Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

A Two-Step Approach to a Glycoazobenzene Macrocycle with Remarkable Photoswitchable Features

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General Information

Methods:
Moisture-sensitive reactions were carried out in flame-dried glassware and under a positive pressure of nitrogen. Analytical thin layer chromatography (TLC) was performed on silica gel plates (GF 254, Merck). Visualization was achieved by UV light and/or with 10% sulfuric acid in ethanol or vanillin (3.0g vanillin and 0.5 mL H2SO4 in 100 mL EtOH), followed by heat treatment at ca. 200 °C. The products were purified by flash chromatography on silica gel columns (Merck, 230–400 mesh, particle size 0.040–0.063 mm). Pyridine was dried over KOH, tetrahydrofuran and *N,N*-dimethylformamide were stored over 3Å molecular sieves under a nitrogen atmosphere.

Instrumentation:

Optical rotations were measured with a PerkinElmer 241 polarimeter with a sodium D-line (589 nm) and a cuvette of 10 cm path length, in the solvents indicated.Circular dichroism spectroscopy was performed on a Jasco J-720 CD spectrometer (Jasco, Tokyo, Japan) with a bandwidth of 1 nm and a cuvette of 10 mm path length. Proton (^{1}H) nuclear magnetic resonance spectra and carbon (^{13}C) nuclear magnetic resonance spectra were recorded on a Bruker DRX-500 and AV-600 spectrometer. Chemical shifts are referenced to internal tetramethylsilane (TMS) or 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS), or to the residual proton of the NMR solvent. Data are presented as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, and br=broad signal), coupling constant in hertz (Hz) and, integration. Full assignment of the signals was achieved by using 2D NMR techniques (^{1}H - ^{1}H COSY and ^{1}H - ^{13}C HSQC and HMBC. Infrared (IR) spectra were measured with a PerkinElmer FTIR Paragon 1000 (ATR) spectrometer and were reported in cm^{-1}. ESI mass spectra were recorded on a Thermo Scientific™ Q Exactive™. *Trans* → *cis* photoisomerization experiments were performed using a LED emitting a 365 nm light from Nichia Corporation with a FWHM of 10 nm and intensity of 25 mW/cm^{2}. *Cis* → *trans* photoisomerization experiments were

performed using a LED emitting a 525 nm light from Nichia Corporation with a FWHM of 45 nm and intensity of 1 mW/cm^2. UV/Vis absorption spectra were measured on PerkinElmer Lambda-241.

Synthesis

Trans-Precursor **4**:

From 2-propynyl α-D-mannopyranoside **2**[1]:

A suspension of **2** (500 mg, 2.29 mmol), 4,4'-dihydroxyazobenzene[2] (**3**) (225 mg, 1.05 mmol) and triphenylphosphine (1.37 g, 5.22 mmol) in dry tetrahydrofurane (11.0 mL) under nitrogen was cooled to 0 °C and diisopropyl azodicarboxylate (1.00 mL, 5.22 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred for 4 d then concentrated under reduced pressure. The crude residue was purified by flash chromatography (dichloromethane/methanol 9:1) to give a mixture of the unprotected product and excess **2**. The crude mixture was dissolved in dry pyridine (2.00 mL) and acetic anhydride (1.00 mL, 10.6 mmol) was added. The mixture was stirred at room temperature for 16 h, then concentrated under reduced pressure and co-evaporated with toluene repeatedly. The crude residue was purified by flash chromatography (cyclohexane/ethyl acetate 3:2) to give **4** (450 mg, 519 µmol, 50% over two steps) as an orange foam.

From 2-propynyl 2,3,4-tri-*O*-acetyl-6-*O*-tosyl-α-D-mannopyranoside **5**[1]:

A suspension of **5** (100 mg, 200 µmol), 4,4'-dihydroxyazobenzene[2] (**3**) (16.0 mg, 75.0 µmol) and Cs_2CO_3 (75.0 mg, 231 µmol) in dry DMF (8.00 mL) under nitrogen was stirred at 70 °C for 16 h then diluted with ethyl acetate (50 mL), washed with 2N HCl, brine and water (50 mL each) and dried over $MgSO_4$, filtered and concentrated to dryness. The crude residue was purified by flash chromatography (cyclohexane/ethyl acetate 3:2) to give **4** (46 mg, 53 µmol, 70%) as an orange foam.

$[\alpha]^{20}_D$= +112 (c= 0.41 in $CHCl_2$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.90 – 7.84 (m, 4 H, Ar-H_{ortho}), 7.05 – 6.96 (m, 4 H, Ar-H_{meta}), 5.45 – 5.37 (m, 4 H, H-3, H-4), 5.32 (dd, $^3J_{2,3}$ = 2.9 Hz, $^3J_{2,1}$ = 1.8 Hz, 2 H, H-2), 5.08 (d, $^3J_{1,2}$ = 1.6 Hz, 2 H, H-1), 4.33 (d, $^4J_{CH2,CH}$ = 2.4 Hz, 4 H, CH_2C≡CH), 4.25 – 4.13 (m, 6 H, H-5, H-6a, H-6b), 2.47 (t, $^4J_{CH,CH2}$ = 2.4 Hz, 2 H, CH_2C≡C*H*), 2.18 (s, 6 H, 2 CH_3C=O), 2.05 (s, 6 H, 2 CH_3C=O), 2.02 (s, 6 H, 2 CH_3C=O); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 170.0 (CH_3*C*=O), 169.9 (2 C, CH_3*C*=O), 160.4 (Ar-C_{para}), 147.3 (Ar-C_{ipso}), 124.4 (Ar-C_{ortho}), 114.9 (Ar-C_{meta}), 96.0 (C-1), 77.9 (CH_2*C*≡CH), 75.6 (CH_2C≡*C*H), 69.5 (C-5), 69.4 (C-2), 68.9 (C-3), 67.5 (C-6), 66.8 (C-4), 54.8 (*C*H_2C≡CH), 20.9 (*C*H_3C=O), 20.8 (*C*H_3C=O), 20.7 (*C*H_3C=O); IR (ATR): ũ = 2363, 1746, 1369, 1215, 1135, 1043, 973, 841, 492 cm^{-1}; ESI-HRMS m/z: calc. 867.28184 for $[C_{42}H_{46}O_{18}N_2+H]^+$; found 867.28077 for $[C_{42}H_{46}O_{18}N_2+H]^+$.

Trans-Macrocycle **6**:

A solution of the precursor **4** (25.0 mg, 28.8 µmol) in dry pyridine (15.0 mL) was stirred at room temperature and irradiated with a LED lamp emitting 365 nm light from a distance of 15 cm for 20 min. After irradiation, the solution was shielded from light and CuBr (62.0 mg, 432 mmol) and N,N,N',N'',N''-pentamethyldiethylenetriamine (90.0 µL, 432 µmol) were added. The mixture was stirred at room temperature for 2 d then concentrated to dryness and coevaporated with toluene. The crude residue was dissolved in ethyl acetate, washed four times with water (50 mL each) and dried over $MgSO_4$. After filtration and concentration under reduced pressure, the residue was

purified by flash chromatography (cyclohexane/ethyl acetate 3:2) to give **6** (12.4 mg, 14.3 µmol, 50%) as an orange foam.

$[\alpha]^{20}_{D}$=-182 (c= 0.12 in $CHCl_2$); ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.90 – 7.84 (m, 4 H, Ar-H_{ortho}), 7.05 – 6.99 (m, 4 H, Ar-H_{meta}), 5.18 (dd, $^3J_{3,2}$ = 10.0 Hz, $^3J_{3,4}$ = 3.4 Hz, 2 H, H-3), 5.09 – 5.02 (m, 4 H, H-2, H-4), 4.53 (s, 2 H, H-1), 4.36 (dd, $^2J_{6a,6b}$ = 13.0 Hz, $^3J_{6a,5}$ = 9.1 Hz, 2 H, H-6a), 4.28 (dd, $^2J_{6b,6a}$ = 13.1 Hz, $^3J_{6b,5}$ = 2.3 Hz, 2 H, H-6b), 4.13 – 4.06 (m, 2 H, H-5), 3.47 (d, $^2J_{CH2,\ CH2}$ = 15.4 Hz, 2 H, CH_2C≡Ca), 3.43 (d, $^2J_{CH2,\ CH2}$ = 15.5 Hz, 2 H, CH_2C≡Cb), 2.07 (s, 6 H, 2 CH_3C=O), 2.06 (s, 6 H, 2 CH_3C=O), 1.91 (s, 6 H, 2 CH_3C=O); ^{13}C NMR (126 MHz, $CDCl_3$) δ = 170.0 (CH_3*C*=O), 169.7 (CH_3*C*=O), 169.6 (CH_3*C*=O), 158.4 (Ar-C_{para}), 147.7 (Ar-C_{ipso}), 124.4 (Ar-C_{ortho}), 117.5 (Ar-C_{meta}), 96.0 (C-1), 72.8 (CH_2*C*≡C), 70.3 (CH_2C≡*C*), 69.2 (C-2), 68.6 (C-3), 67.3 (C-6), 67.2 (2 C, C-4, C-5), 54.8 (*C*H_2C≡C), 20.8 (2 C, *C*H_3C=O), 20.6 (*C*H_3C=O), IR (ATR): ũ = 2923, 2360, 1747, 1209, 1068, 1040, 967, 847, 492 cm^{-1}; ESI-HRMS m/z: calc. 865.26619 for $[C_{42}H_{44}O_{18}N_2+H]^+$; found 865.26543 for $[C_{42}H_{46}O_{18}N_2+H]^+$.

Trans-Macrocycle **1**:

To a solution of the protected macrocycle **6** (55.0 mg, 63.6 µmol) in a mixture of dichloromethane and methanol (1:1, 4.00 mL), NaOMe (5.4 M in methanol, 30 µL) was added and the solution was stirred at room temperature for 16 h. The mixture was neutralized with Amberlite® IR120 H^+ and concentrated to dryness to give the macrocycle **1** (38.8 mg, 63.3 µmol, quantitative) as a yellow amorphous solid.

