Supporting Information

Jellyfish-Inspired Smart Tetraphenylethene Lipids with Unique AIE Fluorescence, Thermal Response, and Cell Membrane Interaction

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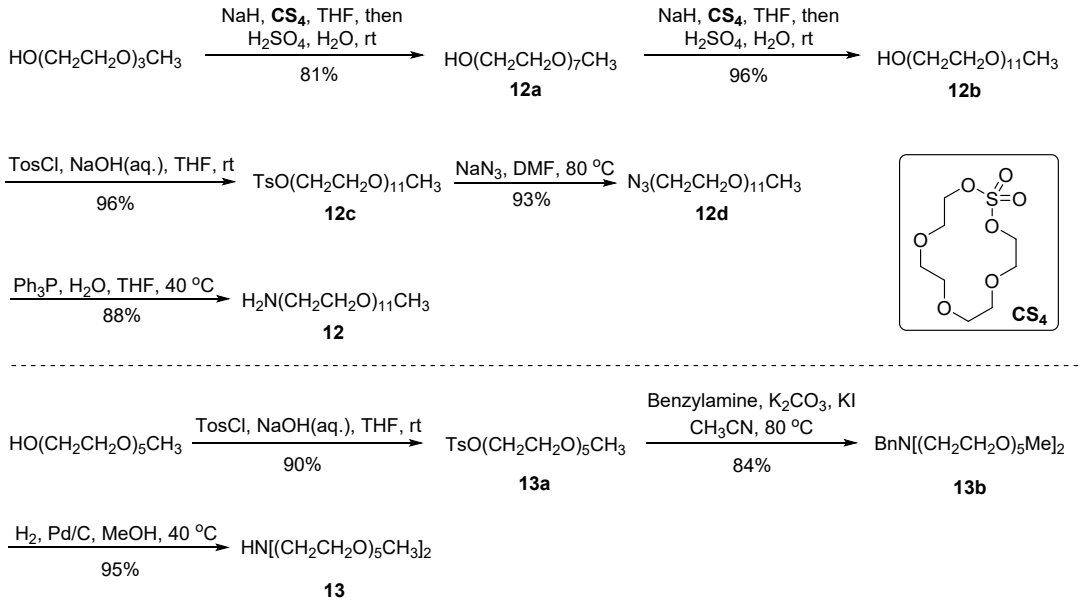
1. General Information

Unless otherwise indicated, all reagents were obtained from commercial suppliers and used without prior purification. Column flash chromatography was performed on silica gel (200-300 mesh) with the eluent as indicated in the procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 or 500 MHz. Chemical shifts are in ppm and coupling constants (*J*) are in Hertz (Hz). ^{1}H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using Chloroform-*d* or Methanol-d_4 as solvent, ^{13}C NMR spectra were referenced to solvent carbons (77.16 ppm for Chloroform-*d* or 49.86 ppm for Methanol-d_4). The splitting patterns for ^{1}H NMR spectra are denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and combinations thereof. MALDI-TOF mass spectra were recorded on a Bruker Ultraflex III TOF/TOF spectrometer.

2. Synthesis of Compounds



Scheme S1. Synthesis of M-PEG amines **12** and **13**

$HO(CH_2CH_2O)_7CH_3$

Heptaethylene glycol monomethyl ether (12a). Sodium hydride (NaH, 3.66 g, 60% in mineral oil, 91.5 mmol) was suspended in anhydrous tetrahydrofuran (THF, 50 mL) under a nitrogen atmosphere. Then, a solution of triethylene glycol monomethyl ether (10.0 g, 61.0 mmol) in anhydrous THF (50 mL) was added at 0 °C. After stirring for 0.5 h, a solution of macrocyclic sulfite (18.8 g, 73.2 mmol) in anhydrous THF (50 mL) was added and the resulting mixture was stirred overnight at 40 °C. Water (3.3 mL) was then added to the reaction, and the pH was adjusted to 3.0 with sulfuric acid, the resulting mixture was stirred at 60 °C. After the reaction was completed, the mixture was neutralized with saturated $NaHCO_3$ solution, and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with methanol/dichloromethane (MeOH/DCM, 1/20) to give heptaethylene glycol monomethyl ether **12a** as a clear oil (16.8 g, 81% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.75 – 3.41 (m, 28H), 3.31 (s, 3H).

$HO(CH_2CH_2O)_{11}CH_3$

Undecaethylene glycol monomethyl ether (12b) was prepared from heptaethylene glycol monomethyl ether (17.0 g, 49.4 mmol) as a clear oil (24.5 g, 96% yield) according to the synthetic procedure for heptaethylene glycol monomethyl ether. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.71 – 3.50 (m, 44H), 3.35 (d, J = 2.2 Hz, 3H).

$TsO(CH_2CH_2O)_{11}CH_3$

2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontan-34-yl 4-methylbenzenesulfonate (12c). To a solution of undecaethylene glycol monomethyl ether (14.8 g, 28.6 mmol) in THF (50 mL) was added a solution of NaOH (4.6 g, 114.2 mmol) in water (20 mL). After the mixture was cooled to 0 °C, a solution of *p*-toluenesulfonyl chloride (10.9 g, 57.1 mmol) in THF (40 mL) was slowly added, and the resulting mixture was stirred overnight at 40 °C. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with MeOH/DCM (1/20) to give compound **12c** as a clear oil (18.3 g, 96% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.70 (d, J = 8.0 Hz, 2H), 7.26 (d, J = 8.0 Hz, 2H), 3.61 – 3.43 (m, 44H), 3.28 (s, 3H), 2.36 (s, 3H).

$Me(OCH_2CH_2)_{11}N_3$

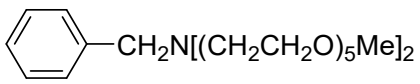
34-azido-2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontane (12d). To a solution of compound **12c** (18.0 g, 26.8 mmol) in N,N-dimethylformamide (DMF, 50 mL) was added sodium azide (2.3 g, 34.8 mmol), and the resulting mixture was stirred at 80 °C overnight. After the reaction was completed, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was washed with water and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel, eluting with MeOH/DCM (1/20) to give compound **12d** as a clear oil (13.5 g, 93% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.70 – 3.63 (m, 42H), 3.57 – 3.53 (m, 2H), 3.38 (s, 3H).

$Me(OCH_2CH_2)_{11}NH_2$

2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontan-34-amine (12). To a solution of compound **12d** (13.0 g, 24.0 mmol) in THF (100 mL) was added triphenylphosphine (9.4 g, 36.0 mmol), and the resulting mixture was stirred for 1 h at 40 ºC. Then H_2O (2.2 mL, 120.0 mmol) was added and the reaction mixture was stirred at 40 ºC overnight. After the reaction was completed, the reaction was concentrated under reduced pressure, the residue was purified by column chromatography on silica gel, eluting with MeOH/DCM (1/15) to give compound **12d** as a clear oil (10.9 g, 88% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.80 – 3.58 (m, 40H), 3.58 – 3.54 (m, 2H), 3.51 (t, *J* = 5.2 Hz, 2H), 3.38 (s, 3H).

$TsO(CH_2CH_2O)_5CH_3$

2,5,8,11,14-pentaoxahexadecan-16-yl 4-methylbenzenesulfonate (13a) was prepared from pentaethylene glycol monomethyl ether (10.0 g, 39.6 mmol) as a clear oil (14.5 g, 90% yield) according to the synthetic procedure for **12c**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.80 (d, *J* = 7.7 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 4.16 (t, *J* = 4.8 Hz, 2H), 3.75-3.47 (m, 18H), 3.38 (s, 3H), 2.45 (s, 3H).

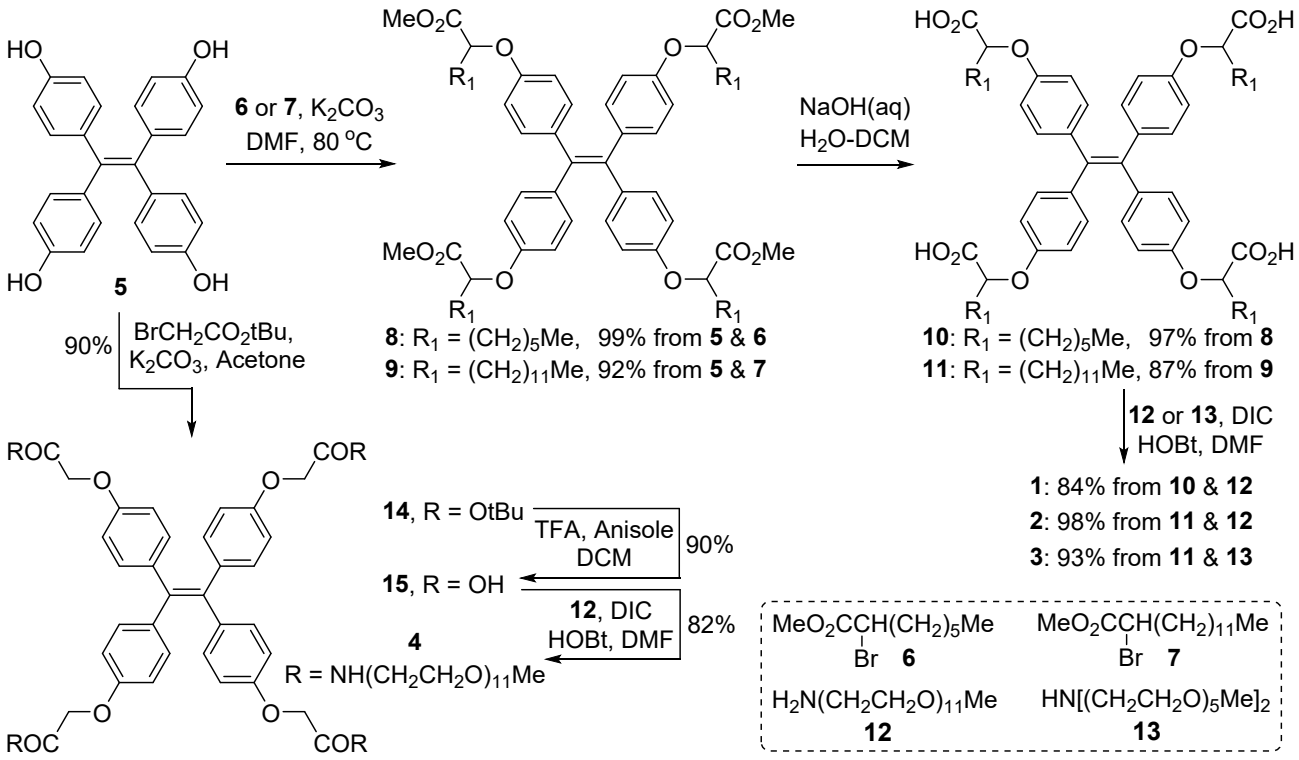


N-benzyl-N-(2,5,8,11,14-pentaoxahexadecan-16-yl)-2,5,8,11,14-pentaoxahexadecan-16-amine

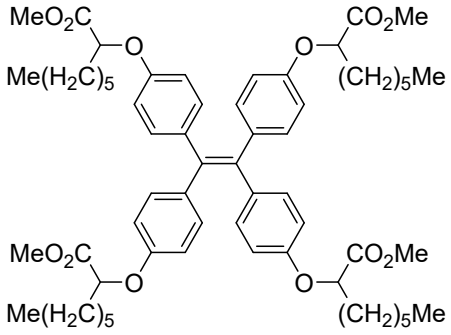
(13b). A suspension of K_2CO_3 (4.0 g, 28.8 mmol), KI (1.2 g, 7.2 mmol), benzylamine (0.8 g, 7.2 mmol), and 2,5,8,11,14-pentaoxahexadecan-16-yl 4-methylbenzenesulfonate (3.5 g, 8.6 mmol) in anhydrous acetonitrile (20 mL) was stirred at 80 ºC for 12 h. Then a solution of **13a** (3.5 g, 8.6 mmol) in anhydrous acetonitrile (20 mL) was added, and the resulting reaction mixture was stirred at 80 ºC for another 12 h. After the reaction was completed, th mixture was filtered through a pad of Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with MeOH/DCM (1/50) to give compound **13b** as a clear oil (3.5 g, 84% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.32 – 7.17 (m, 5H), 3.68 (s, 2H), 3.62 (d, *J* = 5.9 Hz, 20H), 3.59 – 3.57 (m, 4H), 3.56 – 3.50 (m, 12H), 3.35 (s, 6H), 2.72 (t, *J* = 6.3 Hz, 4H).

$[Me(OCH_2CH_2)_5]_2NH$

Di(2,5,8,11,14-pentaoxahexadecan-16-yl)amine (13). Under an atmosphere of H_2, a mixture of compound **13b** (1.0 g, 1.7 mmol) and Pd/C (10% on carbon, 0.20 g) in MeOH (10 mL) was stirred at 40 ºC overnight. After the reaction was completed, the mixture was filtrated through a pad of Celite, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel, eluting with MeOH/DCM (1/20) to give compound **13** as a clear oil (0.78 g, 95% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 3.70 – 3.43 (m, 36H), 3.35 (s, 6H), 2.81 (t, *J* = 5.3 Hz, 4H).

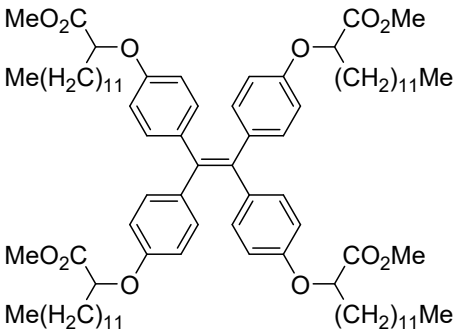


Scheme S2. Synthetic route of TPE lipids **1**-**3** and hydrophilic TPE **4**

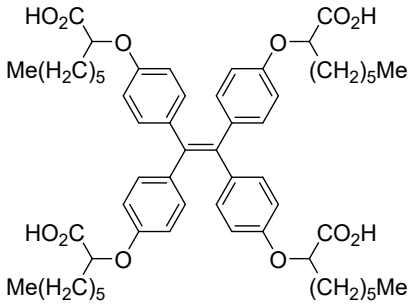


Tetramethyl 2,2',2'',2'''-((ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy)) tetraoctanoate (8). A suspension of K_2CO_3 (0.53 g, 3.83 mmol) and tetrakis(4-hydroxyphenyl)ethylene (0.25 g, 0.64 mmol) in anhydrous DMF (15 mL) was stirred at 80 ºC for 0.5 h. Then, a solution of compound **6** (0.91 g, 3.83 mmol) in anhydrous DMF (15 mL) was added, and the reaction mixture was further stirred at 80 ºC overnight. After the reaction was completed, water was added to quench the reaction. The aqueous layer was then extracted with ethyl acetate (EtOAc)

three times. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography on silica gel, eluting with EtOAc/petroleum ether (PE) (1/10) to give compound **8** as a yellow-green oil (0.65 g, 99% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.86 (d, *J* = 8.3 Hz, 8H), 6.56 (d, *J* = 8.4 Hz, 8H), 4.52 (t, *J* = 6.0 Hz, 4H), 3.72 (s, 12H), 1.97 – 1.83 (m, 8H), 1.52 – 1.38 (m, 8H), 1.35 – 1.26 (m, 24H), 0.88 (t, *J* = 7.5 Hz, 12H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.5, 156.2, 156.2, 138.5, 137.42, 137.39, 137.36, 132.6, 114.2, 114.1, 76.6, 52.2, 32.9, 31.6, 28.9, 25.1, 22.5, 14.0. HRMS (ESI) m/z: $[M+Na]^+$ calcd for $C_{62}H_{84}NaO_{12}^+$ 1043.5860; found 1043.5834.

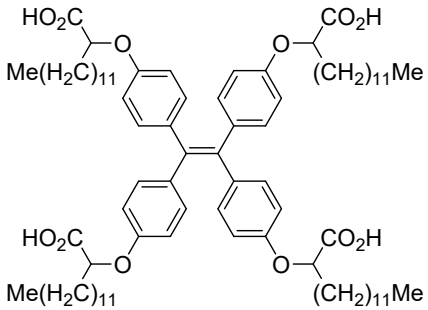


Tetramethyl 2,2',2'',2'''-((ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy)) tetratetradecanoate (9) was prepared as a yellow green oil (0.79 g, 92% yield) according to the synthetic procedure for compound **7**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 7.01 – 6.73 (m, 8H), 6.65 – 6.45 (m, 8H), 4.66 – 4.37 (m, 4H), 3.72 (s, 12H), 2.08 – 1.73 (m, 8H), 1.57-1.15 (m, 80H), 0.88 (t, *J* = 6.8 Hz, 12H). ^{13}C NMR (126 MHz, Chloroform-*d*) δ 172.5, 156.2, 156.2, 137.4, 132.6, 114.2, 114.1, 76.6, 52.1, 32.9, 31.9, 29.7, 29.65, 29.63, 29.5, 29.40, 29.36, 29.3, 25.2, 22.7, 14.1. HRMS (ESI) m/z: $[M+K]^+$ calcd for $C_{86}H_{132}KO_{12}^+$ 1395.9350; found 1395.9377.

