Supporting Information

Photo-responsive liposome composed of spiropyran-containing triazole-phosphotidylcholine: Investigation of merocyanine-stacking effect on liposome-fiber assembly-transition

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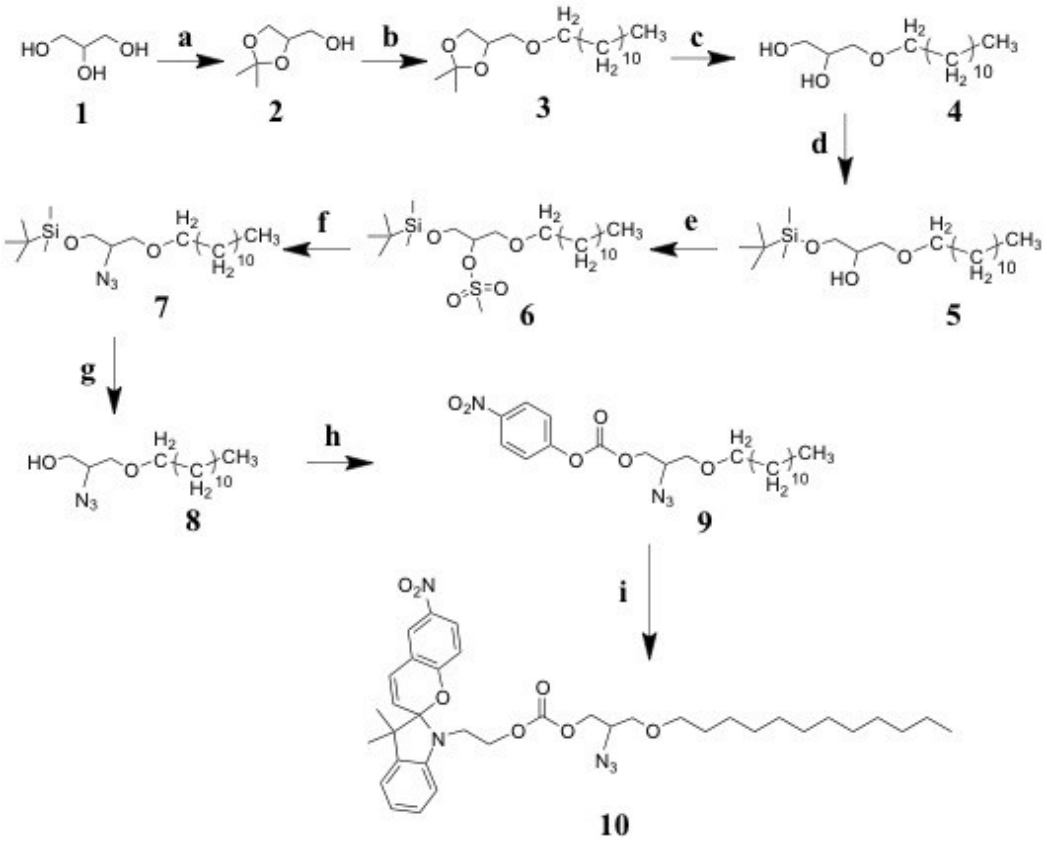
Contents

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 spectra were recorded on Bruker Avance-III 400 spectrometer. Fourier Transform Infrared (FTIR) spectra were recorded on a Nicolet 670 FT-IR spectrometer. Ultraviolet-visible spectra were recorded on a UV-vis spectrometer (Thermo-Fisher Scientific). Cryo-TEM imaging was carried out on FEI Tecnai F30, 300kV FEG-TEM. Liquid crystal phase was observed using Olympus optical microscopy equipped with crossed polarizer, lamda plate and heating plate. Fluorescence images were recorded on a Zeiss Axiovert 200m Wide-field inverted microscope equipped with an EMCCD. Fluorescence microscope with photo-patterning setting was used for selective UV-exposure area. 377 nm laser (8 mW output power) was applied as UV-light source. Fluorescence images were recorded by a Nikon 5300 camera.

2.Experimental

2.1 Synthesis of spiropyran-containing azide precursor



Step a: 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-hour 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)and bromododecane (49.8 g, 0.2 mol) were mixed in round bottle. Into the bottle were added 200mL 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 DCMwas 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 200mL 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-butyldimethylsilyl chloride (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) isopropyl methanesulfonate **(6)**
Methanesulfonate chloride (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) propoxyl dodecane **(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 CH_2Cl_2 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 and step i: spiropyran-containing azide precursor **(10)**
The DCM solution of compound 8 (1 g, 0.0035 mol) and DMAP (0.488 g, 0.004 mol) was added dropwise into DCM solution of 4-nitrophenyl chloroformate (0.806 g, 0.004 mol). The reaction was stirred for two hours and DCM was removed under vaccum. The carbonate intermediate was purified by a silica flash column and was immediately used in next step. DCM solution of N-hydroethyl spiropyran (0.704 g, 0.002 mol) and DMAP (0.024 g, 0.0002 mol) was droplet into carbonate intermediate in DCM. The reaction was stirred overnight. After washed with water and brine, the crude product was purified by a silica flash column. The pure compound 10 was obtained as light yellow solid.

2.2 Synthesis of alkyne lysolipid
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDAC.HCl, 310 mg, 1.616 mmol) was dissolved in dichloromethane, and triethylamine (163 mg, 1.616 mmol) was added. 4-pentynoic acid (158 mg, 1.616 mmol) and 4-dimethylaminopyridine (5 mg, 0.04 mmol) were added with stirring. The mixture was stirred for 20 minutes, and hydroxy-lysolipid (200 mg, 0.404 mmol) was added. After stirring for 12 hours, dichloromethane was completely removed under low pressure, and remaining slurry compound was dissolved into water and transferred into dialysis bag (cutting off MW=2000). After dialysis against water for 24 hours (outside water was changed every 6 hours), the inside aqueous solution was frozen and lyophilized to remove water. The product was a white solid. The impure product was a yellow viscous lipid, and a C18 column was applied to purify the impure product. Eluent was composed of methanol and water. After loading the crude product, 60 v% methanol was used to elute for 4 bed volumns, and a gradient elution was applied from 40 v% to 100% methanol. Product was eluted out when the methanol v% reached 100 v%.

