

Electronic Supplementary Information (ESI)

Microtubes self-assembled from a cholesterol-modified nucleoside

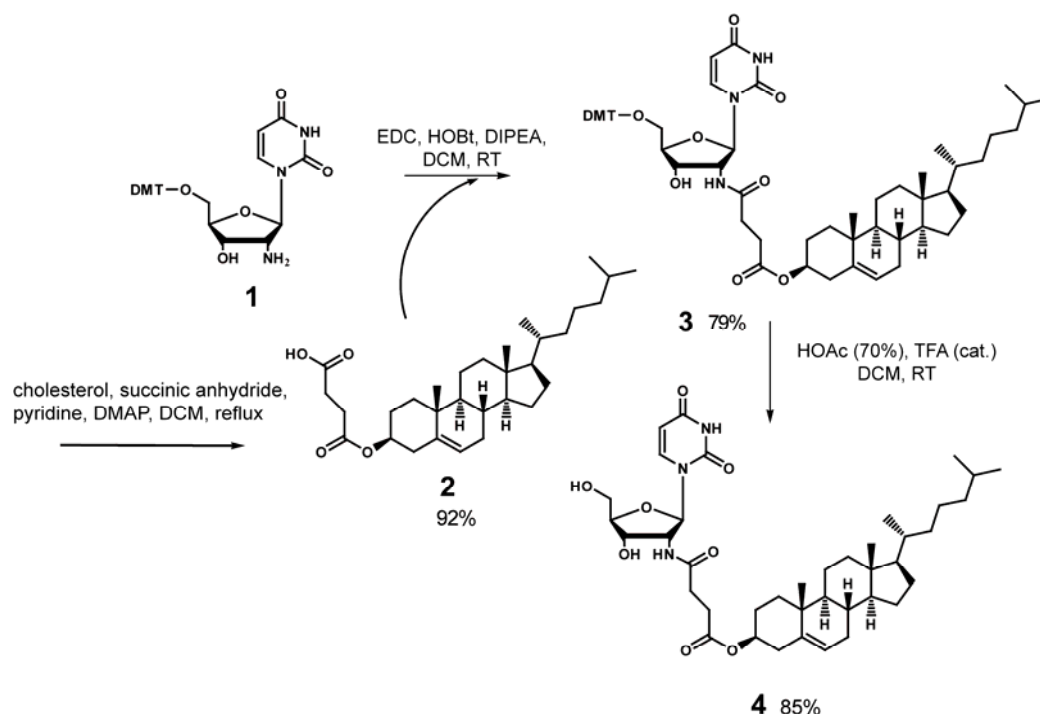
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1) Materials

DOPC (1,2-dioleoyl-*sn*-glycero-3-phosphocholine), NBD-PE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl), ammonium salt) and POPC-*d*₃₁ (1-palmitoyl-*d*₃₁-2-oleoyl-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids (Alabaster, AL). All materials and reagents for synthesis were obtained from commercial suppliers and used without further purification.

2) Synthesis of 2'-N-(2-(cholesteryl)-succinyl)-2'-desoxy-2'-aminouridine

The precursor of the synthesis is 2'-desoxy-2'-aminouridine **1**, which was prepared according to a procedure reported in the literature.¹ Succinylcholesterol **2** was prepared from cholesterol and succinic anhydride in 92% yield. Coupling of **2** with the 2'-amino function of 5'-DMT-protected 2'-dehydro-2'-aminouridine **1** was achieved by a standard protocol of peptide coupling (EDC / HOBt) leading to a 79% yield of **3**. Acidic deprotection provided the final product **4** (85%).



Scheme S1. Synthesis scheme for 2'-N-(2-(cholesteryl)-succinyl)-2'-desoxy-2'-aminouridine (Note: in the manuscript the final product is referred to as compound **1**).

