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Supplementary Information

Thermally-induced lateral assembly of PEG-containing amphiphile triggering vesicle budding

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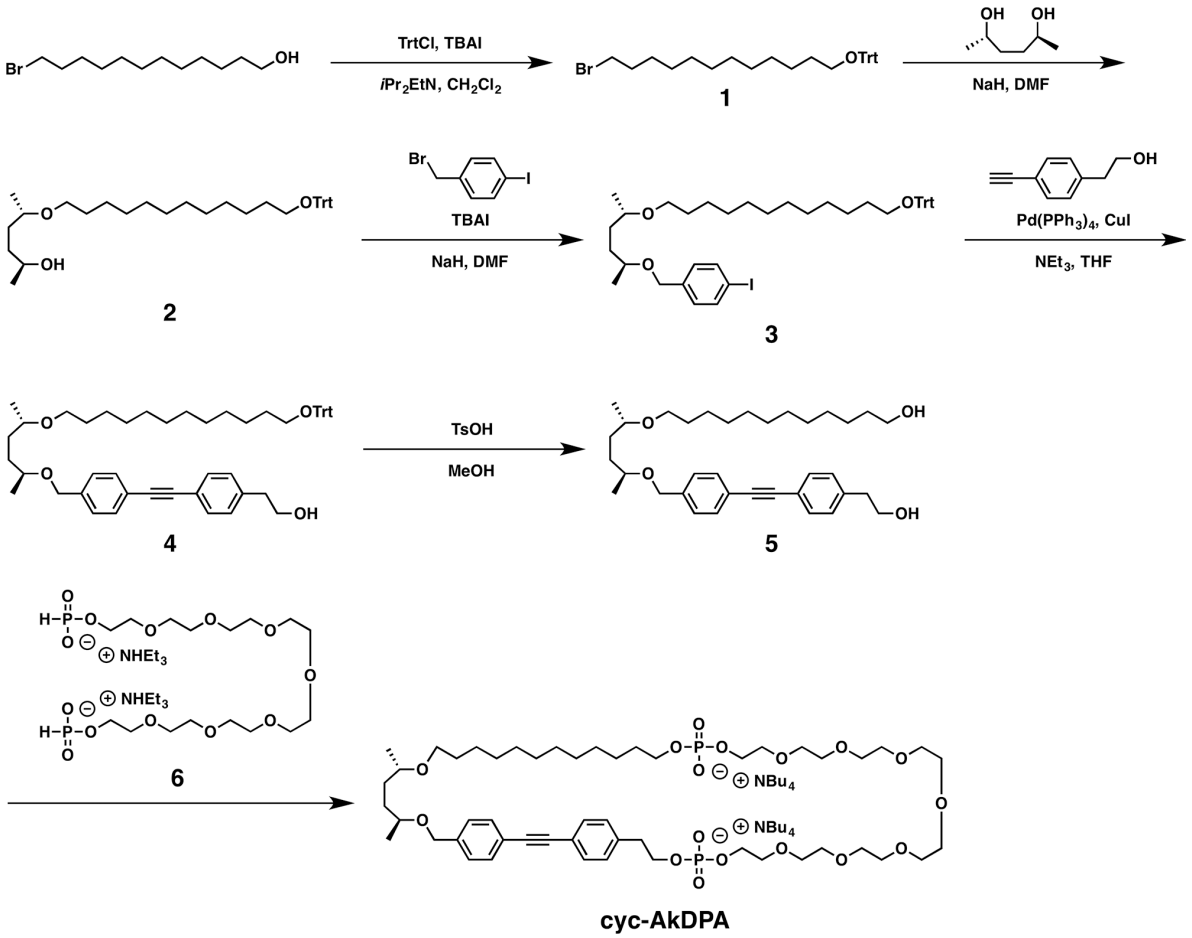
1. Materials

