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# Azo compounds with different sized fluorophores and characterization of their photophysical properties based on *trans* to *cis* conformational change



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## ABSTRACT

We synthesized fluorophores of different sizes attached to azo derivatives, such as thiophene-, naphthalene-, anthracene-, and pyrene-based azo compounds, and studied their photophysical properties. The compounds exhibited strong color and intensity changes in absorption and emission during the structural change from *trans* to *cis*, which also resulted in a large Stokes shift and a difference in quantum yield. Depending on their size, the compounds had different rates of transformation from the *trans* to *cis* structure: less conjugated (smaller) fluorophore azo compounds showed higher transition rates than more conjugated (larger) ones.

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#### Introduction

Photochromic compounds have intriguing spectral properties with many potential applications in photo-responsive materials,<sup>1</sup> non-linear optics,<sup>2</sup> and many areas of biophysics.<sup>3</sup> Azobenzene derivatives, typical photochromic compounds, are very sensitive to light, which induces their structural transition from trans to cis and vice versa.<sup>4</sup> Thus, many research groups have used these compounds for diverse applications including the development of dyes,<sup>5</sup> photochromic surface modification,<sup>6</sup> supramolecular switch system,<sup>7</sup> and molecular machines.<sup>8</sup> Important in biological applications, simple, thermally stable amidoazobenzene derivatives have been developed that undergo a reversible photo-switching from trans to cis without ultraviolet light, which may be harmful to biological systems.<sup>9</sup> They also have developed a more efficient, reversible photo-switching system by optimization of the substitution pattern on the azobenzene derivative.<sup>10</sup> In this respect, adding specifically designed fluorescent properties to the azobenzene compound would enable more diverse applications.

Fluorescent molecules are particularly useful in the recognition of target molecules, such as proteins and enzymes, in the biological systems. They are also highly sensitive to changes in the environment and allow higher resolution than absorption spectroscopy.<sup>11</sup>

Even though azo compounds themselves exhibit some changes in absorption, they are not strong and sensitive enough for detection in complex environments compared to the fluorescence signals.<sup>12</sup> Dramatic changes in fluorescence are important in many diagnostic, molecular imaging, and biophysics areas.<sup>13</sup> Thus, our goal was to develop fluorescent azo compounds that show a strong emission change upon a reversible structural change induced by light. Producing such environment-sensitive fluorescent azo compounds would have diverse applications in many areas, including biology and nanotechnology research.

To develop compounds with efficient fluorescence in the azobenzene system, we screened aromatic molecules of diverse sizes, such as thiophene, naphthalene, anthracene, and pyrene (Scheme 1). Pyrene is the most useful and powerful fluorescent compound that shows a blue emission in the monomeric state, but when close together with another pyrene molecule, a very strong excimer signal is emitted that is resistant to photobleaching.<sup>14</sup> To confer diverse fluorescence properties to azobenzene, we attached anthracene and naphthalene, which, like pyrene, are polyaromatic compounds, only smaller. As another type of a small heteroaromatic group, we chose a thiophene derivative that is commonly used as an electrically induced compound for making conducting and semiconducting materials.<sup>15</sup> We then systematically studied the fluorescence properties of the resulting compounds of different sizes. Here, we report the synthesis of four different fluorescent azobenzene compounds and their physical properties. These new compounds exhibit



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Scheme 1. Designed fluorescent azobenzene compounds.

dramatic changes in absorption and emission, both in color and intensity, upon the conformational change from *trans* to *cis*.

We first synthesized an iodo-azobenzene compound (TA1) as a scaffold material by following a previously published procedure for azobenzene synthesis.<sup>16</sup> Then, we attached different aromatic compounds using Sonogashira coupling<sup>17</sup> to pyrene azobenzene compound (TA5) and Suzuki coupling<sup>18</sup> to thiophene (TA2), naphthalene (TA3), and anthracene azobenzene (TA4) compounds. We confirmed all structures using <sup>1</sup>H NMR; however, we were unable to discriminate between the trans and cis structures using general proton NMR. We scrutinized different solvents from nonpolar to polar with the pyrene-attached azobenzene compound TA5 as a model system (Fig. S1). Four different solvents-toluene, dichloromethane, dimethyl sulfoxide [DMSO], and ethanol-were chosen, and **TA5** at 1 µM was measured to determine the photophysical properties of TA5 depending on the solvent polarity. At the concentration used, the absorption obeyed the Lambert-Beer's law, suggesting that no dimerization or oligomerization occurred within this concentration range.<sup>19</sup>

