Recognition and sequestration of ω-fatty acids by a cavitand receptor

Simone Mosca\textsuperscript{a,b,c}, Dariush Ajami\textsuperscript{b}, and Julius Rebek\textsuperscript{a,b,1}

\textsuperscript{a}Department of Chemistry, Fudan University, Shanghai 200433, China; \textsuperscript{b}The Skaggs Institute for Chemical Biology and Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037; \textsuperscript{c}Department of Biotechnology and Biosciences, University of Milano-Bicocca, 20126 Milano, Italy.

\textsuperscript{1}To whom correspondence should be addressed. Email: jrebek@scripps.edu.

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**General Procedures.** Reactions were performed under a positive pressure of nitrogen, unless otherwise noted. Moisture-sensitive liquids were transferred via glass syringe. Organic solutions were concentrated by rotary evaporation below 55 °C at ca. 25 Torr. Reactions were monitored using reversed-phase HPLC (RP-HPLC). For the NMR spectra of complexes, the cavitands 1 or 2 were dissolved in DMSO -briefly heated up to assure complete dissolution- and the solution diluted with D$_2$O to give 1 mM concentration of cavitand in 5% (vol/vol) DMSO in D$_2$O. In the case of ω-fatty acids and anandamide, the spectra were collected after brief shaking. For the guests 1-adamantanecarbonitrile and n-alkanes, the solutions of the cavitand were overlayered with excess guest and sonicated overnight at 45°C before the spectra were recorded. All molecular modeling and semi-empirical calculations were performed using Guassian 09 (1). All cavitand/guests complexes were optimized - while cavitand coordinates were frozen - first with semi-empirical AM1 and in some cases were further optimized with DFT method B3LYP/6-31G*. The DeepView - Swiss-PdbViewer software was used to identify and calculate volume of cavity.

**Instrumentation.** $^1$H and $^{13}$C NMR spectra were obtained at 600 MHz and 151 MHz on a Bruker DRX-600 spectrometer equipped with a 5 mm DCH cryoprobe. Spectra were recorded at 298 K unless otherwise stated. Chemical shifts are expressed in parts per million ($\delta$ scale) with respect to tetramethylsilane and are referenced to the proton signal of residual, non-deuterated solvent [CHCl$_3$: $\delta$ 7.260 for $^1$H NMR, 77.16 for $^{13}$C NMR; DMSO $\delta$ 2.50 for $^1$H NMR 39.52 for $^{13}$C NMR; D$_2$O: $\delta$ 4.63 for $^1$H NMR. For the mixture of 5% (vol/vol) DMSO-d$_6$ in D$_2$O employed in the binding experiments, the DMSO proton signal was selected as reference and fixed at the average value measured for the fatty acid and alkane series]. Data are presented as follows: 1) chemical shift, 2) multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet and/or multiple resonances, br = broadened signal, app = apparent splitting pattern), 3) coupling constant (Hz), 4) integration. Infrared spectra were recorded as thin films on a Avatar 360 FT-IR spectrophotometer. The following abbreviations are used to indicate the intensities: s = strong, m = middle, w = weak. Analytical RP-HPLC was performed with 214.4 nm and 254.4 nm UV detection on C8 (Agilent Zorbax 300 SB-C8 5 µm 4.6 × 50 mm) analytical columns and a flow rate of 0.5 mL/min. Eluent: (A) water + 0.1% formic acid (FA); (B) acetonitrile (MeCN) + 0.1% FA. The purity was determined by integration of the UV-signal with the software OpenLab for LC from Agilent Technologies. Mass spectrometry was performed at the Scripps Center for Metabolomics and Mass Spectrometry with an Agilent ESI-TOF instrument.

**Materials.** Commercial grade reagents and solvents were used as purchased without additional purification. NMR solvents CDC$_3$, dimethylsulfoxide-d$_6$, acetone-d$_6$ and D$_2$O (100% d, ampules), were purchased from Cambridge Isotope Laboratories, Inc. All other reagents and solvents were purchased from commercial suppliers.
Synthetic Procedures

Scheme S1. Synthesis of the cavitand 1 and the cavitand 2.