2.1) O-Succinyl-cholesterol (2): Cholesterol (2.0 g, 5.2 mmol), succinic acid anhydride (1.17 g, 11.7 mmol) and DMAP (98 mg, 0.8 mmol) were suspended in a mixture of dry dichloromethane (24 ml) and dry pyridine (960 μ l, 11.9 mmol). The reaction mixture was set under argon and refluxed for 3 days. After cooling to room temperature toluene (20 ml) was added. The solvent was removed and the residue was taken up in of DCM (200 ml). The organic phase was washed with 0.25 N aqueous HCl (128 ml) and water (200 ml). After drying over MgSO_4 the solvent was evaporated. The resulting residue was dried under vacuum (2 mbar, 40 $^{\circ}\text{C}$) to obtain compound **2** (2.33 g, 4.79 mmol) as a colorless solid (yield: 92%).

$\text{C}_{31}\text{H}_{50}\text{O}_4$, MW: 486.73

DC: R_f = 0.3 (DCM/MeOH, 30:1);

M.p. 178–180 $^{\circ}\text{C}$.

$^1\text{H-NMR}$ (300 MHz, CDCl_3): δ = 0.67 (s, 3H, $-\text{CH}_3$), 0.80 - 1.62 (m, 33H, $-\text{CH}_2-$, $-\text{CH}_2-$, $-\text{CH}_3$), 1.74 - 2.08 (m, 5H, $-\text{CH}_2-$, $-\text{CH}_2-$), 2.31 (d, $J=7.74$ Hz, 2H, $-\text{C}=\text{C}-\text{CH}_2-$), 2.54 - 2.74 (m, 4H, $-(\text{C}=\text{O})-\text{CH}_2-\text{CH}_2-(\text{C}=\text{O})-$), 4.54 - 4.71 (m, 1H, $-(\text{C}=\text{O})-\text{O}-\text{CH}_2-$), 5.37 (d, $J=3.97$ Hz, 1H, $-\text{C}=\text{C}-\text{H}$) ppm.

$^{13}\text{C-NMR}$ (75.5 MHz, CDCl_3): δ = 11.8 ($-\text{CH}_3$), 18.7 ($-\text{CH}_3$), 19.3 ($-\text{CH}_3$), 21.0 ($-\text{CH}_2-$), 22.5 ($-\text{CH}_3$), 22.8 ($-\text{CH}_3$), 23.8 ($-\text{CH}_2-$), 24.3 ($-\text{CH}_2-$), 27.6 ($-\text{CH}_2-$), 28.0 ($-\text{CH}_2-$), 28.2 ($-\text{CH}_2-$), 29.0 ($-\text{CH}_2-(\text{C}=\text{O})-$), 29.2 ($-\text{CH}_2-(\text{C}=\text{O})-$), 31.8 ($-\text{CH}_2-$), 31.9 ($-\text{CH}_2-$), 35.8 ($-\text{CH}_2-$), 36.2 ($-\text{CH}_2-$), 36.5 ($-\text{CH}_2-$), 36.9 ($-\text{CH}_2-$), 38.0 ($-\text{CH}_2-$), 39.5 ($-\text{CH}_2-$), 39.7 ($-\text{CH}_2-$), 42.3 (Cq), 50.0 ($-\text{CH}_2-$), 56.1 ($-\text{CH}_2-$), 56.6 ($-\text{CH}_2-$), 74.5 ($-\text{CH}-\text{O}-(\text{C}=\text{O})-$), 122.7 ($-\text{CH}=\text{C}-$), 139.5 ($-\text{CH}=\text{C}-$), 171.5 ($-(\text{C}=\text{O})-\text{O}-$), 178.2 ($-(\text{C}=\text{O})-\text{O}-$) ppm.

2.2) 2'-N-(Cholesteryl-succinyl)-2'-deoxy-2'-amino-O-5'-(4,4'-dimethoxytrityl)-uridine (3): The 2'-deoxy-2'-aminouridine **1** (500 mg, 0.9 mmol) and the cholesterol derivative **2** (390 mg, 0.8 mmol) were dissolved in dry DCM (10 ml). EDC (180 μ l, 1.0 mmol), HOBT (185 mg, 1.2 mmol) and DIPEA (160 μ l, 0.9 mmol) were added under stirring. The resulting yellowish mixture was stirred at room temperature for 24 h. The solvent was removed and the residue purified by column chromatography (silica; DCM/MeOH/ Et_3N , 15:1:0.01; R_f = 0.3) giving product **3** (637 mg, 0.63 mmol) as colorless wax in 79% yield.

