

Azobenzene Photoswitching without Ultraviolet Light

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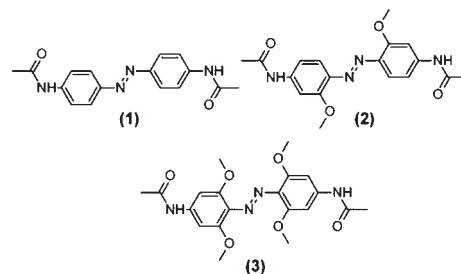
S Supporting Information

ABSTRACT: Most azobenzene-based photoswitches use UV light for photoisomerization. This can limit their application in biological systems, where UV light can trigger unwanted responses, including cellular apoptosis. We have found that substitution of all four ortho positions with methoxy groups in an amidoazobenzene derivative leads to a substantial (~ 35 nm) red shift of the $n-\pi^*$ band of the trans isomer, separating it from the cis $n-\pi^*$ transition. This red shift makes trans-to-cis photoswitching possible using green light (530–560 nm). The cis state is thermally stable with a half-life of ~ 2.4 days in the dark in aqueous solution. Reverse (cis-to-trans) photoswitching can be accomplished with blue light (460 nm), so bidirectional photoswitching between thermally stable isomers is possible without using UV light at all.

Azobenzene compounds have been used for optical control of conformation in diverse settings.^{1–4} In biological systems, optical manipulation of biomolecular structure is a powerful approach for deciphering cellular roles of biomolecules with high spatiotemporal resolution.^{5–13} At equilibrium in the dark, azobenzene exists in the more stable trans conformation. Irradiation with UV light produces a large fraction of the cis isomer, which can revert back to the trans state thermally or upon irradiation with blue light. Azobenzene has been employed as a photoswitch by coupling the photoisomerization event to a change in the structure/function of numerous biomolecular targets, including peptides, proteins, and nucleic acids in vitro and ion channels and receptors in vivo.^{3,14,15} Most of the azobenzene-modified biomolecules developed to date require the use of UV light for photoisomerization. This can limit the use of this molecular switch in vivo. For example, UV light is strongly scattered, making penetration of cells and tissues difficult.¹⁶ Furthermore, UV light can trigger apoptotic events or other responses, complicating studies of cells in culture and of model organisms.^{17–22} Azobenzene derivatives where photoisomerization can occur entirely in the visible region would therefore be desirable for in vivo applications. One approach for achieving long-wavelength switching is to couple azobenzene derivatives to upconverting nanophosphors.²³ The size of these particles, however, may cause interference with biological targets in vivo.

Azobenzene-based photoswitchable cross-linkers and tethered ligands with a 4,4'-diamidoazobenzene core (1) have been used extensively for the photocontrol of biomolecule structure and function.³ The incorporation of electron-donating groups ortho or para to the azo moiety can dramatically red-shift the photoswitching wavelength but also markedly increases the rate

Chart 1. Structures of the Azobenzene Derivatives Studied



of thermal cis-to-trans relaxation.^{24–30} A short-lived cis isomer means that an intense light source is required in order to maintain a substantial fraction of the cis isomer, an undesirable limitation in vivo. Also, while blue-absorbing 2,2'-dimethylaminoazobenzenes were found to undergo thousands of cycles of photoswitching, green-absorbing 2,2'-pyrrolidino derivatives showed photobleaching effects that limited the number of cycles to <100 .²⁹ The mechanism of this photobleaching process is unknown but may involve participation of the *o*-amino group in a photo-oxidation process.^{31,32} To retain the longer cis half-lives observed with ortho substitution and avoid photobleaching, we explored the effect of a different type of electron-donating group: azobenzene switches bearing *o*-methoxy groups on the 4,4'-diacetamidoazobenzene scaffold were synthesized (Chart 1).³³

While the $\pi-\pi^*$ band of *trans*-2,2'-dimethoxy-4,4'-diacetamidoazobenzene (2) showed the expected red shift [see the Supporting Information (SI)], the $\pi-\pi^*$ band of (3) was significantly blue-shifted and weaker ($\lambda_{\max} \approx 338$ nm, $\epsilon = 16\,170$ M⁻¹ cm⁻¹) and the $n-\pi^*$ band significantly red-shifted and stronger (480 nm, ~ 4030 M⁻¹ cm⁻¹) relative to those of the parent compound (1) in dimethyl sulfoxide (DMSO) at 25 °C (Figure 1; also see the SI). In retrospect, this behavior may have been partially predicted on the basis of studies reported by Bunce, Zerner, and colleagues³⁴ and Bisle and Rau³⁵ on azobenzene derivatives bearing alkyl substituents at all four ortho positions where (smaller) blue shifts of $\pi-\pi^*$ bands and red-shifted $n-\pi^*$ bands were also seen (see below).

