

Supporting Information

Partially fluorinated nanoemulsions for ^{19}F MRI-fluorescence dual imaging cell tracking

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1. Experimental section

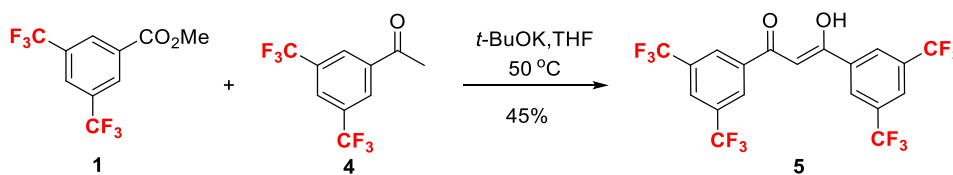
1.1 General information

^1H , ^{19}F , and ^{13}C NMR spectra of compounds were recorded on Bruker AVANCE III 400 MHz or 500 MHz spectrometers. Chemical shifts are in ppm and coupling constants (J) are in Hertz (Hz). ^1H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using acetone- d_6 as solvent. ^{13}C NMR spectra were referenced to solvent carbons (29.84 ppm for acetone- d_6). ^{19}F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in CDCl_3 . The splitting patterns for ^1H NMR spectra are denoted as follows: s (singlet), d (doublet), q (quartet), p (quint).

Phospholipid S75 was purchased from Lipoid GmbH. Pluronic F68 (average MW = 8350) was obtained from Adamas (Shanghai, China). Soybean oil medicinal grade was acquired from Aladdin (Shanghai, China). IR-780 iodide was purchased from J&K Scientific (Beijing, China). DSPE-PEG₂₀₀₀-RGDyC was purchased from Ruixibio (Xian, China). Aza-BODIPY was synthesized in this lab according to the reference (T. Jokic, S.M. Borisov, R. Saf, D.A. Nielsen, M. Kühl, I. Klimant, *Anal. Chem.* 84 (15) (2012) 6723–6730). Human breast adenocarcinoma cell line MCF-7 and Human normal breast epithelial cell line MCF-10A were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Human lung adenocarcinoma cell line A549 was purchased from Beyotime (Shanghai, China). Unless otherwise indicated, all reagents were obtained from commercial supplier and used without prior purification. All solvents were either analytical or HPLC grade. Deionized water was used unless otherwise indicated. THF were dried and freshly distilled prior to use. Column flash chromatography was performed on silica gel (200-300 mesh) with the eluent as indicated in procedures.

Ultrasound bath of 240 W was used for formulation. During the chelation, solutions of chloride of the metal ions were added to the solution of nanoemulsions.

1.2 Synthesis of compound 5



Scheme S1. Synthesis of chelator 5.

Under an argon atmosphere, potassium *tert*-butoxide (1.97 g, 17.58 mmol) was added to a stirring solution of compound **4** (1.5 g, 5.86 mmol) in THF (10 mL). After stirring for 10 min, a solution of compound **1** (1.91 g, 7.03 mmol) in THF (5 mL) was added. The reaction mixture was stirred at 50 °C for 24 h. Subsequently, 2N HCl solution was added to adjust the pH of the solution to neutral. Then THF was evaporated under vacuum and extracted with ethyl acetate (60 mL, 3 times). The combined organic layer was dried over anhydrous Na₂SO₄, concentrated under vacuum and purified by column chromatography on a silica gel (PE/EA=200/1) to give compound **5** as white powder (1.31 g, 45.2% yield). ¹H NMR (500 MHz, Acetone-*d*₆) δ 8.56 (s, 4H), 8.18 (s, 2H), δ 7.74 (s, 1H); ¹⁹F NMR (376 MHz, CDCl₃) δ -66.02 (s); ¹³C NMR (126 MHz, Acetone-*d*₆) δ 184.1, 138.1, 132.9 (q, *J* = 33.7 Hz), 128.9 (d, *J* = 4.0 Hz), 127.1 (p, *J* = 3.7 Hz), 124.2 (q, *J* = 272.2 Hz), 95.9; HRMS (ESI) calcd for C₁₉H₇F₁₂O₂⁻ ([M-H]⁻), 495.0254, found, 495.0263.

