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Lipid cubic phase with an organic-inorganic hybrid structure formed by organoalkoxysilane lipid

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1. Abbreviation

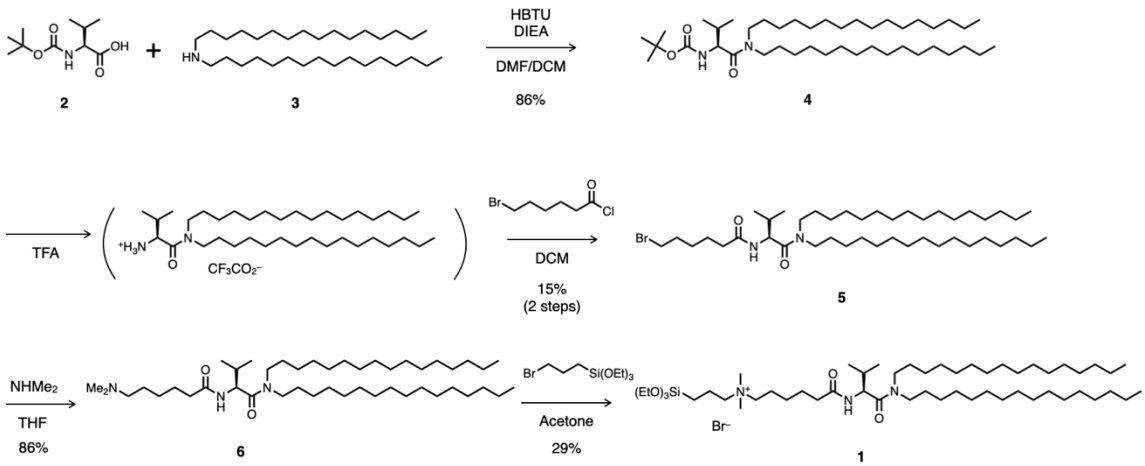
DCM: dichloromethane, DCTB: *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile, DIEA: *N*,*N*-diisopropylethylamine, DMF: *N*,*N*-dimethylformamide, HBTU: hexafluorophosphate benzotriazole tetramethyl uronium, HRMS: high resolution mass spectrometry, MALDI-TOF MS: matrix-assisted laser desorption ionization time-of-flight mass spectrometry, MeCN: acetonitrile, MeOH: methanol, NMR: nuclear magnetic resonance, PEG: polyethylene glycol, POM: polarized optical microscope, SAXS: small angle X-ray scattering, TEA: triethylamine, TEM: transmission electron microscopy, THF: tetrahydrofuran, TMS: tetramethylsilane.

2. Synthesis of organosilane lipid 1

Materials and methods

Organosilane lipid **1** was synthesized according to a literature procedure with a slight modification.[1] All solvents, organic and inorganic reagents are commercially available and were used without further purification unless otherwise stated. Solution state NMR spectroscopy was performed using a JEOL JNM-ECA600 and a JEOL JNM-ECX400P spectrometers. TMS ($Si(CH_3)_4$) = 0 ppm) and $CDCl_3$ ($CDCl_3$ = 77.16 ppm) were used as an internal reference for ^1H and ^{13}C NMR measurements in $CDCl_3$ respectively. HRMS was performed using a JEOL spiral TOF (JMS-S3000) using DCTB as a matrix and PEG as an internal standard. GPC was performed using a JAI JC-908W-C60BW with two polystyrene gel columns connected in series (JAIGEL 1H and 2H) with a mobile phase of $CHCl_3$.

Scheme S1. Synthetic route for **1**.



Preparation of *N*,*N*-dihexadecyl-*N*$^{\alpha}$-(*tert*-butoxycarbonyl)-L-valineamide (4)

N-(*tert*-butoxycarbonyl)-L-valine (**2**) (2.34 g, 10.8 mmol, 1.0 eq), *N,N*-dihexadecylamine (**3**) (4.00 g, 10.7 mmol, 1.0 eq), and HBTU (5.12 g, 13.5 mmol, 1.3 eq) were placed in a two-necked flask. The inner gas was replaced by N_2 and anhydrous DMF (16 mL), anhydrous DCM (20 mL), and DIEA (4.5 mL) were added. After stirring at room temperature for 20 h, the reaction mixture was concentrated by evaporation of solvents at reduced pressure. After adding EtOAc (200 mL), the reaction mixture was washed successively with H_2O (150 mL) and saturated aqueous solution of $NaHCO_3$ (150 mL x 4) followed by brine (100 mL x 3). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure to obtain a crude product as a pale brown oil. The crude product was purified by column chromatography (SiO_2, *n*-hexane/EtOAc = 6/1) to afford **4** (6.13 g, 9.22 mmol, 86%) as a colourless oil.

^{1}H NMR (600 MHz, $CDCl_3$, 293 K, Fig. S1): δ (ppm) = 5.26 (d, 1H, J = 9.1 Hz, CON*H*CH), 4.39 (dd, 1H, J = 9.2, 6.7 Hz, NHC*H*CO), 3.63–3.56 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.40–3.33 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.24–3.17 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.04–2.98 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 1.91 (m, 1H, $(CH_3)_2$C*H*CH), 1.64–1.42 (m, 4H, N$(CH_2$C*H*$_2(CH_2)_{13}CH_3)_2$), 1.42 (s, 9H, $(CH_3)_3$CO), 1.31–1.22 (m, 52H, N$(CH_2CH_2(CH_2)_{13}CH_3)_2$), 0.94 (d, 3H, J = 6.9 Hz, C*H*$_3$CHC*H*$_3$), 0.90 (d, 3H, J = 6.9 Hz, C*H*$_3$CHC*H*$_3$), 0.88 (t, 6H, J = 7.0 Hz, N$((CH_2)_{15}CH_3)_2$).

^{13}C NMR (150 MHz, $CDCl_3$, 293 K, Fig. S2): δ (ppm) = 171.90, 155.81, 100.06, 79.34, 55.10, 48.07, 46.05, 32.30, 32.08, 29.85, 29.81, 29.73, 29.70, 29.56, 29.54, 29.52, 29.41, 28.47, 27.75, 27.17, 26.99, 22.84, 19.78, 17.53, 14.28.

HRMS (MALDI): calcd. for $[C_{42}H_{84}O_3 + Na]^+$: m/z = 687.6371, found: m/z = 687.6375.

