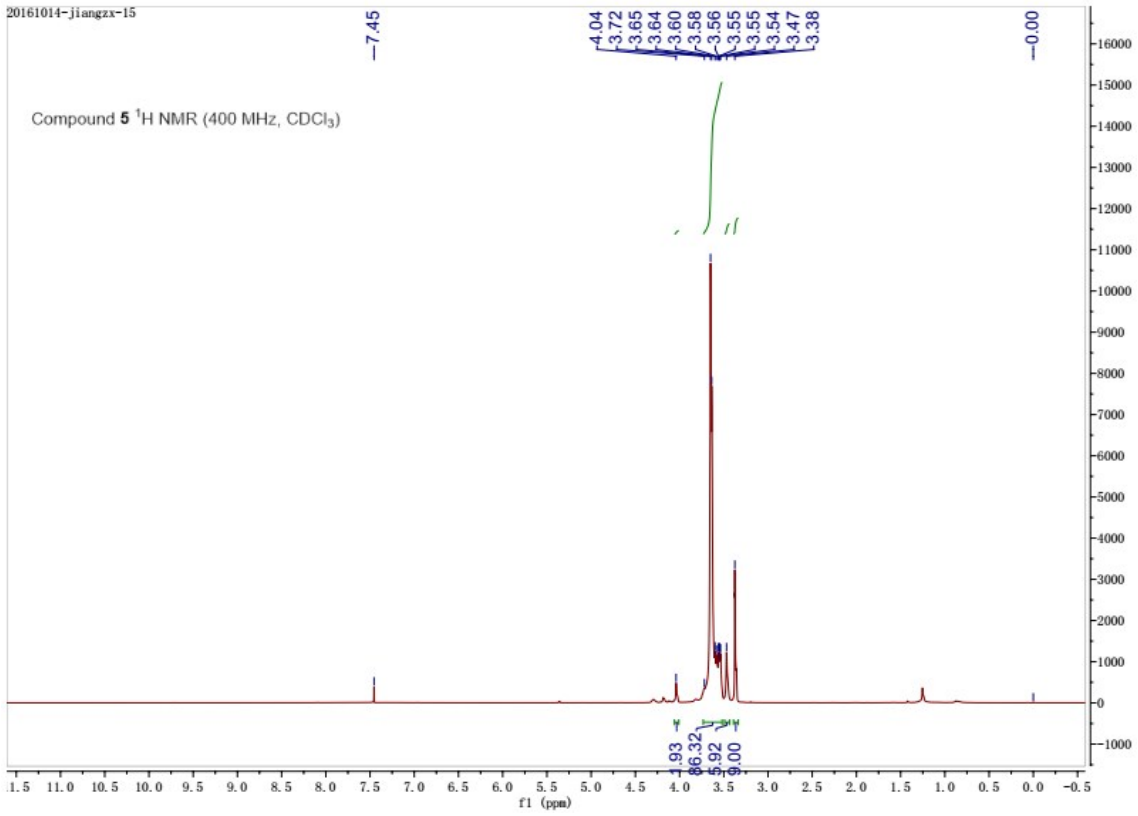
^{13}C NMR spectra of Compound **4** (100 MHz, $CDCl_3$)



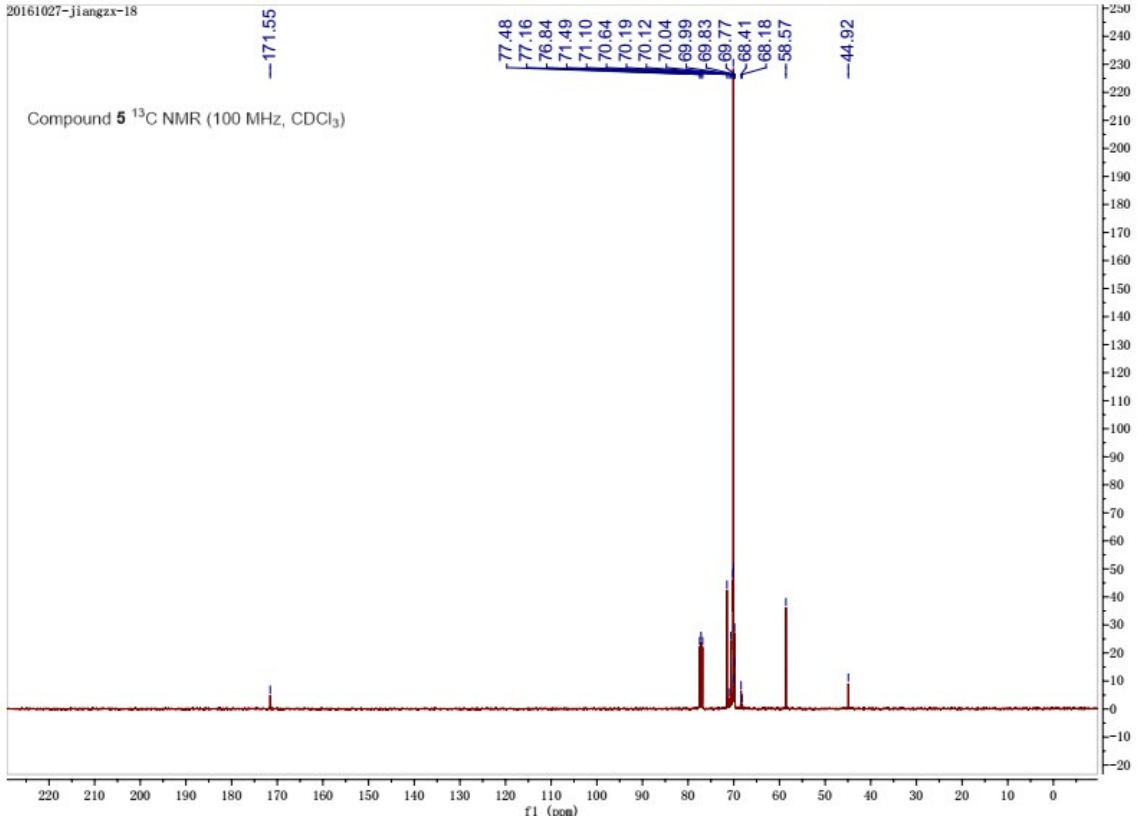
HRMS spectra of Compound **4**



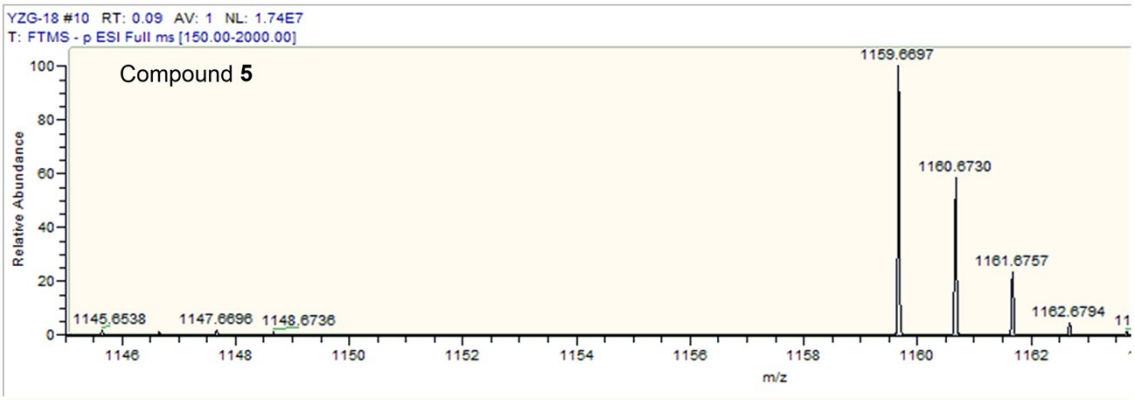
^{1}H NMR spectra of Compound **5** (400 MHz, $CDCl_3$)



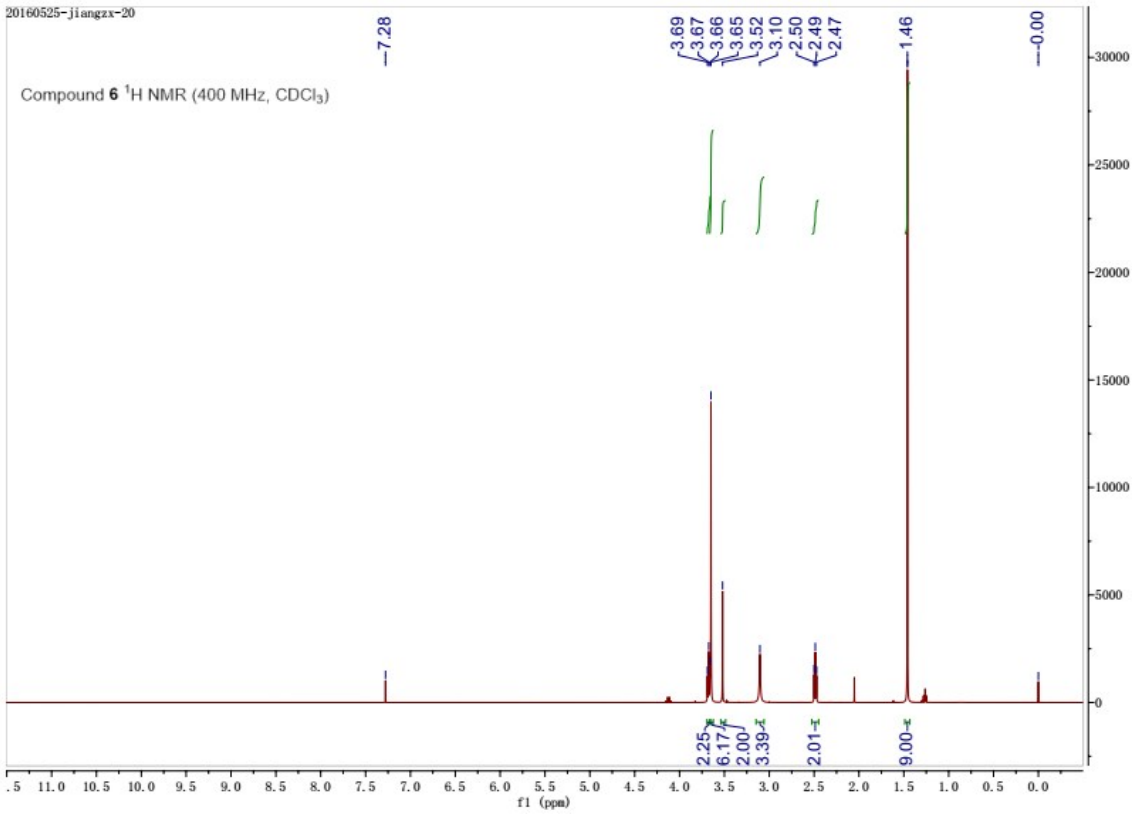
^{13}C NMR spectra of Compound **5** (100 MHz, $CDCl_3$)