Bis(triphenylphosphine)palladium(II) dichloride, 12-bromo-1-dodecanol, (2*S*,5*S*)-2,5-hexanediol and NaH (60% in paraffin), I_2 and pivaloyl chloride were purchased from Tokyo Chemical Industry. Diisopropylethylamine, 1.0 M tetra-*n*-butylammonium fluoride (TBAF) in THF, dry triethylamine, 1.0 M triethylammonium bicarbonate (TEAB) buffer at pH 8.5 and trityl chloride were purchased from Sigma Aldrich. CuI, anhydrous Na_2SO_4, $Na_2S_2O_3$, tetra-*n*-butylammonium iodide (TBAI) and *p*-toluenesulfonic acid monohydrate were purchased from Nacalai Tesque. 4-Iodobenzyl bromide was purchased from Apollo Scientific. Dry pyridine was purchased from Wako Pure Chemical Industries. These commercial reagents were used without further purification. Deuterated solvents were purchased from Acros Organics. Dry CH_2Cl_2, dry *N*,*N*-dimethylformamide (DMF) and dry tetrahydrofuran (THF) was purchased from Kanto Chemical and passed through sequential two or three drying columns on a Glass-Contour system just prior to use. Deionized water (filtered through a 0.22 μm membrane filter, >18.2 MΩ cm) was purified in a Milli-Q system of Millipore. Silica gel column chromatography was carried out with Chromatorex DIOL silica (MB100-75/200, spherical, neutral, particle size: 75–200 μm, pore size: 10 nm), Chromatorex SO3H silica (MB100-75/200, spherical, neutral, particle size: 75–200 μm, pore size: 10 nm) and Chromatorex NH silica (MB100-75/200, spherical, neutral, particle size: 75–200 μm, pore size: 10 nm) purchased from Fuji Silysia Chemical and silica gel 60N (spherical, neutral, particle size: 63–210 μm) purchased from Kanto Chemical. Thin layer chromatography (TLC) was carried out with DIOL TLC, SO3H TLC and NH TLC purchased from Fuji Silysia Chemical and Merck 60 F254. Visualization of the developed chromatogram was performed by UV absorbance, I_2 or basic $KMnO_4$ solution. 2-(4-Ethynylphenyl)ethanol and **6** were synthesized according to the previous reports.[S1,S2]

2. Instrumentation

^{1}H and ^{13}C NMR spectra were recorded on 400 MHz FT NMR Bruker BioSpin AVANCE III 400 spectrometer or 500 MHz FT NMR Bruker BioSpin AVANCE III 500, where the chemical shifts were determined with respect to tetramethylsilane (TMS) or solvent signals. Dynamic light scattering (DLS) measurement was performed with Malvern Zetasizer Nano ZSP light-scattering detector, where a low-volume quartz batch cuvette (ZEN2112) was used. Matrix assisted laser desorption/ionization-time of flight mass (MALDI-TOF MS) measurement was performed with Bruker autoflex speed mass spectrometer with α-cyano-4-hydrocinnamic acid (CHCA) or gentisic acid (GA) as a matrix. UV absorption spectra were recorded on JASCO V-530 UV-Vis spectrophotometer. Circular dichroism (CD) spectra were recorded on JASCO J-820 spectropolarimeter. Fluorescence spectra were recorded on JASCO FP-6500 spectrofluorometer. Fluorescence lifetime was measured with Hamamatsu Photonics Quantaurus-Tau fluorescence lifetime spectrometer. Fluorescent and phase-contrast microscopy were performed with Olympus BX-51 microscope, where U-MWU2 mirror unit (Excitation Filter: 330-385 nm, Emission Filter: 420 nm, Dichroic Mirror: 400 nm) was used for fluorescence observation and Olympus UPLFLN 100XO2PH (magnification: ×100 and ×160) was attached as the objective lens. Surface tension was measured with Kyowa Interface Science Contact Angle Meter DMe-201 by a sessile drop method.

3. Synthesis



(1) Synthesis of 1. To a dry CH_2Cl_2 (1 mL) solution of diisopropylethylamine (200 μL, 0.565 mmol) were added 12-bromo-1-dodecanol (30.0 mg, 0.113 mmol), trityl chloride (63.0 mg, 0.226 mmol) and TBAI (4.2 mg, 11 μmol) at 0 °C under Ar. After being stirred for 10 min at 0 °C, the resulting mixture was stirred for 10 h at room temperature. Then, the reaction mixture was evaporated to dryness under reduced pressure at 30 °C. The residual oil was chromatographed on silica gel (Silica Gel 60) with *n*-hexane as an eluent to isolate **1** in 50% yield (30.0 mg, 59.1 μmol) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS, 23 °C): δ 7.44 (d, J = 7.2 Hz, 6H), 7.31–7.20 (m, 9H), 3.40 (t, J = 6.8 Hz, 2H), 3.04 (t, J = 6.4 Hz, 2H), 1.85 (m, 2H), 1.61 (m, 2H), 1.43–1.25 (m, 16H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 144.68, 128.83, 127.79, 126.90, 86.40, 63.81, 34.14, 32.98, 30.20, 29.65, 28.90, 28.32, 26.41 ppm;

MALDI-TOF MS (CHCA, linear positive mode): *m/z*: calculated for $C_{31}H_{40}BrO$: 507.2263 $[M + H]^+$; found: 507.2103.

