

SUPPORTING INFORMATION FOR:

Stabilization of vesicular and supported membranes by glycolipid oxime polymers

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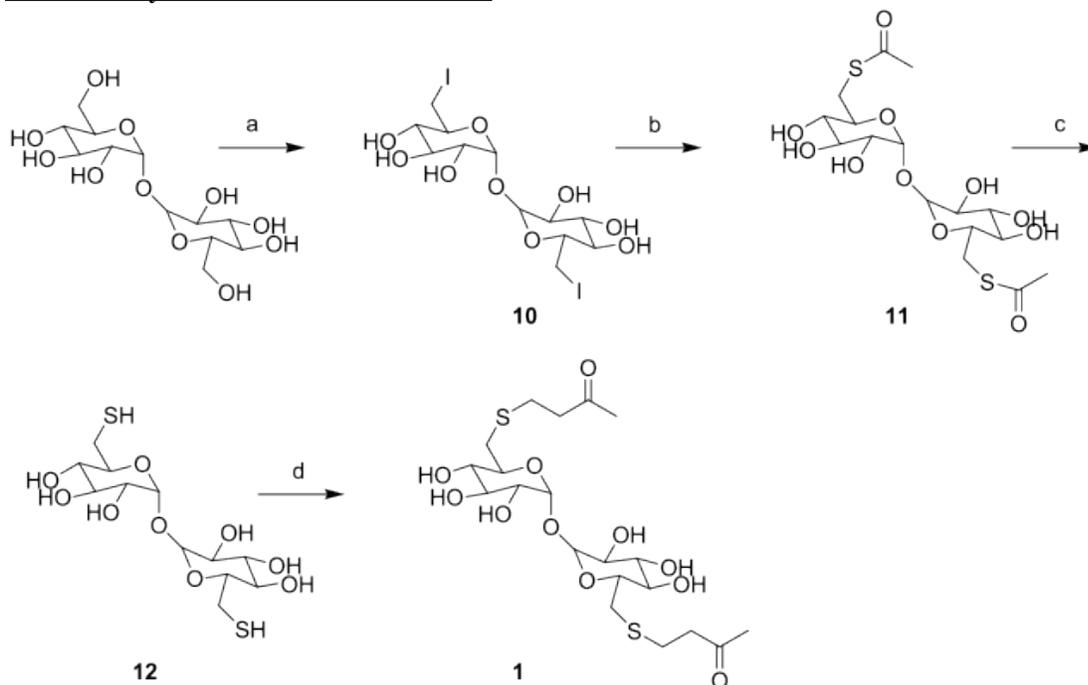
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A. Synthetic procedures and compound characterization data

Materials and characterization methods

All reagents and solvents were purchased from Sigma-Aldrich and Avantilipids. NMR spectra were acquired on a Bruker Avance DPX 400 ultrashield instrument and a Bruker Avance DPX 500 instrument. Electrospray mass spectroscopy was performed on a Bruker MicroTOF equipped with an electrospray ionization source. MALDI mass spectra were acquired on a Bruker Microflex MALDI-TOF instrument using α -cyano-4-hydroxy-cinnamic acid as matrix.

Scheme 1: Synthesis of diketo trehalose 1



a) TPP, I₂, DMF, 1.5 h, 80 °C; b) CH₃COSK, DMF, 7 h, RT; c) 0.2N NaOH, 1 h, RT; d) Methyl vinyl ketone, CH₃CN, saturated Na₂HPO₄, 6 h, RT.

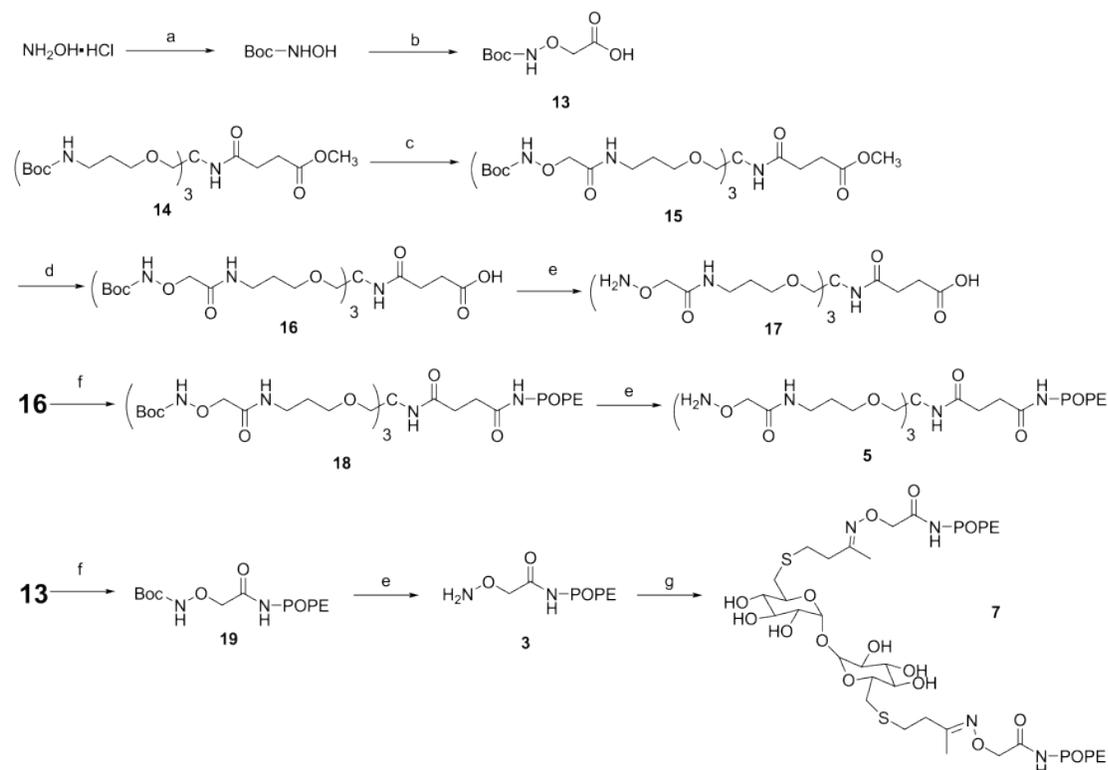
compound 10: To a stirred solution of anhydrous α,α' -D-trehalose (2.5 g, 7.30 mmol) in 50 mL dry DMF, triphenylphosphine (9.57 g, 36.51 mmol) and I₂ (7.41 g, 29.21 mmol) were added under N₂. The mixture was heated to 80 °C for 1.5 h and then evaporated to about 2/3 the volume of DMF under vacuum. 95 mL methanol was added and the solution was adjusted to a pH of 9 with solid sodium methoxide. This mixture was stirred at room temperature for 30 min and neutralized with Amberlyst 15 (H⁺) resin. The resin was filtered off and washed with methanol. The combined filtrates were concentrated, triturated with 77 mL water, and the white precipitate was filtered off. The aqueous solution was evaporated to yield an amorphous solid (mixture of triphenylphosphine oxide and compound 10). This mixture was further purified by using HPLC monitored at 270 nm (SPIRIT PEPTIDE C18 semi-prep column, gradient used 60% solvent A at 6 min to 30% solvent A at 29 min, compound appeared at 20 min. solvent A is 1% methanol in water and solvent B is 10% water in methanol) to give white amorphous solid (1.189 g, 29%) after lyophilization. ¹HNMR (D₂O): δ 3.19 (m, 4H); 3.46 (m, 4H); 3.51 (dd, J = 4, 10 Hz, 2H); 3.69 (t, J = 10 Hz, 2H); 5.09 (d, J = 6 Hz, 2H). Molecular formula: C₁₂H₂₀O₉I₂. Calculated exact mass 561.91, M+Na 584.90, ESI-MS found 584.91.

compound 1: To a solution compound **10** (1 g, 1.78 mmol) in 10 mL dry DMF, potassium thioacetate (0.45 g,

3.91 mmol) was added and the solution was stirred for 7 h at RT (reaction was monitored by TLC). The solvent was removed completely under vacuum to yield yellowish solid (compound **11**) (0.80 g crude). Without further purification, the crude compound **11** was dissolved in 5 mL 0.2 M sodium hydroxide solutions and stirred for 1 h at RT. Water was removed under vacuum and the yellow solid residue (compound **12**) (Calcd. for (M+Na)⁺ 397.06; ESI-MS found 397.1, (M+K)⁺ 413.1) was neutralized using saturated solution of NaH₂PO₄ to maintain the pH 6.8-7.0. Methyl vinyl ketone (0.31 mL, 3.74 mmol) was dissolved in 1 mL acetonitrile and added to the previous solution and stirred for 6 h at RT. Volume of the reaction mixture was reduced to almost 1 mL and purified by HPLC monitored at 235 nm (SPIRIT PEPTIDE C18 semi-prep column, gradient used 70% solvent A at 6 min to 40% solvent A at 29 min, compound appeared at at 18 min. solvent A is 1% methanol in water and solvent B is 10% water in methanol) to yield white solid (compound **1**) after lyophilization (0.64 g, 70% overall yield for 3 steps). ¹HNMR (D₂O): δ 2.06 (s, 6H, 2-Me groups); 2.56 (dd, J = 8, 14 Hz, 2H); 2.64 (m, 4H, 2-CH₂ next to S); 2.74 (m, 4H, 2-CH₂, next to carbonyl); 2.88 (dd, J = 2.5, 14 Hz, 2H); 3.21 (t, J = 10 Hz, 2H); 3.49 (dd, J = 4, 10 Hz, 2H); 3.65 (t, J = 10 Hz, 2H); 3.73 (m, 2H); 5.03 (d, J = 4 Hz, 2H). ¹³CNMR (D₂O): δ 29.52, 32.97, 36.57, 46.42, 74.69, 74.83, 76.14, 76.25, 96.54, 217.46. Molecular formula: C₂₀H₃₄O₁₁S₂. Calculated exact mass 514.15, M+Na 537.14, ESI-MS found 537.16, (M+K)⁺ 553.14.

compound 13: t-Butyl-N-hydroxycarbamate was synthesized following a literature procedure.¹ t-Butyl-N-hydroxycarbamate (2.67 g, 20 mmol) was suspended in 20 mL H₂O containing potassium hydroxide (2.8 g, 50 mmol). Bromoacetic acid (3.48 g, 25 mmol) was dissolved in 10 mL H₂O and added dropwise into the above solution. After stirred for 4 hours at RT, 1 M HCl was added to adjust the pH to be 6-7. The aqueous solution was extracted by CH₂Cl₂ to remove unreacted t-butyl-N-hydroxycarbamate. Then the pH was further adjusted to be 1 by 1 M HCl. NaCl solid was added to saturate the solution. The aqueous solution was extracted by CH₂Cl₂ (20 mL × 4). Washed by H₂O (20 mL × 3), dried on Na₂SO₄, the CH₂Cl₂ solution was evaporated on a rotavapor to give a white solid. The solid was dissolved in a mixture of ethyl acetate and hexane (1:1, about 40 mL), and filtered. The filtrate was evaporated on a rotavapor to give a white solid as pure compound **13** (2.2 g, 58%). ¹HNMR (CDCl₃) δ 1.51 (s, 9H, 3 CH₃); 4.39 (s, 2H, CH₂); 7.64 (s, 1H, NH); 11.7 (br s, 1H, COOH). Molecular formula: C₇H₁₃NO₅. Calculated exact mass 191.08, M-H 190.07, ESI-MS (negative mode) found 190.1.

