Peptidic monodisperse PEG “combs” with fine-tunable LCST and multiple imaging modalities

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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 (δ) were in ppm and coupling constants (*J*) were in Hertz (Hz). ^{1}H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using $CDCl_3$ or CD_3OD as solvent. ^{13}C NMR spectra were referenced to solvent carbons (77.16 ppm for $CDCl_3$; δ 39.52 ppm for DMSO-d_6). ^{19}F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in $CDCl_3$ and 73 mM sodium trifluomethanesulfonate (s, -79.61 ppm) in D_2O. The splitting patterns for ^{1}H NMR spectra were denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were recorded on a Thermo Scientific™ Q Exactive™ Focus mass spectrometer for compounds below 3,000 Da. MALDI-TOF mass spectra were recorded on an autoflex™ speed MALDI-TOF spectrometer using the reflection mode for positive ions with α-cyano-4-hydroxylcinnamic acid as matrix.

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, DCM, Et_3N, MeOH and 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.

For M-PEG peptides HPLC analysis: SPD-20A UV detector (254 nm), a Sunfire C18 column (5 μm, 4.6 × 100 mm), a gradient elution of 70% methanol in water to 100% methanol over 15 min (flow rate 1.0 mL/min). For DOX HPLC analysis: SPD-20A UV detector (480 nm), a Sunfire C18 column (5 μm, 4.6 × 100 mm), 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, 0.35 mL/min) and solvent B (acetonitrile, 0.35 mL/min). For BODIPY HPLC analysis: SPD-20A UV detector (690 nm), a COSMOSIL 5C18-MS-Ⅱ column (5 μm, 4.6 × 250 mm), a gradient elution of 80% acetonitrile in water to 100% acetonitrile over 20 min (flow rate 1.0 mL/min).

Tumor-carrying Balb/c nude mice with tumor volume of 300-800 mm^3 (male, 6-8 week, 23-26 g) were bought from Wuhan Cloud-Clone Corp. During the procedures, mice were anesthetized by 1% pentobarbital sodium (7 μL/g). The animal experimental procedures were performed in accordance

with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local Animal Care and Use Committee.

2. Structure of thermosensitive peptidic M-PEG "combs" (Figure S1)

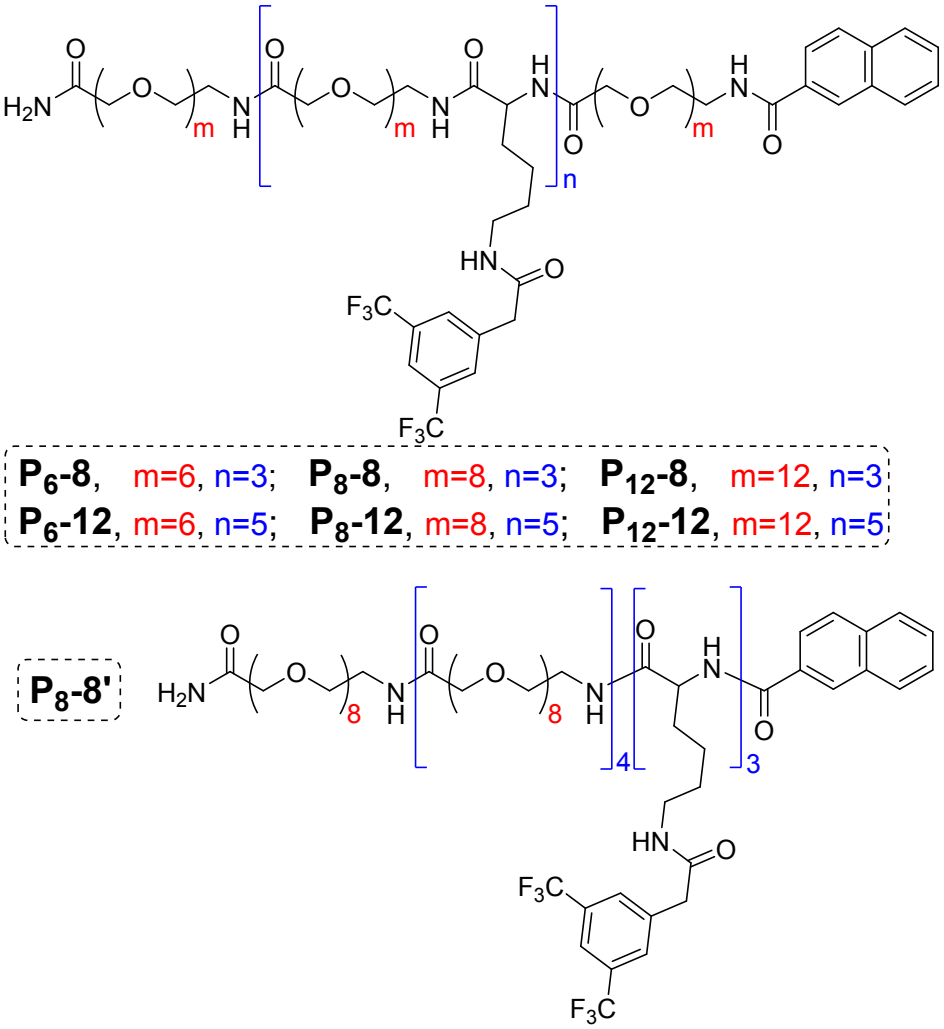


Figure S1. Structure of thermosensitive peptidic M-PEG "combs".

3. Solvent-dependent ^{19}F NMR (Figure S2)

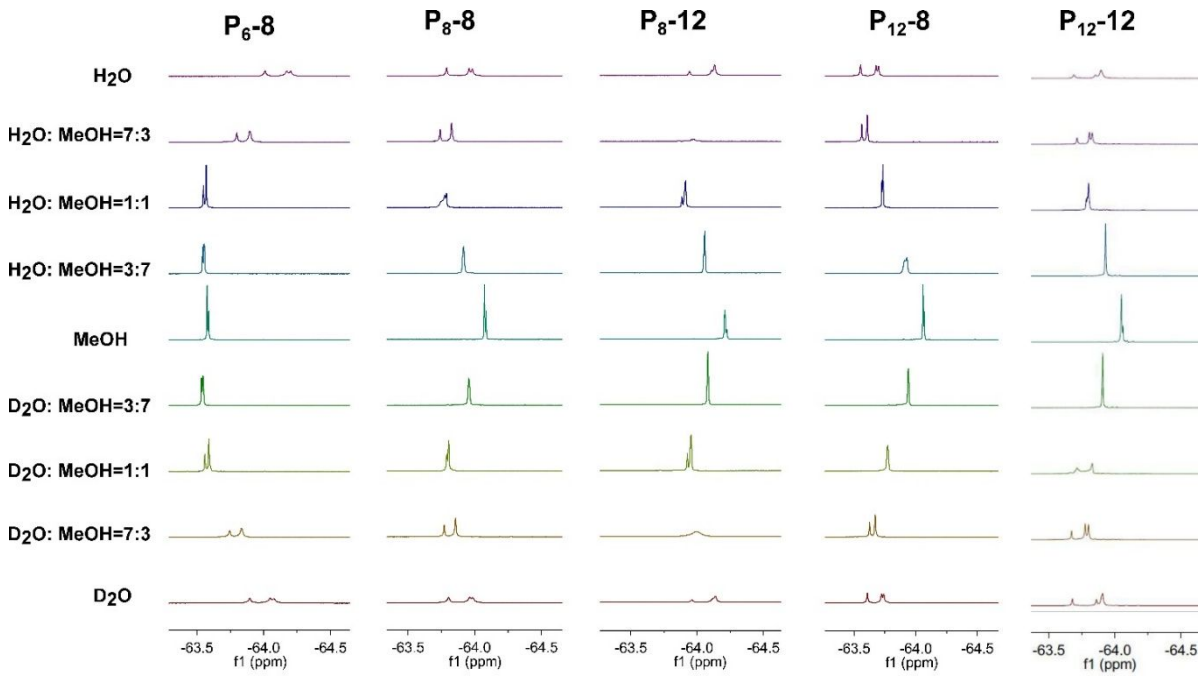


Figure S2. Solvent-dependent ^{19}F NMR spectra of the M-PEG peptides at 10 °C.

4. Turbidity Test (Table S1)

The turbidity test was performed on a UV-visible Lambda 35 spectrometer (Perkin Elmer, USA) at 700 nm. The transmittance was measured between 7 °C and 70 °C through temperature-controlled heating and cooling cycles and the sample was equilibrated for 10 min before measurement.

Table S1. LCSTs of the M-PEG peptides at 3.42 mM and 0.342 mM.

Peptides	LCST at 3.42mM	LCST at 0.342mM
P_6-8	28 °C	31 °C
P_6-12	-	10 °C
P_8-8	43 °C	47 °C
P_8-12	23 °C	26 °C
P_{12}-8	58 °C	62 °C
P_{12}-12	39 °C	43 °C

5. Dynamic light scattering (Figure S3)

The M-PEG peptides in H_2O at 0.342 mM and 3.42 mM were used for DLS analysis. The particle size was measured at an angle of 90° in a 10 mm diameter cell at the desired temperature (from 5 °C to 70 °C) with a Dynamic Light Scattering (DLS) Analyzer (Malvern ZetasizerNano 3690). Eleven scans were run for each measurement and the measurement was repeated 3 times. The particle size and polydispersity index (PDI) were calculated by Malvern software.

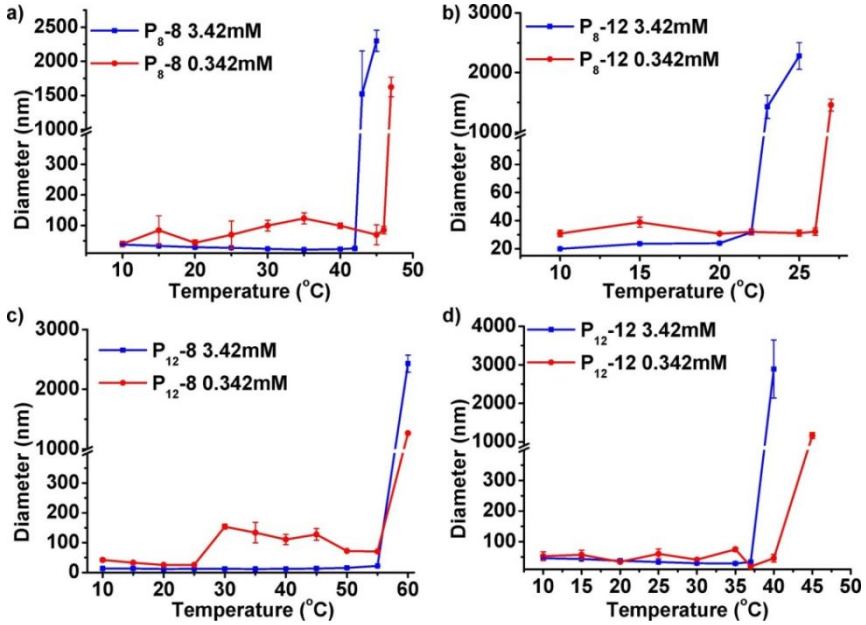


Figure S3. Particle sizes of the M-PEG peptides at 3.42 mM and 0.342 mM measured by DLS.

6. Transmission electron microscopy (Figure S4)

The carbon-coated copper grids, pipette tips, and samples were incubated in an isothermal oven at desired temperature (5 °C, 25 °C, 35 °C, 50 °C or 65 °C) for at least 30 min before sample preparation in the oven. The M-PEG peptides were dissolved in water at a concentration of 0.342 mM. 5 μL of the sample solution was dropped on the grid and blotted after 60 seconds. Then the grids were stained with 1% (wt/vol) uranyl acetate solution for 30 s before taking images. The sample was allowed to dry in the oven at the desired temperature for 30 minutes and then was air-dried for 2 hours. TEM images were taken on a JEM-1230 at an acceleration voltage of 200 kV.

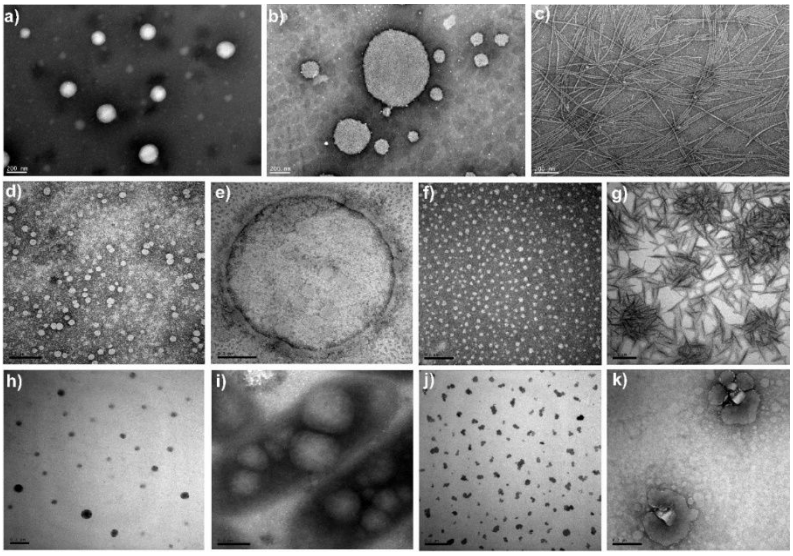


Figure S4. TEM of **P_6-12** (a: 5 °C, b: 25 °C), **P_8-8'** (c: 25 °C), **P_8-8** (d: 25 °C, e: 50 °C), **P_{12}-8** (f: 25 °C, g: 65 °C), **P_8-12** (h: 25 °C, i: 35 °C), **P_{12}-12** (j: 25 °C, k: 50 °C). Figure a, d, f, h, j were images of

M-PEG comb below the corresponding LCST and Figure b, e, g, i, k were images of M-PEG comb above the corresponding LCST, respectively. Scale bars = 200 nm.

7. HPLC chromatograms of the M-PEG peptides (Figure S5)

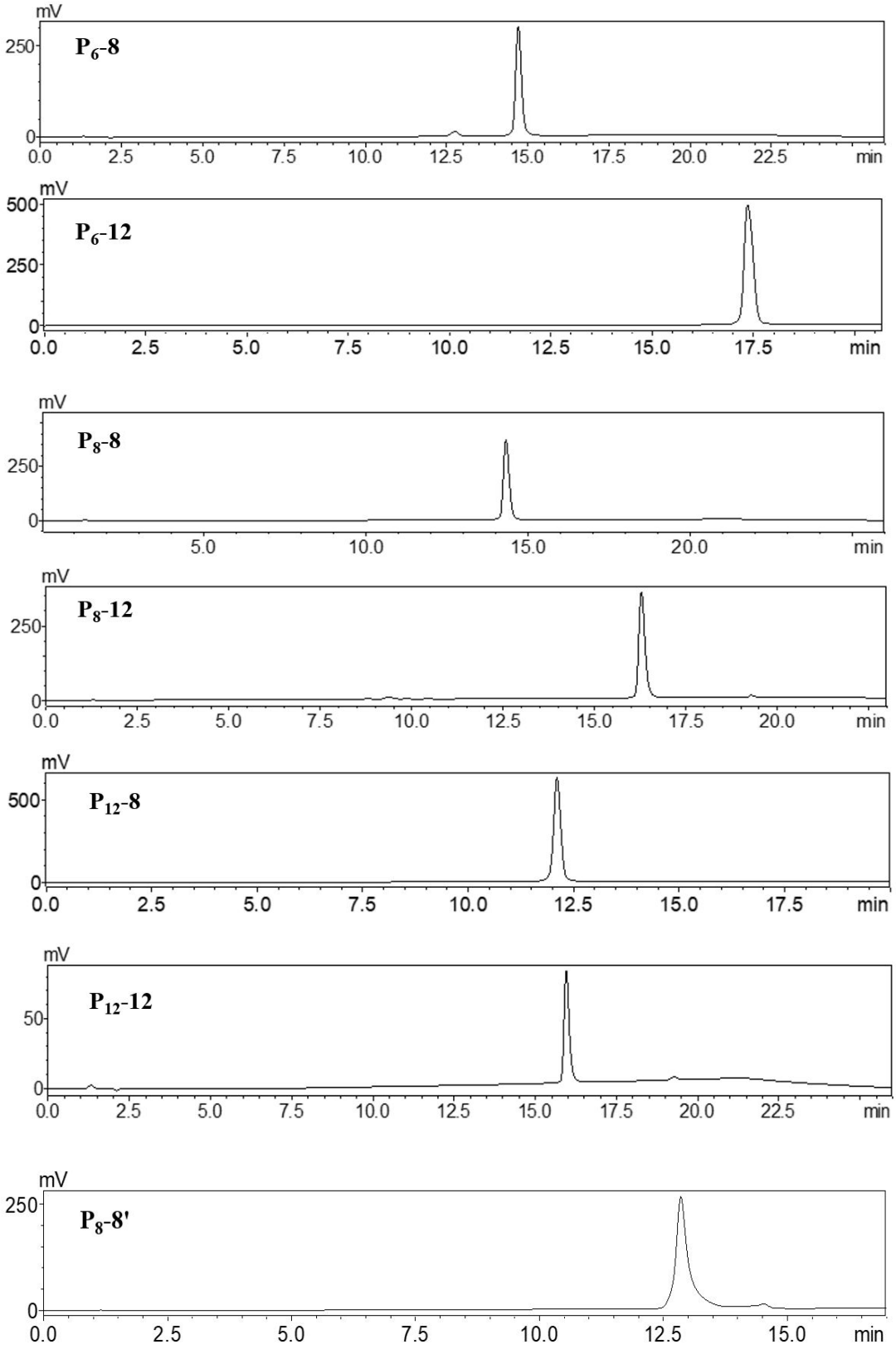


Figure S5. HPLC chromatograms of the M-PEG peptides.

8. Cell culture and cytotoxicity assay

HepG2 cells were cultured in DMEM medium containing 10% FBS. L929 cells were cultured in alpha-MEM medium containing 10% FBS. All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2 and the growth medium was replaced with fresh media every 24 h.

The biocompatibility assay of the M-PEG peptides was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. For the biocompatibility assay, L929 cells were seeded into a 96-well plate and allowed for adherent culture at 37 °C for several hours. Subsequently, a gradient concentration of the M-PEG peptide ranging from 8 μg/mL to 1000 μg/mL were added in a series of wells. Every concentration was set with five wells at least. The wells with 100 μL culture medium alone were used as negative control and wells containing cells alone were used as positive control. After incubation for 24 h, the medium was replaced with 100 μL MTT (1.0 mg/mL) solution and incubated for 4 h. Then the medium was replaced with 100 μL DMSO and the absorbance value was measured at 490 nm using a microplate reader. All of the experiments were repeated in three times at least. Antiproliferation efficiency of the nanoemulsions and DOX on HepG2 cells were performed with MTT assay in the similar fashion

9. IC_{50} of P_{12}-12+DOX and DOX at 37 °C or 40 °C on HepG2 cells

HepG2 cells were seeded into a 96-well plate and allowed for adherent culture at 37 °C for 24 hours. Subsequently, a gradient concentration of free DOX and **P_{12}-12**+DOX nanoemulsion were added and eventual concentration is 0.1, 1, 5, 10, 20, 50 μg/mL, respectively. Then the 96-well plate was cultured at 37 °C or 40 °C for 48 h, followed by replacing the medium with 100 μL MTT (1.0 mg/mL) solution and incubated for another 4 h at 37 °C. Cells treated with normal medium were used as control. Then the medium was replaced with 100 μL DMSO and the absorbance values was measured at 490 nm using a microplate reader. All the experiments were repeated three times.

10. Organ distribution of BODIPY loaded P_{12}-12 nanoemulsion (Figure S6)

The HepG2 tumor-bearing nude mice were injected with 250 μL of **P_{12}-12**-loaded BODIPY nanoemulsion (0.6 μmol/kg BODIPY) via tail vein. Distribution of BODIPY and **P_{12}-12** in kidney, liver and tumor were analyzed on groups of 2 mice. At 4 h and 24 h after iv injection, the mice were euthanized and the kidney, liver and tumor were collected. After tissue homogenization, BODIPY and **P_{12}-12** in tissue samples were extracted with methanol. Then the concentration of BODIPY and **P_{12}-**

12 were determined by HPLC, respectively. The concentration of BODIPY and **P_{12}-12** were expressed as percentage of injected dose per gram tissue (% ID/g tissue).

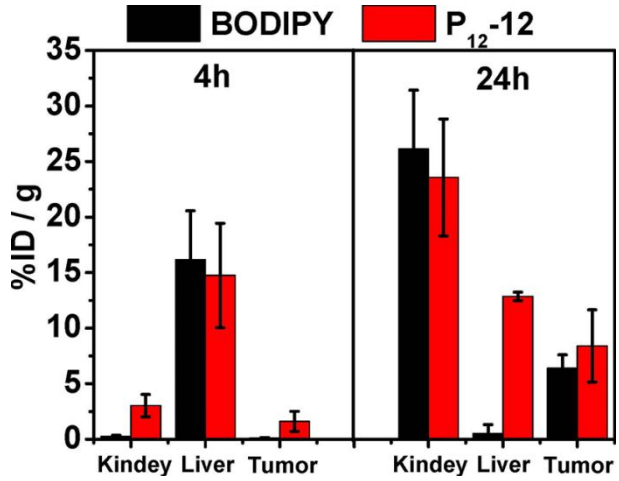


Figure S6. Organ distribution of **P_{12}-12** and BODIPY.

11. LCST range of pure M-PEG combs and their mixtures (Table S2)

Table S2. LCST range of pure M-PEG combs and their mixtures at 0.342 mM

Transmittance between 99% and 5%		
M-PEG combs	Temperature range (°C)	Δ T (°C)
P_6-8	30.5 to 31	0.5
P_6-12	9.5 to 10	0.5
P_8-8	46 to 46.5	0.5
P_8-12	25.5 to 26	0.5
P_{12}-8	61.5 to 62	0.5
P_{12}-12	42.5 to 43	0.5
P_6-8+P_8-8	40 to 44	4
P_6-8+P_{12}-8	49 to 53	4
P_6-8+P_6-12	19 to 23	4
P_6-12+P_8-12	19 to 25	6
P_6-12+P_{12}-12	30 to 35	5
P_8-8+P_{12}-8	53 to 56	3
P_8-12+P_{12}-12	35 to 38	3

12. BODIPY release curves of P_{12}-12+BODIPY nanoemulsion (Figure S7)

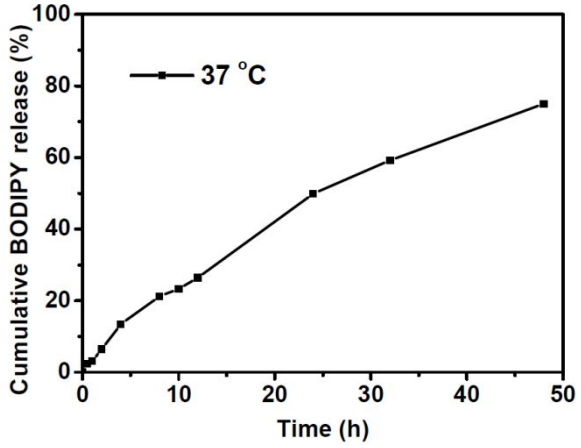
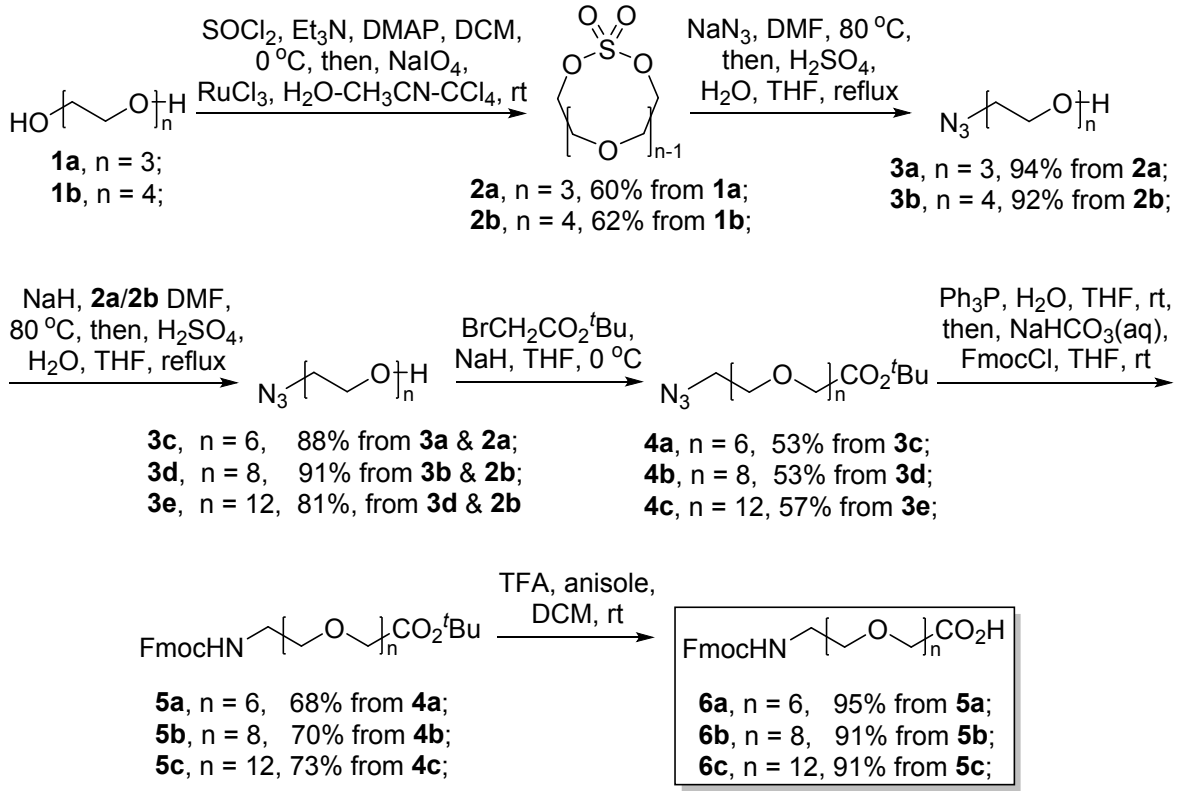


Figure S7 In vitro of BODIPY release curves

13. Synthesis of M-PEGs ω-amino acids



Compound 2a. Under an atmosphere of Ar, $SOCl_2$ (95.2 g, 0.8 mol, in 200 mL CH_2Cl_2) was added over 1 h to a stirring solution of **1a** (60.0 g, 0.4 mol), Et_3N (194.0 g, 1.9 mol) and DMAP (2.4 g, 20.0 mmol) in CH_2Cl_2 (6 L) at 0 °C. After the addition, the mixture was stirred at 0 °C for 2 h and quenched with cold brine (200 mL). The organic layer was collected and washed with H_2O (600 mL, 3 times). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated, purified by flash

chromatography on silica gel (PE: EA = 1:1) to give a residue as brown oil. Subsequently, the residue was dissolved in a mixture of CH_3CN (600 mL), CCl_4 (600 mL) and water (900 mL) at 0 °C. $NaIO_4$ (70.0 g, 0.3 mol) and $RuCl_3 \cdot 3H_2O$ (0.3 g, 1.3 mmol) were sequentially added to the reaction mixture and the resulting mixture was stirred at 0 °C for 2 h. The organic layer was collected and the aqueous layer was extracted with CH_2Cl_2 (500 mL, 2 times). The combined organic layer was dried over anhydrous Na_2SO_4, filtrated through a pad of celite, concentrated, purified by flash chromatography on silica gel (PE: EA = 1:1) to give 51.1 g of **2a** as white solid with a 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65 (s, 4H), 3.85 (t, J = 4.0 Hz, 4H), 4.44 (t, J = 4.0 Hz, 4H).