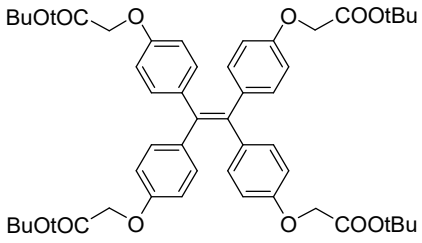


2,2',2'',2'''-((ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy))tetraoctanoic acid (10). To the solution of compound **8** (0.6 g, 0.59 mmol) in a mixed solvent (90 mL, DCM/MeOH =

9/1, v/v) was added NaOH (0.9 g, 23.0 mmol), the reaction mixture was stirred at room temperature for 4 h. After the reaction was completed, 5% HCl was added to adjust the pH to 3.0. The mixture was extracted with DCM, the combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated to give compound **10** as a yellow wax (0.55 g, 97% yield). ^{1}H NMR (500 MHz, Methanol-d_4) δ 6.89 (d, J = 8.2 Hz, 8H), 6.64 (d, J = 8.2 Hz, 8H), 4.58 (s, 4H), 1.90 (d, J = 7.5 Hz, 8H), 1.51 (d, J = 13.2 Hz, 8H), 1.34 (s, 24H), 0.93 (s, 12H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 174.3, 156.5, 138.6, 137.3, 114.0, 31.4, 28.6, 24.8, 22.2, 13.0. MS(MALDI-TOF) *m/z*:[M+Na]$^+$ calcd for $C_{58}H_{76}NaO_{12}^+$ 987.5229; found 987.5802.

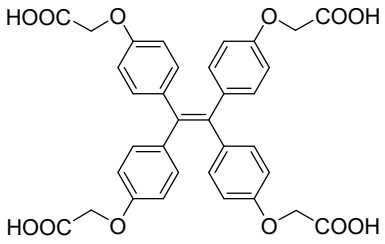


2,2',2'',2'''-((ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy))tetratetradecanoic acid (11) was prepared as a yellow wax (0.66 g, 87% yield) according to the synthetic procedure for compound **7**. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.33 (s, 3H), 6.95 – 6.82 (m, 8H), 6.77 (d, J = 8.3 Hz, 1H), 6.69 (d, J = 8.3 Hz, 2H), 6.62 (dd, J = 13.6, 8.3 Hz, 5H), 4.57 (dt, J = 20.5, 6.6 Hz, 4H), 2.04 – 1.81 (m, 9H), 1.66 – 1.44 (m, 9H), 1.41 – 1.05 (m, 86H), 0.88 (t, J = 6.8 Hz, 12H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 180.1, 158.6, 139.8, 138.3, 133.5, 115.4, 80.6, 34.6, 33.2, 31.0, 30.91, 30.89, 30.87, 30.8, 30.73, 30.71, 30.6, 27.0, 23.8, 14.6. HRMS (ESI) m/z: [M+K]$^+$ calcd for $C_{82}H_{124}KO_{12}^+$ 1339.8724; found 1339.8284.



Tetra-tert-butyl 2,2',2'',2'''-((ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy)) tetraacetate (14). To a suspension of K_2CO_3 (8.3 g, 60.0 mmol) and tetrakis(4-

hydroxyphenyl)ethylene (4.0 g, 10.0 mmol) in anhydrous acetone (15 mL) was added *tert*-Butyl bromoacetate (11.7 g, 60.0 mmol), and the reaction mixture was stirred under reflux overnight. After the reaction was completed, the solvent was removed under vacuum, and the residue was purified by flash column chromatography on silica gel, eluting with EtOAc/PE (1/5) to give compound **14** as a white wax (7.7 g, 90% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.97-6.75 (m, 8H), 6.67-6.50 (m, 8H), 4.43 (s, 8H), 1.46 (s, 36H).



2,2',2'',2'''-((ethene-1,1,2,2-tetrayltetrakis(benzene-4,1-diyl))tetrakis(oxy))tetraacetic acid (15). At room temperature, trifluoroacetic acid (36.4 g, 24.4 mL 318.8 mmol) and anisole (1.3 g, 1.3 mL, 12.0 mmol) were added to a solution of compound **14** (6.8 g, 7.97 mmol) in 100 mL of DCM and the resulting mixture was stirred for 6 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by recrystallization from acetone to give acid **15** as a white solid (4.5 g, 90% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.01-6.81 (m, 8H), 6.74-6.55 (m, 8H), 4.58 (s, 8H).

3. Log P Measurement

The *n*-octanol/water partition coefficients (logP) values of TPE lipids **1-3** and hydrophilic TPE **4** were measured following the shake-flask method.[1] The calibration curve for each compound in water saturated with octanol was plotted using UV–Visible spectrophotometry (Shimadzu UV-2600). Compounds were weighed and dissolved in 4 mL of water and octanol (50/50, v/v), the phases were shaken and left for separation for 24 h. A solution of 0.6 mL of the water phase was transferred and analyzed using the calibration curve method. The log P values were determined by the following equation: logP = lg[(Cs-Cw)/Cw], where Cs and Cw are the concentrations of the starting water solution and the water phase of the compound, respectively.

4. Turbidity Test

The turbidity test was performed using a UV-Visible spectrometer (Perkin Elmer, Lambda 35) at 700

nm. The transmittance was measured between 33 °C and 80 °C through temperature-controlled heating and cooling cycles and the sample was equilibrated for 10 min before the measurement.

5. Relative Fluorescence Quantum Yields Determination

Quinine sulphate in 0.1 M $HClO_4$ was used as the reference solution (QY_R = 0.60 ± 0.02).[4, 5] The quinine sulfate reference solution was prepared at concentrations of 32 μM, 24 μM, 16 μM, and 8 μM. Similarly, TPE **1**-**4** were dissolved in 0.1 M $HClO_4$ to obtain a series of sample solutions at concentrations of 48 μM, 36 μM, 24 μM, and 12 μM, respectively. The UV absorbance at a wavelength of 350 nm of each reference and sample solution was measured using a UV-Visible spectrometer (Thermo Fisher, Evolution 220). Fluorescence emission spectra of each reference and sample solution at an excitation wavelength of 350 nm was measured using a fluorescence spectrometer (HORIBA, Fluoromax-4) with a slit width of 2.5 nm, and the integrated area of the fluorescence emission spectra in the wavelength range of 360 nm to 650 nm was analyzed. The relative quantum yield (QY) can be calculated from the following equation:

$$QY_S = QY_R\left(\frac{I_S}{I_R}\right)\left(\frac{1-10^{-A_R}}{1-10^{-A_S}}\right)\left(\frac{n_S}{n_R}\right)^2$$

where QY_S and QY_R are the photoluminescence QY of the sample and that of quinine sulphate, respectively; I is the integrated emission areas (excitation wavelength: 350 nm); A is the UV absorbance at 350 nm; n is the refractive index of the medium, and the subscripts S and R refer to the measured sample and quinine sulphate, respectively.

6. DLS Measurement and TEM

Dynamic light scattering (DLS) measurement was performed to determine the average hydrodynamic size and the zeta potential of nanoparticles at different concentrations using Malvern Zetasizer (Malvern, Nano ZS 90, UK). Data were given as mean ± standard deviation (SD) based on three independent measurements.

Transmission electron microscopy (TEM, JEM-2100, JEOL) was used to observe the morphology of TPE **1**-**4** of different concentrations (5, 100, and 1000 μM). Samples were prepared by dropping 3 μL

of the solution on 230 mesh carbon support films (copper mesh), and imaged without external staining. Nanoparticles **NP4** was stained with 1% phosphotungstic acid solution for 30 s before taking images.

7. Molecular Dynamics Simulations

The initial structures of TPE molecules were built and optimized by Gaussview.[6] The partial charges of the TPE molecules were fitted to the electrostatic potentials through the restrained electrostatic potential (RESP) method[7] based on the antechamber module.[8] The atom types, bonded interaction parameters, and Van der Waals interaction parameters were defined by the lipids Generalized Amber Force Field (GAFF).[9] For TPEs **1**-**4**, the whole molecules were divided into smaller fragments to calculate the electrostatic interaction parameters. Then the TPE molecules were hydrated in the water box with the minimum distance of atoms on TPE to the border of the box larger than 1.0 nm. The steepest decent method was performed to minimize the system until the root-mean-square of the energy gradient is less than 0.0001 kcal/mol·Å or the maximum iteration steps reached 10,000. The system then was heated to 300 K linearly in the periods of 100 ps in the NVT ensemble with the weak harmonic potential (10 kcal/mol·Å) on the heavy atoms. Subsequently, a 1-ns unrestrained equilibration with Langevin thermostat in the NPT ensemble was performed. The bonds involving hydrogen were constrained with the SHAKE algorithm. The same simulation parameters of NPT equilibration were used in the 200-ns production runs carried out by CUDA-version Amber18.[10]

The initial models of TPEs **1**-**4** in the triglyceride-water interface were built by the Packmol program.[11] 200 triglyceride molecules were put together to be the organic phase and 5000 water molecules composing the aqueous phase. TPEs **1**-**4** were initially put in the aqueous phase. The MD simulation implementation parameters and procedures of TPEs in the triglyceride-water interface are like those in the pure water environments. Finally, 0.5-μs production runs were performed for the TPEs **1**-**4** in the triglyceride water, and the last 200-ns trajectories were utilized in the following analysis.

8. Cytocompatibility and Cytotoxicity Assay

The cell cytotoxicity was evaluated using the Cell Counting Kit-8 (CCK-8) assay. A549 cells, MCF-10A cells, and MCF-7 cells were seeded in 96-well plates at a density of 1×10^4 cells per well, and incubated in 0.1 mL of DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-

streptomycin solution at 37 °C in a humidified 5% CO_2 atmosphere for 24 h, respectively. For each cytotoxicity assay, a range of TPEs **1**-**4** concentrations (100, 80, 60, 40, 20, 10, 5, and 1 μM) were tested. After removing the culture medium, 0.1 mL of the samples in fresh culture medium were added to the well and incubated in dark for 24 h, respectively. Then, the medium was removed, and the cells were washed with phosphate buffered saline (PBS, pH 7.4), and 0.1 mL of fresh medium was added to the wells. Next, 0.1 mL of CCK-8 was added to each well. After incubation for 1.5 h, the absorbance (A) was measured at 450 nm using a microplate reader (BIO-RAD 550). The relative cell viability was calculated as follows:

$$\text{Cell Viability (\%)} = [(A_{sample} - A_{blank})/ (A_{control} - A_{blank})] \times 100$$

Where A_{sample}, $A_{control}$, and A_{blank} represented the absorbance of the cells treated with samples, the cells without treatment, and the PBS solution, respectively. Data were given as mean ± standard deviation (SD) based on three independent measurements.

9. Cellular uptake

MCF-7 cells were seeded in confocal dishs at a density of 1×10^5 cells per dish, and incubated in 1.0 mL of DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution at 37 °C in a humidified 5% CO_2 atmosphere for 24 h. Then, the medium was replaced with 1.0 mL of fresh culture medium containing TPEs **1**-**4** (20 μM), respectively. After 24 h of incubation, the cells were washed with cold PBS (pH 7.4) three times. After stained with Dil, the cells were washed with cold PBS and observed using a laser scanning confocal microscope (A1R/A1, Nikon).

10. Hemolysis Test

Red blood cells (RBCs) were extracted from the BALB/c mice blood sample by removing white blood cells and platelets through centrifugation (4500 rpm, 3.5 min). The remained RBCs were washed in PBS five times. The samples were dissolved in PBS and added to the suspension of RBCs (2 % final in v/v in PBS) at a concentration range from 5 to 160 μM and incubated at 37 °C for 3 h. The hemoglobin release was evaluated by measuring the absorbance (A) of the samples at 540 nm. For negative and positive controls, PBS and water were used, respectively. The percentage of hemolysis was calculated according to the equation.

Percentage of hemolysis (%) = $[(A_{sample} - A_{blank})/ (A_{water} - A_{blank})] \times 100$.

11. Encapsulation Efficiency and Drug Loading Content

Encapsulation efficiency (EE%) and drug loading content (DLC%) of nanoparticles were determined using high performance liquid chromatography (HPLC, Shimadzu LC-20A, an Amethyst C18-H reversed-phase column was used, particle size 5.0 μm, column dimension 4.6 × 250 mm). Briefly, 200 μL of nanoparticles was diluted with 800 μL of MeOH, the mixture was sonicated for 20 minutes, centrifuged at 12,000 rpm for 20 minutes, and the tamoxifen content in the supernatant was determined by HPLC as the total tamoxifen content in nanoemulsions (Wt). Another 200 μL of nanoparticles was diluted with H_2O, the sample was transferred to an ultrafiltration centrifuge tube, and centrifuged at 12,000 rpm for 20 min. MeOH was added to dissolve the trapped nanoparticles, the content of tamoxifen in the MeOH solution was determined by HPLC as the encapsulated tamoxifen content in nanoparticles (Wo). EE% and DLC% were calculated by equations (1) and (2), respectively. Where Wd is the weight of the nanoparticles.

$EE\% = Wo/Wt \times 100\%$ (1)

$DLC\% = Wo/Wd \times 100\%$ (2).

12. Statistical Analysis

The analyzed data are presented as mean ± standard deviation of n = 3 replicates.

13. Supplementary Figures and Tables

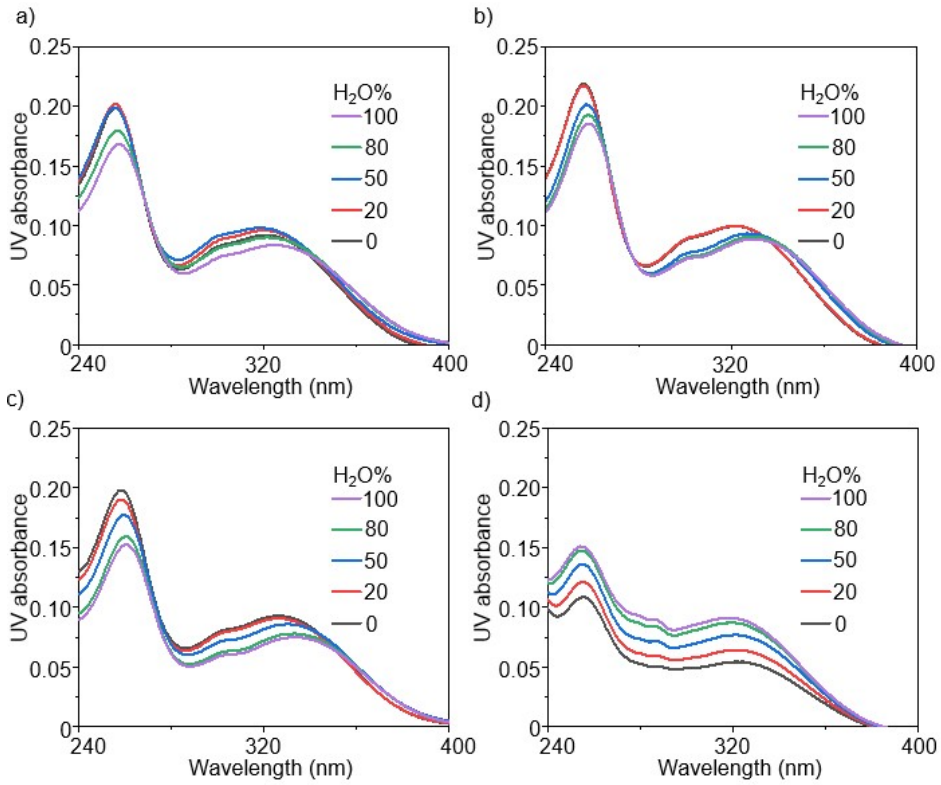


Figure S1 UV absorption spectra of TPE lipid **1** (a), **2** (b), **3** (c), and TPE **4** (d) (solvent changed from methanol to water).

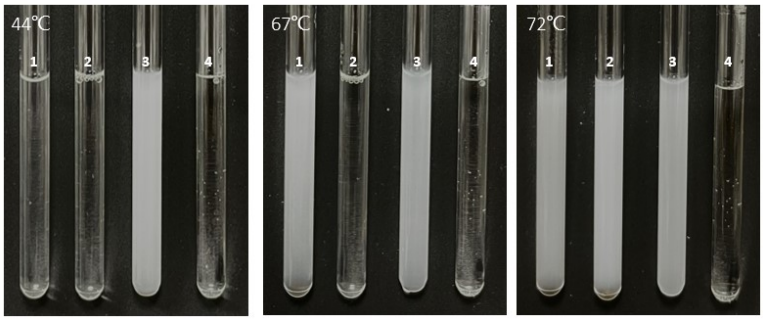


Figure S2 Images of TPE lipids **1**-**3** and hydrophilic TPE **4** solutions (1.0 mM) under the indicated temperatures.

