Vesicle Formation from Reactive Surfactants

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Synthesis

Reagents and solvents were from Fluka, Aldrich and Sigma.

The syntheses of \(N\)-\(N\) dimethyldodecylammonium bromide 3a (\(B=12\)) and \(N\)-(2-bromoethyl)-\(N\)-\(N\) dimethyltetradecylammonium bromide 3b (\(B=14\)) have been described.\(^{[81]}\)

\[ \text{H}_2\text{C}-(\text{CH}_2)_n \text{OH} \xrightarrow{\text{PSCl}_3} \text{H}_2\text{C}-(\text{CH}_2)_n \text{OPSCl}_2 \]

\((n=11), 4a\)  ; \((n=13), 4b\)  

Dodecyl- and tetradecylthiophosphoric dichloride were prepared from thiophosphoryl chloride and the corresponding alcohol following the procedure of Dvolaitzky and Guedeau.\(^{[82]}\)

**Dodecylthiophosphoric dichloride (4a):** To a stirred solution of thiophosphoryl chloride (6.77 g, 40 mmol) in 5 ml of hexane at 5°C was added triethylamine (4.04 g, 40 mmol) dissolved in 25 ml of trichloroethylen. Dodecanol (5 g, 27 mmol) in 40 ml of trichloroethylen was added dropwise over 50 min. The mixture was warmed to room temperature, and stirring was continued for 1 hour. The precipitated triethylammonium chloride was removed and 25 ml toluene added. The solvents were removed under reduced pressure. The residue was taken up in toluene and the solution was filtered. The solvent was evaporated to obtain crude 4a as an oil. Purification by flash chromatography (hexane) gave 5.24 g (61 %) of a colourless oil.

\[ \text{H}_2\text{C}-(\text{CH}_2)_n \text{OH} \xrightarrow{\text{NaOAc/NEt}_3} \text{H}_2\text{C}-(\text{CH}_2)_n \text{OPO}_2\text{S}_2- \]

\((n=11), 2a\)  ; \((n=13), 2b\)

1H-NMR (500 MHz, CDCl_3): 0.81 (t, 3H, CH_3), 1.19 (s, 16H, (CH_2)_n), 1.34 (m, 2H, CH_2), 1.77 (m, 2H, CH_2), 2.26 (m, 2H, CH_2), 4.26 (m, 2H, CH_2)

13C-NMR (125 MHz, CDCl_3): 14.11 (CH_3), 22.68 (CH_2), 25.33 (CH_2), 28.97 (CH_2), 29.34 (CH_2), 29.38 29.40 (CH_2), 29.47 (CH_2), 29.50 (CH_2), 29.55(CH_2), 29.60 (CH_2), 31.90 (CH_2), 72.58 (d, CH_2)

31P-NMR (202 MHz, CDCl_3): 59.5 (s).

**Tetradecylthiophosphoric dichloride (4b):** This compound was prepared similarly to 4a from tetradecanol (5 g, 23 mmol). Purification by flash chromatography (hexane) gave 4.54 g (57 %) of a colourless oil.

1H-NMR (500 MHz, CDCl_3): 0.86 (t, 3H, CH_3), 1.24 (s, 20H, (CH_2)_10), 1.40 (m, 2H, CH_2), 1.77 (m, 2H, CH_2), 4.31 (m, 2H, CH_2)

13C-NMR (125 MHz, CDCl_3): 14.11 (CH_3), 22.68 (CH_2), 25.34 (CH_2), 28.98 (CH_2), 29.33 (CH_2), 29.38 (CH_2), 29.47 (CH_2), 29.50 (CH_2), 29.54 (CH_2), 29.59 (CH_2), 29.63 (CH_2), 29.66 (CH_2), 31.91 (CH_2), 72.57 (d, CH_2)

31P-NMR (202 MHz, CDCl_3): 59.5 (s).