$\text{C}_{61}\text{H}_{79}\text{N}_3\text{O}_{10}$, MW: 1014.29

M.p. 136–139 $^{\circ}\text{C}$.

HRMS: calculated for $\text{C}_{61}\text{H}_{80}\text{N}_3\text{O}_{10}^+$ 1014.5838; found 1014.5841

$^1\text{H-NMR}$ (300 MHz, CDCl_3): δ = 0.56 (s, 3H, $-\text{CH}_3$), 0.61 - 1.57 (m, 33H, $-\text{CH}_2-$, $-\text{CH}_2-$, $-\text{CH}_3$), 1.59 - 1.96 (m, 5H, $-\text{CH}_2-$, $-\text{CH}_2-$), 2.16 (d, $J=7.55$ Hz, 2H, $-\text{C}=\text{C}-\text{CH}_2-$), 2.30 - 2.66 (m, 4H, $-(\text{C}=\text{O})-\text{CH}_2-\text{CH}_2-(\text{C}=\text{O})-$), 3.13 - 3.36 (m, 2H, CH_2-5'), 3.64 (s, 6H, $-\text{O}-\text{CH}_3$), 3.98 - 4.15 (m, 1H, $\text{CH}-4'$), 4.23 - 4.33 (m, 1H, $\text{CH}-3'$), 4.38 - 4.49 (m, 1H, $-(\text{C}=\text{O})-\text{O}-\text{CH}_2-$), 4.54 - 4.70 (m, 1H, $\text{CH}-2'$), 5.16 - 5.22 (m, 1H, $-\text{C}=\text{C}-\text{H}$), 5.28 (d, $J=8.12$ Hz, 1H, $\text{CH}-5$), 5.99 (d, $J=8.50$ Hz, 1H, $\text{CH}-1'$), 6.71 (d, $J=9.06$ Hz, 4H, CHar), 7.02 - 7.35 (m, 9H, CHar), 7.50 (d, $J=8.12$ Hz, 1H, $\text{CH}-6$) ppm.

$^{13}\text{C-NMR}$ (75.5 MHz, CDCl_3): δ = 11.2 ($-\text{CH}_3$), 18.1 ($-\text{CH}_3$), 18.7 ($-\text{CH}_3$), 20.4 ($-\text{CH}_2-$), 21.9 ($-\text{CH}_3$), 22.2 ($-\text{CH}_3$), 23.3 ($-\text{CH}_2-$), 23.7 ($-\text{CH}_2-$), 27.1 ($-\text{CH}_2-$), 27.4 ($-\text{CH}_2-$), 27.7 ($-\text{CH}_2-$), 29.2 ($-\text{CH}_2-(\text{C}=\text{O})-$), 30.0 ($-\text{CH}_2-(\text{C}=\text{O})-$), 31.2 ($-\text{CH}_2-$), 31.3 ($-\text{CH}_2-$), 35.2 ($-\text{CH}_2-$), 35.6 ($-\text{CH}_2-$), 36.0 ($-\text{CH}_2-$), 36.4 ($-\text{CH}_2-$), 37.4 ($-\text{CH}_2-$), 38.9 ($-\text{CH}_2-$), 39.2 ($-\text{CH}_2-$), 41.7 (Cq), 49.4 ($-\text{CH}_2-$), 54.6 ($-\text{O}-\text{CH}_3$), 55.6 ($\text{CH}-2'$), 55.7 ($-\text{CH}_2-$), 56.1 ($-\text{CH}_2-$), 63.3 (CH_2-5'), 70.7 ($\text{CH}-3'$), 73.8 ($-\text{CH}-\text{O}-(\text{C}=\text{O})-$), 84.6 ($\text{CH}-4'$), 85.0 ($\text{CH}-1'$), 86.3 (Cq), 102.3 ($\text{CH}-5$), 112.7 (CHar), 122.0 ($-\text{CH}=\text{C}-$), 126.5 (CHar), 127.5 (CHar), 127.6 (CHar), 129.6 (CHar), 134.6 (Car), 134.8 (Car), 139.1 ($-\text{CH}=\text{C}-$), 139.6 ($\text{CH}-6$), 143.8 (Car), 150.9 ($-(\text{C}=\text{O})-$), 158.1 (Car), 162.9 ($-(\text{C}=\text{O})-$), 172.2 ($-(\text{C}=\text{O})-\text{N}-$), 172.3 ($-(\text{C}=\text{O})-\text{O}-$) ppm.