Scheme 2: synthesis of s-NO POPE 3, t-NO POPE 5 and POPE dimer 7



a) H₂O, Na₂CO₃, Boc₂O, Et₂O. b) KOH, H₂O, bromoacetic acid. c) (i) TFA, CH₂Cl₂; (ii) **13**, HATU, DIEA, DMF. d) (i) LiOH, MeOH, H₂O; (ii) diluted HCl. e) TFA, H₂O, CH₂Cl₂. f) (i) HATU, DIEA, DMF; (ii) POPE, CHCl₃. g) compound **1**, H₂O, MeOH, acetic acid, CHCl₃.

compound 15: Compound **14** was synthesized following a literature procedure.² Compound **14** (0.71 g, 1 mmol) was dissolved in 2 mL CH₂Cl₂, and 1 mL TFA and 1 drop of H₂O was added. The solution was stirred at RT for 30 min. All solvent was removed on a rotavapor and the yellow oil residue was dissolved in 5 mL DMF. Compound **13** (0.63 g, 3.3 mmol) was dissolved in 10 mL DMF. *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 1.22 g, 3.2 mmol) and diisopropylethylamine (DIEA, 0.75 mL, 4 mmol) was added and stirred for 30 min at RT. Then the two solutions were mixed and more DIEA (about 0.8 mL) was added to neutralize the residue TFA. A small aliquot of mixed solution was diluted into 10 times volume of H₂O, and the pH was checked to be about 7-8. The reaction was stirred at RT for overnight. Then all solvent was evaporated under vacuum. The residue was dissolved in 20 mL CH₂Cl₂ and washed by 0.1 M HCl (20 mL × 3) and H₂O (20 mL × 2), and then dried on Na₂SO₄. The crude product was purified on a silica gel flash column, using CH₂Cl₂:MeOH = 20:1 as eluent. Compound **15** (0.48 g, 52%) was collected as a light-yellow oil. ¹HNMR (CDCl₃) δ 1.45 (s, 27H, 9 CH₃); 1.76 (m, 6H, 3CH₂); 2.51 (m, 4H, 2 CH₂); 3.38 (m, 6H, 3 CH₂); 3.45 (m, 6H, 3 CH₂); 3.68 (s, 6H, 3 CH₂); 4.14 (s, 3H, CH₃); 4.33 (s, 6H, 3 CH₂); 6.53 (s, 1H, NH); 7.65 (br s, 3H, 3 NH), 7.81 (br s, 3H, 3 NH). Molecular formula: C₃₉H₇₁N₇O₁₈. Calculated exact mass 925.49, M+Na 948.48, ESI-MS found 948.5.

compound 16: Compound **15** (0.46 g, 0.5 mmol) was dissolved in 2 mL methanol. LiOH (60 mg, 2.5 mmol)

was dissolved in 2 mL water and added into the methanol solution. After stirred at RT for 30 min, 2 M HCl was added to adjust the pH to be 2. Half volume of the solvent was removed by rotavapor. The aqueous solution was extracted by CH₂Cl₂ (10 mL×6). The organic layer was dried over Na₂SO₄ and evaporated to yield a white solid as the pure compound **16** (0.42 g, 92%). ¹HNMR (CDCl₃) δ 1.45 (s, 27H, 9 CH₃); 1.75 (m, 6H, 3CH₂); 2.55 (m, 4H, 2 CH₂); 3.38 (m, 6H, 3 CH₂); 3.45 (m, 6H, 3 CH₂); 3.68 (s, 6H, 3 CH₂); 4.32 (s, 6H, 3 CH₂); 6.83 (s, 1H, NH); 8.15 (br s, 3H, 3 NH), 8.61 (br s, 3H, 3 NH). ¹³CNMR (CDCl₃) 173.6, 172.9, 169.6, 157.9, 82.8, 75.7, 69.5, 68.7, 60.1, 53.5, 36.5, 29.0, 28.1. Molecular formula: C₃₈H₆₉N₇O₁₈. Calculated exact mass 911.47, M-H 910.46, ESI-MS (negative mode) found 910.4.

compound 17: Compound **16** (0.137 g, 0.15 mmol) was dissolved in 1 mL CH₂Cl₂, and 0.5 mL TFA and 1 drop of H₂O was added. The solution was stirred at RT for 30 min. All solvent was removed on a rotavapor. The residue was dissolved in 2 mL H₂O, and frozen and lyophilized to give a sticky solid film as a TFA salt of compound **17** (94 mg, contains TFA and H₂O). ¹HNMR (D₂O) δ 1.71 (m, 6H, 3 CH₂); 2.50 (m, 4H, 2 CH₂); 3.22 (m, 6H, 3 CH₂); 3.45 (m, 6H, 3 CH₂); 3.60 (s, 6H, 3 CH₂); 4.53 (s, 6H, 3 CH₂). Molecular formula: C₂₃H₄₅N₇O₁₂. Calculated exact mass: 611.31, M-H 610.30, ESI-MS (negative mode) found 610.3.

compound 18: Compound **16** (100 mg, 0.11 mmol) was dissolved in 2 mL DMF. *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 42 mg, 0.11 mmol) and diisopropylethylamine (DIEA, 22 uL, 0.12 mmol) was added and stirred for 30 min at RT. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE, 72 mg, 0.1 mmol) in 4 mL CHCl₃ was added. The solution was stirred for 2 h at RT. 0.3 mL 1 M HCl was added. Then all the solvent was evaporated on a rotavapor. The residue was dissolved in 10 mL CHCl₃. After wash by 1 M HCl (10 mL×3) and water (10 mL×2), the CHCl₃ layer was dried on Na₂SO₄ and evaporated to yield a solid film. The crude product was purified on a silica gel flash column, using CHCl₃:MeOH:H₂O = 82:18:2 as the eluent. Compound **18** (103 mg, 64%) was collected as a colorless solid film. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 0.82 (t, J = 6.5 Hz, 6H, 2 CH₃); 1.21 (br s, 44H, 22 CH₂); 1.40 (s, 27H, 9 CH₃); 1.52 (m, 4H, 2 CH₂); 1.71 (m, 6H, 3 CH₂); 1.93 (m, 4H, 2 CH₂); 2.22 (m, 4H, 2 CH₂); 2.41 (m, 4H, 2 CH₂); 3.30 (m, 8H, 3CH₂ from compound 16 moiety, 1 CH₂ from POPE); 3.40 (m, 6H, 3 CH₂); 3.66 (s, 6H, 3 CH₂), 3.85 (m, 4H, 2 CH₂ from POPE), 4.08 (m, 1H); 4.21 (s, 6H, 3CH₂); 4.30 (m, 1H); 5.15 (br s, 1H, chiral OCH); 5.28 (m, 2H, CH=CH). ¹³CNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 13.91, 22.55, 24.75, 27.09, 27.97, 28.98, 29.01, 29.05, 29.11, 29.14, 29.19, 29.24, 29.40, 29.54, 29.56, 29.58, 29.64, 31.26, 31.80, 33.98, 34.12, 36.14, 36.27, 40.27, 59.84, 62.53, 63.44, 64.05, 68.62, 69.29, 70.34, 75.30, 82.37, 129.5, 129.9, 158.05, 169.62, 173.03, 173.15, 173.32, 173.72. Molecular formula: C₇₇H₁₄₃N₈O₂₅P. Calculated exact mass 1610.99, M-H, 1609.98, ESI-MS (negative mode) found 1609.9.

compound 5: Compound **18** (80 mg, 0.05 mmol) was dissolved in 1 mL CH₂Cl₂, and 0.5 mL TFA and 1 drop of H₂O was added. The solution was stirred at RT for 30 min. All the solvent was removed on a rotavapor. The residue was dissolved in 5 mL CHCl₃, and washed by 1 M NaHCO₃ (5 mL×2) and water (5 mL×2). Dried on Na₂SO₄, the solvent was evaporated. The crude product was purified on a silica gel flash column, using CHCl₃:MeOH:H₂O = 65:25:4 as the eluent. Compound **5** was collected as a colorless solid film (55 mg, 85%). ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) 0.84 (t, J = 6.5 Hz, 6H, 2 CH₃); 1.25 (br s, 44H, 22 CH₂); 1.55 (m, 4H, 2 CH₂); 1.74 (m, 6H, 3 CH₂); 1.98 (m, 4H, 2 CH₂); 2.24 (m, 4H, 2 CH₂); 2.48 (m, 4H, 2 CH₂); 3.33 (m, 8H, 3CH₂ from compound 16 moiety, 1 CH₂ from POPE); 3.49 (m, 6H, 3 CH₂); 3.65 (s, 6H, 3 CH₂), 3.90 (m,

4H, 2 CH₂ from POPE), 4.10 (m, 1H); 4.16 (s, 6H, 3CH₂); 4.40 (m, 1H); 5.20 (br s, 1H, chiral OCH); 5.32 (m, 2H, CH=CH). ¹³CNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 14.04, 22.64, 24.83, 27.19, 29.03, 29.08, 29.12, 29.16, 29.24, 29.27, 29.29, 29.32, 29.49, 29.63, 29.67, 29.72, 31.86, 31.89, 34.06, 34.21, 36.37, 40.22, 59.93, 62.65, 63.60, 64.28, 65.84, 69.15, 69.78, 70.30, 74.33, 129.62, 130.07, 170.35, 173.04, 173.15, 173.42, 173.80. Molecular formula: C₆₂H₁₁₉N₈O₁₉P. Calculated exact mass 1310.83, M-H, 1309.82, ESI-MS (negative mode) found 1309.9.