1.3 ¹⁹F NMR of agent **1** and chelator **5** mixture

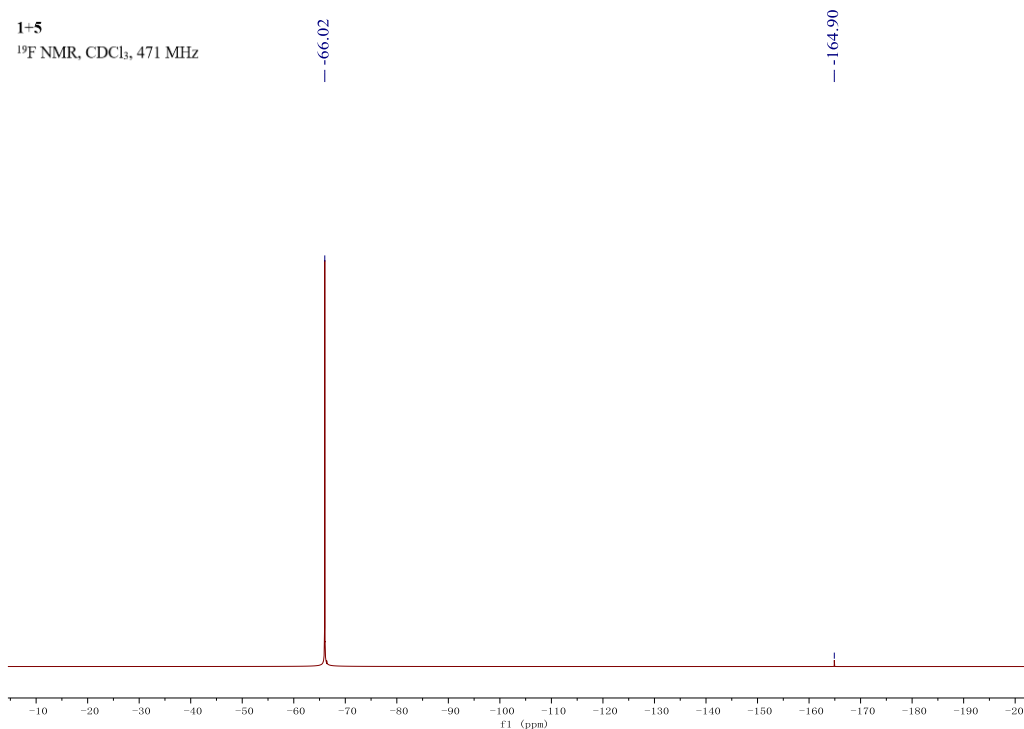


Figure S1. ¹⁹F NMR of imaging agent **1** and chelator **5** mixture.

1.4 Preparation and characterization of E1-E20

Preparation of **E1-E8**. Lecithin, Egg Yolk Lecithin E80, Soybean oil, Saffron oil, Phospholipid lipid S75 and Pluronic F68 in dichloromethane were prepared. To a solution of Lecithin (or E80, Soybean oil, Saffron oil, S75) and F68 in dichloromethane was added compound **1** (109 mg). De-

ionized water (4 mL) was added to this mixture under stirring. The mixture was stirred for 4 h and repeated three times the process of 10 min ultrasound bath and 1 h stirring. During this process, dichloromethane was volatilized. The nanoemulsion was obtained by filtered through a 0.2 μm syringe filter.

Preparation of **E9-E13**. Stock solutions of Phospholipid lipid S75 and Pluronic F68 in dichloromethane were prepared. The solution of compound **7** (IR-780) was prepared in dichloromethane. Compound **5** was dissolved in compound **1**. To a solution of Phospholipid lipid S75 (80 mg) and Pluronic F68 (40 mg) in dichloromethane was added compound **1** (109 mg) with compound **5** (1 mg) and **7** (2 mg). De-ionized water (4 mL) was added to this mixture under stirring. The mixture was stirred for 4 h and repeated 3 times the process of 10 min ultrasound bath and 1 h stirring. The nanoemulsion was filtered through a 0.2 μm syringe filter. The nanoemulsion chelated with metal ions was obtained by adding an aqueous solution of ferric chloride ($n_5 : n_{\text{Fe}^{3+}} = 4 : 1$) to the nanoemulsion and stirring for 24 hours. **E12** were incubated with DSPE-PEG₂₀₀₀-RGDyC ($n_{\text{phospholipid}} : n_{\text{DSPE-PEG}_{2000}\text{-RGDyC}} = 20 : 1$) on a rotary shaker at 25 °C for 1 h to provide RGD modified nanoemulsion **E13**.

Preparation of **E14-E18**. Stock solutions of Pluronic F68 (1% w/w) in deionized water and Phospholipid lipid S75 (20% w/v) in dichloromethane were prepared. The solution of compound **8** (aza-BODIPY) was prepared in dichloromethane. To a solution of Lipoid S75 (40 mg) in dichloromethane was added compound **1** (109 mg) with compound **5** (1 mg) and aza-BODIPY (1 mg). The mixture was transferred to a 100 mL round bottom flask with soybean oil (100 mg) and subjected to solvent removal under vacuum at 37 °C and 50 rpm for 10 min and form a thin film. The deionized water (4 mL) of Pluronic F68 (40 mg) was added to the reaction flask under ultrasound. The crude nanoemulsion was sonicated in a water bath for 10 min and stirred for 24 h, followed by filtration through a 0.2 μm syringe filter. The nanoemulsion **E16** and **E18** chelated with metal ions was obtained by adding an aqueous solution of ferric chloride ($n_5 : n_{\text{Fe}^{3+}} = 4 : 1$) to the crude nanoemulsion and stirring for 24 hours.

