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Supplementary Information

Production of Dynamic Lipid Bilayers Using the Reversible Thiol-Thioester Exchange Reaction

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Materials and Methods

General. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra for product verification were gathered on a Bruker Ascend 400 spectrometer. High-resolution mass spectrometry (HRMS) was performed on a Waters Synapt G2-Si (q-TOF) mass spectrometer. Reactions were monitored on an Agilent 1100 Series HPLC fitted with an Agilent Zorbax 5um 4.6x50mm C8 column coupled with Agilent G1946D Mass Spectrometer and SedeX Evaporative Light Scattering Detector.

Synthesis of Thiol Lysolipid (SHPC):

To a dry 10mL round-bottomed flask equipped with magnetic stir bar was added 70mg (0.14mmol, 1 equiv.) 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine and 3-3.5mL chloroform. Following stirring for 30 minutes under argon the solution was cooled to $0^{o}C$ and 60mg (0.28mmol, 2 equiv.) 3,3'-dithiodipropionic acid, 80mg (0.42mmol, 3 equiv.) EDAC and 5mg (0.042mmol, 0.3 equiv.) DMAP was added while stirring. The solution was allowed to react overnight under argon. Following reaction, the $CHCl_3$ was removed and products were dissolved in H_20 for 30 minutes. This solution was transferred to 1,000MW cutoff dialysis tubing and dialyzed overnight. Following dialysis, the solution was transferred to a vial equipped with stir bar and 200mg (0.7mmol, 5 equiv.) TCEP-HCl was added. 1M NaOH was added dropwise to neutralize the solution followed by spinning overnight. This solution was then transferred to fresh 1,000MW cutoff dialysis tubing for overnight dialysis. The product was lyophilized to produce a white powder. Yield = 71% ^{1}H NMR (400mHz, $CDCl_3$) δ: 5.24 (m, 1H), 4.36 (dd, J=12.1, 3.0 Hz, 1H), 4.27 (m, 2H), 4.15 (dd, J=12.1, 7.4 Hz, 1H), 3.95 (m, 2H), 3.75 (m, 2H), 3.32 (s, 9H), 2.74 (m, 2H), 2.67 (m, 2H), 2.28 (m,

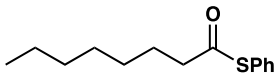
2H), 1.80 (t, J=8.2 Hz, 1H), 1.57 (m, 2H), 1.25 (m, 24H), 0.86 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 173.7, 171.2, 71.3, 66.3, 63.6, 62.9, 59.4, 54.4, 38.6, 34.2, 32.1, 29.8, 29.8, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 29.5, 29.3, 25.0, 22.8, 19.8, 14.3. HRMS *m/z*: calcd. for $[C_{27}H_{54}NO_8PS+Cl]^-$ 618.3002; found 618.3032.

Synthesis of *S*-phenyl thioester:

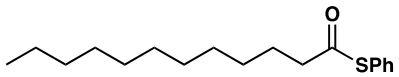
S-phenyl thiooctanoate[1] and S-phenyl dodecanoate[2] have been documented previously and were synthesized here using the below stated procedures.

***S*-phenyl thiooctanoate.**

To a 100 mL round-bottomed flask equipped with a magnetic stir bar was added 0.57 mLs (0.61 grams, 5.54 mmol, 1.1 equiv) of thiophenol, 0.98 mLs (0.71 grams, 7 mmol, 1.4 equiv) of triethylamine (Et_3N), and was diluted with 25 mLs (0.2 M) of anhydrous CH_2Cl_2. This solution was cooled to 0°C and the octanoyl chloride 0.85 mLs (0.81 grams, 5 mmol, 1equiv.) was added drop-wise over a period of 15 min. The mixture was allowed to react for another 16 hours at room temperature. After this period, the contents of the reaction mixture were diluted with 100 mLs of CH_2Cl_2 and then water (30 mLs). This biphasic mixture was transferred to a separatory funnel, the organic layer was removed and the aqueous layer was further extracted with CH_2Cl_2 (~50 mLs, 2X), the combined organics were washed with 1N HCl solution(~50 mLs, 1X), water (~50 mLs, 1X), (brine (~25 mLs, 1X), dried over Na_2SO_4, filtered, and concentrated to give 1.08 grams (92%) of the title compound (***S*-phenyl thiooctanoate**) as a colorless liquid which was used directly with no further purifications.



***S*-phenyl thiooctanoate**: colorless liquid; 92% yield; NMR (400 MHz, $CDCl_3$) δ = 7.41(m, 5H), 2.67 – 2.64 (m, 2H), 1.75-1.68 (m, 2H), 1.35-1.27(m, 8H), 0.91-0.87 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 197.8, 134.6, 129.4, 129.3, 128.1, 43.9, 31.8, 29.1, 29.1, 25.7, 22.7, 14.2.