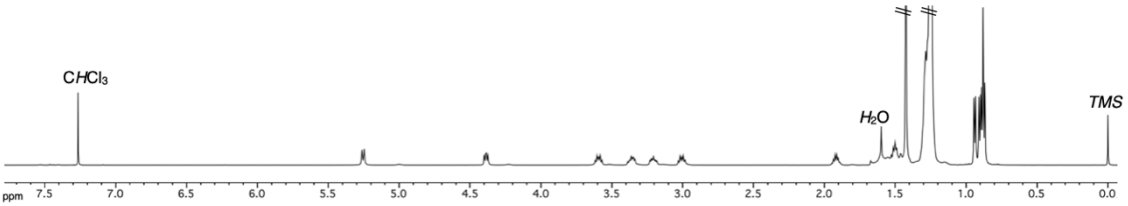


Fig. S1 ^{1}H NMR spectrum of **4** (600 MHz, $CDCl_3$, 293 K).

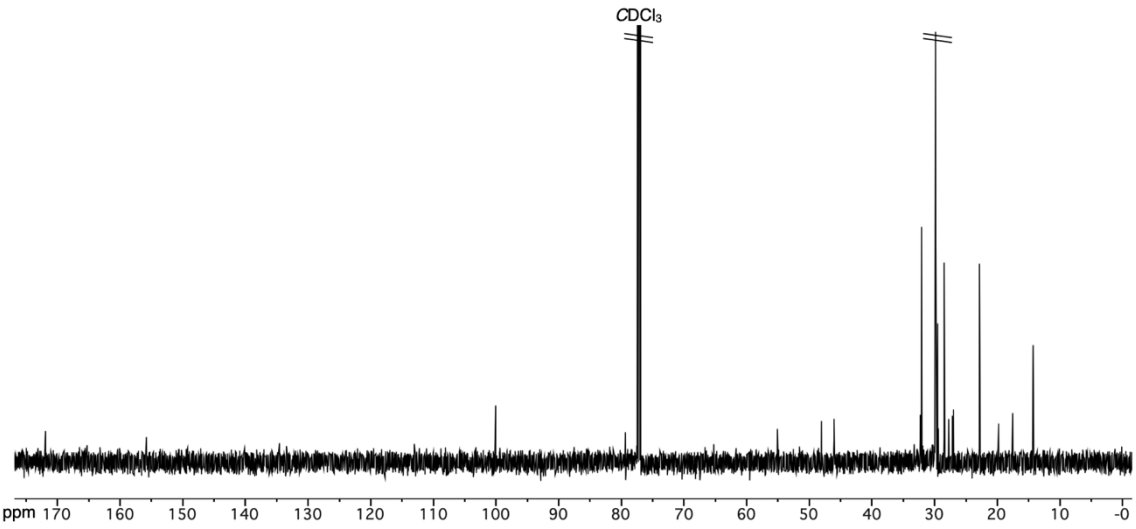


Fig. S2 ^{13}C NMR spectrum of **4** (150 MHz, $CDCl_3$, 293 K).

Preparation of *N*,*N*-dihexadecyl-N^{α}-(6-bromohexanoyl)-L-valineamide (5)

4 (6.05 g, 9.11 mmol, 1.0 eq) was placed in a flask. After adding TFA (8.5 mL), the mixture was stirred at room temperature for 15.5 h. After adding DCM (20 mL), the solvent was evaporated under reduced pressure to obtain a colourless oil. The inner gas of the flask was replaced by N_2 and anhydrous DCM (40 mL) and TEA (10.5 mL) were added. After cooling down to 0 °C, 6-bromohexanoyl chloride (10 mL, 9.8 g, 74.3 mmol, 8.2 eq) was added dropwise over 15 min. After stirring at room temperature for 20 h, H_2O (20 mL) and DCM (100 mL) were added to the mixture. The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (100 mL x 4) followed by brine (100 mL x 3). The organic layers were combined and dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to obtain a crude product as a brown oil. The crude product was purified by column chromatography (SiO_2, *n*-hexane/DCM = 1/0–0/1) followed by reprecipitation from cold MeOH to afford **5** (2.42 g, 1.35 mmol, 15%) as a colourless solid.

^{1}H NMR (600 MHz, $CDCl_3$, 293 K, Fig. S3): δ (ppm) = 6.18 (d, 1H, J = 8.8 Hz, CON*H*CH), 4.77 (dd, 1H, J = 8.6, 6.6 Hz, NHC*H*CO), 3.60–3.53 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.40 (t, 2H, J = 6.8 Hz, BrC*H*$_2(CH_2)_4$CO), 3.38–3.33 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.26–3.20 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.05–3.99 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 2.22 (t, 2H, J = 7.5 Hz, Br$(CH_2)_4$-C*H*$_2$CO), 1.97 (m, 1H, $(CH_3)_2$C*H*CH), 1.87 (tt, J = 7.2, 7.2 Hz, 2H, BrCH$_2$C*H*$_2(CH_2)_3$CO), 1.71–1.62 (m, 2H, N$(CH_2)_3$C*H*$_2$CH$_2$CO), 1.61–1.54 (m, 6H, NCH$_2$C*H*$_2(CH_2)_3$CO, N(CH$_2$C*H*$_2(CH_2)_{13}CH_3)_2$), 1.36–1.22 (m, 54H, N(CH$_2$CH$_2$(C*H*$_2)_{13}CH_3)_2$, N$(CH_2)_2$C*H*$_2$CH$_2$CO),

0.95 (d, 3H, J = 6.5 Hz, CH_3CHCH$_3$), 0.90 (d, 3H, J = 6.5 Hz, CH_3CHCH$_3$), 0.88 (t, 6H, J = 7.1 Hz, N((CH$_2$)$_{15}$CH_3)$_2$).

^{13}C NMR (150 MHz, CDCl$_3$, 293 K, Fig. S4): δ (ppm) = 172.31, 171.54, 53.42, 48.08, 46.14, 36.65, 33.69, 32.61, 32.28, 32.08, 29.85, 29.81, 29.73, 29.52, 29.47, 29.37, 27.92, 27.74, 26.16, 26.98, 24.95, 22.85, 19.89, 17.71, 14.28.

