Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.
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Syn-cryptophanes: macrocyclic compounds with optimized characteristics for the design of ^{129}Xe NMR-based biosensors

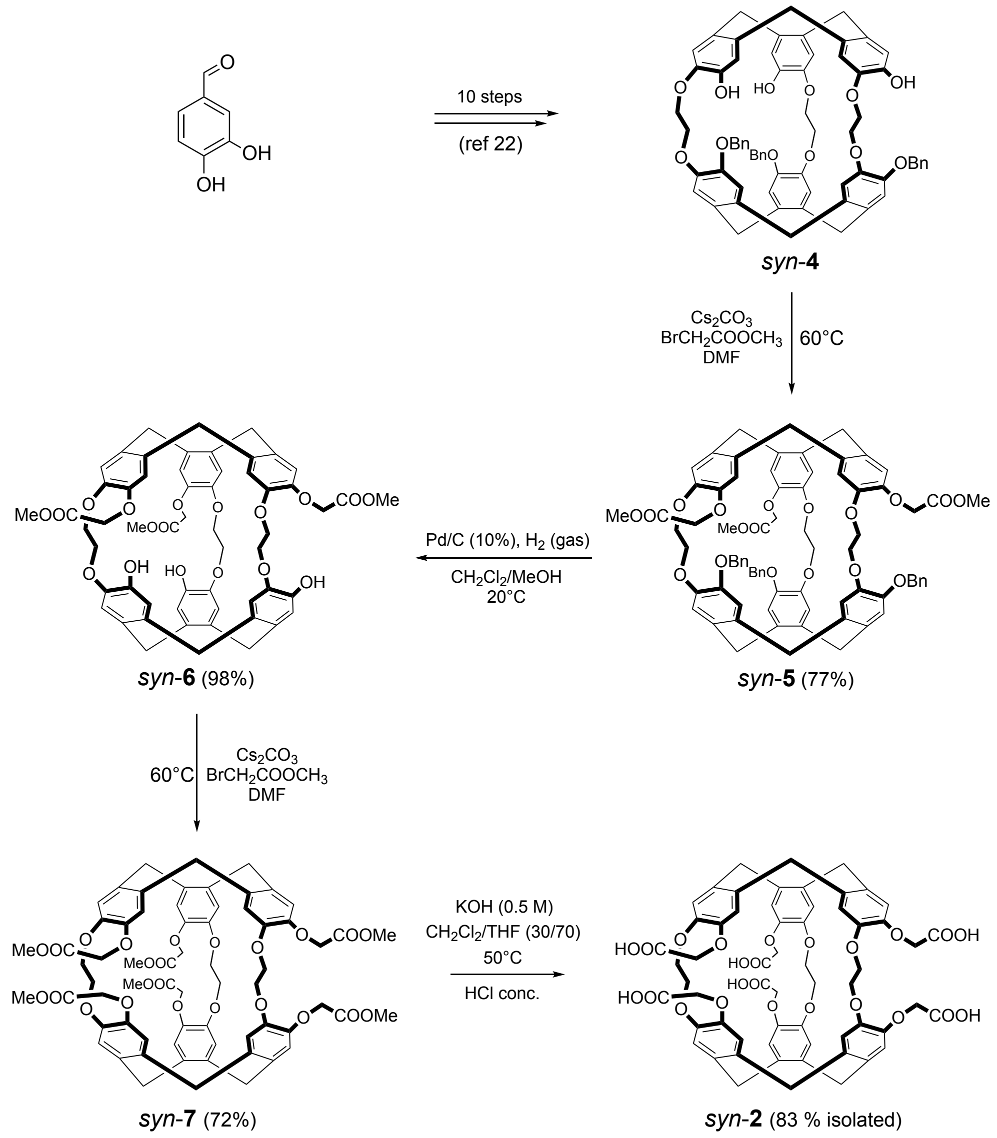
Estelle Léonce, Thierry Brotin, and Patrick Berthault

NIMBE, CEA, CNRS, Université de Paris Saclay, CEA Saclay, 91191 Gif-sur-Yvette, France.
ENSL, CNRS, Laboratoire de Chimie UMR 5182, 46 allée d'Italie, 69364 Lyon France

ELECTRONIC SUPPLEMENTARY INFORMATION

Table of Content:

Scheme S1:



Synthesis of cryptophane *syn*-**2**.

Text S2: Experimental procedures for the synthesis of cryptophanes *syn*-2, 4, 5, 6 and 7.

Mass spectra (HRMS) were performed by the Centre de Spectrométrie de Masse, University of Lyon. Analyses were performed with a hybrid quadrupole-time of flight mass spectrometer, microToF QII equipped with an Electrospray ion source. Data Analysis® 4.0 was used for instrument control, data collection, and data treatment. HRMS analyses were performed in full scan MS with a mass range from 50 to 2000 Da at an acquisition rate of 1 Hz. Transfer parameters were: RF Funnel 1, 200 V; RF Funnel 2, 200 V; hexapole, 50 V; transfer time, 70 µs and PrePulse storage time, 1 µs Before each acquisition batch, external calibration of the instrument was performed with a sodium formate clusters solution. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75.5 MHz for compound **5**-**7**. ^{1}H, ^{13}C, HMQC and HMBC NMR spectra of compound **2** were recorded at 500 and 126.7 MHz, respectively. Chemical shifts are referenced to Me_4Si (^1H, ^{13}C). Column chromatographic separations were carried out over Merck silica gel 60 (0.040-0.063 mm). Analytical thin layer chromatography (TLC) was performed on MERCK silica gel TLC plates F-254. The solvents were distilled prior to use: CH_2Cl_2 from CaH_2, THF from Na/benzophenone.

Synthesis of cryptophane *syn*-**5**: methyl-bromoacetate (0.27 g, 0.17 mL) was added under an argon atmosphere to a solution of compound *syn*-**4** (0.32 g, 0.30 mmol) and cesium carbonate (0.29 g, 0.9 mmol) in DMF (5 mL). The mixture was stirred overnight at 60°C under an argon atmosphere. Then, the mixture was poured in CH_2Cl_2 (20 mL) and water (20 mL). The aqueous phase was extracted two times with CH_2Cl_2 (10 mL). The combined organic layers were then dried over sodium sulfate, filtrated and evaporated under reduced pressure to give a residue. Purification of this residue over silica gel (CH_2Cl_2/Acetone: 90/10) gives rise to compound *syn*-**5** (C_3-symmetry) as a white glassy solid (0.3 g; 77 %). ^1H NMR (300 MHz, CD_2Cl_2, 25°C): $\delta = 7.52 - 7.30$ (m, 15 H), 6.74 (s, 3 H), 6.70 (s, 3 H), 6.69 (s, 3 H), 6.57 (s, 3 H), 5.06 (m, 6 H), 4.52 (d, $J = 13.5$ Hz, 3 H), 4.49 (d, $J = 13.5$ Hz, 3 H), 4.35 (d, $J = 16.6$ Hz, 3 H), 4.40 – 4.18 (m, 6 H), 4.15 (d, $J = 16.6$ Hz, 3 H), 4.06-3.94 (m, 6 H), 3.72 (s, 9 H), 3.32 (2×d, $J = 13.5$ Hz, 6 H). $^{13}C\{^1H\}$ NMR (75.5 MHz, CD_2Cl_2, 25°C) $\delta = 170.0$ (3 C), 149.7 (3 C), 148.9 (3 C), 147.6 (3 C), 147.4 (3 C), 138.5 (3 C), 135.1 (3 C), 135.0 (3 C), 133.5 (3 C), 133.0 (3 C), 129.1 (6 C), 128.2 (3 C), 127.4 (6 C), 122.7 (3 C), 122.4 (3 C), 117.6 (3 C), 117.2 (3 C), 71.9 (3 C), 71.1 (3 C), 70.6 (3 C), 66.6 (3 C), 52.3 (3 C), 36.6 (3 C), 36.4 (3 C). HRMS(ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{78}H_{72}O_{18}Na$ 1319.4616, found 1319.4604.