$[\alpha]^{24}_{D}$=-146 (c= 0.08 in DMSO); ^{1}H NMR (500 MHz, DMSO-*d6*) δ = 7.90 – 7.83 (m, 4 H, Ar-H_{ortho}), 7.19 – 7.11 (m, 4 H, Ar-H_{meta}), 5.23 (bs, 2 H, 2 OH), 4.99 (bs, 4 H, 4 OH), 4.74 (dd, $^2J_{6a,6b}$ = 12.8 Hz, $^3J_{6a,5}$ = 2.0 Hz, 2 H, H-6a), 4.44 (d, $^3J_{1,2}$ = 1.3 Hz, 2 H, H-1), 4.19 (dd, $^2J_{6b,6a}$ = 12.8 Hz, $^3J_{6b,5}$ = 10.0 Hz, 2 H, H-6b), 3.70 (ddd, $^3J_{5,6a}$ = $^3J_{5,6b}$ = 9.5 Hz, $^3J_{5,4}$ = 1.6 Hz, 2 H, H-5), 3.65 (d, $^2J_{CH2,CH2}$ = 15.3 Hz, 2 H, CH_2C≡Ca), 3.49 (d, $^3J_{1,2}$ = 0.9 Hz, 2 H, H-2), 3.32 – 3.28 (m, 4 H, H-3, H-4), 3.25 (d, $^2J_{CH2,CH2}$ = 15.4 Hz, 2 H, CH_2C≡Cb); ^{13}C NMR (126 MHz, DMSO-*d6*) δ = 158.7 (Ar-C_{para}), 146.4 (Ar-C_{ipso}), 123.8 (Ar-C_{ortho}), 117.6 (Ar-C_{meta}), 99.0 (C-1), 74.4 (CH_2*C*≡C), 70.6 (C-3), 69.6 (C-2), 68.5 (CH_2C≡*C*), 67.9 (C-6), 67.7 (C-4), 67.6 (C-5), 53.2 (*C*H_2C≡C), IR (ATR): ũ = 3371, 2916, 2363, 1595, 1210, 1065, 1040, 851, 556, 485 cm^{-1}; ESI-HRMS m/z: calc. 613.20280 for $[C_{30}H_{32}O_{12}N_2+H]^+$; found 613.20237 for $[C_{30}H_{32}O_{12}N_2+H]^+$.

Cis-Macrocycle **1**:

$[\alpha]^{24}_{D}$=-576 (c= 0.08 in DMSO); ^{1}H NMR (500 MHz, DMSO-*d6*) δ = 6.90 – 6.85 (m, 4 H, Ar-H_{meta}), 6.84 – 6.78 (m, 4 H, Ar-H_{ortho}), 5.22 (bs, 2 H, 2 OH), 4.99 (bs, 4 H, 4 OH), 4.71 (s, 2 H, H-1), 4.36 (d, $^2J_{6a,6b}$ = 10.8 Hz, 2 H, H-6a), 4.29 (d, $^2J_{CH2,CH2}$ = 16.5 Hz, 2 H, CH_2C≡Ca), 4.16 (d, $^2J_{CH2,CH2}$ = 16.6 Hz, 2 H, CH_2C≡Cb), 4.03 (dd, $^2J_{6b,6a}$ = 11.3 Hz, $^3J_{6b,5}$ = 8.0 Hz, 2 H, H-6b), 3.68 (dd, $^3J_{5,6a}$ = $^3J_{5,6b}$ = 8.0 Hz, 2 H, H-5), 3.61 (s, 2 H, H-2), 3.46 - 3.43 (m, 2 H, H-3, H-4); ^{13}C NMR (126 MHz, DMSO-*d6*) δ = 157.2 (Ar-C_{para}), 146.5 (Ar-C_{ipso}), 122.1 (Ar-C_{ortho}), 114.3 (Ar-C_{meta}), 100.2 (C-1), 76.2 (CH_2*C*≡C), 71.8 (C-5), 70.6 (C-3), 69.7 (C-2), 69.6 (CH_2C≡*C*), 67.6 (C-6), 66.8 (C-4), 54.7 (*C*H_2C≡C).

NMR spectra of the synthesized compounds

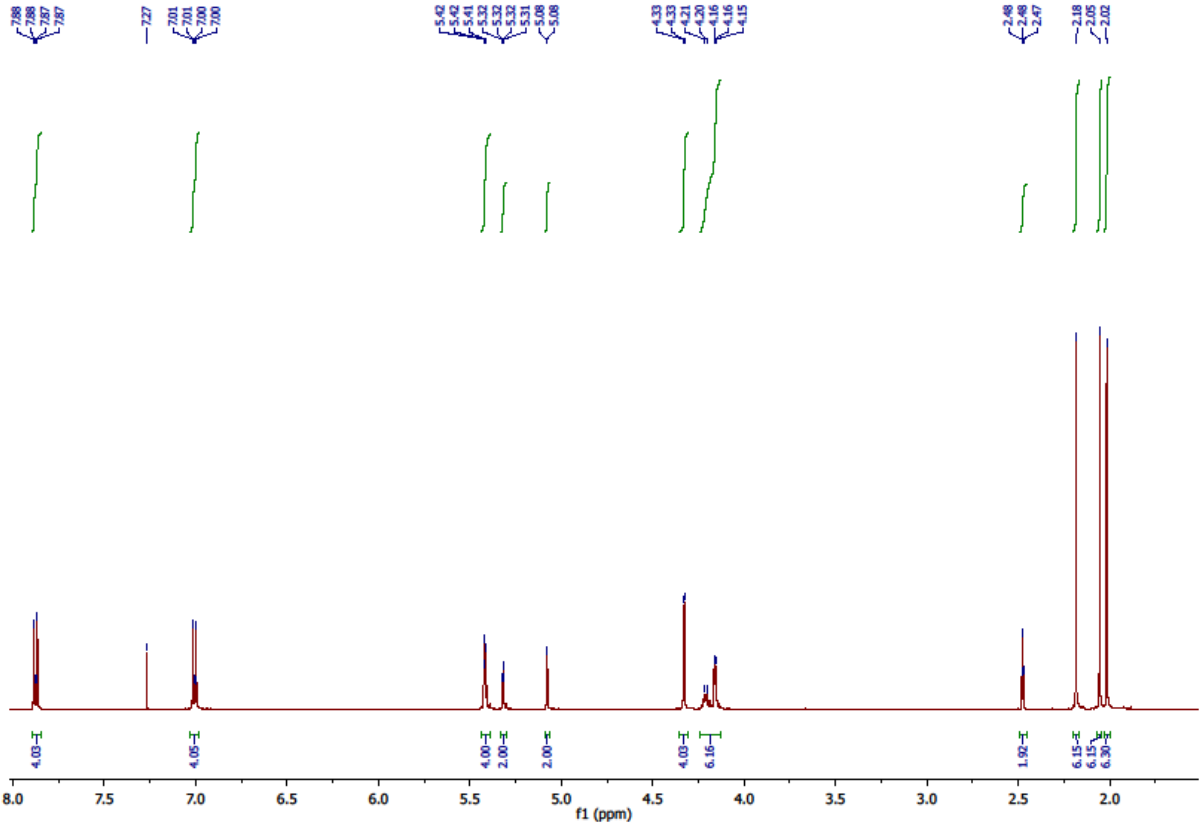

Figure S1: ^{1}H NMR spectrum of *trans*- **4** (500 MHz, $CDCl_3$, 300 K).

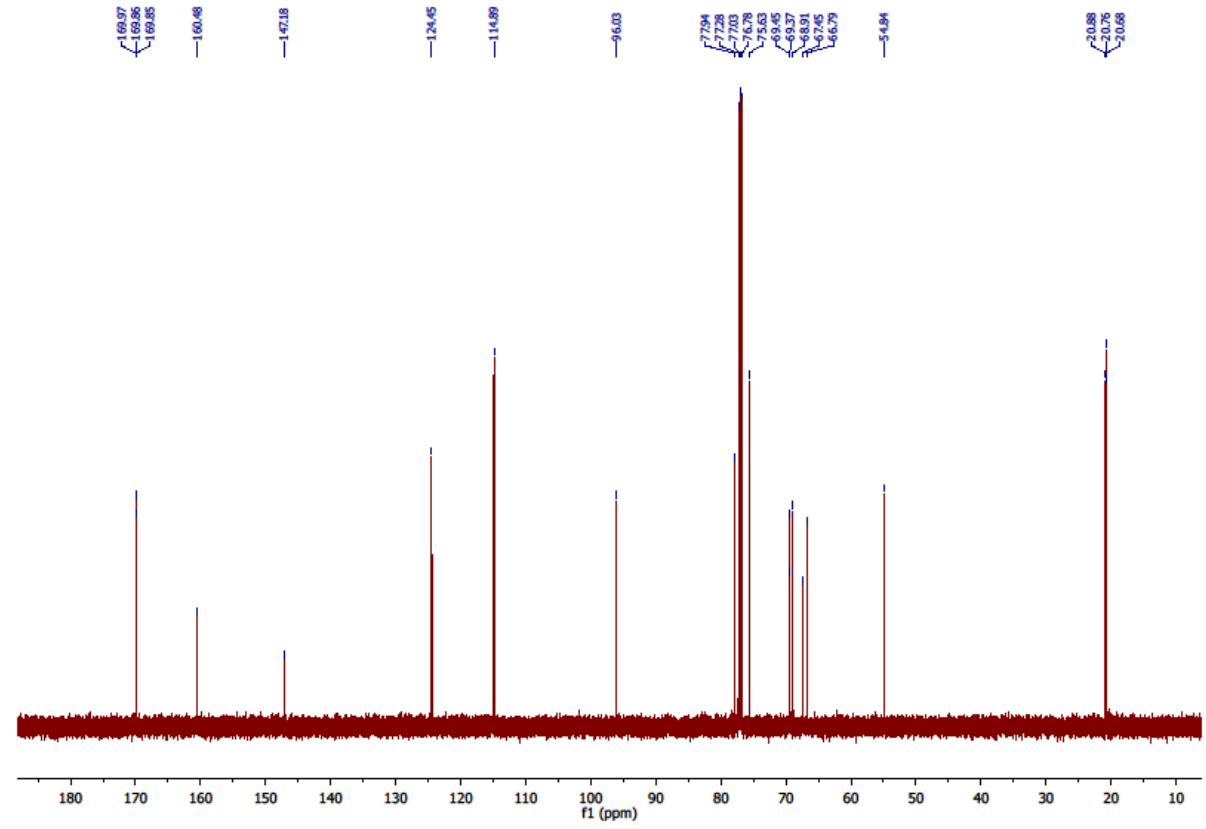

Figure S2: ^{13}C NMR spectrum of *trans*- **4** (126 MHz, $CDCl_3$, 300 K).