Preparation of **E19-E20**. Stock solutions of Pluronic F68 (1% w/w) in deionized water and Phospholipid lipid S75 (20% w/v) in dichloromethane were prepared. The solution of compound **8** (aza-BODIPY) was prepared in dichloromethane. Perfluoro-15-crown-5 (109 mg), compound **5** (1

mg) and aza-BODIPY (1 mg) were successively added to a solution of Lipoid S75 (40 mg). The mixture was transferred to a 100 mL round bottom flask with soybean oil (100 mg) and subjected to solvent removal under vacuum at 37 °C and 50 rpm for 10 min to remove the solvent and form a thin film. The deionized water (4 mL) of Pluronic F68 (40 mg) was added to the reaction flask under ultrasound. The crude nanoemulsion was sonicated in a water bath for 10 min and cell disruptor for 5min, followed by filtration through a 0.2 μm syringe filter. The nanoemulsion **E20** chelated with metal ions was obtained by adding an aqueous solution of ferric chloride ($n_5 : n_{Fe^{3+}} = 4 : 1$) to the crude nanoemulsion **E19** and stirring for 24 hours.

The size distribution of **E1-E20** were measured by DLS (Nano ZS 90, Malvern, UK) and TEM (negative staining with phosphotungstic acid at 1%, w/v). All nanoemulsions were diluted at 1:100 v/v ratio with water and measurements were taken after equilibrating at room temperature for at least 30 min prior to each measurement. Nanoemulsion **E17** were monitored by DLS dispersed in water and cell culture medium (DMEM) containing 10% fetal bovine serum at room temperature for 14 days.

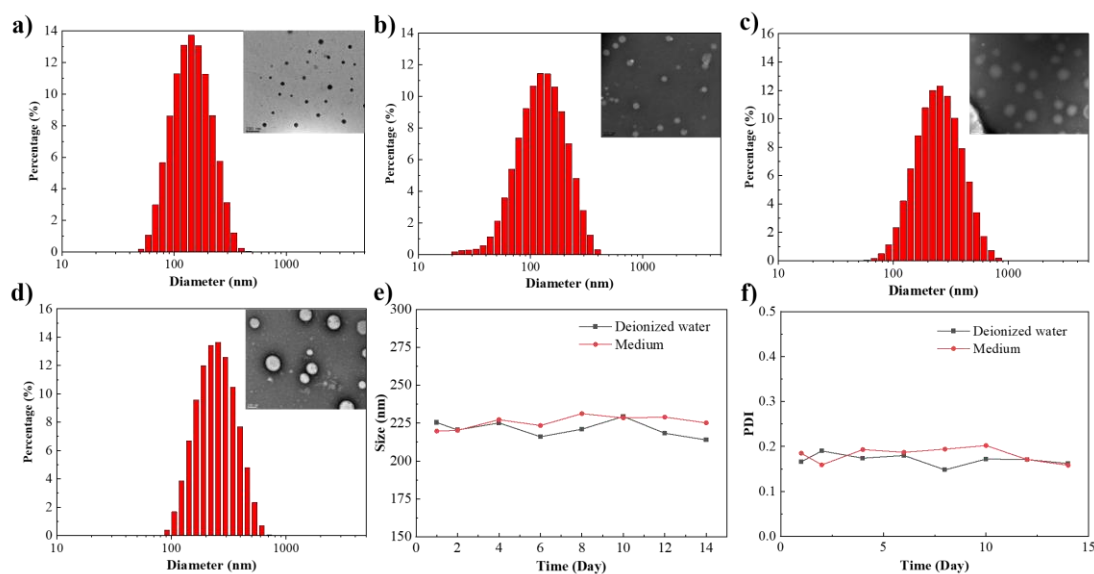


Figure S2. DLS and TEM images of **E10** (a), **E12** (b), **E17** (c) and **E18** (d), and the plots of **E17** particle size (e) and PDI (f) over 14 days at deionized water and medium.

1.5 ^{19}F relaxation times of nanoemulsions E14-E20.

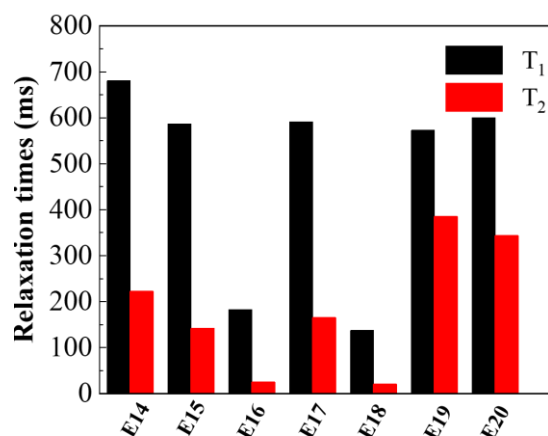


Figure S3. ^{19}F relaxation times of nanoemulsions E14 to E20.

