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Cucurbit[7]uril Complexation Drives Thermal *trans–cis*-Azobenzene Isomerization and Enables Colorimetric Amine Detection

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Abstract: Complexation of yellow diaminoazobenzenes **1** and **3** inside cucurbit[7]uril (CB[7]) results in the formation of purple-colored CB[7]•*cis*-**1**•2 H^+ and CB[7]•*cis*-**3**•2 H^+ complexes, respectively. The high binding affinity and selectivity displayed by CB[7] toward **1** and **3** pays the >10 kcal mol^{-1} thermodynamic cost for this isomerization. We investigated the behavior of these complexes as a function of pH and observed large pK_a shifts and high pH responsiveness, which are characteristic of cucurbit[*n*]uril molecular containers. The remarkable yellow to purple color change was utilized in the construction of an indicator displacement assay for biologically active amines **4**–**10**. This indicator displacement assay is capable of quantifying the pseudoephedrine (**5**) content in Sudafed tablets over the 5–350 μM range.

Keywords: azo dyes · cucurbiturils · indicator displacement assay · isomerization · supramolecular chemistry

Introduction

Azobenzenes are prized components of photochemically responsive systems because of the substantial geometric and colorimetric changes that occur upon *trans*-azobenzene to *cis*-azobenzene isomerization. For example, azobenzenes have been employed for optical data storage, light to mechanical energy transduction, and as biological photoswitches.[1] In these systems, light is required to pay the thermodynamic cost (>10 kcal mol^{-1}) associated with establishing and maintaining a photostationary state containing thermodynamically unstable *cis*-azobenzene.[2] In contrast, the use of chemical stimuli to populate the *cis*-azobenzene form at thermodynamic equilibrium is exceedingly rare.[3] In this paper, we expand the range of chemically responsive azobenzenes with the ultimate goal of attaining finer control over their applications by dual photochemical and chemical stimuli responsiveness.

To create chemically responsive azobenzenes, we turned to the cucurbit[*n*]uril (CB[*n*]) family of molecular containers.[4] CB[*n*] supramolecular chemistry is undergoing rapid expansion fueled by their extraordinary recognition properties toward cationic species in water (K_a up to 10^{15} M^{-1}),[5] and their high stimuli responsiveness (e.g. pH, electrochemical, chemical), which makes CB[*n*] ideal components for molecular machines and devices.[4b,6] For example, Nau and co-workers[7] have utilized the substantial pK_a shifts that occur within CB[*n*]•chromophore complexes to devise (fluorescence) displacement assays for enzyme activity and enantiomeric excess.[8] Herein, we report the remarkable ability of CB[7] to thermally populate the *cis*-isomer form of azobenzenes **1** and **3**, and the use of CB[7]•*cis*-**1** and CB[7]•*cis*-**3** in indicator displacement assays for cationic drugs (**4**–**10**) in water (Scheme 1).[9]

Results and Discussion

CB[7] induces *trans–cis* isomerization of azobenzenes 1 and 3: Several years ago, while measuring the affinity of a variety of guests toward CB[6]–CB[8][5b] we observed the instantaneous formation of an unusual purple color when CB[7] was added to a solution of *trans*-azobenzene **1** or *trans*-azobenzene **3** in water.[10] In sharp contrast, compound **2** that contains a cyclohexylammonium-ion binding region does not exhibit a similar purple color when combined with CB[7]. The upfield shifts observed for the cyclohexyl ring of **2** in the ^{1}H NMR spectrum recorded for CB[7]•**2** (see the Supporting Information) established that CB[7] binds to the cyclohexylammonium tail of **2**. In combination, these results