***S*-phenyl thiododecanoate**: colorless liquid; 91% yield; NMR (400 MHz, $CDCl_3$) δ = 7.41(m, 5H), 2.67 – 2.63 (m, 2H), 1.75-1.67 (m, 2H), 1.40-1.23(m, 16H), 0.90-0.86 (m, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 197.8, 134.6, 129.4, 129.3, 128.1, 43.9, 32.1, 29.7, 29.7, 29.6, 29.5, 29.4, 29.1, 25.8, 22.8, 14.3.

All other compounds were purchased from standard sources and were used as received.

Preparation of Lipid Solutions. Prior to use, SHPC was reduced overnight in an equimolar mixture of TCEP-HCl and NaOH in a 2:1 SHPC:TCEP solution. For monitoring of the phospholipid coupling reaction *via* LCMS, a 100uL solution was prepared of aliphatic phenyl thioester in water (5mM), reduced SHPC mixture (5mM SHPC), and TEA (10mM) in HPLC vials fitted with 350uL Supelco glass inserts. The solution was then left at room temperature to react.

Microscopy samples were prepared similarly ([reduced SHPC]=[thioester tail] = 5mM, [TEA]= 10mM) with the addition of 2uM rhodamine-DHPE for fluorescence microscopy samples. Following mixing, fluorescence microscopy samples were mixed and sealed under coverslips for development of liposomal structures. C7

thioester samples were developed at room temperature for 48 hours. C11 samples were developed at 30°C for 24 hours prior to imaging *via* fluorescence microscopy or 40°C prior to freezing for cryo-TEM. Photobleaching samples were prepared the same as the fluorescence microscopy samples, with the addition of 1mM HPTS in the reaction mixture.

Exchange reactions began the same as above ([reduced SHPC]=[thioester tail] = 5mM, [TEA]= 10mM, as well as [rhodamine-DHPE]=2uM if sample was to be used for fluorescence microscopy). C11 thioester samples were reacted for 24 hours and C7 thioester samples were reacted for 48 hours. Reaction was followed by addition of equimolar C7 thioester tail in C11 samples or up to 10 equivalents of C11 thioester tail into C7 samples. Microscopy samples for C11 thioester product exchange with C7 thioester tails were developed at 30°C.

Microscope Imaging. Fluorescent imaging was performed using a Nikon A1R laser scanning confocal microscope equipped with Nikon Elements software version 4.20-4.6. In all cases, a 561nm laser was employed to excite the rhodamine-DHPE while a 488nm laser was additionally used for photobleaching assays. For acquisition of TEM images, 4 µl of sample solution was transferred onto a lacey holey-carbon grid, blotted for 1-2 seconds, and then plunge-frozen using an FEI-Vitrobot Mark IV at room temperature. The resulting vitrified sample was imaged on an FEI Tecnai F20 FEG-TEM, operating at 200kV and 25,000x magnification, using a Gatan US4000 CCD camera. The electron dose per image was limited to 20 electrons/$\mathbf{Å^2}$, with a defocus of -2µm, using the low-dose mode of Serial EM acquisition software.

LC-MS of Product Formation. Phospholipid formation was conducted in HPLC vials fitted with 350uL glass inserts as described above with 5uL samples taken over the course of the reaction. Samples were injected onto an analytical 1100 Series Agilent LC-MS with Agilent G1946D Mass Spectrometer and SedeX Evaporative Light Scattering Detector controlled by ChemStation software and fitted with Agilent Zorbax 5um 4.6x50mm C8 column. A binary solvent system of 0.1% formic acid in 5:4:1 isopropanol:water:methanol and chloroform was used at a flow rate of 0.9mL/minute. To quantify conversion, SHPC peak area was normalized to the area measured at 0 hours while products were normalized to areas measured at 24 hours (C11 system) or 48 hours (C7 system). For phospholipid **1b** to phospholipid **1c** exchange, peak areas were normalized to 0 hours for phospholipid **1b** and 96 hours for phospholipid **1c**.

Photobleaching Experiments. Vesicle formation was conducted in sealed slides in the presence of 1mM HPTS and 2uM rhodamine-DHPE as described above. Following self-assembly of bilayers, select liposomes were imaged for 5 seconds, exposed to high intensity 488nm light for 15 seconds to bleach encapsulated HPTS, then imaged for one minute to monitor for restoration of fluorescence. Mean internal fluorescence of photobleached liposomes was measured using Nikon Elements software and values normalized to initial fluorescence intensity were averaged across 6 liposomes for phospholipid **1c** and 3 liposomes for phospholipid **1b**.

