Supporting Information

Efficient temperature-feedback liposome for ^{19}F MRI signal enhancement

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1.Chemicals and reagents

1-Ethynyl-4-(trifluoromethyl) benzene was purchased from Energy Chemical (Shanghai, China). 4-Bromo-1,8-naphthalic anhydride was purchased from J&K Scientific (Chengdu, China). lyso-PC(1-stearoyl-2-hydroxy-sn-glycero-3-phosphocholine) was purchased from A.V.T. (Shanghai,China). DPPC(1,2-dipalmitoyl-sn-glycero-3-) and MPEG-2000-DSPE (N-(Carbonyl-methoxypolyethyleneglycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine, sodium salt) were purchased from Corden Pharma (Liestal, Switzerland). MPPC(1-myristoyl-2-palmitoyl-sn- glycero-3phosphocholine was obtained form Avanti (Alabama,USA). PFOB(perfluorooctyl bromide) was purchased from Sigma Aldrich (Darmstadt, Germany).The non-small-cell lung cancer cell A549 was purchased from cell bank of Chinese academy of sciences (Shanghai, China). IMDM (Iscove's Modi ed Dulbecco's Medium was purchased from Boster (Wuhan, China).

2. Synthesis of fluorinated lipid F-PC

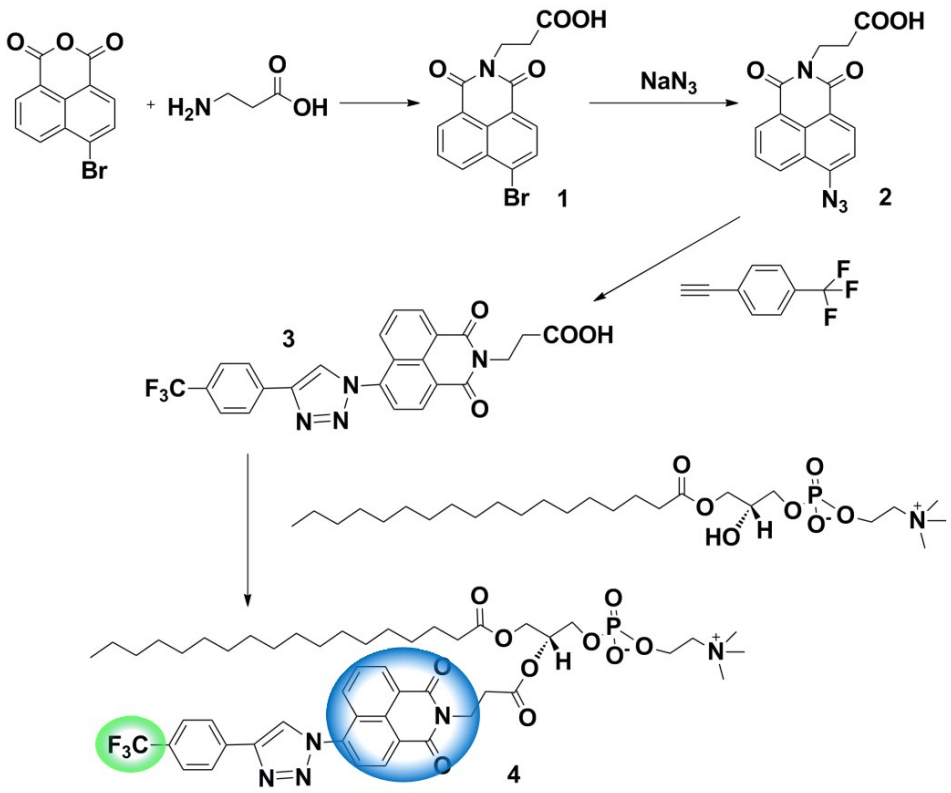


Figure S1. Synthetic route of fluorinated lipid F-PC.

F-PC was dissolved in $CHCL_3$-$CDCL_3$(90/10, v/v) at a concentration of 5.7 mM. The T_1 and T_2 of F-PC was detected by ^{19}F NMR recorded on a 500MHz spectrometer. The T_1 of this F-PC solution was measured using a standard inversion-recovery pulse sequence at 25℃. The T_2 of F-PC was measured using CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence at 25℃. UV-Vis and fluorescence spectra of F-PC were collected on a UV-Vis spectrometer (Thermo Scientific evolution 220, USA) and a fluorescence spectrophotometer (Edinburgh FS5,UK), respectively.

3. Preparation and characterization of TSL

The lipids used here were DPPC , MPEG-2000-DSPE and MPPC. Temperature-sensitive liposome TSL were composed of DPPC/MPPC/F-PC/PEG2000-DSPE (90:10:10:4, molar ratio) and prepared by the thin film hydration method, followed by membrane extrusion for size control. Specifically, Lipids were dissolved in chloroform and dried under vacuum overnight to form a film. The film was hydrated with PBS (pH 7.0) and sonicated in a water bath for 10 min. Large multilamellar TSLs were obtained. Then the liposome was extruded 10 times using 0.2-μm pore size filters and lyophilized for use. PFOB liposomal nanoemulsion (PFOB-TSL) was prepared using the same

procedure as TSL. PFOB was added during the hydration period (1:1, molar ratio, PFOB/lipids). Non-encapsulated PFOB was removed by centrifuging for 5 min at 1000 rpm.

The particle size and zeta potential of the various TSL were determined by DLS (dynamic light scattering with a particle analyzer, ZS, Malvern, UK). TEM studies were performed using a JEM2010 electron microscope (JEOL, Japan) with samples of 1% phosphotungstic acid negative staining. The outlook of large TSL was observed by CLSM (confocal laser scanning microscopy, A1R/A1, Nikon, Japan). To verify the single peak of this TSL, ^{19}F NMR was recorded at room temperature by 500MHz NMR spectrometer. The amount of fluorine atoms of liposome was calculated from ^{19}F NMR spectroscopy after adding 10 μl of 5% Triton X-100. Sodium trifluoromethanesulfonate (-78.69 ppm) was added as internal for F content detection by ^{19}F NMR.

The size variation of liposome at different temperatures was monitored by DLS. Temperatures tested here included 25,37,38,39,40,41 and 42℃. The heating time of each point was 10 min. Liposomal water solution at 25℃ was dripped to copper net as sample 1. Liposomal water solution incubated at 42℃ for 10 min, was rapidly dripped to a new copper net as sample 2. The two samples were stained with 1% phosphotungstic acid and then visualized by TEM.

4.*In vitro* ^{19}F NMR

^{19}F NMR signal variation of TSL at different temperatures: In order to confirm this liposome can turn the ^{19}F signal on at mild temperature, ^{19}F signal was monitored on a 500MHz NMR spectrometer at different temperatures (25,37,38,39,40,41 and 42℃). Liposomes at a concentration of 0.71 mM of fluorine in water(90% H_2O +10% D_2O) were kept in the spectrometer for 10 min at each temperature. Then ^{19}F NMR spectrum was recorded. Liposome with 10 μl of 5% Triton X-100 was set as control. The ^{19}F NMR integral ratio was calculated as I_T/I_c*100%, in which I_T is the integral at temperature T, I_C is the integral of control(Figure S2). Liposomes at different concentrations of fluorine(0.18, 0.36, 0.71, 1.07 and 1.43 mM) at 42℃ were detected by ^{19}F NMR, with incubation time of 5 min (Figure S3). As a comparison, ^{19}F NMR spectrum of fluorine-encapsulated TSL (PFOB-TSL) at different temperatures was recorded at the same conditions as above (Figure S4).

