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Supporting Information

Disulfide Phosphatidylcholines: Alternative Phospholipids for the Preparation of Functional Liposomes

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1. Experiment section

1.1. Materials

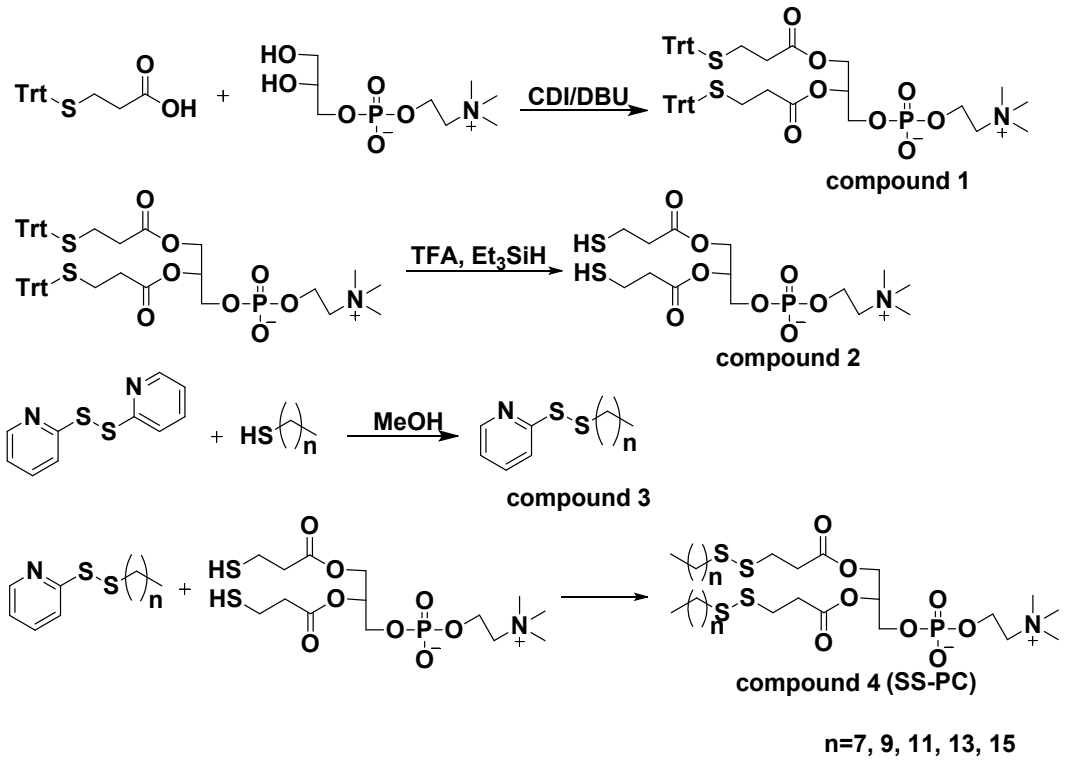
3-(Tritylthio)propanoic acid (>98%) was purchased from GL Biochem Ltd. (Shanghai, China). L-α-glycerophosphorylcholine (GPC) (>98%, stored in vacuum drier) was provided by Suzhou Fushilai Pharmaceutical Co. (Suzhou, China). 1-Octanethiol (>98.5%), 1-decanethiol (>95%), 1-dodecanethiol (>95%), 1-tetradecanethiol (>95%), 1-hexadecanethiol (>95%), 1,2-di(pyridin-2-yl)disulfane (>98%) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (>99%) were afforded by Aladdin Co. (Shanghai, China). *N*,*N*'-carbonyldiimidazole (CDI) (>99%), triethylsilane (Et_3SiH) (>98%) and trifluoroacetic acid (TFA) (>99%) were supplied by Jiahua Energy Co. (Jiaxing, China). Doxorubicin (DOX) (pharmaceutical grade) was provided from HVSF Co. (Beijing, China). DSPE-PEG_{2000} (pharmaceutical grade) was obtained from Nanocs Inc. (New York, NY). Solvents (analytically pure and chromatographic grade) were supplied by Sinopharm Co. (Shanghai, China). All reagents could be used directly without further purification.

1.2. Instruments

Mass spectra (MS) were recorded by Agilent 1100 MSD mass spectrometer and high-resolution mass spectra (HRMS) were recorded by Agilent 1260-6224 LC–MS Time of Flight Mass Spectrometry using electro-spray ionization (ESI). ^{1}H NMR (300 MHz) spectra were recorded on Bruker AVANCE AV-300 equipment. ^{1}H NMR (600 MHz), ^{13}C NMR (150 MHz) and ^{31}P NMR (243 MHz) spectra were recorded on Bruker AVANCE III HD equipment. The particle size and zeta-potential were measured by Brookhaven NanoBrook Omni Particle Size Analyzer. The fluorescence spectra were recorded by HORIBA FluoroMax®-4 Spectrofluorometer. The thermal behavior was studied by Netzsch-Gerätebau GmbH 200F3 analyzer differential scanning calorimetry (DSC). The transmission electron microscopy (TEM) and cryo-TEM images were obtained using JEOL JEM-2100 TEM system and FEI Tecnai G2 F20 200 kV cryogenic transmission electron microscope, respectively. Confocal laser scanning microscopy (CLSM) images were obtained by Olympus FV3000 microscope.

1.3. Synthesis of redox-sensitive phosphatidylcholines (SS-PCs)

Scheme S1. Synthesis of SS-PCs.



Synthesis of compound 1: compound 1 was synthesized from 3-(tritylthio)propanoic acid and GPC using CDI/DBU catalytic system[1]. 3-(tritylthio)propanoic acid (1.04 g, 3 mmol) and CDI (0.64 g, 3.9 mmol) were dissolved in 20 mL of dimethylsulfoxide and stirred for 2 h. Dimethylsulfoxide (20 mL) containing GPC (0.33 g, 1.3 mmol) and DBU (0.20 g, 1.3 mmol) were added into the reaction mixture and stirring was continued overnight at room temperature. The reaction mixture was directly separated by silica gel column chromatography using gradient elution (A: dichloromethane/methanol, 5/1; B: dichloromethane/methanol/water, 65/25/4) to obtain the compound as a white powder (0.95 g, yield: 80%). MS m/z $[M+H]^+$ calculated 918.3, found 918.3; $[M+Na]^+$ calculated 940.3, found 940.1. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (d, 12H, *J*=7.8 Hz), 7.16 (t, 12H, *J*=7.8 Hz), 7.08 (t, 6H, *J* = 7.2 Hz), 5.02 (br, 1H), 4.09 (br, 4H), 3.80-3.73 (m, 2H), 3.51 (br, 2H), 3.09 (s, 9H), 2.33-2.25(m, 4H), 2.11-2.02 (m, 4H).

Synthesis of compound 2: compound 2 was obtained by thiol deprotection of compound 1[2]. Compound 1 (0.92 g, 1 mmol) was dissolved in methanol (20 mL). Then, TFA (0.34 g, 3 mmol) was added dropwise and the colorless mixture turned to yellow. Et_3SiH (0.35 g, 3 mmol) was added subsequently and the mixture became colorless. The crude solution of compound 2 could be used without further separation (yield ~ 100% by TLC).

Synthesis of compound 3: 1,2-di(pyridin-2-yl)disulfane (0.27 g, 1.2 mmol) and thiol (1 mmol) were dissolved in 20 mL of methanol and stirred 2 h at room temperature. After concentration under reduced pressure, the crude compound was separated by silica gel column chromatography using methanol/dichloromethane (10:1) as eluant to give compound 3 as a liquid. **Compound 3 (n=7):** MS m/z $[M+Na]^+$ calculated 278.1, found 278.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.78 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.25 (m, 8H), 0.87 (m,3H). **Compound 3 (n=9):** MS m/z $[M+Na]^+$ calculated 306.1, found 306.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.77 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.30 (t, 2H, J=4.5 Hz), 1.25 (m, 10H), 0.87 (m,3H). **Compound 3 (n=11):** MS m/z $[M+Na]^+$ calculated 334.2, found 334.1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.76 (t, 2H, *J*=4.5 Hz), 1.77 (d, 2H, J=3.0 Hz), 1.29

(t, 2H, J=4.5 Hz), 1.24 (m, 12H), 0.88 (m,3H). **Compound 3 (n=13):** MS m/z $[M+Na]^+$ calculated 362.2, found 362.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.76 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.24 (m, 14H), 0.87 (m,3H). **Compound 3 (n=15):** MS m/z $[M+Na]^+$ calculated 390.2, found 390.2. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.76 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.25 (m, 16H), 0.87 (m,3H).

Table S1. The quantity and yield of the synthesis step of compound 3

	thiols	quantity of thiols	quantity and yield of compound 3
n=7	1-octanethiol	0.15 g	0.21 g, 82%
n=9	1-decanethiol	0.17 g	0.25 g, 87%
n=11	1-dodecanethiol	0.20 g	0.30 g, 95%
n=13	1-tetradecanethiol	0.23 g	0.31 g, 91%
n=15	1-hexadecanethiol	0.26 g	0.34 g, 94%

Synthesis of SS-PCs: compound 3 (3 mmol) was added to the solution of compound 2 (0.43 g, 1 mmol) and stirred at room temperature overnight. The reaction mixture was separated by silica gel column chromatography using a gradient elution (A: dichloromethane/methanol, 5/1; B: dichloromethane/methanol/water, 65/25/4) to obtain the product as a white powder. The SS-PCs with tail length (C and S) of 13, 15, 17, 19 and 21 were named as SS13-PC, SS15-PC, SS17-PC, SS19-PC and SS21-PC (see Table S2), respectively. **SS13-PC:** HRMS m/z $[M+H]^+$ calculated 722.29, found 722.30271; $[M+Na]^+$ calculated 744.28, found 744.28597. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.40 (m, 1H), 4.28 (s, 2H), 4.21 (m, 1H), 3.94 (s, 2H), 3.73 (s, 2H), 3.29 (s, 9H), 2.86 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.27 (m, 16H), 0.88 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.74, 70.89, 66.22, 63.36, 59.48, 39.45, 34.04, 31.73, 29.15, 28.38, 22.40, 14.07. **SS15-PC**, HRMS m/z $[M+H]^+$ calculated 778.36, found 778.36582; $[M+Na]^+$ calculated 800.35, found 800.34915. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 2H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 24H), 0.88 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.03, 70.91, 66.27, 63.43, 59.49, 38.52, 33.56, 31.93, 29.62, 28.38, 22.62, 14.07. **SS17-PC:** HRMS m/z $[M+H]^+$ calculated 834.42, found 834.41872; $[M+Na]^+$ calculated 856.41, found 856.40300. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 1H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 32H), 0.88 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.23, 70.80, 66.32, 63.24, 59.52, 38.54, 33.57, 31.83, 29.64, 28.36, 22.62, 14.07. **SS19-PC:** HRMS m/z $[M+H]^+$ calculated 890.48, found 890.49077; $[M+Na]^+$ calculated 912.47, found 912.47373. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 1H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 40H), 0.88 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.25, 70.77, 66.23, 63.29, 59.32, 38.59, 33.74, 31.64, 29.54, 28.39, 22.62, 14.10. **SS21-PC:** HRMS m/z $[M+Na]^+$ calculated 968.53, found 968.53411. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 1H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 48H), 0.88 (m, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.21, 70.92, 66.42, 63.35, 59.31, 38.44, 33.69, 31.23, 29.63, 28.38, 22.61, 14.07.

Table S2. The quantity and yield of the synthesis step of compound 4

	quantity of compound 3	quantity and yield of SS-PCs

SS13-PC	0.77 g	0.51 g, 71 %
SS15-PC	0.86 g	0.58 g, 75 %
SS17-PC	0.94 g	0.72 g, 87 %
SS19-PC	1.02 g	0.76 g, 85 %
SS21-PC	1.09 g	0.84 g, 89 %

1.4. Critical aggregation concentration (CAC) measurement

The critical aggregation concentration (CAC) of SS-PC was investigated by pyrene fluorescence probe method[3]. Briefly, pyrene was dissolved in acetone to the concentration of 6.08 mg/L as the stock solution. SS-PC suspension of 500 μg/mL was prepared, and then diluted to 250, 125, 62.5, 31.25, 15.62, 7.81, 3.91, 1.95, 0.97, 0.49, 0.24, 0.12 μg/mL by deionized water. After that, 10 mL SS-PC suspension of each sample was transferred into different centrifuge tubes with 0.04 mL pyrene stock solution. After shaking at 50 ^{o}C for 4 h, all samples were detected using fluorescence spectrophotometer with excitation wavelength of 334 nm. The peak values of I_3 (384 nm)/I_1(373 nm) (I_3/I_1) in emission spectra were recorded.

1.5. Differential scanning calorimetry (DSC) measurement

The phase transition temperature (T_c) was detected by measuring the thermal behavior of SS-PC using DSC analyzer (Netzsch-Gerätebau GmbH, Germany) using SS-PC sample with heating rate 5 ^{o}C/min from -40 ^{o}C to 80 ^{o}C.

1.6. Preparation of liposomes

Liposomes were prepared by thin-film dispersion method. Lipid composition of the liposomes was SS-PCs (or DSPC), DSPE-PEG_{2000} and cholesterol in a 9:1:3 molar ratios[4]. The thin-film was formed under under reduced pressure. Then, the thin-film was further hydrated at 50 ^{o}C for 30 min. The obtained mixture was homogenized by ultrasonic probe. The obtained mixture was homogenized by ultrasonic probe. The liposomes assembled by SS13-PC, SS15-PC, SS17-PC, SS19-PC and SS21-PC were named as SS13-LP, SS15-LP, SS17-LP, SS19-LP and SS21-LP, respectively.

For DOX loaded liposomes, ammonium sulfate gradient method was used[5]. Firstly, thin-film was formed under under reduced pressure (with total lipids of 50 mg). Then the thin-film was further hydrated at 50 ^{o}C for 30 min using 5 mL of 250 mM ammonium sulfate. Then unloaded ammonium sulfate was removed by dialysis. After that, the ammonium sulfate loaded liposomes were sired by adding DOX solution (to final volume of 10 mL with DOX concentration of 0.5 mg/mL) at 60 ^{o}C for 2 h. Finally, the unloaded DOX was removed by dialysis for 2 h.

1.7. Characterization of liposomes

The size and zeta-potential of liposomes were analyzed by dynamic light scattering (DLS) technique using NanoBrook Omni Particle Size Analyzer (Brookhaven, Holtsville, NY). The morphology of liposomes was observed by TEM (JEOL, Japan). In addition, the storage and serum stability of SS17-LP was detected by recording the change of size and polydispersity index (PDI). For storage stability SS-LP was stored and checked at 4 ^{o}C for 1 month. For serum stability, SS17-LP was incubated in PBS containing (or not) 5% bovine serum with the concentration of 0.1 mg/mL. The size of liposomes was recorded at 0, 1, 3, 6 h. After addition of 20 mM DTT at 6 h, the size was further measured at 6.5 and 12 h.

The liposomal characteristic lamellarity morphology of SS-LPs was assessed by cryo-TEM. SS-

LPs solution was dropped onto hydrophilized carbon support films. The cryo-TEM samples were prepared by a vitrobot Mark IV automated blotting device (FEI, OR) and then observed by Tecnai G2 F20 200 kV cryogenic transmission electron microscope (FEI, OR).

