Photoswitches

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Spectral Tuning of Azobenzene Photoswitches for Biological Applications**

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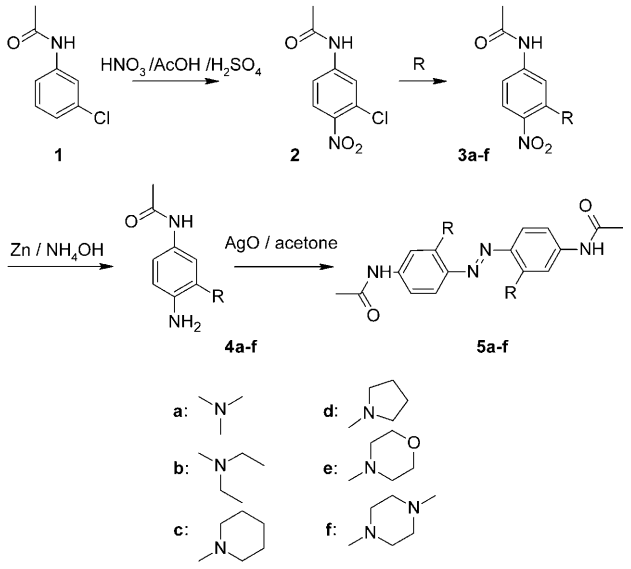
Photoisomerization of azobenzene has been used in diverse fields to enable photocontrol of molecular processes. In biology, the introduction of azobenzene photoswitches has led to the development of photocontrolled ion channels and enzymes, powerful tools for studying living systems.[1–4] Currently, a limited range of switching wavelengths in the UV region and/or sensitivity to the reducing intracellular environment limits broad applicability of these photoswitches for in vivo applications.[5,6] We report a series of azobenzene derivatives in which longer switching wavelengths (up to 530 nm) are combined with good photochemical yields and stabilities of the *cis* isomers. These derivatives can be used for photocontrol of biomolecular structures in intracellular environments.

A large number of azobenzene derivatives are known in which enhanced electron-donating nature of ring substituents increases both the wavelength of absorption of the *trans* isomer and the rate of thermal back-isomerization from *cis* to *trans*.[7,8] This phenomena has been attributed to similarities between the electronic excited state of the *trans* isomer and the thermal transition state for back-relaxation, that is, that both species have substantial dipolar character.[7,9] The dipolar nature of the transition state also results in strong solvent sensitivity of the half-life for thermal relaxation. For instance, the half-life of *cis*-4-diethylamino-4′-nitroazobenzene changes from 1 ms in DMSO to 100 s in cyclohexane.[9] Of course, photoswitches intended for use in a biological application must operate in water. In general, azobenzene photoswitches that absorb at long wavelengths in water relax very quickly back to the *trans* state so that only vanishingly small amounts of the *cis* isomer can be produced under low-power steady-state illumination.

Here we report the synthesis of a series of *ortho*-amino-substituted azobenzene derivatives in which long switching wavelengths are combined with relatively slow thermal relaxation rates and high *cis*-state yields. As a result, these molecules can be used as effective long wavelength photoswitches to drive conformational photocontrol in biochemical systems.

The 4,4′-diamido-substituted azobenzene unit (structure **5** with R = H) has served as a useful core in several studies in which switches were used to control conformational changes in peptides and proteins, as well as gating behavior in ion channels.[4,10] The *trans*-to-*cis* isomerization of this unit produces a change in mean end-to-end distance of about 4 Å that can lead to effective control of biomolecules linked through the 4,4′-amido arms. To preserve the conformational change associated with this unit, we introduced electron-donating amino substituents in the 2,2′-positions. Despite the very large number of azo dyes known, only a limited variety of *ortho*-amino-substituted azobenzenes has been previously reported.[11–13] The key step in the synthesis was the use of silver oxide in acetone for formation of the azo group (Scheme 1). A wide variety of other oxidizing agents and solvents was tried without success. The effectiveness of AgO and the requirement for acetone as solvent in this case indicate participation of a solvent-stabilized radical intermediate.[14]



Scheme 1. Synthesis of 4,4′-diacetamido azobenzenes bearing amino substituents in the 2,2′-positions.