Using UV light at 350 nm, we induced the conformational change from *trans* to *cis*, which resulted in a hypsochromic shift with decreasing absorption intensity (Fig. S1). Among the solvents tested, DMSO showed the highest differences in the photophysical properties induced by the *trans* to *cis* transition of **TA5**. Thus, we choose DMSO as a solvent for all of our fluorescent azobenzene compounds.

Next, we measured the absorption for all of the fluorescent azobenzene compounds. In trans conformation compared with iodoazobenzene (TA1), all other fluorescent compounds-thiophene (TA2), naphthalene (TA3), anthracene (TA4), and pyrene (TA5)-exhibited a strong, red shift absorption band pattern; the strength of the red shift depended on the size: thiophene < naphthalene < anthracene < pyrene (Fig. 1). Incorporation of the electron donating groups capable of extending the pi-conjugation in the para position of the azobenzene moiety can produce a different conjugation state and may induce a dramatic red shift of the absorbance wavelength.<sup>20</sup> Compared to **TA1** with iodine in this position, the fluorophores in **TA2** through **TA5** are more  $\pi$ -conjugated because of the attachment of an aromatic group, where the  $\pi$ -electron donating groups in TA2-**TA5** provide an electron to the structured  $\pi$ - $\pi$ \* electronic transition state, which induces a red shift. In Figure 1, all compounds exhibited the  $\lambda_{max}$  of the absorption band between the 380 nm and 420 nm (TA1, 383 nm; TA2, 399 nm; TA3, 392 nm; TA4, 405 nm; TA5, 408 nm). Due to overlap, the characteristic band of the general azo



**Figure 1.** Absorption spectra of fluorescent azo compounds in *trans* conformation (**TA1** to **TA5**). All fluorescent azobenzene compounds were prepared at a concentration of 1  $\mu$ M in DMSO at 20 °C.

compound  $n-\pi^*$  (400–500 nm) was not distinguishable from the  $\pi-\pi^*$  electronic transition band.

Further, we studied how the absorption properties depend on the structural transition from *trans* to *cis*. Upon transition to the *cis* form, the absorbance pattern of each fluorescent azo compound changed dramatically.

**TA2** showed a very distinct  $\pi$ - $\pi^*$  absorption band ( $\lambda_{max}$ , 399 nm); in **CA2**, the  $\pi$ - $\pi^*$  band almost disappeared, while a new blue-shifted band appeared at 310 nm (Fig. 2a). When **TA3** and **TA4** compounds changed to **CA3** and **CA4**, a marked decrease in the bands at 392 nm and 405 nm was observed, respectively, and a new band at 320 nm appeared, which was similar to that of the **TA2** compound (Fig. 2b & c, respectively). However, when **TA5** 



Figure 2. Absorption spectra of fluorescent azo compounds: (a) TA2, (b) TA3, (c) TA4, and (d) TA5 before and after *trans* to *cis* transition (black and green lines, respectively) at 1  $\mu$ M concentration in DMSO at 20 °C. All samples were irradiated using 350 nm light source.

was transformed to **CA5**, a dramatic blue shift in  $\lambda_{max}$  from 420 nm to 377 nm was observed without the notable change in intensity and with a new band appearing at 290 nm (Fig. 2d), possibly caused by a change in the electron transition state as seen in the crystal structure of a *trans* and *cis* azo compound.<sup>2a,21</sup> According to the X-ray and computational data, trans azo compounds adopt a planar structure, while cis azo compounds exhibit a non-planar conformation,<sup>22</sup> severely distorting the structure and inducing a low  $\pi$ -delocalization relative to planar arrangement. In the *trans* azo conformation, the planar structure of the attached aromatic ring with respect to the azo benzene ring supports the preferred delocalization of the  $\pi$ -electrons, and thus all compounds exhibit intense  $\pi$ -conjugate band at the longer wavelength (Fig. 2). In the cis conformation, TA2, TA3, and TA4 are expected to experience a significant torsional strain due to the proximity of the aromatic and azobenzene rings, and each compound exhibited a featureless absorption band, possibly owing to some vibrational couplings. However, in the case of cis-TA5, the pyrene ring is attached through acetylene, which may provide enough distance resulting in less steric hindrance with the azobenzene ring. The pyrene ring can readily conjugate with the azobenzene ring through the acetylene link, and the conjugated structure would absorb at a longer wavelength.