(2) Synthesis of 2. To a dry DMF (0.5 mL) suspension of NaH (washed for three times with dry *n*-hexane in advance to remove paraffin; 7.3 mg, 0.18 mmol) was added (2*S*,5*S*)-2,5-hexanediol (20.0 mg, 0.169 mmol) at 0 °C under Ar, and the resulting mixture was stirred for 1 h at 0 °C. Then, to the reaction mixture was added a dry DMF (0.5 mL) solution of **1** (30.0 mg, 59.1 μmol) at 0 °C, and the resulting mixture was stirred for 12 h at 70 °C. Then, to the resulting mixture was added water (1 mL) at 0 °C. The resulting mixture was extracted with ethyl acetate (5 mL, three times), and the organic extract was washed with brine (5 mL) and dried over anhydrous Na_2SO_4. After filtration to remove insoluble substances, the filtrate was evaporated to dryness under reduced pressure. The residual oil was chromatographed on silica gel (Silica Gel 60) with a mixture of EtOAc and *n*-hexane (20/80) as an eluent to isolate **2** in 62% yield (19.8 mg, 0.0364 mmol) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS, 23 °C): δ 7.44 (d, J = 8.0 Hz, 6H), 7.31–7.20 (m, 9H), 3.78 (m, 1H), 3.50 (m, 1H), 3.40 (m, 1H), 3.31 (m, 1H), 3.04 (t, J = 6.8 Hz, 2H), 2.63 (s, 1H), 1.63–1.48 (m, 8H), 1.35–1.24 (m, 16H), 1.18 (d, J = 6.0 Hz, 3H), 1.14 (d, J = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 144.70, 128.85, 127.80, 126.91, 86.41, 75.82, 68.77, 68.33, 63.84, 35.91, 33.67, 30.21, 30.16, 29.75, 29.67, 29.62, 26.43, 26.35, 23.67, 19.68 ppm. MALDI-TOF MS (CHCA, reverse positive mode): *m/z*: calculated for $C_{37}H_{52}NaO_3$: 567.3814 $[M + Na]^+$; found: 567.5301.

(3) Synthesis of 3. To a dry DMF (10 mL) suspension of NaH (washed for three times with dry *n*-hexane in advance to remove paraffin; 132 mg, 5.50 mmol) was added a dry DMF (2 mL) solution of **2** (300 mg, 0.551 mmol) at 0 °C under Ar, and the resulting mixture was stirred for 1 h. Then, to the resulting mixture was added a dry DMF (2 mL) solution of 4-iodobenzyl bromide (164 mg, 0.551 mmol) and TBAI (102 mg, 0.551 mmol) at 0 °C. After being stirred for 12 h at 60 °C, the resulting mixture was cooled to 0 °C. After addition of water (10 mL), the resulting mixture was extracted with ethyl acetate (5

mL, four times), and the organic extract was washed with brine (5 mL) and dried over anhydrous Na_2SO_4. After filtration of the resulting mixture to remove insoluble substances, the filtrate was evaporated to dryness under reduced pressure. The residual oil was chromatographed on silica gel (Silica Gel 60) with a mixture of EtOAc and *n*-hexane (1/50) as an eluent to isolate **3** in 60% yield (250 mg, 0.328 mmol) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS, 23 °C): δ 7.65 (d, J = 7.2 Hz, 2H), 7.44 (d, J = 8 Hz, 6H), 7.31–7.20 (m, 9H), 7.08 (d, J = 7.2 Hz, 2H), 4.49 (d, J = 12 Hz, 1H), 4.39 (d, J = 12 Hz, 1H), 3.49 (m, 1H), 3.44 (m, 1H), 3.31 (m, 2H), 3.04 (t, J = 6.7 Hz, 2H), 1.64–1.45 (m, 8H), 1.35–1.24 (m, 16H), 1.18 (d, J = 6.0 Hz, 3H), 1.14 (d, J = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 144.67, 138.97, 137.48, 129.57, 128.83, 127.79, 126.89, 92.82, 86.38, 75.24, 75.17, 69.65, 68.64, 63.82, 32.40, 30.33, 30.20, 29.75, 29.67, 26.42, 19.85, 19.70 ppm; MALDI-TOF MS (CHCA, reverse positive mode): *m/z*: calculated for $C_{44}H_{57}IO_3Na$: 783.3250 $[M + Na]^+$; found: 783.2698.