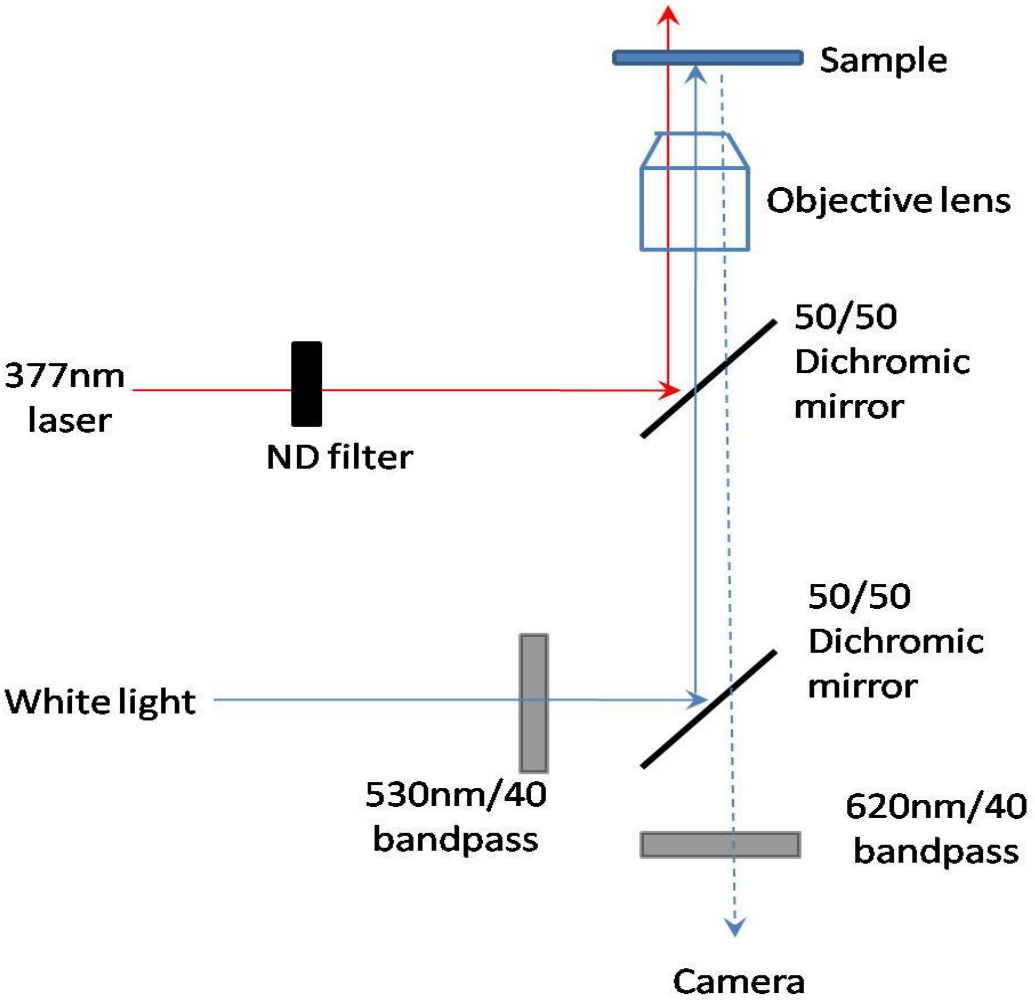


Fig. S1 Light-path of fluorescence microscope with photo-patterning setting

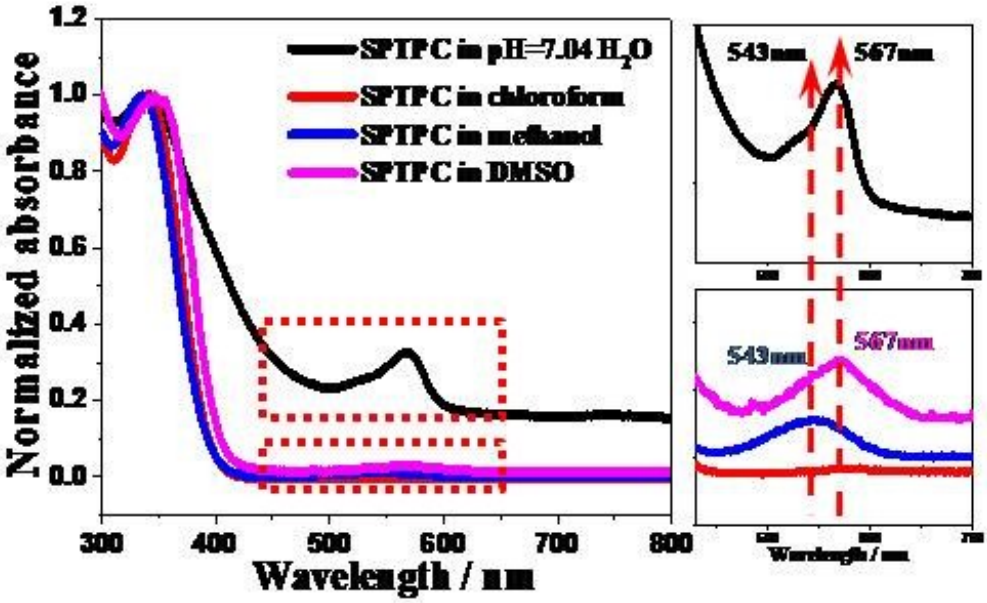


Fig. S2 Absorption spectra of SPTPC in different organic solvents and SPTPC-liposome.

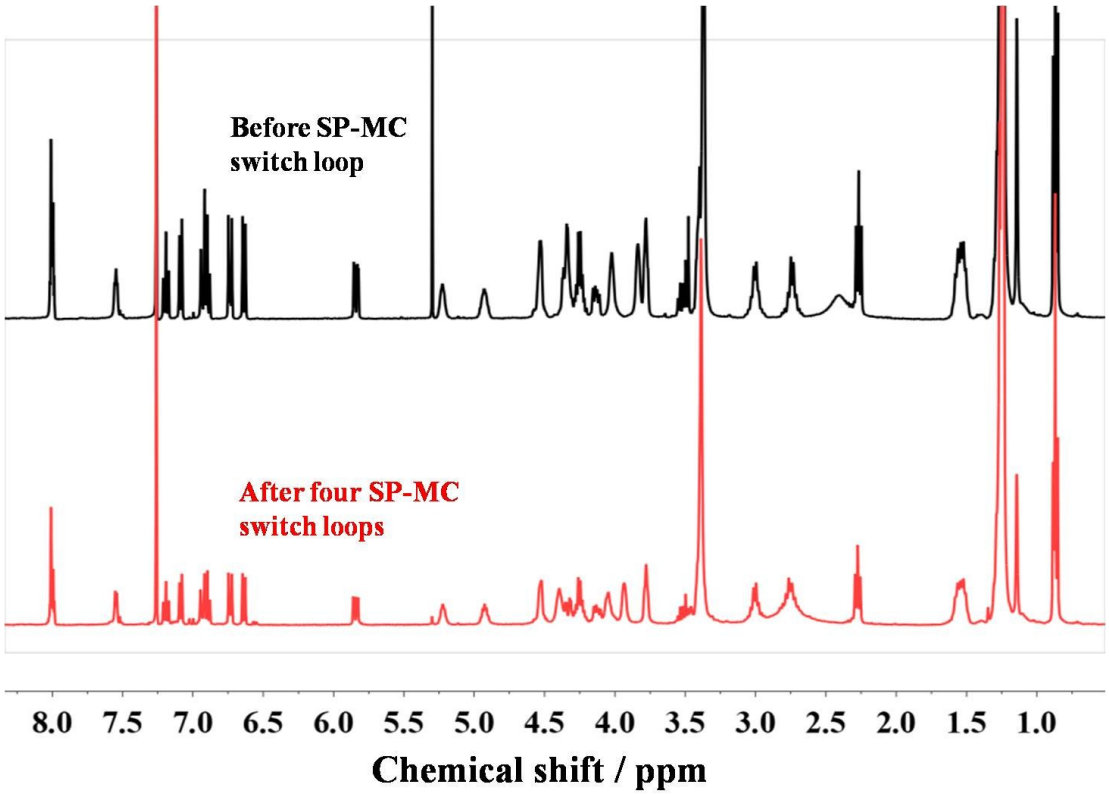


Fig. S3 NMR spectrum of SPTPC-liposome sample before and after four SP-to-MC switch loops. (samples were lyophilized and dissolve in $CDCl_3$)

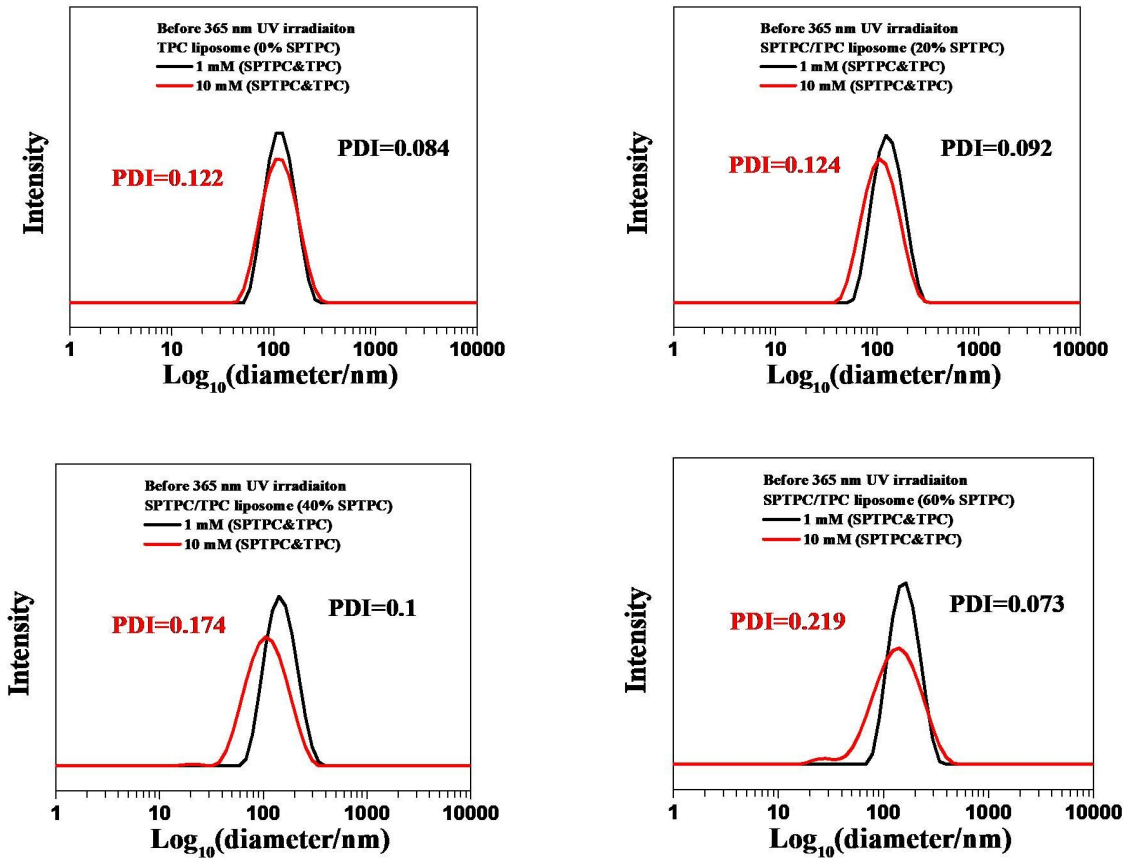


Fig. S4 Before 365 nm UV irradiation, comparison of size distribution curve of SPTPC/TPC liposomes (sonicated&extruded) with different total phospholipid concentration.