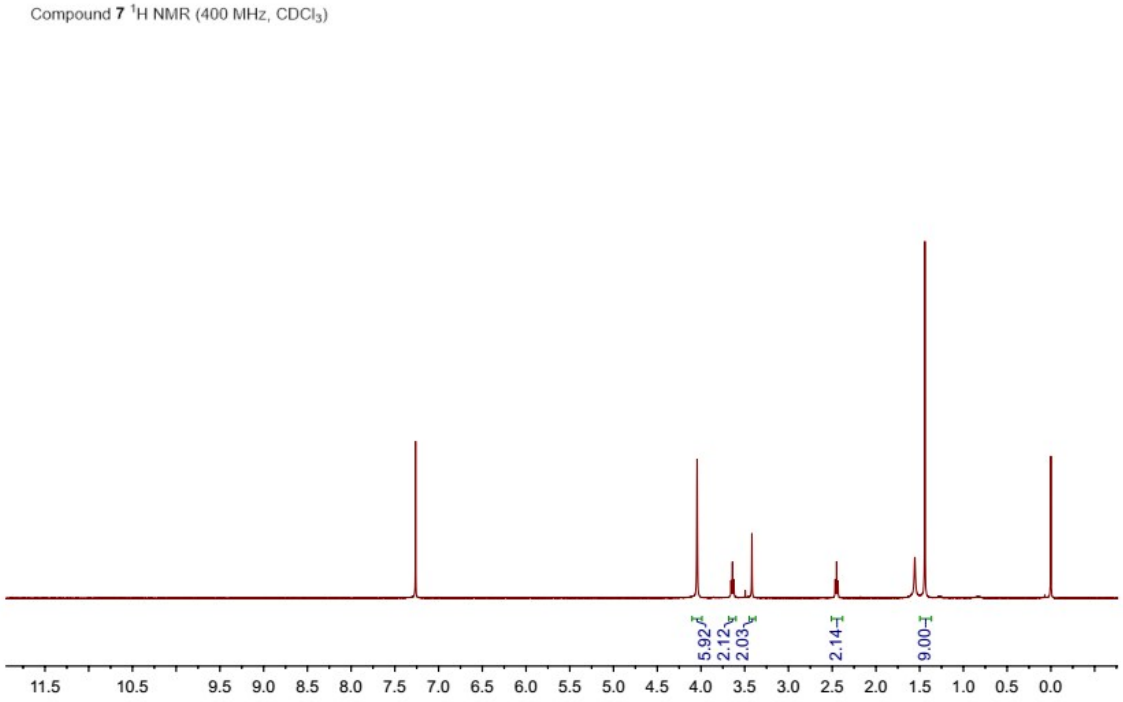
HRMS spectra of Compound **5**



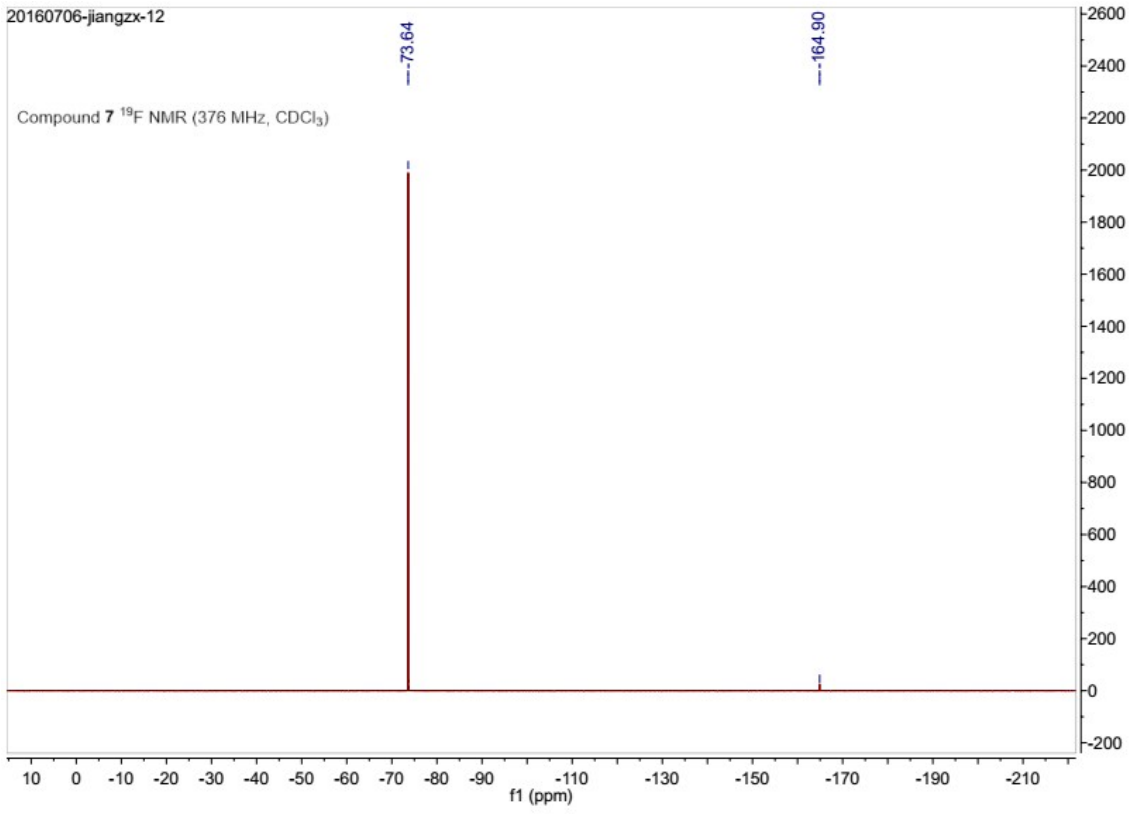
^{1}H NMR spectra of Compound **6** (400 MHz, $CDCl_3$)



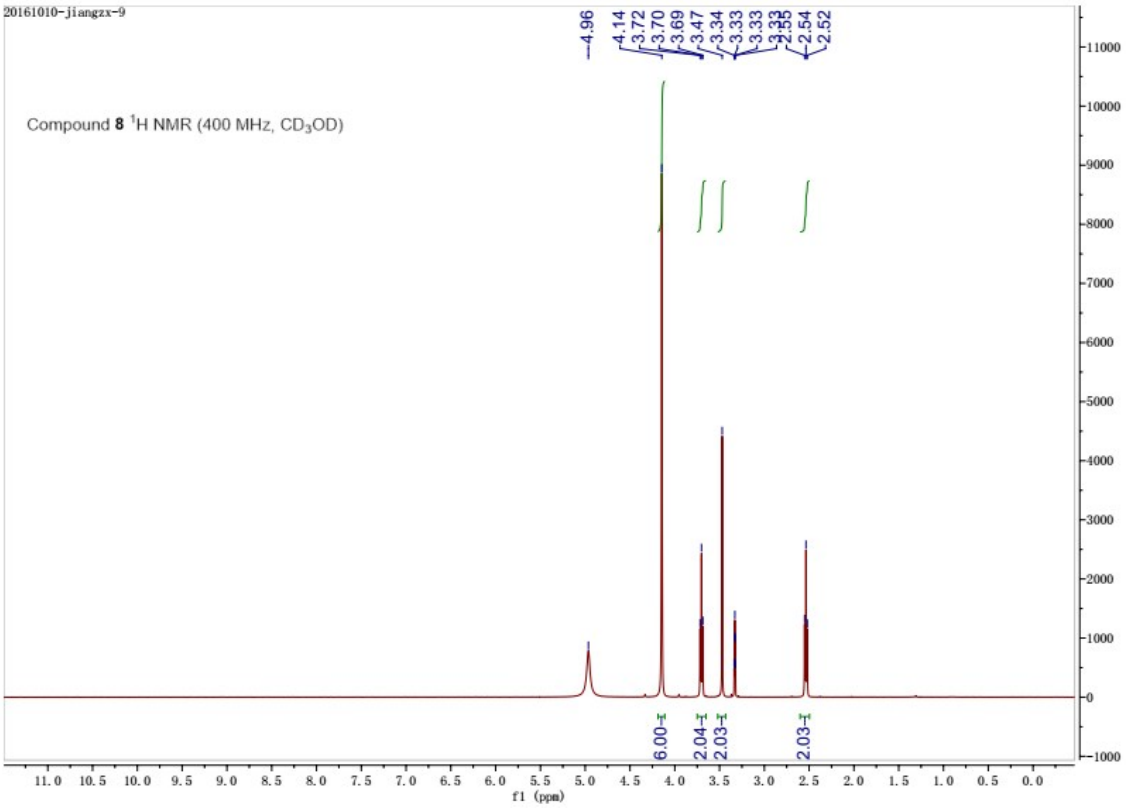
^{1}H NMR spectra of Compound **7** (400 MHz, $CDCl_3$)



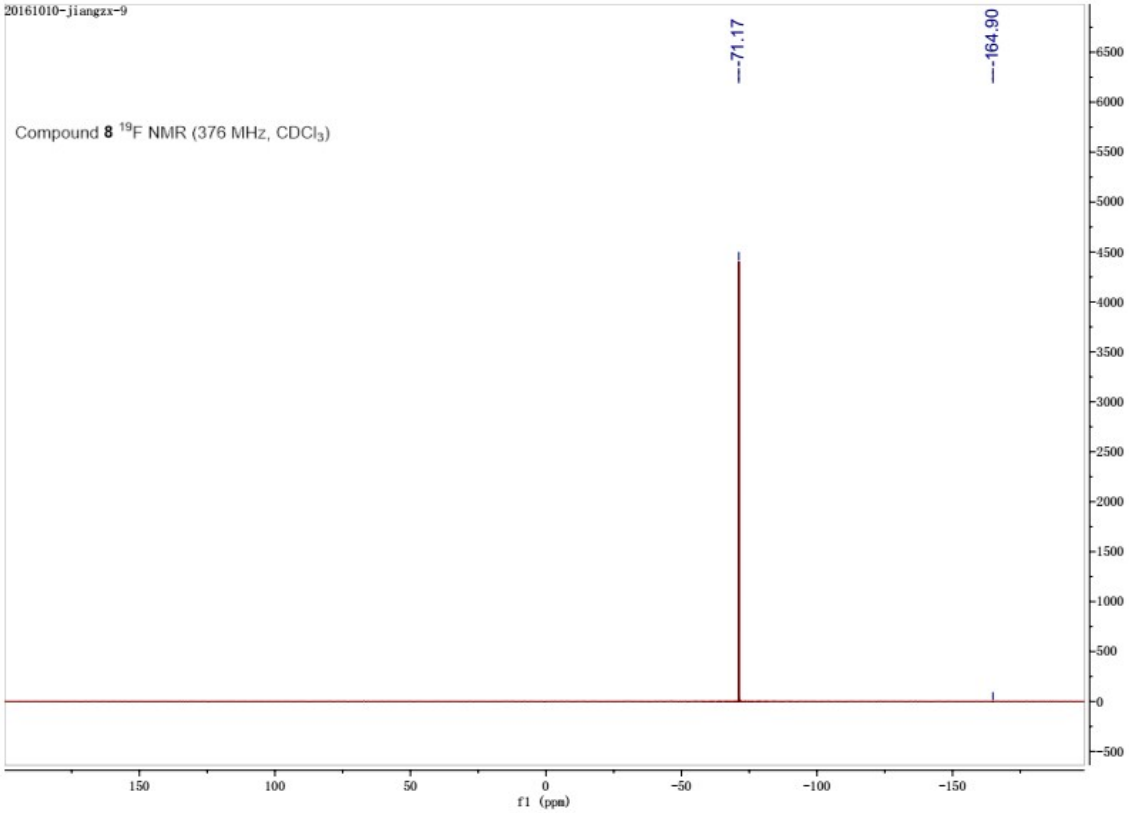
^{19}F NMR spectra of Compound **7** (376 MHz, $CDCl_3$)



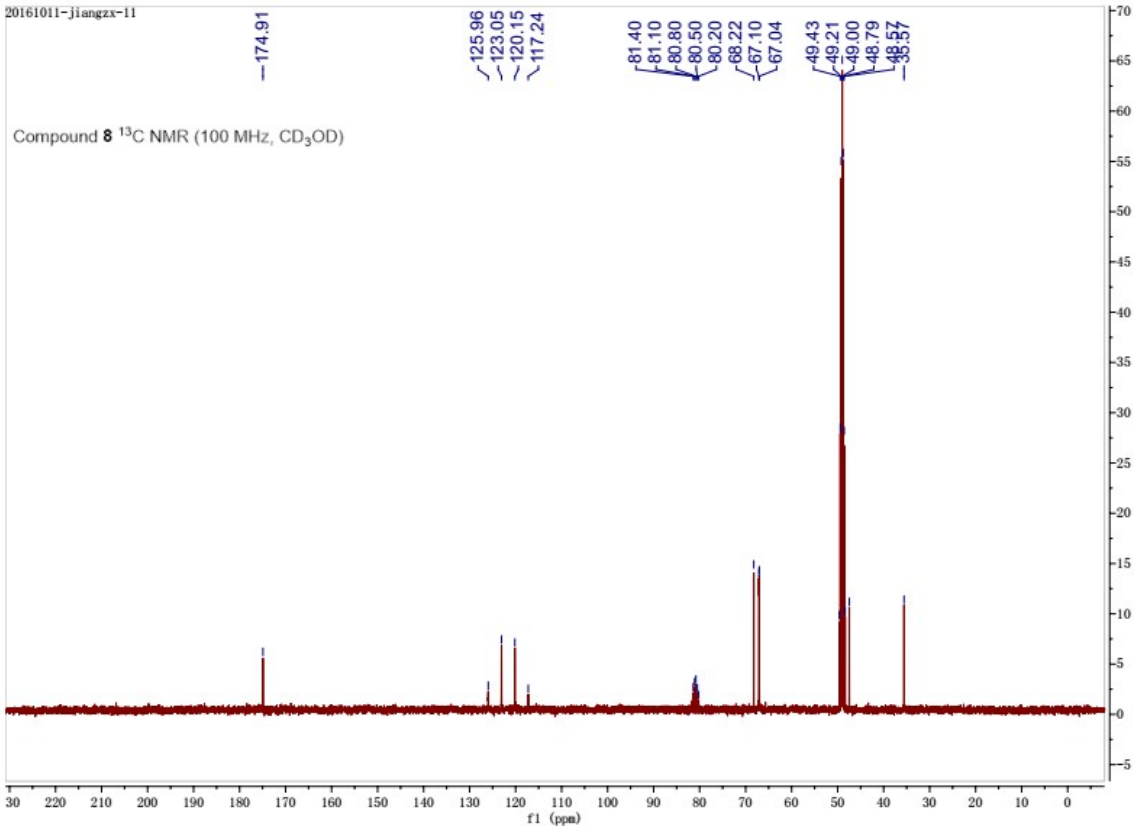
^{1}H NMR spectra of Compound **8** (400 MHz, CD_3OD)