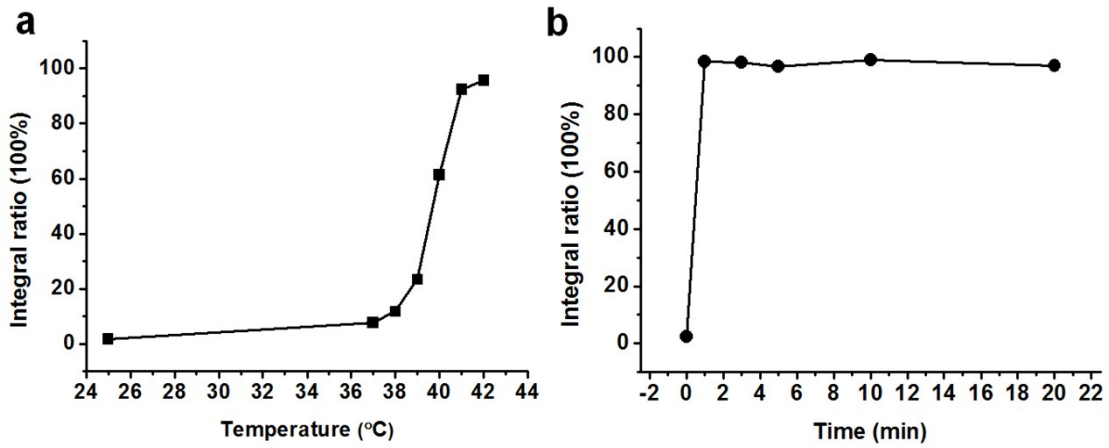


Figure S2. a) the corresponding integral ratio variation of Figure 4a. b) The corresponding integral ratio variation of Figure 4b.

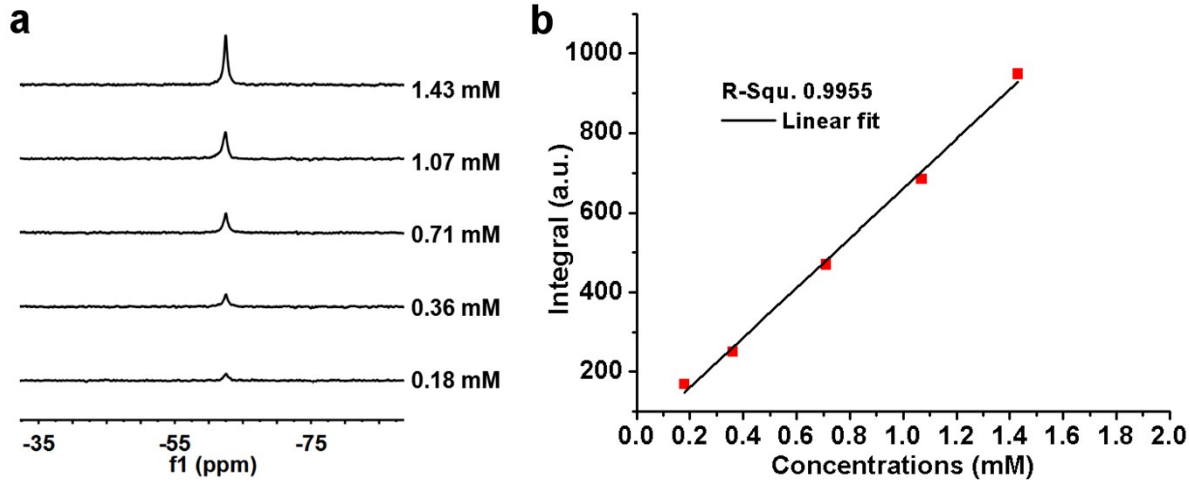


Figure S3. ^{19}F NMR studies of TSL. a) ^{19}F NMR of TSL at different concentrations at 42℃. b) Linear fit of integral of ^{19}F NMR signal according to a.

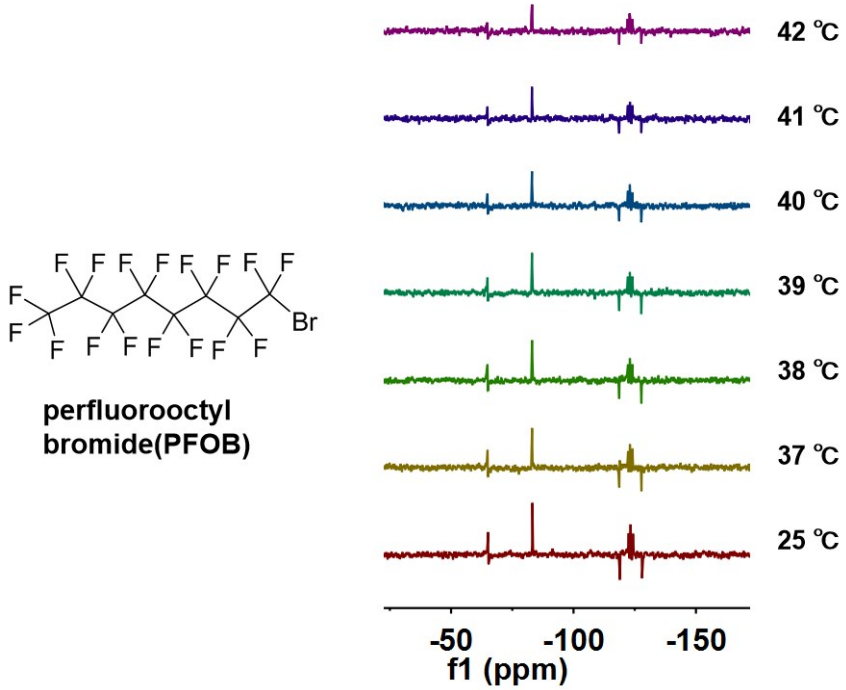


Figure S4. ^{19}F NMR of PFOB-encapsulated TSL at different temperatures. PFOB had single peaks at -64.82 ppm and -83.03 ppm, and multiple peaks at -122.95 ppm.

The response rate of TSL at 42℃ was detected by 500 MHz NMR spectrometer. Spectra of samples (0.71 mM fluorine, 90% H_2O +10% D_2O) in water were collected at 0, 1,3,5,10 and 20 min at 42℃, respectively. Liposomes with 10 μl of 5% Triton X-100 was set as control. The ^{19}F NMR integral ratio was calculated as I_t/I_c*100%, in which I_t is the integral at time t, I_C is the integral of control. All ^{19}F NMR spectra above was collected with one scan.

Reversible ^{19}F NMR signal control at mild temperature:^{19}F NMR of liposomes, which was dissolved in water(90%H_2O +10%D_2O) or serum(10% serum+80%H_2O+10%D_2O) or cell lysates (10% cell lysates+80%H_2O+10%D_2O) at a concentration of 0.36 mM of fluorine, was recorded for 6 cycles by repeated warming at 42℃and cooling at 37℃. Incubation time was 10 min at each temperature. All ^{19}F NMR spectra was collected with one scan (Figure S5 and S6). Cell lysates was prepared as following. The non-small-cell lung cancer cell A549 was cultured in two 250 ml culture bottles in IMDM with 10% fetal bovine serum, 100 U/ml penicillin and 100U/ml streptomycin at 37℃. Cells($1*10^7$) were collected in 2 ml PBS when cell confluence reached above 90%. After 3 cycles of freezing at -80℃ and thawing at 37℃, homogeneous cell lysates was obtained.