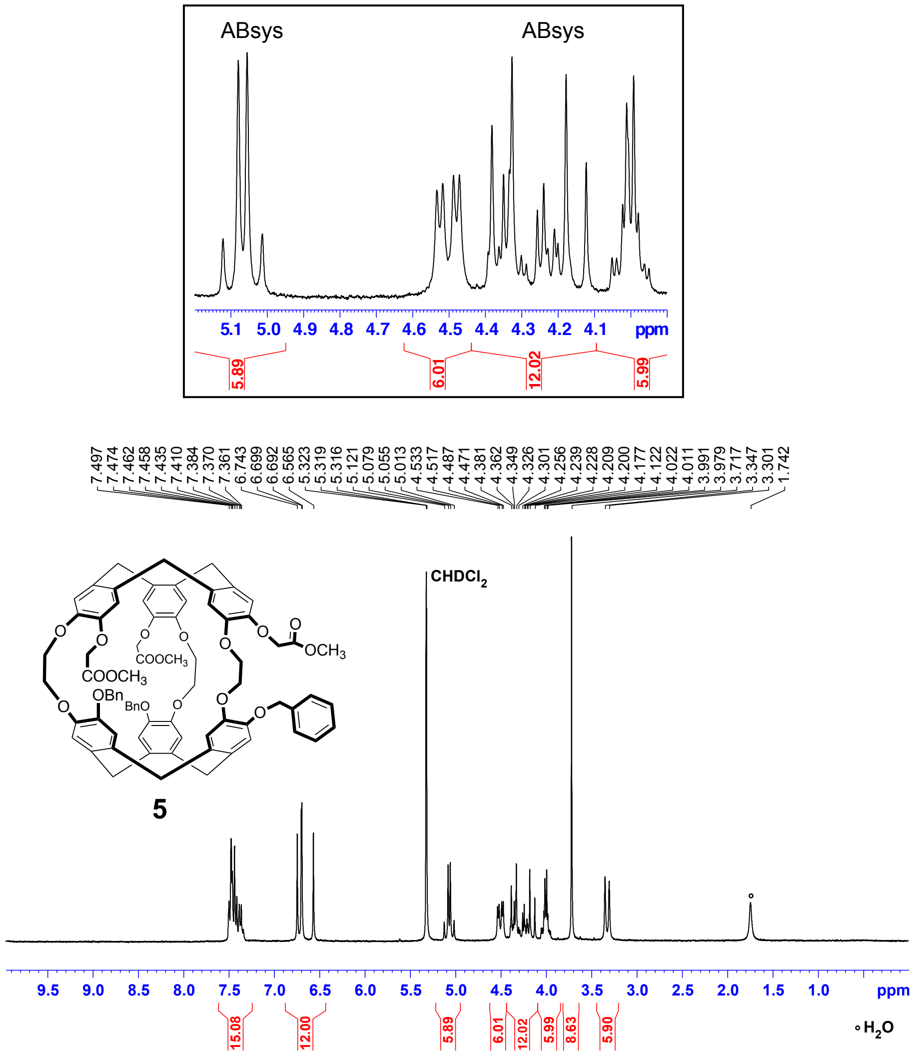
Synthesis of cryptophane *syn*-**6**: a small amount of Pd/C (10%) was added to a stirred solution of cryptophane *syn*-**5** (0.23 g, 0.18 mmol) in a mixture of CH_2Cl_2 (20 mL) and methanol (15 mL). Hydrogen gas was slowly bubbled for one hour. The mixture was then stirred for 18 hours at room temperature. The solution was filtrated over Celite and the solvents were evaporated under reduced pressure. A ^1H NMR spectrum showed complete removal of the benzyl groups under these conditions. A small silica column (CH_2Cl_2/Acetone: 90/10) and evaporation of the solvents give cryptophane *syn*-**6** (C_3-symmetry) as a white glassy solid (0.18 g, 98 %). ^1H NMR (300 MHz, DMSO-d_6, 25°C): $\delta = 7.49$ (s, 3 H), 6.95 (s, 3H), 6.87 (s, 3H), 6.74 (s, 3H), 6.68 (s, 3H), 4.64 (m, 6H, AB_{sys}), 4.52 (d, $J = 13.7$ Hz, 3 H), 4.43 (d, $J = 13.7$ Hz, 3 H), 4.30-4.00 (m, 9 H), 3.76 (m, 12 H), 3.32 (d, $J = 13.7$ Hz, 3 H), 3.23 (d, $J = 13.7$ Hz, 3 H). $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO-d_6, 25°C) $\delta = 169.7$ (3 C), 147.6 (3 C), 146.4 (3 C), 145.6 (3 C), 143.5 (3 C), 134.7 (3 C), 133.6 (3 C), 132.8 (3 C), 130.3 (3 C), 120.1 (3 C), 119.6 (3 C), 117.1 (3 C), 116.8 (3 C), 68.8 (3 C), 68.0 (3 C), 66.5 (3 C), 51.7 (3 C), 34.9 (3 C), 34.7 (3 C). HRMS(ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{57}H_{54}O_{18}Na$ 1049.3202, found 1049.3194.

Synthesis of cryptophane *syn*-**7**: methyl-bromoacetate (0.24 g, 0.15 mL) was added under an argon atmosphere to a solution of compound *syn*-**6** (0.16 g, 0.13 mmol) and cesium carbonate (0.13 g, 0.4 mmol) in DMF (4 mL). The mixture was stirred overnight at 80°C under an argon atmosphere. Then, the mixture was poured in CH_2Cl_2 (10 mL) and water (10 mL). The aqueous phase was extracted two times with CH_2Cl_2 (10 mL). The combined organic layers were washed twice with water and then dried over sodium sulfate. Filtration and evaporation of the solution under reduced pressure give *syn*-**7** as a white solid. The solid is then washed with diethyl ether on a frit to give rise to compound *syn*-**7** (C_{3h}-symmetry) as a white glassy solid (0.14 g, 72 %). ^1H NMR (300 MHz, CD_2Cl_2, 25°C): $\delta = 6.72$ (s, 6H), 6.64 (s, 6H), 4.64 (m, 12 H, AB_{sys}), 4.53 (d, $J = 13.8$ Hz, 6 H), 4.30 (m, 6 H), 3.98 (m, 6 H), 3.82 (s, 18 H), 3.36 (d, $J = 13.8$ Hz, 6 H). $^{13}C\{^1H\}$ NMR (75.5 MHz, CD_2Cl_2, 25°C) $\delta = 170.6$ (6 C), 149.0 (6 C), 147.6 (6

C), 134.9 (6 C), 133.9(6 C), 122.0 (6 C), 117.9 (6 C), 70.5 (6 C), 67.5 (6 C), 52.7 (6 C), 36.7 (6 C). HRMS(ESI-TOF) *m/z* $[M + Na]^+$ calcd for $C_{66}H_{66}O_{24}Na$ 1265.3836, found 1265.3835.