HRMS (MALDI) mass: calcd. for [$C_{43}H_{85}N_2BrO_2$ + Na]$^+$: m/z = 763.5687, found: m/z = 763.5620.

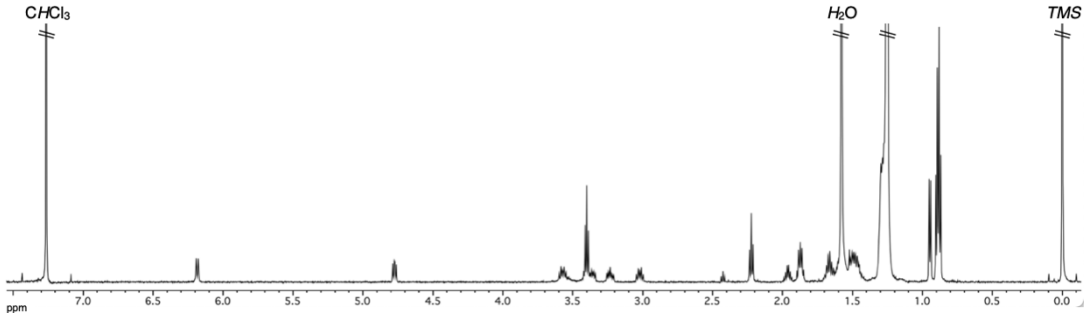


Fig. S3 ^{1}H NMR spectrum of **5** (600 MHz, CDCl$_3$, 293 K).

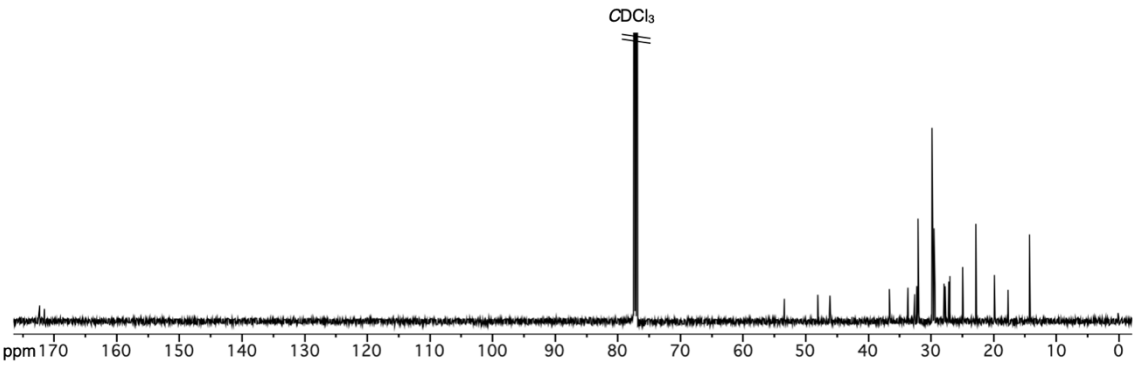


Fig. S4 ^{13}C NMR spectrum of **5** (150 MHz, CDCl$_3$, 293 K).

Preparation of *N*,*N*-dihexadecyl-*N*$^{\alpha}$-[6-(dimethylamino)hexanoyl]-L-valineamide (6)

A THF solution of NHMe$_2$ (ca. 2 M, 20 mL, ca. 40 mmol, ca. 15 eq) was added in a flask containing **5** (2.01 g, 2.71 mmol, 1.0 eq). The mixture was stirred at room temperature for 17.5 h. The reaction mixture was evaporated under N$_2$ flow to obtain a crude product as a colourless solid. The crude product was purified by reprecipitation in MeCN to afford **6** (1.65 g, 2.34 mmol, 86%) as a colourless solid.

^{1}H NMR (600 MHz, $CDCl_3$, 293 K, Fig. S5): δ (ppm) = 6.17 (d, 1H, J = 8.9 Hz, CON*H*CH), 4.77 (dd, 1H, J = 9.0, 6.4 Hz, NHC*H*CO), 3.60–3.51 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.40–3.32 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.28–3.21 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.06–2.99 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 2.25–2.19 (m, 4H, NC$H_2(CH_2)_3$CH_2CO), 2.19 (s, 6H, $(CH_3)_2$N$(CH_2)_5$CO), 1.96 (m, 1H, $(CH_3)_2$C*H*CH), 1.69–1.62 (m, 2H, N$(CH_2)_3$CH_2CH_2CO), 1.61–1.44 (m, 6H, NCH_2C$H_2(CH_2)_3$CO, N$(CH_2$C$H_2(CH_2)_{13}CH_3)_2$), 1.36–1.22 (m, 54H, N$(CH_2)_2(CH_2)_{13}CH_3)_2$, N$(CH_2)_2CH_2(CH_2)_2$CO), 0.94 (d, 3H, J = 6.5 Hz, CH_3CHCH_3), 0.90 (d, 3H, J = 6.5 Hz, CH_3CHCH_3), 0.88 (t, 6H, J = 6.8 Hz, N$((CH_2)_{15}CH_3)_2$).

^{13}C NMR (150 MHz, $CDCl_3$, 293 K, Fig. S6): δ (ppm) = 172.62, 171.58, 59.77, 53.38, 48.07, 46.13, 45.62, 36.92, 32.26, 32.06, 29.84, 29.80, 29.72, 29.51, 29.46, 29.37, 27.73, 27.59, 27.26, 27.16, 26.98, 25.83, 22.83, 19.87, 17.73, 14.27.

HRMS (MALDI): calcd. for $[C_{45}H_{91}N_3O_2 + H]^+$: m/z = 706.7184, found: m/z = 706.7164.

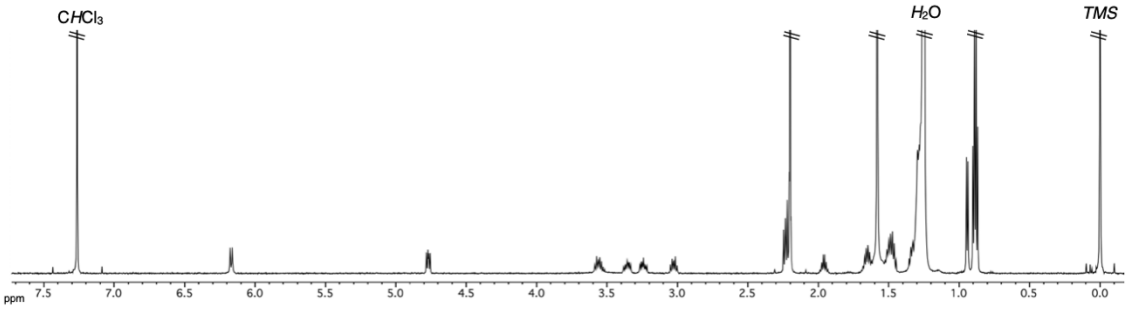


Fig. S5 ^{1}H NMR spectrum of **6** (600 MHz, $CDCl_3$, 293 K).

