Embedding resorcinarene cavitands in lipid vesicles

Supporting Information

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1. General considerations

$^1$H NMR was acquired on a Bruker Apollo 400 MHz at 298 K, $^{13}$C was acquired at 100 MHz, 298 K. For $^1$H NMR binding experiments 128 scans were acquired. Data was processed (iNMR 3.5.1) using a Fourier transform with exponential weighting (0.50 Hz). NMR solvents were purchased from Cambridge Isotope Laboratories and residual solvent peak was used to as an internal standard. Confocal Microscope images were acquired using an Olympus Fluoview 1000 confocal laser scanning system mounted on an inverted microscope (Olympus IX-81) equipped with differential interference contrast (DIC) optics – (National Science Foundation DBI 0722757). Lipids were purchased from Avanti Polar Lipids and used as received. SiliaFlash® P60 ACADEMIC Silica Gel, 40-63μm, 60A was purchased from Silicycle.

2. Synthesis of new compounds

![Chemical structure](image)

Resorcinarene 1 was prepared according to literature procedure.$^1$

Cavitand A. Resorcinarene 1 (0.44 g, 0.58 mmol) and 1,2-difluoro-4,5-dinitrobenzene (0.47 g, 2.3 mmol) was dissolved in DMF (14.6 mL, anhydrous) under argon atmosphere. Triethylamine (1.33 mL, 9.54 mmol) was added dropwise and the reaction was heated to 65 °C for 18 hours.$^2$ The reaction was allowed to cool to room temperature and poured into a mixture of water (50 mL) and 1M HCl (12 mL). The reaction mixture was filtered on a medium glass sintered frit, rinsed with water (15 mL) transferred to a 100 mL round bottom flask and placed under vacuum (1 mmHg) for 24 hours to give 0.744 g of A, that was used without further purification.
Cavitand B. Cavitand A (0.739 g, 0.52 mmol), SnCl₂ (2.48 g, 13.1 mmol) and 4:1 Ethanol:conc. HCl was added (26 mL) to a 100 mL flask. The mixture was heated to 75 °C for 25 hours. The reaction mixture was rotavapped to dryness and then suspended in ethyl acetate (75 mL). To this suspension was added K₂CO₃ in water (125 mL of 30g in 150 mL) until the pH was basic. Propionyl chloride (3 X 1 mL at 20 minute intervals) was added with stirring. The reaction was stirred an additional 30 minutes, separated, and the organic layer was washed with water (2 X 75 mL). The reaction mixture was dried over anhydrous sodium sulfate, gravity filtered, and concentrated. The material was purified by flash chromatography two times (1 X 20:1 CH₂Cl₂:MeOH, 40 g silica gel; 1 X 50:1 CH₂Cl₂:MeOH, 20 g silica gel) to give the pure product (221 mg, 26 % yield). ¹H NMR (400 MHz, Acetone-d₆) δ 0.92 (t, 9H); 1.22 (t); 1.38 (m); 1.58 (quintet, 2H); 2.48 (m); 3.59 (t, 1H); 3.66 (t, 2H), 5.83 (t, 4H), 7.51 (s, 4H), 7.72 (s, 8H), 7.94 (d, 4H), 9.50 (s, 8H). Solvent impurities indicated with “X.”
$^1$H NMR of Cavitand B.
Cavitand B (0.215 g, 0.133 mmol) and CH₂Cl₂ (4 mL) were cooled to 0 °C. Triethylamine (86 uL, 0.644 mmol) and methanesulfonyl chloride (12.3 uL, 0.159 mmol) were added. After 4 hours the reaction was incomplete by TLC (20:1 CH₂Cl₂:MeOH) and an additional portion of methanesulfonyl chloride (20 uL) was added. After 1 additional hour of stirring all starting material was consumed. The reaction mixture was diluted with CH₂Cl₂ (20 mL), washed with 1M HCl (5 mL), Sat. NaHCO₃ (10 mL), brine (10 mL), dried over sodium sulfate, gravity filtered, concentrated and used without further purification. To the mesylate (0.226 g, 0.133 mmol) was added DMF:THF (4:1, 10 mL) and sodium azide (26 mg, 0.400 mmol). The reaction mixture was heated to 70 °C under argon for 20 hours. TLC indicated complete consumption of starting material (20:20:1, Hexane:Ethyl acetate:methanol). The reaction mixture was diluted with ethyl acetate (30 mL) and washed with 1 M HCl (10 mL), Sat. NaHCO₃ (10 mL), Sat. CuSO₄ (10 mL), brine (10 mL), dried with sodium sulfate, filtered, concentrated and analyzed to give 210 mg of pure 2 (quantitative). ¹H NMR (400 MHz, Acetone-d₆) δ 0.92 (t, 9H); 1.22 (t); 1.38 (m); 2.49 (m); 3.48 (t, 2H); 5.83 (m, 4H), 7.51 (d, 4H), 7.72 (s, 8H), 7.91 (s, 4H), 9.50 (s, 8H). ¹³C NMR (100 MHz, Acetone-d₆) δ 10.47, 14.34, 23.45, 28.35, 28.71, 31.32, 32.69, 32.77, 34.30, 34.53, 52.11, 117.62, 117.70, 122.29, 130.09, 137.12, 137.49, 137.64, 137.92, 151.06, 156.46, 156.50, 156.56, 156.59, 175.21. Solvent impurities indicated with “X.”
$^1$H NMR of Cavitand 2.