The entrapment efficiency (EE) and drug loading (DL) of DOX/SS-LPs were determined by UV-vis spectrophotometer (Shimadzu, Japan) at 490 nm. The amount of DOX was calculated using the concentration-absorbance fitting equation of PTX solution obtained previously. The EE% and DL% were obtained by formulas as follows:

$$EE\% = \frac{M_{loaded}}{M_{total}} \times 100\%$$

$$DL\% = \left(\frac{M_{loaded}}{M_{lipid} + M_{loaded}} \right) \times 100\%$$

wherein, M_{loaded} is the amount of loaded DOX, M_{total} is the total amount of DOX added, and M_{lipid} is the amount of all the lipid materials.

1.8. Evaluation of reduction sensitivity

The reduction sensitivity of SS-PCs or SS-LPs was studied by investigating of the changes of HRMS, DLS, TEM, and release behavior. To detect the cleavage of disulfide bonds under reduction condition, SS-PC was dissolved in deionized water (10 μg/mL) with the 10 mM DTT for 3 h. After that, equal volume of methanol/acetonitrile (1/1, v/v) mixed solvent was added to the mixture. After vortex and filtration by 200 nM filter, the resulting mixture was detected by MS.

After preparation into liposomes, the size of morphology change of DOX/SS-LP were further investigated by DLS and TEM. DOX/SS-LPs with DOX concentration of 0.1 mg/mL was incubated under reduction condition of 20 Mm DTT for 2 h. The size distribution was measured by DLS and the morphology was observed by TEM.

The redox-sensitive release behavior of DOX from DOX/SS-LPs was studied. The PBS solution (pH 7.4) without DTT or with 10 mM DTT were set as the release medium of DOX/SS-LPs. DOX/LP was used as control. Liposome solution (2 mL) was transferred into dialysis bags (MWCO 8000) and immersed in 200 mL of different release medium at 37 °C. At predetermined time points, 1 mL of external release buffer was collected. Then, 1 mL of fresh release media was added into the external buffer. The concentration of DOX of each sample was measured by UV-vis to calculate the release rate.

1.9. Internalization

The cell internalization of DOX/SS-LP towards cancer cells were studied by confocal laser scanning microscopy (CLSM). Briefly, MCF-7 cells were seed in confocal dishes and incubated with DOX/LP and DOX/SS17-LP for 3 h, cells were washed by PBS for tree times and fixed by 4% paraformaldehyde for 30 min. After that, DAPI was added to stain the nuclei for another 10 min. Finally, the cells were were observed by a CLSM (Olympus, Japan).

For uptake mechanism research, co-localization of endosome/lysosome and DOX/SS17-LP was further explored. Briefly, MCF-7 cells were incubated with DOX/S17-LP and LysoBlue Tracker (KeyGen, China) for 1 h. Finally, the cells were observed by a CLSM (Olympus, Japan).

1.10. *In vitro* cytotoxicity test

The cytotoxicity of DOX, DOX/LP and DOX/SS-LPs were evaluated against A549 and MCF-7 cells using MTT assay. Briefly, cells were seeded into 96-well plates (1×10^4 cells/well). Then, the cells were incubated with medium containing DOX, DOX/LP and DOX/SS-LPs at 37°C for 24 h.

The medium of each well was removed and 20 μL of MTT solution was added. After incubation for another 4 h, MTT solution was removed and 150 μL of DMSO was added. The optical density (OD) of each well was measured by a spectrophotometer (Thermo Fisher, MA) at a wavelength of 490 nm. The cell viability was calculated as follows:

$$\text{Cell viability (\%)} = \frac{OD_{drug} - OD_{blank}}{OD_{\text{control}} - OD_{blank}} \times 100\%$$

wherein, OD_{drug} is the OD value of cells treated with DOX, DOX/LP and DOX/SS-LPs, while $OD_{control}$ is the OD value of the cells only treated with blank medium, and OD_{blank} is the OD value of DMSO.

1.11. *In vivo* anticancer activity evaluation

In vivo anticancer activity was evaluated by intravenous injection of DOX, DOX/LP, DOX/SS17-LP and DOX/SS21-LP using 4T1 tumor-bearing BALB/c nude mice model. The animals were divided into 4 groups randomly (n=5). The tumor-bearing BALB/c nude mice model was established by KeyGen Co. (Nanjing, China). DOX, DOX/LP, DOX/SS17-LP and DOX/SS21-LP were injected every 3 d with the DOX dose of 5 mg/kg, saline was used as a blank control. The tumor size and body weight of each group was measured every 2 d. After 21 d of therapy, all the animals were sacrificed by cervical dislocation. The tumors were separated and weighed.

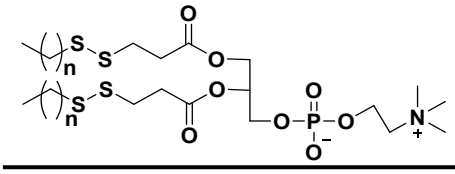
1.12. Statistical analysis

All results were showed as mean ± standard deviation unless otherwise mentioned. Statistical analysis was performed using independent-samples T-test. The statistical significance was considered as $P<0.05$.

2. Characterization of compounds

2.1. Synthesis of redox-sensitive phosphatidylcholine

Table S3. Characteristics of SS-PCs.



PCs	n	Appearance	CAC/μg mL^{-1}	T_c/°C	cLogP	LogS
SS13-PC	7	oiliness	5.25	<-30	-0.506	-6.87
SS15-PC	9	oiliness	4.68	15.1	1.610	-8.22
SS17-PC	11	powder	2.04	37.8	3.726	-9.56
SS19-PC	13	powder	0.93	48.9	5.842	-10.93
SS21-PC	15	powder	0.68	58.8	7.958	-12.28

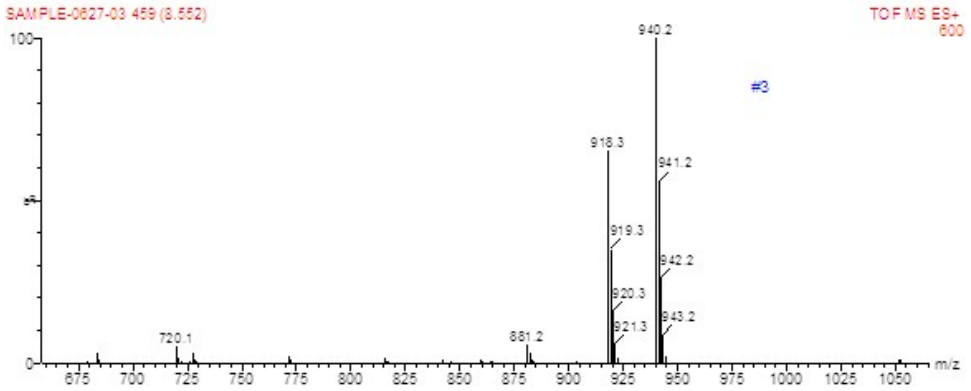


Figure S1. MS of compound 1, m/z $[M+H]^+$ calculated 918.3, found 918.3; $[M+Na]^+$ calculated 940.3, found 940.1.

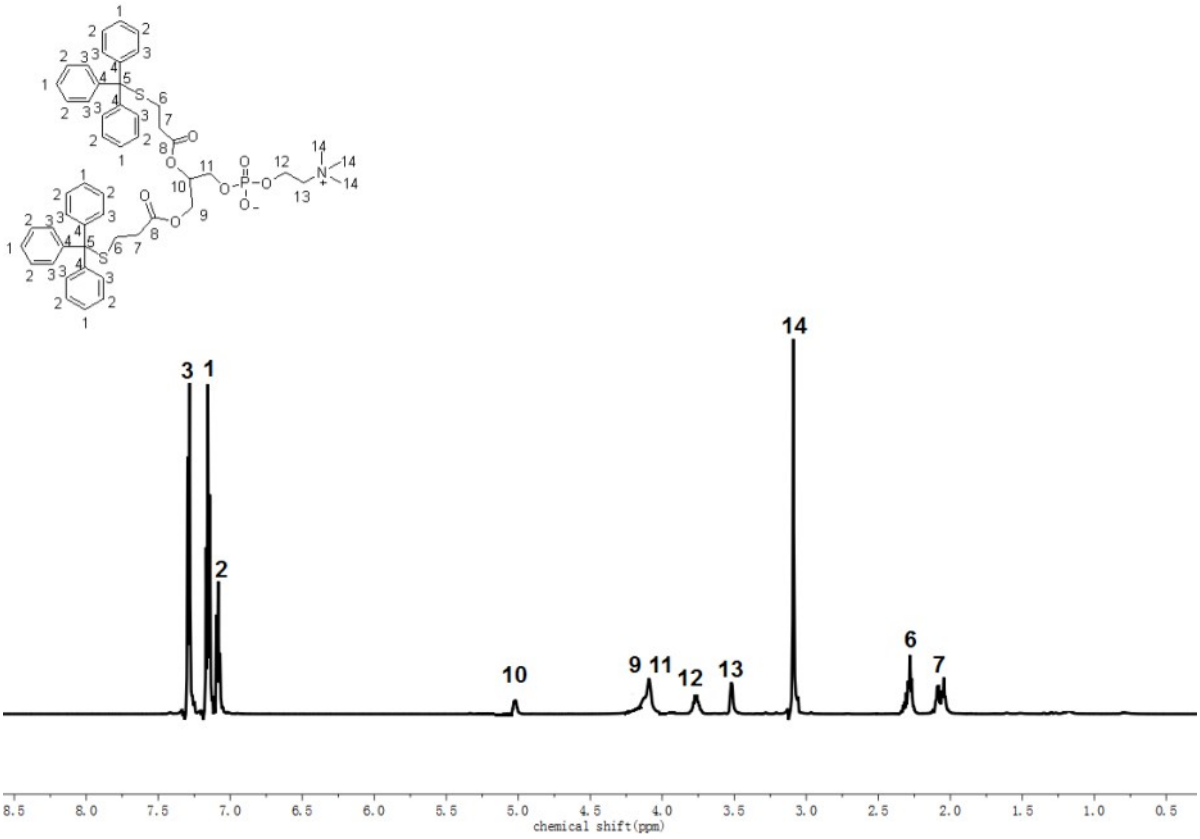


Figure S2. ^{1}H NMR of compound 1. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (d, 12H, *J*=7.8 Hz), 7.16 (t, 12H, *J*=7.8 Hz), 7.08 (t, 6H, *J* = 7.2 Hz), 5.02 (br, 1H), 4.09 (br, 4H), 3.80-3.73 (m, 2H), 3.51 (br, 2H), 3.09 (s, 9H), 2.33-2.25(m, 4H), 2.11-2.02 (m, 4H).

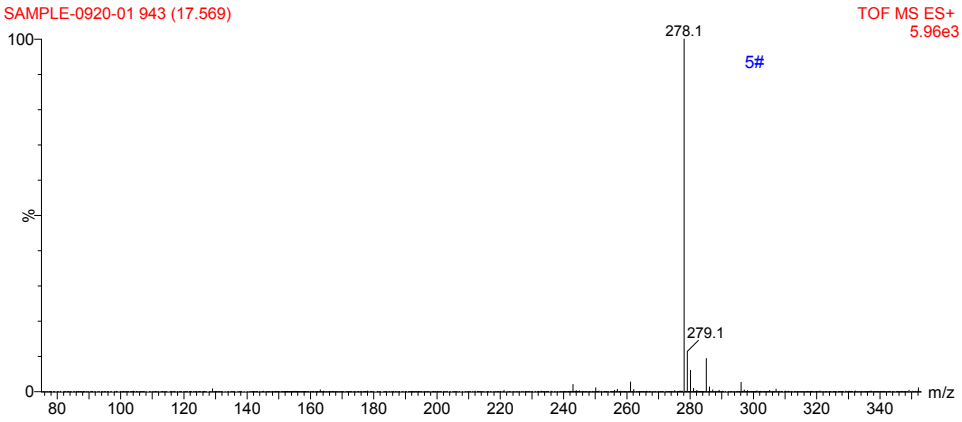
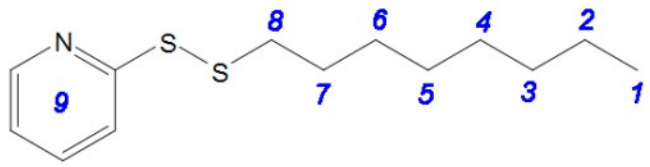


Figure S3. MS of compound 3 (n=7), m/z $[M+Na]^+$ calculated 278.1, found 278.1.



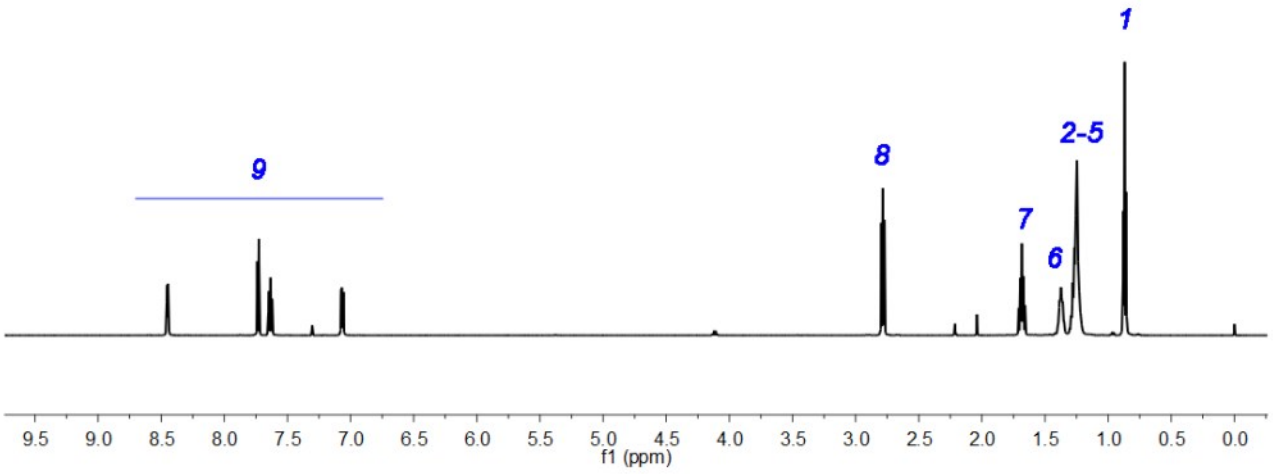


Figure S4. ^{1}H NMR of compound 3 (n=7). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.78 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.25 (m, 8H), 0.87 (m,3H).

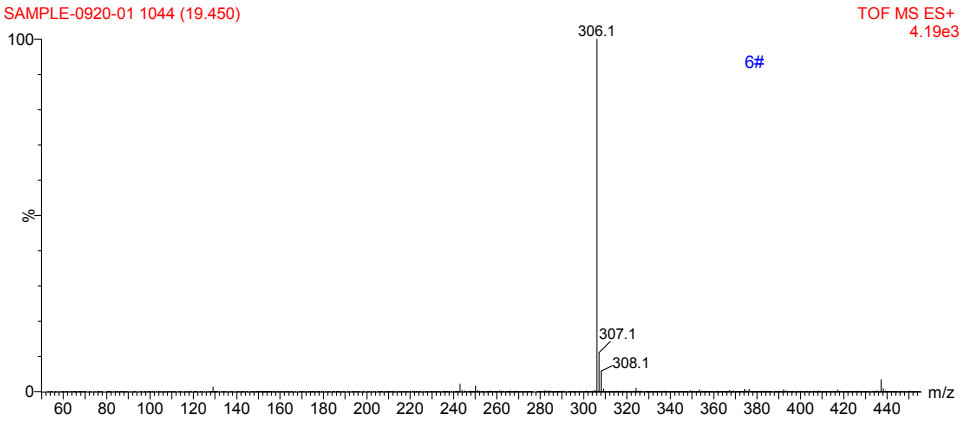


Figure S5. MS of compound 3 (n=9), m/z $[M+Na]^+$ calculated 306.1, found 306.1.