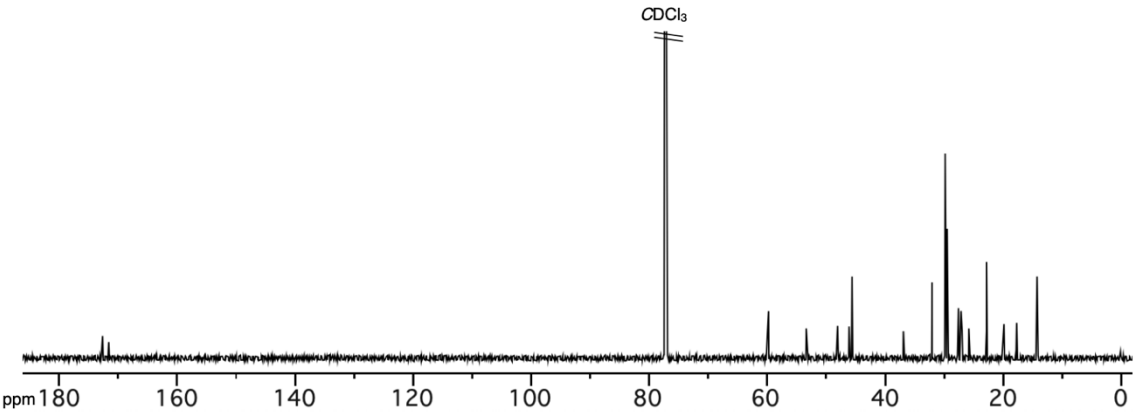


Fig. S6 ^{13}C NMR spectrum of **6** (150 MHz, $CDCl_3$, 293 K).

Preparation of *N*,*N*-dihexadecyl-*N*$^{\alpha}$-[6-[(3-triethoxysilyl)propyldimethylammonio]-hexanoyl]-L-valineamide bromide (1)

In a flask containing a solution of **6** (2.74 g, 3.89 mmol, 1.0 eq) in anhydrous acetone (8.0 mL) under N_2 was added (3-bromopropyl)triethoxysilane (5.1 mL, 6.63 g, 27.3 mmol, 7.0 eq). After stirring the mixture at room temperature for 6 days, the solvent was removed by evaporation under reduced pressure to obtain a crude product as a colourless oil. The crude product was purified by GPC ($CHCl_3$) to afford **1** (1.13 g, 1.16 μmol, 29%) as a colourless oil.

^{1}H NMR (600 MHz, $CDCl_3$, 293 K, Fig. S7): δ (ppm) = 6.42 (d, 1H, J = 8.5 Hz, CON*H*CH), 4.71 (dd, 1H, J = 8.4, 6.8 Hz, NHC*H*CO), 3.83 (q, 6H, J = 7.1 Hz, $(CH_3CH_2O)_3Si$), 3.61–3.46 (m, 5H, NC*H*H$(CH_2)_{14}CH_3$, $CH_2CH_2N^+CH_2CH_2$), 3.34 (s, 3H, $CH_2N^+(CH_3)(CH_3)CH_2$), 3.33 (s, 3H, $CH_2N^+(CH_3)(CH_3)CH_2$), 3.33 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.25 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 3.00 (m, 1H, NC*H*H$(CH_2)_{14}CH_3$), 2.35 (dt, 1H, J = 7.2 and 14.4 Hz, $N^+(CH_2)_4CHHCO$), 2.24 (dt, 1H, J = 7.2 and 14.4 Hz, $N^+(CH_2)_4CHHCO$), 1.98 (m, 1H, $(CH_3)_2CHCH$), 1.86–1.76 (m, 2H, $N^+CH_2CH_2(CH_2)_3CO$), 1.76–1.69 (tt, 2H, J = 7.4 and 7.4 Hz, $N^+CH_2CH_2(CH_2)_3CO$), 1.66–1.52 (m, 2H, $N^+(CH_3)_2CH_2CH_2CO$), 1.52–1.47 (m, 2H, $NCH_2CH_2(CH_2)_{13}CH_3$), 1.47–1.40 (m, 2H, $NCH_2CH_2(CH_2)_{13}CH_3$), 1.32–1.20 (m, 54H, $N(CH_2CH_2(CH_2)_{13}CH_3)_2$, $N^+(CH_2)_2CH_2CH_2$), 1.23 (t, 9H, J = 7.2 Hz, $(CH_3CH_2O)_3Si$), 0.95 (d, 3H, J = 6.8 Hz, CH_3CHCH_3), 0.91 (d, 3H, J = 6.8 Hz, CH_3CHCH_3), 0.88 (t, 6H, J = 7.0 Hz, $N((CH_2)_{15}CH_3)_2$), 0.67 (t, 2H, J = 7.8 Hz, $SiCH_2$).

^{13}C NMR (150 MHz, $CDCl_3$, 293 K, Fig. S8): δ (ppm) = 172.17, 171.43, 65.73, 64.00, 58.81, 53.73, 51.36, 48.10, 46.20, 35.78, 32.03, 31.90, 29.81, 29.71, 29.68, 29.51, 29.47, 29.42, 29.33, 27.73, 27.17, 27.02, 25.70, 24.92, 22.80, 22.49, 19.90, 18.45, 17.82, 16.79, 14.24, 6.95.

HR-MS (MALDI): calcd. for $[C_{54}H_{112}N_3O_5Si]^+$: m/z = 910.8368, found: m/z = 910.8388.

