Electronic supplementary information

Supramolecular hydrogel prepared from thymine-containing artificial nucleolipid: study of assembly and lyotropic mesophases

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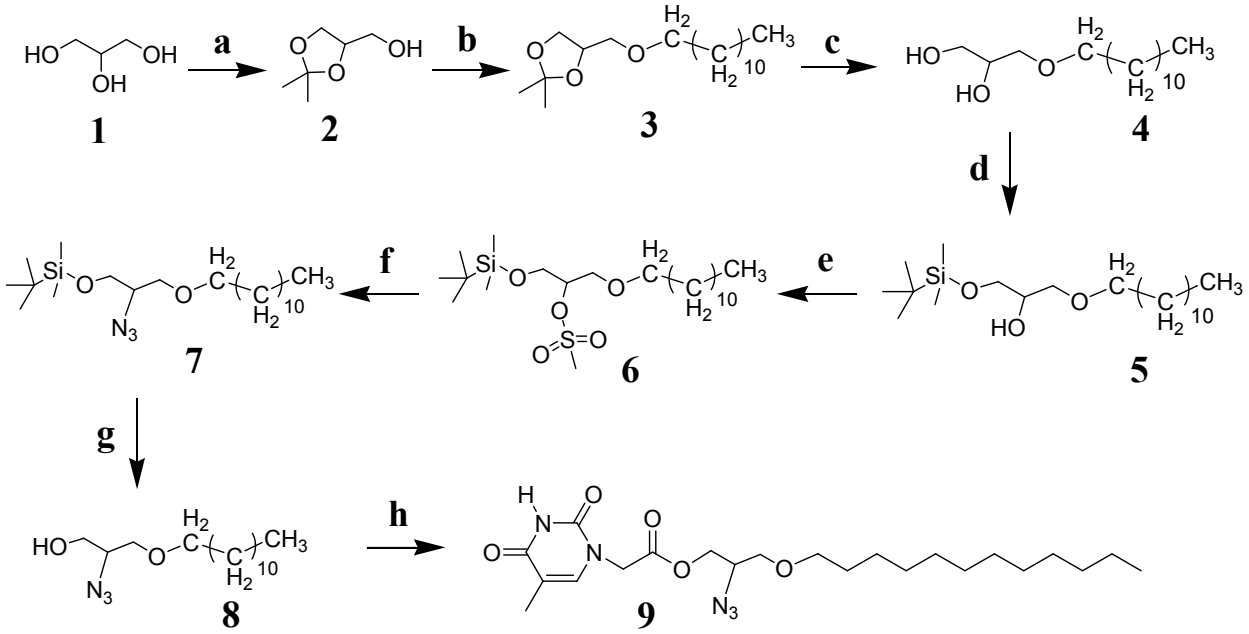
Contents

1.General Information

Unless otherwise noted, all reactions were run under air and room temperature. All chemical reagents were obtained commercially without further purification. ^{1}H, ^{13}C NMR and 2-D heteronuclear single-quantum correlation (HSQC) NMRspectra were recorded on Bruker Avance-III 400 spectrometer. Temperature varied 1H NMR were recorded on Varian Inova 400 spectrometer with air-flow heating system.Fourier Transform Infrared (FTIR) spectra were recorded on a NIicolet 670 FT-IR spectrometer. Fluorescence excitation ratios were recorded on QM-6 steady-state spectrofluorimeter (Photon Technology International).Oscillatory rheology was performed on a TA Instruments DHR-3 rheometer with a 40mm stainless steel parallel plate geometry and a water-proof cover. Negatively stained TEM imaging was carried out on FEI Tecnai T12 Spirit, 120 kV LaB6 filament TEM. Cryo-TEM imaging was carried out on FEI Tecnai F30, 300 kV FEG-TEM. Liquid crystal phase was observed using Olympus optical microscopy equipped with crossed polarizer, lamda plate and heating plate. Fluorescence images were recorded onOlympus laser scanning confocal fluorescence microscopy equipped with a laser source and a polarizer.

2.Experimental

2.1 Synthesis of thymine-containing azide precursor



Stepa: 2,2-Dimethyl-1,3-dioxolane-4-methanol **(2)**
Glycerol (21.16 g, 0.23 mol), 2,2-dimethoxypropane (14.88 g, 0.143 mol)and 300 mL acetone were added into round bottle. *p*-toluene sulfonic acid (1.23 g, 0.007 mol) was added as catalyst. After 10 hours stirring, acetone was removed under vaccum, and the left liquid was dissolved in 500 mL dichloromethane (DCM), washed with water (100 mL×3). After evaporation of DCM, product is colorless liquid (18.5 g, 0.14 mol, yield: 98%).

Step b:4-[(Dodecyloxy)methyl]-2,2-dimethyl-1,3-dioxolane **(3)**
Compound **2** (13.2 g, 0.1 mol)andbromododecane (49.8 g, 0.2 mol) were mixed in round bottle. Into the bottle were added 200 mL NaOH aqueous solution (50 wt%) and tetrabutylammonium iodide (1.845 g, 0.005 mol) as catalyst. Reaction system was placed into 80 ℃ oil-bath and stirred for 12 hours. After cooling down, 200 mL DCM was added. Organic phase was washed with HCl solution, $NaHCO_3$ solution and brine, dried by anhydrous Na_2SO_4. After evaporation of DCM, pure compound 3 was obtained using column chromatography (hexane: ethyl acetate=20:1), viscous liquid (28.5 g, 0.095 mol, yield: 95%).

Step c: 3-(Dodecyloxy)-1,2-propanediol **(4)**
Compound **3** (28.5 g, 0.095 mol) was dissolved into 200 mL methanol, strong acid cationic ion-exchanging resin (4 g) was added into solution. After stirring for 24 hours, reaction solution was filtered and condensed. Crude compound was purified by column chromatography (hexane: ethyl acetate=1:1). Compound 4 was gained as colorless and viscous liquid (24 g, 0.093 mol, yield: 98%).

Step d: 1-(tertbutyldimethylsilyl)oxy-3-(dodecyloxy)-2-Propanol **(5)**
Compound 4 (24 g, 0.093 mol), triethylamine (13.13 g, 0.13 mol) and 4-dimethylaminopyridine (DMAP, 0.61 g, 0.005 mol) were dissolved into 250 mL DCM. Into this solution was droplet tert-butyldimethylsilylchloride (16.6 g, 0.11 mol) in DCM. The reaction system was stirred overnight, washed with HCl solution, $NaHCO_3$ solution and brine, dried by anhydrous Na_2SO_4. After evaporation of DCM, crude compound was purified using column chromatography (hexane:ethyl acetate=5:1). Compound 5 was obtained as colorless and viscous liquid (31.25 g, 0.084 mol, yield: 90%).

Step e: 1-(tertbutyldimethylsilyl)oxy-3-(dodecyloxy)isopropylmethanesulfonate **(6)**
Methanesulfonatechloride (19.24 g, 0.168 mol) was droplet into the DCM solution of compound 5 (31.25 g, 0.084 mol) and N,N-diisopropylethylamine (21.67 g, 0.168 mol). Reaction solution was stirred overnight, washed with HCl solution, $NaHCO_3$ solution and brine, dried by anhydrous Na_2SO_4. After evaporation of DCM, crude compound was purified using column chromatography (hexane: ethyl acetate=5:1). Compound 6 was obtained as light-yellow and viscous liquid (34.2 g, 0.076 mol, yield: 90%).

Step f: 1-(2-azido-3-(tertbutyldimethylsilyl)oxy)propoxydodecane **(7)**
Compound 6 (6.840 g, 0.0152 mol) and sodium azide (3 g, 0.046 mol) were added into round bottle with a condenser. 150 mL N,N-dimethylformamide (DMF) was added as solvent. Reaction system was placed into 90 ℃ oil-bath and stirred for 24 hours. After evaporation of DMF under vaccum, DCM was added, and organic phase was washed with water, $NaHCO_3$ solution and brine, dried by anhydrous Na_2SO_4. Removal of DCM gives pure compound 7 (5.95 g, 0.015 mol, yield: 98.7%).

Step g: 2-azido-3-(dodecyloxy)-1-propanol **(8)**
Compound 7 (5.95 g, 0.015 mol) was dissolved into tetrahydrofuran (THF), and tetrabutylammonium fluoride (5.75 g, 0.022 mol) was added under stirring. After 12 hours, THF was removed under vaccum and DCM was added. Organic phase was washed with saturated $NaHCO_3$ and brine, dried by anhydrous Na_2SO_4. DCM was evaporated under vaccum, and crude compound was purified by column chromatography (hexane: ethyl acetate=1:1). Compound 8 was obtained as colorless and viscous liquid (3.7 g, 0.013 mol, yield: 86.7%).

Step h: 2-azido-3-(dodecyloxy)-1-propyl 3,4-Dihydro-5-methyl-2, 4-dioxo-1(2*H*)-pyrimidineacetate**TN_3 (9)**
N-(3-dimethylaminopropyl)-N'-ethyl carbodiimidehydrochloride (EDAC, 1.344 g, 0.007 mol) and triethylamine (0.707 g, 0.007 mol) were dissolved in DCM and stirred for 10 minutes. Thymine acetic acid (1.288 g, 0.007 mol), DMAP(43 mg, 0.35 mmol) and compound 8 (1 g, 0.0035 mol) were added in sequence. After 12 hours, DCM solution was washed using HCl solution, $NaHCO_3$ solution and brine, dried by anhydrous Na_2SO_4. DCM was removed under vaccum, crude compound was purified by column chromatography (hexane: ethyl acetate=1:3). Compound 9 was obtained as white solid (1.21 g, 0.0027 mol, yield: 77%).

LC-MS monitoring of *in situ*CuAAC
(AL, 1 eq) and TN_3 (1 eq) were dispersed in water to obtain 5mM precursors dispersion. $CuSO_4$ (0.05 eq) and sodium ascorbate (0.1 eq) were added subsequently to trigger CuAAC reaction. At different time point, 10 uL solution was sampled and diluted with 40 uL methanol. Samples were analyzed by LC-MS coupled with an evaporative light scattering detector (ELSD). For all LC-MS runs, the flow-rate was 1mL/min and elution-phase consisted of HPLC-grade water with 0.1% formic acid and HPLC-grade methanol with 0.1% formic acid.

Preparation of TTPC nanofiber assembly (*in situ* CuAAC) and supermolecular hydrogel
(AL, 1 eq) andTN_3 (1 eq) were dispersed in water to obtain 5mM precursors dispersion. $CuSO_4$ (0.05 eq) and sodium ascorbate (0.1 eq) were added subsequently to trigger CuAAC reaction. The reaction system was placed statically for 12 hours. For gel preparation, the TTPC nanofiber dispersion was stirred and placed on heating plate to evaporate water until a designed TTPC weight percentage.

Measurement of critical aggregation concentration (CAC) of TTPC in water
Using the aqueous TTPC nanofiber assembly as stock dispersion (5 mM TTPC), samples with different TTPC concentration were prepared, with constant concentration of pyrene as fluorescent probe (0.001 mM). Samples were stirred for 6 hours and placed statically overnight. Steady-state fluorescence excitation spectrum (Ex=300~360 nm, Em=395 nm) were measured for each sample. The intensity ratio of I_{338}/I_{333} of pyrene excitation spectrum was plot as a function of concentration of TTPC in water.