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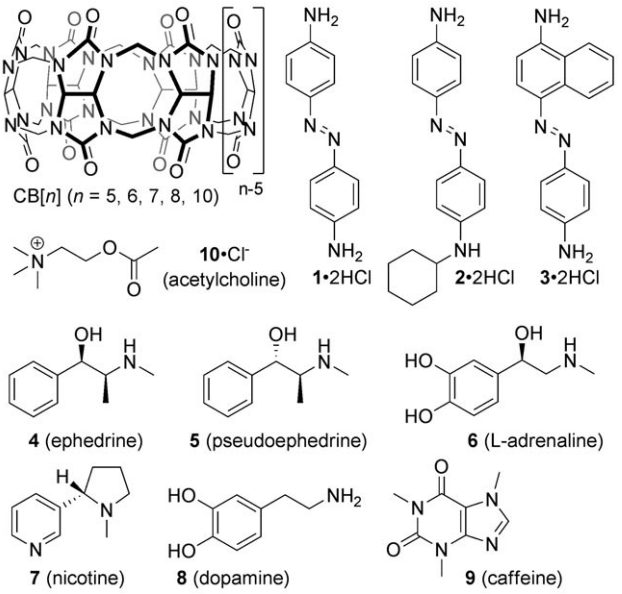


Scheme 1. Compounds used in this study.

suggested to us that CB[7] promoted the thermal *trans*-**1** to *cis*-**1** isomerization by binding to the entire azobenzene region of **1**.[11]

UV/Vis titration for CB[7]·1: Therefore, we decided to investigate the interaction between CB[7] and **1** in detail. Figure 1 shows the UV/Vis titration curves of **1** with CB[7],

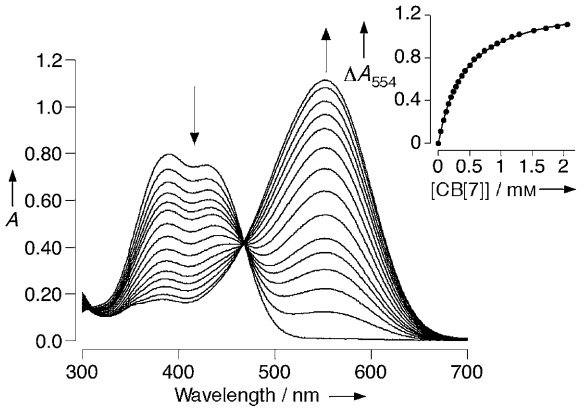


Figure 1. Selected UV/Vis spectra from the titration of **1** (26.6 μM) with CB[7] (0–2.2 mM) in 50 mM NaOAc buffer (pH 4.76). The inset shows a plot of ΔA_{554} as a function of CB[7] concentration. The solid line represents the best non-linear fit of the data to a 1:1 binding model.

which exhibit an isosbestic point at $\lambda = 468$ nm—indicative of a well defined two-state equilibrium—along with a large increase in absorbance at $\lambda = 554$ nm. The titration data fit well to a 1:1 binding model that allowed us to determine the binding constant ($K_a = 2.26 \pm 0.03 \times 10^3\,M^{-1}$) for the CB[7]·**1** complex. A Job plot based on UV/Vis measurements ([CB[7]] + [**1**] = 35 μM) also confirmed the 1:1 stoichiometry (see the Supporting Information).

pH dependence of the UV/Vis spectra of CB[7]·*1*: Next, we performed a pH titration of **1** alone to determine whether the purple form was accessible by changes in pH. Over the range of pH 0.5–12.5 we did not observe this purple color (see the Supporting Information). The three pK_a values for the ionization of **1**·3H$^+$ (−0.5), **1**·2H$^+$ (2.11), and **1**·H$^+$ (3.54) can be derived from the pH titration and have been reported previously.[12] Next, we performed a pH titration of CB[7]·**1** over the range of pH 0.18–13.35 range (see the Supporting Information). At high pH values (pH > 6.5) we did not observe significant changes in the UV/Vis spectra. Between pH 6.5 and 4.32, however, large changes in absorbance were observed across the spectrum with a particularly dramatic increase at $\lambda = 554$ nm (Figure 2). This change in