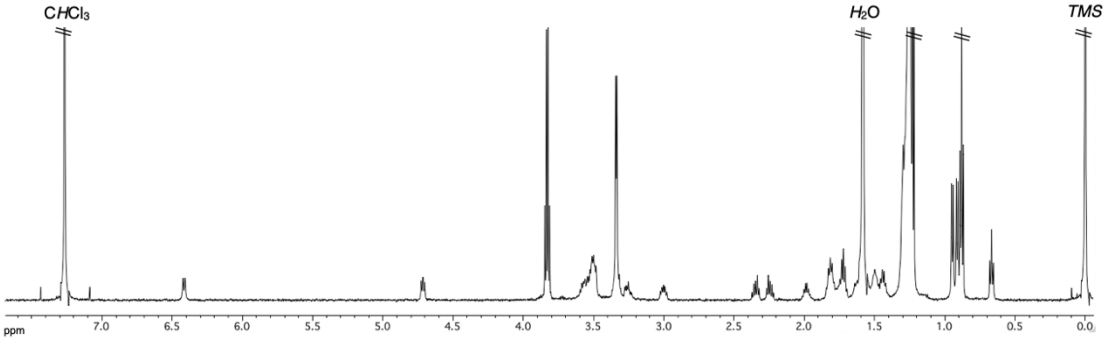


Fig. S7 ^{1}H NMR spectrum of **1** (600 MHz, $CDCl_3$, 293 K).

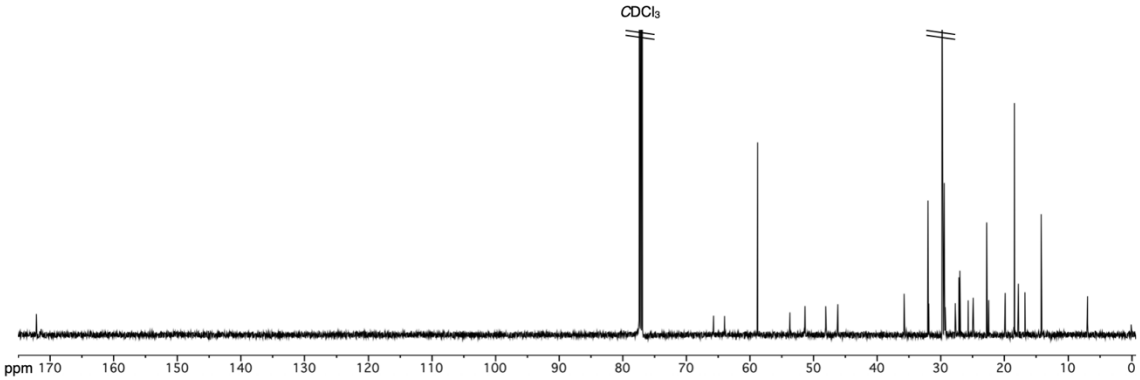


Fig. S8 ^{13}C NMR spectrum of **1** (150 MHz, $CDCl_3$, 293 K).

3. Preparation and characterization of organic-inorganic hybrid lipid cubic phase

Experimental procedures

Typical procedure for the preparation of lipid-water mixture ([**1**] = 90 w/w%)

1 (26.89 mg) and water (2.99 mg) were independently introduced into two different syringes which were connected by a coupler (Fig. S9a). The samples were mixed by repeatedly transferring the content between syringes for 500 cycles at 35 °C. The resulting transparent viscous lipid-water mixture was loaded into the bottom of the glass capillary tube (1 mm diameter). After centrifugation to remove air bubbles in the mixture, the open end of the capillary was sealed by flame fusing of the glass with a burner and protected by coating with melted wax to avoid the unexpected evaporation of water (Fig. S9b).

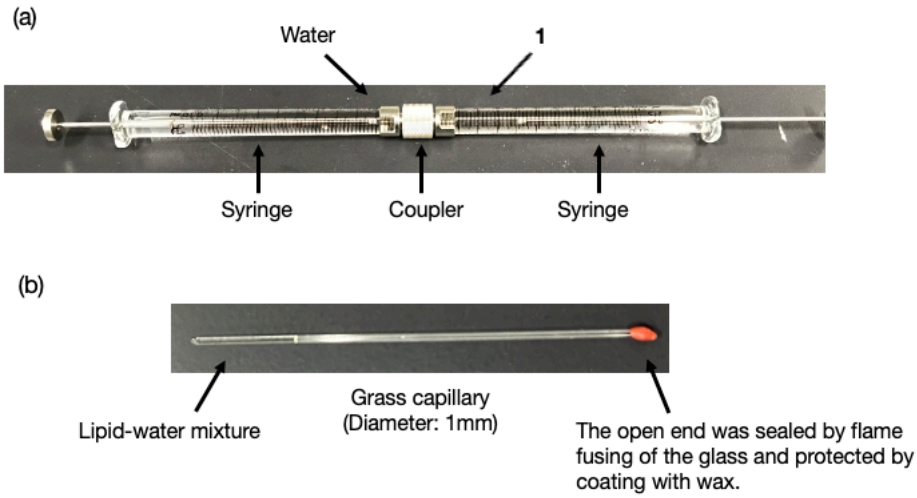


Fig. S9 (a) Two syringes each containing water and **1** which were connected by a coupler. (b) A sealed capillary containing a lipid-water mixture ([**1**] = 90 w/w%).

Solution state ^{1}H NMR spectroscopy of the lipid-water mixture

Lipid-water mixtures ([**1**] = 90 w/w%) in different sealed capillaries were subjected to the high-temperature incubation at 60 °C for a certain time. The resulted samples were removed from the capillaries and dissolved in $CDCl_3$. ^{1}H NMR spectra of the resulted solutions were measured (Fig. S10).

MALDI-TOF MS of the lipid-water mixture

Lipid-water mixture ([**1**] = 90 w/w%) in a sealed capillary was subjected to the high-temperature incubation at 60 °C for 2 days. The resulted sample was removed from the capillary and dissolved in $CHCl_3$. MALDI-TOF MS of the resulted solution was performed with a Bruker Autoflex II using DCTB as a matrix (Fig. 2a in the main text).

POM observation of the lipid-water mixture

Lipid-water mixtures ([**1**] = 90 w/w%) in different sealed capillaries were subjected to the high-temperature incubation at 60 °C using a heating stage (TOKAI HIT TPi-SZX2X) equipped with an Olympus binocular stereomicroscope (SZX2-ILLTQ). The time courses of the POM image traces of the samples during high-temperature incubation were recorded (Fig. S12).

