Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Deep Cavitand Vesicles - Multicompartmental Hosts

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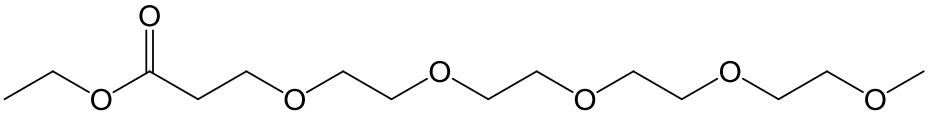
1 Experimental procedures

General remarks: Thin layer chromatography (TLC) was performed using glass plates coated with SiO_2 60 F_{254} obtained from Merck. Column chromatography was performed with SiO_2 60 (0.04-0.063 mm, 230-400 mesh) obtained from Fluka. NMR spectra were recorded using a Bruker DRX-600 [^{1}H-NMR (600 MHz), ^{13}C-NMR (151 MHz)] equipped with a 5 mm QNP probe at 300 K with tetramethylsilane (δ_H = 0.00 ppm) and $CDCl_3$ (δ_C = 77.01 ppm) as internal standard. Mass spectrometry was performed with an Agilent ESI-TOF instrument or on an Applied Biosystems Voyager STR mass spectrometer for MALDI-TOF using α-cyano-4-hydroxycinnamic acid, 2,5-dihydroxybenzoic acid (DHB) or 2',4',6'-trihydroxyacetophenone monohydrate (THAP) as matrix. Infrared (IR) spectra were recorded on a Perkin-Elmer 100 FT-IR spectrometer. The sonication of the vesicle samples was performed using a Fischer Scientific FS60 Ultrasound bath delivering 100 W of output power at 42 kHz.

1.1 Synthesis of ethyl 2,5,8,11,14-pentaoxaheptadecan-17-oate 3



3

Under nitrogen atmosphere, tetraethylene glycol monomethyl ether (**1**) (5.00 g, 24.0 mmol), ethyl acrylate (**2**) (3.00 g, 30.0 mmol) and sodium ethoxide (30.0 mg, 0.44 mmol) were stirred for 48 h at 80 °C. The excess ethyl acrylate was evaporated and the remaining liquid was purified by column chromatography (silica gel, ethyl acetate), yielding ethyl 2,5,8,11,14-pentaoxaheptadecan-17-oate (**3**) (3.40 g, 11.0 mmol, 46 %) as a colorless liquid.

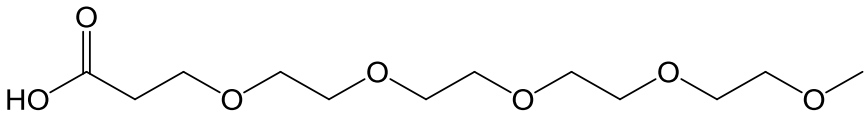
^{1}H-NMR (600 MHz, $CDCl_3$): δ = 4.16 (q, ^3J = 7.3 Hz, 2 H), 3.76 (t, ^3J = 6.5 Hz, 2 H), 3.69 - 3.61 (m, 14 H), 3.58 - 3.54 (m, 2 H), 3.39 (s, 3 H), 2.60 (t, ^3J = 6.5 Hz, 2 H), 1.27 (t, ^3J = 7.1 Hz, 3 H) ppm.

^{13}C-NMR (151 MHz, $CDCl_3$): δ = 171.98, 72.35, 71.02, 71.00, 70.99, 70.92, 70.89, 70.81, 67.05, 60.85, 59.42, 35.51, 14.59 ppm.

ESI-MS calcd. for $[M+H]^+$ ($C_{14}H_{29}O_7^+$): 309.19; found: 309.19, calcd. for $[M+Na]^+$ ($C_{14}H_{28}O_7Na^+$): 331.17, found: 331.18.

IR (ATR): ν = 2872 (m), 2360 (w), 1732 (s), 1454 (w), 1371 (w), 1350 (w), 1250 (m), 1185 (m), 1099 (vs), 1028 (m), 944 (m), 852 (m) cm^{-1}.

1.2 Synthesis of 2,5,8,11,14-pentaoxaheptadecan-17-oic acid 4



4

Ethyl 2,5,8,11,14-pentaoxaheptadecan-17-oate (**3**) (2.10 g, 6.82 mmol) was stirred at room temperature in an aqueous potassium hydroxide solution (10 %, 15 mL) for 16 h. The mixture was washed with dichloromethane. The aqueous layer was acidified by adding conc. hydrochloric acid and extracted with dichloromethane. After evaporation of the solvent, 2,5,8,11,14-pentaoxaheptadecan-17-oic acid (**4**) (1.45 g, 5.19 mmol, 76 %) was obtained as a colorless liquid.

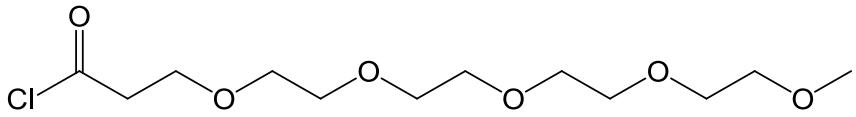
^{1}H-NMR (600 MHz, $CDCl_3$): δ = 3.79 (t, ^{3}J = 6.2 Hz, 2 H), 3.70 - 3.64 (m, 14 H), 3.60 - 3.56 (m, 2 H), 3.40 (s, 3 H), 2.64 (t, ^{3}J = 6.1 Hz, 2 H) ppm.

^{13}C-NMR (151 MHz, $CDCl_3$): δ = 175.36, 72.28, 71.06, 71.02, 70.98, 70.90, 70.87, 70.79, 70.70, 66.89, 59.34, 35.36 ppm.

