Electronic Supplementary Information

Structural self-sorting of pseudopeptide homo and heterodimeric disulfide cages in water: mechanistic insights and cation sensing.

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Table of contents:

Materials and methods

All chemicals and solvents were purchased from commercial sources. NMR solvents were purchased from Deutero GmbH (Germany).

NMR spectra were acquired on a Bruker Fourier 300 spectrometer equipped with $^{1}H/^{13}C$ 5 mm DUAL EasyProbe, Bruker Ascend 600 MHz equipped with a $^{1}H/^{13}C$ 5 mm probe and Bruker AVANCE III 700MHz equipped with a $^{1}H/^{13}C$ 5 mm probe, and referenced on solvent residual peaks or TMSP-Na internal reference for D_2O measurements.

ESI-MS spectra were recorded on a Bruker Impact HD Q-TOF spectrometer. HPLC measurements were performed on a Hewlett Packard 1050 Series HPLC system coupled to a diode array detector.

LC-MS measurement were performed on a UHPLC UltiMate 3000 Thermo Scientific/Dionex conjugated with Bruker Impact HD Q-TOF spectrometer. All LC separations were performed on a Symmetry C8 Column, 100Å, 5 µm, 4.6 mm X 250 mm, 1/pkg, with a flow rate 1 mL/min in a solvent gradient of 40% MeOH in 60% H_2O to 100% MeOH in 20 min. Solvents (water and MeOH) were acidified with 0.1% HCOOH. Chromatograms were monitored using 254 nm absorption.

The semipreparative separations for obtaining heterodimeric cages were performed on a Eurospher II 100-5, column C8, 250x8mm, with a flow rate 5 mL/min. in a solvent gradient of 40% MeOH in 60% H_2O to 100% MeOH in 20 min.

A typical analytical DCL was prepared in a 0.5 mL scale by dissolving an equimolar mixture of thiol components (5 mM) in 10 mM aqueous NaOH, followed by titration with 100 mM aqueous 0.1M NaOH/HCl to pH = 8. The DCL was stirred in a close-capped HPLC vial at room temperature until being analysed after 5 days. The pH of each library was checked before and after equilibration process to make sure it remained unchanged. The HPLC traces remained unchanged after 7 days indicating that a final state had been reached.

Due to the high structural diversity in the chromophores of the three building blocks, the molar extinction coefficients were determined for each of them. The appropriate conversion factor was then used in quantitative calculations, to obtain the real amount of species in each DCL.

The UV-Vis measurements were performed on a Jasco V750 Spectrophotometer in 1cm quartz cuvettes.

The emission spectra were recorded on an Agilent Cary Eclipse fluorescence spectrophotometer in 1cm quartz cuvettes with 345 nm excitation wavelength, with 5 mm slits and PMT 550 V.

The solid-state emission spectra were recorded on a Hitachi F-7000 FL Spectrophotometer in solid sample holder with 345 nm excitation wave, on 2.5 mm slits open and PMT 600 V.

The ICP-MS measurements were performed on a NexION 300D instrument. A 0.0308 g sample was dissolved in the mineralizer, aqua-regia. Then it was transferred quantitatively to a 250 mL volumetric flask and diluted to volume with distilled water.

The NCHS elemental analysis was performed on a Thermo Scientific FLASH 2000 instrument. Each sample was measured twice.

TGA/DGA scans were performed on a PerkinElmer TGA 4000 instrument in the 30 – 600°C range.

Synthesis and characterisation of thiol components

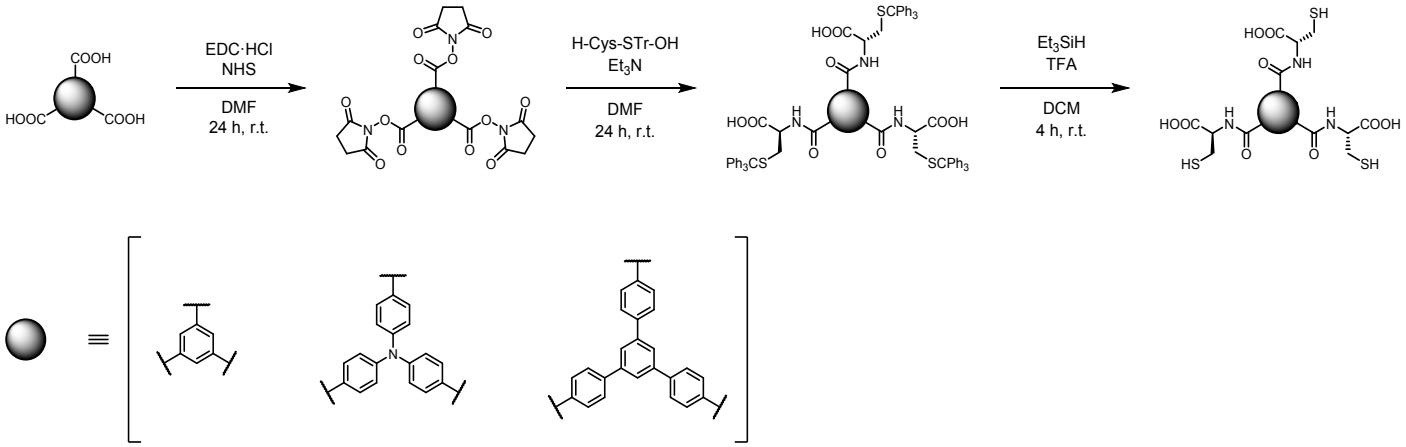
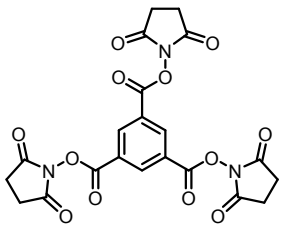


Figure S1: The main scheme for the synthesis of thiol components and structures of the organic platforms used. General synthesis methods were taken from previously reported work.[1]

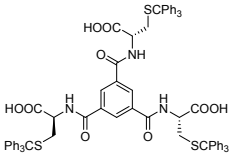
Synthesis of **1_AE**.



1,3,5-benzenetricarboxylic acid (210 mg, 1 mmol, 1 equiv.), N-hydroxy succinimide (NHS) (580 mg, 5 mmol, 5 equiv.) and EDC·HCl (960 mg, 5 mmol, 5 equiv.) were dissolved in anhydrous DMF (30 mL) and stirred at room temperature for 24h under argon atmosphere. The solvent was removed and acetone (2 mL) was added into the oily residue and stirred to give a clear solution before adding it to 1M HCl (100 mL). A white solid precipitated. It was filtered off, washed with H_2O and Et_2O, and dried under high vacuum. Yield 84%.

^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.93 (s, 3H), 2.92 (s, 12H). **^{13}C NMR** (125.75 MHz, DMSO-d_6) δ: 170.33, 160.12, 136.72, 127.77, 25.99. **ESI-MS**: m/z calc. for $[M-H]^-$ 501.0661, found 501.0511.

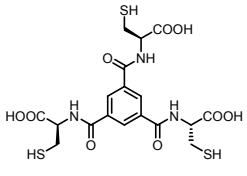
Synthesis of **1_STr**.



Activated ester **1_AE** (501 mg, 1 mmol, 1 equiv.) and *L*-Cys-STr-OH (1820 mg, 5 mmol, 5 equiv.) were dissolved in anhydrous DMF (80 mL), and Et_3N (1.0 mL, excess) was added. The reaction mixture was stirred for 24 h at room temperature before the solvent was removed and acetone (2 mL) added to the oily residue. The resulting solution was poured dropwise into 1M HCl (100 mL) causing precipitation of a white solid. It was filtered off, washed with H_2O, and dried under high vacuum. Yield 72%.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.83 (s, 3H), 9.30 – 9.01 (d, 3H), 7.35 – 7.20 (m, 45H), 4.35 (m, 3H), 2.81 (m, 3H), 2.57 (m, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ: 171.54, 144.26, 129.12, 128.11, 126.84, 66.39, 52.34, 39.52. **ESI-MS**: m/z calc. for: $[M-H]^-$ 1244.3650, found 1244.9631.

Synthesis of **1**.



The **1_STr** (1245 mg, 1 mmol, 1 equiv.) was placed in an argon-purged flask and dissolved in 10 mL of DCM. 3 mL of TFA was added and after 30 min. of stirring, Et_3SiH (1.0 mL, 6 mmol, 6. equiv.) was added and stirring was continued for an additional 5 h. The liquids were removed under high vacuum and the solid residue suspended in 10 mL of Et_2O, sonicated and filtered off. It was washed well with additional portions of Et_2O (5 x 20 mL) and dried under high vacuum. Yield 93%.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 12.95 (s, 3H), 9.04 (d, *J* = 7.8 Hz, 3H), 8.52 (s, 3H), 4.58 (td, *J* = 8.4, 4.5 Hz, 3H), 3.12 – 2.82 (m, 6H), 2.62 (t, *J* = 8.4 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ: 171.65, 165.84, 134.42, 129.37, 55.73, 39.52, 25.12. **ESI-MS**: m/z calc. for: $[M-H]^-$ 518.1286, found 518.0360.

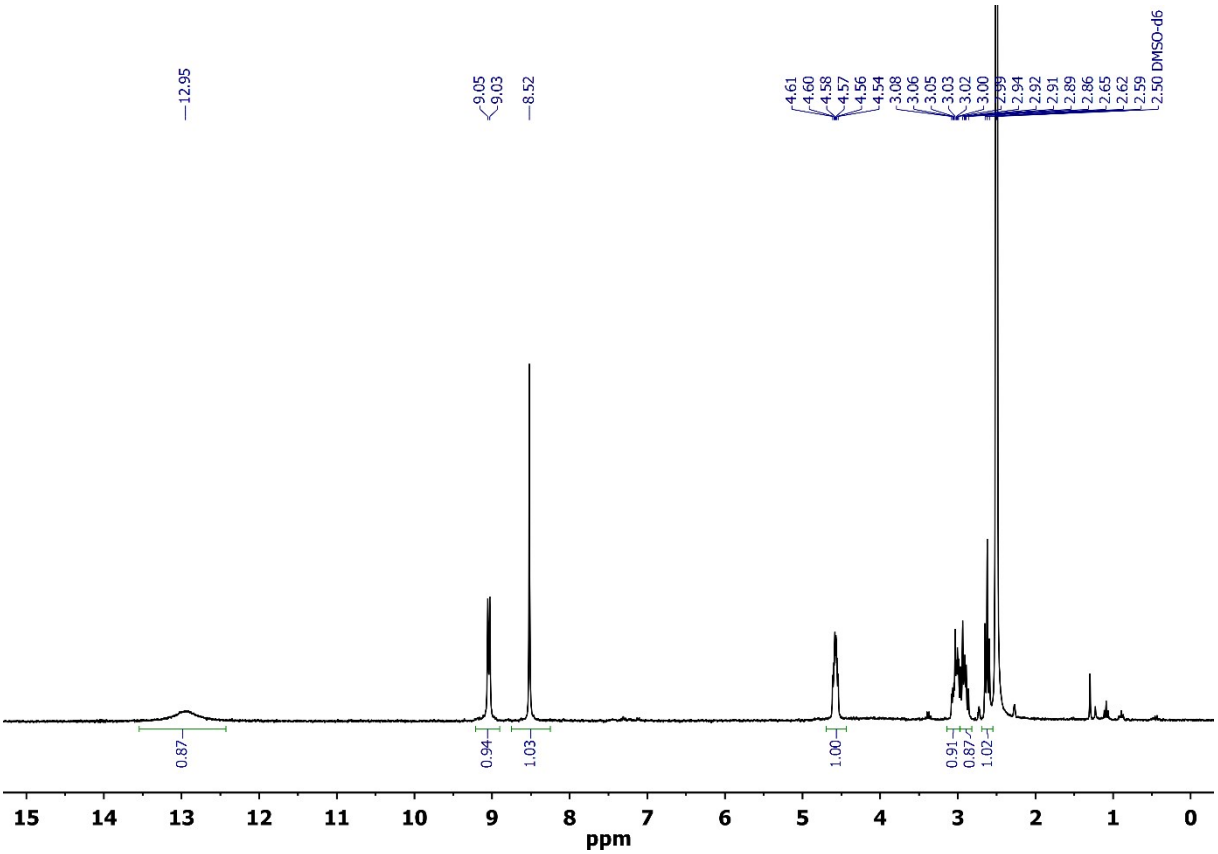


Figure S2: ^{1}H NMR spectrum of component **1** in DMSO-d_6 at 298 K (300 MHz).

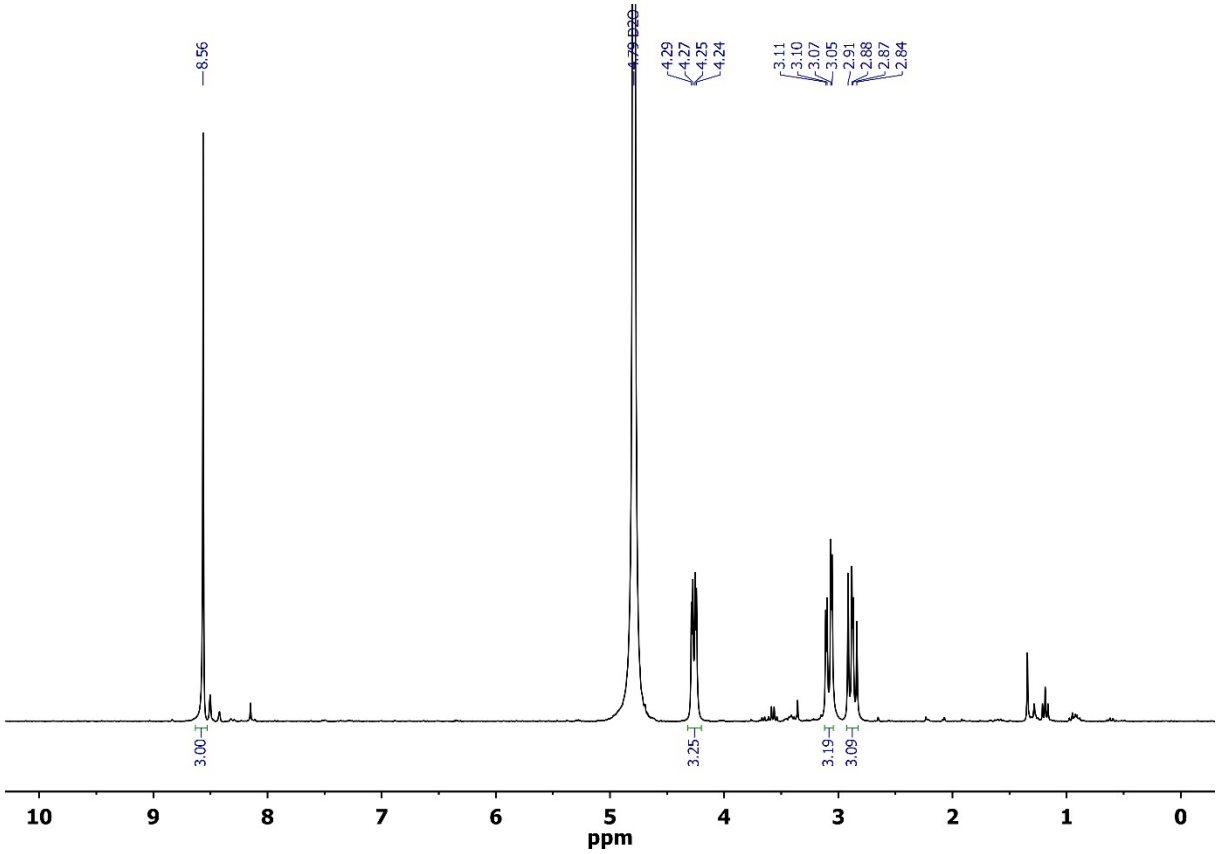


Figure S3: ^{1}H NMR spectrum of component **1** in DMSO-d_6 at 298 K (300 MHz).

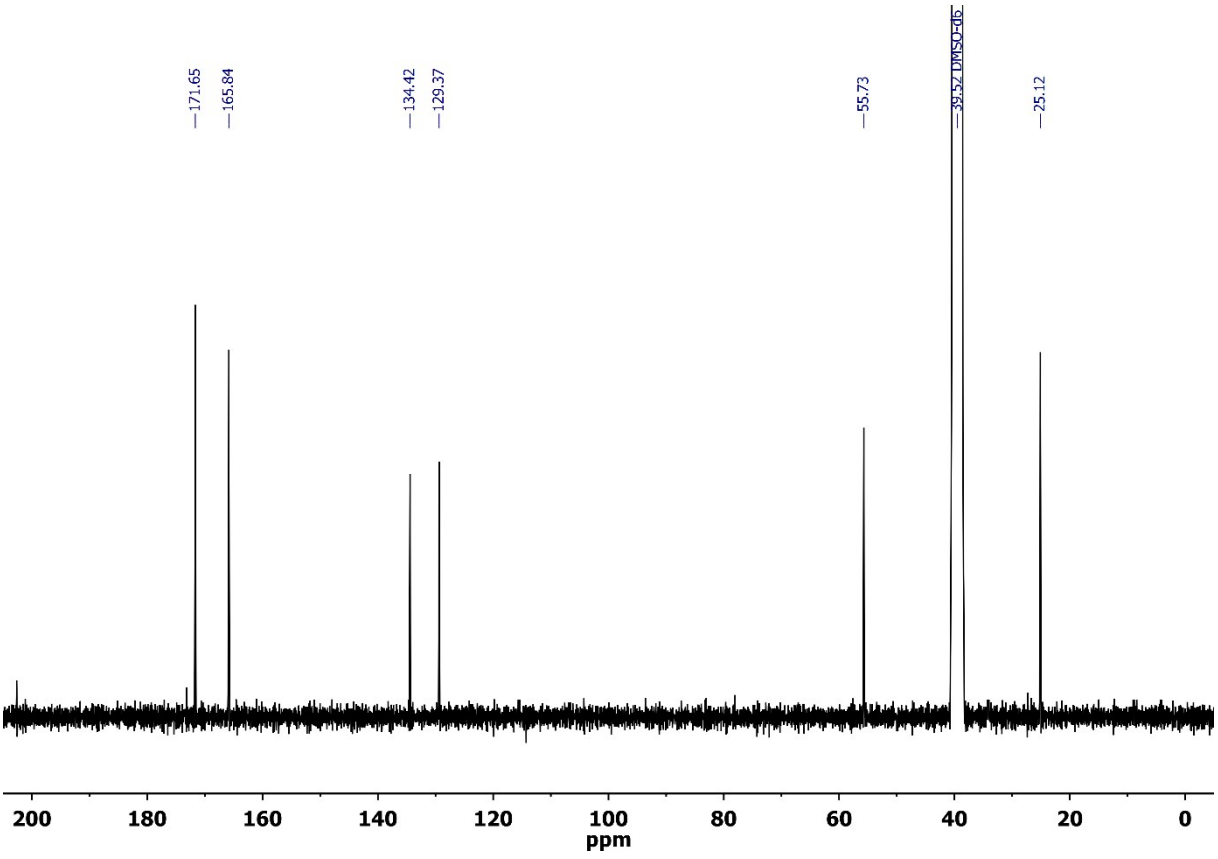
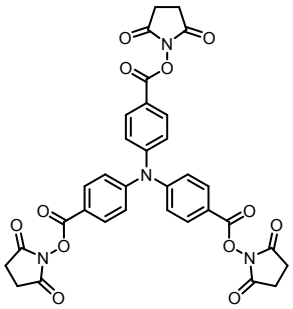


Figure S4: ^{13}C NMR spectrum of component **1** in DMSO-d_6 at 298 K (75 MHz).

Synthesis of **2_AE**.



4,4',4''-nitrilotribenzoic acid (378 mg, 1 mmol, 1 equiv.), N-hydroxy succinimide (NHS) (580 mg, 5 mmol, 5 equiv.) and EDC·HCl (960 mg, 5 mmol, 5 equiv.) were dissolved in anhydrous DMF (50 mL) and stirred at room temperature for 24h under argon. The solvent was evaporated off and acetone (2 mL) added into the oily residue. After stirring to give a clear solution, it was poured into 1M HCl (100 mL). A white solid precipitated and was filtered off, washed with H_2O and Et_2O, and dried under high vacuum. Yield 74%.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (d, *J* = 8.8 Hz, 6H), 7.40 (d, *J* = 8.8 Hz, 6H), 2.89 (s, 12H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ(ppm) 170.38, 151.22, 132.12, 124.86, 119.83, 39.52, 25.57. **ESI-MS**: m/z calc. for $[M+H]^+$ 669.1463, found 669.2107.

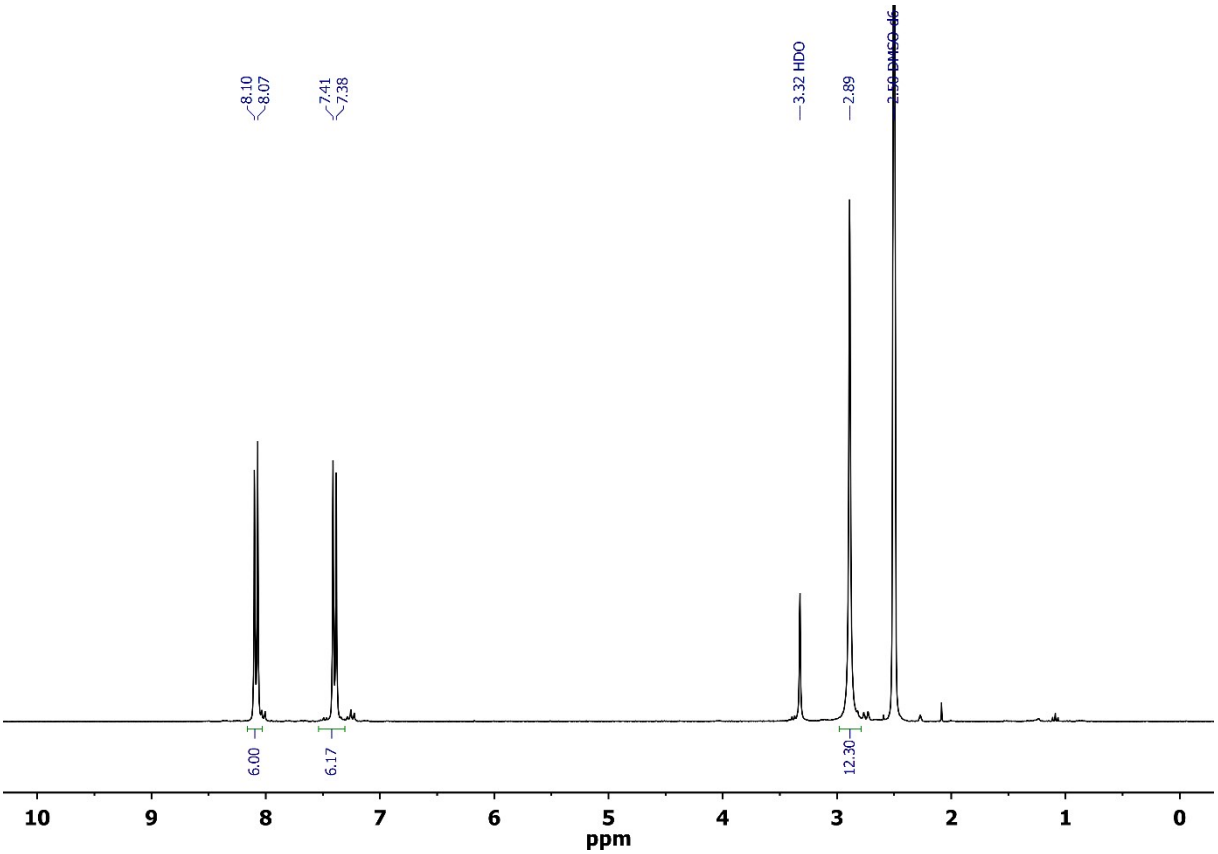


Figure S5: ^{1}H NMR spectrum of component **2_AE** in DMSO-d_6 at 298 K (300 MHz).

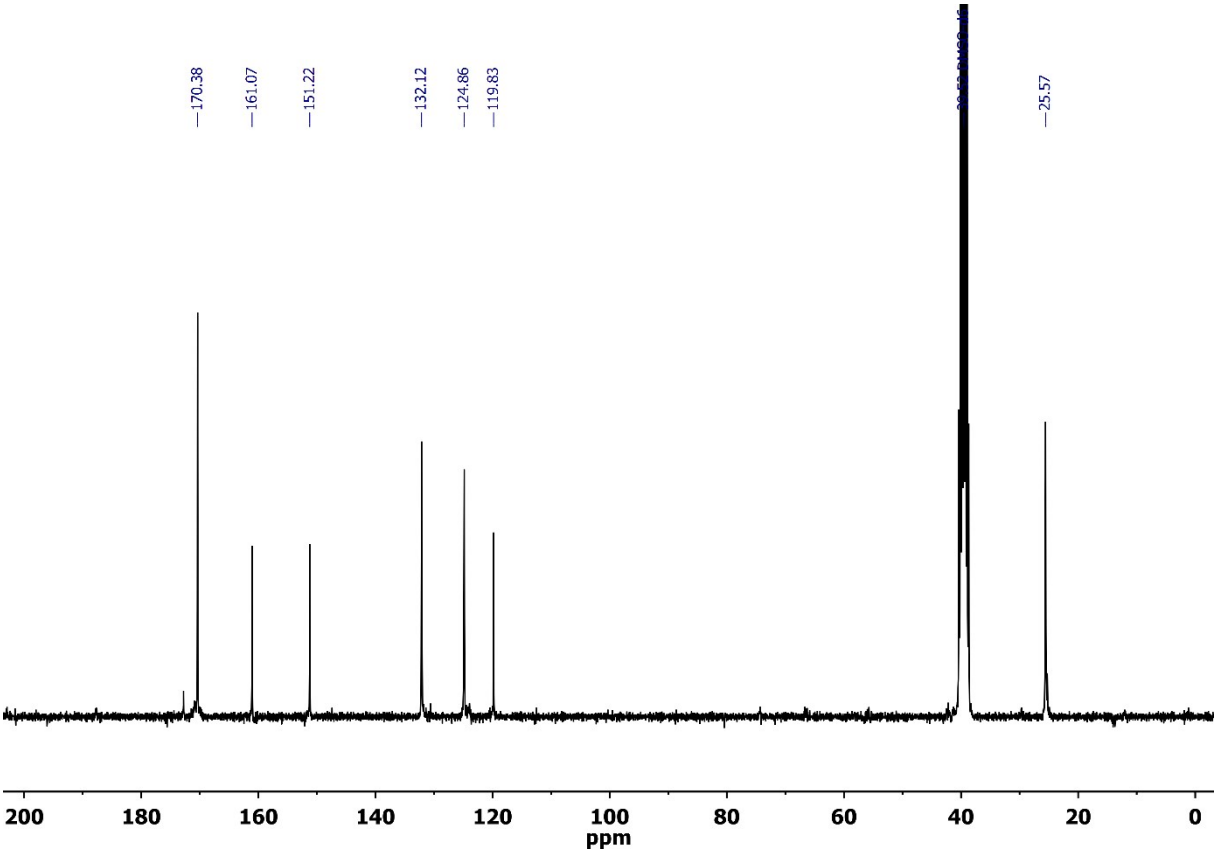
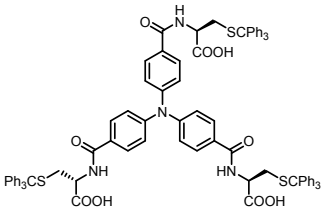


Figure S6: ^{13}C NMR spectrum of component **2_AE** in DMSO-d_6 at 298 K (75 MHz).

Synthesis of **2_STr**



Activated ester **2_AE** (669 mg, 1 mmol, 1 equiv.) and *L*-Cys-STr-OH (1820 mg, 5 mmol, 5 equiv.) were dissolved in anhydrous DMF (100 mL), and Et_3N (1.0 mL, excess) was added. The reaction mixture was stirred overnight at room temperature, then the solvent was removed and acetone (2 mL) was added to the oily residue. The resulting solution was poured dropwise into 1M HCl (100 mL) causing precipitation of a white solid. It was filtered off, washed with H_2O, and dried under high vacuum. Yield 74%.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.64 (d, *J* = 7.1 Hz, 3H), 7.85 (d, *J* = 8.5 Hz, 6H), 7.40 – 7.20 (m, 45H), 7.15 (d, *J* = 8.5 Hz, 6H), 4.31 (dt, *J* = 13.0, 6.9 Hz, 3H), 2.79 – 2.68 (m, 3H), 2.55 (m, *J* = 7.5 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-*d6*) δ 171.82, 165.22, 148.96, 144.35, 129.11, 128.05, 127.77, 127.52, 126.77, 126.63, 66.09, 59.54, 39.52, 26.53. **ESI-MS**: m/z calc. for: $[M-H]^-$ 1412.6405, found 1412.4407.