(4) Synthesis of 4. To a dry THF (1 mL) solution of triethylamine (1.0 mL, 7.16 mmol) and **3** (60.0 mg, 78.9 μmol) were added bis(triphenylphosphine)palladium(II) dichloride (2.80 mg, 3.99 μmol), CuI (1.5 mg, 7.88 μmol) and a dry THF (1 mL) solution of 2-(4-ethynylphenyl)ethanol[S1] (11.5 mg, 78.7 μmol) at 0 °C under Ar. After being stirred for 10 h at room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The residual oil was chromatographed on silica gel (Silica Gel 60) with a mixture of EtOAc and *n*-hexane (20/80) as an eluent to isolate **4** in 70% yield (43.0 mg, 55.2 μmol) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS, 23 °C): δ 7.50–7.43 (m, 8H), 7.33–7.20 (m, 11H), 4.57 (d, J = 12 Hz, 1H), 4.46 (d, J = 12 Hz, 1H), 3.87 (m, 2H), 3.52 (m, 1H), 3.46 (m, 1H), 3.31 (m, 2H), 3.03 (t, J = 6.6 Hz, 2H), 2.88 (t, J = 6.6 Hz, 2H), 1.62–1.47 (m, 8H), 1.38–1.24 (m, 16H), 1.20 (d, J = 6.0 Hz, 3H), 1.13 (d, J = 6.0 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 144.74, 139.59, 139.03, 131.94, 131.68, 129.19, 128.88, 127.80, 127.57, 126.92, 122.44, 121.68, 89.41, 89.25, 86.46, 75.29, 75.23, 70.03, 68.68, 63.87, 63.58, 39.29, 32.46, 30.38, 30.23, 29.77, 29.69, 26.45, 19.88, 19.76 ppm;

MALDI-TOF MS (CHCA, linear positive mode): *m/z*: calculated for $C_{54}H_{67}O_4$: 779.5039 $[M + H]^+$; found: 779.3632.

(5) Synthesis of 5. To a MeOH (2 mL) solution of **4** (43.0 mg, 55.2 μmol) was added *p*-toluenesulfonic acid monohydrate (2.1 mg, 11 μmol) at room temperature. After being stirred for 4 h at room temperature, the reaction mixture was evaporated to dryness under reduced pressure at 30 °C. The residual oil was chromatographed on silica gel (Silica Gel 60) with a mixture of EtOAc and *n*-hexane (50/50) as an eluent to isolate **5** quantitatively (29.6 mg, 55.2 μmol) as a colorless solid.

^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS, 23 °C): δ 7.48 (m, 4H), 7.32 (d, J = 8.0 Hz, 2H), 7.22 (d, J = 8.0 Hz, 2H), 4.57 (d, J = 12 Hz, 1H), 4.47 (d, J = 12 Hz, 1H), 3.87 (t, J = 6.4 Hz, 2H), 3.62 (t, J = 6.6 Hz, 2H), 3.55–3.43 (m, 4H), 3.37–3.27 (m, 2H), 2.88 (t, J = 6.5 Hz, 2H), 1.70–1.27 (m, 24H), 1.20 (d, J = 6.0 Hz, 3H), 1.13 (d, J = 6.0 Hz, 3H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 139.57, 139.11, 131.89, 131.65, 129.17, 127.55, 122.45, 121.63, 89.37, 89.26, 75.29, 75.23, 70.01, 68.64, 63.50, 63.17, 39.29, 32.95, 32.46, 30.35, 29.72, 29.64, 29.56, 26.43, 25.88, 19.85, 19.73 ppm. MALDI-TOF MS (CHCA, reverse positive mode): *m/z*: calculated for $C_{35}H_{52}O_4Na$: 559.3763 $[M + Na]^+$; found: 559.6476.

(6) Synthesis of cyc-AkDPA. To a dry pyridine (20 mL) solution of **5** (110 mg, 0.205 mmol) and **6**[S2] (172 mg, 0.246 mmol) was added pivaloyl chloride (1.0 mL, 5.6 mmol) at 0 °C under Ar, and the resulting mixture was stirred for 1 h at 0 °C in the dark. To the resulting mixture was added I_2 (300 mg, 1.18 mmol) in a mixture of pyridine (5 mL) and water (2 mL), followed by saturated $Na_2S_2O_3$ aqueous solution (8 mL) and 1.0 M TEAB buffer (9 mL) at room temperature. After being stirred for 1.5 h at room temperature, the reaction mixture was evaporated to dryness under reduced pressure at 30 °C. To the residue was added CH_2Cl_2 (20 mL), and the resulting mixture was filtered off from insoluble substances and evaporated to dryness under reduced pressure at 30 °C. The residue was chromatographed on silica gel (Chromatorex DIOL silica) with a mixture of EtOAc and MeOH (80/20) as an eluent to allow isolation of cyc-AkDPA in a form of a

