ADVANCED HEALTHCARE MATERIALS

Supporting Information

for *Adv. Healthcare Mater.*, DOI: 10.1002/adhm.201901331

Peptidic Monodisperse PEG "Comb" as Multifunctional "Add-On" Module for Imaging-Traceable and Thermo-Responsive Theranostics

*Junfei Zhu, Huaibin Zhang, Kexin Chen, Yu Li, Zhigang Yang, Shizhen Chen, Xing Zheng, Xin Zhou, and Zhong-Xing Jiang**

Supporting Information

Peptidic monodisperse PEG "comb" as multifunctional "add-on" module for imaging-traceable and thermo-responsive theranostics

Junfei Zhu, Huaibin Zhang, Kexin Chen, Yu Li, Zhigang Yang, Shizhen Chen, Xing Zheng, Xin Zhou and Zhong-Xing Jiang[*]

Dr. J. Zhu, Mr. H. Zhang, Prof. Z. Yang, Prof. Z.-X. Jiang
Hubei Province Engineering and Technology Research Center for Fluorinated Pharmaceuticals
School of Pharmaceutical Sciences
Wuhan University, Wuhan 430071, China
E-mail: zxjiang@whu.edu.cn

Mr. H. Zhang, Dr. Y. Li, Prof. S. Chen, Prof. X. Zhou
State Key Laboratory for Magnetic Resonance and Atomic and Molecular Physics
National Center for Magnetic Resonance in Wuhan
Wuhan Institute of Physics and Mathematics
Chinese Academy of Sciences, Wuhan 430071, China

Ms. K. Chen, Prof. X. Zheng
Group of Lead Compound, Institute of Pharmacy & Pharmacology
Hunan Province Cooperative Innovation Center for Molecular Target New Drug Study
University of South China, Hengyang 421001, China.

Table of contents

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.

Tumor-carrying Balb/c nude mice with tumor volume of 100-150 mm^3 (male, 6-8 week, 20-25 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. "Comb" 1 and L1 ^{19}F NMR (Figure S1)

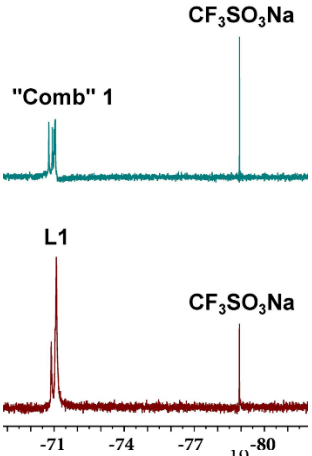


Figure S1. "Comb" **1** and **L1** ^{19}F NMR at 25 °C.

3. 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 45 °C and 59 °C through temperature-controlled heating and cooling cycles and the sample was equilibrated for 10 min before measurement.

Table S1. LCSTs of "comb" **1** at 1 mg/mL, 10 mg/mL and 20 mg/mL.

Peptides	LCST at 1 mg/mL	LCST at 10 mg/mL	LCST at 20 mg/mL
"Comb" 1	55 ^{o}C	50 ^{o}C	49 ^{o}C

4. Dynamic Light Scattering

"Comb" **1** in H_2O at 10 mg/mL was used for DLS analysis. The particle size was measured at an angle of 90° in a 10 mm diameter cell at the desired temperature (25 °C and 50 °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.

5. Synthesis and Purification of "Comb" 1 (Figure S2)

"Comb" **1** was manually synthesized through the Fmoc-strategy in a sintered glass reaction funnel fitted with a three-way stopcock on Rink amide-AM resin. Coupling reactions were performed in DMF for 2 hours with 2.5 equiv of Fmoc-protected amino acid which was activated in situ with either 2.5 equiv of HATU and 5.0 equiv of DIPEA in DMF or 2.5 equiv of HOBt, 2.5 equiv of TBTU and 5.0 equiv of DIPEA. Double coupling reactions were carried out on each residue. The coupling efficiency was assessed by TNBS test (1% picrylsulfonic acid in DMF and 10% *N,N*-diisopropylethylamine in DMF) for 5 min. Activation reactions were performed by treating the resin 5 min with a cocktail of either piperidine/DMF (2:8) or piperidine/DBU/DMF (2:2:96) for several times. The target peptide was released from the resin with a solution of TFA/TES/DCM (20:1:20). The crude peptide was purified with preparative reverse phase HPLC (UV detection at 210 nm, RP C18 column (10 µm; 30 mm × 250 mm), gradient elution of 60% methanol in water to 100% methanol over 60 min, flow rate 10 mL/min). The purity of "comb" **1** was evaluated on reverse phase HPLC (SPD-20A UV detector (254 nm), a Sunfire C18 column (5 µm, 4.6 × 250 mm), a gradient elution of 50% methanol in water to 100% methanol over 30 min, flow rate 0.7 mL/min).

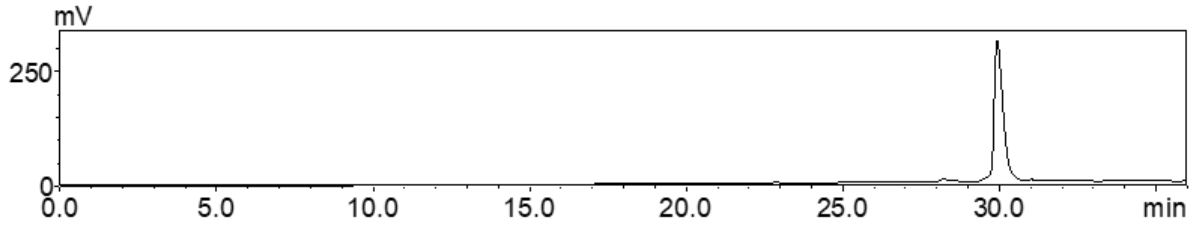


Figure S2. HPLC chromatograms of "comb" **1**.

6. Preparation of Liposomes L0 and L1 (Table S2)

The liposome **L0** was prepared with the film dispersion method. A mixture of "comb" **1** /Lecithin (180 mg/60 mg, mass ratio of 3:1) was dissolved in chloroform. The chloroform solution was added to the bottom of a 10 mL round-bottom glass flask, followed by rotary evaporation of the solvent. This formed a thin lipid biofilm, which was further dried under a vacuum overnight. The film was subsequently hydrated in 2 mL of sodium citrate-hydrochloric acid buffer solution (pH = 4, 200 mM) at 50^{o}C before probe sonication for 15 min, using a 1/2 s on/off cycle at a power output of 40%. Liposome **L0** was collected by filtration through a 0.45-μm polycarbonate membrane and a 0.22-μm polycarbonate membrane. To achieve DOX loaded liposome **L1**, the pH of liposome **L0** solution was adjusted to 7.5-7.8 with sodium hydroxide solution (1 M) and incubated with 10 mg/mL DOX for 30 min at 45 °C. The liposomes were comprehensively characterized for size, ζ-potential, loading capacity, and morphology using DLS, HPLC and TEM, respectively.

An ultrafiltration technique was used to separate the unencapsulated DOX from liposomes. A total of 0.5 ml drug containing liposomes, which was diluted 10 times, was placed in the upper chamber of a centrifuge tube matched with an ultrafilter (Millipore 0.5 mL, 3kDa) and was centrifuged for 20 min at 4000 rpm. The ultrafiltrate in the ultrafilter containing the unencapsulated drug was determined by HPLC (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 (MeOH, 0.35 mL/min).). The total drug in **L1** was determined through MeOH disruption by HPLC after making a standard curve with DOX. The entrapment efficiency (EE%) was calculated using the following equation:

$$EE\% = (W_{\text{total drug}} - W_{\text{free drug}}) / W_{\text{total drug}} * 100\%$$

Where $W_{\text{total drug}}$ and $W_{\text{free drug}}$ represent the total drug in **L1** and the amount of free drug in the ultrafiltrate, respectively.

Table S2. characterization of liposomes **L0** & **L1**.

	Size (nm)	Zeta (mV)	PDI	EE%
L0	96.3	-38.1	0.187	
L1	70.6	-37.5	0.169	95%

7. *In Vitro* Release of DOX from L1

In vitro drug release from **L1** in PBS was established at 37 and 42°C. Add **L1** 0.1 mL into a little centrifuge tube which had 1.9 ml PBS (42°C or 37°C) under stirring. The drug release was measured over time (at 0.5, 2, 4, 8, 10, 12, 24, 48, 72, 96 h), extracting 100 μL **L1** suspension and ultrafiltration, then add additional 100 μL normal PBS. Dissolved **L1** (by adding MeOH) were considered as a positive control. The accumulated drug release (A%) was calculated using the following equations:

$$A\,(\%) = \frac{\boldsymbol{C}_i \times 10 + (\boldsymbol{C}_{i-1} + \boldsymbol{C}_{i-2} + \ldots + \boldsymbol{C}_1) \times 0.5}{\boldsymbol{C}_{control} \times 0.1} \times 100\%$$

i-The sample number; C_i-The concentration of ultrafiltration filtrate; $C_{control}$-The concentration of dissolved **L1**.

8. *In vitro* ^{19}F MRI Experiments (Table S3)

All magnetic resonance imaging (MRI) experiments were performed on a 400 MHz Bruker BioSpec MRI system. The temperature of the magnet room was maintained at 25 ^{o}C during the entire MRI experiment. "comb" **1** solutions (3.5, 1.8, 0.88, 0.44, 0.22, 0.11 mM) were prepared by sequential dilution with deionized water. The ^{19}F *in vitro* images were acquired using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32 × 32, SI = 37 mm, FOV = 3.7 cm, TR = 4000 ms, TE = 5.5 ms, scan time = 32 s.

Table S3. T_1 and T_2 of "comb" **1** and **L1**

	T_1 (ms)	T_2 (ms)
"comb" **1**	542	152
L1	538	51

9. H&E staining assay results of major organs (Figure S3)

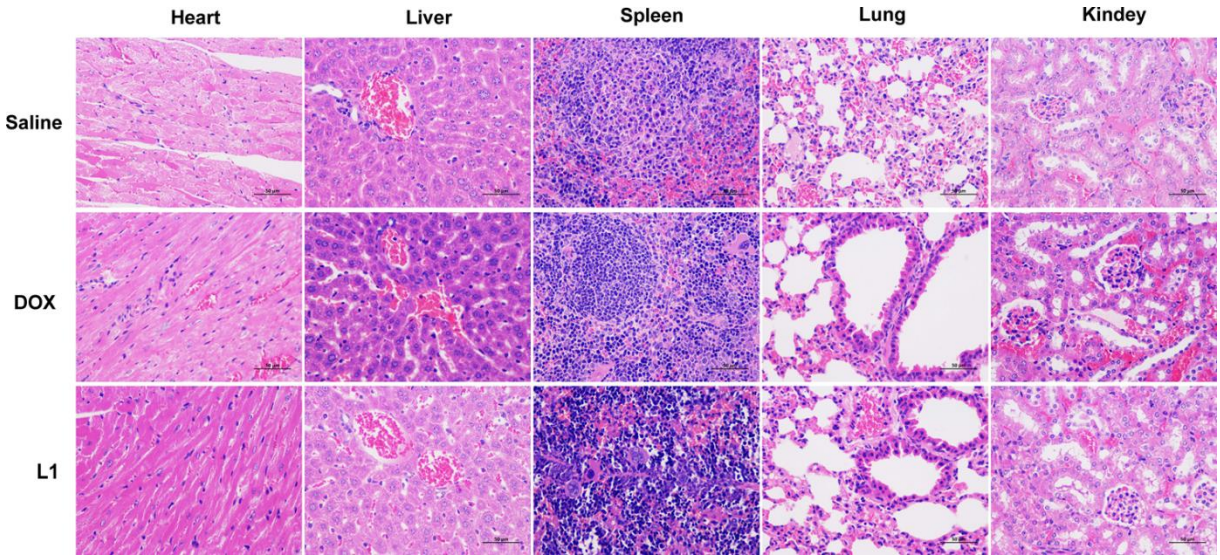
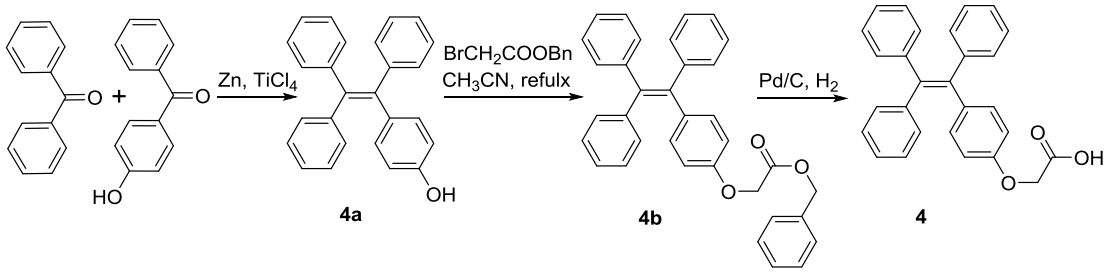