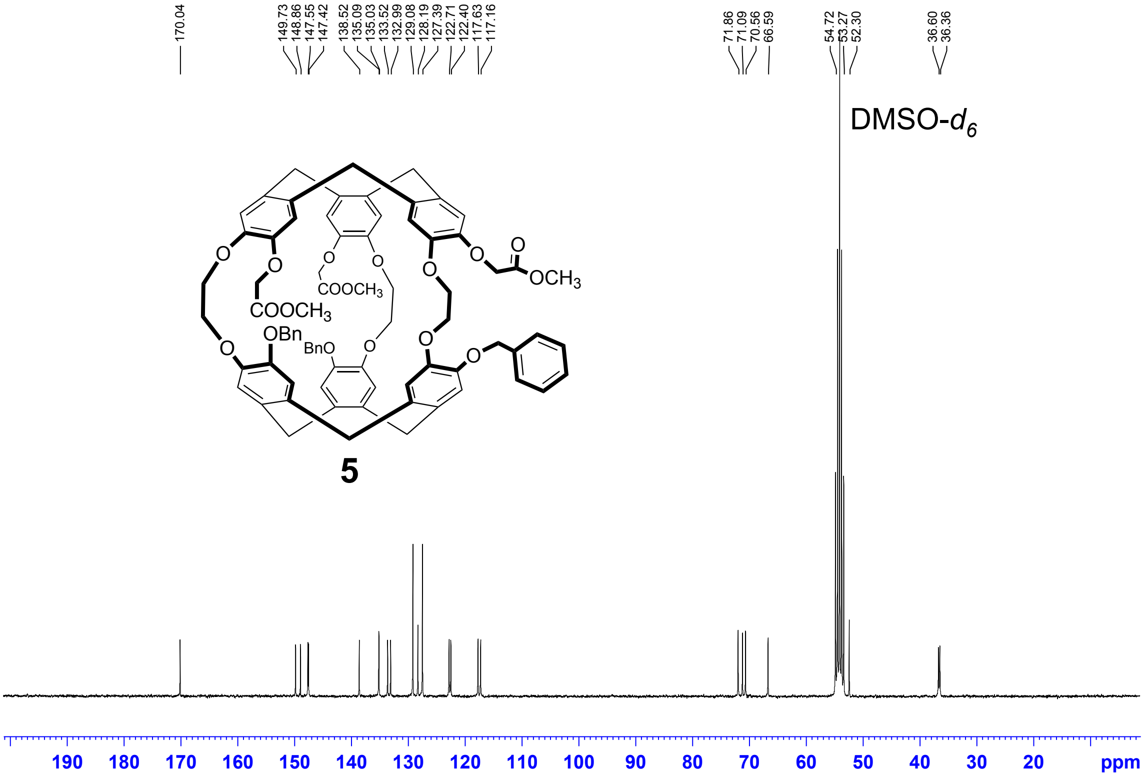
Synthesis of cryptophane *syn*-**2**: compound *syn*-**7** (0.13 g, 0.10 mmol) was dissolved in 10mL of a CH_2Cl_2/THF (7/3) mixture. The Solution was added to a 50 mL round bottom flask equipped with a reflux condenser. Then, 10 mL of a KOH (0.5 M) solution was added and mixture was stirred under argon for 18 hours at 50°C. The organic solvents were removed under reduced pression and 10 mL of water was added. The balloon was cooled at 0°C with ice and the solution was acidified with few drops of conc. HCl. The resulting white precipitate was then filtrated on a frit and washed several times with distilled water. The solid was dried on air and then washed several times with diethyl ether to give rise to compound *syn*-**2** (C_{3h}-symmetry) as a white solid (0.1 g, 83 %). See main text for its characterization.

Figure S3:



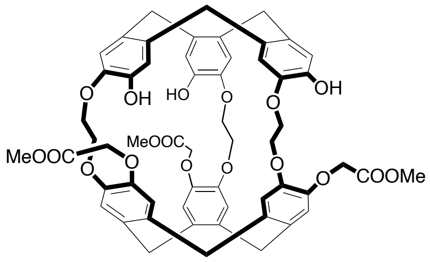
^{1}H NMR (300 MHz) spectrum of compound *syn*-**5** recorded in CD_2Cl_2 at 298 K.

Figure S4:



^{13}C{^{1}H} NMR (75.5 MHz) spectrum of compound *syn*-**5** recorded in CD_2Cl_2 at 298 K.

Figure S5:



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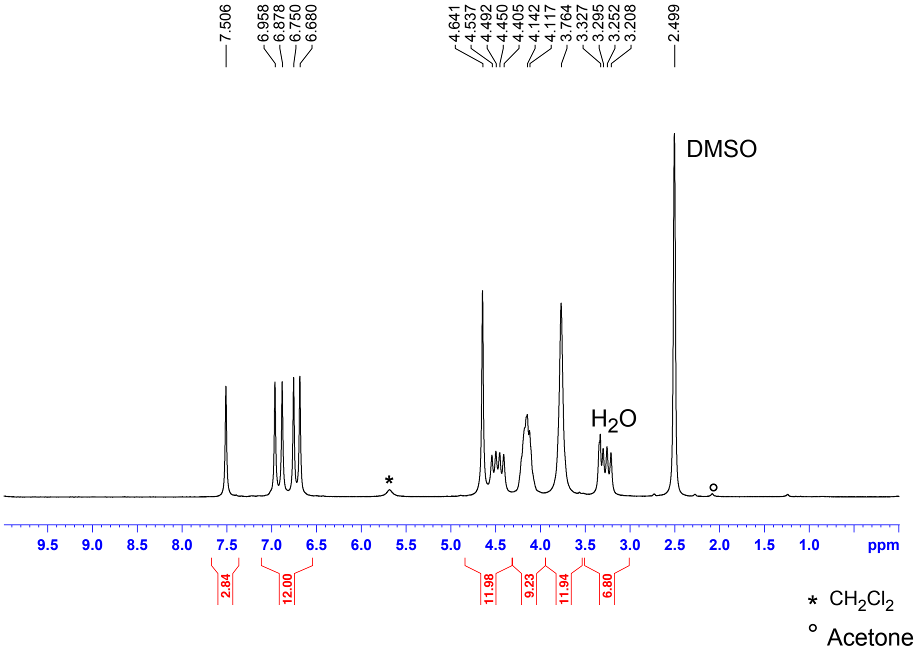
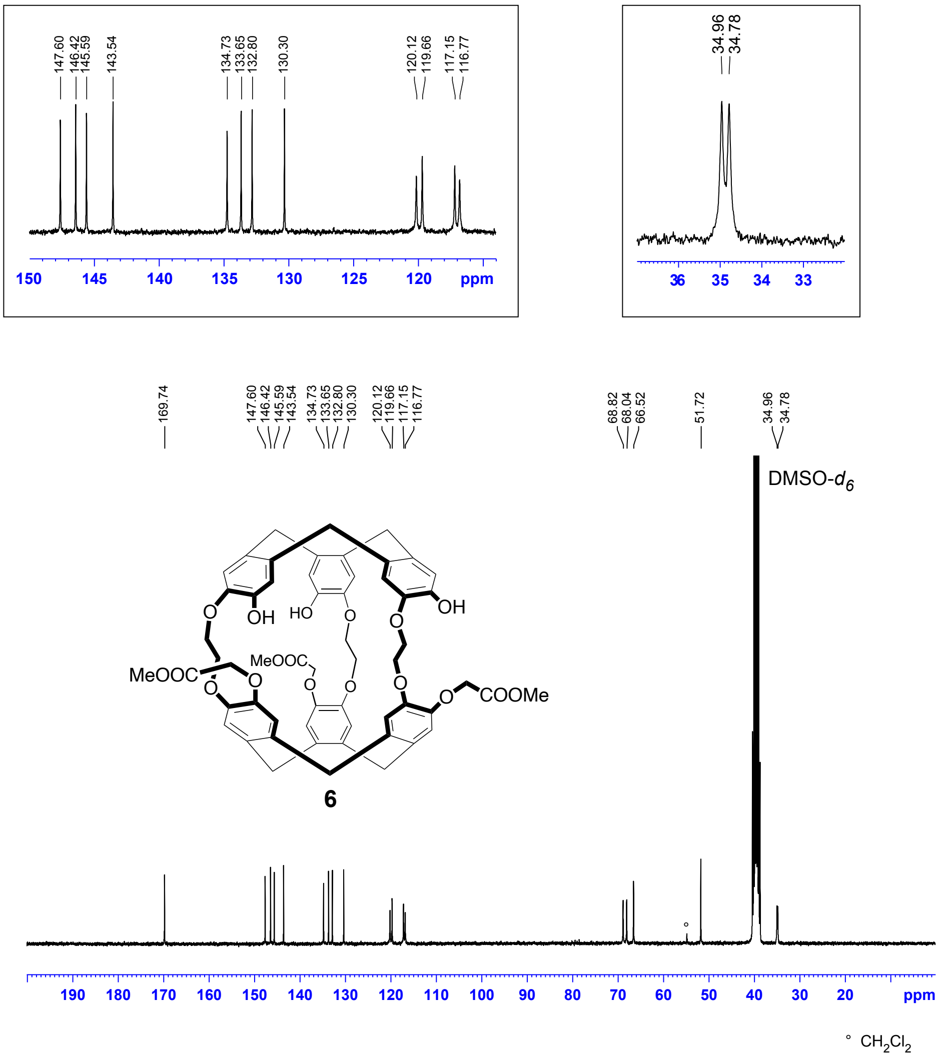


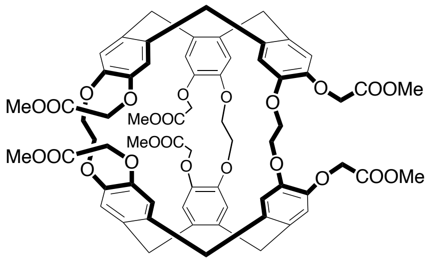
^{1}H NMR (300 MHz) spectrum of compound *syn*-**6** recorded in CD_2Cl_2 at 298 K.