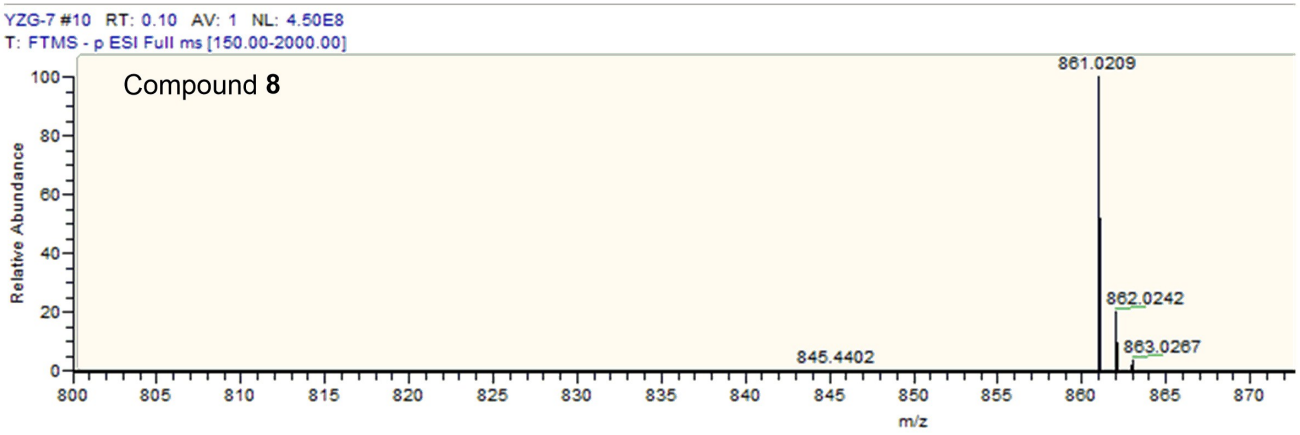
^{19}F NMR spectra of Compound **8** (376 MHz, $CDCl_3$)



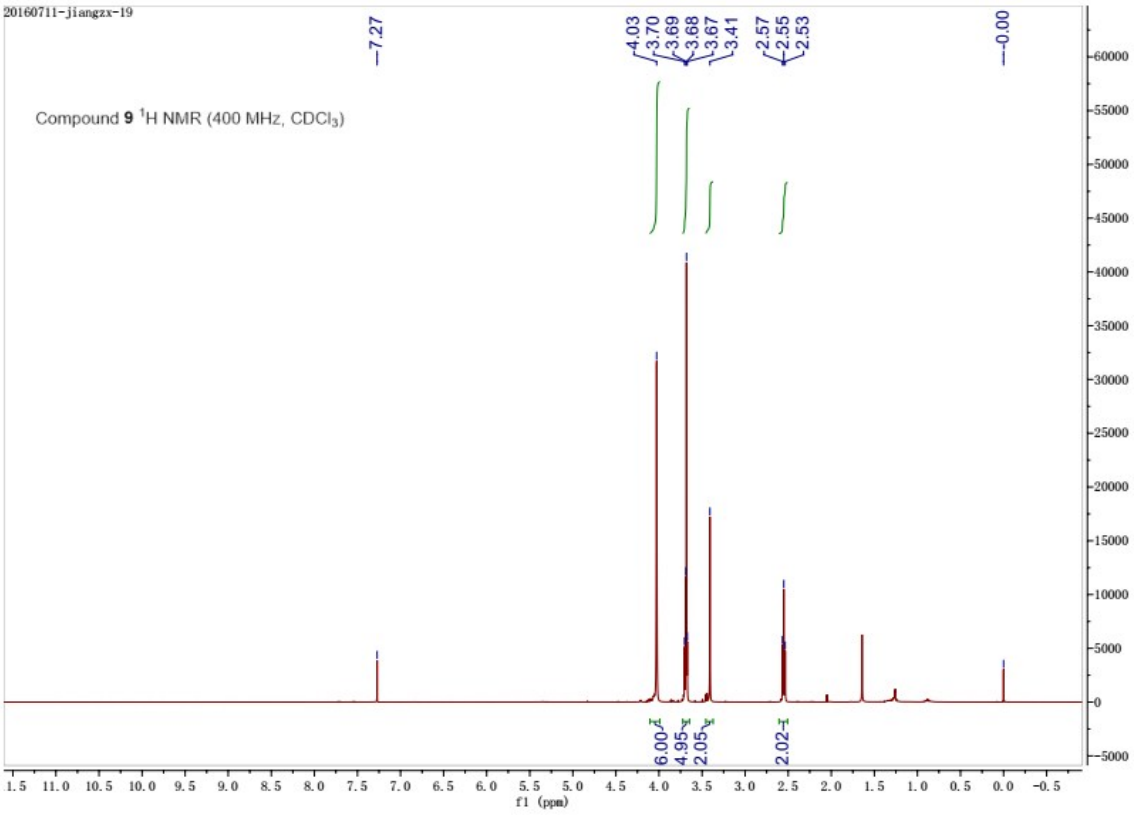
^{13}C NMR spectra of Compound **8** (100 MHz, CD_3OD)



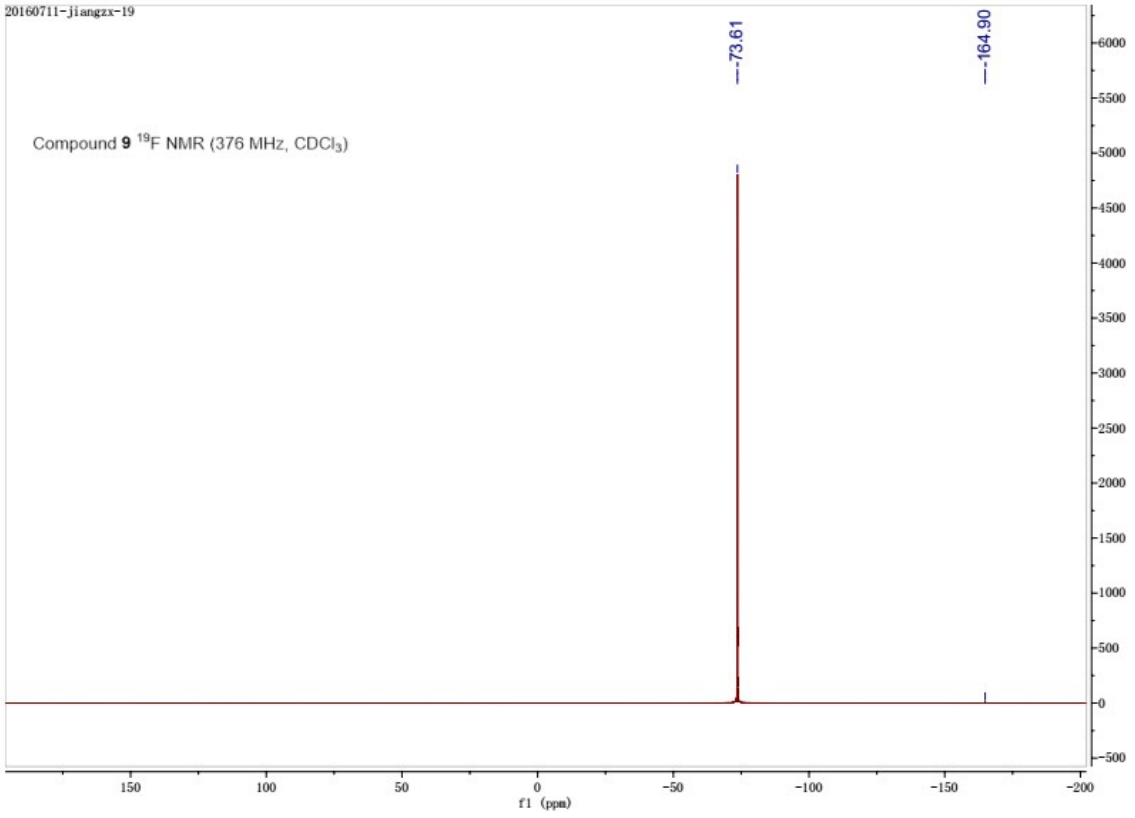
HRMS spectra of Compound **8**



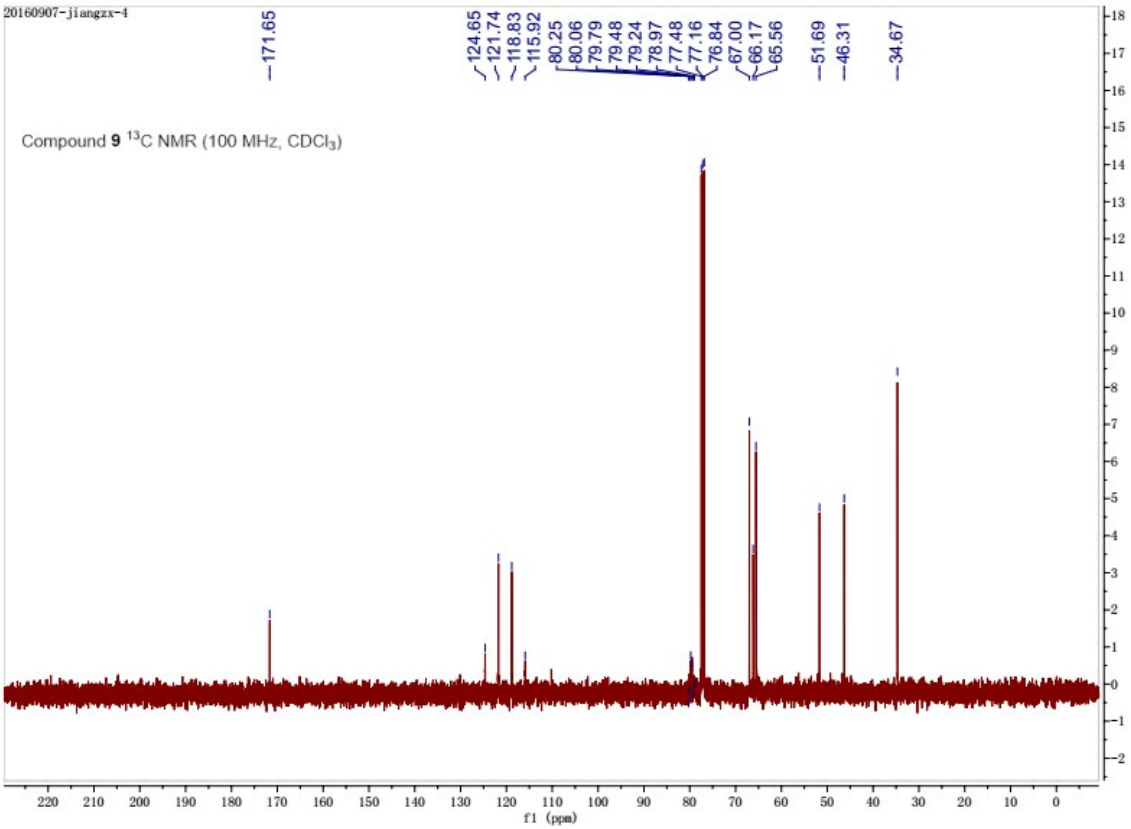
^{1}H NMR spectra of Compound **9** (400 MHz, $CDCl_3$)



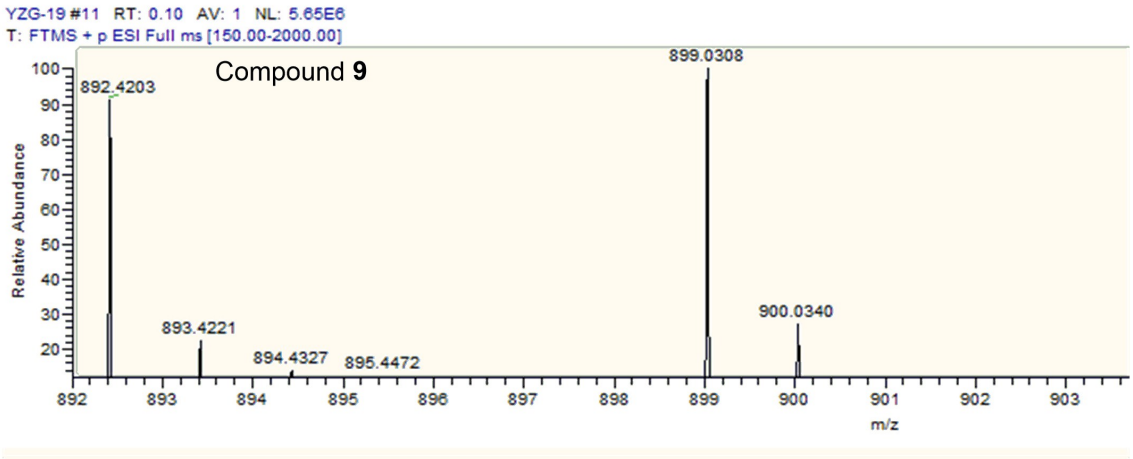
^{19}F NMR spectra of Compound **9** (376 MHz, $CDCl_3$)