The spectrum of *cis*-(3) in DMSO was then determined by producing a mixture of cis and trans isomers by UV irradiation and determining the cis content by quantitative NMR methods (see the SI). Remarkably, the spectrum of the cis isomer showed a large (36 nm) blue shift of the $n-\pi^*$ band relative to the trans isomer and an obvious color change (Figure 1). For most azobenzenes, including the parent compound, the trans and cis

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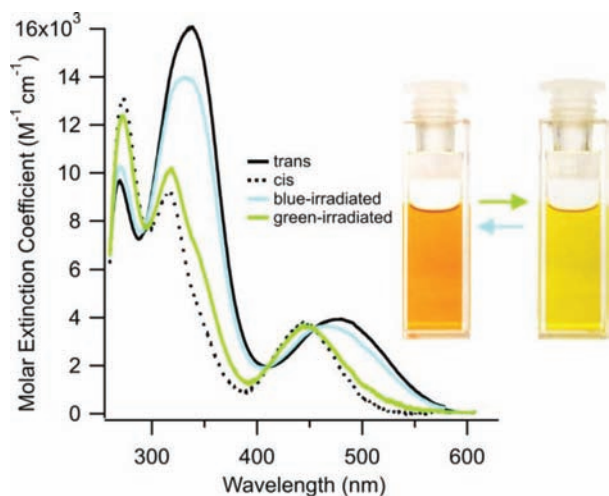


Figure 1. Photoisomerization of (3) measured in DMSO at 25 °C.

isomers have $n-\pi^*$ bands at similar wavelengths.³⁶ To our knowledge, an $n-\pi^*$ shift larger than this has been seen only with the bridged azobenzene derivative recently reported by Siewertsen et al.,³⁶ where the $n-\pi^*$ band of the trans isomer occurs at 490 nm and the cis isomer at 404 nm (see below). The shifts in the $n-\pi^*$ transitions between cis and trans isomers in the tetra-*o*-alkyl-substituted azo derivatives studied by Bunce, Zerner and colleagues³⁴ were substantially smaller (4–9 nm). This observation of a separation of the trans and cis $n-\pi^*$ bands in (3) indicated that trans-to-cis isomerization should be possible not only with UV light (excitation in the trans $\pi-\pi^*$ band) but also with green light (excitation in the trans $n-\pi^*$ band), a wavelength that would normally lead to photoisomerization in the opposite direction. Indeed, irradiation of *trans*-(3) with green light (530–560 nm) was found to produce a mixture with an ~80% fraction of the cis isomer (Figure 1), which is comparable to the fraction produced using UV light with the parent 4,4'-diamidoazobenzene (1). Photochemical cis-to-trans isomerization of (3) could be then induced by excitation of the cis $n-\pi^*$ band using blue light. Irradiation with a 450–460 nm light-emitting diode resulted in a large fraction of the trans isomer (~85%) (Figure 1). Similar photoswitching wavelengths and isomer yields were also observed in other nonpolar solvents such as acetonitrile, dioxane, and dichloromethane (see the SI).

Since biological applications require the use of the photo-switch in aqueous solution, the photoswitching properties were also determined in 25 mM phosphate buffer (pH 7.0). While the parent compound (1) and the 2,2'-derivative (2) are only sparingly soluble in water, (3) is very soluble. Interestingly, in aqueous solutions, the position of the $\pi-\pi^*$ band of *trans*-(3) was found to be affected by temperature. At higher temperatures, the $\pi-\pi^*$ band was blue-shifted relative to that of (1), as in DMSO. Conversely, lowering the temperature to 4 °C caused a red shift ($\lambda_{\max} \approx 380$ nm) and a hyperchromic effect. The spectrum of the cis isomer did not appear to be significantly affected by temperature; as a result, there was a smaller separation of the trans and cis $n-\pi^*$ bands in aqueous solution. Nevertheless, irradiation with green wavelengths (530–560 nm) led to production of a large fraction of the cis isomer (~70%) that could be switched back to the trans form with blue light (~80%) at 25 °C (see the SI).