Negatively stained TEM
5 uL sample was dropped on a carbon-coated copper grid, excess solution was wicked away using filter paper. 2 uL 1% phosphotungsticacid aqueous solution was dropped on sample and the sample was left and dried in air. Prepared sample was observed and imaged using FEI Tecnai T12 Spirit, 120 kV LaB6 filament TEM.

Cryo-TEM
2 uL sample was dropped on a carbon-coated copper grid, and copper grid was frozen using FEI Vitrobot Mark IV manual plunge freeze device. After careful transportation of sample to the holder, sample was observed and imaged using FEI Tecnai F30, 300 kV FEG-TEM.

Characterization in driving forces of TTPC nanofiber assembly
480 uL TTPC d6-DMF solution was prepared and scanned under ^1H NMR model. After addition of 20 uL H2O, NMR tube loading sample was vortex for 1 minute and placed statically for 10 minutes before next ^1H NMR scanning. Addition of H_2O and NMR scanning were repeated until the complete attenuation of characteristic proton signals.

Thermodynamic analysis in TTPC assembly process (Temperature varied ^{1}H NMR)
200 uL TTPC nanofiber assembly (5 mM) was loaded into NMR tube (5 mm diameter), and coaxial inner tube (50 mm-stem hight) loading Sodium Trimethylsilylpropionate was inserted into sample-loading NMR tube. ^{1}H NMR was scanned and recorded on Varian Inova 400 spectrometer with air-flow heating system. Temperature interval was 5 ℃.

Characterization of self recovery of TTPC supramolecular hydrogel
8 wt% TTPC gel sample was placed on the rheometer with 300 um gap. Water-proof cover was set up. For frequency scan, measurements were taken with an oscillatory shear strain of 1%. For step-stain scan, frequency was kept at 100 rad.s^{-1}, and the lower strain was 0.1% and recording time was 5 minutes; higher strain was 20% and recording time was 30 s.

Preparation of gold nanorods (GNRs) and TTPC/GNRs composite supermolecular hydrogel
CTAB-protected gold seed particles were prepared by the chemical reduction ofthe gold (III) chloride trihydrate ($HAuCl_4.3H_2O$, Sigma-Aldrich, 0.25 mM) with the surfactant hexadecyltrimethylammoniumbromide based solution (CTAB, Sigma-Aldrich, 0.1 M) in a clean glass bottle with a total volume of 10 mL. This was followed by 0.6 mL of freshly prepared ice-cold sodium borohydride ($NaBH_4$, Sigma-Aldrich, 10 mM). The solution was kept under vigirous stirring for 2 mins to force uniform seed particles. Note that the precision of the $NaBH_4$ amount added to the seed solution is crucial to get high quality of the seed particles. Meanwhile the growth solution is synthesized on a separate vile where 50 mL of Deionized Water (DI) added toboth 1.4 g CTAB and 246 mg of the additive sodium oleate (NaOL > 97.0%, TCI America) and kept under mixing at high temperature (50-70 ℃) until it is fully dissolved. The growth solution was then cooled to room temperature before adding 4.8 mL of silver nitrate ($AgNO_3$, Sigma-Aldrich, 4 mM) and kept undisturbed for 15 minutes. Then 50 mL of $HAuCl_4$ of 1mM is added and kept under stirring at 700 rpm for 90 minutes. 0.42 mL of of HCl (37 wt% in water, 12.1 M) was then added to tune the pH of the solution to 1.36 ±0.03 and the stirring speed was decreased to 400 rpm for 15 mins. After which 0.25 mL of L-ascorbic Acid (BioUltra ≥ 99.5%, Sigma-Aldrich, 64 mM) was added and kept under vigorous stirring for 30 seconds, then the growth solution was injected with 0.16 mL of the seed solution and was stirred for another 30 seconds before it was left undisturbed at 30 ℃ for 12 hrs. The growth solution was then centrifuged at 7000 rpm for 30 mins and excess supernatants were removed from the precipitates, mainly GNRs, and the resultant were re-dispersed in DI water and was ready to use.

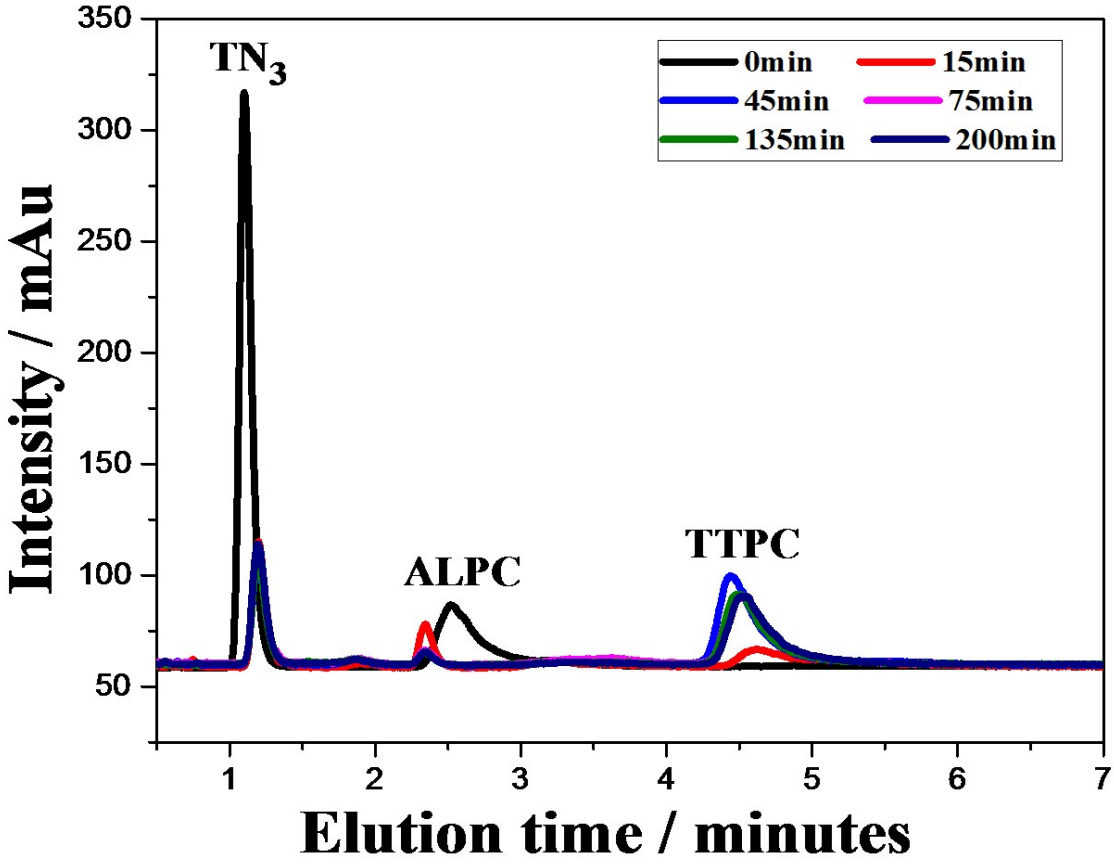


Fig. S1 LC-MS-ELSD traces of CuAAC reaction between ALPC and TN_3

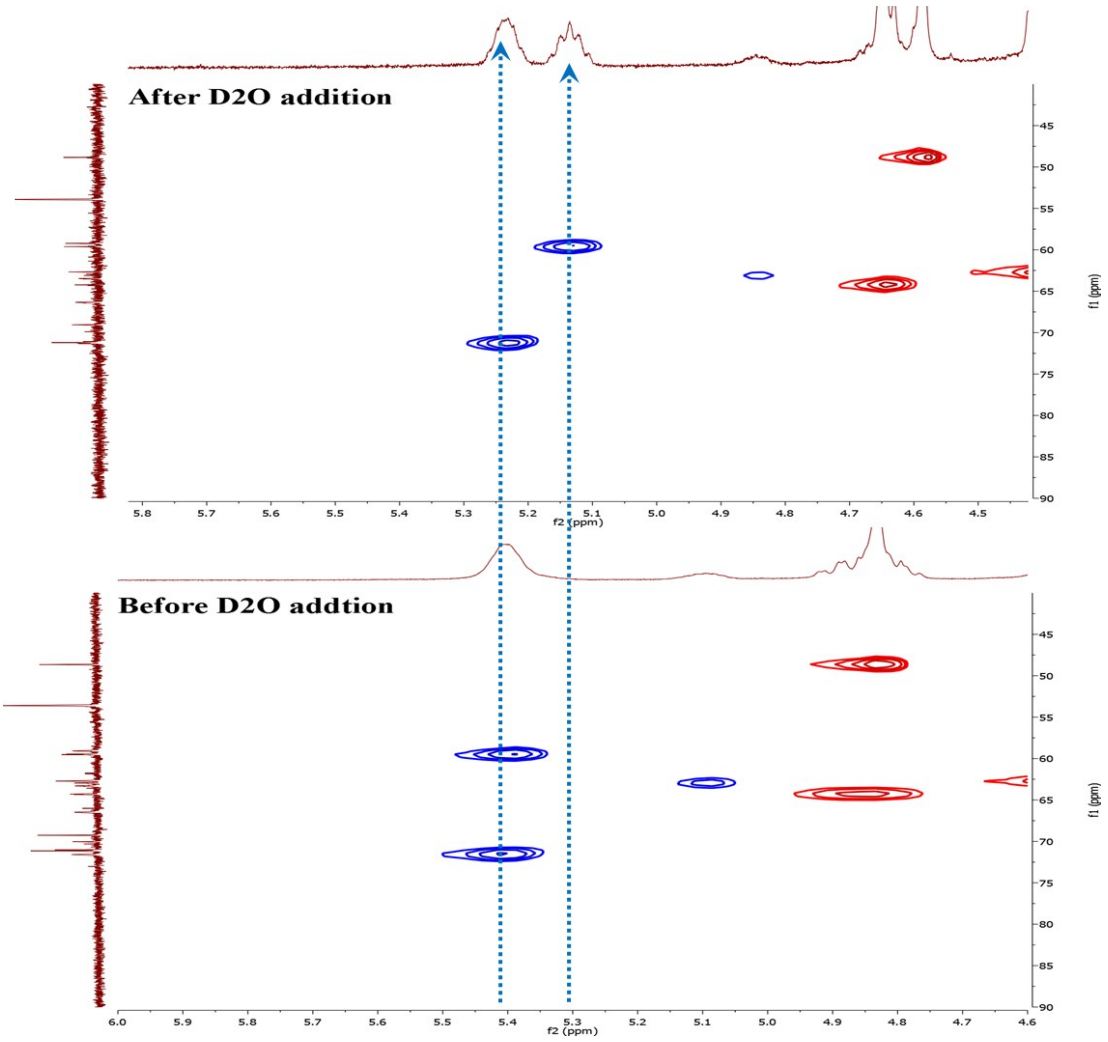


Fig. S2 Change of 2-D HSQC NMR signals of glycerol methane protons before and after addition of D_2O into TTPC *d*6-DMF solution