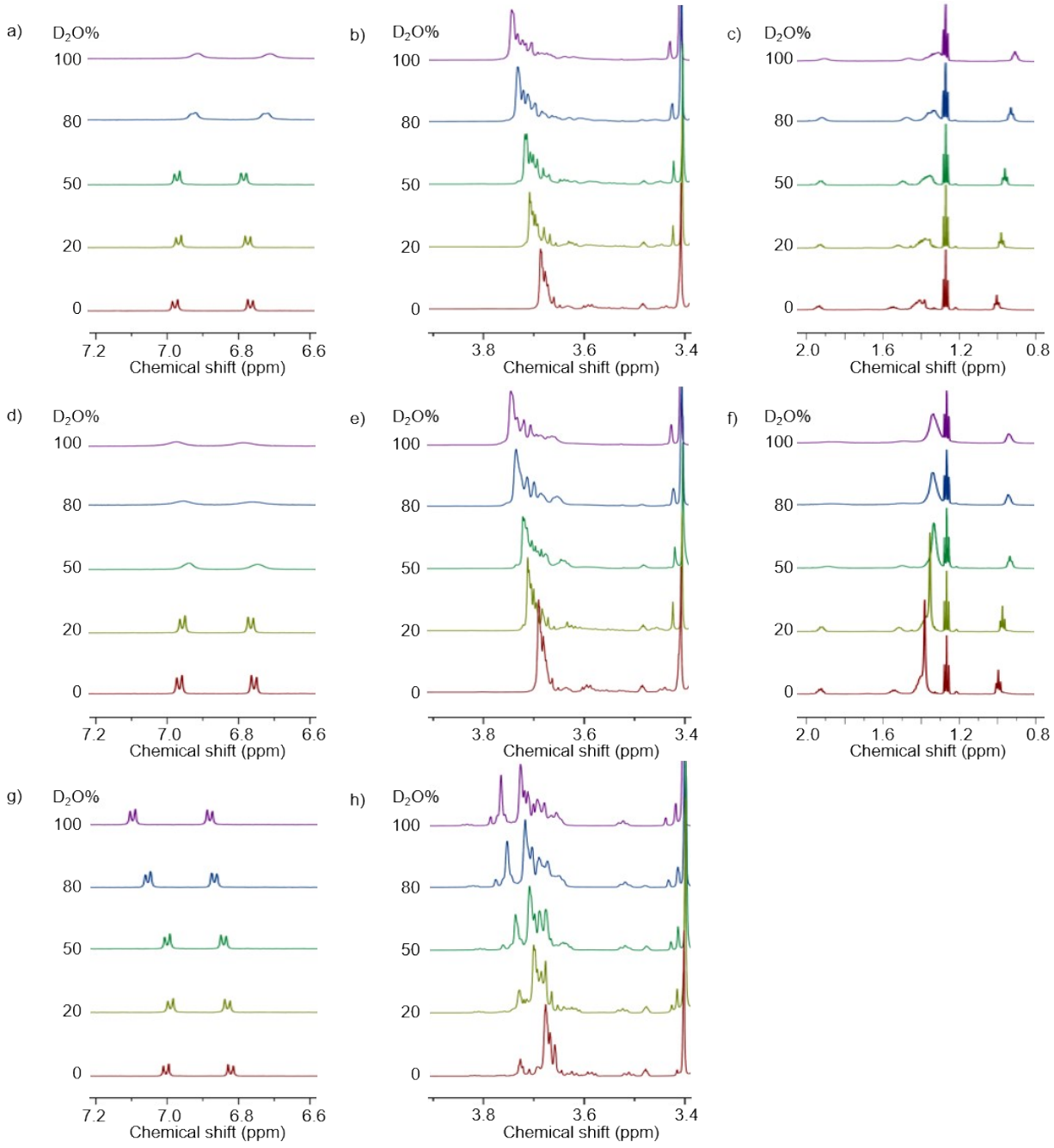


Figure S3 The partial ^{1}H NMR spectra of TPE lipid **1** (a-c), **2** (d-f) and TPE **4** (g-h) (a, d, g: aromatic protons, b, e, h: M-PEG protons, c, f: alkyl protons, when the solvent was changed from deuterated methanol to deuterium oxide at 50 μM).

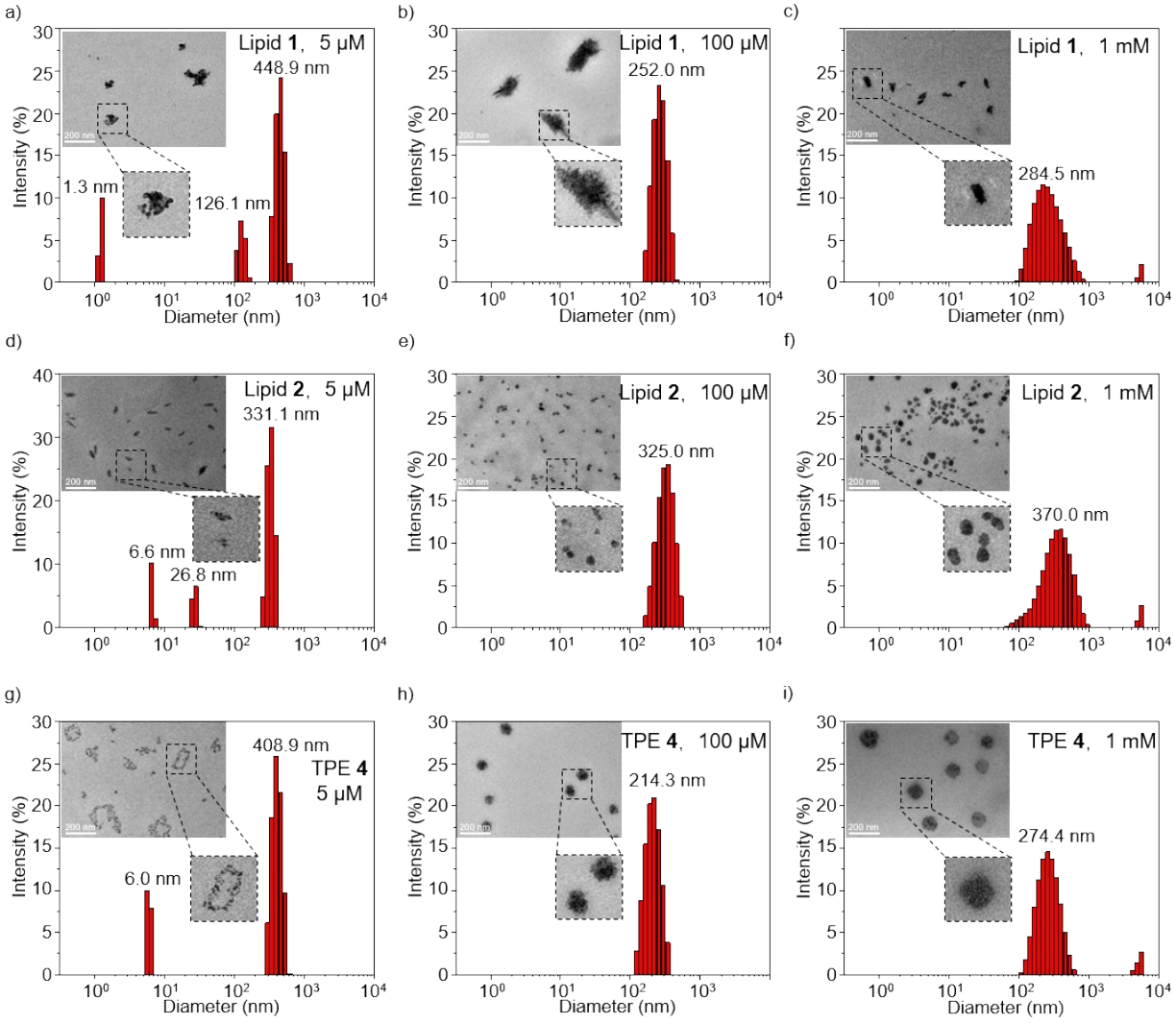


Figure S4 DLS with inset TEM images of lipid **1** (a-c), **2** (d-f) and TPE **4** (g-i) (a, d, g: 5 μM, b, e, h: 100 μM, c, f, i: 1000 μM).

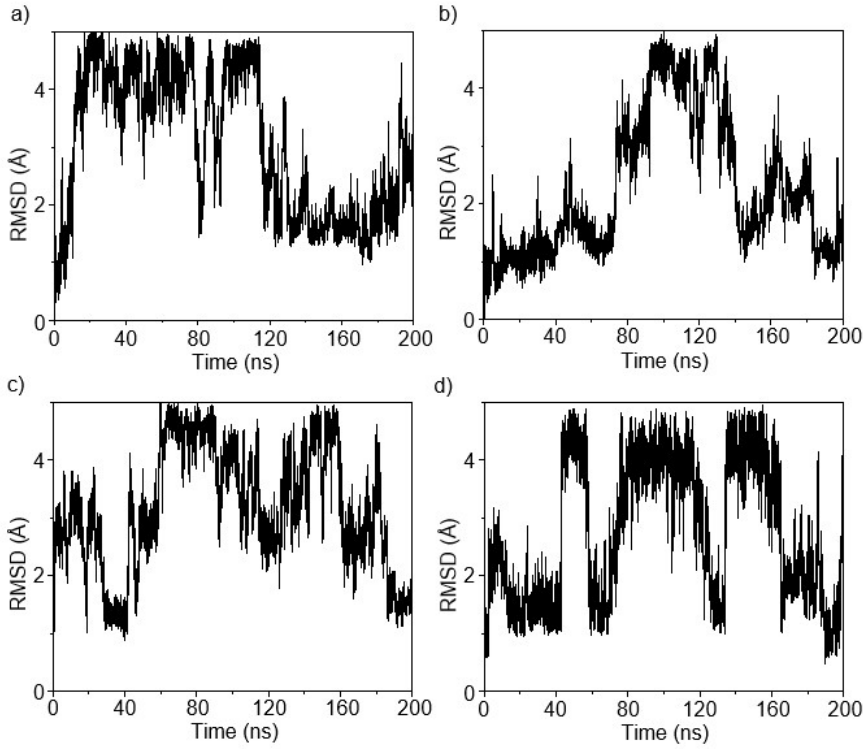


Figure S5 The molecular dynamic simulations of the aromatic groups' RMSD in TPE lipid **1** (a), **2** (b), **3** (c), and TPE **4** (d).

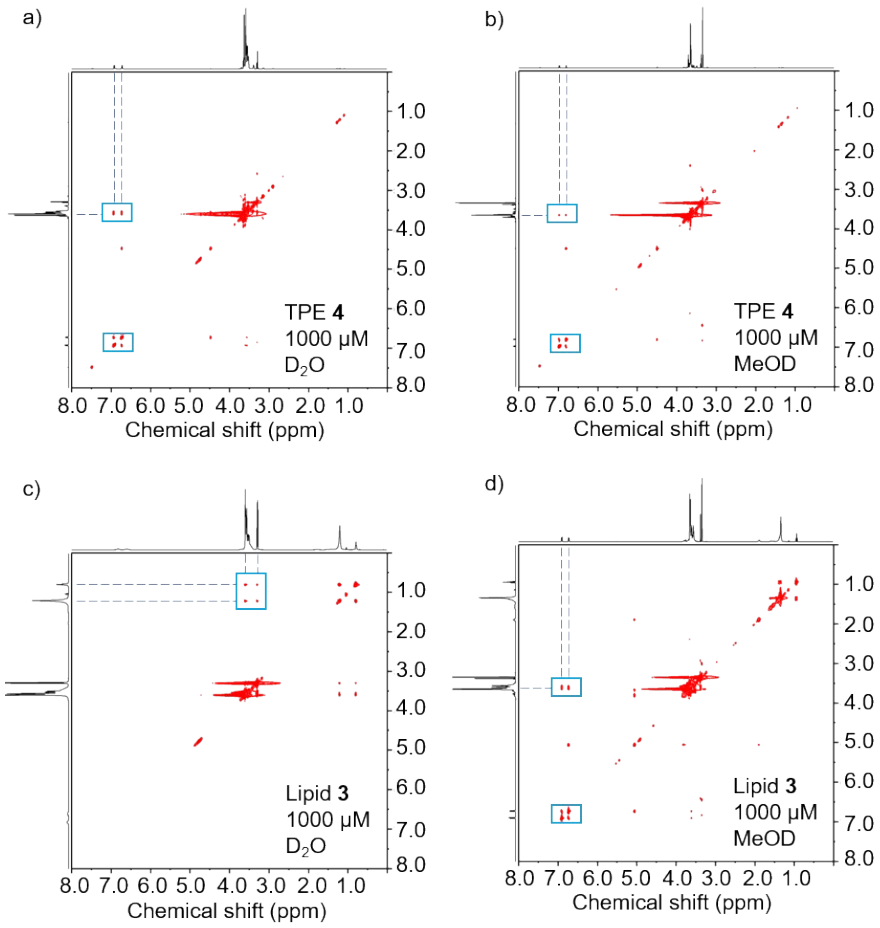


Figure S6 2D ^{1}H-^{1}H ROESY spectra of TPE **4** at 1000 μM in D_2O (a), and in MeOD (b). 2D ^{1}H-^{1}H ROESY spectra of lipid **3** at 1000 μM in D_2O (c), and in MeOD (d).

Table S1 IC_{50}[a] values of TPEs against different cell lines.

Compound	Cell lines [IC_{50}, μM]		
	A549	MCF-7	MCF-10A
1	19.10±0.74	35.18±3.11	19.12±0.87
2	42.66±6.24	203.03±16.62	403.57±10.87
3	ND[b]	ND	ND
4	ND	ND	ND

[a]IC_{50} value, defined as the concentrations corresponding to 50% growth inhibition for 24 h by the CCK-8 assay which was calculated from three independent experiments.

[b]ND, not determined.

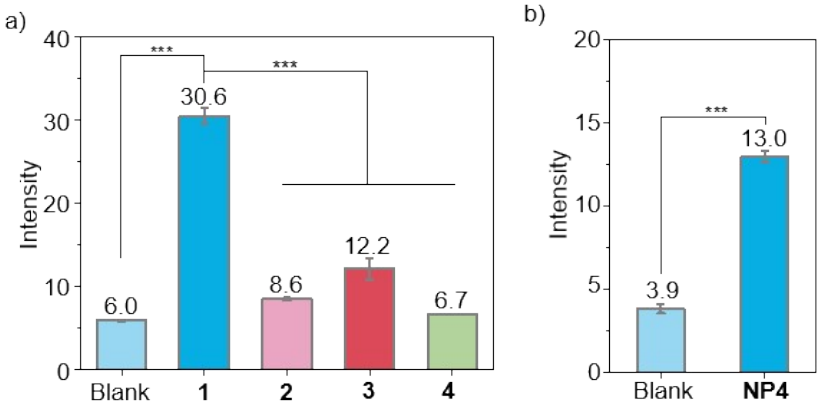


Figure S7 Quantification of the mean fluorescence intensity signal of MCF-7 cells uptake of lipids **1**-**3**, TPE **4** (a), and **NP4** (b), Statistical significance: ***p < 0.001.

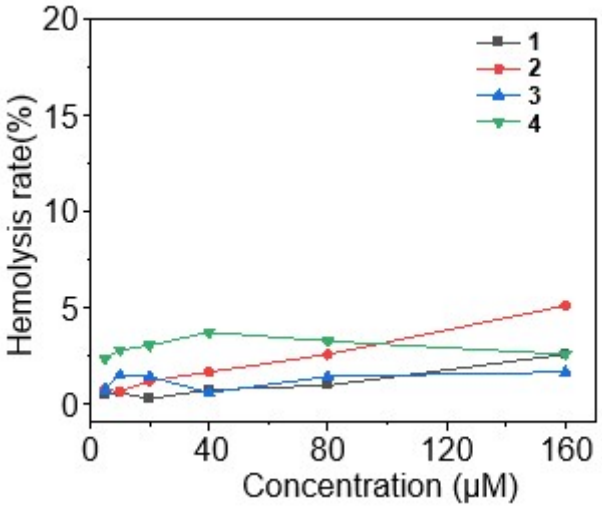


Figure S8 Percentage of hemolysis of RBCs incubated with TPE lipids **1**-**3** and hydrophilic TPE **4**.

Table S2 The ingredients, particle size, and PDI of the nanoparticles **NP1-1**, **NP1-2**, and **NP1**.

lipid:MCT oil:TAM=3:1:1	**Size (PDI)**
NP1-1	498.4 nm (0.76)
NP1-2	349.9 nm (0.445)
NP1	153.0 nm (0.24)

Table S3 The ingredients, particle size, and PDI of the nanoparticles.

lipid:S75:TAM=1:5:0.2	**Size (PDI)**
lipid3:S75:TAM	152.8 nm (0.106)
DSPE-PEG_{2000}:S75:TAM	203.9 nm (0.435)
HSPC:S75:TAM	369.8 nm (0.618)

Table S4 The ingredients, particle size, PDI, drug loading content (DLC%), and encapsulation efficiency (EE%) of nanoparticles.

TPE3:S75:TAM	**Size (PDI)**	**DLC%**	**EE%**
1:5:0.2	152.8 nm (0.106)	3.0	96
1:5:0.4	155.0 nm (0.247)	4.9	96
1:5:0.6	156.3 nm (0.201)	6.4	89
1:5:0.8	208.4 nm (0.387)	5.9	60
1:5:1	739.5 nm (0.925)	---	---

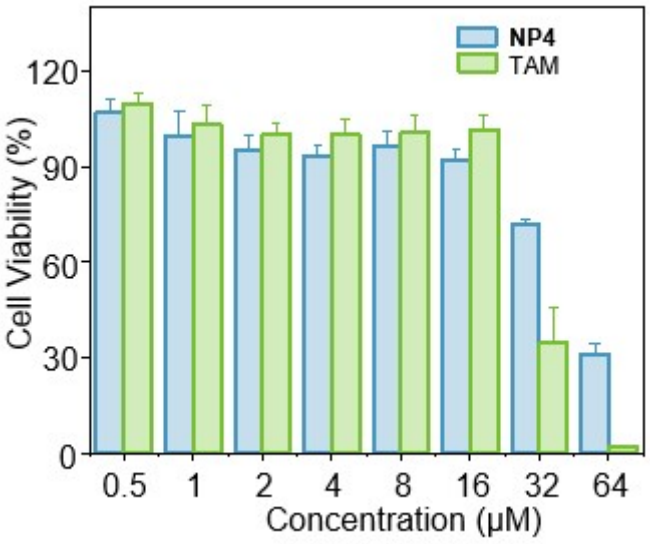


Figure S9 Cytotoxicity assay in MCF-10A of nanoparticles **NP4** and tamoxifen (TAM).

Table S5 IC_{50}[a] values of tamoxifen and **NP4** against different cell lines.