Supplementary Figures

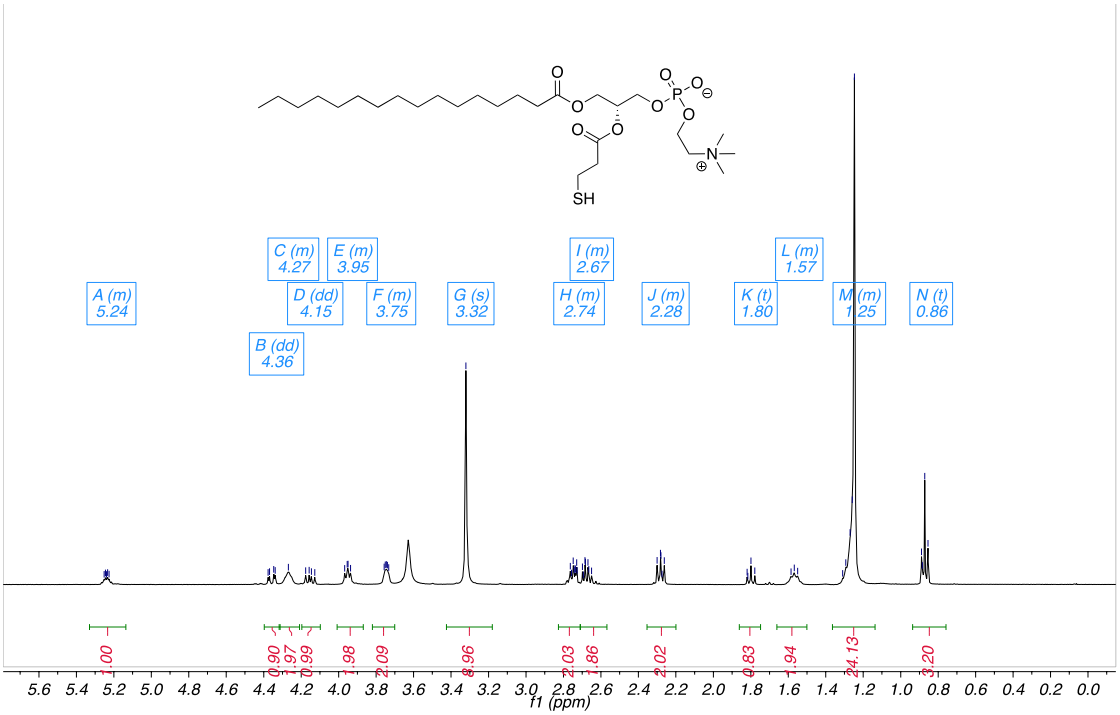


Figure S1. ^{1}H NMR of thiol-functionalized lysolipid.

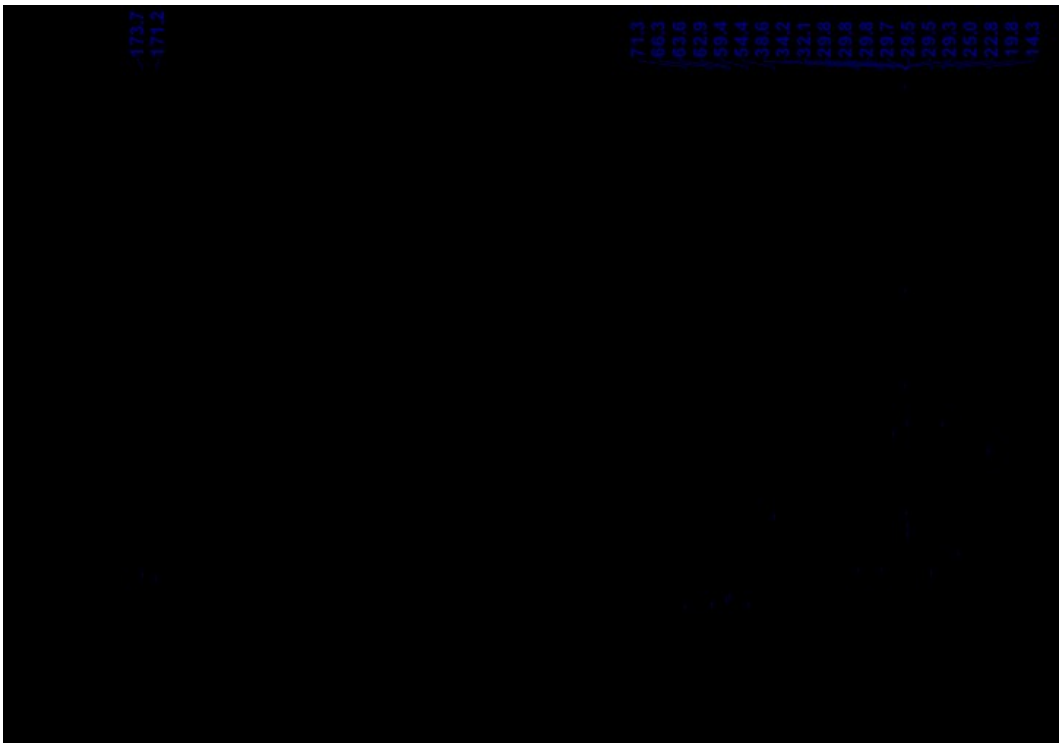


Figure S2. ^{13}C NMR of thiol-functionalized lysolipid.