1.6 Quantification of fluorine content in the nanoemulsion

The fluorine content of nanoemulsions were determined by the ^{19}F -NMR spectrum internal standard method. The nanoemulsion E10 was diluted with deionized water to a series of concentrations, adding 10% sodium trifluoromethanesulfonate in D_2O solution (120 mM) as an internal standard to calibrate the concentration of the nanoemulsion sample. Comparing the integrals of the peaks, it was possible to assay the fluorine content of the sample.

1.7 ^{19}F MRI Phantom experiments

All ^{19}F MRI phantom experiments were performed on a 400 MHz Bruker BioSpec MRI system at 24 °C. Nanoemulsions E10, E12, E17 and E18 were serially diluted with water to give a series of ^{19}F concentrations: 160 mM, 80 mM, 40 mM, 20 mM, 10 mM, 5 mM, and 2.5 mM, respectively.

For E10 and E12, the ^{19}F density-weighted ^{19}F MRI phantom images were acquired by using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32×32 , SI = 20 mm, FOV = 3.0×3.0 cm, TR = 4000 ms, TE = 3 ms, NS=8, scan time = 256 s. For T₁-weighted ^{19}F MRI phantom images, a gradient-echo (GRE) pulse sequence was used with the following parameters (method = RARE, matrix size = 32×32 , SI = 20 mm, FOV = 3.0×3.0 cm, TR = 100 ms, TE = 3 ms, NS=256, scan time = 819 s).

For **E17** and **E18**, the ^{19}F density-weighted ^{19}F MRI phantom images were acquired by using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32×32 , SI = 20 mm, FOV = 3.0×3.0 cm, TR = 2500 ms, TE = 3.0 ms, NS = 8, scan time = 160 s. For T_1 -weighted ^{19}F MRI phantom images, a gradient-echo (GRE) pulse sequence was used with the following parameters (method = RARE, matrix size = 32×32 , SI = 20 mm, FOV = 3.0×3.0 cm, TR = 100 ms, TE = 3 ms, NS=200, scan time = 160 s).

1.8 Cellular uptake study and cytotoxicity assay

A549 cells, MCF-10A cells and MCF-7 cells were cultured in DMEM-High glucose medium with fetal bovine serum and 1% penicillin-streptomycin. All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2 .

About 2×10^5 cells (A549 and MCF-7) were seeded on 2 cm cell culture dishes for 24 h. Culture medium containing the IR-780 (10 $\mu\text{g}/\text{mL}$) or BODIPY (10 $\mu\text{g}/\text{mL}$) labelled nanoemulsions (**E12**, **E13** and **E18**) was added. After 0.5 h (1h, 2h, 6h and 12h) of incubation, cells were washed with PBS, fixed with 4% paraformaldehyde for 15 min. Then fixed cells were stained by 200 μL 4,6-diamino-2-phenylindole (DAPI) for 10 min and washed with PBS at least three times. Finally, cells were imaged by CLSM.

The *in vitro* cell cytotoxicity was evaluated by cell counting (CCK-8) assay. About 1×10^4 cells (A549, MCF-7 and MCF-10A, respectively) were seeded per well in 96-well plates ($n = 3$) and cultured for 24 h. Nanoemulsions (**E12** and **E17-E20**) were diluted with the medium to certain concentration and added to each well, respectively. After incubated for 12 h, cells were washed with PBS (pH 7.4) two times. 100 μL of CCK-8 (10% v/v) solution was added to each well and incubated for another 2 h. Finally, the absorbance at 450 nm was measured with a microplate reader.