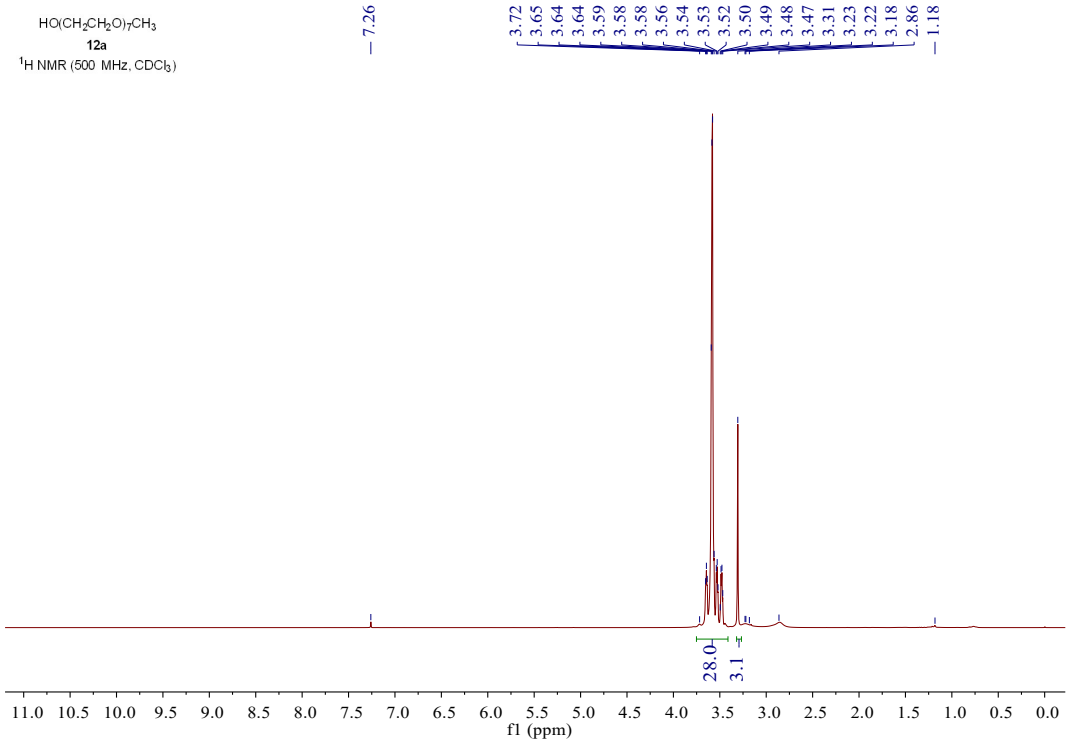
Compound	Cell lines [IC_{50}, μM]		
	MCF-7	MCF-10A	SI[b]
Tamoxifen	20.16±1.37	29.47±2.11	1.46
NP4	10.01±0.24	46.25±2.70	4.62

[a]IC_{50} value, defined as the concentrations corresponding to 50% growth inhibition for 24 h by the CCK-8 assay which was calculated from three independent experiments.

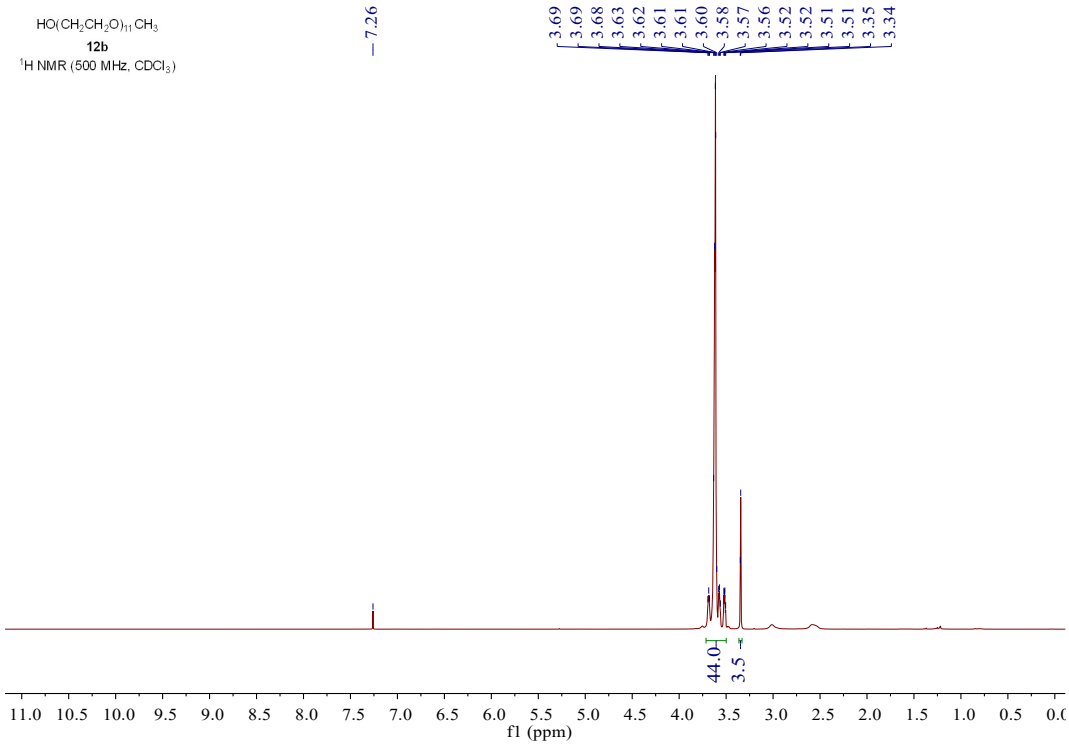
[b]SI, selective index, IC_{50} (MCF-10A)/IC_{50} (MCF-7).