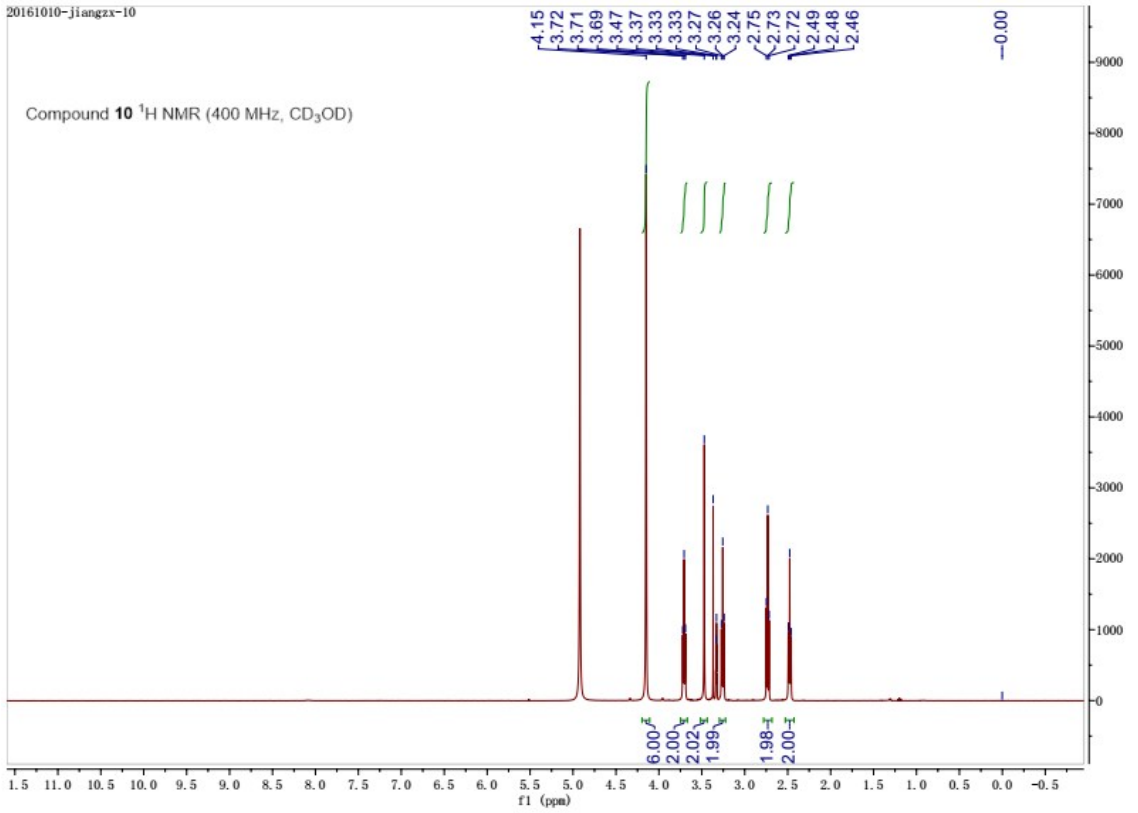
^{13}C NMR spectra of Compound **4** (100 MHz, $CDCl_3$)



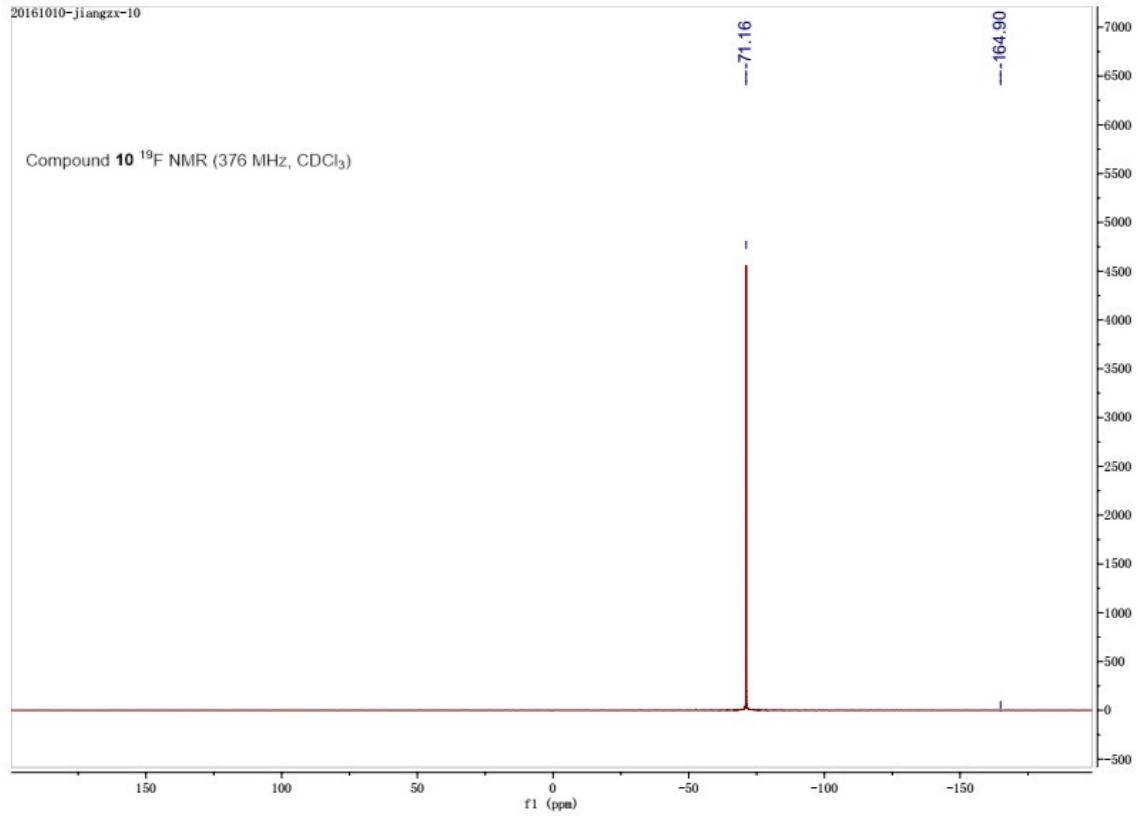
HRMSspectra of Compound **9**



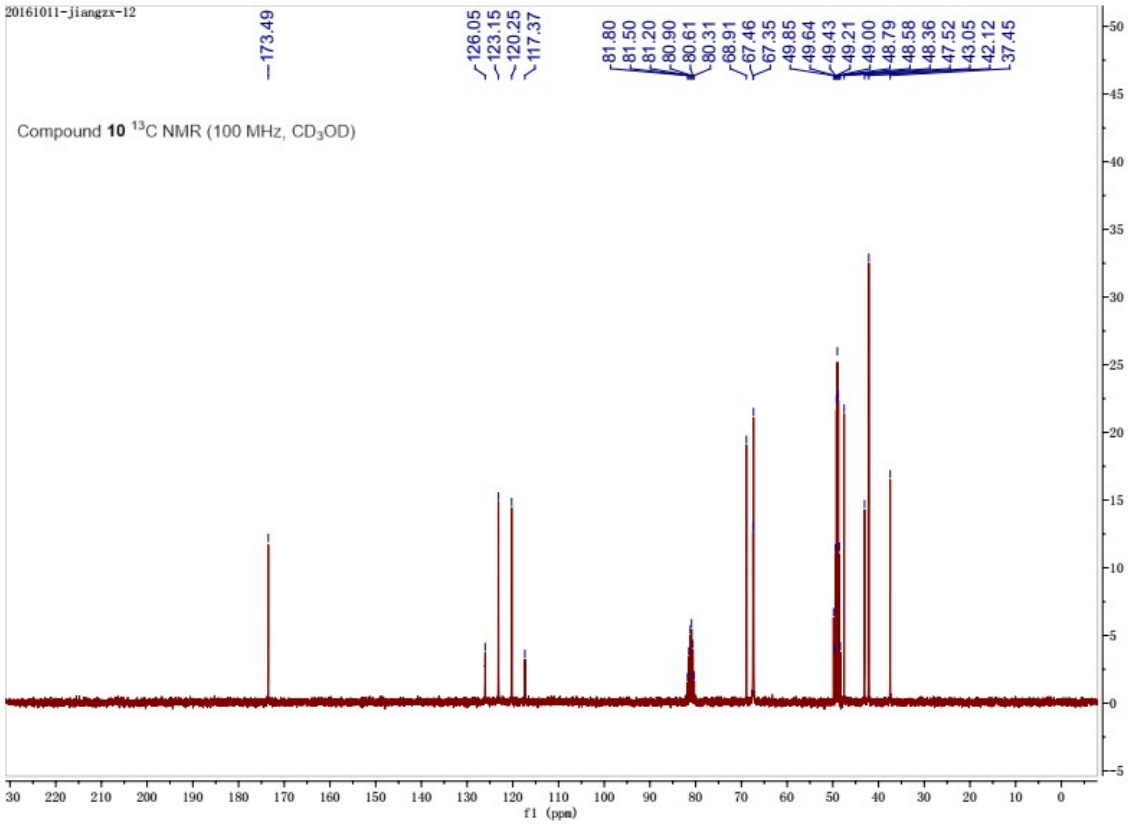
^{1}H NMR spectra of Compound **10** (400 MHz, CD_3OD)



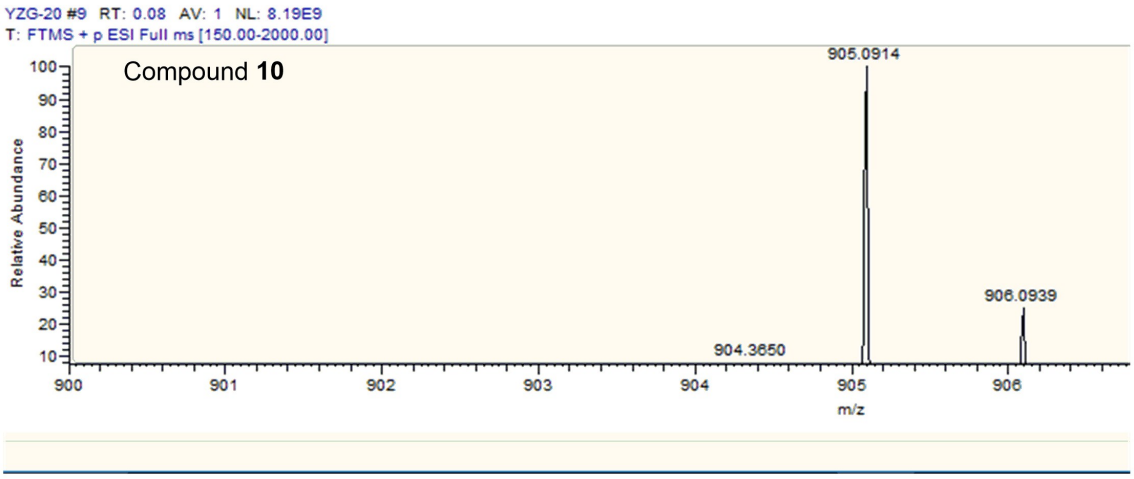
^{19}F NMR spectra of Compound **10** (376 MHz, $CDCl_3$)



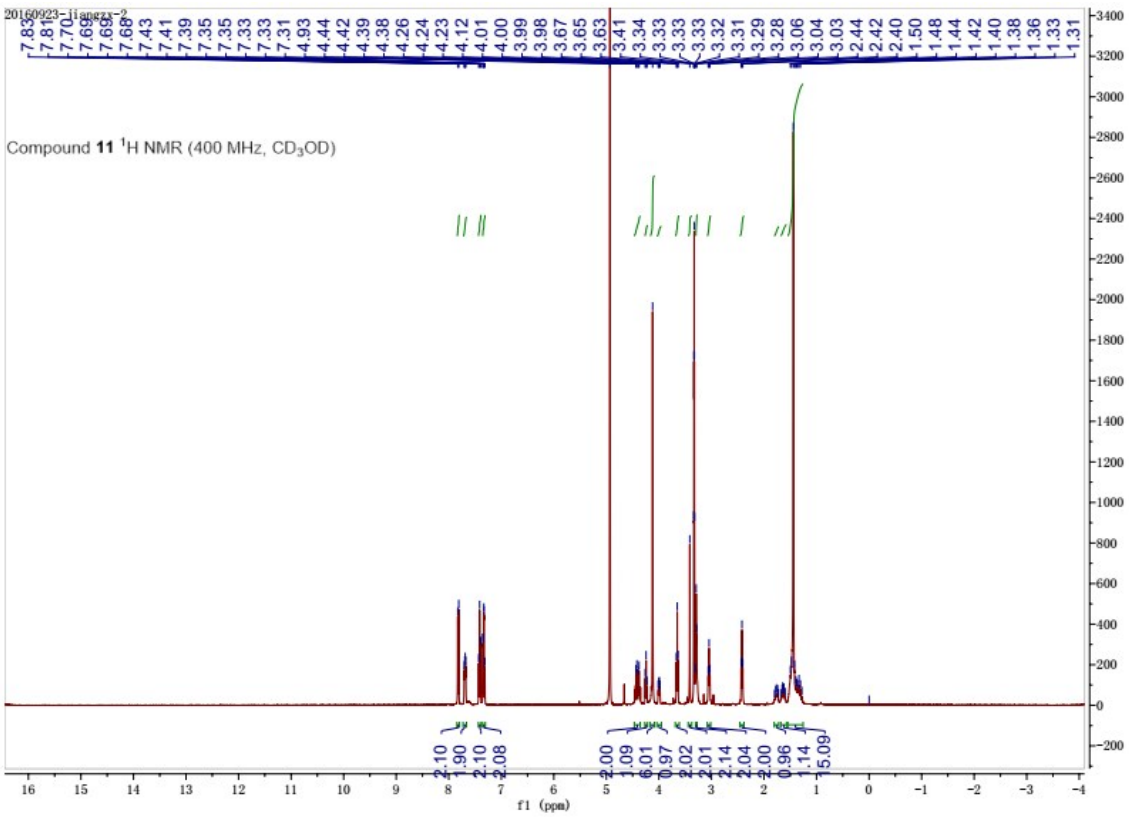
^{13}C NMR spectra of Compound **10** (100 MHz, CD_3OD)