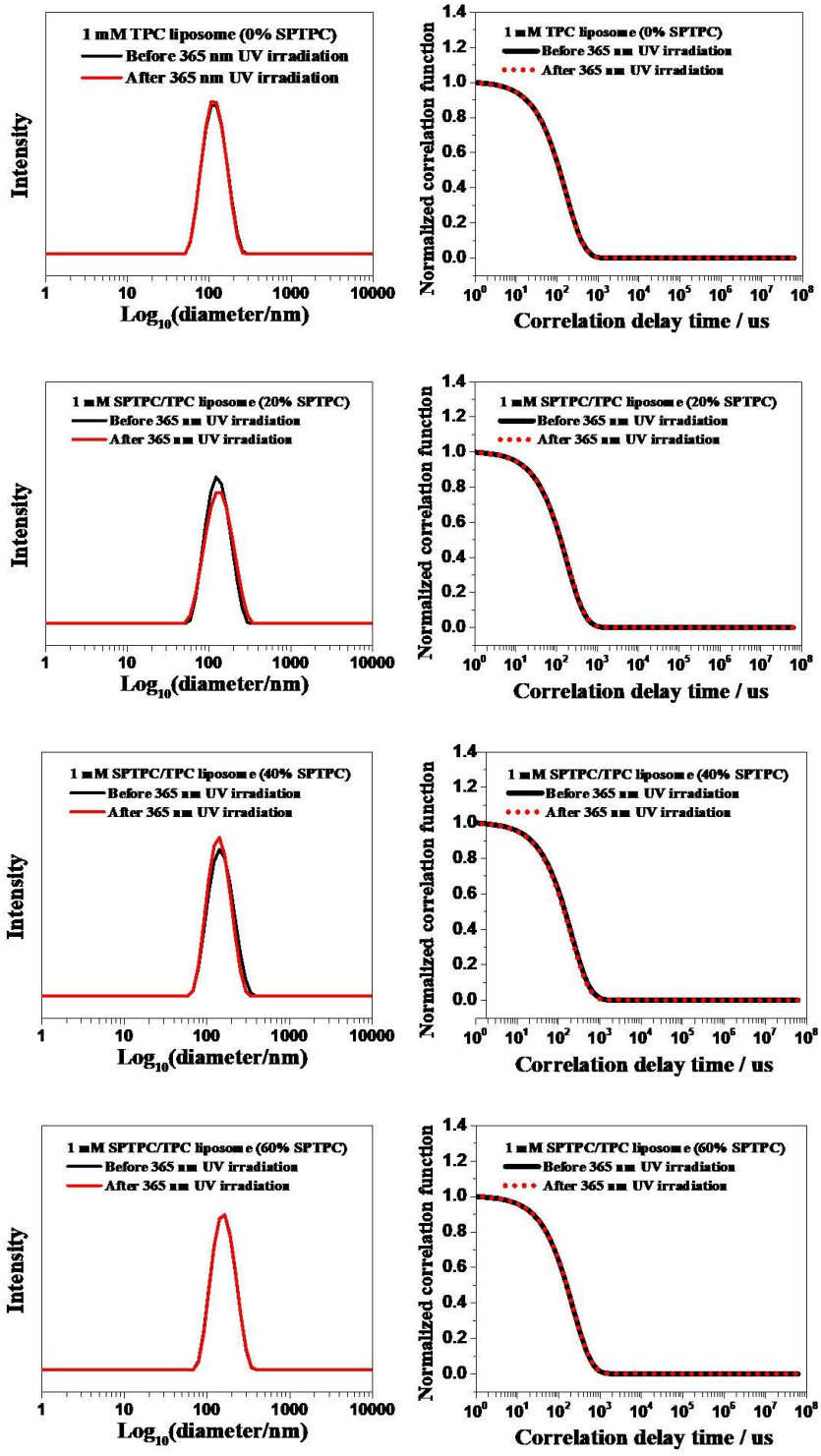


Fig. S5 Before and after 365 nm UV irradiation, comparison of DLS data of 1mM SPTPC/TPC liposomes (sonicated&extruded) with different SPTPC%.

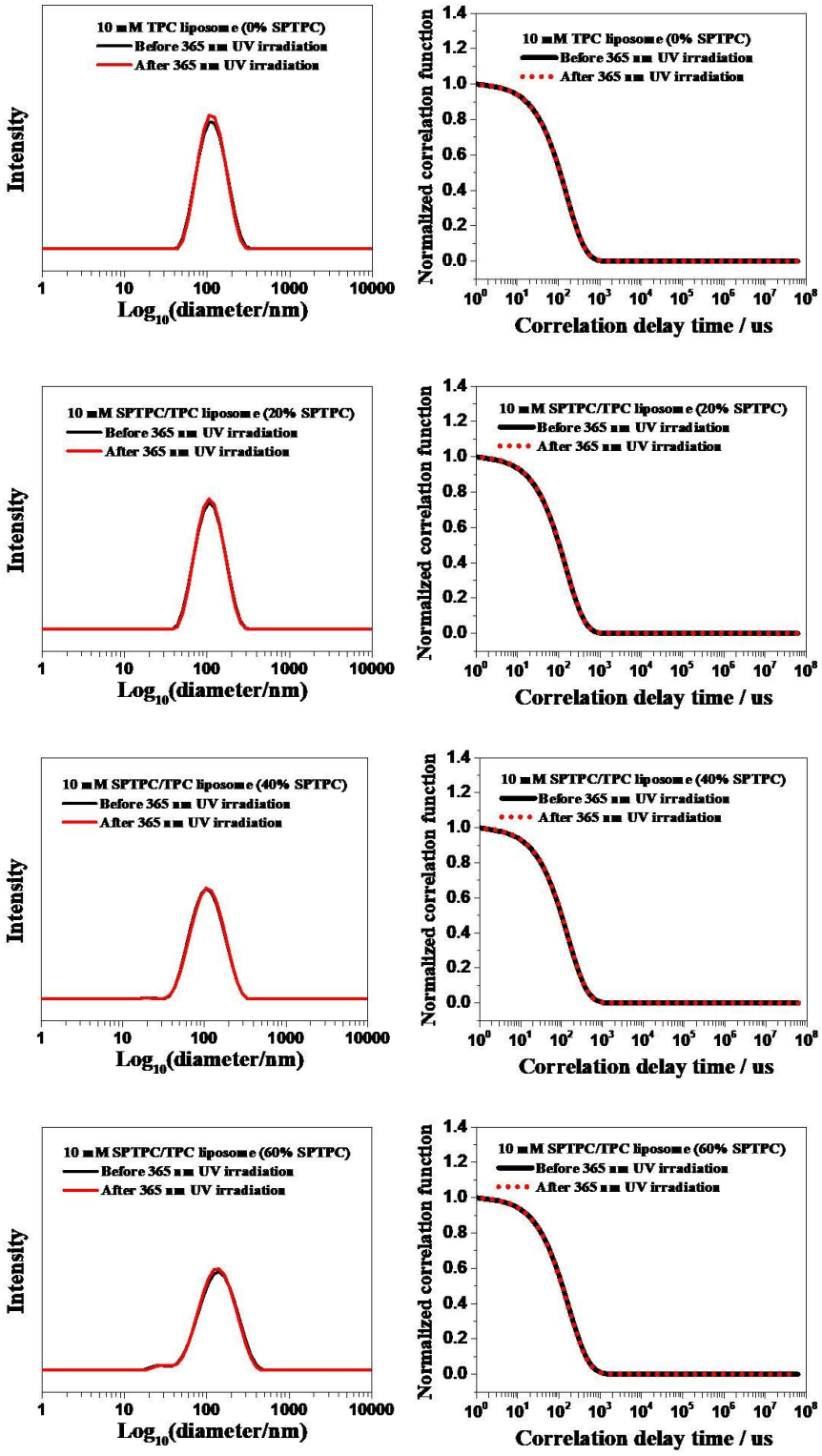


Fig. S6 Before and after 365 nm UV irradiation, comparison of DLS data of 10mM SPTPC/TPC liposomes (sonicated&extruded) with different SPTPC%.

Movie S1. Time lapse fluorescence microscopy of photo-inert TPC-liposome upon exposure to 365nm UV-light. Membrane staining dye (Rh-DHPE, 0.005 mM).