Figure S2. H&E staining assay results of major organs (Scale bar = 50 μm)

10. Synthesis of Compound 4

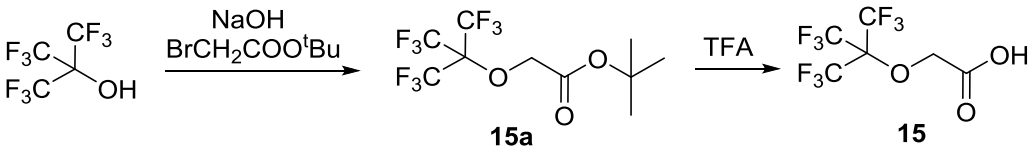


Compound 4a. Benzophenone (3.5 g, 19.1 mmol), 4- hydroxybenzophenone (3.8 g, 19.1mmol), and zinc powder (5.0 g, 76.5 mmol) were added to a three-necked flask, which was then vacuum evacuated and flushed 3 times with dry nitrogen. A 100 mL dry THF was added, and then $TiCl_4$ (7.2 g, 38.2 mmol) was added dropwise using a syringe at -78 ^oC. After refluxing overnight, the reaction was quenched by addition of 10% K_2CO_3 solution. The organic phase was washed 2 times with brine and then dried over anhydrous Na_2SO_4. The crude product was filtered, concentrated, and passed through a silica gel column. The final product **4a** was a white solid (3.3 g, yield: 50%).

Compound 4b. To a solution of **1a** (2.6 g, 7.5 mmol) in CH_3CN (50 mL) was added $BrCH_2COOBn$ (3.4 g, 15.0 mmol) at room temperature. After stirring overnight, the reaction mixture was concentrated under vacuum to give a residue. The residue was purified by flash column chromatography on silica gel to give the desired product **4b** (2.9 g, yield: 78%) as white solid. ^1H NMR (400 MHz, $CDCl_3$) δ 4.60 (s, 9H), 5.24 (s, 2H) 6.64 (d, J = 8 Hz, 2H), 6.97 (d, J = 8 Hz, 2H), 7.04-7.12 (m, 15H), 7.36-7.39 (m, 15H).

Compound 4. A mixture of **4b** (2.9 g, 5.8 mmol) and Pd/C (0.6 g, 10% on carbon) in THF was stirred at room temperature under H_2 (1 atm) for 12 h. The mixture was filtered through Cite and the filtrate was concentrated to give **4** as white solid (2.2 g, yield: 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.61 (s, 2H), 6.65 (d, J = 8 Hz, 2H), 6.97 (d, J = 8 Hz, 2H), 7.00-7.12 (m, 15H).

11. Synthesis of Amino Acid 3



Compound 15a. To a solution of NaOH (847 mg, 21.2 mmol) in H_2O (3 mL) was added perfluoro-tert-butanol (5.0 g, 21.2 mmol) at 0 °C. After stirring for 1 h, the reaction mixture was lyophilized and the solid was dissolved in dry DMF. Then $BrCH_2COO^tBu$ (10.5 g, 53.8 mmol) was added under stirring, followed heating to 50 °C. After reacting overnight, the reaction was added to H_2O (200 mL), the organic phase was purified by flash column chromatography on silica gel to give **15a** as colorless oil (3.1 g, yield: 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 3.52 (s, 2H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -70.43.

Compound 15. To a solution of **15a** (3.1 g, 8.9 mmol) in dry DCM was added anisole (2.9 g, 26.6 mmol) and TFA (20.2 g, 178.0 mmol) at rt and the reaction mixture was stirred for 1 h at room temperature. The mixture was concentrated and purified by flash column chromatography on silica gel to give the **15** as white solid (1.8 g, yield: 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.59 (s, 2H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -70.54.

Compound 13. To a solution of **12** (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 **12** 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 **13** 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).

Compound 14. To a solution of **13** (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 **13** was consumed completely on TLC (PE: EA = 3:1), the reaction mixture was concentrated under reduced pressure to give **14** as yellow foam (1.7 g, yield: 99%).

Compound 16. A mixture of **15** (0.5 g, 1.7 mmol), DIPEA (323.1 mg, 2.6 mmol) and EDCl (488.8 mg, 2.6 mmol) in dry DMF (20 mL) was stirred at 0 ^{o}C under N_2. HOBt (351.3 mg, 2.6 mmol) in DMF was added slowly into the mixture at 0 ^{o}C and the mixture was stirred for 30 min at this temperature. Then **14** (1.3 g, 2.2 mmol) in DMF was added slowly to the reaction mixture. After addition, the resulting mixture was stirred at 50 ^{o}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 **16** (786.3 mg, yield: 63%) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28-1.55 (m, 4H), 1.67-1.92 (m, 2H), 3.26-3.31 (m, 2H), 4.19-4.23 (m, 1H), 4.33-4.45 (m, 3H), 4.50 (s, 2H), 5.15-5.22 (m, 2H), 5.39 (d, 2H), 5.26 (s, 1H) 7.29-7.42 (m, 9H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.76 (t, *J* = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.3, 29.0, 32.1, 38.8, 41.3, 47.1, 53.7, 67.1 67.3, 67.6 120.1, 118.5 (q, *J* = 290 Hz), 120.0, 125.1, 127.1, 127.7, 128.6, 128.7, 135.2, 141.3, 143.7, 143.9, 156.0, 165.6, 172.2. ^{19}F NMR (376 MHz, $CDCl_3$) δ -70.40. HRMS (ESI) calcd for $C_{34}H_{31}F_9N_2NaO_6^+$ 757.1931 ($(M+Na)^+$), found 757.1925.

Compound 3. A mixture of **16** (3.5 g, 4.8 mmol) and Pd/C (0.7 g, 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 **3** as white solid (2.7 g, yield: 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30-1.39 (m, 4H), 1.57-1.69 (m, 2H), 3.08-3.09 (m, 2H), 4.21-4.27 (m, 4H), 4.49 (s, 2H), 7.32 (t, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 8.0 Hz, 2H), 7.70 (d, *J* = 8.0 Hz, 1H), 7.87 (d, *J* = 8.0 Hz, 2H), 8.06 (s, 1H). ^{13}C NMR (100MHz, $CDCl_3$) δ 22.3, 28.9, 31.8, 38.9, 47.1, 53.5, 67.1, 67.5, 120.0, 121.4 (q, *J* = 290 Hz), 125.1, 127.1, 127.7, 141.3, 143.7, 143.8, 156.3, 166.2, 175.7. ^{19}F NMR (376 MHz, $CDCl_3$) δ -70.42. HRMS (ESI) calcd for $C_{27}H_{25}F_9N_2NaO_6^+$ 667.1461 ($(M+Na)^+$), found 667.1462.

12. Synthesis of M-PEGs ω-Amino Acid 2

Compound 6. 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 **5** (77.3 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 65.8 g of **6** as white solid with a 62% yield. ^{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 7. To a mixture of cyclic sulfate **6** (24.1 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 **7** (19.0 g, yield: 92%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.39 (t, J = 5.2 Hz, 2H), 3.59-3.72 (m, 14H).

Compound 8. 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 **7** (19.4 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 **6** (31.7 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 **8** (30.7 g, yield: 88%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.38 (t, J = 4.8 Hz, 2H), 3.58-3.72 (m, 30H).

Compound 9 was prepared from **8** and cyclic sulfate **6** by following the same procedure for **8** 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 10. 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 **9** (40.9 g, 71.6 mmol) in THF (150 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 **10** as yellowish wax (26.0 g, 53%). ^{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 11. Compound **10** (26.0 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 **11** as yellowish oil (22.8 g, 68%). ^{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).

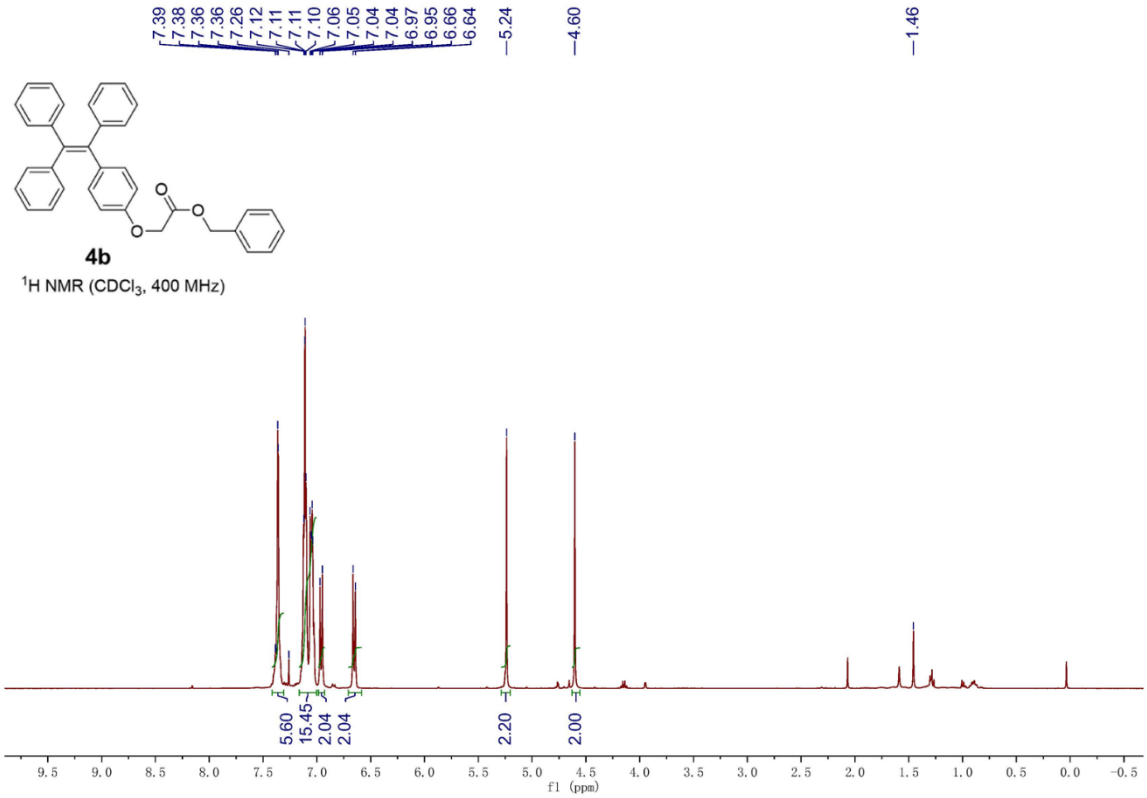
Compound 2. A solution of compound **11** (16.3 g, 18.5 mmol), anisole (4.0 mL, 36.9 mmol) and TFA (27.5 mL, 370.1 mmol) in DCM (150 mL) was stirred at 25 °C over 4 h. After concentrated under vacuum, the residue was dissolved in ether (100 mL) and washed with water (100 mL). The organic layer was discarded 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 **2** (13.9 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).