ESI-MS calcd. for $[M+H]^+$ ($C_{12}H_{25}O_7^+$): 281.16, found: 281.18; calcd. for $[M+Na]^+$ ($C_{12}H_{24}O_7Na^+$): 303.14, found 303.14.

IR (ATR): ν = 3460 (m), 2873 (m), 1722 (s), 1453 (w), 1351 (m), 1247 (m), 1196 (m), 1088 (vs), 943 (m), 842 (m) cm^{-1}.

1.3 Synthesis of 2,5,8,11,14-pentaoxaheptadecan-17-oyl chloride 5



5

Under a nitrogen atmosphere, 2,5,8,11,14-pentaoxaheptadecan-17-oic acid (**4**) (700 mg, 2.50 mmol) was dissolved in 8 mL dichloromethane. After addition of oxalyl chloride (0.60 mL, 6.99 mmol), the mixture was stirred for 16 h at room temperature. The solvent and excess oxalyl chloride were evaporated yielding 2,5,8,11,14-pentaoxaheptadecan-17-oyl chloride (**5**) (710 mg, 2.38 mmol, 95 %) as a colorless oil.

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 3.81 (t, ^{3}J = 5.9 Hz, 2 H,), 3.69 - 3.63 (m, 14 H), 3.58 - 3.55 (m, 2 H), 3.39 (s, 3 H), 3.15 (t, ^{3}J = 6.0 Hz, 2 H) ppm.

^{13}C-NMR (151 MHz, $CDCl_3$): δ = 172.24, 72.34, 71.12, 71.05, 71.01, 70.98, 70.90, 66.27, 59.41, 47.77 ppm.

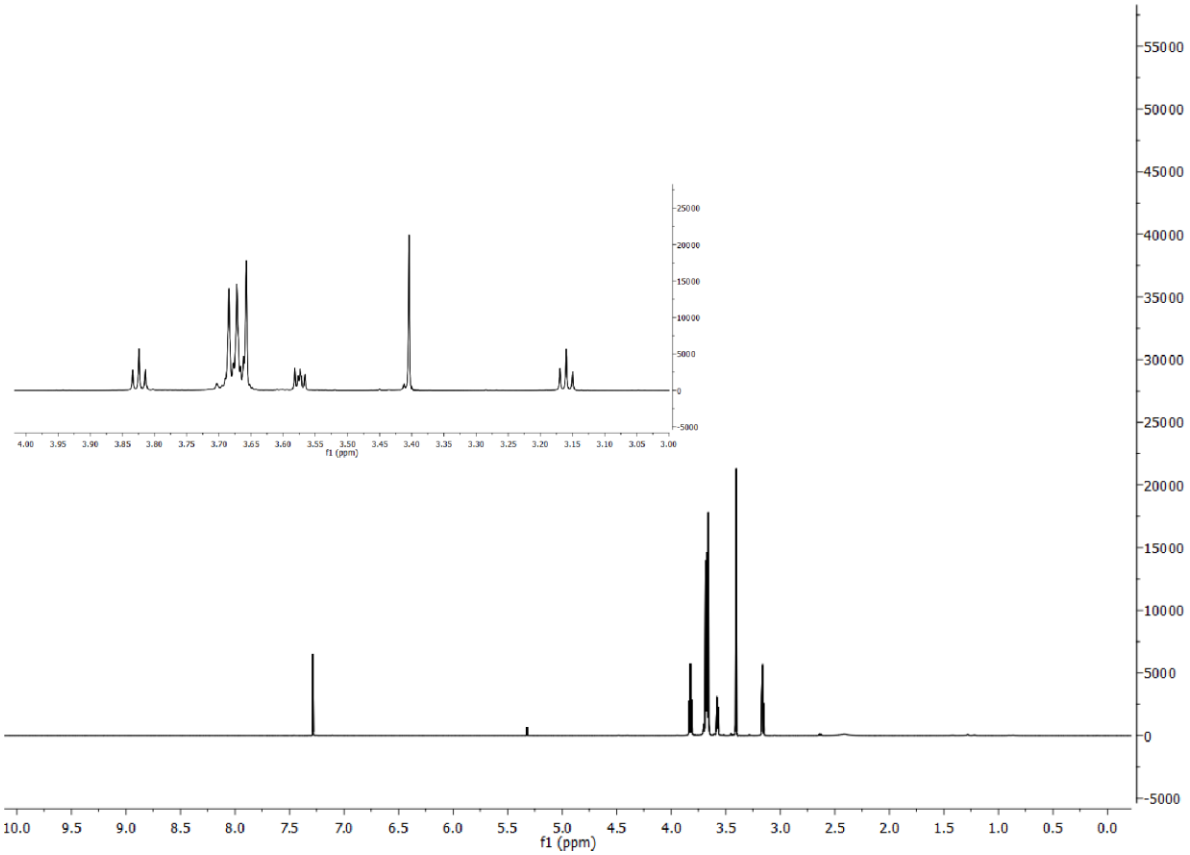
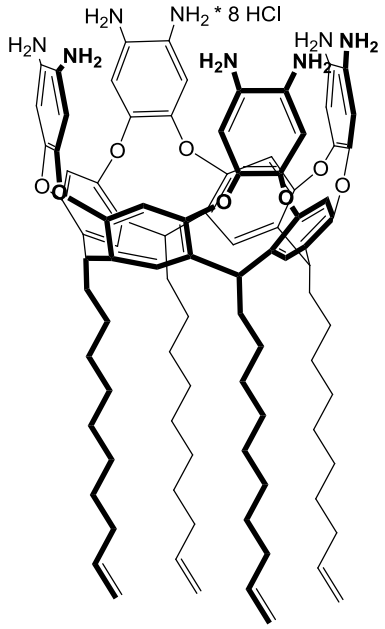


Figure S1: ^{1}H-NMR (600 MHz, $CDCl_3$) of 2,5,8,11,14-pentaoxaheptadecan-17-oyl chloride (**5**).