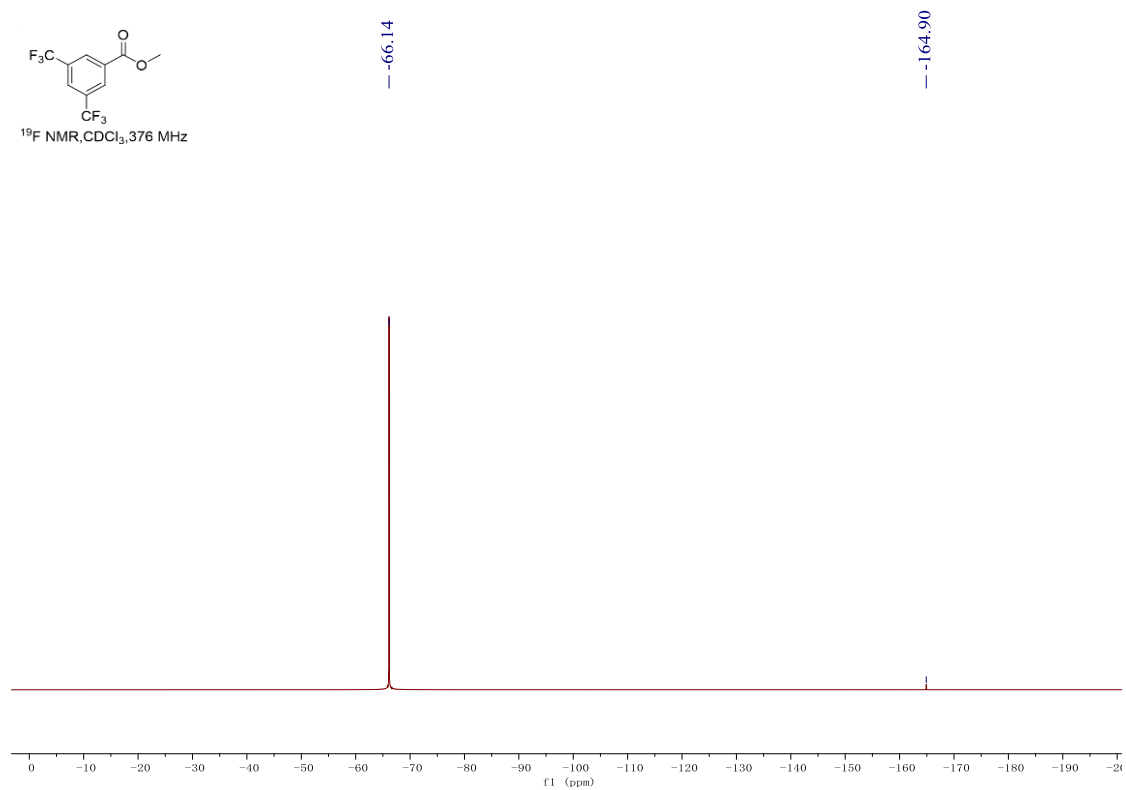
Cell viability (%) was calculated as the formula:

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

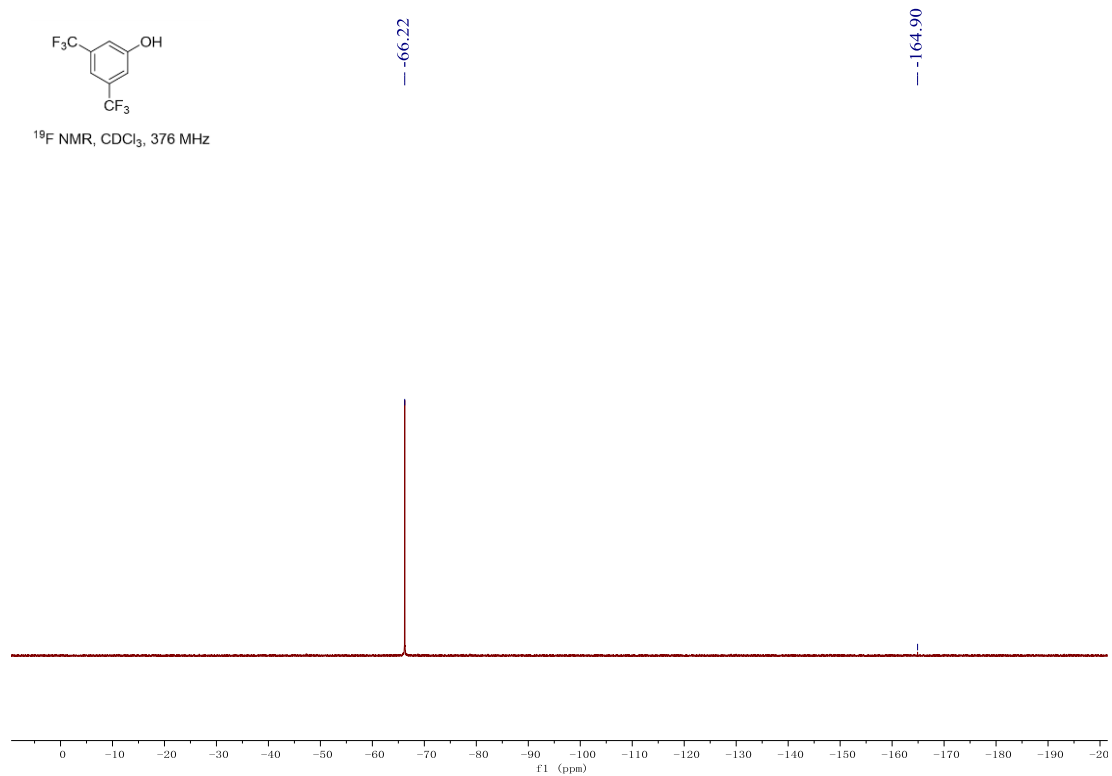
A_{Test} , A_{Control} and A_{Blank} represented the absorbance of cells with different treatments, untreated cells and PBS buffer solution, respectively.