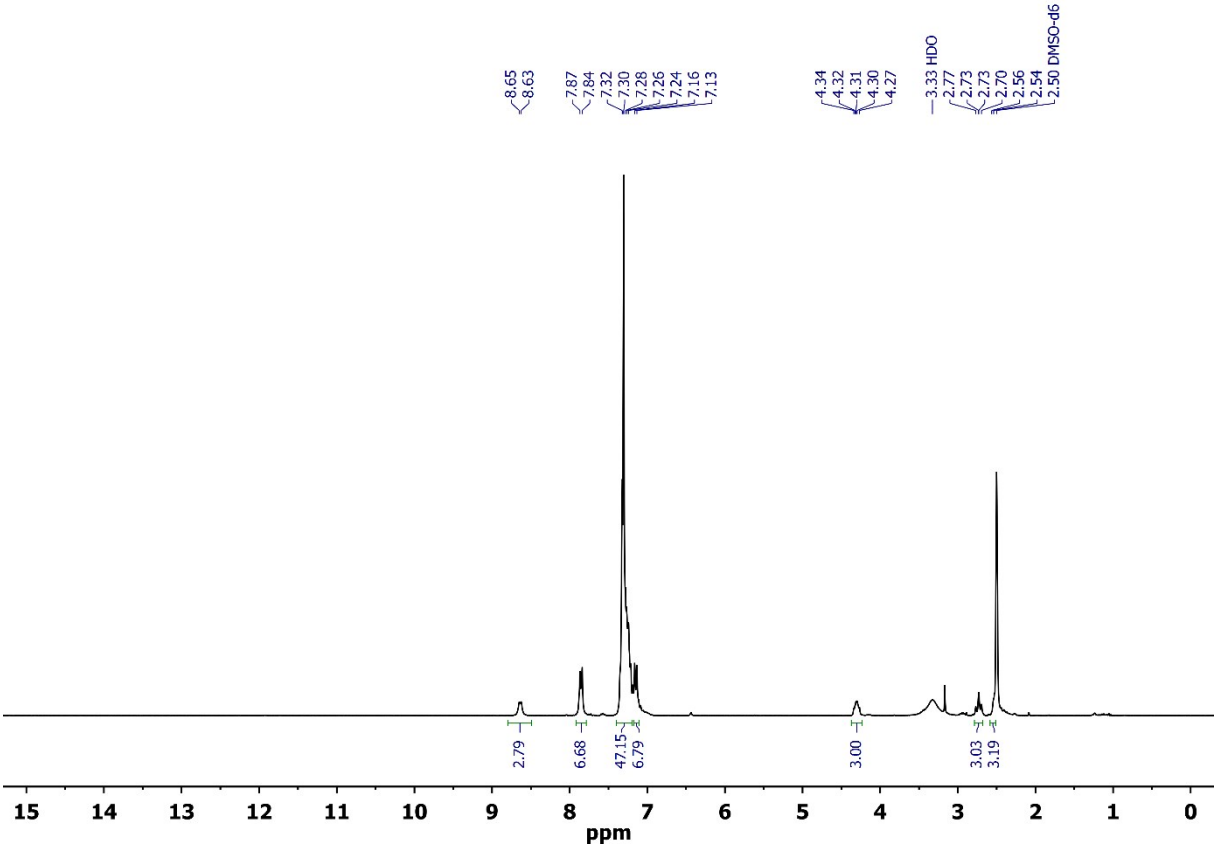


Figure S7: ^{1}H NMR spectrum of component **2_STr** in DMSO-d_6 at 298 K (300 MHz).

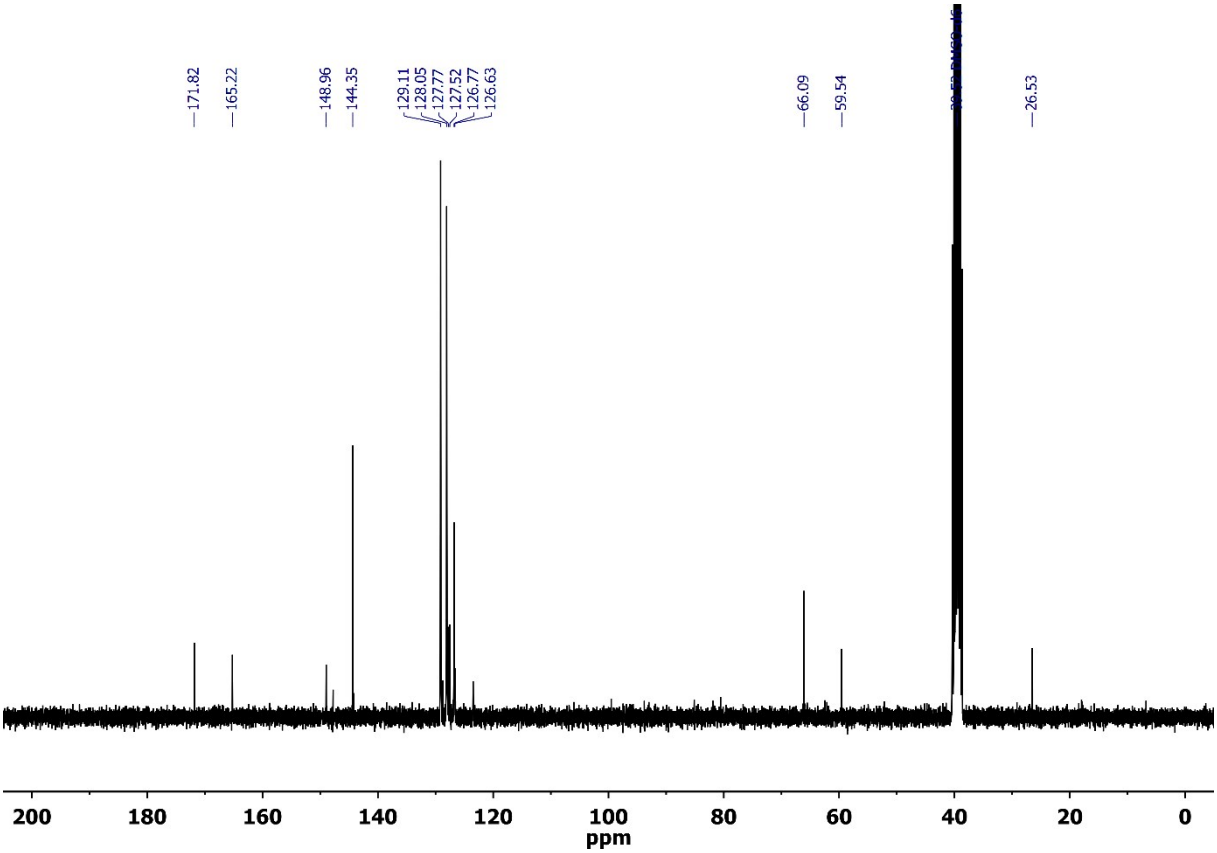
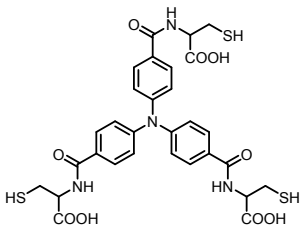


Figure S8: ^{13}C NMR spectrum of component **2_STr** in DMSO-d_6 at 298 K (75 MHz).

Synthesis of **2.**



2 was synthesized according to procedure **1.** The **2_STrt** (1414 mg, 1 mmol, 1 equiv.) was placed in an argon-purged flask and dissolved in 10 mL of DCM. 4 mL of TFA was added and after 30 min. of stirring, Et_3SiH (1.0 mL, 6 mmol, 6. equiv.) was added and stirring was continued for an additional 5 hThe liquids were removed under high vacuum and the voluminous solid residue was suspended in 20 mL of Et_2O, sonicated and filtered off. It was washed well with additional portions of Et_2O (5 x 20 mL) and dried under high vacuum. Yield 91%.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.60 (d, *J* = 7.8 Hz, 3H), 7.90 (d, *J* = 8.6 Hz, 6H), 7.15 (d, *J* = 8.5 Hz, 6H), 4.52 (td, *J* = 8.4, 4.6 Hz, 3H), 3.01 – 2.83 (m, 6H), 2.63 – 2.53 (m, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ: 171.89, 165.75, 148.98, 129.21, 128.74, 123.42, 55.50, 25.18. **ESI-MS**: m/z calc. for: $[M-H]^-$ 685.1301, found 685.1103.

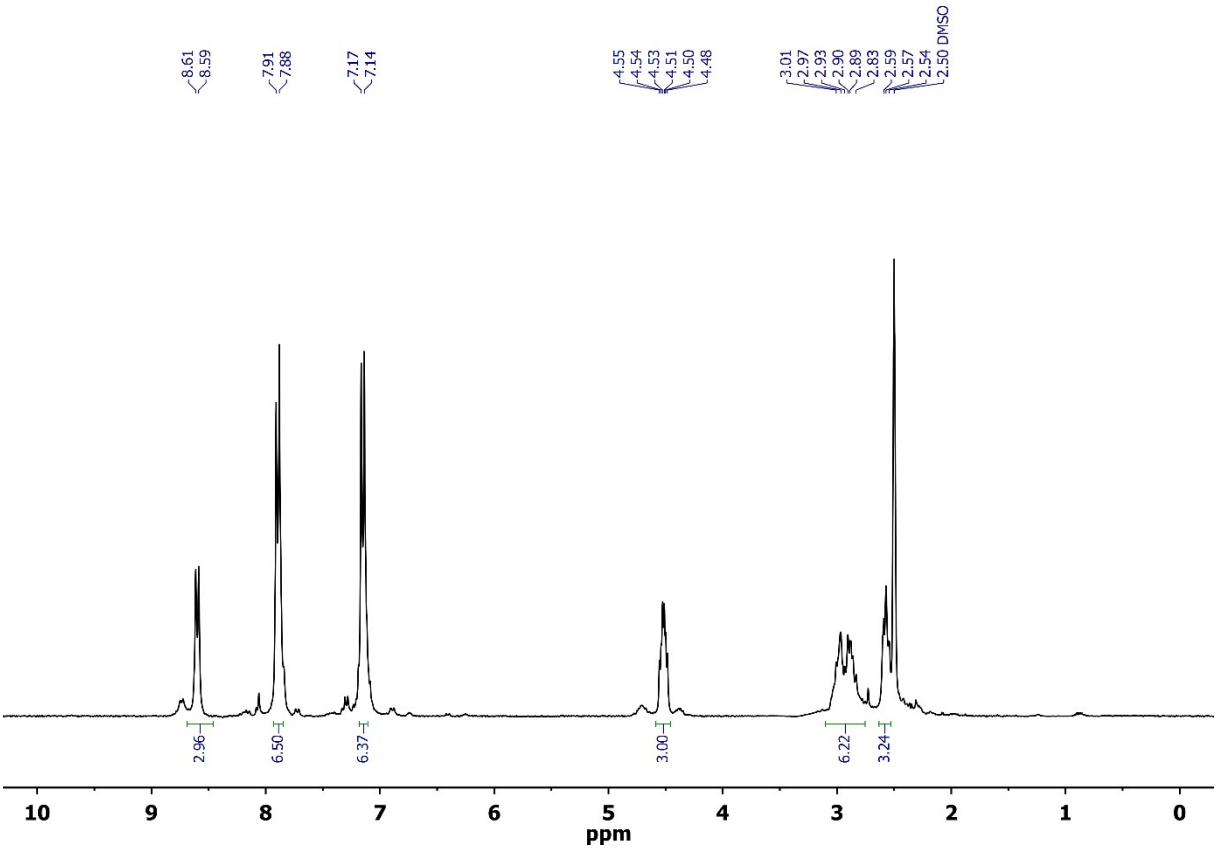


Figure S9: ^{1}H NMR spectrum of component **2** in DMSO-d_6 at 298 K (300 MHz).

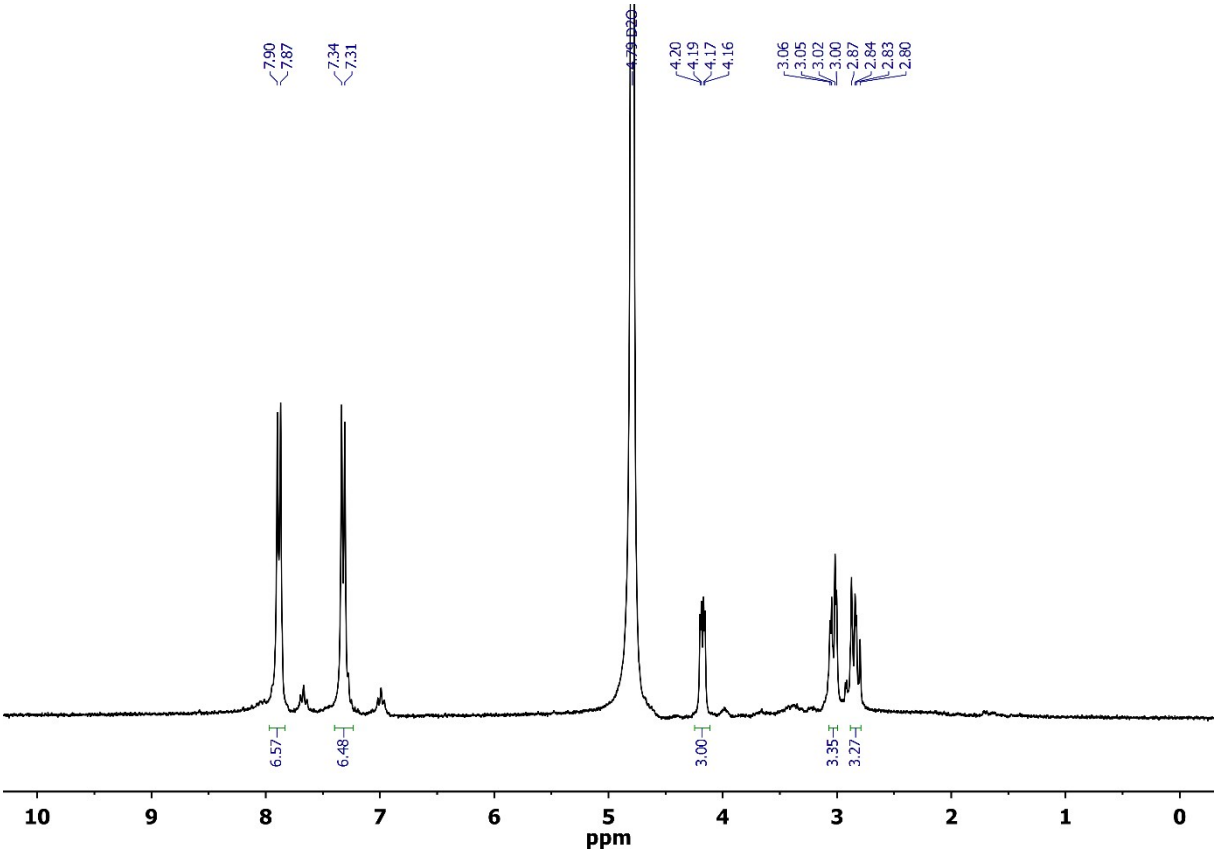


Figure S10: ^{1}H NMR spectrum of component **2** in D_2O at 298 K (300 MHz).

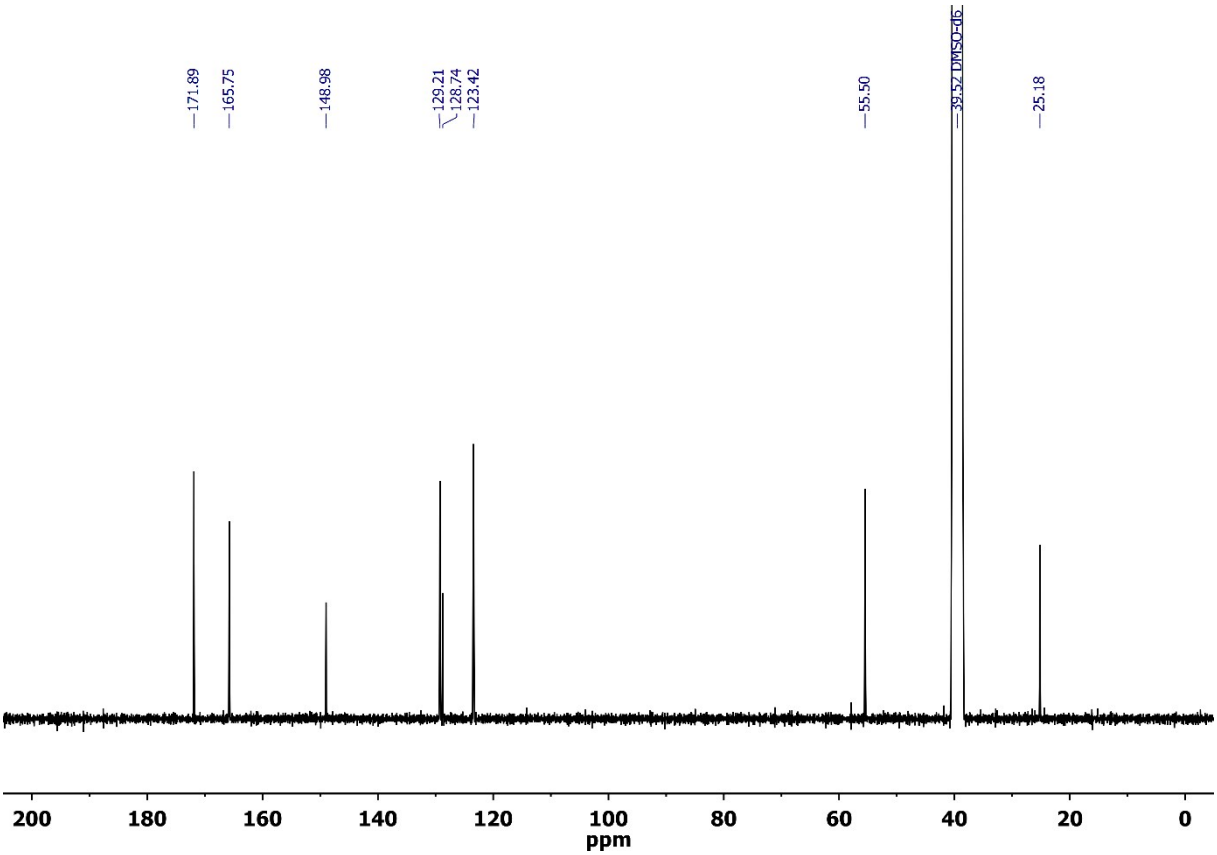
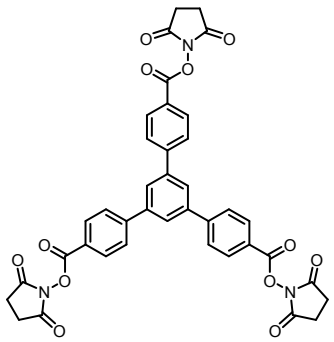


Figure S11: ^{13}C NMR spectrum of component **2** in DMSO-d_6 at 298 K (75 MHz).

Synthesis of **3_AE.**



3_AE was synthesized according to procedure **1_AE.** 5'-(4-carboxyphenyl)-[1,1':3',1''-terphenyl]-4,4''-dicarboxylic acid (377 mg, 1 mmol, 1 equiv.), N-hydroxy succinimide (NHS) (580 mg, 5 mmol, 5 equiv.) and EDC·HCl (960 mg, 5 mmol, 5 equiv.) were dissolved in anhydrous DMF (40 mL) and stirred at room temperature for 24 h under argon. The solvent was removed and acetone (4 mL) was added into the oily residue and stirred to give a clear solution before being poured into 1M HCl (200 mL). A white, voluminous solid precipitated. It was filtered off, washed with H_2O and Et_2O, and dried under high vacuum. Yield 82%.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.24 (m, 15H), 2.92 (s, 12H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ: 170.37, 161.64, 146.00, 140.19, 130.59, 128.29, 126.48, 123.71, 25.60. **ESI-MS**: m/z calc. for: $[M-H]^-$ 728.3754, found 728.1324.

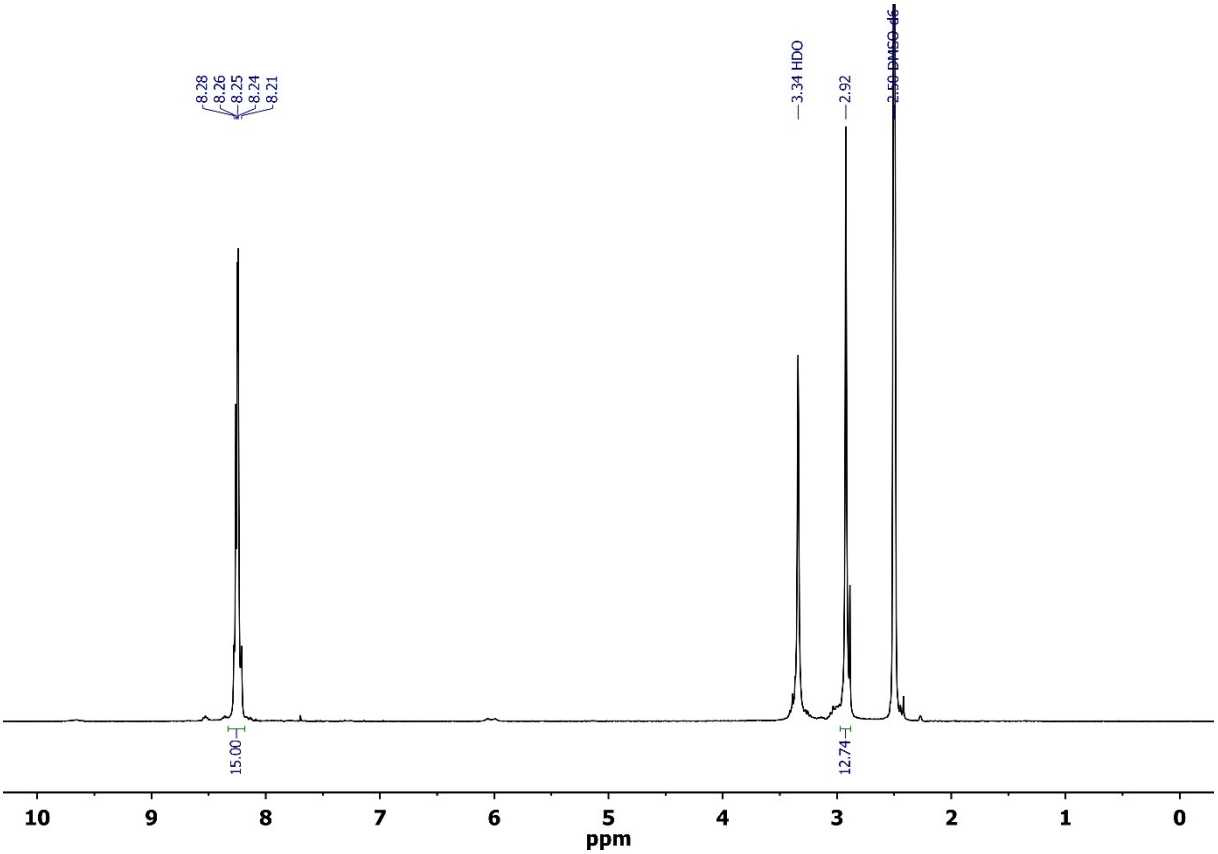


Figure S12: ^{1}H NMR spectrum of component **3_AE** in DMSO-d_6 at 298 K (300 MHz).

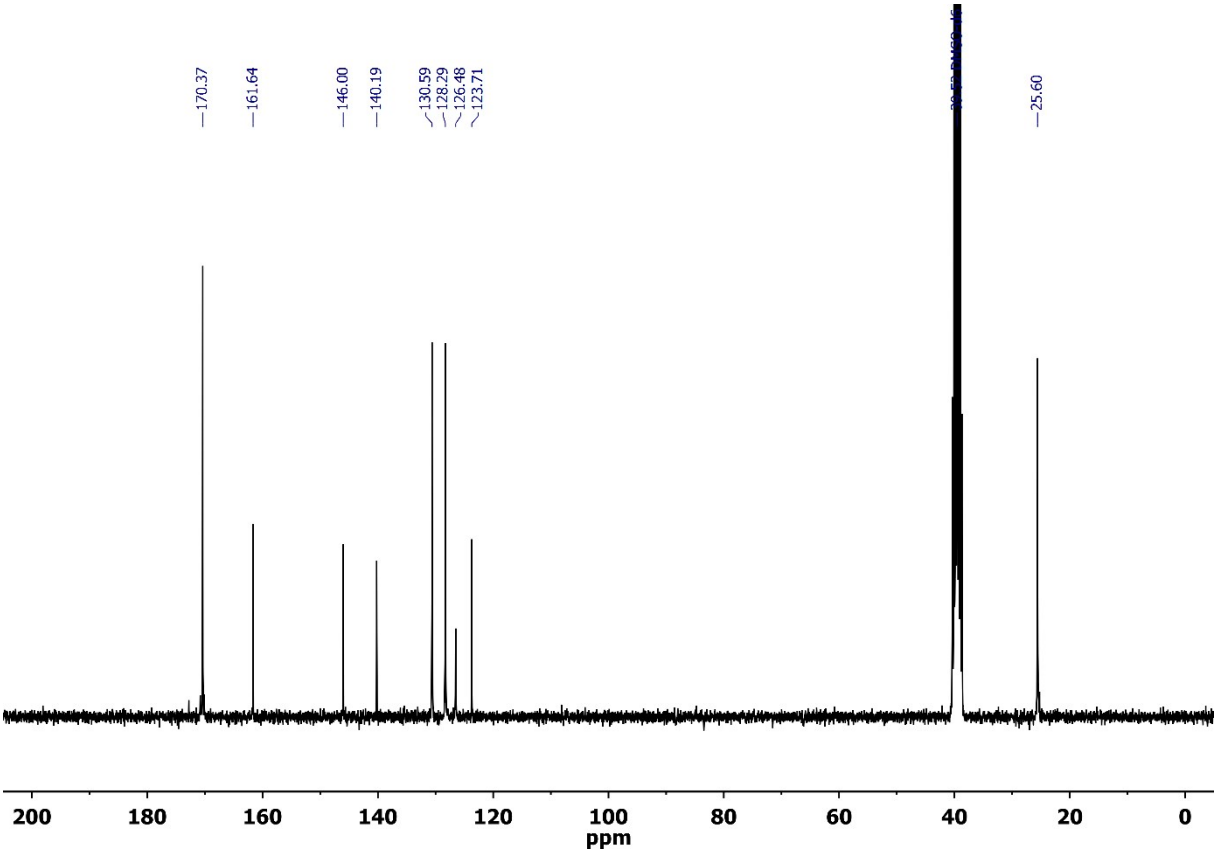
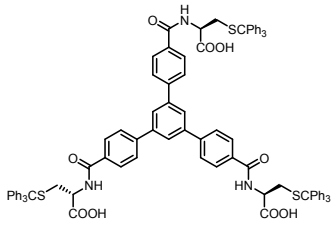


Figure S13: ^{13}C NMR spectrum of component **3_AE** in DMSO-d_6 at 298 K (75 MHz).

Synthesis of **3_STr**



3_STr was synthesized according to procedure **1_STrt** using **3_AE.**
Activated ester **3_AE** (730 mg, 1 mmol, 1 equiv.) and *L*-Cys-STr-OH (1820 mg, 5 mmol, 5 equiv.) were dissolved in anhydrous DMF (100 mL), and Et_3N (1.0 mL, excess) was added. The reaction mixture was stirred overnight at room temperature before the solvent was removed and acetone (5 mL) added to the oily residue. The resulting mixture was poured dropwise into 1M HCl (100 mL) causing precipitation of a voluminous white solid. It was filtered off, washed with H_2O, and dried under high vacuum. Yield 82%.