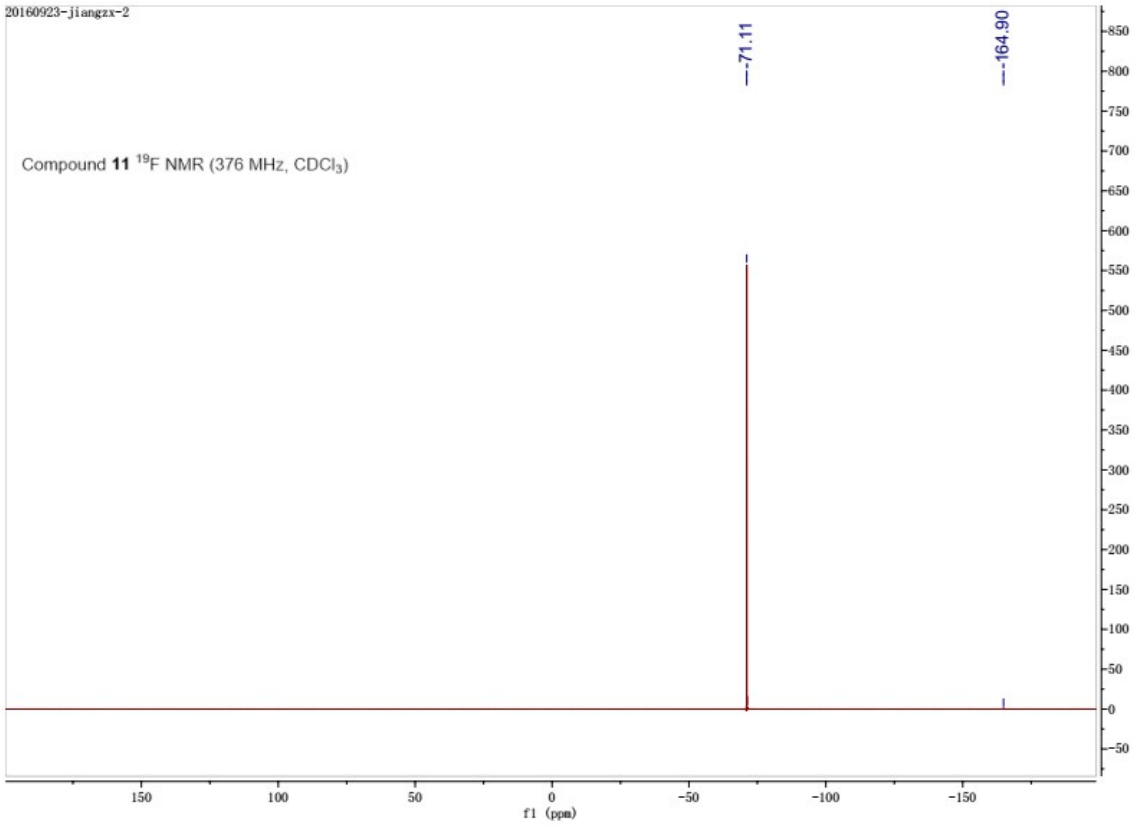
HRMS spectra of Compound **10**



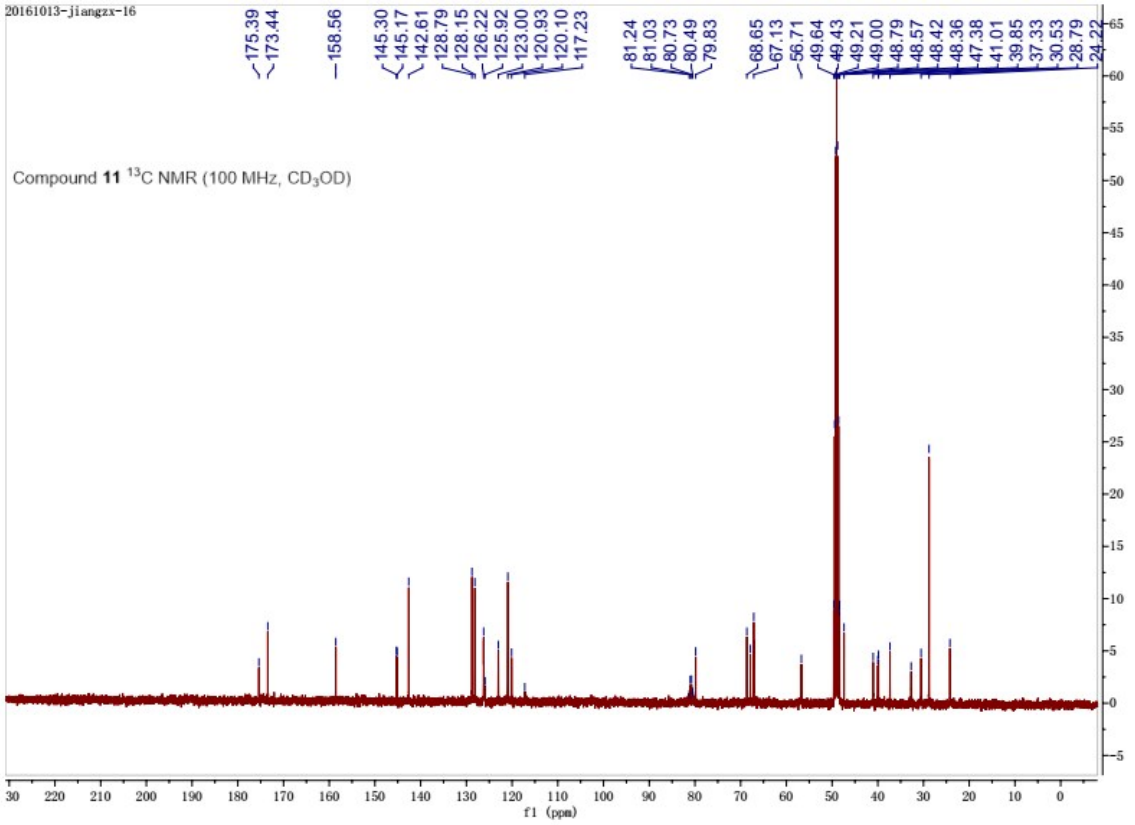
^{1}H NMR spectra of Compound **11** (400 MHz, CD_3OD)



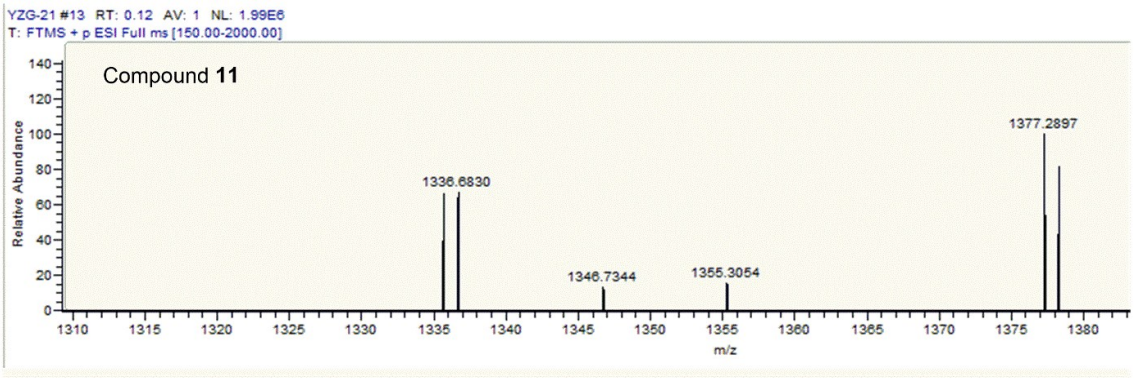
^{19}F NMR spectra of Compound **11** (376 MHz, $CDCl_3$)



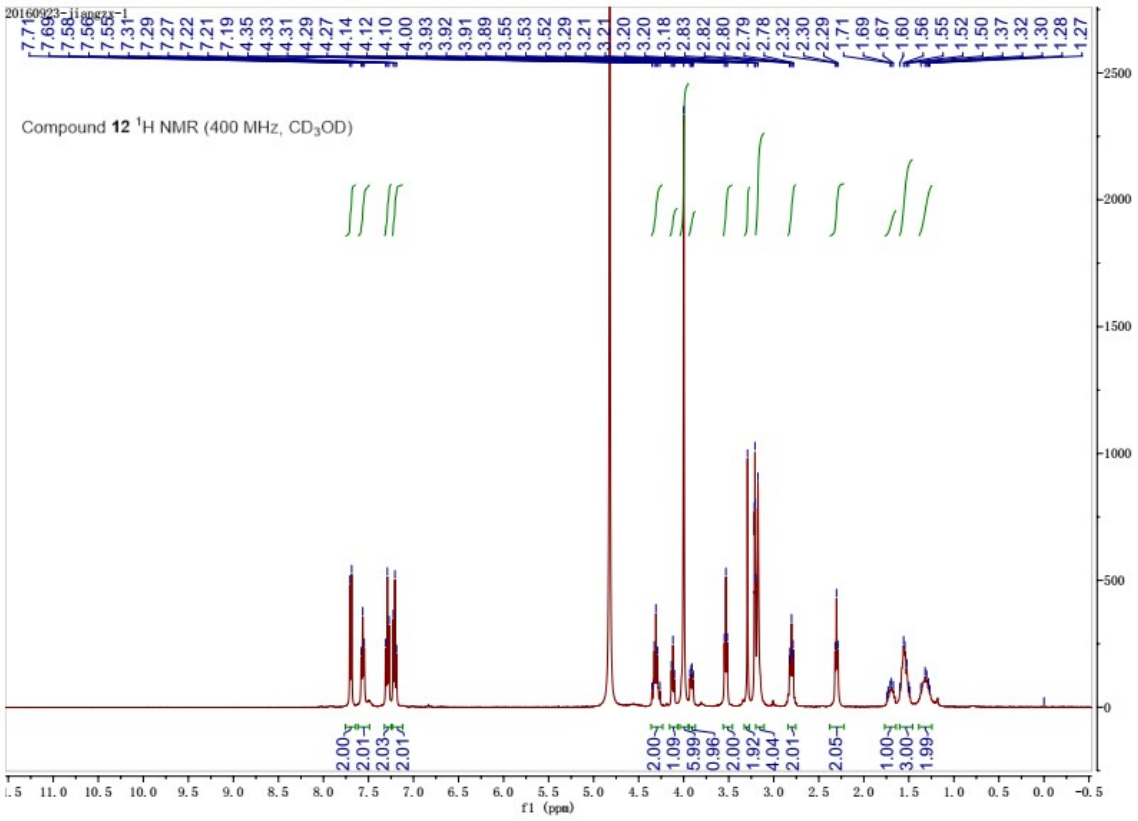
^{13}C NMR spectra of Compound **11** (100 MHz, CD_3OD)