The absorption change was accompanied by a distinct change in color from yellowish-orange to colorless in all fluorophore azo compounds. The most pronounced color change was observed for transitions of **TA2**, **TA3**, and **TA4** to **CA2**, **CA3**, and **CA4**, respectively. Such strong visual signal change may be useful for diverse applications that depend on structural changes.

Next, we measured fluorescence spectra to determine the emission properties of each compound (**TA2–TA5**) (Fig. S2). We choose the  $\lambda_{max}$  of the absorption band as the excitation wavelength. In the *trans* structure, **TA2** and **TA3** showed an emission  $\lambda_{max}$  around 470 nm and 460 nm, respectively, with corresponding large Stokes shifts of around 80 nm and 60 nm, respectively. **TA4** and **TA5** showed  $\lambda_{max}$  around 520 nm and 510 nm, respectively, with a very large Stokes shift of around 134 nm for **TA4** and 124 nm for **TA5** with a pyrene exciplex signal. These large Stokes shifts might originate from  $\pi$  electron transfer to the  $\pi$ - $\pi$ \*. As expected, the **TA4** and **TA5** and **TA3** due to more  $\pi$ -electron conjugation. All trends in the changes of emission bands are consistent with those for the absorption bands.

Next, we measured the emission spectra during the trans to cis transition induced by irradiation of the solution at 350 nm (Fig. 3). The *trans*-isomers, which have a planar structure, exhibited a very weak fluorescence because of photoisomerization mechanism of azo dyes, where the  $\pi$ - $\pi$ <sup>\*</sup> excited single state readily transfers the excited state electron to that of  $n-\pi^*$  triplet state, thereby the azo dyes are weakly emissive. However, in the case of cis-isomers, which have a distorted structure that is expected to inhibit the intramolecular charge transfer, exhibited strong fluorescence.<sup>22,2</sup> Interestingly, the TA3 compound showed a dramatic emission change upon this transition: the emission signal of the cis structure was almost 30-fold stronger than that of the trans structure, and was characterized by a blue shift in  $\lambda_{max}$  (446 nm to 430 nm). The **TA2** compound exhibited a change in the emission pattern similar to that of **TA3**. but with a smaller increase in the band at 470 nm. **TA5** exhibited an even more dramatic change in the emission pattern upon transition from trans to cis: the emission peak color changed from greenish ( $\lambda_{max}$ , 510 nm) to a stronger blue peak ( $\lambda_{max}$ , 435 nm). The latter may result from the interference of electron transfer from pyrene to the azo group, which was more characteristic for a pyrene monomer signal. In addition, the anthracene compound (TA4) showed a similar pattern of green to blue shift during the trans to cis transition.



**Figure 3.** Fluorescence spectra of fluorescent azo compounds: (A) **TA2**, (B) **TA3**, (C) **TA4**, and (D) **TA5** before and after *trans* to *cis* transition (black and green lines, respectively) at 1  $\mu$ M concentration in DMSO at 20 °C; All samples were irradiated using 350 nm light source.

We also measured the fluorescence quantum yield for all fluorophore compounds using 9,10-diphenylanthracene as a reference compound (Table 1). Most of the *trans* fluorescent azobenzene compounds showed low quantum yield, while corresponding *cis* compounds had a relatively high quantum yield (2.4, 8.7, 42.0, and 48.9 fold increase in CA2, CA3, CA4, and CA5, respectively. In the *trans* conformation, the fluorescent excited state of the chromophore moiety is strongly quenched by the azobenzene unit which is a well known property of azobenzene with intramolecular charge transfer. In the *cis* conformation, the distorted structure causes low intramolecular charge transfer of  $\pi$ -electrons, resulting in recovered fluorescence. This large change in the quantum yield depending on the conformational change may be useful for diverse material and biological applications.