complex with triethylammonium cation (157 mg, 0.127 mmol) as a yellowish oil. The obtained product was passed through silica gel (Chromatorex SO3H silica) with a mixture of $CHCl_3$ and MeOH (90/10) as an eluent to exchange the cation with proton, which was then dissolved in $CHCl_3$ (10 mL) and mixed with a 1.0 M THF solution of TBAF (0.50 mL, 0.50 mmol). After being stirred for 20 min at room temperature, the resulting mixture was washed with water (10 mL, twice) and evaporated to dryness under reduced pressure at 30 °C to yield cyc-AkDPA in 20% yield (60.0 mg, 39.6 μmol) as a yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$ containing 0.03% TMS, 23 °C): δ 7.47 (d, J = 7.7 Hz, 2H), 7.42 (d, J = 7.4 Hz, 2H), 7.31 (d, J = 8.3 Hz, 2H), 7.24 (m, 2H), 4.58 (m, 1H), 4.44 (m, 1H), 4.12 (m, 2H), 4.04–3.44 (m, 38H), 3.29 (m, 16H), 2.97 (m, 2H), 1.65 (m, 16H), 1.44 (m, 16H), 1.26–1.11 (m, 30H), 0.99 (t, J = 7.2 Hz, 24H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$, 23 °C): δ 139.37, 139.13, 131.56, 129.31, 127.54, 122.40, 121.09, 89.45, 89.07, 75.21, 75.09, 70.65, 69.92, 68.58, 66.11, 64.89, 58.84, 37.04, 32.34, 30.80–29.61, 26.41, 25.88, 24.04, 19.79, 19.68, 13.81 ppm; MALDI-TOF MS (GA, reverse negative mode): *m/z*: calculated for $C_{51}H_{82}NaO_{17}P_2$: 1051.4936 $[M - 2NBu_4 + Na]^-$; found: 1051.8703.

4. Preparation of vesicles

(1) Preparation of giant unilamellar vesicles (GUVs). To a test tube was added $CHCl_3$ solutions of cyc-AkDPA (1.0 mM, 4 μL) and DOPC (1.0 mM, 36 μL), and the resulting mixture was gently evaporated by Ar flow. The resulting thin film on the bottom of the test tube was further dried under vacuum for 4 h at 25 °C, to which was added HEPES buffer (20 mM, 100 μL, pH 7.45) containing 200-mM sucrose. Then, the mixture was incubated for 10 h at 37 °C. For the phase-contrast microscopic observations, HEPES buffer (20 mM, 100 μL, pH 7.45) containing 200-mM glucose was added to the GUV suspension to enhance the contrast between the inside and outside of the GUVs by a large difference in the refractive indices.

(2) Preparation of large unilamellar vesicles (LUVs). To a test tube was added $CHCl_3$ solutions of cyc-AkDPA (1.0 mM, 5 μL) and DOPC (1.0 mM, 45 μL), and a mixture of $CHCl_3$ and MeOH (2:1 v/v, 50 μL), and the resulting mixture was gently evaporated by Ar flow. The resulting thin film on the bottom of the test tube was further dried under vacuum for 1.5 h at 25 °C, to which was added HEPES buffer (20 mM, 1.0 mL, pH 7.45). Then, the mixture was shaken on a shaker at 200 min^{-1} for 1 h at 37 °C, followed by freezing-and-thawing for three times, vortex mixing for 10 s and incubation at 37 °C for 10 h. Finally the mixture was passed through a polycarbonate membrane (200 nm pore size) attached to a LiposoFast-Basic device by pushing the sample back and forth between the two gastight syringes over 13 times.

5. Surface tension of cyc-AkDPA

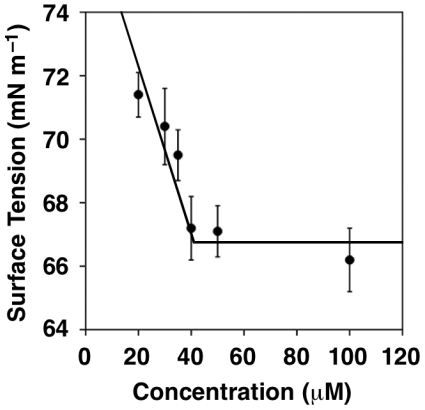


Fig. S1 Surface tension change of cyc-AkDPA depending on the concentration in water at 25 °C, indicating that the critical aggregation concentration (CAC) of cyc-AkDPA in water is 40 μM.

6. DLS of cyc-AkDPA

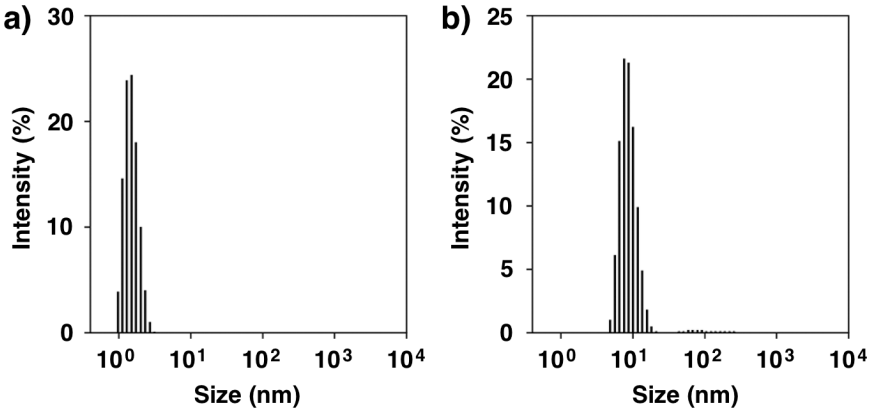


Fig. S2 DLS profiles with volume-based distribution of cyc-AkDPA (50 μM) in a) THF and b) water at 20 °C, after extrusion through a 200-nm pore size polycarbonate membrane.