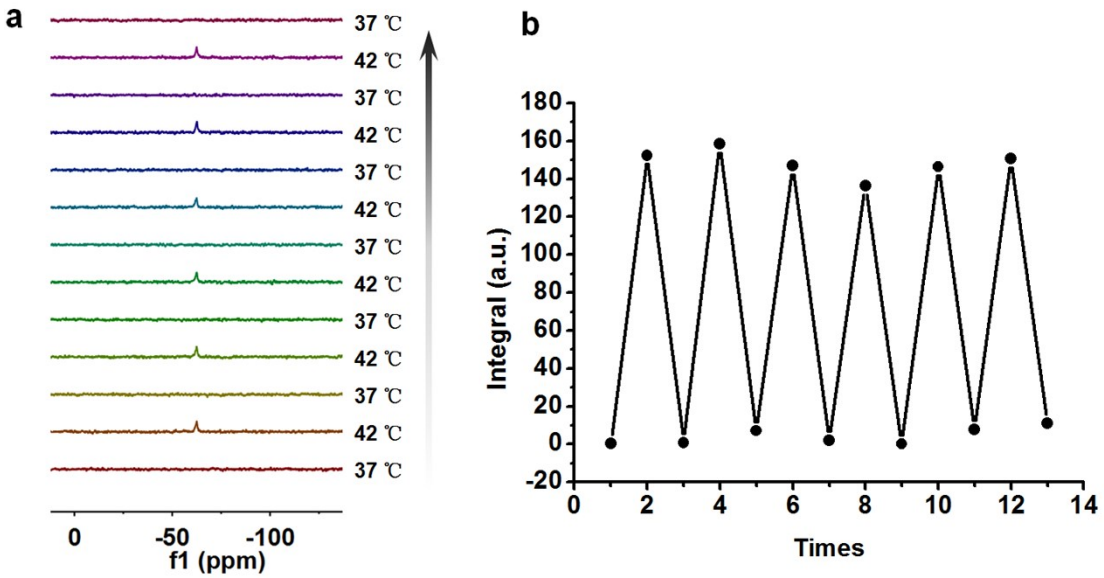


Figure S5. Reversible ^{19}F NMR control at body temperature and mild high temperature. a) ^{19}F NMR of TSL in serum under repeated warming at 42℃and cooling at 37℃. b) Integral curve of ^{19}F NMR signal corresponding to a, in which 1,3,5,7,9,11,13 were the integral of ^{19}F NMR signal at 37℃, 2,4,6,8,10,12 were the integral of ^{19}F NMR signal at 42℃.

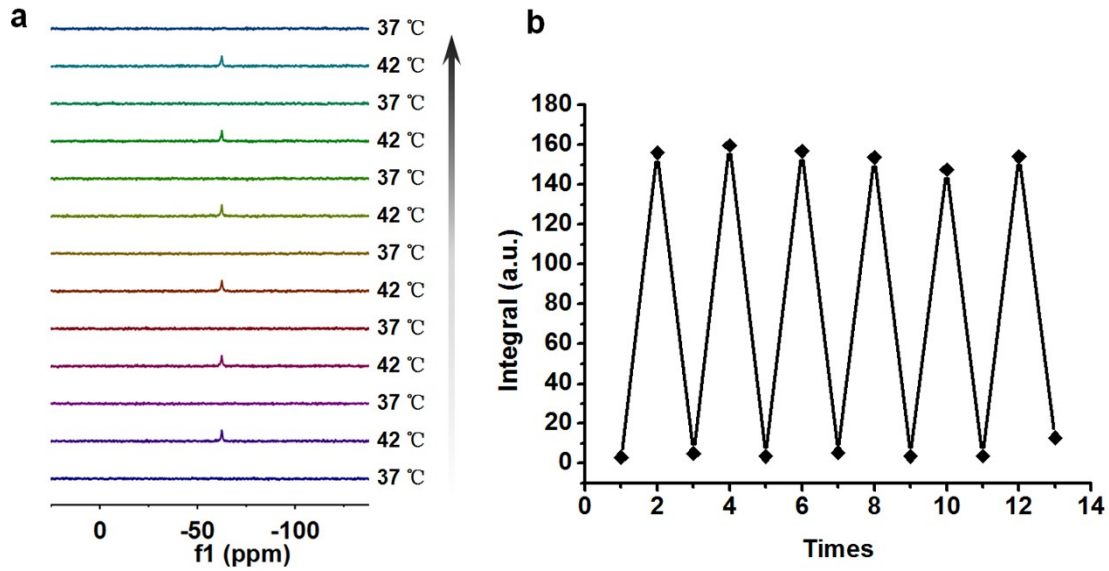


Figure S6 a) ^{19}F NMR of TSL in cell lysates under repeated warming at 42℃and cooling at 37℃. b) Integral curve of ^{19}F NMR signal corresponding to a, in which 1,3,5,7,9,11,13 were the integral of ^{19}F NMR signal at 37℃, 2,4,6,8,10,12 were the integral of ^{19}F NMR signal at 42℃.

5.*In vitro* ^{19}F MRI

^{19}F MR images at different temperatures were acquired on a 400MHz micro-imaging system (Bruker Biospin, Ettlingen, Germany) with 10 mm ^{19}F coil. Liposomes were dispersed in water (90% H_2O +10% D_2O) or cell lysates (10% cell lysate+ 80%H_2O+ 10%D_2O) at a concentration of 1.43 mM fluorine. ^{1}H MR images were recorded for localization of the samples using a RARE sequence. ^{19}F MR images were taken by the same sequence as above with an echo time (TE) of 3 ms and a repetition time (TR) of 1000 ms. The field of view was 4*4 cm^2, RARE factor was 4, number of averages was 256, matrix size was 32 *32, acquisition time was 2048 s and slice thickness was 24 mm.

6.Cellular uptake of TSL

In order to detect the fluorescence imaging of tumor cells by TSL, the non-small-cell lung cancer cell A549 was incubated with liposome, followed by confocal laser scanning. About $1*10^5$ cells were seeded on cover slips placed in cell culture dishes. Liposome (10mg/ml with 0.18 mM fluorine) in IMDM was added to the cells when cell confluence reached to 30%. After 4 h incubation, cells were stained with RedDot for 20 min. The fluorescence images were obtained by confocal laser scanning microsopy (A1R/A1, Nikon, Japan).