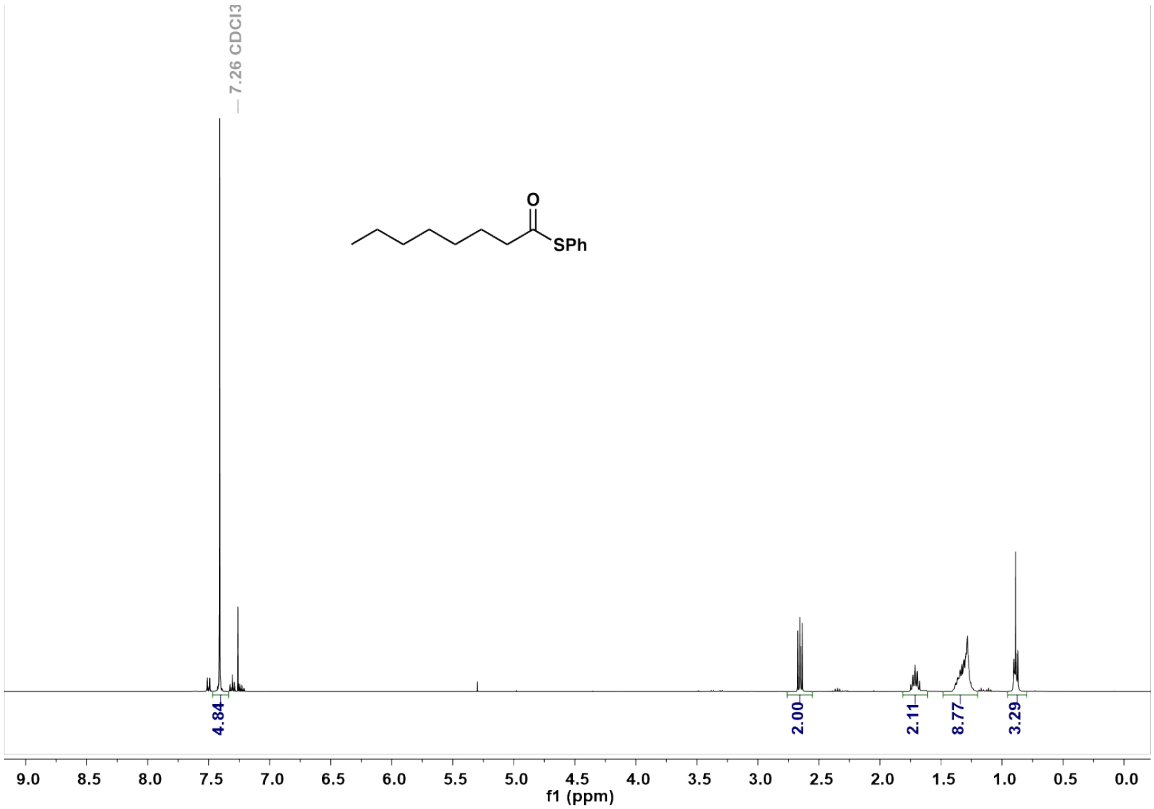


Figure S3. ^{1}H NMR of *S*-phenyl thiooctanoate

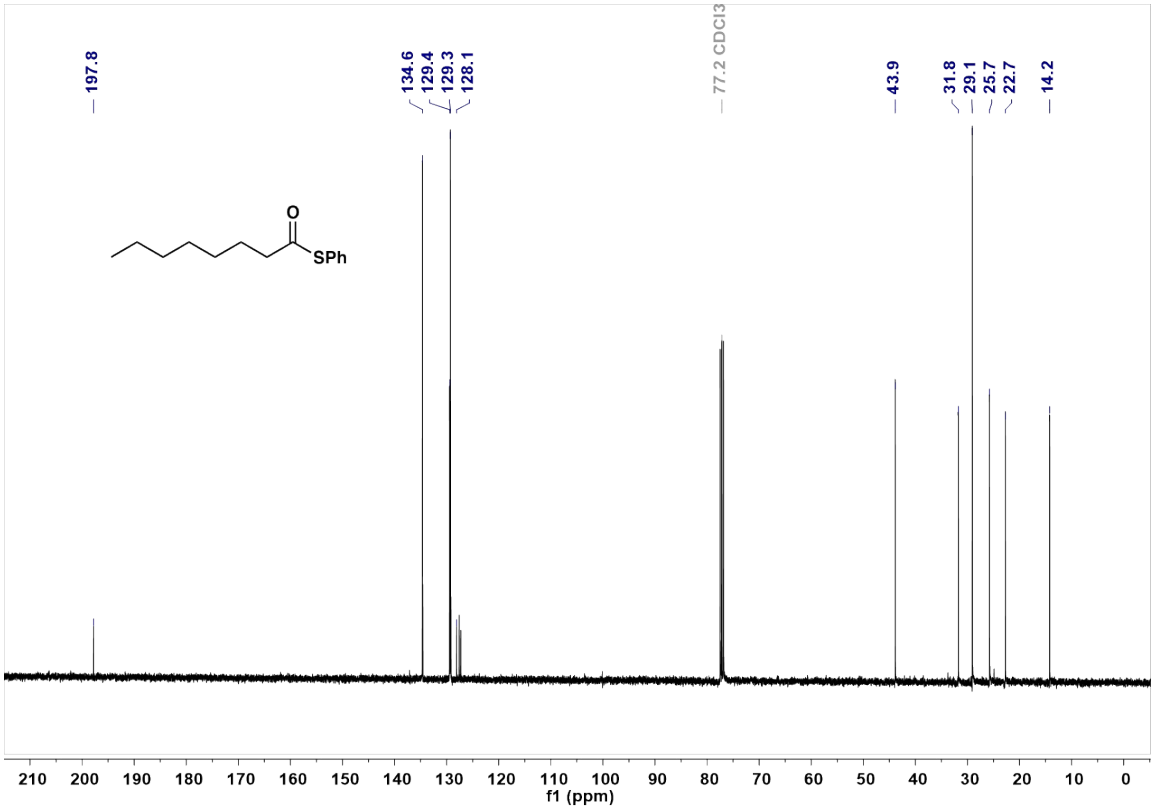


Figure S4. ^{13}C NMR of *S*-phenyl thiooctanoate

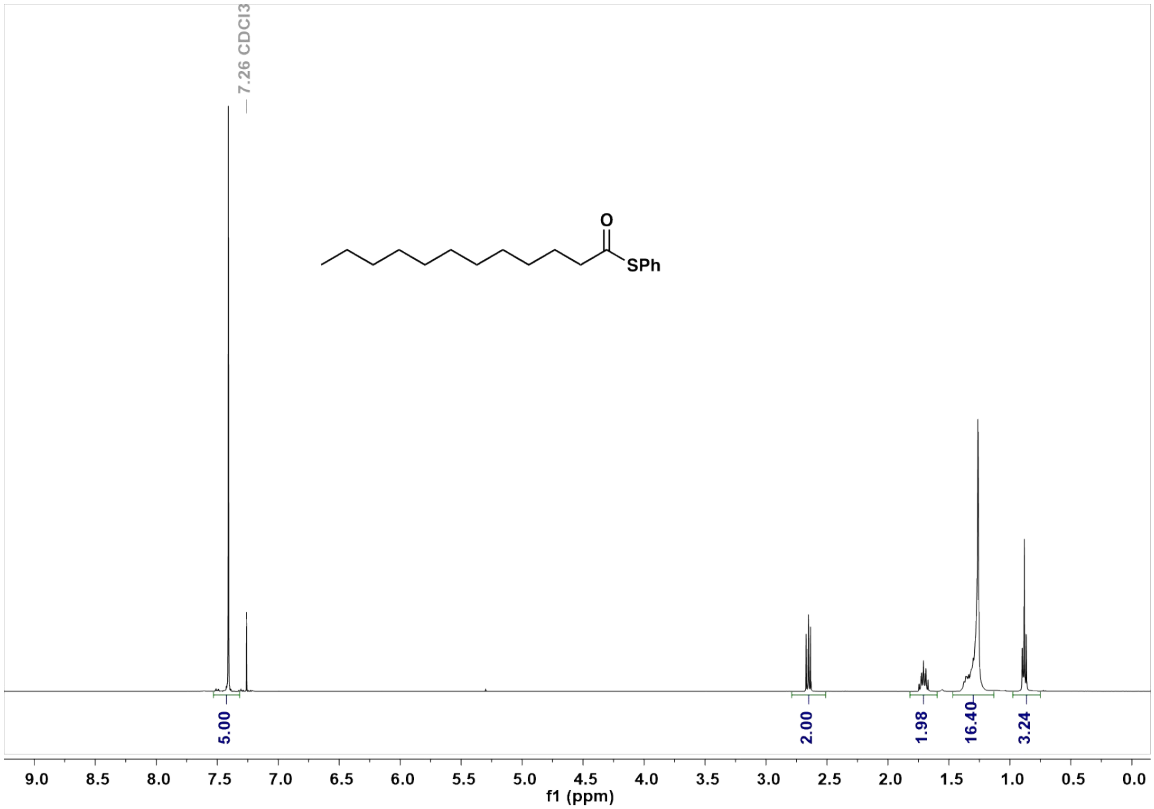