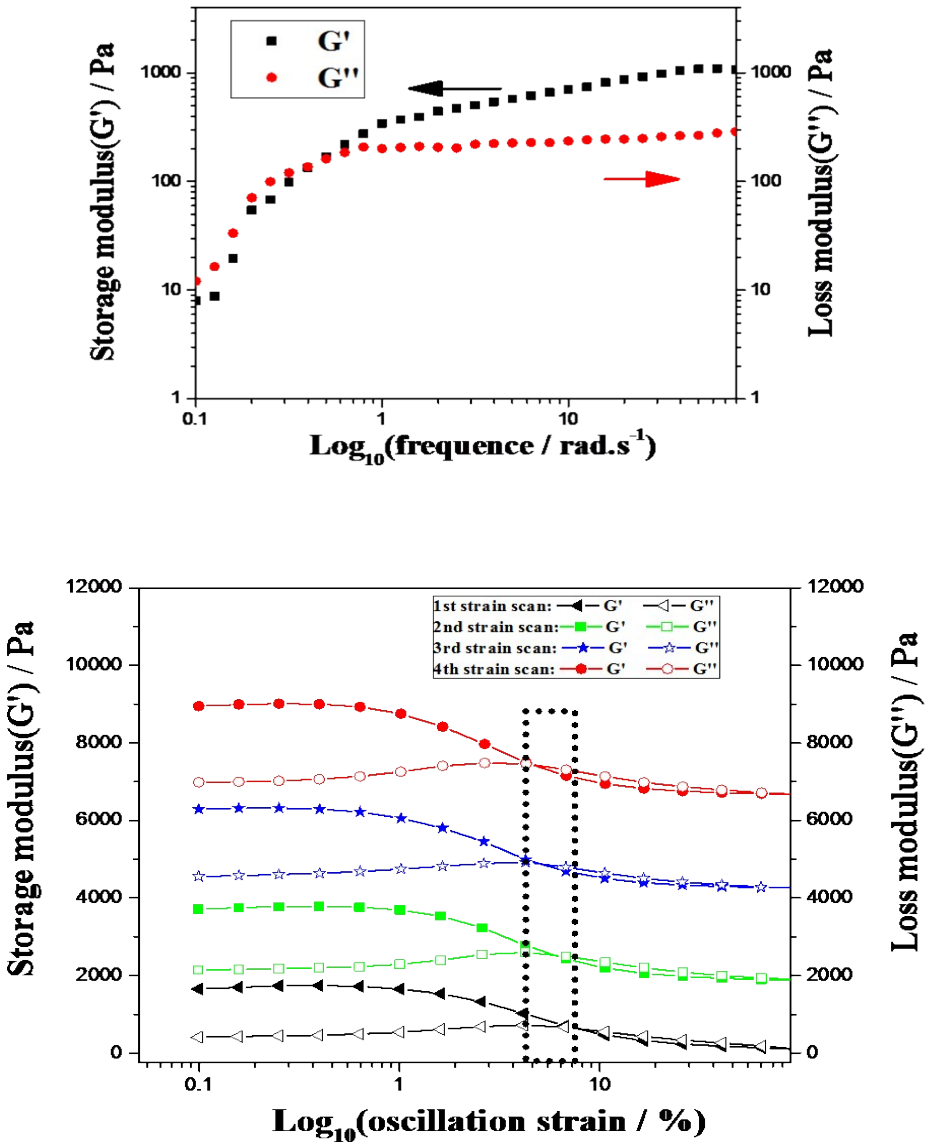


Fig. S3 Single frequency scan and continuous strain scans of TTPC (8 wt %) supramolecular hydrogel

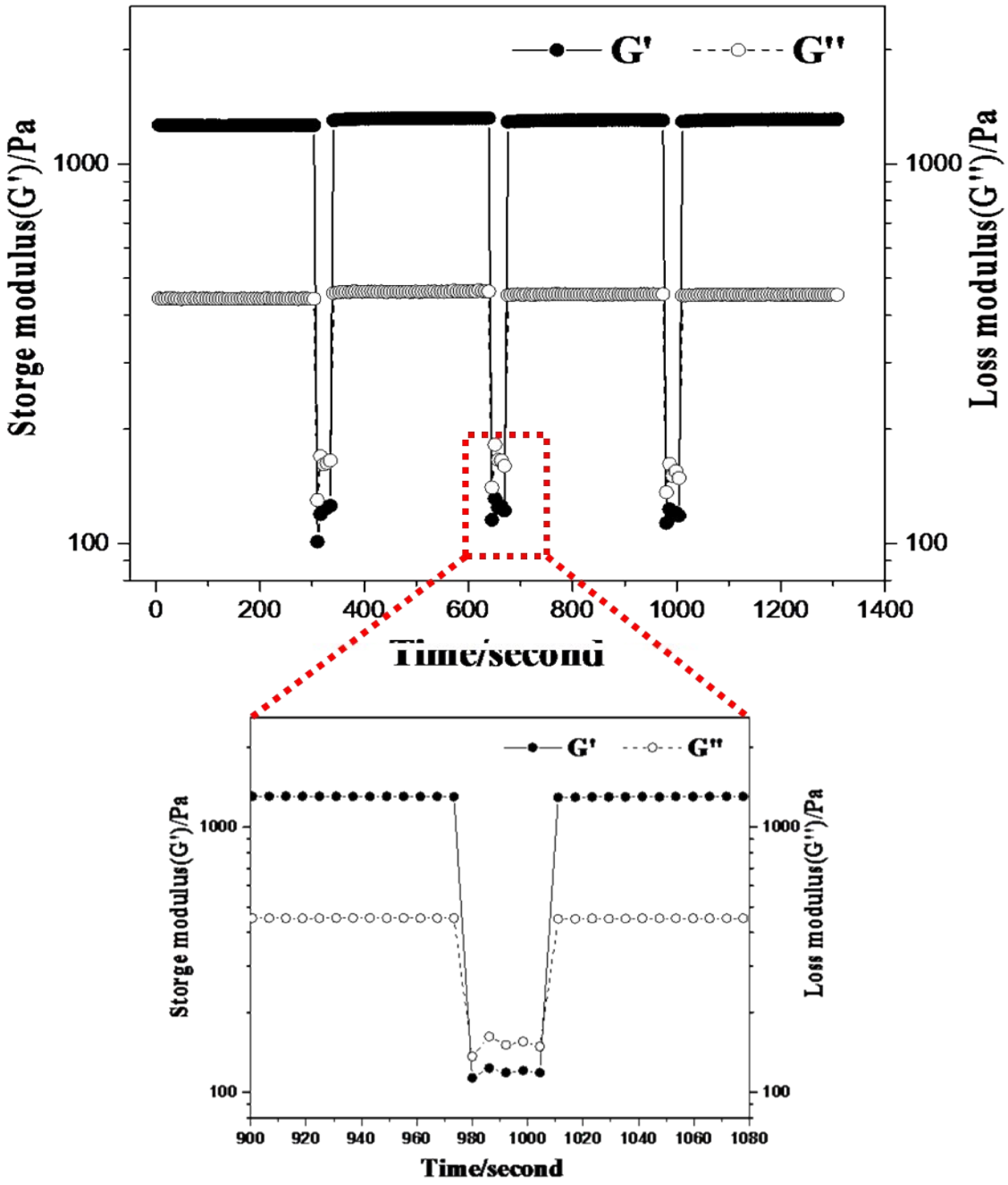
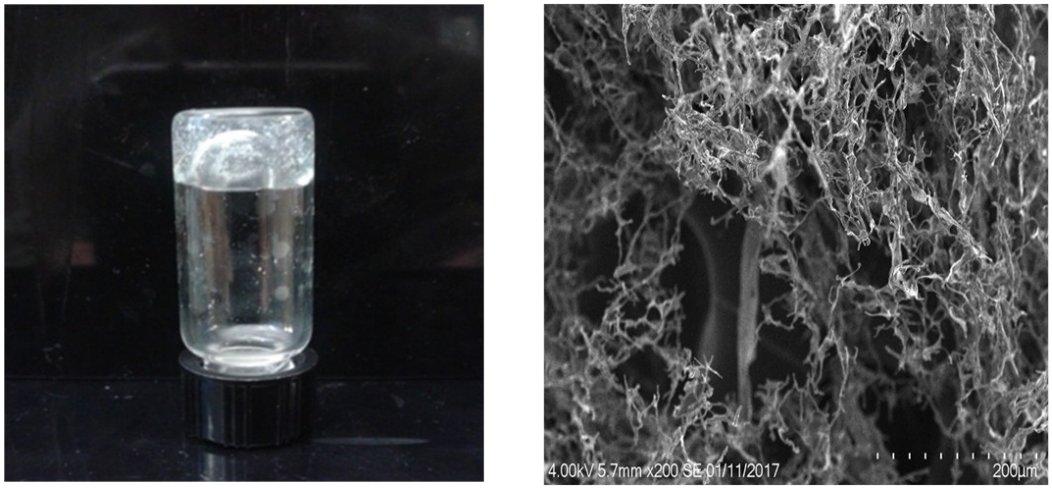


Fig. S4 Step-strain scans of TTPC (8 wt %) supramolecular hydrogel



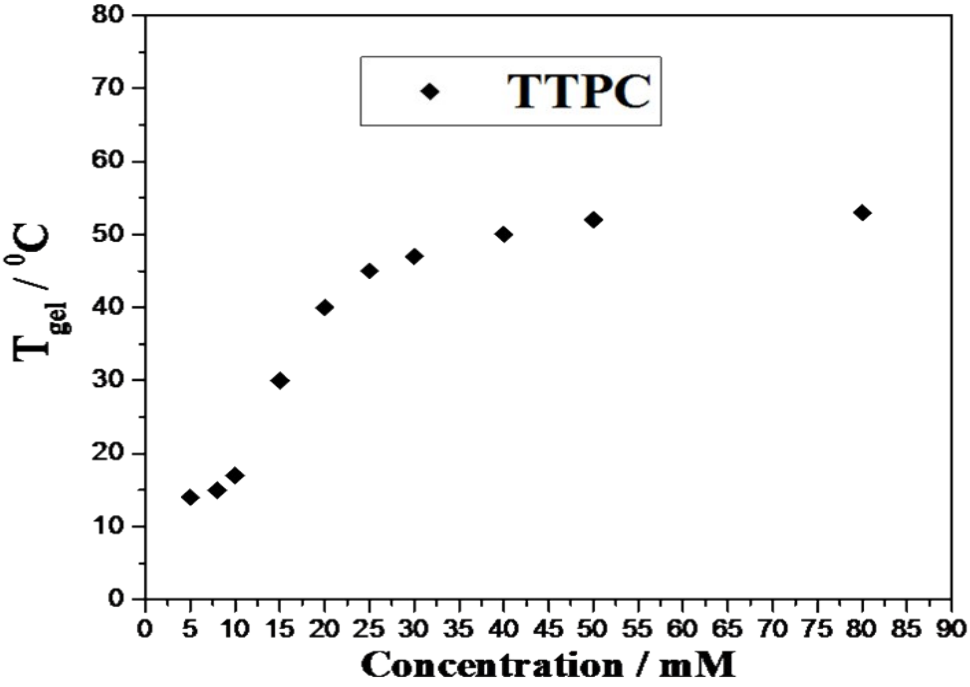


Fig. S5 Appearance of TTPC supramolecular hydrgel and the scanning electron microscopy image of gel-network after lyophilization (8 wt %)
Sol-gel trainsition temperature of TTPC supramolecular hydrogel with different TTPC concentration