Compared with the parent compound, 4,4′-diamido-substituted azobenzene ($\lambda_{max} = 370$ nm), these derivatives exhibit substantial redshifts (Figure 1). A number of factors are expected to affect the electron-donating ability of the 2,2′ substituents. The presence of a six-membered ring (**5c, 5e**, **5f**) introduces steric interactions that lead to loss of sp^2 character on the N atom, whereas a five-membered ring (**5d**), or no ring at all (**5a**, **5b**) produces enhanced N delocalization.[15,16] In addition, compounds **5e** and **5f** have heteroatoms in the 2,2′

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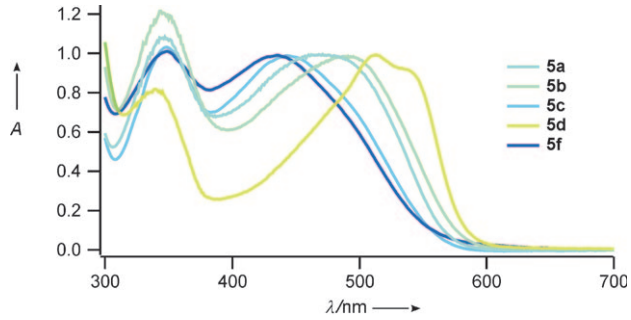

Figure 1. UV/Vis spectra of the *trans* isomers of **5a–f** in a mixed aqueous solvent (acetonitrile/water 7/3 with 1 mM sodium phosphate buffer, pH 7.0) in which all compounds are soluble.

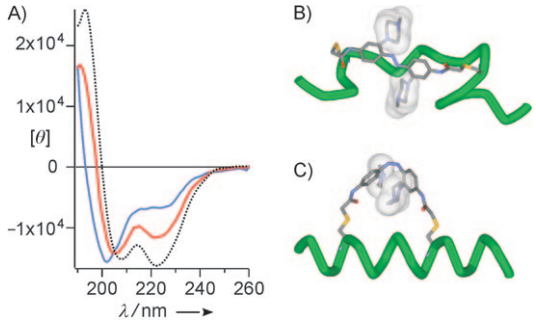

Figure 2. A) CD spectra of the JRK-W peptide intramolecularly cross-linked via Cys residues with the chloroacetyl derivative of **5f**. Dark-adapted (blue), irradiated (red), and calculated 100% *cis* spectra (dashed) indicate increased helix content on *trans*-to-*cis* isomerization. Models of the disordered peptide with a *trans* cross-linker (B) and the helical *cis*-linked species (C). The *ortho* substituents are shown in space-filling form.

substituents that are expected to decrease the basicity of the N atoms.[17] In general the expected relative electron-donating ability of the 2,2′ substituents correlates with the wavelength of maximal absorption; species with the most electron donating pyrrolidino substituents (**5d**) exhibit the longest wavelength absorption.

Despite the long wavelength absorption of the *trans* isomers, these compounds relax from *cis* to *trans* on time-scales of seconds to minutes, and substantial fractions of *cis* isomers can be produced by low-power LED illumination (Table 1). A previously reported photoswitch that absorbs in this wavelength range relaxes with a time constant of about 10 ms under comparable conditions.[18]

Table 1: Properties of photoswitches **5a–f**.

Photoswitch	λ_{max} [nm][a]	ε[a] [$M^{-1}cm^{-1}$]	$\tau_{1/2}$[a] [s]	$\tau_{1/2}$[b] [s]
5a	470	13900	3.3 ± 0.3	–
5b	488	13000	0.8 ± 0.1	–
5c	445	10180	6.0 ± 0.2	–
5d	513/537	–	0.7 ± 0.1	–
5e	435	9610	302 ± 4	8.1 ± 0.2
5f	437	10440	207 ± 10	27 ± 1

[a] In 70% acetonitrile/water, 1 mM sodium phosphate buffer, pH 7.0, 20°C. [b] In 10 mM sodium phosphate buffer, pH 7.0, 20°C.