14. Copies of $^{1}H/^{13}C$ NMR and MS spectra of compounds.

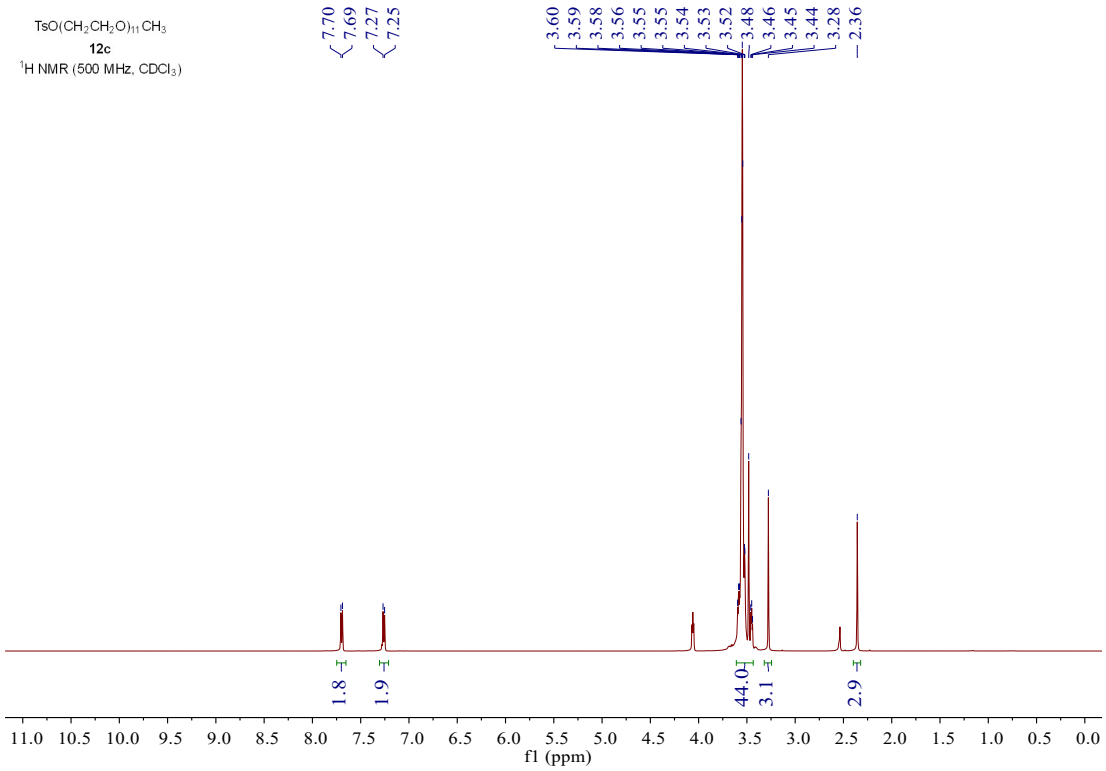
^{1}H NMR of compound **12a**



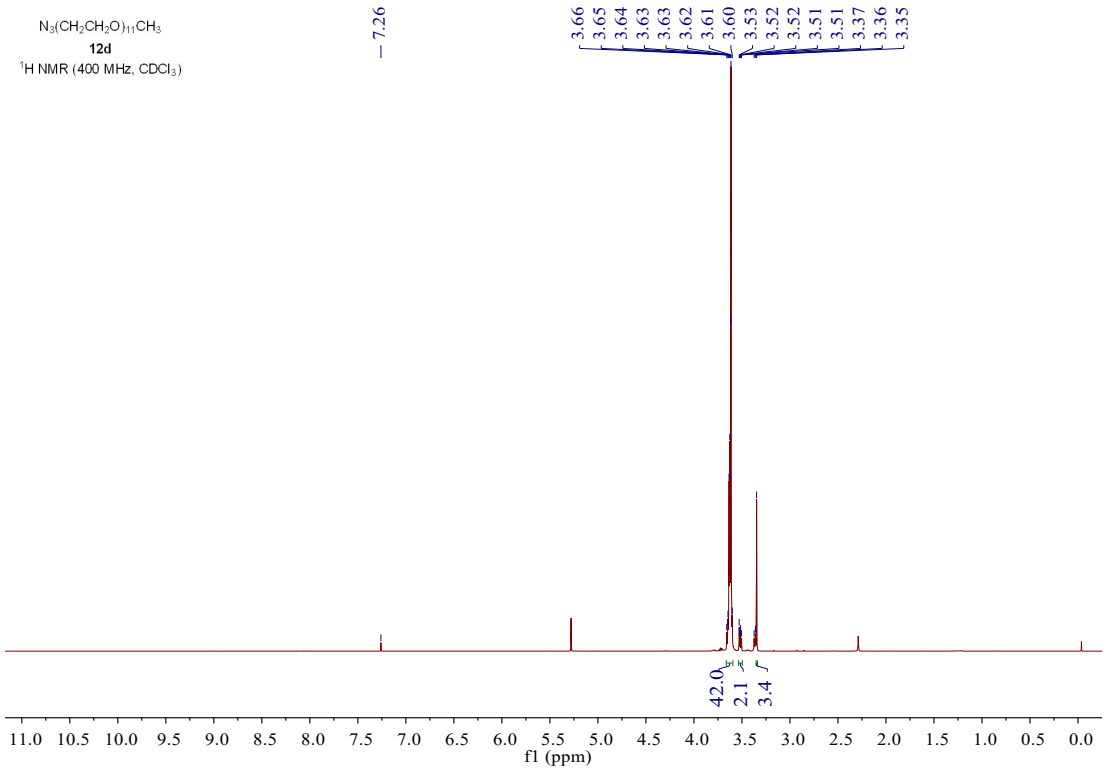
^{1}H NMR of compound **12b**



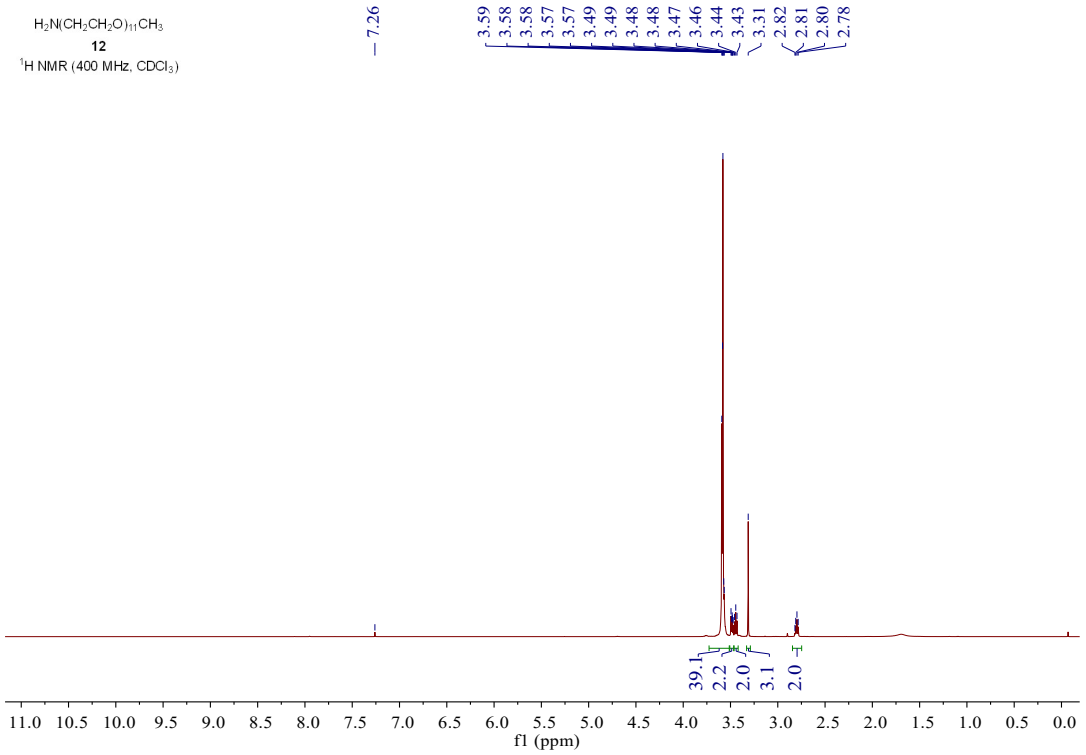
^{1}H NMR of compound **12c**



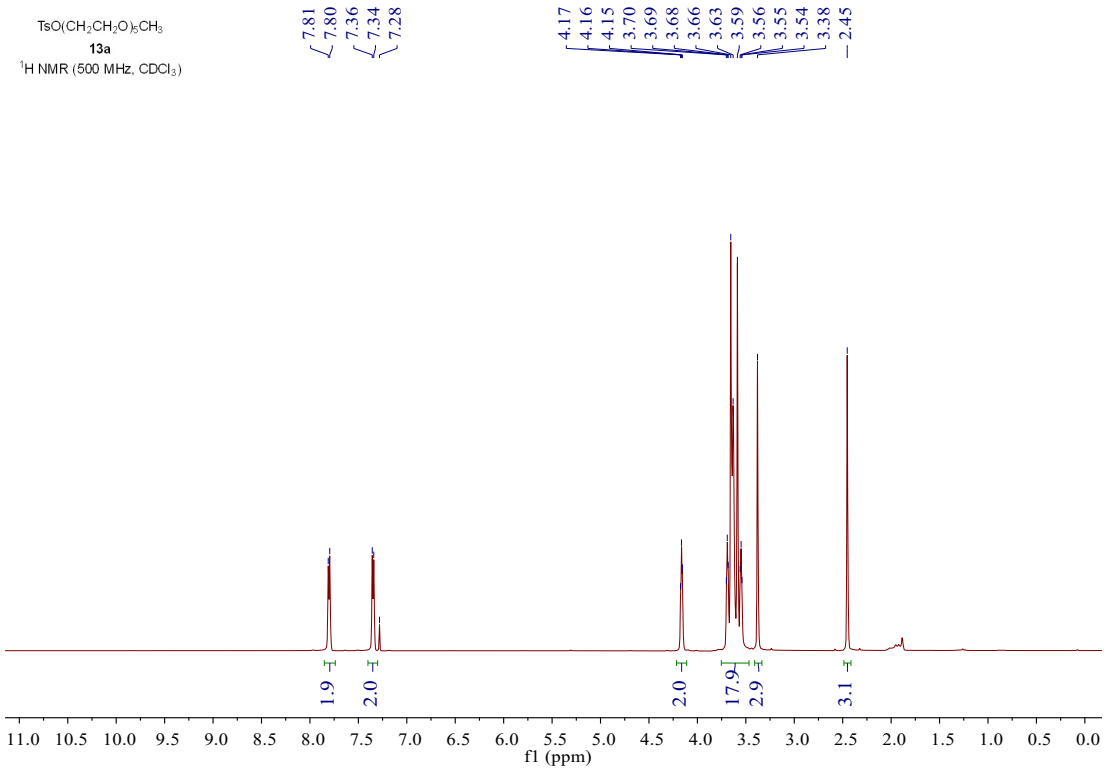
^{1}H NMR of compound **12d**



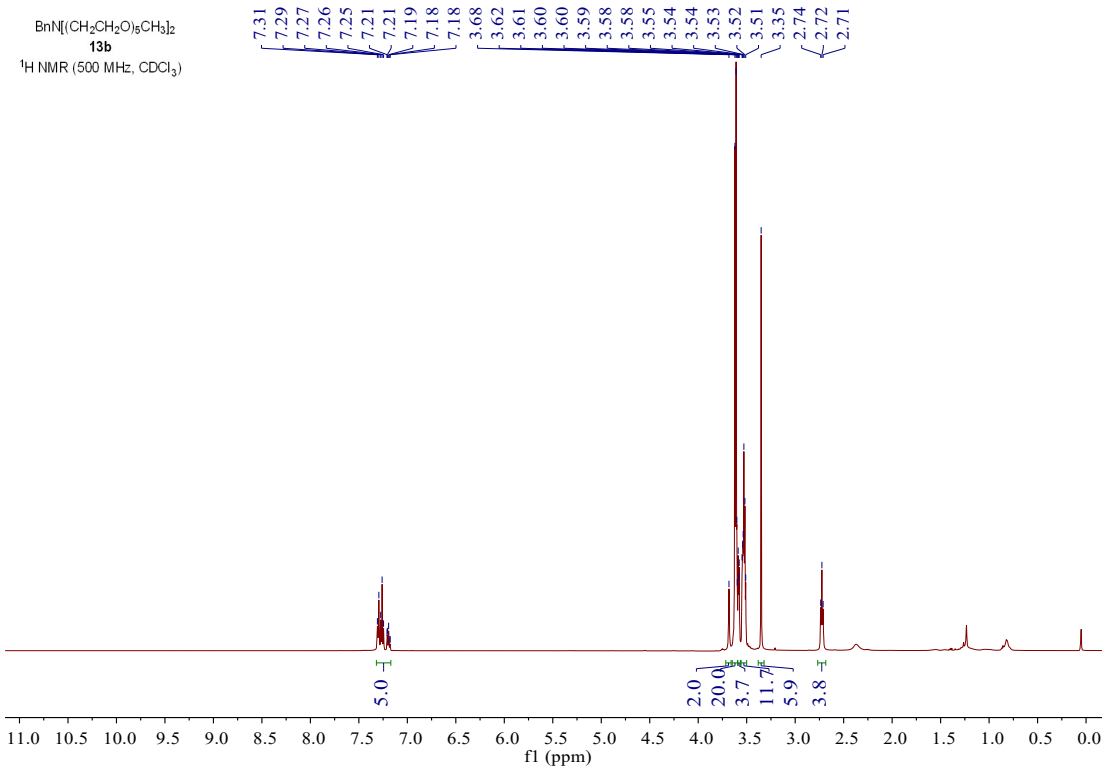
^{1}H NMR of compound **12**



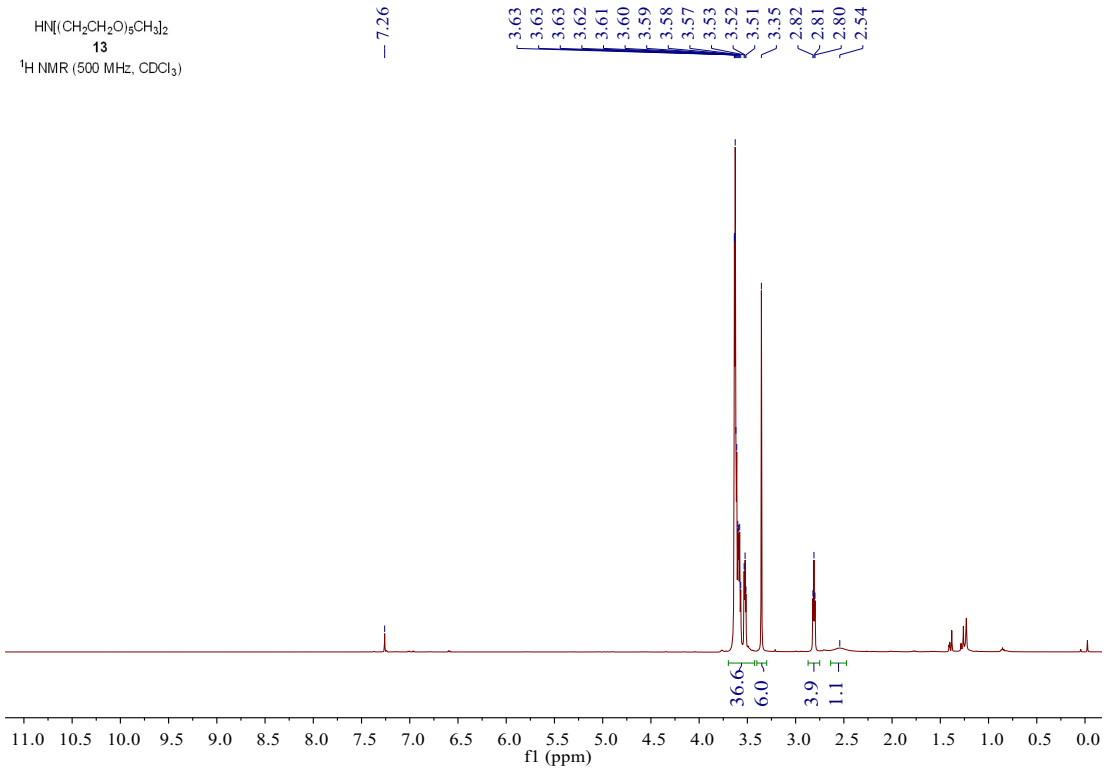
^{1}H NMR of compound **13a**



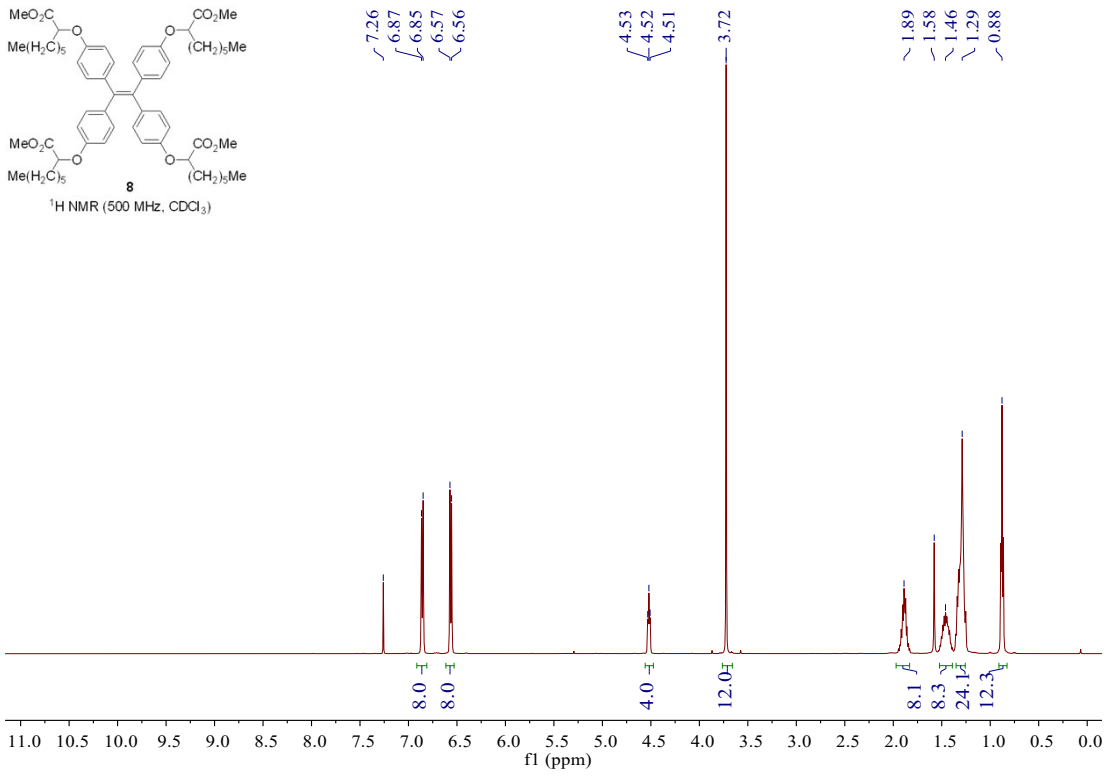
^{1}H NMR of compound **13b**



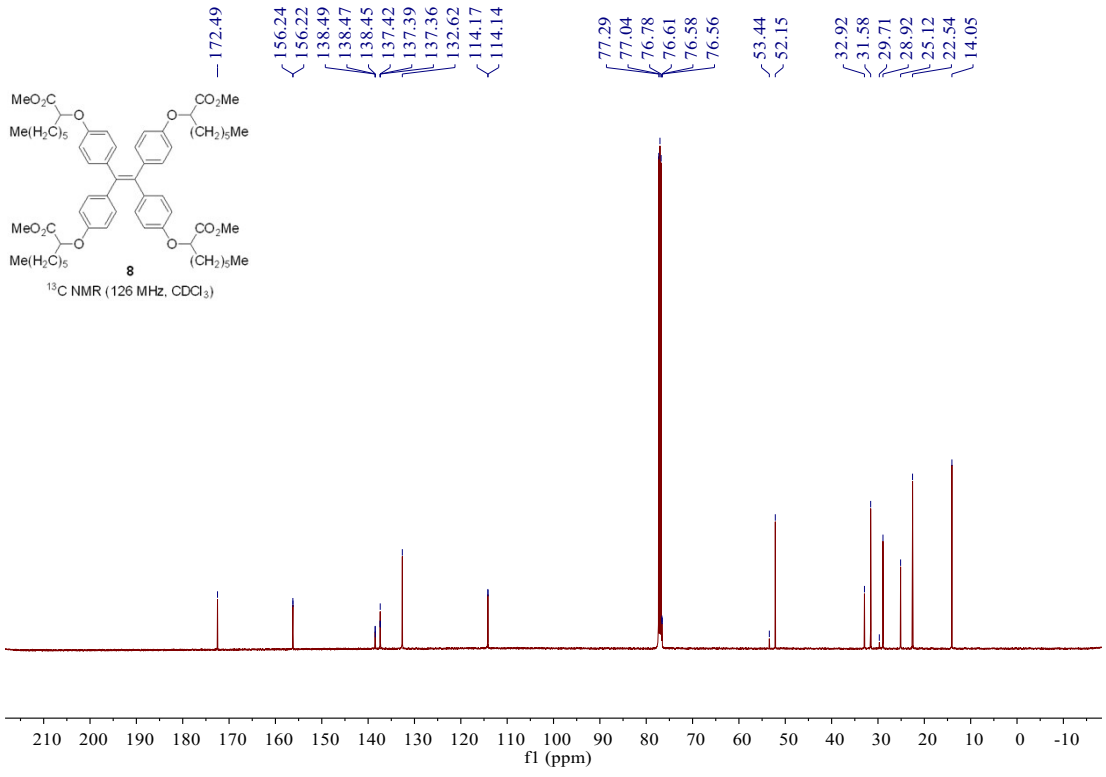
^{1}H NMR of compound **13**