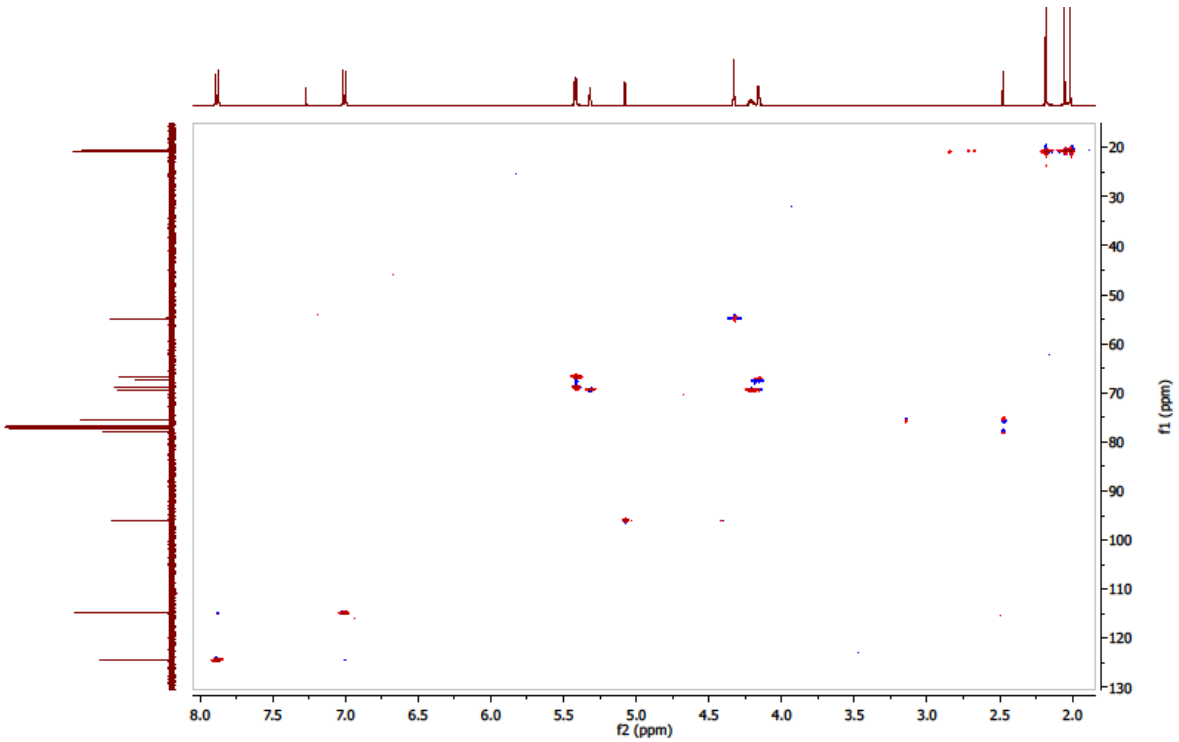

Figure S3: HSQC spectrum of *trans*- **4** ($CDCl_3$, 300 K).

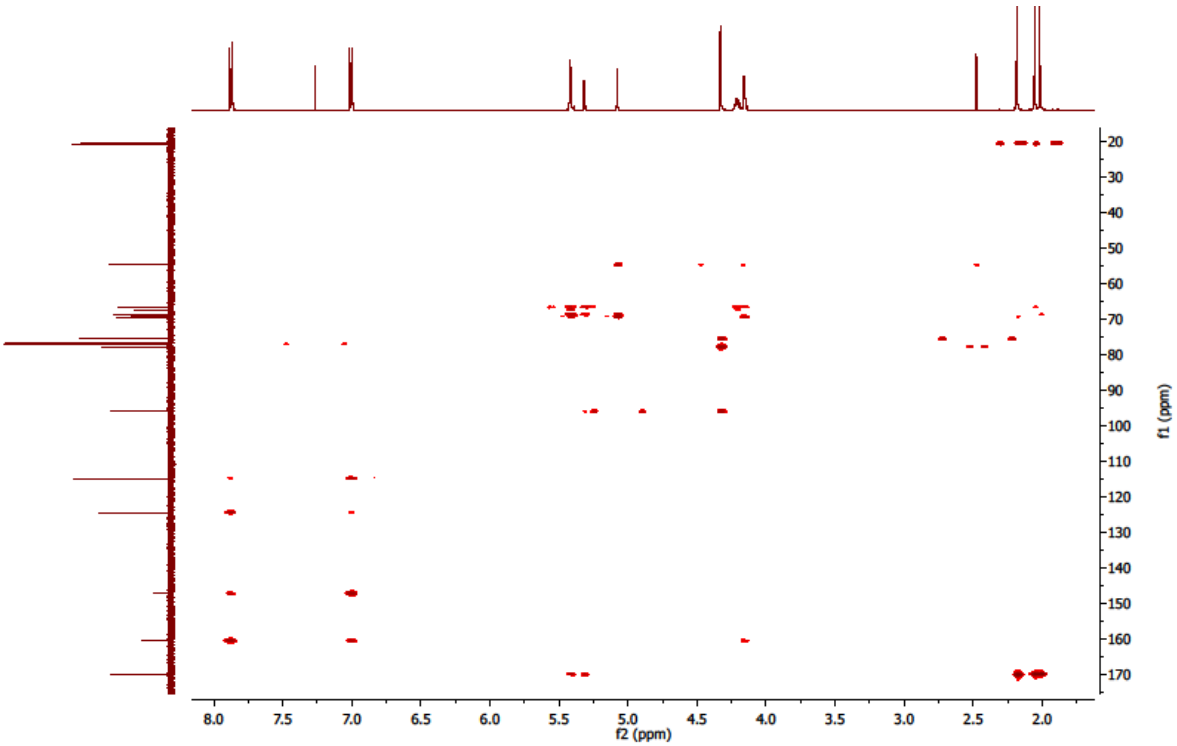

Figure S4: HMBC spectrum of *trans*- **4** ($CDCl_3$, 300 K).

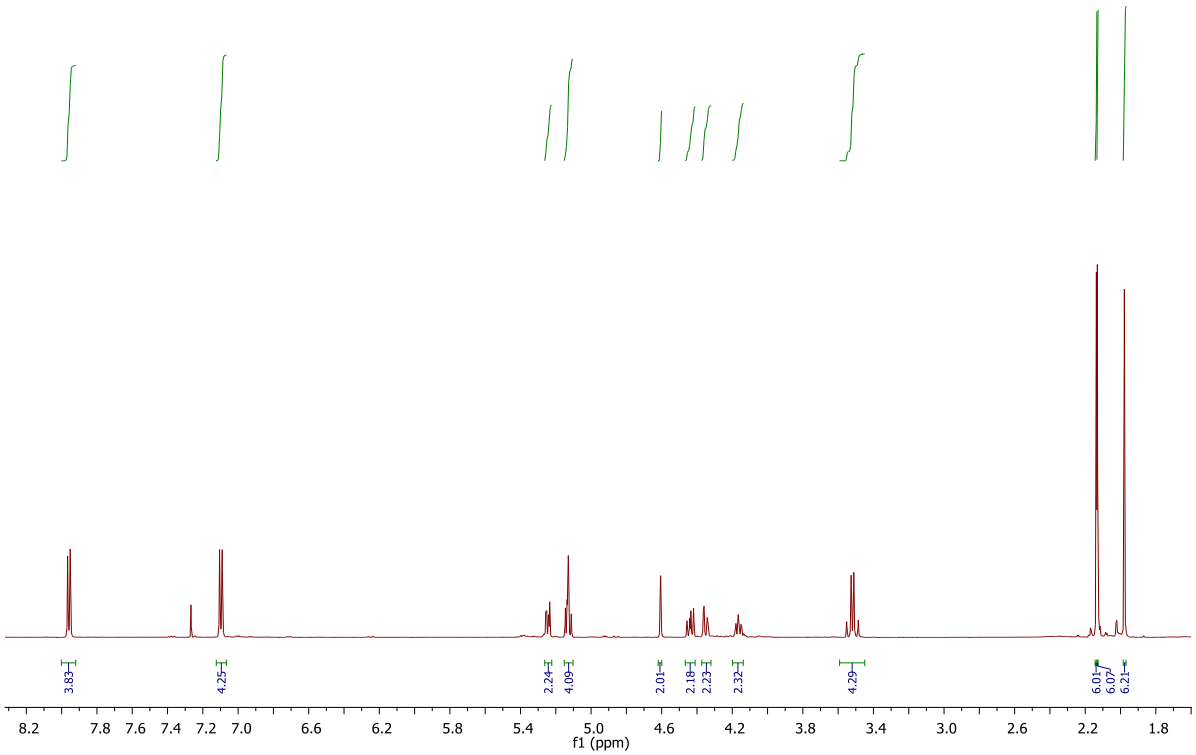

Figure S5: ^{1}H NMR spectrum of *trans*-macrocycle **6** (600 MHz, $CDCl_3$, 300 K).

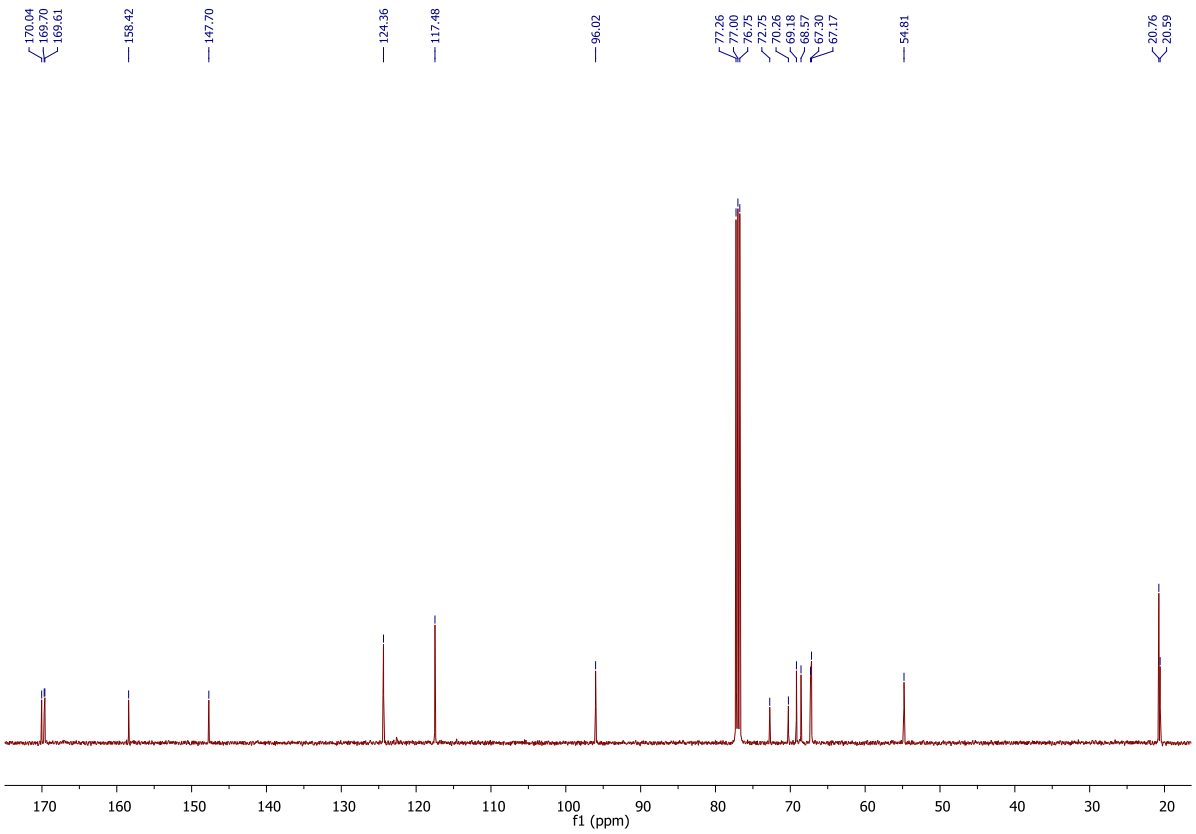

Figure S6: ^{13}C NMR spectrum of *trans*-macrocycle **6** (125 MHz, $CDCl_3$, 300 K).