Compound 2b was prepared from **1b** by following the same procedure for **2a** as white solid (65.8 g, yield: 62%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65-3.71 (m, 8H), 3.85 (t, J = 8.0 Hz, 4H), 4.47-4.50 (m, 4H).

Compound 3a. To a mixture of cyclic sulfate **2a** (25.0 g, 94.2 mmol) in DMF (200 mL) was added sodium azide (9.2 g, 141.3 mmol) and the resulting mixture was stirred at 80 °C for 5 h. After cooled to room temperature, excess sodium azide was filtrated by a pad of celite. DMF was removed under vacuum and the resulting residue was dissolved in THF (250 mL). Then, water (2.6 mL, 141.3 mmol) was added and the pH was adjusted to 2-3 with H_2SO_4, after addition, the resulting mixture was refluxed for 1 h. The reaction was quenched with saturated $NaHCO_3$ solution and the resulting mixture was extracted with DCM (200 mL × 3). The organic layers were combined, dried over anhydrous Na_2SO_4, concentrated under vacuum, purified by flash chromatography on silica gel (PE: EA = 1:1) to give **3a** (15.5 g, yield: 94%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.41 (t, J = 5.2 Hz, 2H), 3.61-3.64 (m, 2H), 3.67-3.71 (m, 6H), 3.75 (t, J = 4.8 Hz).

Compound 3b was prepared from cyclic sulfate **2b** by following the same procedure for **3a** as clear oil (28.2 g, yield: 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (t, J = 5.2 Hz, 2H), 3.59-3.72 (m, 14H).

Compound 3c. Under an atmosphere of Ar, to a suspension of NaH (3.0 g, 60% in mineral oil, 123.8 mmol) in dry THF (100 mL) was added a solution of compound **3a** (15.5 g, 88.4 mmol) in THF (10 mL) at 0 °C and the resulting mixture was stirred for 20 min at this temperature. Then a solution of cyclic sulfate **2a** (26.3 g, 123.8 mmol) in THF (100 mL) was added and the resulting mixture was stirred for 12 h at rt. Then, water (2.6 mL, 141.3 mmol) was added and the pH was adjusted to 2-3 with H_2SO_4, after addition, the resulting mixture was refluxed for 1 h. The reaction was quenched with saturated $NaHCO_3$ solution and the resulting mixture was extracted with DCM (200mL × 3). The

organic layers were combined, dried over anhydrous Na_2SO_4, concentrated under vacuum, purified by flash chromatography on silica gel (DCM: MeOH = 20:1) to give **3c** (24.0 g, yield: 88%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (t, *J* = 5.2 Hz, 2H), 3.60-3.74 (m, 22H).

Compound 3d was prepared from **3b** and cyclic sulfate **2b** by following the same procedure for **3c** as clear oil (29.0 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38 (t, *J* = 4.8 Hz, 2H), 3.58-3.72 (m, 30H).

Compound 3e was prepared from **3d** and cyclic sulfate **2b** by following the same procedure for **3c** as clear oil (33.5 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (t, *J* = 5.2 Hz, 2H), 3.64-3.71 (m, 46H), 4.74 (s, 1H).

Compound 4a. Under an atmosphere of Ar, to a suspension of NaH (4.3 g, 60% in mineral oil, 107.4 mmol) in dry THF (200 mL) was added a solution of azide **3c** (22.0 g, 71.6 mmol) in THF (80 mL) at 0 °C and the mixture was stirred for 30 min at this temperature. Then *tert*-butyl bromoacetate (41.9 g, 214.8 mmol) was added and the resulting mixture was stirred for 24 h at 25 °C. The mixture was quenched with water (200 mL). The resulting mixture was extracted with EtOAc (300 mL, 3 times). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum, purified by flash chromatography on silica gel (DCM: MeOH = 30:1) to give **4a** as yellowish wax (15.9 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.48 (s, 9H), 3.40 (t, *J* = 5.2 Hz, 2H), 3.66-3.71 (m, 22H), 4.02 (s, 2H).

Compound 4b was prepared from **3d** by following the same procedure for **4a** as yellowish wax (24.3 g, 53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46(s, 9H), 3.38 (t, *J* = 5.2 Hz, 2H), 3.63-3.71 (m, 30H), 4.00 (s, 2H).

Compound 4c was prepared from **3e** by following the same procedure for **4a** as yellowish wax (12.5 g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 9H), 3.30 (t, *J* = 5.2 Hz, 2H), 3.55-3.65 (m, 46H), 3.93 (s, 2H).

Compound 5a. Compound **4a** (16.0 g, 38.0 mmol) was dissolved in THF (200 mL) and then triphenylphosphine (14.9 g, 56.9 mmol) was added and the mixture was stirred for 5 h at 25 °C. H_2O (3.4 μL, 189.8 mmol) was then added to the reaction mixture and the mixture was stirred for an additional hour. The reaction mixture was concentrated under vacuum and the residue was purified by flash chromatography on silica gel to give the crude product. Then the crude product was dissolved in THF (150 mL) and added to a mixture of saturated $NaHCO_3$ (3.8 g, 45.6 mmol in 15 mL H_2O) at 0 °C. The reaction mixture was then slowly added a solution of 9-fluorenylmethyl chloroformate (11.1 g,

43.7 mmol) in THF (50 mL) over 1 h. The reaction mixture was stirred at 25 °C for 4 h. Brine (200 mL) was added to quench the reaction. EtOAc (200 mL) was added to the reaction mixture and the organic layer was collected. The aqueous layer was extracted with EtOAc (300 mL, 3 times). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum, purified by chromatography on silica gel (DCM: MeOH = 30:1) to give **5a** as yellowish oil (15.5 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 3.37-3.44 (m, 2H), 3.56 (t, *J* = 5.2 Hz, 2H), 3.62-3.71 (m, 20H), 4.00 (s, 2H), 4.22 (t, *J* = 6.8 Hz, 1H), 4.38 (d, *J* = 6.8 Hz, 2H), 7.29-7.76 (m, 8H).

Compound 5b was prepared from **4b** by following the same procedure for **5a** as yellowish oil (12.9 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 3.39-3.41 (m, 2H), 3.57 (t, *J* = 4.8 Hz, 2H), 3.61-3.72 (m, 28H), 4.01 (s, 2H), 4.22 (t, *J* = 6.8 Hz, 1H), 4.39 (d, *J* = 7.2 Hz, 2H), 7.31-7.77 (m, 8H).

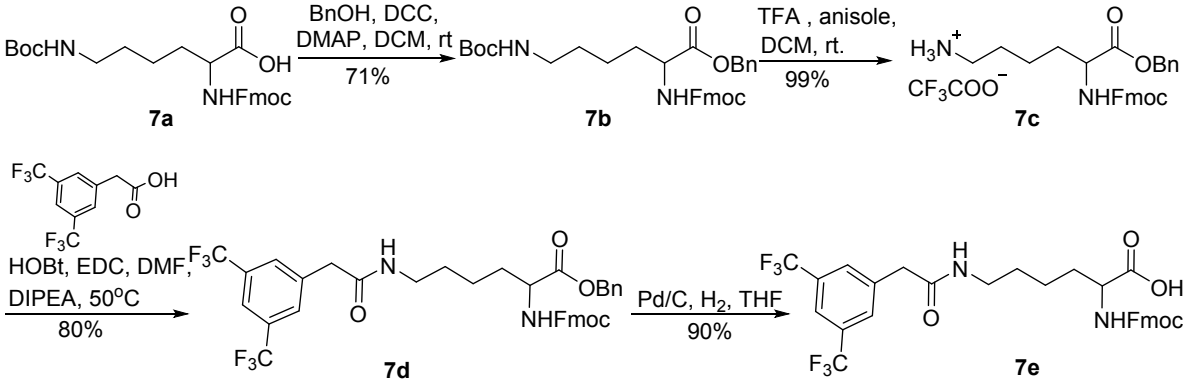
Compound 5c was prepared from **4c** by following the same procedure for **5a** as yellowish oil (13.7 g, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 3.36-3.40 (m, 2H), 3.56-3.69 (m, 46H), 4.00 (s, 2H), 4.21 (t, *J* = 6.8 Hz, 1H), 4.38 (d, *J* = 6.8 Hz, 2H), 7.28-7.76 (m, 8H).

Compound 6a. A solution of compound **5a** (11.4 g, 18.5 mmol), anisole (4.0 mL, 36.9 mmol) and TFA (27.5 mL, 370.1 mmol) in DCM (150 mL) was stirred at 25 °C over 4 h. After concentrated under vacuum, the residue was dissolved in ether (100 mL) and washed with water (100 mL). The organic layer was discarded and the aqueous layer was extracted with DCM (200 mL, 3 times). The combined organic layer was dried over anhydrous Na_2SO_4, concentrated under vacuum to give **6a** (9.9 g, 95% yield) as yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.35-3.39 (m, 2H). 3.54-3.69 (m, 22H), 4.12 (s, 2H), 4.20 (t, *J* = 6.9 Hz, 1H), 4.39 (d, *J* = 7.0 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.4 Hz, 2H), 7.73 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 175.1, 156.6, 143.9, 141.13, 127.5, 127.0, 125.0, 119.8, 70.3-69.5(m), 69.3 (d, *J* = 23.5 Hz), 66.3, 47.1, 40.6. HRMS (ESI) calcd for $C_{29}H_{39}NNaO_{10}^+$ [$(M+Na)^+$] 584.2466, found 584.2436.

Compound 6b was prepared from **5b** by following the same procedure for **6a** (11.4 g, 91% yield) as yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39-3.40 (m, 2H), 3.48-3.73 (m, 30H), 4.14 (s, 2H), 4.22 (t, *J* = 6.4 Hz, 1H), 4.39 (d, *J* = 6.8 Hz, 2H), 7.29-7.77 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 156.7, 141.3, 127.7, 127.1, 125.1, 120.0, 71.0, 70.4 (dt, *J* = 25.1, 14.4 Hz), 70.1-69.8 (m), 68.8, 66.5, 47.3, 40.9. HRMS (ESI) calcd for $C_{33}H_{47}NNaO_{12}^+$ [$(M+Na)^+$] 672.2990, found 672.2997.

Compound 6c was prepared from **5c** by following the same procedure for **6a** (10.2 g, 91% yield) as yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38-3.41 (m, 2H), 3.55-3.65 (m, 46H), 3.93 (s, 2H), 4.21 (t, *J* = 6.4 Hz, 1H), 4.38 (d, *J* = 6.8 Hz, 2H), 7.28-7.76 (m, 8H).

14. Synthesis of amino acid 7e



Compound 7b. To a solution of **7a** (2.0 g, 4.3 mmol) in dry DCM (100 mL) was added DCC (1.3 g, 6.4 mmol) and DMAP (26.1 mg, 0.2 mmol) at 0 °C. After the reaction mixture was stirring for 15 min, phemethylol (1.4 g, 12.8 mmol) in DCM was added drop-wise over 5 min at 0 °C. Then the resulting mixture was stirring at rt for 2 h. The reaction mixture was filtered after **7a** was completely consumed on TLC (DCM: MeOH = 10:1). The filtrate was concentrated and purified by flash column chromatography on silica gel (PE: EA = 6:1 to 5:1) to give **7b** as white foam (1.7 g, yield: 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24-1.35 (m, 2H), 1.43 (s, 11H), 1.62-1.75 (m, 1H), 1.83-1.91 (m, 1 H), 3.05-3.08 (m, 2H), 4.21 (t, *J* = 8.0 Hz, 1H), 4.35-4.42 (m, 3H), 4.52 (s, 1H), 5.17 (q, *J* = 12.2 Hz, 2H), 5.43 (d, *J* = 8.0 Hz, 1H), 7.29-7.42 (m, 9H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.77 (d, *J* = 4.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 28.4, 29.5, 32.0, 40.0, 47.1, 53.8, 67.0, 67.1, 79.1, 119.9, 125.1, 127.1, 127.7, 128.3, 128.5, 128.6, 135.3, 141.3, 143.7, 143.9, 156.1, 172.4. HRMS (ESI) calcd for $C_{33}H_{38}N_2NaO_6^+$ 581.2622 ($(M + Na)^+$), found 581.2625.

Compound 7c. To a solution of **7b** (1.7 g, 3.0 mmol) in dry DCM was added anisole (0.7 g, 6.0 mmol) and TFA (10.3 g, 90.1 mmol) at rt and the reaction mixture was stirred for 1 h at room temperature. After **7b** was consumed completely on TLC (PE: EA = 3:1), the reaction mixture was concentrated under reduced pressure to give **7c** as yellow foam (1.7 g, yield: 99%).

Compound 7d. A mixture of 2-(3,5-Bis(trifluoromethyl)phenyl) acetic acid (1.3 g, 4.8 mmol), DIPEA (0.7 mL, 12.1 mmol) and EDCl (1.4 g, 7.2 mmol) in dry DMF (20 mL) was stirred at 0 °C under N_2.

HOBt (1.0 g, 7.2 mmol) in DMF was added slowly into the mixture at 0 °C and the mixture was stirred for 30 min at this temperature. Then **7c** (2.3 g, 4.0 mmol) in DMF was added slowly to the reaction mixture. After addition, the resulting mixture was stirred at 50 °C for 2 h. The mixture was extracted with DCM (100 mL × 3) after H_2O (80 mL) was added. The combined organic layers were dried over anhydrous sodium sulfate and concentrated to give a residue. The residue was purified by flash column chromatography on silica gel (PE: EA = 6:1 to 2:1) to give the desired product **7d** (2.3 g, yield: 80%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29-1.41 (m, 4H), 1.64-1.73 (m, 2H), 3.04-3.05 (m, 2H), 3.66 (s, 2H), 4.04-4.09 (m, 1H), 4.22 (t, *J* = 8.0 Hz, 1H), 4.27-4.36 (m, 2H), 5.12 (s, 2H), 7.29-7.34 (m, 9H), 7.38-7.42 (t, *J*=8.0 Hz, 2H), 7.72 (d, *J* = 4.0 Hz, 2H), 7.87 (t, *J* = 8.0 Hz, 2H), 7.95 (s, 3H), 8.20 (t, *J* = 4.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.0, 28.6, 30.4, 38.2, 41.3, 46.7, 54.0, 65.8, 65.9, 120.1, 122.1 (q, *J* = 271 Hz), 125.3, 127.7, 127.8, 128.0, 128.4, 129.9, 130.2, 136.0, 139.9, 140.8, 143.8, 143.9, 156.3, 168.8, 172.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -66.02. HRMS (ESI) calcd for $C_{38}H_{34}F_6N_2NaO_5^+$ 735.2264 ($(M+Na)^+$), found 735.2268.

Compound 7e. A mixture of **7d** (4.5 g, 6.3 mmol) and Pd/C (450 mg, 10% on carbon) in THF was stirred at rt under H_2 (1 atm) for 12 h. The mixture was filtered through Cite and the filtrate was concentrated to give **7e** as white solid (3.5 g, yield: 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29-1.43 (m, 4H), 1.55-1.70 (m, 2H), 3.04-3.06 (m, 2H), 3.66 (s, 2H), 3.87-3.91 (m, 1H), 4.20-4.28 (m, 3H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.61 (d, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 8.0 Hz, 2H), 7.88 (d, *J* = 8.0 Hz, 2H), 7.95-7.97 (d, 3H), 8.19 (t, *J* = 8.0 Hz, 2H). ^{13}C NMR (100MHz, DMSO-d_6) δ 23.2, 28.7, 30.7, 38.8, 41.4, 46.8, 54.1, 65.7, 120.2, 122.2 (q, *J* = 271 Hz), 125.4, 127.2, 127.7, 130.1, 140.0, 140.9, 143.9, 144.0, 156.3, 168.9, 174.4. ^{19}F NMR (376 MHz, DMSO-d_6) δ -61.27. HRMS (ESI) calcd for $C_{31}H_{29}F_6N_2O_5^+$ 623.1975 (M^+), found 623.1998.

Peptide **P_6-8**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.35-1.39 (m, 6H), 1.51-1.56 (m, 6H), 1.67-1.70 (m, 3H), 1.80-1.83 (m, 3H), 3.17-3.19 (m, 6H), 3.34-3.70 (m, 128H), 3.96 (s, 2H), 3.99-4.03 (m, 6H), 4.38-4.42 (m, 3H), 7.54-7.61 (m, 2H), 7.81-7.99 (m, 13H), 8.40 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ -64.17, -64.18. MS (MALDI-TOF) m/z 2948.2 ($[M + Na]^+$, expected mass for $C_{129}H_{192}F_{18}N_{12}NaO_{42}^+$, 2947.3).

Peptide **P_6-12**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.29-1.41 (m, 10H), 1.51-1.56 (m, 10H), 1.67-1.74 (m, 5H), 1.78-1.85 (m, 5H), 3.15-3.18 (m, 10H), 3.33-3.72 (m, 180H), 3.96 (s, 2H), 4.00-4.03 (m, 11H), 4.38-4.42 (m, 5H), 7.56-7.59 (m, 2H), 7.86-7.99 (m, 19H), 8.40 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD)

δ -64.16, -64.18. MS (MALDI-TOF) m/z 4351.7 ([M + Na]$^+$, expected mass for $C_{189}H_{278}F_{30}N_{18}NaO_{60}^+$, 4352.9).

Peptide **P_8-8**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.29-1.39 (m, 6H), 1.53-1.54 (m, 6H), 1.65-1.74 (m, 3H), 1.80-1.83 (m, 3H), 3.17-3.19 (m, 6H), 3.35-3.72 (m, 168H), 3.97 (s, 2H), 4.00-4.03 (m, 7H), 4.39-4.42 (m, 3H), 7.55-7.61 (m, 2H), 7.86-7.98 (m, 13H), 8.41 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ -64.14. MS (MALDI-TOF) m/z 3388.6 ([M + Na]$^+$, expected mass for $C_{149}H_{232}F_{18}N_{12}NaO_{52}^+$, 3387.6).

Peptide **P_8-12**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.38-1.39 (m, 10H), 1.53-1.56 (m, 10H), 1.65-1.72 (m, 5H), 1.80-1.83 (m, 5H), 3.18-3.19 (m, 10H), 3.31-3.70 (m, 236H), 3.97 (s, 2H), 4.00-4.03 (m, 11H), 4.39-4.42 (m, 5H), 4.63 (s, 4H), 7.55-7.61 (m, 2H), 7.86-7.94 (m, 19H), 8.41 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ -64.11. MS (MALDI-TOF) m/z 4969.8 ([M + Na]$^+$, expected mass for $C_{217}H_{334}F_{30}N_{18}NaO_{74}^+$, 4971.2).

Peptide **P_{12}-8**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.36-1.41 (m, 6H), 1.51-1.56 (m, 6H), 1.66-1.72 (m, 3H), 1.78-1.83 (m, 3H), 3.17-3.20 (m, 6H), 3.35-3.70 (m, 248H), 3.98 (s, 2H), 4.01-4.04 (m, 8H), 4.38-4.42 (m, 3H), 4.63 (s, 2H), 7.57-7.62 (m, 2H), 7.87-8.00 (m, 13H), 8.41 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ -64.12, -64.13. MS (MALDI-TOF) m/z 4266.1 ([M + Na]$^+$, expected mass for $C_{189}H_{312}F_{18}N_{12}NaO_{72}^+$, 4267.1).

Peptide **P_{12}-12**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.32-1.41 (m, 10H), 1.51-1.56 (m, 10H), 1.65-1.75 (m, 5H), 1.78-1.89 (m, 5H), 3.17-3.21 (m, 10H), 3.34-3.70 (m, 348H), 3.97 (s, 2H), 4.00-4.03 (m, 12H), 4.38-4.42 (m, 5H), 7.55-7.62 (m, 2H), 7.87-8.00 (m, 19H), 8.41 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ -64.13. MS (MALDI-TOF) m/z 6204.7 ([M + Na]$^+$, expected mass for $C_{273}H_{446}F_{30}N_{18}NaO_{102}^+$, 6204.0).

Peptide **P_8-8'**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.39-1.61 (m, 12H), 1.68-1.92 (m, 6H), 3.14-3.24 (m, 6H), 3.35-3.73 (m, 168H), 3.97 (s, 2H), 3.99-4.00 (m, 7H), 4.29-4.36 (m, 3H), 7.53-7.60 (m, 2H), 7.84-7.97 (m, 13H), 8.43 (s, 1H). ^{19}F NMR (376 MHz, CD_3OD) δ -64.19. MS (MALDI-TOF) m/z 3401.7 ([M + K]$^+$, expected mass for $C_{149}H_{232}F_{18}N_{12}KO_{52}^+$, 3402.5).

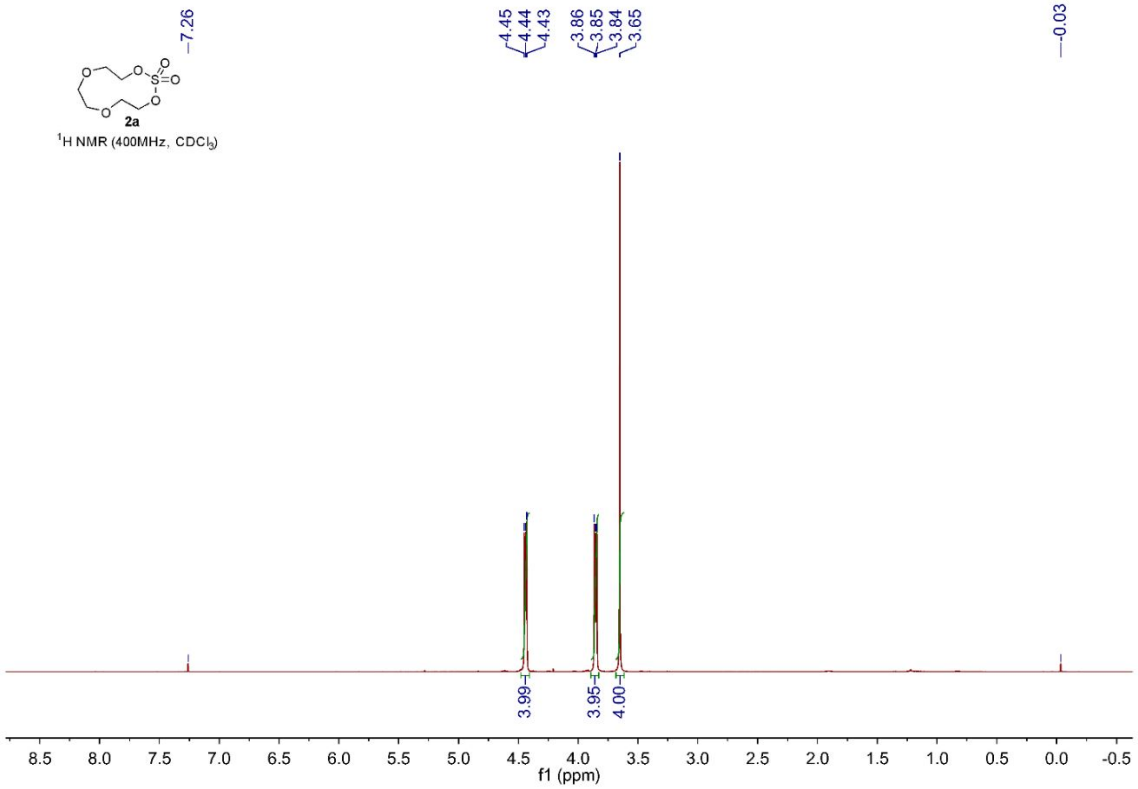
15. Table of known compounds (Table S3)

Table S3. The known compounds

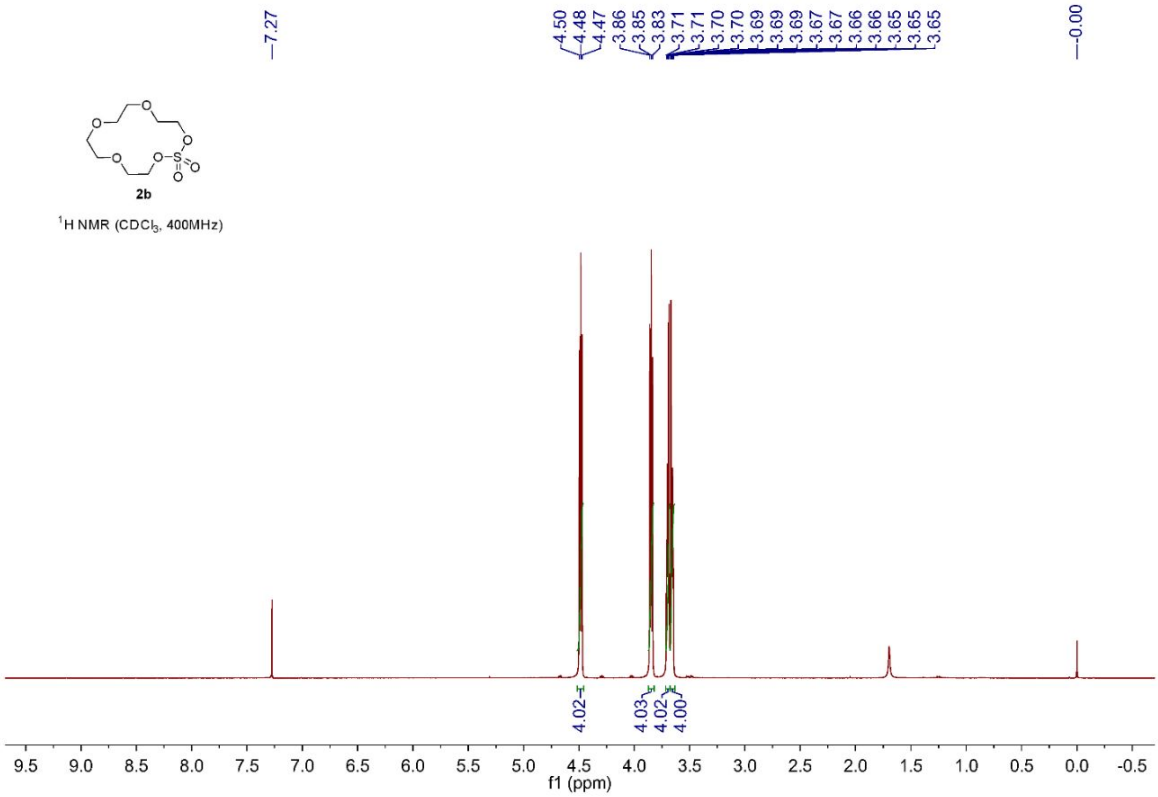
Compound	Reference
2a-b; **3a-e**; **4a-c**; **5a-c**; **6c**	*Org. Biomol. Chem.* **2016**, *14*, 7912-7919.