1.4 Synthesis of octaamino cavitand 6



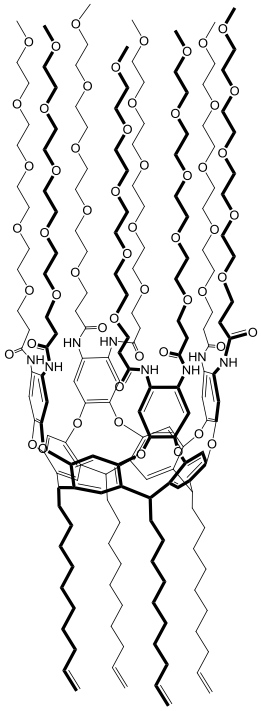
6

Under nitrogen atmosphere, octanitro cavitand with alkene feet[23] (600 mg, 0.35 mmol) and tin(II) chloride dihydrate (4.62 g, 20.5 mmol) were dissolved in 20 mL ethanol. After addition of 5 mL conc. hydrochloric acid, the mixture was stirred for 16 h at 70 °C. Ethanol was evaporated and after addition of water a colorless solid precipitated. The obtained solid was washed with water and dried under vacuum, yielding octaamino cavitand **6** (527 mg, 0.30 mmol, 86 %) as hydrochloride.

^{1}H-NMR (600 MHz, DMSO-d_6): δ = 7.72 (s, 4 H), 7.44 (s, 8 H), 7.19 (s, 4 H), 5.90 - 5.71 (m, 4 H), 5.55 (br s, 4 H), 4.99 (dd, ^3J = 17.1 Hz, ^2J = 1.6 Hz, 4 H), 4.96 - 4.86 (m, 4 H), 2.37 - 2.27 (m, 8 H), 2.01 (m, 8 H), 1.45 - 1.17 (m, 48 H) ppm.

MALDI-TOF-MS calcd. for $[M+H^+]^+$ ($C_{92}H_{111}N_8O_8^+$): 1455.85, found: 1457.

1.5 Synthesis of octa-TEG cavitand 7



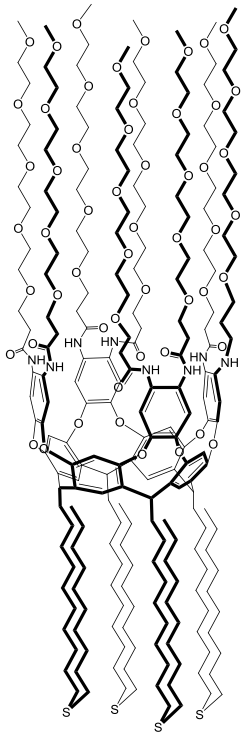
7

Octaamino cavitand **6**[23] (433 mg, 0.25 mmol) was dissolved in 80 mL ethyl acetate and a solution of potassium carbonate (19.0 g, 137 mmol) in 80 mL water was added. After addition of 2,5,8,11,14-pentaoxaheptadecan-17-oyl chloride **5** (710 mg, 2.38 mmol), the mixture was stirred for 3 d at room temperature. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried with $MgSO_4$. After evaporation of the solvent octa-TEG cavitand **7** (909 mg, 0.25 mmol, 100 %) was obtained as colorless oil, which was used in the next step without further purification.

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 9.44 (s, 8 H), 7.52 (s, 4 H), 7.29 (s, 4 H), 7.24 (s, 4 H), 5.84 (ddt, ^3J = 16.9, 10.1, 6.7 Hz, 4 H), 5.78 (t, ^3J = 8.0 Hz, 4 H), 5.02 (dd, ^3J = 17.1 Hz, ^2J = 1.9 Hz, 4 H), 4.96 (dd, ^3J = 10.2 Hz, ^2J = 0.9 Hz, 4 H), 3.87 - 3.79 (m, 16 H), 3.71 - 3.60 (m, 112 H, OCH_2), 3.57 - 3.54 (m, 16 H), 3.38 (s, 24 H), 2.75 - 2.55 (m, 16 H), 2.31 - 2.22 (m, 8 H), 1.76 - 1.62 (m, 8 H), 1.52 - 1.31 (m, 48 H) ppm.

MALDI-TOF-MS calcd. for $[M+Na^+]^+$ ($C_{188}H_{288}N_8O_{56}Na^+$): 3576.98, found: 3577, calcd. for $[M+K^+]^+$ ($C_{188}H_{288}N_8O_{56}K^+$): 3592.96, found: 3593.

1.6 Synthesis of octa-TEG cavitand 8



8

Under nitrogen atmosphere, octa-TEG cavitand **7** with alkene feet (900 mg, 0.25 mmol) was dissolved in 20 mL THF. At 0 °C 1-decanethiol (1.20 mL, 5.78 mmol) and a 0.5 M solution of 9-borabicyclo(3.3.1)nonane (9-BBN) in THF (1.20 mL, 0.60 mmol) were added. The mixture was stirred at room temperature for 48 h. The solvent was evaporated and the obtained light yellow oil was purified by preparative thin layer chromatography (silica gel, dichloromethane/methanol, 95:5), yielding octa-TEG cavitand **8** with thioether feet (436 mg, 0.10 mmol, 41 %) as colorless oil.

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 9.44 (s, 8 H), 7.51 (s, 4 H), 7.29 (s, 8 H), 7.23 (s, 4 H), 5.76 (br s, 4 H), 3.90 - 3.76 (m, 16 H), 3.70 - 3.60 (m, 112 H), 3.57 - 3.53 (m, 16 H), 3.38 (s, 24 H), 2.74 - 2.56 (m, 16 H), 2.52 (t, ^{3}J = 7.4 Hz, 16 H), 2.30 -2.20 (m, 8 H), 1.63 - 1.56 (m, 16 H), 1.50 - 1.24 (m, 112 H), 0.90 (t, ^{3}J = 7.0 Hz, 12 H).