There are a number of possible origins of the relatively slow thermal relaxation rates. Possibly, the *ortho* substituents selectively stabilize the *cis* isomer relative to the transition state for thermal relaxation as compared to analogous *para*-substituted analogues. Examining molecular models of *cis* isomers shows that the *ortho* substituents can pack closely around the azo group and may form a local hydrophobic cage that stabilizes the structure (Figure 2C). Such a local hydrophobic environment around the azo group would also tend to destabilize isomerization transition states that involve buildup of negative charge on the azo nitrogen atoms.[7] The *ortho* substituents may thus mimic the effects of solvents on relaxation rates. In addition, *ortho* substituents are expected to lead to twisting of the rings about the azo group so that resonance stabilization of dipolar transition states is decreased.[8]

Inclusion of polar groups (**5e**, **5f**) on the *ortho* substituents distal to their attachment points to the azo chromophore retains the wavelength and switching properties but greatly enhances water solubility. For this reason, we chose the *N*-methylpiperazino derivative **5f** for reaction with a test peptide in aqueous solution. The peptide JRK-W, a model helix-forming peptide that has been studied extensively,[19] was intramolecularly cross-linked with the chloroacetyl derivative of **5f**, as shown in Figure 2. As expected, based on previous work with the parent cross-linker[19] the *trans* isomer destabilized the JRK-W helix. Irradiation with a 400-nm LED produced about 50% *cis* isomer and a substantial increase in helix content as judged by circular dichroism spectrometry (Figure 2). Since thermal reversion generates about 100% *trans* isomer, production of 50% *cis* isomer under low-power irradiation constitutes a large change in the population of this *cis* isomer.[20] Attachment of the photoswitch to the peptide slowed the relaxation rate even further. The half-life for thermal relaxation of this photoswitchable peptide was about 40 min at 4°C, and about 5 min at 20°C.

A variety of pathways for the in vivo modification of azo compounds that could lead to loss of photoswitching are known.[21] The detailed structure and route of administration of an azo compound can determine whether it is metabolized quickly or not at all.[21] In general, the electron-rich nature of these photoswitches makes them less susceptible to reduction (e.g., by intracellular thiols) than azobenzene photoswitches studied previously.[6] Compound **5f** was incubated in 10 mM reduced glutathione/5 mM reduced DTT (dithiothreitol) for 24 h at 37°C, an environment more reducing than typically found in the eukaryotic cytosol,[22] and no change in UV/Vis absorption or switching kinetics was observed (see Supporting Information).

By combining long-wavelength switching with good photochemical yields and stabilities of the *cis* isomers in reducing aqueous environments, this series of azobenzene photoswitches thus significantly expands the possibilities for directed conformational control in biological settings.

Experimental Section

Compounds **3a–f**, **4a–f**, and **5a–f** were prepared as outlined in Scheme 1. Representative reaction procedures: **3f**: A solution of 3′-chloro-4′-nitroacetanilide (**2**; 5 g, 23 mmol) in 1-methylpiperazine (25 mL) was heated at 100 °C with stirring for 12 h and then diluted with water (150 mL). The solid was collected and used for the next step without additional purification. Yield: 5.24 g (81 %).

4f: Zn dust (3 g) was added to **3f** (1 g, 3.6 mmol) in 28 % ammonium hydroxide (100 mL) over 1 min. The mixture was stirred at room temperature (not higher than 35 °C) for 30 min, concentrated under reduced pressure to 30 % of its initial volume, and filtered. The aqueous solution was washed with chloroform, and the organic layer collected and dried with Na_2SO_4. The chloroform was evaporated to give the product **4f** (0.7 g, 78 %), which could be used directly in the next step.