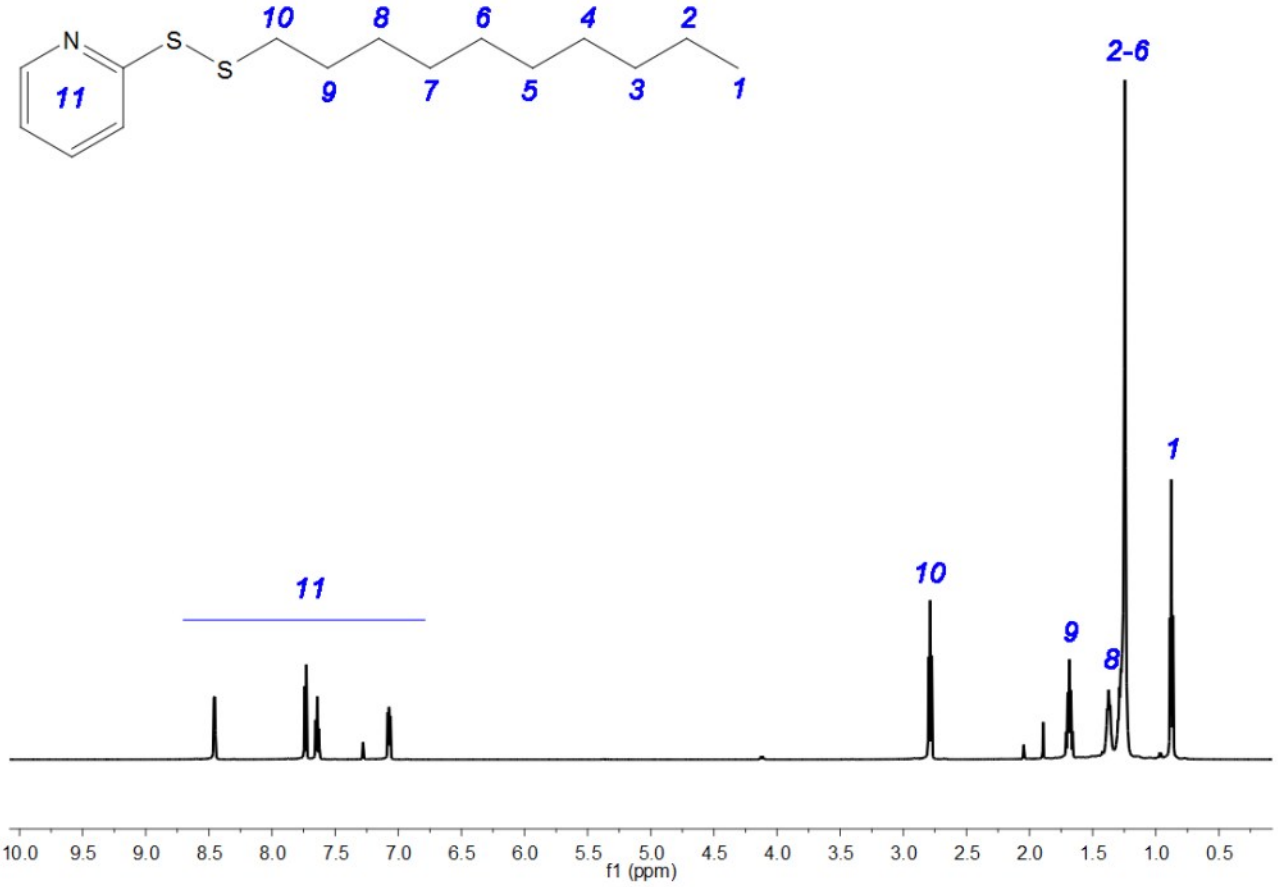


Figure S6. ^{1}H NMR of compound 3 (n=9). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.77 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.30 (t, 2H, J=4.5 Hz), 1.25 (m, 10H), 0.87 (m,3H).

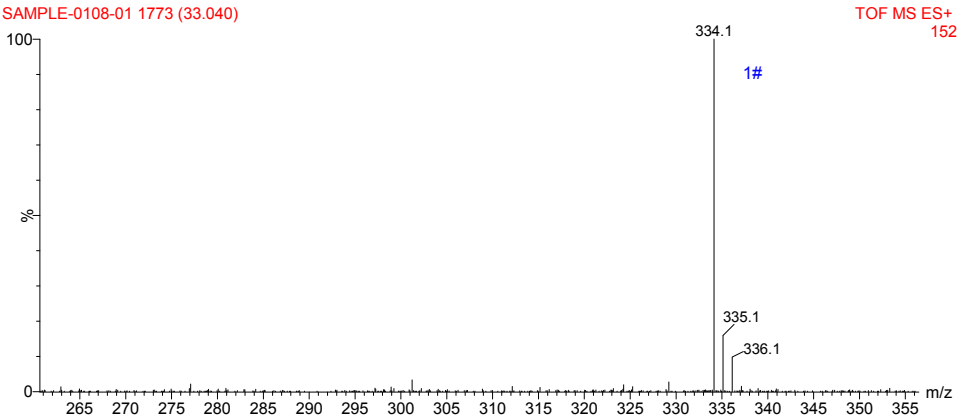


Figure S7. MS of compound 3 (n=11), m/z $[M+Na]^+$ calculated 334.2, found 334.1.

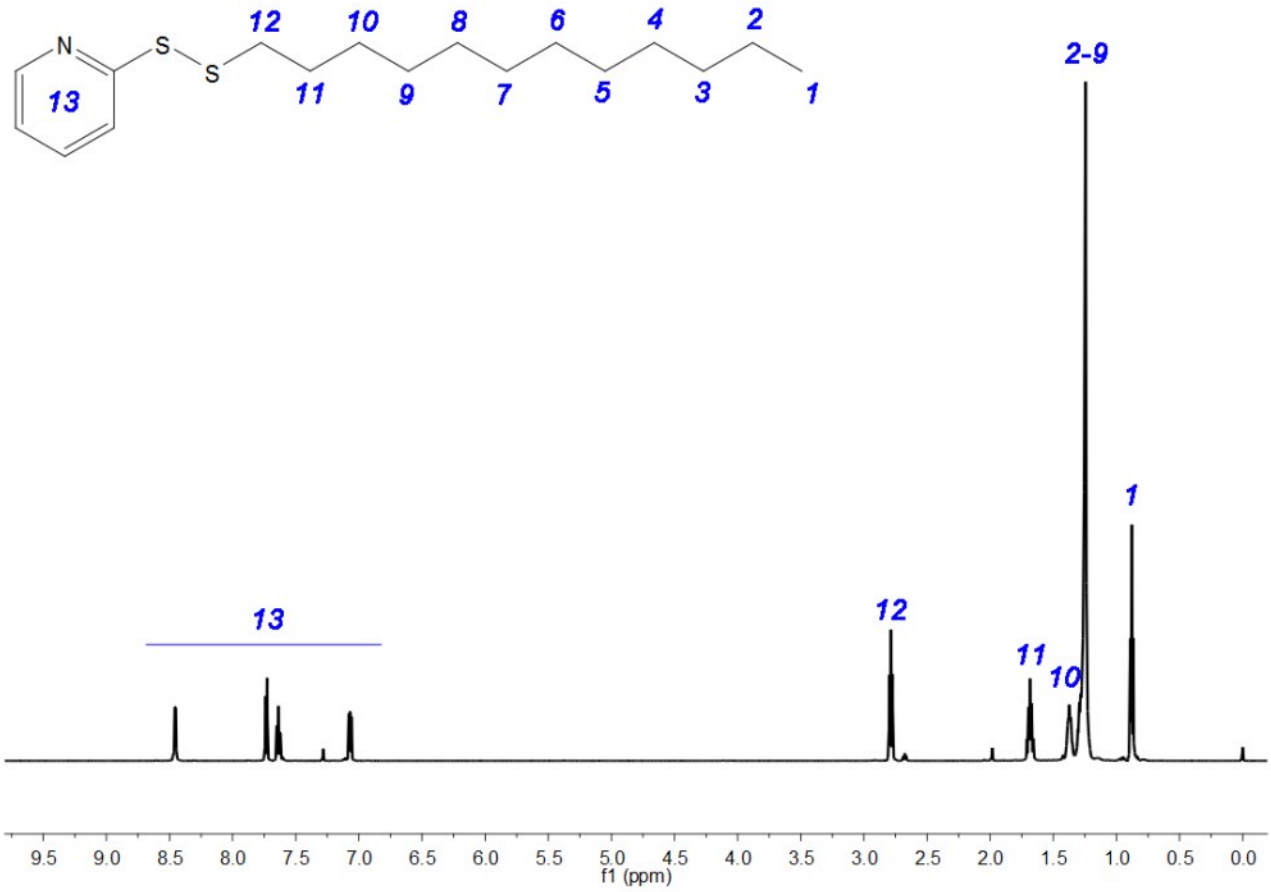


Figure S8. ^{1}H NMR of compound 3 (n=11). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.76 (t, 2H, *J*=4.5 Hz), 1.77 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.24 (m, 12H), 0.88 (m,3H).

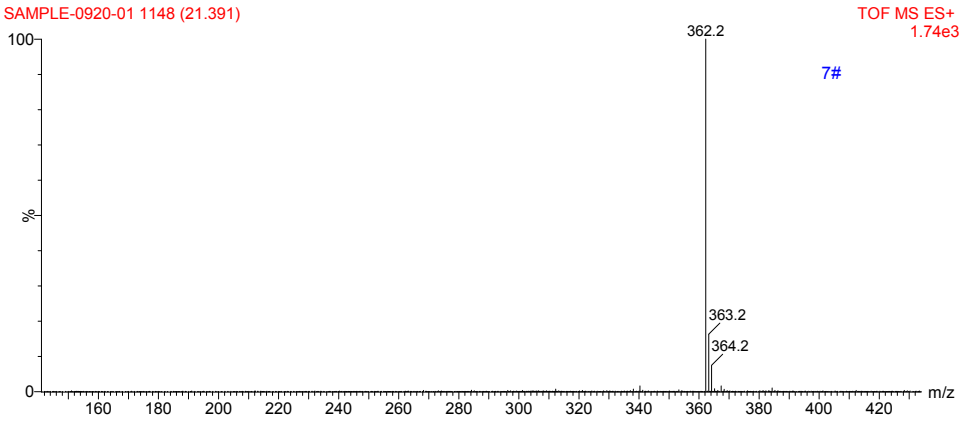


Figure S9. MS of compound 3 (n=13), m/z $[M+Na]^+$ calculated 362.2, found 362.2.

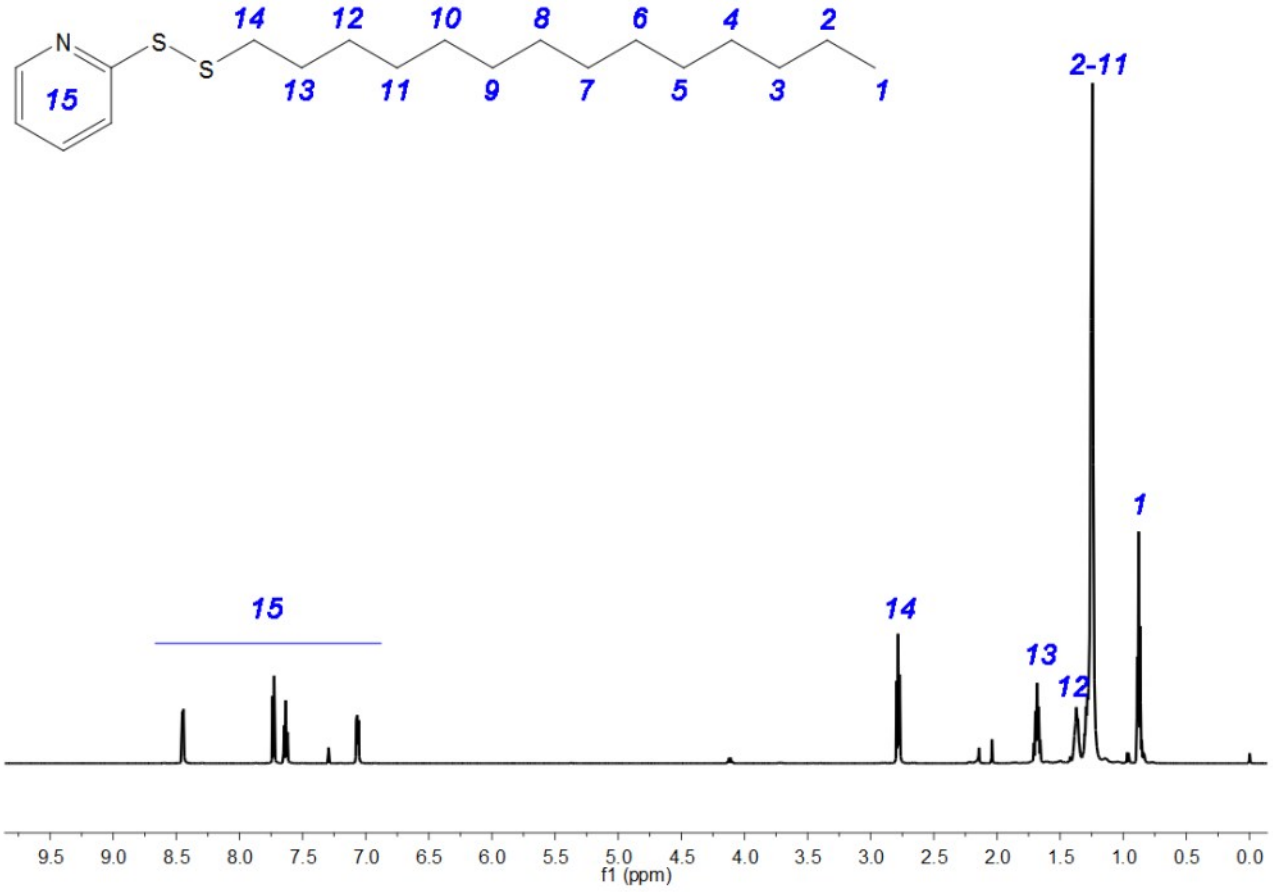


Figure S10. ^{1}H NMR of compound 3 (n=13). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.76 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.24 (m, 14H), 0.87 (m,3H).