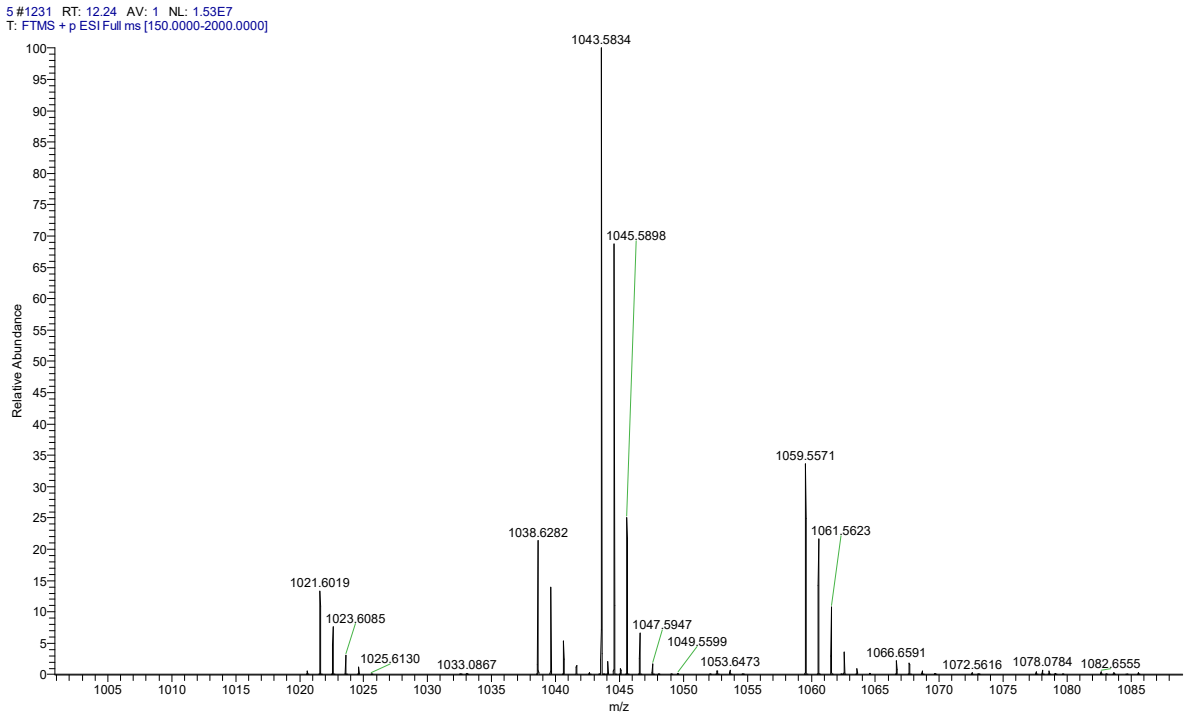
^{1}H NMR of compound **8**



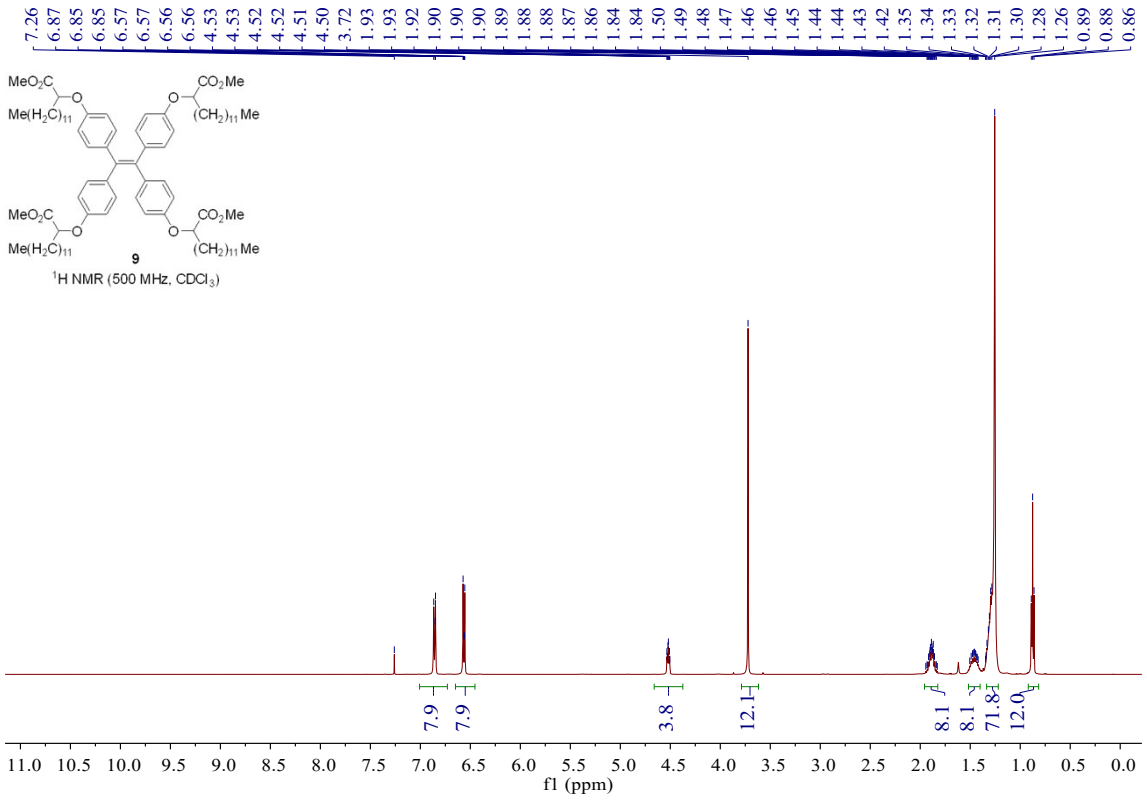
^{13}C NMR of compound **8**



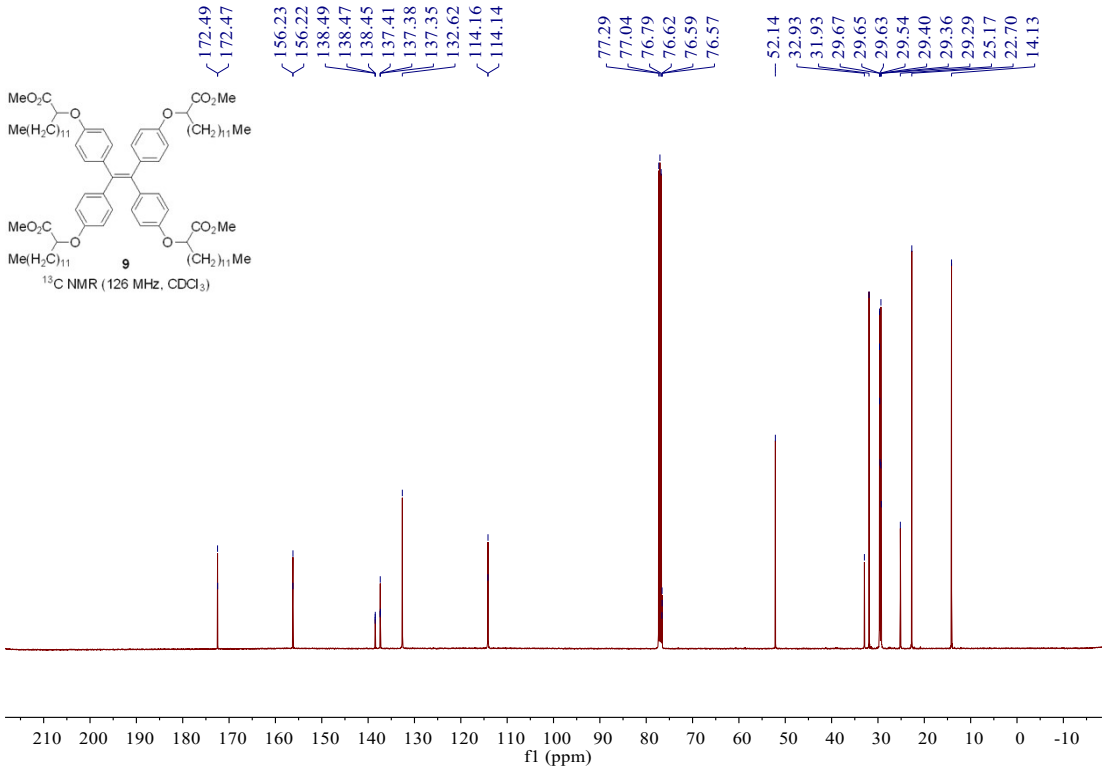
HRMS(ESI) of compound **8**



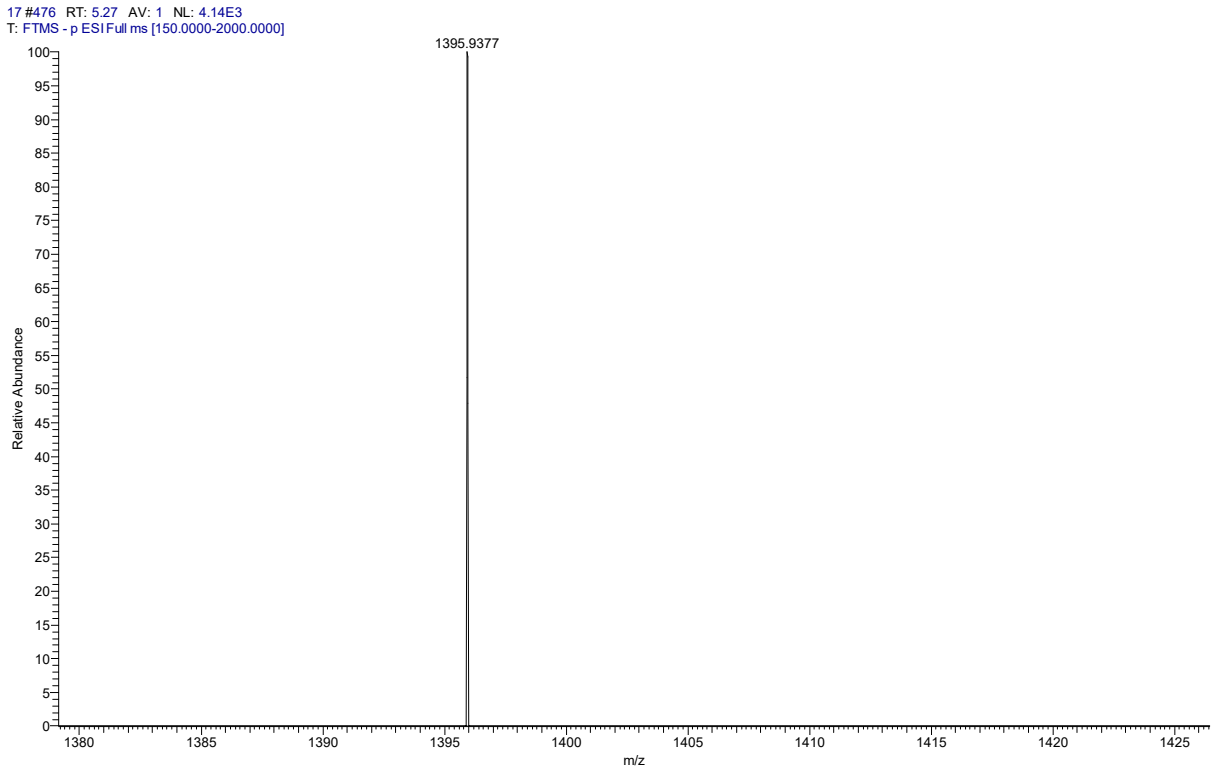
^{1}H NMR of compound **9**



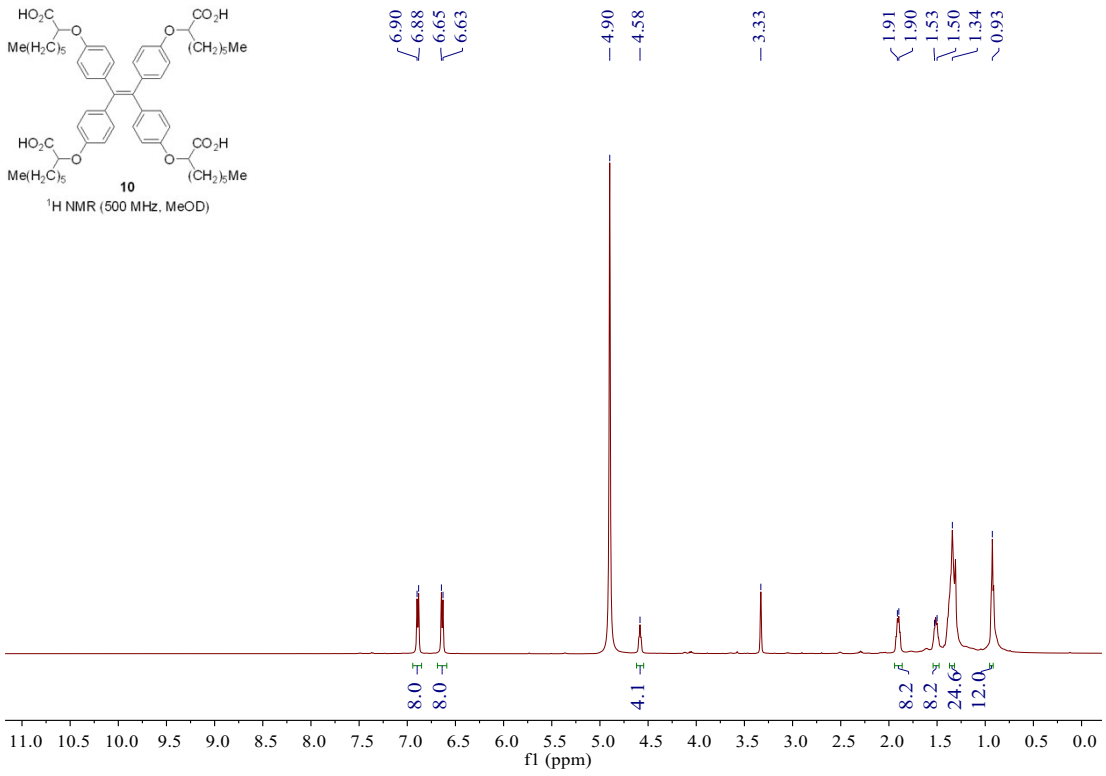
^{13}C NMR of compound **9**



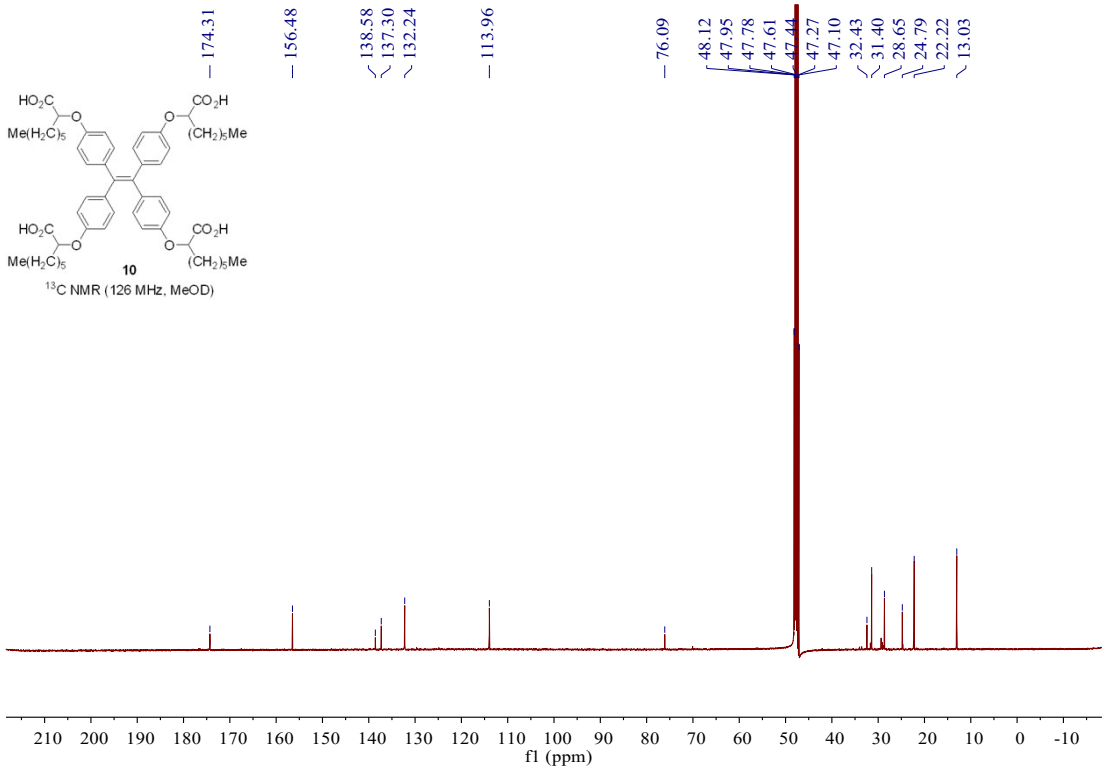
HRMS(ESI) of compound **9**



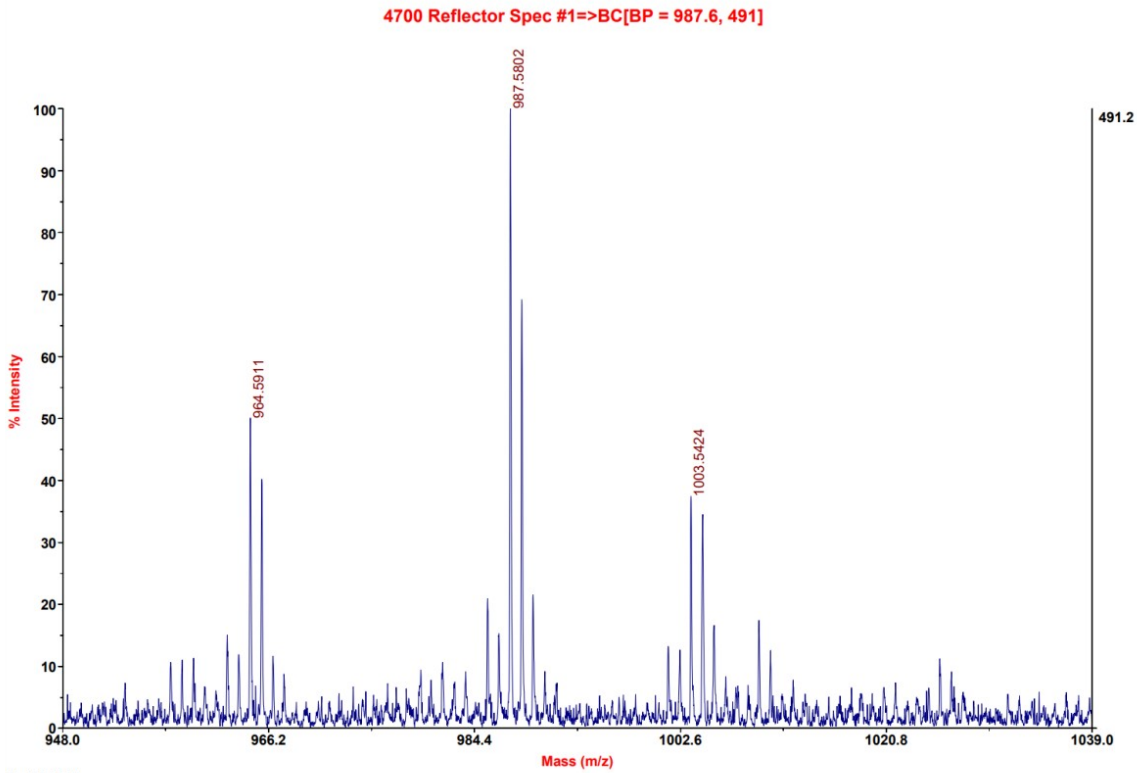
^{1}H NMR of compound **10**



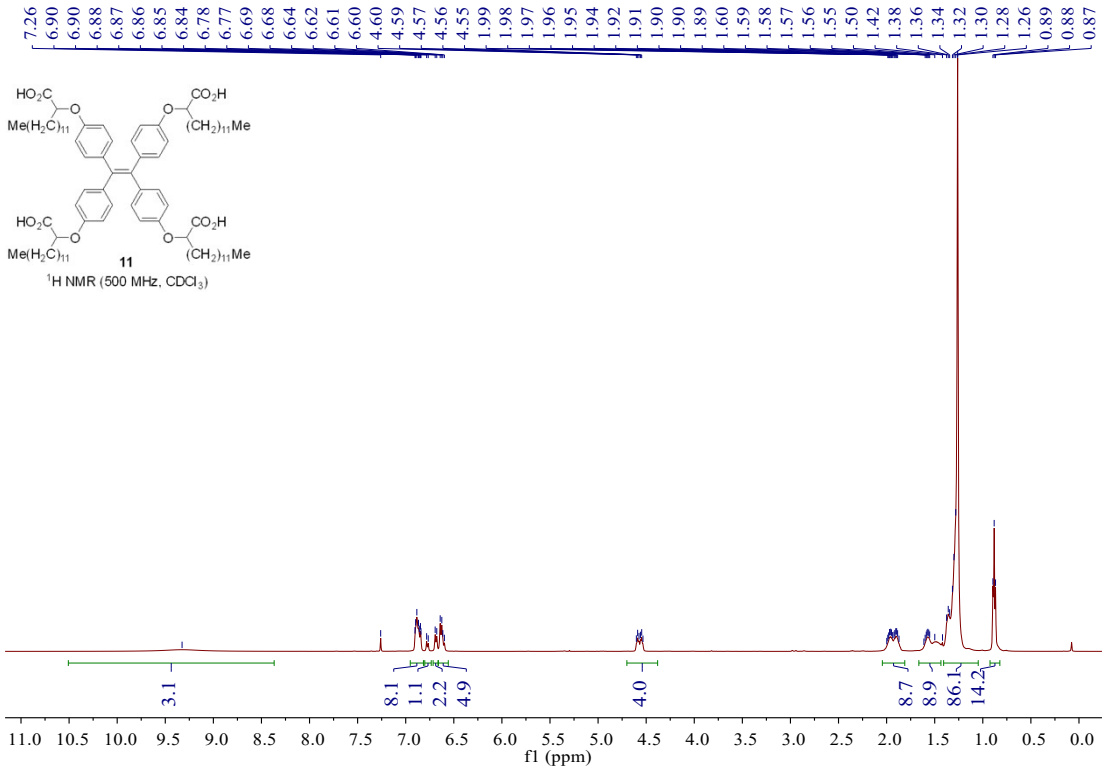
^{13}C NMR of compound **10**



MS(MAIDI-TOF) of compound **10**