7.Synthetic procedures of F-PC

As shown in Figure S1, firstly, 4-Bromo-1,8-naphthalic anhydride(1.0 eq.) and alanine(1.0 eq.) were dissolved in ethanol and heated to reflux for 8 h at 80℃. The crude product was filtered and washed with ethanol for 3 times. Then the product 1 was collected and dried under vacuum overnight. Then, compound 1 was dissolved in DMF and stirred for 0.5 h. After adding NaN_3(1.5 eq.), the mixture was heated to reflux around 100℃ for 0.5 h. The crude product was filtered and washed with water and methanol sequentially for 2 times, respectively. Compound 2 was obtained after drying under vacuum. Then a click reaction was performed. Compound 2 was dissolved in DMF and stirred for 0.5 h. Vc (10 eq.), $CuSO_4$(5 eq.) and 1-Ethynyl-4-(trifluoromethyl) benzene were added in sequence. The mixture was stirred for 10 h at room temperature under a N_2 atmosphere, evaporated under reduced pressure which resulted in a brown gum that was purified with gel chromatography, eluted with 1% MeOH/CH_2CL_2. The white powder was compound 3. Finally, Compound 3, EDC (2 eq.), DMAP (2 eq.), HOBT (2 eq.) and lyso-PC (1 eq.) were dissolved in $CHCL_3$. The reaction mixture was stirred for 72 h at room temperature. After evaporating the solvent, the residue was purified by thin layer chromatography (20*20 cm precoated silica gel TLC plate with fluorescence indicator, 1.5 mm in thickness). CH_2CL_2-methano (10:1, v/v) was used as the

developing system. An off-white powder was obtained as fluorinated lipid tag (F-PC, compound 4) and dried for the following application.

The products of each step above were verified by ESI-TOF MS (Agilent 6530 Accurate-Mass Q-TOF spectrometer, USA), ^{1}H NMR and ^{13}C NMR(recorded on Bruker AVANCE 500 spectrometer(USA), using TMS as internal standard). In addition, compound 3 and 4 were detected by ^{19}F NMR recorded on a 500MHz spectrometer with a 5 mm broadband inverse probe(BBO5), using Tetrafluoronitrobenzene (-101.99 ppm) as an internal standard. The sample volume was 500ul in the nuclear magnetic tube with inner diameter of 5 mm.The ^{19}F NMR spectra were collected using only one scan.

Synthesis of compound 1. ^{1}H NMR (500 MHz, DMSO-*d6*, δ): 8.55 (dd, *J* = 7.3 Hz, 1Hz, 1H), 8.52 (dd, *J* = 8.4, 0.7 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 8.20 (d, *J* = 7.9 Hz, 1H), 7.98 (dd, *J* = 8.3, 7.5 Hz, 1H), 4.28 -4.20 (t, *J*= 7.5 Hz, 2H), 2.60 (t, *J* = 3.5 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-*d6*, δ): 172.95, 163.10, 163.05, 132.99, 131.92, 131.70, 131.28, 130.06, 129.59, 129.12, 128.52, 122.98, 122.20, 36.35, 32.61; HRMS (ESI) *m/z*: $[M - H]^-$ calcd for $C_{15}H_{10}BrNO_4$, 345.9720; found, 345.9629 .

Synthesis of compound **2**. ^{1}H NMR (500 MHz, DMSO-*d6*,δ): 8.51 (d, *J* = 7.2 Hz, 1H), 8.46 (d, *J* = 8.0 Hz, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 7.85 (t, *J* = 7.9 Hz, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 4.21 (t, *J* = 7.9 Hz, 2H), another CH_2 signal was overlay by the signal of DMSO; ^{13}C NMR (126 MHz, DMSO-*d6*,δ):173.24, 163.58, 163.12, 143.31, 132.05, 131.97, 128.83, 128.78, 127.77, 123.99, 122.65, 118.66, 116.42, 36.72, 33.51; HRMS (ESI)*m/z*: $[M - H]^-$ calcd for $C_{15}H_{10}N_4O_4$, 309.0629; found, 309.0628.

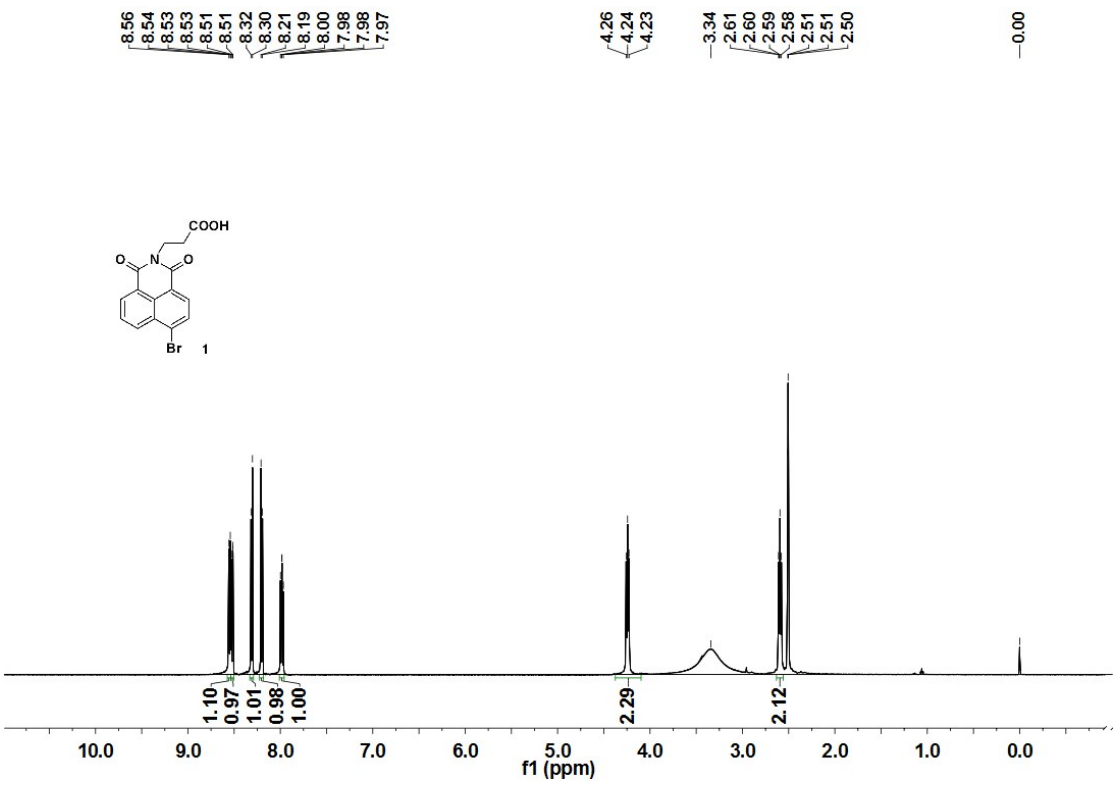
Synthesis of compound **3**. ^{1}H NMR (500 MHz, DMSO-*d6*, δ): 12.41 (s, 1H), 9.50 (s, 1H), 8.67 (d, *J* = 7.8 Hz, 1H), 8.62 (dd, *J* = 7.2, 0.9 Hz, 1H), 8.32 (dd, *J* = 8.6, 0.9 Hz, 1H), 8.24 (d, *J* = 8.1 Hz, 2H), 8.18 (d, *J* = 7.8 Hz, 1H), 7.98 (dd, *J* = 8.5, 7.3 Hz, 1H), 7.91 (d, *J* = 8.2 Hz, 2H), 4.30 (t, *J* = 10 Hz, 2H), 2.65 (t, *J* = 10 Hz, 2H); ^{13}C NMR (126 MHz, DMSO-*d6*, δ): 172.93, 163.55, 163.03, 146.^{19}, 137.97, 134.47, 132.10, 130.9, 129.93, 129.47, 129.13, 128.87, 128.84, 126.56, 126.20, 126.00, 125.78, 124.78, 124.08, 123.62, 123.11, 36.42, 32.56; ^{19}F NMR (470 MHz, DMSO-*d6*, δ): −60.96; HRMS (ESI)*m/z*: $[M - H]^-$ calcd for $C_{24}H_{15}N_4O_4F_3$, 479.0973; found, 479.0909 .