^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.75 (d, *J* = 7.5 Hz, 3H), 8.08 – 8.04 (m, 15H), 7.43 – 7.09 (m, 45H), 4.36 (q, *J* = 8.5 Hz, 3H), 2.76 (t, *J* = 10.5 Hz, 3H), 2.64 – 2.51 (m, 3H). **^{13}C NMR** (75 MHz, DMSO-*d6*) δ: 171.75, 165.81, 144.29, 142.70, 140.82, 132.85, 129.11, 128.09, 127.77, 127.51, 127.17, 126.83, 67.02, 66.26, 25.14. **ESI-MS**: m/z calc. for: $[M-H]^-$ 1473.8150, found 1473.4588.

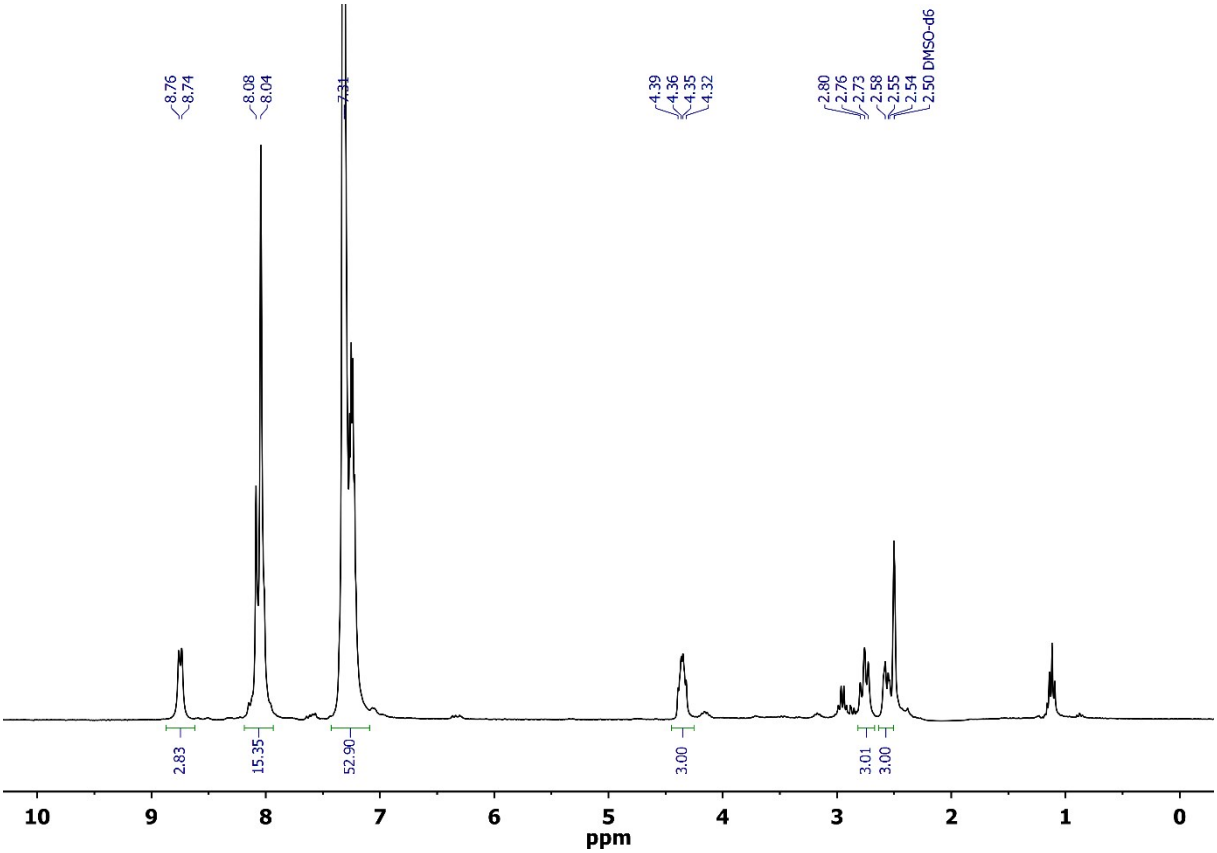


Figure S14: ^{1}H NMR spectrum of component **3_STr** in DMSO-d_6 at 298 K (300 MHz).

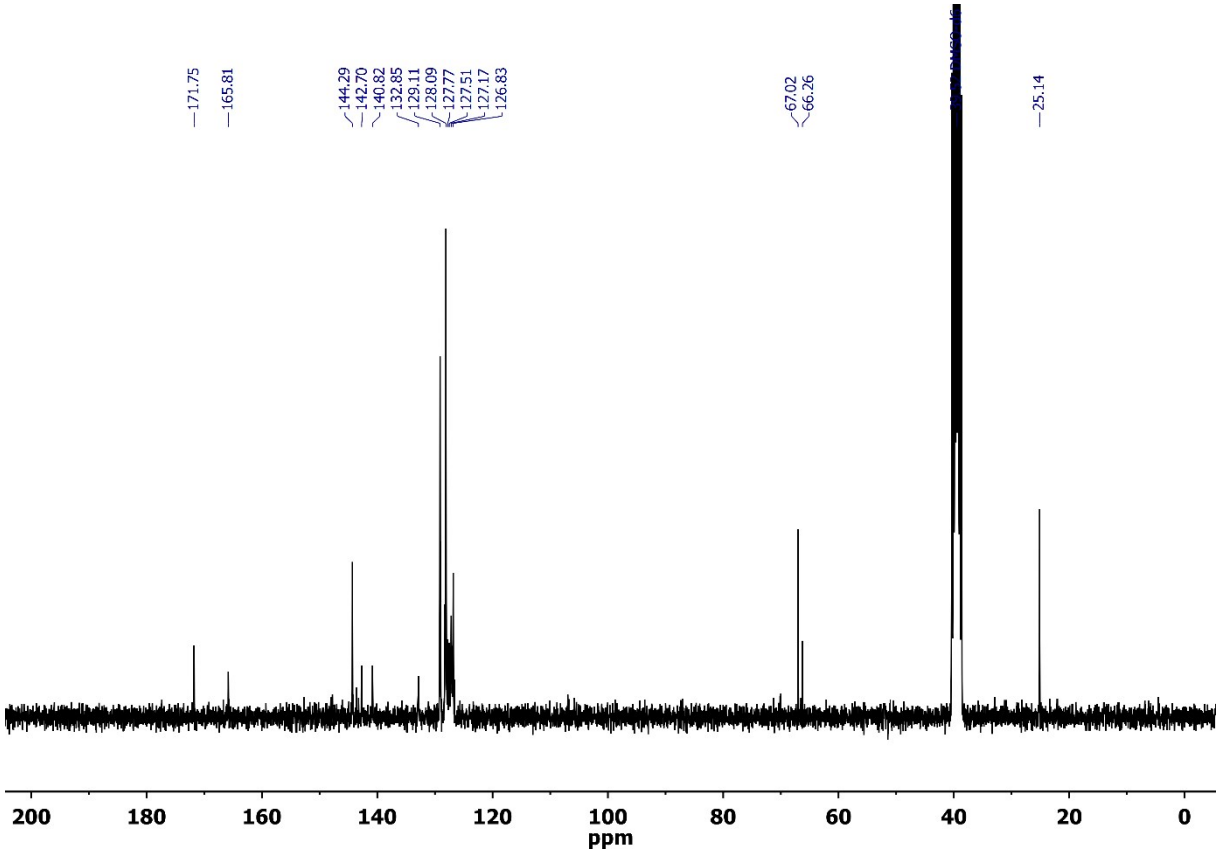
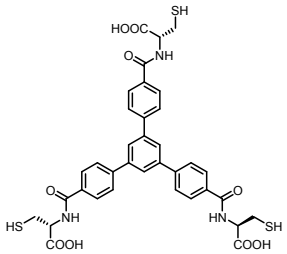


Figure S15: ^{13}C NMR spectrum of component **3_STr** in DMSO-d_6 at 298 K (75 MHz).

Synthesis of **3**.



3 was synthesized according to procedure **1** using **3_STr**

3_STrt (1475 mg, 1 mmol, 1 equiv.) was placed in an argon-purged flask and dissolved in 10 mL of DCM. 4 mL of TFA was added and after 30 min. of stirring, Et_3SiH (1.0 mL, 6 mmol, 6. equiv.) was added and stirring was continued for another 5 h. The liquids were removed under high vacuum and the voluminous solid residue was suspended in 20 mL of Et_2O, sonicated and filtered off. It was washed well with additional portions of Et_2O (5 x 20 mL) and dried under high vacuum. Yield 89%.

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^{1}H NMR (300 MHz, DMSO-d_6) δ 12.88 (s, 3H), 8.77 (d, *J* = 7.8 Hz, 3H), 8.07 (d, *J* = 6.7 Hz, 15H), 4.57 (td, *J* = 8.5, 4.7 Hz, 3H), 3.09 – 2.90 (m, 6H), 2.61 (t, *J* = 8.4 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-*d6*) δ: 171.90, 166.25, 142.74, 140.90, 133.03, 129.05, 128.18, 127.20, 64.95, 25.26. **ESI-MS**: m/z calc. for: $[M-H]^-$ 746.3564, found 746.1309.

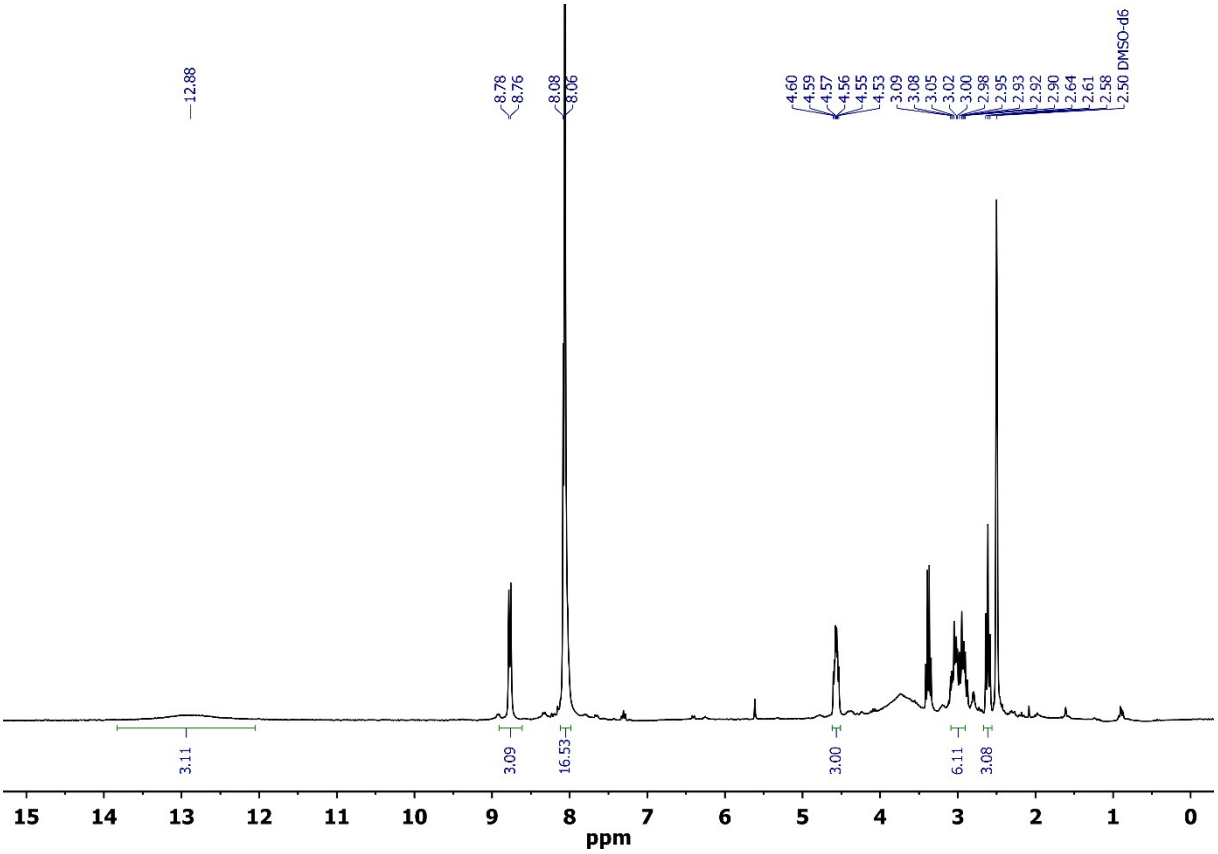


Figure S16: ^{1}H NMR spectrum of component **3** in DMSO-d_6 at 298 K (300 MHz).

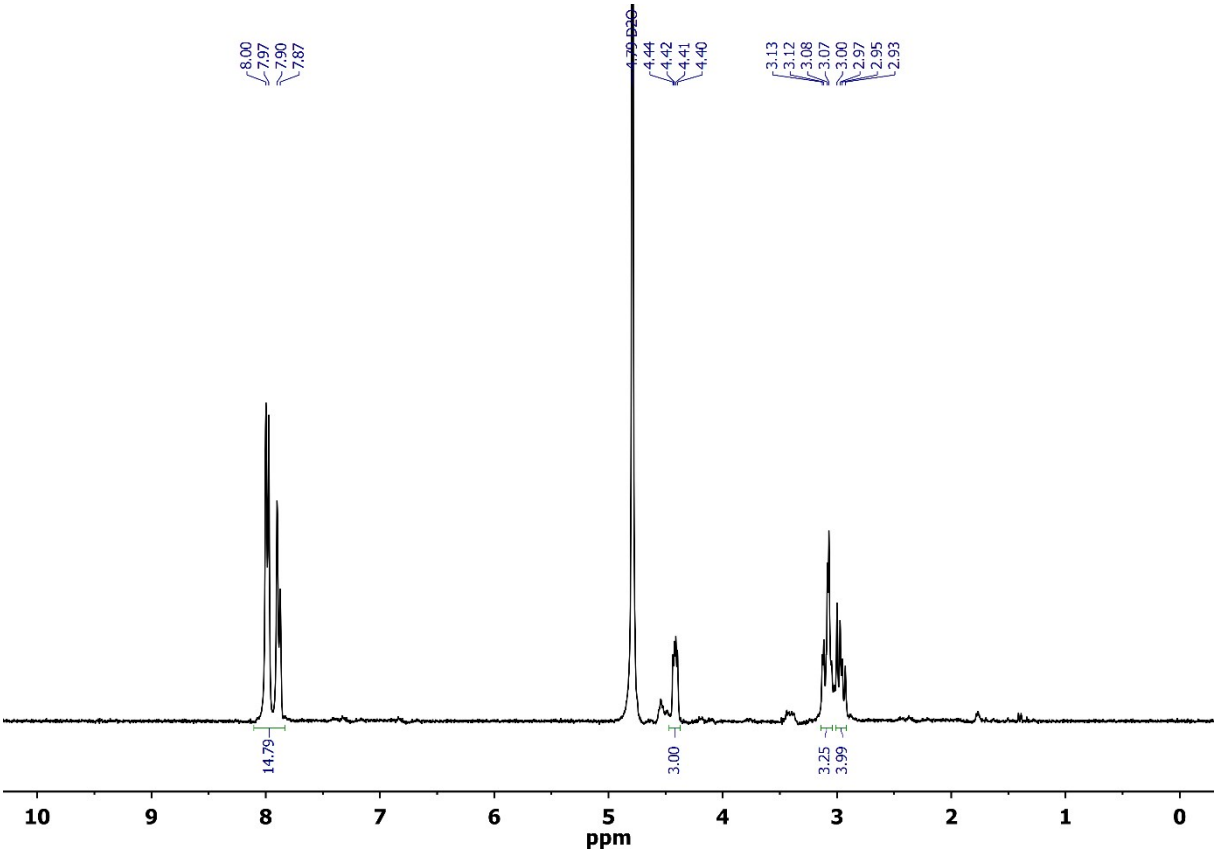


Figure S17: ^{1}H NMR spectrum of component **3** in D_2O at 298 K (300 MHz).

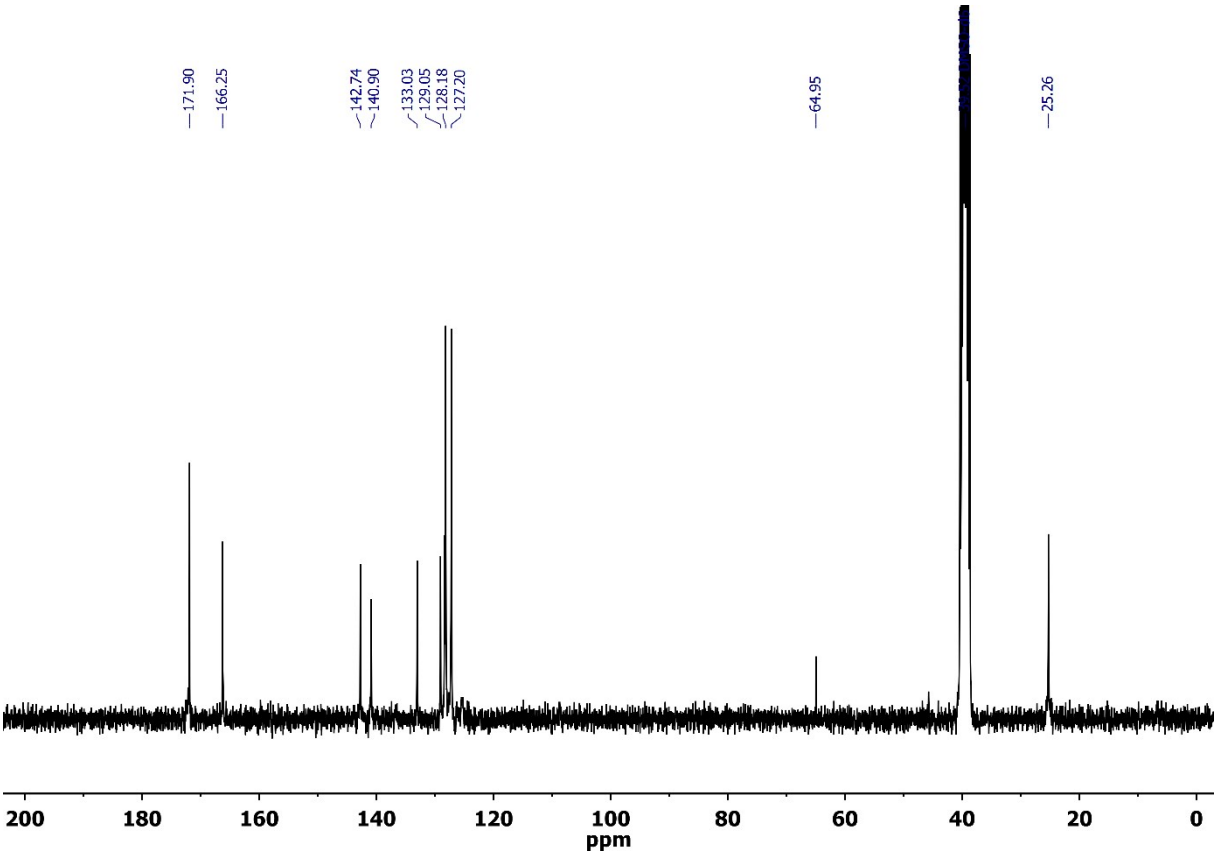
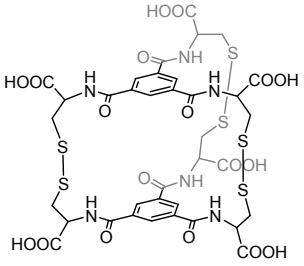


Figure S18: ^{13}C NMR spectrum of component **3** in DMSO-d_6 at 298 K (75 MHz).

Synthesis and characterisation of homodimeric cages

Homodimeric cage **1-1**.



Component **1** (52 mg, 0.1 mmol) was dissolved in aqueous NaOH (0.01 M, 20 mL) and the pH adjusted to 8 before stirring the solution for 3 days in a loosely capped vessel. The solution was then was filtered through a Celite pad and acidified with 1M HCl to pH = 2, which caused the solution to become turbid. MeCN (20 mL) was added to give a clear solution before vacuum evaporation was used to reduce the volume to 10 mL, at which point a white solid precipitated. It was filtered off and dried under high vacuum. Yield 87%.

^{1}H NMR (300 MHz, D_2O) δ: 8.14 (s, 6H), 4.68 (t, *J* = 6.8 Hz, 6H), 3.39 (dd, *J* = 13.9, 6.3 Hz, 6H), 3.04 (dd, *J* = 13.8, 7.6 Hz, 6H). **^{13}C NMR** (150 MHz, DMSO-*d6*) δ: 171.92, 165.12, 132.96, 129.01, 51.53, 38.56. **HR-MS** in positive ion mode, calc for: $[M+H]^+$ 1033.0483 m/z, found: $[M+H]^+$ 1033.0449 m/z.

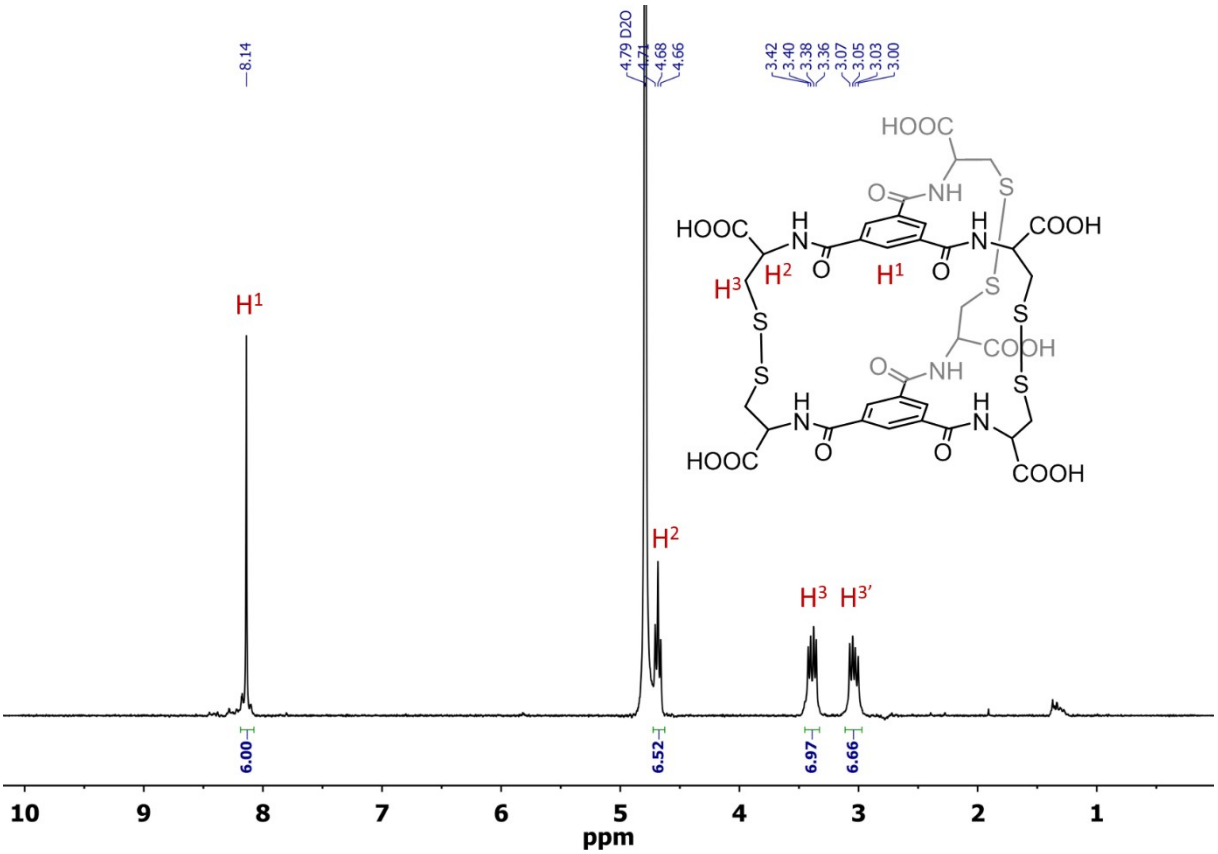


Figure S19: ^{1}H NMR spectrum of cage **1-1** in D_2O at 298 K (300 MHz).

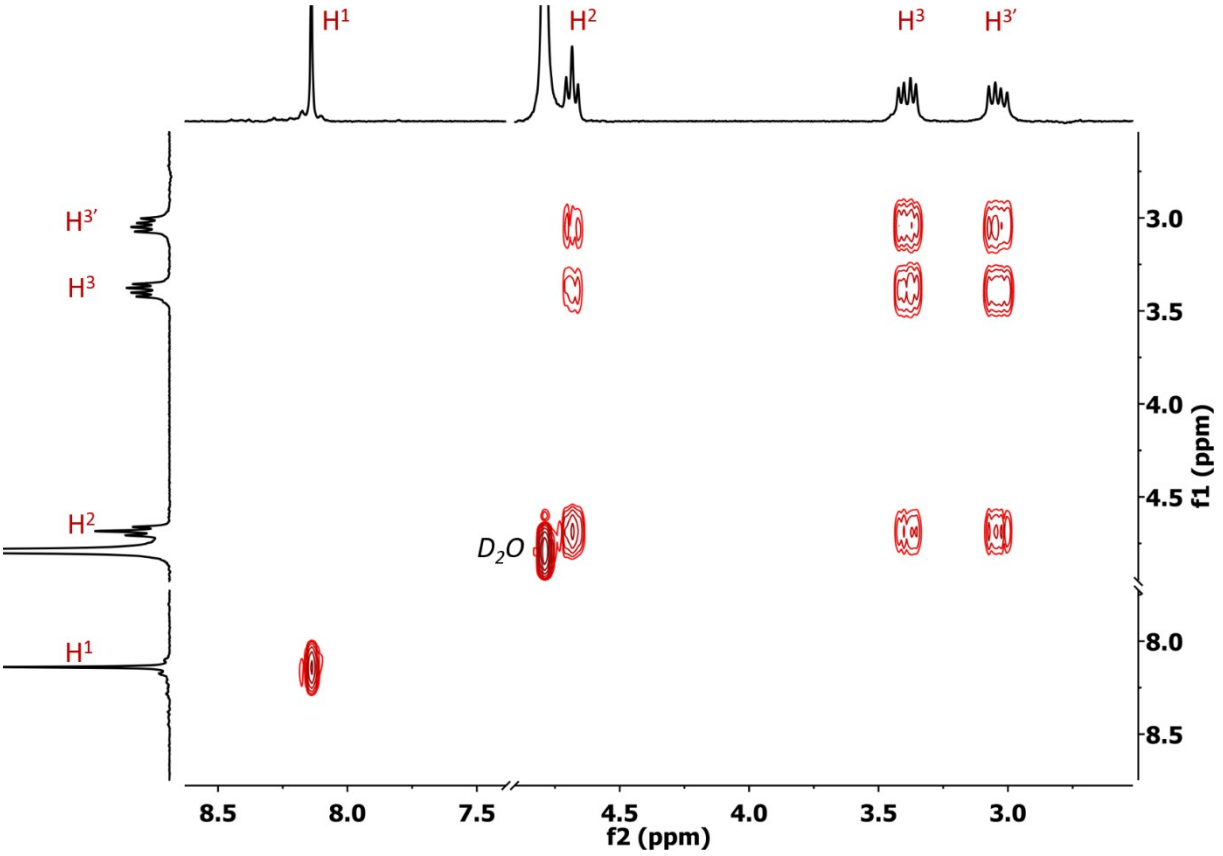


Figure S20: COSY NMR spectrum of cage **1-1** in D_2O at 298 K (300 MHz).