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



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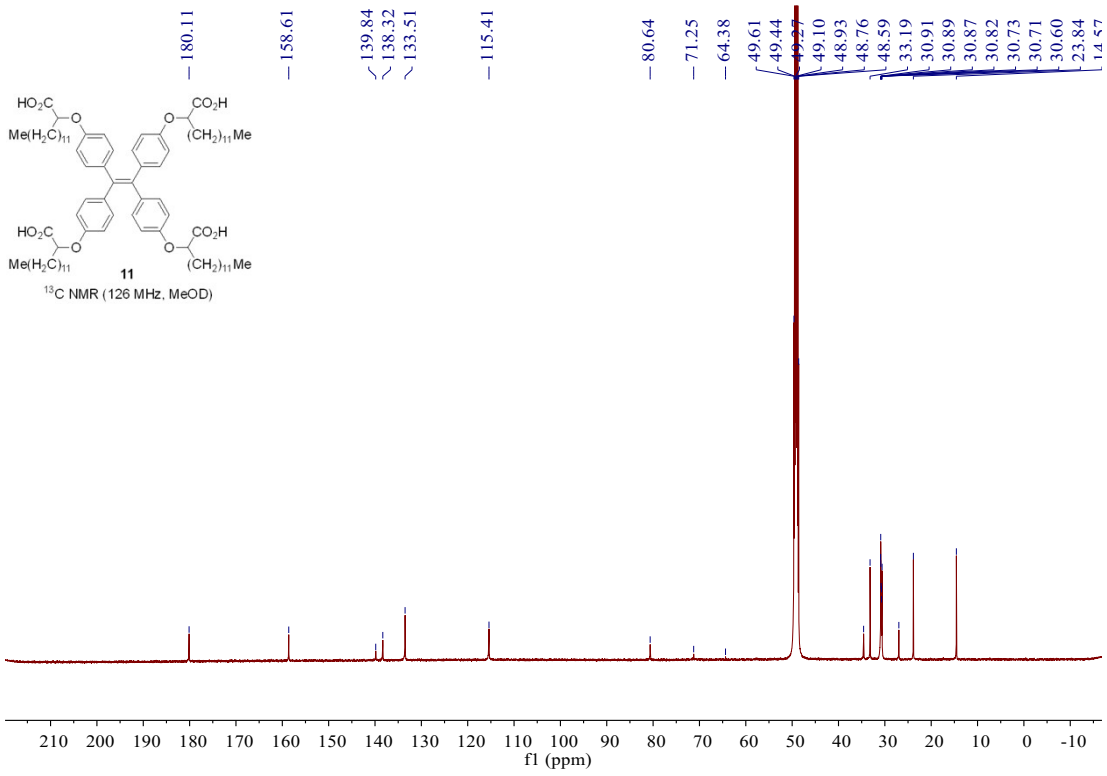
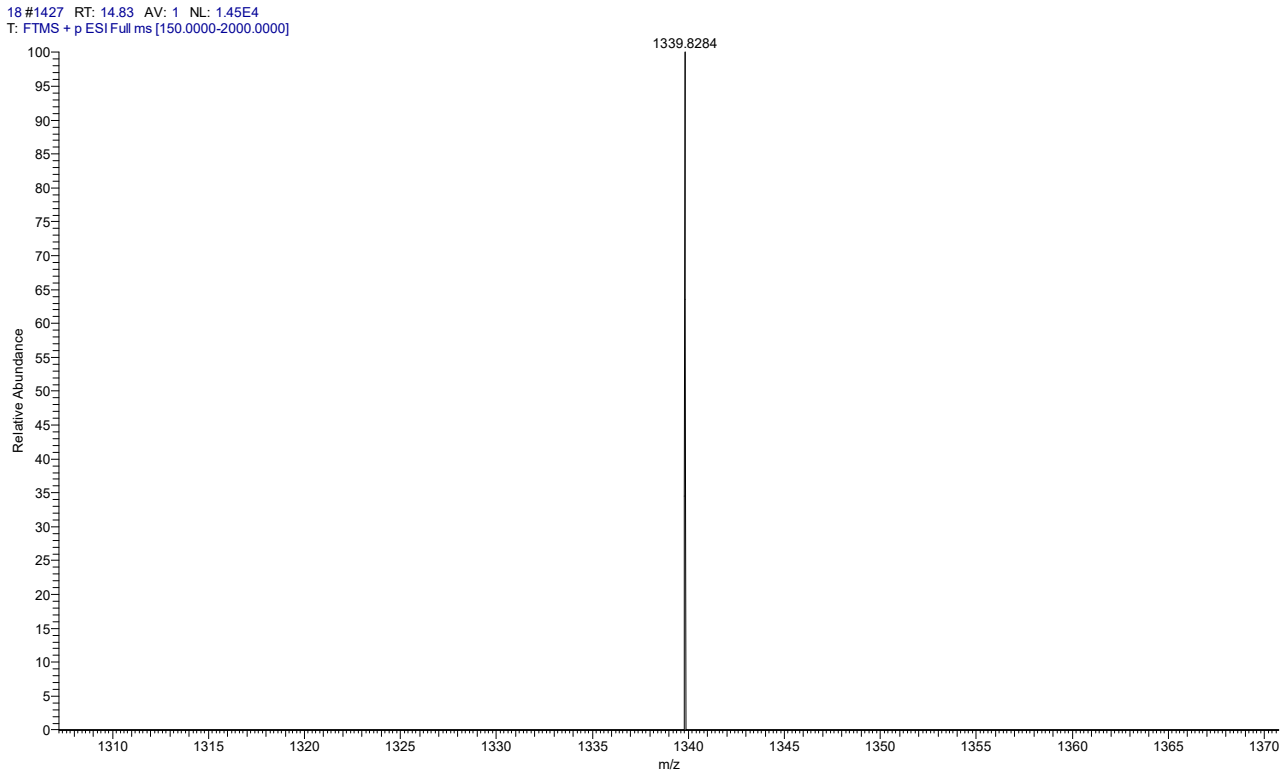
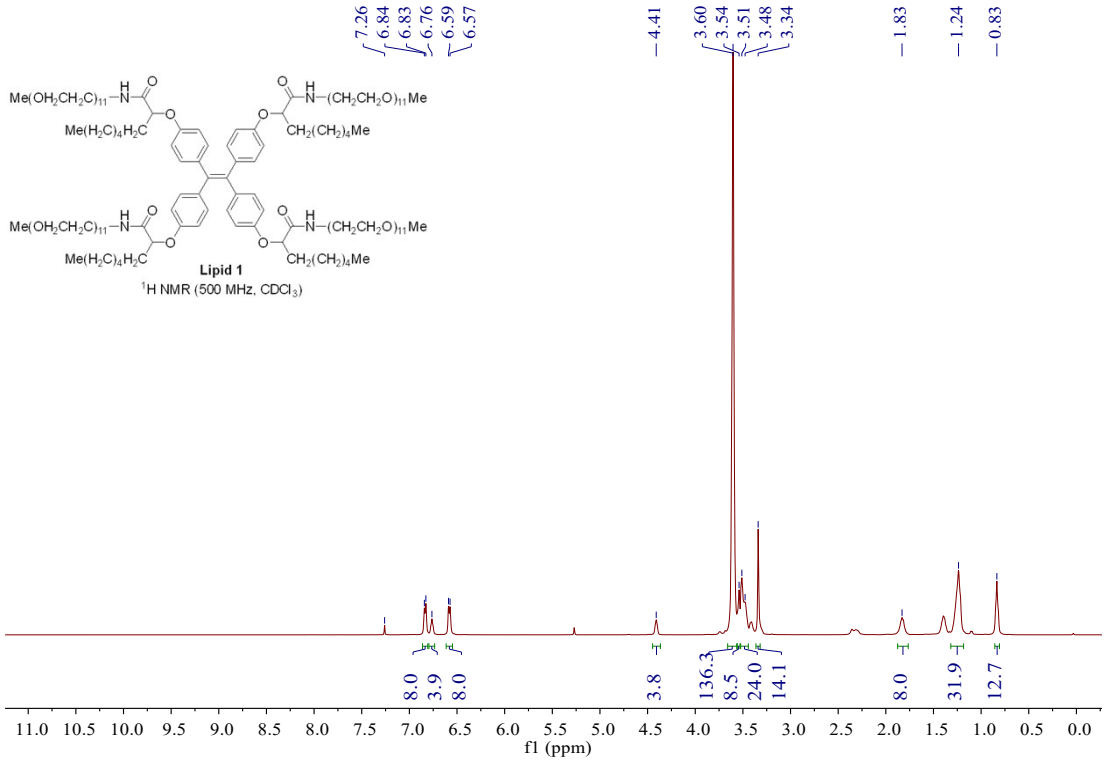


HRMS(ESI) of compound **11**



^{1}H NMR of **Lipid 1**



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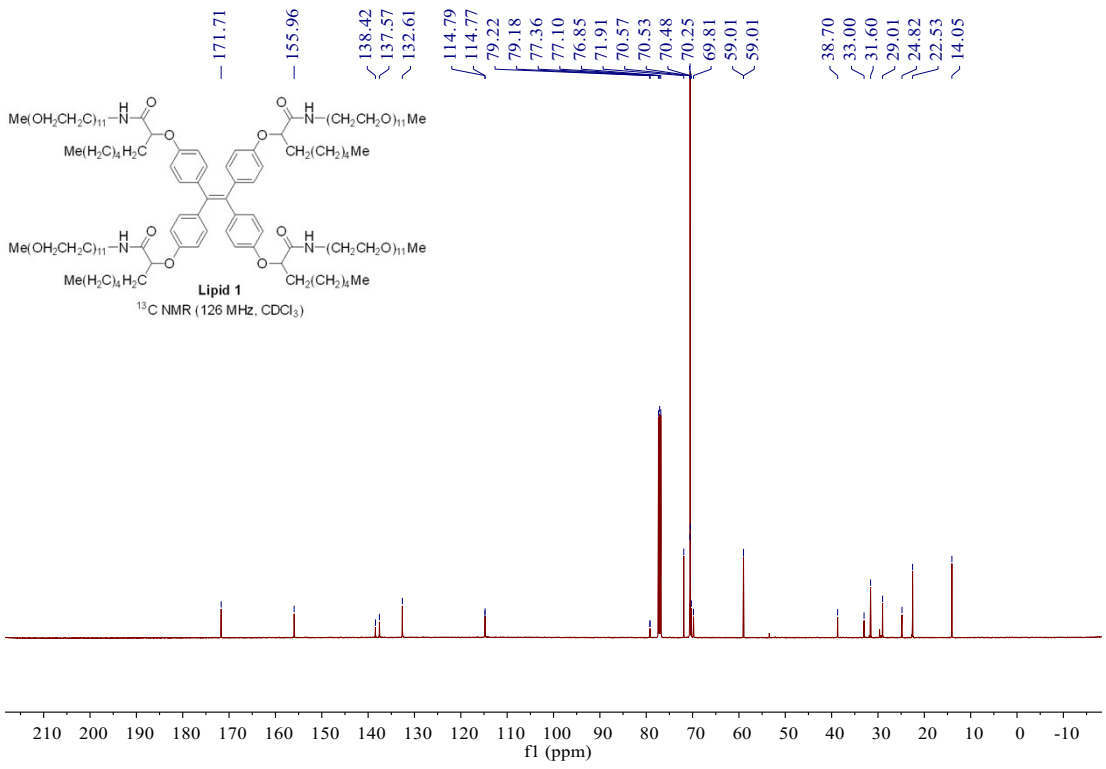
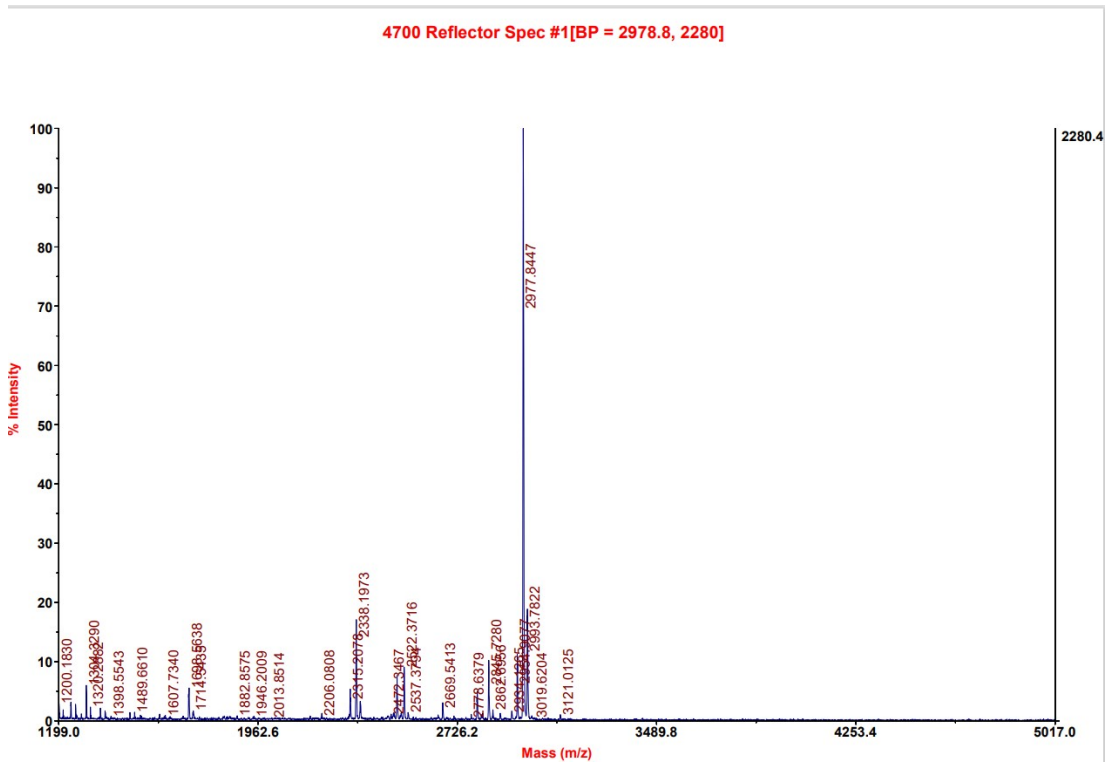
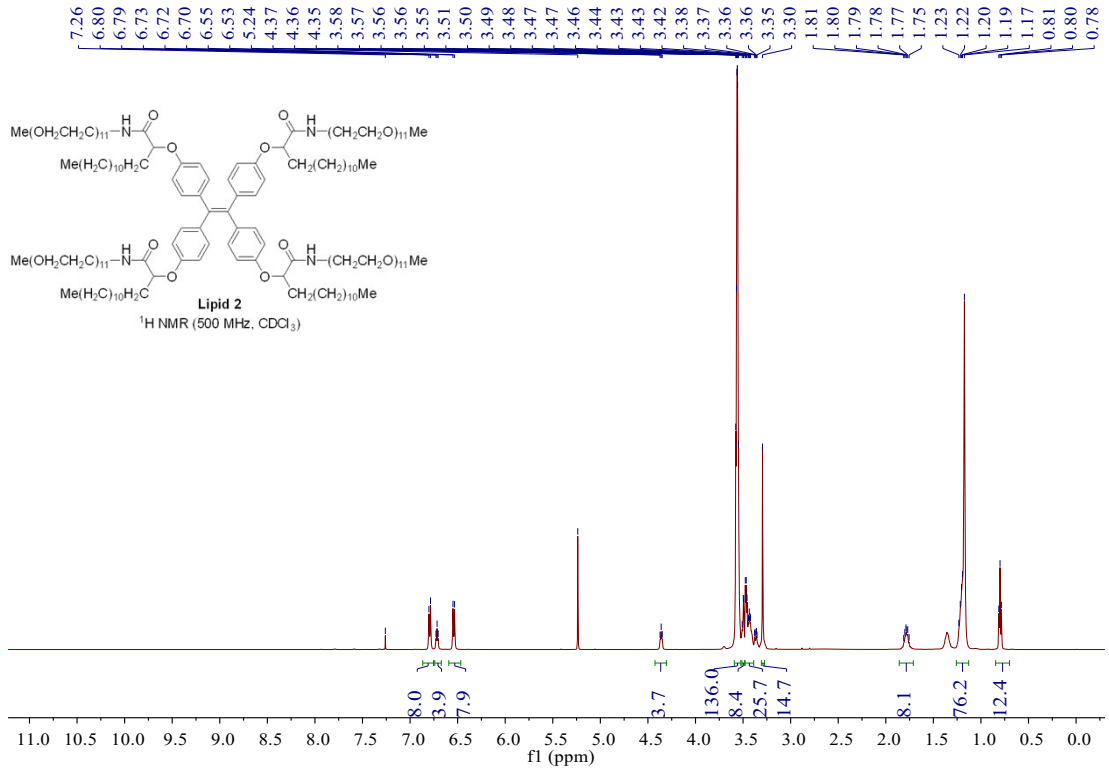


MS(MAIDI-TOF) of **Lipid 1**



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



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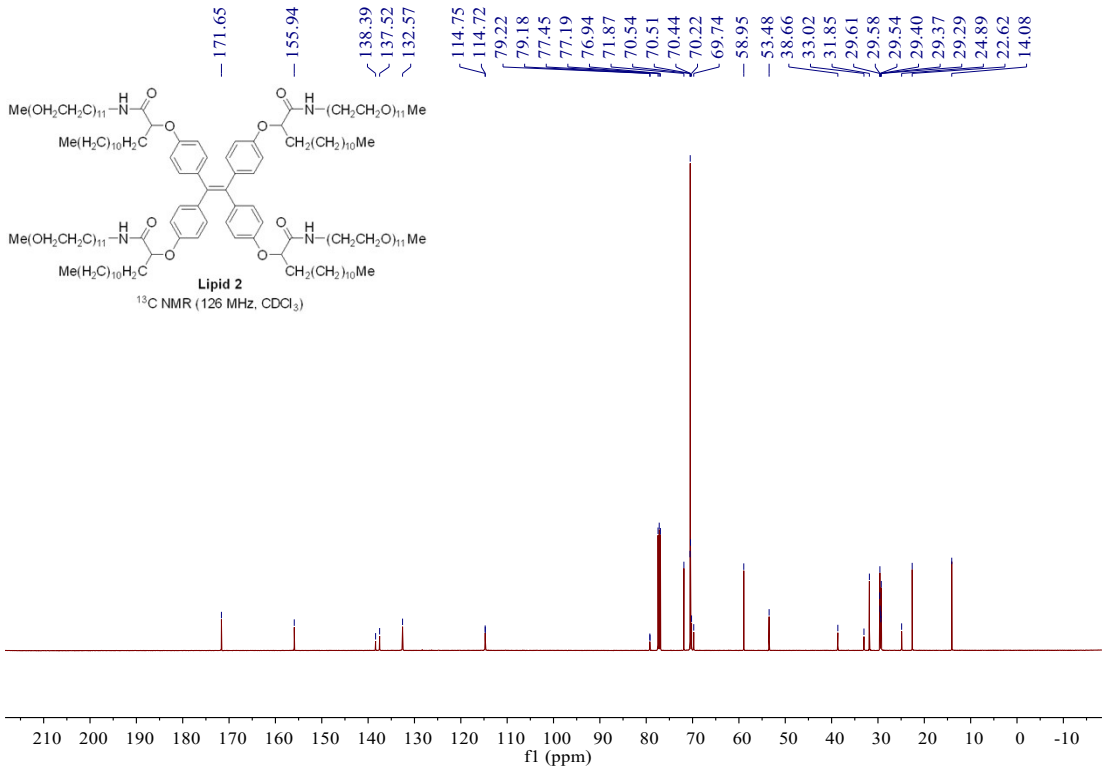