Figure S6:



$^{13}C\{^1H\}$ NMR (75.5 MHz) spectrum of compound *syn*-**6** recorded in CD_2Cl_2 at 298 K.

Figure S7:



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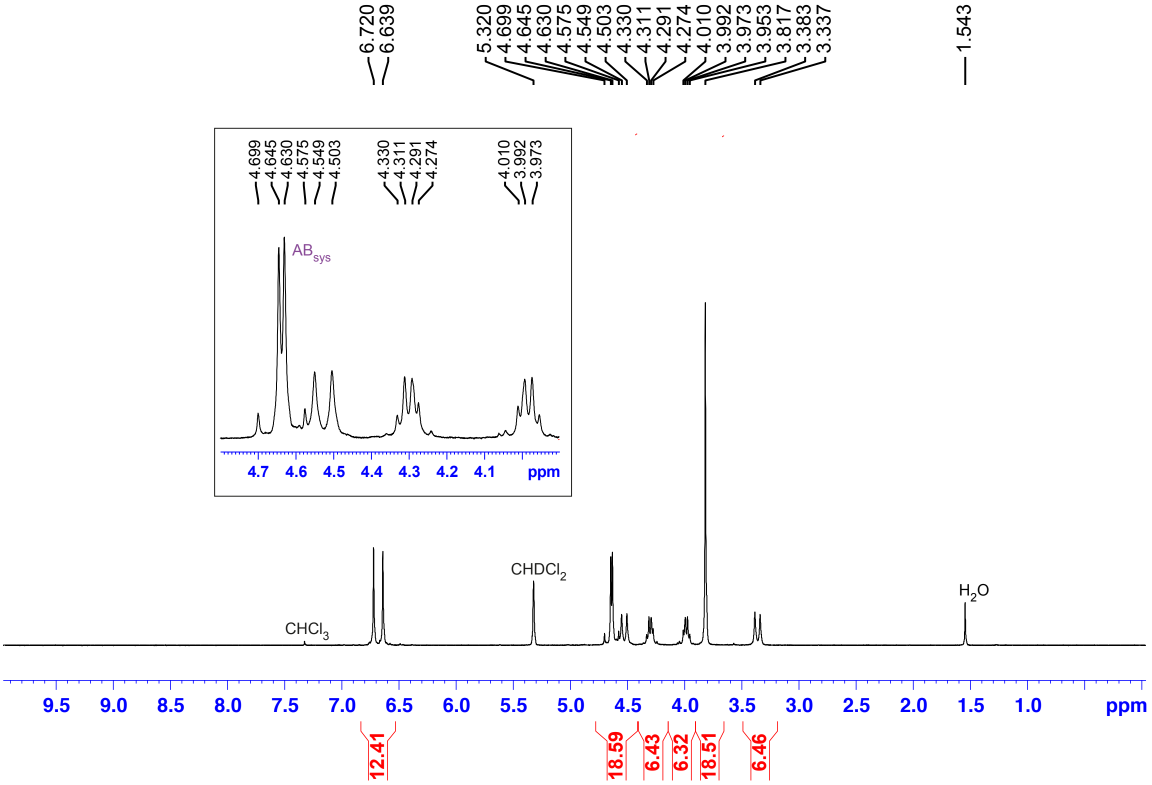
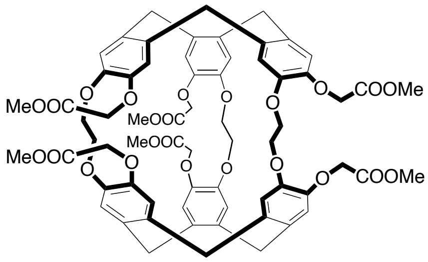


^{1}H NMR (300 MHz) spectrum of compound *syn*-**7** recorded in CD_2Cl_2 at 298 K.

Figure S8:



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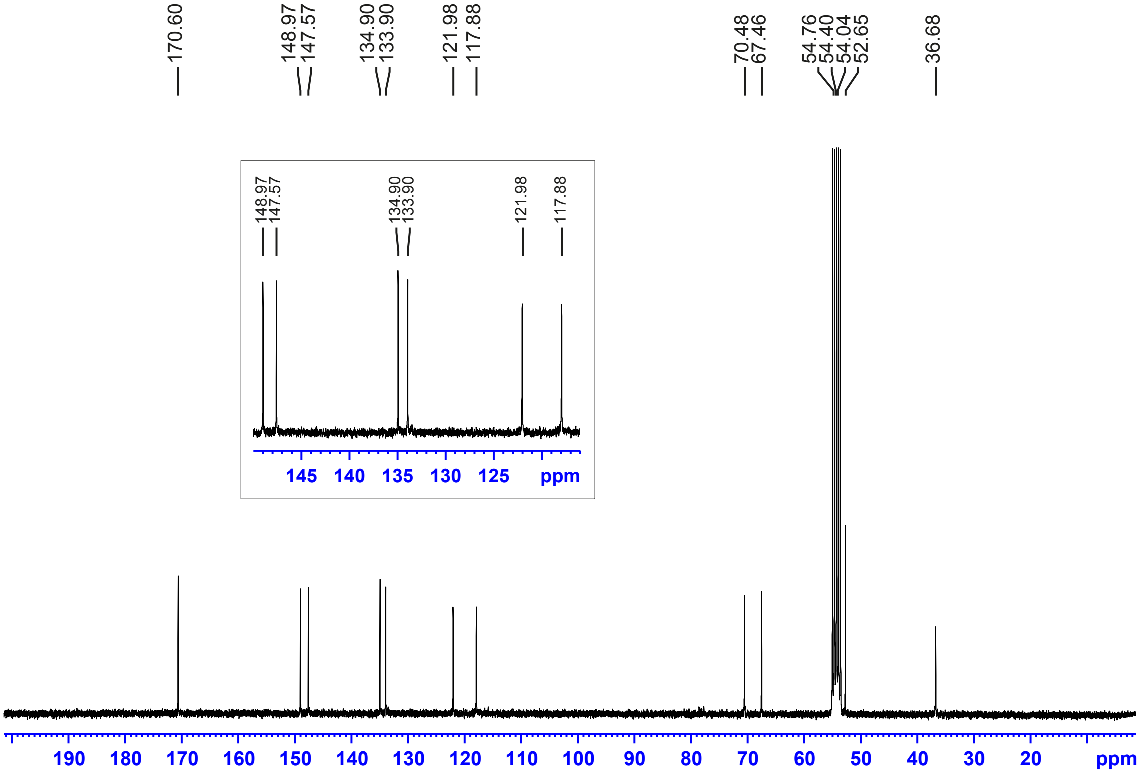
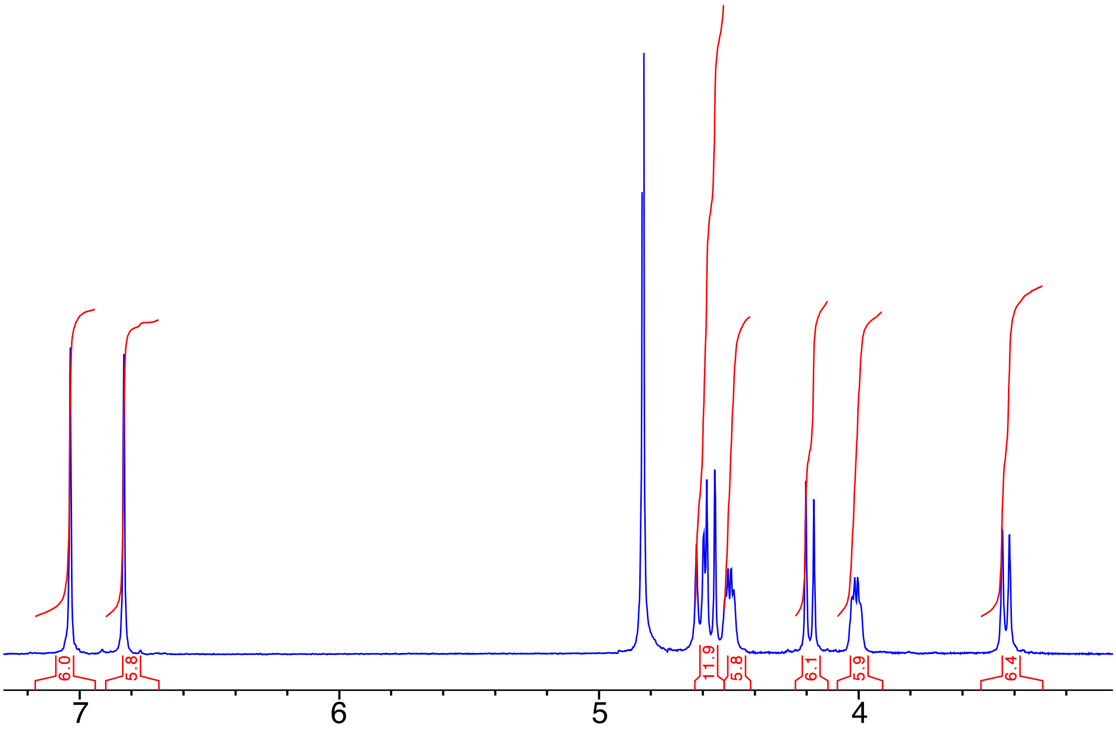