Synthesis of compound **4**. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.56 (m , 3H), 8.24 (d, *J* = 8.5 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 7.7 Hz, 1H), 7.75 (t, *J* = 7.9 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 2H), 4.45-4.35 (m, 2H), 4.32 (s, 2H), 4.29-4.24 (m, 2H), 4.09 (m, 1H), 3.95 (s, 2H), 3.91 (s, 2H), 3.42 (s, 9H), 2.75 (m, 2H), 2.^{19} (t, *J* = 7.6 Hz, 2H), 1.55-1.42 (m, 2H), 1.15 (m, 28H), 0.79 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$,δ): 173.50, 170.56, 163.38, 162.89,

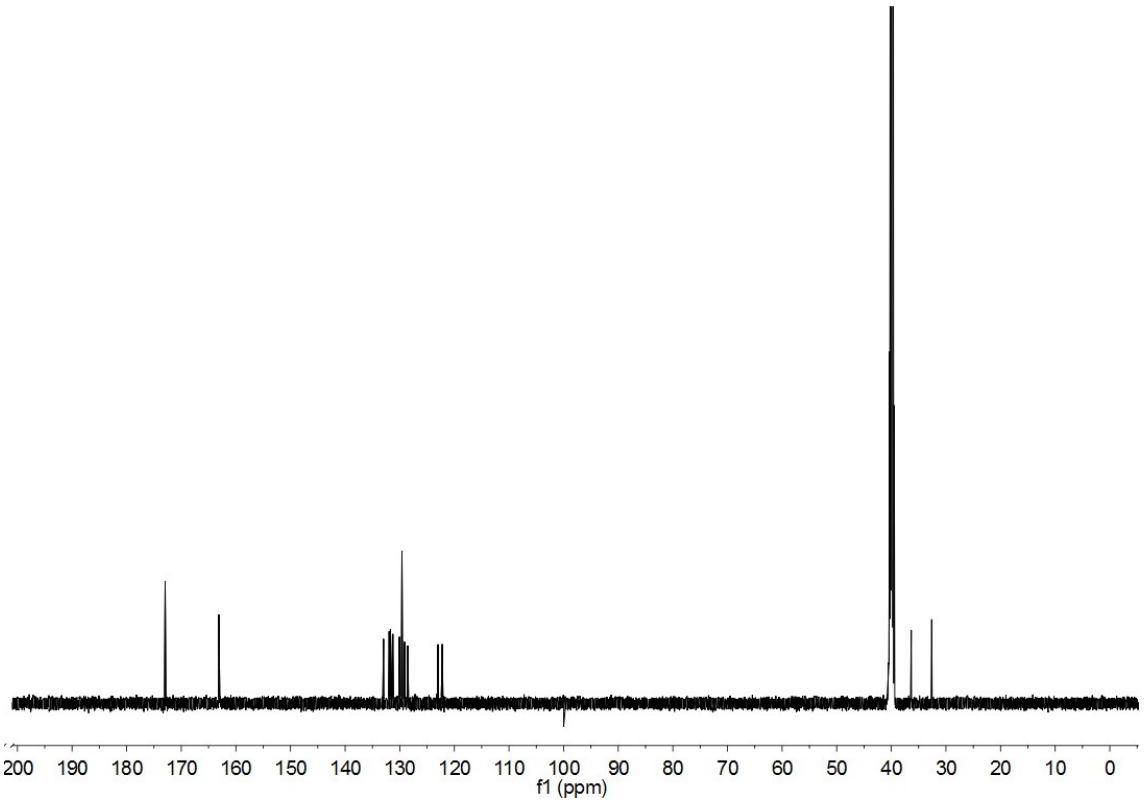
159.72, 146.98, 138.07, 133.14, 132.36, 130.98, 130.52, 130.26, 129.83, 128.95, 128.71, 126.30, 126.15, 126.01-125.81 (m), 125.05, 124.28, 123.79, 123.72, 123.45, 123.22, 122.88, 122.50, 118.45, 110.94, 71.11, 71.06, 66.22, 66.17, 63.68, 63.63, 62.33, 59.57, 59.53, 55.81, 54.34, 43.36, 36.9, 36.14, 34.86, 33.93, 32.51, 31.82, 31.49, 29.60, 29.57, 29.55, 29.45, 29.25, 29.23, 29.07, 26.10, 24.76, 22.59, 22.56, 15.52, 14.05; ^{19}F NMR (470 MHz, CDCl3,δ): −62.60; HRMS (ESI)*m/z*: $[M + H]^+$ calcd for $C_{50}H_{67}N_5O_{10}PF_3$, 986.4650; found, 986.4661 .

8.^{1}H NMR, ^{19}F NMR, ^{13}C NMR and HRMS spectra of compounds

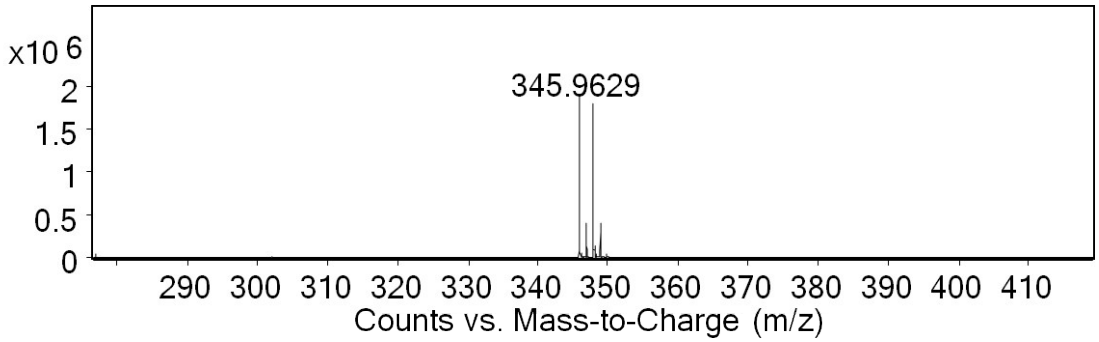
^{1}H NMR of compound 1(500 MHz, DMSO-*d6*)