2.3) 2'-N-(Cholesteryl-succinyl)-2'-deoxy-2'-amino-uridine (4): To a solution of compound **3** (637 mg, 0.63 mmol) in DCM (3.5 ml) 70 % HOAc (1.1 ml) and TFA (158 μ l) were added under stirring. The red emulsion was stirred at room temperature for 30 min and then neutralized with saturated NaHCO₃ solution (2 ml). The solvent was removed under vacuum and toluene (10 ml) was added. The solvent was removed again and the resulting residue was purified by column chromatography (silica; DCM/MeOH, 15:1; R_f = 0.4) providing the final product **4** (382 mg, 0.54 mmol) as colorless solid (yield: 85%).

C₄₀H₆₁N₃O₈, MW: 711.93

M.p. 234 °C.

HRMS: calculated for C₄₀H₆₂N₃O₈⁺ 712.4531; found 712.4538

¹H-NMR (300 MHz, CD₃OD/CDCl₃, 1:2): δ = 0.46 (s, 3H, -CH₃), 0.54 - 1.47 (m, 33H, -CH-, -CH₂-, -CH₃), 1.49 - 1.89 (m, 5H, -CH-, -CH₂-), 1.99 - 2.16 (m, 2H, -C=C-CH₂-), 2.18 - 2.53 (m, 4H, -(C=O)-CH₂-CH₂-(C=O)-), 3.45 - 3.71 (m, 2H, CH₂-5'), 3.79 - 3.94 (m, 1H, CH-4'), 4.00 - 4.12 (m, 1H, CH-3'), 4.26 - 4.41 (m, 1H, -(C=O)-O-CH-), 5.01 - 5.23 (m, 1H, CH-2'), 5.36 - 5.60 (m, 1H, CH-5), 5.73 - 5.89 (m, 1H, CH-1'), 7.61 - 7.80 (m, 1H, CH-6) ppm.

¹³C-NMR (75.5 MHz, CD₃OD/CDCl₃, 1:2): δ = 11.3 (-CH₃), 18.2 (-CH₃), 18.7 (-CH₃), 20.6 (-CH₂-), 21.9 (-CH₃), 22.2 (-CH₃), 23.4 (-CH₂-), 23.8 (-CH₂-), 27.2 (-CH), 27.5 (-CH₂-), 27.8 (-CH₂-), 29.1 (-CH₂-(C=O)-), 29.8 (-CH₂-(C=O)-), 31.4 (-CH-), 31.5 (-CH₂-), 35.4 (-CH-), 35.7 (-CH₂-), 36.1 (-CH₂-), 36.5 (-CH₂-), 37.5 (-CH₂-), 39.1 (-CH₂-), 39.3 (-CH₂-), 41.9 (Cq), 49.6 (-CH-), 55.7 (CH-2'), 56.3 (-CH-), 61.8 (CH₂-5'), 70.5 (CH-3'), 74.3 (-CH-O-(C=O)-), 86.4 (CH-4'), 86.6 (CH-1'), 102.3 (CH-5), 122.3 (-CH=C-), 139.1 (-CH=C-), 140.8 (CH-6), 151.0 (-(C=O)-), 164.0 (-(C=O)-), 172.5 (-(C=O)-N-), 172.8 (-(C=O)-O-) ppm.