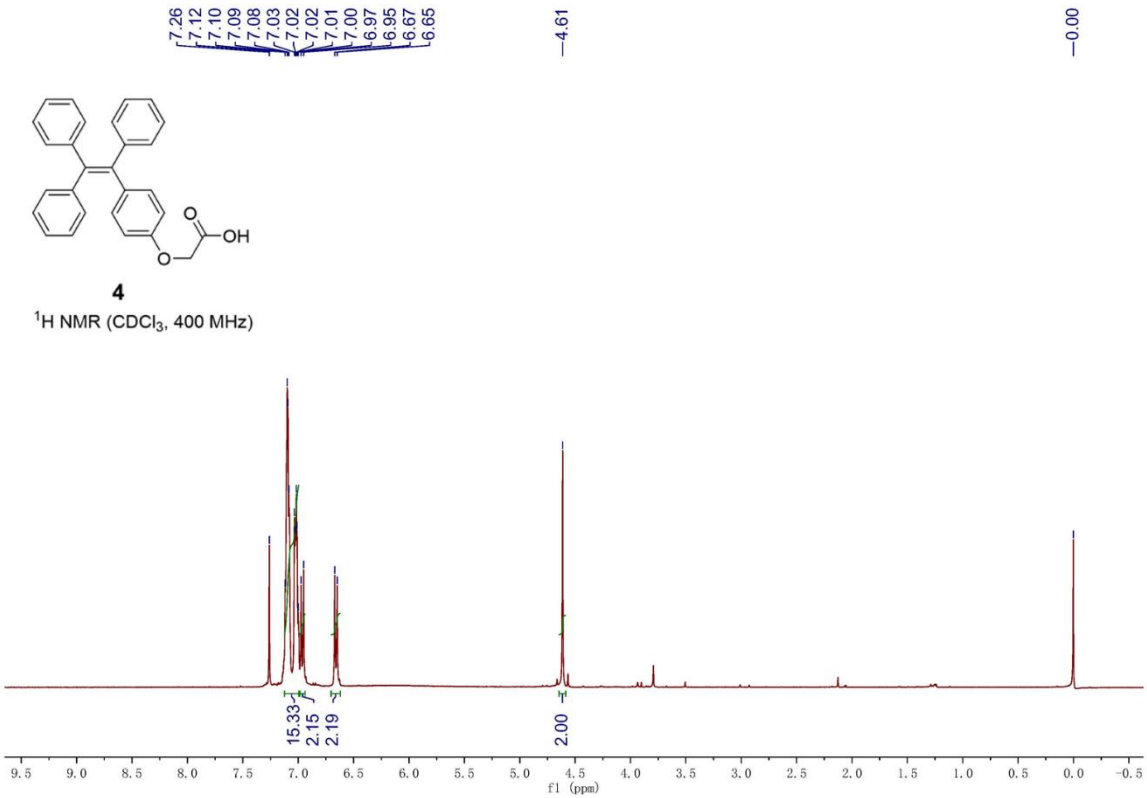
"Comb" **1**. ^{1}H NMR (400 MHz, CD_3OD) δ 1.32-1.37 (m, 10H), 1.51-1.54 (m, 10H), 1.66-1.72 (m, 5H), 1.76-1.83 (m, 5H), 3.20-3.24 (m, 10H), 3.31-3.70 (m, 337H), 3.93 (s, 2H), 3.97 (s, 3H), 4.00 (s, 9H), 4.36-4.39 (m, 5H), 4.42 (s, 2H), 4.51 (s, 10H) 6.71 (d, *J* = 8Hz, 2H), 6.90 (d, *J* = 8Hz, 2H), 6.93-6.99 (m, 6H), 7.03-7.09 (m, 9H). ^{19}F NMR (376 MHz, CD_3OD) δ -71.50. MS (MALDI-TOF) m/z 6554.1 ($[M + Na]^+$, expected mass for $C_{270}H_{445}F_{45}N_{18}NaO_{108}^+$, 6547.9).