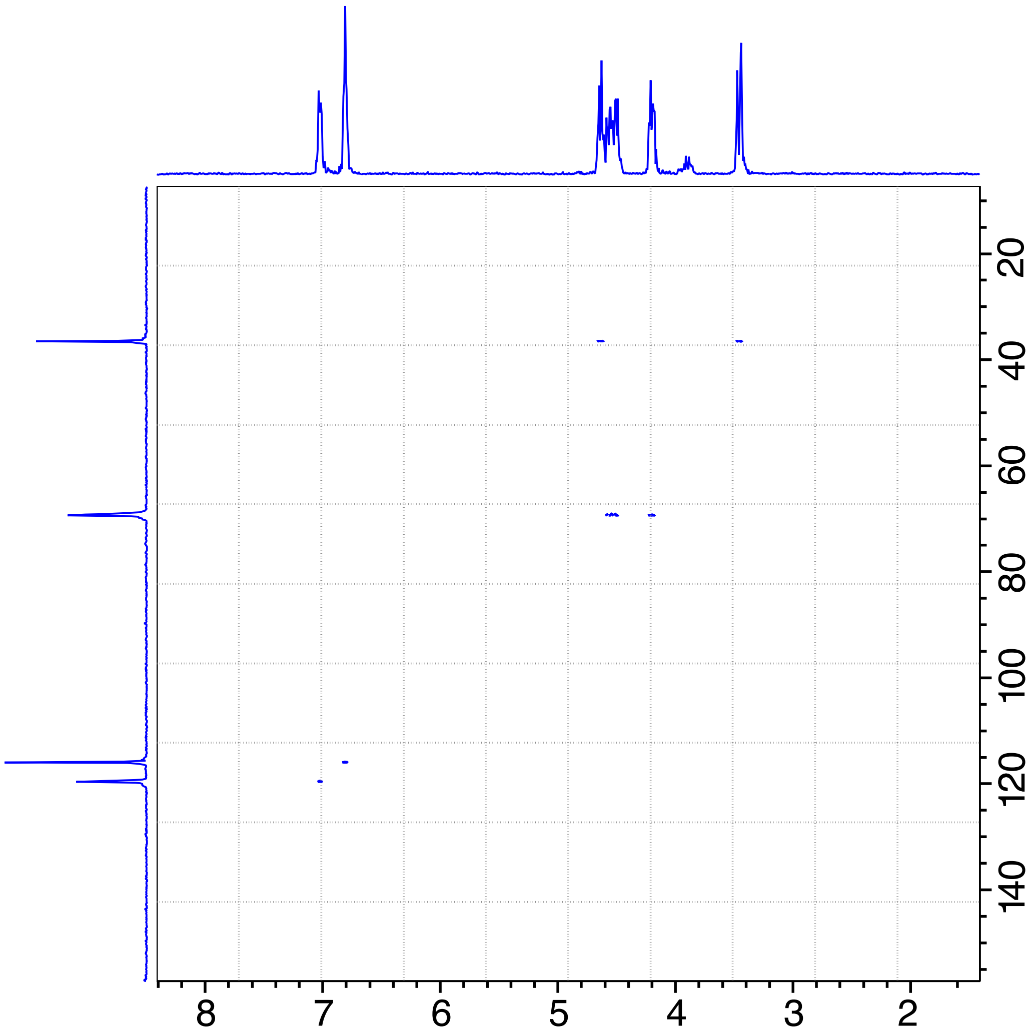
^{13}C{^{1}H} NMR (75.5 MHz) spectrum of compound *syn*-**7** recorded in CD_2Cl_2 at 298 K.

Figure S9:



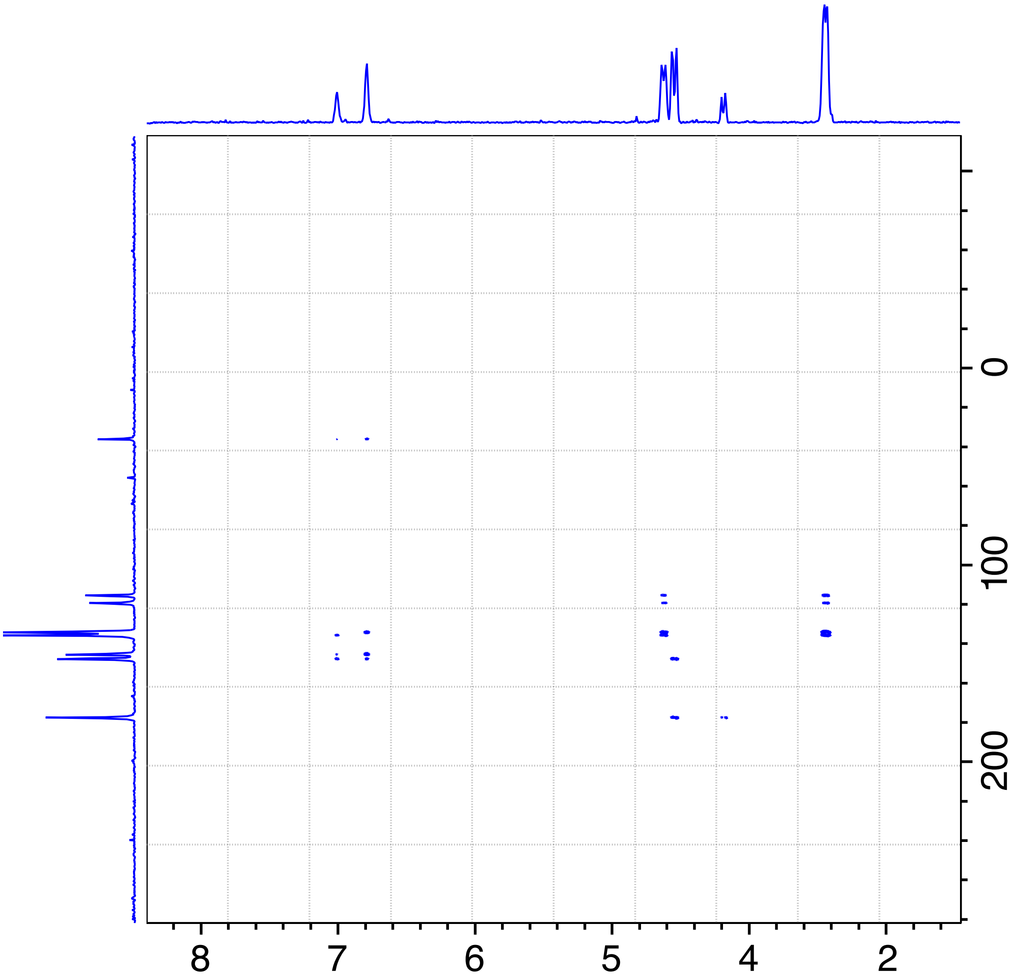
^{1}H NMR (500 MHz) spectrum of compound *syn*-**2** in D_2O at 298 K with integrals.