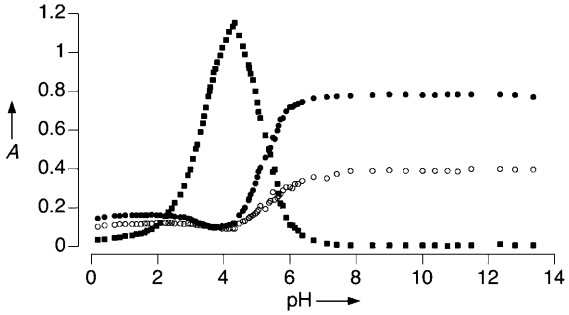
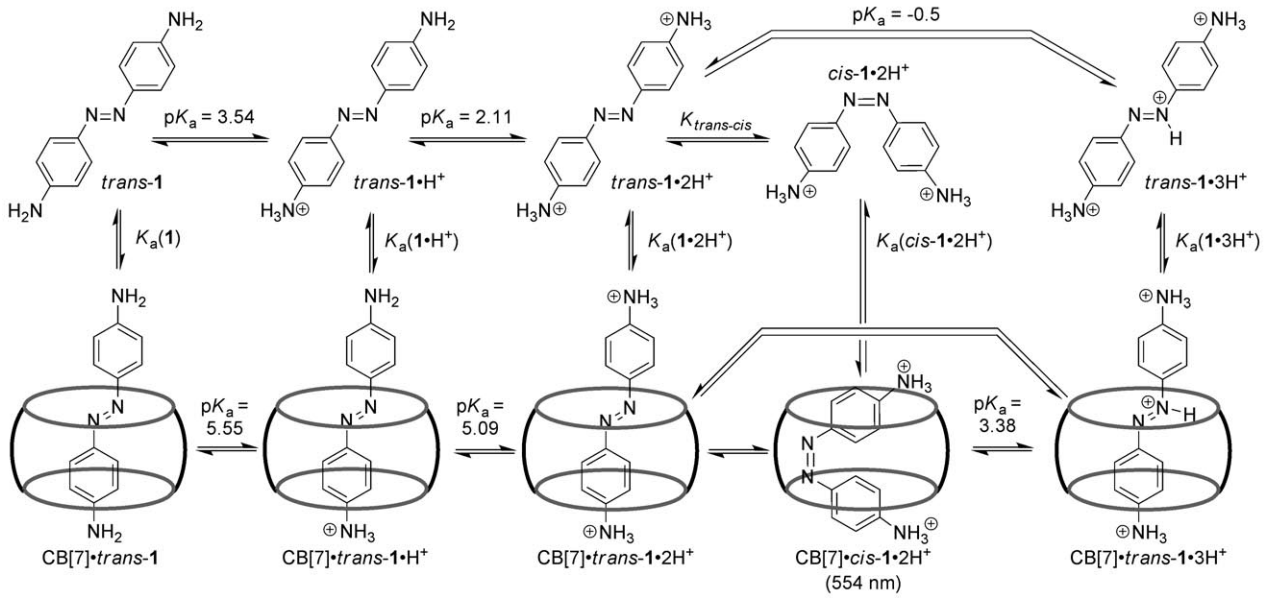


Figure 2. Plot of absorbance versus pH used in the pK_a determinations. (■ = 554 nm; ● = 391 nm; ○ = 320 nm).

absorbance at $\lambda = 554$ nm—unique to the CB[7]·*cis*-**1**·2H$^+$ complex—allowed a determination of p$K_a = 5.09$ for CB[7]·*cis*-**1**·2H$^+$ corresponding to a pK_a shift of 2.98 units upon complexation. Between pH 4.32 and 2.0 the purple color is lost with a concomitant decrease in absorbance at $\lambda = 554$ nm corresponding to formation of the CB[7]·*trans*-**1**·3H$^+$ complex. The change in absorbance at $\lambda = 554$ nm was analyzed to yield p$K_a = 3.38$ for the CB[7]·*trans*-**1**·3H$^+$ complex. In this case, complexation induced a very large pK_a shift (3.88 units) due to preferential binding of the CB[7]·*trans*-**1**·3H$^+$ form. Similarly dramatic pK_a shifts have been previously observed by Nau et al. for CB[*n*] complexes[7b] and Raymond et al. in self-assembled capsules.[13] The pK_a of the CB[7]·*trans*-**1**·H$^+$ was challenging to determine because of the small UV/Vis-absorption change for this protonation event; we estimate pK_a (CB[7]·*trans*-**1**·H$^+$) = 5.55 from the titration data at $\lambda = 320$ nm.