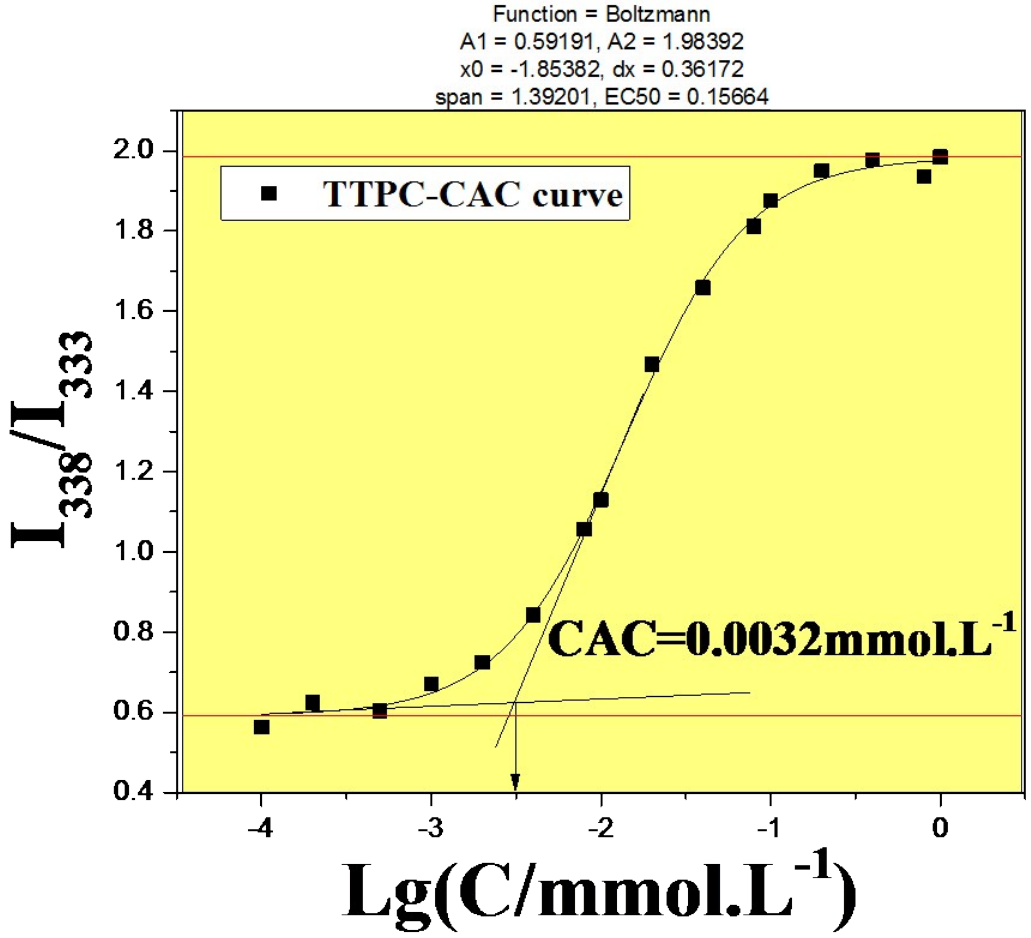


Fig. S6 Determination of critical aggregation concentration (CAC) of TTPC in water by steady fluorescence measurement

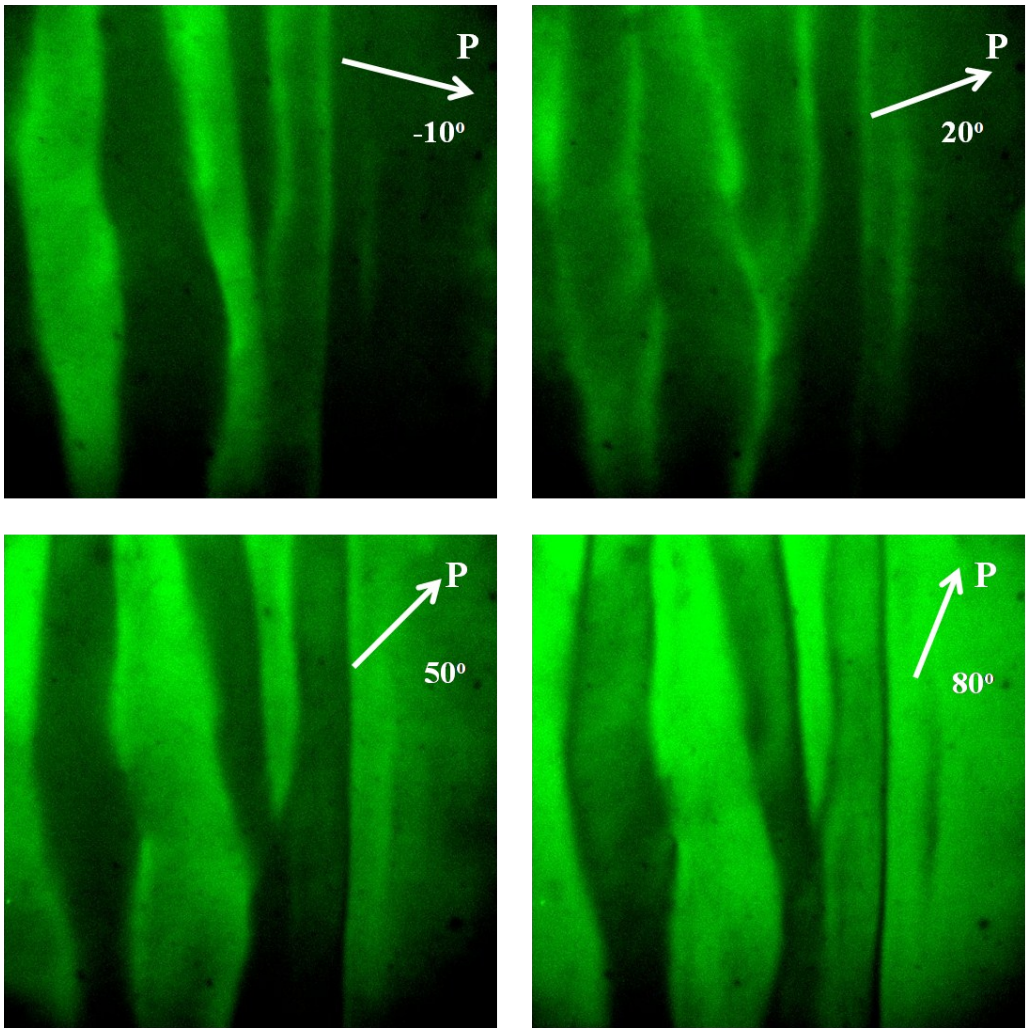


Fig. S7 Polarization-angle-related 2PEFPM images of liquid crystal phase in TTPC (25 wt %) supramolecular hydrogel

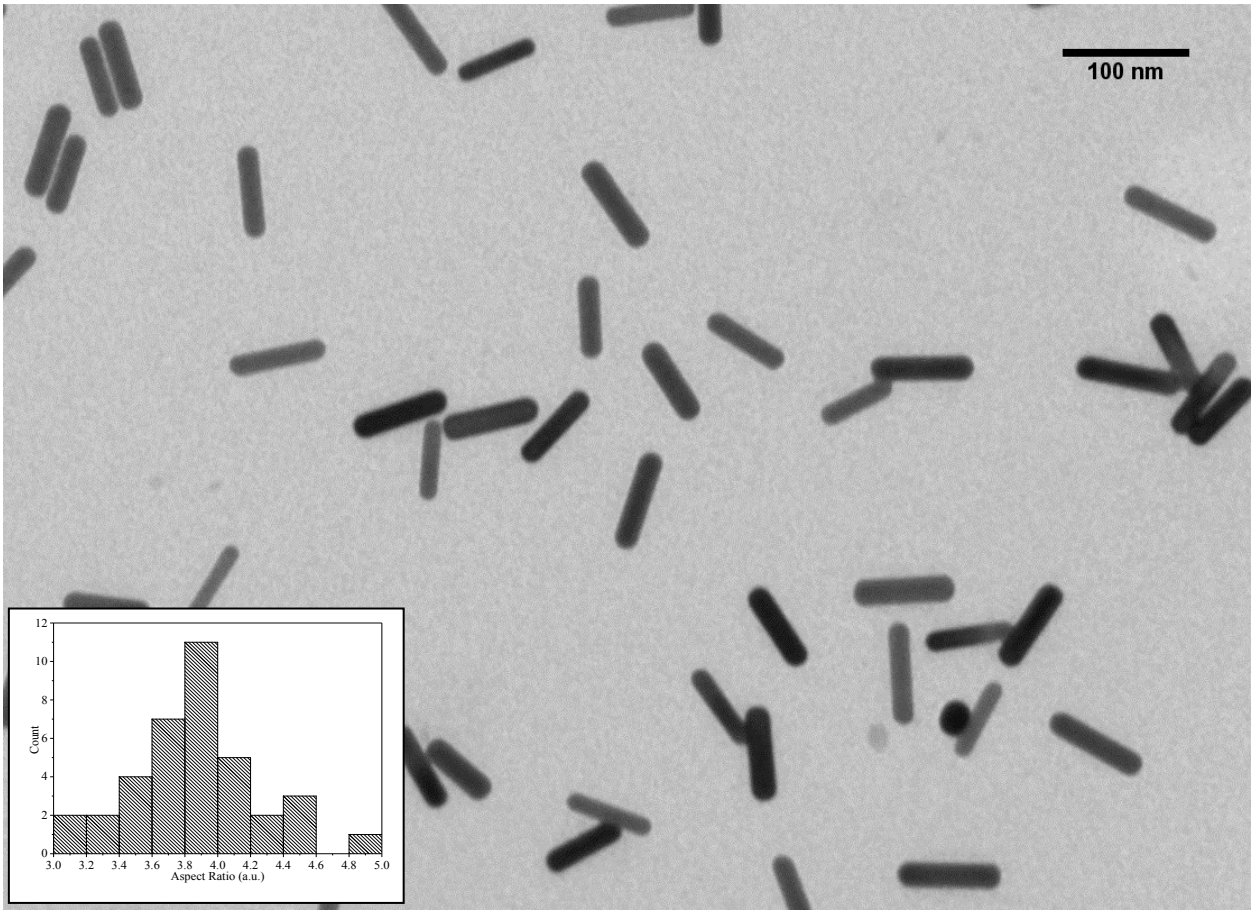
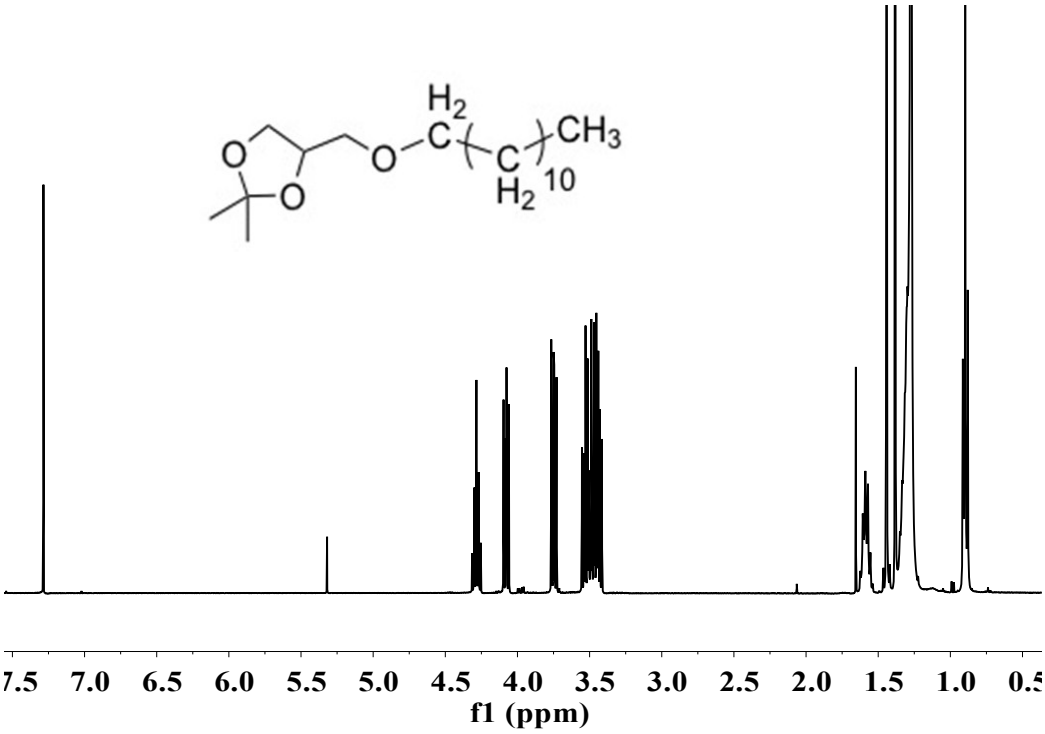