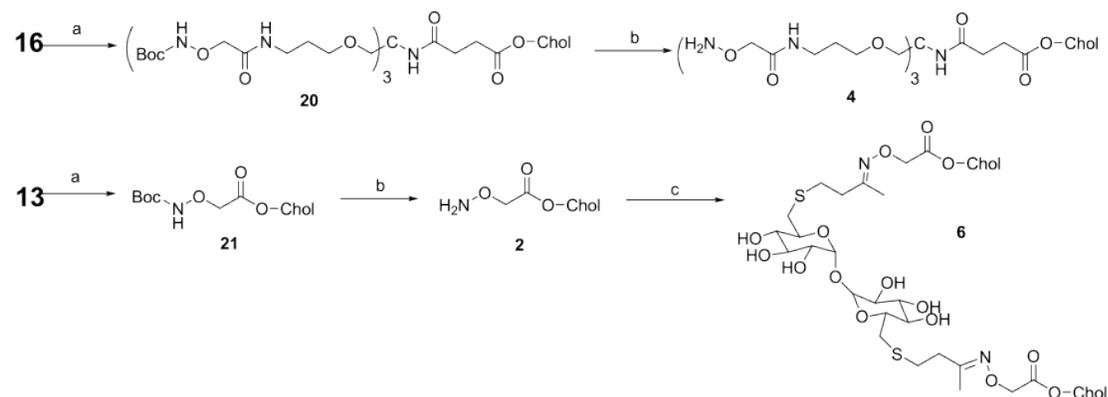
compound 19: Compound **13** (42 mg, 0.22 mmol) was dissolved in 2 mL DMF. *O*-(7-Azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU, 80 mg, 0.21 mmol) and diisopropylethylamine (DIEA, 40 μL, 0.22 mmol) was added and stirred for 30 min at RT. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE, 72 mg, 0.1 mmol) in 4 mL CHCl₃ was added. The solution was stirred for 2 h at RT. 0.3 mL 1 M HCl was added, and all the solvent was evaporated by on a rotavapor. The residue was dissolved in 10 mL CHCl₃. After wash by 1 M HCl (10 mL×3) and water (10 mL×2), the CHCl₃ layer was dried on Na₂SO₄ and evaporated to yield a solid film. The crude product was purified on a silica gel flash column, using CHCl₃:MeOH:H₂O = 82:18:2 as the eluent. Compound **19** (67 mg, 75%) was collected as a colorless solid film. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 0.83 (t, J = 6.5 Hz, 6H, 2 CH₃); 1.24 (br s, 44H, 22 CH₂); 1.40 (s, 9H, 3 CH₃); 1.54 (m, 4H, 2 CH₂); 1.98 (m, 4H, 2 CH₂); 2.24 (m, 4H, 2 CH₂); 3.45 (m, 2H); 3.93 (m, 4H, 2 CH₂), 4.10 (m, 1H); 4.18 (s, 2H, CH₂); 4.38 (m, 1H); 5.19 (br s, 1H, chiral OCH); 5.33 (m, 2H, CH=CH). ¹³CNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 13.90, 22.58, 24.77, 27.09, 27.98, 28.11, 29.01, 29.04, 29.06, 29.16, 29.24, 29.31, 29.43, 29.58, 29.61, 29.66, 31.82, 33.89, 34.12, 40.83, 62.43, 63.72, 64.15, 70.40, 82.50, 129.22, 129.85, 159.15, 169.86, 173.47, 173.86. Molecular formula: C₄₆H₈₇N₂O₁₂P. Calculated exact mass 890.6, M-H, 889.59, ESI-MS (negative mode) found 889.6.

compound 3: Compound **19** (45 mg, 0.05 mmol) was dissolved in 2 mL CH₂Cl₂, and 0.5 mL TFA and 1 drop of H₂O was added. The solution was stirred at RT for 30 min. All solvent was removed on a rotavapor. The residue was dissolved in 5 mL CHCl₃, and washed by 1 M NaHCO₃ (5 mL×2) and water (5 mL×2). Dried on Na₂SO₄, the solvent was evaporated. The crude product was purified on a silica gel flash column, using CHCl₃:MeOH:H₂O = 82:18:2 as eluent. Compound **3** was collected as a colorless solid film (34 mg, 86%). ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) 0.82 (t, J = 6.5 Hz, 6H, 2 CH₃); 1.24 (br s, 44H, 22 CH₂); 1.54 (m, 4H, 2 CH₂); 1.98 (m, 4H, 2 CH₂); 2.24 (m, 4H, 2 CH₂); 3.45 (m, 2H); 3.93 (m, 4H, 2 CH₂), 4.10 (m, 1H); 4.19 (m, 1H); 4.23 (s, 2H, CH₂ONH₂); 5.20 (br s, 1H, chiral OCH); 5.32 (m, 2H, CH=CH). ¹³CNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 13.90, 22.58, 24.77, 27.09, 28.11, 29.0, 29.03, 29.06, 29.16, 29.22, 29.27, 29.43, 29.56, 29.61, 29.66, 31.82, 33.89, 34.12, 40.79, 62.40, 63.68, 64.14, 70.24, 129.15, 129.95, 169.86, 173.47, 173.86. Molecular formula: C₄₁H₇₉N₂O₁₀P. Calculated exact mass 790.55, M+Na, 813.54, ESI-MS found 813.5.

compound 7: Compound **3** (40 mg, 0.05 mmol) was dissolved in 2 mL CHCl₃. Compound **1** (13 mg, 0.025 mmol) was dissolved in 0.5 mL water containing 5 μL acetic acid. The two solutions were mixed together and methanol (1-2 mL) was added to give a clear solution. After stirred at RT for overnight, the solvent was evaporated on a rotavapor. 5 mL CHCl₃ was added to dissolve the residue. After washed by water (5 mL×3) and dried on Na₂SO₄, CHCl₃ was evaporated to give a colorless solid film. The crude product was purified on a silica gel flash column, using CHCl₃:MeOH:H₂O = 82:18:2 as the eluent. Compound **7** (27 mg, 53%) was

collected as a colorless solid film. Because the oxime could exist in *cis*- and *trans*- configuration, the compound **7** is actually a mixture of di-*cis*, di-*trans* and *cis,trans* three compounds. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 0.83 (12H, 4 CH₃); 1.38 (br s, 88H, 44 CH₂); 1.57 (m, 8H, 4 CH₂); 1.84 and 1.90 (2 singlets, 6H, CH₃ from compound **1**, converted to *trans* and *cis* oxime, integration area ratio A_{1.84} : A_{1.90} = 1:2.5); 1.99 (m, 8H, 4 CH₂); 2.29 (m, 8H, 4CH₂); 2.47 (m, 2H, 2 CH); 2.53 (m, 4H, 2 CH₂); 2.76 (m, 4H, 2 CH₂); 3.01 (m, 2H, 2 CH); 3.40 (m, 2H, 2 CH); 3.45-3.60 (br, 8H); 3.94 (m, 10H); 4.10 (m, 2H); 4.35 (m, 2H); 4.48 (s, 4H); 5.13 (m, 2H); 5.19 (br s, 2H); 5.32 (m, 4H, 2 CH=CH). Molecular formula: C₁₀₂H₁₈₈N₄O₂₉P₂S₂. Calculated exact mass 2059.23, M+2Na, 2105.21, MALDI found 2105.4.

Scheme 3: synthesis of *s*-NO cholesterol **2**, *t*-NO cholesterol **4** and cholesterol dimer **6**



a) Cholesterol, DCC, DMAP, CHCl₃. b) TFA, H₂O, CH₂Cl₂. c) compound **1**, H₂O, MeOH, acetic acid, CHCl₃.

compound 20: Compound **16** (183 mg, 0.2 mmol) and cholesterol (100 mg, 0.26 mmol) was dissolved in 5 mL CHCl₃. *N,N'*-Dicyclohexylcarbodiimide (DCC, 42 mg, 0.2 mmol) and 4-(dimethylamino)pyridine (DMAP, 4 mg, 0.003 mmol) was added. The solution was stirred at RT for overnight. The dicyclohexylurea precipitate was filtered off. After washed by 1 M HCl (10 mL×3) and water (10 mL×2), the CHCl₃ layer was evaporated on a rotavapor. The crude was purified on a silica gel flash column. First washed by ethyl acetate : hexane = 2:1 to remove excess cholesterol and some impurities, and then the product was eluted using CH₂Cl₂:MeOH = 20:1. Compound **20** (44 mg, 17%) was collected as a colorless solid film. One possible reason for the low yield is the steric hindered carboxylate, which was hard to be activated by DCC. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 0.59 (s, 3H); 0.70-1.58 (m, 62H, contains a singlet from Boc group); 1.60-1.95 (m, 11H, contains 3CH₂ from compound **16** moiety); 2.21 (m, 2H); 2.40-2.50 (m, 4H); 3.16 (m, 1H); 3.26 (m, 6H, 3CH₂ from compound **16** moiety); 3.35 (m, 6H, 3CH₂ from compound **16** moiety); 3.56 (s, 6H, 3CH₂ from compound **16** moiety); 4.18 (s, 6H, 3CH₂ from compound **16** moiety); 5.26 (d, 1H *J* = 4 Hz). Molecular formula: C₆₅H₁₁₃N₇O₁₈. Calculated exact mass 1279.81, M+Na, 1302.80, ESI-MS found 1302.8.

compound 4: Compound **20** (39 mg, 0.03 mmol) was dissolved in 2 mL CH₂Cl₂, and 0.5 mL TFA and 1 drop of H₂O was added. The solution was stirred at RT for 30 min. All solvent was removed on a rotavapor. The residue was dissolved in 1 mL MeOH:H₂O = 5:1, and purified by HPLC monitored at 230 nm (Phenomenex Jupiter C4 semi-prep column), using 10% water and 90% methanol as eluent. Compound **4** was collected as white solid (17 mg, 57%) after lyophilization. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 0.64 (s, 3H); 0.78-1.62 (m, 35H); 1.70-2.03 (m, 11H, contains 3CH₂ from compound **16** moiety); 2.26 (m, 2H); 2.45-2.55

