Push-pull azobenzene chromophores with negative halochromism

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ARTICLE INFO

ABSTRACT

This work describes the synthesis and properties of monoazobenzene compounds carrying multiple electron-donating and electron-withdrawing substituents. The donors are methoxy and dialkylamine groups. The acceptors are cyano and nitro groups. The position of these groups has a strong influence on the absorption spectrum. When the nitro and cyano groups are located at the *ortho* and *para* positions to the azo bond, respectively, then the absorption maximum (λ_{max}) can be found at 484 nm. However, switching this arrangement leads to a 57 nm red-shift ($\lambda_{max} = 541$ nm). This shift can be enhanced further ($\lambda_{max} = 584$ and 604 nm) by moving one of the methoxy groups from the *ortho* to the *meta* position and by encompassing the nitrogen atom in a five-membered ring. Interestingly, under acidic conditions, a reversible blue-shift (negative halochromism) is observed.

1. Introduction

Azobenzenes are a class of organic compounds that are useful in a variety of disciplines [1–7]. One particular avenue is their sensitivity towards chemical and biological reducing agents. When exposed to such reagents, the azo bond cleaves into two aniline fragments. This dissociation concept has given rise to linkers useful in proteomics and imaging applications and led to the development of sulfa drugs [8,9]. We have harnessed this cleavage process to assemble and disassemble polymer nanoparticles and design colon-specific imaging agents [10–14]. During this work, we became aware that generally the azo bond scission is a relatively sluggish process that either requires a high concentration of the reductant or long reaction times. In proteomics applications, for example, these parameters can result in side reactions and background signals. This led us to examine simple molecular designs in which electron-withdrawing and electron-donating substituents were placed on the azobenzene scaffold. We observed that in a donor-acceptor arrangement, an increase in the number of donor substituents led to an enhancement of the cleavage rate. Due to the rapidity of the cleavage reaction, we called the structures 'hypersensitive azobenzenes' [15,16]. In these structures only one acceptor group (CO_2CH_3, CF_3, CN, or NO_2) was used. Therefore, a logical next question was: what will happen when we increase the number of electron-withdrawing groups in the system. In considering monoazobenzene scaffolds with multiple donors and acceptors, the study from Mustroph and Gussmann is particularly noteworthy [17]. This study utilized 2- and 3-methoxy-substituted-4-*N*,

N-diethylaminoazobenzenes and showed that in such chromophores, an optimum bathochromic shift could be obtained when the strength of the electron withdrawing groups increases in the diazo component of the molecule. They employed a combination of cyano and nitro groups to achieve a maximum red shift. Hallas and coworkers pursued a slightly different line of investigation [18]. They considered cyclic analogues of the 4-dialkylamino group in the monoazo dyes. This study showed that the lone pair of the nitrogen atom interacts most efficiently with the rest of the aromatic system when incorporated into a five-membered ring. Inspired by these studies, 6 compounds carrying two electron-withdrawing groups (cyano and nitro) were prepared. Among these, one compound carried the amine donor in a five-membered ring. This study produced unexpected red and blue-shift trends in the absorption spectra of the azobenzene nucleus as described in the next section along with their sensitivities towards chemical and biological reducing agents.

2. Results and discussion

The synthesis is practical and begins with copper-catalyzed amination of the commercially available iodobenzenes with ethylethanolamine to afford the multi-donor fragments in 78–81% yield (Scheme 1). In the second step, these fragments are combined with electron-deficient anilines possessing cyano (CN) and nitro (NO_2) groups through a diazotization reaction [19,20]. The isolated yields for this step range from 60 to 68%. In this way, the azobenzenes **1–3** can be prepared in two
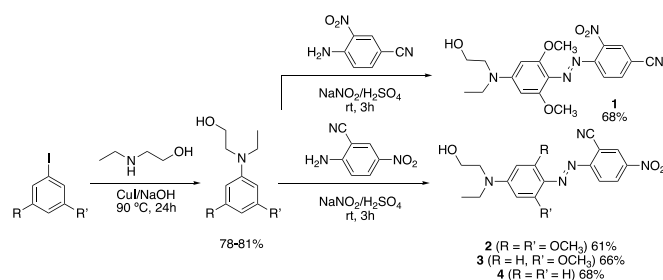
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Scheme 1. Synthesis of **1**–**4**.