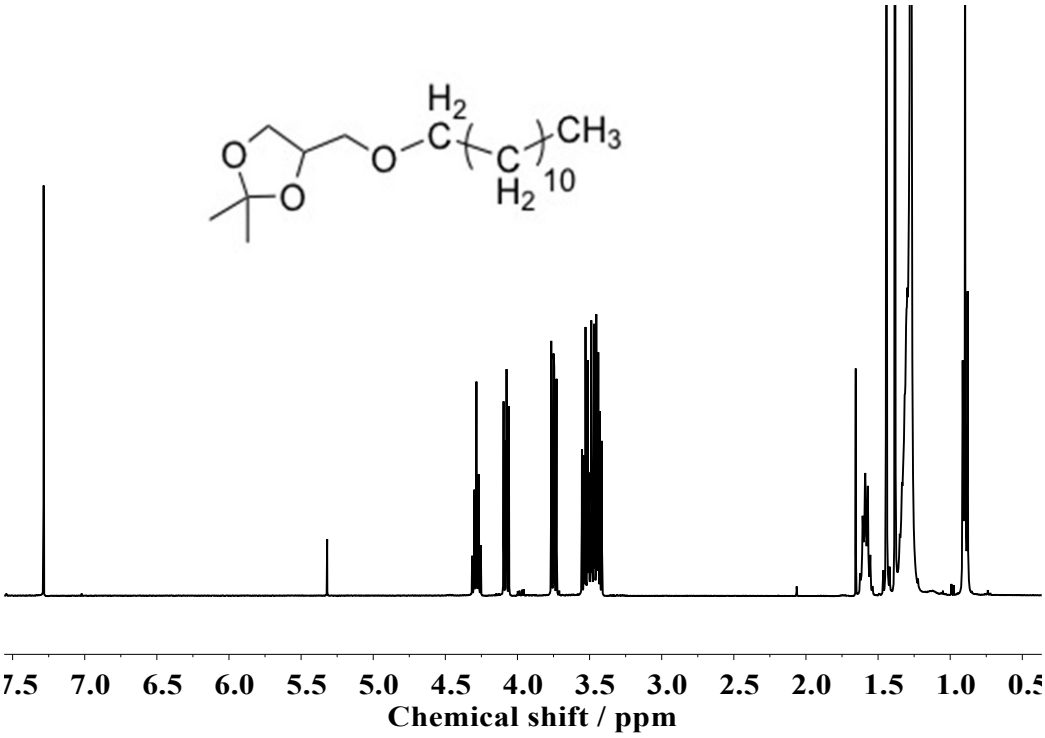
Movie S2. Time lapse fluorescence microscopy of SPTPC/TPC-liposome with **20 mol%** SPTPC upon exposure to 365 nm UV-light. Membrane staining dye (Rh-DHPE, 0.005 mM).

Movie S3. Time lapse fluorescence microscopy of SPTPC/TPC-liposome with **30 mol%** SPTPC upon exposure to 365 nm UV-light. Membrane staining dye (Rh-DHPE, 0.005 mM).

Movie S4. Time lapse fluorescence microscopy of SPTPC/TPC-liposome with **40 mol%** SPTPC upon exposure to 365 nm UV-light. Membrane staining dye (Rh-DHPE, 0.005 mM).

Movie S5. Time lapse fluorescence microscopy of SPTPC/TPC-liposome with **60 mol%** SPTPC upon exposure to 365 nm UV-light. Membrane staining dye (Rh-DHPE, 0.005 mM).

Movie S6. Time lapse fluorescence microscopy of SPTPC/TPC-liposome with **80 mol%** SPTPC upon exposure to 365 nm UV-light. Membrane staining dye (Rh-DHPE, 0.005 mM).



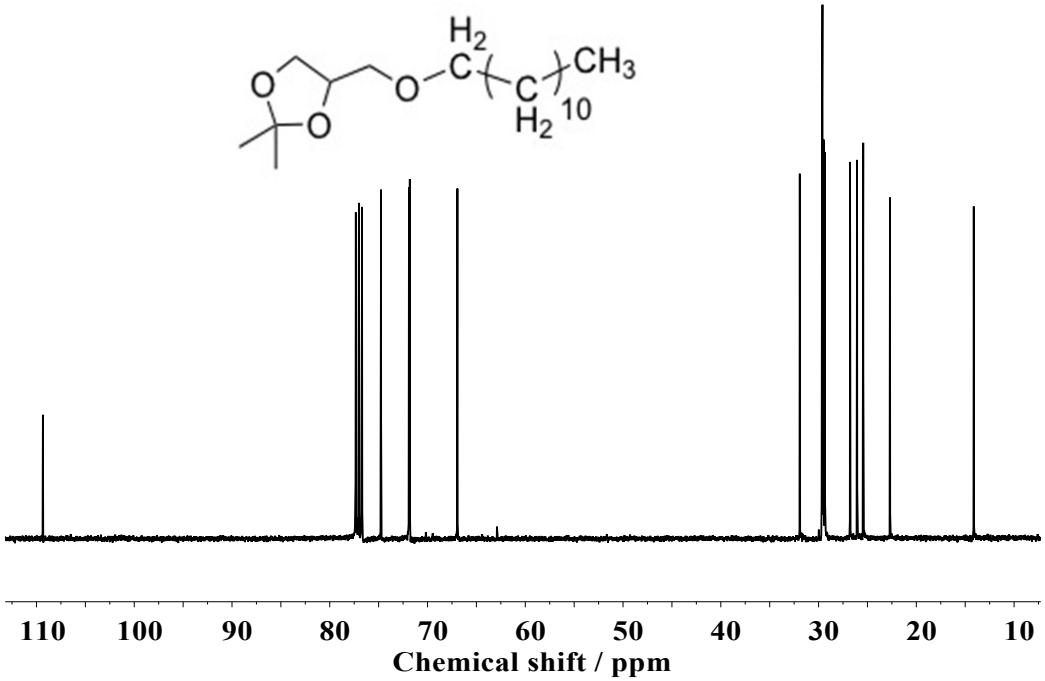
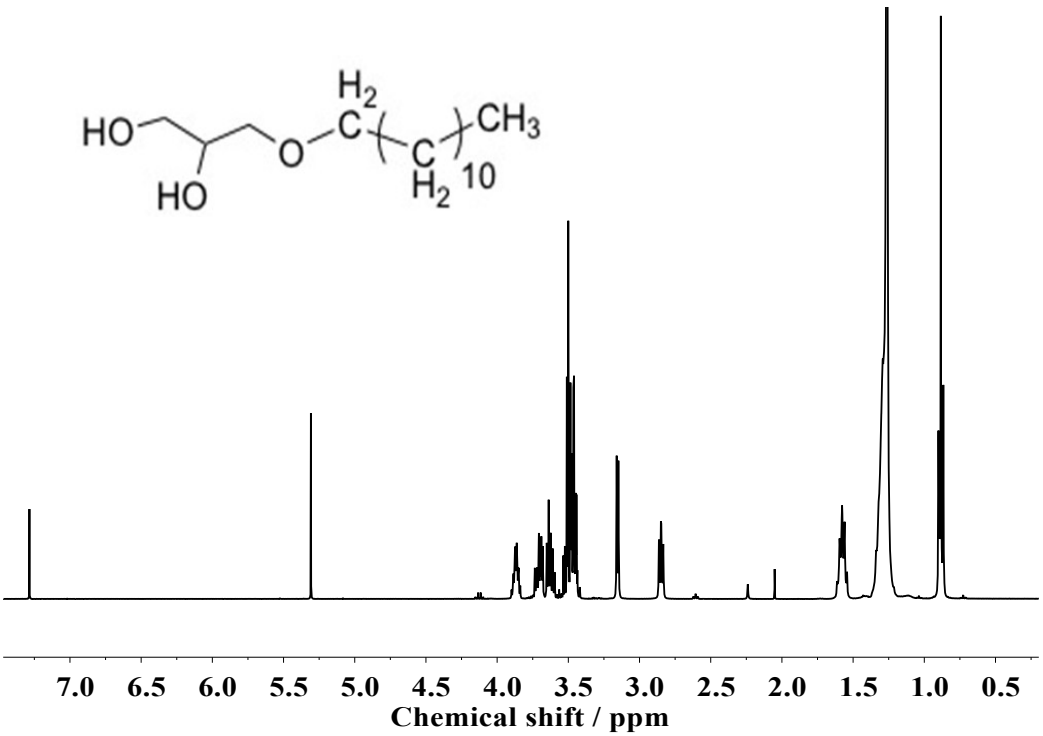