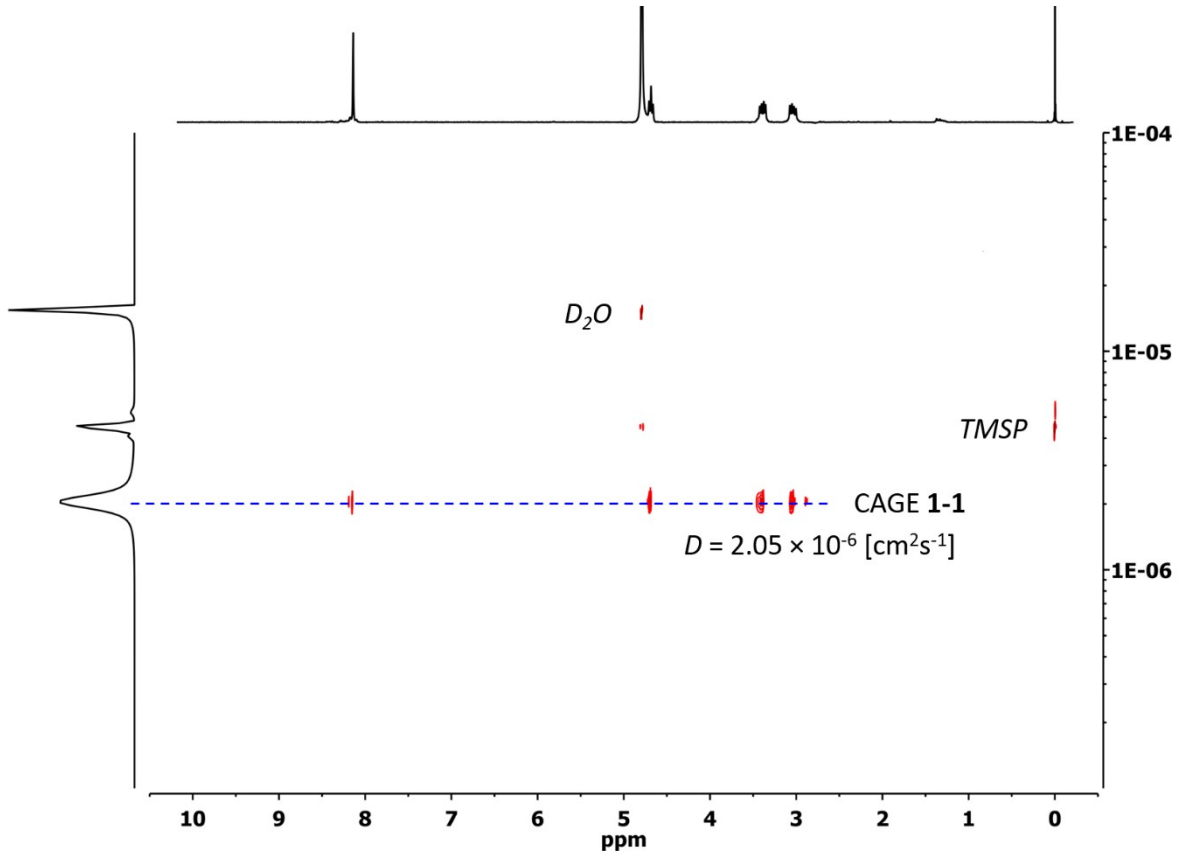


Figure S21: DOSY spectrum of cage **1-1** in D_2O at 298 K (700 MHz).

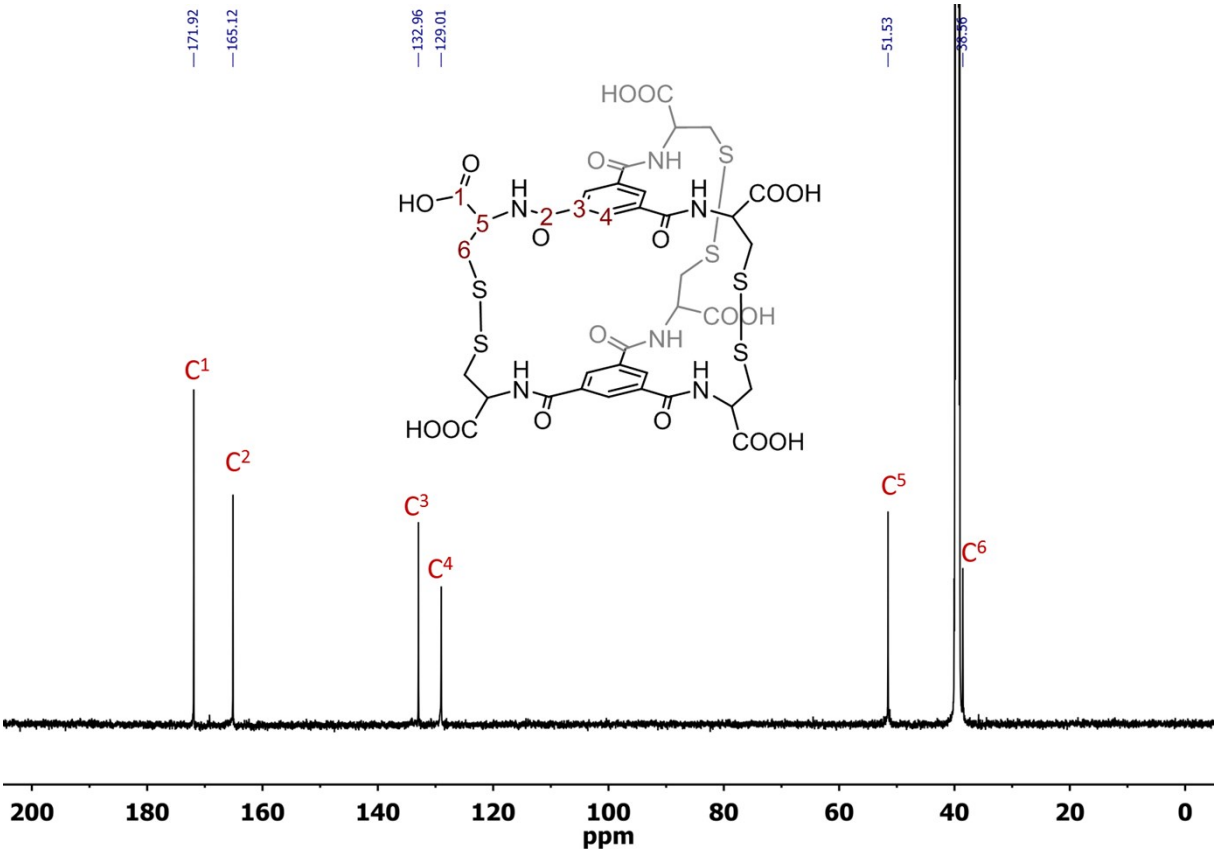


Figure S22: ^{13}C NMR spectrum of cage **1-1** in DMSO-d_6 at 298 K (600 MHz).

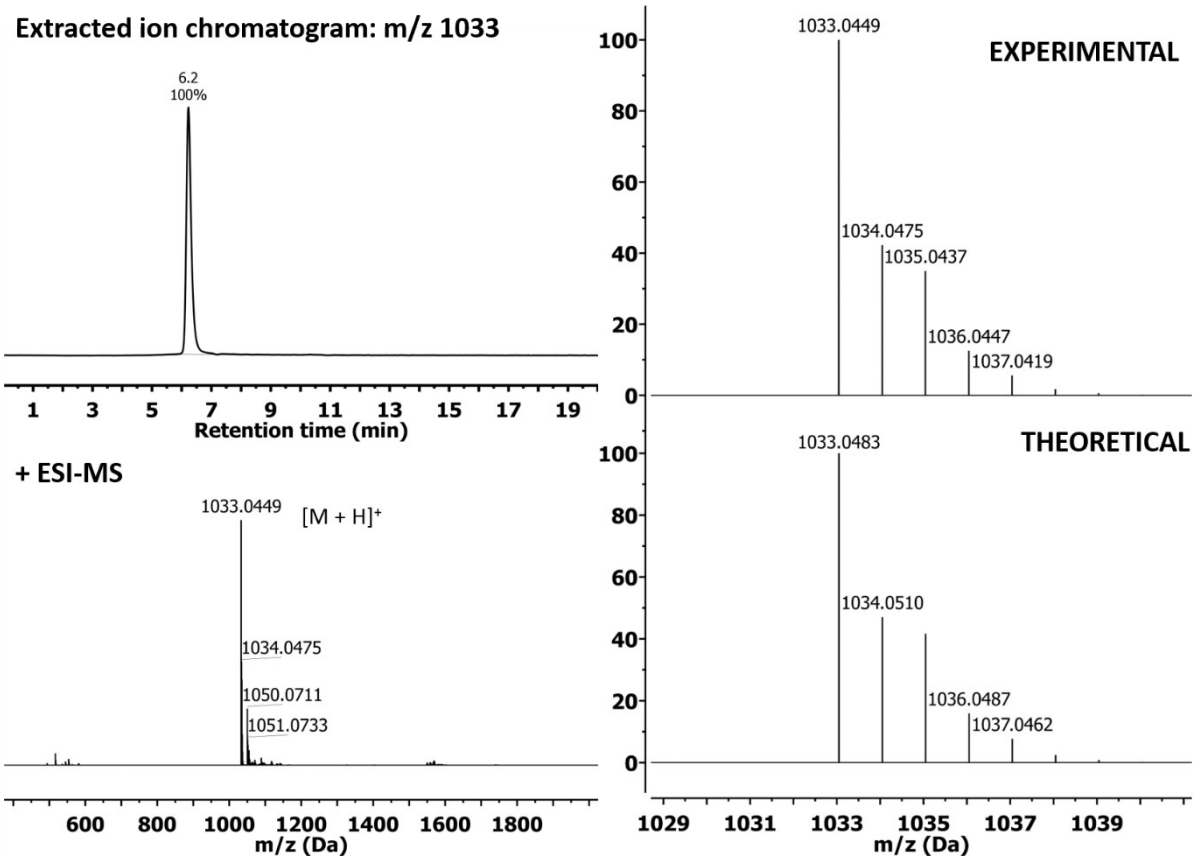
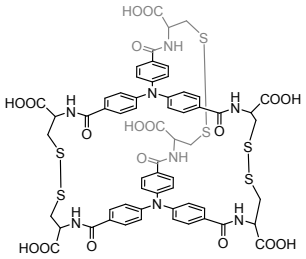


Figure S23: LC-MS analysis of cage **1-1**.

Homodimeric cage **2-2**.



2-2 was synthesized according to procedure **1-1** using **2.**

2 (68 mg, 0.1 mmol) was dissolved in aqueous NaOH (0.01 M, 20 mL) and the pH adjusted to 8 before stirring the solution for 3 days in a loosely capped vessel. The solution was then was filtered through a Celite pad and acidified with 1M HCl to pH = 2 which caused the solution to become turbid. MeCN (20 mL) was added to give a clear solution before vacuum evaporation was used to reduce the volume to 10 mL, at which point a white solid precipitated. It was filtered off and dried under high vacuum. Yield 85%.

^{1}H NMR (300 MHz, D2O) δ 7.63 (d, *J* = 8.7 Hz, 6H), 7.00 (d, *J* = 8.7 Hz, 6H), 4.72 – 4.65 (m, 3H), 3.39 (dd, *J* = 13.8, 5.6 Hz, 3H), 3.16 (dd, *J* = 14.0, 7.9 Hz, 3H). **^{13}C NMR** (150 MHz, DMSO-*d6*) δ 172.01, 165.67, 148.41, 129.10, 128.66, 122.98, 52.49, 40.06. **HR-MS** in positive ion mode, calc for: $[M+H]^+$ 1367.1953 m/z, found: $[M+H]^+$ 1367.1895 m/z.

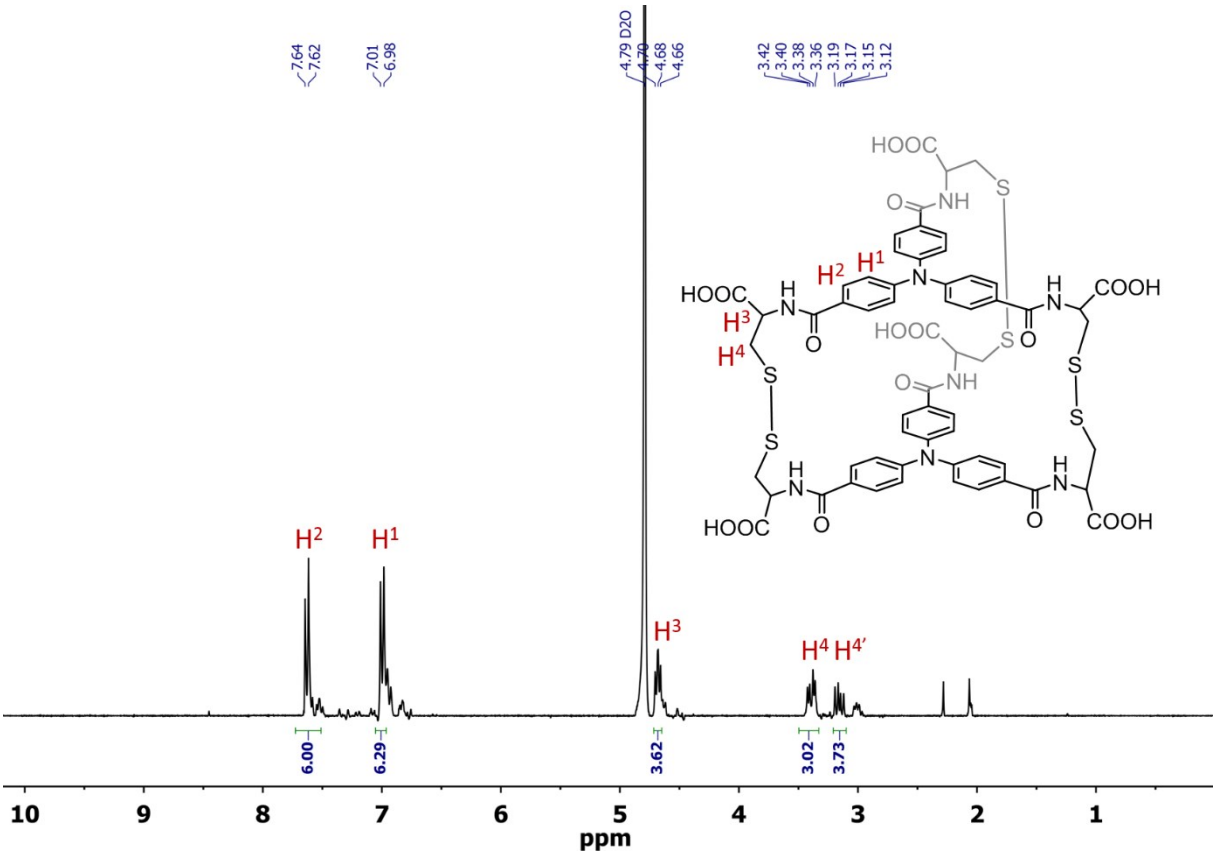


Figure S24: ^{1}H NMR spectrum of cage **2-2** in D_2O at 298 K (300 MHz).

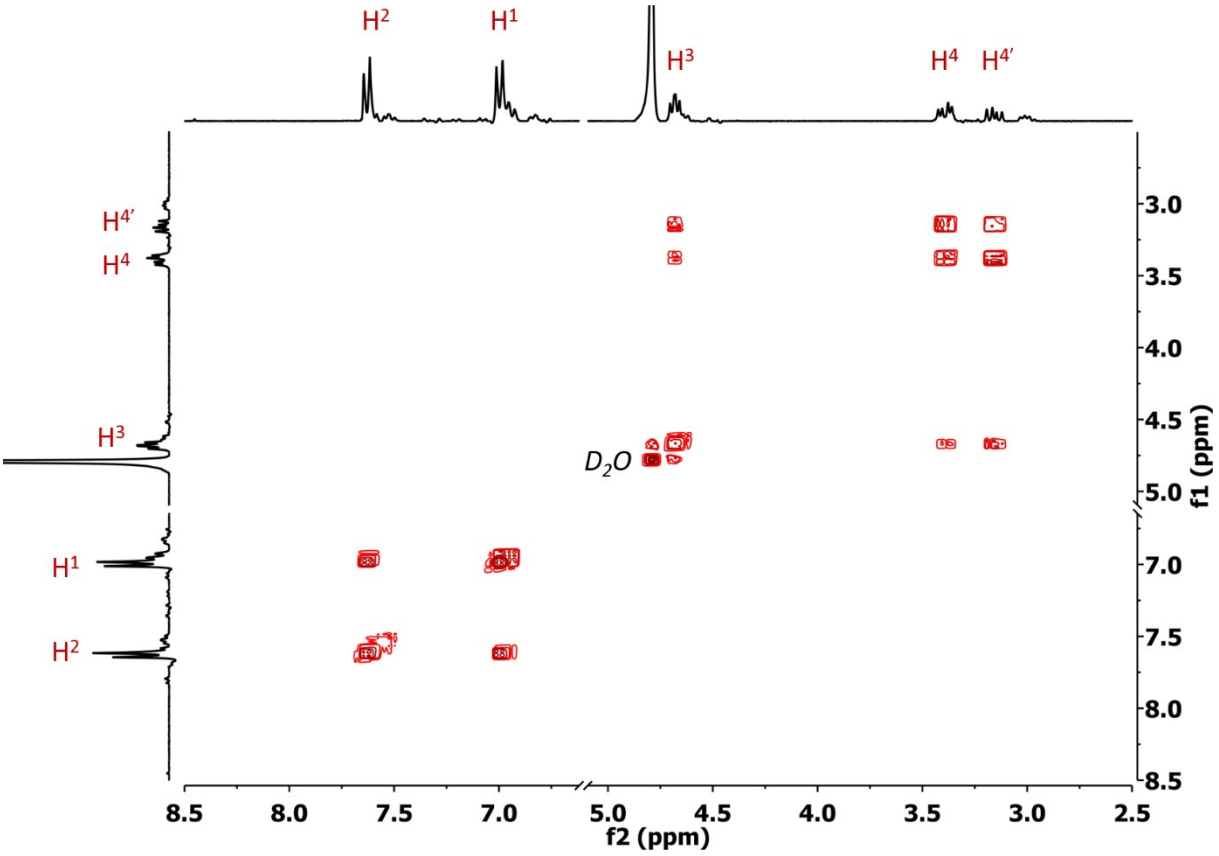


Figure S25: COSY NMR spectrum of cage **2-2** in D_2O at 298 K (300 MHz).

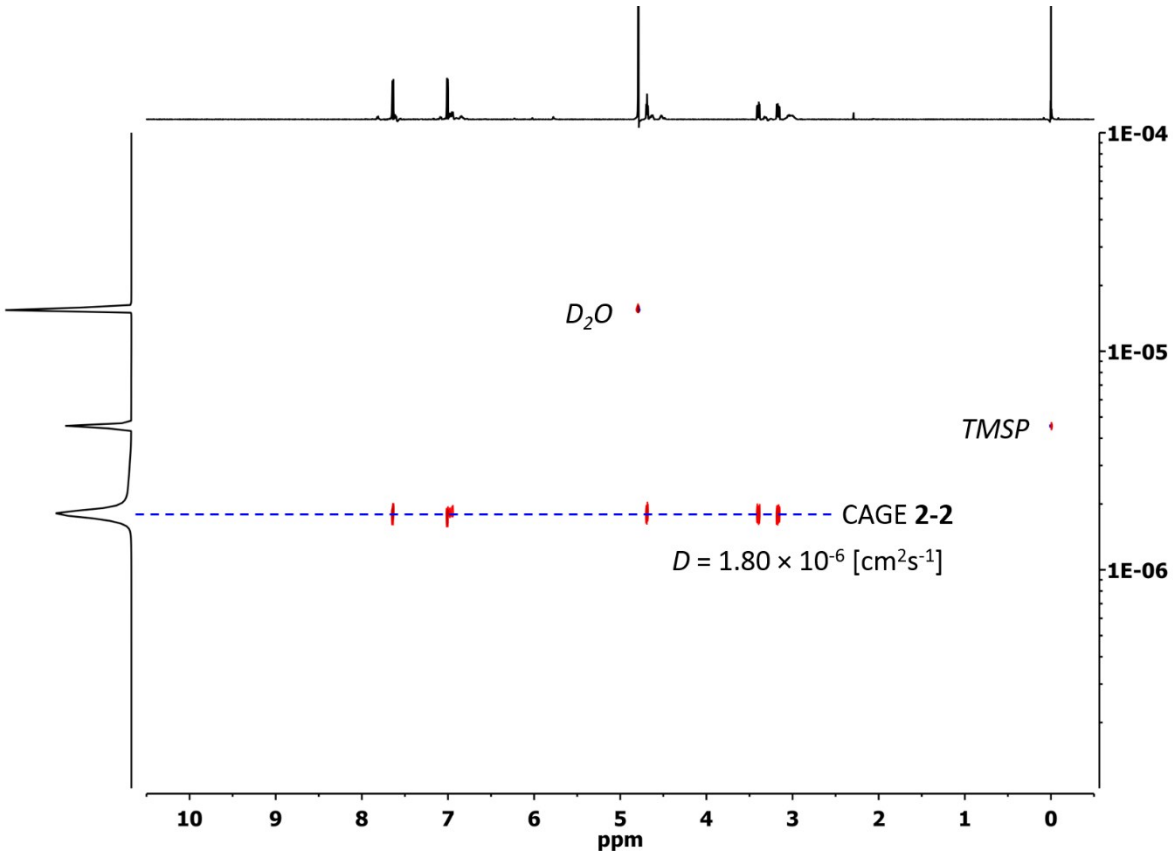


Figure S26: DOSY NMR spectrum of cage **2-2** in D_2O at 298 K (700 MHz).

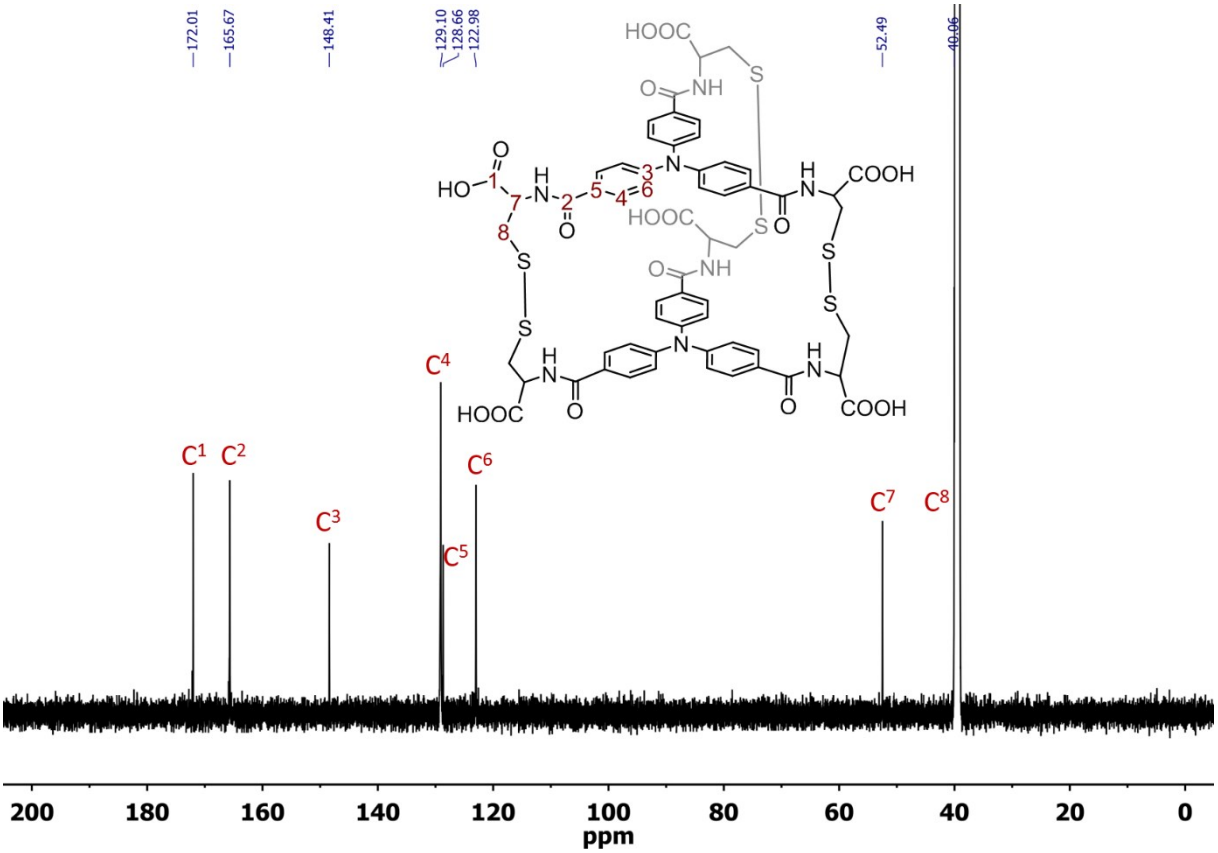


Figure S27: ^{13}C NMR spectrum of cage **2-2** in DMSO-d_6 at 298 K (150 MHz).

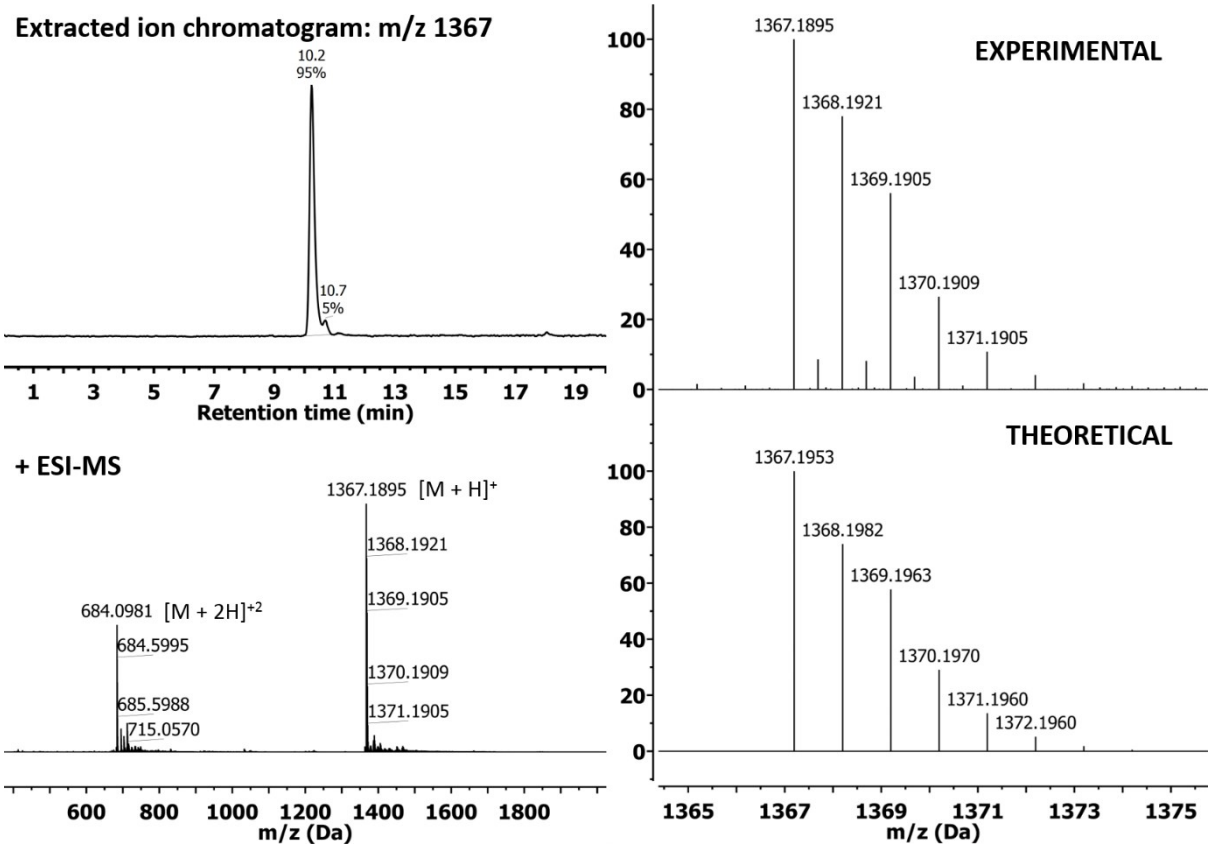
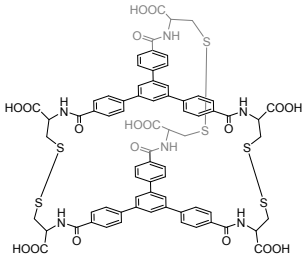


Figure S28: LC-MS analysis of cage **2-2**.

Homodimeric cage **3-3**.