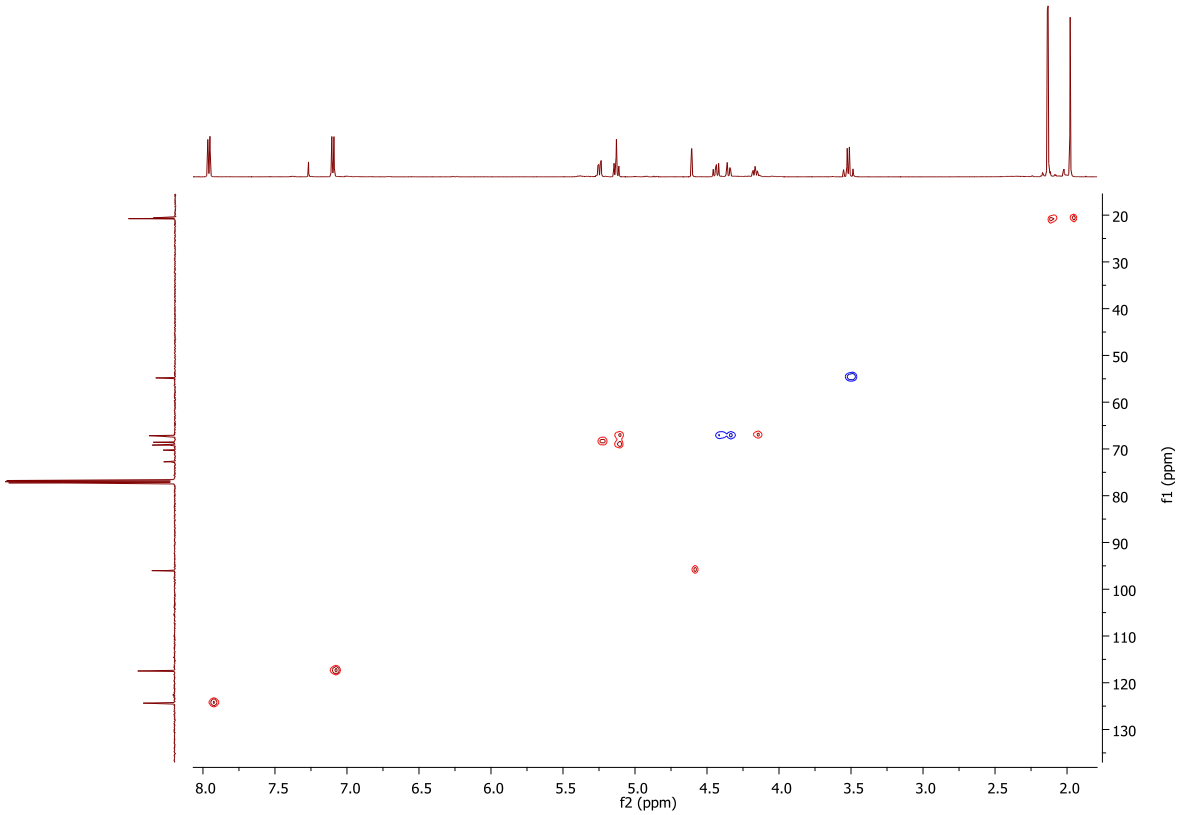

Figure S7: HSQC spectrum of *trans*-macrocycle **6** ($CDCl_3$, 300 K).

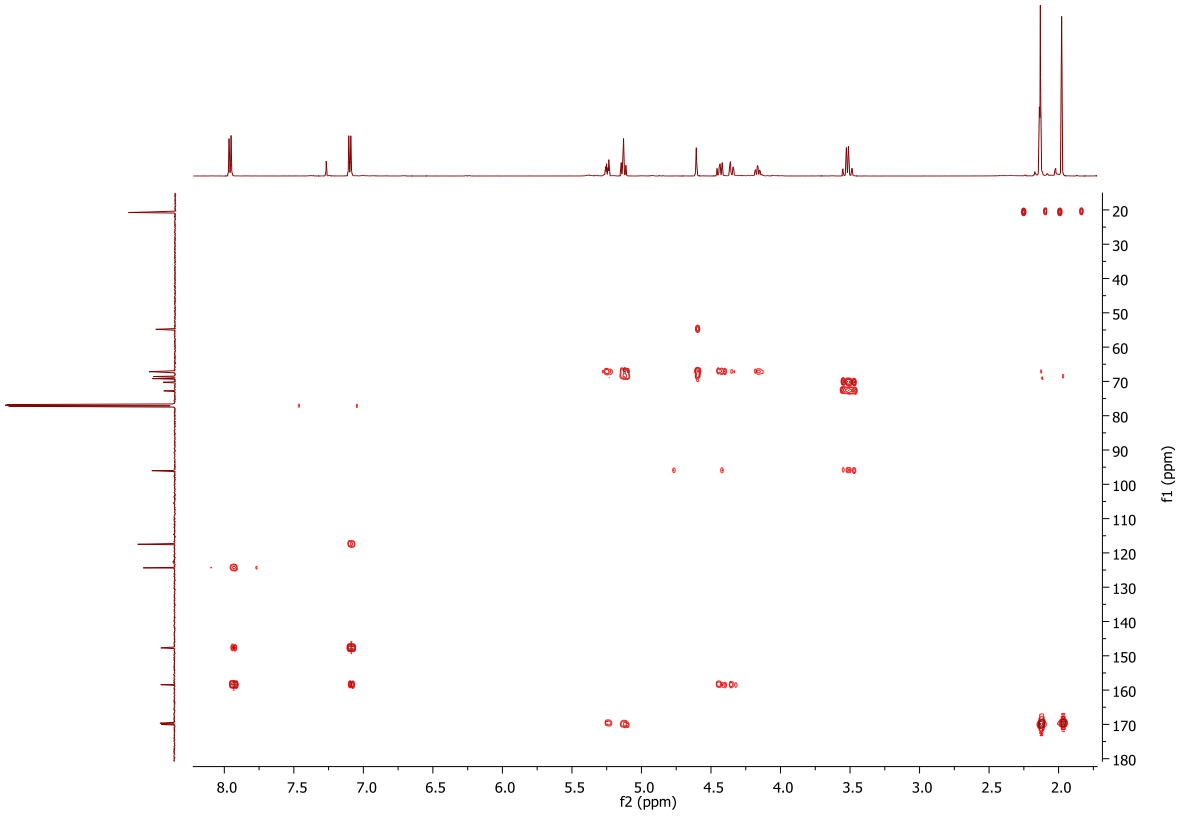

Figure S8: HMBC spectrum of *trans*-macrocycle **6** ($CDCl_3$, 300 K).

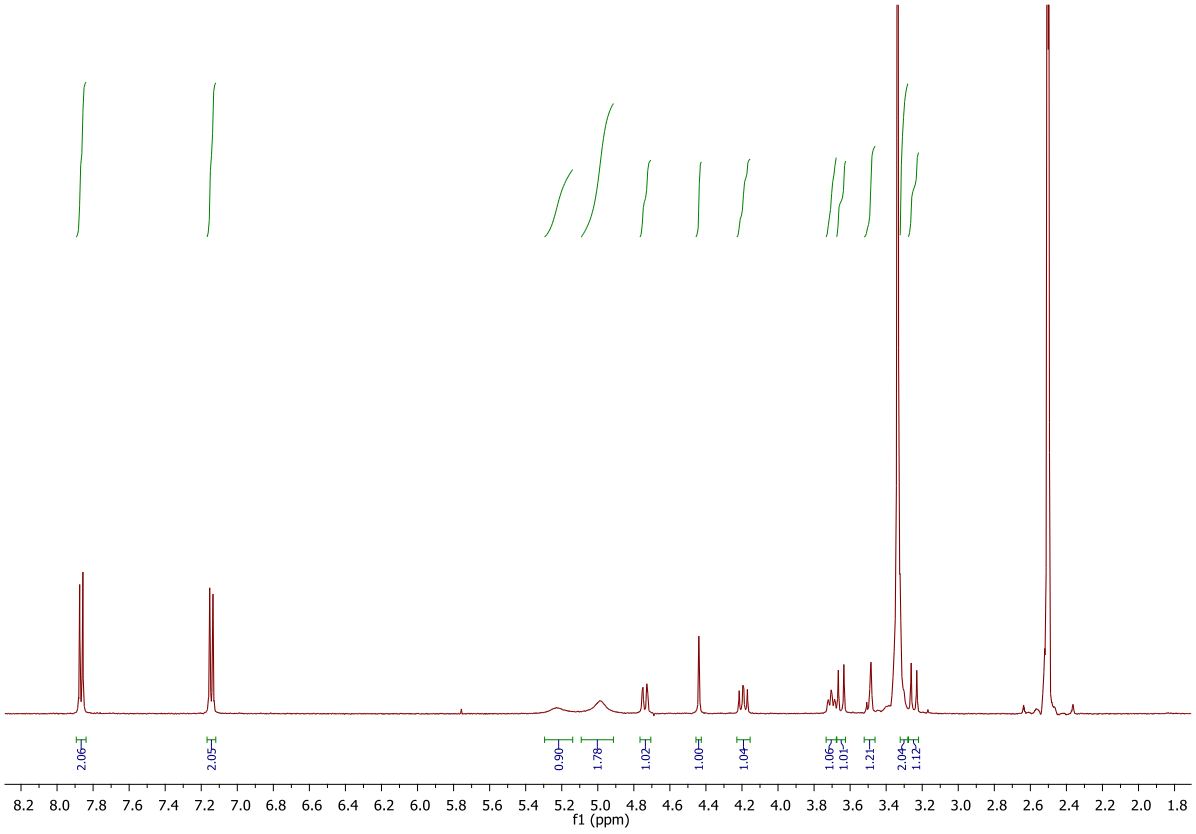

Figure S9: ^{1}H NMR spectrum of *trans*-macrocycle **1** (500 MHz, DMSO-d_6, 300 K).