3) Sample preparation

Mixtures of **4** and DOPC were prepared in chloroform/methanol (1/1 v/v). After removing the solvent by rotary evaporation, the lipid film was suspended in ultrapure water at a total lipid concentration of 7 mg/ml and stirred for 30 minutes to obtain multilamellar vesicles (MLVs). The samples were then dried (either by vacuum centrifugation or by freeze-drying) and resuspended in buffer (10 mM HEPES, pH 7.2) at a concentration of 0.9 mg/ml. The lipid suspensions were heated at a rate of approx. 5°C/min using a thermomixer incubator (Eppendorf, Germany) and held for 30 minutes at the final temperature. Finally, the samples were cooled down to room temperature at approximately 2°C/min. When the dry films were directly hydrated with buffer at the final concentration and then heated without the lyophilization step, the formation of tubular structures was also observed; however a significant fraction of the lipid mixture remained attached to the glass surface. Introducing the lyophilization step allowed to detach the lipids (especially the cholesteryl-uridine conjugate) from the glass surface, and also led to a higher yield of tubes. To eliminate the remaining vesicles and obtain homogeneous microtube preparations, 0.16% Triton X-100 was added to the samples, followed by short mixing. The microtubes were then washed two times in HEPES buffer and collected by centrifugation at 4°C (600 rcf, 15 min).

4) Confocal laser scanning microscopy

Images were acquired with an inverted CLSM (LSM 510Meta, Carl Zeiss MicroImaging GmbH, Jena, Germany). Imaging of the NBD-PE labeled microtubes was carried out using the 488 nm line of an Ar⁺ laser with a dichroic mirror (reflective below 488 nm), a 505 nm long pass emission filter and a C-Apochromat 63 $\times$ /1.2 water immersion objective (Zeiss).

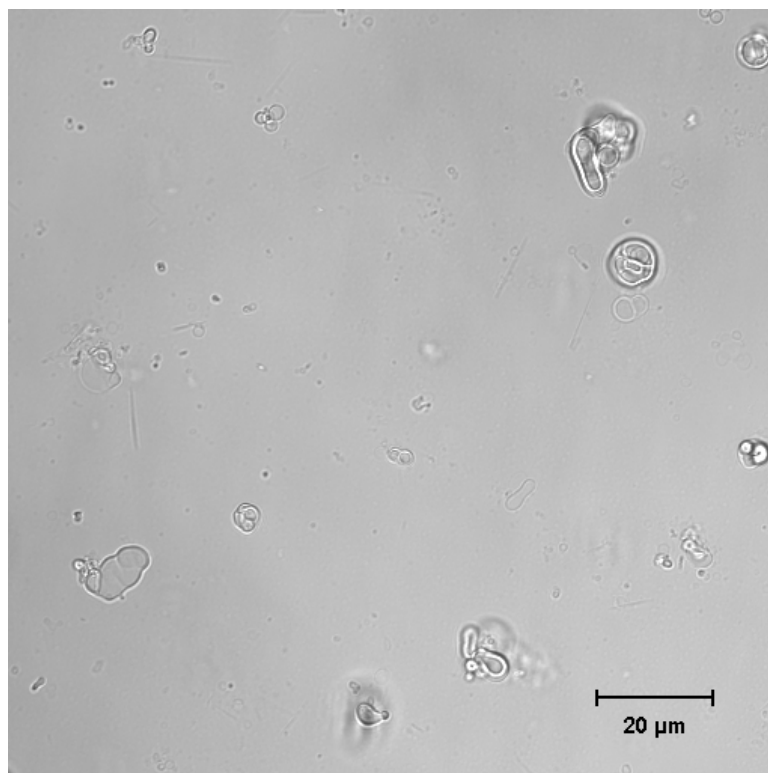


Figure S1. Differential interference contrast image of a sample after hydration of the lipid film at 50 °C and subsequent cooling to room temperature. Under these conditions, a heterogeneous mixture of vesicles and filament-like structures was found in the samples, but no tube formation was observed.

5) Electron microscopy

5.1) Scanning electron microscopy (SEM)

For the direct visualization of tubules in the scanning electron microscope a drop (5 μ l) of a tubules suspension was dropped onto the glass and air dried. The samples were sputter-coated for 7 seconds with gold-palladium (4-5 nm layer of Au-Pd, Polaron Sputter Coating Unit E 5100, GaLa Instrumente, Bad Schwalbach). The samples were examined using a LEO 1530 Scanning Electron Microscope (Carl Zeiss SMT AG, Oberkochen) operated at 3 kV.