Finally, we measured reversible photoisomerization of each compound. While irradiation of the *trans* compounds at the wavelengths of the  $\pi$ - $\pi$ \* absorption bands induced a transition to *cis*, irradiation at the wavelengths of the n- $\pi$ \* absorption bands induced the *trans* structure as a major component. In the simple **TA1** compound, *trans* to *cis* transition was reversible (Fig. S3): irradiation at 350 nm induced transition from *trans* to *cis*, while irradiation at 420 nm induced transition from *cis* to *trans*. Interestingly, however, all other compounds (**TA2-TA5**) did not show the reverse *cis* to *trans* transition. We could not detect the n- $\pi$ \* absorption bands because

Table 1				
Quantum yield and ph	notophysical data	of TA2 to TA5	and CA2 to C	A5 compounds

Compound	Absorbance $(\lambda_{max})$ (nm)	Emission $(\lambda_{max})$ (nm)	Quantum yield (QY) <sup>a</sup>
TA2	399	477	0.010
TA3	392	446	0.0035
TA4	405	520	0.00039
TA5	408	510	0.00056
CA2	321	470	0.0238
CA3	312	430	0.0305
CA4	324	467	0.0164
CA5	386	435	0.0274

 $^a$  Emission quantum yield was measured in 1  $\mu M$  solution of respective compounds in DMSO at different  $\lambda_{max}$  wavelengths for each compound.

of their overlap with the  $\pi$ - $\pi^*$  absorption bands. We tried to achieve the *cis* to *trans* transition using general n- $\pi^*$  irradiation light at 420 nm, but this approach was not successful even after a long 6 h exposure. Thus, we tried other stimuli, such as heating to 90 °C for 6 h, pH change from basic to acidic, different solvents including acetonitrile, different wavelengths 254, 300, and 450 nm, and long incubation in the dark. However, none of these conditions induced the structural change from *cis* to *trans*. At present, there is no explanation of the non-reversibility of the *cis* to *trans* transition but we are trying to understand and clarify of this phenomenon in our fluorescent azobenzene compounds.

Another interesting phenomenon was that the transition rate from *trans* to *cis* depended on the size of the attached aromatic group. **TA2** showed the fastest transition. The transition rate decreased as follows: **TA2** > **TA3** > **TA4** > **TA5** (Fig. S4). The pyrene-based azo compound **TA5** showed the slowest transition relative to others and thiophene **TA2** showed the fastest transition. These differences may be explained by the conjugation theory: more conjugated compounds with higher aromaticity have a more stable structure and therefore a slower structural change from *trans* to *cis*.

## Conclusions

We synthesized and characterized spectroscopically four azobenzene derivatives with different size fluorophores attached, namely thiophene (TA2), naphthalene (TA3), anthracene (TA4), and pyrene (TA5). Upon induction with UV light, all compounds demonstrated significant changes in spectroscopic properties, both absorbance and fluorescence, due to the conformational change from trans to cis. All of them had superior spectroscopic properties in comparison with the original iodo azobenzene precursor compound (TA1) and showed strong differences in absorption between the trans and cis forms, accompanied with a strong color change from yellowish-orange to colorless. In the fluorescence spectra, TA3 exhibited signal quenching in the trans form, however the emission signal was almost 30 times stronger in the cis form. TA5 also exhibited a strong emission change from a quenched red-shifted band in the trans form to strong blue-shifted emission in the *cis* form. The *trans* to *cis* transition rate was depended on the size of the attached fluorophores. However, the reverse photoisomerization was not achieved with the fluorescent azobenzene derivatives under various conditions. Only the iodo azobenzene compound TA1 exhibited reversible photoisomerization. Even though the mechanism behind these observations is as yet unclear, the dramatic changes in absorption and fluorescence of these new compounds upon conformational changes induced by UV light may be useful in diverse applications that need a strong signal change with unidirectional structural change from trans to cis.

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## Supplementary data

Supplementary data (experimental procedures for the synthesis of the fluorescent azo derivatives, UV absorption, and fluorescence spectra for each compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet. 2014.07.120.

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