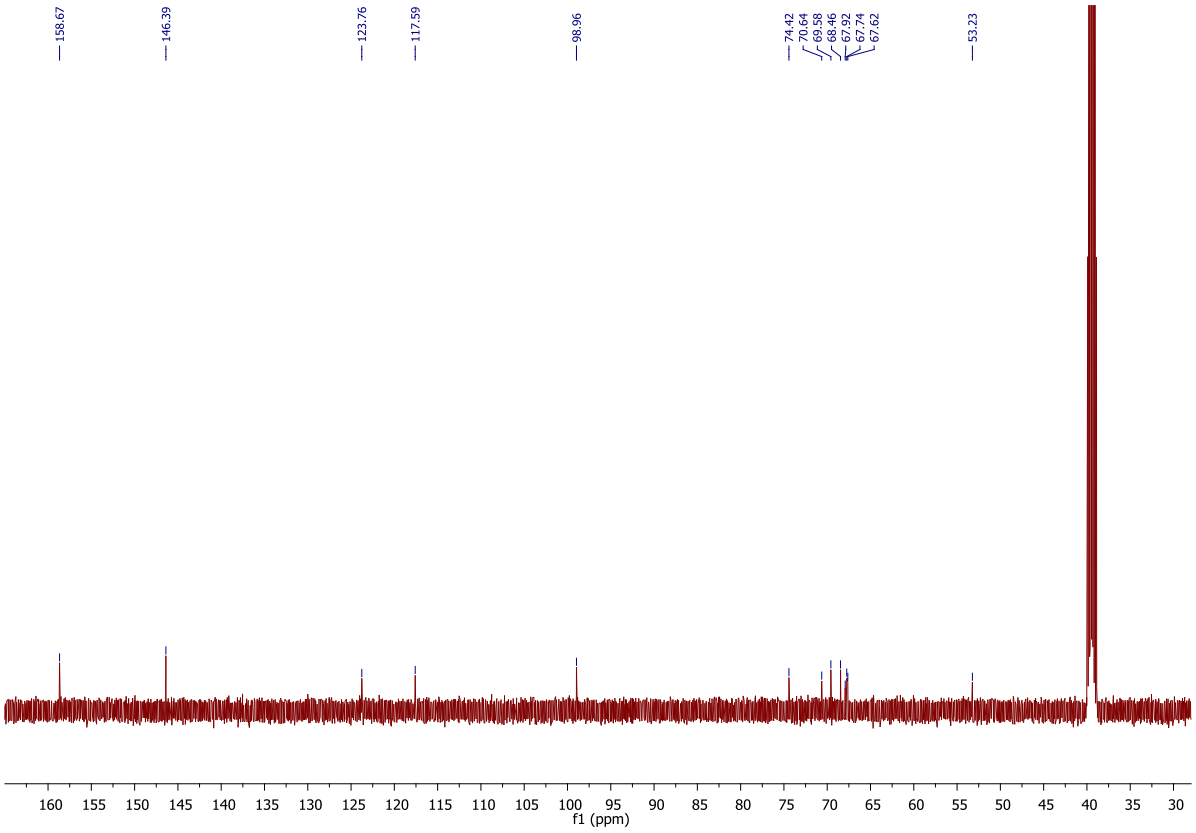

Figure S10: ^{13}C NMR spectrum of *trans*-macrocycle **1** (126 MHz, DMSO-d_6, 300 K).

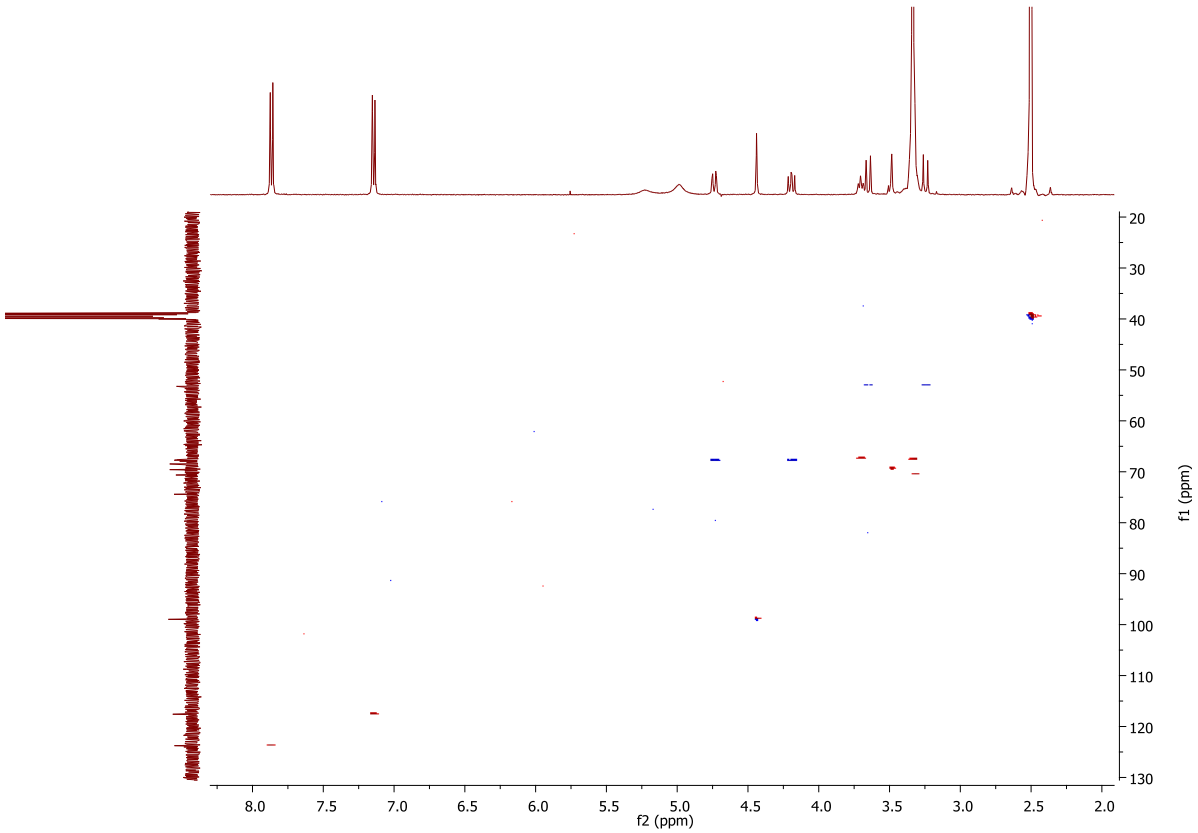

Figure S11: HSQC spectrum of *trans*-macrocycle **1** (DMSO-d_6, 300 K).

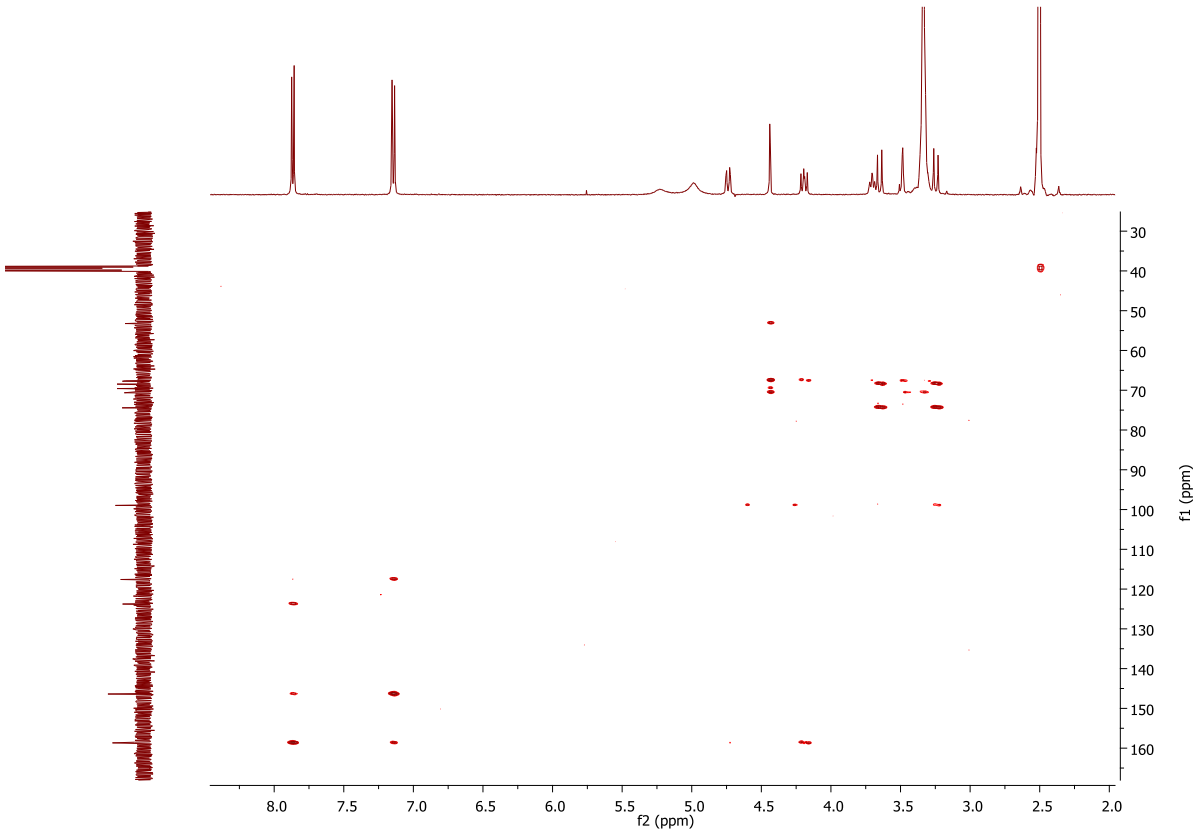

Figure S12: HMBC spectrum of *trans*-macrocycle **1** (DMSO-d_6, 300 K).