5f: Freshly prepared, dry AgO (1.04 g, 8.4 mmol) was added to a solution of **4f** (0.7 g, 2.8 mmol) in dry acetone (25 mL) at room temperature with vigorous stirring. After stirring in the dark for 1 d, a fresh portion of AgO (1.04 g) was added, stirring was continued for another 1–4 d, and the presence of the initial amine was monitored by thin-layer chromatography. The solid was collected by filtration, washed with methanol, and the filtrates were combined and evaporated. The product was further purified by column chromatography on silica gel (MeOH/EtOAc 1/1; $R_f = 0.1$) to give 0.17 g (24 %) of a fine orange solid. Full experimental details of characterization of compounds **3a–f**, **4a–f**, and **5a–f** and the chloroacetyl derivative of **5f** can be found in the Supporting Information. Peptide synthesis and cross-linking were performed as described previously.[10] UV/Vis and CD spectra were obtained and analyzed as detailed in the Supporting Information.

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[1] C. Renner, L. Moroder, *ChemBioChem* **2006**, *7*, 868 – 878.
[2] M. Volgraf, P. Gorostiza, R. Numano, R. H. Kramer, E. Y. Isacoff, D. Trauner, *Nat. Chem. Biol.* **2006**, *2*, 47 – 52.
[3] M. Bose, D. Groff, J. Xie, E. Brustad, P. G. Schultz, *J. Am. Chem. Soc.* **2006**, *128*, 388 – 389.
[4] D. L. Fortin, M. R. Banghart, T. W. Dunn, K. Borges, D. A. Wagenaar, Q. Gaudry, M. H. Karakossian, T. S. Otis, W. B. Kristan, D. Trauner, R. H. Kramer, *Nat. Methods* **2008**, *5*, 331 – 338.
[5] R. H. Kramer, J. J. Chambers, D. Trauner, *Nat. Chem. Biol.* **2005**, *1*, 360 – 365.
[6] C. Boulègue, M. Loweneck, C. Renner, L. Moroder, *ChemBioChem* **2007**, *8*, 591 – 594.
[7] N. Nishimura, T. Tanaka, M. Asano, Y. Sueishi, *J. Chem. Soc. Perkin Trans. 2* **1986**, 1839 – 1845.
[8] N. Nishimura, T. Sueyoshi, H. Yamanaka, E. Imai, S. Yamamoto, S. Hasegawa, *Bull. Chem. Soc. Jpn.* **1976**, *49*, 1381 – 1387.
[9] K. S. Schanze, T. F. Mattox, D. G. Whitten, *J. Org. Chem.* **1983**, *48*, 2808 – 2813.
[10] D. C. Burns, F. Zhang, G. A. Woolley, *Nat. Protoc.* **2007**, *2*, 251 – 258.
[11] H. Bisle, H. Rau, *Chem. Phys. Lett.* **1975**, *31*, 264 – 266.
[12] H. Mustroph, *Dyes Pigm.* **1991**, *15*, 129 – 137.
[13] B. Ortiz, F. Walls, P. Villanue, *J. Org. Chem.* **1972**, *37*, 2748 – 2750.
[14] M. Hájek, P. Silhavy, J. Malek, *Tetrahedron Lett.* **1974**, *15*, 3193 – 3196.
[15] G. Hallas, R. Marsden, J. D. Hepworth, D. Mason, *J. Chem. Soc. Perkin Trans. 2* **1986**, 123 – 126.
[16] J. D. Hepworth, D. Mason, G. Hallas, R. Marsden, *Dyes Pigm.* **1985**, *6*, 389 – 396.
[17] H. Mustroph, *Dyes Pigm.* **1991**, *16*, 223 – 230.
[18] L. Chi, O. Sadovski, G. A. Woolley, *Bioconjugate Chem.* **2006**, *17*, 670 – 676.
[19] D. C. Burns, D. G. Flint, J. R. Kumita, H. J. Feldman, L. Serrano, Z. Zhang, O. S. Smart, G. A. Woolley, *Biochemistry* **2004**, *43*, 15329 – 15338.
[20] D. A. James, D. C. Burns, G. A. Woolley, *Protein Eng.* **2001**, *14*, 983 – 991.
[21] M. A. Brown, S. C. DeVito, *Crit. Rev. Environ. Sci. Technol.* **1993**, *23*, 249 – 324.
[22] H. R. Lopez-Mirabal, J. R. Winther, *Biochim. Biophys. Acta* **2008**, *1783*, 629 – 640.