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

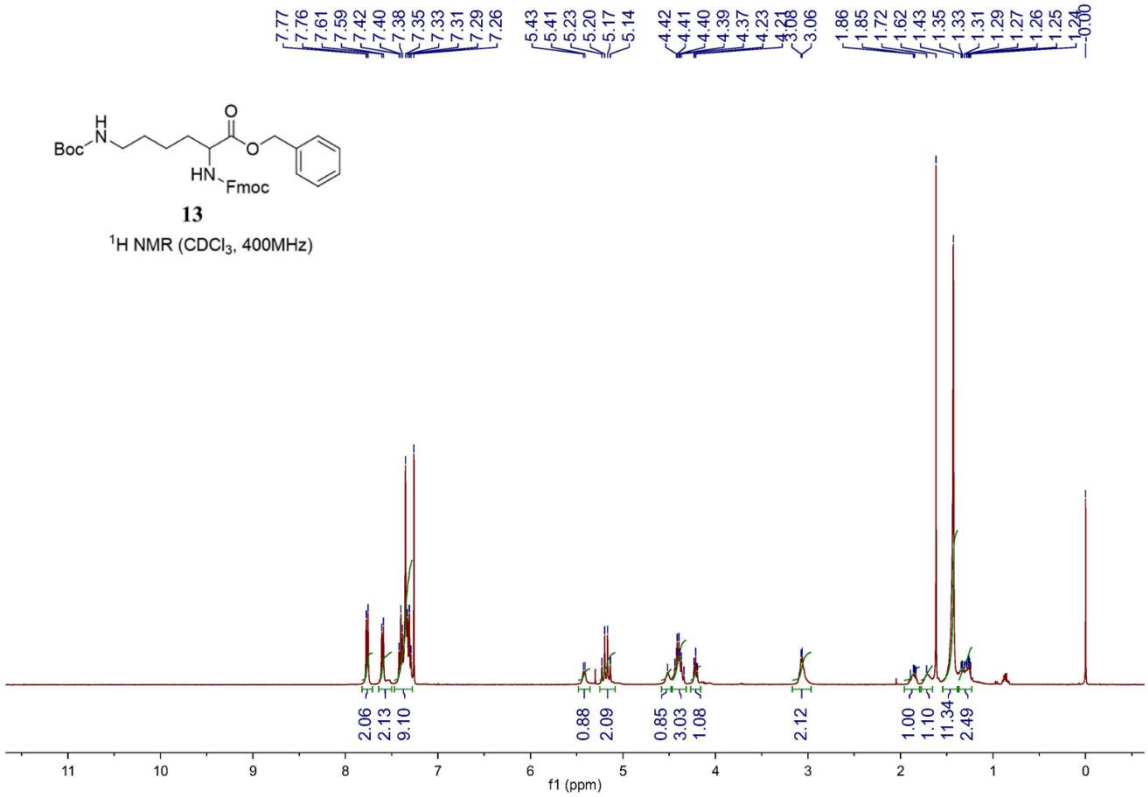
^{1}H NMR of compound **4b**



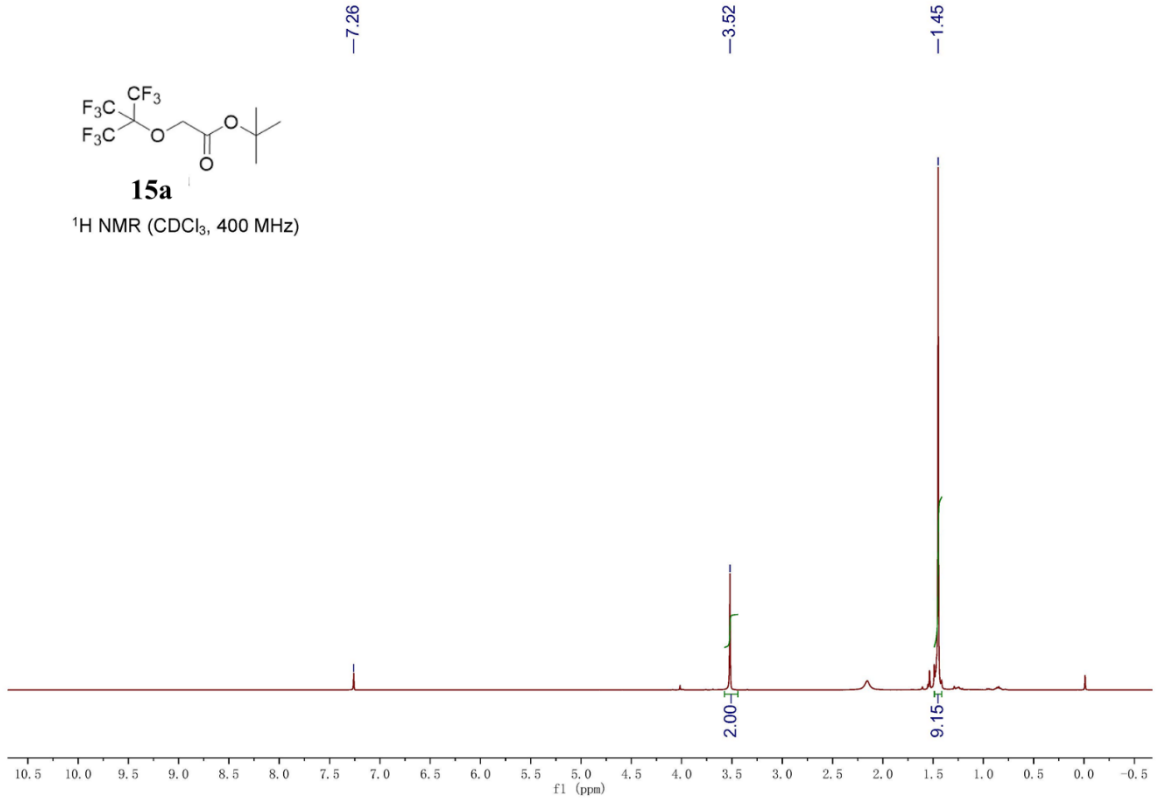
^{1}H NMR of compound **4**



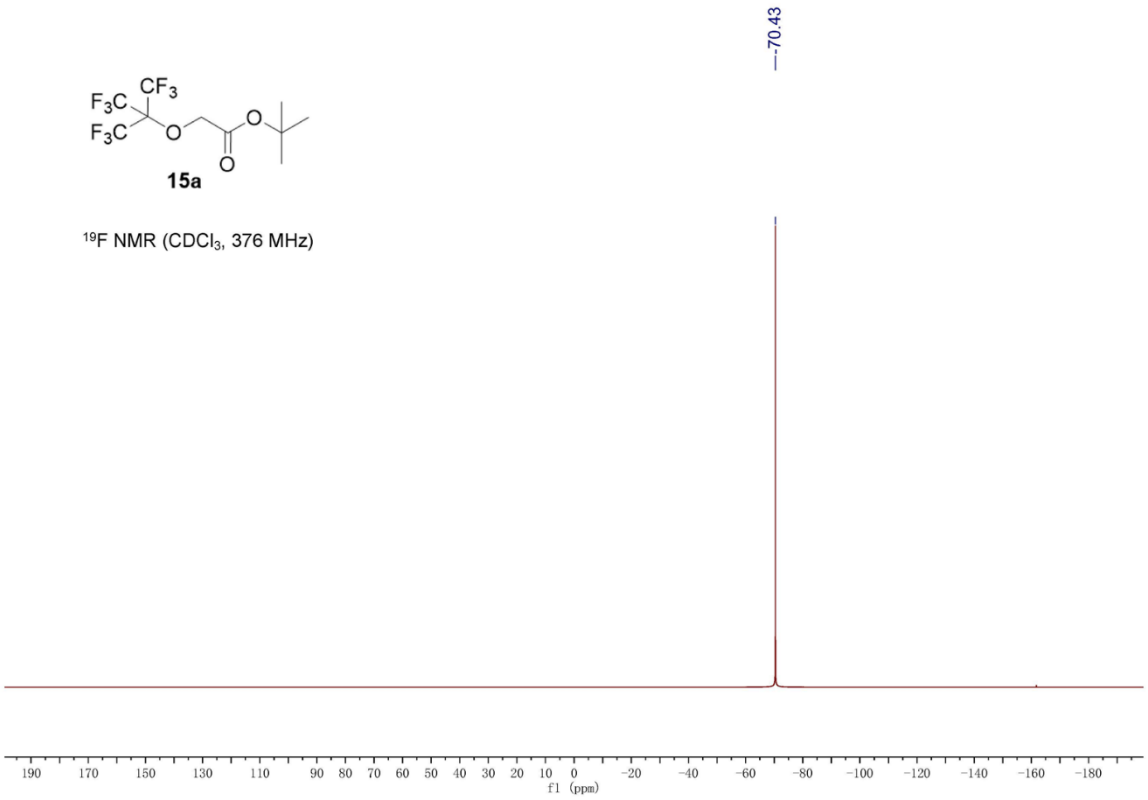
^{1}H NMR of compound **13**



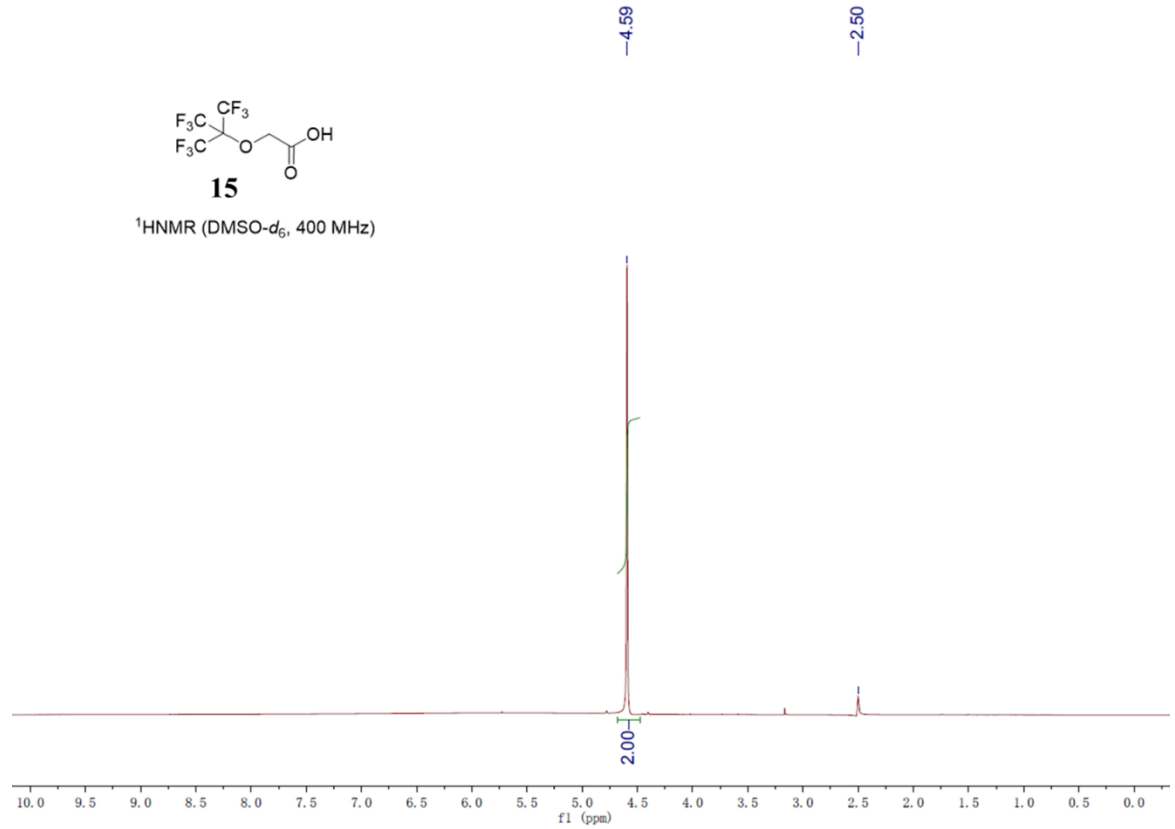
^{1}H NMR of compound **15a**