Figure S5. ^{1}H NMR of *S*-phenyl thiododecanoate

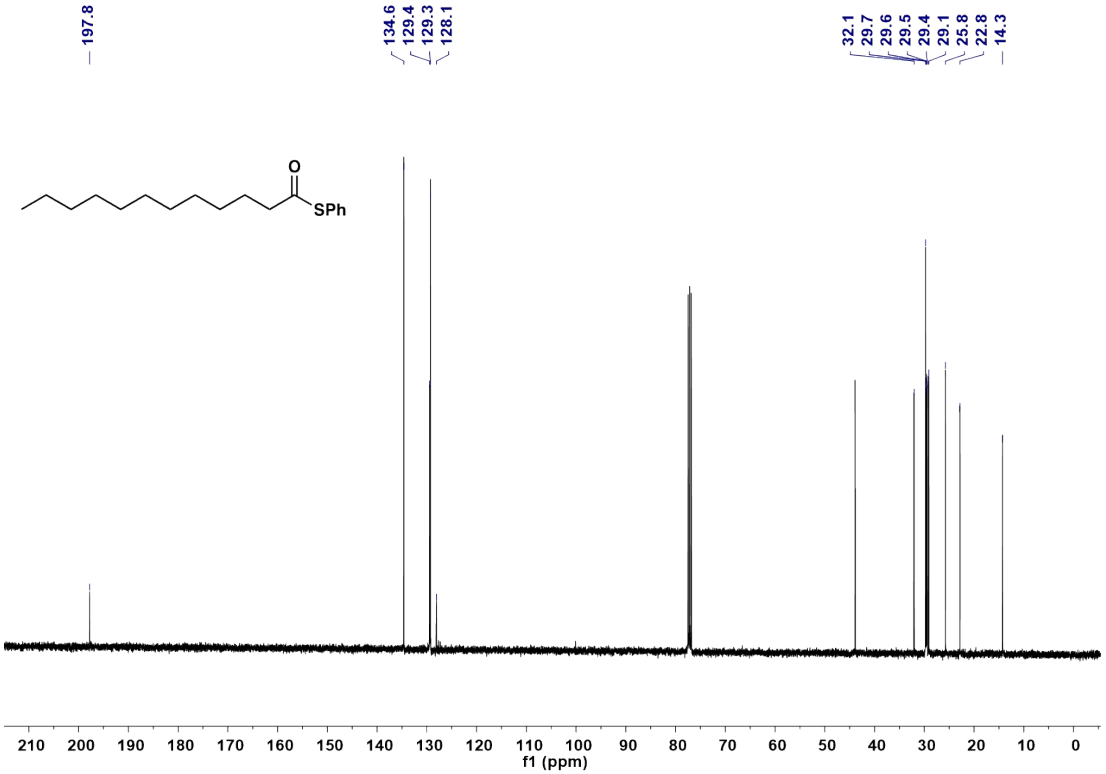


Figure S6. ^{13}C NMR of *S*-phenyl thiododecanoate

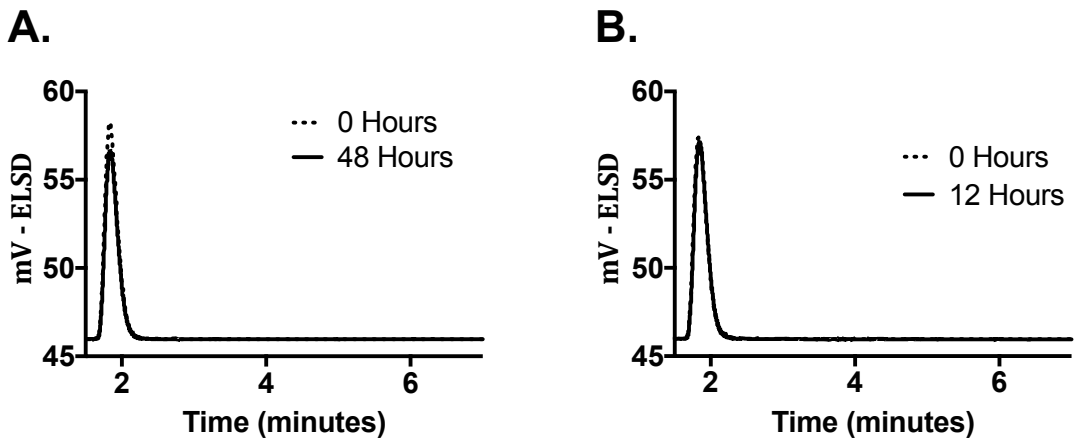


Figure S7. HPLC-MS-ELSD of catalyst-free control reactions. SHPC (5mM) and either C7 phenyl thioester (A) or C11 phenyl thioester (B) (5mM) were mixed and allowed to react at room temperature. Samples were collected at various time points and injected onto the LC-MS-ELSD. Over the time monitored, no conversion was apparent.