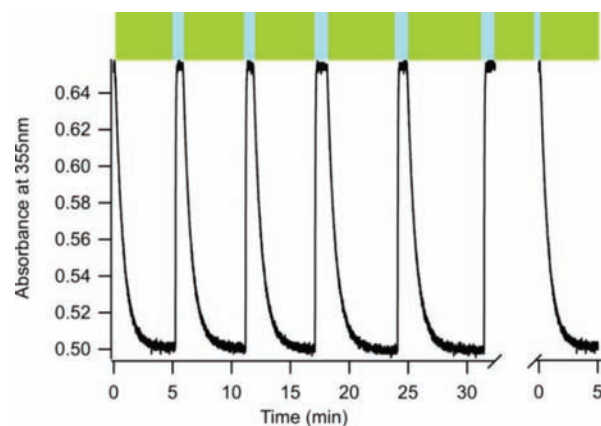


Figure 2. Multiple rounds of photoswitching of (3) with alternating green (530 nm) and blue (450–460 nm) light in aqueous solution at 25 °C. The gap on the time axis corresponds to 1 h of continuous high-intensity (70 mW/cm²) irradiation.

Thermal cis-to-trans isomerization was followed by acquiring UV–vis spectra as a function of time using a light source too weak to cause significant photoisomerization. Half-lives are collected in Table S1 in the SI. Notably, the half-life of (3) is substantially longer than that of the parent compound (1) (53 h vs ~12 min in aqueous solution³⁷). Decreasing the solvent polarity further extended the lifetime of the cis isomer, an effect that has been attributed to a dipolar transition state.^{24,28} A half-life of 2.4 days means that both the cis and trans isomers can be rapidly photogenerated and maintained in the dark for the duration of most biological process where spatiotemporal control is of interest.

The pH dependence of the UV–vis absorbance spectra in water was also investigated. The apparent pK_a (~3.8) for the protonated (3) species was higher than the pK_a for the unsubstituted compound (1) (see the SI), but the spectra were essentially unaffected by pH above pH 5.0. Thus, protonation of the photoswitch should not be a problem for biomolecular targets operating near pH 7.

Multiple rounds of photoisomerization, and even constant high-intensity irradiation with green light (70 mW/cm²) for ~1 h, gave no evidence of photobleaching or photo-oxidation (Figure 2). Conversely, (3) was found to be more sensitive to reduction than the parent compound. Whereas (1) is stable for extended periods in 1 mM reduced glutathione,³⁸ (3) was bleached with a half-life of ~1.5 h (see the SI). This may limit the application of (3) to more oxidizing environments in vivo.³⁹

In an effort to understand the structural basis for the spectroscopic behavior of (3), we carried out computational modeling of the compound in its cis and trans forms. We employed density functional theory (DFT) using the B3LYP hybrid functional⁴⁰ and the 6-31G* basis set since this method successfully reproduces the relative energies of the isomers as well as the spectra of many azobenzene derivatives, including the parent compound and bridged azobenzenes.^{41–44} A conformational search was carried out as described in the SI; the lowest-energy conformers for *trans*- and *cis*-(3) are shown in Figure 3. The trans isomer is highly twisted in comparison with the parent compound (1), which exhibits a nearly planar geometry (see the SI). The 2,2'-dimethoxy species (2) can also adopt a planar structure by having the substituents on opposite sides of the azo moiety

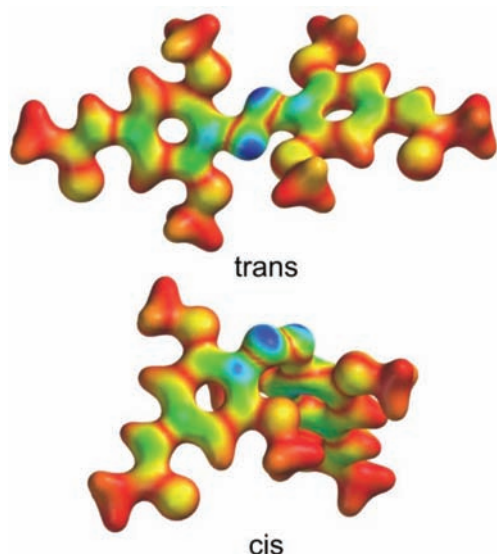


Figure 3. Calculated structures of *trans*- and *cis*-(**3**). The absolute value of the HOMO of each isomer is mapped onto the bond density surface (blue is the maximum value of the HOMO).