Fig. S3 ^{1}H and ^{13}C NMR spectra of compound **3**



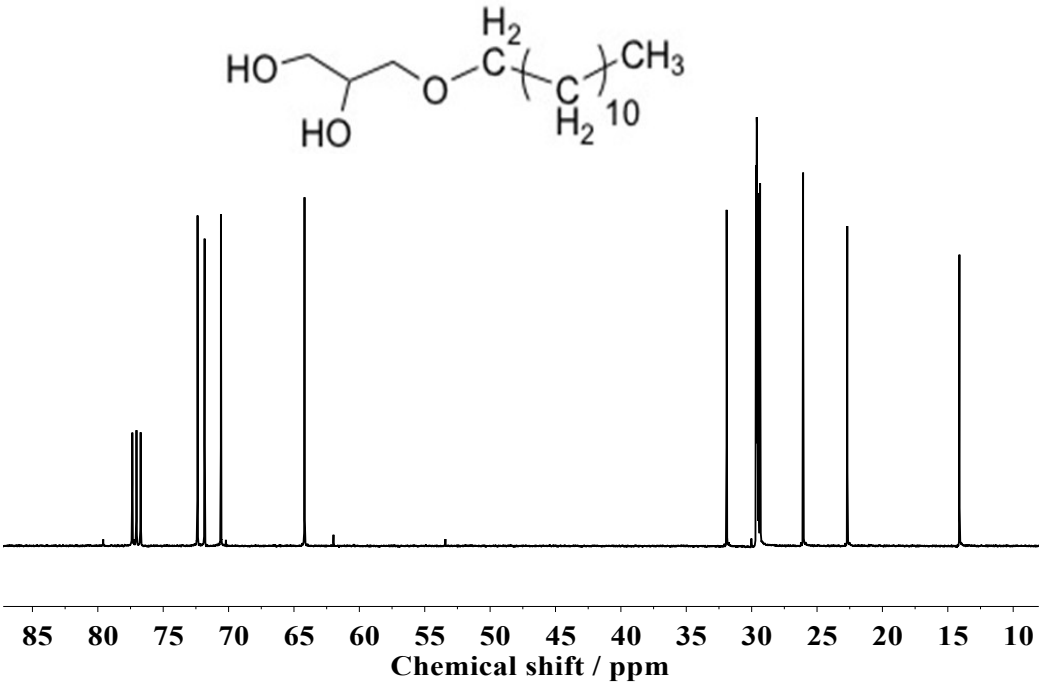
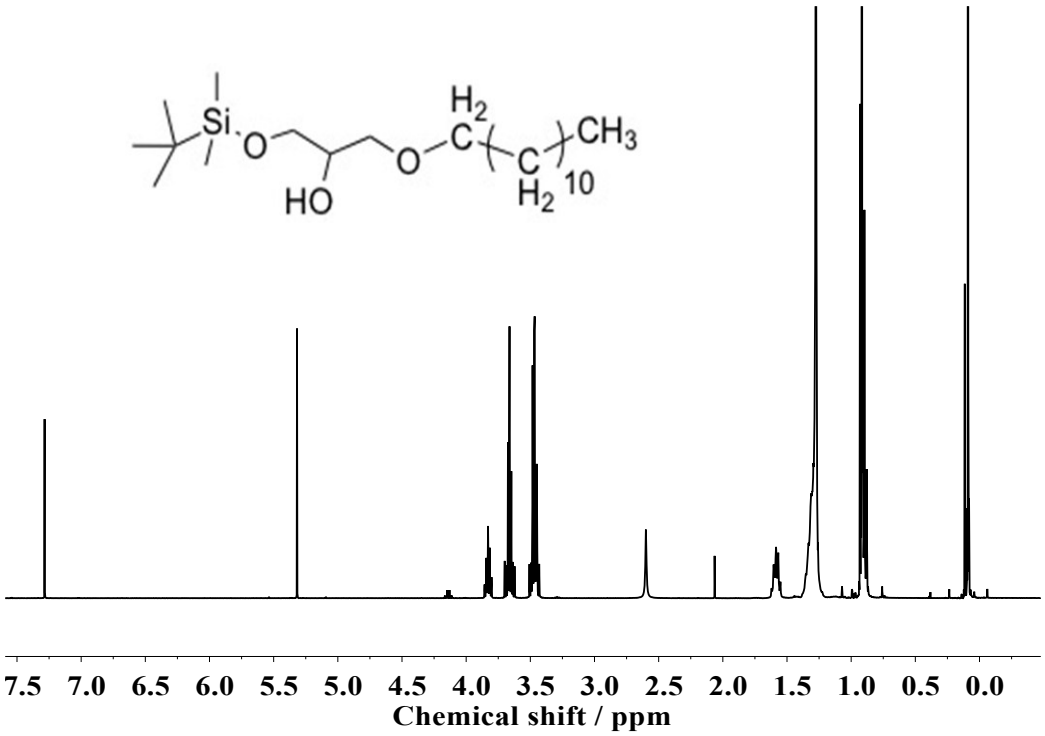


Fig. S4 ^{1}H and ^{13}C NMR spectra of compound **4**



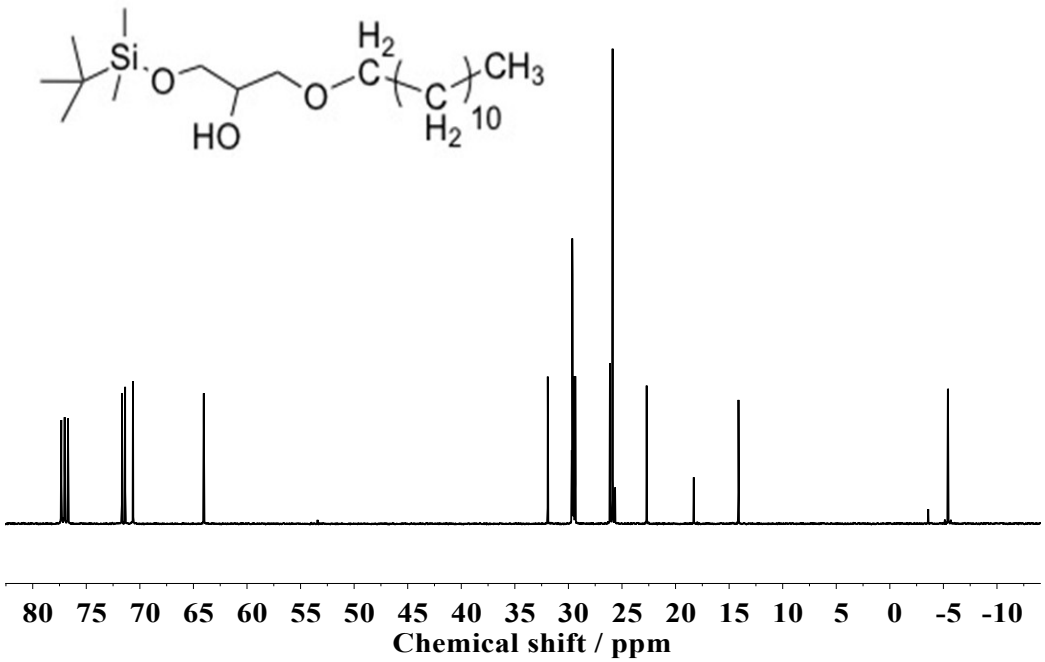
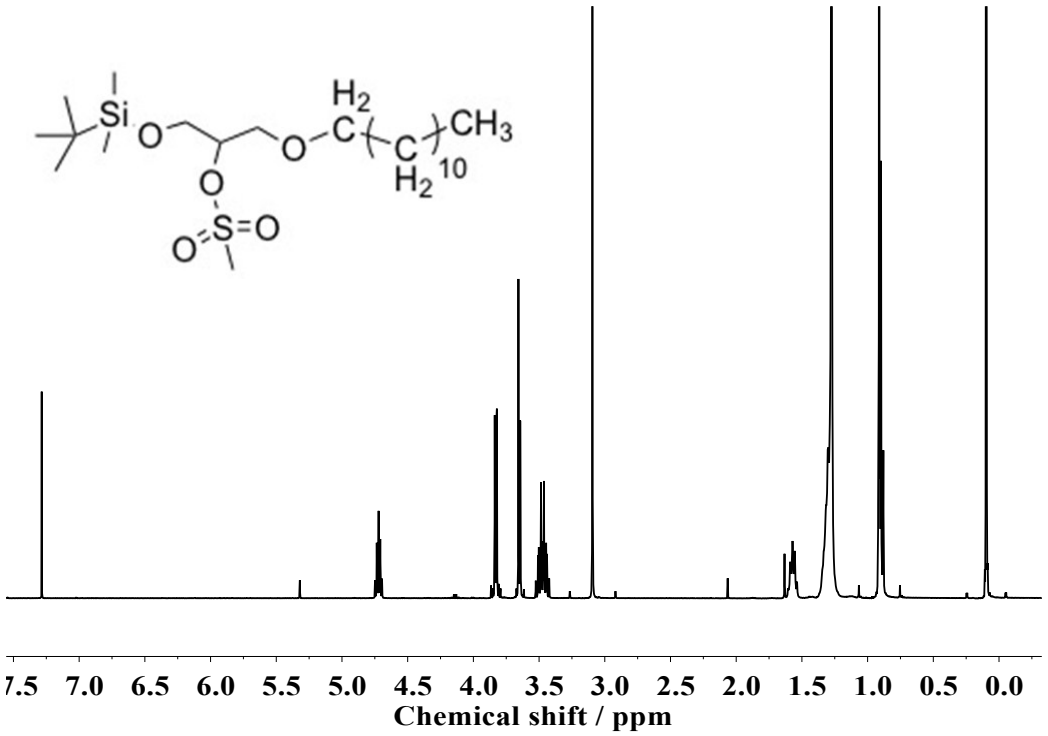