Synthesis of the cavitand 1.

Compound b. To an ice-cold solution of nitrobenzoyl chloride (0.629 g, 3.39 mmol, 8.2 equiv) in AcOEt (80 mL) were added compound a (2) (0.6 g, 0.41 mmol, 1 equiv) and slowly an aqueous solution (80 mL) of K₂CO₃ (20 g). The mixture was degassed by brief exposure to vacuum and stirred vigorously at room temperature for 3 days under a nitrogen atmosphere. After NaCl (12 g) was added to the yellow suspension. The organic layer was separated and allowed to stand at 4°C overnight. The suspended solid was collected by vacuum filtration and washed with H₂O (2 × 10 mL), 0°C cold MeOH (3 × 10 mL), and Et₂O (3 × 10 mL). After drying in vacuo compound b was obtained as light yellow flaky solid (0.67 g, 0.28 mmol, 67%). ¹H NMR [600 MHz, 5% (vol/vol) D₂O in aceton-d₆] δ 8.13 (br s, 8H), 8.05 (br s, 4H), 7.79 – 7.69 (m, 32H), 7.67 (br s, 4H), 5.95 (t, J = 8.2 Hz, 4H), 3.78 (t, J = 6.4 Hz, 8H), 2.65 (q, J = 8.0 Hz, 8H), 1.92 (quint, J = 6.5 Hz, 8H). ¹³C NMR [151 MHz, 5% (vol/vol) D₂O in aceton-d₆] δ 164.11, 154.87, 148.88, 148.46, 139.97, 135.27, 129.38, 128.23, 124.52, 122.47, 120.23, 116.18, 44.39, 32.88, 30.93. HRMS (ESI-TOF): Calcd for C₁₃₂H₁₃₂N₆O₁₂ [M-H⁺]+ 2399.4113, Found: 2399.4187 (monoisotopic); [M-2H⁺]+ 1199.2027 (monoisotopic).

Cavitand 1. The solution of b (0.16 g, 0.067 mmol) in 1-methylimidazole (16.7 mL) was heated at 90°C for 14 h under a nitrogen atmosphere. Then the mixture was cooled to room temperature and kept under N₂ atmosphere. Et₂O (100 mL) was added slowly through the septum whilst stirring fast. The resulting suspension was allowed to stand at to 0°C and the supernatant removed with a syringe. MeOH (80 mL) was added and the suspension was heated up to 60°C and stirred until complete dissolution was achieved. The solution was concentrated at 60°C at rotavapor until the first solid appeared and slowly added to stirred, ice-cold Et₂O (400 mL). The mixture was stirred overnight. The precipitate was collected by centrifugation, washed with Et₂O (3 × 40 mL) and then dried under high vacuum to give 1 as pale orange solid (0.136 g, 0.05 mmol, 75%). ¹H NMR [600 MHz, DMSO-d₆/D₂O 9:1 (vol/vol)] δ 9.28 (br s, 4H), 8.40 – 7.29 (m, 56H), 5.58 (br s, 4H), 4.38 (br s, 8H), 3.88 (br s, 12H), 2.71 (br s, 8H), 1.81 (br s, 8H); ¹³C NMR [151 MHz, DMSO-d₆/D₂O 9:1 (vol/vol)] δ 163.89, 154.24, 148.86, 148.45, 139.34, 136.56, 135.55, 128.87, 128.66, 126.23, 123.45, 122.98, 122.42, 121.87, 115.88, 49.07, 35.79, 33.68, 28.28; IR (film): ν = 1656 (m), 1598 (m), 1518 (s), 1484 (s), 1402 (m), 1346 (s), 1282 (s), 1186 (m), 1161 (m), 1091 (m), 1013 (w), 869 (m), 832 (cm)⁻¹; HRMS (ESI-TOF): Calcd for C₁₃₆H₁₃₆N₆O₁₈ [M-4Cl⁺]⁺ 647.1890, Found: 647.1884 (monoisotopic); [M-3Cl⁺]⁺ 874.5750, Found: 874.5754 (monoisotopic); [M-2Cl⁺]²⁺ 1329.3469, Found: 1329.3531 (monoisotopic); RP-HPLC analysis (Agilent C8 column) 2% to 98% (vol/vol) MeCN in 10 min, Tr = 9.1 min; 25% to 98% (vol/vol) MeCN in 30 min, Tr = 19.0 min.