Solid-state NMR spectroscopy of the lipid-water mixture

Lipid-water mixture ([**1**] = 90 w/w%) in a sealed syringe was subjected to high-temperature incubation at 60 °C for 7 days. The resulting sample was removed from the syringe and sealed in a zirconium cell. Solid-state ^{29}Si and ^{13}C NMR spectra (60 °C, Fig. 2b in the main text and Figs. S11 and S15) were measured using a Bruker Avance III 600 (Bruker Biospin, AG, Switzerland) equipped with a narrow-bore magnet operated at a ^{1}H resonance frequency of 600 MHz. Data were recorded using a VTN double-resonance 4 mm magic angle spinning (MAS) probe, at a spinning speed of 5 kHz. The ^{29}Si spectra were collected with a 4 μs excitation pulses and a 250 s recycle delay. The ^{29}Si NMR spectrum was also measured with sample tube alone and subtracted to exclude background signals. The ^{29}Si chemical shifts were externally referenced to hexamethylcyclotrisiloxane (-9.5 ppm).[2] The ^{13}C spectra were acquired using a 5 μs excitation pulses with 50 kHz proton decoupling and a 5 s recycle delay. ^{13}C chemical shifts were externally referenced to the methylene carbon of adamantane (38.48 ppm).[3] For the measurement of the penetration of paramagnetic Mn^{2+} into the cubic phase sample, 2 μL of saturated $MnCl_2 \cdot 4H_2O$ in D_2O (1, 980 g・L^{-1}) was added to approximately 100 mg of lipid-water

mixture. The spectrum in the addition of Mn^{2+} was acquired 5 days after the addition.

SAXS measurement of the lipid-water mixture

Lipid-water mixtures ([**1**] = 90 w/w%) in sealed capillaries were subjected to high-temperature incubation at 60 °C for a certain time. SAXS of the resulting samples were measured at a controlled temperature using a synchrotron X-ray beam (λ = 1.00 Å) of BL-10C at the Photon Factory, Japan, equipped with a customized temperature controller (INSTEC HCS302-LN190) and a Dectoris model PIRATUS 2M detector (Fig. 4 in the main text and Figs. S13 and S14).

Experimental Data

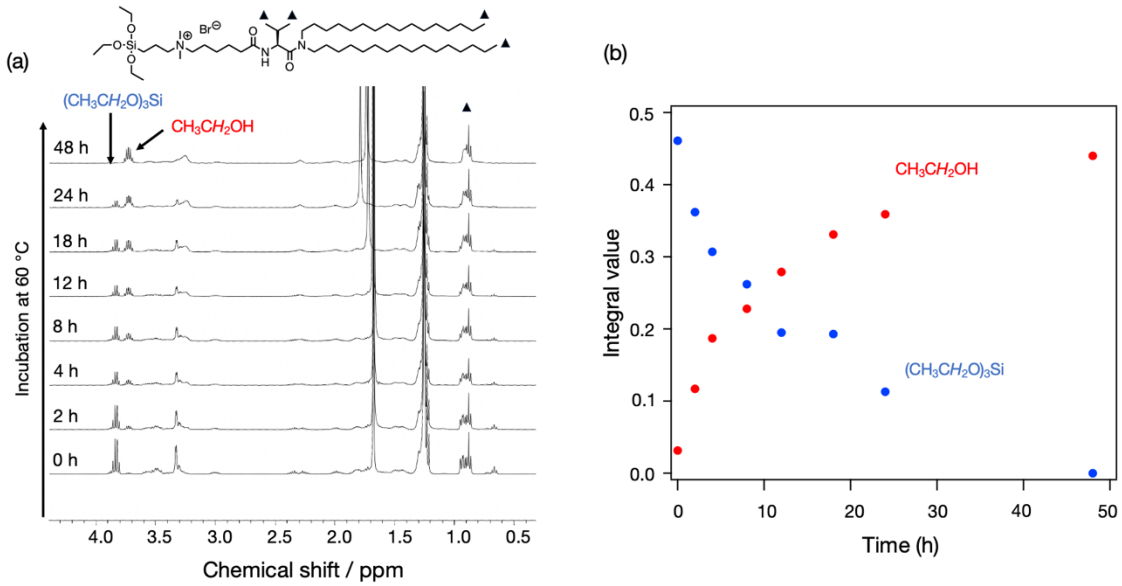


Fig. S10 (a) ^{1}H NMR (400 MHz, $CDCl_3$, 293 K) spectra of the lipid-water mixture ([**1**] = 90 w/w%) acquired after the high-temperature incubation for a certain time as indicated. (b) Time course change in the integral values of methylene (CH_2) signals at 3.83 ppm and 3.73 ppm assigned as triethoxysilyl group ($(CH_3CH_2O)_3Si$) and ethanol (CH_3CH_2OH). The integral values were evaluated by taking the sum of the integral values of peaks at 0.95–0.85 ppm as 1.00 as a standard which are assigned as the CH_3 groups of the hexadecyl chains and isopropyl group of **1** and its derivatives (labelled with filled triangles in the panel a).