(m, 4H); 3.20 (m, 1H); 3.32 (m, 6H, 3CH₂ from compound 16 moiety); 3.43 (m, 6H, 3CH₂ from compound 16 moiety); 3.63 (s, 6H, 3CH₂ from compound 16 moiety); 4.35 (s, 6H, 3CH₂ from compound 16 moiety); 5.26 (d, 1H, $J = 4$ Hz). Molecular formula: C₅₀H₈₉N₇O₁₂. Calculated exact mass 979.66, M+Na, 1002.65, ESI-MS found 1002.6.

compound 21: Compound **13** (0.19 g, 1 mmol) and cholesterol (0.46 g, 1.2 mmol) was dissolved in 20 mL CHCl₃. *N,N'*-Dicyclohexylcarbodiimide (DCC, 0.25 g, 1.2 mmol) and 4-(dimethylamino)pyridine (DMAP, 13 mg, 0.1 mmol) was added. The solution was stirred at RT for overnight. The white solid dicyclohexylurea was filtered off. After washed by 1 M HCl (10 mL×3) and water (10 mL×2), the CHCl₃ layer was evaporated on a rotavapor. The crude was purified on a silica gel flash column. First washed by ethyl acetate : hexane = 1:2 to remove excess cholesterol and some impurities, and then the product was eluted using CH₂Cl₂:MeOH = 30:1. Compound **21** (0.37 g, 67%) was collected as a white solid. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) δ 0.54 (s, 3H); 0.60-1.55 (m, 44H, containing a singlet from the Boc group); 1.60-2.0 (m, 5H); 2.15 (m, 2H); 3.26 (m, 1H); 4.29 (s, 2H); 5.24 (d, 1H, $J = 4$ Hz). Molecular formula: C₃₄H₅₇NO₅. Calculated exact mass 559.42, M+Na, 582.41, ESI-MS found 582.4.

compound 2: Compound **21** (56 mg, 0.1 mmol) was dissolved in 2 mL CH₂Cl₂, and 0.5 mL TFA and 1 drop of H₂O was added. The solution was stirred at RT for 30 min. All solvent was removed on a rotavapor. The residue was dissolved in 5 mL CHCl₃, and washed by 1 M NaHCO₃ (5 mL×2) and water (5 mL×2). Dried on Na₂SO₄, the solvent was evaporated. The crude product was purified by silica gel flash column, using CH₂Cl₂:MeOH = 15:1 as the eluent. Compound **2** was collected as a white solid (38 mg, 82%). ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) 0.56 (s, 3H); 0.65-1.60 (m, 35H); 1.65-2.0 (m, 5H); 2.05-2.3 (m, 2H); 3.30 (m, 1H); 3.65 (s, 2H); 5.29 (d, 1H, $J = 4$ Hz). Molecular formula: C₂₉H₄₉NO₃. Calculated exact mass 459.37, M+Na, 482.36, ESI-MS found 482.4.

compound 6: Compound **2** (23 mg, 0.05 mmol) was dissolved in 2 mL CHCl₃. Compound **1** (13 mg, 0.025 mmol) was dissolved in 0.5 mL water containing 5 μ L acetic acid. The two solutions were mixed together and methanol (1-2 mL) was added to give a clear solution. After stirred at RT for overnight, the solvent was evaporated on a rotavapor. 5 mL CHCl₃ was added to dissolve the residue. After washed by water (5 mL×3) and dried on Na₂SO₄, CHCl₃ was evaporated to give a colorless solid film. The crude product was purified on a silica gel flash column, using CHCl₃:MeOH = 10:1 as the eluent. Compound **6** (22 mg, 62%) was collected as a colorless solid film. Because the oxime could exist in *cis*- and *trans*- configuration, the compound **6** is actually a mixture of di-*cis*, di-*trans* and *cis,trans* three compounds. ¹HNMR (90% CDCl₃ and 10% *d*₄-methanol) 0.51 (s, 6H); 0.70-1.70 (m, 66H); 1.72-2.05 (m, 20H, contains 2 singlets from the Me on *cis* and *trans* oxime); 2.30 (m, 4H, 2 CH₂); 2.42 (m, 2H, 2 CH); 2.50-2.75 (m, 4H, 2 CH₂); 2.98 (m, 2H, 2 CH); 3.25-3.40 (m, 2H, 2 CH); 3.52 (m, 2H, 2 CH); 3.79 (m, 2H, 2 CH); 3.90 (m, 2H, 2 CH); 4.52 (m, 4H); 5.13 (br s, 2H); 5.39 (br, 2H). Molecular formula: C₇₈H₁₂₈N₂O₁₅S₂. Calculated exact mass 1396.88, M+Na, 1419.87, ESI-MS found 1419.9.

Polymerization of keto-trehalose and t-NO acid followed by ¹HNMR: Compound **17** (12.2 mg, 0.02 mmol) was dissolved in 1 mL D₂O to be solution A. Compound **1** (15.5 mg, 0.03 mmol) was dissolved in 1 mL D₂O to be solution B. 0.25 mL solution A and 0.25 mL solution B was mixed together into a 5 mm NMR tube, and 0.1 mL PBS in D₂O (pD = 6.0) was added to acidify the solution. The reaction was carried on at RT and

monitored by ^1H NMR. The reaction was complete after about 18 h (Figure S1). The final polymer was characterized by COSY and 2D-NOESY (Figure S2). The HNMR peaks were assigned by COSY crossing peaks. Two crossing peaks on 2D-NOESY indicate the formation of oxime leakage (the peaks in red circle).

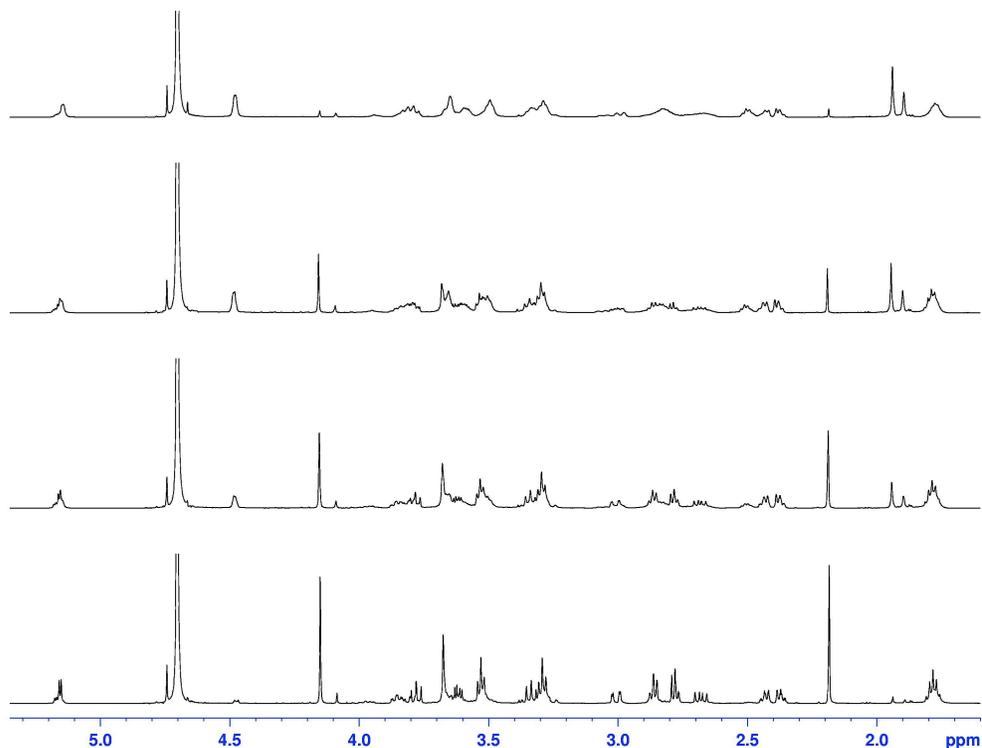
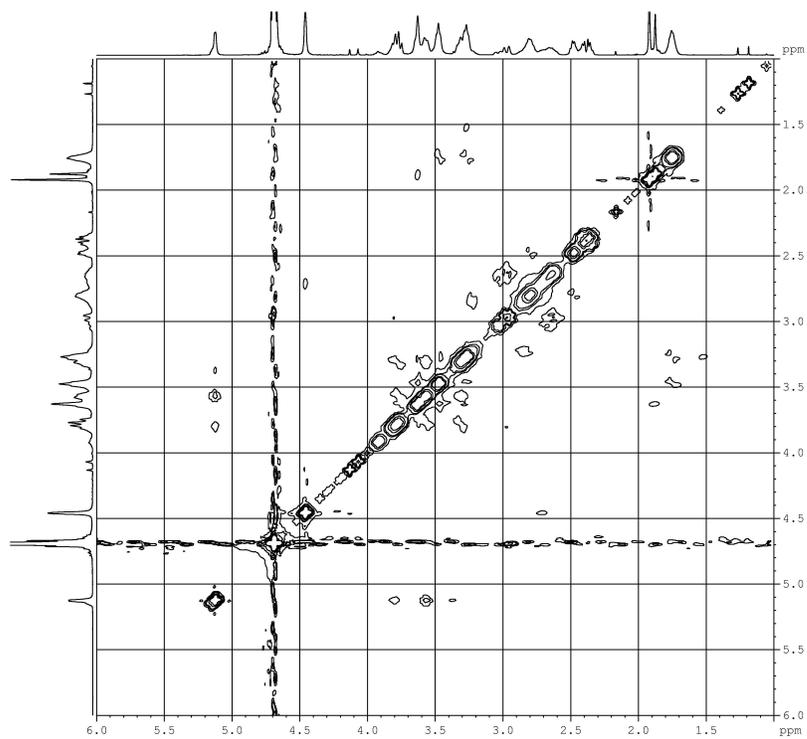
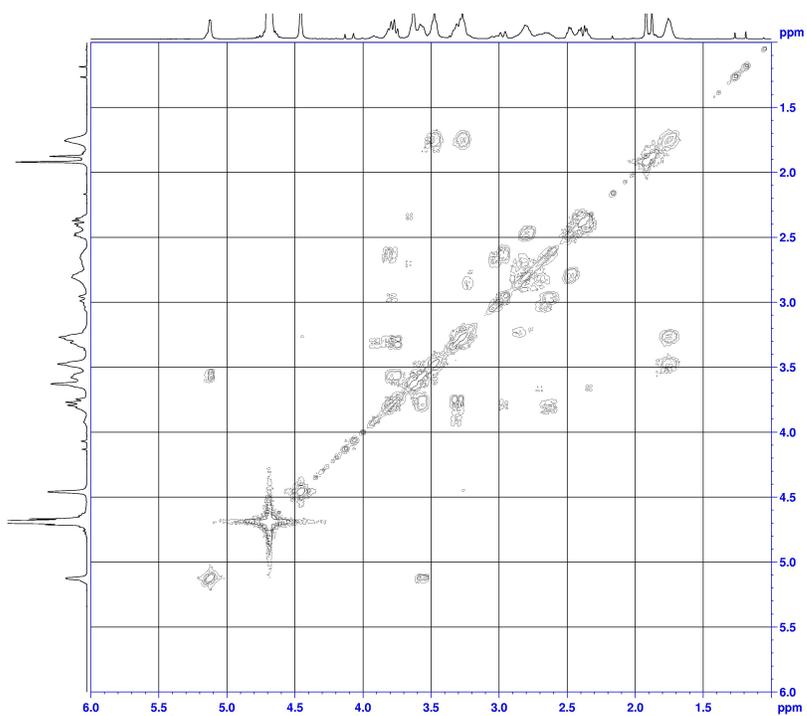