16. Copies of $^{1}H/^{13}C/^{19}F$ NMR, MS and HRMS spectra of compounds

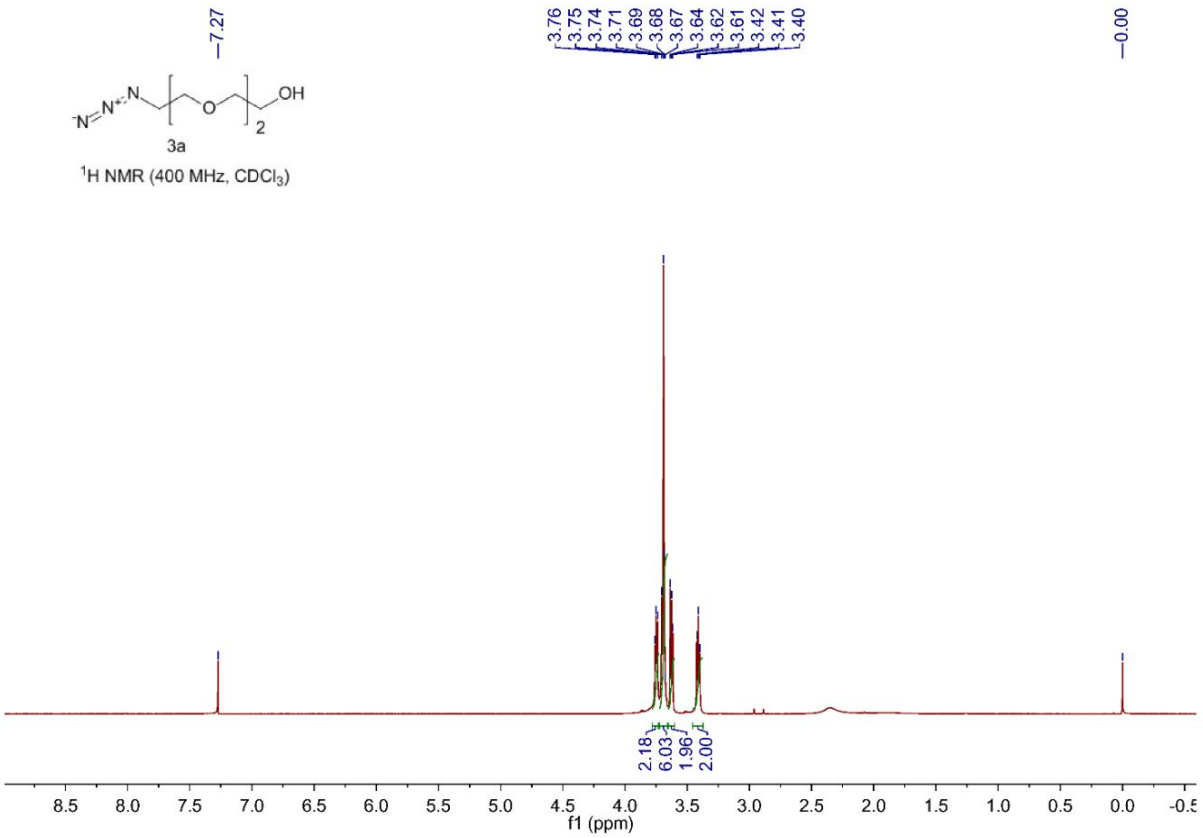
^{1}H NMR of compound **2a**



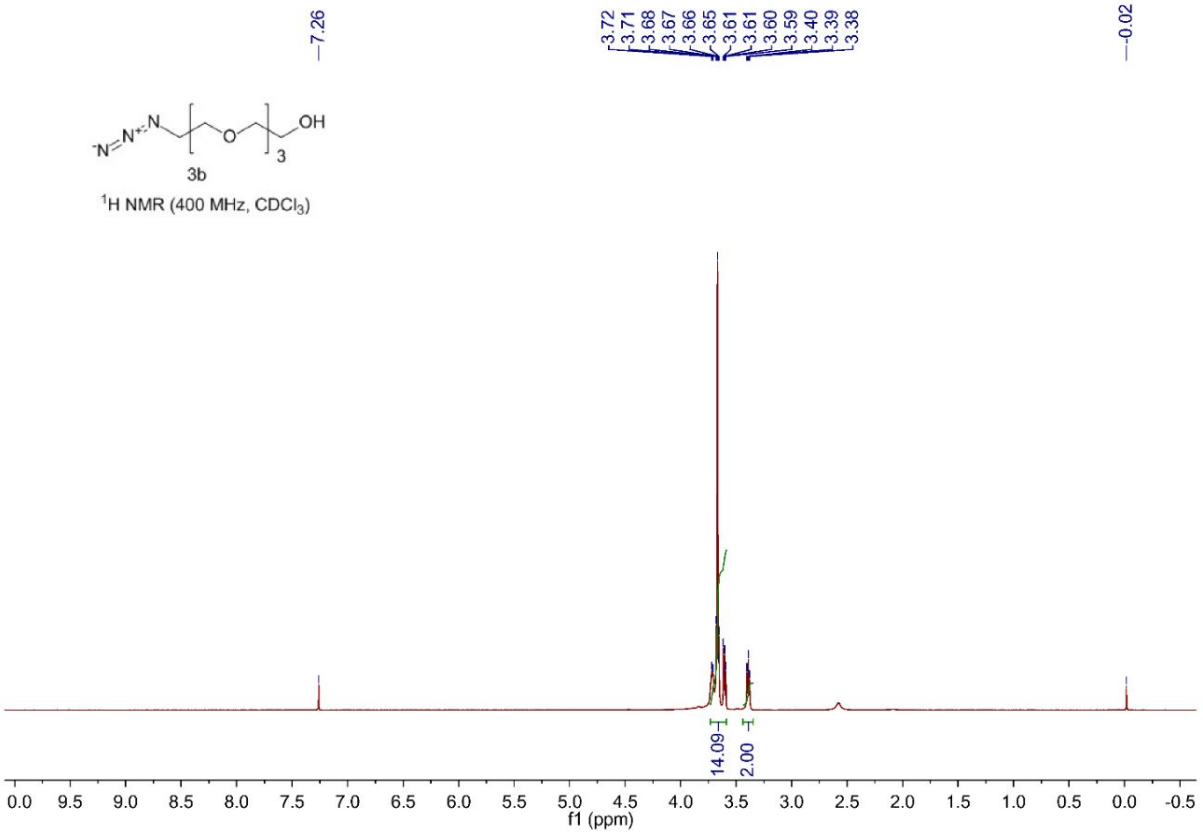
^{1}H NMR of compound **2b**



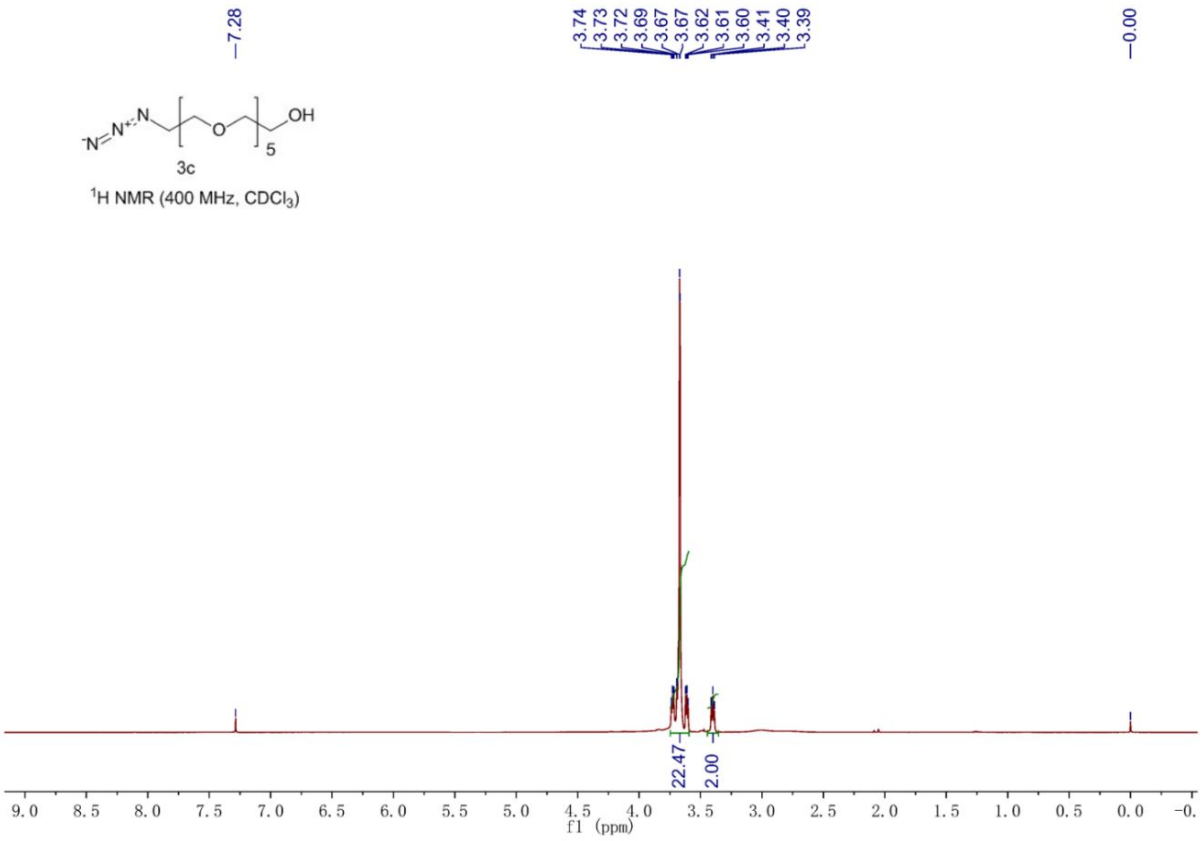
^{1}H NMR of compound **3a**



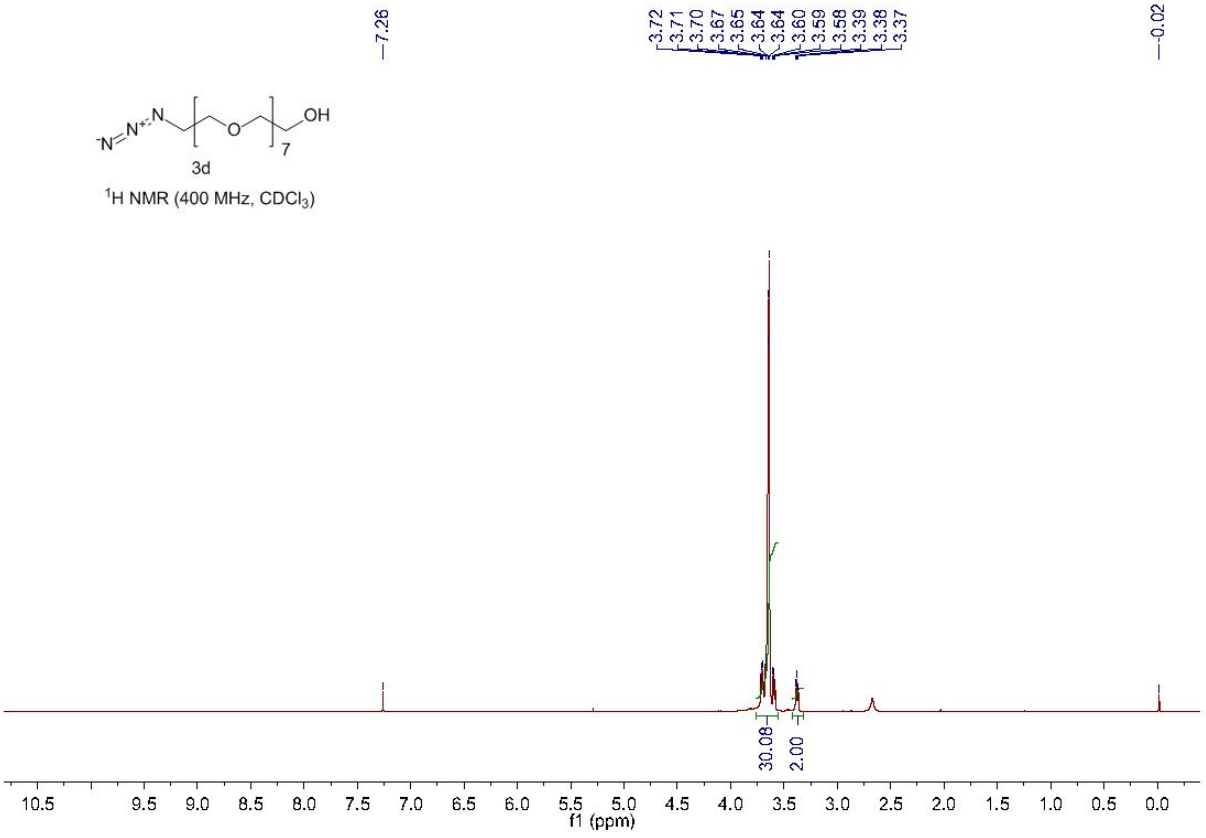
^{1}H NMR of compound **3b**



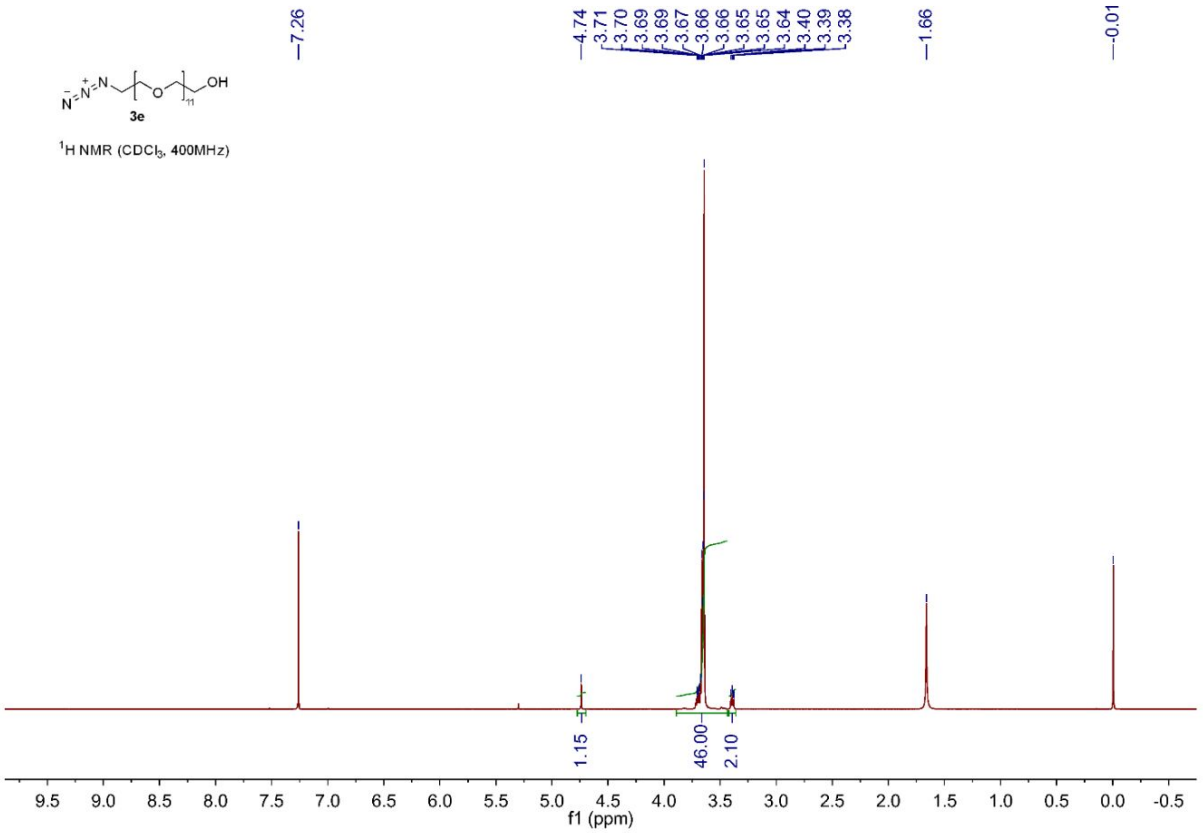
^{1}H NMR of compound **3c**



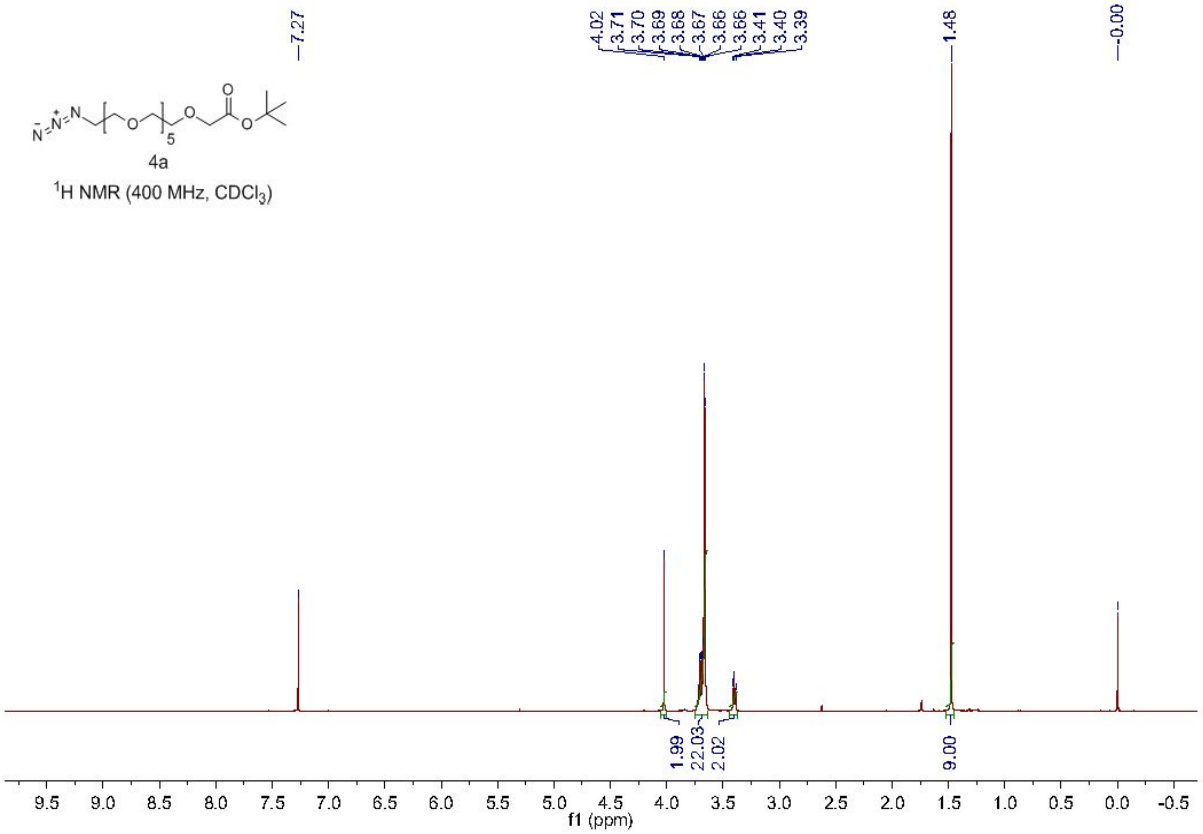
^{1}H NMR of compound **3d**



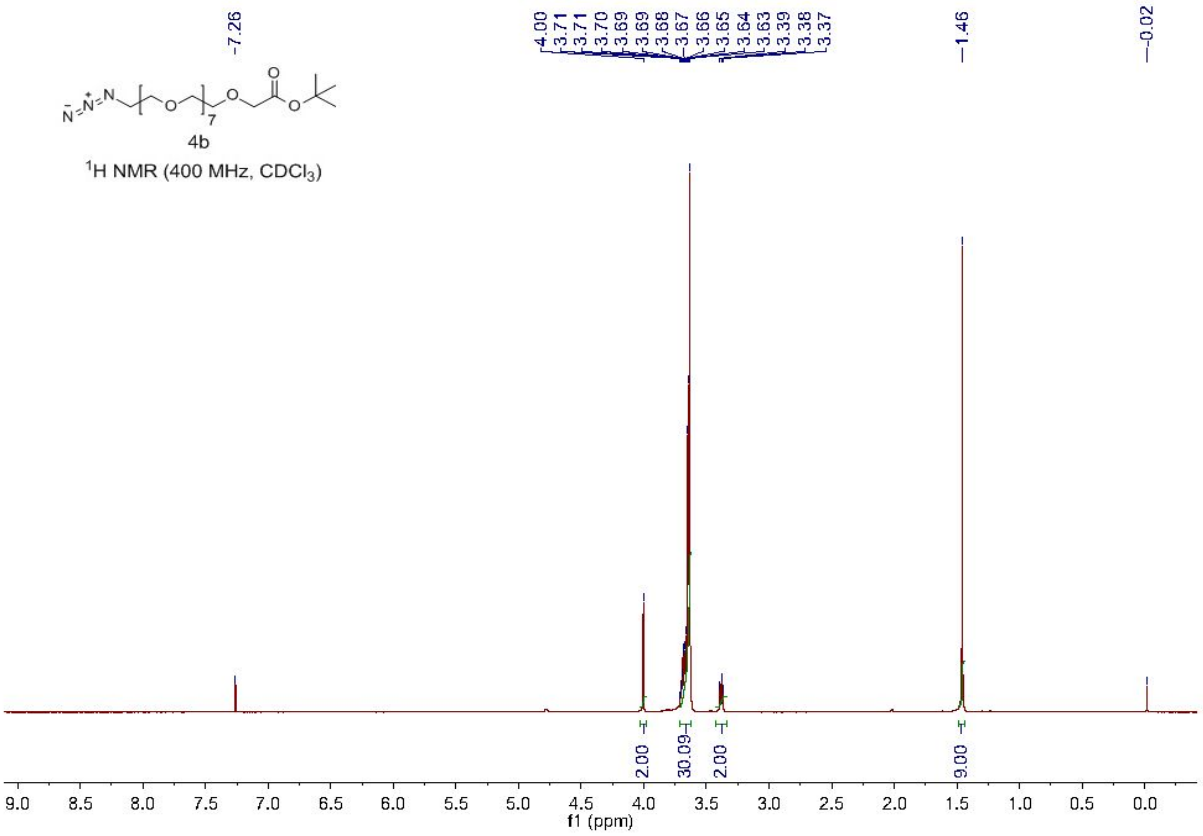
^{1}H NMR of compound **3e**



^{1}H NMR of compound **4a**



^{1}H NMR of compound **4b**



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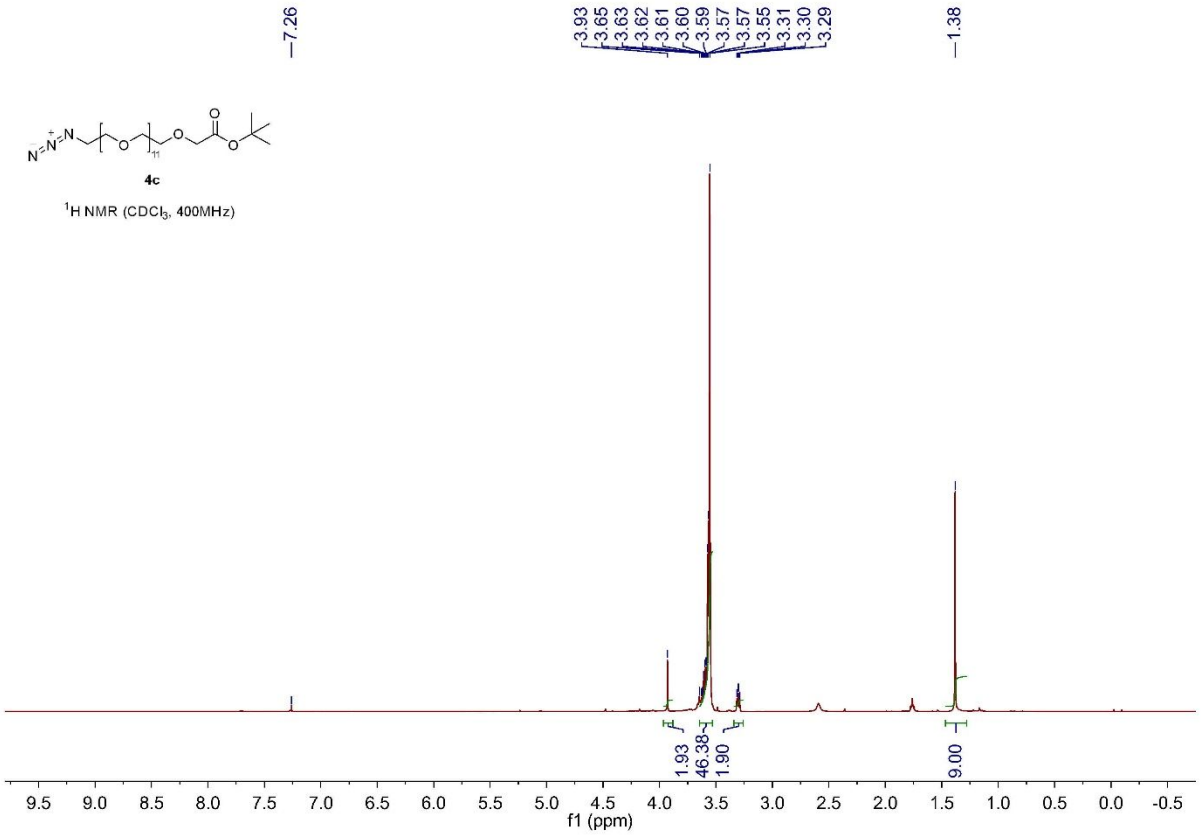
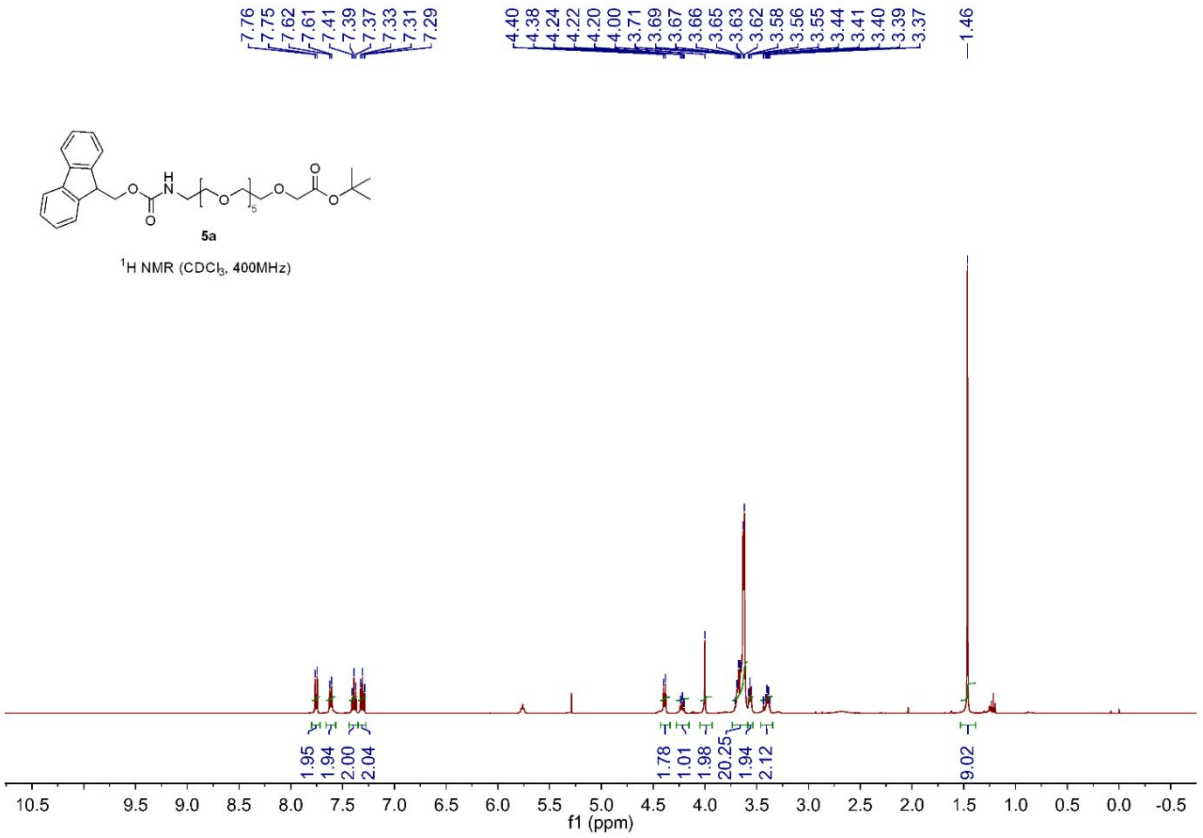
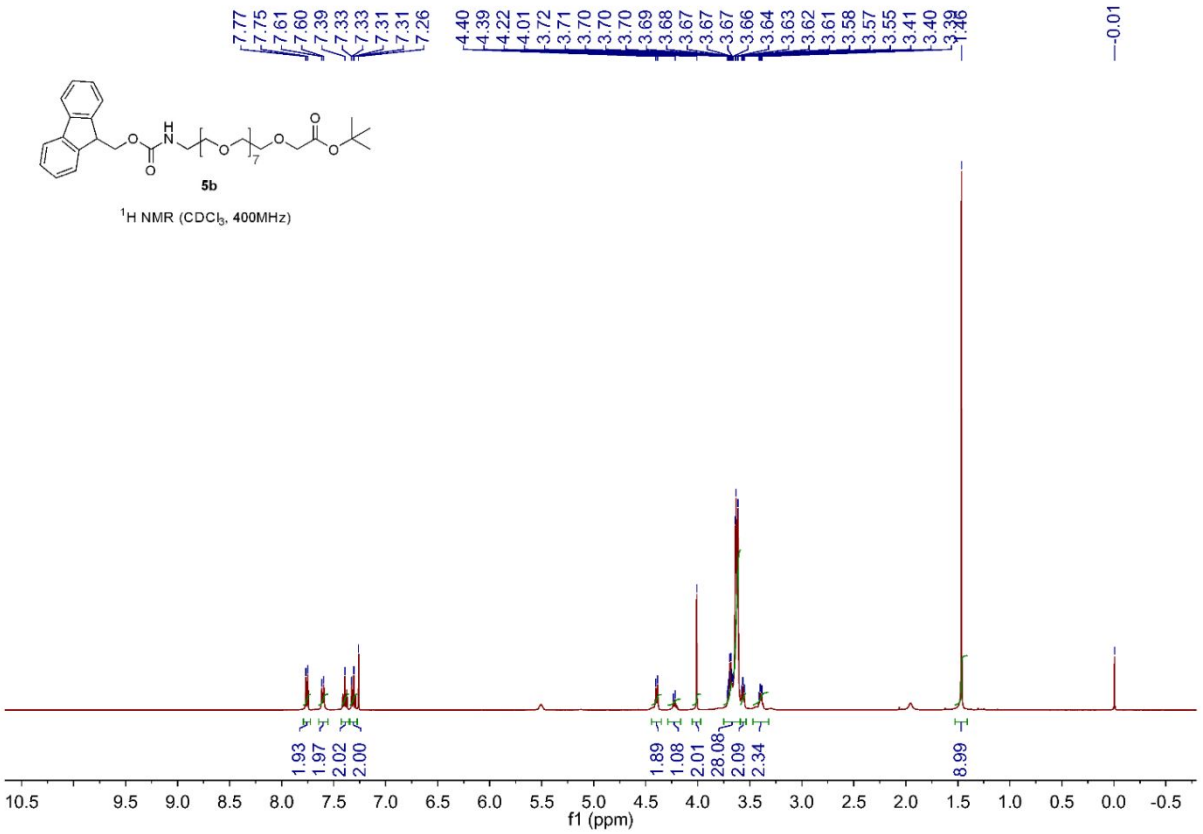