(see the SI). This arrangement is seen in X-ray crystal structures of related 2,2'-alkoxy-substituted azobenzenes.^{45,46} The calculated energies of the isomers of (**3**) were sensitive to the incorporation of a solvation term. In water and DMSO, the *trans* isomer was calculated to be 8–9 kcal/mol more stable than the *cis* form. This is consistent with the *cis* isomer being undetectable by NMR spectroscopy in a dark-adapted sample of *trans*-(**3**) (see the SI).

Time-dependent DFT calculations reproduced the blue shift of the π – π^* band and the red shift of the n – π^* band of *trans*-(**3**) relative to the parent compound (**1**) (see the SI). Moreover, the large separation of the n – π^* bands of the *trans* and *cis* isomers was also predicted, with the *trans* isomer having $\lambda_{\text{max}} = 520$ nm (slightly higher energy conformers had $\lambda_{\text{max}} = 505$ – 515 nm) and the *cis* isomer having λ_{max} near 460 nm (a separation of 40–60 nm; the experimental separation was 36 nm).

A possible origin of this separation of the *trans* and *cis* n – π^* bands, originally suggested by Bunce and Zerner,³⁴ can be appreciated by comparing the absolute value of the highest occupied molecular orbital (HOMO) of each isomer mapped onto the bond density surface (Figure 3). The HOMO is centered on the lone pairs of the azo N atoms in each case. However, for the *trans* isomer, the HOMO is close to the electron-rich oxygen atoms of the methoxy groups, which may be expected to raise its relative energy. Isomerization to the *cis* form relieves this interaction, and the azo N atom lone pairs are exposed to solvent in the same manner as in the parent compound. Indeed, the n – π^* transition of the *cis* isomer is similar in energy to that of the parent compound.

As noted above, Siewertsen et al.³⁶ reported a bridged azobenzene derivative in which a large separation of n – π^* bands was also observed. In that case, the n – π^* transition of the *trans* isomer was red-shifted, but the n – π^* transition of the *cis* isomer was also significantly blue-shifted relative to unmodified azobenzene (the corresponding parent compound), indicating that the origin of the n – π^* separation is different. Moreover, in that case, the *cis* isomer was the more stable isomer by 6–7 kcal/mol. In both the bridged azobenzene case reported by Siewertsen and the tetra-*o*-methoxy case described here, the large separation of

the *cis* and *trans* n – π^* bands makes possible efficient switching using n – π^* excitation. While for the bridged azobenzene near-UV light at 385 nm was still required for optimal *cis*-to-*trans* switching and green light (520 nm) for *trans*-to-*cis*, in the present work, blue light (460 nm) and green light (530–560 nm) are optimal for *cis*-to-*trans* and *trans*-to-*cis* switching, respectively. In the bridged azobenzene case, however, the magnitude of the n – π^* separation is larger, so more complete switching is possible. Although incomplete photoconversion of *cis* and *trans* isomers can enable photocontrol of biomolecule function in specific cases,^{30,47} more complete switching is generally preferable. By increasing the separation of the n – π^* bands, more complete switching should be possible. A mechanism of selective destabilization of the *trans*-isomer n – π^* HOMO by ortho substituents suggests an approach to the design of azobenzene derivatives with even better (i.e., more complete) switching behavior that do not require the use of UV light. Further manipulation of the ring substituents may also permit the sensitivity to reduction by glutathione to be mitigated.³⁸

The synthesis of (**3**) is straightforward, and derivatives could be readily linked to other molecules via the *p*-amido substituents. The solvent dependence observed may cause the *cis*/*trans* ratio at the photostationary state to be dependent on the local environment of the target molecule incorporation site, although effective switching is still possible under fully hydrated conditions. Slow thermal relaxation means only brief pulses of low-intensity light can be used for photoswitching in either direction. In this manner, a biological target could be maintained in either an “off” or “on” state as required and could be interconverted with brief light pulses. Related tetra-*o*-methoxy-substituted derivatives could also find applications in materials science and supramolecular chemistry, where reversible conformational control using visible light is desired.²³

■ ASSOCIATED CONTENT

S Supporting Information. Synthetic procedures and UV–vis spectra in other solvents; pH and temperature dependence of the spectra, and calculated structures and spectra of compounds (**1**)–(**3**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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