Figure S1. ^1H NMR of polymerization reaction of keto-trehalose and t-NO acid. From bottom to up: 20 min, 2.5 h, 6.5 h, 18 h. The singlet at 2.19 ppm was the Me group on keto-trehalose, which converts to two singlets at 1.89 and 1.94 ppm. These two new singlets are Me groups in cis and trans oxime. The singlet at 4.14 ppm was the methylene group of $\text{CH}_2\text{-ONH}_2$ on t-NO acid, which converts to a broad singlet (actually two singlets, seen in the bottom spectra) at 4.48 ppm. The new broad singlet is the methylene group in cis and trans oxime. All other peaks get broader upon the polymerization.



t-NO cholesterol trehalose polymer 8: Compound **4** (5 mg, 5 μ mmol) was dissolved in 5 mL CHCl_3 . Compound **1** (3.9 mg, 7.5 μ mmol) was dissolved in 5 mL 20 mM NaAc-HAc buffer (pH = 4). The two solutions were mixed together and vortexed to give a milky emulsion. After intensively stirred at RT for overnight, the solvent was evaporated under vacuum. The solid residue was washed by H_2O to remove salt. The remaining solid was dissolved in a solvent mixture (CHCl_3 :MeOH = 9:1, stirred at RT for 1h) and centrifuged to remove any insoluble particles. Polymer **8** was collected as a white solid (6.2 mg, 70%) ^1H NMR were acquired using 90% CDCl_3 and 10% d_4 -methanol as solvent.

t-NO POPE trehalose polymer 9: Compound **5** (6.5 mg, 5 μ mmol) was dissolved in 1 mL CHCl_3 . Compound **1** (3.9 mg, 7.5 μ mmol) was dissolved in 1 mL 20 mM NaAc-HAc buffer (pH = 4). The two solutions were mixed together and vortexed to give a milky emulsion. After intensively stirred at RT for overnight, the solvent was evaporated under vacuum. The solid residue was washed by H_2O to remove salt. The remaining solid was dissolved in a solvent mixture (CHCl_3 :MeOH: H_2O = 13:6:1, stirred at RT for 1h) and centrifuged to remove any insoluble particles. Polymer **9** was collected as a solid film (8.5 mg, 83%). ^1H NMR was acquired using 70% CDCl_3 and 30% d_4 -methanol as solvent.

B. Assays of studying the trehalose-lipid polymer stabilized lipid membranes

PEG-DSPE was purchased from Aldrich and used as provided. In all experiments, PEG-DSPE (PEG 2000) was incorporated at 5 mole percent, as reported in the literature.

Determination of the molecular weight of trehalose polymer by DOSY

Four poly(methyl methacrylate) (PMMA) standard polymers were purchased from American Polymer Standard Corporation. PMMA standards were dissolved in CDCl_3 (12 mg/mL). Polymer **8** was dissolved in a mixture of 90% CDCl_3 and 10% d_4 -methanol (4 mg/mL). Polymer **9** was dissolved in a mixture of 70% CDCl_3 and 30% d_4 -methanol (5 mg/mL). The DOSY NMR experiment was performed on a Bruker Avance DPX 500 instrument at 27°C. The diffusion time for all samples was set as 100 ms. The gradient pulse width was adjusted for each sample. The residue CHCl_3 in CDCl_3 was used as an internal standard to ensure the comparability of different samples. The M_w of two polymers was calculated based on a literature procedure.³ The water soluble polymer of keto-trehalose (compound **1**) and t-NO acid (compound **17**) was dissolved in D_2O (15 mg/mL). Its $\log(D/\text{m}^2\text{s}^{-1}) = 9.985$ and its M_w was around 9 kD based on the standard curve of PEO samples dissolved in D_2O .³

	PMMA 7 kD	PMMA 20 kD	PMMA 34 kD	PMMA 45 kD	Polymer 8	Polymer 9
M_n	6450	17000	32400	40700		
M_w	7630	20300	34500	45300	~7000	~20000
Gradient pulse (ms)	4.4	5.5	5.9	6.5	4.4	5.5
$\log(D/\text{m}^2\text{s}^{-1})_{\text{sample}}$	-9.591	-9.754	-9.861	-9.979	-9.58	-9.75
$\log(D/\text{m}^2\text{s}^{-1})_{\text{CHCl}_3}$	-8.583	-8.578	-8.578	-8.617	-8.578	-8.606

Vesicle preparation and characterization by DLS and zeta potential

Large unilamellar vesicles were prepared by extrusion method. Lipids were dissolved in chloroform or chloroform/methanol mixture to make a stock solution. Desired lipid solutions were mixed and evaporated under nitrogen flow to form a lipidic film, and dried under vacuum overnight. Calcein sodium salt (0.36 g) was dissolved in 10 mL 50 mM sodium phosphate (tribasic), and the pH was adjusted to 7.4 by 50% NaOH solution. The final calcein concentration (51 mM) was determined by measuring the absorbance (494 nm) of diluted sample at pH 9. The dried lipid film was hydrated by 0.5 mL calcein solution at 40°C (for eggPC samples) or 70°C (for DPPC and Hydrogenated eggPC samples) under nitrogen with intermittent vortex. Then the solution was extruded 10 times both ways through a polycarbonate film with 100 nm pore size at 40°C (for eggPC samples) or 70°C (for DPPC and Hydrogenated eggPC samples). Excess calcein was removed by eluting the solution from a Sephadex G-50 column using PBS buffer (50 mM sodium phosphate, 120 mM sodium chloride, pH 7.4). Typically, the final total lipid concentration was 1 mM. For the preparation of the supported lipid bilayers, the dried lipid film containing 1.5% DHPE Oregon Green (Invitrogen) was hydrated by 0.5 mL PBS at 40°C under nitrogen with intermittent vortex. Then the solution was extruded 10 times both ways through a polycarbonate film with 100 nm pore size at 40°C. The vesicle containing Oregon Green was kept in dark before use. The size and zeta potential of the vesicles were measured on a Malvern Instruments Zeta Sizer Nano-ZS (backscatter detection at 173°).

eggPC vesicles	5% POPG	10% POPG	5% 7	10% 7	5% 9	10% 9		
size (nm)	112±4	110±3.5	112±3.5	99±4	135±10	150±10		
Zeta potential (mV)	-10.3±0.3	-14.7±0.4	-12.7±0.2	-16.0±0.2	-11.2±0.2	-13.9±0.3		
eggPC vesicles	30% chol	10% chol	20% chol	30% 6	5% 8, 25% chol	10% 8, 20% chol	20% 8, 10% chol	30% 8
Size (nm)	120±8.5	117±9	116±9.5	107±7	95±8	88±9.5	87±8.5	89±9.5
Zeta potential (mV)	-5.3±0.2	-6.7±0.2	-5.8±0.2	-6.4±0.3	-5.8±0.2	-6.9±0.2	-7.2±0.3	-6.9±0.2

Calcein leakage induced by 30% FBS

Vesicle sample with calcein encapsulated (100 µL) was diluted by 30% fetal bovine serum (fetal bovine serum was diluted by PBS buffer containing 2 mM NaN₃) to a total volume of 2 mL. Samples were then incubated at 37 °C. A small aliquot of sample was withdrawn at different time points and fluorescence intensities were measured on a Perkin Elmer LS-50B fluorimeter (excitation 495 nm, emission 518 nm). Vesicles were finally lysed by adding 20 µL of 10% Triton X-100 to 150 µL sample to completely release calcein. The final fluorescence was used as 100% signal ($F_{100\%}$). The calcein release percentage was defined as $(F_{\text{signal}} - F_{\text{ini}}) / (F_{100\%} - F_{\text{ini}})$, where F_{signal} is the fluorescence intensity of the sample at different time points and F_{ini} is the fluorescence intensity immediately after mixing with fetal bovine serum.

Lyophilization and rehydration of trehalose lipid polymer vesicles

Vesicle samples with calcein encapsulated (100 µL) were placed in a 4-mL glass vial and frozen in liquid N₂.

Then these samples were lyophilized on a Labconco benchtop lyophilizer. 100 μL DI water was added into the lyophilized samples at RT. The samples were briefly vortexed before the DLS measurement and cryo-TEM visualization.

Cryo-TEM

Generally, ~ 20 μL of vesicle solution (1 mM or higher lipid concentration) was applied to a lacey Formvar carbon support film EM grid (cat No. 01881-F) from TED PELLA. The specimen was blotted from behind with a filter paper and then plunged into ethane slurry maintained at liquid nitrogen temperature. The vitrified specimens were placed into the Tecnai G2 Spirit (FEI Company) electron microscope operating at 120 kV. Images were recorded using a Gatan CCD camera. The pixel size was determined by imaging a standard sample grid (gold shadowed latex spheres with average size of 204 nm from TED PELLA) at several different magnifications.