1.9 Copies of ^1H , ^{13}C , ^{19}F NMR and HRMS spectra of compounds 1-5

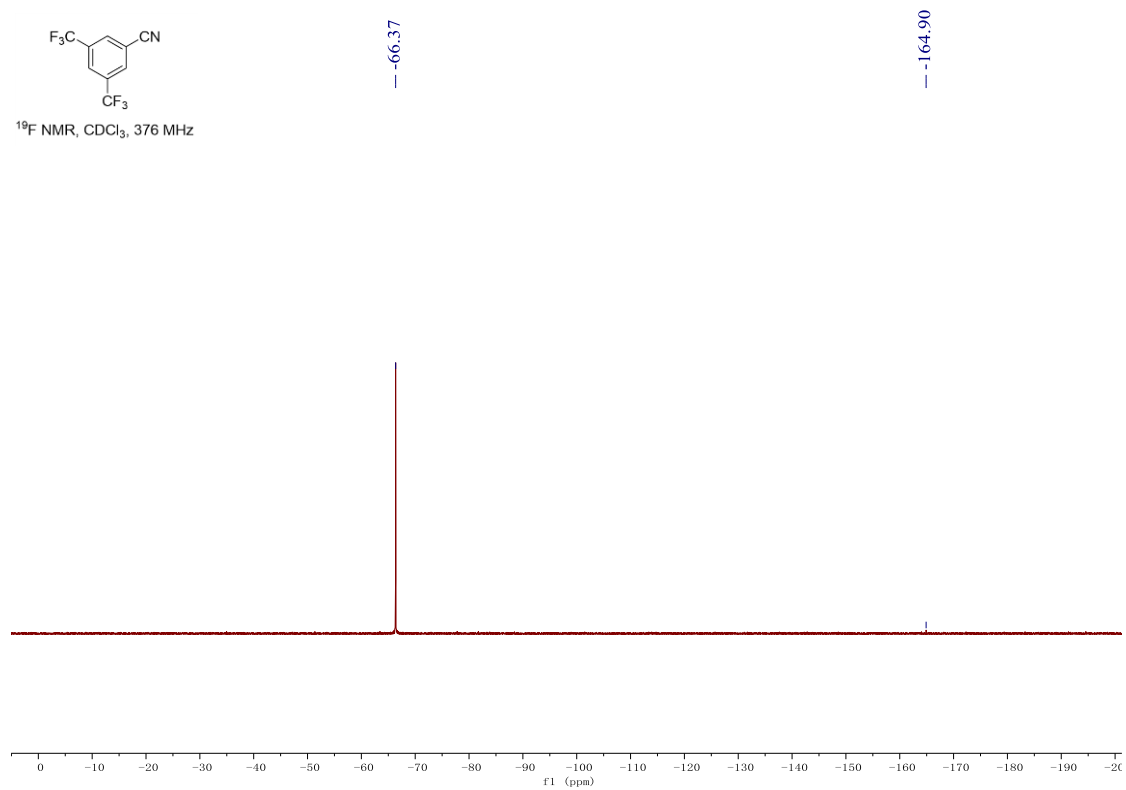
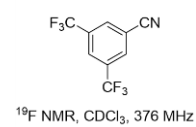
^{19}F NMR of compound 1



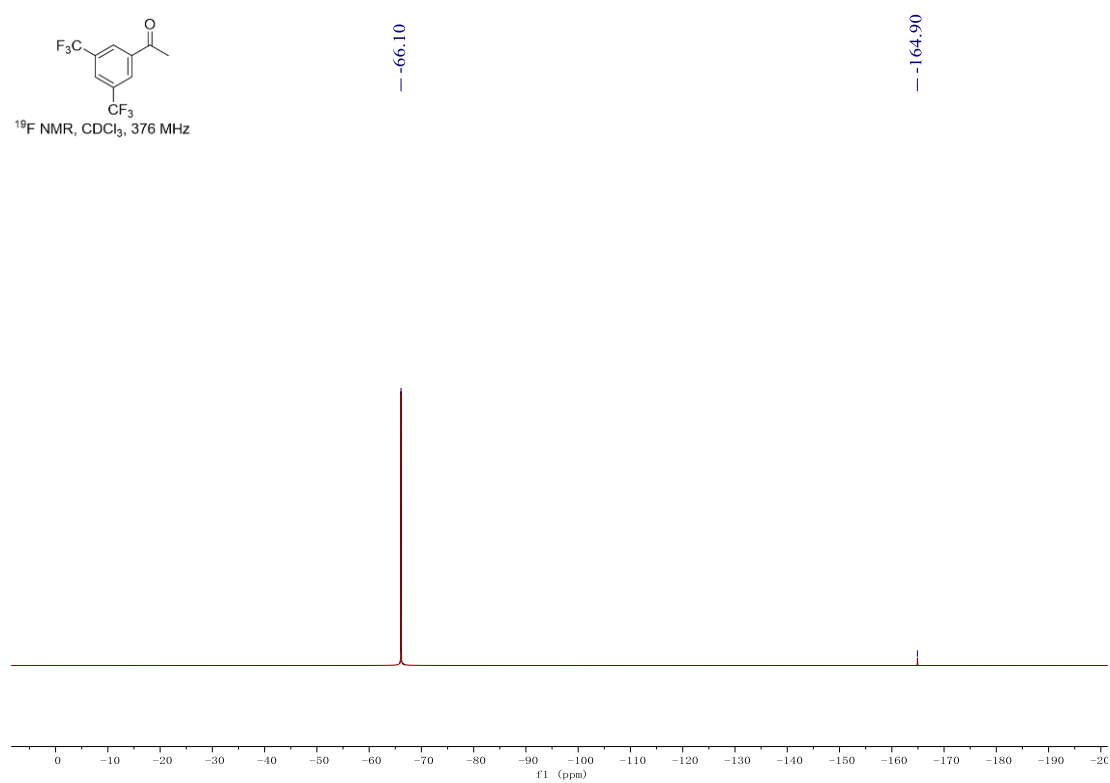
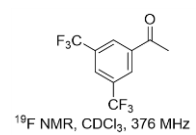
^{19}F NMR of compound 2