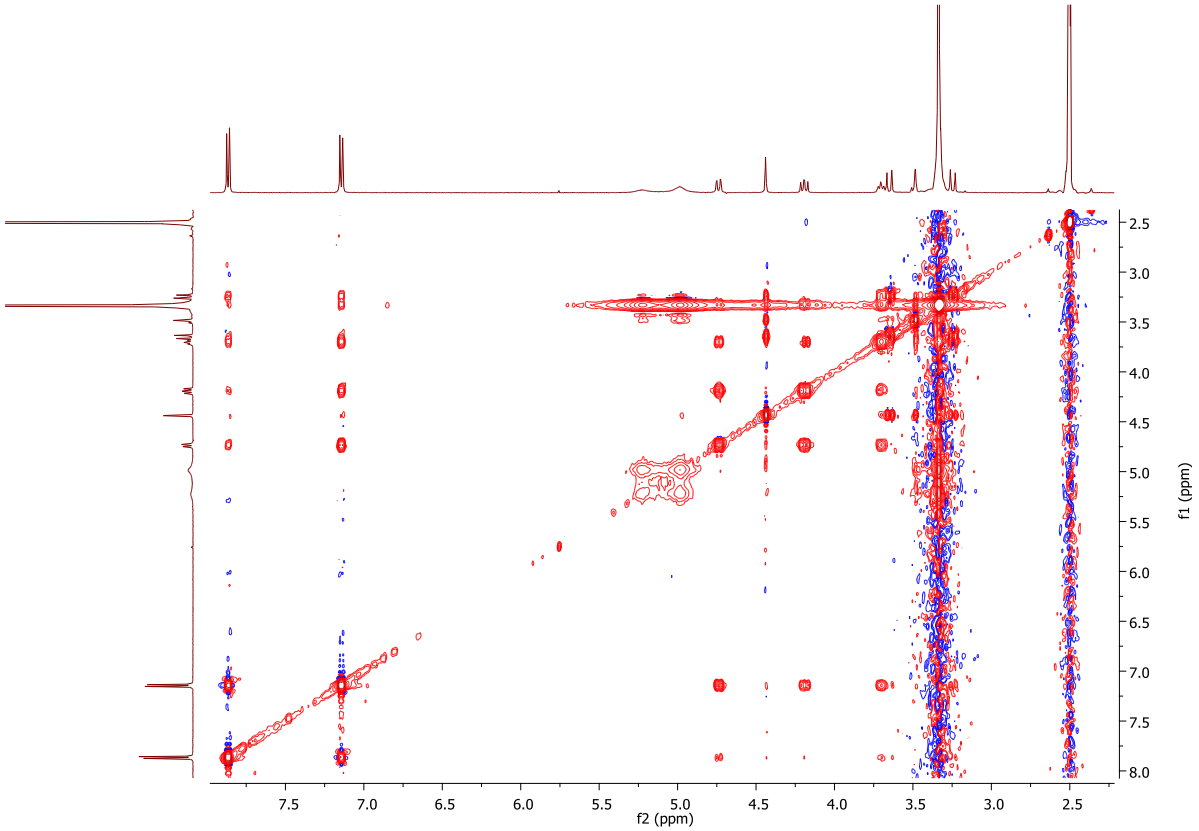

Figure S13: NOESY spectrum of *trans*-macrocycle **1** (DMSO-d_6, 300 K).

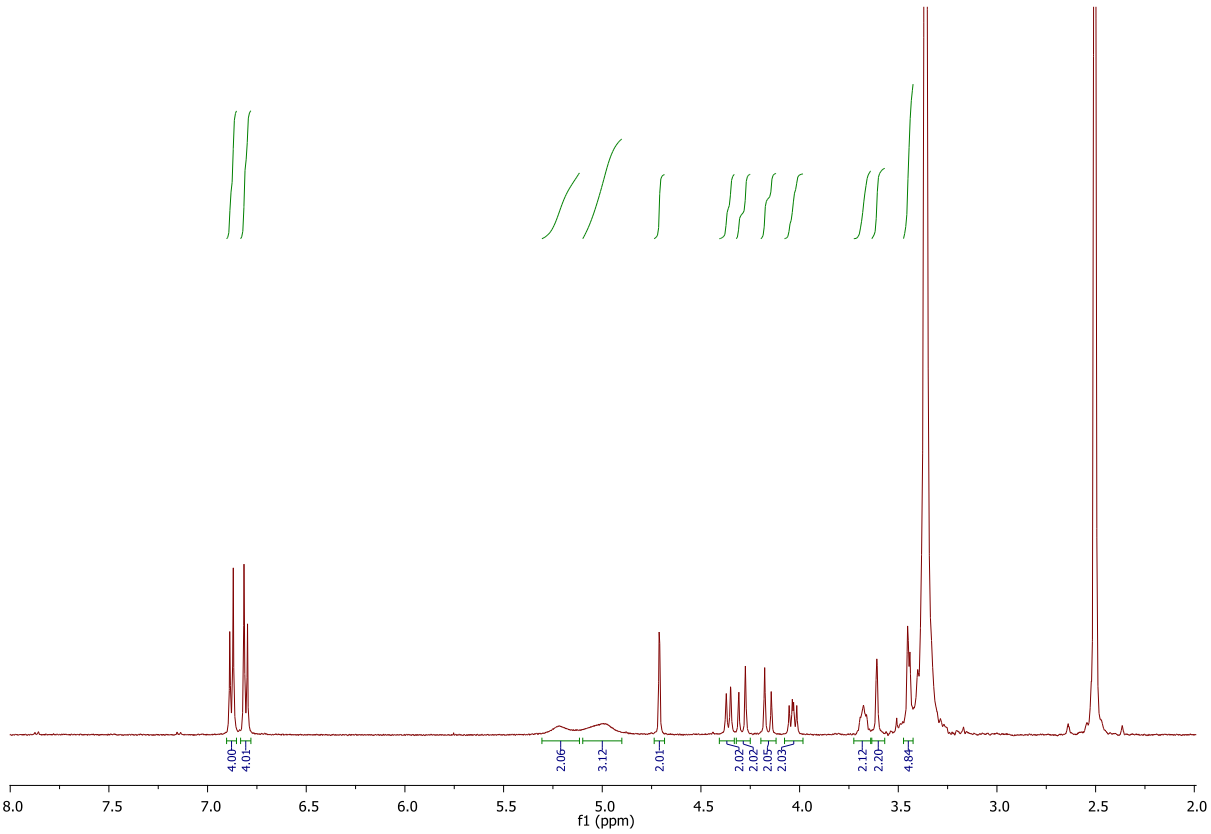

Figure S14: ^{1}H NMR spectrum of *cis*-macrocycle **1** (500 MHz, DMSO-d_6, 300 K).

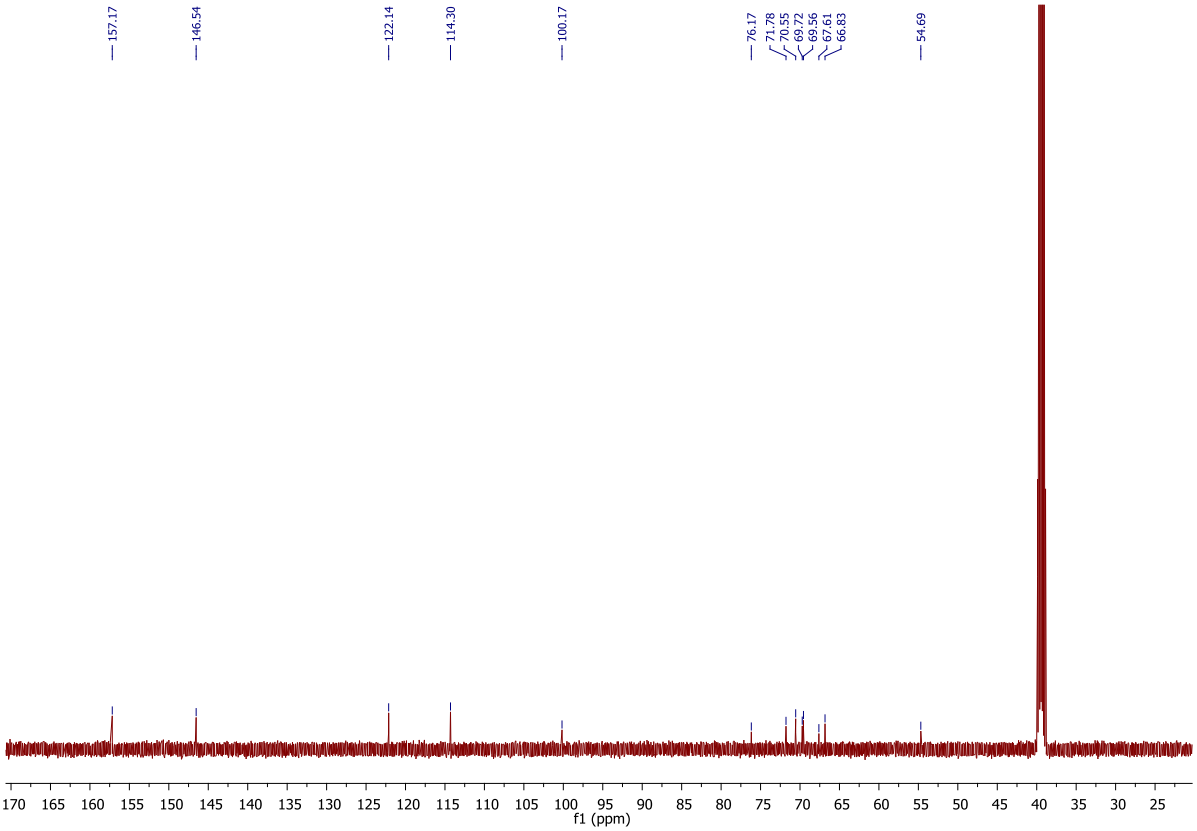

Figure S15: ^{13}C NMR spectrum of *cis*-macrocycle **1** (125 MHz, DMSO-d_6, 300 K).