3-3 was synthesized according to procedure **1-1** using **3.**

3 (75 mg, 0.1 mmol) was dissolved in aqueous NaOH (0.01 M, 20 mL) and the pH adjusted to 8 before stirring the solution for 3 days in a loosely capped vessel. The solution was then was filtered through a Celite pad and acidified with 1M HCl to pH = 2, which caused the solution to become turbid. MeCN (20 mL) was added to give a clear solution before vacuum evaporation was used to reduce the volume to 10 mL, at which point a white solid precipitated. It was filtered off and dried under high vacuum. Yield 82%.

^{1}H NMR (300 MHz, D2O) δ: 7.65 (d, *J* = 7.9 Hz, 6H), 7.49 (d, *J* = 7.8 Hz, 6H), 7.40 (s, 3H), 4.73 (m, 3H), 3.40 (dd, *J* = 13.6, 6.1 Hz, 3H), 3.05 (dd, *J* = 13.5, 7.3 Hz, 3H). **^{13}C NMR** (150 MHz, DMSO-*d6*) δ: 172.16, 166.04, 141.88, 139.57, 128.10, 127.75, 127.04, 126.25, 51.97, 40.05. **HR-MS** in positive ion mode, calc for: $[M+H]^+$ 1489.2361 m/z, found: $[M+H]^+$ 1489.2295 m/z.

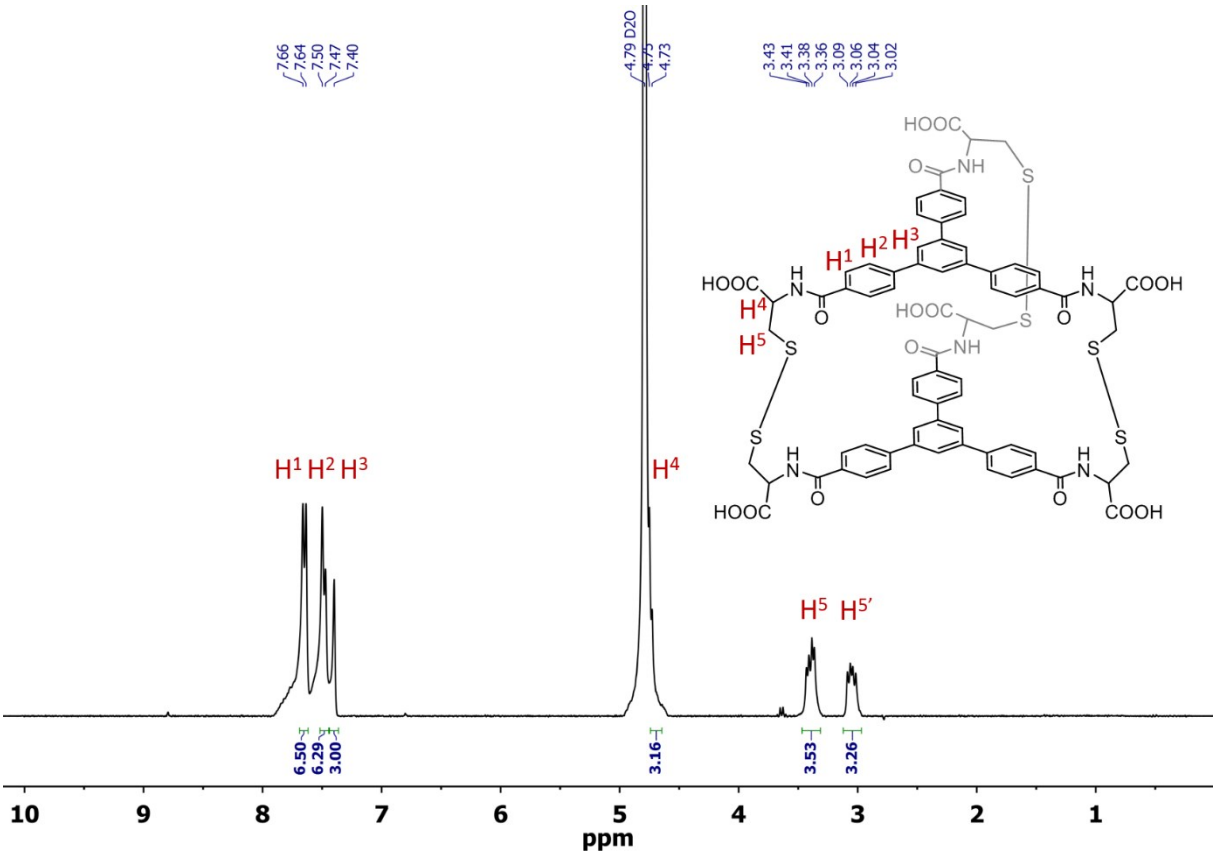


Figure S29: ^{1}H NMR spectrum of cage **3-3** in D_2O at 298 K (300 MHz).

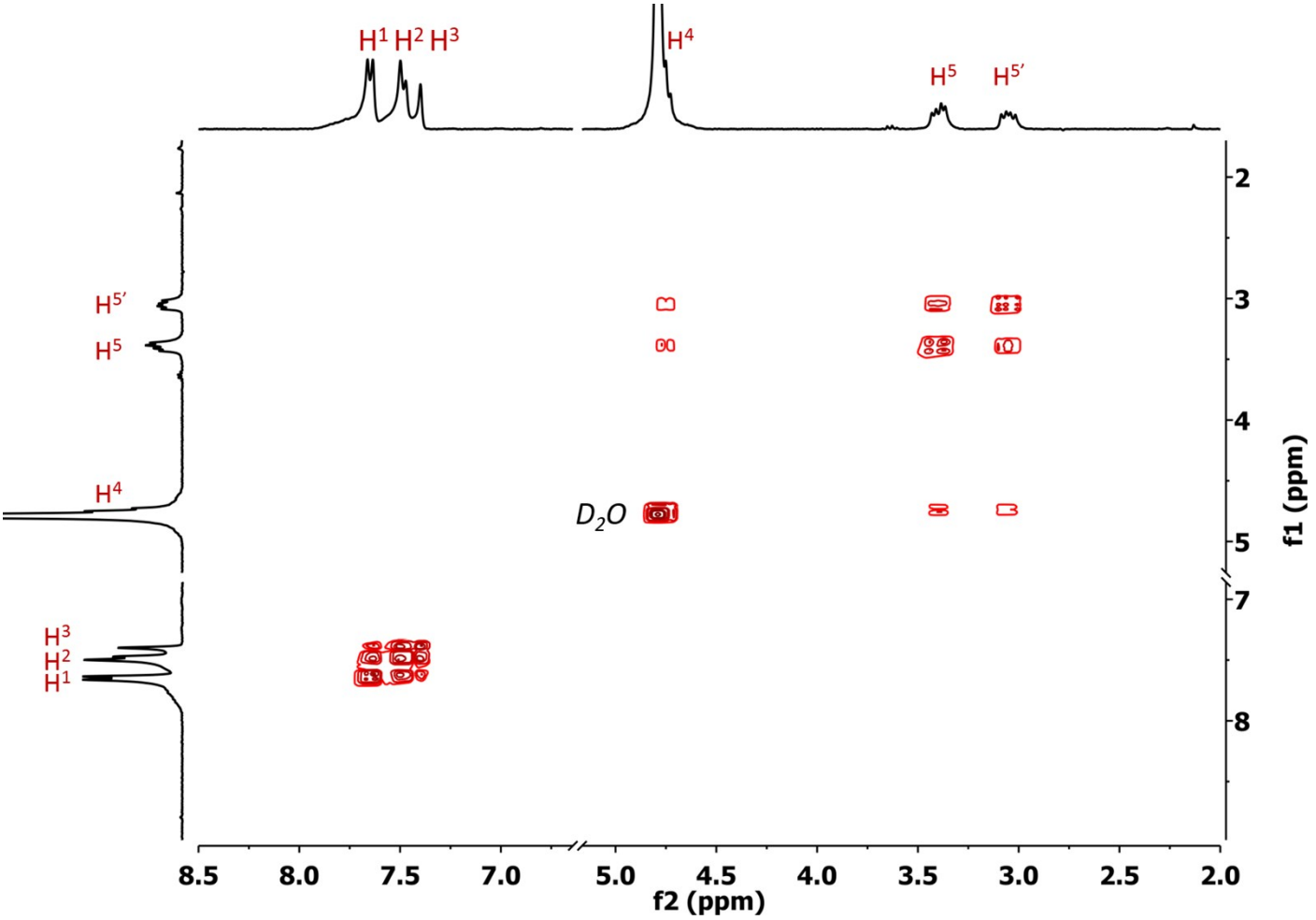


Figure S30: ^{1}H NMR spectrum of cage **3-3** in D_2O at 298 K (300 MHz).

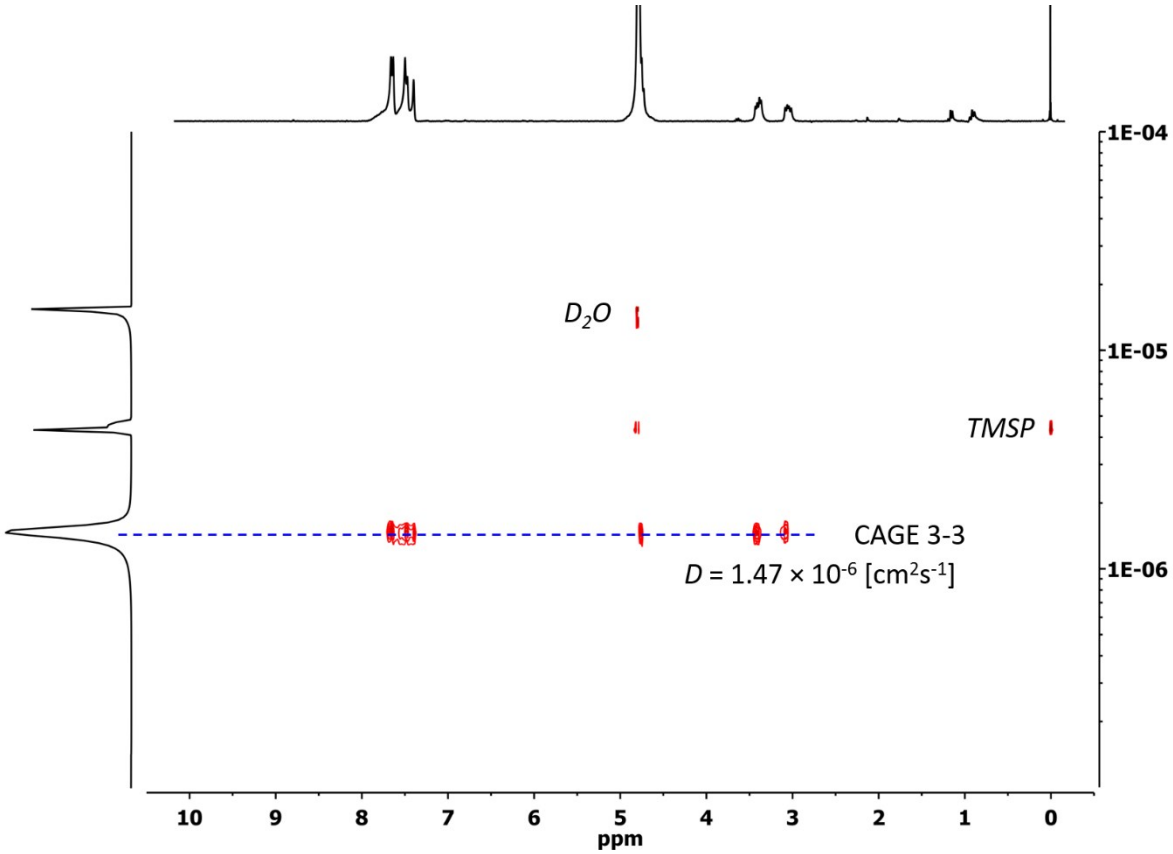


Figure S31: ^{1}H NMR spectrum of cage **3-3** in D_2O at 298 K (300 MHz).

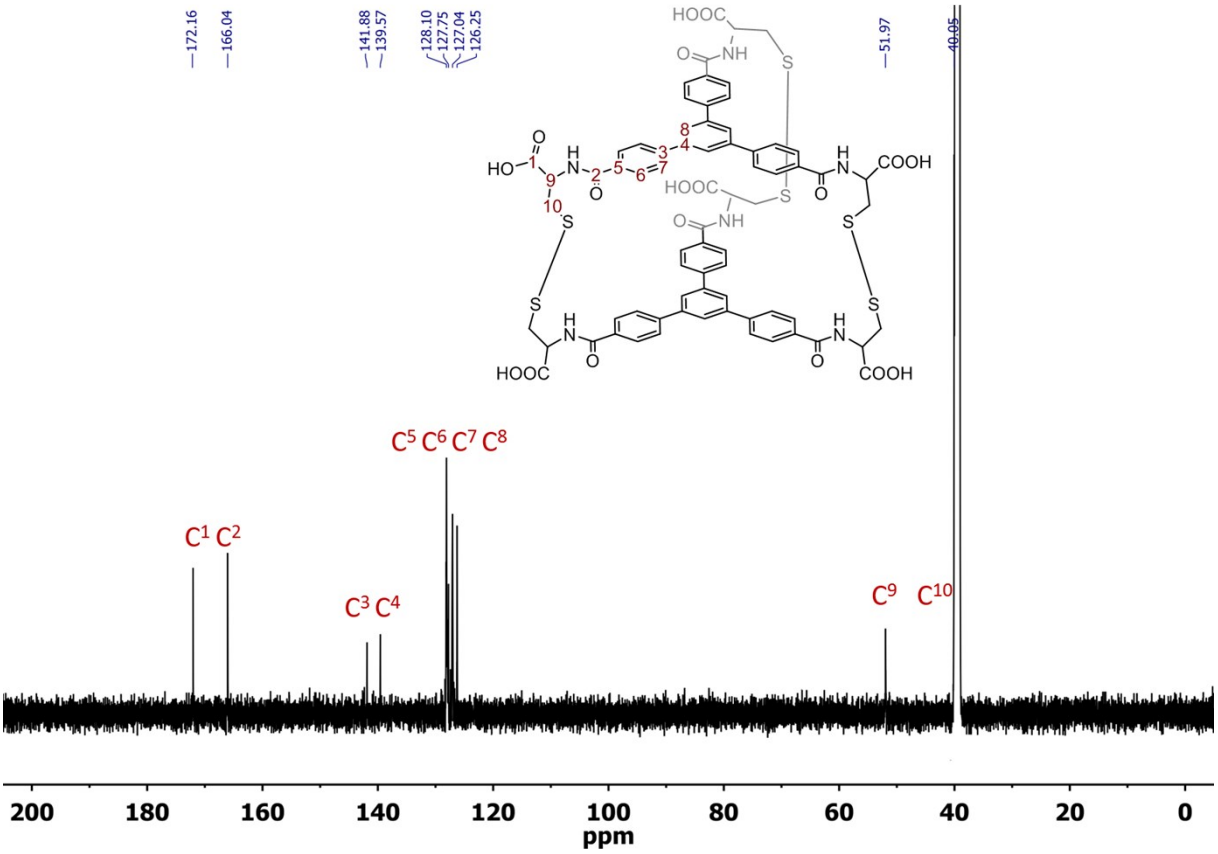


Figure S32: ^{13}C NMR spectrum of cage **3-3** in DMSO-d_6 at 298 K (150 MHz).

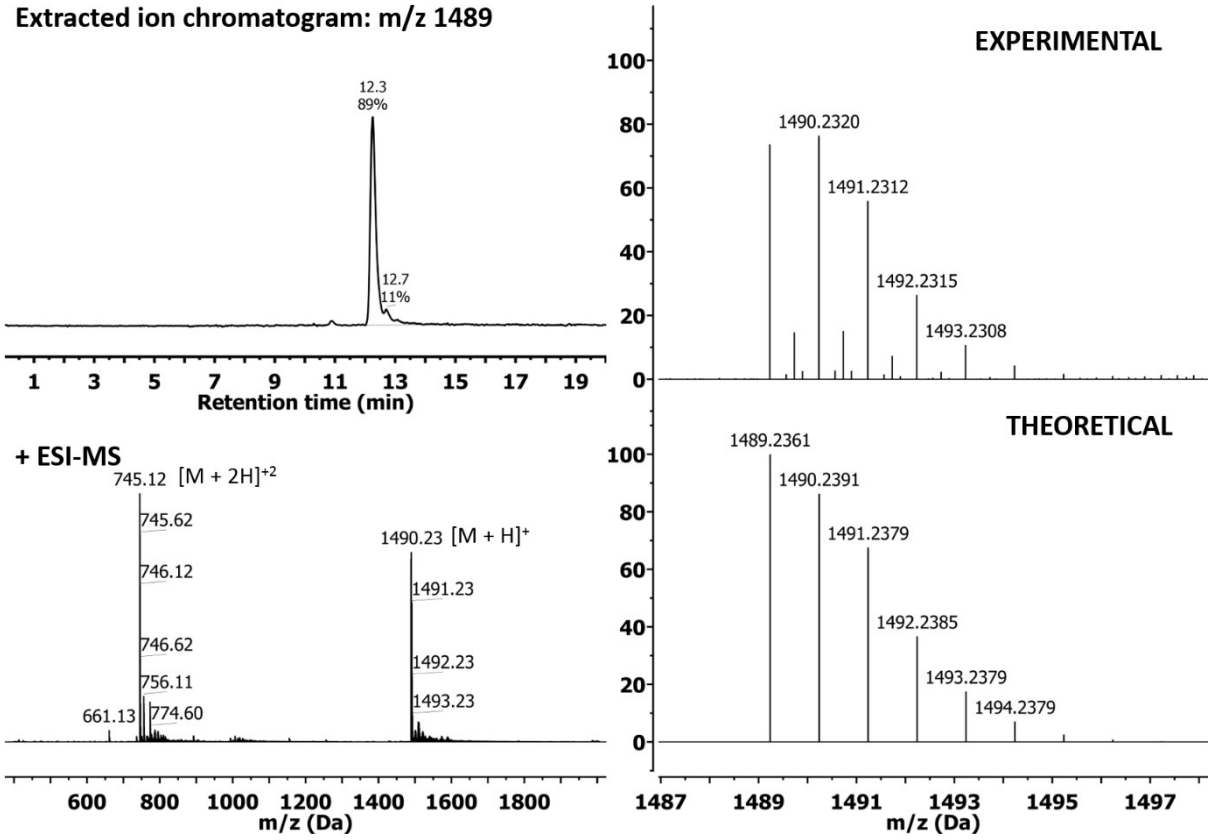
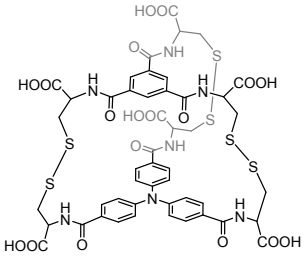


Figure S33: LC-MS analysis of cage **3-3**.

Synthesis and characterisation of heterodimeric cages

Heterodimeric cage **1-2**.



Component **1** (52 mg, 0.1 mmol) and component **2** (68 mg, 0.1 mmol) were dissolved in aqueous NaOH (0.01 M, 10 mL, pH adjusted to 8) and then stirred in a loosely capped vial for 5 days. The desired cage **1-2** was obtained from the post-reaction mixture via semipreparative HPLC.

^{1}H NMR (600 MHz, D_2O) δ: 8.20 (s, 3H), 7.76 (d, *J* = 8.1 Hz, 6H), 6.84 (d, *J* = 8.0 Hz, 6H), 4.57 (dd, *J* = 10.4, 5.3 Hz, 3H), 3.45 – 3.37 (m, 3H), 3.18 (dd, *J* = 14.8, 9.4 Hz, 3H), 3.04 (q, *J* = 10.6, 8.9 Hz, 3H), 2.78 (dd, *J* = 12.8, 5.8 Hz, 3H). **HR-MS** in positive ion mode, calc for: $[M+H]^+$ 1200.1218 m/z, found: $[M+H]^+$ 1200.1474 m/z.

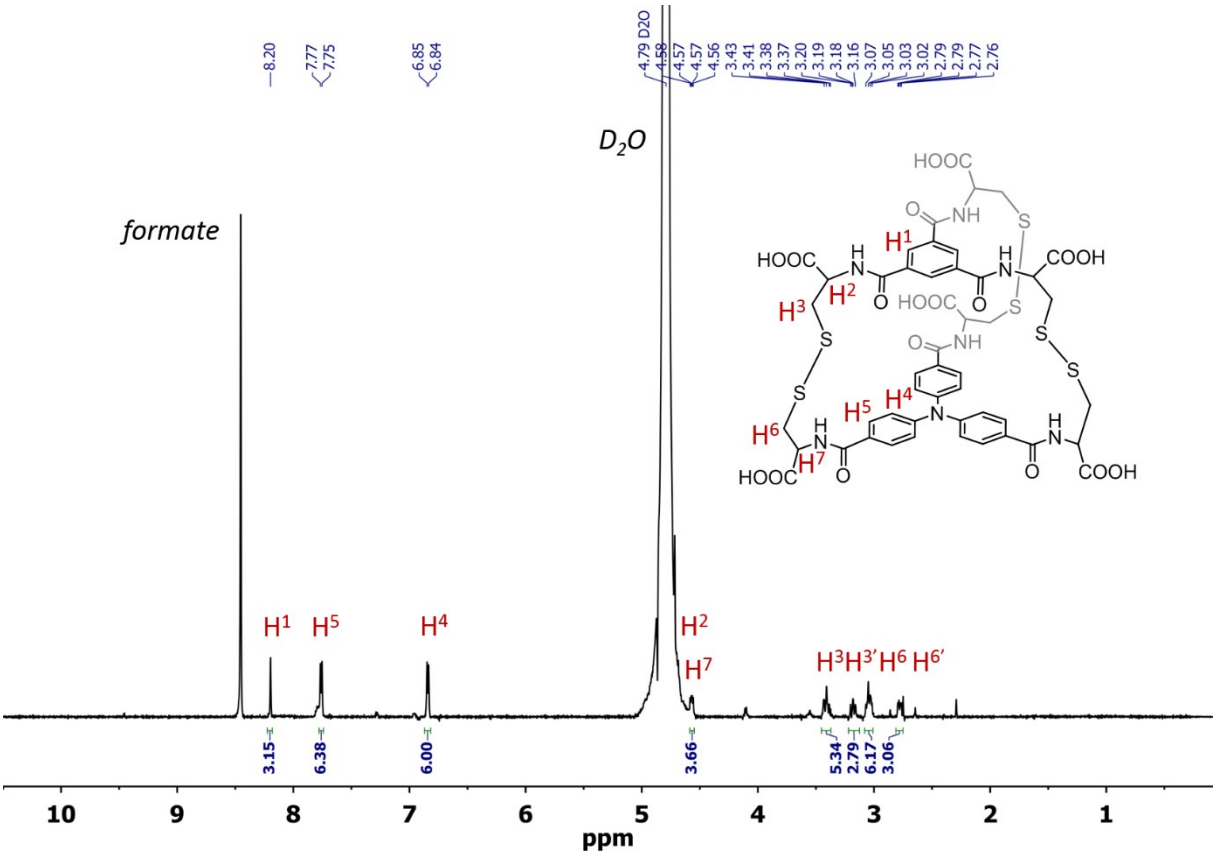


Figure S34: ^{1}H NMR spectrum of cage **1-2** in D_2O at 298 K (600 MHz).

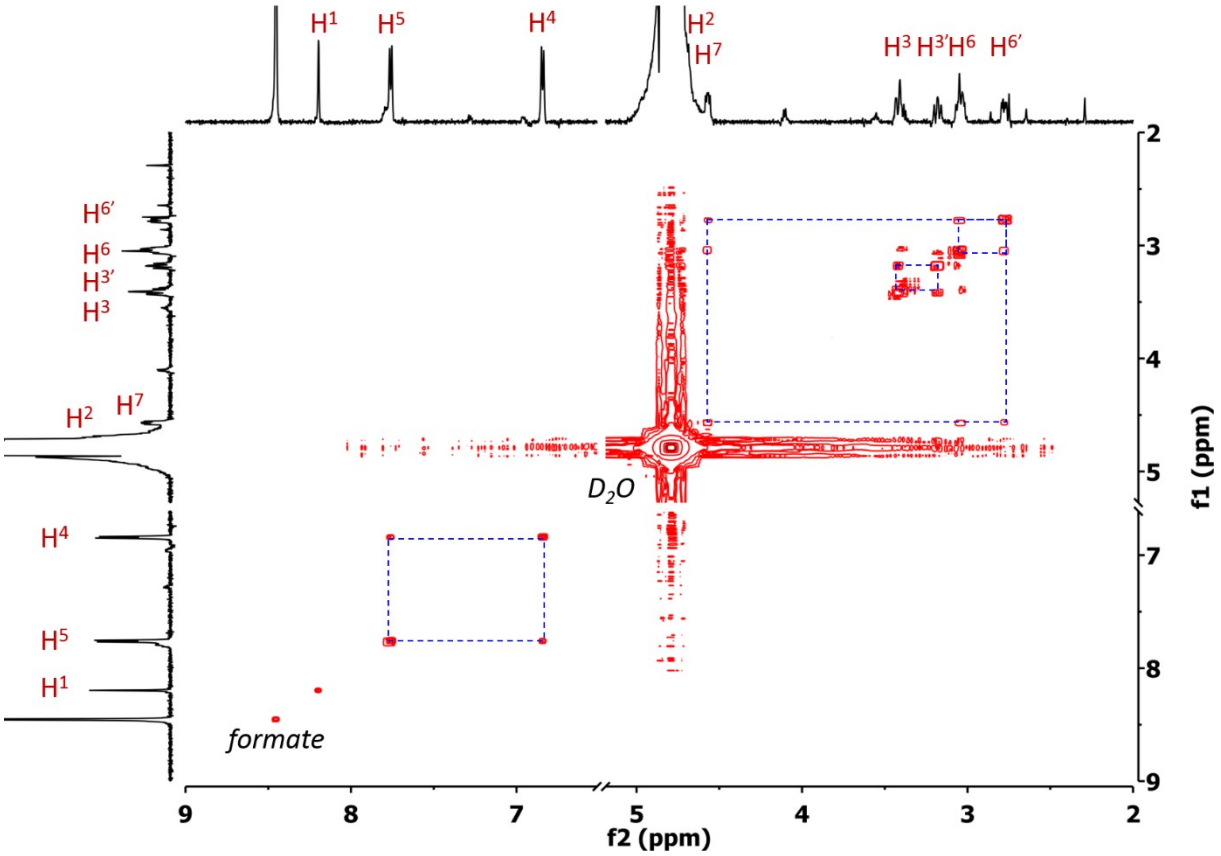


Figure S35: COSY spectrum of cage **1-2** in D_2O at 298 K (600 MHz).

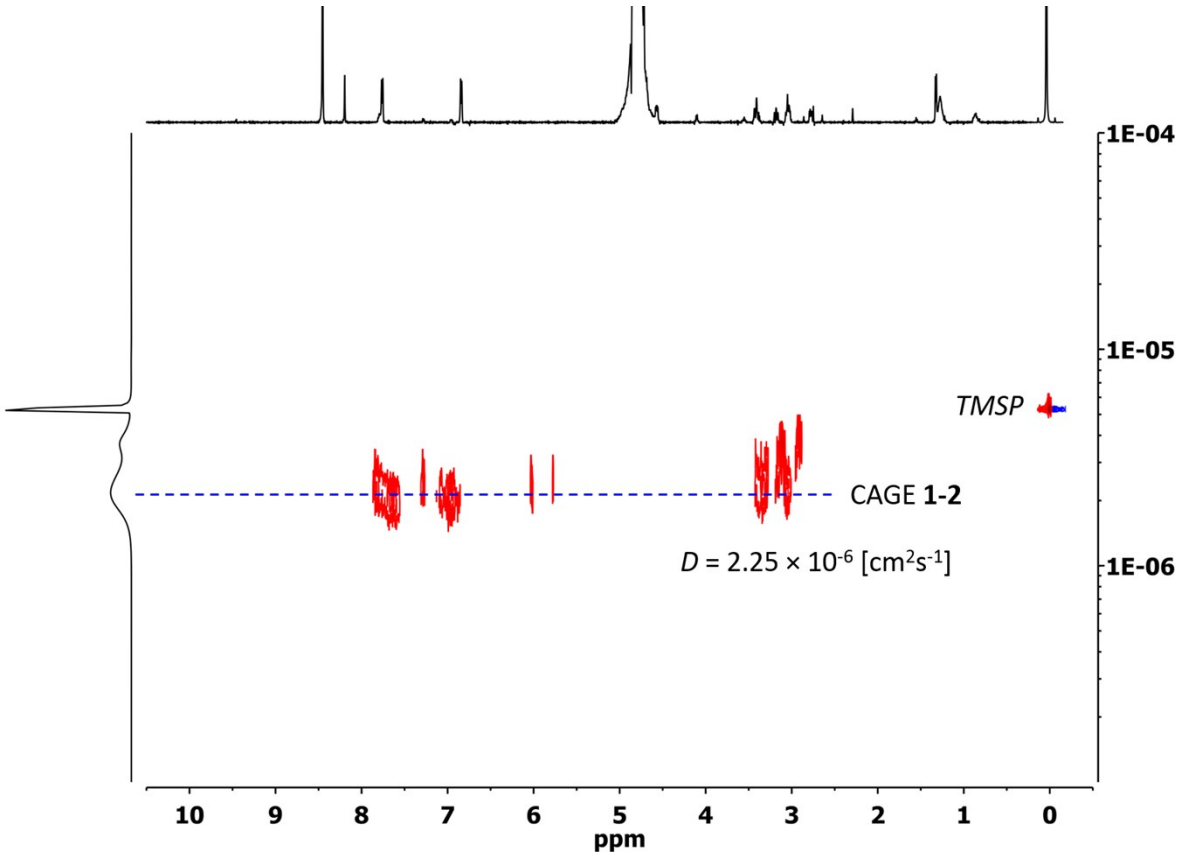


Figure S36: DOSY spectrum of cage **1-2** in D_2O at 298 K (500 MHz).