Fig. S8 TEM image of GNRs with an aspect ratio of 3.9.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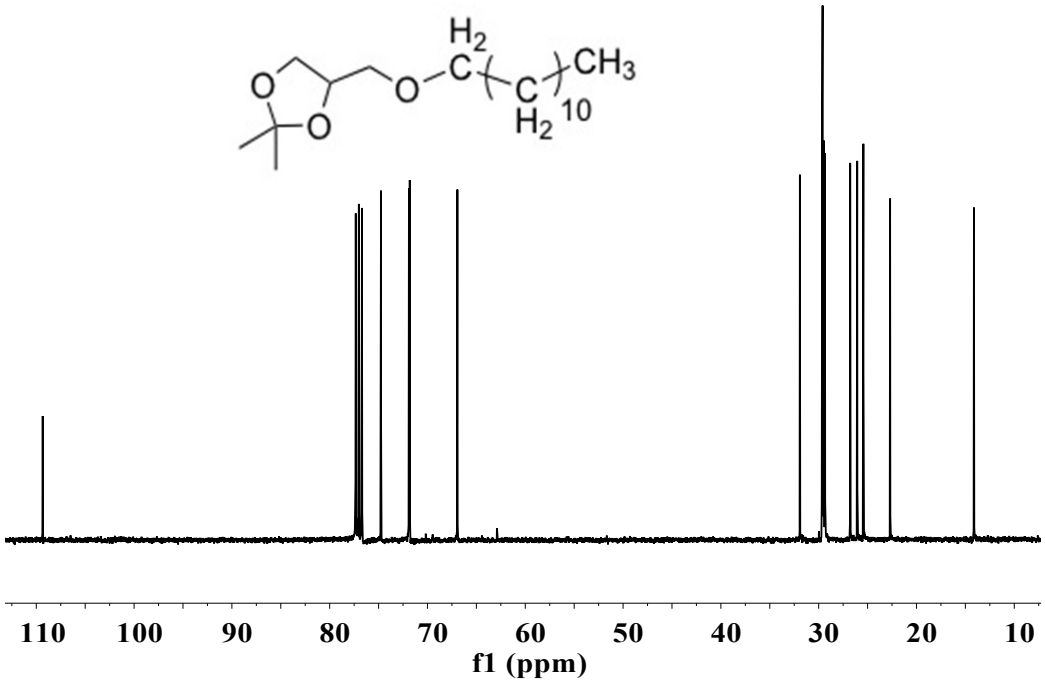
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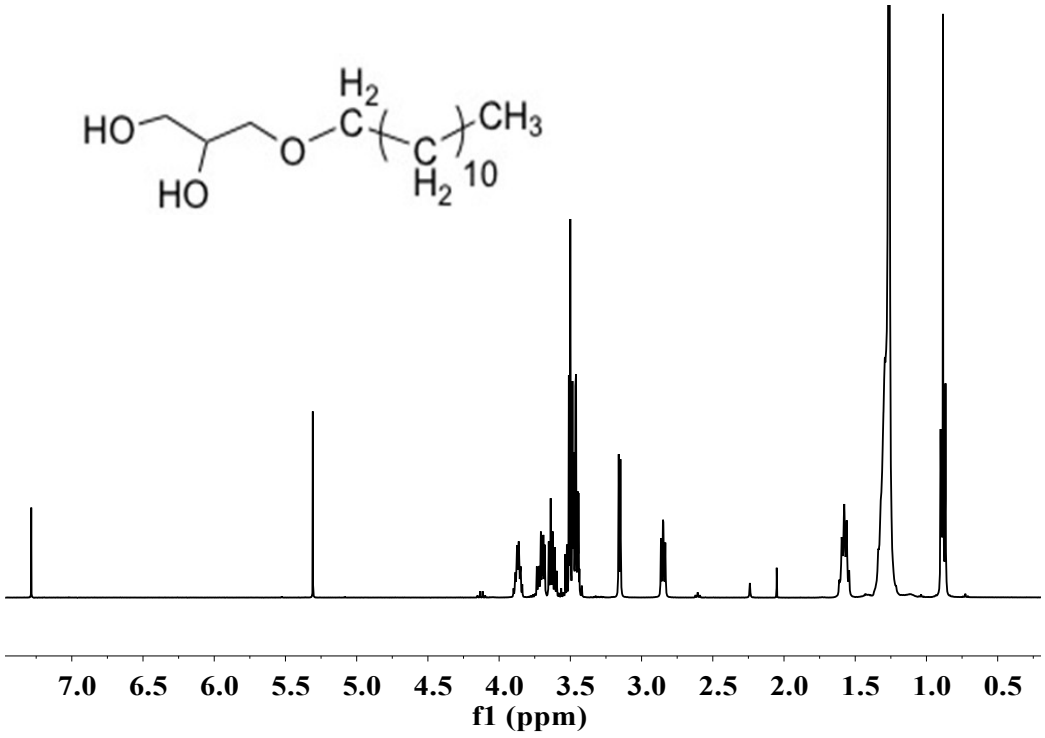
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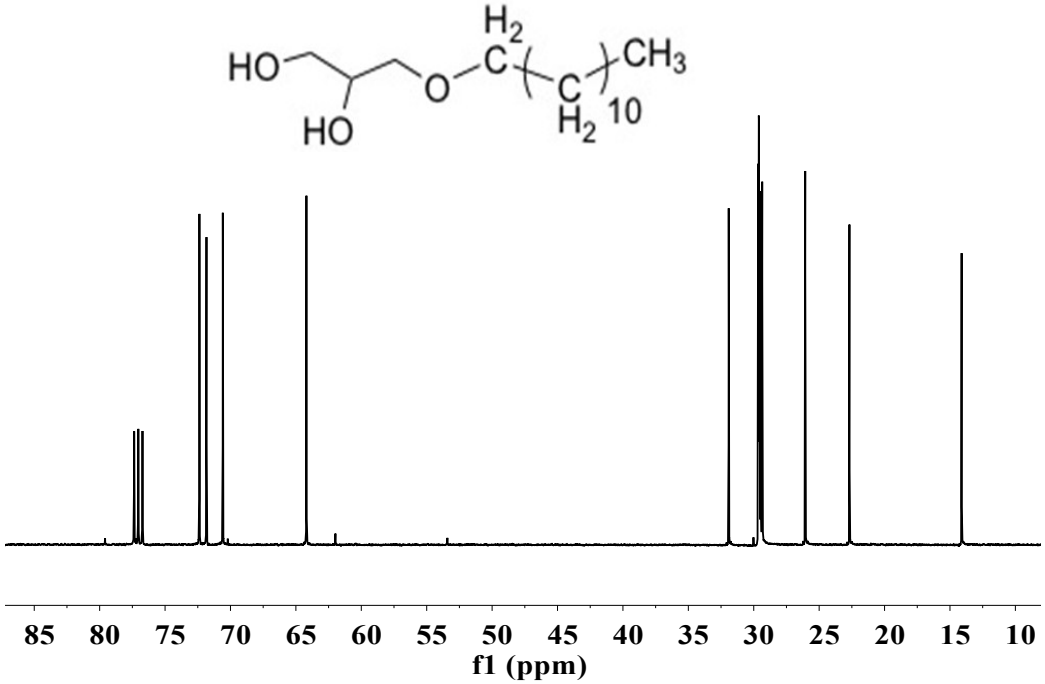
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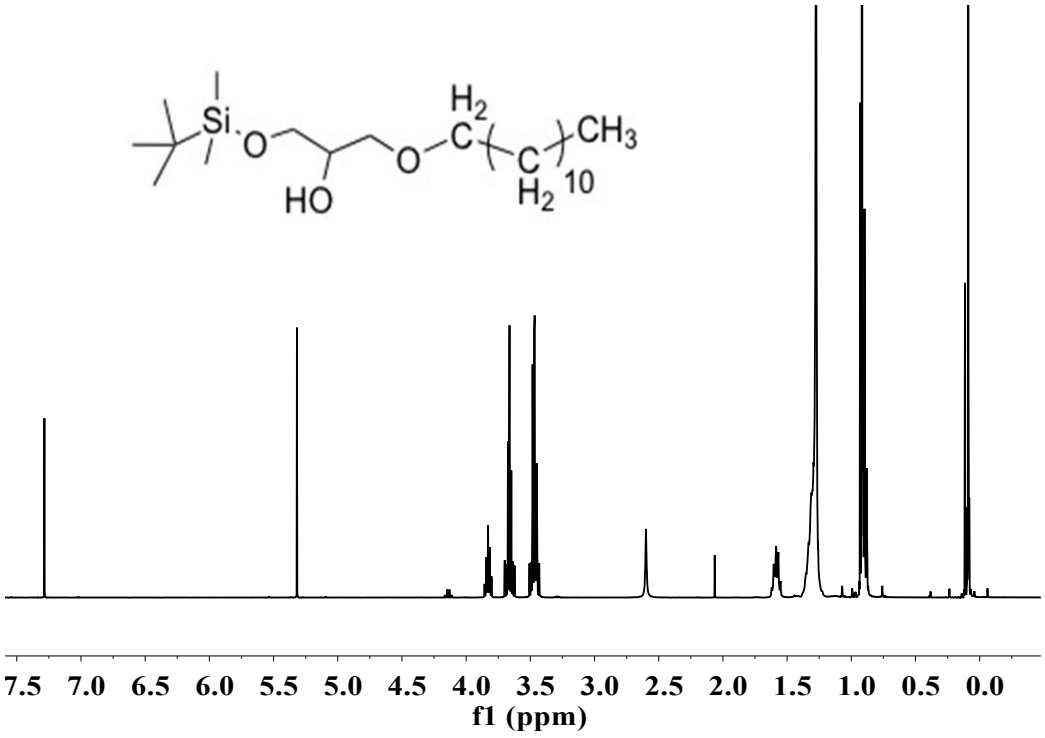