Fig. S5 ^{1}H and ^{13}C NMR spectra of compound **5**



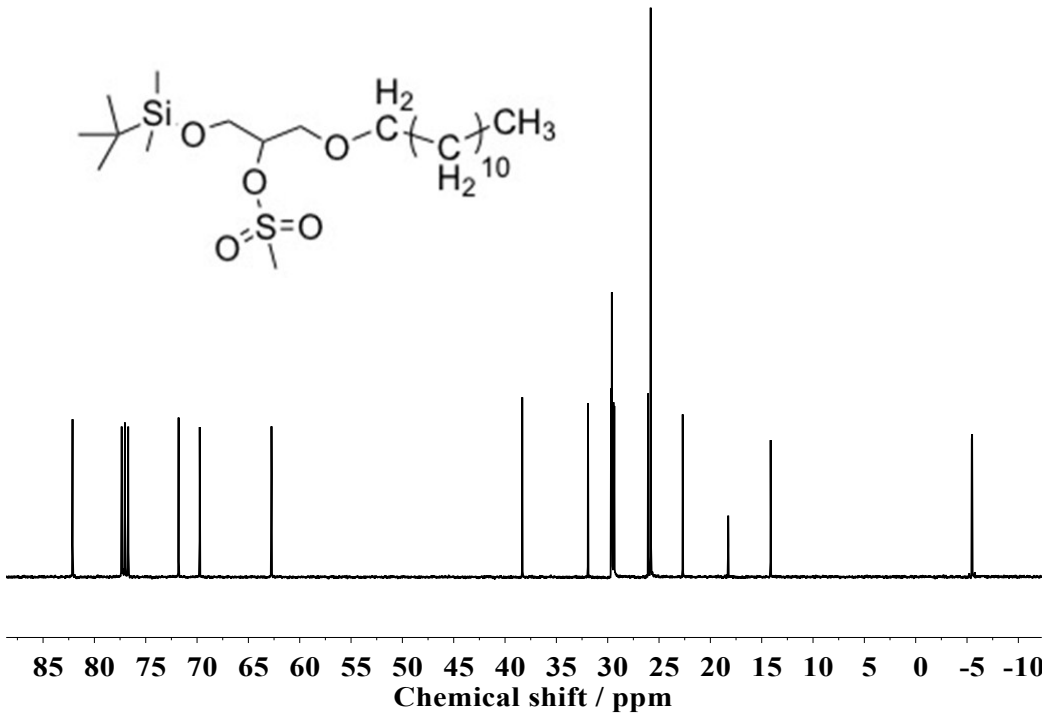
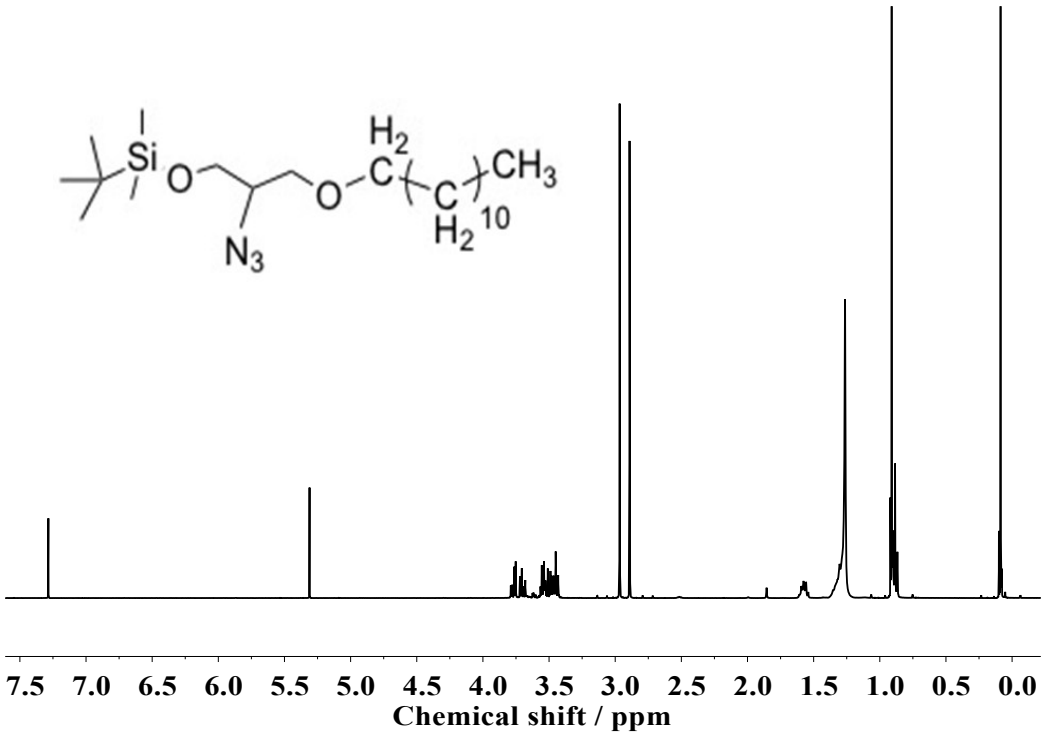


Fig. S6 ^{1}H and ^{13}C NMR spectra of compound **6**



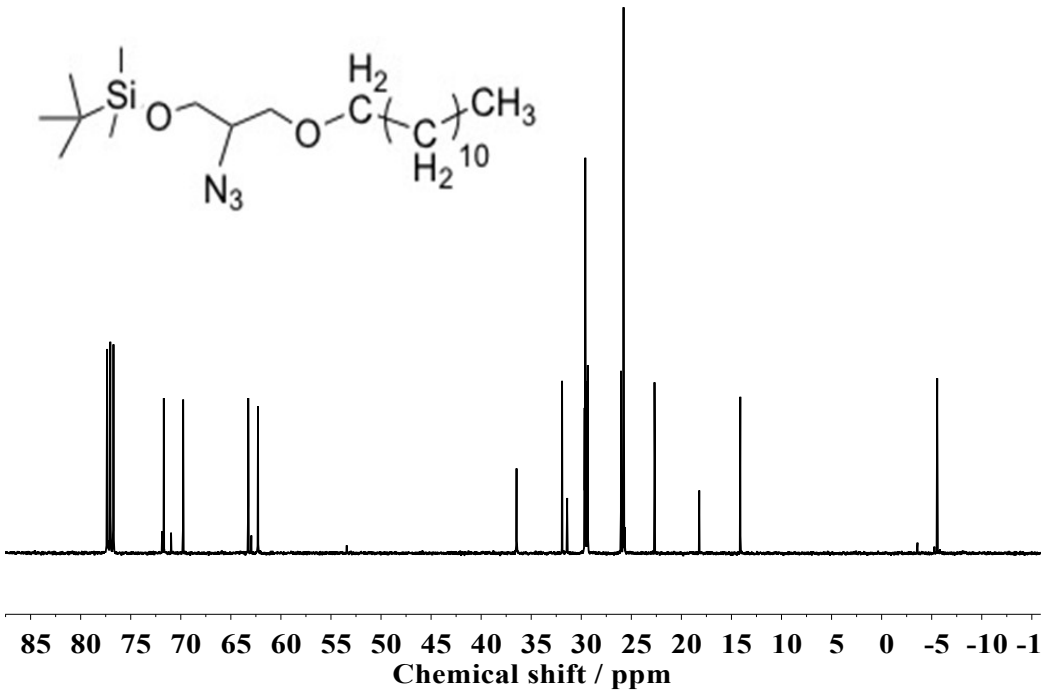
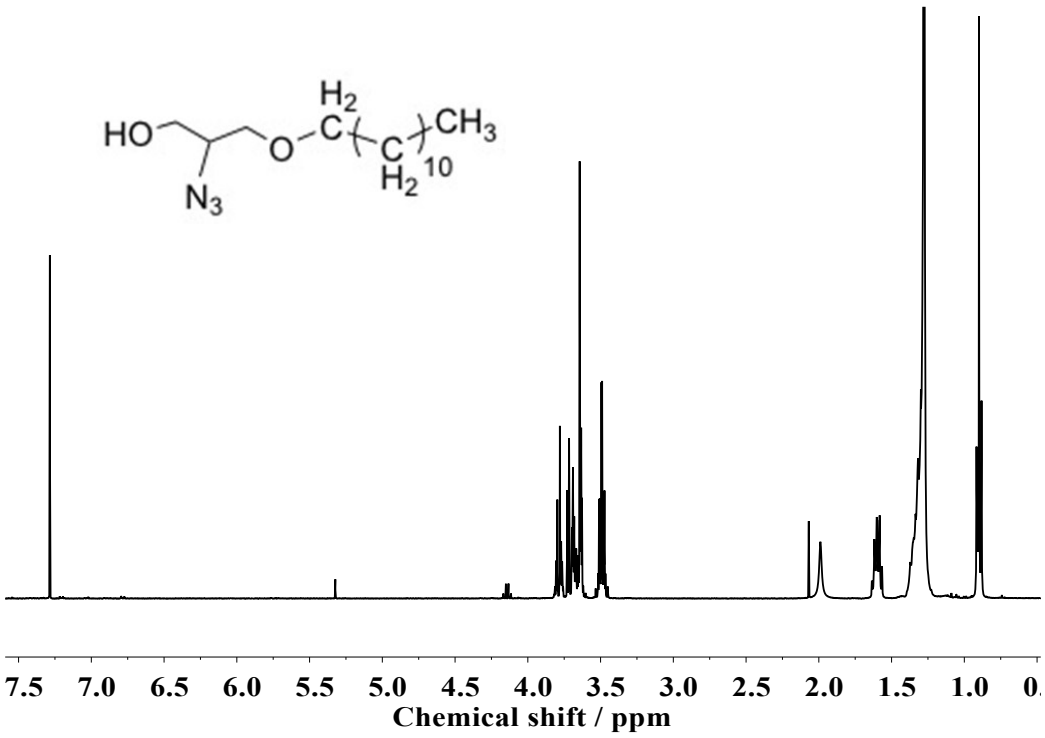