Alkylthiophosphoric dichloride 4 were hydrolyzed to the corresponding alkylphosphorothioatoes 2 following the procedure of Bäuerlein and Gaugler.\(^{[83]}\)

**Dodecylphosphorothioato (2a):**

Dodecylthiophosphoric dichloride 4a (1g, 3.1 mmol) was emulsified of sodium acetate (1.12 g, 13.6 mmol) in 10 ml water at 0°C. Under vigorous stirring triethylamine (1.38 g, 13.6 mmol) was added dropwise and the mixture was stirred for 1 hour below 5°C. The reaction mixture was then allowd to warm up to room temperature and neutralized with dilute hydrochloric acid. Barium chloride (910 mg, 3.7 mmol) dissolved in 6 ml H_2O was added to precipitate the dodecylphosphorothioato. The precipitate was filtered and washed with water and acetone, and then resuspended into 3 ml of water. Sodium sulfate (500 mg, 3.5 mmol) was added, the precipitated barium sulfate was filtered off, and the remaining solution freeze-dried. The crude product was finally purified by chromatography over silica gel and eluted with 2-propanol/NH_3OH/water (7:1:1 v/v/v) to obtain 595 mg (61 %) of the diammonium salt.
1H-NMR (500 MHz, DMSO-d6): 0.84 (s, 3H, CH3), 1.23 (s, 18H, (CH2)18), 1.46 (s, 2H, CH2), 3.65 (q, 2H, CH2-OH).
13C-NMR (125 MHz, DMSO-d6): 13.93 (CH3), 22.08 (CH2), 25.54 (CH2), 28.71 (CH2), 28.92 (CH2), 29.02 (CH2), 29.07 ((CH2)9), 29.10 (CH2), 30.25 (CH2), 31.28 (CH2), 63.75 (d, CH2-O).
P-NMR (202 MHz, DMSO-d6): 51.54 (s).
HRMS (ESI, neg.): 281.1347 (C12H26O2PS; calc. 281.1346)

**Tetradecylphosphorothioate (2b):**
This substance was prepared similarly to 2a from tetradecylthiophosphoric dichloride 4b (1g, 2.9 mmol). Purification by flash chromatography (2-propanol/NH4OH/water (7:1:1 v/v/v)) gave 540 mg (54 %) of the dication salt.

1H-NMR (500 MHz, DMSO-d6): 0.84 (s, 3H, CH3), 1.23 (s, 18H, (CH2)18), 1.46 (s, 2H, CH2), 3.64 (q, 2H, CH2-O).
13C-NMR (125 MHz, DMSO-d6): 13.91 (CH3), 22.06 (CH2), 25.54 (CH2), 28.68 (CH2), 28.91 (CH2), 28.99 (CH2), 29.05 ((CH2)9), 29.09 (CH2), 30.24 (CH2), 31.27 (CH2), 63.73 (d, CH2-O).
P-NMR (202 MHz, DMSO-d6): 51.47 (s).
HRMS (ESI, neg.): 309.1654 (C14H28O2PS; calc. 309.1659)

**S-2-(dimethyl(dodecyl)ammonio)ethyl O-dodecyl phosphorothioate 1a:**
Dodecylphosphorothioate 2a (80 mg, 0.25 mmol) and N-(2-bromoethyl)-N,N-dimethyl dodecylammonium bromide (100 mg, 0.25 mmol) were dissolved each in 10 ml sodium bicarbonate (0.1 M). The solutions were transferred into syringes and continuously mixed over 2 hours with the help of a syringe pump into 2 ml sodium bicarbonate (0.1 M). After complete mixing, the resulting suspension was stirred for one hour, saturated with NaCl and extracted with ethyl acetate (3x20 ml). The organic phase was dried over MgSO4, filtered and the solvent evaporated under reduced pressure. The residue was taken up in 4 ml chloroform, filtered and the solvent was removed under reduced pressure. The residue was recrystallized from acetonitrile. Yield 121 mg (93%).

M.p. 192-193°C
Anal. found: C, 64.38; H, 11.73; N, 2.56; Calcd for C28H60NO2PS: C, 64.45; H, 11.59; N, 2.68.
HRMS (MALDI): 522.4110 (calc. 522.4104 (MH+))
1H-NMR (500 MHz, CDCl3): 0.85 (t, 6H, 2xCH3), 1.18-1.37 (b, 36H, 18xCH2), 1.58 (m, 2H, CH2), 1.71 (b, 2H, CH2), 2.90-3.03 (m, 4H, 2xCH2), 3.21 (s, 6H, 2xCH2N+), 3.27 (m, 2H, CH2), 3.80-3.90 (m, 2H, CH2)
13C-NMR (125 MHz, CDCl3): 14.07, 22.65, 22.71, 25.98, 26.37, 29.19, 29.34, 29.45, 29.49, 29.60, 29.65, 29.69, 30.76, 30.82, 31.9, 51.14, 64.55, 65.01, 65.60, 65.66
P-NMR (202 MHz, CDCl3): 14.68

**S-2-(dimethyl(tetradecyl)ammonio)ethyl O-tetradecyl phosphorothioate 1b:**
This molecule was prepared similarly to 1a using 2b (50 mg, 0.15 mmol) and 3b (62 mg, 0.15 mmol) except that the mixing flask was kept at 35°C. Yield 76 mg (91%).