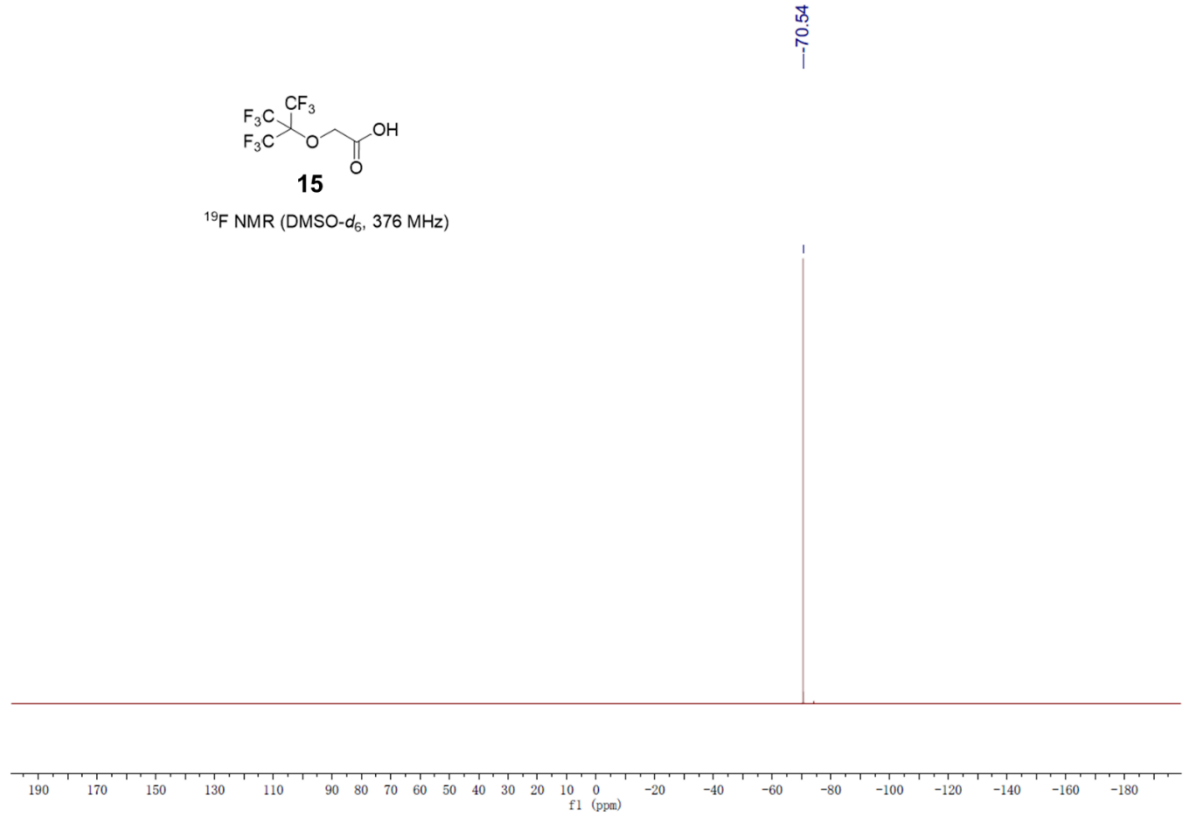
^{19}F NMR of compound **15a**



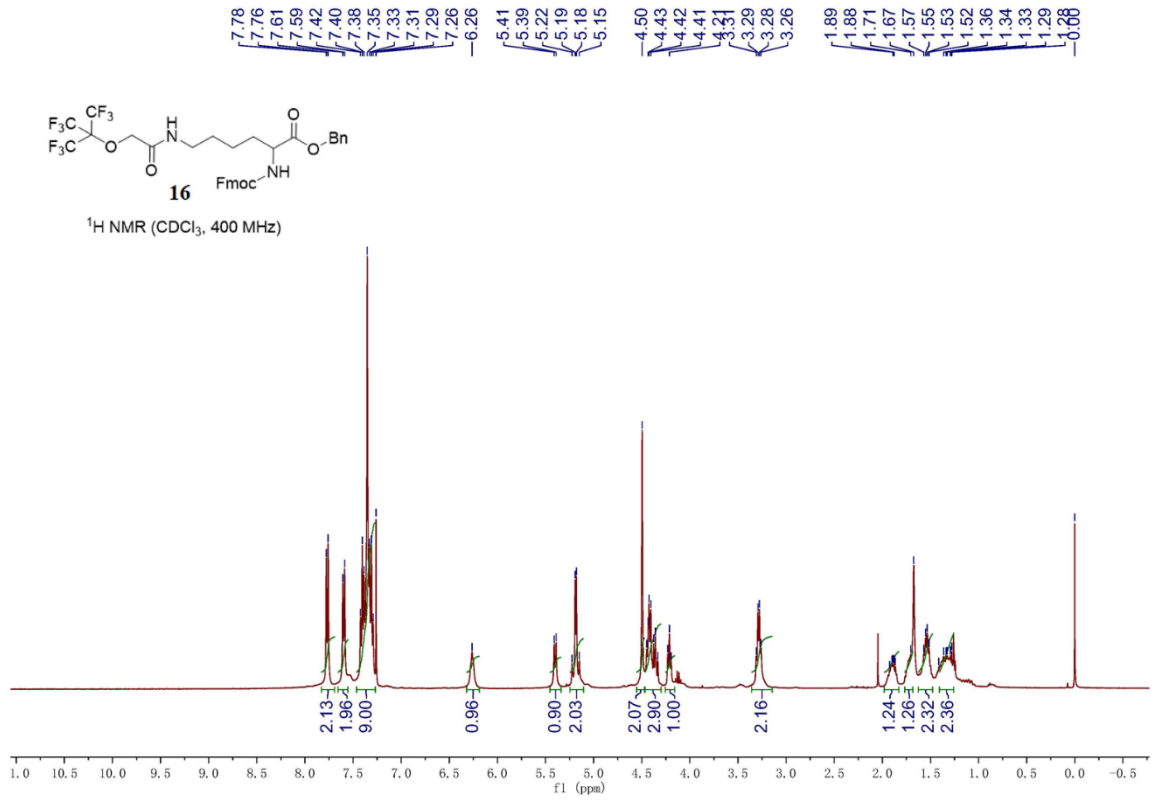
^{1}H NMR of compound **15**



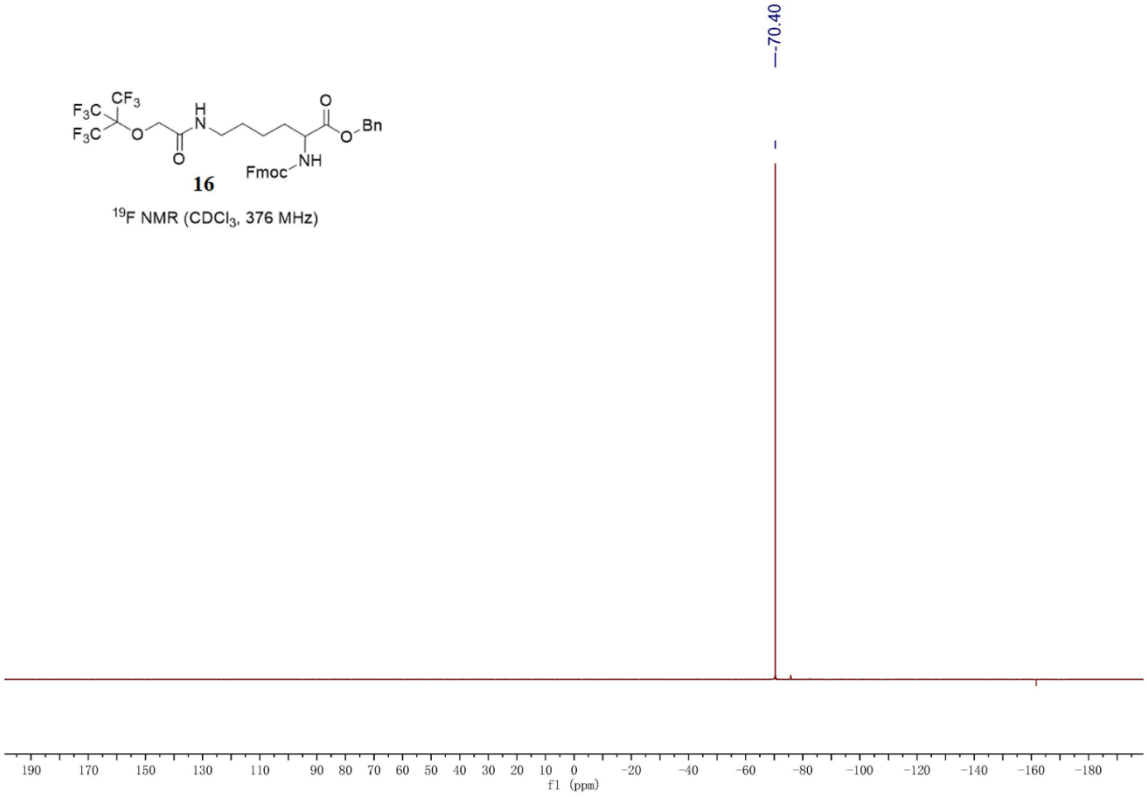
^{19}F NMR of compound **15**



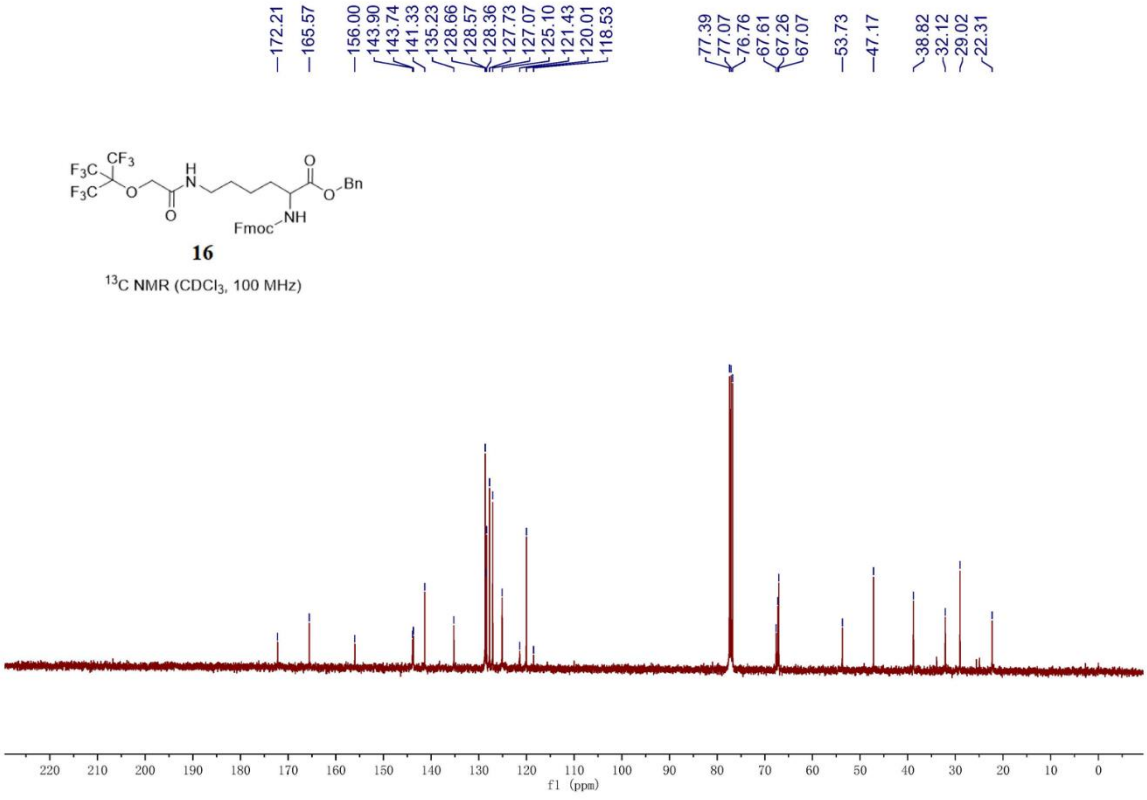
^{1}H NMR of compound **16**



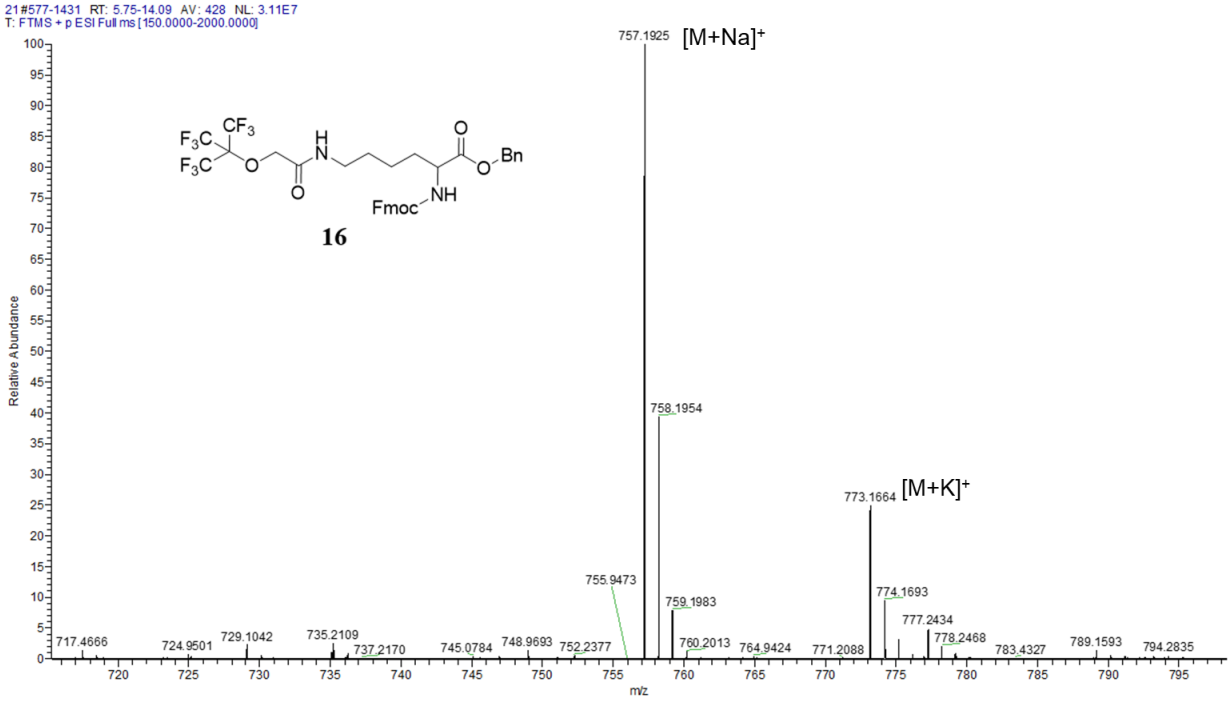
^{19}F NMR of compound **16**