Fig. S7 ^{1}H and ^{13}C NMR spectra of compound **7**



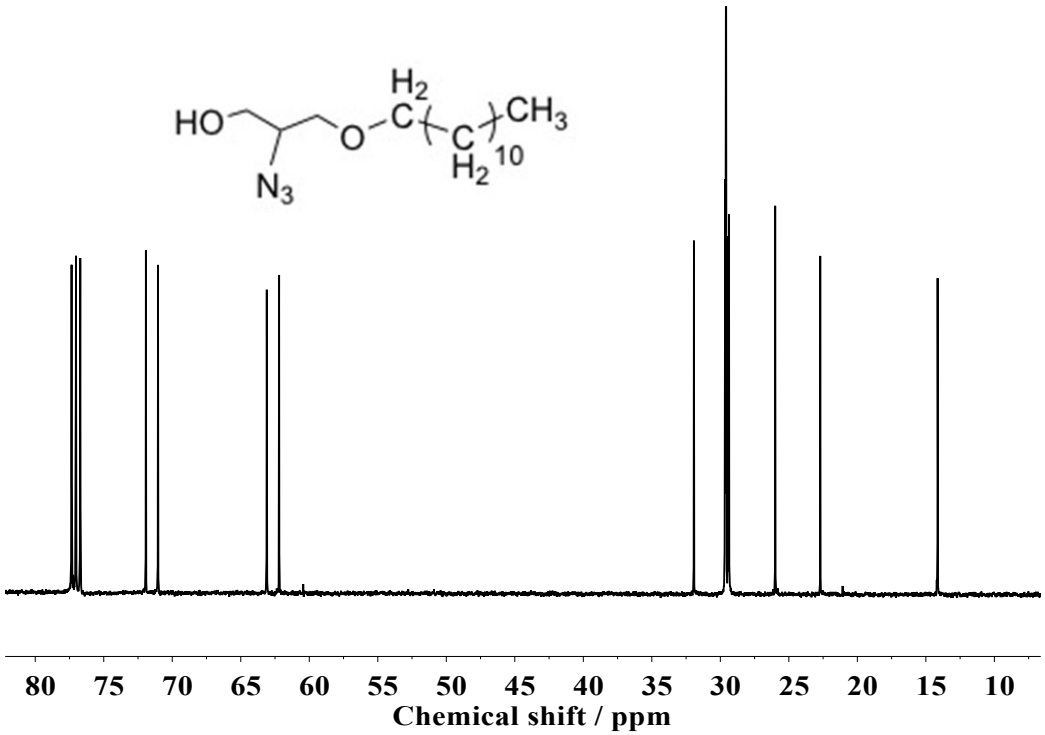


Fig. S8 ^{1}H and ^{13}C NMR spectra of compound **8**

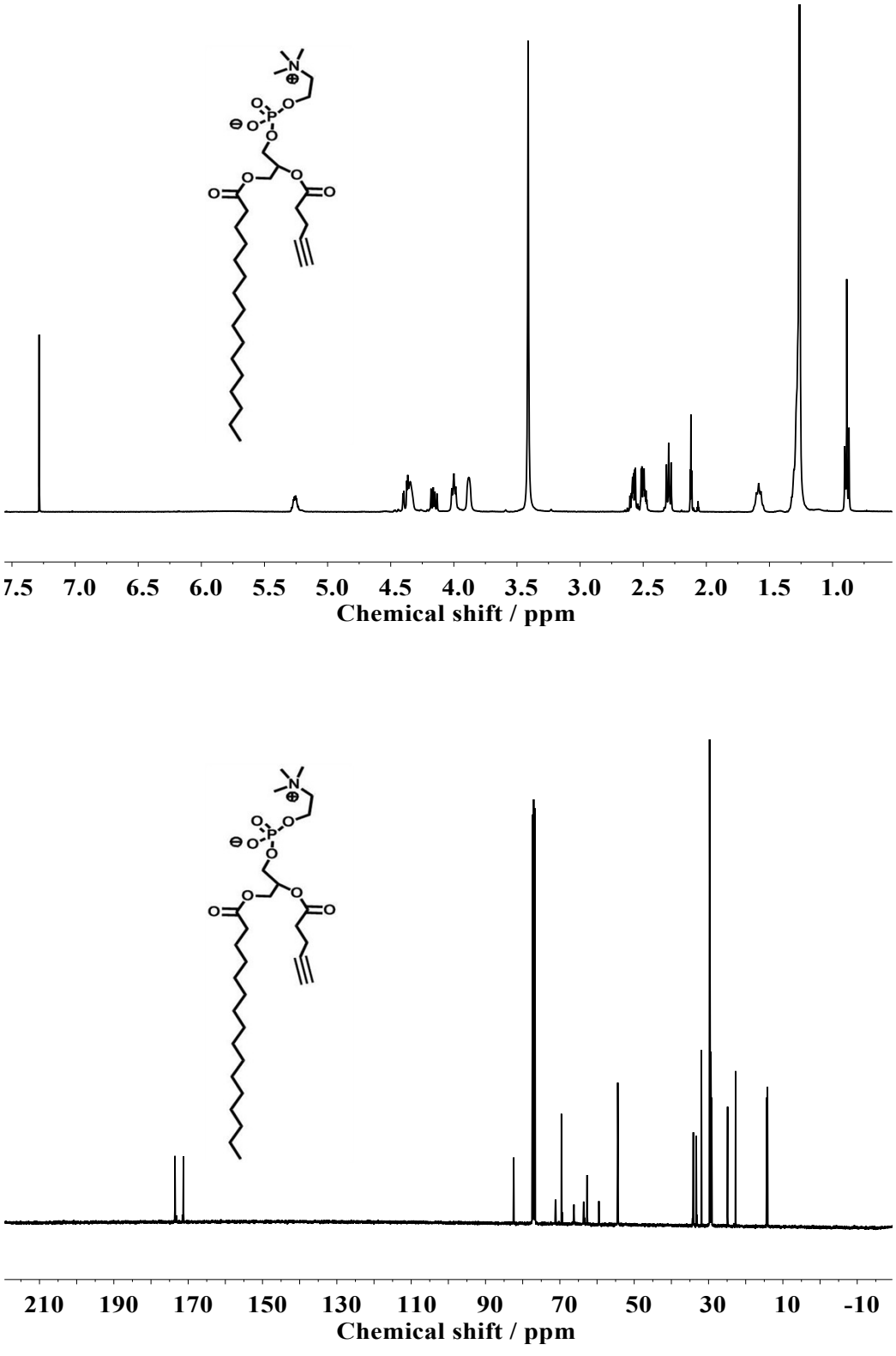
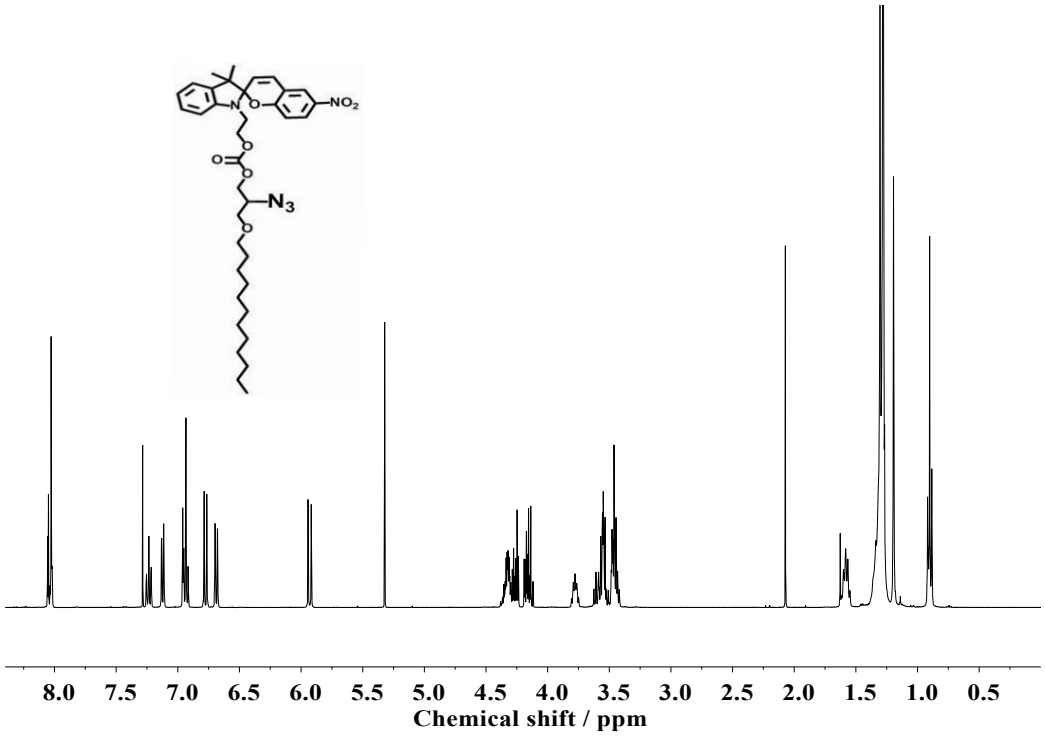