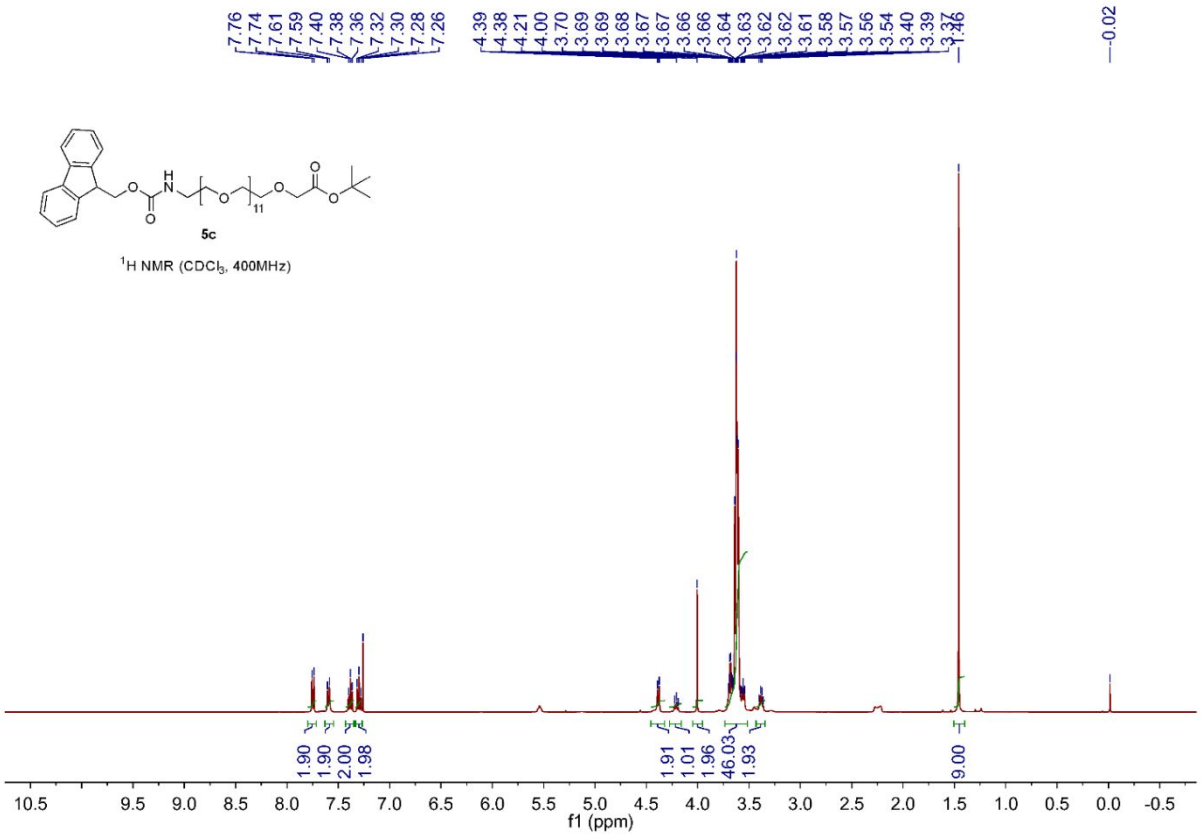
^{1}H NMR of compound **5a**



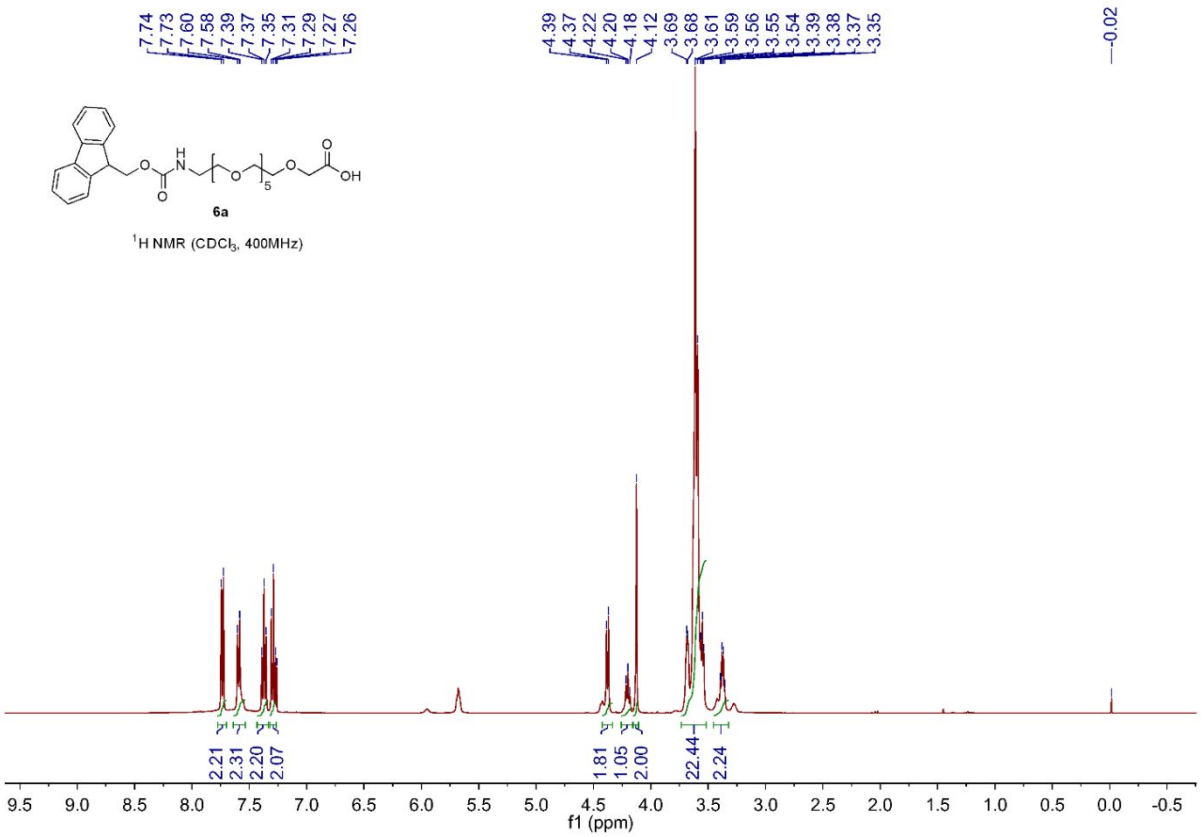
^{1}H NMR of compound **5b**



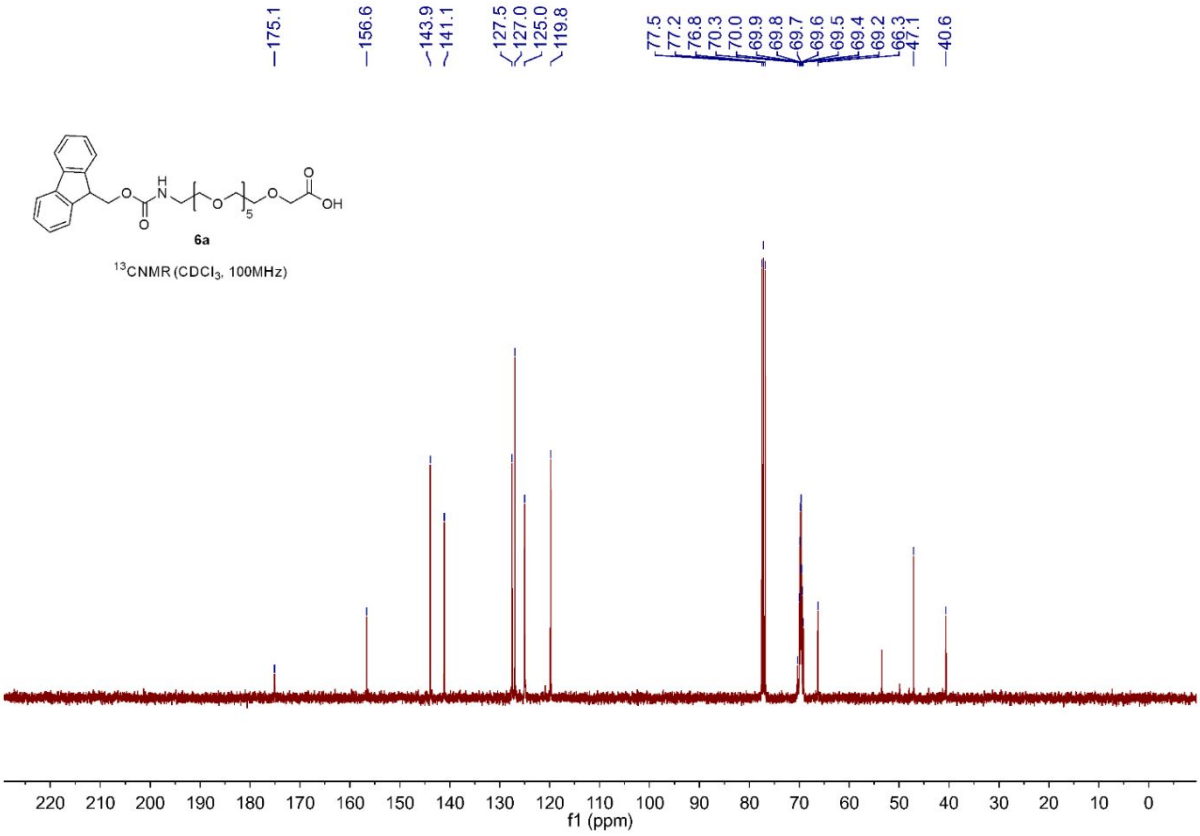
^{1}H NMR of compound **5c**



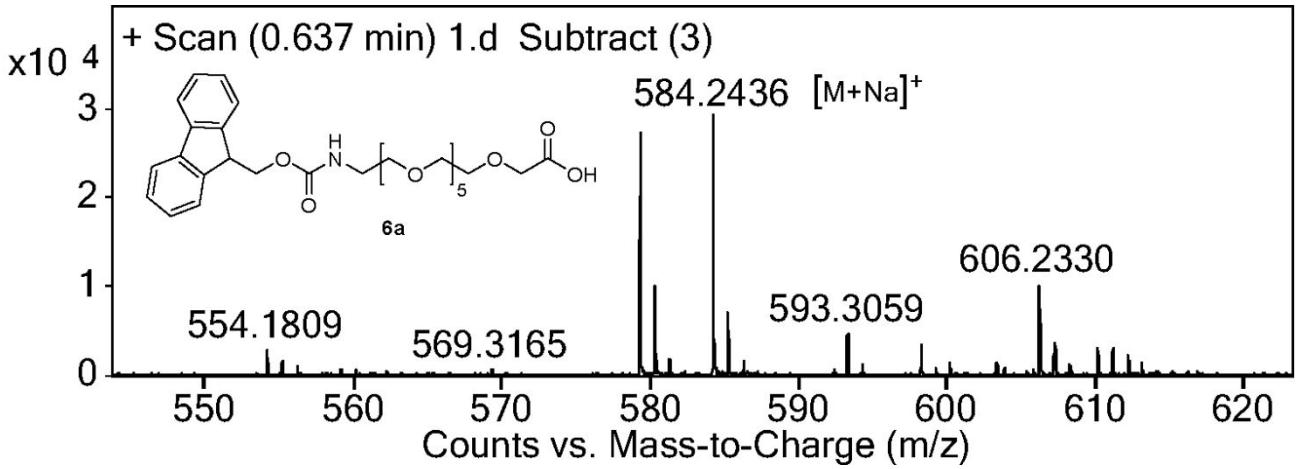
^{1}H NMR of compound **6a**



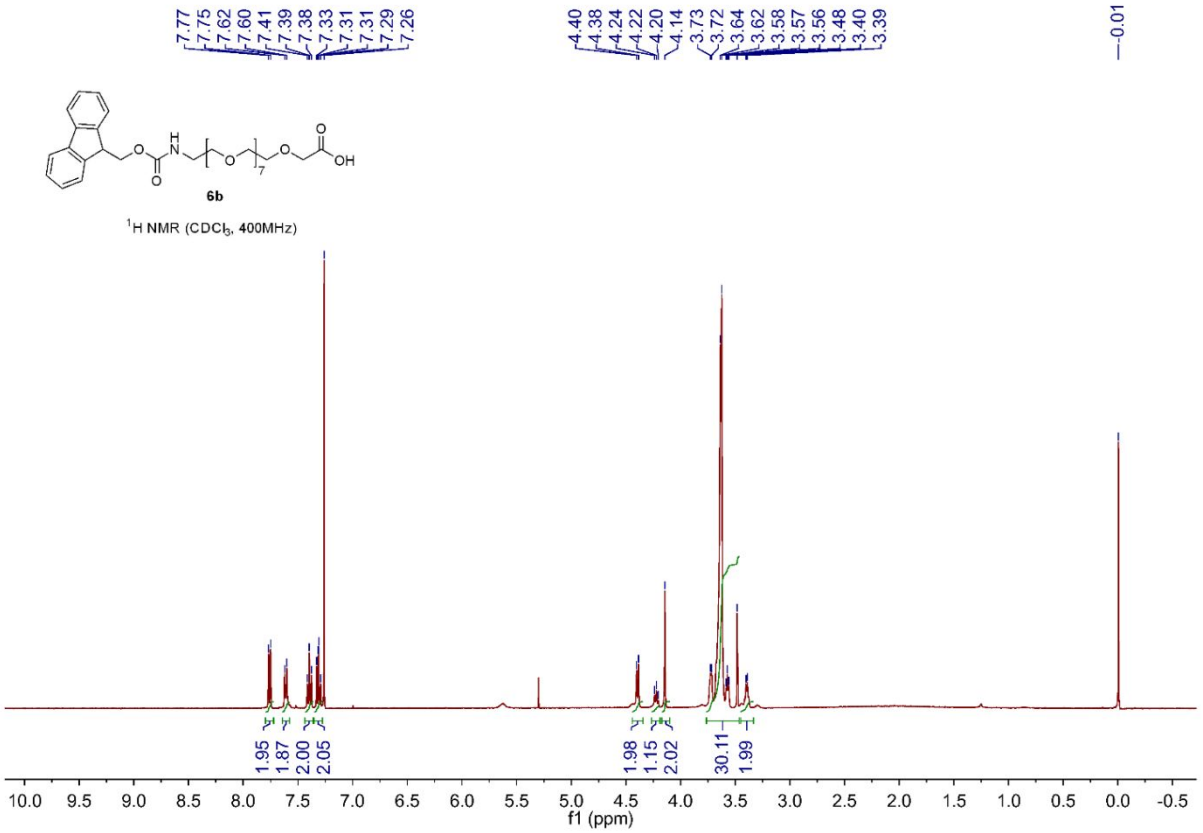
^{13}C NMR of compound **6a**



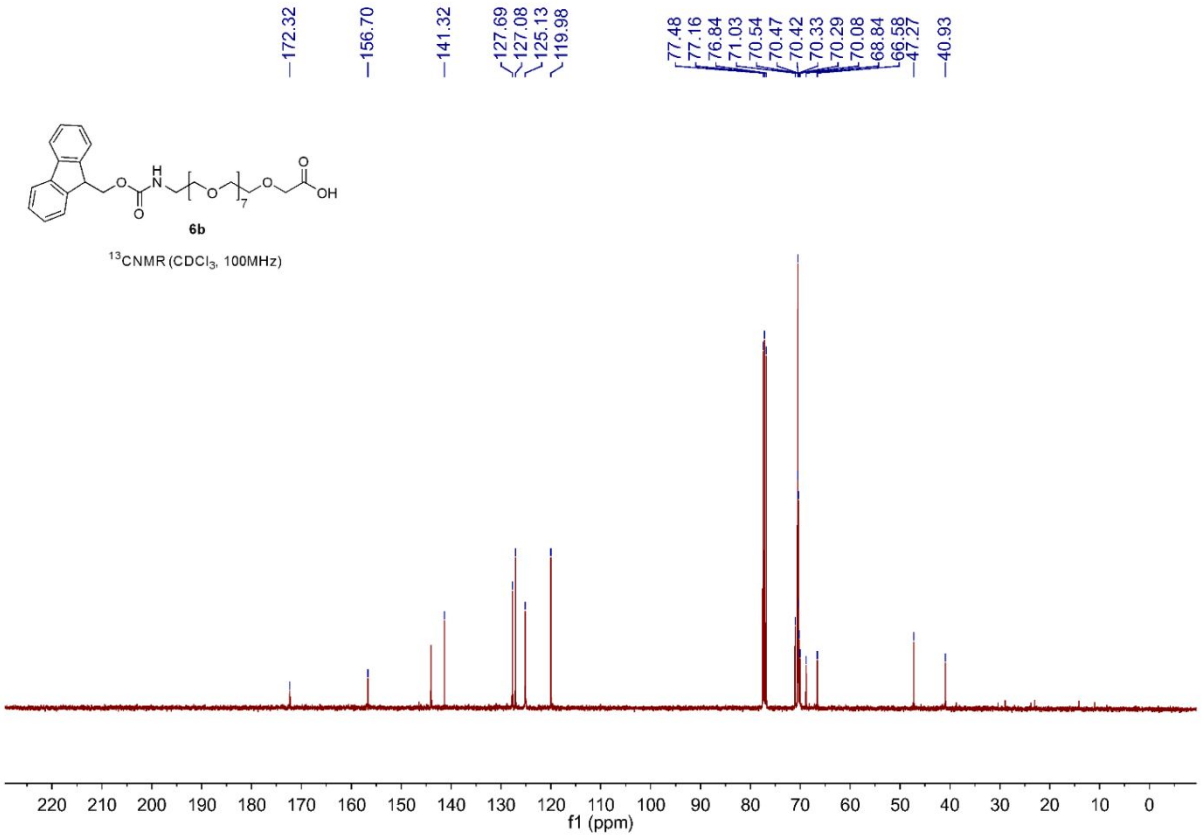
Mass spectrum of compound **6a**



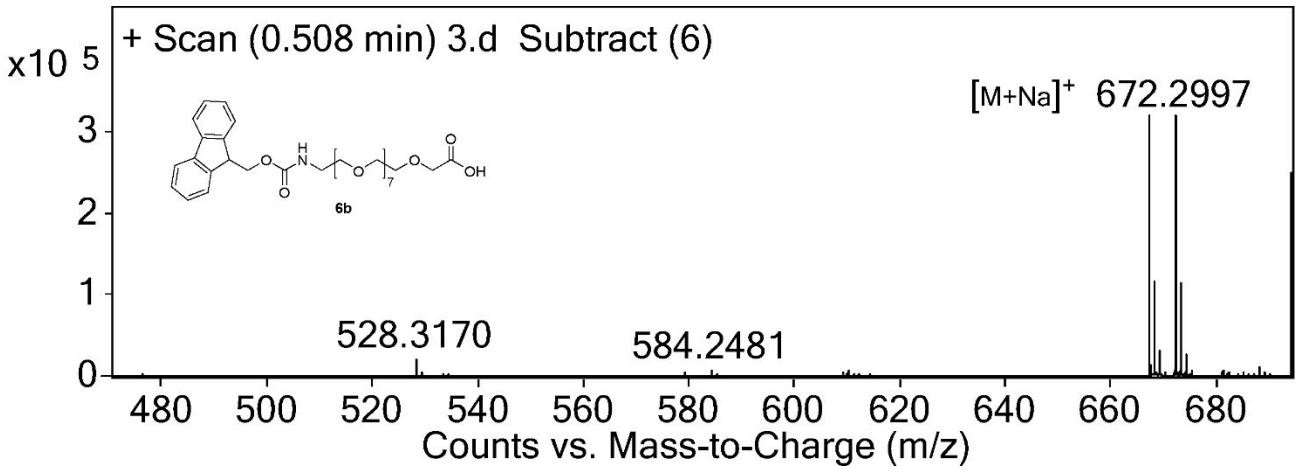
^{1}H NMR of compound **6b**



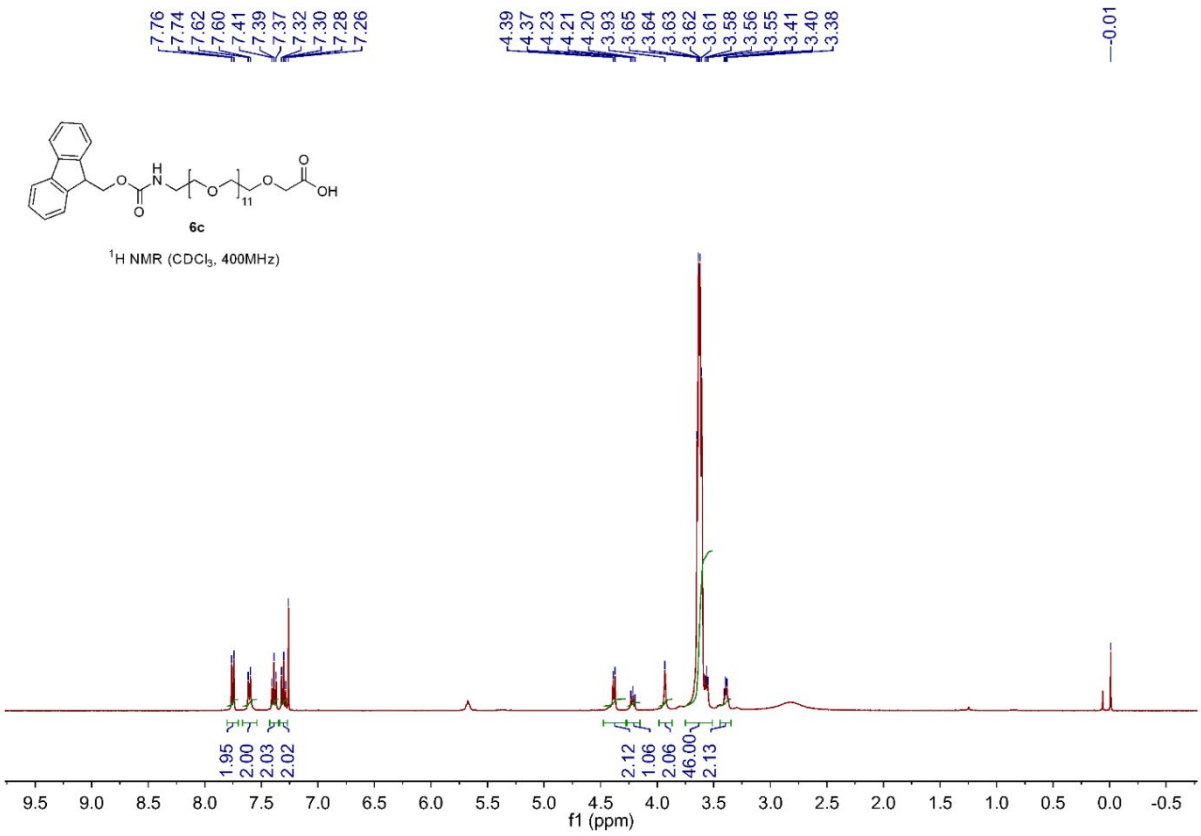
^{13}C NMR of compound **6b**