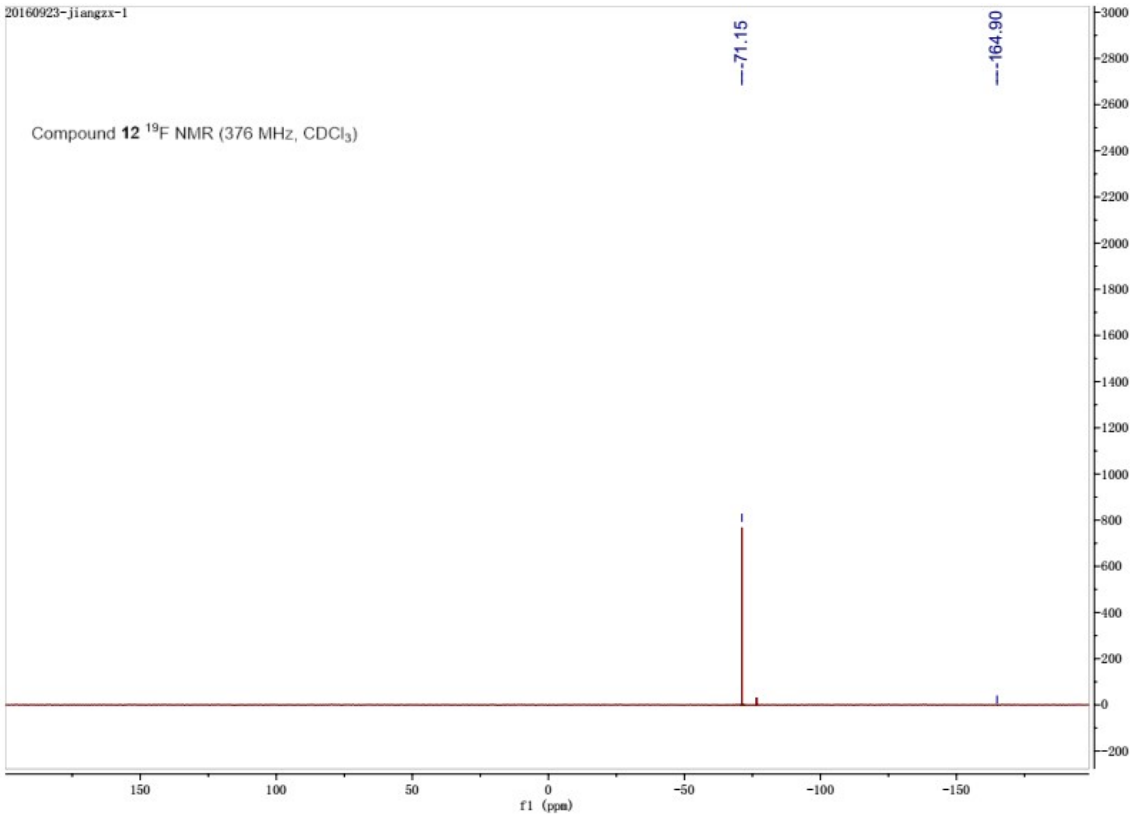
HRMS spectra of Compound **11**



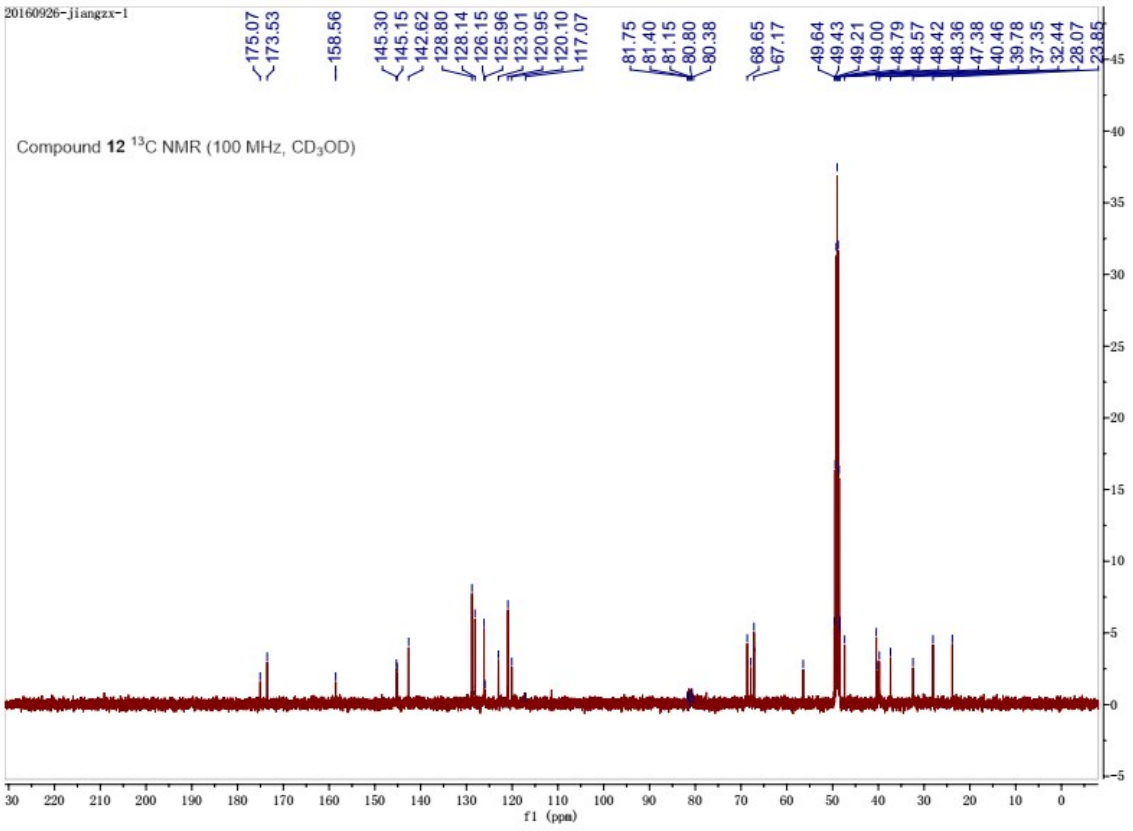
^{1}H NMR spectra of Compound **12** (400 MHz, CD_3OD)



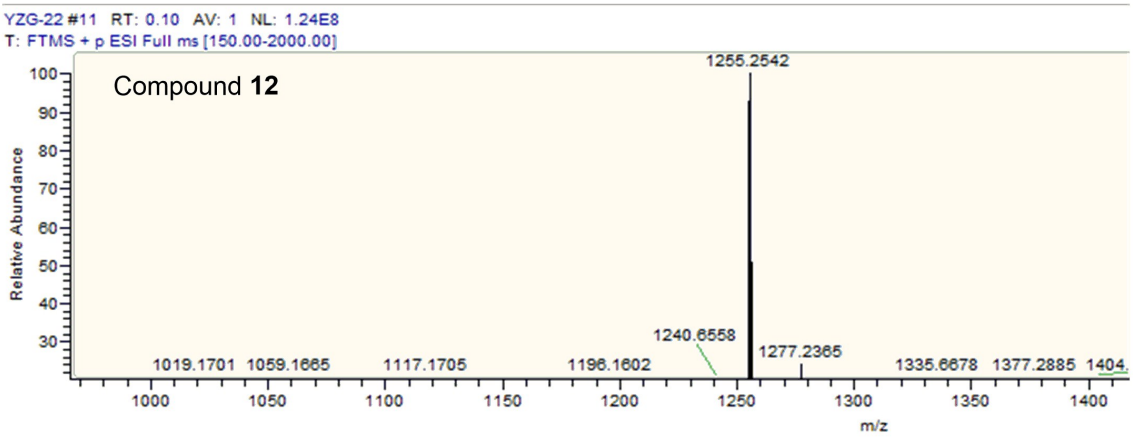
^{19}F NMR spectra of Compound **12** (376 MHz, $CDCl_3$)



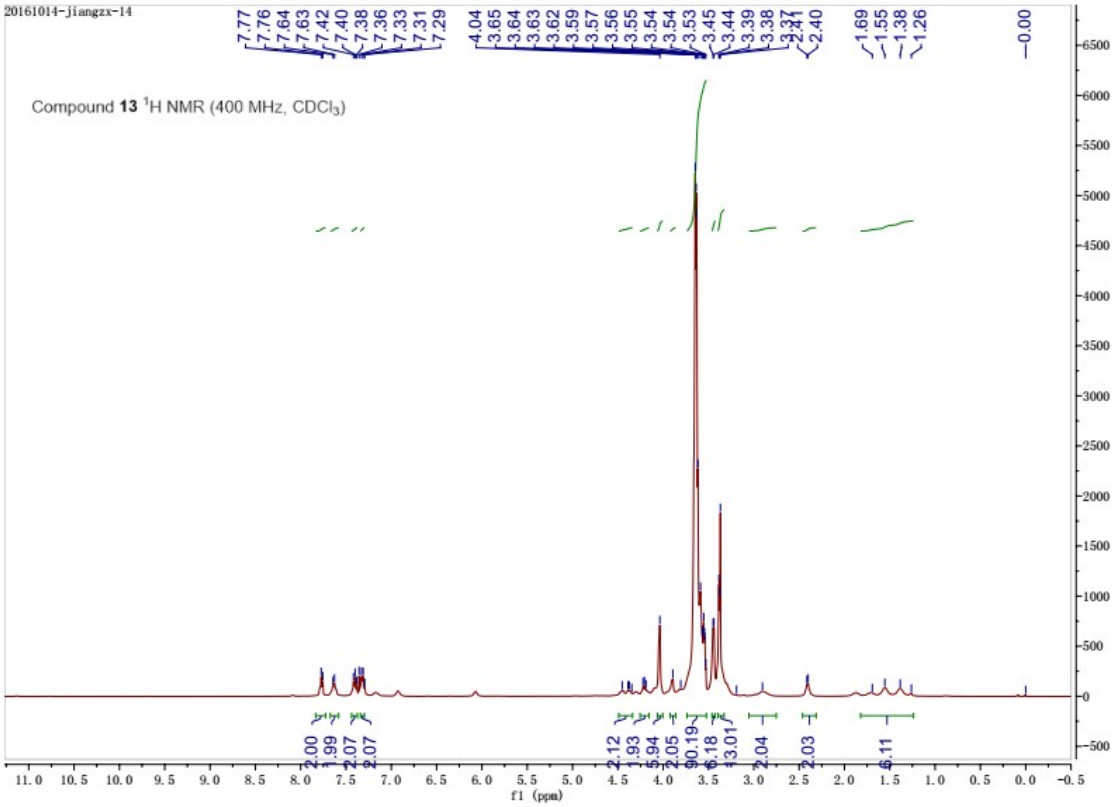
^{13}C NMR spectra of Compound **12** (100 MHz, CD_3OD)