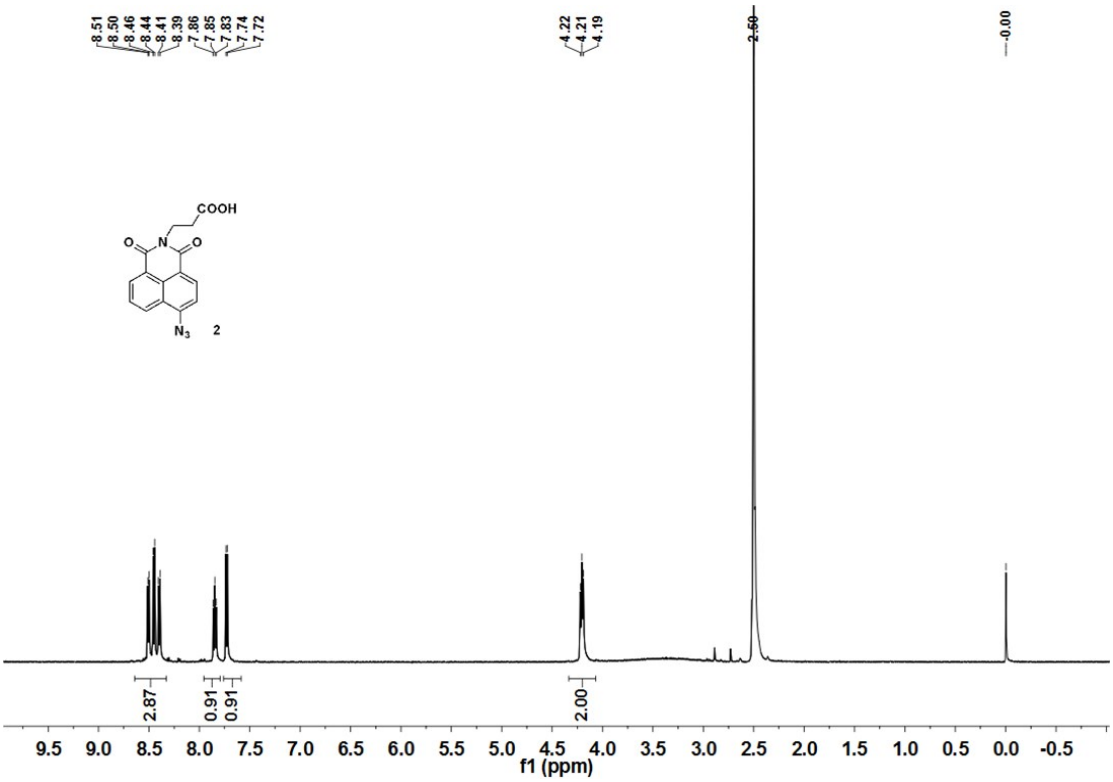
^{13}C NMR of compound 1(126 MHz, DMSO-*d6*)



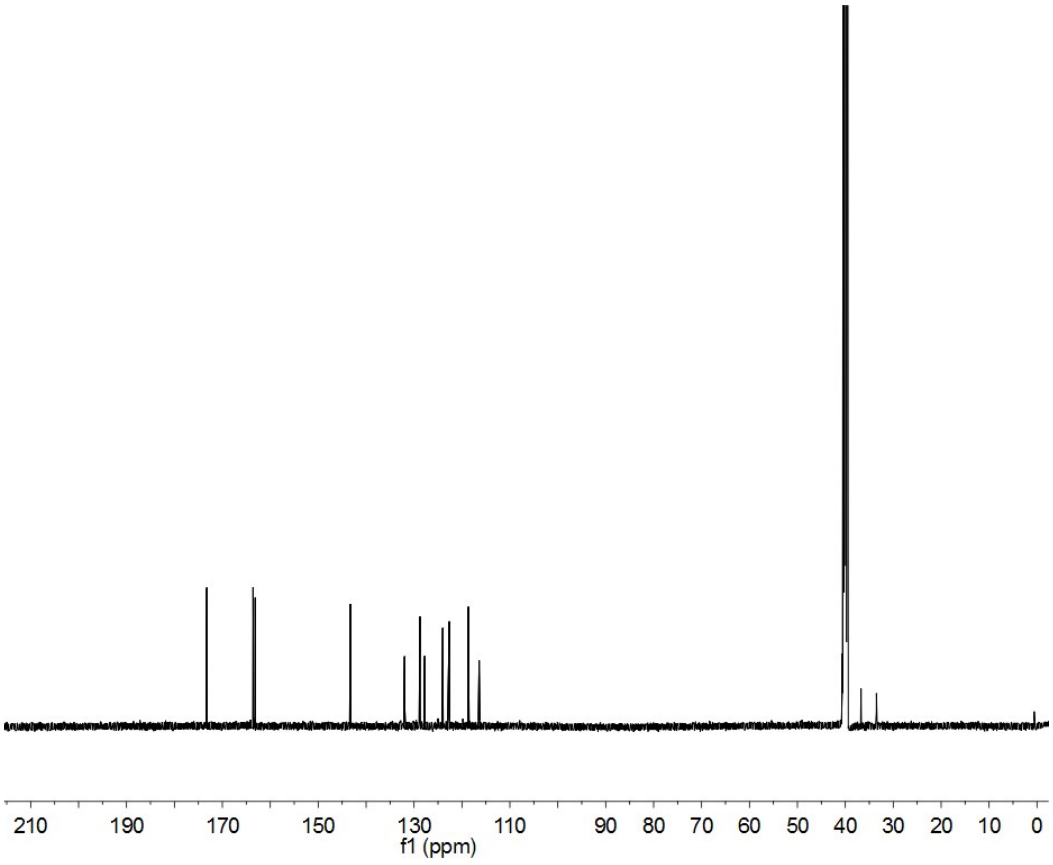
ESI-HRMS of compound 1.



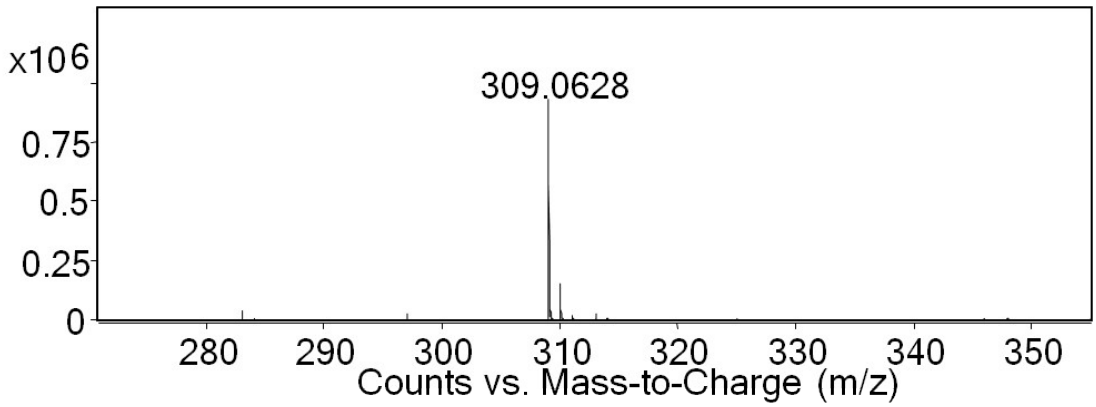
^{1}H NMR of compound 2(500 MHz, DMSO-*d6*)