Binding model for the interaction of CB[7] and 1: The overall interaction model between CB[7] and **1** including the values of pK_a is summarized in Scheme 2. Initially, the equilibrium between *trans*-**1** and *cis*-**1** greatly favors the *trans* form ($\Delta E_{trans-cis} > 10$ kcal mol^{-1}).[2] The presence of CB[7]—with its remarkable binding energies (K_a up to $10^{15}\,M^{-1}$)—is able to pay the thermodynamic cost (> 10 kcal mol^{-1}) for population of the *cis*-**1** form in the range of pH 3–6. At pH < 3, *cis* to *trans* isomerization occurs with preferential formation of CB[7]·*trans*-**1**·3H$^+$ (Scheme 2). Similar pH ti-



Scheme 2. Model proposed for the formation of CB[7]•*cis*-**1**•2H$^+$.

trations were performed with **3** and CB[7]•**3** (see the Supporting Information) but the analysis of this data was complicated by the fact that all four N atoms of dye **3** are chemically distinct.

Use of CB[7]•*cis*-3 to construct an indicator displacement assay (IDA): To demonstrate an application of the thermally driven *trans*- to *cis*-azobenzene isomerization we decided to use it to construct an indicator displacement assay[14] for biologically relevant amines **4**–**10**.[15] Despite the remarkable color change (393 to 554 nm) and increased molar extinction coefficient ($\varepsilon_{CB[7]\text{-}cis\text{-}1\cdot2H^+(554\ nm)} = 6.6\times10^4\,M^{-1}cm^{-1}$), **1** is not a suitable indicator for the preparation of IDAs because K_a for the CB[7]•**1** complex [$(2260\pm30)\,M^{-1}$] is lower than the affinity of drugs **4**–**10** towards CB[7]. Compound **3** with its larger, more hydrophobic naphthalene ring also exhibits the purple color indicating a similar CB[7] induced *trans–cis* isomerization. A UV/Vis titration yielded $K_a = (1.03\pm0.02)\times10^4\,M^{-1}$ for the CB[7]•**3** complex (see the Supporting Information). By using this value of K_a we were able to determine the affinity of CB[7] towards seven biologically important drugs (**4**–**10**) by competitive IDA (see the Supporting Information).[16] Figure 3 illustrates that good levels of selectivity are readily achieved during the IDA by using CB[7]•*cis*-**3** towards this set of analytes.

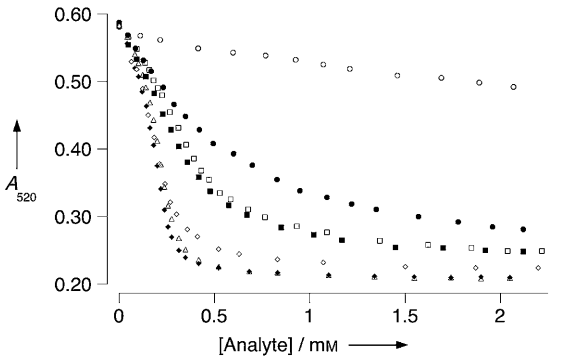


Figure 3. Change in absorbance at $\lambda = 520$ nm for a solution of **3** (34 μM) and CB[7] (236 μM) in NaOAc buffer (pH 4.76, [Na$^+$] = 50 mM) versus increasing concentration of analyte **4**–**10**. Legend: ○ = **9**; ● = **6**; □ = **8**; ■ = **7**; ◇ = **10**; △ = **5**; ◆ = **4**.

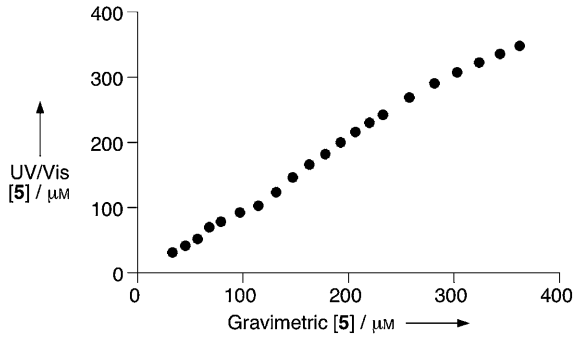


Figure 4. Plot of the content of **5** in Sudafed tablets measured by gravimetric methods versus the value determined by the IDA. Conditions: [**3**] = 30 μM, [CB[7]] = 231 μM (50 mM NaOAc, pH 4.76).