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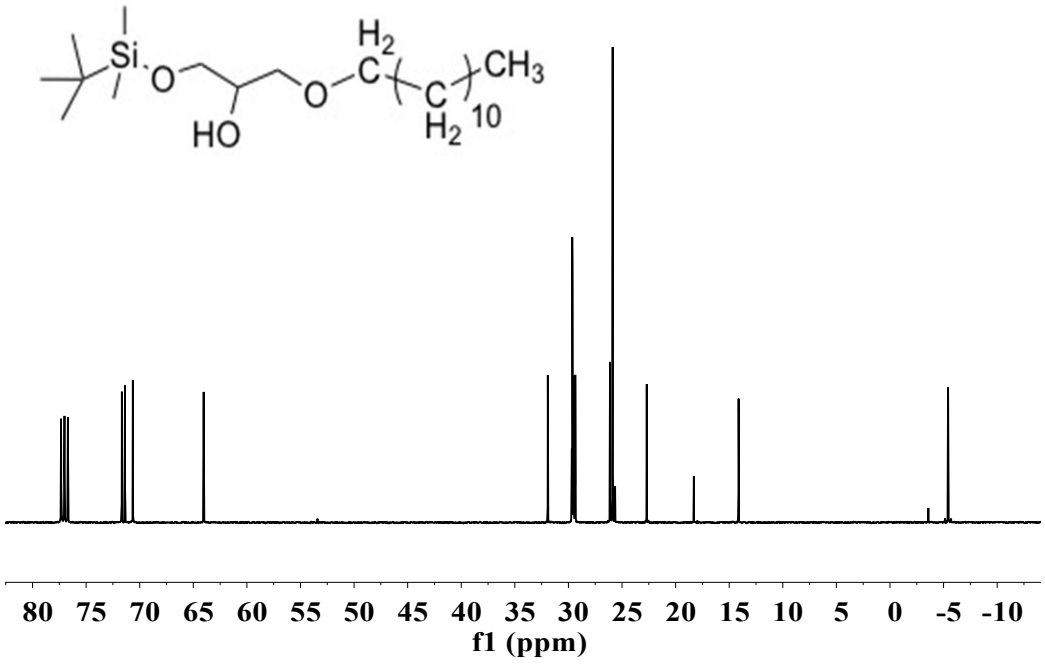
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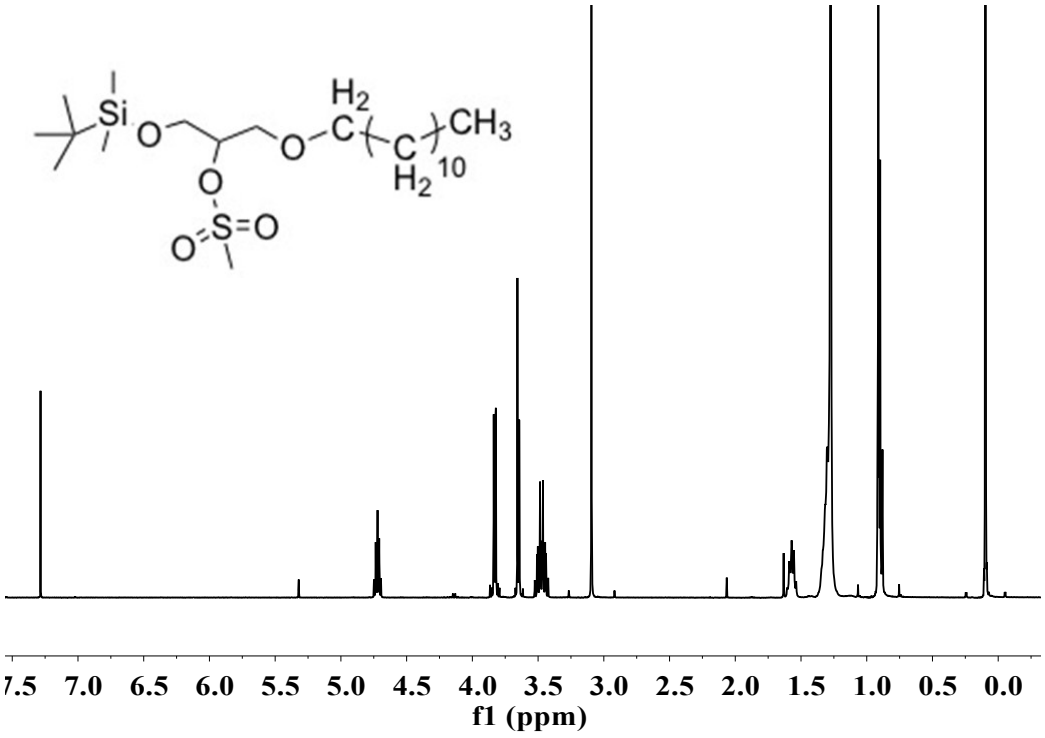
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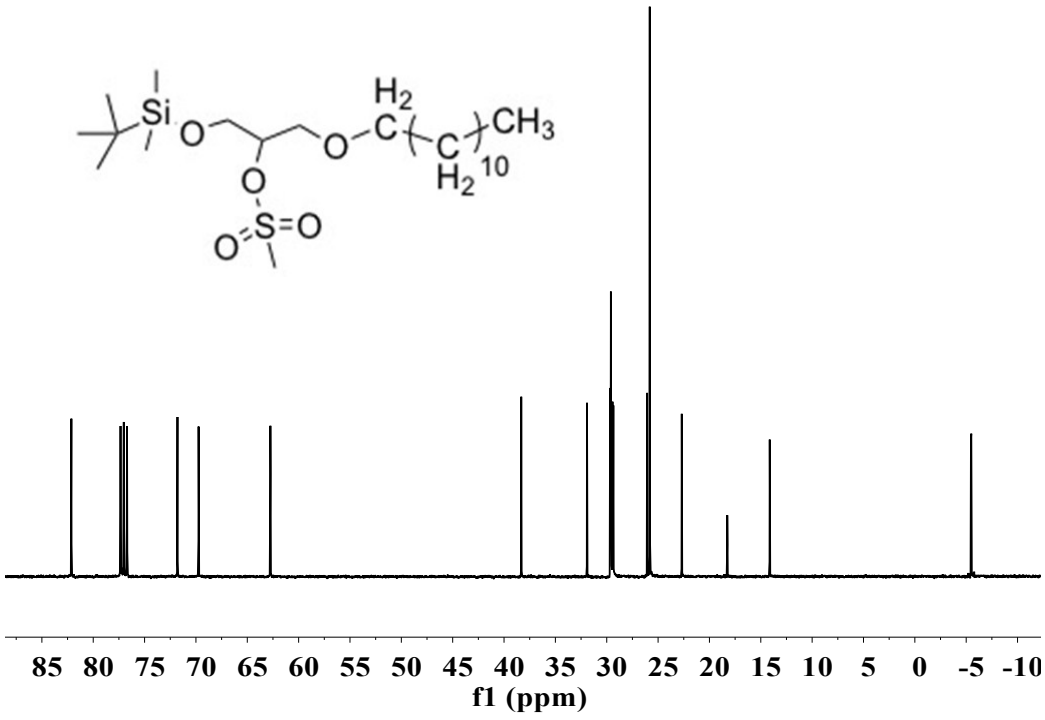
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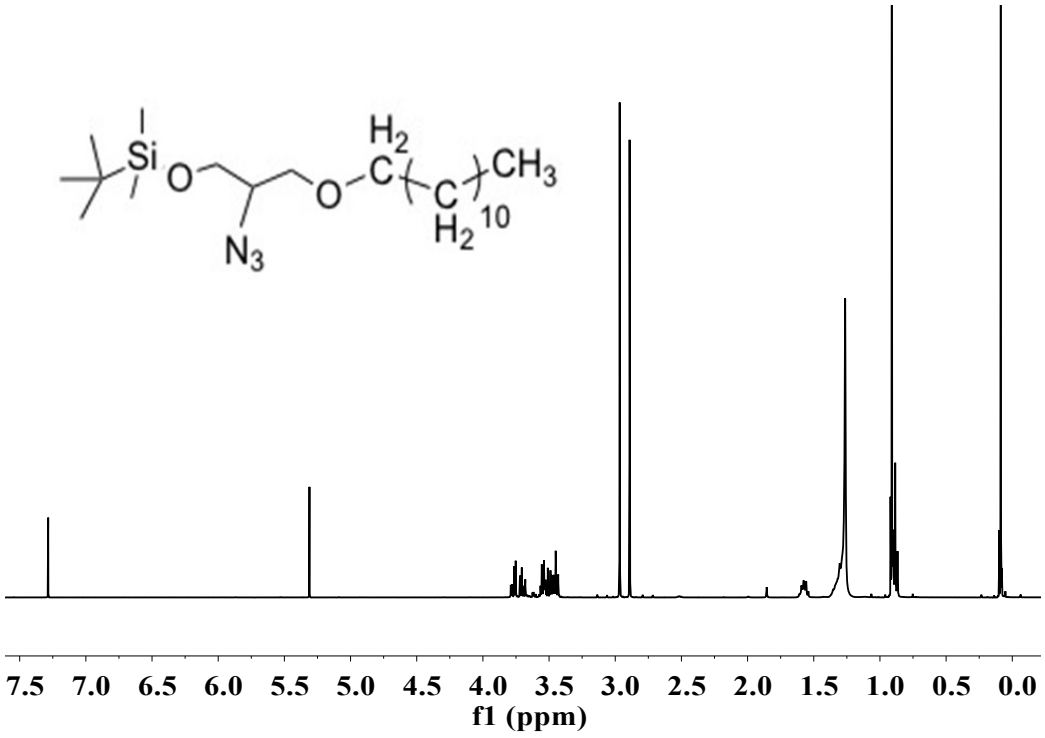