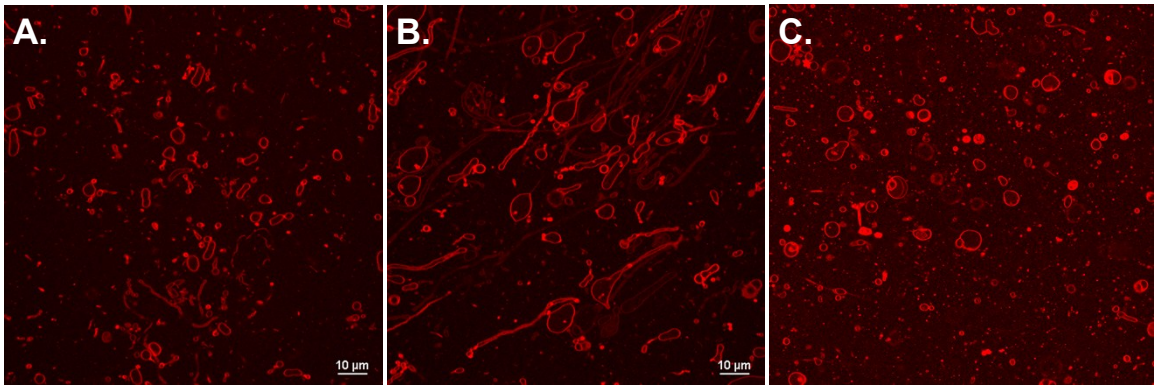


Figure S8. Fluorescence microscopy displays no significant dependence upon temperature for liposome assembly in mixtures of SHPC (5mM), C7 phenyl thioester (5mM), TEA (10mM), and rhodamine-DHPE (2uM) reacted in sealed glass slides for 48 hours at 4°C (A), room temperature (B), or 40°C (C) prior to imaging.

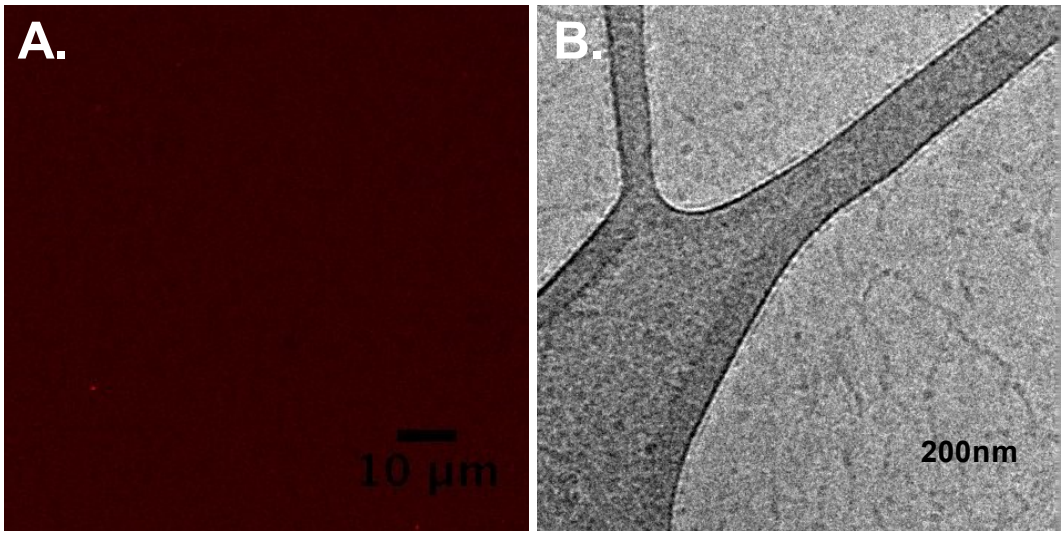
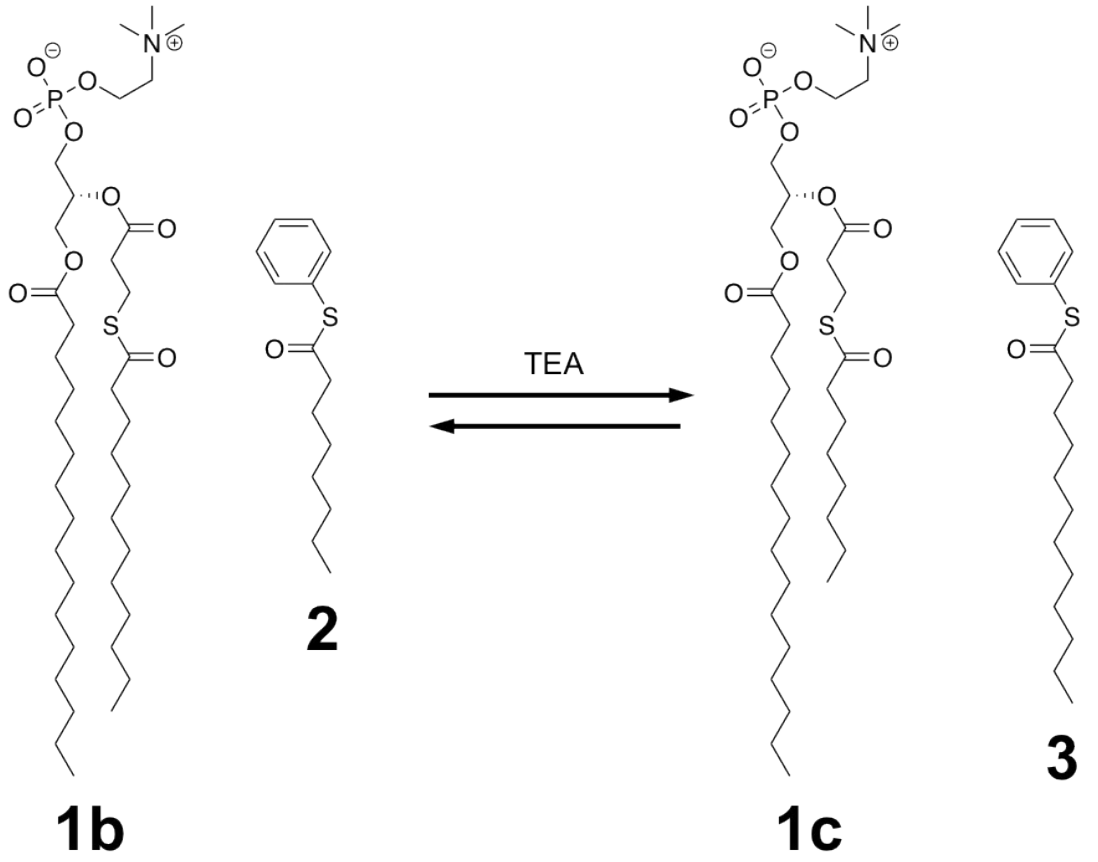