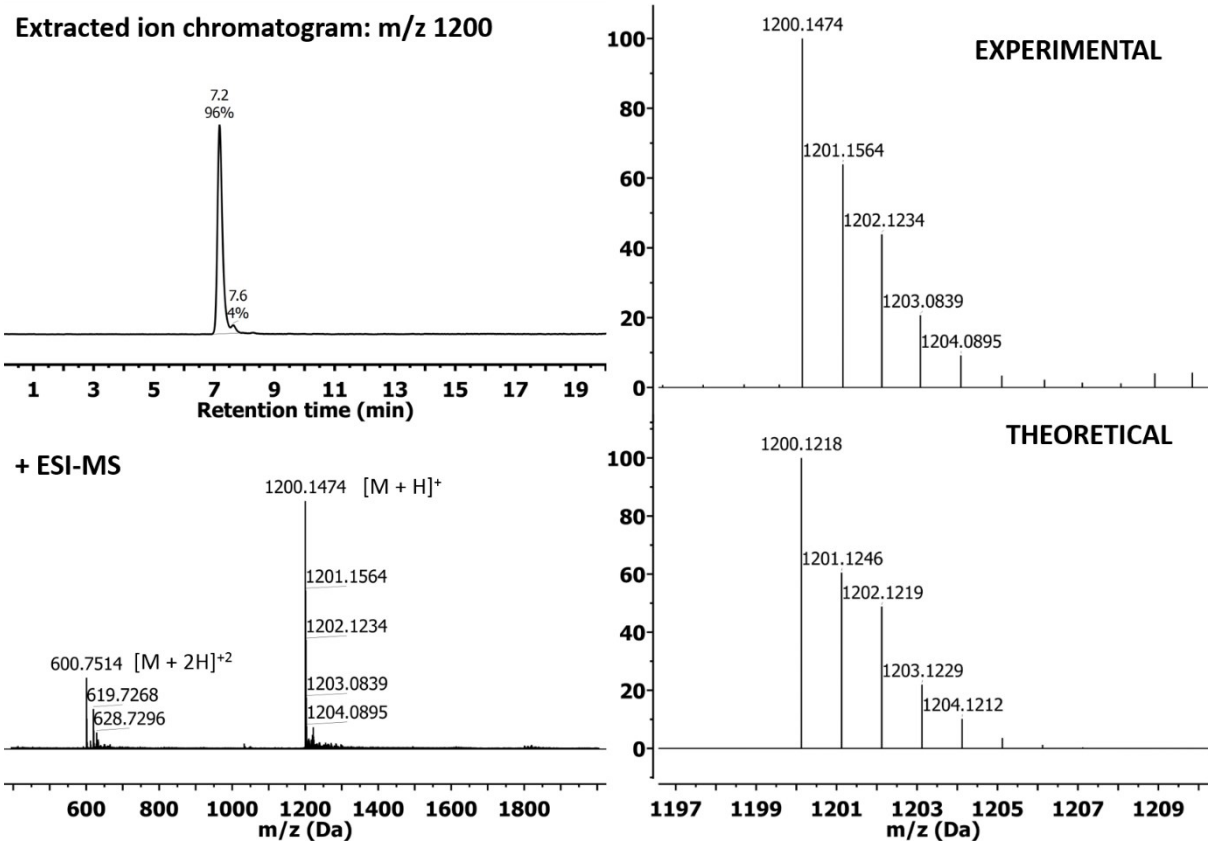
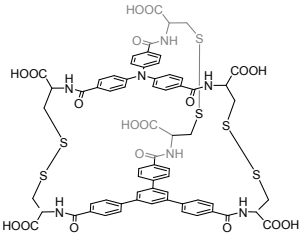


Figure S37: LC-MS analysis of cage **1-2**.

Heterodimeric cage **2-3**.



Component **2** (68 mg, 0.1 mmol) and component **3** (75 mg, 0.1 mmol) were dissolved in aqueous NaOH (0.01 M, 10 mL, pH adjusted to 8) and then stirred in a loosely capped vial for 5 days. Then the desired cage **1-2** was obtained from the post-reaction mixture via semipreparative HPLC.

^{1}H NMR (600 MHz, D2O) δ: 7.95 (s, 3H), 7.87 (d, *J* = 8.0 Hz, 6H), 7.80 (d, *J* = 8.1 Hz, 6H), 7.40 (d, *J* = 8.3 Hz, 6H), 6.56 (d, *J* = 8.3 Hz, 6H), 4.75 (d, *J* = 4.4 Hz, 3H), 4.69 – 4.65 (m, 3H), 3.46 (dd, *J* = 13.9, 3.9 Hz, 3H), 3.25 (dd, *J* = 13.3, 8.7 Hz, 3H), 3.18 (dd, *J* = 13.8, 9.9 Hz, 3H), 2.88 (dd, *J* = 13.3, 5.6 Hz, 3H). **HR-MS** in positive ion mode, calc for: $[M+H]^+$ 1428.2157 m/z, found: $[M+H]^+$ 1428.2106 m/z.

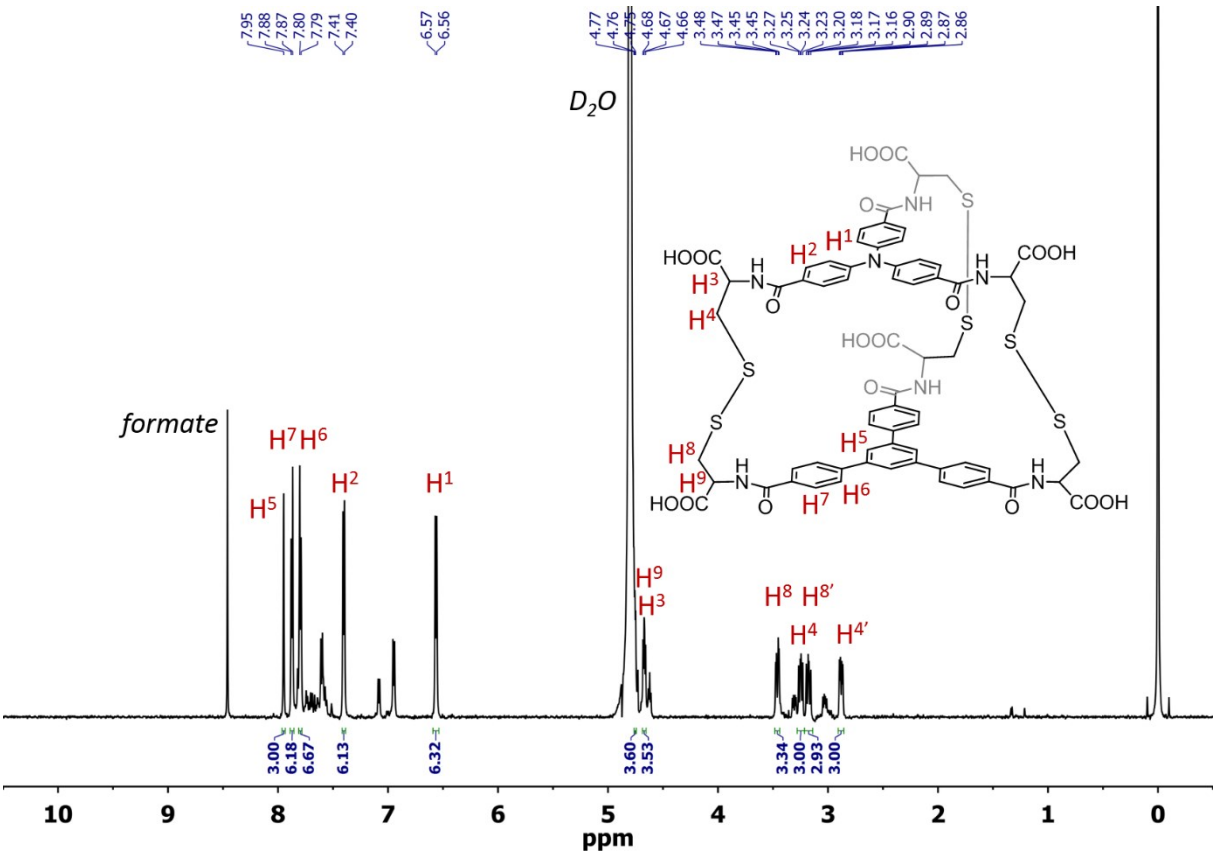


Figure S38: ^{1}H NMR spectrum of cage **2-3** in D_2O at 298 K (600 MHz).

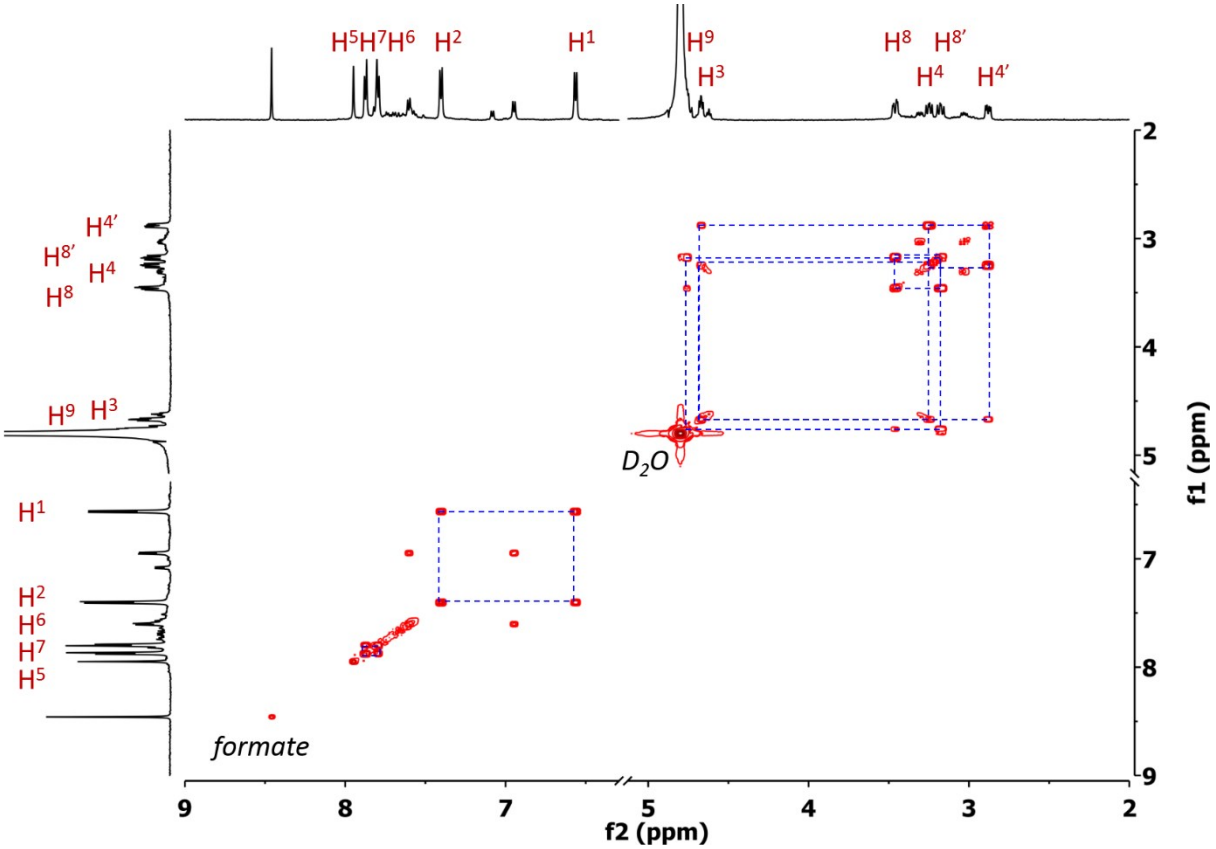


Figure S39: COSY spectrum of cage **2-3** in D_2O at 298 K (600 MHz).

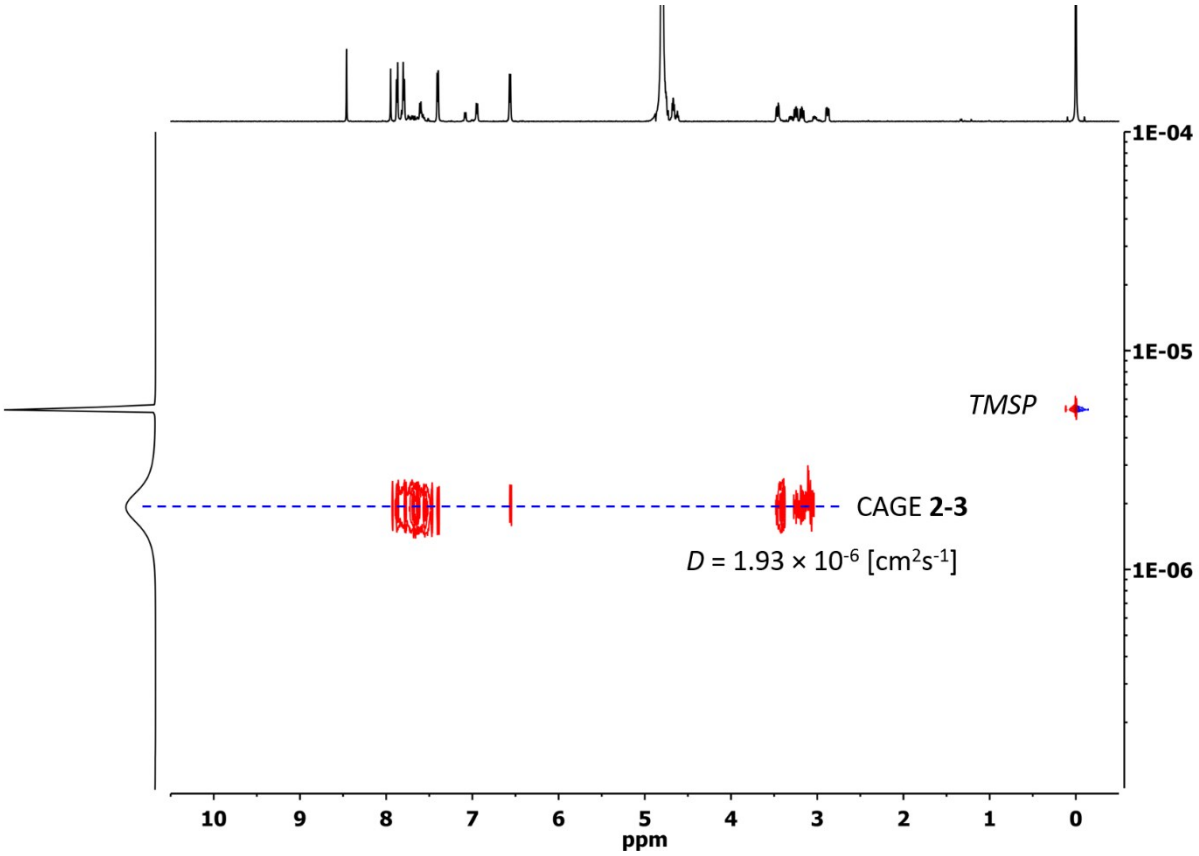


Figure S40: DOSY spectrum of cage **2-3** in D_2O at 298 K (500 MHz).

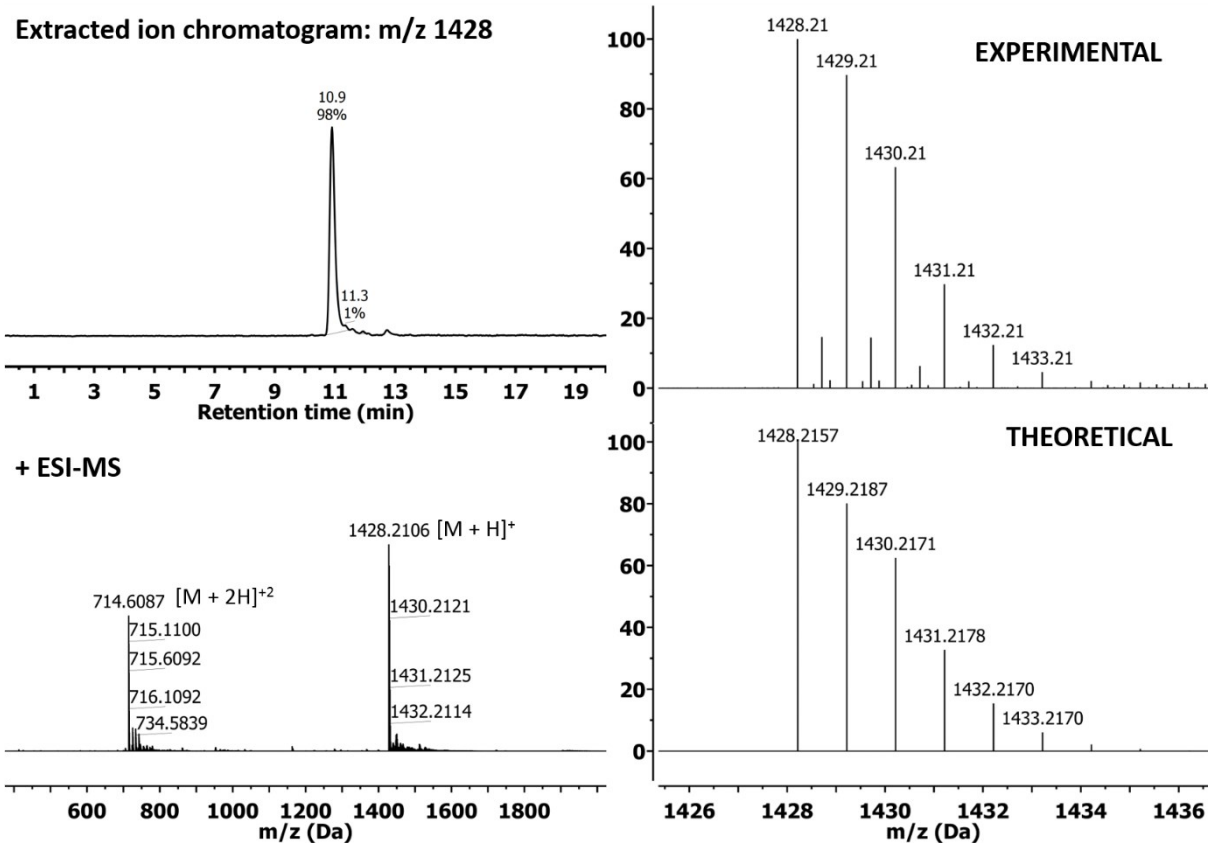


Figure S41: LC-MS analysis of cage **2-3**.

HPLC-MS Data

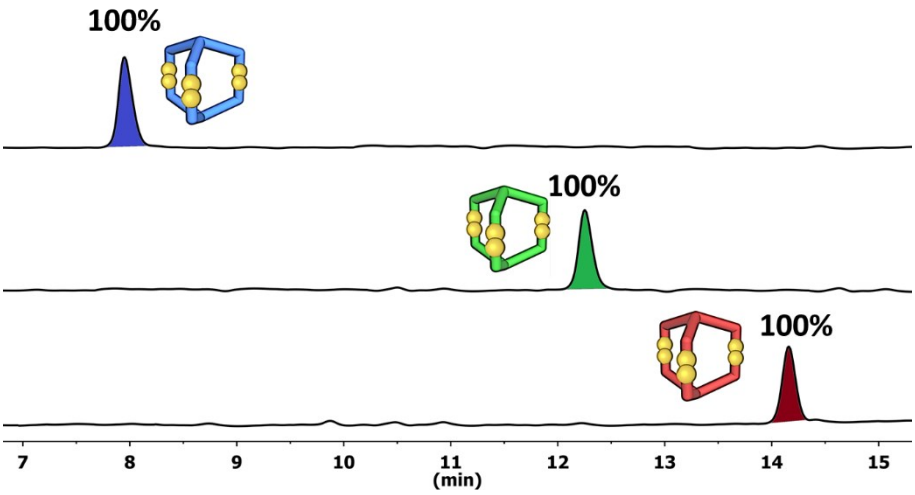


Figure S42: HPLC chromatograms of homodimeric cages post-reaction mixtures (254 nm) showing the exclusive formation of a single cage-like product.

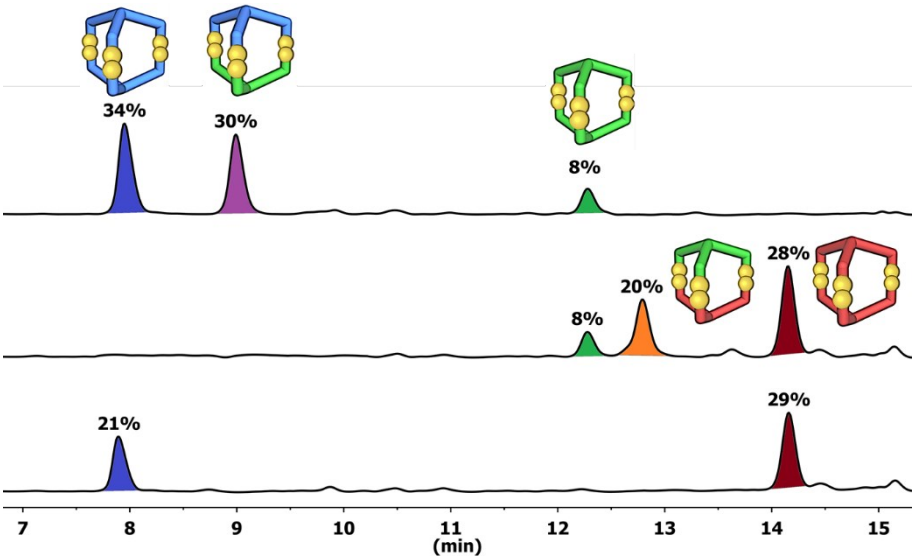


Figure S43: HPLC chromatograms of post-reaction mixtures (254 nm) showing formation of homo and hetero-dimeric cages. The bottom chromatogram shows no formation of a potential **1-3** cage.

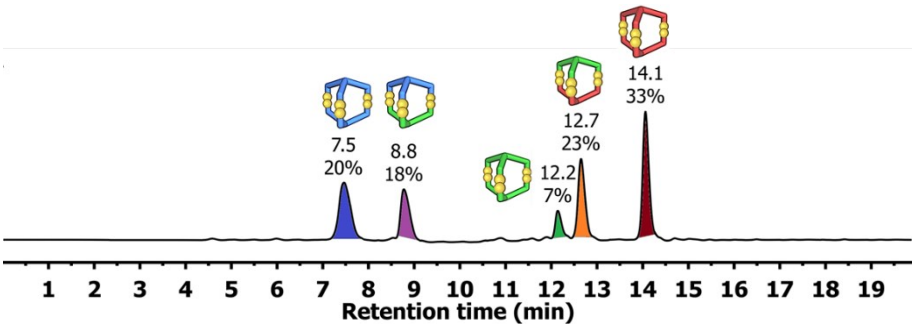


Figure S44: HPLC chromatograms (254 nm) of post-reaction DCL containing an equimolar mixture of all five cages.

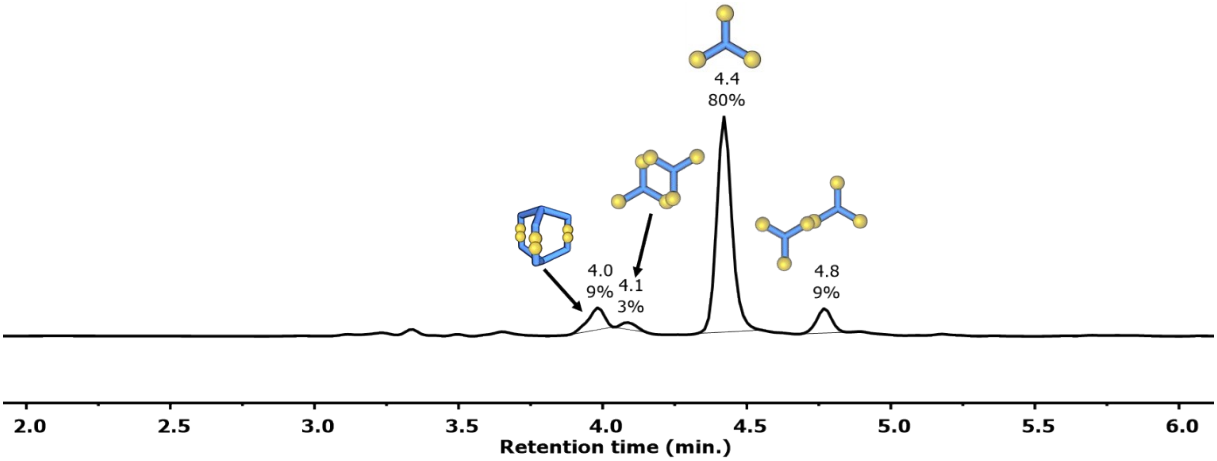


Figure S45: HPLC chromatogram (254 nm) of reaction DCL mixture containing **1** after 60 min.

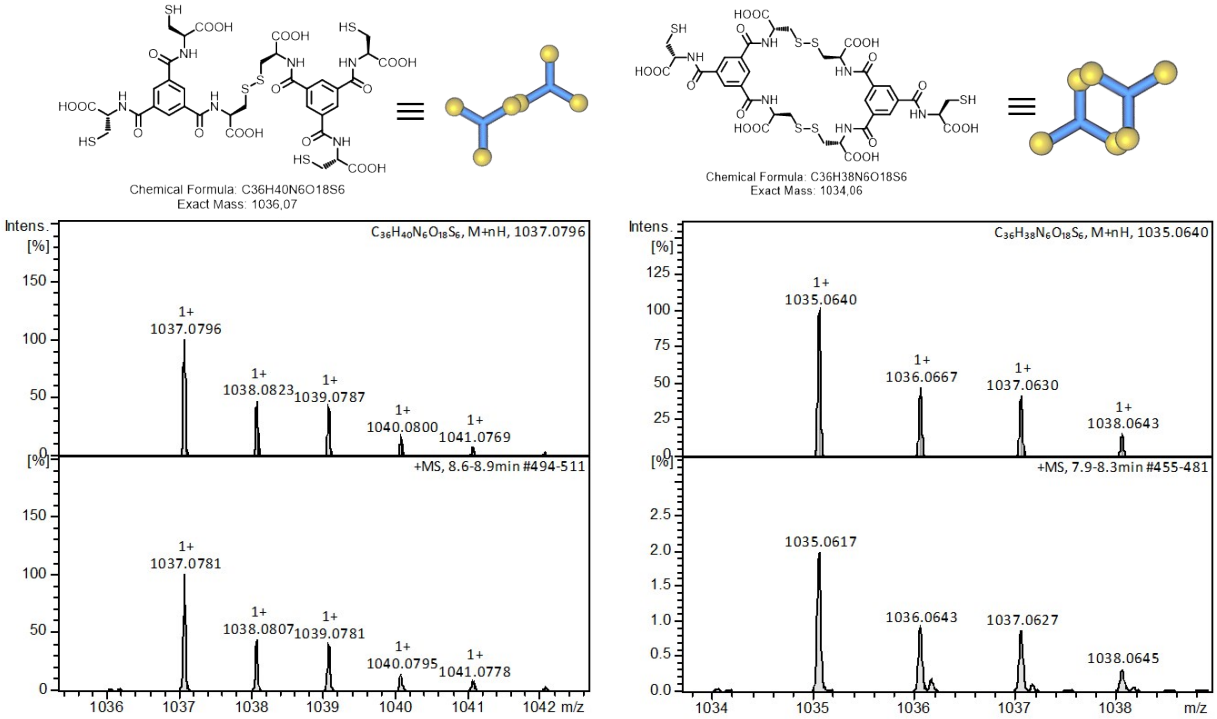


Figure S46: Mass analysis of **1** intermediates during reaction after 60 min.