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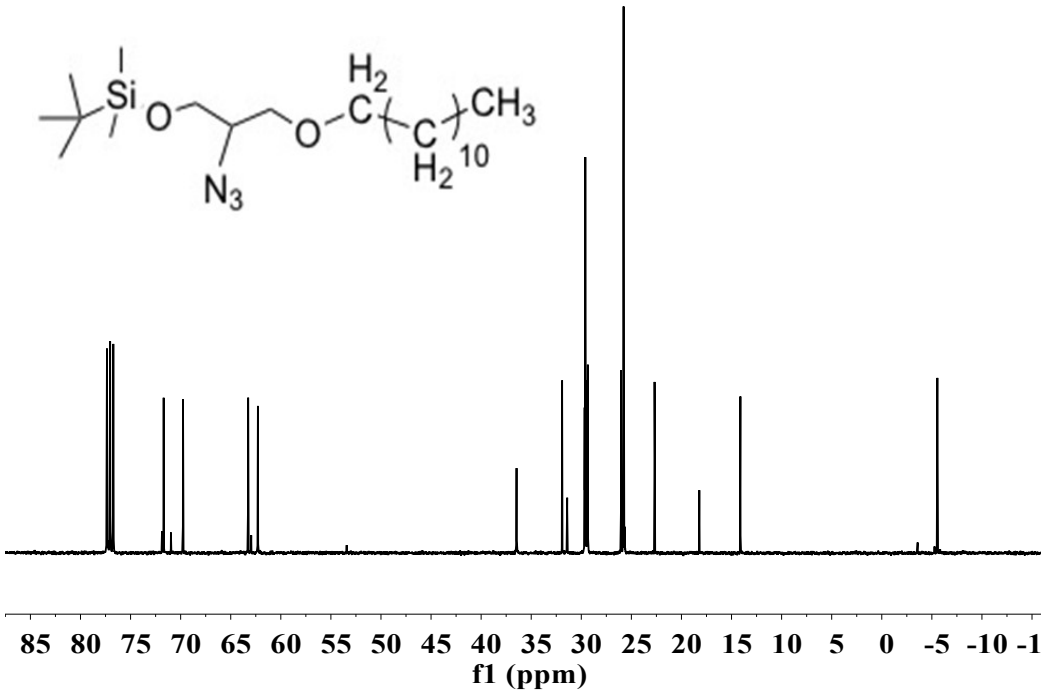
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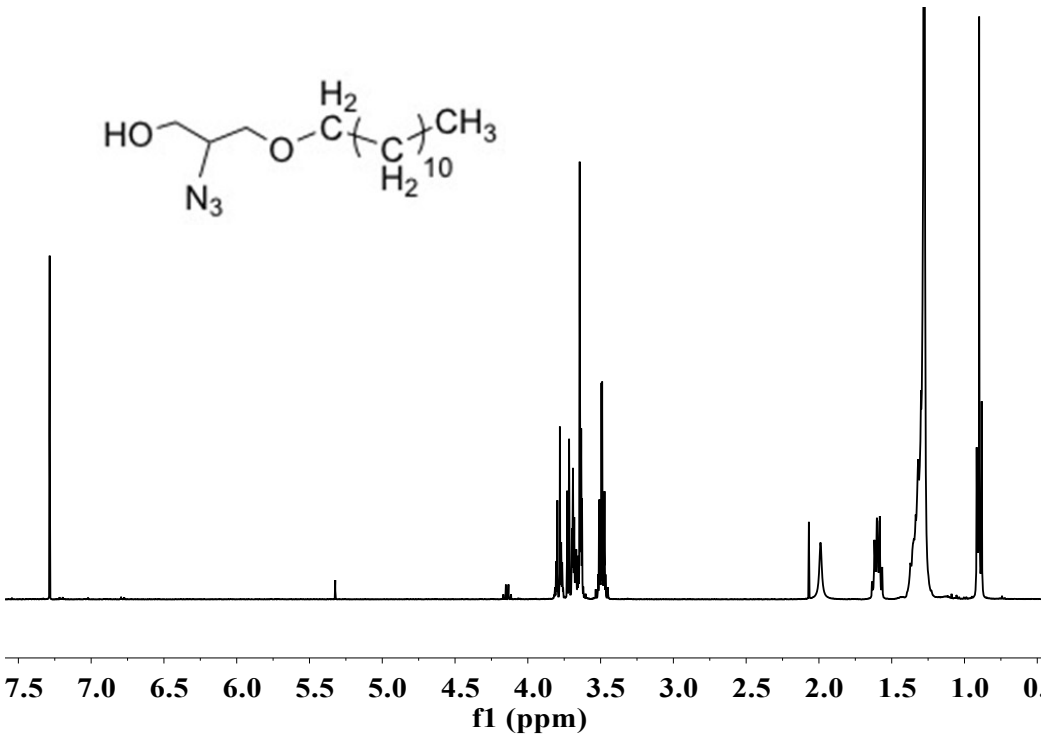
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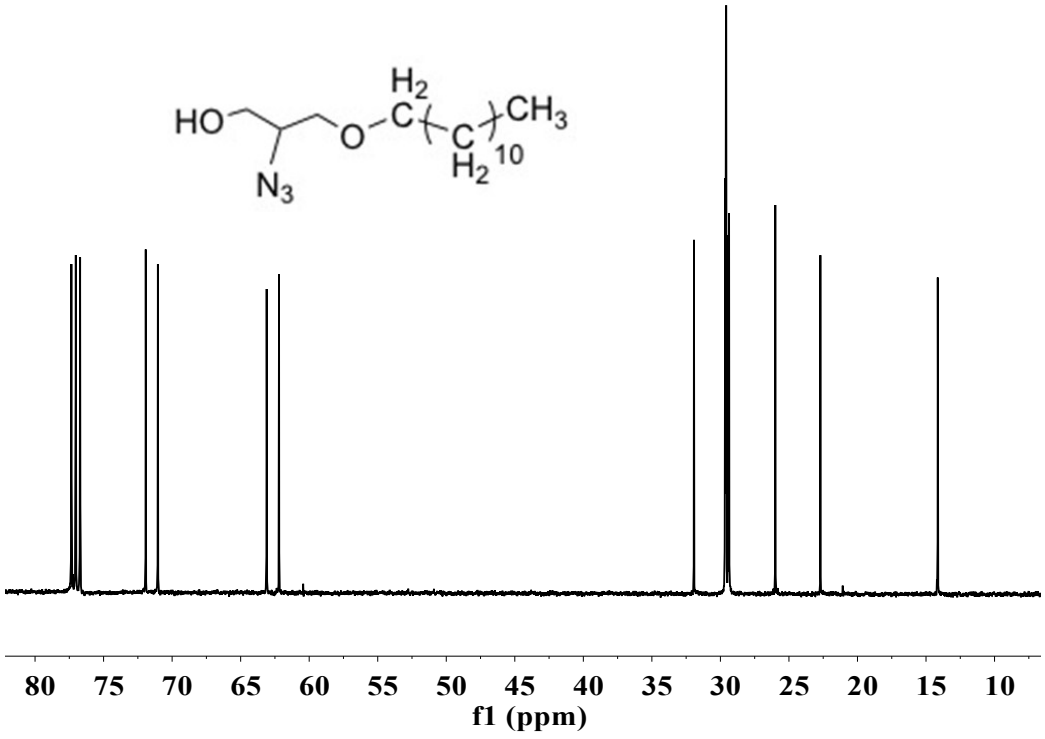
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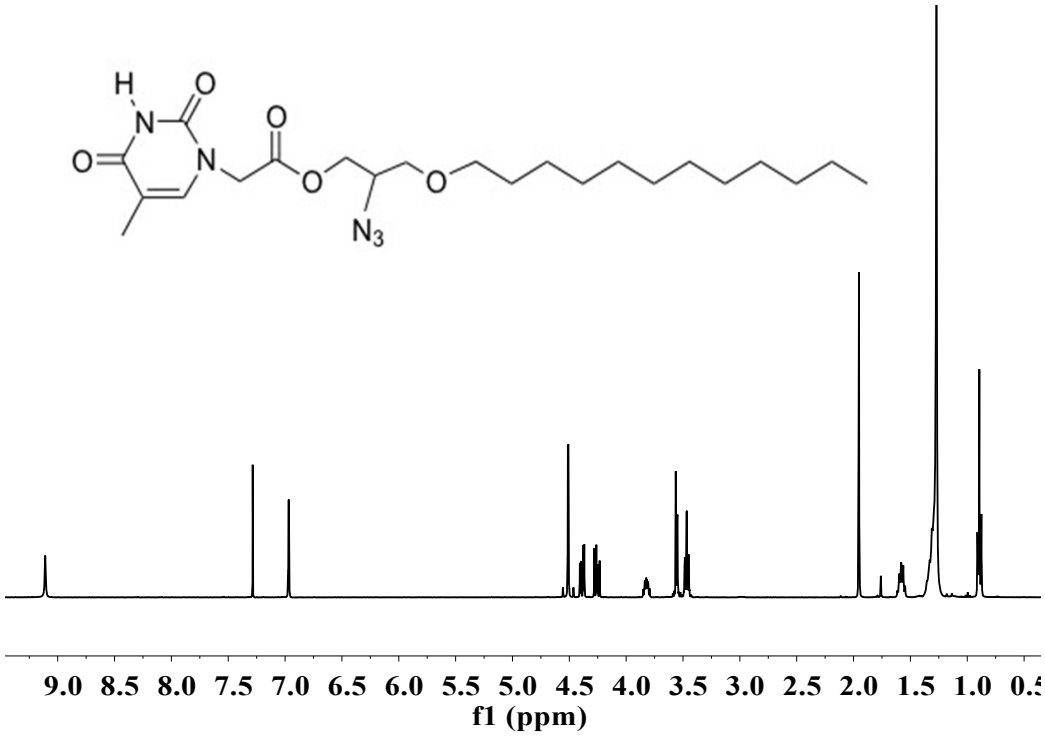
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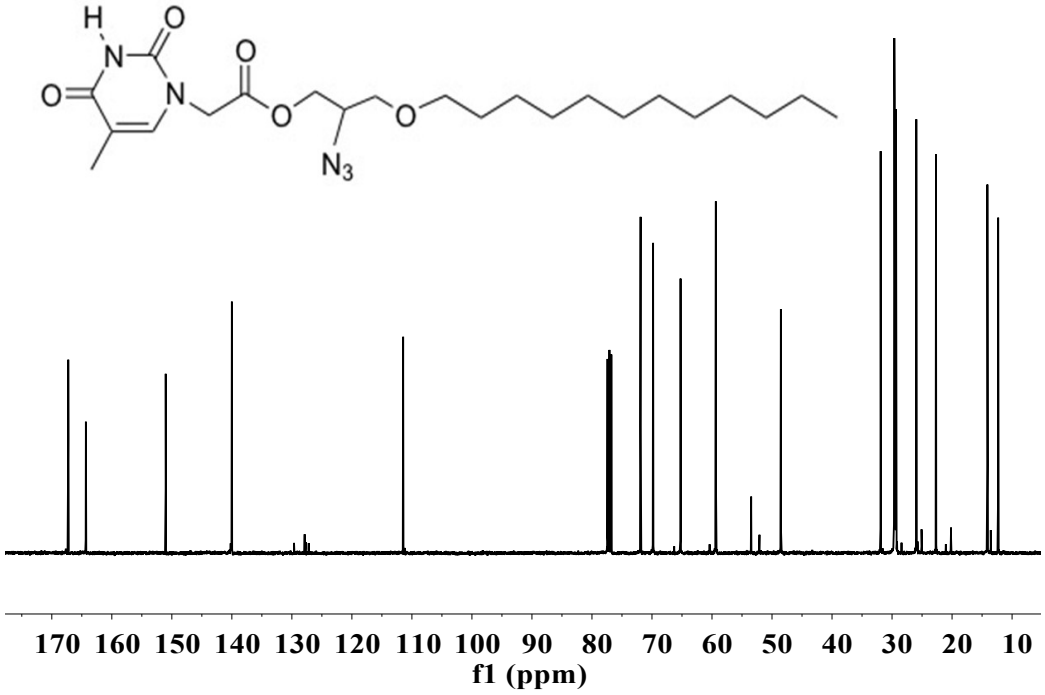