7. Fluorescence lifetime of cyc-AkDPA

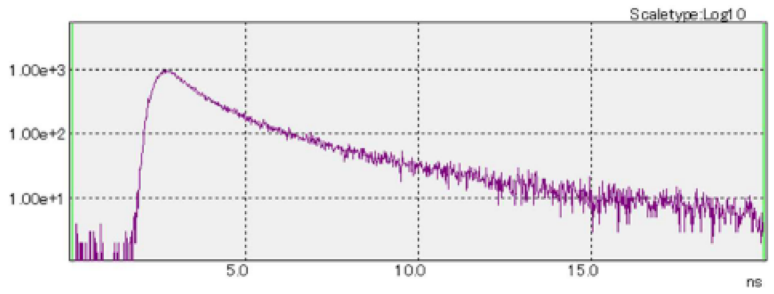


Fig. S3 Fluorescence decay profile of cyc-AkDPA in water at 20 °C at the 382-nm emission light. Excitation at 288 nm. The decay profile at the 320-nm emission light is not shown because of its too short lifetime (<0.1 ns) for analysis. The sample was extruded through a 200-nm pore size polycarbonate membrane.

8. CD spectrum of cyc-AkDPA

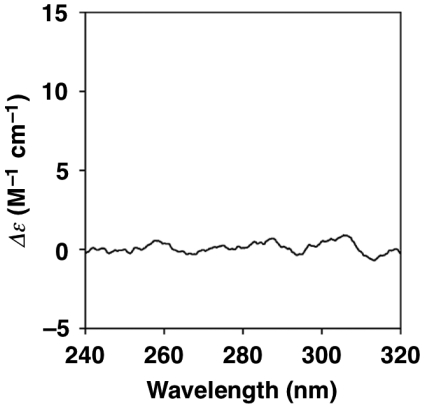


Fig. S4 CD spectrum of cyc-AkDPA in water at 20 °C (50 μM in 1-mm thick quartz cuvette). The sample was extruded through a 200-nm pore size polycarbonate membrane.

9. Variable temperature ^{13}C NMR spectra of cyc-AkDPA

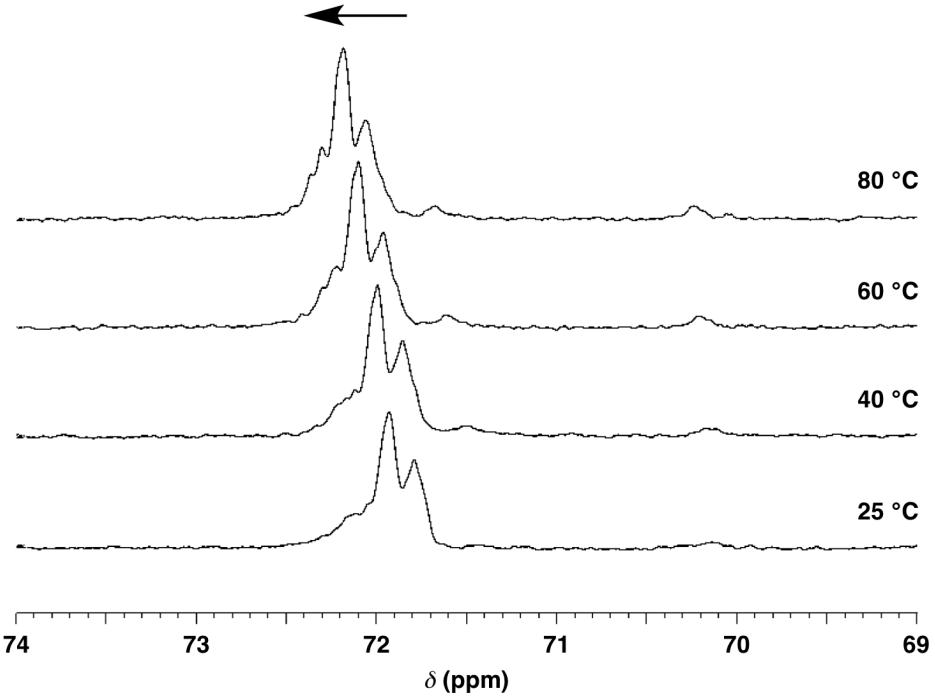


Fig. S5 Variable temperature ^{13}C NMR spectra of cyc-AkDPA in D_2O at 11 mM at 25, 40, 60 and 80 °C.