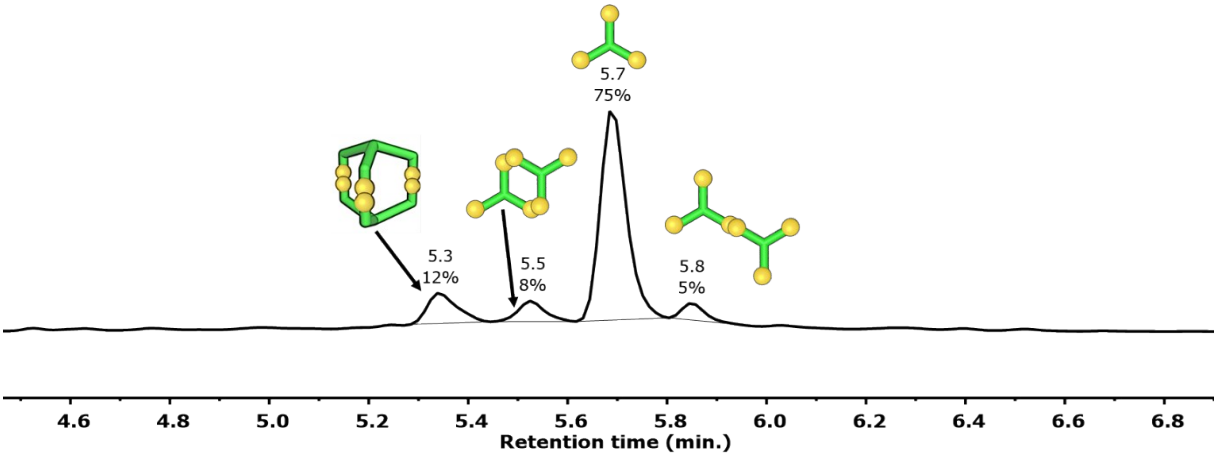


Figure S47: HPLC chromatogram (254 nm) of reaction DCL mixture containing **2** after 60 min.

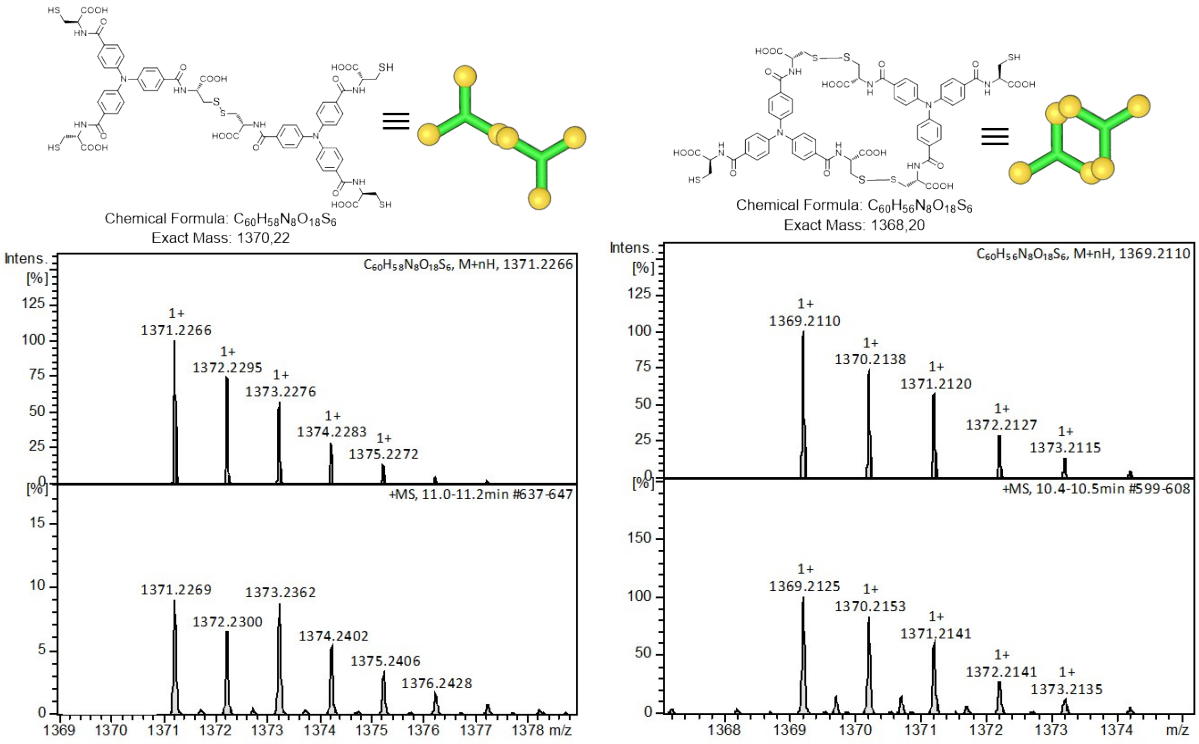


Figure S48: Mass analysis of **2** intermediates during reaction after 60 min.

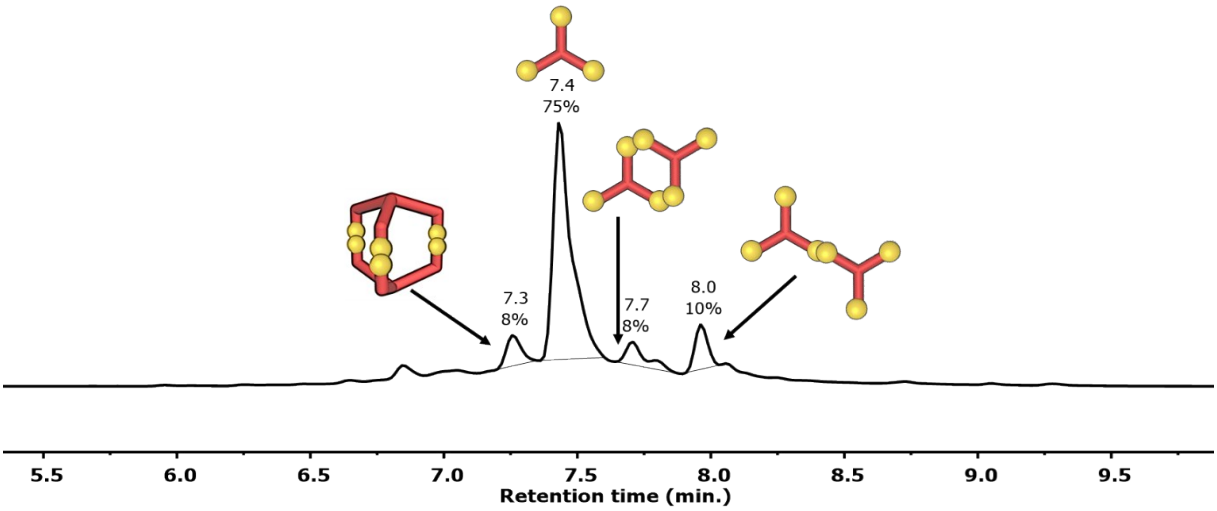


Figure S49: HPLC chromatogram (254 nm) of reaction DCL mixture containing **3** after 60 min.

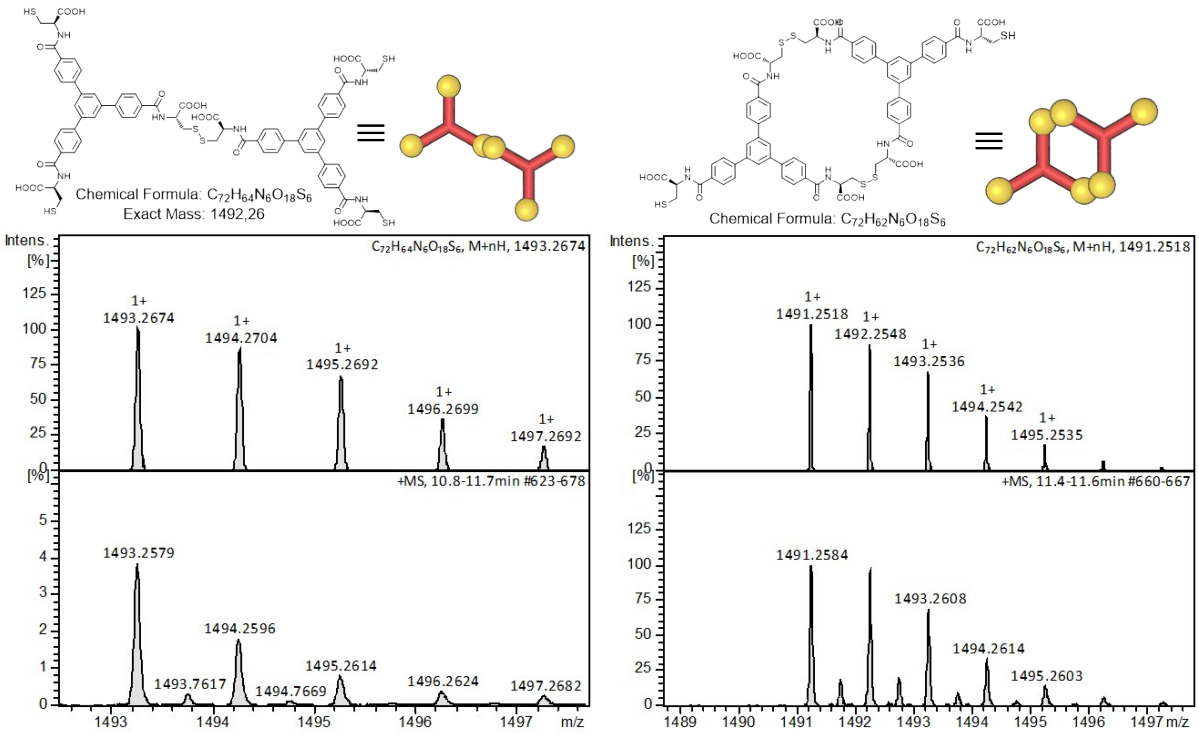


Figure S50: Mass analysis of **3** intermediates during reaction after 60 min.

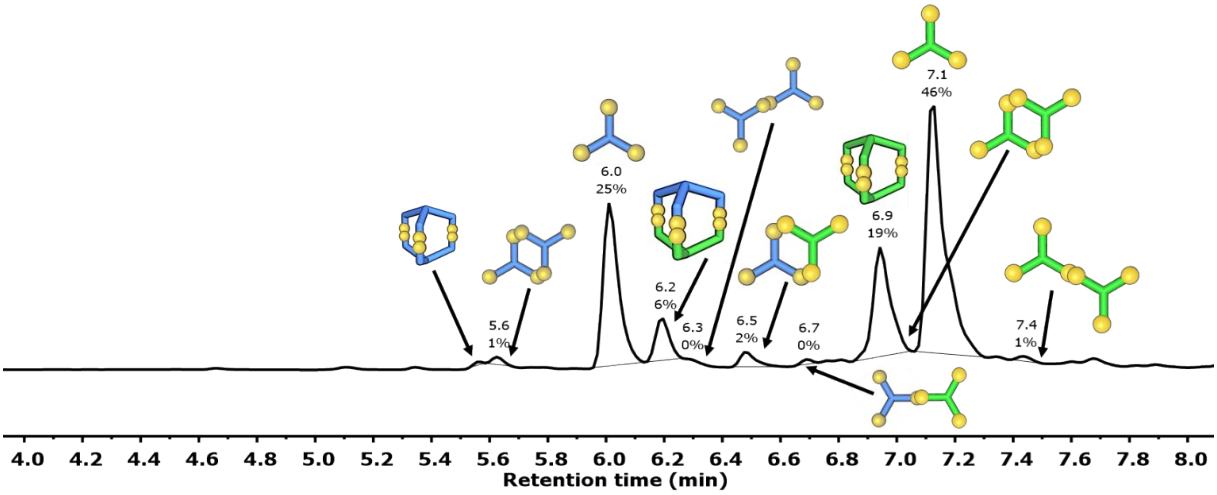


Figure S51: HPLC chromatogram (254 nm) of reaction DCL mixture containing equimolar **1**+**2** after 60 min.

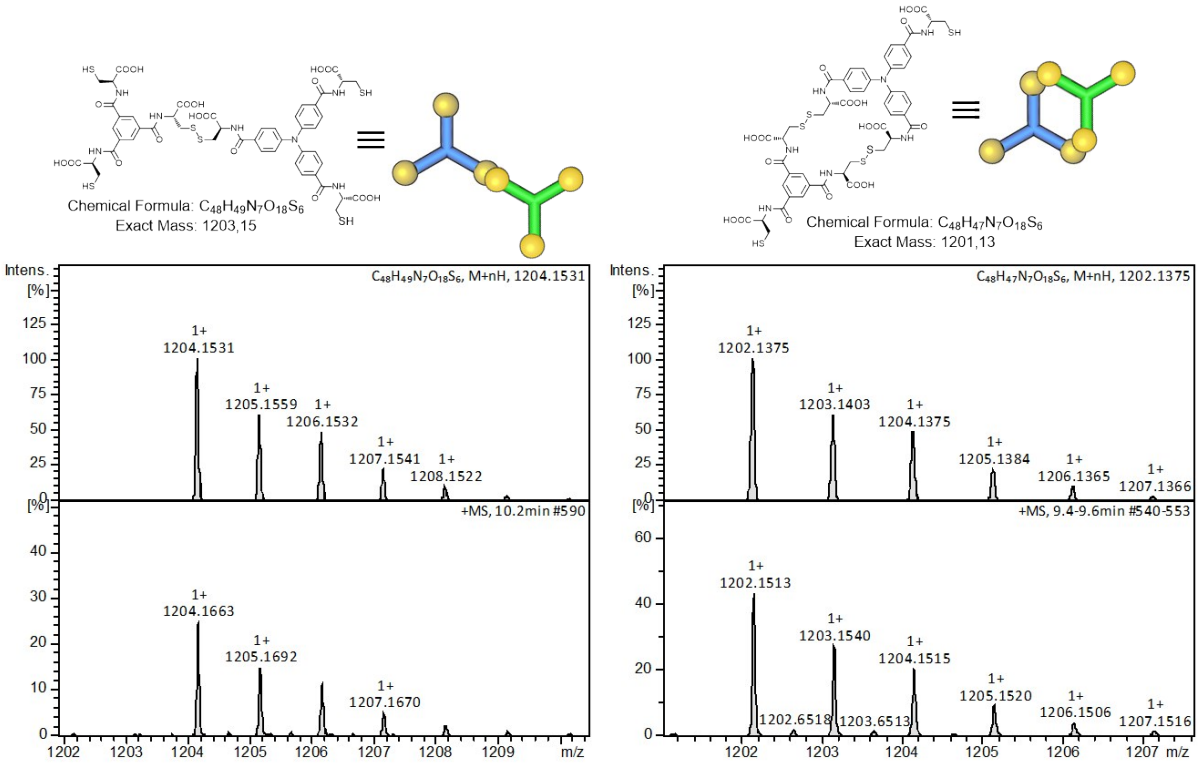


Figure S52: Mass analysis of **1+2** mixture intermediates during reaction after 60 min.

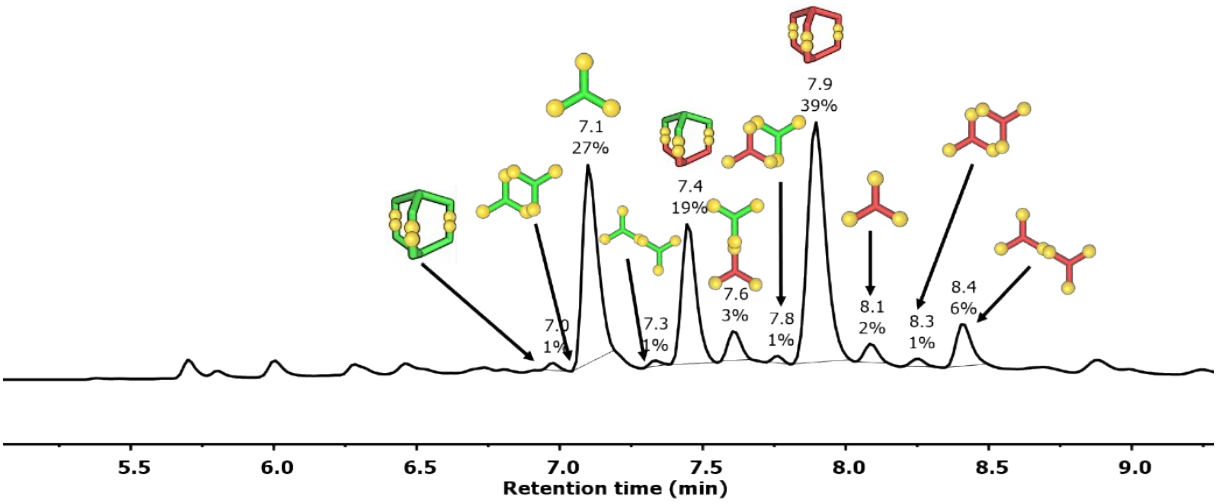


Figure S53: HPLC chromatogram (254 nm) of reaction DCL mixture containing equimolar **2+3** after 60 min.

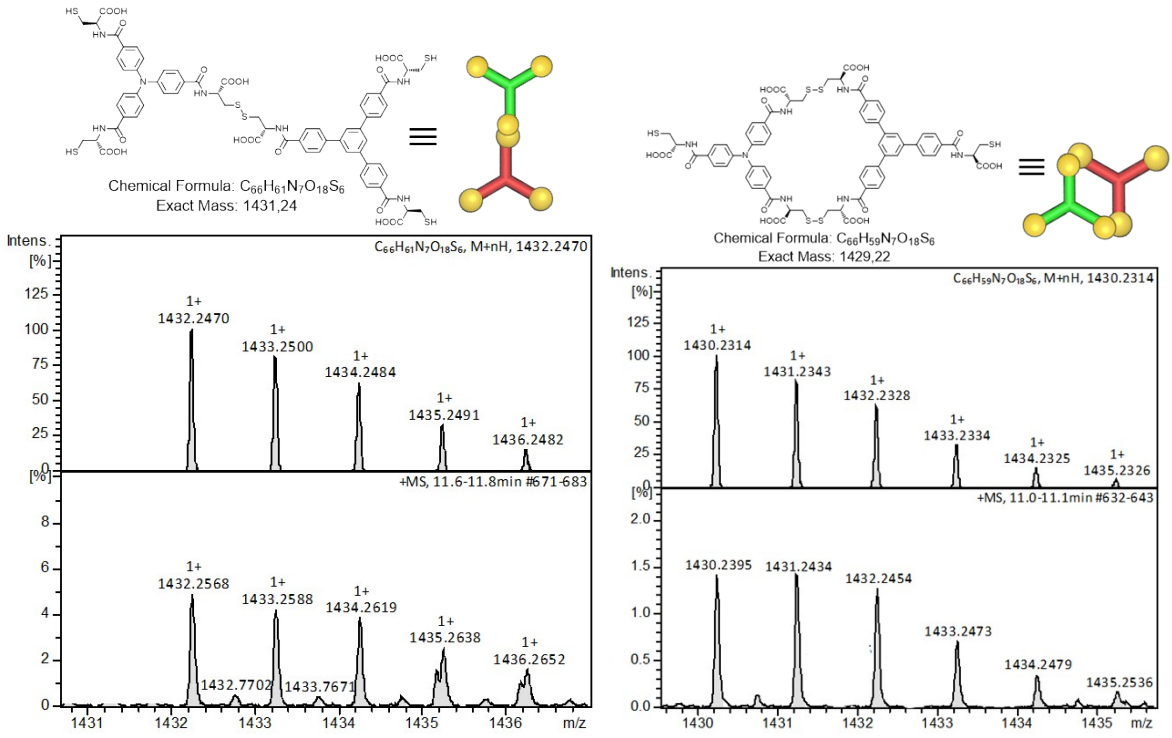


Figure S54: Mass analysis of **2+3** mixture intermediates during reaction after 60 min.

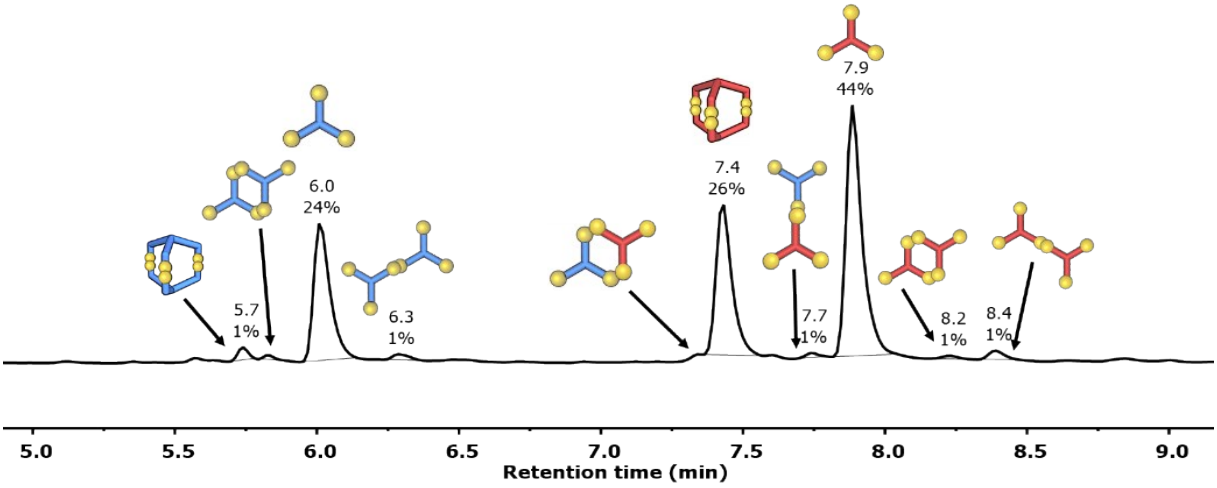


Figure S55: HPLC chromatogram (254 nm) of reaction DCL mixture containing equimolar **1+3** after 60 min.

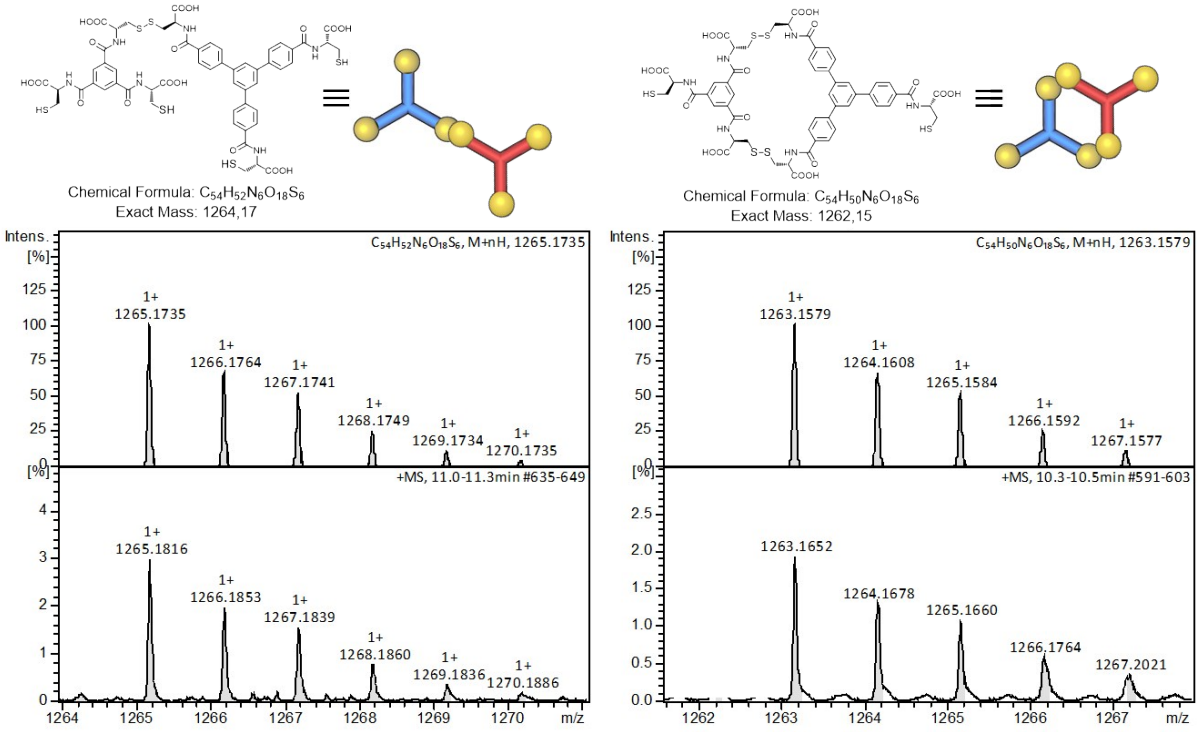


Figure S56: Mass analysis of **1+3** mixture intermediates during reaction after 60 min.

UV-Vis Data

Relative peak area [%] (HPLC, 254 nm)

cage	L50-01	L50-02	L50-03	L50-04
c1-1	27	18	21	8
c1-2	23	31	29	28
c2-2	11	19	13	26
c2-3	17	18	21	31
c3-3	22	14	16	7

Relative peak area corrected for molar extinction coefficients

cage	1:1:1	1:2:1	1:3:1	1:8:1
c1-1	20	16	13	6
c1-2	20	25	27	24
c2-2	22	26	38	52
c2-3	19	23	20	34
c3-3	20	15	13	6

Normalized relative peak area

cage	1:1:1	1:2:1	1:3:1	1:8:1
c1-1	0,20	0,15	0,12	0,05
c1-2	0,20	0,24	0,24	0,20
c2-2	0,20	0,25	0,34	0,42
c2-3	0,20	0,22	0,18	0,28
c3-3	0,20	0,14	0,11	0,05

% change

cage	1:2:1	1:3:1	1:8:1
1-1	-0,0514	-0,0799	-0,1519
1-2	0,0410	0,0428	-0,0022
2-2	0,0484	0,1423	0,2225
2-3	-0,0230	0,0198	0,0799
3-3	-0,0610	-0,0854	-0,1483

Figure S57: Supplementary table for Fig 5. Calculations of percentage changes in DCLs distribution depending on various concentration of component **2**.

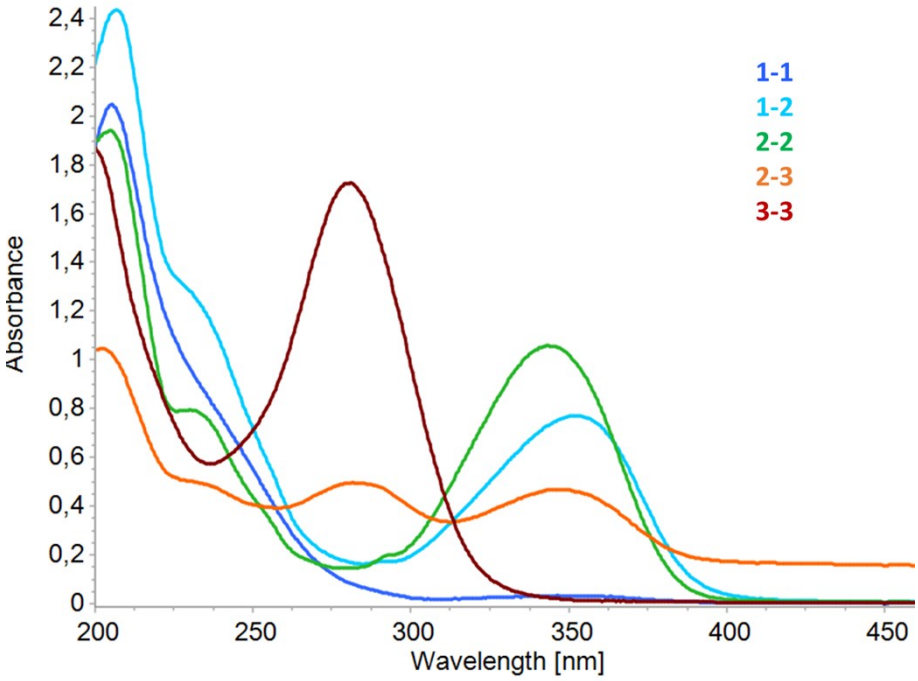


Figure S58: UV-VIS spectra of five dimeric cages in water (pH 8.0, conc. 5×10^{-5}M, 1×1 cm).