Use of CB[7]•*cis*-3 to quantify the pseudoephedrine content in Sudafed tablets: We next wanted to confirm the applicability of this colorimetric IDA based on CB[7]•*cis*-**3** for the detection of pseudoephedrine (**5**) content in the over-the-counter nasal decongestant Sudafed. Figure 4 shows a plot of the concentration of **5** measured by the IDA by using CB[7]•*cis*-**3** and that determined gravimetrically as a function of absorbance at $\lambda = 520$ nm. A wide range of concentrations (20–350 μM) could be detected by the IDA with very good agreement ($\leq 5\,\%$) between both methods. It is possi-

ble to extend the detection range (5–20 μM) by decreasing the CB[7] concentration or by using indicator **1** (see the Supporting Information). The sensitivity and selectivity of this IDA for **5** in Sudafed tablets is notable given that each pill (145 mg total weight) contains only 30 mg of **5**.

The high pH sensitivity of CB[7]·guest complexes leads to high dispersion in principal component analysis (PCA) score plots: Although we were pleased that the IDA based on CB[7]·*cis*-**3** was capable of quantifying the amount of **5** in Sudafed tablets over a broad range of concentrations we were concerned that this IDA might have difficulties differentiating between analytes given the similar values of K_a for CB[7] towards certain pairs of drugs (e.g. **4** vs. **5**, **4** vs. **10**, and **7** vs. **8**). Given the fact that CB[7], **1** or **3**, and analytes **4**–**9** undergo protonation events in water—and given the importance of cation-dipole interactions in determining the magnitude of K_a values for CB[*n*]·guest complexes—we suspected that conducting IDAs as a function of pH would lead to excellent analyte differentiation.

We first conducted UV/Vis binding studies between CB[7] and **3** at five values of pH between 4.00–5.01. Quite interestingly, the value of K_a changes by 200-fold ($7.02\times10^5\,M^{-1}$ to $3.17\times10^3\,M^{-1}$) and obeys a linear free energy relationship ($\log K_a$ vs. pH) with a slope of −2.36 over this range of pH. The absorbance versus pH profile (see the Supporting Information) indicates that two protonation events are accessible over this range of pH that drive formation of the CB[7]·*cis*-**3**·$2H^+$ complex at lower values of pH. Accordingly, we measured the response of a sensing ensemble comprising CB[7] (46 μM) and **3** (200 μM) to the addition of compounds **4**–**10** at four different pH values (pH 4.00, 4.32, 4.50, and 4.76) and analyzed this data by using PCA.[17] Figure 5 shows the PCA plots of the data. The excellent dispersion between the clusters of data and their tight distribution highlights the discrimination ability of this sensing ensemble, which we attribute to the high pH dependence of K_a values for CB[*n*] complexes.[7,18] For example, compounds **7** and **8** that are barely distinguishable at pH 4.76 (Figure 3) become well differentiated in the PCA score plot (Figure 5) due to the accessibility of the pyridinium form of **7** over this range of pH. Similarly, compounds **4** and **10** that are poorly differentiated at pH 4.76 are well separated in the PCA score plot (Figure 5). The clustering observed for diastereomers **4** and **5** in Figure 5 illustrates one of the limitations of this system. Compounds that exhibit similar values of K_a toward CB[7] and that contain functional groups of comparable pK_a will be difficult to differentiate by pH changes alone.

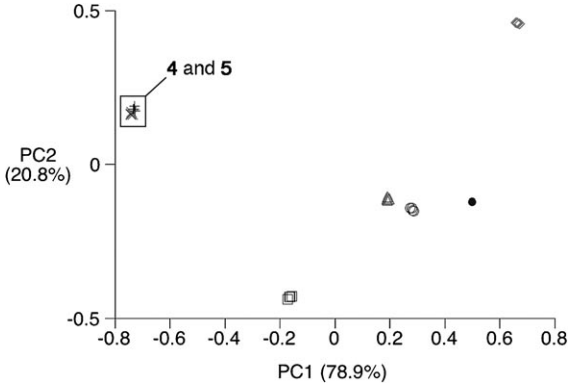


Figure 5. PCA plot for drugs **4**–**10** constructed from IDA data at pH 4.76, 4.52, 4.30, and 4.00 run in quadruplicate. Legend: ×=**4**; +=**5**; ●, **6**; △=**7**; ○=**8**; ◇=**9**; □=**10**.