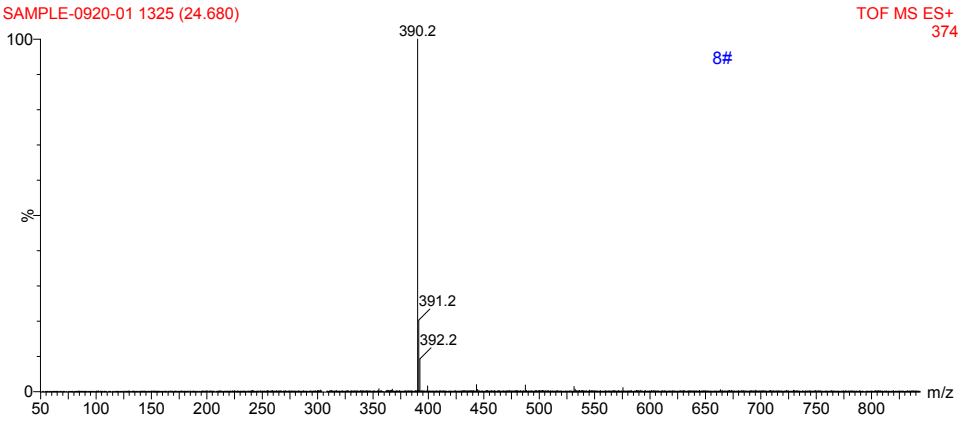


Figure S11. MS of compound 3 (n=15), m/z $[M+Na]^+$ calculated 390.2, found 390.2.

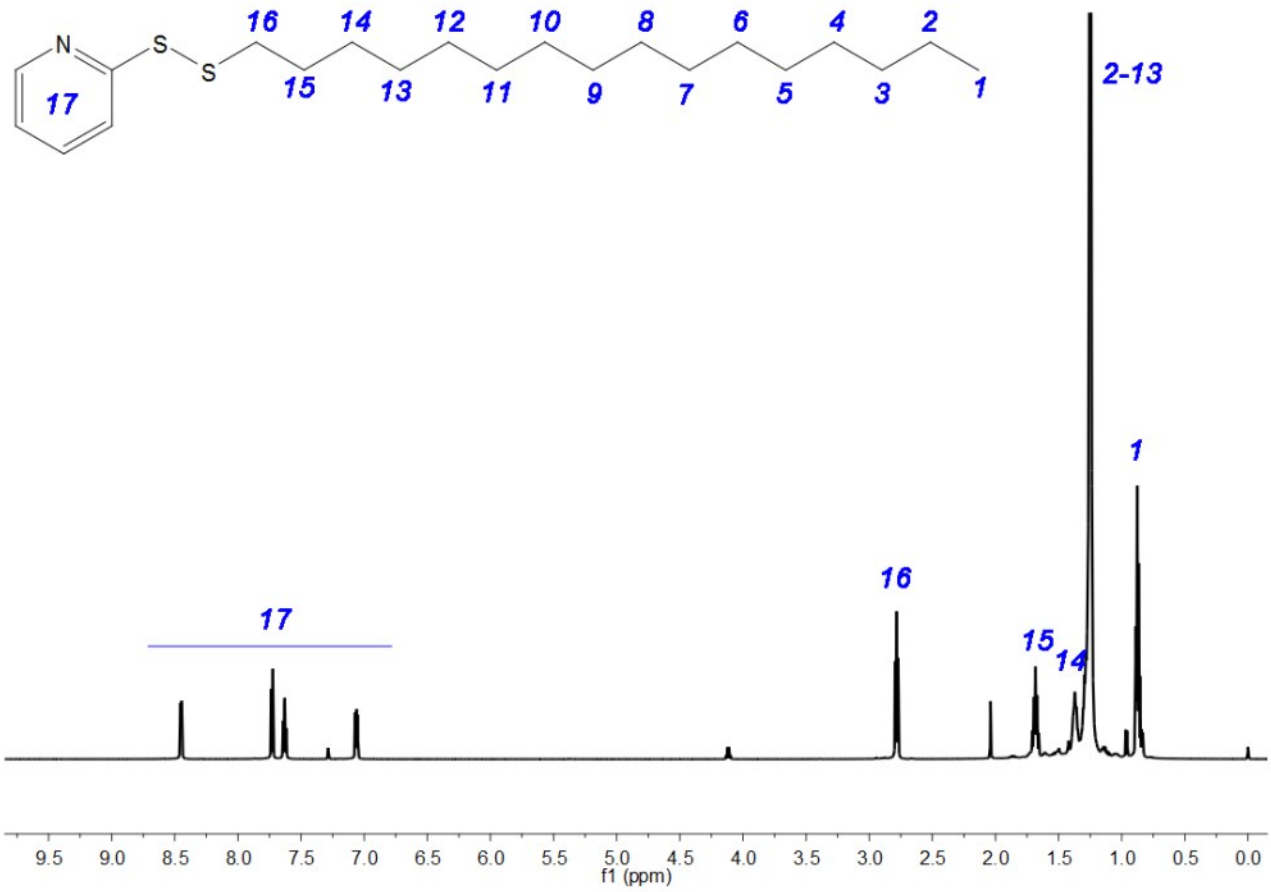


Figure S12. ^{1}H NMR of compound 3 (n=15). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45-7.05 (m, 4H), 2.76 (t, 2H, *J*=4.5 Hz), 1.76 (d, 2H, J=3.0 Hz), 1.29 (t, 2H, J=4.5 Hz), 1.25 (m, 16H), 0.87 (m,3H).

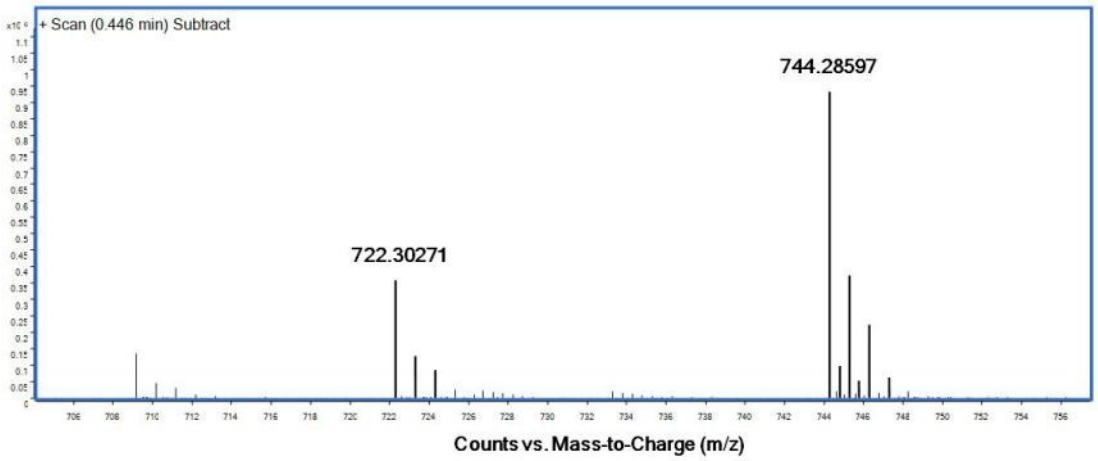


Figure S13. HRMS of SS13-PC, m/z $[M+H]^+$ calculated 722.29, found 722.30271; $[M+Na]^+$ calculated 744.28, found 744.28597.

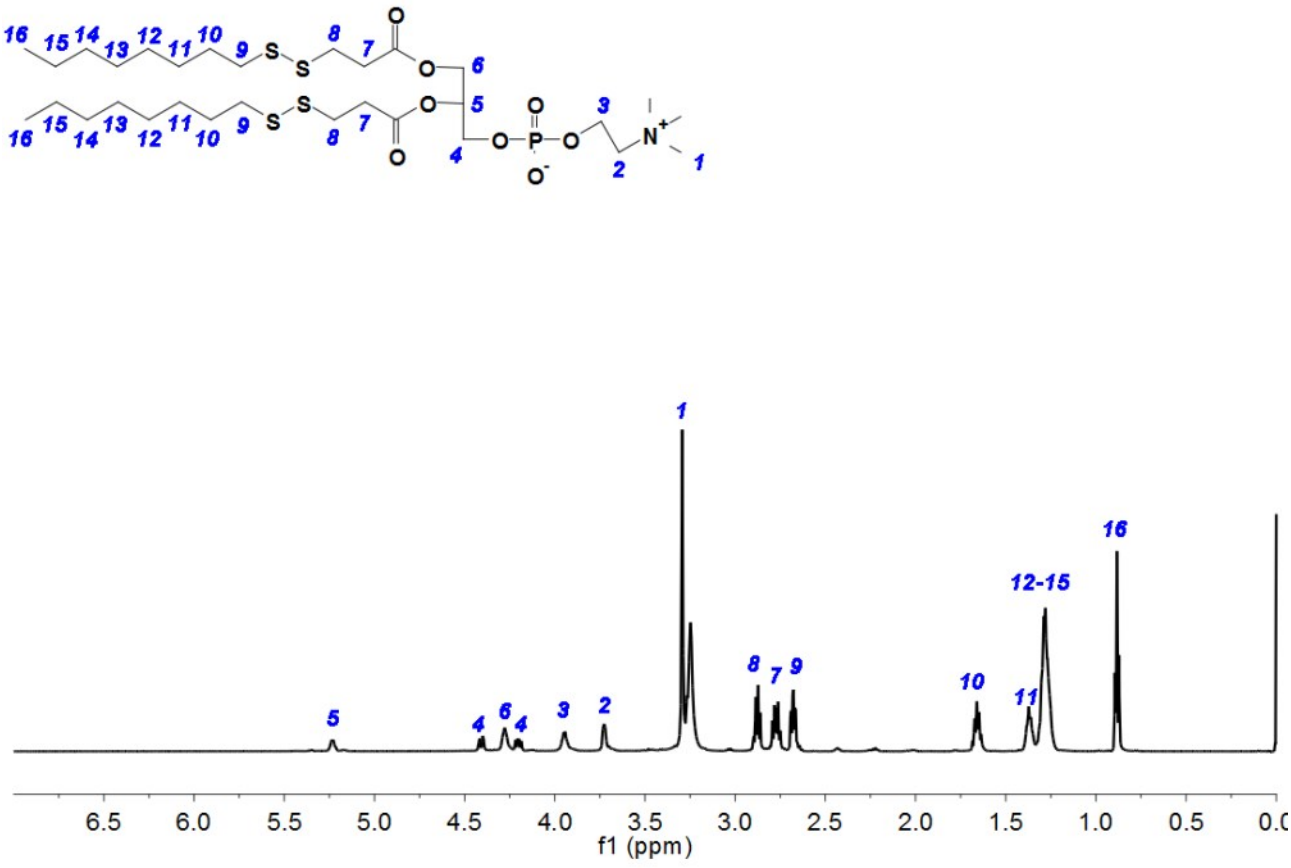


Figure S14. ^{1}H NMR of SS13-PC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.40 (m, 1H), 4.28 (s, 2H), 4.21 (m, 1H), 3.94 (s, 2H), 3.73 (s, 2H), 3.29 (s, 9H), 2.86 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.27 (m, 16H), 0.88 (m, 6H).

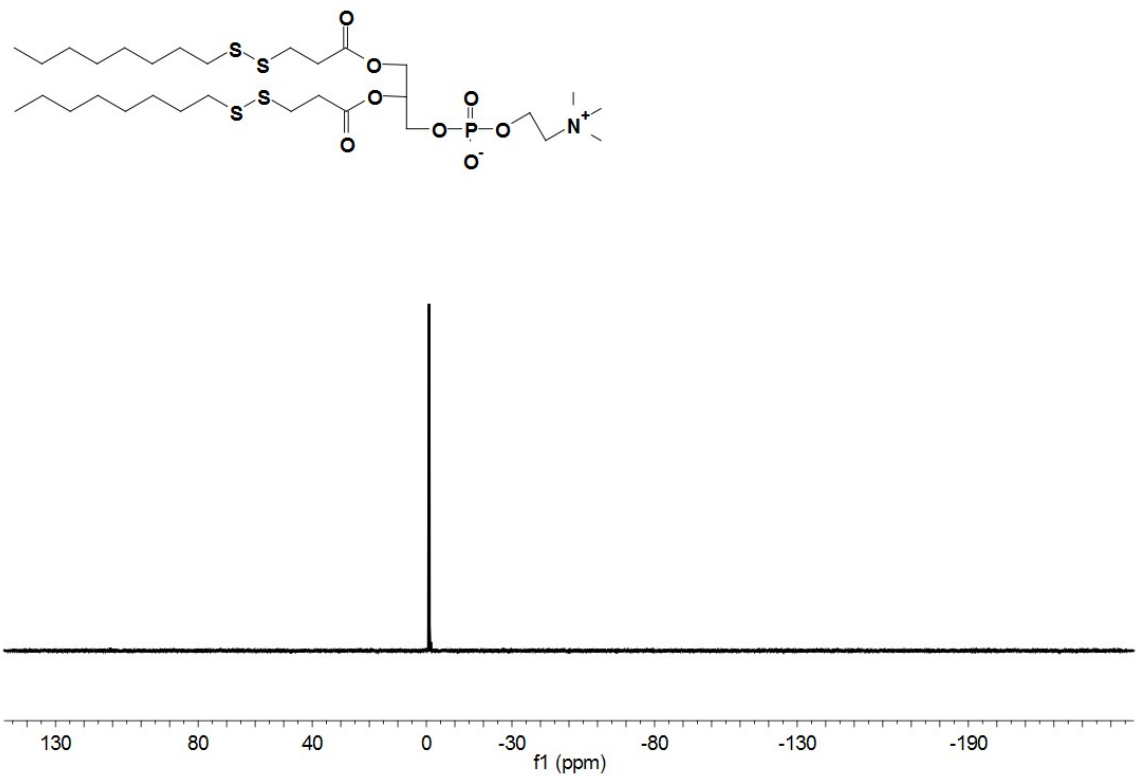


Figure S15. ^{31}P NMR of SS13-PC.