Supported lipid bilayer formation⁴

Microscopic glass slides were boiled in detergent solution (Linbro 7X Lab Glass Cleaner, ICN Pharmaceuticals) for 20 min, followed by extensive rinsing by DI water, and then baked at 450 $^{\circ}\text{C}$ for 4 h. CoverWellTM perfusion chamber gasket (9 mm diameter, 0.5 mm deep, from Invitrogen) was attached on a glass slide. A desired vesicle solution (lipid concentration not less than 0.25 mM, ~ 35 μL) was added into the chamber and incubated for 5 min at RT. The formed supported lipid bilayer was rinsed with excess PBS buffer.

Microscopy imaging and fluorescence recovery after photobleaching (FRAP)

Epifluorescence microscopy was performed on a Zeiss Axio Observer A1 inverted microscope equipped with a mercury light source and an AxioCam ICc1 Camera (Zeiss). Images were acquired with AxioVision software. Dry objective 10x was used for SLB adhesion, dehydration and rehydration studies. The field of view in Figure 4 images is ~ 600 μm diameter. Dry objective 40x was used for FRAP studies. The SLB was bleached by the highest intensity light for 10s. The bleached spot is ~ 40 -45 μm diameter. Images were acquired before bleaching, immediately after bleaching and 1min after bleaching. All images were taken under the same intensity and focus.

SLB dehydration and rehydration

SLBs were dehydrated by removing the PBS buffer from the well, followed by 30 min of drying under vacuum at RT. Rehydration was performed by adding PBS buffer to fill up the well and incubate for 5 min. Microscopy images were taken before dehydration and after rehydration.

Reference

1. Jérôme, D.; Alan, M.H.; John, W.; *etc. Tetrahedron*, **2004**, *60*, 2559-2567
2. Ma, M.M.; Gong, Y.; Bong, D. *J. Am. Soc. Chem.* **2009**, *131*, 16919-16926
3. Chen, A.D.; Wu, D.H.; Johnson, C.S. *J. Am. Soc. Chem.* **1995**, *117*, 7965-7970
4. Kam, L. and Boxer, S. G. *J. Am. Chem. Soc.* **2000**, *122*, 12901– 12902

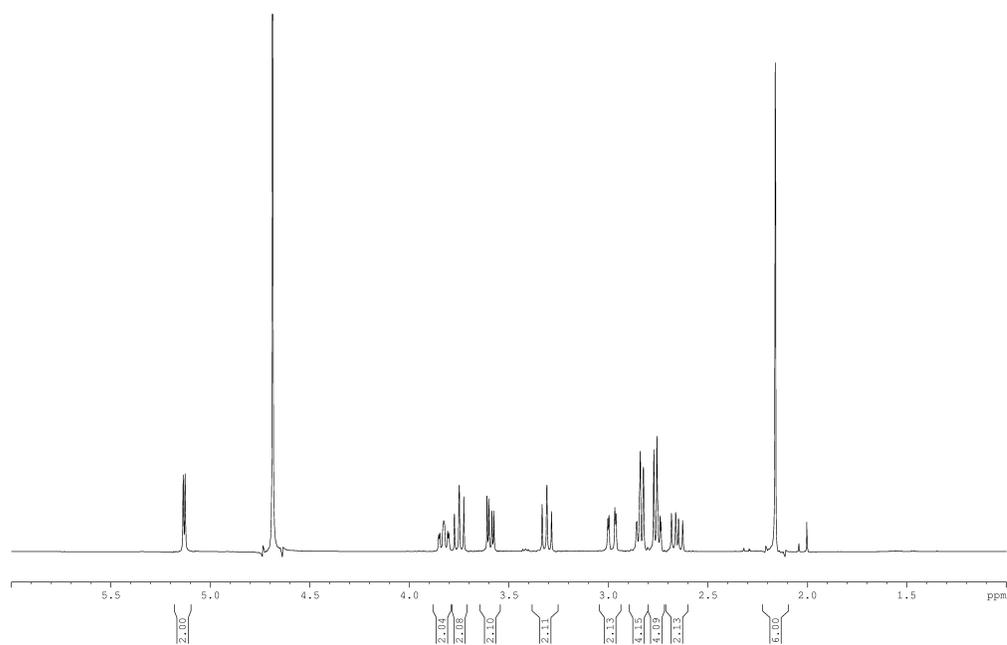


Figure S3. ¹H NMR of keto-trehalose (compound **1**) in D₂O.

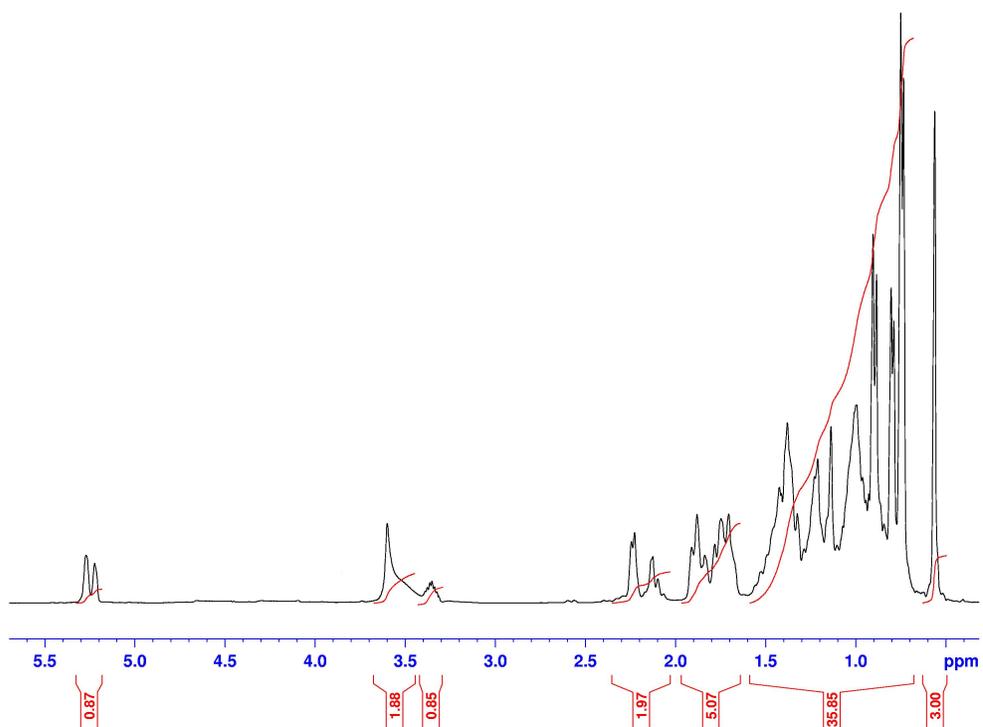


Figure S4. ¹H NMR of s-NO cholesterol (compound **2**) in 90% CDCl₃ and 10% d₄-methanol.

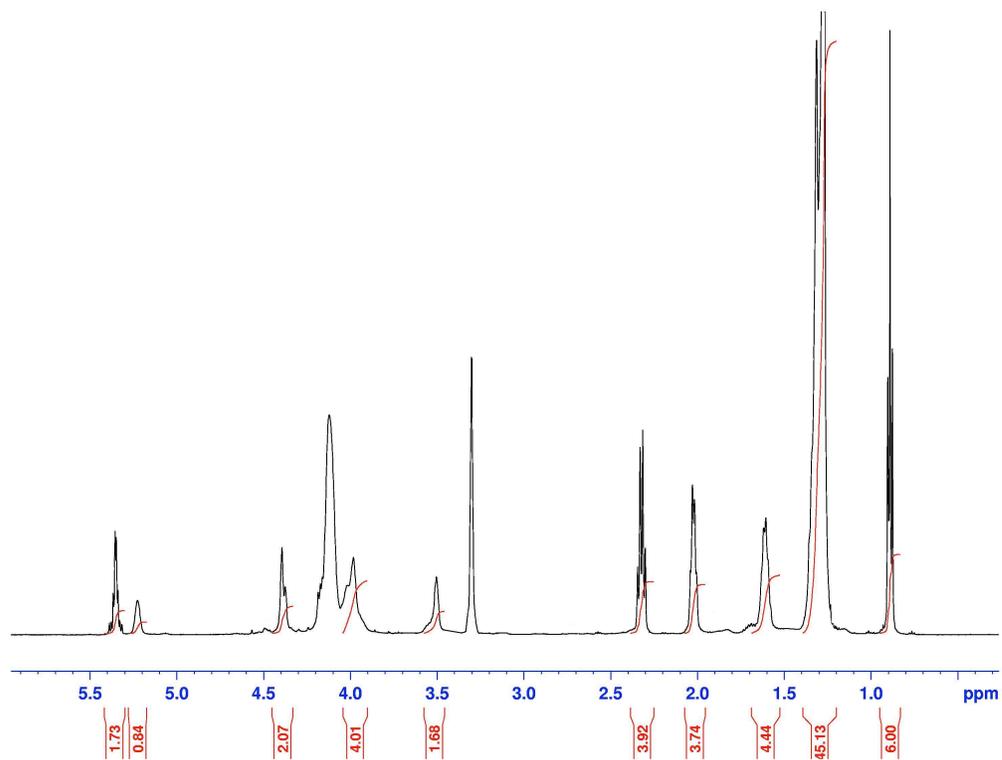


Figure S5. ¹H NMR of s-NO POPE (compound 3) in 70% CDCl₃ and 30% d₄-methanol.