Conclusion

In conclusion, we have shown that CB[7] pays the thermodynamic cost ($>10\,kcal\,mol^{-1}$) associated with thermal *trans* to *cis* isomerization of the azobenzene dyes **1** and **3**. The CB[7]·*cis*-**1**·$2H^+$ and CB[7]·*cis*-**3**·$2H^+$ complexes are intensely purple colored and can be used to construct indicator displacement assays (IDA) for drugs **4**–**10** and to quantify the content of pseudoephedrine (**5**) within the background matrix of Sudafed tablets. A particularly notable feature of this IDA is the high pH sensitivity of CB[*n*]·guest complexes that allows efficient analyte differentiation by principal component analysis (PCA) over a narrow pH range. In combination, the high K_a values and selectivity achieved by CB[*n*] receptors, and their high pH responsiveness suggests great potential for their future use in sensor arrays. Although this study highlighted the ability of CB[7] to drive thermal azobenzene isomerization and demonstrated a typical azobenzene application—sensing—in thermally rather than photochemically driven mode we believe the implications of the research are broader. For example, the ability to address azobenzene systems by dual photochemical and chemical means promises to extend the range of control that can be exerted in applications like data storage and liquid crystal technologies. Perhaps equally exciting is the potential to interface azobenzenes and CB[*n*] molecular containers with biological systems where they would function as non-natural photochemically and chemically activated switches.

Experimental Section

General experimental details: Starting materials, dye **1**, and drug molecules were purchased from commercial suppliers and were used without further purification. CB[7] was prepared according to the literature procedures.[19] Compounds **2** and **3** were prepared as described below. TLC analysis was performed by using precoated plates from EM Science. Column chromatography was performed by using silica gel (230–400 mesh, 0.040–0.063 μm) from Sorbent Technologies by using eluents in the indicated v:v ratio. Hexane and ethyl acetate were distilled before use as chromatographic solvents. Melting points were measured on a Meltemp apparatus in open capillary tubes and are uncorrected. NMR spectra were measured on spectrometers operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm relative to the deuterated solvent as the internal standard. IR was performed on FT/IR-4100 Fourier Transform Infrared Spectrometer. Mass spectrometry analysis was performed by using a JEOL AccuTOF CS instrument by electro-

spray ionization (ESI). CsI cluster ions were used as internal standard for high resolution MS measurement.

4-(4-Nitrophenylazo)phenylcyclohexylamine (11): Sodium nitrite (0.118 g, 1.71 mmol) was added to a stirred solution of concentrated sulfuric acid (1 mL). The mixture was heated until the temperature reached 70 °C and then cooled to 0 °C. To this mixture was added a solution of 4-nitroaniline (0.236 g, 1.71 mmol) dissolved in a mixture of propionic acid/acetic acid/DMF (0.43 mL:2.14 mL:1.71 mL). The mixture was stirred at 0 °C for 2 h. A DMF solution (4.28 mL) of cyclohexylphenylamine (0.15 g, 0.856 mmol) was added to this mixture, which then was stirred at 0 °C for 1 h, then warmed to room temperature and stirred for 24 h. The product solution was basified with aqueous ammonium hydroxide (30 %) and a crude dark red solid was obtained after vacuum filtration. The residue was purified by column chromatography (SiO_2, toluene/hexane, 5:1) to give **11** (0.172 g, 0.53 mmol) in 62 % yield. M.p. 146–147 °C, $R_f = 0.28$ (toluene/hexane 5:1); IR: $\tilde{\nu} = 3392$ (m), 2926 (m), 2853 (m), 1599 (s), 1514 (s), 1333 (s), 1105 cm^{-1} (m); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.30$ (d, $J = 8.9$ Hz, 2H), 7.89 (d, $J = 8.9$ Hz, 2H), 7.83 (d, $J = 8.9$ Hz, 2H), 6.61 (d, $J = 8.9$ Hz, 2H), 4.28 (d, $J = 7.4$ Hz, 1H), 3.45–3.30 (m, 1H), 2.15–2.05 (m, 2H), 1.85–1.75 (m, 2H), 1.75–1.65 (m, 1H), 1.45–1.35 (m, 2H), 1.30–1.15 ppm (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 156.8$, 151.3, 147.3, 144.3, 126.5, 124.7, 122.6, 112.4, 51.5, 33.1, 25.6, 24.8 ppm; ES-MS: *m/z* (%): 325 (100, $[M+H]^+$); HR-MS: *m/z*: calcd for $C_{18}H_{21}N_4O_2$: 325.1665; found: 325.1670 ($[M+H]^+$.