^{13}C-NMR (151 MHz, $CDCl_3$): δ = 171.01, 155.05, 150.18, 135.87, 128.36, 123.95, 121.49, 116.59, 72.33, 70.97, 70.94, 70.90, 70.87, 67.56, 59.41, 52.90, 38.58, 33.71, 32.64, 32.30, 30.21, 30.17, 30.11, 30.06, 29.98, 29.96, 29.80, 29.73, 29.69, 29.48, 29.39, 28.50, 23.08, 14.52.

MALDI-TOF-MS calcd. for $[M+Na]^+$ ($C_{228}H_{376}N_8O_{56}S_4Na^+$): 4273.56; found: 4275.

IR (ATR): ν = 3256 (w), 2921 (s), 2852 (s), 1660 (m), 1599 (w), 1513 (m), 1482 (s), 1402 (m), 1350 (w), 1275 (m), 1196 (m), 1106 (vs), 895 (m) cm^{-1}.

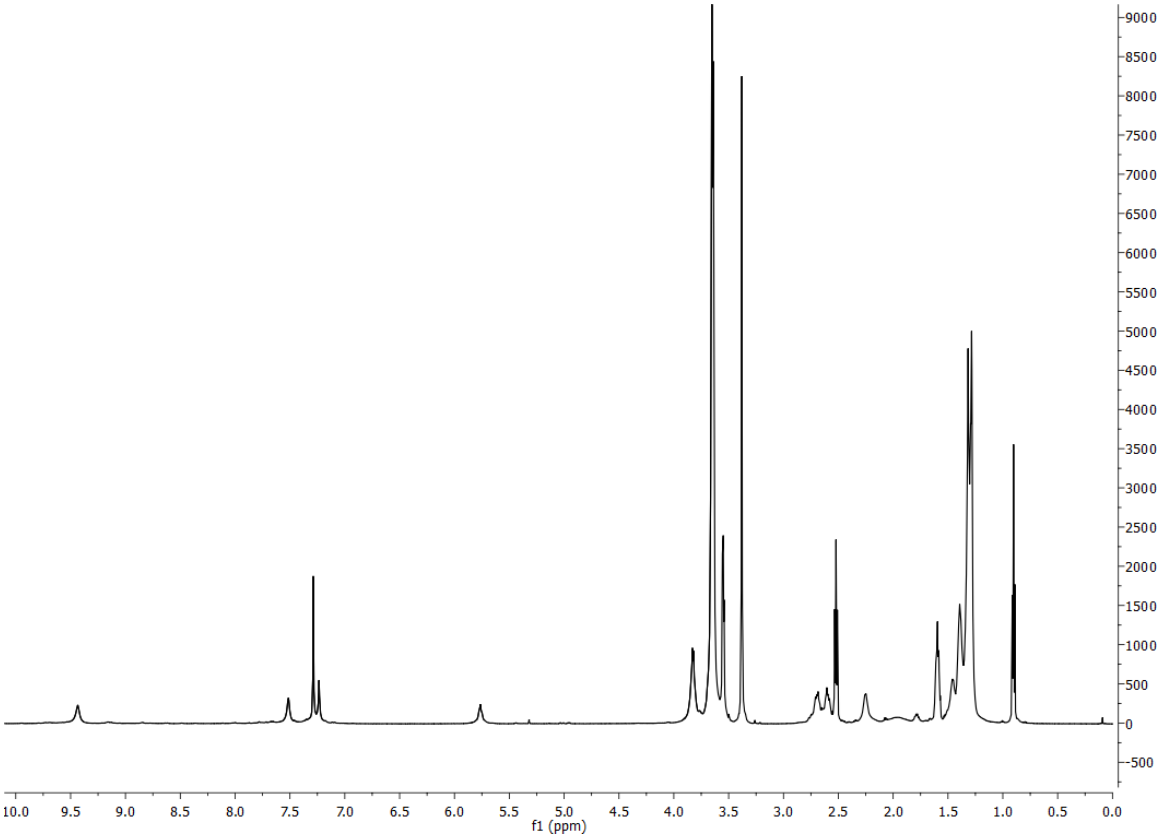
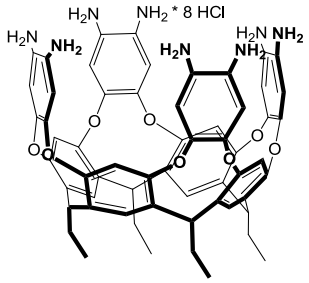


Figure S2: ^{1}H-NMR (600 MHz, $CDCl_3$) of octa-TEG cavitand **8** with thioether feet.

1.6 Synthesis of octaamino cavitand with C_2-feet

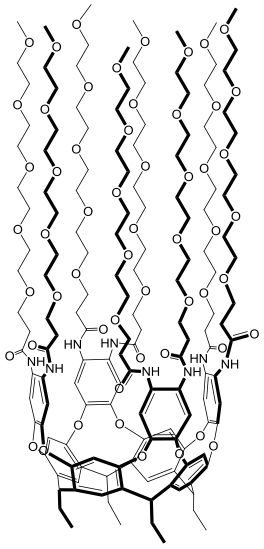


Under nitrogen atmosphere, octanitro cavitand with C_2-feet (440 mg, 0.35 mmol) and tin(II) chloride dihydrate (4.62 g, 20.5 mmol) were dissolved in 20 mL ethanol. After addition of 5 mL conc. hydrochloric acid, the mixture was stirred for 16 h at 70 °C. Ethanol was evaporated and after addition of water a colorless solid precipitated. The obtained solid was washed with water and dried under vacuum, yielding octaamino cavitand with C_2-feet (304 mg, 0.23 mmol, 67 %) as hydrochloride.