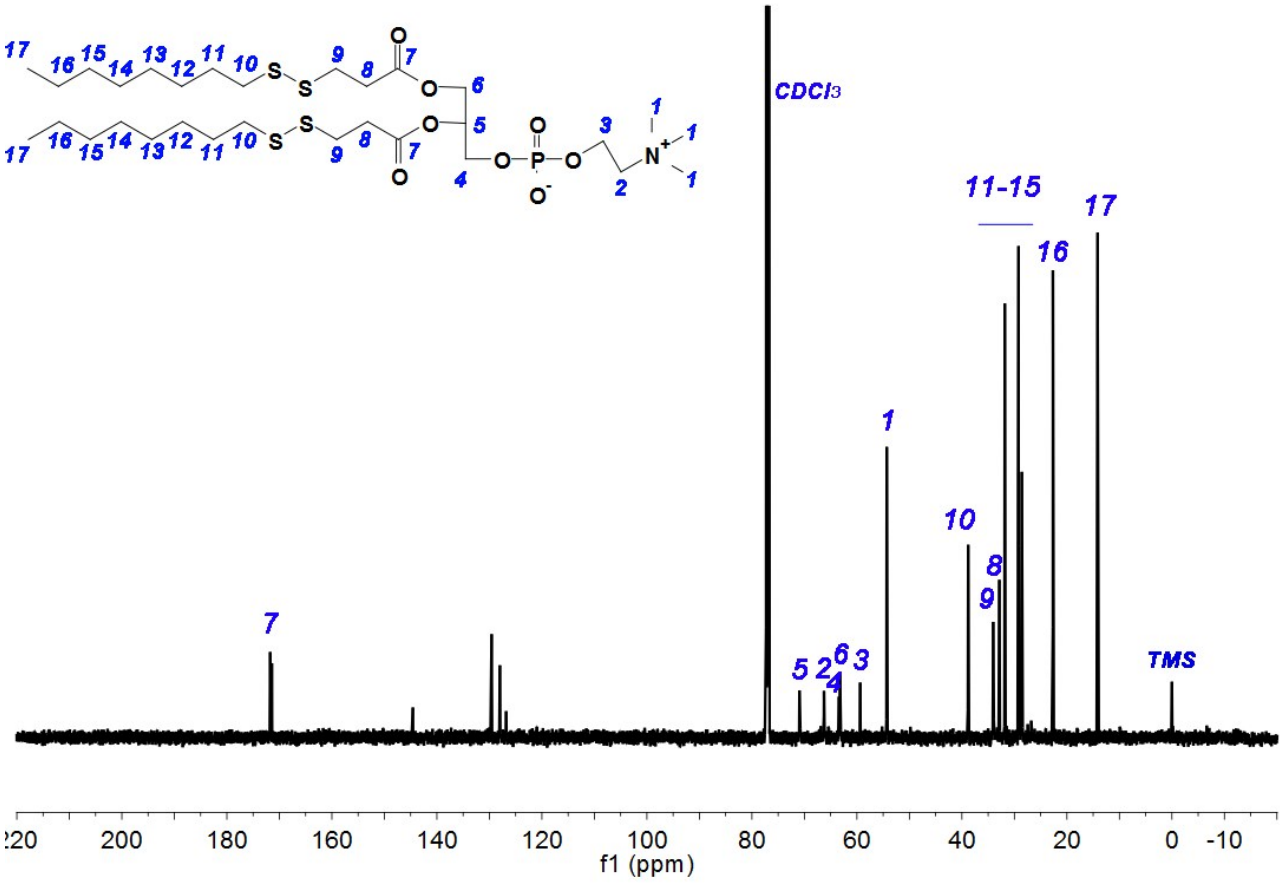


Figure S16. ^{13}C NMR of SS13-PC. ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.74, 70.89, 66.22, 63.36, 59.48, 39.45, 34.04, 31.73, 29.15, 28.38, 22.40, 14.07.

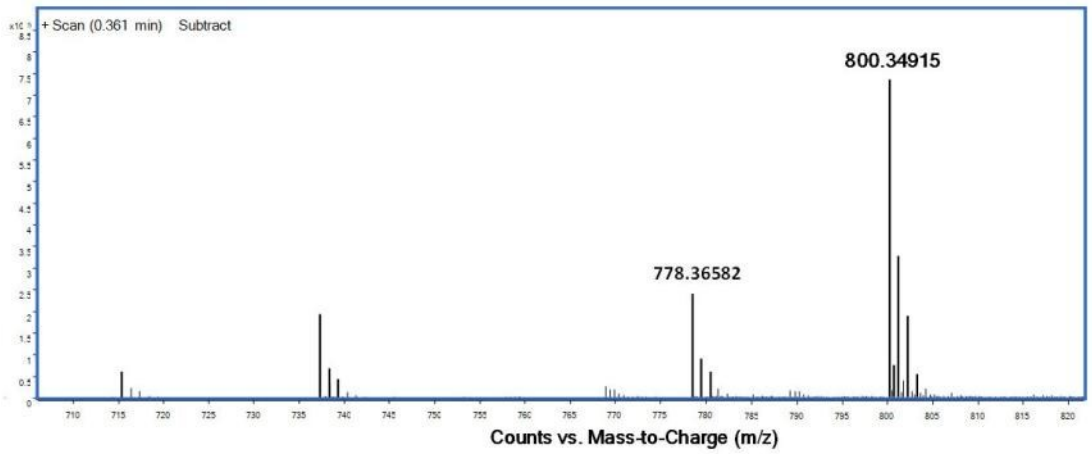
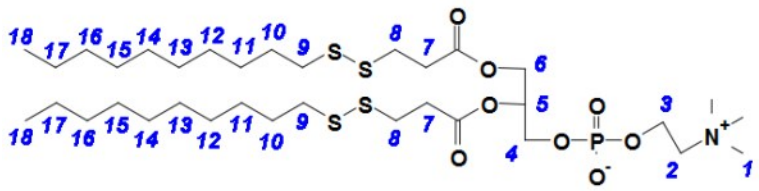


Figure S17. HRMS of SS15-PC, m/z $[M+H]^+$ calculated 778.36, found 778.36582; $[M+Na]^+$ calculated 800.35, found 800.34915.



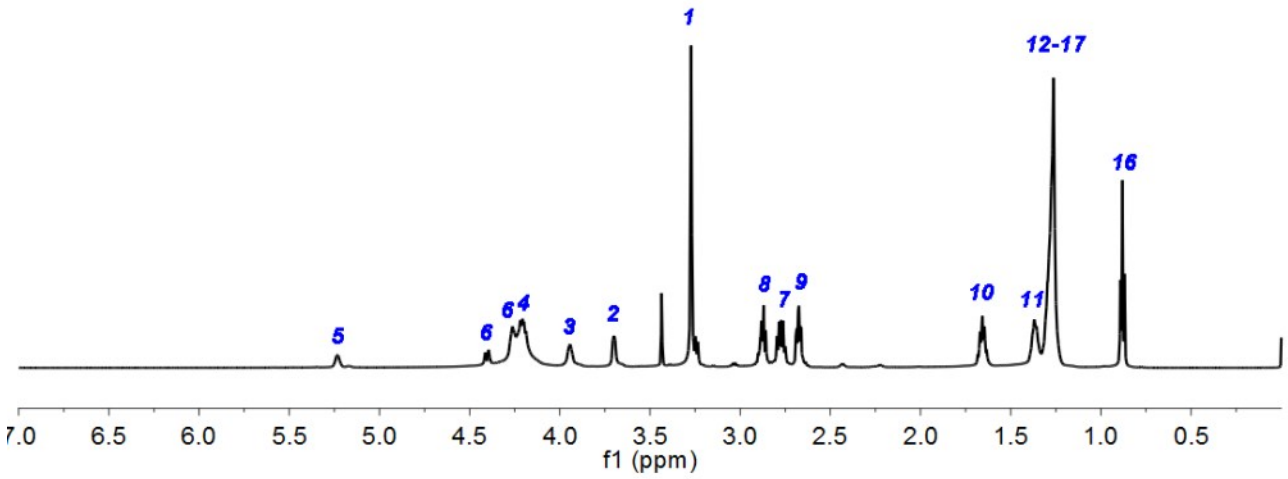


Figure S18. ^{1}H NMR of SS15-PC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 2H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 24H), 0.88 (m, 6H).

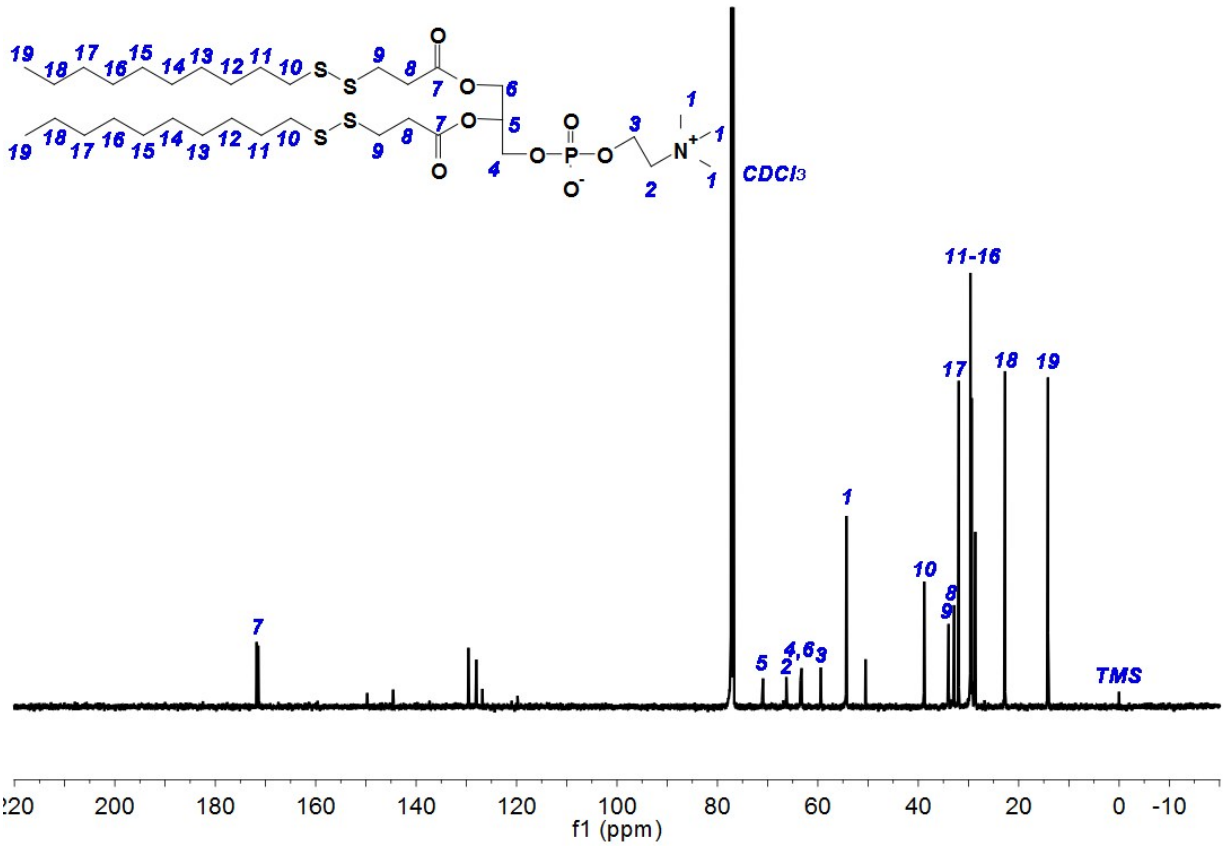


Figure S19. ^{13}C NMR of SS15-PC. ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.03, 70.91, 66.27, 63.43, 59.49, 38.52, 33.56, 31.93, 29.62, 28.38, 22.62, 14.07.

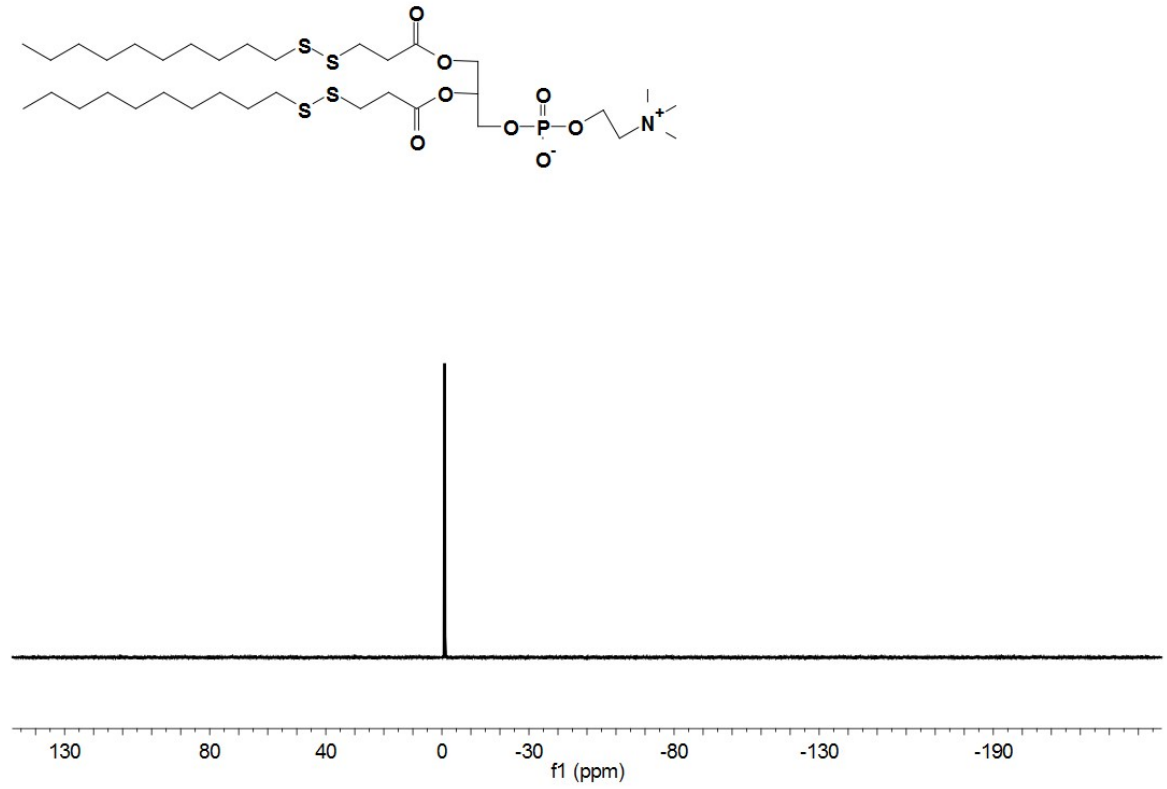


Figure S20. ^{31}P NMR of SS15-PC.

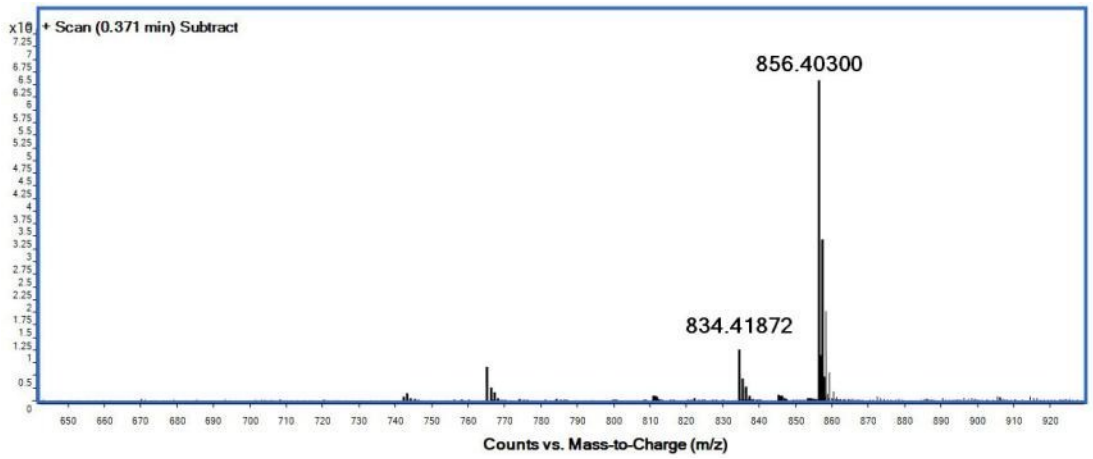


Figure S21. HRMS of SS17-PC, m/z $[M+H]^+$ calculated 834.42, found 834.41872; $[M+Na]^+$ calculated 856.41, found 856.40300.