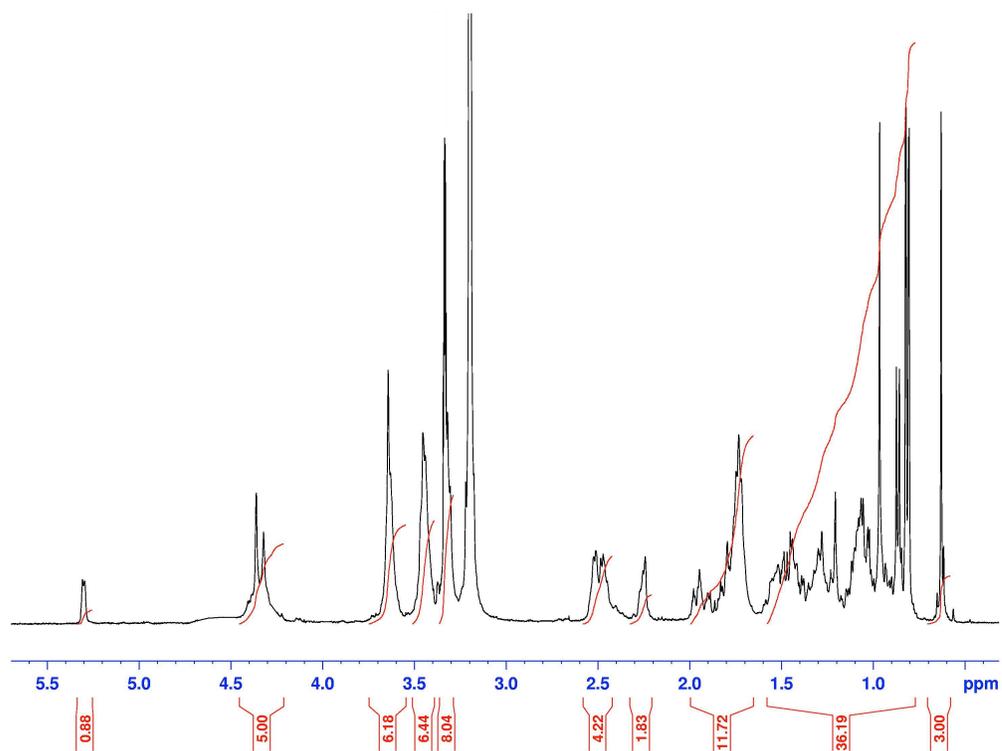


Figure S6. ¹H NMR of t-NO cholesterol (compound 4) in 90% CDCl₃ and 10% d₄-methanol.

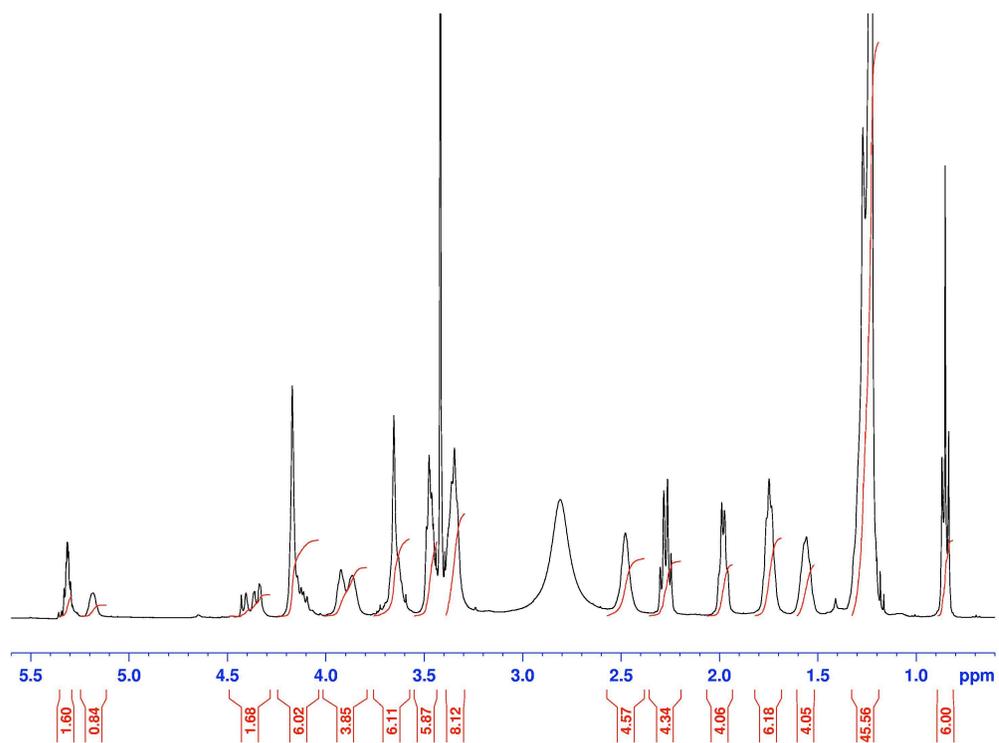


Figure S7. ¹H NMR of t-NO POPE (compound 5) in 70% CDCl₃ and 30% d₄-methanol.

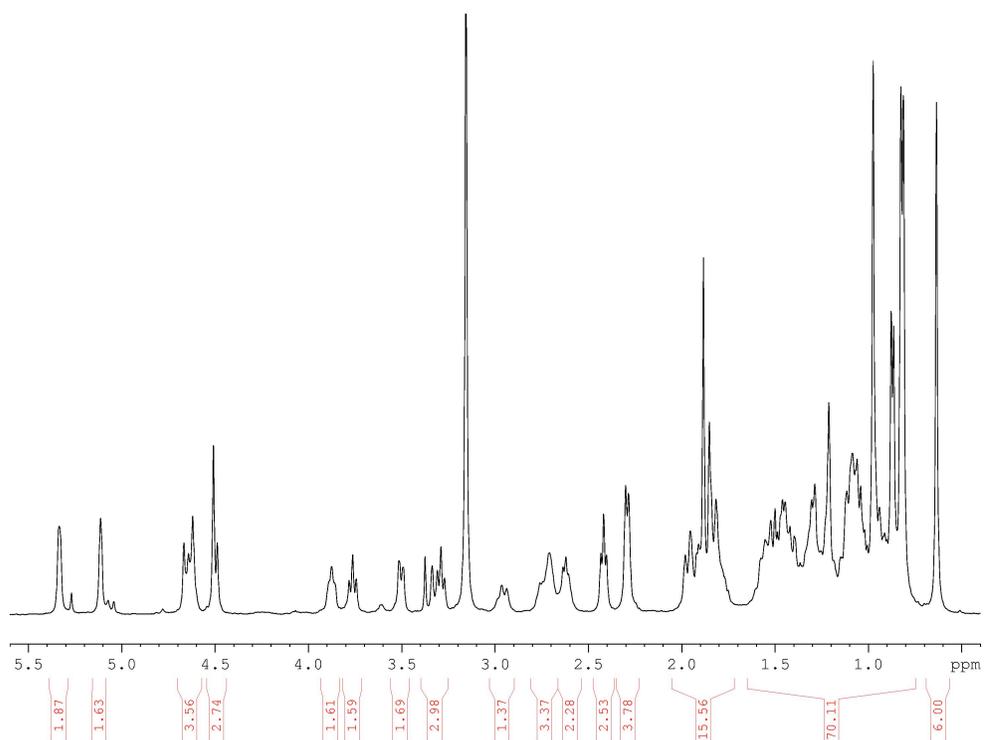


Figure S8. ¹H NMR of s-NO cholesterol dimer (compound 6) in 90% CDCl₃ and 10% d₄-methanol.

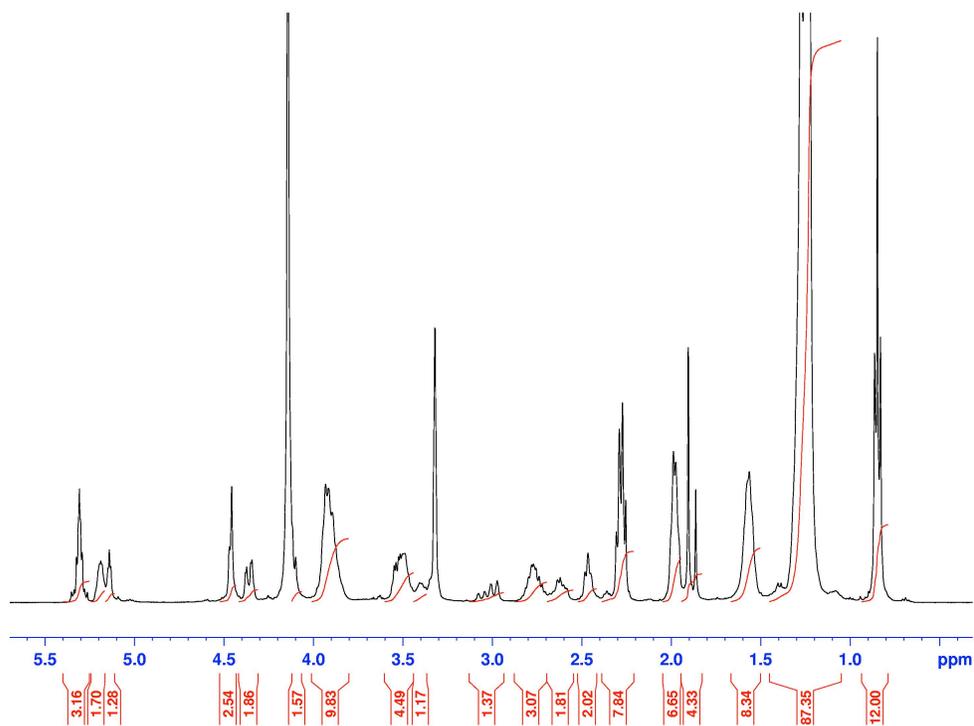


Figure S9. ¹H NMR of s-NO POPE dimer (compound 7) in 70% CDCl₃ and 30% d₄-methanol.