4-(4-Aminophenylazo)phenylcyclohexylamine (12): A solution of **11** (0.172 g, 0.53 mmol) in EtOH (20 mL) was refluxed with sodium sulfide nonahydrate (0.264 g, 1.1 mmol) for 8 h. After the reaction was complete the mixture was poured into water (80 mL). The product was extracted with ethyl acetate (3×30 mL), dried over $MgSO_4$ and concentrated by rotary evaporation. The residue was purified by column chromatography (SiO_2, toluene/ethyl acetate, 10:1) to give **12** (0.141 g, 0.48 mmol) in 90 % yield. M.p. 145–146 °C; $R_f = 0.23$ (toluene/ethyl acetate 10:1); IR: $\tilde{\nu} =$ 3459 (m), 3373 (m), 3208 (m), 2925 (m), 2848 (m), 1592 (s), 1506 (s), 1306 (m), 1144 (s), 830 cm^{-1} (s); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $J = 8.7$ Hz, 2H), 7.73 (d, $J = 8.5$ Hz, 2H), 6.69 (d, $J = 8.5$ Hz, 2H), 6.61 (d, $J = 8.7$ Hz, 2H), 3.96 (d, $J = 7.8$ Hz, 1H), 3.89 (d, $J = 9.2$ Hz, 1H), 3.35–3.25 (m, 1H), 2.10–2.00 (m, 2H), 1.80–1.70 (m, 2H), 1.70–1.60 (m, 1H), 1.45–1.30 (m, 2H), 1.30–1.10 ppm (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 149.1$, 148.0, 145.8, 144.3, 124.5, 124.0, 114.7, 112.4, 51.3, 33.1, 25.7, 24.8 ppm; ES-MS: *m/z* (%): 295 (100, $[M+H]^+$); HR-MS: *m/z*: calcd for $C_{18}H_{23}N_4$: 295.1923; found: 295.1940 ($[M+H]^+$).

Dye 2: HCl gas was passed through a solution of **12** (0.118 g, 0.40 mmol) in anhydrous Et_2O (300 mL) for 5 min. The resulting precipitate was separated from the mother liquor by centrifugation, the supernatant decanted, and the solid dried under high vacuum to give **2** (0.146 g, 0.40 mmol) in 99 % yield. M.p. 166–167 °C; IR: $\tilde{\nu} = 3459$ (w), 3390 (w), 3325 (w), 2930 (m), 2848 (m), 1585 (s), 1507 (m), 1304 (s), 1256 (m), 1144 (s), 1082 (m), 831 cm^{-1} (s); ^1H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.75$–7.65 (m, 4H), 7.08 (d, $J = 8.6$ Hz, 2H), 6.87 (d, $J = 8.6$ Hz, 2H), 3.35–3.25 (m, 1H), 1.95–1.85 (m, 2H), 1.75–1.65 (m, 2H), 1.60–1.50 (m, 1H), 1.40–1.05 ppm (m, 5H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 149.1$, 147.6, 144.8, 143.7, 125.4, 124.5, 119.3, 115.0, 53.1, 32.6, 26.2, 25.2 ppm;. ES-MS: *m/z* (%): 295 (100, $[M+H]^+$); HR-MS: *m/z*: calcd for $C_{18}H_{23}N_4$: 295.1923; found: 295.1931 ($[M+H]^+$).