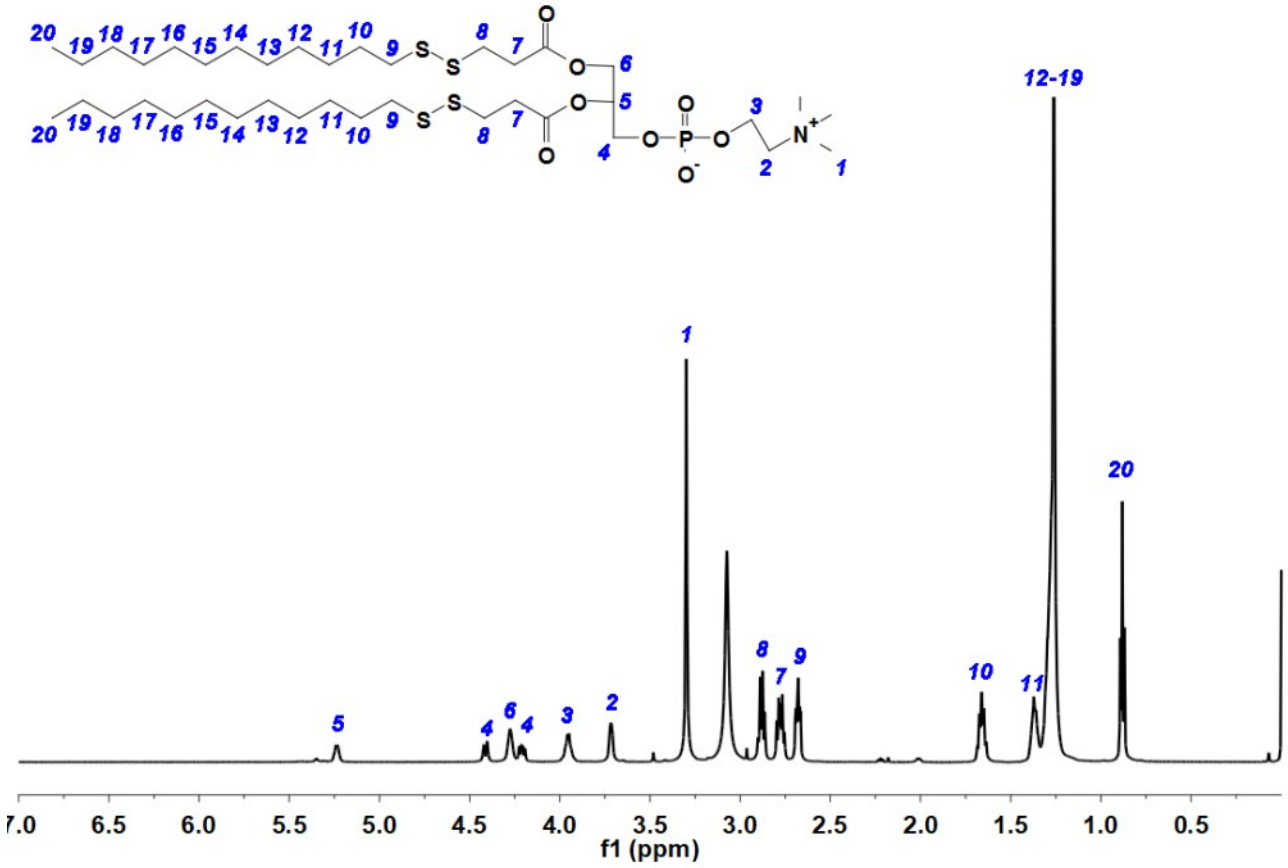


Figure S22. ^{1}H NMR of SS17-PC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 1H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 32H), 0.88 (m, 6H).

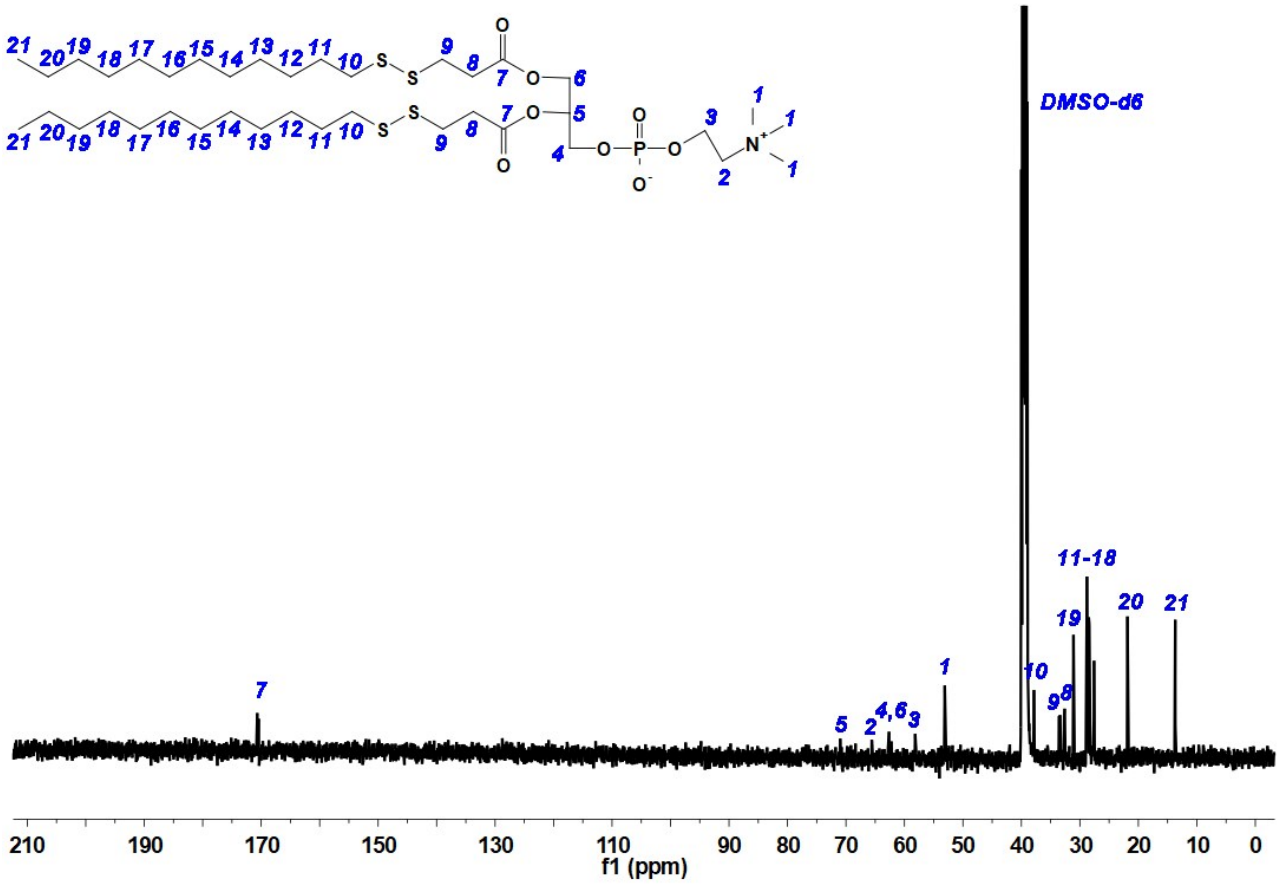


Figure S23. ^{13}C NMR of SS17-PC. ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.23, 70.80, 66.32, 63.24, 59.52, 38.54, 33.57, 31.83, 29.64, 28.36, 22.62, 14.07.

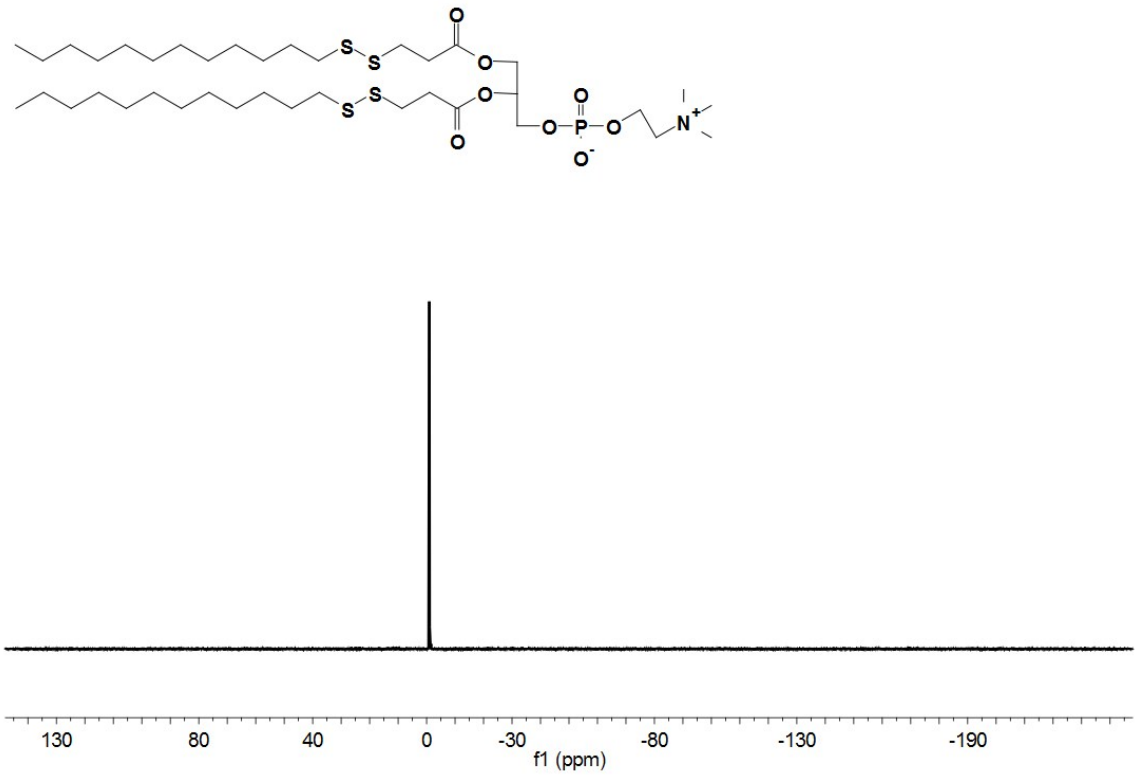


Figure S24. ^{31}P NMR of SS17-PC.

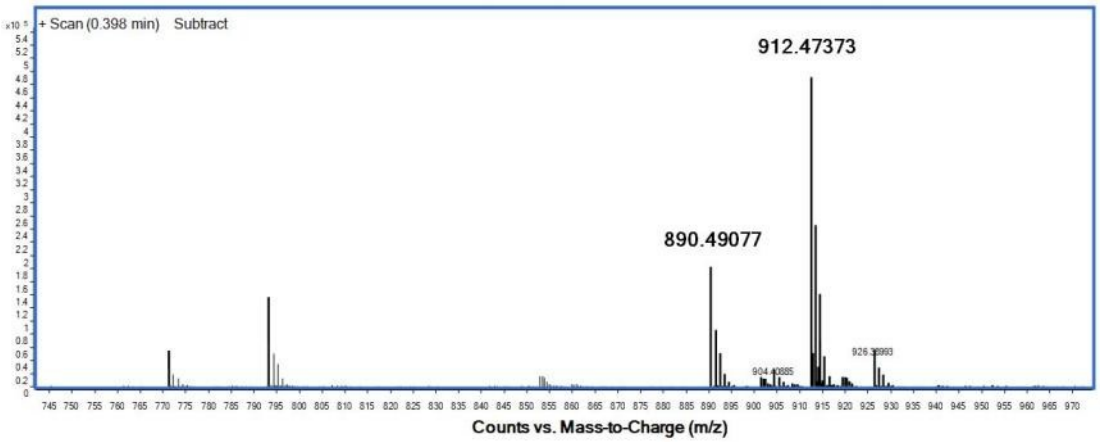


Figure S25. HRMS of SS19-PC, m/z $[M+H]^+$ calculated 890.48, found 890.49077; $[M+Na]^+$ calculated 912.47, found 912.47373.

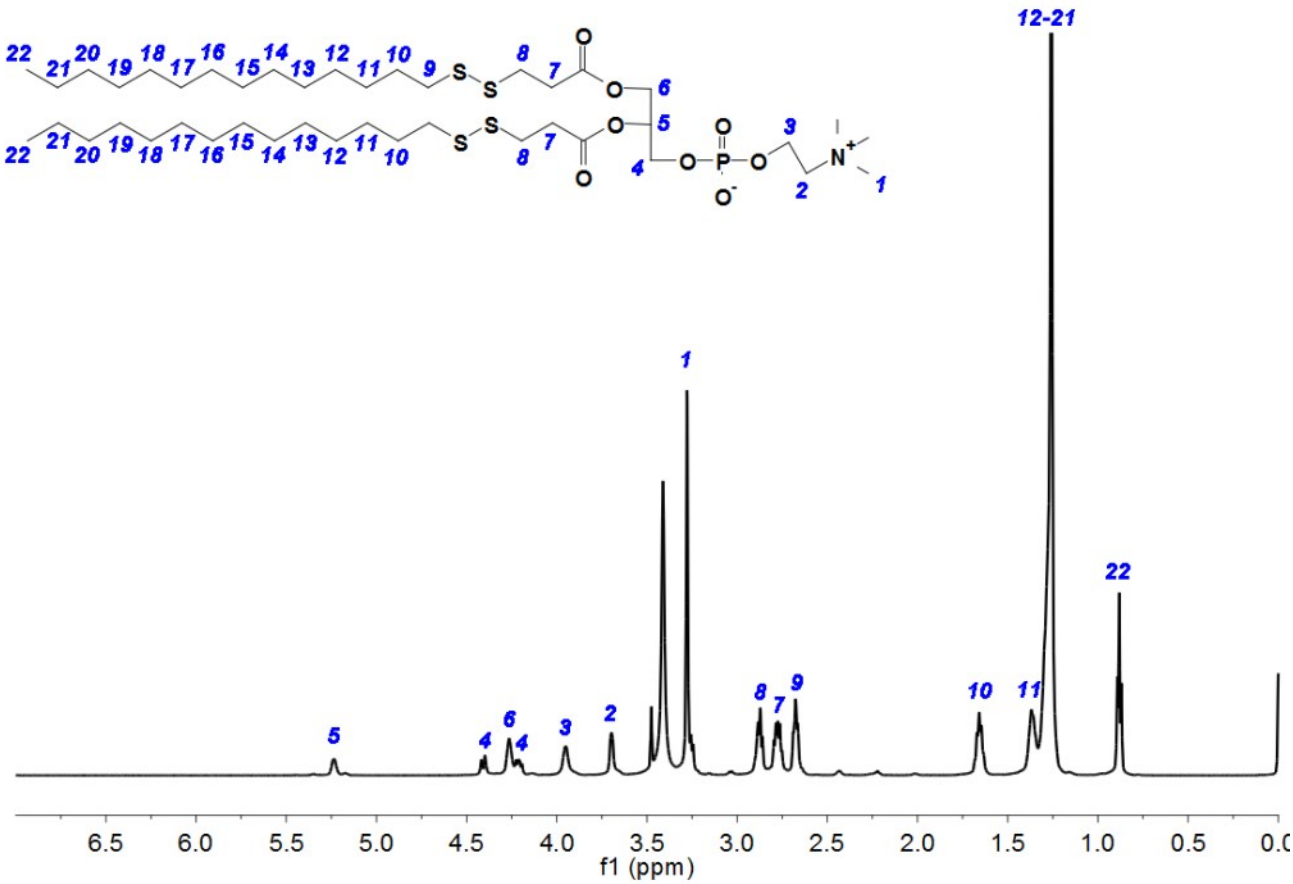


Figure S26. ^{1}H NMR of SS19-PC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 1H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 40H), 0.88 (m, 6H).