$^{13}$C NMR of Cavitand 2.
Propargyl Amine appended nitrobenzoxadiazole C. 4-Chloro-7-nitrobenzofurazan (NBD-Cl) (200 mg, 1.0 mmol), ethyl acetate (5 mL), propargyl amine (55.2 mg, 1.0 mmol) and NaHCO₃ (253 mg, 3.0 mmol) were added sequentially to a 15 mL round bottom flask and stirred for 24 hr. The reaction mixture was filtered through a course sintered funnel under vacuum and to the filtrate was added 1 M HCl (20 mL) and CH₂Cl₂ (20 mL). The mixture was shaken, the organic layer was dried over Na₂SO₄, filtered, evaporated and purified by column chromatography (1:1 ethyl acetate:hexane) to give 139 mg (54 % yield) of the title compound C. ¹H NMR (400 MHz, CDCl₃) δ 2.43 (t, 1H); 4.29 (dd, 2H); 6.30 (broad, 1H, NH), 6.33 (d, 1H); 8.53 (d, 1H). In CD₃OD the NH signal is absent, the peak at 4.29 becomes a broad singlet, and the overall spectra is more sharply resolved.

¹H NMR of Cavitand C.
Cavitand 3. Cavitand 2 (42.3 mg, 0.025 mmol) and propargyl amine appended nitrobenzoxadiazole C (5.5 mg, 0.025 mmol) were dissolved in THF (1 mL). To this was added CuI (0.7 mg, 3.68x10^{-3} mmol) and TBTA ligand (1.4 mg, 2.64x10^{-3} mmol). The reaction mixture was heated in oil bath at 65 °C with stirring. An additional portion of THF (1 mL) was added and the reaction was monitored by TLC after 12 hours of stirring (3:3: 0.2 hexane: ethyl acetate: methanol), indicating consumption of starting material and formation of a new compound. The reaction mixture was transferred to a separatory funnel with CH2Cl2 (20 mL) and washed with water (10 mL), Sat. NH4Cl (10 mL), dried over Na2SO4, gravity filtered, and concentrated. The title compound was purified by column chromatography, and concentrated, to give 30 mg (64 % yield) of analytically pure 3. 1H NMR (400 MHz, acetone-d6) δ 0.87- 0.93 (m); 1.21 (t); 1.29- 1.39 (m); 2.41-2.54 (m); 4.51 (t, 2H); 4.92 (s, 2H); 5.81 (t, 4H); 6.58 (d, 1H); 7.48 (d, 4H); 7.71 (s, 8H); 7.88 (d, 4H); 8.09 (s, 1H); 8.50 (d, 1H); 9.48 (s, 8H).13C NMR (100 MHz, acetone-d6) δ 10.46, 14.33, 23.40, 23.45, 28.63, 31.31, 32.63, 32.74, 33.89, 34.48, 40.12, 50.49, 117.64, 122.22, 124.04, 126.24, 126.30, 130.05, 136.75, 137.36, 137.56, 137.94, 138.25, 144.59, 145.88, 146.32, 150.97, 156.34, 156.45, 156.49, 156.56, 175.20. Solvent impurities indicated with “X.” ESI mass spectrum for C103H113N15O19Na 1887.83 (m/z) calculated, 1887.8 found (base peak). Accurate mass acquired using an Agilent 6210 LCToF instrument, operated in "Multimode" mode (UC Riverside High Resolution Mass Spectrometry Facility) for C103H113N15O19Na 1886.8229 (exact mass) calculated, 1886.8178 found.
$^1$H NMR of cavitand 3.