Figure S10:



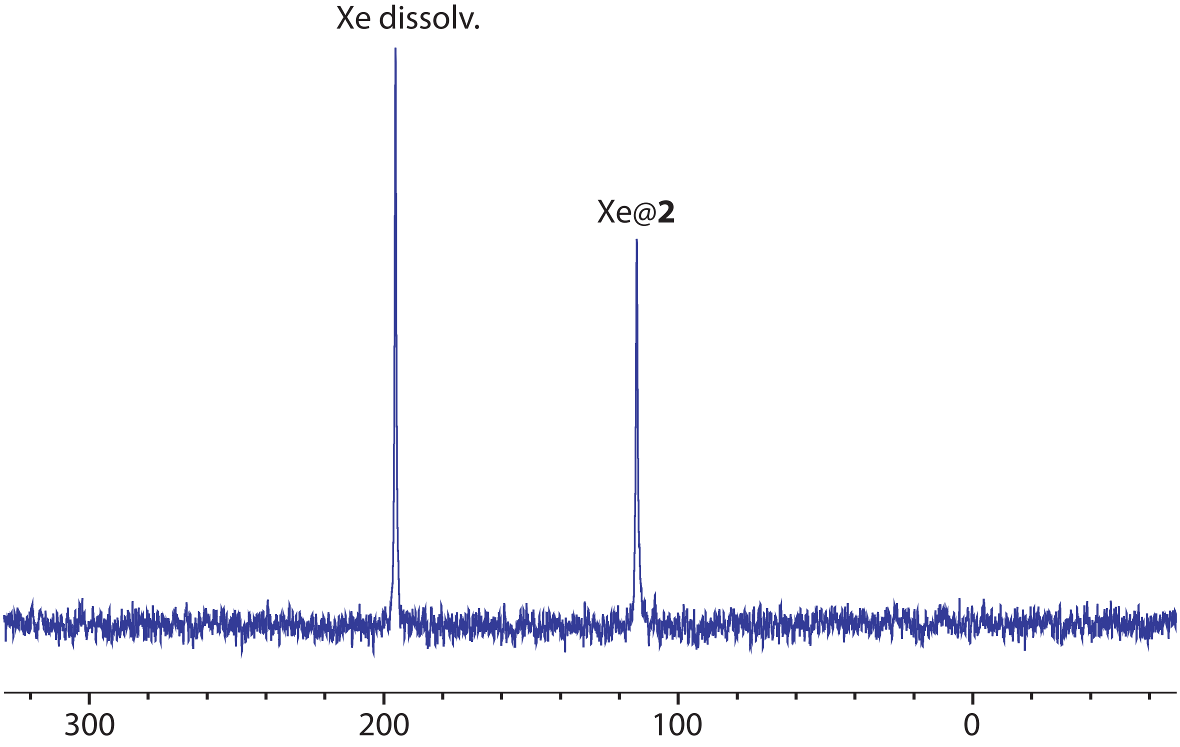
^{1}H-^{13}C HSQC of compound **2** (11.7 T, 298K).

Figure S11:



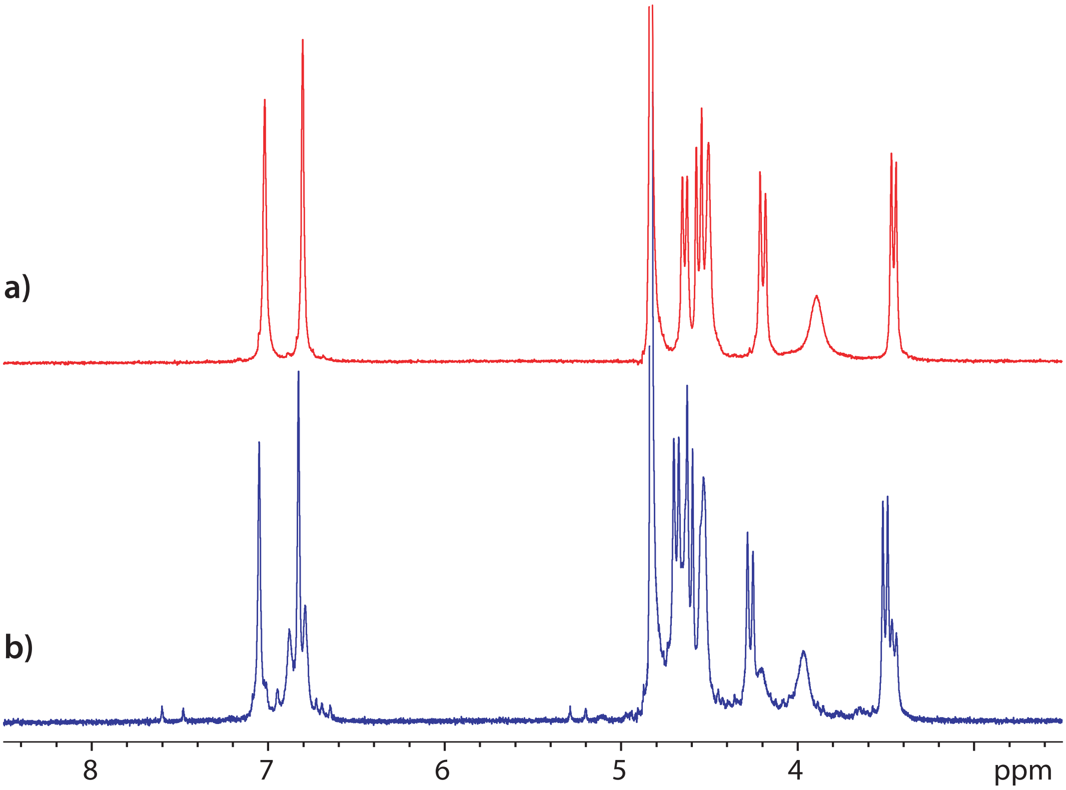
^{1}H-^{13}C HMBC of compound **2** (11.7 T, 298K).

Figure S12:



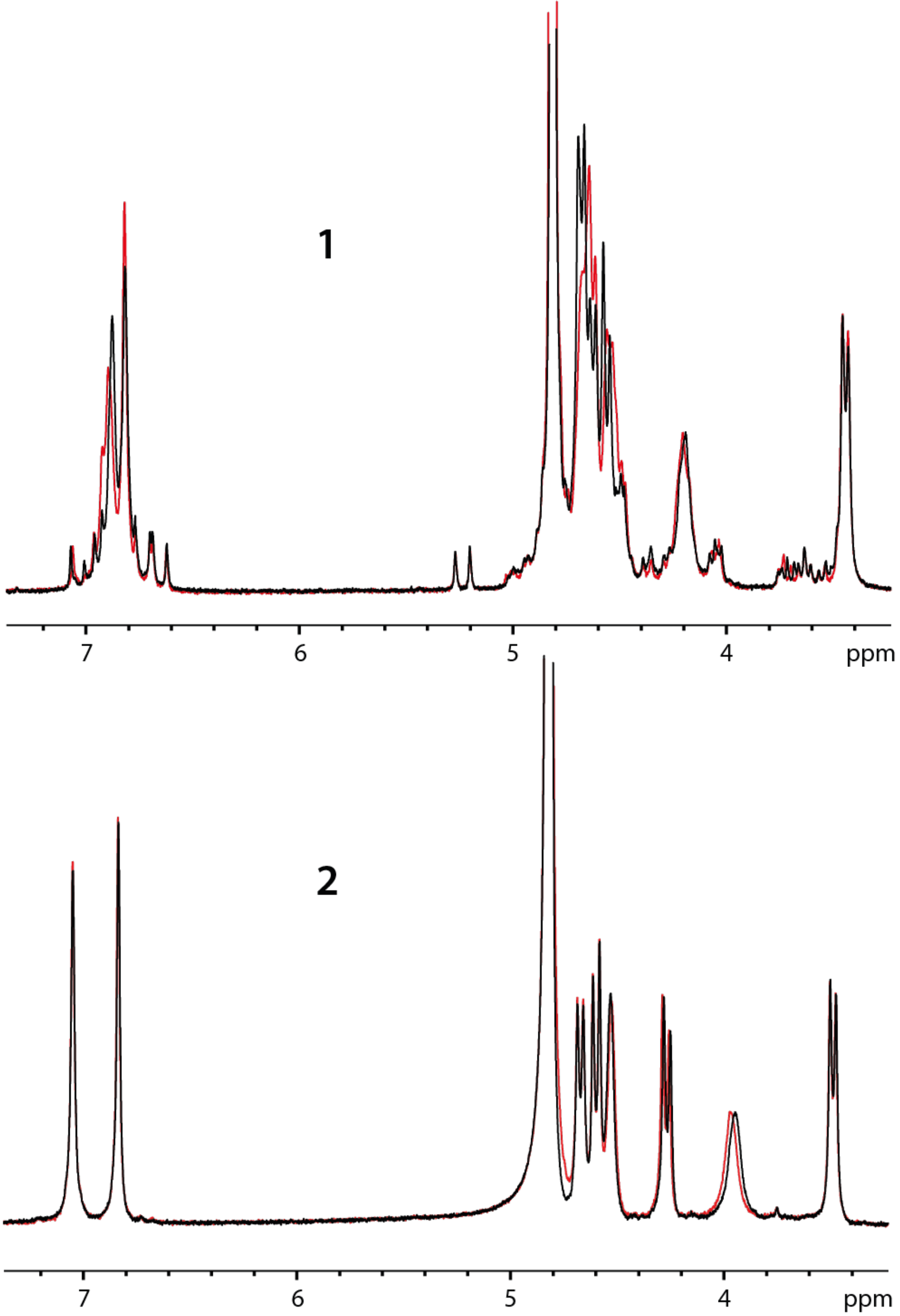
^{129}Xe NMR spectrum of a 948 µM solution of compound **2** (11.7 T, 298K) in D_2O + ε NaOD.

Figure S13:



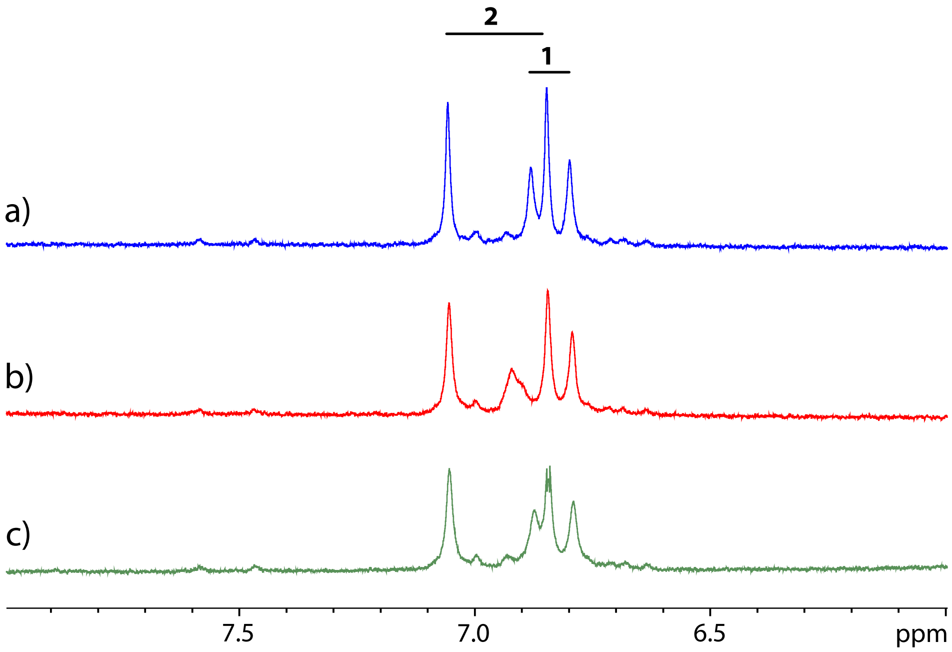
^{1}H spectrum of a) syn-cryptophane **2** in water; b) an equimolar mixture of anti-cryptophane **1** and syn-cryptophane **2** just after dissolution in D_2O + ε NaOD (11.7 T, 298K).

Figure S14:



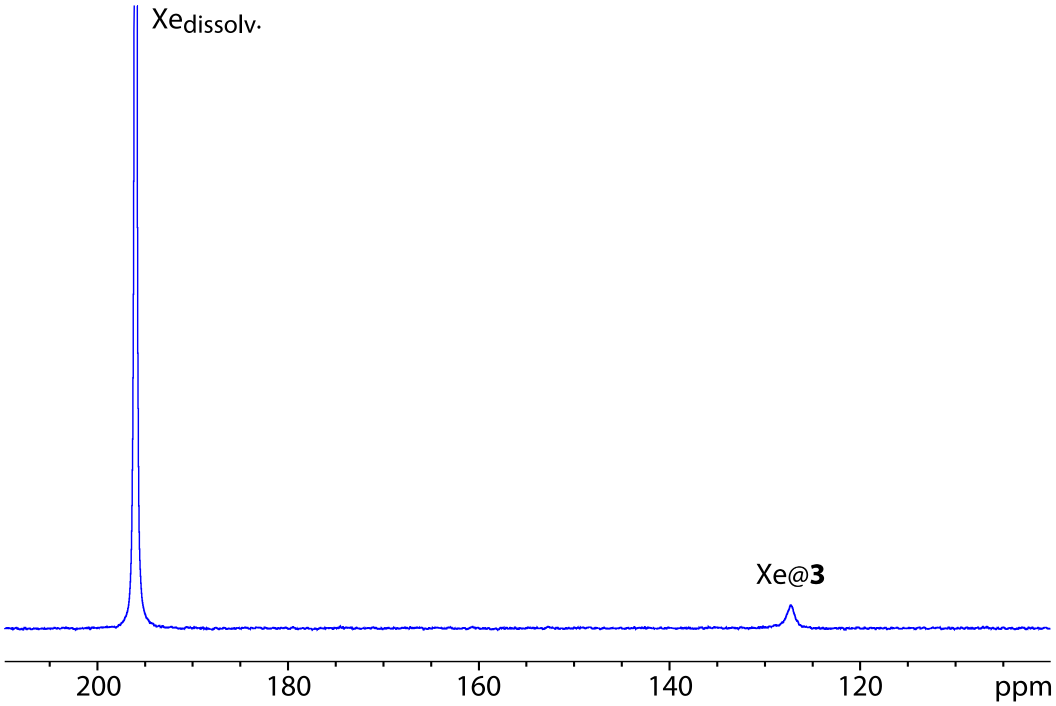
Comparison of the ^1H NMR spectra (11.7 T, 298K) of a) cryptophane **1** degassed (black) and in the presence of oxygen (red); b) cryptophane **2** degassed (black) and in the presence of oxygen (red).