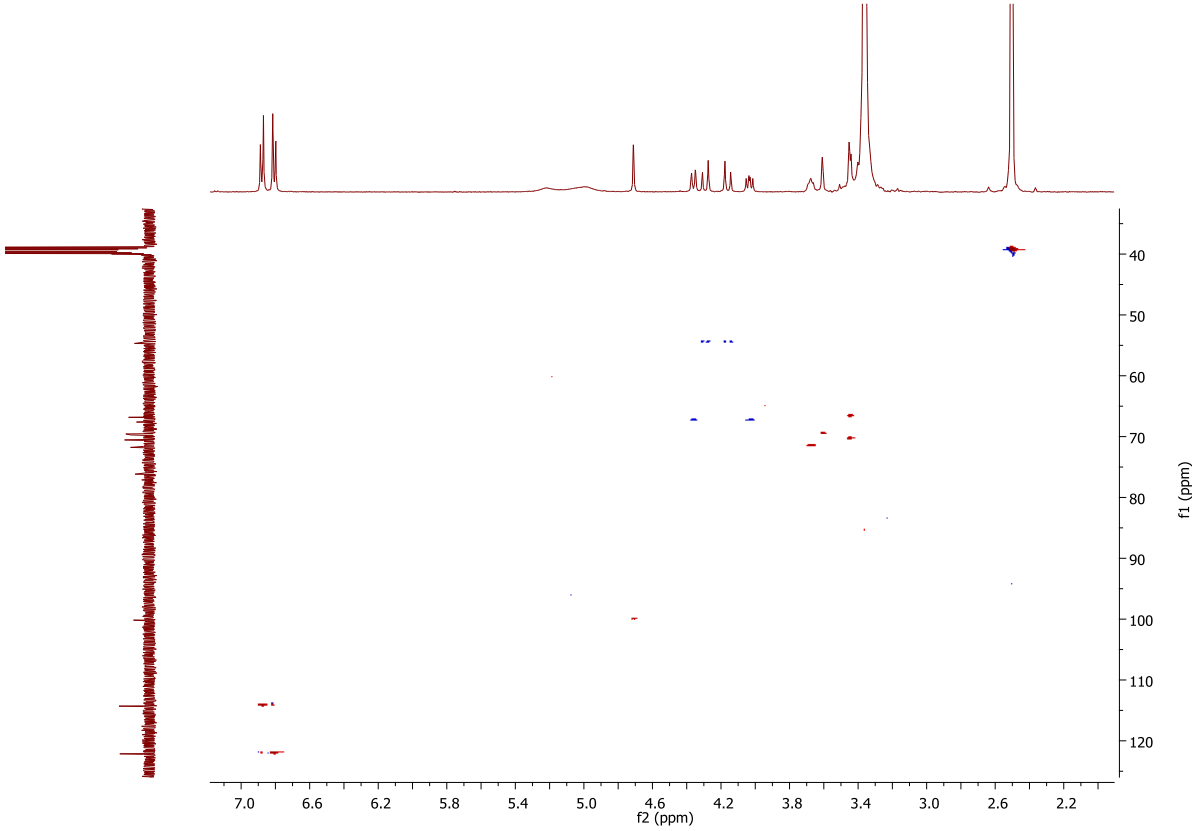

Figure S16: HSQC spectrum of *cis*-macrocycle **1** (DMSO-d_6, 300 K).

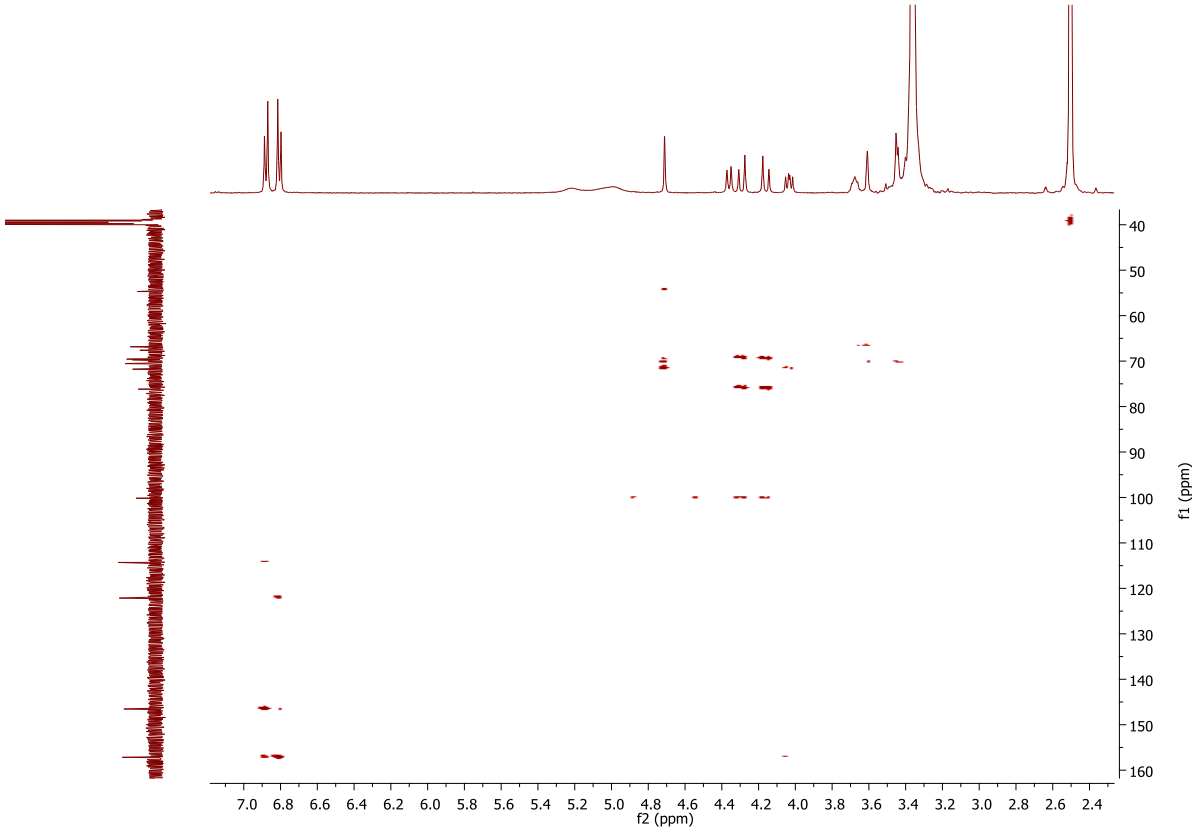

Figure S17: HMBC spectrum of *cis*-macrocycle **1** (DMSO-d_6, 300 K).

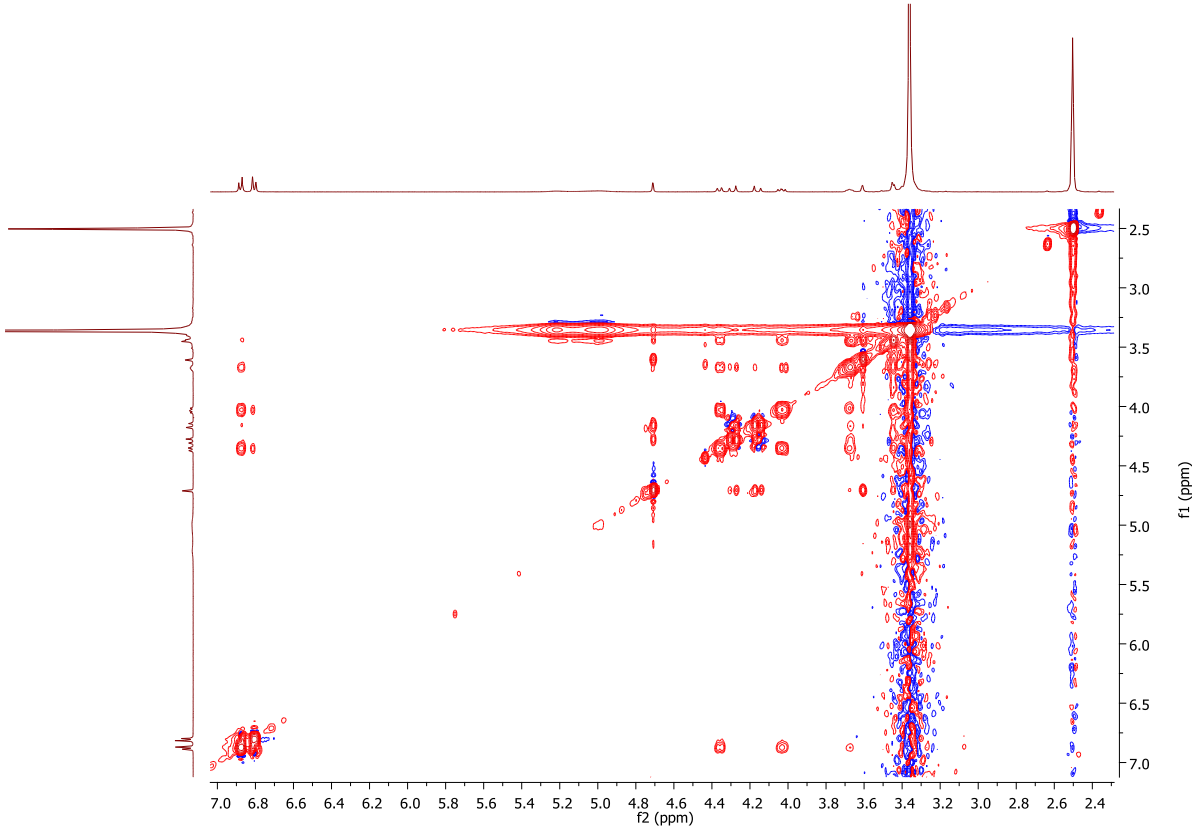

Figure S18: NOESY spectrum of *cis*-macrocycle **1** (DMSO-d_6, 300 K).

Irradiation Experiments

Each sample was heated at 45 °C in the dark for 20 h prior to the beginning of each experiment in order to fully relax the azobenzene to its *trans* form. The respective sample was irradiated in the dark, the distance between the lamp and the sample being about 5 cm, then the respective measurement was performed immediately afterwards.