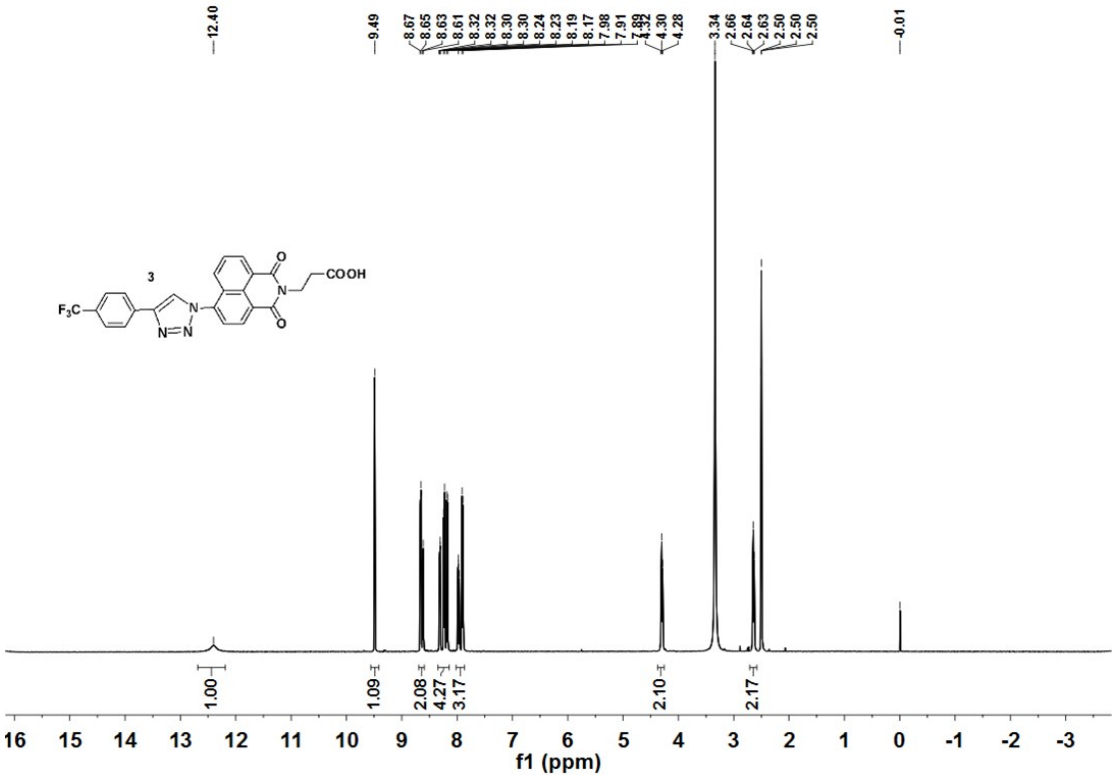
^{13}C NMR of compound 2(126 MHz, DMSO-*d6*)



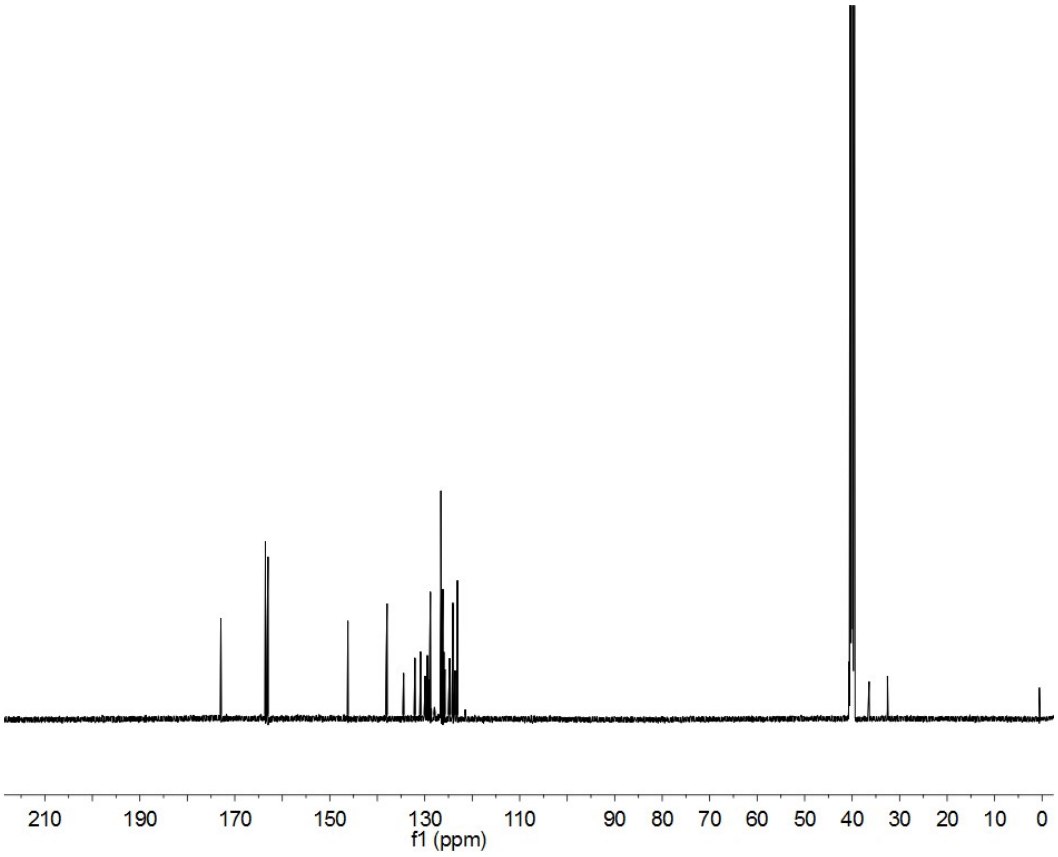
ESI-HRMS of compound 2.



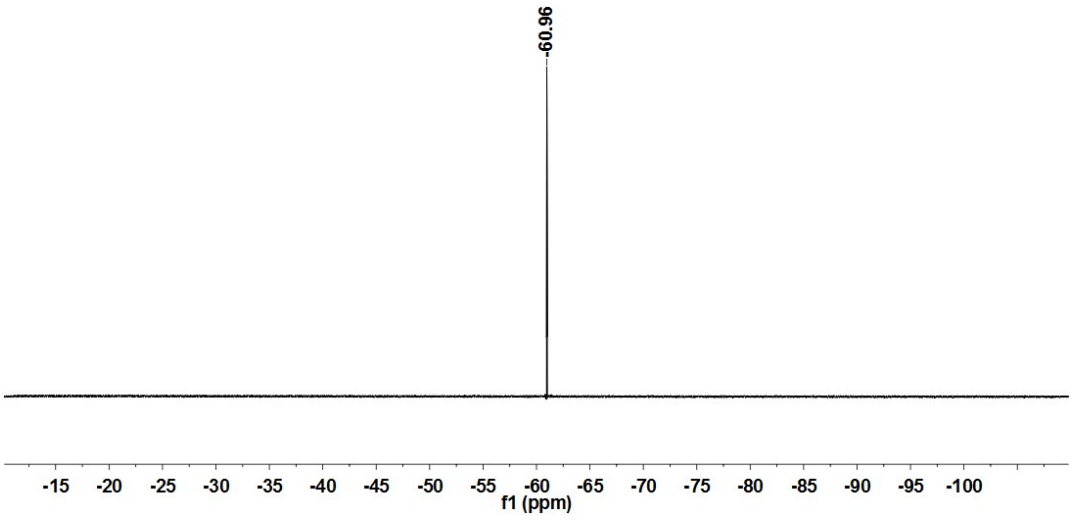
^{1}H NMR of compound 3(500 MHz, DMSO-*d6*)