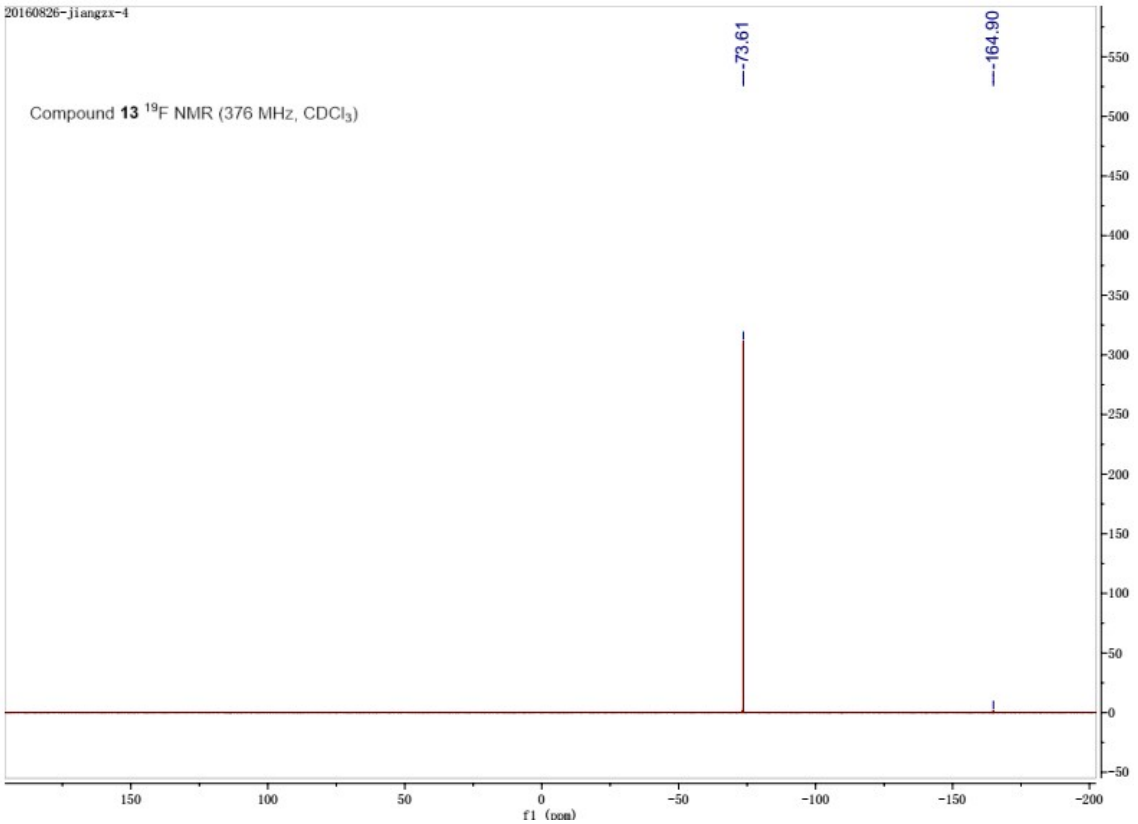
HRMS spectra of Compound **12**



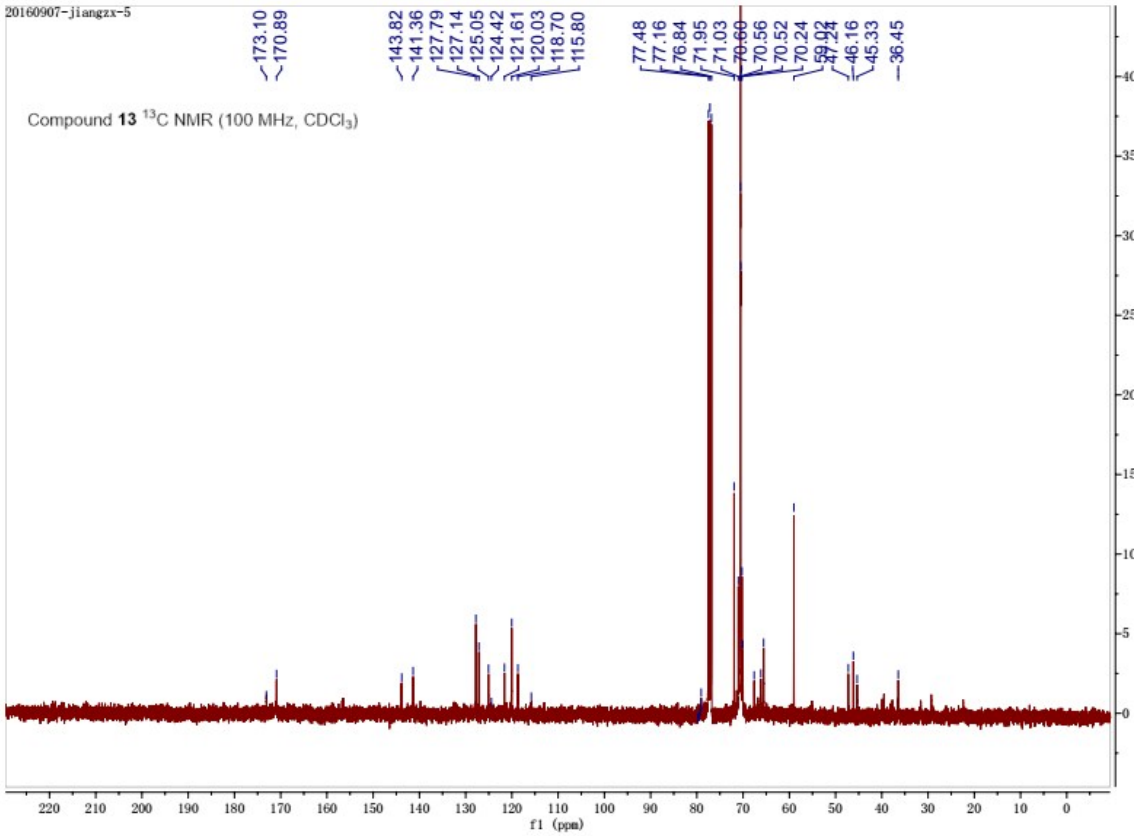
^{1}H NMR spectra of Compound **13** (400 MHz, $CDCl_3$)



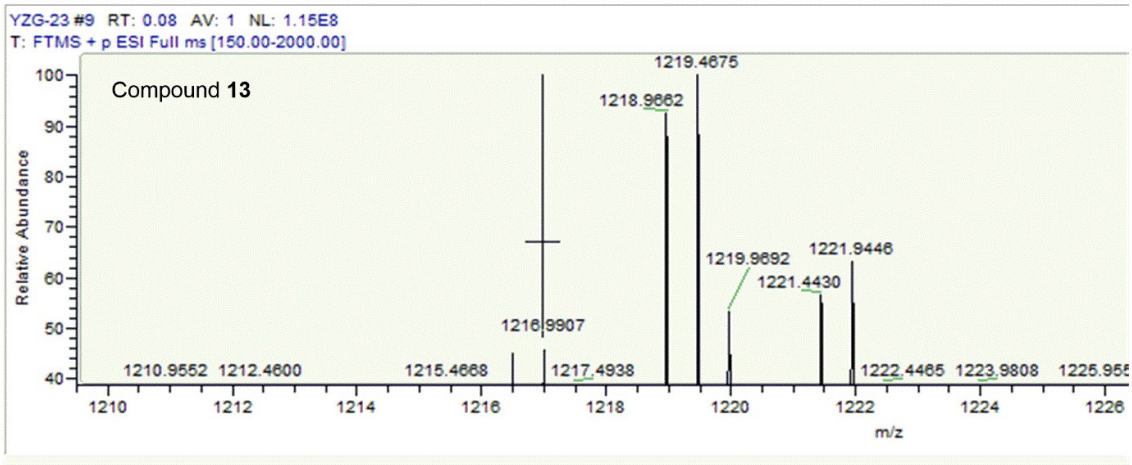
^{19}F NMR spectra of Compound **13** (376 MHz, $CDCl_3$)



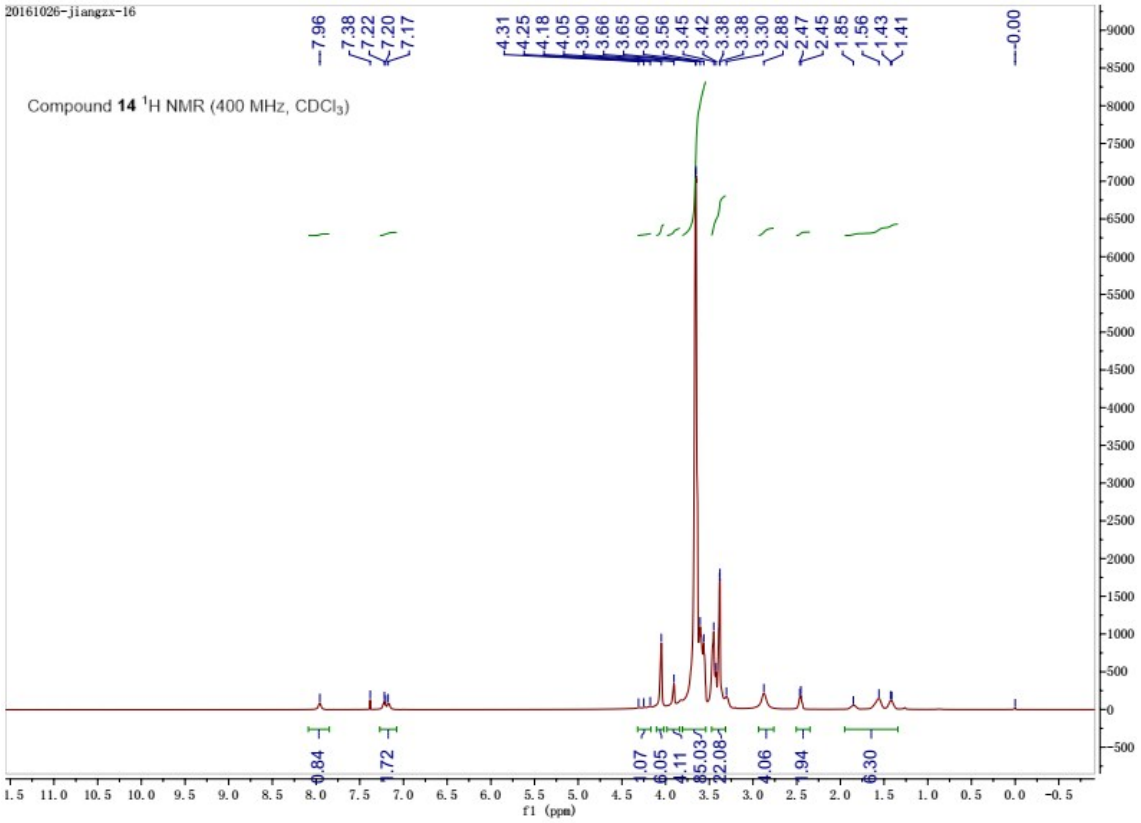
^{13}C NMR spectra of Compound **13** (100 MHz, $CDCl_3$)



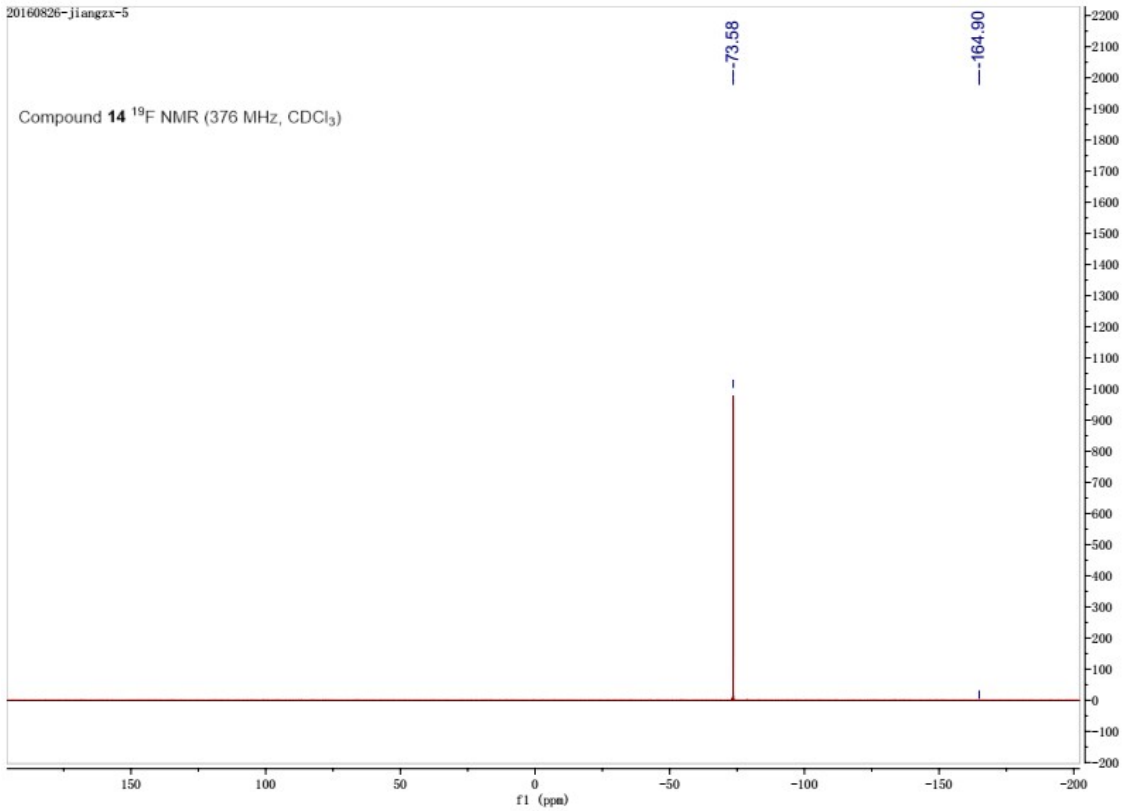
HRMS spectra of Compound **13**



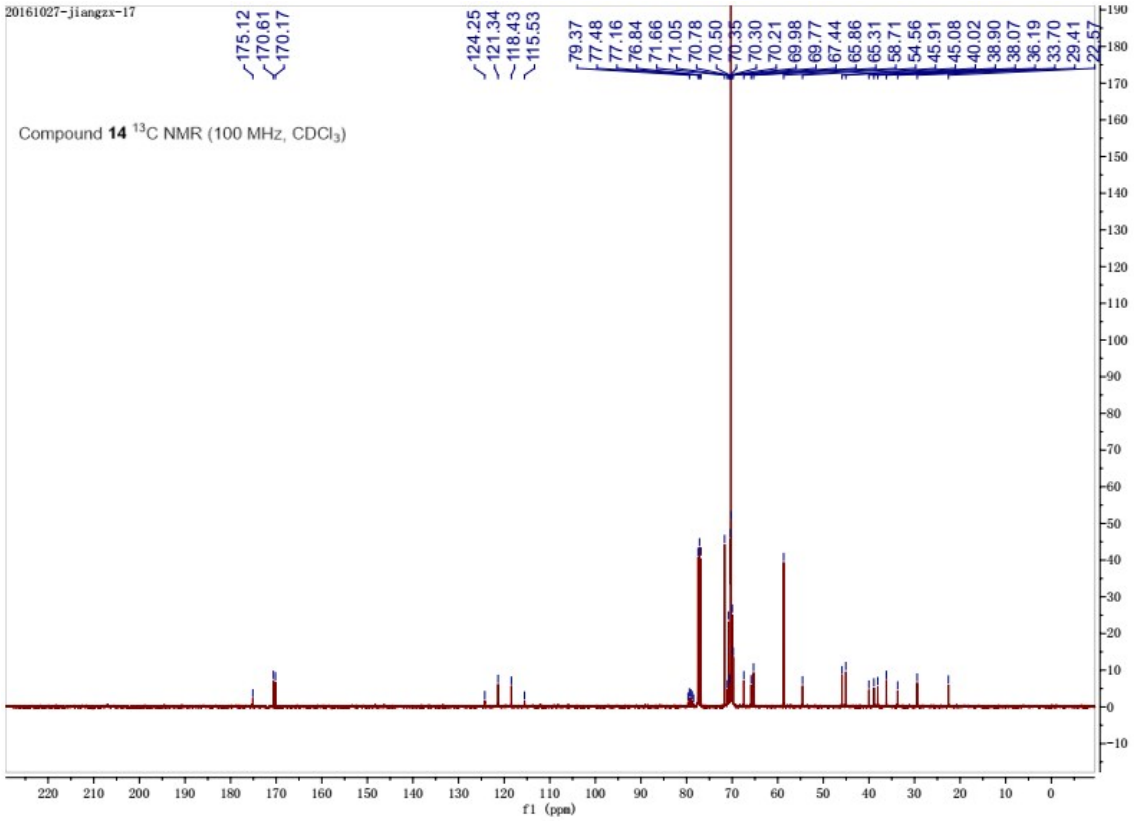
^{1}H NMR spectra of Compound **14** (400 MHz, $CDCl_3$)