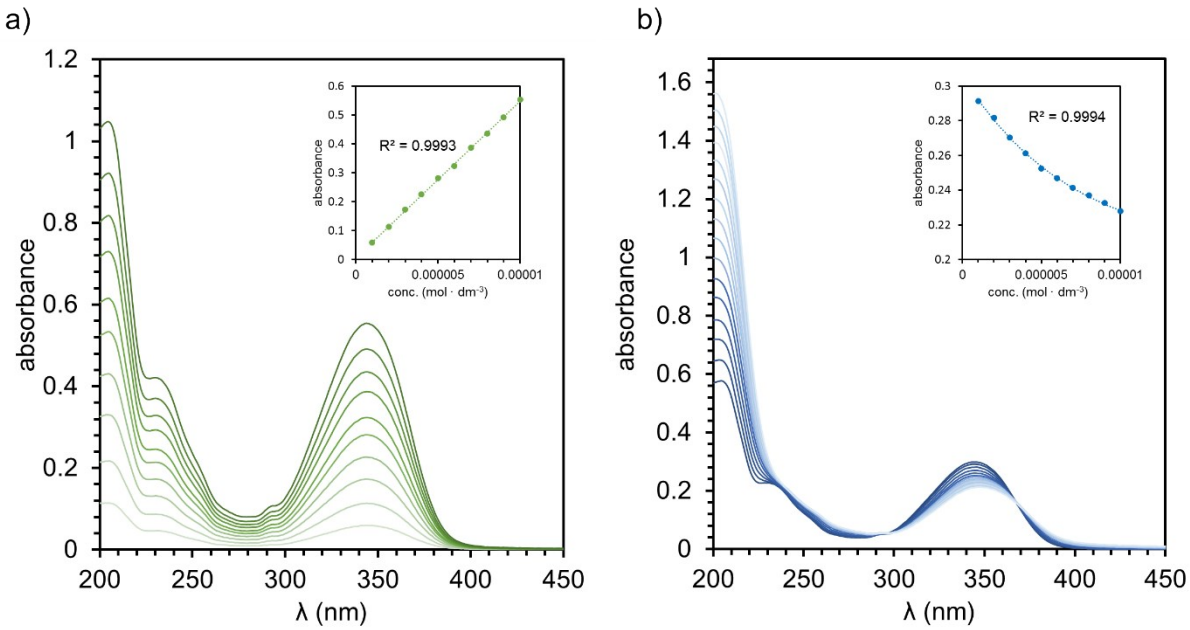


Figure S59: a) UV-VIS spectra of **2-2** (pH 8.0, conc. 10^{-6} M - 10^{-5} M, 1×1 cm), b) UV-VIS titration of cage **2-2** with La^{3+} in water (pH 8.0, conc. 5×10^{-5}M, 1×1 cm, 0-3 equiv., 0.3 equiv./step).

Computational data

We started with conformational analysis performed under the Spartan'14 software, using Monte Carlo methods and, where necessary, with more relaxed conformer search conditions. Candidates for further calculations were selected from the results and initially optimised with the semiempirical RM1 method (Recife Model 1).[2] The decisive factors in choosing a given structure were the lowest energy and the proper symmetry. This last condition was due to the NMR data that clearly showed the formation of highly symmetrical species. This optimization revealed that the homodimeric cages assume D_3 symmetry, while heterodimeric cages assume C_3 symmetry, in line with the experimental observations. Then, the selected conformers were further optimised by DFT b3lyp calculations using Grimme dispersion functions (GD3) and Becke-Johnes damping (BJ) functions to predict long-range interactions.[3] The basis set used was Pople's 6-31g+(d), with additional diffusion functions to correctly represent the behavior of anions, and polarization functions for heavy atoms.

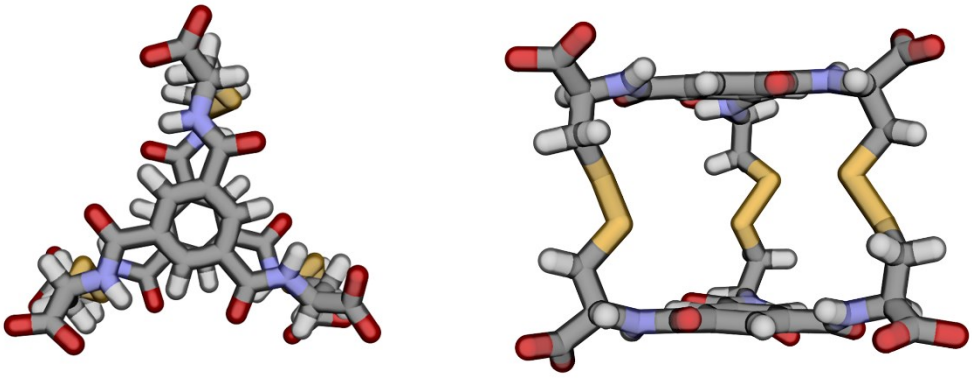


Figure S60: Optimised structure of cage **1-1**, top and side view.

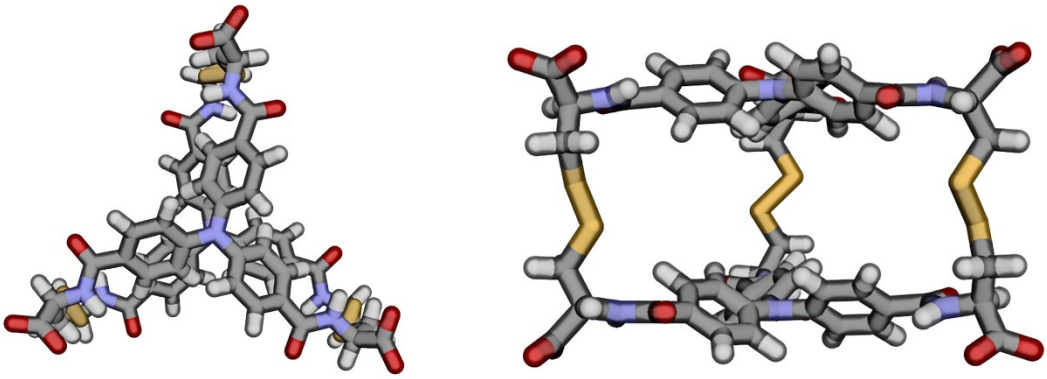


Figure S61: Optimised structure of cage **2-2**, top and side view.

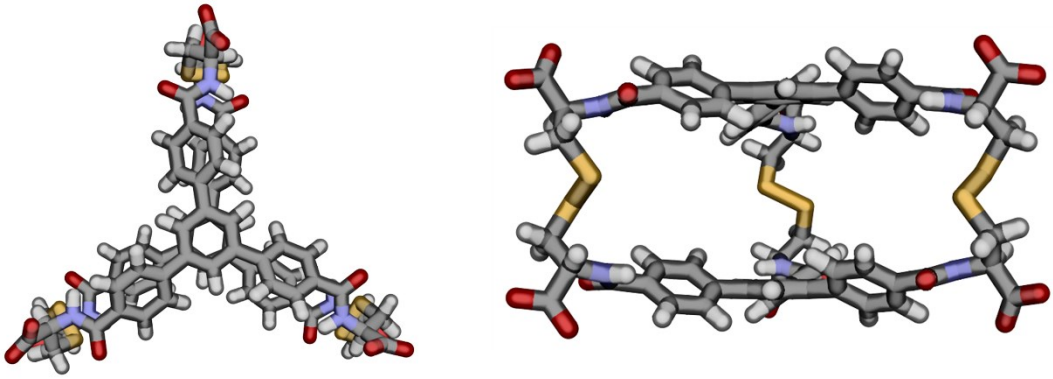


Figure S62: Optimised structure of cage **3-3**, top and side view.

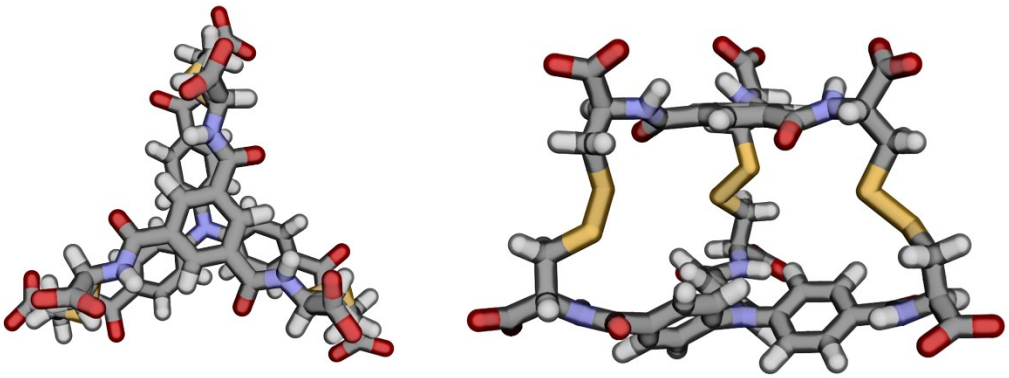


Figure S63: Optimised structure of cage **1-2**, top and side view.

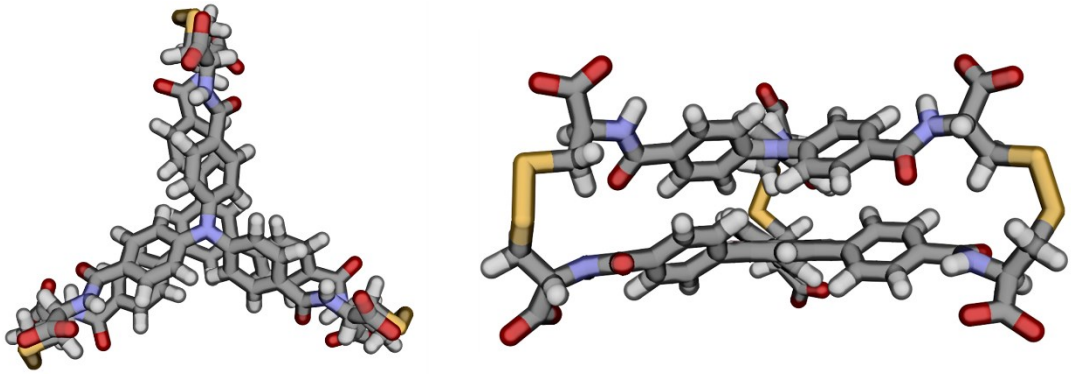


Figure S64: Optimised structure of cage **2-3**, top and side view.

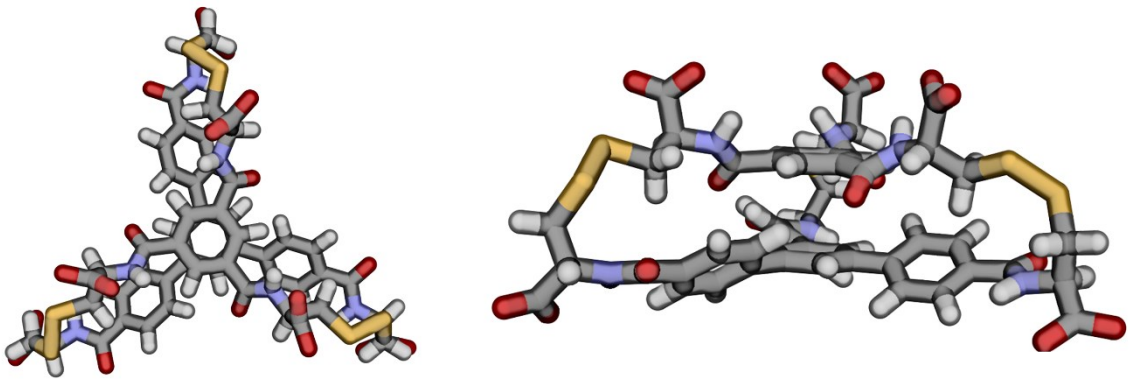


Figure S65: Optimised structure of cage **1-3**, top and side view.

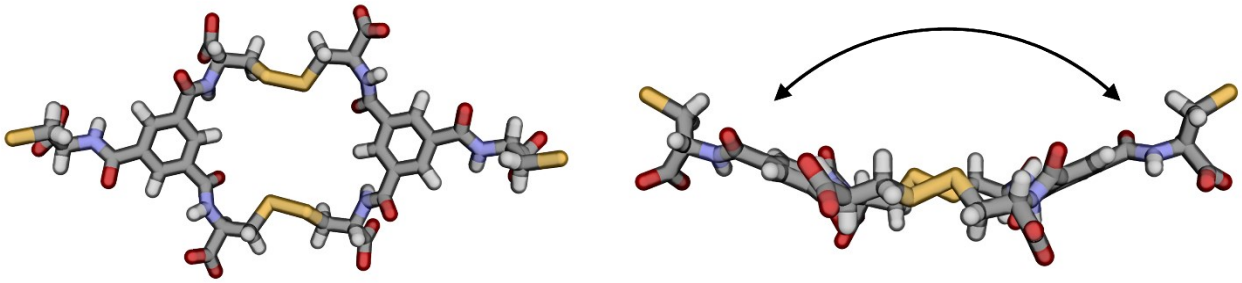


Figure S66: Optimised structure of **1B**, top and side view.

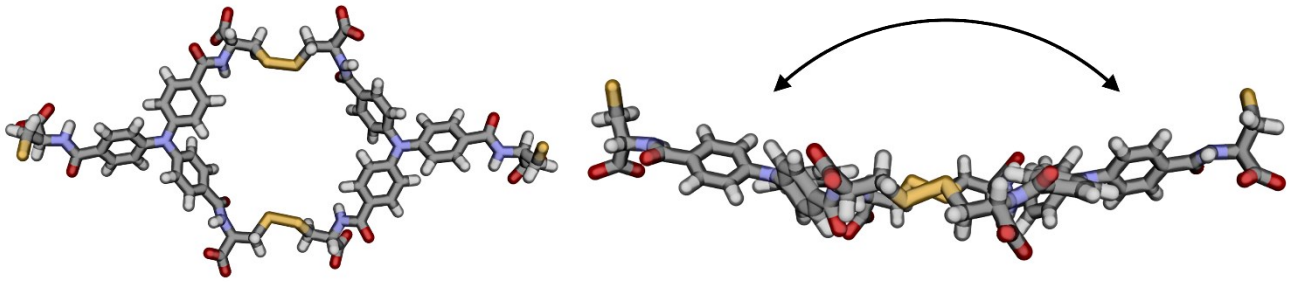


Figure S67: Optimised structure of **2B**, top and side view.

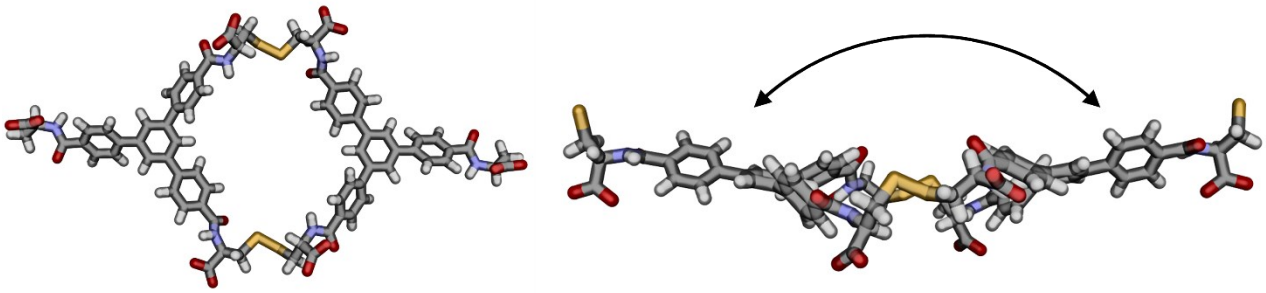


Figure S68: Optimised structure of **3B**, top and side view.

Compound	Energy [Ha]	Relative energy* [Ha]	Relative energy* [kcal/mol]	Energy difference between cage and macrocyclic intermediate, ΔE [kcal/mol]
1B	-5461,4	0,2	127	-127
1-1	-5461,6	0	0	
2B	-6496,7	0,4	248	-248
2-2	-6497,1	0	0	
3B	-6848,2	0,3	210	-210
3-3	-6848,6	0	0	

* energies relative to corresponding cage total energy = 0 kcal/mol.

Figure S69: Comparison of calculated relative energies of cages and macrocyclic intermediate products.

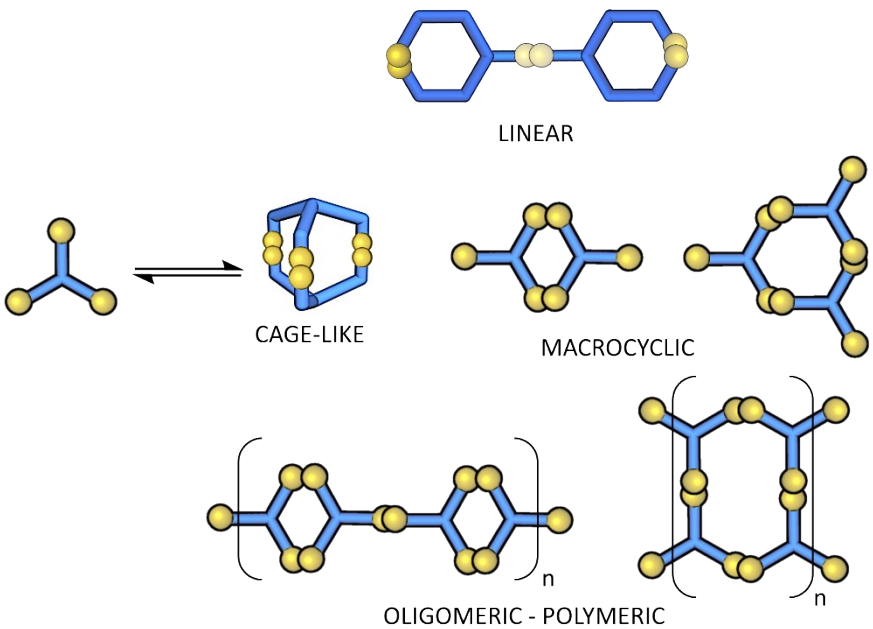


Figure S70: Numerous possibilities of combinatorial connections between the three trifunctional components.

Solid state analysis of 2-2-La

Synthesis of **2-2La**

The 68 mg ($5x10^{-5}$ mol) of **2-2** was dissolved in water (2 mL) via NaOH (0.1M) titration to pH 8. Then the solution of $La(NO_3)_3$ x $6H_2O$ (44 mg, 10^{-4} mol, 2 equiv., 2 mL) was added with vigorous stirring. The pale-yellow solid immediately precipitated. The mixture was then stirred for another 15 min at r.t. and centrifuged for 10 min. The supernatant was removed and the solid material was dried under a high vacuum. Yield 78 mg.

Elemental microanalysis (NCHS) calculated for **$LaNa_3$(2-2) · $La(OH)_3$ · 24 H_2O**, $C_{60}H_{99}La_2N_8Na_3O_{45}S_6$: C 32.88%, H 4.55%, La 12.68%, N 5.11%, Na 3.15%, O 32.85%, S 8.78%, found: C 32.32%, H 3.39%, N 3.67%, S 8.90%.

ICP-MS (La) calculated for **$LaNa_3$(2-2) · $La(OH)_3$ · 24 H_2O**, $C_{60}H_{99}La_2N_8Na_3O_{45}S_6$: La 12.68%, found 10.66%.

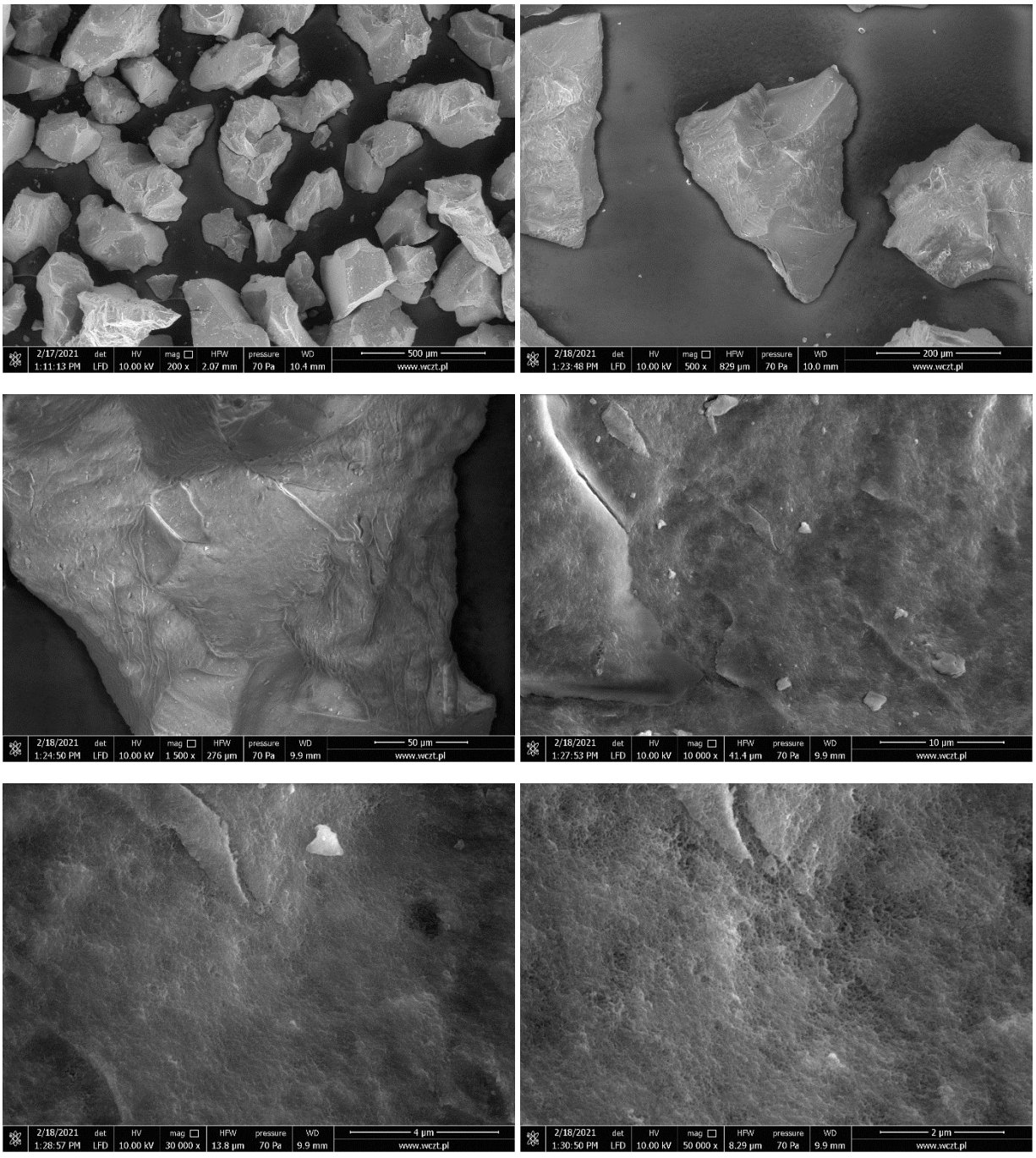


Figure S71: SEM imaging of **2-2-La**.

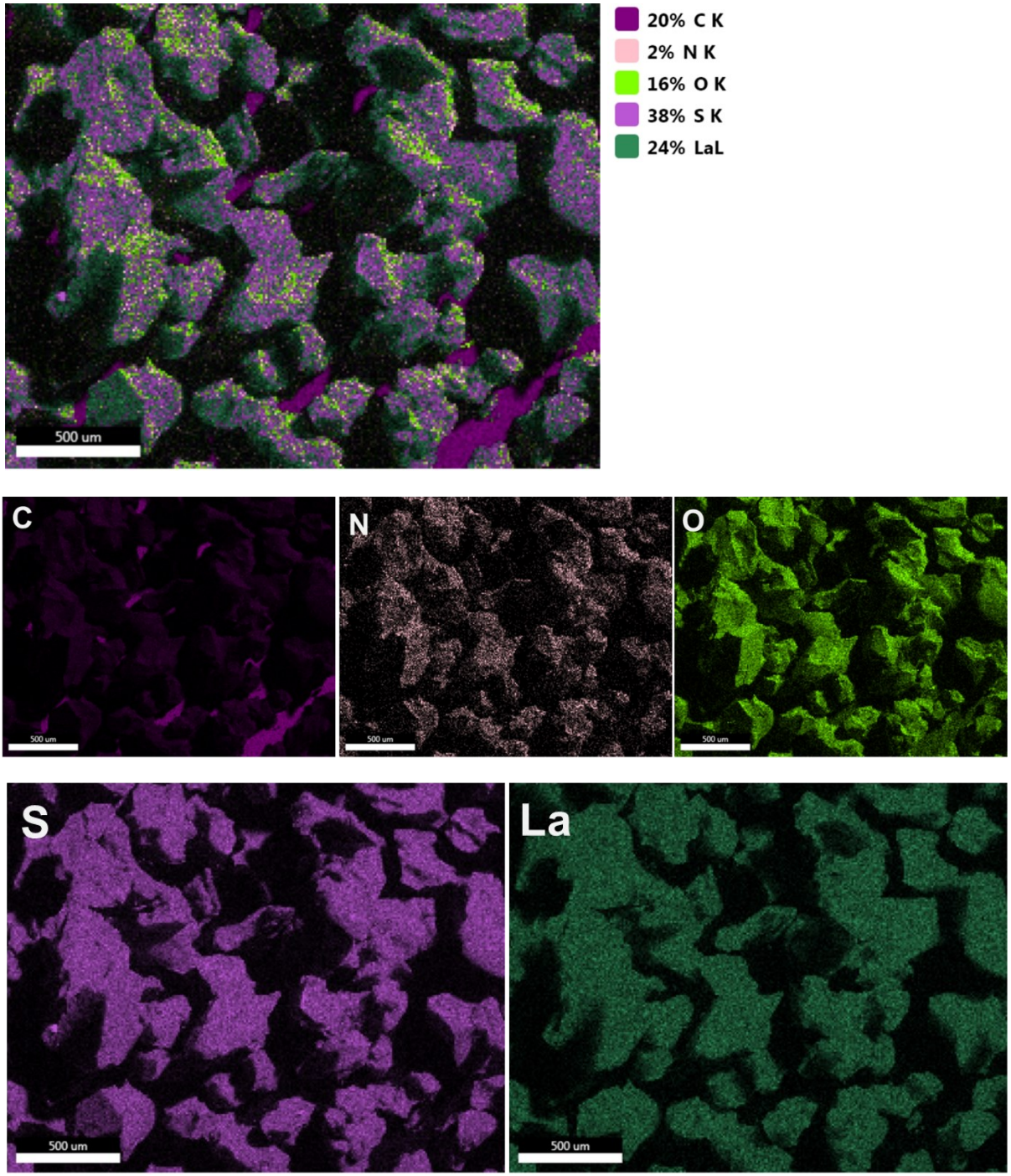


Figure S72: Energy Dispersive Spectroscopy (SEM-EDS) analysis of **2-2-La**.

References

[1] A. R. Stefankiewicz, M. R. Sambrook, J. K. M. Sanders, *Chem. Sci.* **2012**, *3*, 2326.

[2] a) G. B. Rocha, R. O. Freire, A. M. Simas, J. J. Stewart, *J. Comput. Chem.* **2006**, *27*, 1101-1111; b) J. J. Stewart, *J. Mol. Model.* **2007**, *13*, 1173-1213.

[3] S. Grimme, S. Ehrlich, L. Goerigk, *J. Comput. Chem.* **2011**, *32*, 1456-1465.