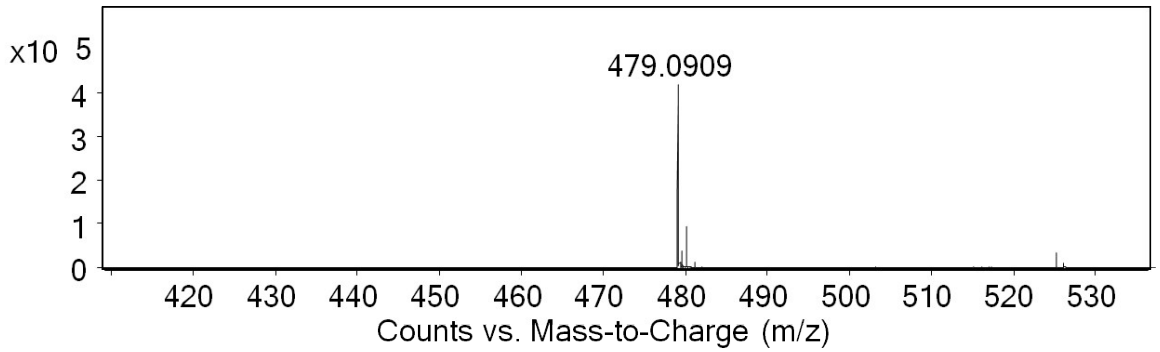
^{13}C NMR of compound 3(126 MHz, DMSO-*d6*)



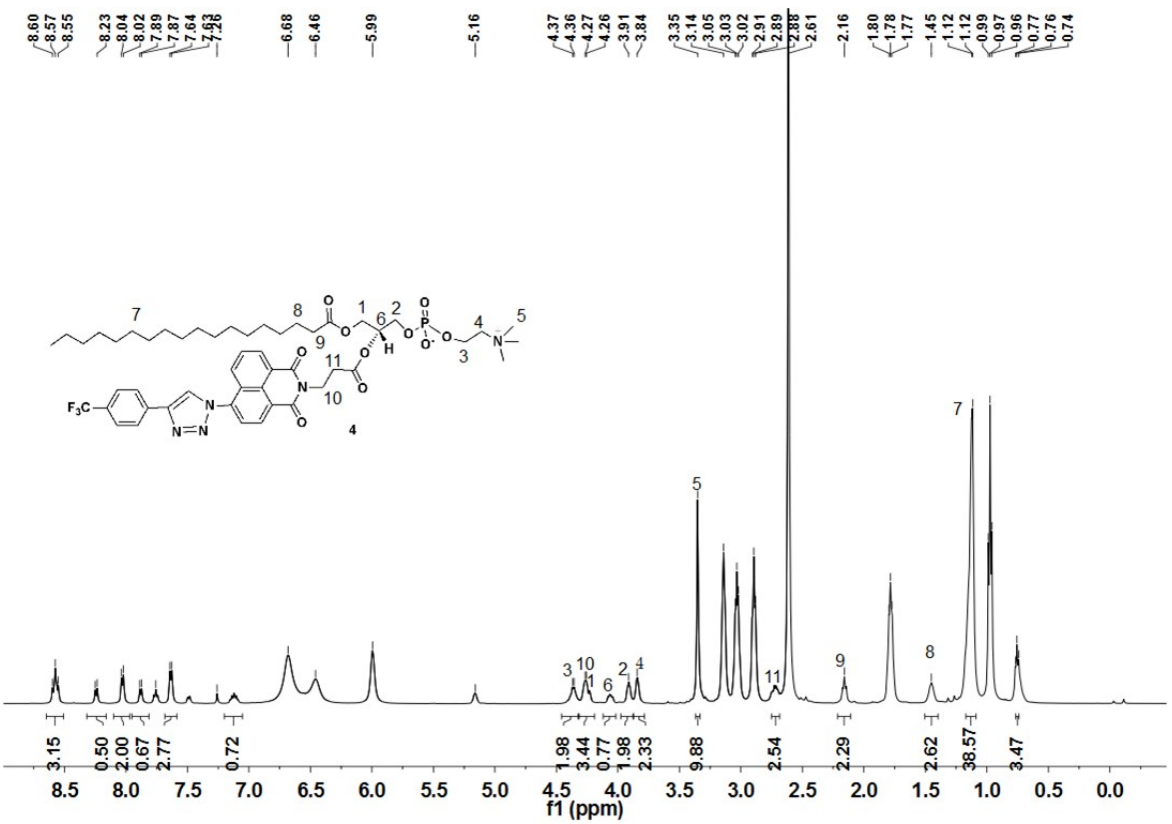
^{19}F NMR of compound 3(470 MHz, DMSO-*d6*,)



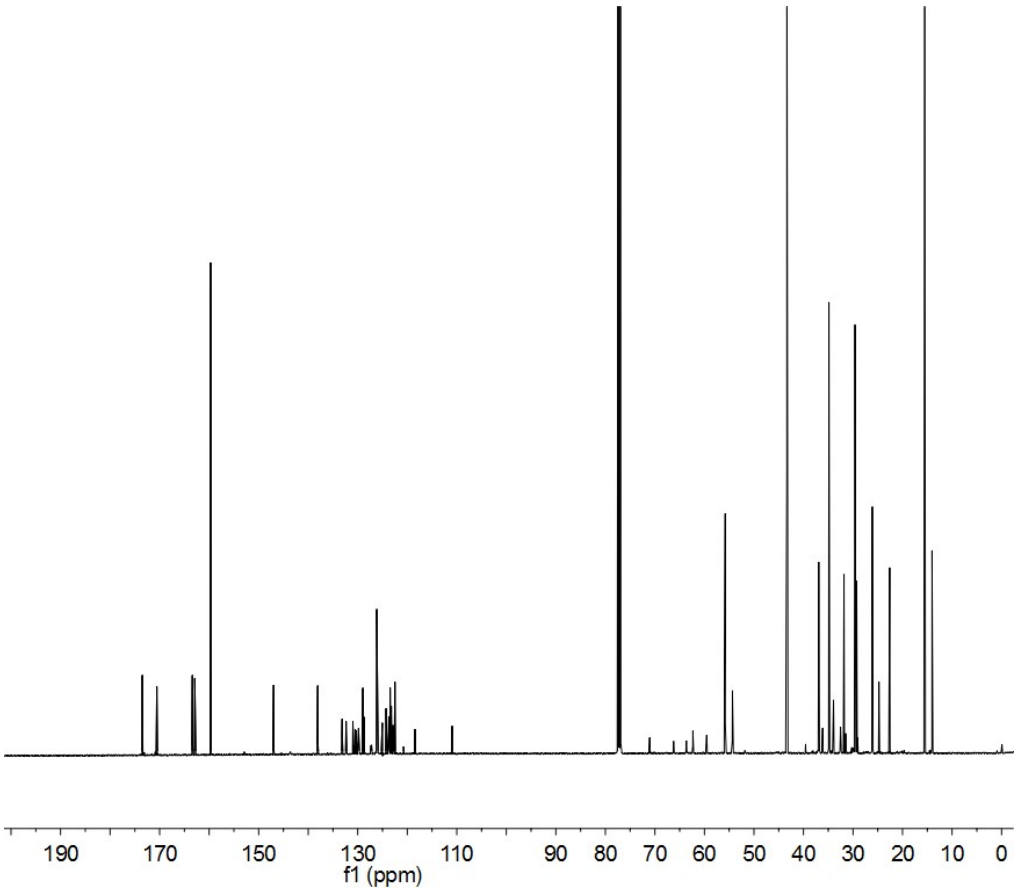
ESI-HRMS of compound 3



^{1}H NMR of compound 4(500 MHz, $CDCl_3$)



^{13}C NMR of compound 4(126 MHz, $CDCl_3$)



ESI-HRMS of compound 4