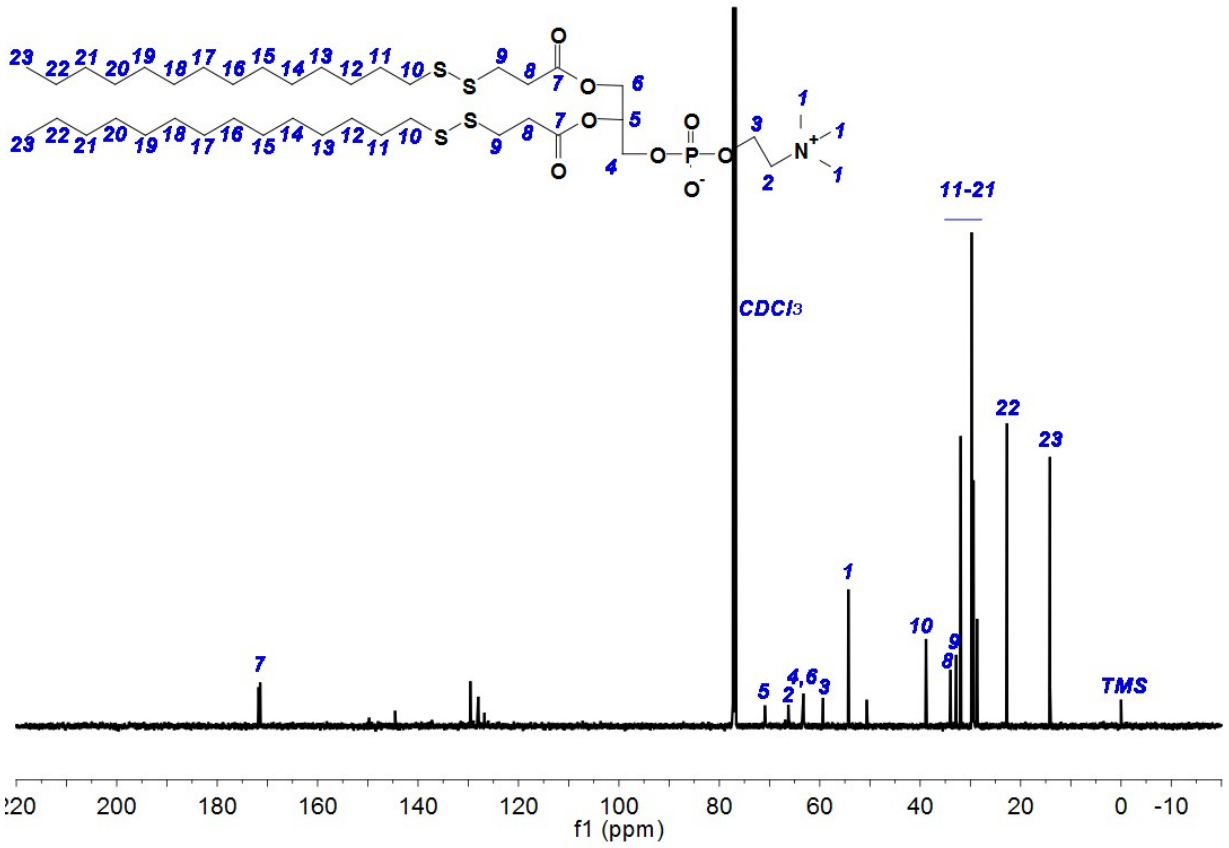


Figure S27. ^{13}C NMR of SS19-PC. ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.25, 70.77, 66.23, 63.29, 59.32, 38.59, 33.74, 31.64, 29.54, 28.39, 22.62, 14.10.

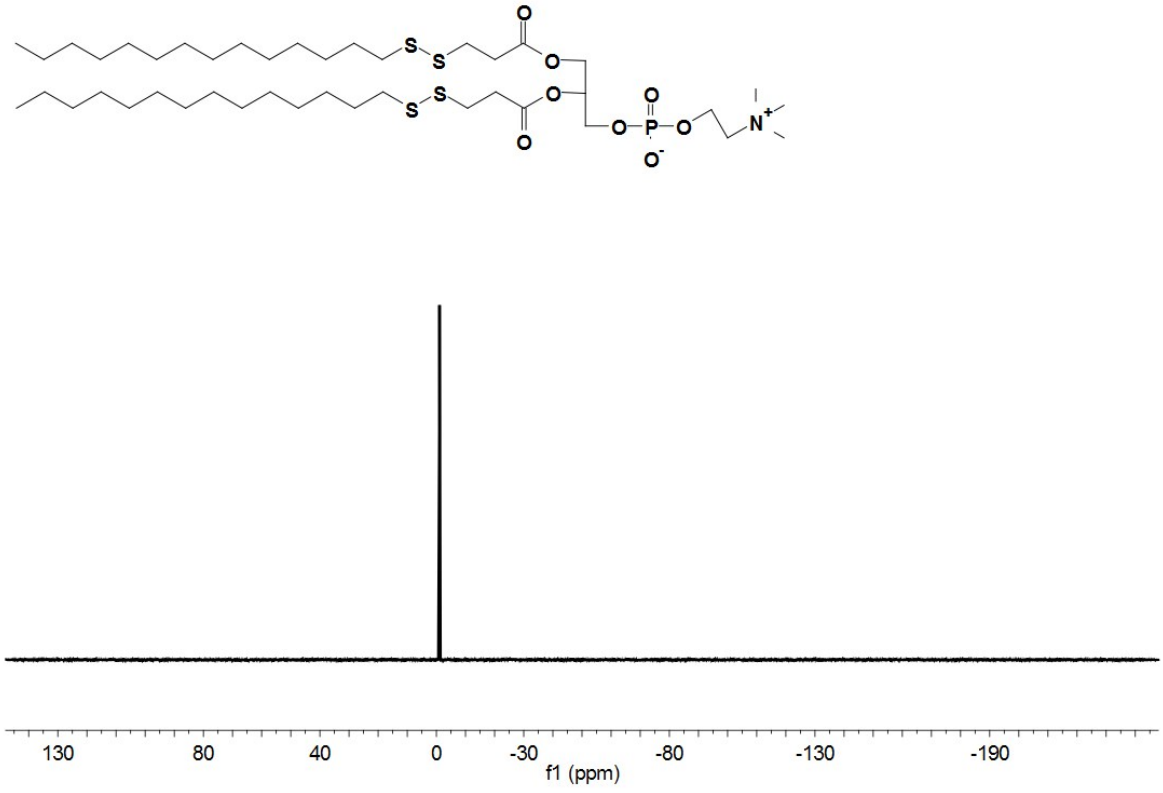


Figure S28. ^{31}P NMR of SS19-PC.

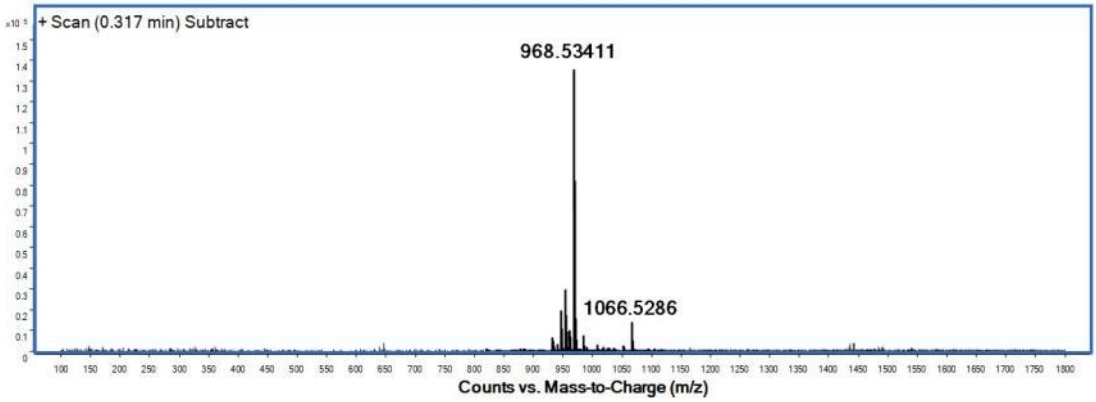


Figure S29. HRMS of SS21-PC, m/z $[M+Na]^+$ calculated 968.53, found 968.53411.

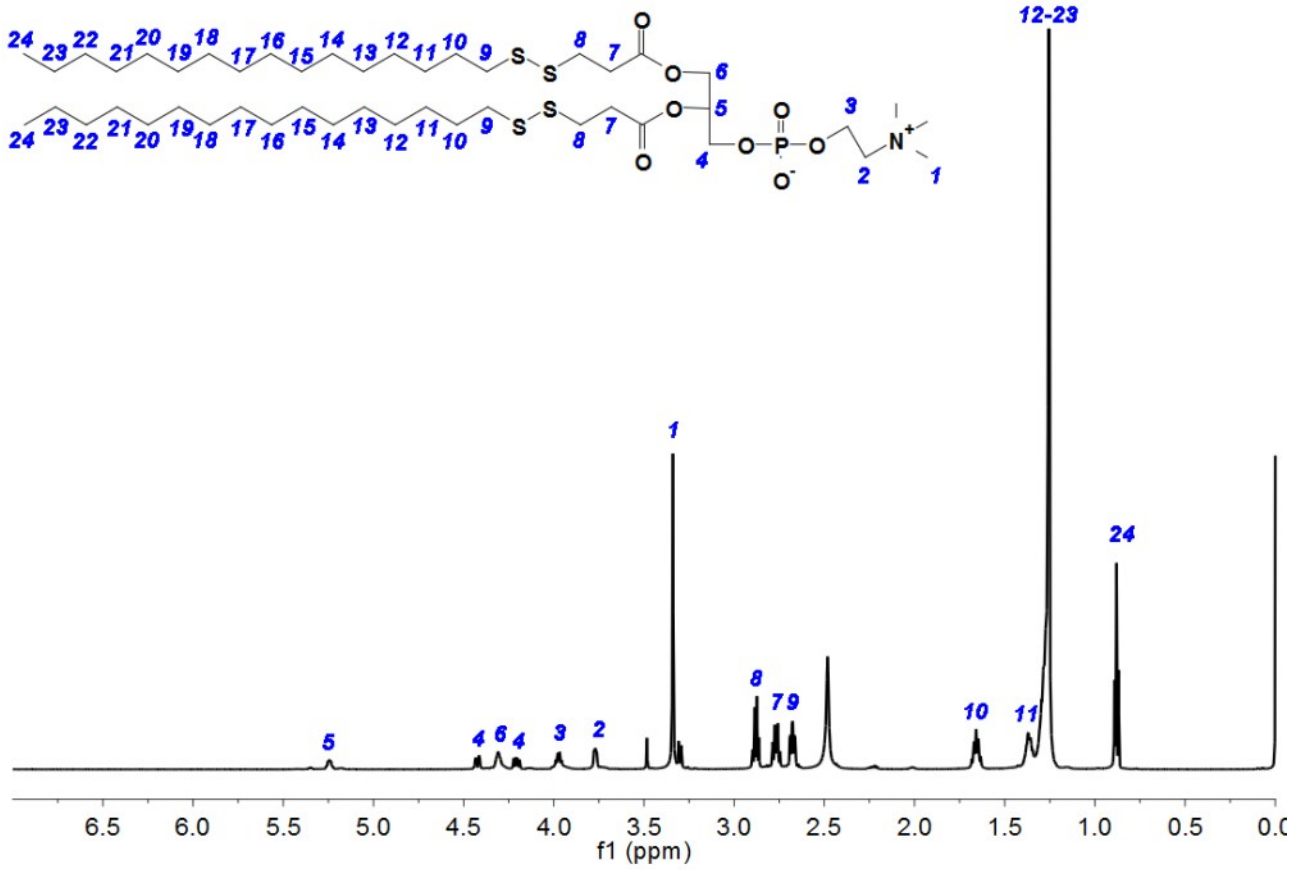


Figure S30. ^{1}H NMR of SS21-PC. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (br, 1H), 4.41 (m, 1H), 4.26 (m, 1H), 4.20 (m, 2H), 3.94 (s, 1H), 3.70 (s, 2H), 3.27 (s, 9H), 2.87 (m, 4H), 2.76 (m, 4H), 2.66 (m, 4H), 1.66 (t, 4H, *J*= 6.0 Hz), 1.29 (m, 4H), 1.28 (m, 48H), 0.88 (m, 6H).

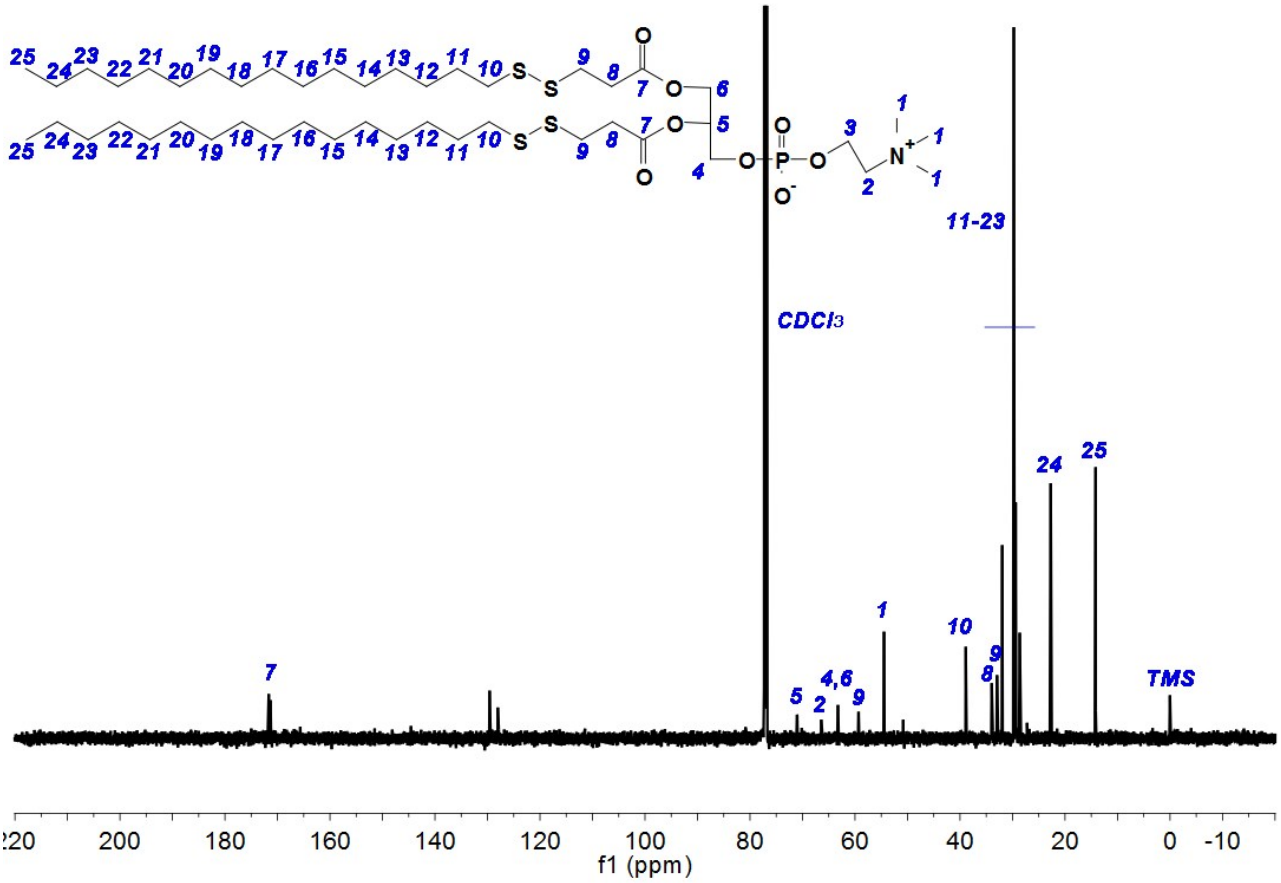
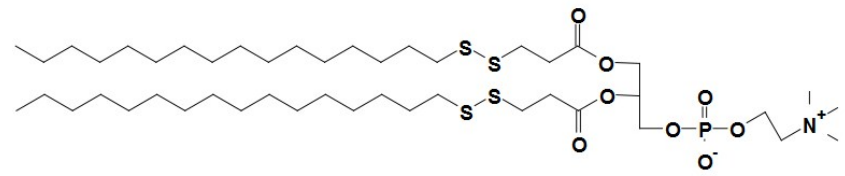


Figure S31. ^{13}C NMR of SS21-PC. ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.21, 70.92, 66.42, 63.35, 59.31, 38.44, 33.69, 31.23, 29.63, 28.38, 22.61, 14.07.