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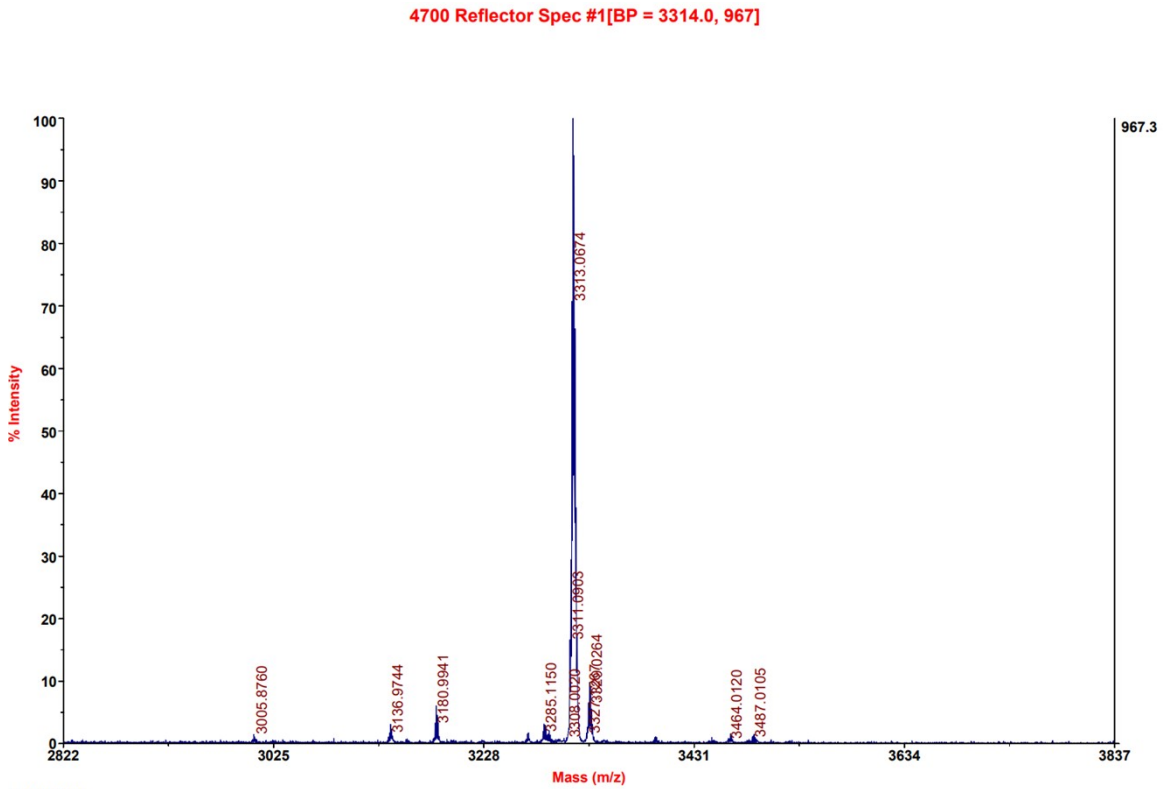
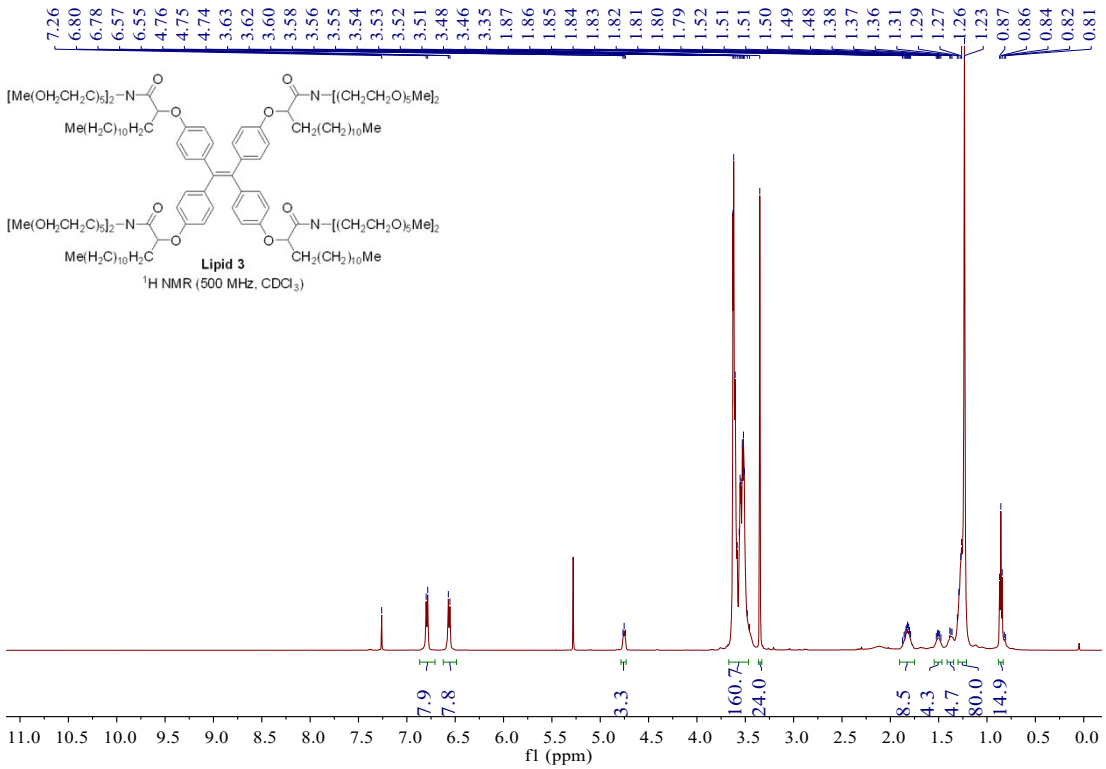
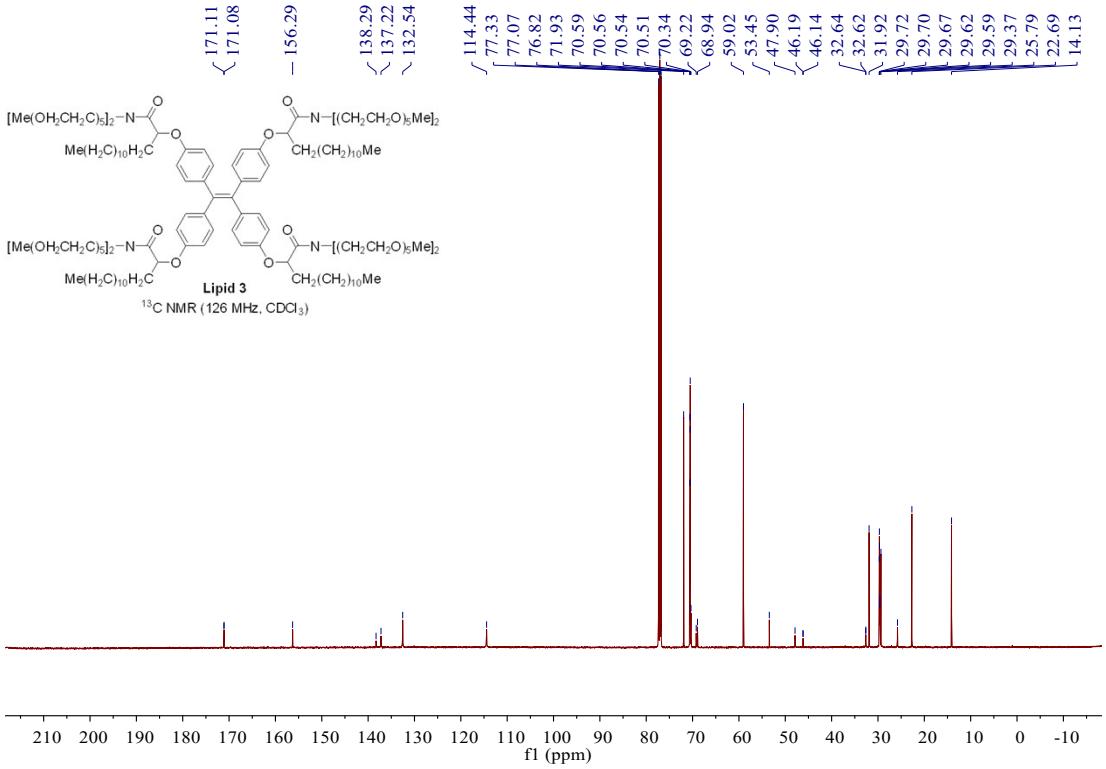


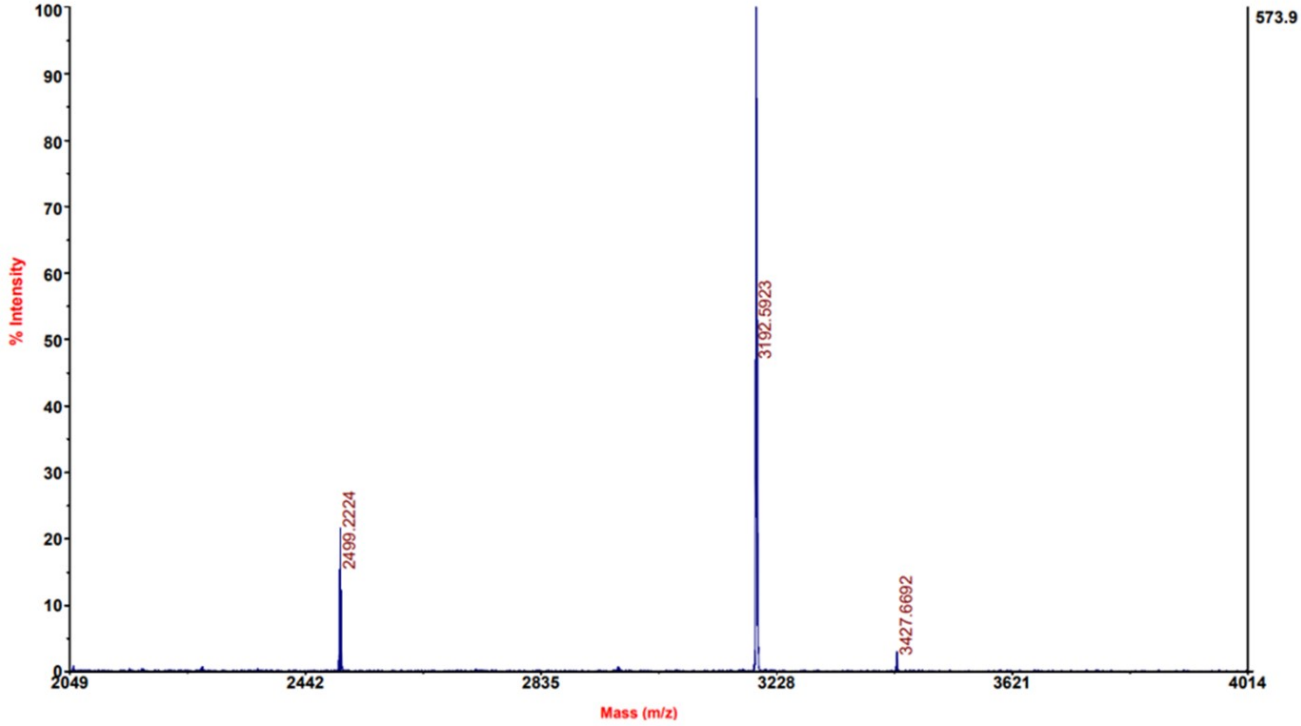
^{1}H NMR of **Lipid 3**



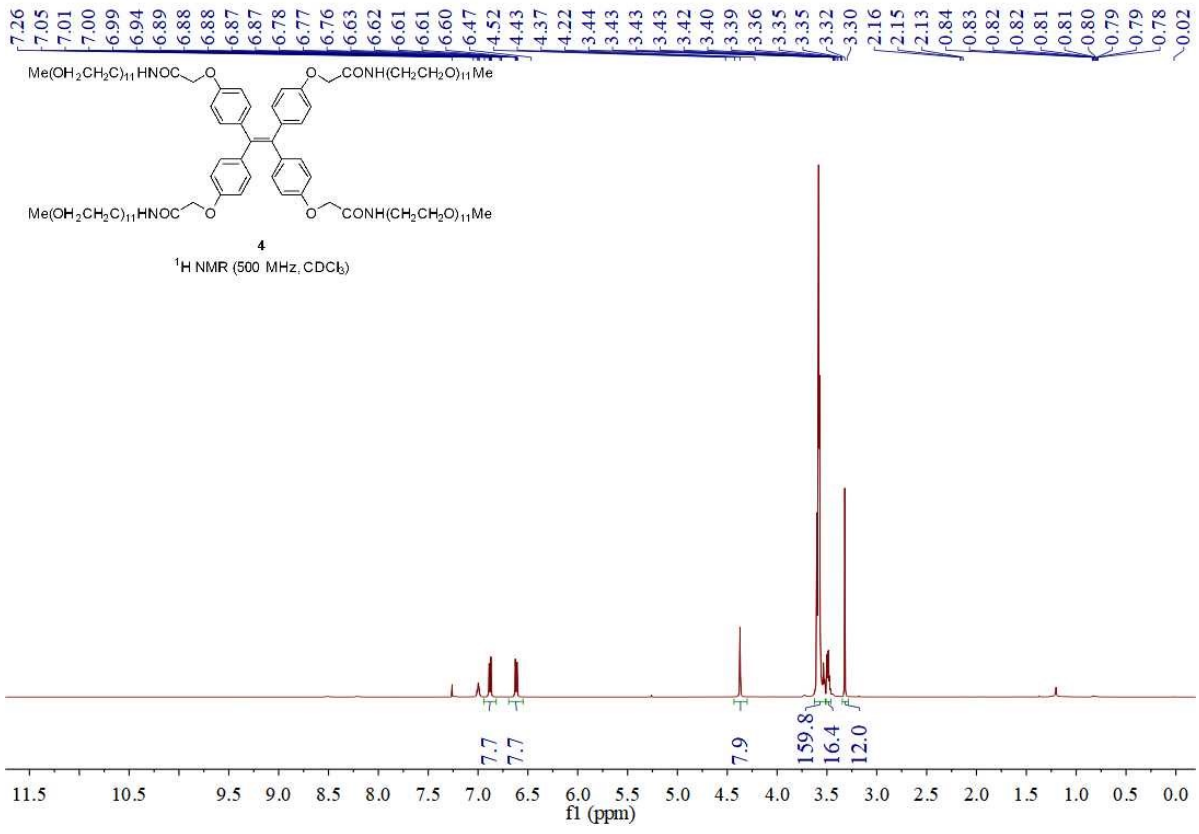
^{13}C NMR of **Lipid 3**



MS(MAIDI-TOF) of **Lipid 3**



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



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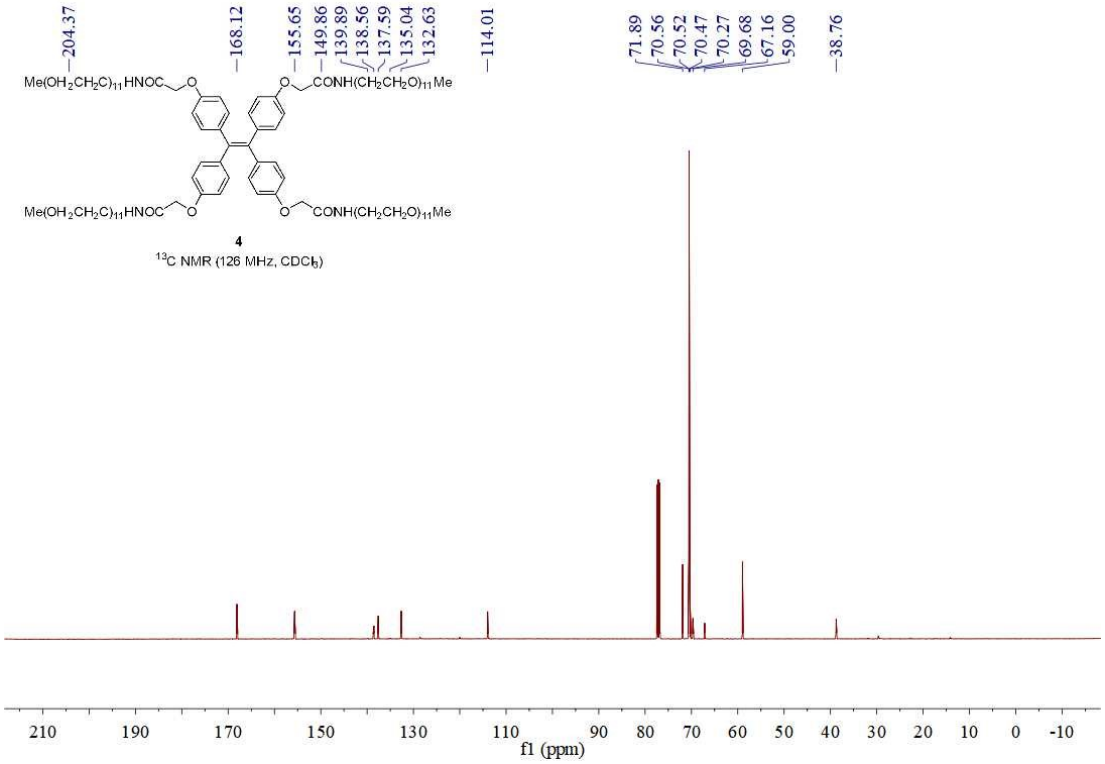
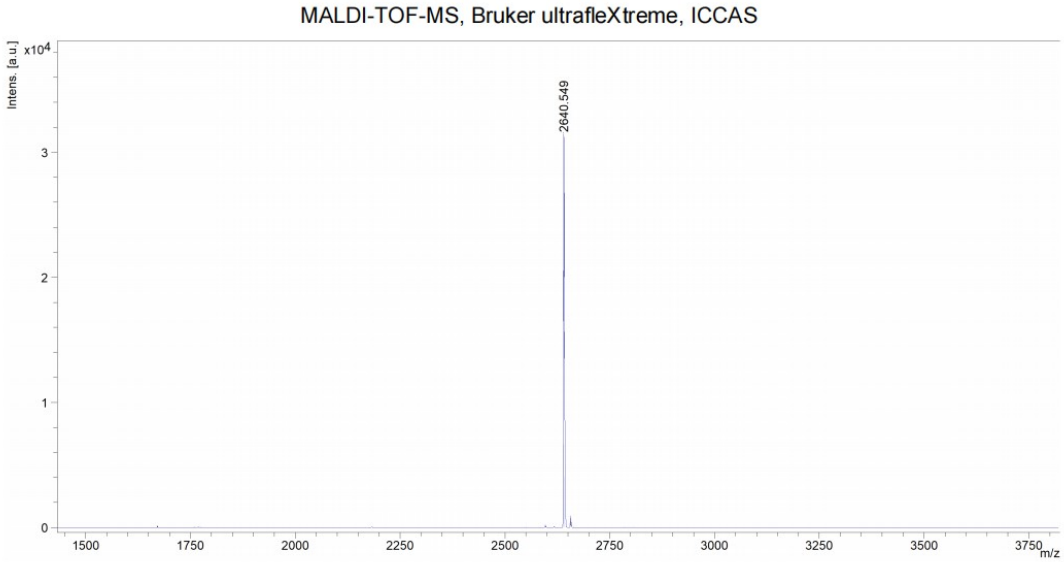


MS(MAIDI-TOF) of **TPE 4**



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