Fig. S9 ^{1}H and ^{13}C NMR spectra of alkyne lysolipid



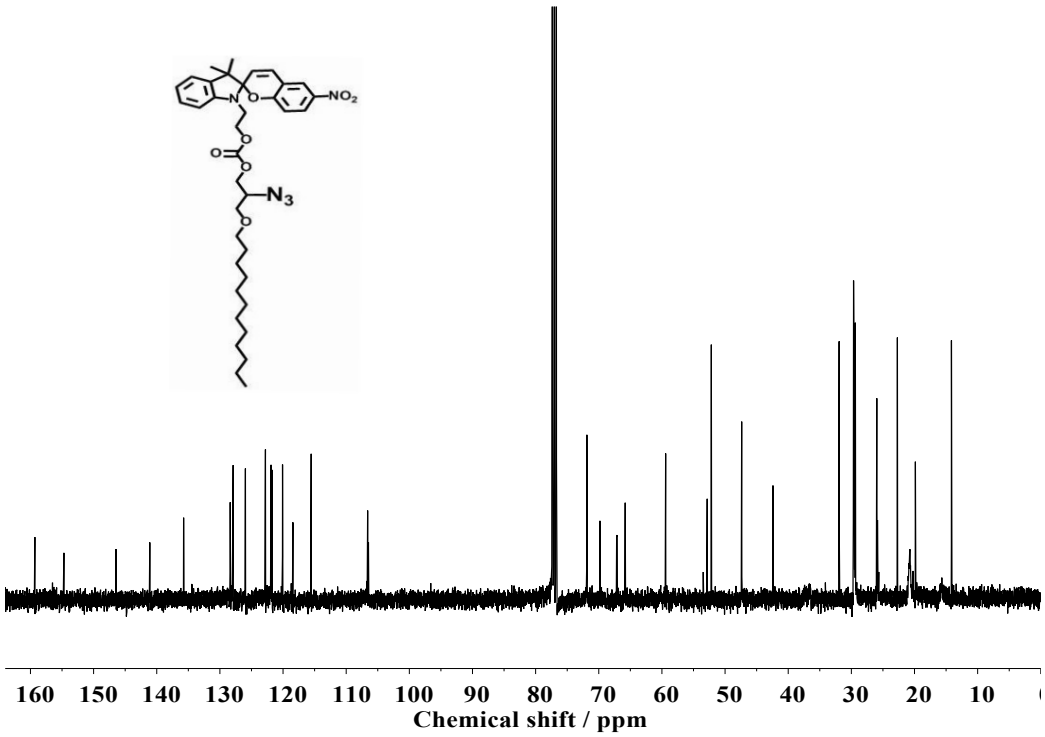
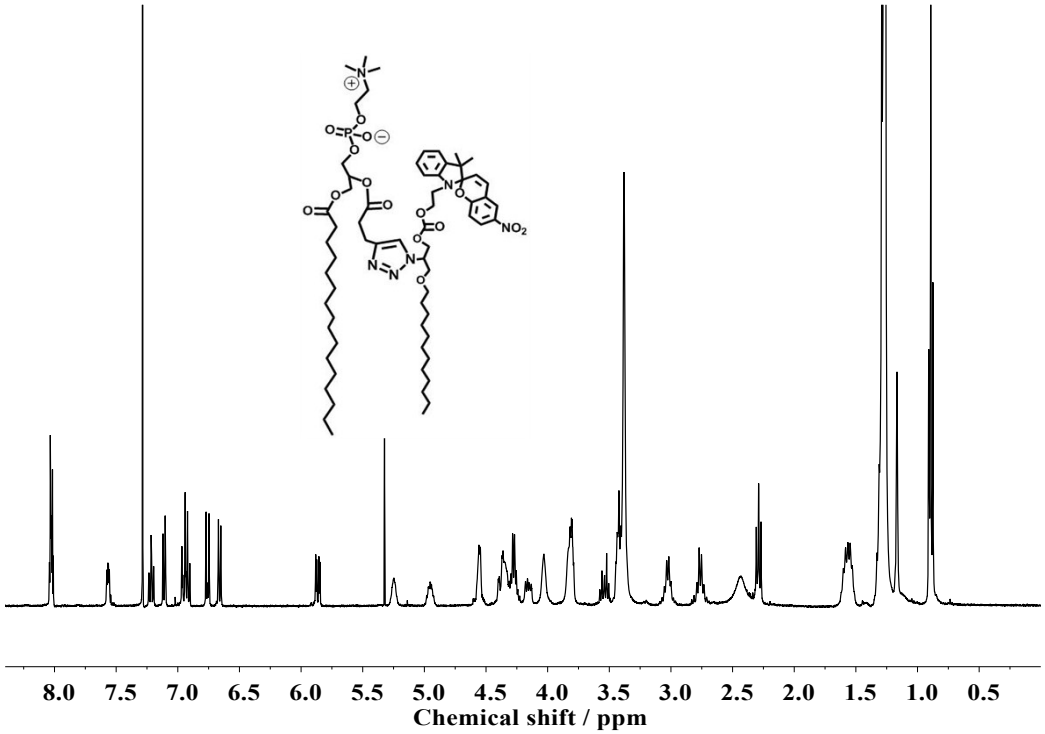


Fig. S10 ^{1}H and ^{13}C NMR spectra of SPN_3



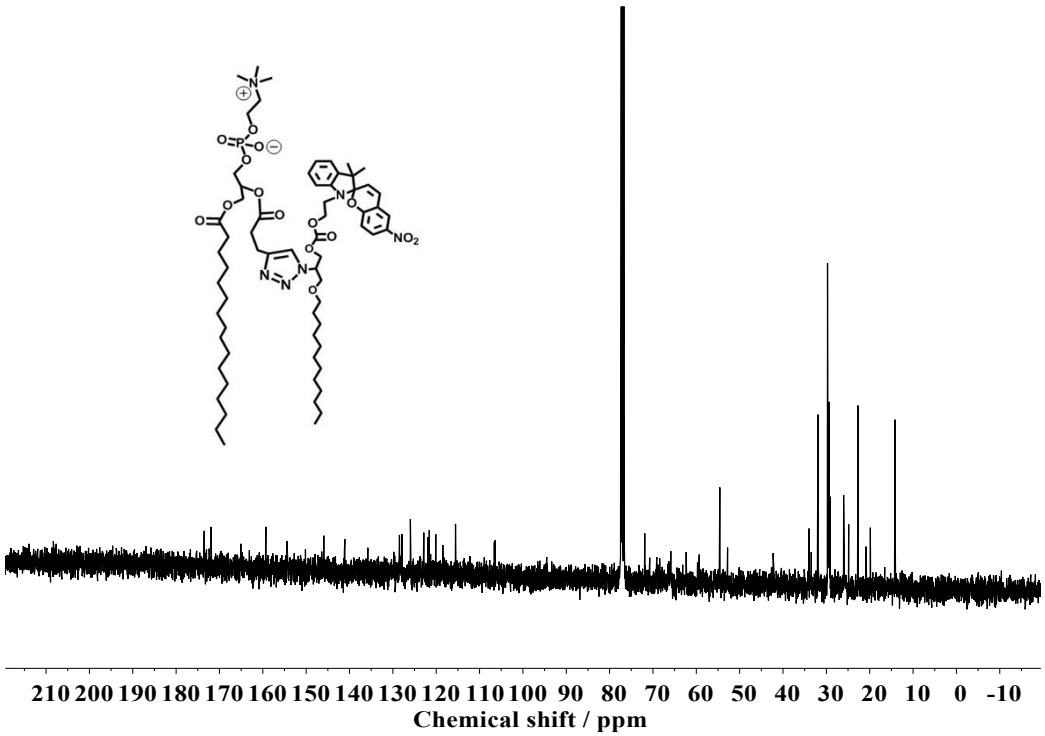


Fig. S11 ^{1}H and ^{13}C NMR spectra of spiropyran-containing triazole-phospholipid (SPTPC)