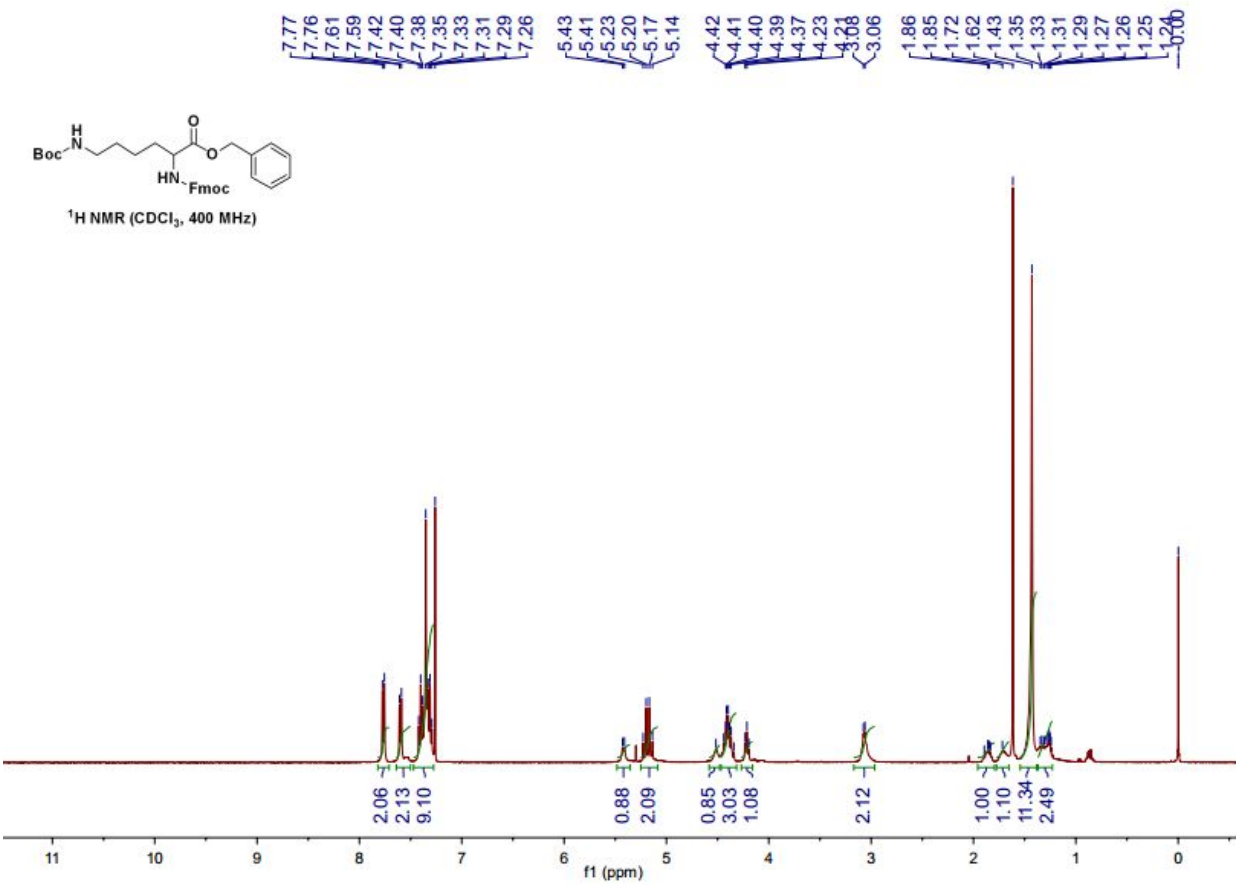
Mass spectrum of compound **6b**



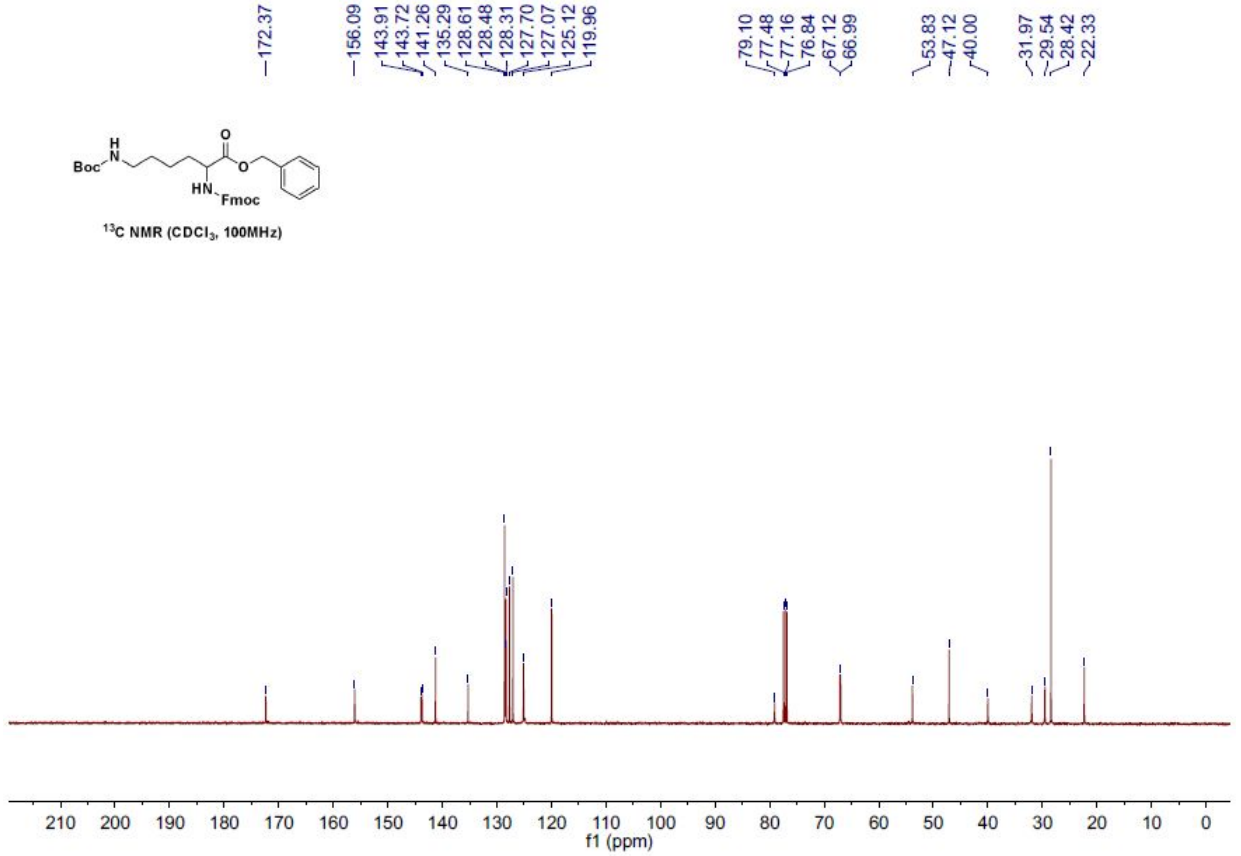
^{1}H NMR of compound **6c**



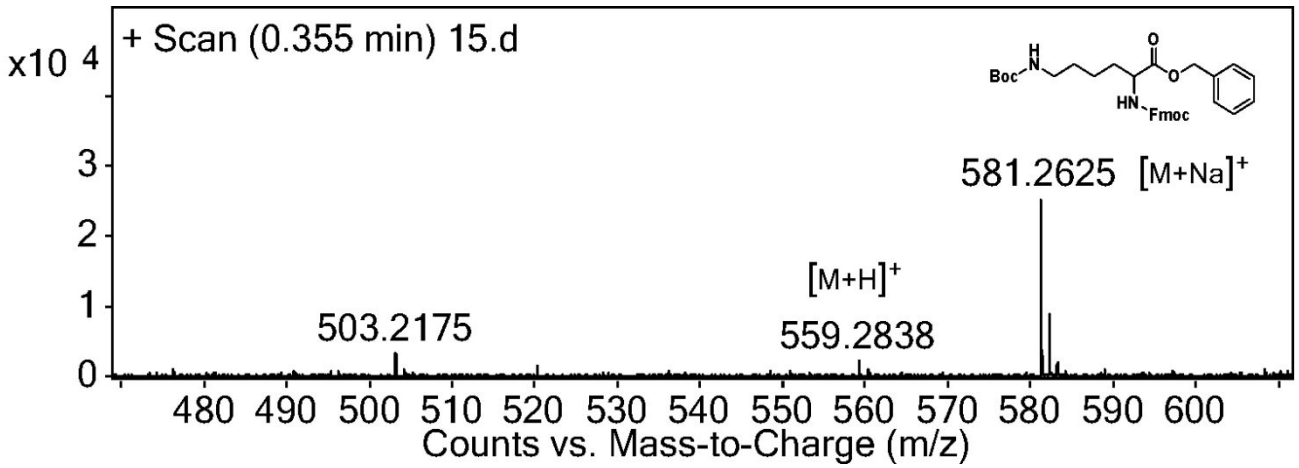
^{1}H NMR of compound **7b**



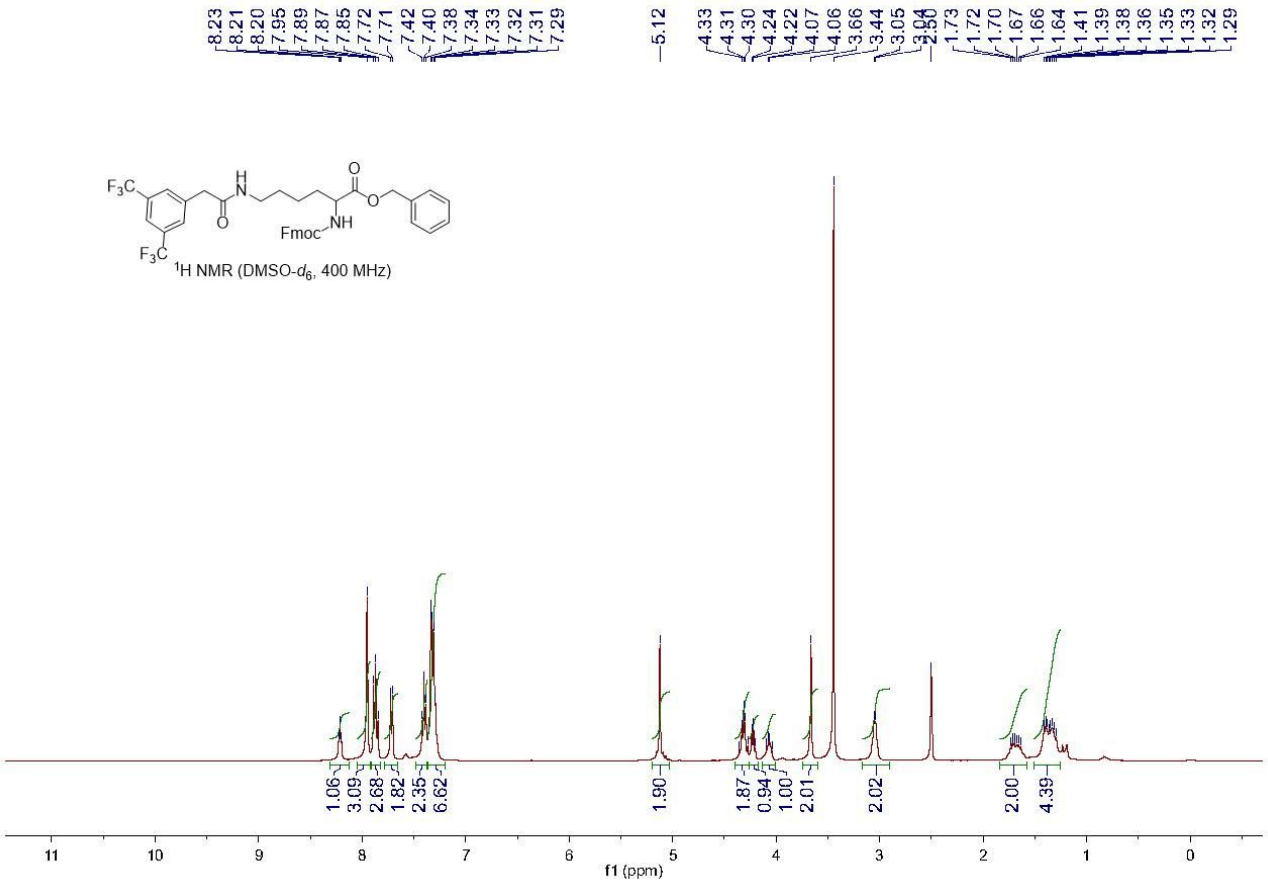
^{13}C NMR of compound **7b**



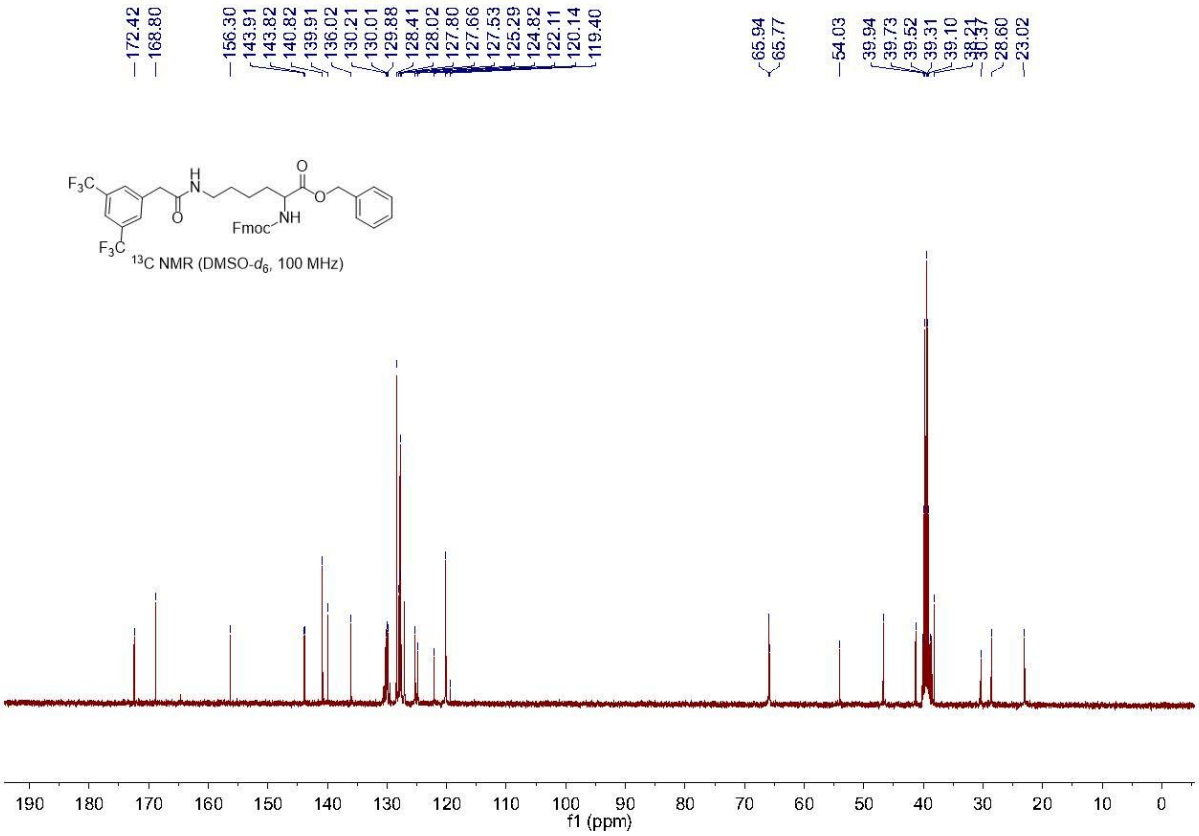
Mass spectrum of compound **7b**



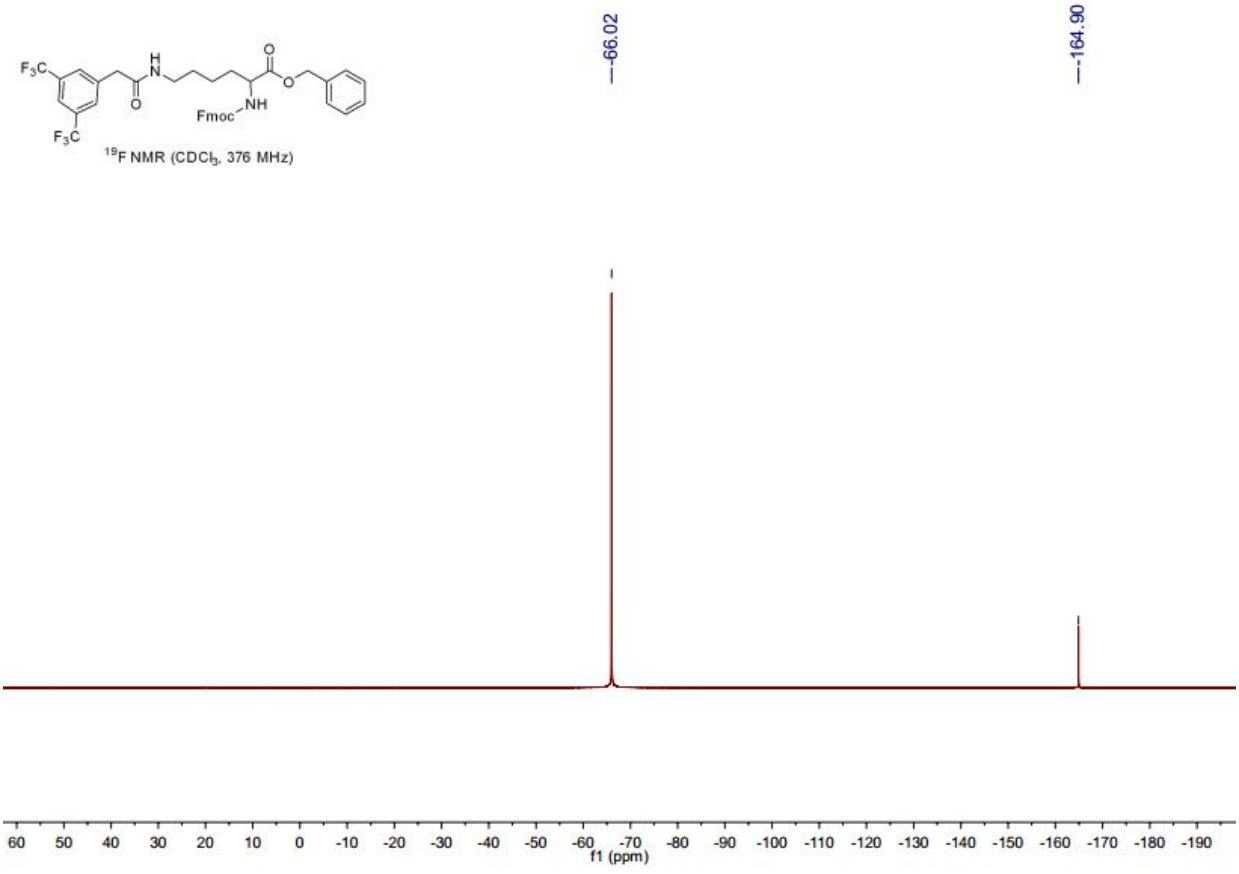
^{1}H NMR of compound **7d**



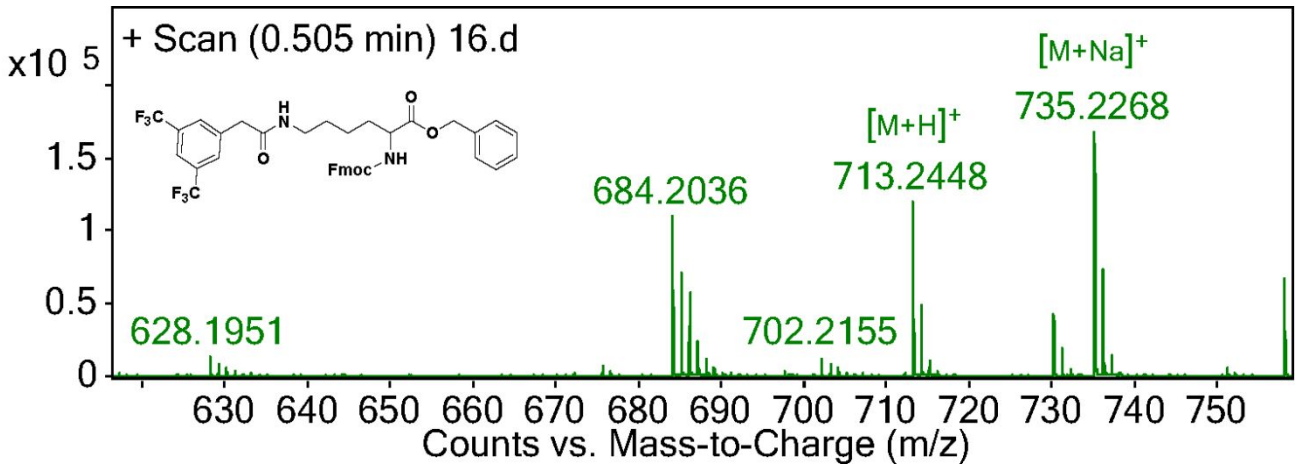
^{13}C NMR of compound **7d**



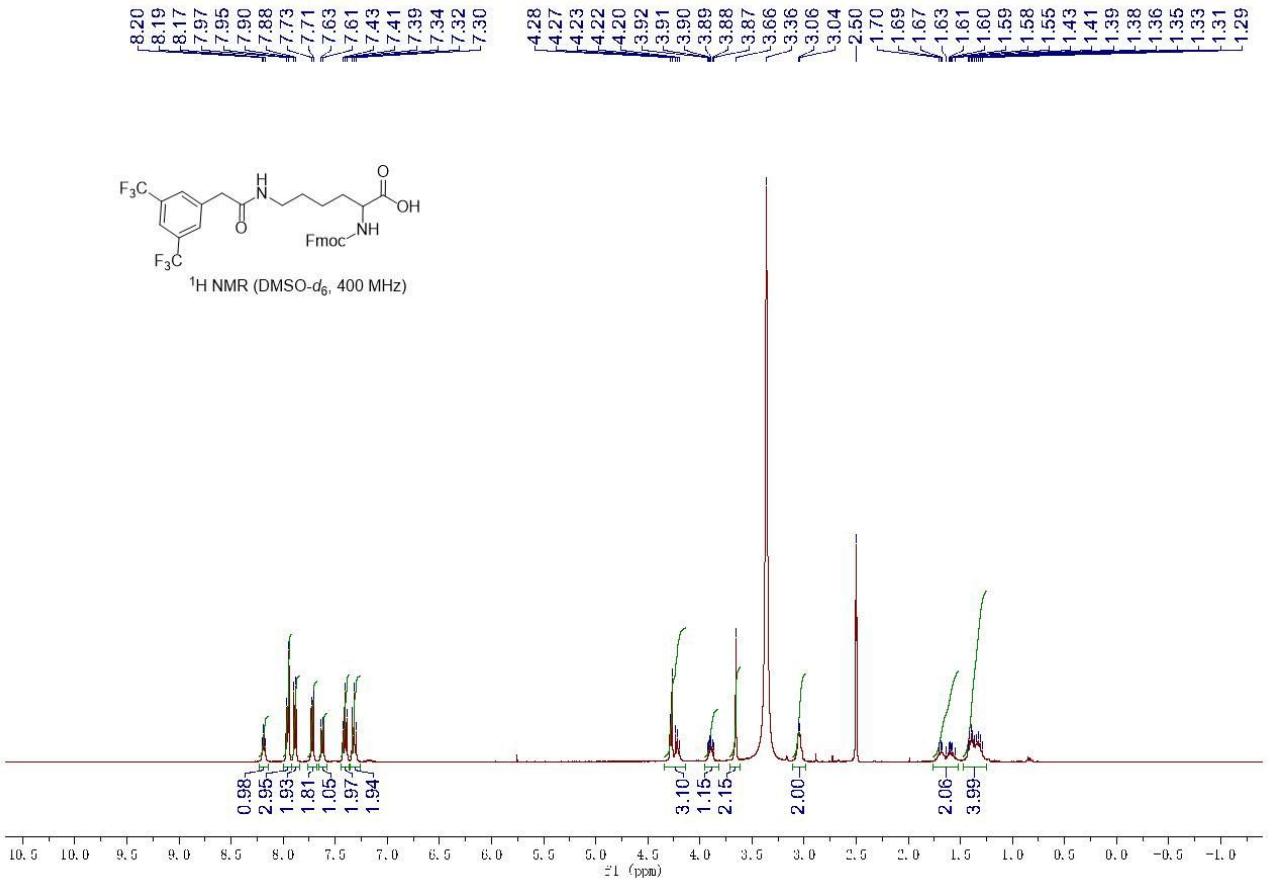