Figure S15:



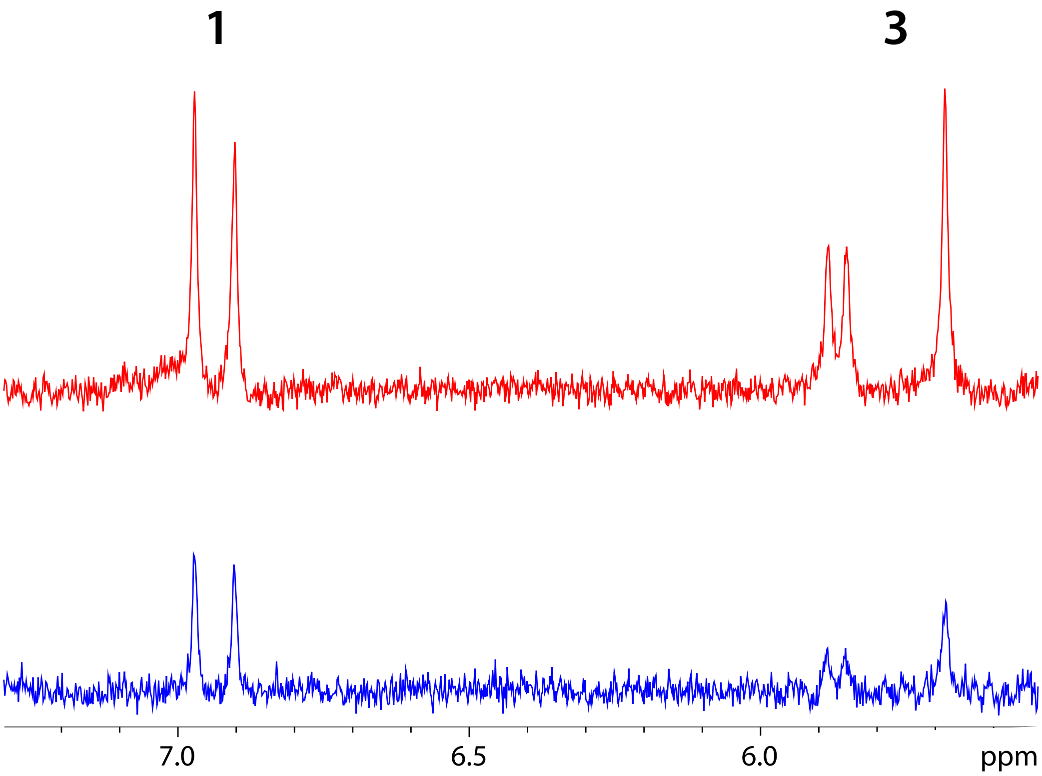
Comparison of the ^{1}H NMR spectra of a mixture of compounds **1** and **2** (11.7 T, 298K), a) just after dissolution in D_2O + ε NaOD and degassing, b) after addition of oxygen gas in the tube, c) after subsequent degassing and introduction of nitrogen in the tube. The signals of the canonical 'crown-crown' forms are indicated.

Figure S16:



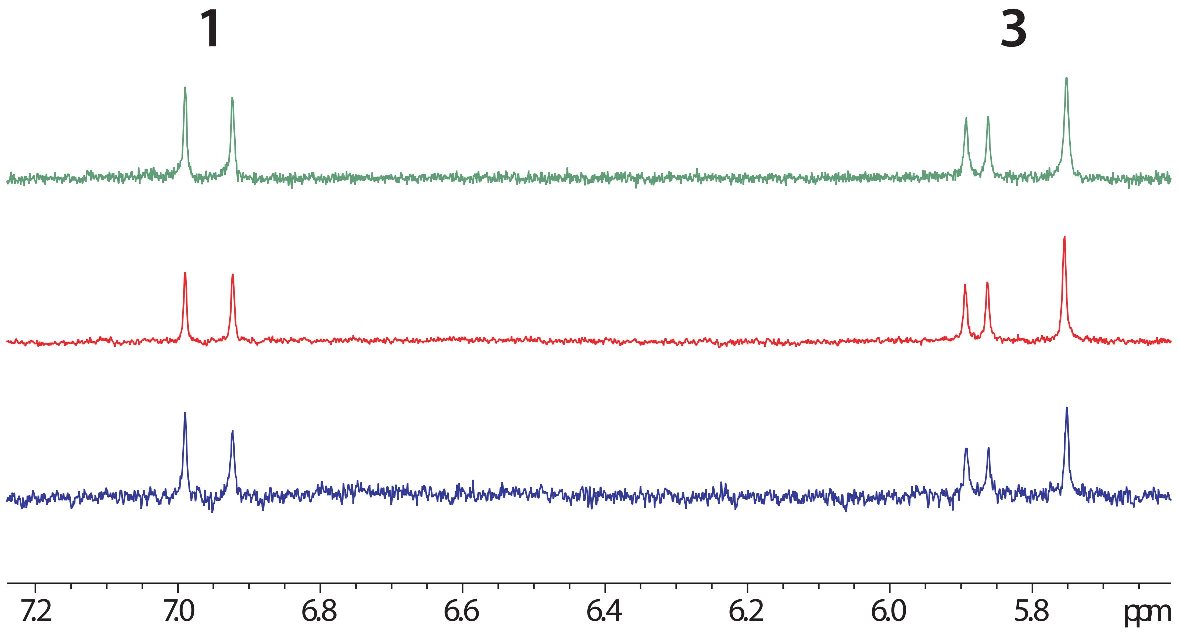
One-scan ^{129}Xe NMR spectrum of **3** at 635 µM in a solution of D_2O with Na_2SO_4 0.2 M (11.7 T, 298K).

Figure S17:



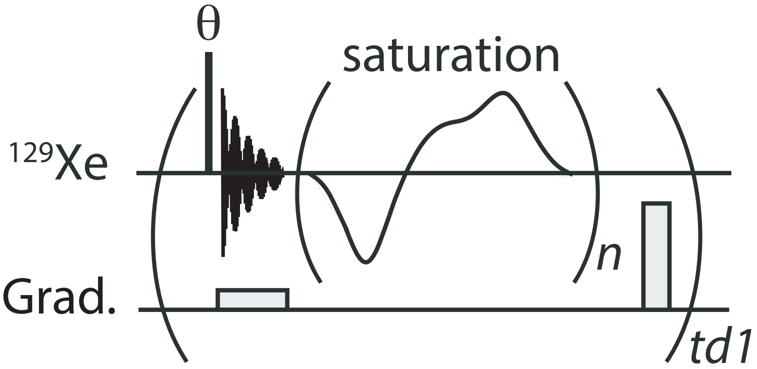
Low-field region of the ^1H NMR spectra of a mixture of **1** (3.9 µM) and **3** (3.3 µM, in red) and after dilution in D_2O by a factor 2 (blue) (11.7 T, 298K).

Figure S18:



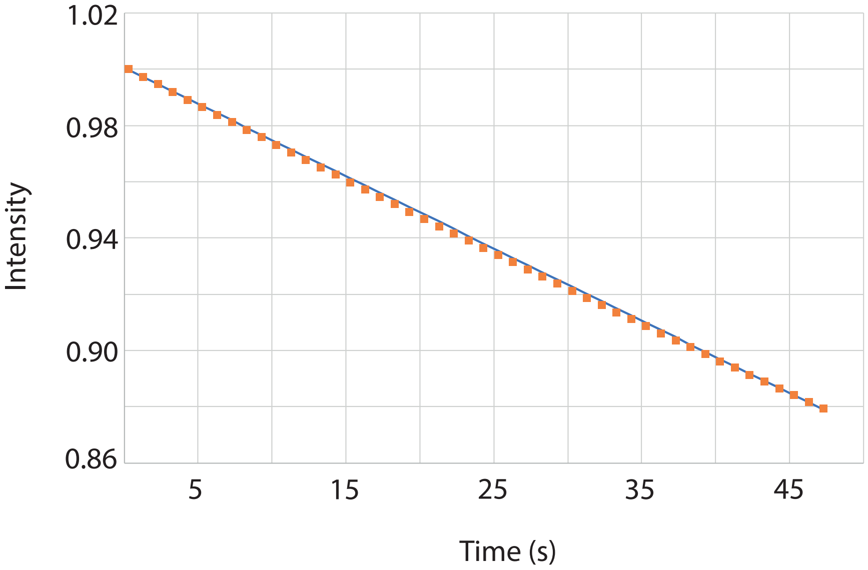
Low-field region of the ^{1}H NMR spectra of an equimolar mixture of **1** and **3** after dilution in D_2O with 0.2 M NaCl (11.7 T, 298K).

Figure S19:



^{129}Xe pulse sequence used to measure the depolarization rate. θ denotes a small (4°) flip angle pulse. The saturation is made of a succession of *n* Dsnob pulses.

Figure S20:



Simulation of the effect of the rf pulses on the ^{129}Xe magnetization (situation of the depolarization curve in the absence of exchange). The squares follow the equation $y_t = y_{t-1} \cos\theta$ for t in the range 1:48, $y_0 = 1$, and $\theta = 4°$. The blue line is the best fit straight line (R coefficient = 0.9998). Its slope is -0.00257.