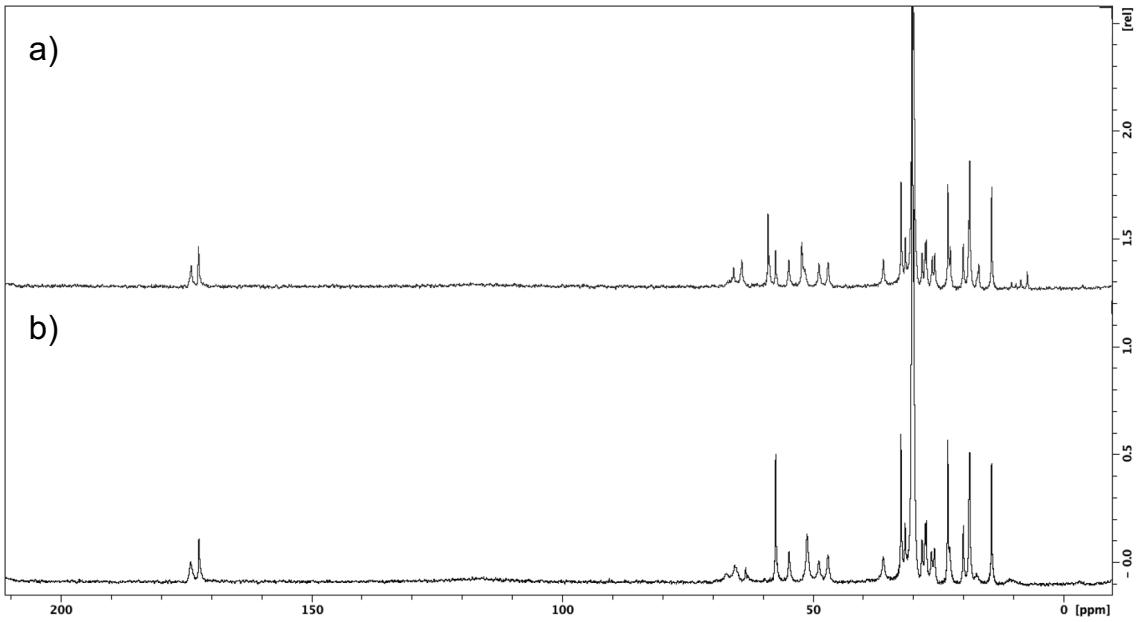


Fig. S11 ^{13}C solid-state MAS NMR spectra of the lipid-water mixture ([**1**] = 90 w/w%). The spectra were acquired at 1 hour (a) and 6 days (b) after the sample preparation.

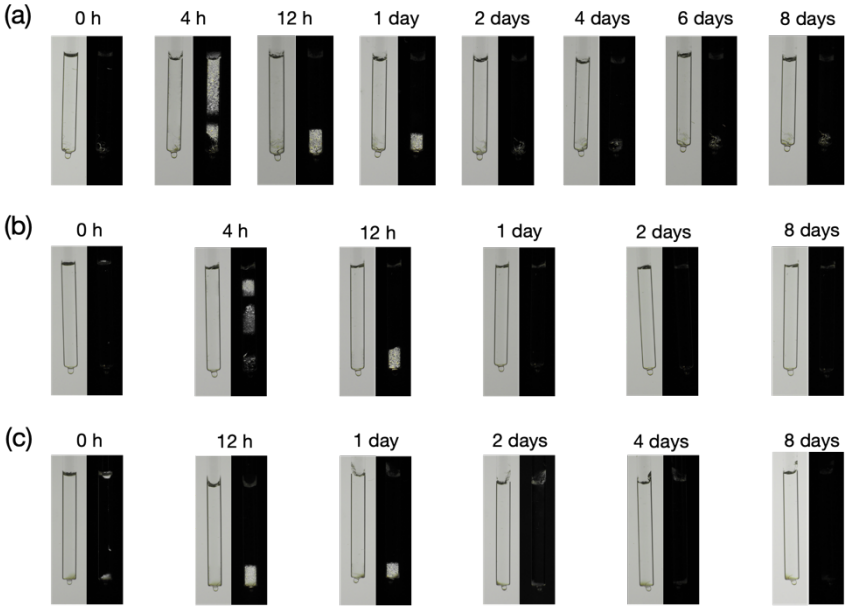


Fig. S12 Time courses of the POM image traces of the lipid-water mixtures ([**1**] = 90 w/w%) in sealed capillaries during high-temperature incubation (recorded at 60 °C). The left images are bright field images of the samples without polarizer and the dark images on the right are crossed-Nicole images of the samples recorded at a certain incubation time as indicated. Three different samples (a–c) were observed independently.

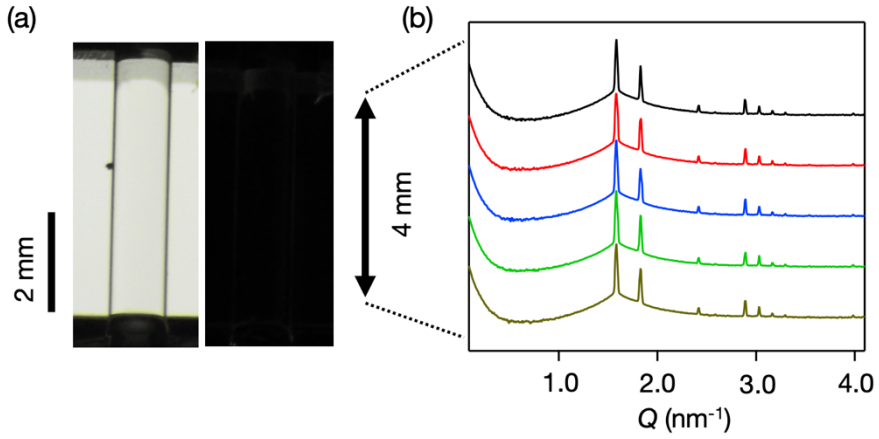


Fig. S13 (a) POM image of the lipid-water mixture ([**1**] = 90 w/w%) in a sealed capillary after high-temperature incubation for 6 days (recorded at 60 °C). The left is a bright field image of the sample without a polarizer and the right is a crossed-Nicole image of the sample. (b) SAXS patterns of the lipid-water mixture ([**1**] = 90 w/w%) in a sealed capillary acquired after the high-temperature incubation for 6 days (recorded at 60 °C). X-ray was irradiated at different locations on the sample shown in panel (a).

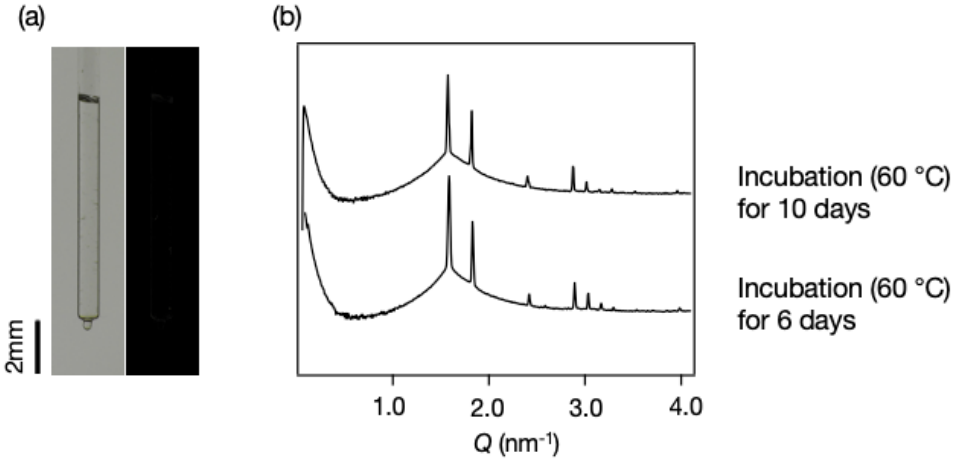


Fig. S14 (a) POM image of the lipid-water mixture ([**1**] = 90 w/w%) in a sealed capillary after high-temperature incubation for 10 days (recorded at 60 °C). The left is a bright field image of the sample without a polarizer and the right is a crossed-Nicole image of the sample. (b) SAXS patterns of the lipid-water mixture ([**1**] = 90 w/w%) in a sealed capillary acquired after the high-temperature incubation for 10 days (top) and 6 days (bottom) (recorded at 60 °C).