4-(4-Nitrophenylazo)naphthalen-1-amine (13): Sodium nitrite (1.035 g, 15.0 mmol) was added to a stirred solution of concentrated sulfuric acid (10 mL). The mixture was heated until the temperature reached 70 °C and then cooled to 0 °C. To this mixture was added a solution of 4-nitroaniline (2.07 g, 15.0 mmol) dissolved in propionic acid/acetic acid/DMF (3.5 mL:19 mL:15 mL). The mixture was stirred at 0 °C for 2 h. A DMF solution (30 mL) of aminonaphthalene (0.716 g, 5.00 mmol) was added to this mixture, which then was stirred at 0 °C for 1 h, then warmed to room temperature and stirred for 24 h. The solution was basified with aqueous ammonium hydroxide (30 %). The crude dark red solid was obtained by vacuum filtration. The crude solid was purified by column chromatography (SiO_2, toluene/ethyl acetate, 10:1) to give **13** (0.789 g, 2.70 mmol) in 54 % yield. M.p. 249–250 °C; $R_f = 0.16$ (toluene/ethyl acetate 1:25); IR: $\tilde{\nu} = 3424$ (w), 3320 (w), 3227 (w), 3220 (w), 1571 (m), 1508 (s), 1321 (s), 1189 (m), 1141 (m), 1102 (m), 854 (m). 821 (m), 758 cm^{-1} (s); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.03$ (d, $J = 8.4$ Hz, 1H), 8.36 (d, $J = 9.0$ Hz, 2H), 8.04 (d, $J = 9.0$ Hz, 2H), 8.03 (d, $J = 8.4$ Hz, 1H), 7.81 (d, $J = 8.4$ Hz, 1H), 7.68 (t, $J = 7.3$ Hz, 1H), 7.56 (t, $J = 7.3$ Hz, 1H), 6.81 (d, $J = 8.4$ Hz, 1H), 4.83 ppm (brs, 2H). ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 157.0$, 152.9, 146.2, 137.2, 134.0, 128.5, 125.1, 124.8, 122.9, 122.7, 122.3, 120.9, 117.1, 108.2 ppm; ES-MS: *m/z* (%): 293 (100, $[M+H]^+$); HR-MS: *m/z*: calcd for $C_{16}H_{13}N_4O_2$: 293.1039; found: 293.1033 ($[M+H]^+$).

Dye 3: A solution of **11** (0.400 g, 1.37 mmol) in EtOH (60 mL) was refluxed with sodium sulfide nonahydrate (0.650 g, 2.72 mmol) for 20 h. The mixture was poured into water (400 mL). The product was extracted with ethyl acetate (3×100 mL), dried over $MgSO_4$ and concentrated by rotary evaporation. The residue was purified by column chromatography (SiO_2, toluene/ethyl acetate, 6:1) to give **3** (0.322 g, 1.23 mmol) in 90 % yield. M.p. 170–171 °C; $R_f = 0.11$ (toluene/ethyl acetate 6:1); IR: $\tilde{\nu} = 3432$ (w), 3393 (w), 3350 (w), 3313 (w), 3210 (w), 1598 (m), 1571 (m), 1501 (m), 1462 (m), 1398 (m), 1340 (m), 1282 (m), 1255 (m). 1139 (m), 834 (s), 755 cm^{-1} (s); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 9.00$ (d, $J = 8.3$ Hz, 1H), 7.86 (d, $J = 8.7$ Hz, 2H), 7.81 (d, $J = 8.2$ Hz, 1H), 7.80 (d, $J = 8.4$ Hz, 1H), 7.65–7.55 (m, 1H), 7.55–7.45 (m, 1H), 6.81 (d, $J = 8.3$ Hz, 1H), 6.80–6.70 (m, 2H), 4.46 (brs, 2H), 3.96 ppm (brs, 2H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 151.0$, 147.9, 144.0, 137.2, 132.4, 126.5, 124.1, 122.9, 122.5, 121.5, 113.6, 113.4, 107.3 ppm (only 13 of the 14 expected resonances were observed); ES-MS: *m/z* (%): 263 (100, $[M+H]^+$); HR-MS: *m/z*: calcd for $C_{16}H_{15}N_4$: 263.1297; found: 263.1308 ($[M+H]^+$,.

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