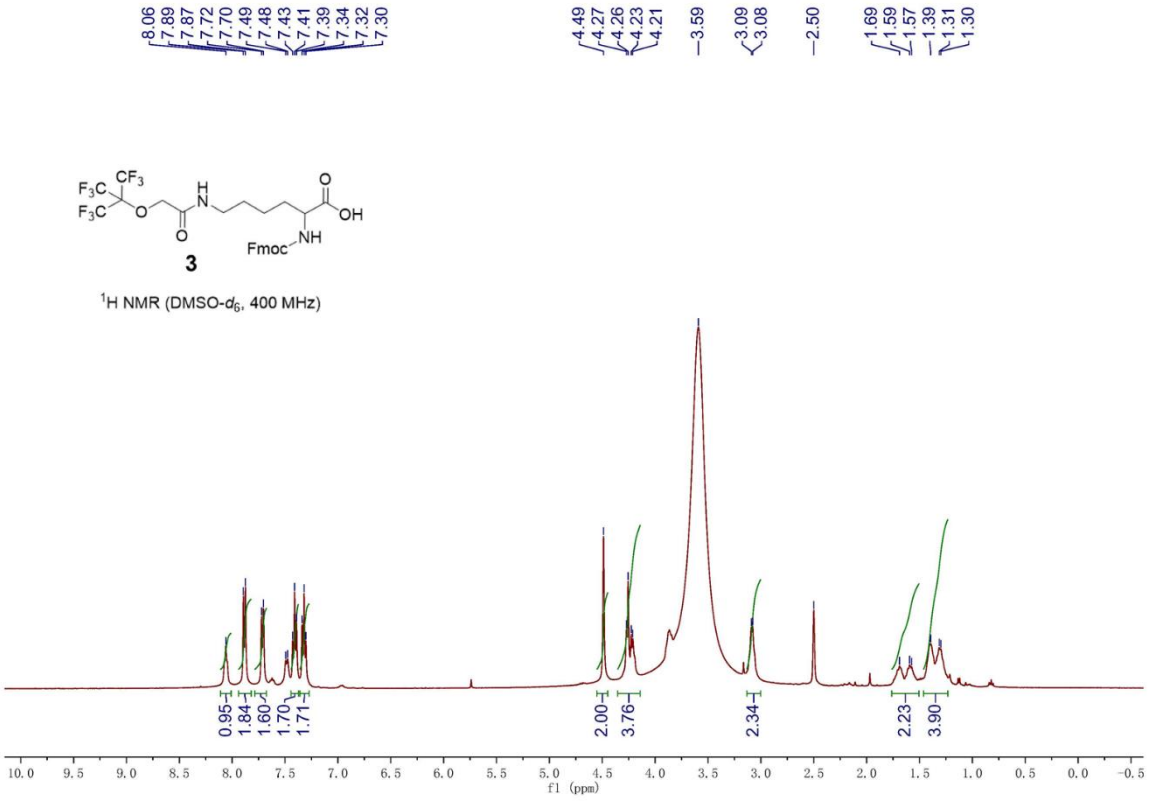
^{13}C NMR of compound **16**



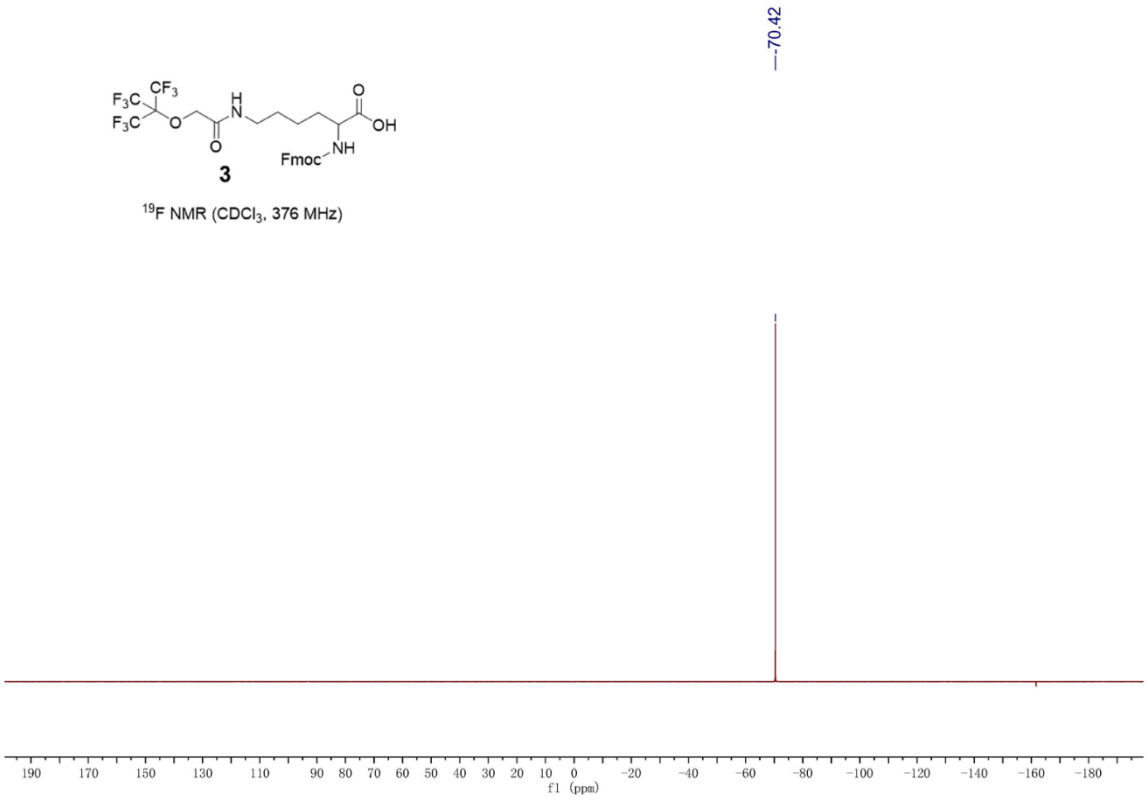
Mass spectrum of compound **16**



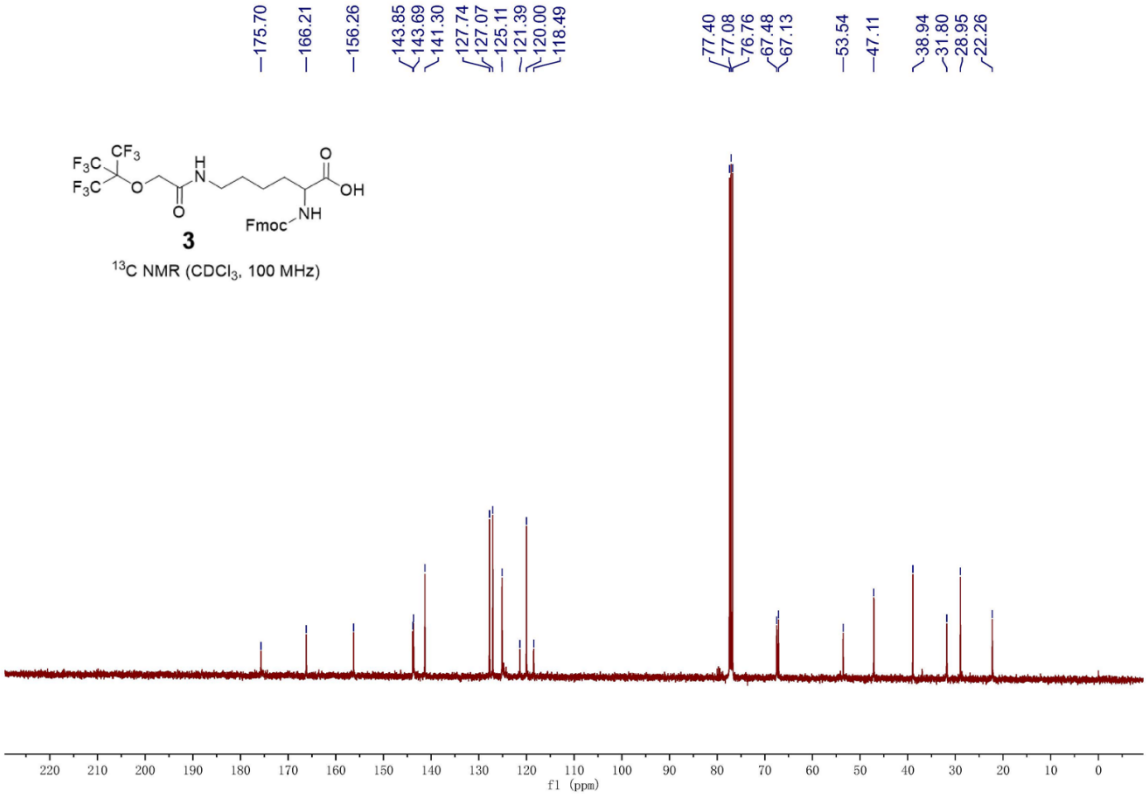
^{1}H NMR of compound **3**



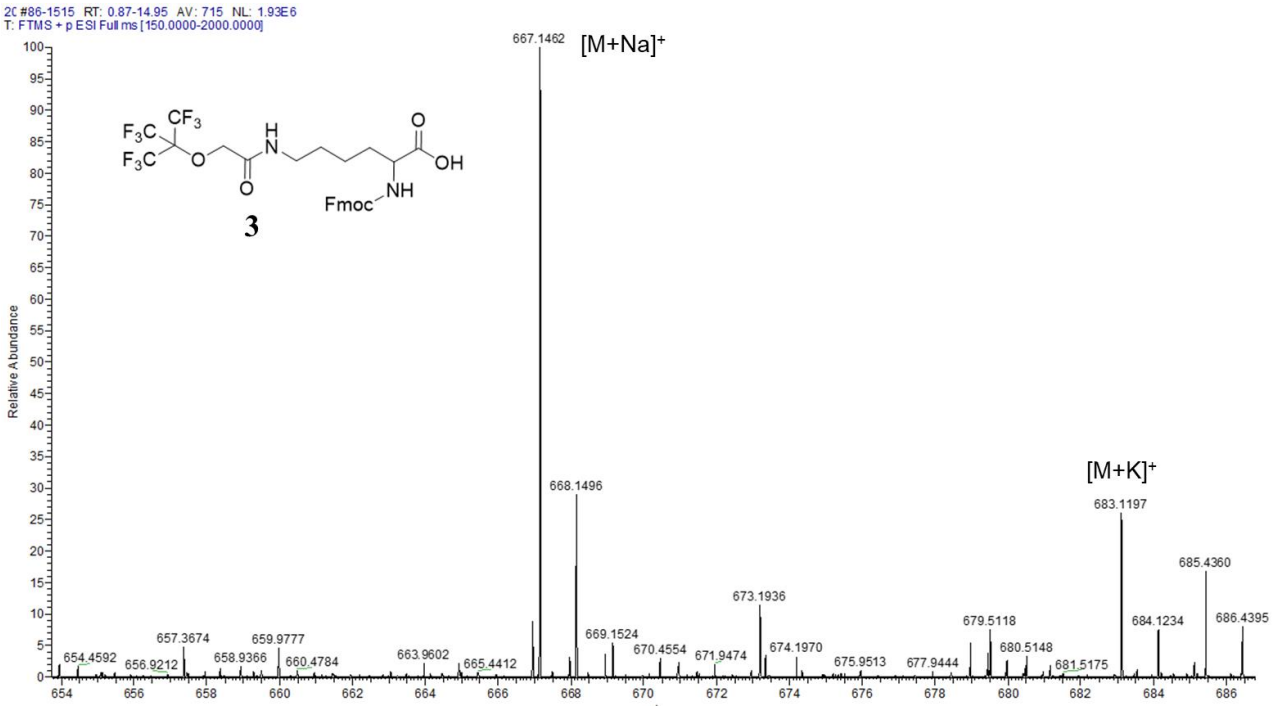
^{19}F NMR of compound **3**



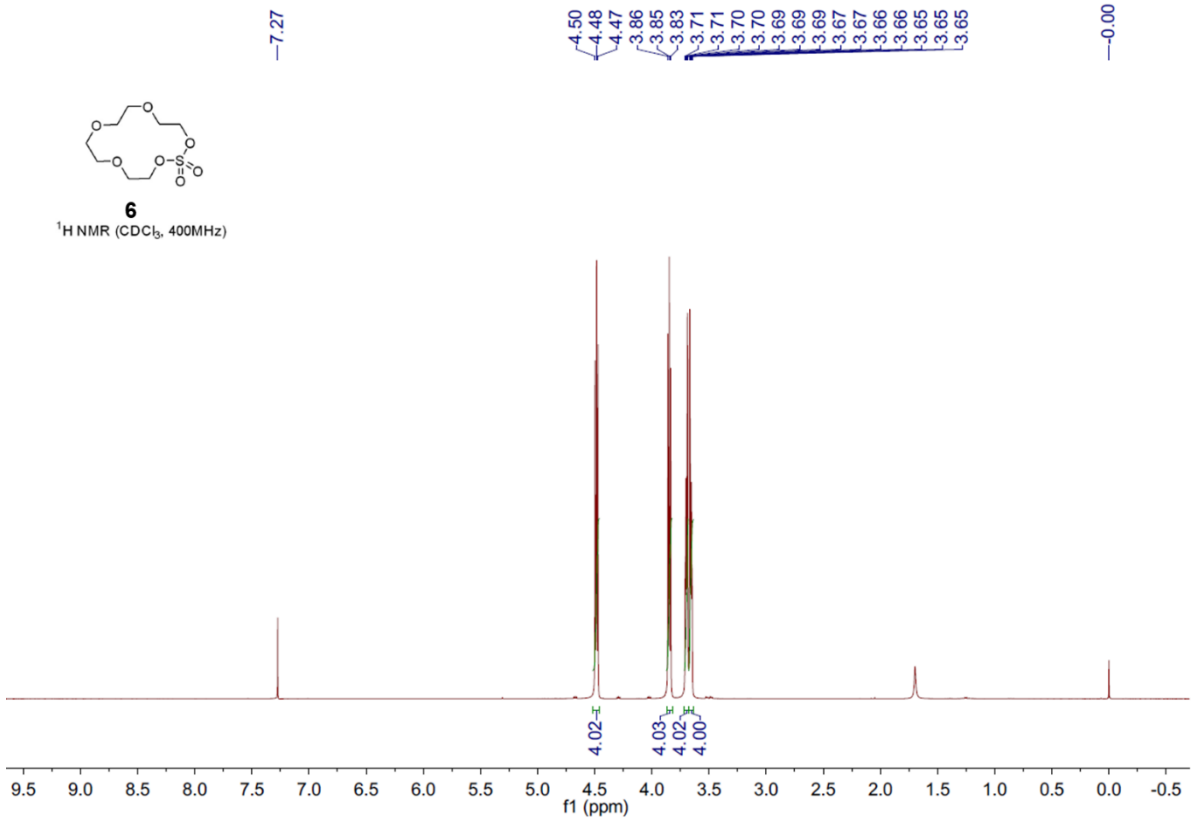