^{1}H-NMR (600 MHz, DMSO-d_6): δ = 7.75 (s, 4 H), 7.33 (s, 8 H), 7.19 (s, 4 H), 6.22 (br s, 16 H), 5.41 (br s, 4 H), 2.43 - 2.28 (m, 8 H), 0.89 (t, ^3J = 7.1 Hz, 12 H) ppm.

MALDI-TOF-MS calcd. for $[M+H]^+$ ($C_{60}H_{57}N_8O_8^+$)1017.43, found: 1017; $[MH^+]$, calcd. for $[M+K]^+$ ($C_{60}H_{56}N_8O_8K^+$): 1055.39, found: 1055.

1.7 Synthesis of octa-TEG cavitand with C_2-feet



Octaamino cavitand with C_2-feet (274 mg, 0.21 mmol) was dissolved in 80 mL ethyl acetate and a solution of potassium carbonate (19.0 g, 137 mmol) in 80 mL water was added. After addition of 2,5,8,11,14-pentaoxaheptadecan-17-oyl chloride (**5**) (588 mg, 1.97 mmol), the

mixture was stirred for 48 h at room temperature. The layers were separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried with $MgSO_4$. The solvent was evaporated and the obtained yellow oil was purified by preparative thin layer chromatography (silica gel, dichloromethane/methanol, 95:5), yielding octa-TEG cavitand with C_2-feet (264 mg, 0.09 mmol, 40 %) as colorless oil.

^{1}H-NMR (600 MHz, $CDCl_3$): δ = 9.44 (s, 8 H), 7.53 (s, 8 H), 7.30 (s, 4 H), 7.26 (s, 4 H), 5.68 (br s, 4 H), 3.89 - 3.78 (m, 16 H), 3.73 - 3.60 (m, 112 H), 3.59 - 3.52 (m, 16 H), 3.38 (s, 24 H), 2.77 - 2.54 (m, 16 H), 2.36 - 2.25 (m, 8 H), 1.04 (t, ^3J = 6.9 Hz, 12 H) ppm.

^{13}C-NMR (151 MHz, $CDCl_3$): δ = 171.02, 155.10, 150.16, 135.79, 128.38, 123.90, 121.46, 116.63, 72.33, 70.96, 70.93, 70.89, 70.87, 67.57, 59.41, 38.59, 35.72, 25.78, 12.84.

MALDI-TOF-MS calcd. for $[M+Na]^+$ ($C_{156}H_{232}N_8O_{56}Na^+$): 3136.54; found: 3136.

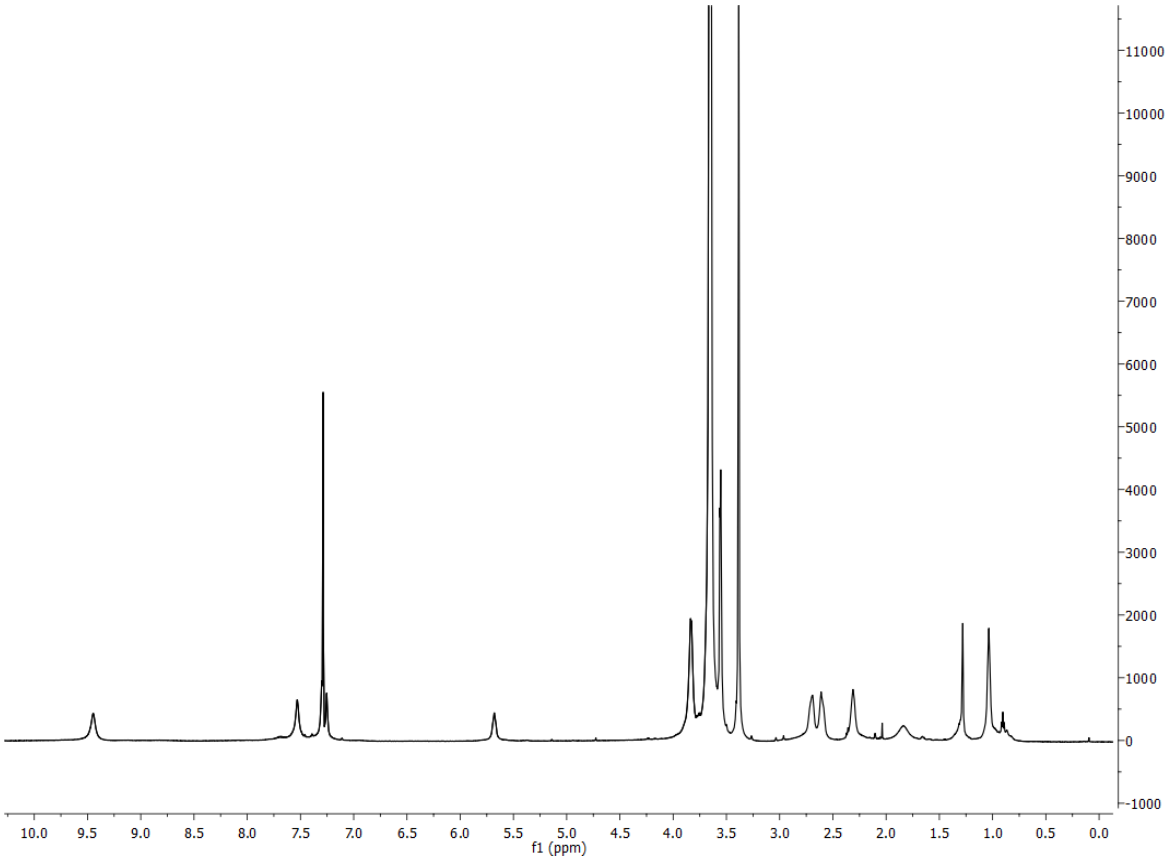
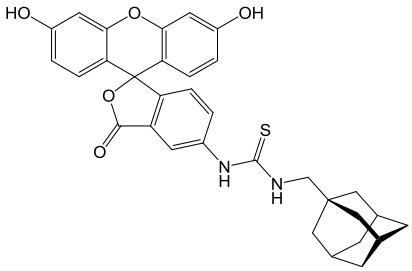


Figure S3: ^{1}H-NMR (600 MHz, $CDCl_3$) of octa-TEG cavitand **8** with C_2-feet.