^{19}F NMR of compound **7d**



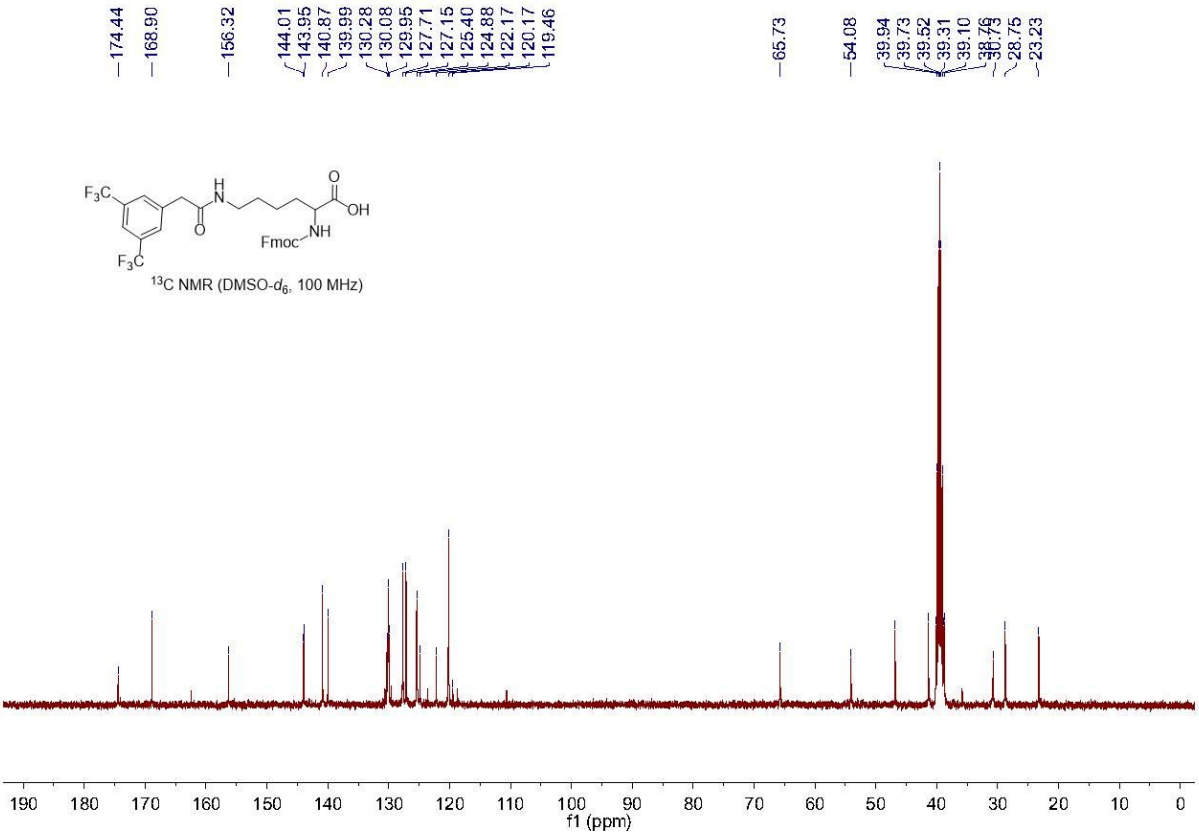
Mass spectrum of compound **7d**



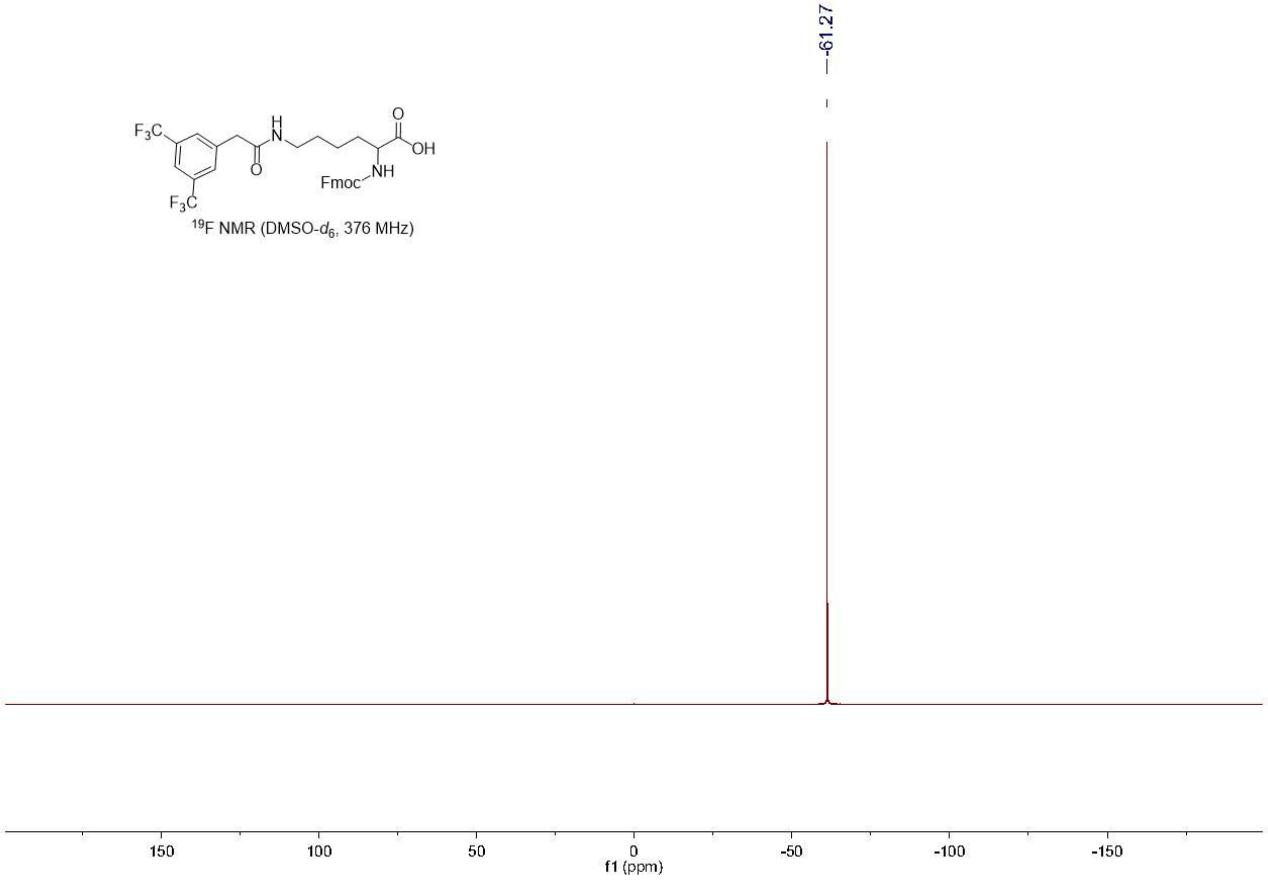
^{1}H NMR of compound **7e**



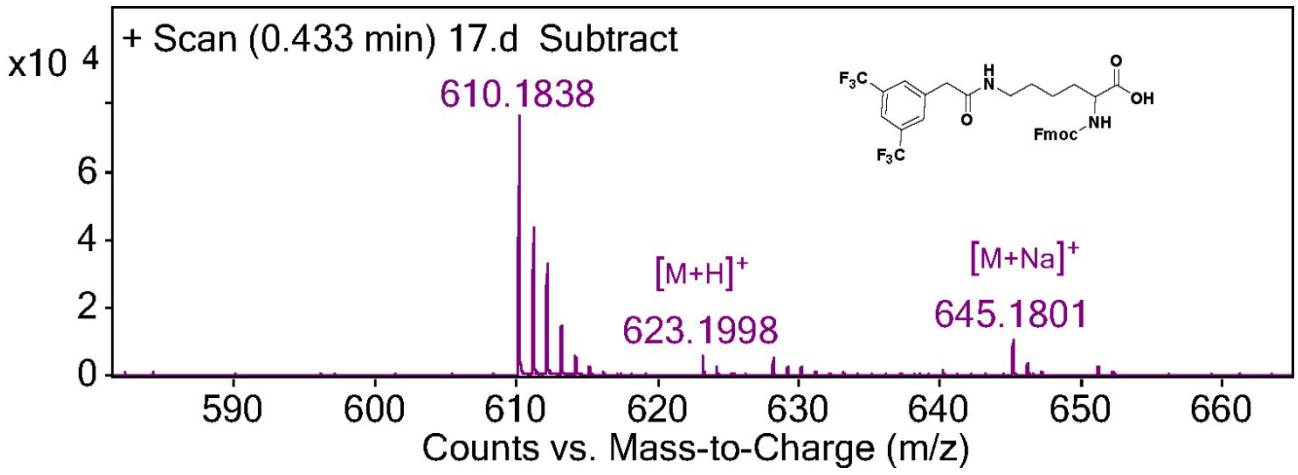
^{13}C NMR of compound **7e**



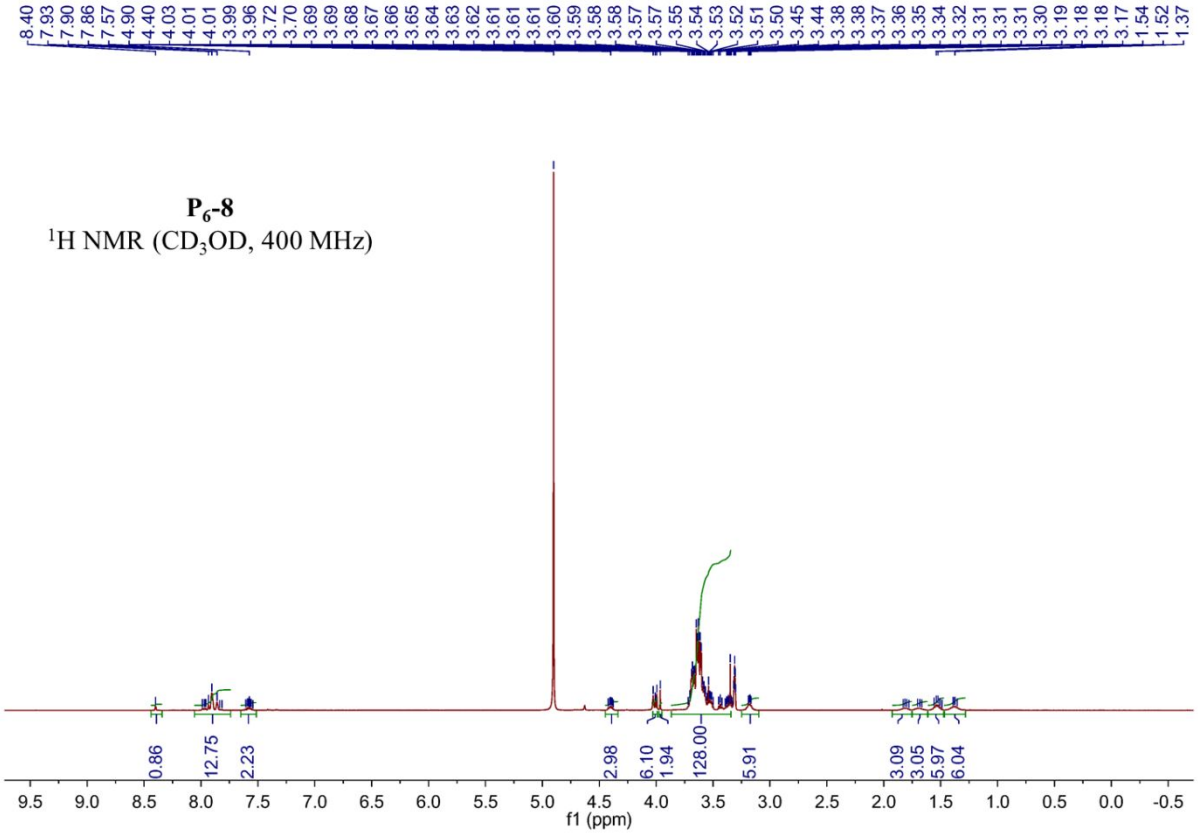
^{19}F NMR of compound **7e**



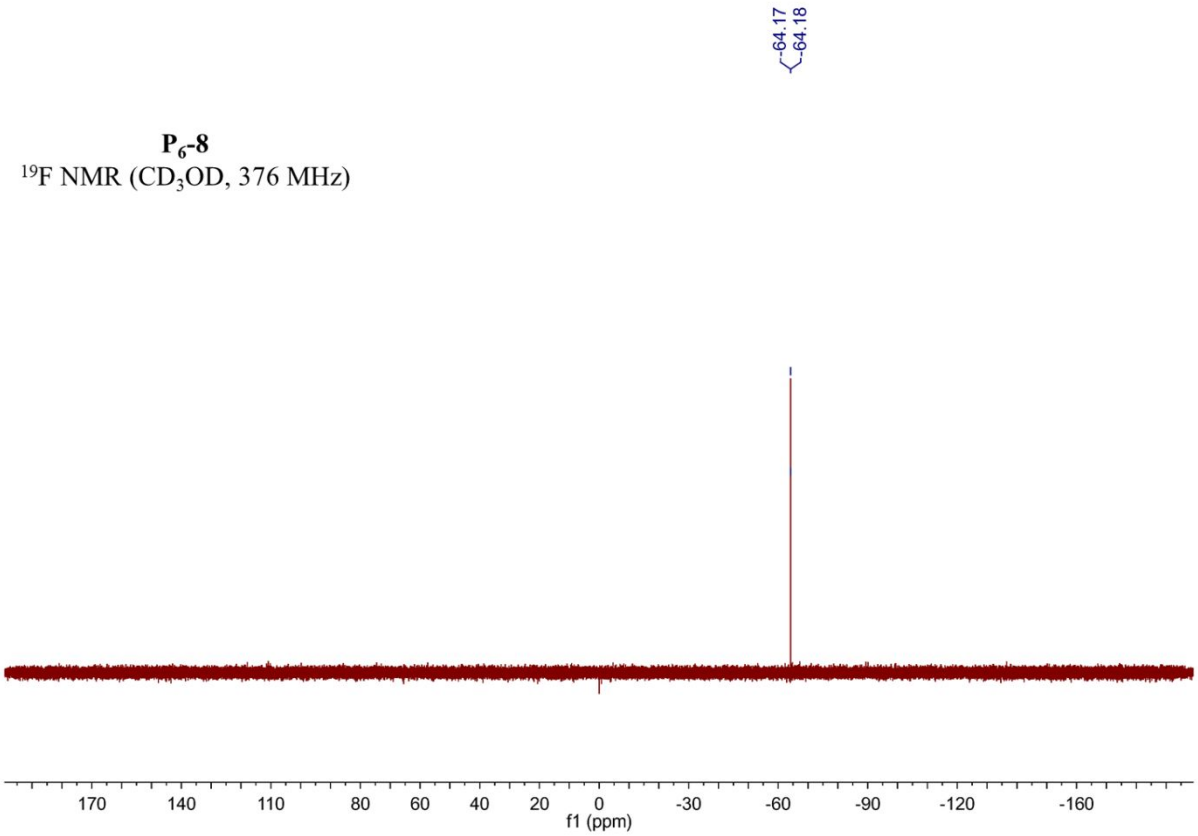
Mass spectrum of compound **7e**



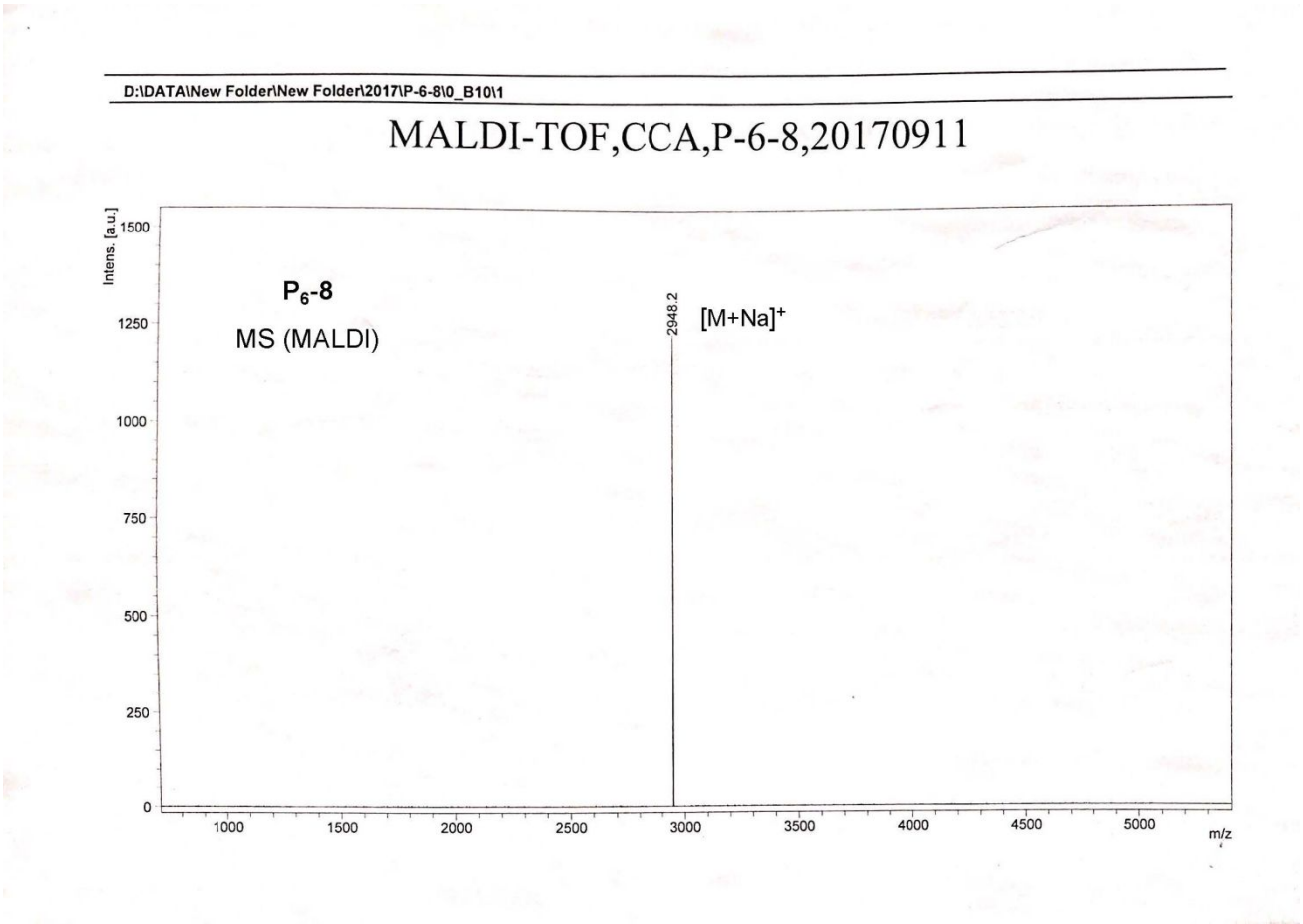
^{1}H NMR of **P_6-8**



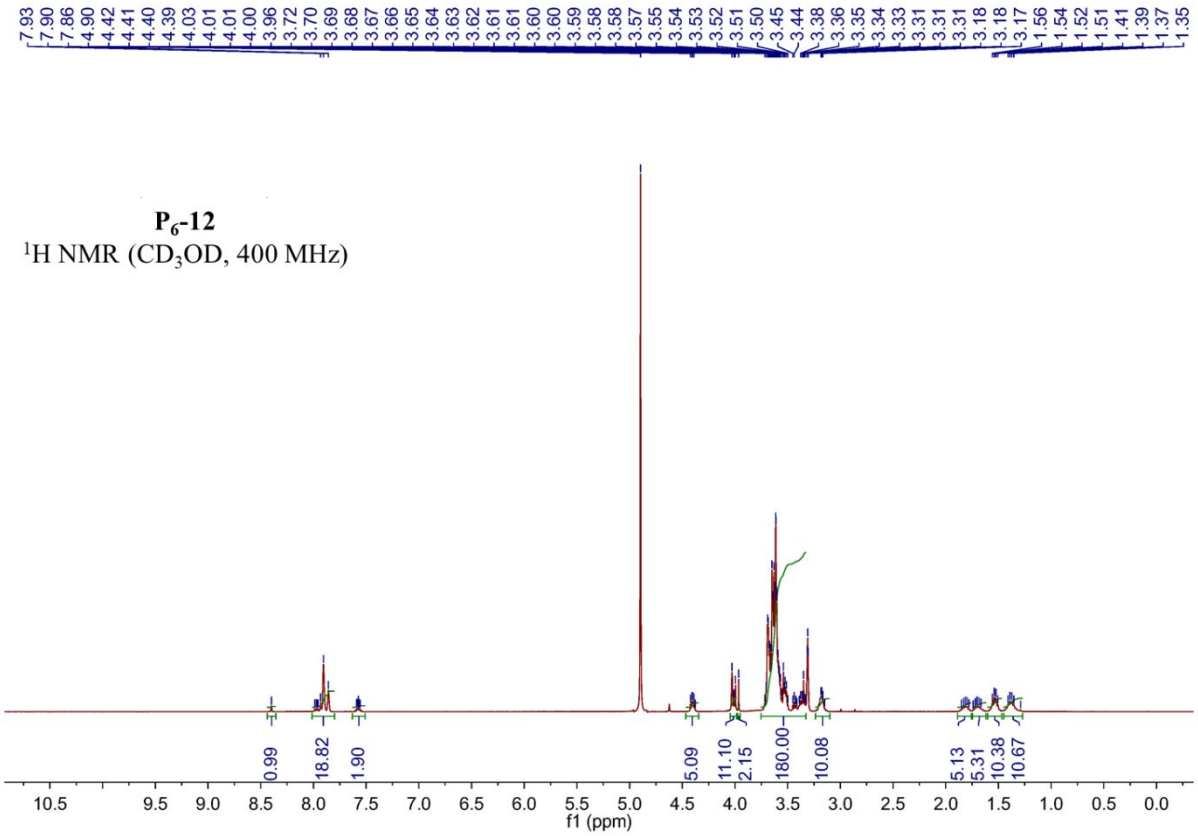
^{19}F NMR of **P_6-8**



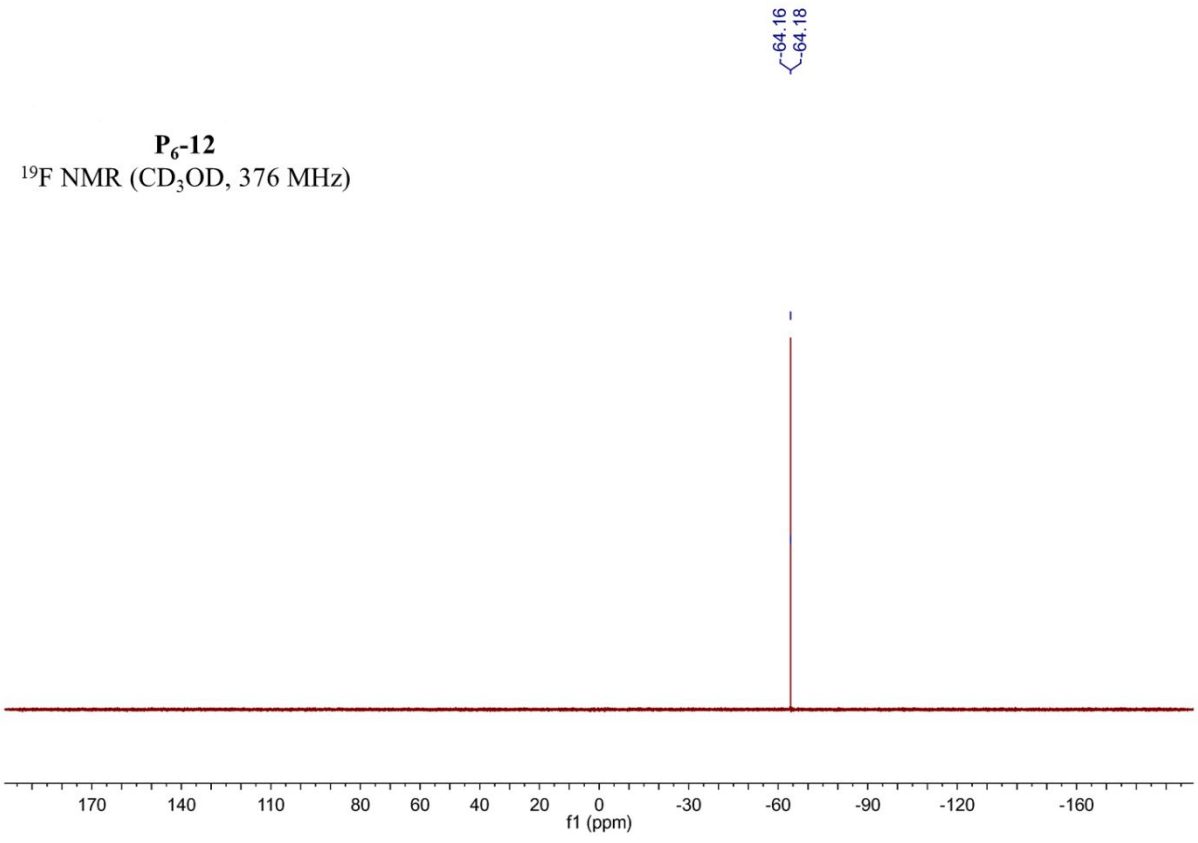
