Supporting Information

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

Julia Hain and Guillaume Despras*
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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 (^1H) nuclear magnetic resonance spectra and carbon (^13C) 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 (^1H - ^1H COSY and ^1H - ^13C 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™QM 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/cm². 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/cm². UV/Vis absorption spectra were measured on PerkinElmer Lambda-241.

Synthesis

Trans-Precursor 4:

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

A solution 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 Cs₂CO₃ (75.0 mg, 231 µmol) in dry DMF (8.00 mL) under nitrogen was stirred at 70 °C for 16 h then diluted with ethanol (50 mL), washed with 2 N HCl, brine and water (50 mL each) and dried over MgSO₄. 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.

\[\alpha\]₀ = +112 (c = 0.41 in CHCl₃); ¹H NMR (500 MHz, CDCl₃) δ = 7.90 – 7.84 (m, 4 H, Ar-Hortho), 7.05 – 6.96 (m, 4 H, Ar-Hmeta), 5.45 – 5.37 (m, 4 H, H-3, H-4), 5.32 (dd, 3J₂,₃ = 2.9 Hz, 3J₁₁ = 1.8 Hz, 2 H, H-2), 5.08 (d, 3J₁₁ = 1.6 Hz, 2 H, H-1), 4.33 (d, 2J₃CH₂CH = 2.4 Hz, 4 H, CH₂C=CHH), 4.25 – 4.13 (m, 6 H, H-5, H-6a, H-6b), 2.47 (t, 2J₃CH₂CH = 2.4 Hz, 2 H, CH₂C=CH), 2.18 (s, 6 H, 2 CH₂C=O), 2.05 (s, 6 H, 2 CH₂C=O), 2.02 (s, 6 H, 2 CH₂C=O); ¹³C NMR (126 MHz, CDCl₃) δ = 170.0 (CH₃C=O), 169.9 (2 C, CH₃C=O), 160.4 (Ar-Cpar), 147.3 (Ar-Cipso), 124.4 (Ar-Cortho), 114.9 (Ar-Cmeta), 96.0 (C-1), 77.9 (CH₂C=CH), 75.6 (CH₂C=CH), 69.5 (C-5), 69.4 (C-2), 68.9 (C-3), 67.5 (C-6), 66.8 (C-4), 54.8 (CH₂C=CH), 20.9 (CH₃C=O), 20.8 (CH₃C=O), 20.7 (CH₃C=O); IR (ATR): β = 2363, 1746, 1369, 1215, 1135, 1043, 973, 841, 492 cm⁻¹; ESI-HRMS m/z: calc. 867.28184 for [C₄₂H₄₅O₁₈N₂+H⁺]; found 867.28077 for [C₄₂H₄₅O₁₈N₂+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 co-evaporated with toluene. The crude residue was dissolved in ethyl acetate, washed four times with water (50 mL each) and dried over MgSO₄. 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.