5.2) Negative staining transmission electron microscopy (TEM)

For the direct visualization of tubules in the transmission electron microscope a drop (3 μ l) of a tubule suspension was added to a filmed grid surface. After 10 min incubation at room temperature excess suspension was removed by blotting with a filter paper and a drop of 0.25% aqueous uranyl acetate was added onto the grid, subsequently the grid was blotted dry. Negatively stained² tubules were visualized with an EM 902 (ZEISS, Germany) at 80 kV and images were taken with a slow-scan CCD camera (Proscan, Germany).

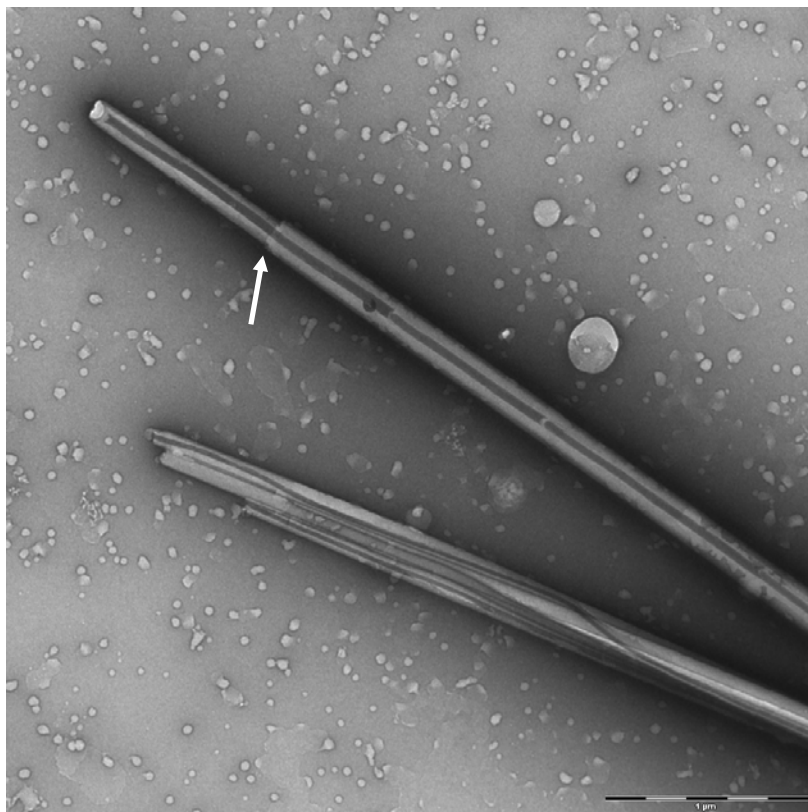


Figure S2. Negative staining TEM image of a sample containing nanometer-diameter tubules. Scale bar is 1 μm. One thin straight rod with an outer diameter of about 200 nm is filled with the staining solution, indicating that the structure is an open tubule with an inner diameter of about 60 nm. The white arrow indicates an edge of an extra layer rolled on the tubule.