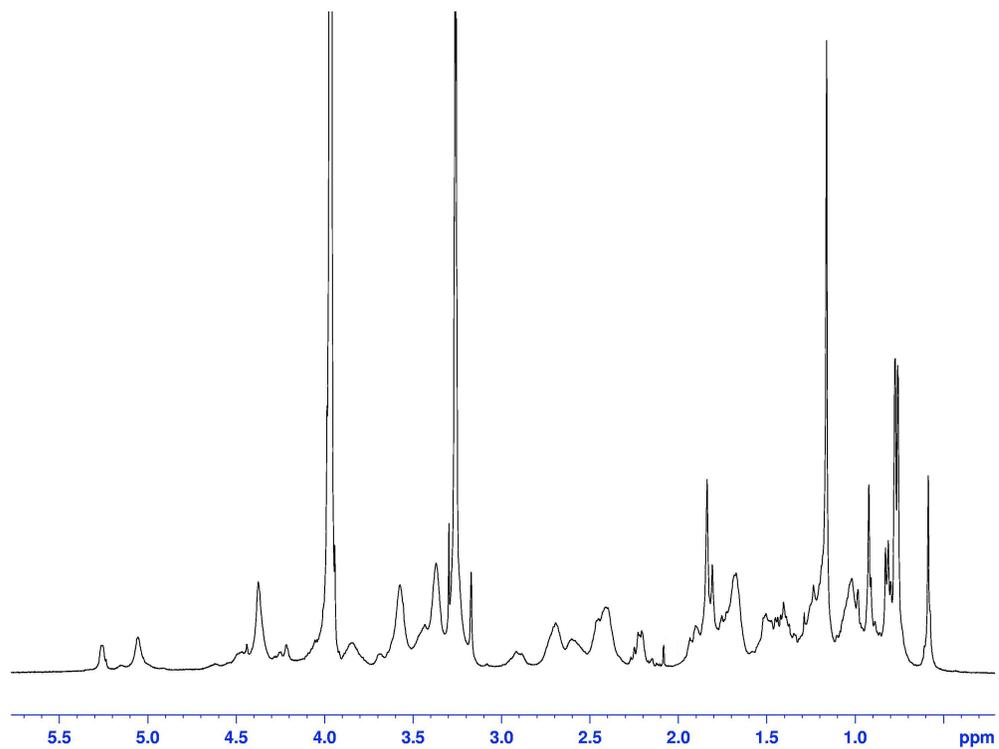


Figure S10. ¹H NMR of t-NO cholesterol polymer (**8**) in 90% CDCl₃ and 10% *d*₄-methanol.

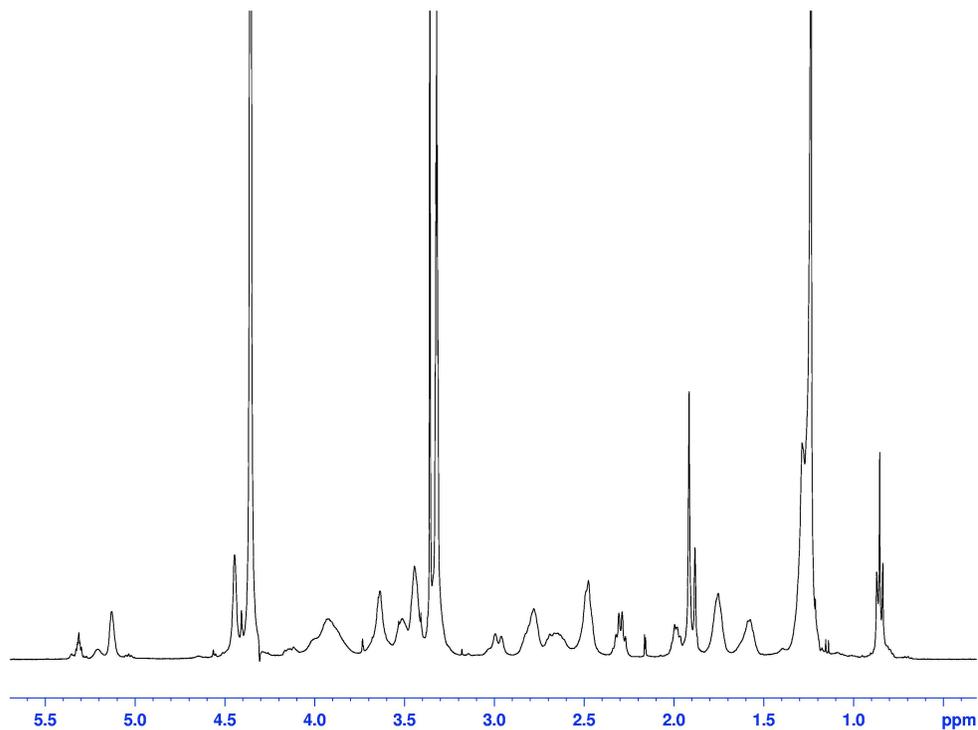


Figure S11. ¹H NMR of t-NO POPE polymer (**9**) in 70% CDCl₃ and 30% *d*₄-methanol.

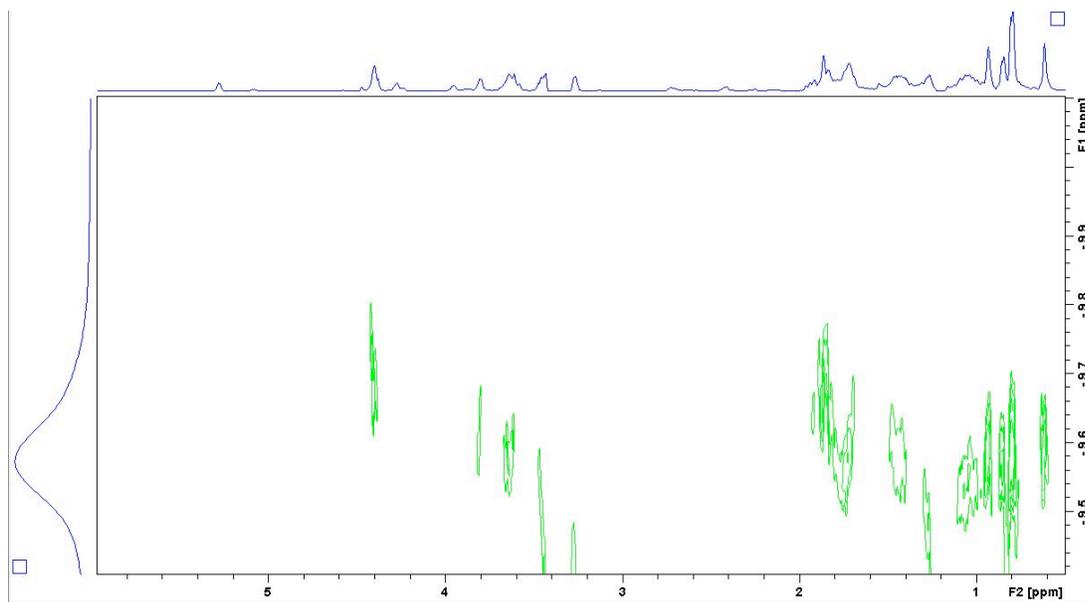


Figure S12. DOSY NMR of t-NO cholesterol polymer (**8**) in 90% CDCl₃ and 10% *d*₄-methanol.

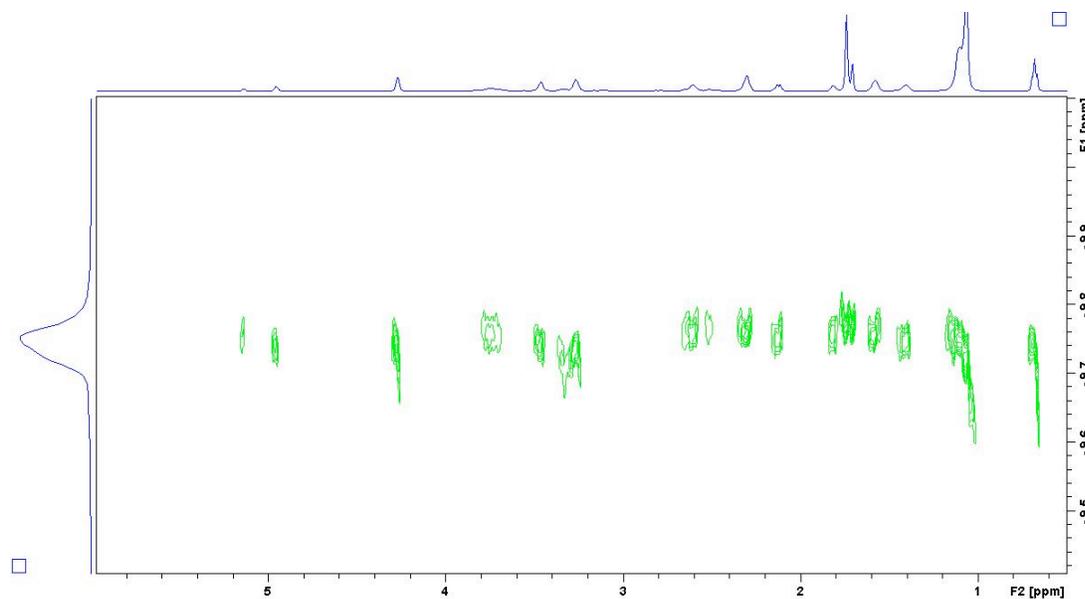


Figure S14. DOSY NMR of t-NO POPE polymer (**9**) in 70% CDCl_3 and 30% d_4 -methanol.

Additional DLS data.

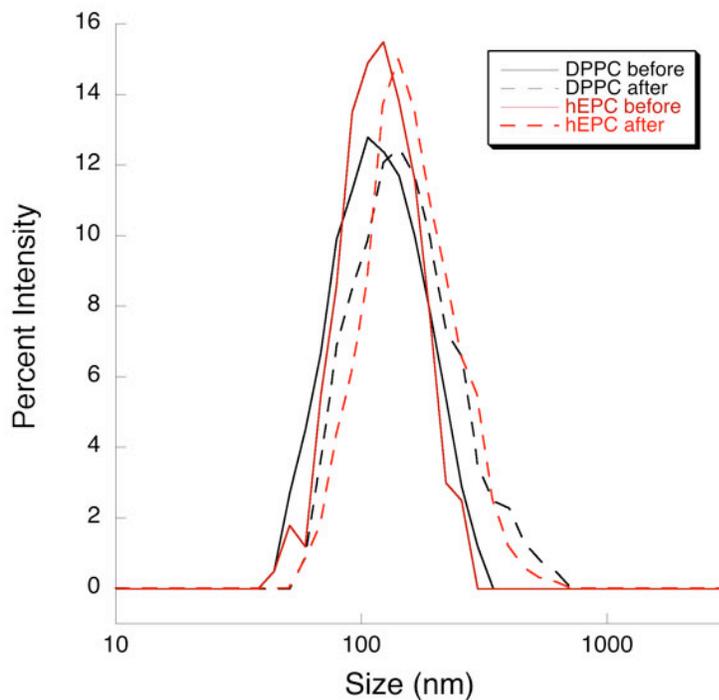


Figure S15. DLS data (backscatter 173°) of calcein loaded LUVs before and after freeze-drying/rehydration. Data is identical to Fig. 3 (manuscript) but is provided in terms of scattering intensity instead of volume. LUVs contain 30% polymer **8** (mol/mol) and the lipid indicated in the legend.