^{13}C NMR of compound **3**



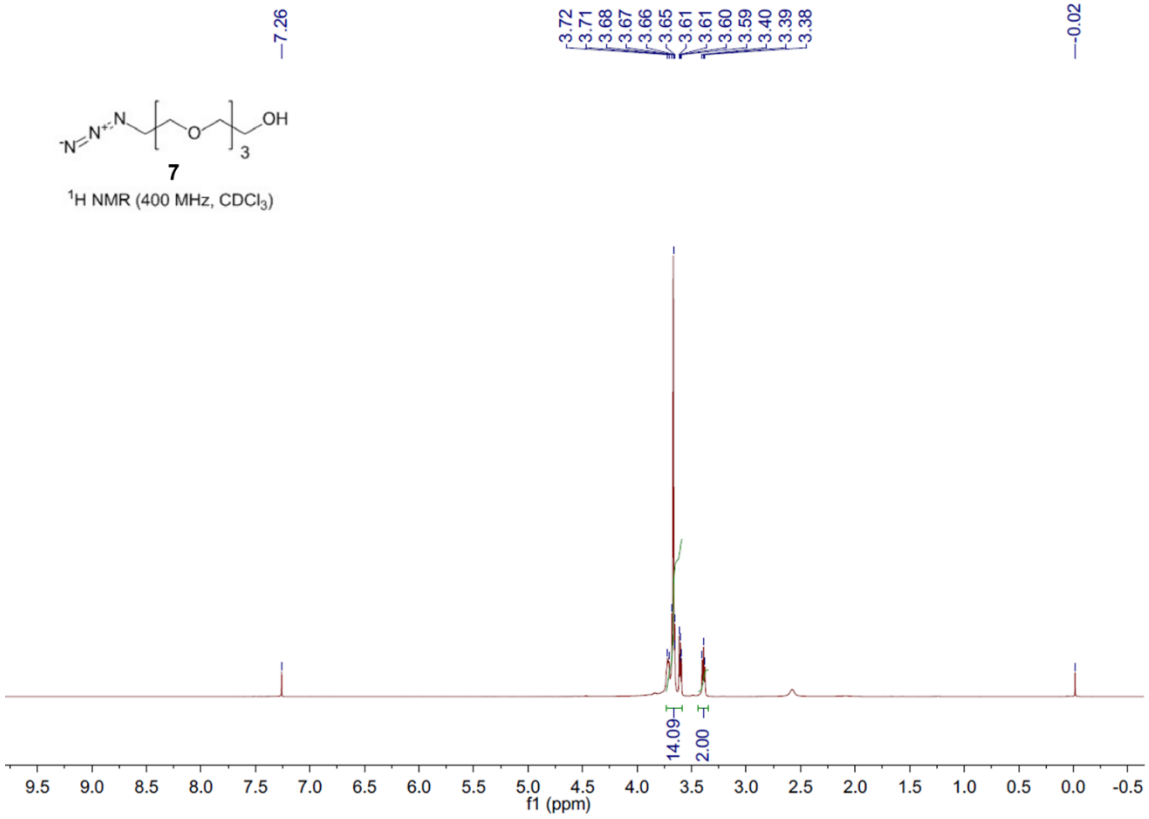
Mass spectrum of compound **3**



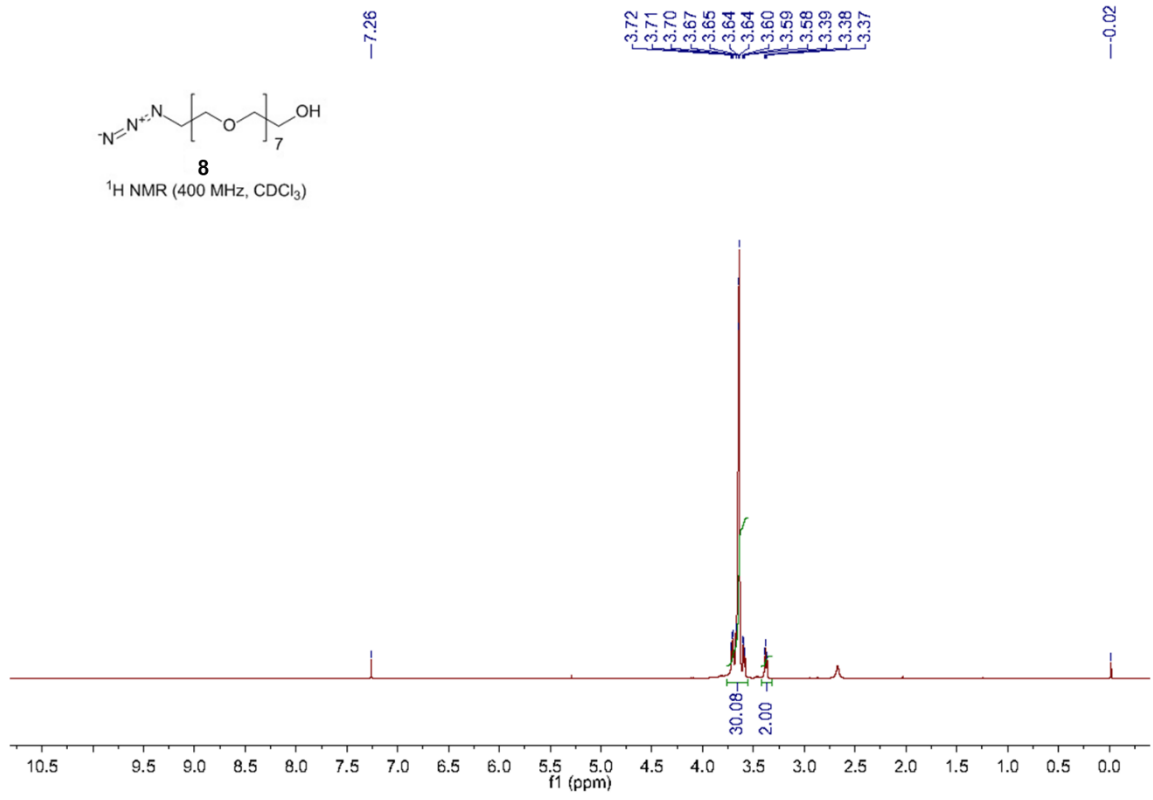
^{1}H NMR of compound **6**



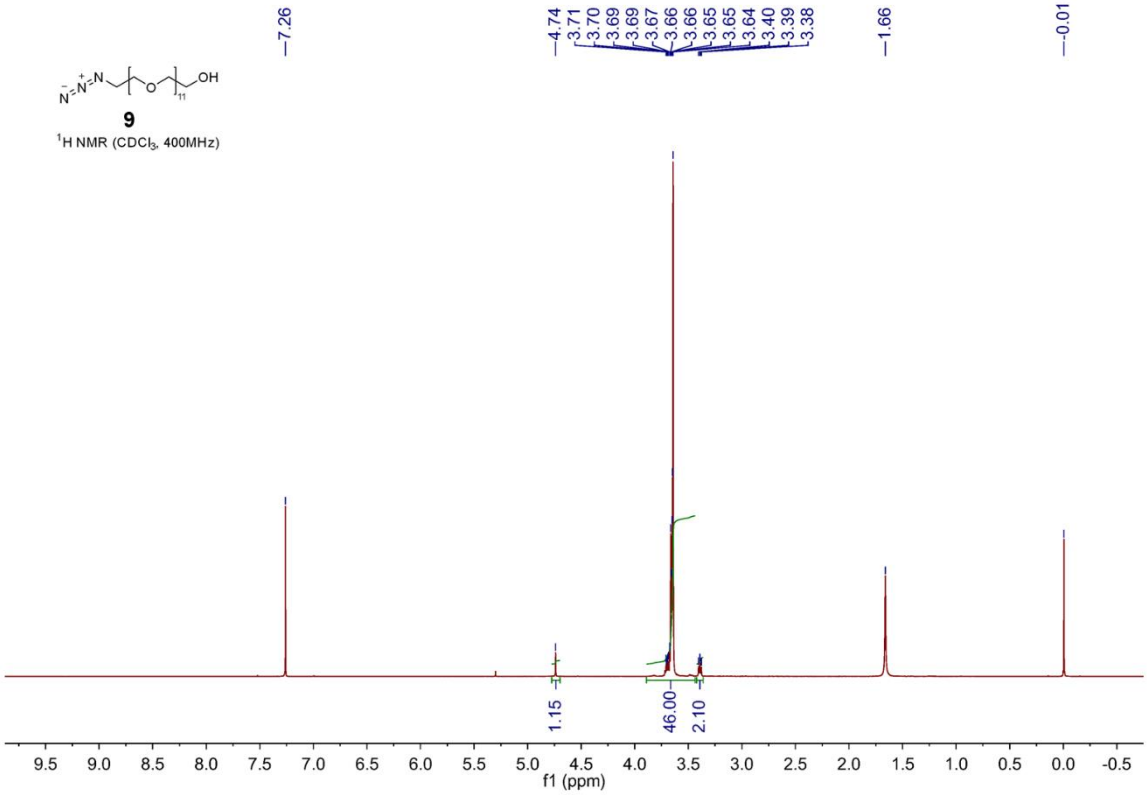
^{1}H NMR of compound **7**



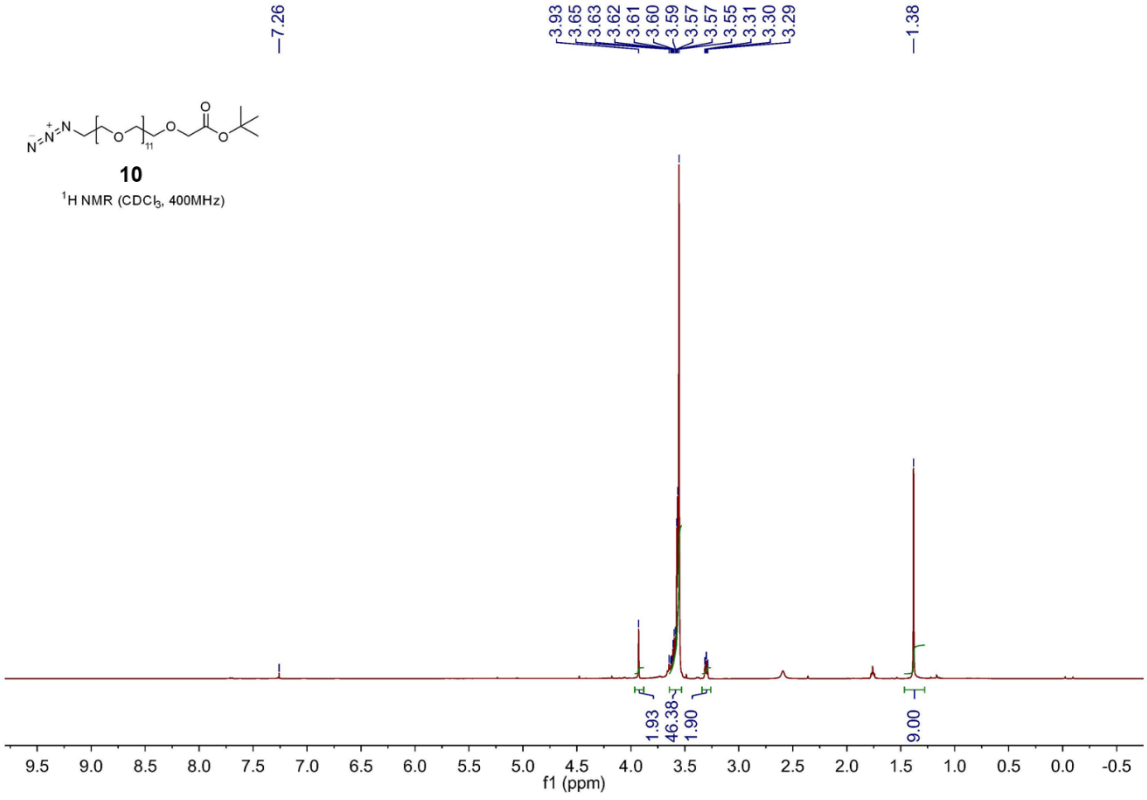
^{1}H NMR of compound **8**



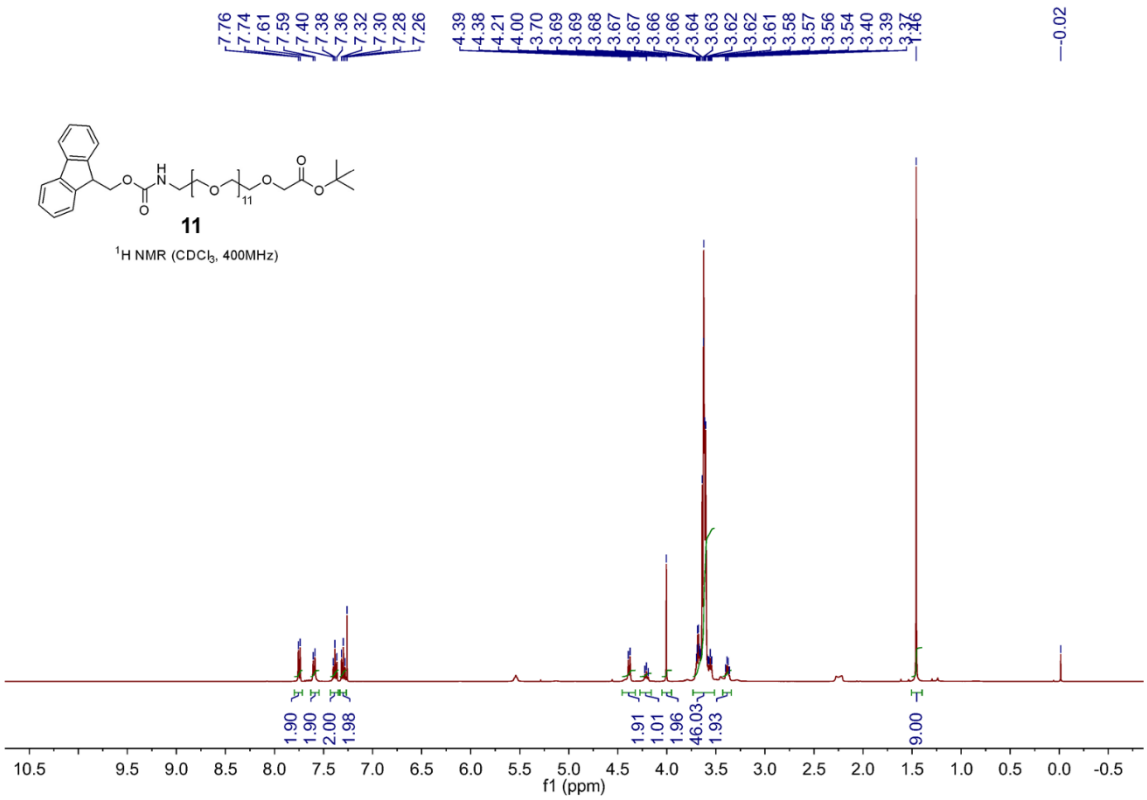