Absorption spectroscopy

Photostationary states (PSS) were reached after irradiating the respective sample for 3 min at 365 nm or 14 min at 525 nm. Extinction coefficients (ε) were calculated using the Beer-Lambert law (see equation below), plotting the absorbance at the respective λ_{max} versus the concentration (6 - 8 different concentrations in a range of 2.10^{-5} to 10^{-4} mol·L^{-1}). In the case of PSS 365 nm, the λ_{max} of the n-π* band was used. A linear fitting gave the value of ε as the slope of the linear plot.

$$A = \varepsilon \cdot c \cdot l$$

A = absorbance
ε = extinction coefficient = slope of the plot
c = molar concentration in mol·L^{-1}
l = optical path length in cm

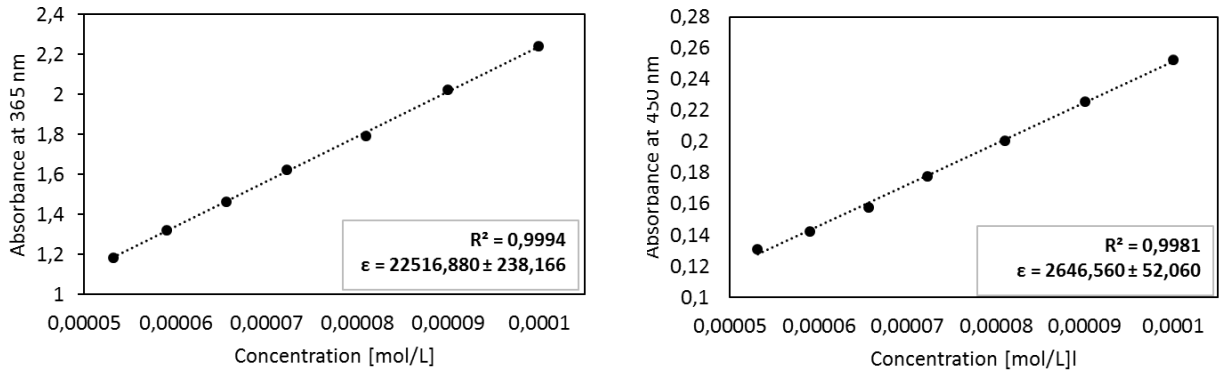

Figure S19: Plot of the absorbance at the λ_{max} versus the concentration; the slope of the linear curve gives the value of the molar extinction coefficient ε. Measured at 298 K in DMSO in concentrations from 5.10^{-5} to 10^{-4} mol·L^{-1} (a) *trans*-**1**, absorbance at 365 nm; (b) PSS after irradiation at 365 nm, absorbance at 450 nm.

Switching cycle experiments were performed by irradiating the sample alternatively for 3 min at 365 nm and 14 min at 525 nm, within 19 cycles. The value of the absorbance at $\lambda_{max(trans)}$ was plotted against the number of times the sample was irradiated.

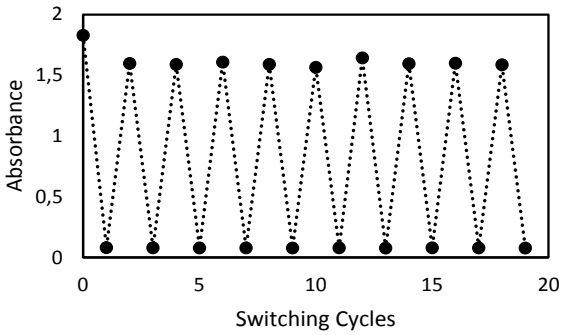

Figure S20: Measured absorbance after alternating photoirradiation with 365 nm and 525 nm. Measured at 298 K in DMSO at a concentration of 100 µM.

NMR spectroscopy

PSS was reached after irradiating the respective sample for 3 min with 365 nm or 17 min with 525 nm and the *cis*:*trans* ratios were measured by ^{1}H NMR spectroscopy. The PSS were determined by integration of one aromatic signal of each isomer.

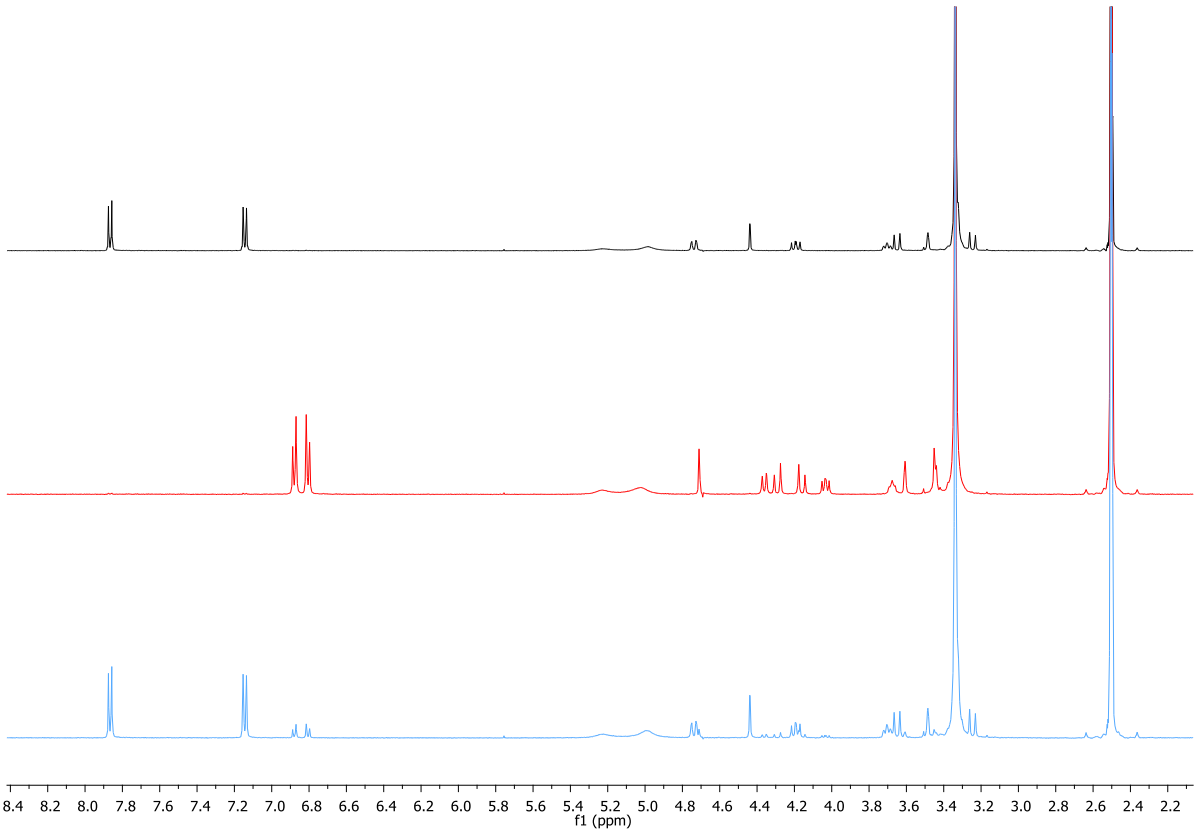

Figure S21: NMR spectra of trans-1 after heating at 45 °C in the dark for 20h (black), after irradiation with 365 nm for 3 min (red) and after irradiation with 525 nm (blue).

Thermal *cis*→*trans* relaxation

The kinetics of thermal *cis*→*trans* relaxation process was determined by ^{1}H NMR. After irradiation, the spectra of the samples were recorded in regular intervals, keeping the sample inside the probe at a constant temperature of 300 K, over a period of 7 days. The decay of the integral of the *cis* form was plotted versus the time, and an exponential decay of first order fitted to the data, according to the following equation:

$$I = I_{inf} + A \cdot \exp^{-k \cdot t}$$

I = integral of the *cis* isomer
I_{inf} = integral of the *cis* isomer at infinite time
A = pre-exponential factor proportional to the initial integral of the *cis* isomer
k = rate of thermal isomerization
t = time

A linear fitting gave the value of *k* as the slope of the following plot:

$$\ln(I - I_{inf}) = \ln(A) - kt$$

The half-life of the *cis* isomer ($\tau_{1/2}$) was determined as $\tau_{1/2} = \ln 2/k$.

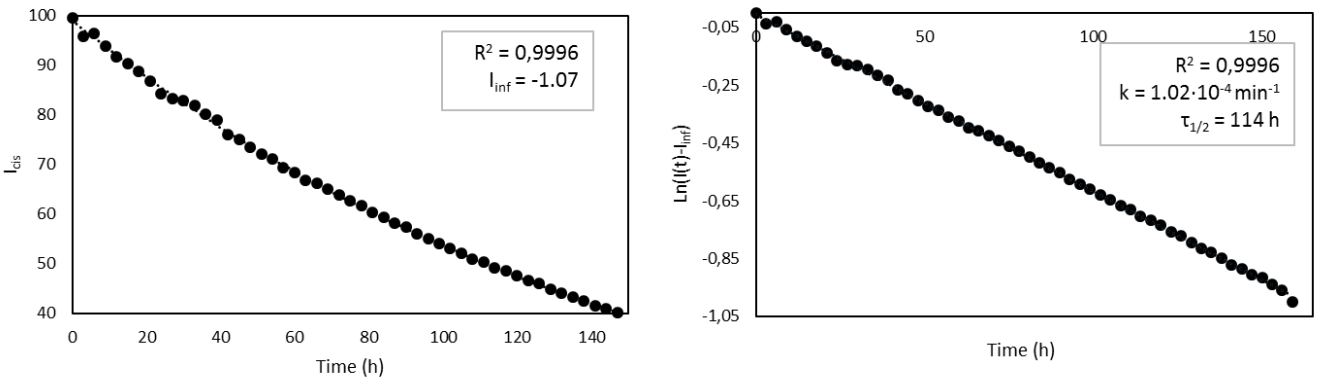

Figure S22: Kinetics of the *cis*→*trans* thermal relaxation process at 300 K investigated by 1H NMR spectroscopy. (a) Exponential decay of the integral of an aromatic signal of *cis*-**1** (300 MHz, DMSO-d6); (b) Linearization of the exponential decay of *cis*-**1**.

Specific rotation and circular dichroism

The optical rotation and the circular dichroism spectra were measured at 293 K after irradiating the respective sample at 365 nm for 10 min. The CD signal was recorded as ellipticity (θ), expressed in units of millidegrees (mdeg). The ellipticity was converted into difference in molar absorption coefficient (Δε), expressed in $M^{-1} \cdot cm^{-1}$, by using the following equation:

$\Delta\varepsilon = \theta / (32980 \cdot c \cdot l)$
Δε = difference in molar absorption coefficient
θ = ellipticity
c = molar concentration in $mol \cdot L^{-1}$
l = optical path length in cm

Literature References

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[2] H. J. Jung, H. Min, H. Yu, T. G. Lee and T. D. Chung, *Chem. Comm.* **2010**, *46*, 3863-3865.