MALDI-TOF mass of **P_6-8**



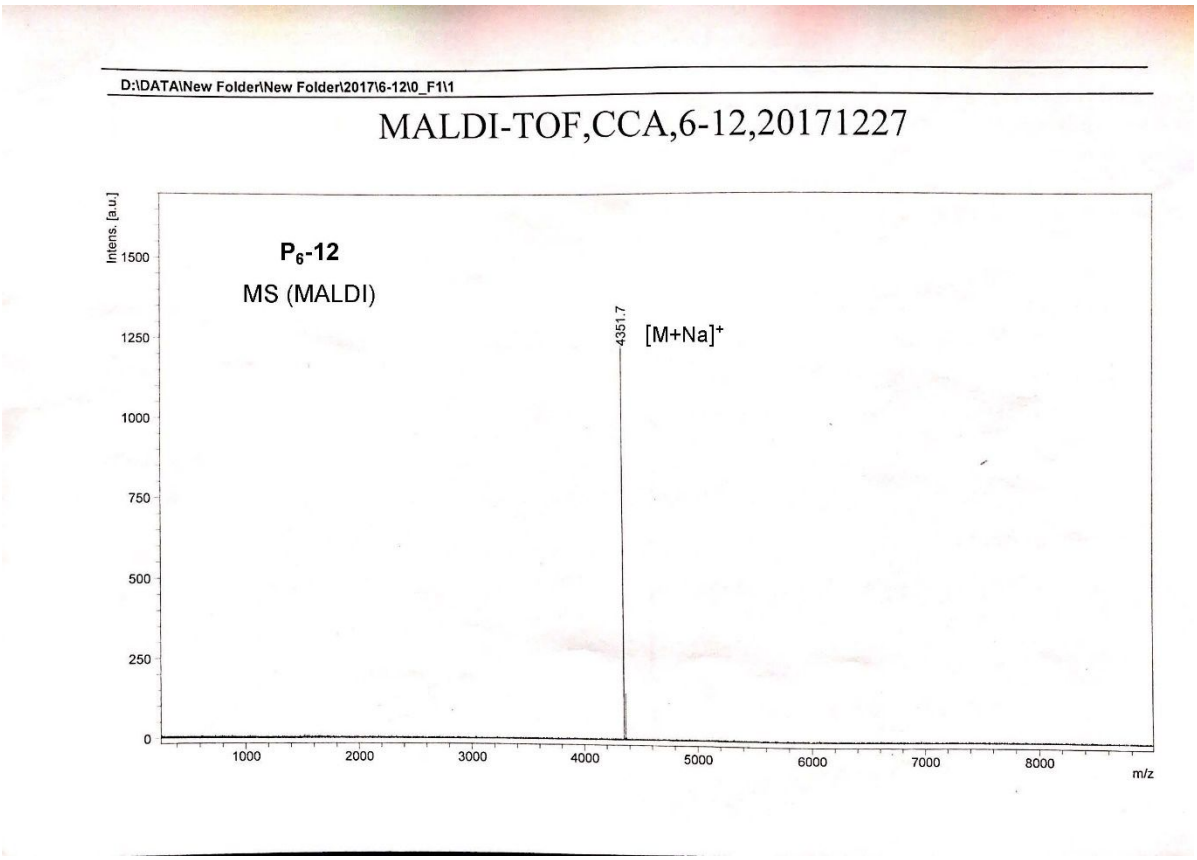
^{1}H NMR of **P_6-12**



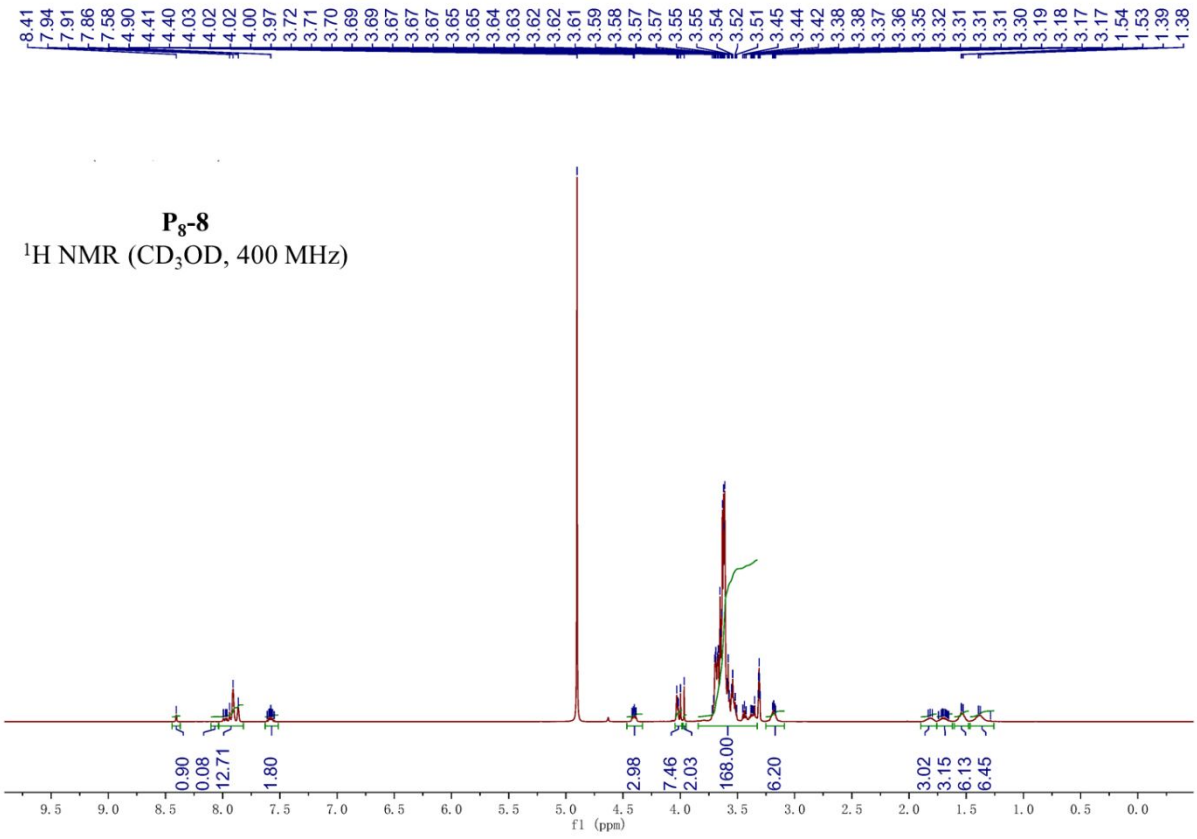
^{19}F NMR of **P_6-12**



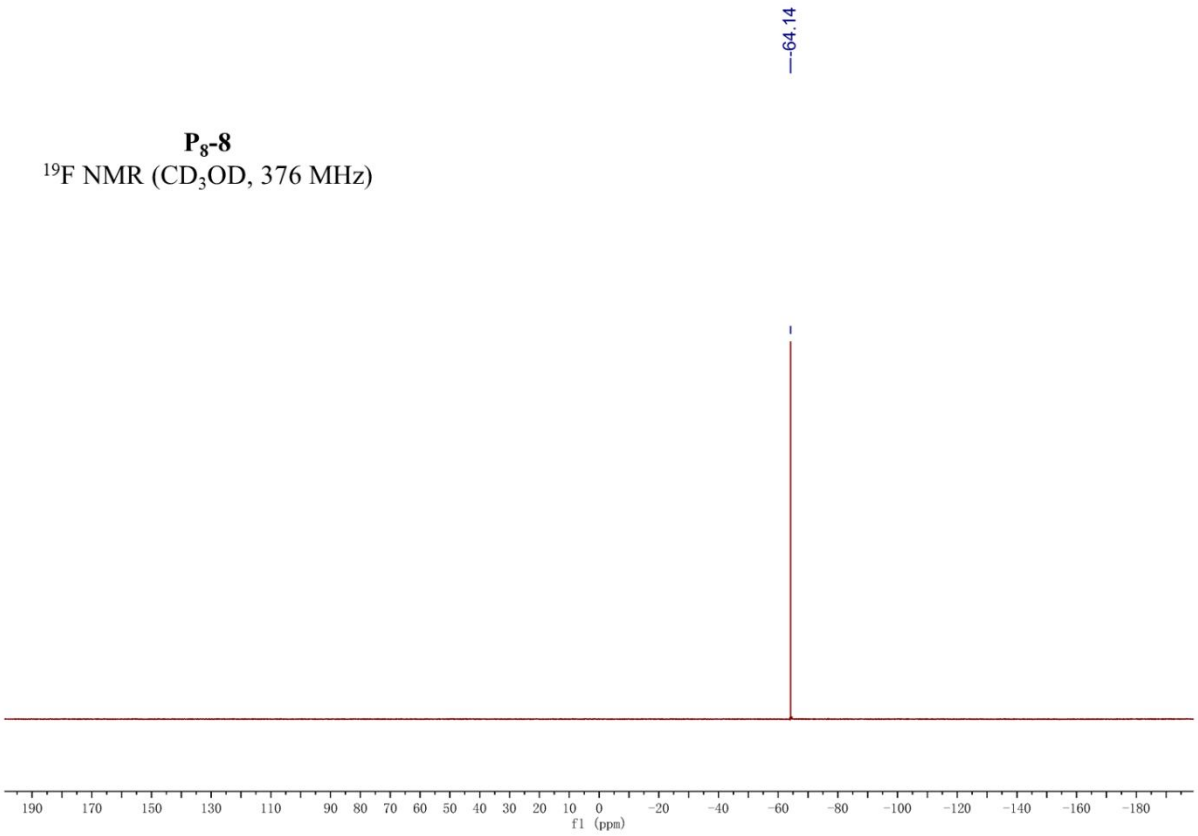
MALDI-TOF mass of **P_6-12**



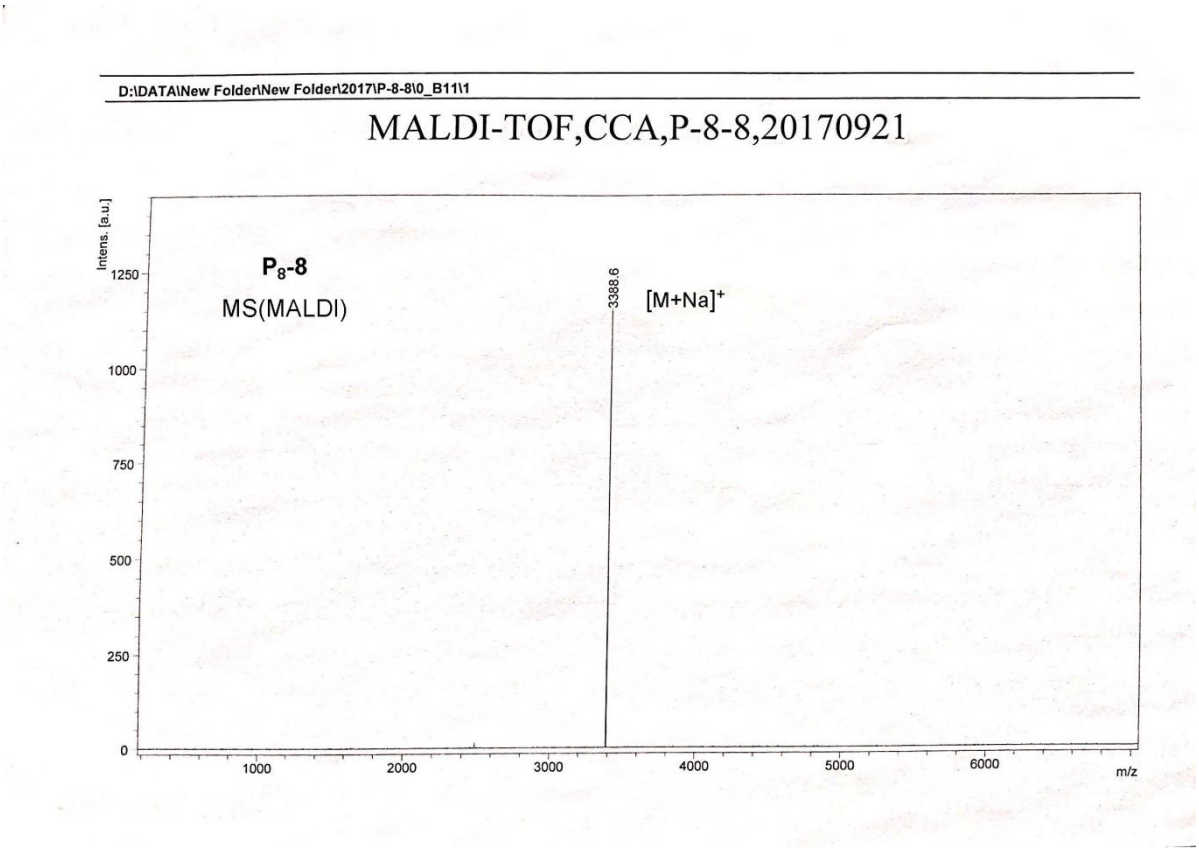
^{1}H NMR of **P_8-8**



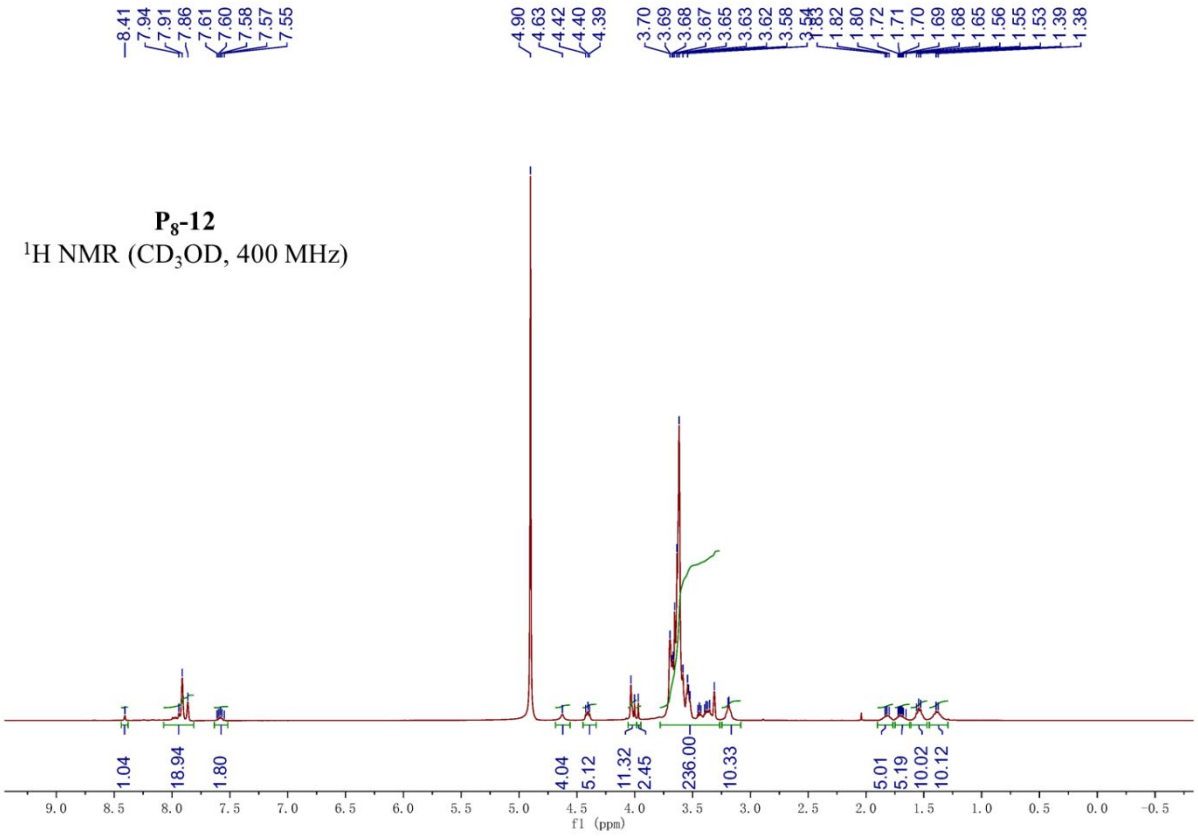
^{19}F NMR of **P_8-8**



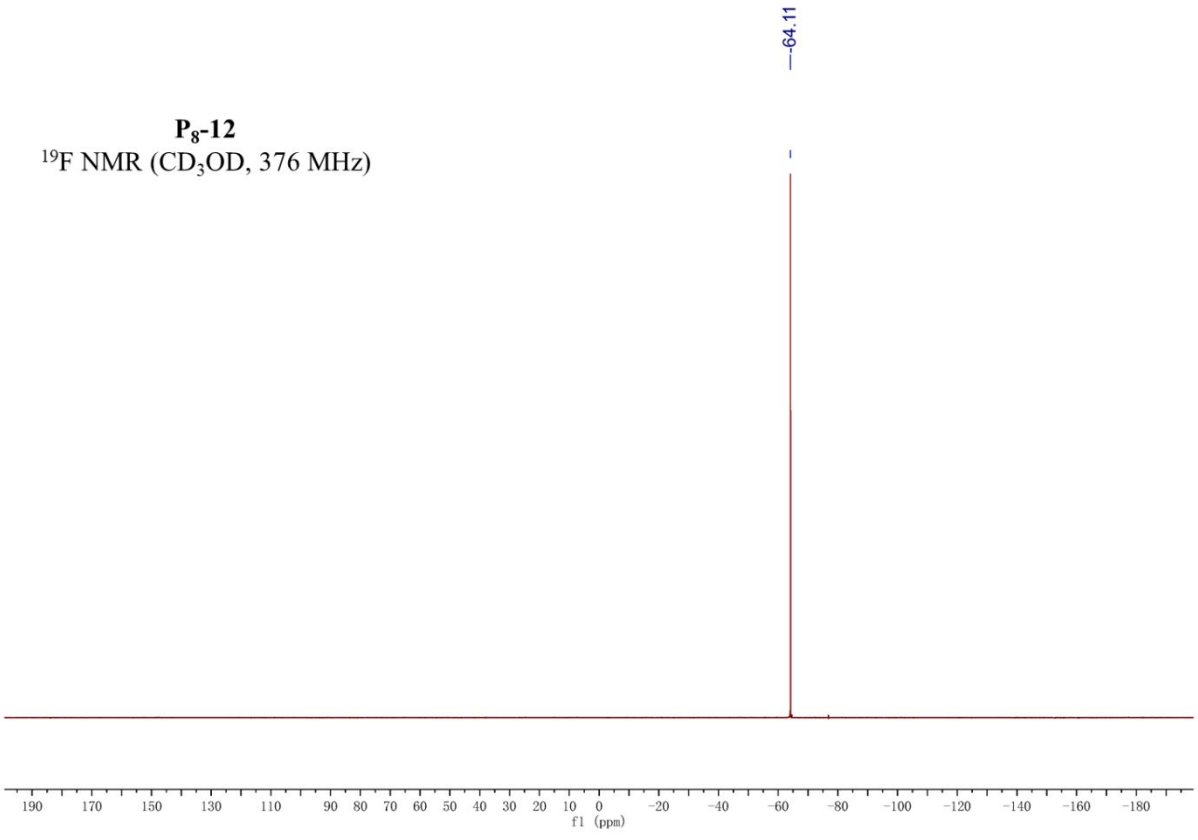
MALDI-TOF mass of **P_8-8**



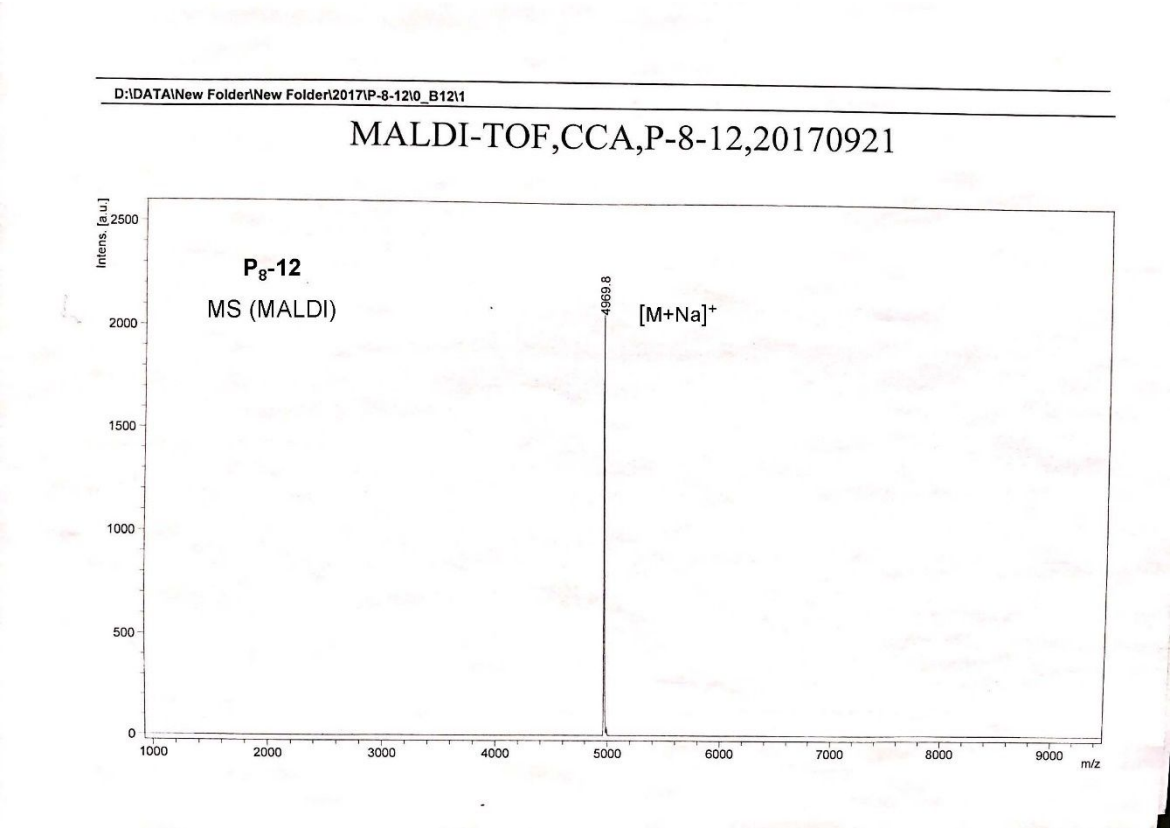
^{1}H NMR of **P_8-12**



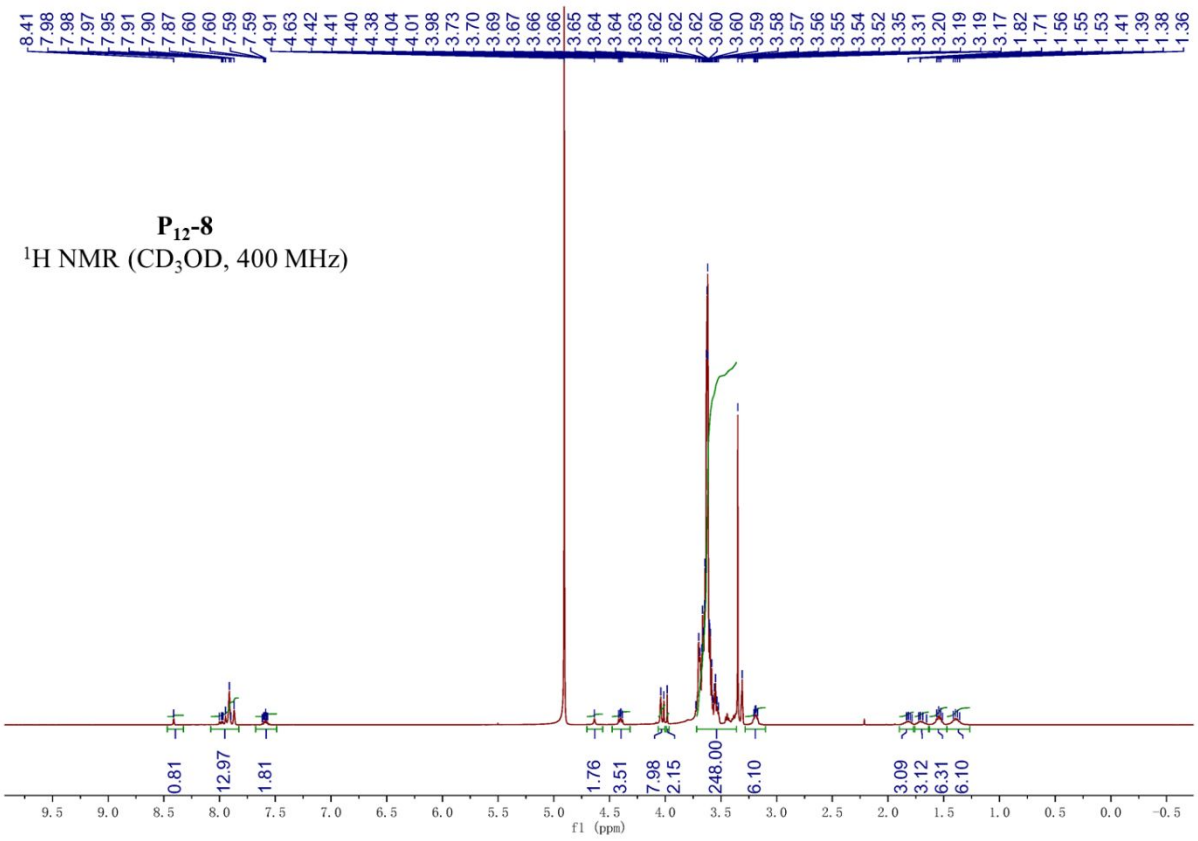
^{19}F NMR of **P_8-12**