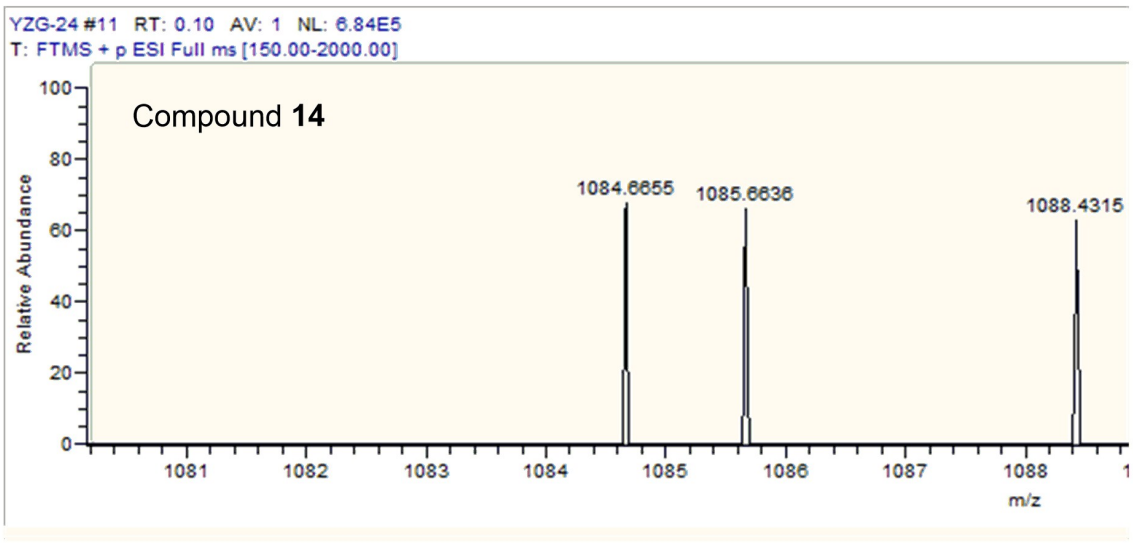
^{19}F NMR spectra of Compound **14** (376 MHz, $CDCl_3$)



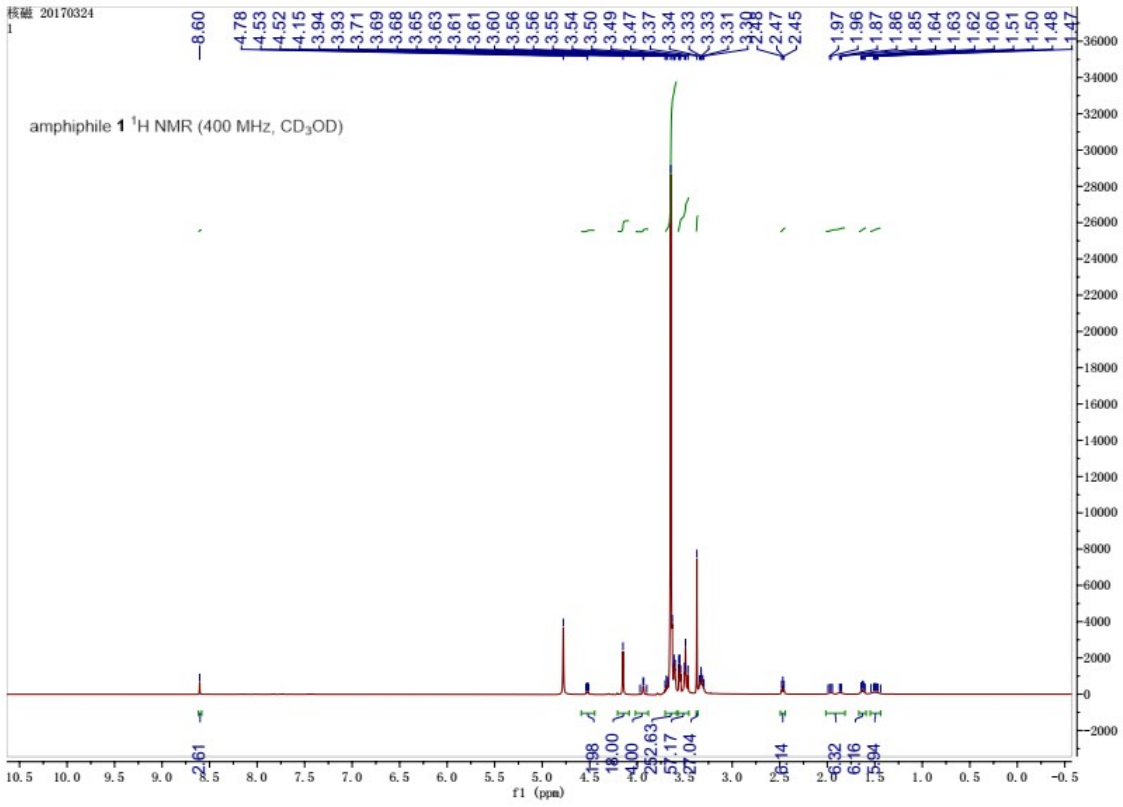
^{13}C NMR spectra of Compound **14** (100 MHz, $CDCl_3$)



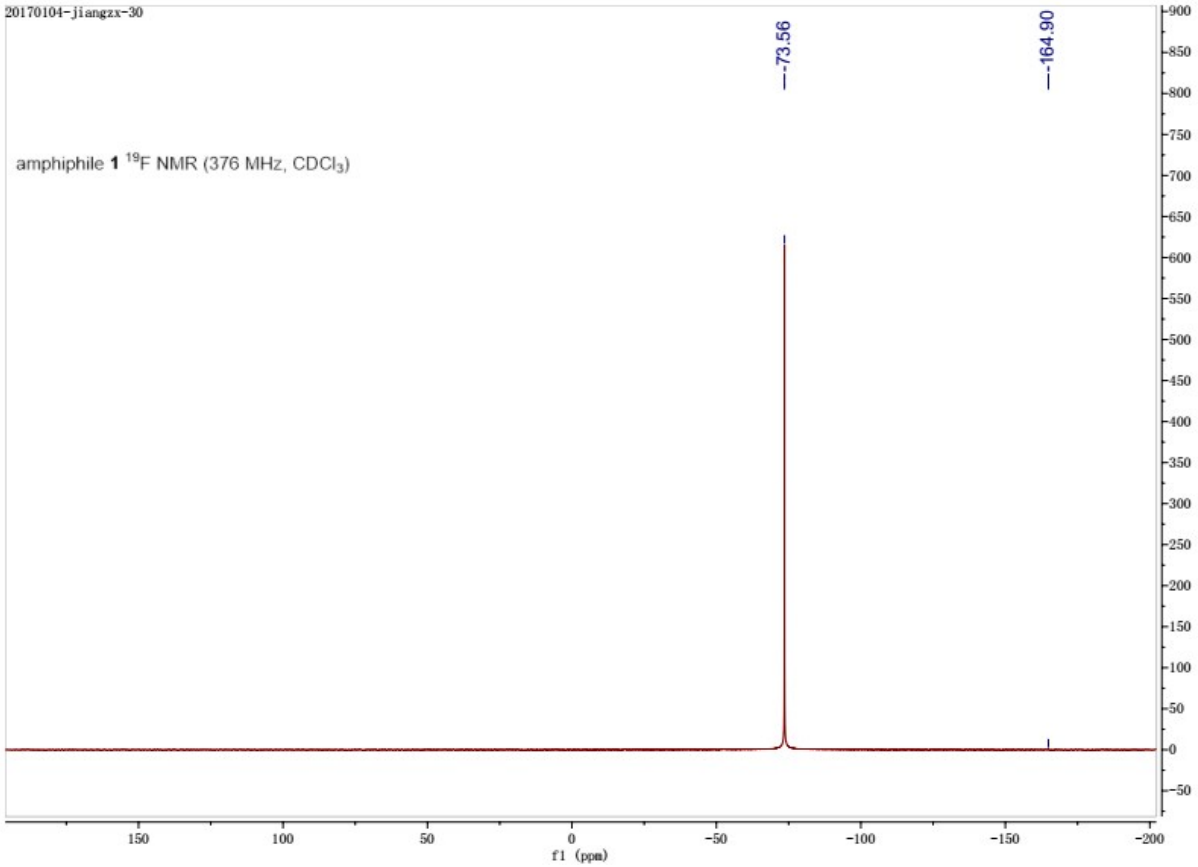
HRMS spectra of Compound **14**



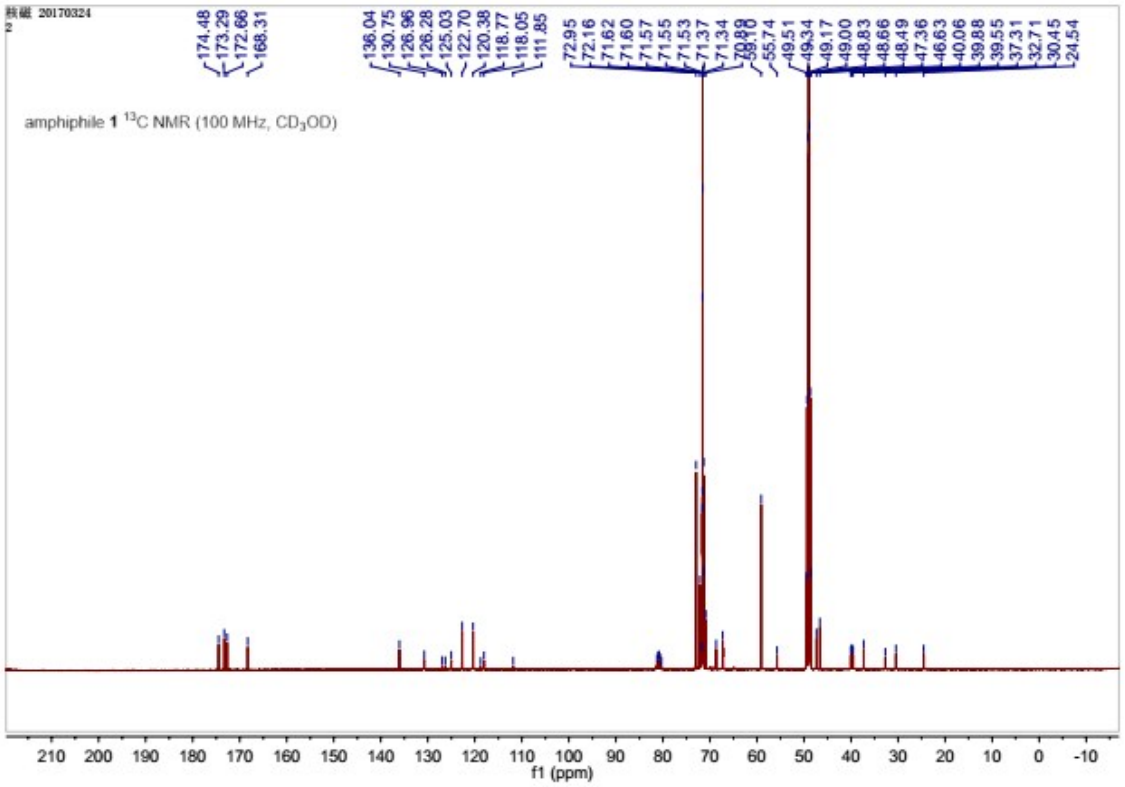
^{1}H NMR spectra of amphiphile **1** (400 MHz, CD_3OD)



^{19}F NMR spectra of amphiphile **1** (376 MHz, $CDCl_3$)



^{13}C NMR spectra of amphiphile **1** (100 MHz, CD_3OD)



MALDI-TOF MS spectra of amphiphile **1**