Synthesis of the cavitand 2.

Compound c. SnCl₂ dihydrate (1,408 g, 6.24 mmol) was added to a 0°C cold suspension of compound b (0.1 g, 0.04 mmol) in EtOH (8 mL) and concentrated aqueous HCl (2 mL) was slowly added. The reaction mixture was degassed and set under a nitrogen atmosphere. The mixture was heated to 110°C for 1 h and then cooled to room temperature. EtOH was evaporated and ice-cold 3N aqueous HCl (30 mL) was added. After filtration, the solid obtained was washed with 0°C cold MeCN (3 × 5 mL), Et₂O (3 × 10 mL), and dried under vacuum, yielding compound c (102 mg, 0.04 mmol, quant) as hydrochloride that was taken directly to the next step.

Cavitand 2. The solution of c (0.12 g, 0.049 mmol) in 1-methylimidazole (12.1 mL) was heated at 90°C for 14 h under a nitrogen atmosphere, then the mixture was cooled to room temperature. The resulting suspension was taken into a syringe and added dropwise to 0°C Et₂O (100 mL) stirred under a nitrogen atmosphere. The precipitate was collected by centrifugation and washed with Et₂O (3 × 40 mL) and then MeCN (2 × 25 mL). The residue was dissolved with 60°C hot H₂O (20 mL) and after lyophilization the cavitand 2 was obtained as a pale ocher solid (0.104 g, 0.042 mmol, 86%). ¹H NMR [600 MHz, DMSO-d₆/D₂O 6:4 (vol/vol)] δ 9.02 (br s, 4H), 8.18 – 7.06 (m, 40H), 6.42 (br s, 16H), 5.63 (br s, 4H), 4.39 (br s, 8H), 3.90 (br s, 12H), 2.70 (br s, 8H), 1.87 (br s, 8H); ¹³C NMR [151 MHz, DMSO-d₆/D₂O 6:4 (vol/vol)] δ 166.27, 154.13, 151.56, 148.20, 135.79, 128.79, 125.52, 123.39, 121.89, 120.84, 119.91, 115.86, 113.28, 48.85, 35.56, 33.35, 27.91; IR (film): ν = 1603 (s), 1504 (m), 1484 (s), 1401 (m), 1280 (s), 1189 (s), 1157 (m), 1136 (m), 1090 (w), 836 (cm)⁻¹; HRMS (ESI-TOF): Calcd for C₁₃₆H₁₃₆N₆O₁₂ [M-4Cl⁺]⁴⁺ 587.2406, Found: 587.2411 (monoisotopic); [M-3Cl⁺]⁴⁺ 794.6438, Found: 794.6432 (monoisotopic); [M-2Cl⁺]²⁺ 1209.4502, Found: 1209.4463 (monoisotopic); RP-HPLC analysis (Agilent C8 column) analysis 2% to 95% (vol/vol) MeCN in 10 min, Tr = 5.8 min.
Figure S1. Top, $^1$H NMR spectrum [600 MHz, DMSO-d$_6$/D$_2$O 9:1 (vol/vol), 298 K] of the cavitand 1. The characteristic methine peak is marked with a blue dot. Bottom, $^{13}$C NMR spectrum [151 MHz, DMSO-d$_6$/D$_2$O 9:1 (vol/vol), 298 K] of 1.
Figure S2. Mass spectrum of the experimental isotope pattern of [1-4Cl]+, [1-3Cl]2+, [1-2Cl]3+.