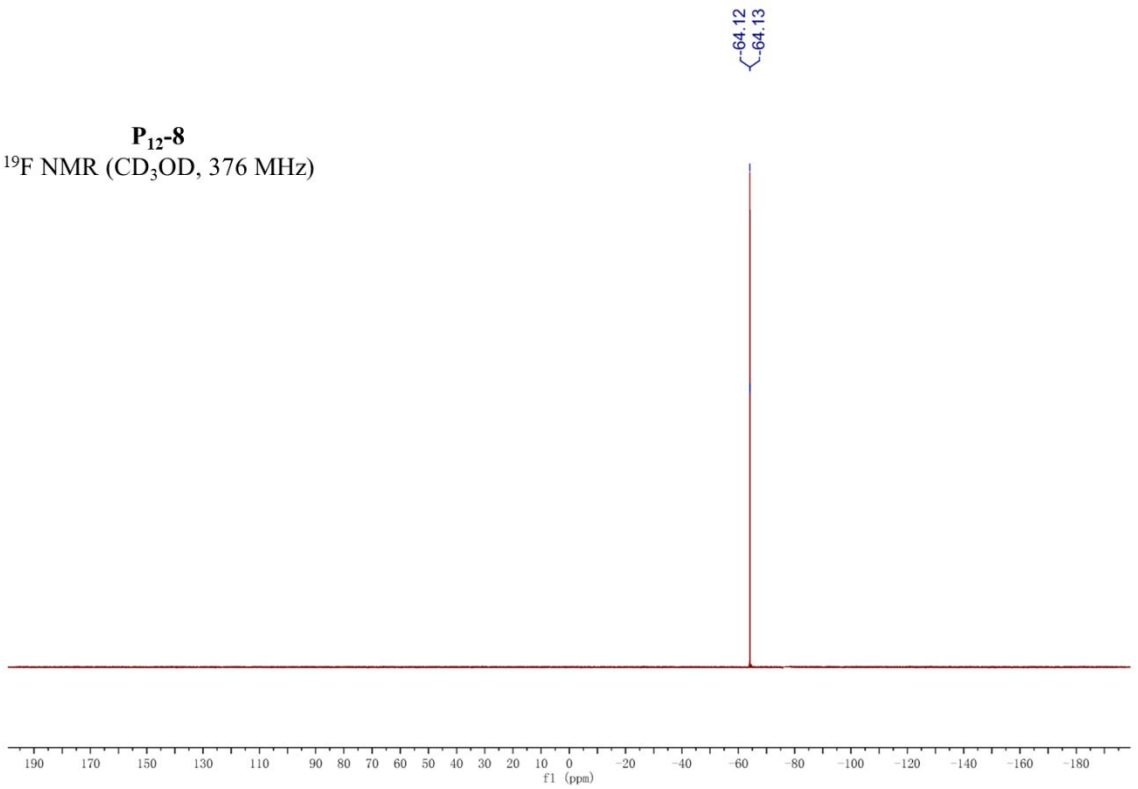
MALDI-TOF mass of **P_8-12**



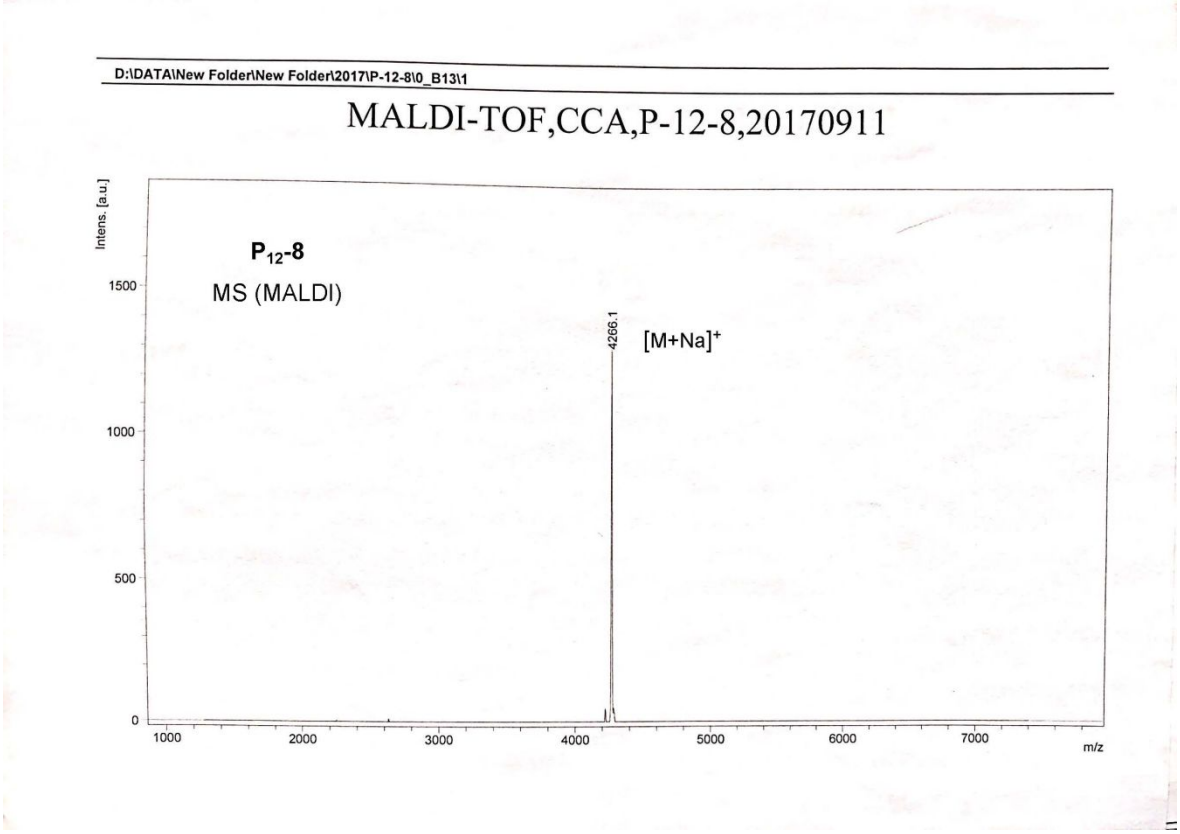
^{1}H NMR of **P_{12}-8**



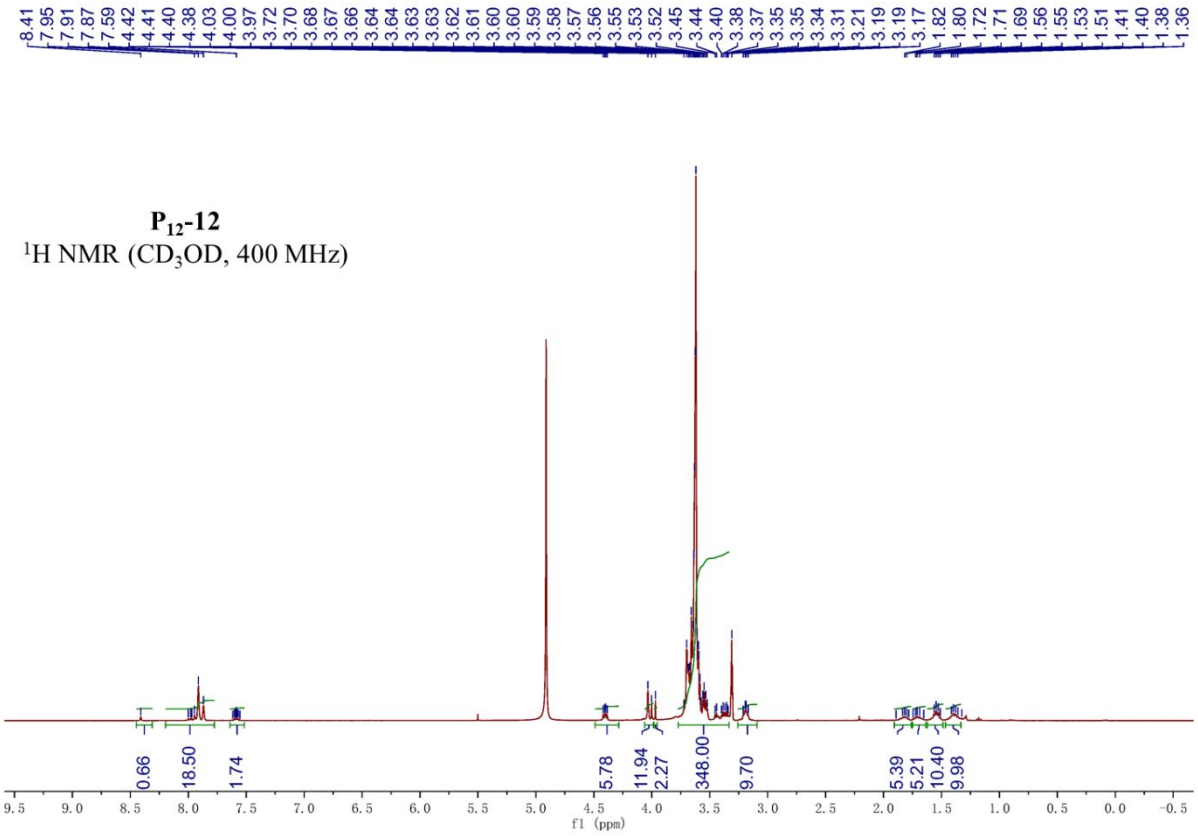
^{19}F NMR of **P_{12}-8**



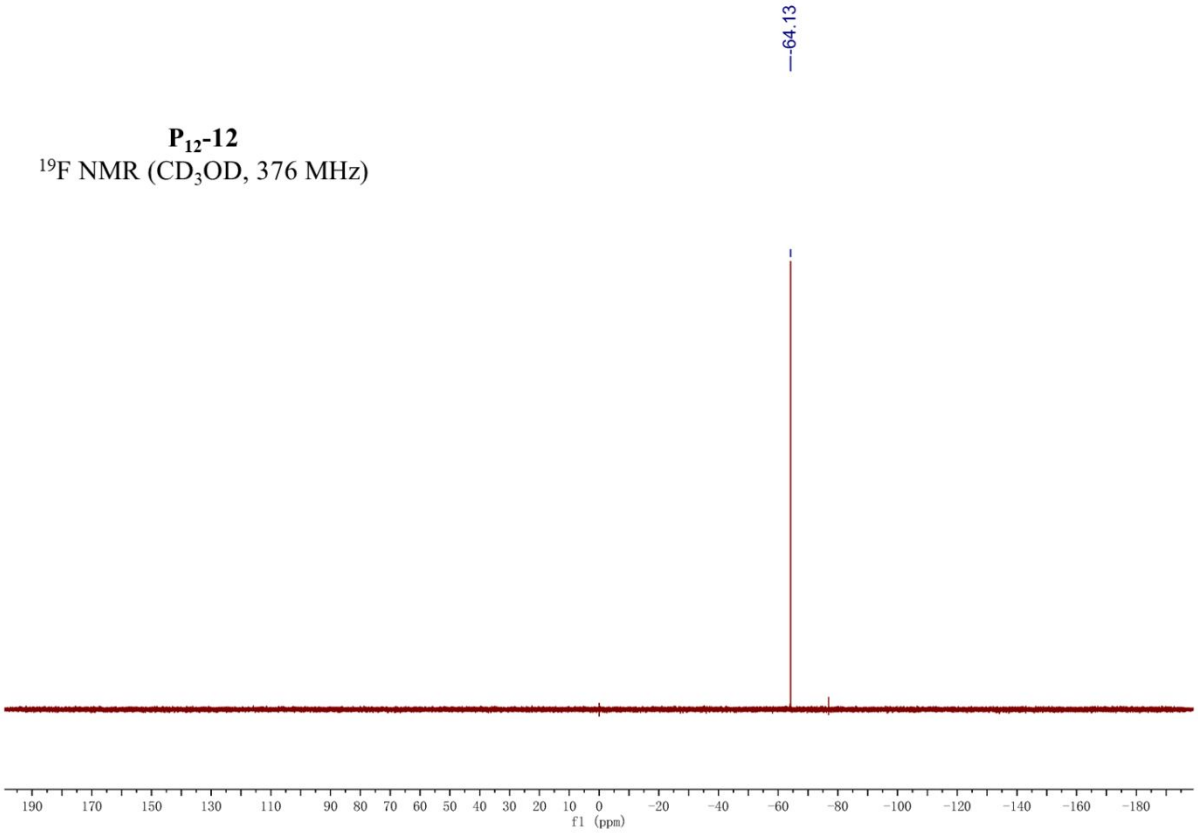
MALDI-TOF mass of **P_{12}-8**



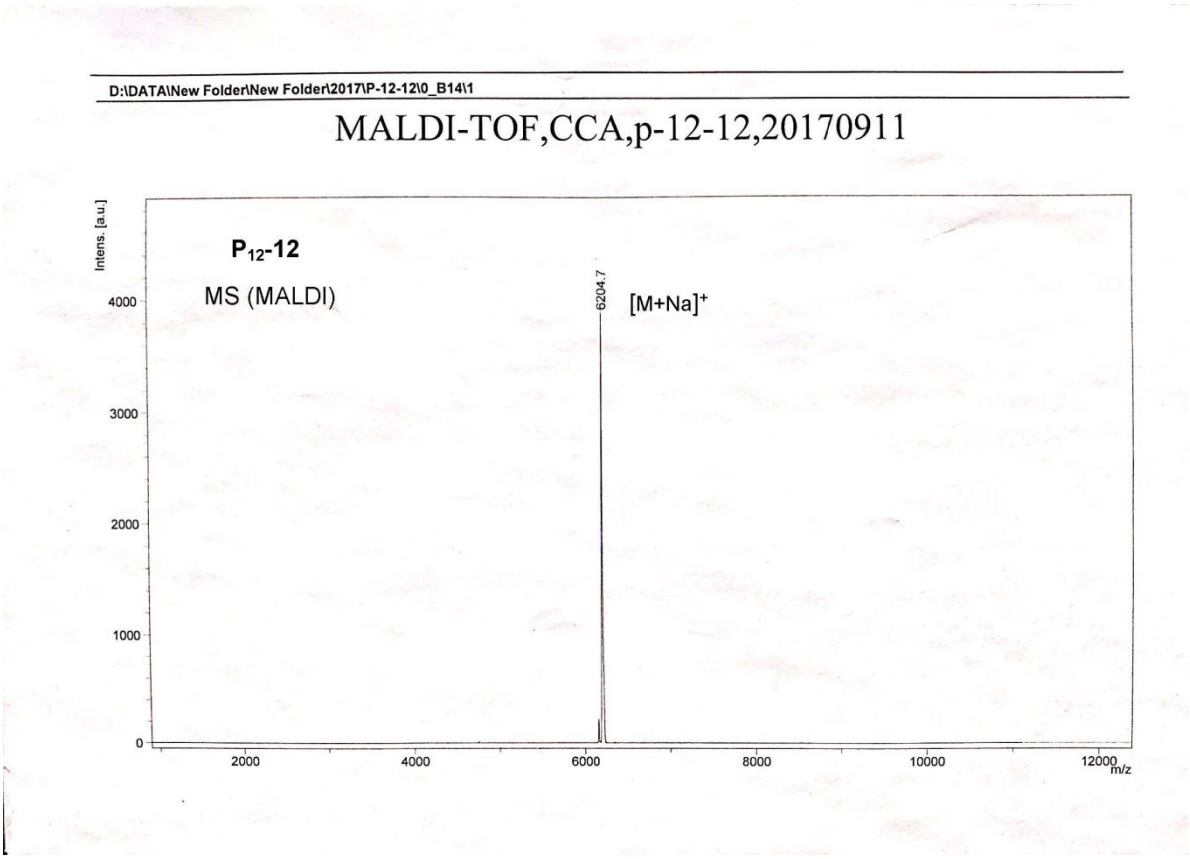
^{1}H NMR of **P_{12}-12**



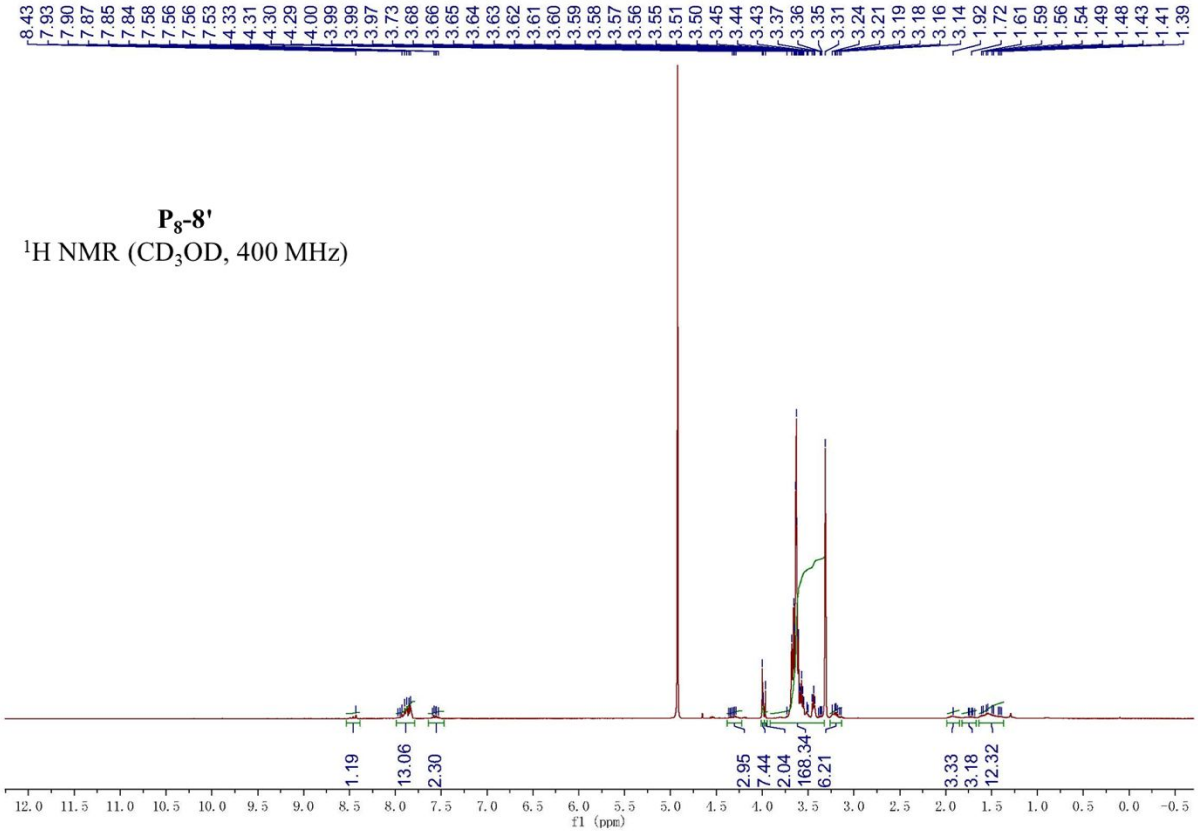
^{19}F NMR of **P_{12}-12**



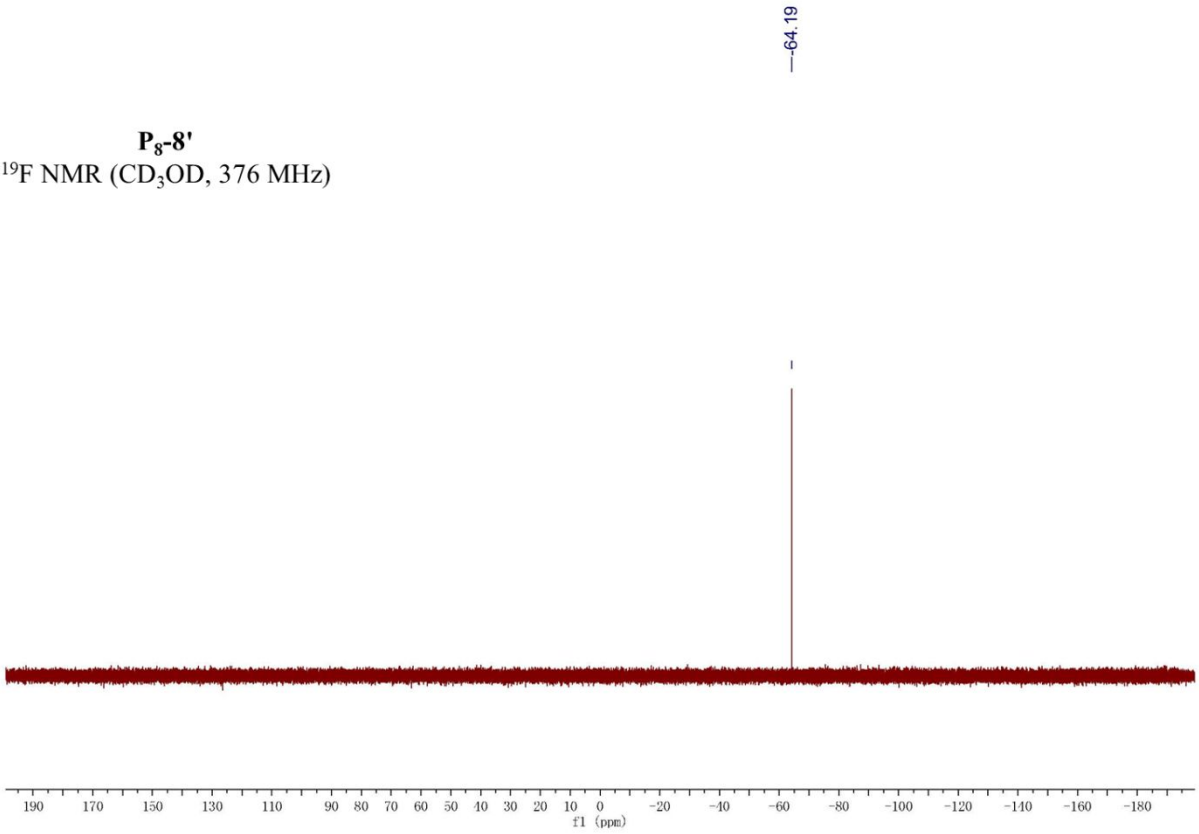
MALDI-TOF mass of **P_{12}-12**



^{1}H NMR of **P_8-8'**



^{19}F NMR of **P_8-8'**



MALDI-TOF mass of **P_8-8'**