M.p. 190.5-191°C
Anal. found: C, 66.39; H, 11.91; N, 2.50; Calcd for C32H68NO2PS: C, 66.50; H, 11.86; N, 2.42.
HRMS (MALDI): 578.4721 (calc. 578.4730 (MH+))
1H-NMR (500 MHz, CDCl3): 0.85 (t, 6H, 2xCH3), 1.17-1.38 (m, 44H, 22xCH2), 1.57 (m, 2H, CH2), 1.71 (b, 2H, CH2), 2.90-3.00 (m, 2H, CH2), 3.03 (b, 2H, CH2), 3.22 (s, 6H, 2xCH2N+), 3.27 (m, 2H, CH2), 3.8-3.9 (m, 2H, CH2)
13C-NMR (125 MHz, CDCl3): 14.09, 22.66, 22.72, 23.24, 25.98, 26.36, 29.20, 29.34, 29.45, 29.50, 29.61, 29.65, 29.67, 29.70, 30.76, 30.82, 31.9, 51.14, 64.53, 64.99, 65.65
P-NMR (202 MHz, CDCl3): 14.74

**Methods**
Preparation of liposomes: The appropriate amount of lipid 1 was dissolved in chloroform in a round bottom flask and the solvent was removed under reduced pressure and dried. Sodium bicarbonate solution (0.1 M) was added and vortexed. After 5 freeze/thaw-cycles the dispersion was extruded through polycarbonate membranes (Nucleopore).

Encapsulation experiments: To FITC-dextran (Mw 20’000, Sigma) (2.5 mg) in 0.5 ml NaHCO3 (0.1 M) at 35°C was added 2b (3.4 mg in 1 ml NaHCO3 0.1 M) and 3b (4.3 mg in 1 ml NaHCO3 0.1 M) with the help of a syringe pump in the course of 2 hours. After an additional hour at 35°C 0.5 ml of the lipid suspension was chromatographed over Sepharose 4b (0.6 cm x 10 cm). The vesicle-containing fractions were examined on a Zeiss 2000M (inverse) spinning disk mikroskop (argon krypton laser 488 nm, 100x objective).

Dynamic Light Scattering (DLS): the mean diameter of liposomes was measured at 90° with a Zetasizer 3000 HSA (Malvern Instruments).

Cvc determination: using DLS the count rate in kilocounts/sec was recorded for suspensions of diC12-ZG from 1mM to 1 nM and plotted in double-logarithmic plot.
Growth experiments: 2 ml of a suspension of liposomes of 1a (2 mM in 0.1 M sodium bicarbonate), extruded to 100 nm, was stirred slowly at room temperature. To that was simultaneously added 1 ml of 2a (4 mM) and 1ml of 3a (4 mM) with the aid of a syringe pump (Thermo Orion M361) continuously over a period of 2 hours. At intervals an aliquot of 50 µl was diluted with sodium bicarbonate (0.1 M) and the mean diameter determined with DLS.

Differential Scanning Calorimetry: DSC measurements were performed on a Perkin-Elmer DSC 7 instrument in the range of -10 to 50°C.

Cryo-transmission electron microscopy: sample specimen of 3.5 ul each were first transferred on holey carbon grids (Quantifoil, Germany), then blotted to remove excess probe suspension. The grids were then plunged into liquid ethane using a Vitrobot apparatus (FEI, Eindhoven). The grids were examined at the temperature of liquid nitrogen using a cryo-holder (626, Gatan, USA) and a Tecnai G2 F20 microscope (FEI) (EMEZ, ETH Zurich) equipped with a field emission gun and energy filter (Gatan) that operated with an accelerating voltage of 200 kV.

![Image](image.png)

Figure SI1: Cryo-TEM picture of vesicles prepared from 1a.

References