$^{13}$C NMR of cavitand 3.
3. Binding of adamantyl amine with cavitand 3

To a 20 mL scintillation vial was added cavitand 3 (5 mg), adamantyl amine (10 mg), and Mesitylene-d$_{12}$ (0.550 mL, 98 % D, Cambridge Isotope Laboratories). The mixture was sonicated for 10 minutes to aid in dissolution.

The upfield region of the $^1$H NMR is shown, indicating the presence of bound adamantyl amine, consistent with literature precedent (see figure 9 of reference). The region downfield of 3 ppm is increasingly complex, as the cavitand lacks C$_{4v}$ symmetry and this appears to be accentuated upon guest binding. The control experiment of adamantyl amine in Mesitylene-d$_{12}$ follows. The chemical shift difference between the peak integrating to 6Hs is Δ$\partial$ 2.40. The alkane portion of the guest is clearly surrounding by the shielding environment of the cavitand interior. Cavitand alone in Mesitylene-d$_{12}$ gave broad unresolved peaks in the absence of a suitable guest.

$^1$H NMR of cavitand 3 (5 mg), adamantyl amine (10 mg) in Mesitylene-d$_{12}$ (0.55 mL).
H NMR of adamantyl amine (10 mg) in Mesitylene-d$_{12}$ (0.55 mL).
4. Preparation and characterization of vesicles (from manuscript)

1,2-dilauroyl-sn-glycero-3-phosphocholine (15 μL, 0.0804 M in chloroform, Avanti®
Polar Lipids), methanol (200 μL), chloroform (1mL), and Hepes (3 mL, 0.01 M in 18.2
MΩ H₂O) were placed in a 50 mL pear-shaped flask. The solution was slowly rotovapped
at 10 mmHg, 40° C, 40 rpm for 4-5 minutes. After two distillation events were observed
an opaque liquid resulted. When using cavitand 3, 1 mol % was added prior to vesicle
formation. When using fluorescein-choline 4, 2.5 mol % was added prior to or post
vesicle formation. When using carboxyfluorescein, 5 mol % was added prior to or post
vesicle formation (see E.S.I.). For gel filtration we employed Bio Rad Econo-Pac® 10
DG 10 mL Disposable Chromatography Columns and for dialysis Spectra/Por® Dialysis
Membrane, MWCO: 6-8,000, Nominal Flat Width: 10 mm, Diameter: 6.4 mm,
Vol/Length: 0.32 mL/cm, Lot # 3248269, using a minimum of 3 buffer exchanges.
Deepwell slides were prepared using 80 μL of GUV solutions. Slides were viewed on an
inverted Olympus Fluoview 1000 confocal laser scanning system utilizing a Olympus IX-
81 microscope with a 40x oil objective and 2x digital zoom. The dyes were excited by a
488 nm argon ion laser, and emission was captured at 559 nm. The dimensions of the
images taken were 1024 x 1024 pixels and a z-scan analysis was performed capturing a
minimum of 10 planes. On average vesicles imaged were 10 +/- 2 microns in diameter.
Figure 2 (from manuscript) Fluorescent Confocal images (top), DIC (bottom) (Olympus Fluoview 1000, IX-81; ex: 488; em 559; 40 X) of prepared DLPC vesicles that were then treated with 2-(Thioureidofluorescein)ethyltrimethylammonium Chloride 4 (A), DLPC vesicles that were prepared with 4 present prior to vesicle formation (B), and that were then subjected to dialysis (C). Vesicles are approximately 10 microns.

Carboxyfluorescein (as referenced in manuscript) Fluorescent Confocal images (Olympus Fluoview 1000, IX-81; ex: 488; em 559; 40 X) of prepared DLPC vesicles that were treated with carboxyfluorescein (A), and DLPC vesicles that were prepared with carboxyfluorescein that were then subjected to size exclusion gel chromatography (B). Vesicles are approximately 10 microns.
Figure 3 (from manuscript) Fluorescent Confocal images (top), DIC (bottom) (Olympus Fluoview 1000, IX-81; ex: 488; em 559; 40 X) of prepared DLPC vesicles that were treated with 1 % cyclohexylamine-NBD (A), and DLPC vesicles that were prepared with 1 % 1-acyl-2-(12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl)-sn-glycero-3-phosphocholine (B). Vesicles are approximately 10 microns.
Figure 4 (additional images) Fluorescent Confocal images (Olympus Fluoview 1000, IX-81; ex: 488; em 559; 40 X) of prepared DLPC vesicles that had 1 mol % of Cavitand 3 present, differential interference contrast image (top frames), and fluorescent image (bottom frames). Z-scan analysis with dimensions reported, each slice represents a 1.18 micron adjustment in the z-scan height.
References (numbered as found in E.S.I.):