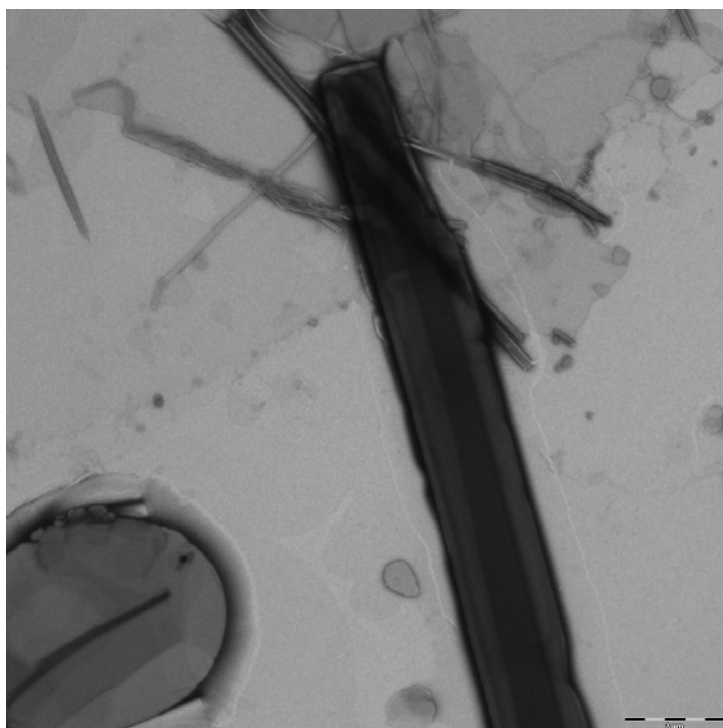


Figure S3. Negative staining TEM image of a tubule sample. Scale bar is 2 μm. One straight rod with an outer diameter of about 2 μm is surrounded by thinner tubules.

6) NMR experiments

6.1) Solid-state NMR

Microtube samples were prepared as described above. The composition of the initial mixture was 60:30:10 mol % DOPC:4:POPC- d_{31} . For control measurements with MLVs, a 60:30:10 mol% DOPC:4:POPC- d_{31} mixture was prepared in 1/1 v/v chloroform/methanol. After evaporating the solvent, the lipid film was resolved in cyclohexane and lyophilized overnight. The obtained fluffy powder was rehydrated with 50 wt% deuterium-depleted water, homogenized by several freeze-thaw cycles and finally transferred into 4 mm MAS rotors for NMR measurements. Static ^2H NMR spectra were measured on a Bruker Avance 750 MHz NMR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany) operating at a resonance frequency of 115.1 MHz for ^2H . A single-channel solids probe equipped with a 5 mm solenoid coil was used. The ^2H NMR spectra were accumulated with a spectral width of ± 250 kHz using quadrature detection and a phase-cycled quadrupolar echo sequence.³ The length of a 90° pulse was 2.9 μs , and a relaxation delay of 1 s was applied. The measurements were conducted at a temperature of 303 K. The ^2H NMR spectra were dePaked⁴ using the algorithm of McCabe and Wassall⁵ and the order parameter profiles of the acyl chains were determined by a numerical spectral fitting procedure from the observed quadrupolar splitting $\Delta\nu_Q(n)$:

$$\Delta\nu_Q(n) = \frac{3}{4} \frac{e^2 q Q}{h} S(n), \quad (\text{S2})$$

where $e^2 q Q/h$ is the quadrupolar-coupling constant (167 kHz for ^2H in a C- ^2H bond) and $S(n)$ the chain order parameter for the n^{th} carbon position in the chain.

Static ^{31}P NMR spectra were acquired on a Bruker DRX 600 NMR spectrometer operating at a resonance frequency of 242.8 MHz for ^{31}P using a Hahn-echo pulse sequence. A ^{31}P 90° pulse length of 9.8 μs , a Hahn-echo delay of 50 μs , a spectral width of 100 kHz, and a recycle delay of 2.5 s were used. Continuous-wave low power proton decoupling was applied during signal acquisition. All measurements were conducted at a temperature of 303 K.

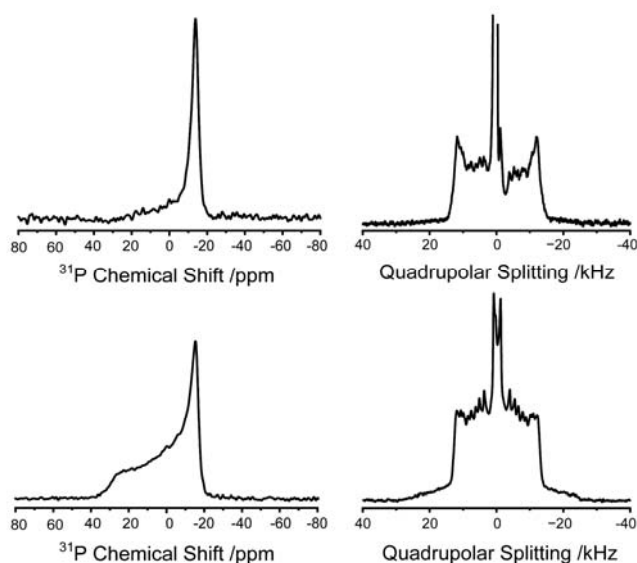


Figure S4. ^{31}P (left) and ^2H (right) spectra obtained from 4:DOPC microtubes (upper curves). The typical powder spectra for multilamellar vesicles prepared from the same material are shown for comparison (lower curves). The samples for ^2H NMR contained 10 mol% *sn*-1 chain perdeuterated POPC- d_{31} .