Figure S3. RP-HPLC [C8 column; eluent A: H2O + 0.1% FA, eluent B: MeCN + 0.1% FA; Gradient: Linear 25-98% (vol/vol) B in 30 min] traces of 1, T = 19.0 min, with 214.4 nm (top) and 254.4 nm (bottom) UV detection.
Figure S4. $^1$H NMR spectrum [600 MHz, D$_2$O/DMSO-d$_6$ 95:5 (vol/vol), 298 K] of the cavitand 1 (1.0 mM); 1 features a kinetically stable (on the NMR time scale) vase conformation in aqueous solutions and broadened aryl C–H signals between 6.0 and 6.5 ppm are not observed even in absence of hydrophobic guests. The characteristic methine peak is marked with a blue dot.

Figure S5. $^1$H NMR [600 MHz, D$_2$O/DMSO-d$_6$ 95:5 (vol/vol), 298 K] spectrum of the complexes of 1 (1.0 mM) with hexadecane (C$_{16}$). The pronounced peak splitting of upfield signals for the bound long alkane guests is extended to the host structure, and the aromatic region in particular. The cavitand 1 exists in different isomeric forms that interconvert slowly on the NMR timescale; the guests in inverted J-shaped conformations can produce hydrophobic interactions with the “prestacked” aromatic system and stabilize the complex. This can reduce the number of conformers.
Figure S6. Upfield region of the $^1$H NMR [600 MHz, D$_2$O/DMSO-$d_6$ 95:5 (vol/vol), 298 K] spectrum of the complexes of 1 (1.0 mM) with heptadecyne. Only the saturated alkyl end of this asymmetric guest binds near the resorcinarene floor and shows an extended conformation for the first four carbons of the chain.

Figure S7. Upfield region of the $^1$H NMR [600 MHz, D$_2$O/DMSO-$d_6$ 95:5 (vol/vol), 298 K] spectrum of the complexes of 1 (1.0 mM) with 1-adamantanecarbonitrile.
Figure S8. Top, $^1$H NMR spectrum [600 MHz, DMSO-$d_6$/D$_2$O 6:4] of the cavitand 2. The characteristic methine peak is marked with a blue dot. Bottom, $^{13}$C NMR spectrum [151 MHz, DMSO-$d_6$/D$_2$O 6:4 (vol/vol), 298 K] of 2.

Figure S10. RP-HPLC [C8 column; eluent A: H₂O + 0.1% FA, eluent B: MeCN + 0.1% FA; Gradient: Linear 2-98% (vol/vol) B in 10 min] traces of Z, Tₓ = 5.7 min, with 214.4 nm (top) and 254.4 nm (bottom) UV detection.
Figure S11. Comparative RP-HPLC (214.4 nm UV detection on C8 column; eluent A: H2O + 0.1% FA, eluent B: MeCN + 0.1% FA; Gradient: Linear 2-98% (vol/vol) B in 10 min) traces of 1 (top, T_R = 9.1 min) and 2 (bottom, T_R = 5.7 min).
Figure S12. Top, $^1$H NMR spectrum [600 MHz, D$_2$O/DMSO-d$_6$, 95:5 (vol/vol), 298 K] of the cavitand 2 (1.0 mM); mostly the kite form is observed, with broadened aryl C−H signals and low methine C−H signal at ~ 5.5 ppm. Bottom, $^1$H NMR spectrum [600 MHz, D$_2$O/DMSO-d$_6$, 95:5 (vol/vol), 298 K] of the cavitand 2 (1.0 mM) in presence of excess 1-adamantanecarbonitrile. The cavitand 2 mainly shows the vase form. The characteristic methine peak is marked with a blue dot, the signals of the free 1-adamantanecarbonitrile with green dots, and the bound guest with red dots.
Figure S1. Left, $^1$H NMR [600 MHz, D$_2$O/DMSO-d$_6$ 95:5 (vol/vol), 298 K] spectra of (a) 2 (1.0 mM) and in presence of (b) excess 1-adamantanecarbonitrile; (c) C$_{17}$; (d) excess linoleic acid; (e) approximately stoichiometric amount of arachidonic acid; (e) excess arachidonic acid. Right, upfield region of the spectra with increased intensity to highlight the eventual presence of complexes. Only 1-adamantanecarbonitrile (b) shifts significantly the dynamic equilibrium of 2 to the vase form and produces NMR spectra with less broadened aryl C–H signals and sharpened upfield signals of bound guest.

References