1.8 Synthesis of an adamantane substituted fluorescein derivative 15



Fluorescein isothiocyanate (100 mg, 0.26 mmol) was added to a solution of 1-adamantane methylamine hydrochloride (52 mg, 0.26 mmol) and potassium carbonate (108 mg, 0.78 mmol) in 5 mL water. The mixture was stirred for 16 h at room temperature. After evaporation of the solvent, the residue was purified by column chromatography (silica gel, dichloromethane/methanol, 5:1), yielding adamantane substituted fluorescein **15** (127 mg, 0.23 mmol, 88 %) as an orange solid.

^{1}H-NMR (600 MHz, CD_3OD): δ = 8.26 (s, 1 H), 7.84 (d, ^{3}J = 8.2 Hz, 1 H), 7.17 (d, ^{3}J = 8.1 Hz, 1 H), 6.73 (d, ^{3}J = 8.6 Hz, 2 H), 6.70 (d, ^{4}J = 2.3 Hz, 2 H), 6.58 (dd, ^{3}J = 8.7 Hz, ^{4}J = 2.3 Hz, 2 H), 3.37 (s, 2 H), 2.03 (s, 3 H), 1.84 - 1.73 (m, 6 H), 1.67 (s, 6 H).

^{13}C-NMR (151 MHz, CD_3OD): δ = 182.65, 170.25, 160.32, 153.21, 148.38, 141.90, 130.64, 129.31, 127.78, 124.45, 118.50, 112.61, 110.54, 102.53, 56.24, 40.56, 37.06, 34.44, 28.82.

MALDI-TOF-MS calcd. for $[M+H]^+$ ($C_{32}H_{31}N_2O_5S^+$): 555.19; found: 555, calcd. for $[M+Na]^+$ ($C_{32}H_{30}N_2O_5SNa^+$): 577.18; found: 577.

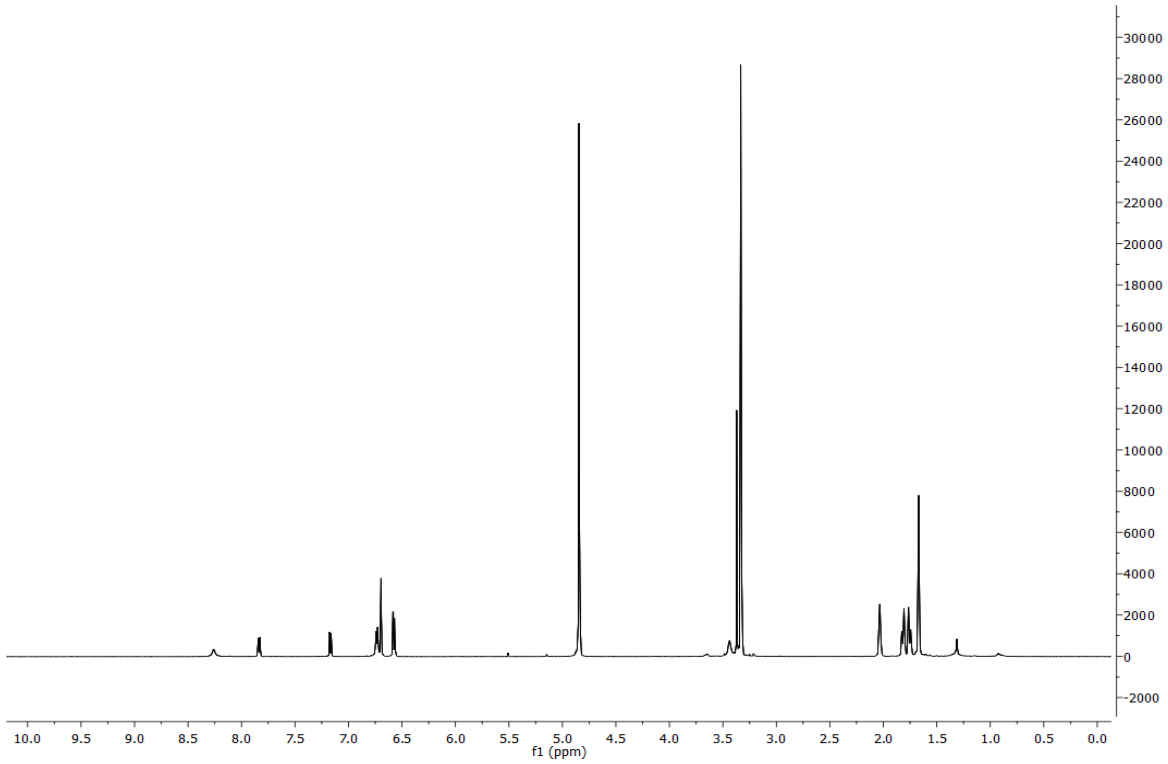


Figure S4: ^{1}H-NMR (600 MHz, CD_3OD) of adamantane substituted fluorescein derivative **15**.

2 Determination of the solubility at 300 K using NMR

The solubility of the TEG substituted cavitands with thioether and ethyl feet was determined for saturated solutions in D_2O by integration of ^{1}H-NMR signals relative to a calibrated external reference. The reference consisted of a sealed capillary containing a solution of sodium 3-(trimethylsilyl)-propionate-2,2,3,3-d_4 at an apparent concentration of 44.6 μM in D_2O placed in the NMR tube. The apparent concentration of the NMR reference was determined by titration using solutions of 2-adamantanone of known concentrations. To ensure quantitative spin relaxation, all the solubility experiments were performed with a D1 delay time of 20 s corresponding to > 5 T1 relaxation time. A T1 of 3.7 s was determined for the reference compound. All the other components of the system, being larger, have in principle shorter T1 values under identical conditions.