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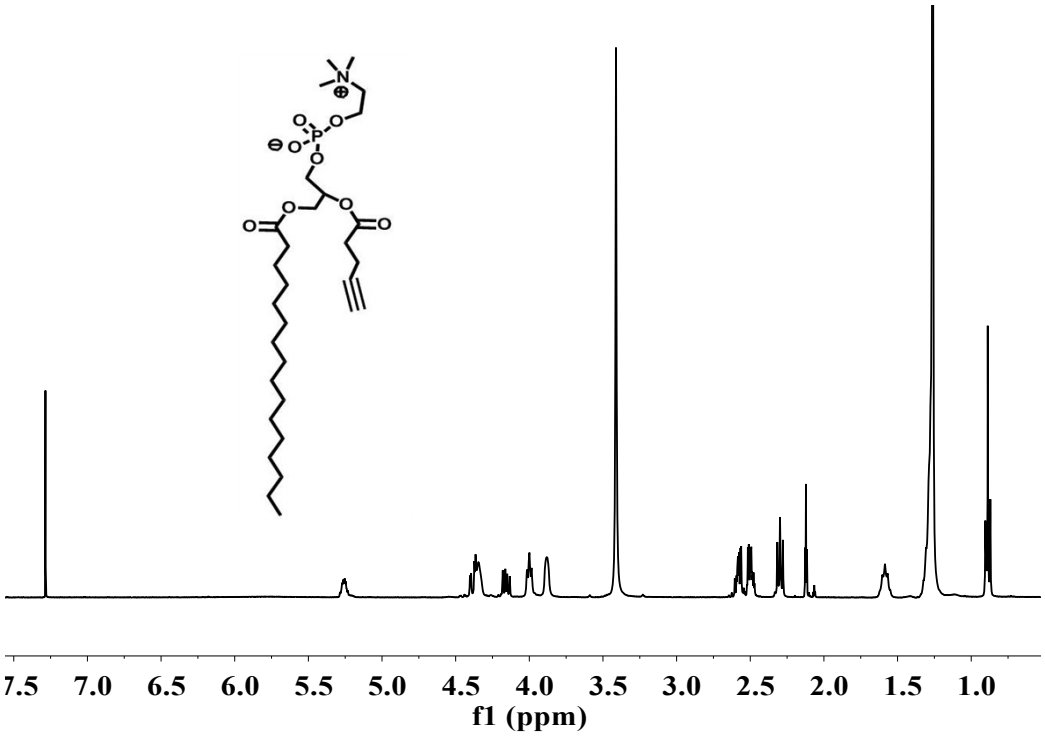
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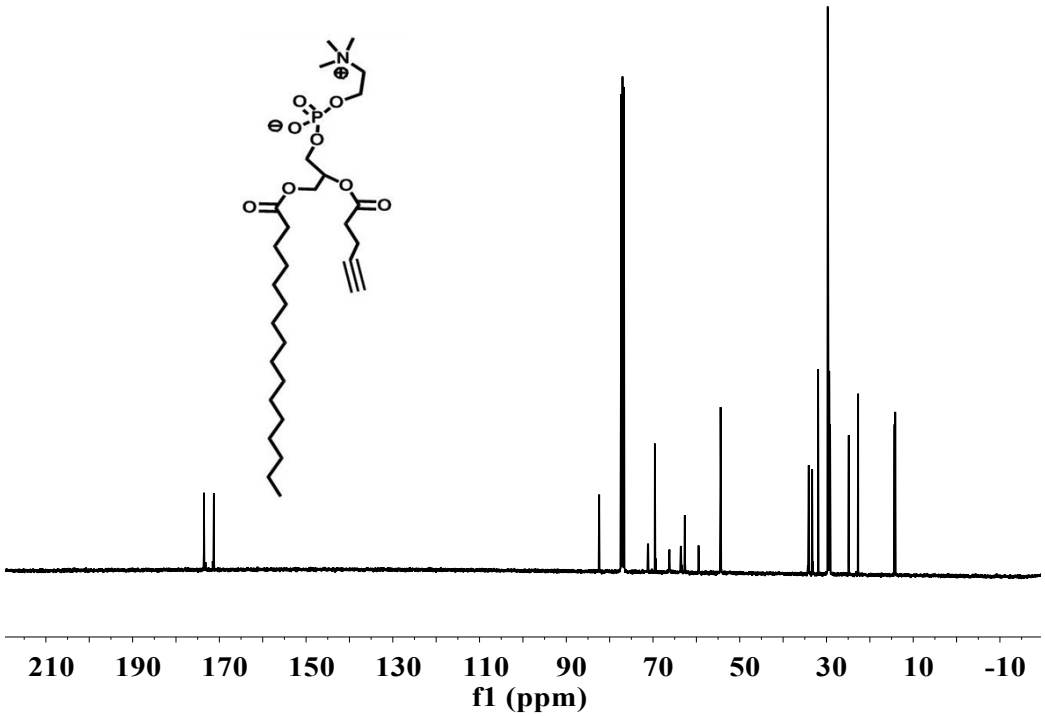
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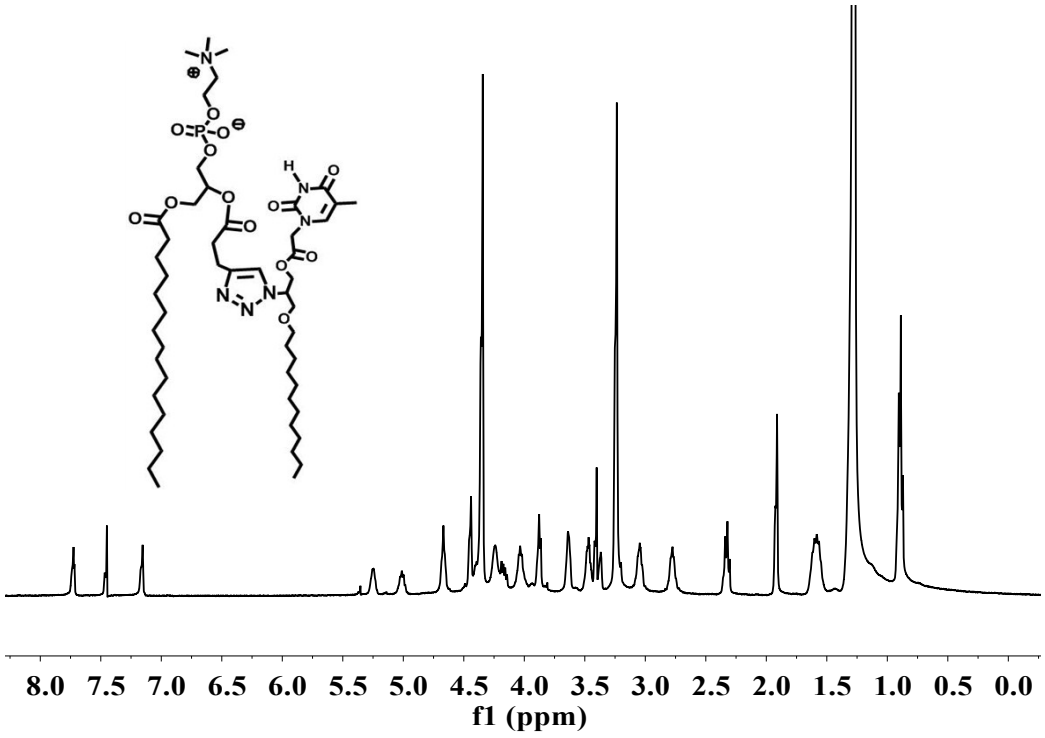
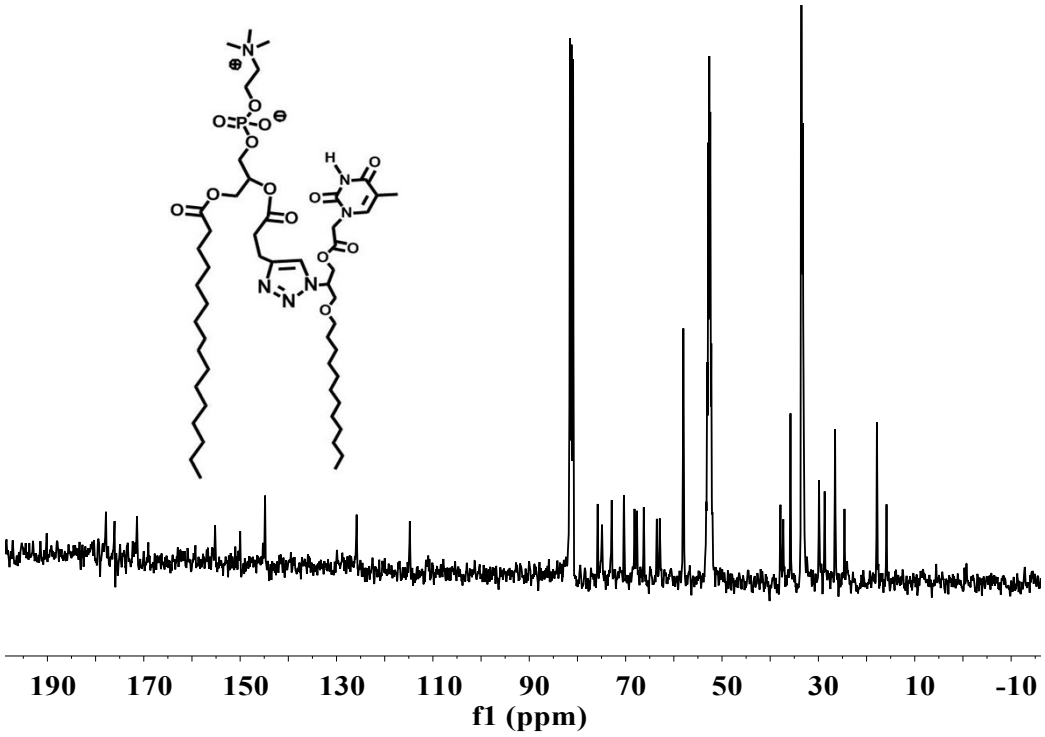

8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


190 170 150 130 110 90 70 50 30 10 -10
f1 (ppm)