10. DLS of $LUV_{DOPC \cdot cyc\text{-}AkDPA}$

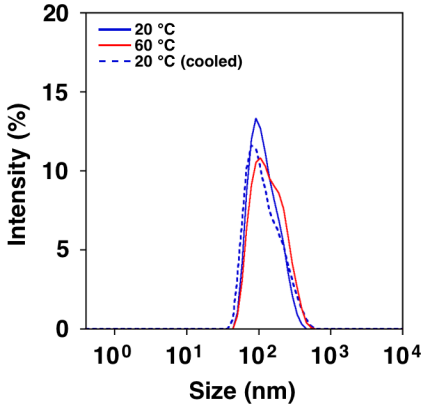


Fig. S6 DLS profiles with volume-based distribution of $LUV_{DOPC \cdot cyc\text{-}AkDPA}$ in water at 20 °C (blue solid line), 60 °C (red solid line), and 20 °C after cooling (blue broken line). The sample was prepared by extrusion through a 200-nm pore size polycarbonate membrane prior to the heating. [DOPC] = 45 μM, [cyc-AkDPA] = 5.0 μM.

11. Fluorescence depth quenching study of $LUV_{DOPC \cdot cyc\text{-}AkDPA}$

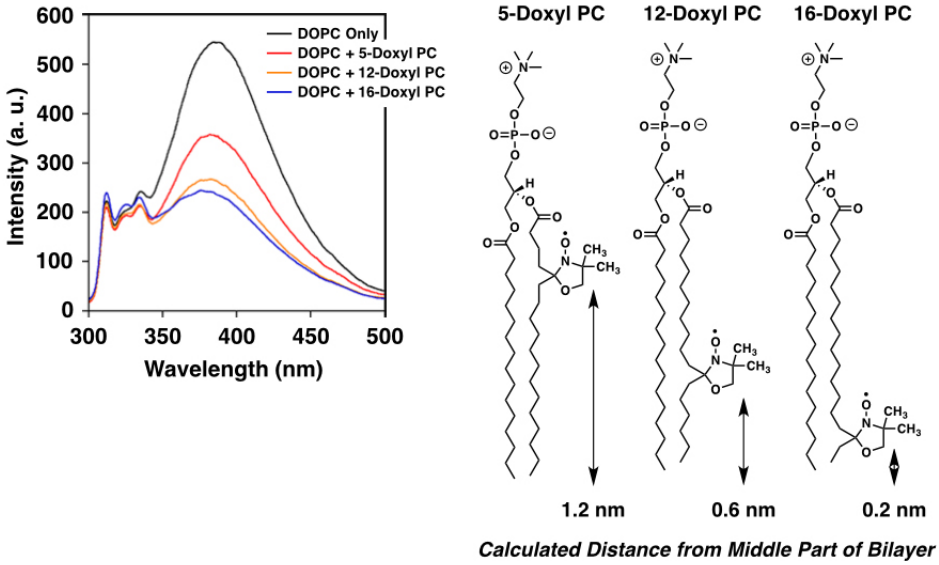


Fig. S7 Fluorescence spectra of LUVs consisting of a mixture of phosphocholine and cyc-AkDPA in water at 20 °C upon excitation at 288 nm. The phosphocholines used for the LUVs were only DOPC (black line), or mixtures of DOPC with 9-mol% 5-, 12- or 16-Doxyl PC (red, orange, and blue lines, respectively). [Total phosphocholines] = 45 μM, [cyc-AkDPA] = 5.0 μM. Molecular structures of 5-, 12- and 16-Doxyl PCs are shown with the calculated distances of the doxyl groups from the center of the bilayer.

12. TEM of $LUV_{DOPC \cdot cyc\text{-}AkDPA}$

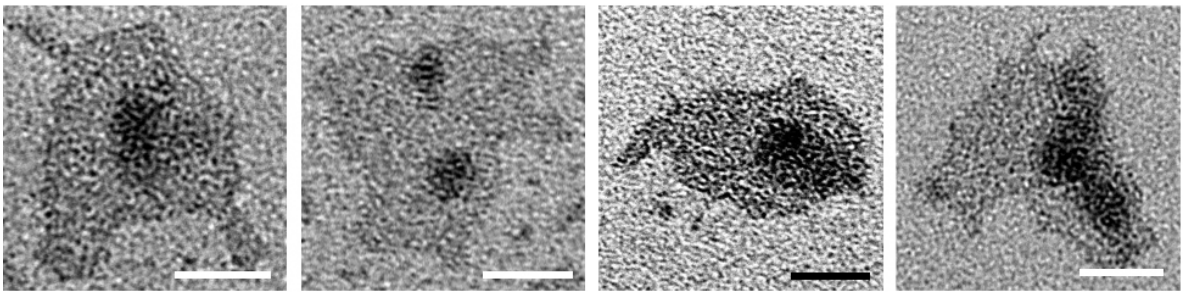


Fig. S8 Transmission electron micrographs of $LUV_{DOPC \cdot cyc\text{-}AkDPA}$ prepared at 25 °C after heating to 60 °C in water ([DOPC] = 45 μM, [cyc-AkDPA] = 5.0 μM). Stainer: uranyl acetate. Scale bars: 50 nm.