3 Determination of binding constants using NMR

The association constants K_a were determined for the systems at equilibrium by integration of free guest, free cavitand and caviplex signals relative to the external reference signals. A delay time D1 of 2 s was used for all the association constant determinations. This delay time was chosen after checking that there was no significant (< 10 %) differences between K_a values obtained with a 2 s delay and the same experiment using a D1 of 20 s. In the titration experiments, the relative concentrations of diverse species were adjusted such as the cavitand (usually ~ 0.5 mM) had a guest-occupancy in the range of 20 – 80% (*i.e.* guest concentration in the millimolar range);

4 Transmission Electron Microscopy (TEM)

For TEM measurements 4.7 mmolar solutions of the respective compound in Milli-Q water were used. When indicated, guest molecules were added in a 1:1 mixture. Sodium silicotungstate was used as a negative stain. Copper grids (carbon coated 400 mesh (Electron Microscopy Sciences, Hatfield PA)) were glow discharged and immediately inverted onto 7 μl aliquots of sample on parafilm. After 3 minutes, excess liquid was wicked off and the grids immediately placed onto individual droplets of freshly prepared and filtered, 1% aqueous sodium silicotungstate (SST) pH 8.0. After 2 minutes excess stain was removed and the grids were allowed to dry thoroughly. Each sample was examined on a Philips CM100 electron microscope (FEI, Hillsbrough OR) at 80 kV and images collected using a Megaview III ccd camera (Olympus Soft Imaging Solutions, Lakewood CO).

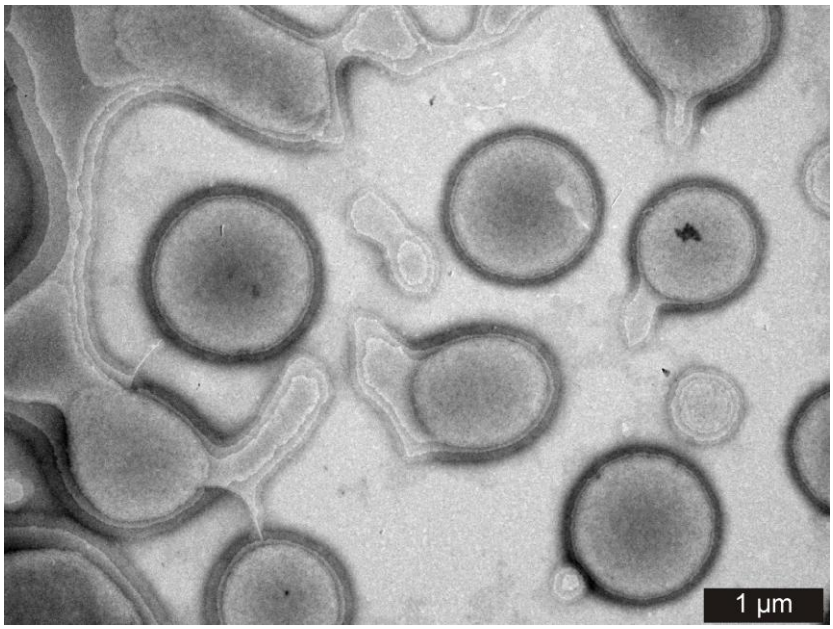


Figure S5: TEM image of vesicles and larger structures of cavitand **8**, showing that the vesicles may undergo fusion or fission, respectively.

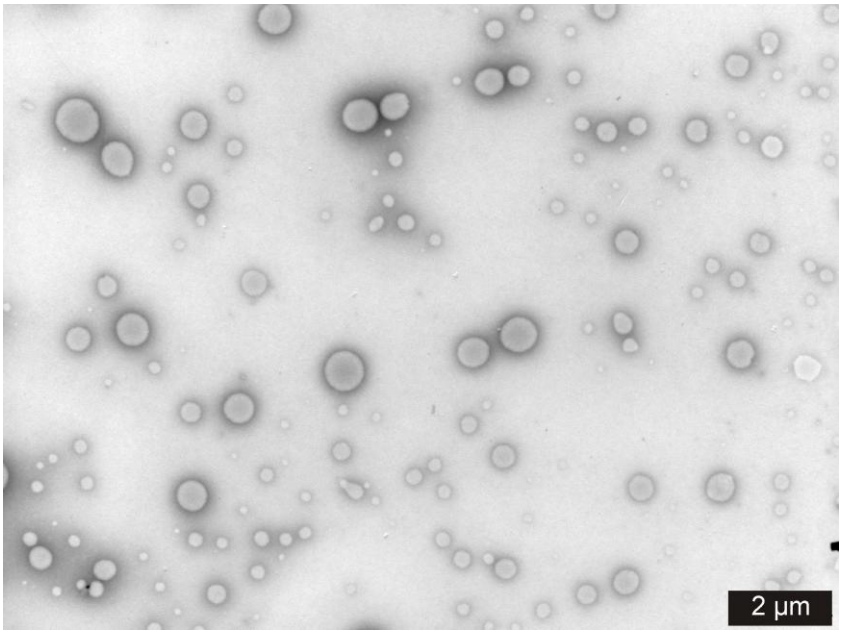


Figure S6: TEM image of vesicles of octa-TEG cavitand with short ethyl feet.