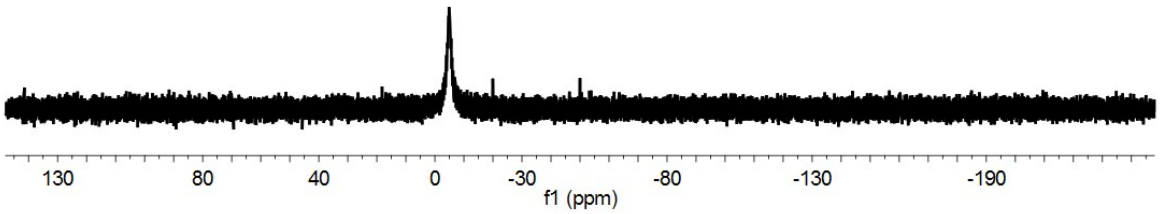


Figure S32. ^{31}P NMR of SS21-PC.

3. Preparation of SS-LP

Table S4. Size and polydispersity index (PDI) and zeta-potentials of liposomes

Liposomes	Size/nm	PDI	Zeta-potential/mV	EE*/%	DL*/%
DSPC-LP	107.15	0.257	-10.51	94.31	8.61
SS13-LP	146.54	0.313	-9.76	85.57	7.83
SS15-LP	115.53	0.302	-12.31	90.63	8.31
SS17-LP	102.03	0.308	-11.42	93.05	8.51
SS19-LP	99.71	0.276	-12.52	95.98	8.76
SS21-LP	99.03	0.284	-13.79	94.70	8.66

*detected by loading DOX.

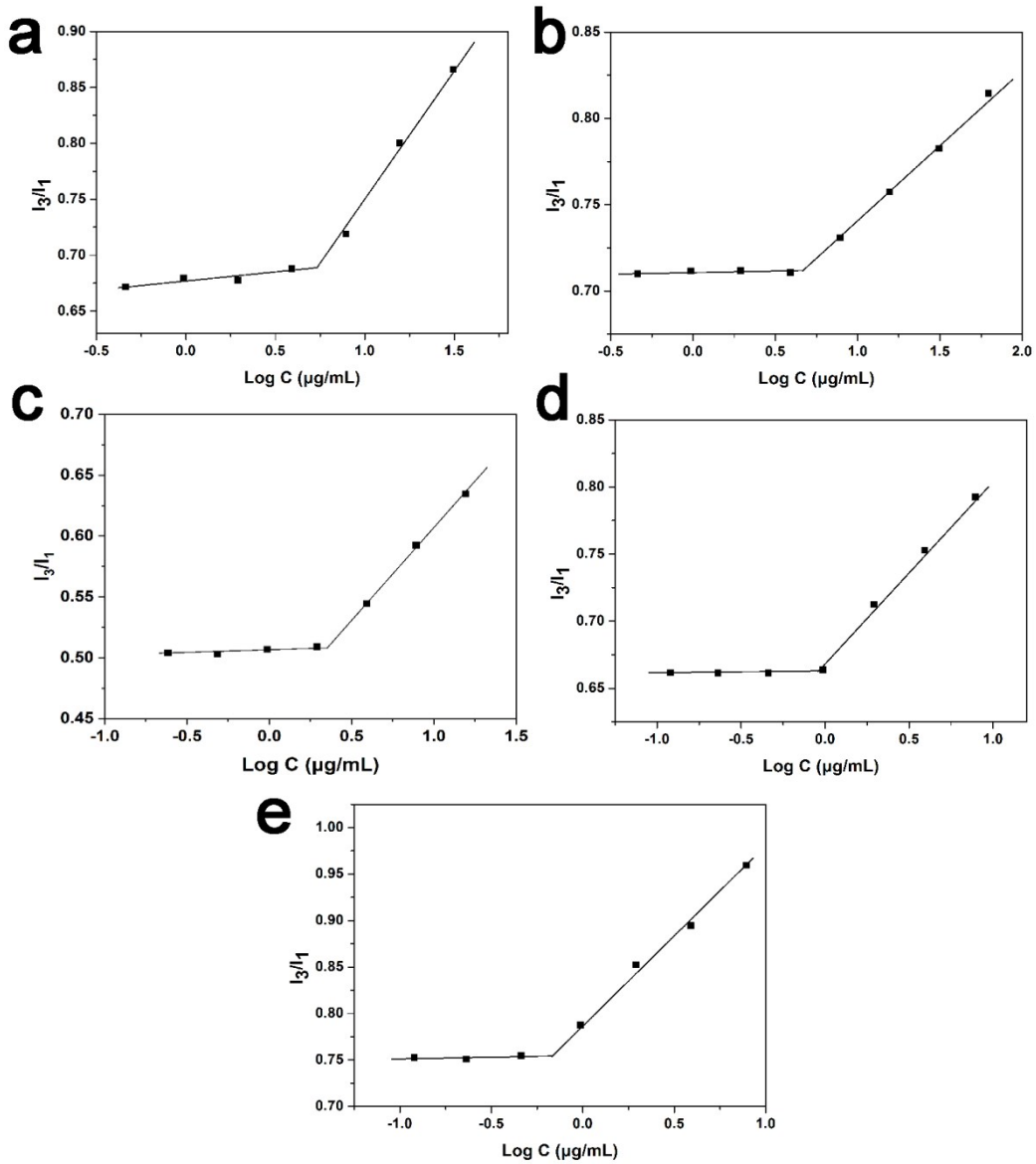


Figure S33. CAC of SS-PC was determined using the crossing point of the plot: (a) SS13-PC, 5.25 μg/mL; (b) SS15-PC, 4.68 μg/mL; (c) SS17-PC, 2.04 μg/mL; (d) SS19-PC, 0.93 μg/mL; (e) SS21-PC, 0.68 μg/mL.

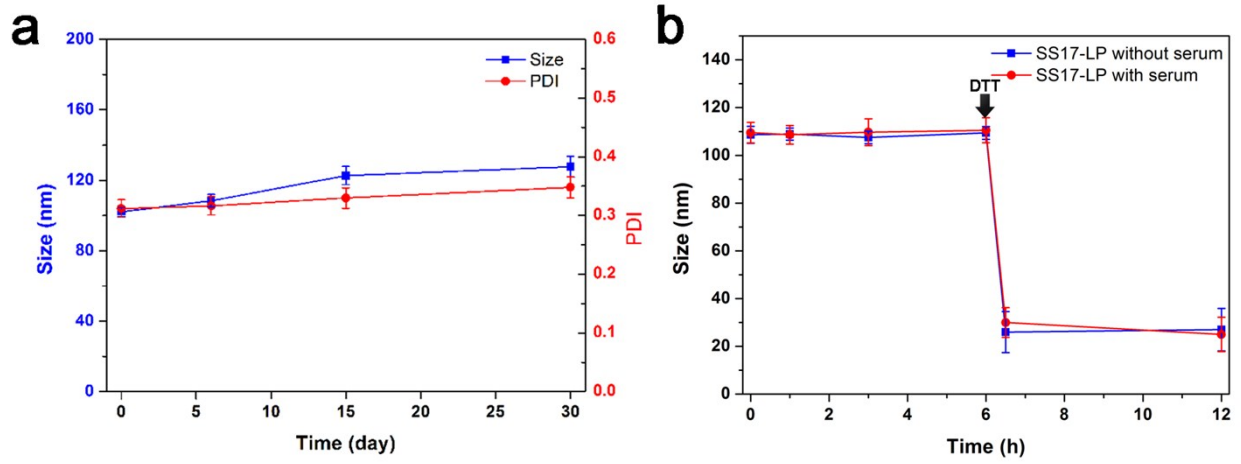


Figure S34. Storage (a) and serum (b) stability of SS17-LP. For serum stability assay, 20 mM DTT was added as a stimulated tumor reduction environment. The data showed the great storage and serum stability of liposomes, as well as the rapid sensitivity of DTT in serum containing medium.

4. Redox-sensitive assay

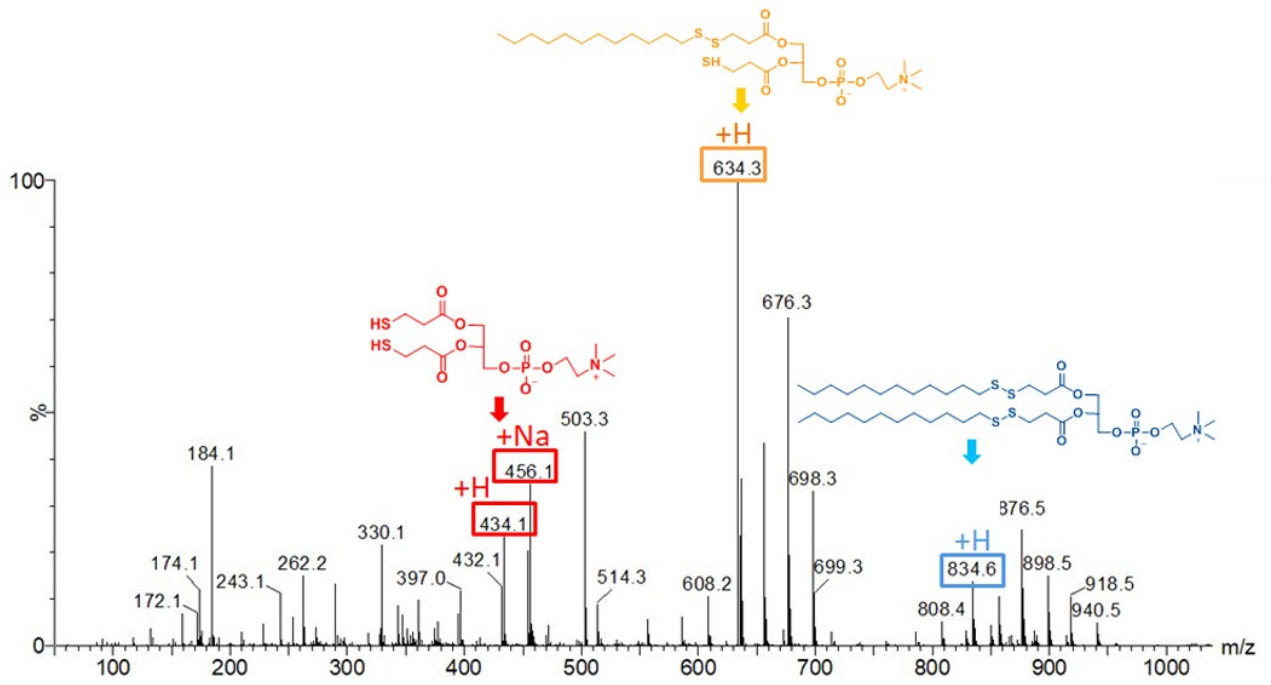


Figure S35. Degradation mechanism of SS17-LP under DTT treatment using MS technique: the molecular ion peaks of SS-PC with the two disulfide bonds cleaved structure (red) were detected as m/z: $[M+H]^+$ calculated 434.1, found 434.1; $[M+Na]^+$ calculated 456.1, found 456.1, and with one disulfide bond cleaved structure (orange) were detected as m/z: $[M+H]^+$ calculated 634.3, found 634.3.

5. Cellular uptake

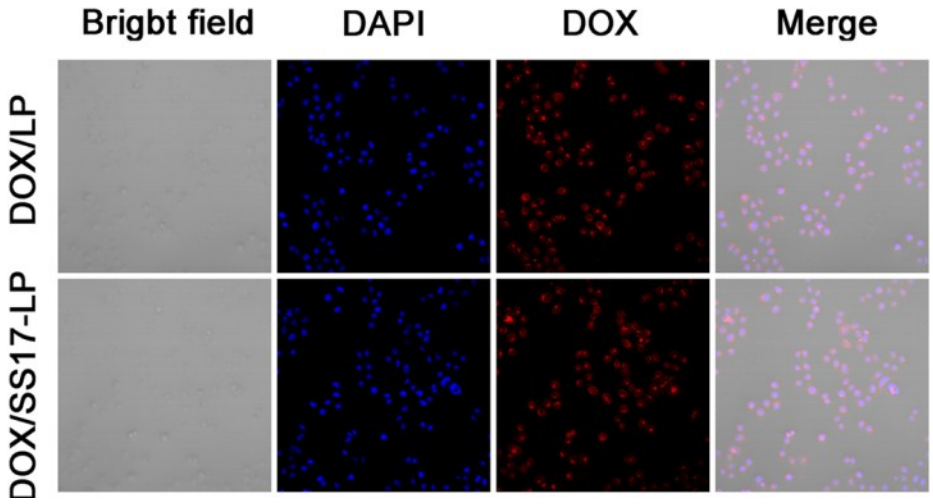


Figure S36. Cellular uptake of DOX/SS17-LP was studied in MCF-7 cells using CLSM.

References

1 A. K. Hinderliter, J. Huang and G. W. Feigenson, *Biophysical journal*, 1994, **67**, 1906.

2 J. P. Badyal, A. M. Cameron, N. R. Cameron, D. M. Coe, R. Cox, B. G. Davis, L. J. Oates, G. Oye and P. G. Steel, *Tetrahedron Letters*, 2001, **42**, 8531.

3 J. Gao, J. Ming, B. He, Z. Gu and X. Zhang, *Biomedical Materials*, 2008, **3**, 015013.

4(a) G. Ren, D. Liu, W. Guo, M. Wang, C. Wu, M. Guo, X. Ai, Y. Wang and Z. He, *Drug delivery*, 2016, **23**, 1272; (b) V. Kannan, P. Balabathula, M. K. Divi, L. A. Thoma and G. C. Wood, *Journal of liposome research*, 2015, **25**, 308.

5 C. Sanson, C. Schatz, J.-F. Le Meins, A. Soum, J. Thévenot, E. Garanger and S. Lecommandoux, *Journal of Controlled Release*, 2010, **147**, 428.