\[ \text{[\alpha]}^{20}_D = -182 \text{ (c = 0.12 in CHCl}_3\) \]; \(^1\text{H NMR (500 MHz, CDCl}_3\) \( \delta = 7.90 – 7.84 \text{ (m, 4 H, Ar-H} \text{ortho}), 7.05 – 6.99 \text{ (m, 4 H, Ar-H} \text{meta}), 5.18 \text{ (dd,} \text{ J}_{\text{H,2}} = 10.0 \text{ Hz,} \text{ J}_{\text{H,4}} = 3.4 \text{ Hz, 2 H, H-3), 5.09 – 5.02 \text{ (m, 4 H, H-2, H-4), 4.53 \text{ (s, 2 H, H-1), 4.36 \text{ (dd,} \text{ J}_{\text{H,6a,b}} = 13.0 \text{ Hz,} \text{ J}_{\text{H,6a,b}} = 9.1 \text{ Hz, 2 H, H-6a), 4.28 \text{ (dd,} \text{ J}_{\text{H,6b,5}} = 2.3 \text{ Hz, 2 H, H-6b), 4.13 – 4.06 \text{ (m, 2 H, H-5), 3.47 \text{ (d,} \text{ J}_{\text{CH2,CH2}} = 15.4 \text{ Hz, 2 H, CH}_2\text{C=C=Ca), 3.43 \text{ (d,} \text{ J}_{\text{CH2,CH2}} = 15.5 \text{ Hz, 2 H, CH}_2\text{C=C=Cb), 2.07 \text{ (s, 6 H, 2 CH}_2\text{C=C=O), 1.91 \text{ (s, 6 H, 2 CH}_2\text{C=C=O), 1}^{13}\text{C NMR (126 MHz, CDCl}_3\) \( \delta = 170.0 \text{ (CH}_2\text{C=O), 169.7 \text{ (CH}_2\text{C=O), 169.6 \text{ (CH}_2\text{C=O), 158.4 \text{ (Ar-C} \text{para}), 147.7 \text{ (Ar-C} \text{para}), 124.4 \text{ (Ar-C} \text{ortho), 117.5 \text{ (Ar-C} \text{meta), 96.0 \text{ (C-1), 72.8 \text{ (CH}_2\text{C=C=Ca), 70.3 \text{ (CH}_2\text{C=C=Cb), 69.2 \text{ (C-2), 68.6 \text{ (C-3), 67.3 \text{ (C-4, C-5), 54.8 \text{ (CH}_2\text{C=C=Ca), 20.8 \text{ (C, CH}_2\text{C=C=O), 20.6 \text{ (CH}_2\text{C=C=O), IR (ATR):} \text{ } \delta = 2923, 2360, 1747, 1209, 1068, 1040, 967, 847, 492 \text{ cm}^{-1}; \text{ ESI-HRMS m/z: calc. 865.26619 for} \text{ [C}_4\text{H}_4\text{O}_2\text{N}_2\text{H}^+]^+, \text{ found 865.26543 for} \text{ [C}_4\text{H}_4\text{O}_2\text{N}_2\text{H}^+]^+}.\text{ Trans-Macrocycle 1:} \text{ 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}^+ \text{ and concentrated to dryness to give the macrocycle 1 (38.8 mg, 63.3 µmol, quantitative) as a yellow amorphous solid.} \text{ [\alpha]}^{24}_D = -146 \text{ (c = 0.08 in DMSO);} \text{ [\alpha]}^{24}_D = -146 \text{ (c = 0.08 in DMSO);} \text{ [\alpha]}^{24}_D = -146 \text{ (c = 0.08 in DMSO);} \text{ [\alpha]}^{24}_D = -576 \text{ (c = 0.08 in DMSO);} \text{ [\alpha]}^{24}_D = -576 \text{ (c = 0.08 in DMSO);} \text{ [\alpha]}^{24}_D = -576 \text{ (c = 0.08 in DMSO);} \text{ [\alpha]}^{24}_D = -576 \text{ (c = 0.08 in DMSO);}
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 (CDCl₃, 300 K).

Figure S4: HMBC spectrum of trans-4 (CDCl₃, 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-\textsubscript{d\textsubscript{6}}, 300 K).

Figure S14: \textsuperscript{1}H NMR spectrum of cis-macrocycle 1 (500 MHz, DMSO-\textsubscript{d\textsubscript{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 ($\varepsilon$) were calculated using the Beer-Lambert law (see equation below), plotting the absorbance at the respective $\lambda_{\text{max}}$ versus the concentration (6 - 8 different concentrations in a range of $2 \times 10^{-5}$ to $10^{-4}$ mol·L$^{-1}$). In the case of PSS 365 nm, the $\lambda_{\text{max}}$ of the n-$\pi^*$ band was used. A linear fitting gave the value of $\varepsilon$ as the slope of the linear plot.

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

A = absorbance  
$\varepsilon$ = 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 $\lambda_{\text{max}}$ versus the concentration; the slope of the linear curve gives the value of the molar extinction coefficient $\varepsilon$. Measured at 298 K in DMSO in concentrations from $5 \times 10^{-5}$ 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 $\lambda_{\text{max(\text{trans})}}$ 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 1H 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:

\[ I = I_{\text{inf}} + A \cdot \exp^{-k \cdot t} \]

\( I \) = integral of the cis isomer
\( I_{\text{inf}} \) = 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:

\[ \ln(I - I_{\text{inf}}) = \ln(A) - k \cdot t \]

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

![Figure S22](image)

*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 spectras were measured at 293 K after irradiating the respective sample at 365 nm for 10 min. The CD signal was recorded as ellipticity (\( \theta \)), expressed in units of millidegrees (mdeg). The ellipticity was converted into difference in molar absorption coefficient (\( \Delta \varepsilon \)), expressed in M\(^{-1}\)·cm\(^{-1}\), by using the following equation:

\[ \Delta \varepsilon = \frac{\theta}{(32980 \cdot c \cdot l)} \]

\( \Delta \varepsilon \) = difference in molar absorption coefficient
\( \theta \) = ellipticity
\( c \) = molar concentration in mol·L\(^{-1}\)
\( l \) = optical path length in cm
Literature References