13. Optical micrographs of $GUV_{DOPC \cdot cyc\text{-}AkDPA}$ incubated at 25 °C

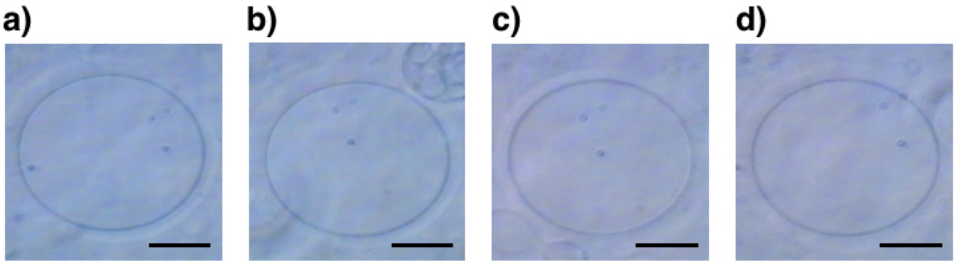


Fig. S9 Phase-contrast micrographs of $GUV_{DOPC \cdot cyc\text{-}AkDPA}$ taken at 25 °C at a) 0, b) 1, c) 3 and d) 6 h after the preparation. [DOPC] = 0.18 mM, [cyc-AkDPA] = 0.020 mM. Scale bars: 20 μm.

14. Optical micrographs of $GUV_{DOPC \cdot cyc\text{-}AkDPA}$ upon temperature changes

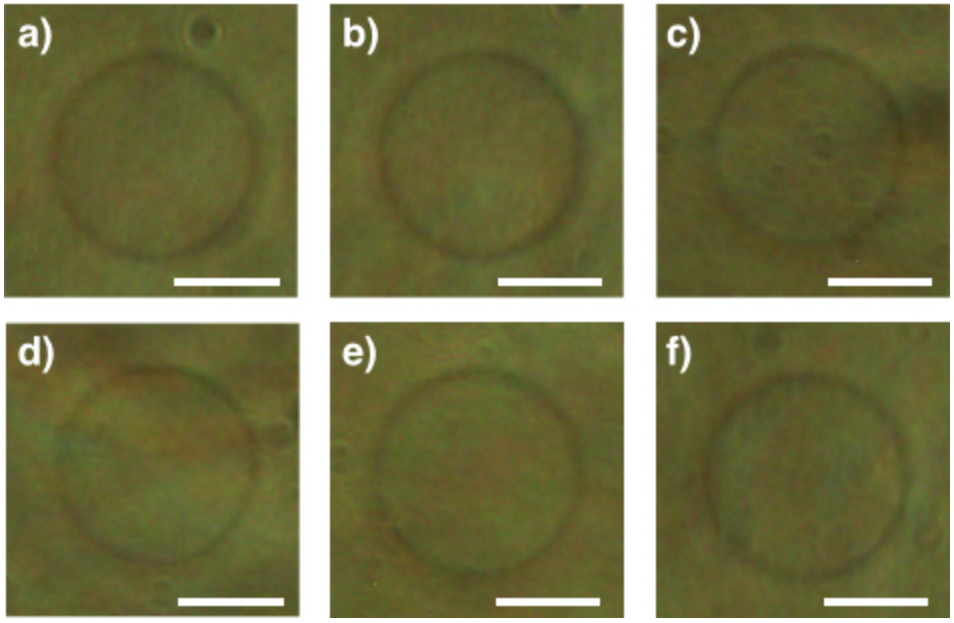


Fig. S10 Phase-contrast micrographs of $GUV_{DOPC \cdot AkDPA}$ taken at a) 25, b) 35, c) 45, d) 55, e) 60 °C upon temperature elevation and at f) 25 °C after cooling. [DOPC] = 0.198 mM, [cyc-AkDPA] = 0.002 mM ([DOPC]/[cyc-AkDPA] = 99/1). Scale bars: 5.0 μm.

15. References

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S2 R. Li, T. Muraoka and K. Kinbara, *Langmuir*, 2016, **32**, 4546.