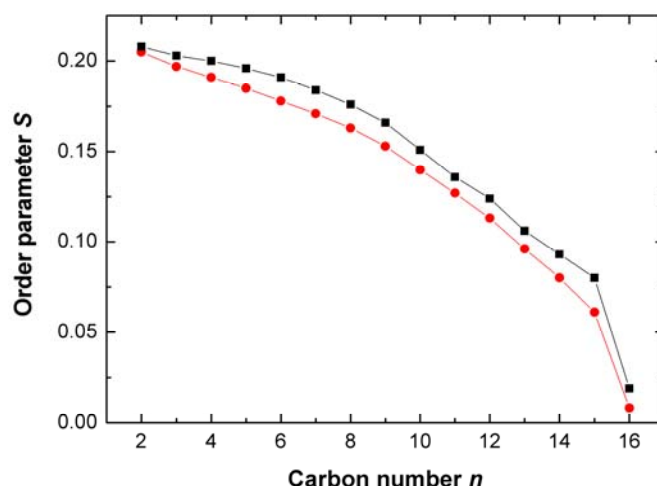


Figure S5. ^2H NMR order parameter plot for multilamellar vesicles (squares) and microtubes (circles) made of 2'-N-(2-(cholesteryl)-succinyl)-2'-desoxy-2'-aminouridine/DOPC/POPC- d_{31} . Order parameters were determined from the ^2H NMR spectra shown in Fig. S4.

5.2) High-resolution NMR

^1H high resolution NMR spectra of chloroform- d solutions of 4:DOPC microtubes and of pure DOPC vesicles (control sample) were measured on a Bruker DRX 600 NMR spectrometer with a 5 mm standard probe head. The 90° pulse length was 9.4 μs .

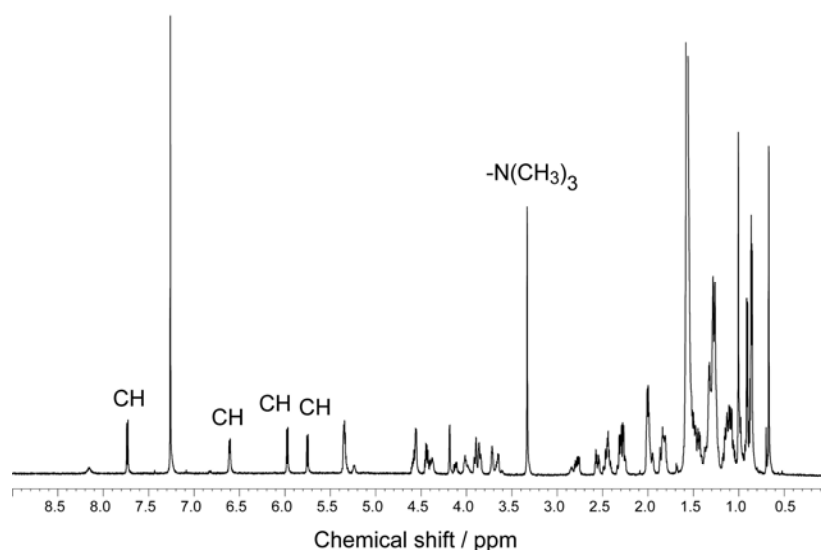


Figure S6. High resolution NMR spectrum of a typical microtube sample. The chemical composition of the microtubes was calculated from the integration of the labeled peaks. CH refers to single hydrogens from either the nucleobase or the deoxyribose; $-\text{N}(\text{CH}_3)_3$ refers to the choline headgroup from DOPC and comprises 9 hydrogens. From the peak ratio, the true composition of the sample could be determined.

References

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