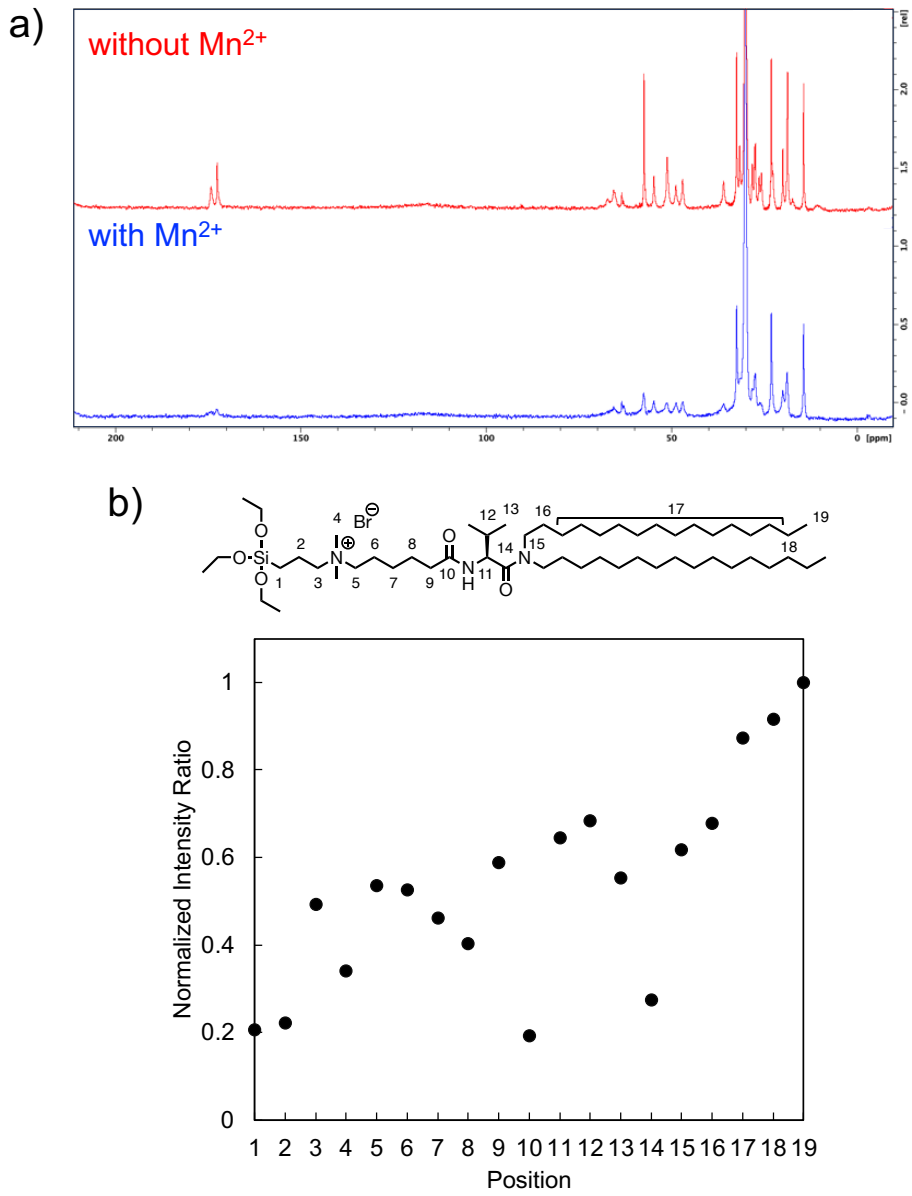


Fig. S15 (a)^{13}C MAS NMR spectra of the lipid-water mixture ([**1**] = 90 w/w%) in the absence and presence of paramagnetic Mn^{2+} ion. The Mn^{2+}-free spectrum is the same as shown in Fig. S11. The spectrum in the presence of Mn^{2+} was acquired 5 days after the addition of aqueous $MnCl_2$ to the sample, which had been incubated for 6 days prior to the addition. (b) Carbon position-dependent effect of Mn^{2+} ion on the peak intensity. Normalized Intensity Ratio is defined as {(intensity of each peak in the presence of Mn^{2+}) / (intensity of each peak in the absence of Mn^{2+})} / {(intensity of the peak at position 19 in the presence of Mn^{2+}) / (intensity of the peak at position 19 in the absence of Mn^{2+})}. Marked low intensities for carbonyl carbons (positions 10 and 14) suggest the interaction of Mn^{2+} with amide groups.

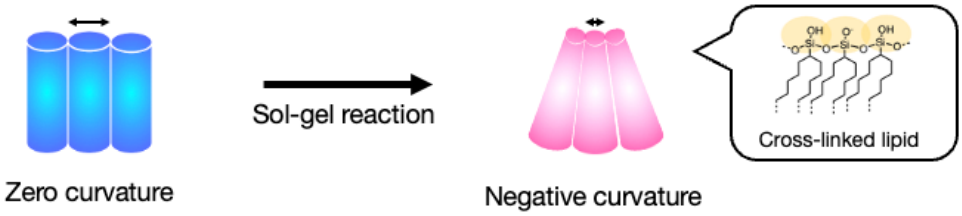


Fig. S16 Schematic image explaining the plausible mechanism of lipid cubic phase formation by sol-gel reaction. Hydrolysis and cross-linking of the lipid headgroup decreases the apparent cross-sectional area of the lipid head group whereas the volume of the hydrophobic chain was maintained, resulting in the negative spontaneous curvature of lipid assembly.

4. Reference

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