^{1}H NMR of compound **9**



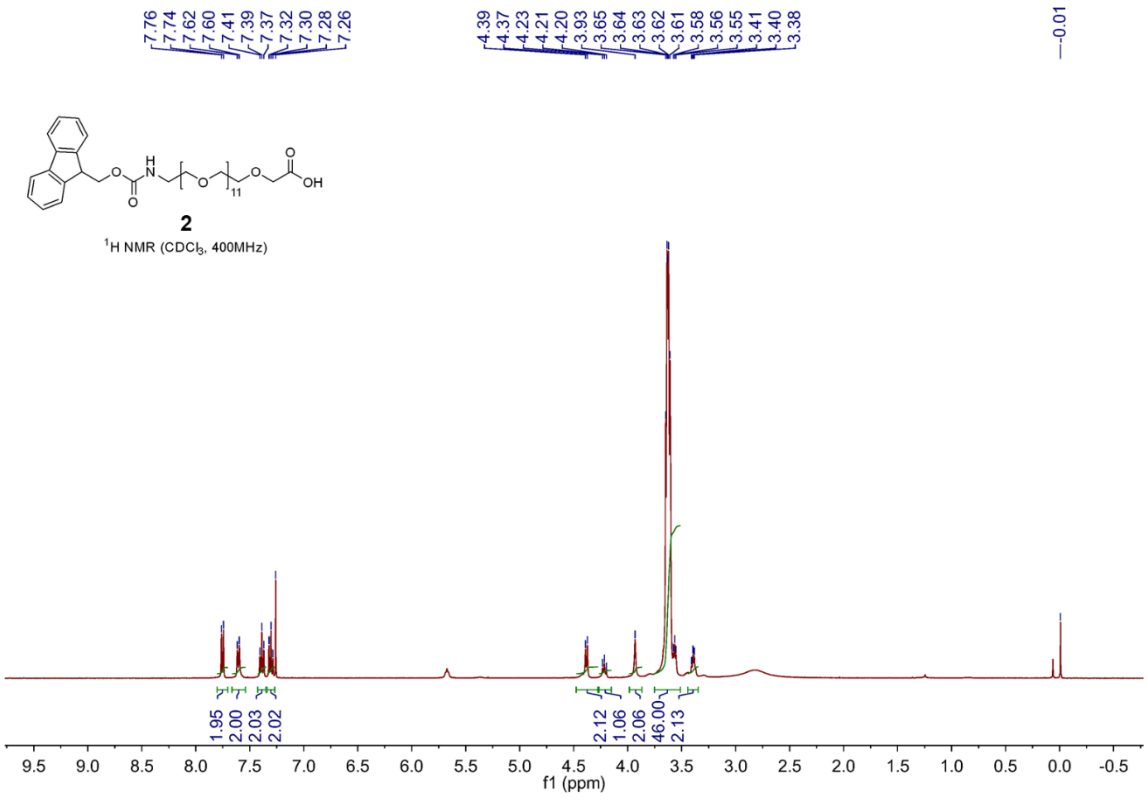
^{1}H NMR of compound **10**



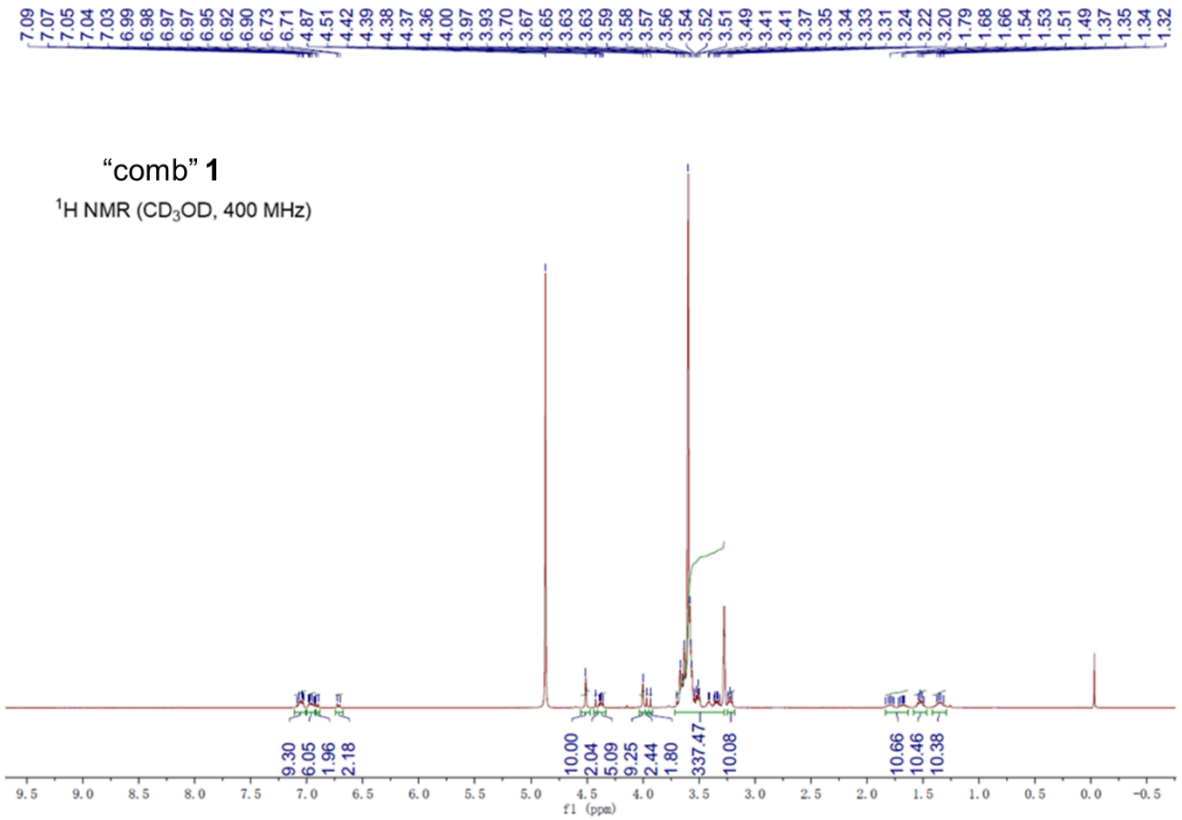
^{1}H NMR of compound **11**



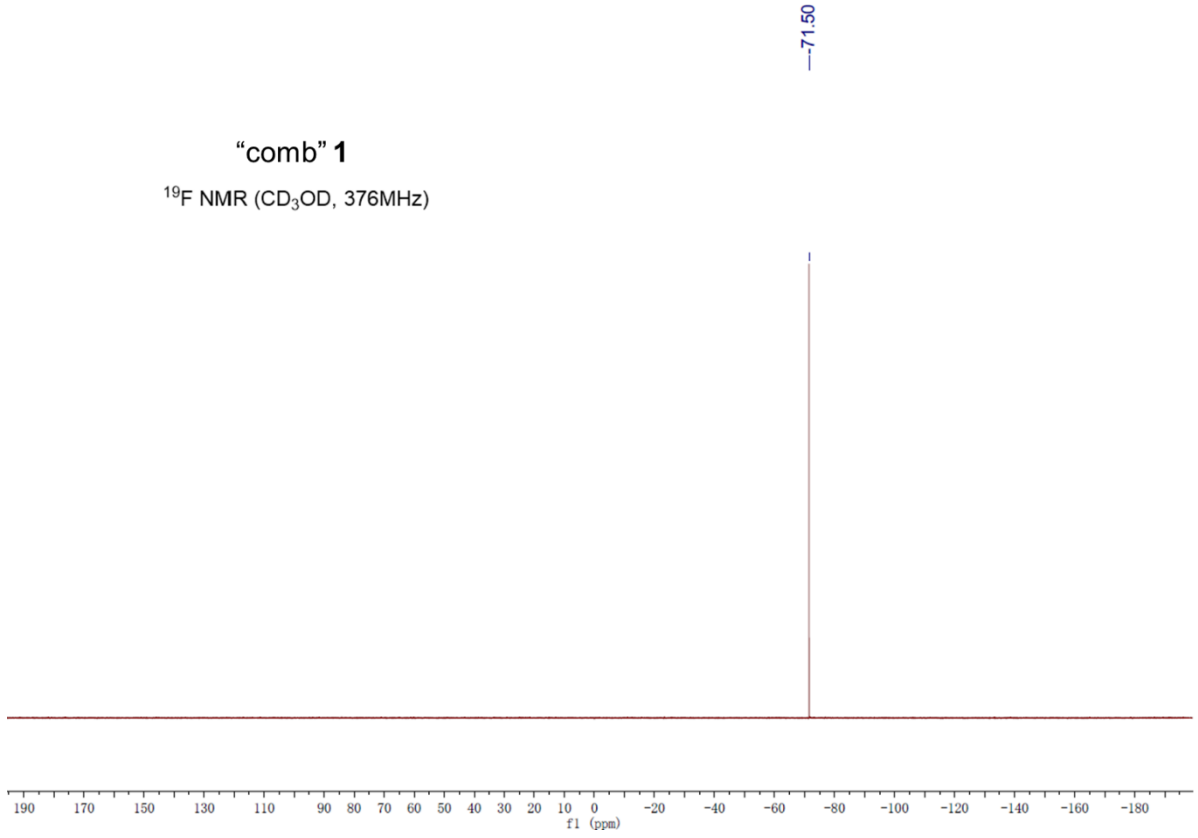
^{1}H NMR of compound **2**



^{1}H NMR of "comb" **1**



^{19}F NMR of "comb" **1**



MALDI-TOF mass of "comb" **1**