Figure S9. Fluorescence microscopy displays no giant vesicle formation when mixtures of SHPC (5mM), C11 phenyl thioester (5mM), TEA (10mM), and rhodamine-DHPE (2uM) were reacted in a sealed glass slide at room temperature for 24 hours (A). However, worm-like micelles are evident using cryo-TEM (B).



Scheme S1. Schematic illustrating the exchange of phospholipid **1b** with C7 thioester tails [2] to form new phospholipid **1c** products. The exchange of a phenyl thioester tail into a pre-existing thioester-containing phospholipid demonstrates the ability to remodel the lipid membrane and the future ability to introduce new functionalities into pre-formed membranes.

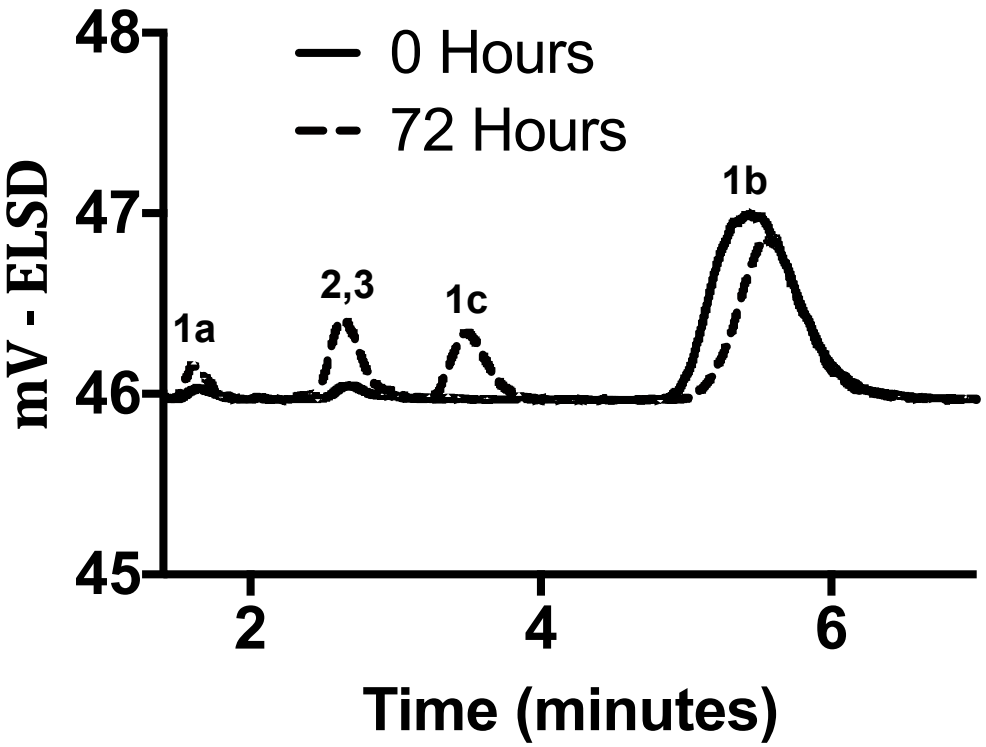


Figure S10. LC-MS-ELSD displays exchange of phospholipid **1b** with C7-phenyl thioester tails [**2**] to form new phospholipid **1c**. Following reaction of SHPC [**1a**] and C11-phenyl thioester tails [**3**] over the course of 24 hours, resulting in nearly 100% conversion to phospholipid **1b**, equimolar C7-thioester tails [**2**] were added. Exchange of the two tails progressed over the course of 96 hours to convert over 30% of phospholipid **1b** to phospholipid **1c**.

References:

1 Development of Activity-Based Chemical Probes for Human Sirtuins, https://pubs.acs.org/doi/pdf/10.1021/acschembio.7b00754, (accessed June 10, 2018).
2 H. Nambu, K. Hata, M. Matsugi and Y. Kita, *Chem. – Eur. J.*, **11**, 719–727.