5 Confocal Microscopy

The samples for confocal microscopy measurements were prepared as follows:

- A 1:1 mixture of a 4.7 mmolar solution of cavitand **8** and fluorescein derivative **15** in Milli-Q water was chromatographed on a Sephadex® G-50 column. The first fluorescent fraction was directly used to prepare the microscope slides.
- Cavitand **8** was dissolved in an aqueous solution of doxorubicin hydrochloride yielding a 5 mmolar solution (1:1 mixture). The solution was dialyzed for 3 d using a Spectra-Por® Float-A-Lyzer® G2 black, MWCO 3.5-5 kDa. The dialyzed solution was directly used to prepare the microscope slides.

The measurements were carried out using a Zeiss 710 Laser Scanning Confocal Microscope (Zeiss Inc).

6 Fluorescence Spectroscopy

For fluorescence measurements a FluoroLog®-3, Jobin Yvon Inc., Horiba Group was used.

The formation of hydrophobic domains in an aqueous solution of cavitand **8** was evidenced using pyrene fluorescence as described in "D. López-Díaz, M. M. Velázquez, *Chem. Educator* **2007**, *12*, 327-330." The ratio of the I_1 (~372 nm) and I_3 (~385 nm) emission bands was considered to assess the local microenvironment of pyrene. The formation of hydrophobic domains by aggregation of cavitand **8** was evidenced by the typical change in the I_1/I_3 ratio from ~1.5 to ~1.1 and was determined by titration at various concentrations of cavitand **8**. ^{1}H-NMR analysis confirmed that pyrene was not a guest for the cavity of **8**. The change of fluorescence was therefore attributed to the formation of hydrophobic domains by aggregation upon reaching the critical aggregation concentration determined at ~1 μM.

Procedure: 2 μL of 0.4 mM solution of pyrene in methanol was introduced in the cuvette and the solvent was evaporated in a stream of compressed air. Milli-Q water (2 mL) was added and the fluorescence spectrum recorded (ex. 320 nm, slit 5 nm and em. 340–440 nm, slit 5 nm). The solution was then titrated using a 17.4 μM solution of **8** in Milli-Q water and the ratio between the I_1 (~372 nm) and I_3 (~385) emission bands plotted against the concentration of **8**. The critical aggregation concentration was determined by the intersection of the linear regressions of the two linear domains of the graph.

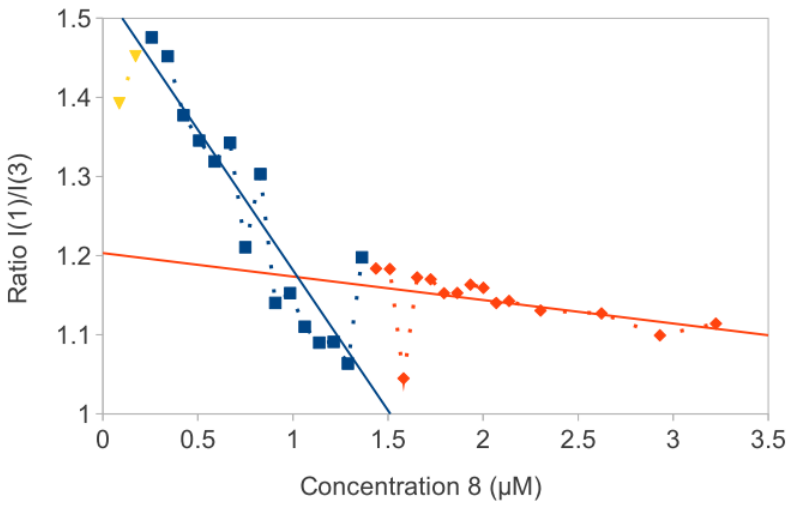


Figure S7. Ratio of the I_1/I_3 emission bands of the pyrene fluorescence in presence of various concentrations of cavitand **8**. Yellow data points were measured at very low concentration of **8** and were not used in the analysis. In blue are the data points, and the linear fit of, at concentrations approaching the critical aggregation concentration (CAC). In red are the data points, and the linear fit of, at concentrations above the CAC. The concentration at the intercept of the two extrapolation lines corresponds to the CAC.

To investigate the release of doxorubicin hydrochloride, encapsulated inside the vesicles of compound **8**, the fluorescence spectrum ($\lambda_{excitation}$ = 479 nm) of a sample of dialyzed (20 h) solution of cavitand **8** and doxorubicin hydrochloride (diluted to 10 μmol/l, prepared at 5 mmol/l) was measured. After addition of Triton X-165, the typical fluorescence emission bands (λ_1 = 554 nm, λ_2 = 592 nm) of doxorubicin hydrochloride became visible, confirming a release of the encapsulated drug.

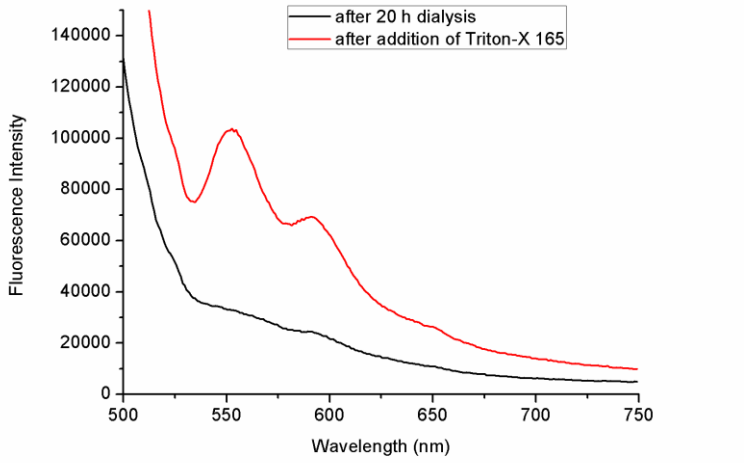


Figure S8: Fluorescence spectrum ($\lambda_{excitation}$ = 479 nm) of a dialyzed solution of cavitand **8** and doxorubicin hydrochloride in water before (black) and after (red) addition of Triton X-165.