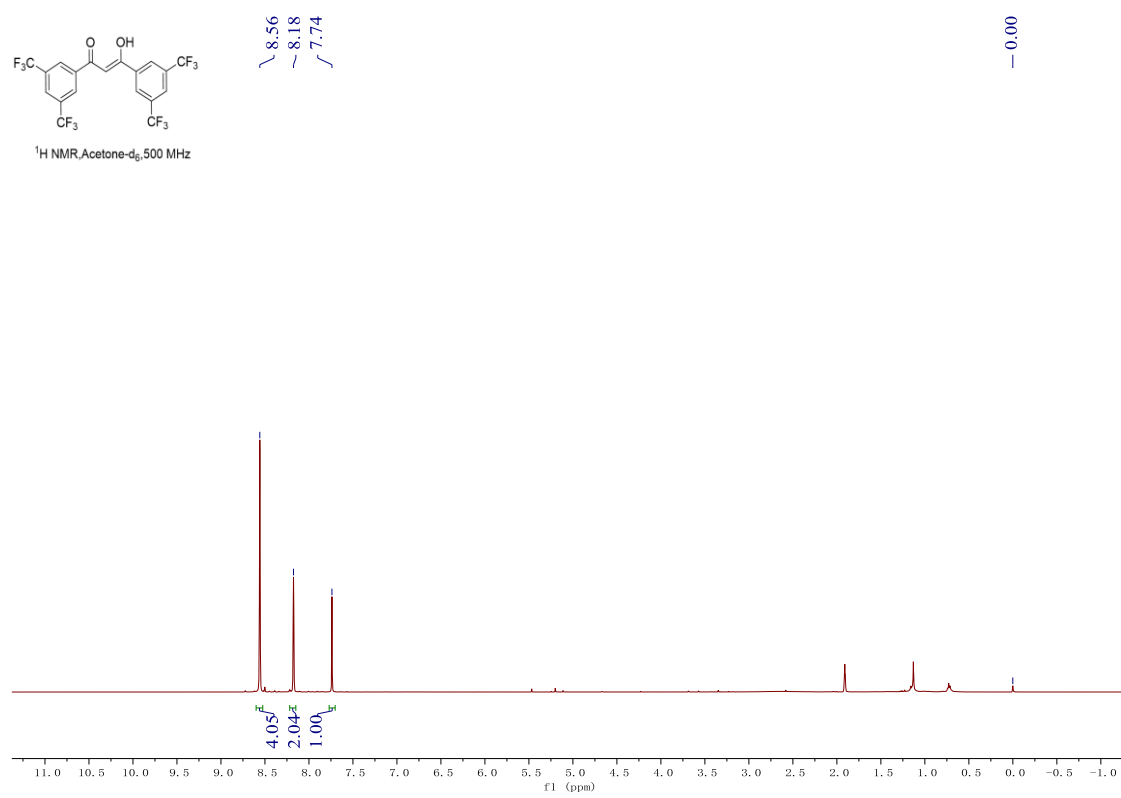
¹⁹F NMR of compound 3



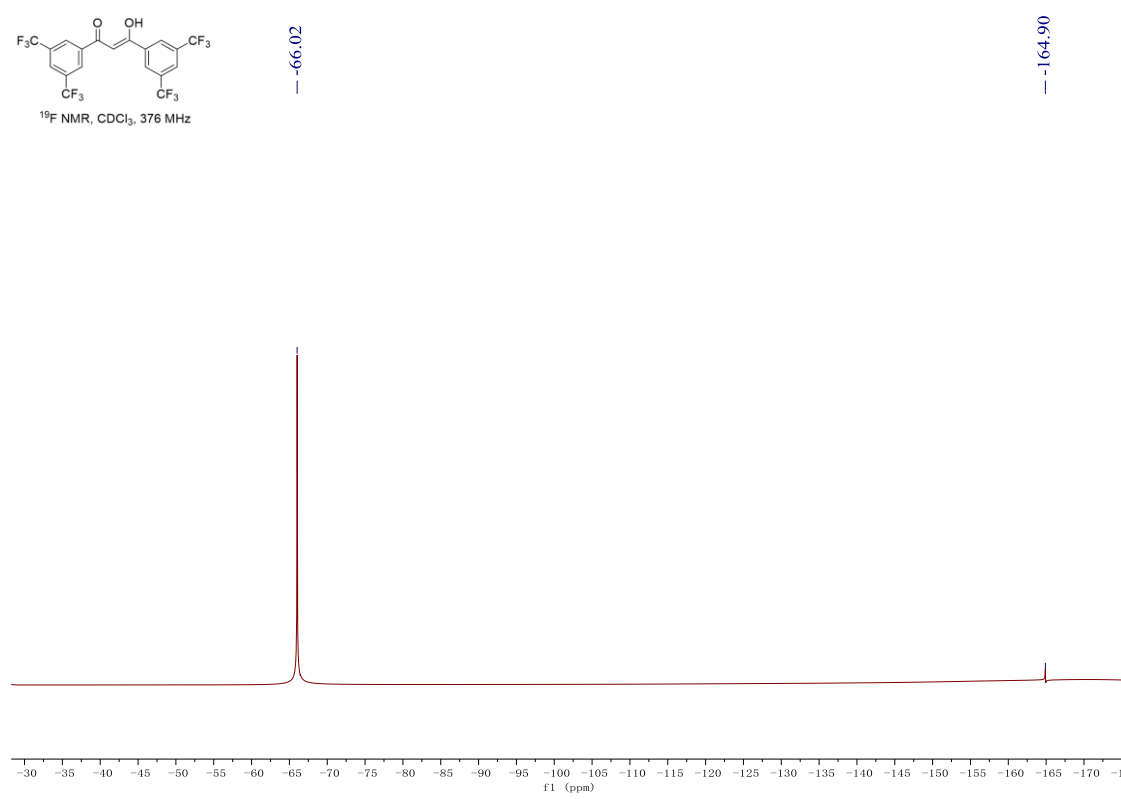
¹⁹F NMR of compound 4



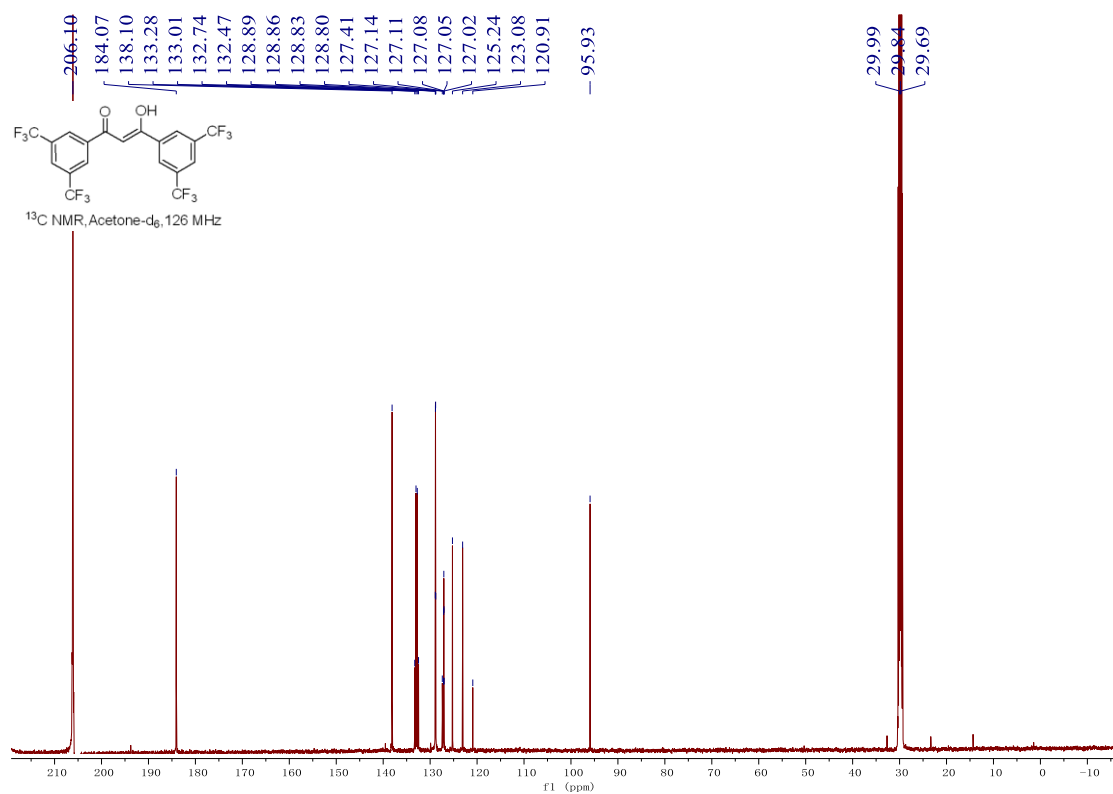
¹H NMR of compound 5



¹⁹F NMR of compound 5



¹³C NMR of compound 5



HRMS of compound 5