synthetic steps beginning with commercially available starting materials (Figs. S1-S6). Compound **4** [21] with only one donor group could be prepared in one synthetic step from commercially available *N*-ethyl-anilinoethanol in 68% isolated yield (Figs. S7-S8). The hydroxyl group was included in the molecular structure to aid water-solubility of the compounds. Despite this, use of dimethylsulfoxide (DMSO) was necessary as a co-solvent to prepare aqueous solutions for UV–Vis spectroscopy.

The UV–Vis absorbance spectra revealed many interesting features (Fig. 1). Compound **1** having the nitro group at the *ortho* and the cyano group at the *para* positions to the azo bond absorbed at 484 nm. However, compound **2** in which their positions were switched showed a red-shift of 57 nm. This meant that the electron withdrawing capabilities of the cyano and the nitro groups were optimum at the *ortho* and *para* positions, respectively. Interestingly, however, compound **3** with only one methoxy group showed the maximum shift of 71 nm. Compound **4** with no methoxy group showed a shift of 58 nm.

The fact that compound **3** exhibited the maximum red-shift indicated that the second methoxy group was not required at the *ortho* position. Therefore, inspired by the studies of Woolley and coworkers [22], compound **5** and **6** were prepared (Scheme 2 and Figs. S9-S16). In these compounds one of the methoxy group is located at the *meta* position to the azo bond. The difference between **5** and **6** is that in **6** the amine donor is part of a five-membered ring. From Hallas studies [18], a red-shift is expected with the five-membered cyclic donor. Indeed, the λ_{max} in compounds **5** and **6** are located at 584 and 604 nm, respectively. These results indicated that by properly placing the acceptors and the donors, red absorbing azo compounds could be successfully accessed

with synthetic ease. In considering azo compounds with a blue appearance (absorption in the red region), our design differs from Wooley's design [22] in terms of structural symmetry and from commercial azo blue/trypan blue in terms of compactness of the structure and underlines the importance of multiple donor/acceptors moieties and their relative arrangement on the aromatic scaffold.

To further red-shift the absorption spectrum, we hypothesized that protonation of the azo bond under acidic conditions would be a useful strategy [23]. However, we observed that the addition of a drop of hydrochloric acid (solution pH = 1–2) shifted the absorption spectrum to the blue region (Fig. 1). The shift could be reversed by neutralization of the acid in the solution. This meant that we were observing a rare negative halochromism property in the monoazobenzene scaffolds [24]. The extent of the blue-shift depended upon the molecular structure. Compound **1** exhibited a minimum shift of 18 nm while compound **6** displayed a maximum shift of 114 nm.

Next, changes in the absorption spectra as a function of pH was studied with the help of buffer solutions (Fig. 2 and Figs. S17-S21). The pH was varied from near neutral (7.5) to acidic (2.5). This study produced complex results. For example, compounds **1** and **2** displayed a complete transition to the shorter wavelengths. However, in the case of **1**, the transition was very fast and a change of 1 pH unit (7.5–6.5) was enough to completely shift the spectra. Compound **2** had a gradual change and each unit of the pH change produced a significant change in the absorption spectrum and a complete shift was observed only at pH 4. Compound **3**, **5**, and **6** exhibited incomplete and slow transition and resisted any shift until the pH became lower than 4. Interestingly, compound **4** displayed no change in its absorption spectrum. Except for compound **4** (Fig. S19), a well-defined isosbestic point was observed in all cases indicating that no secondary reactions occurred under acidic conditions.

The stability of the produced azo compounds against a biologically reducing agent was studied next (Figs. S22-S27). The compounds were exposed to 10 mM glutathione concentration as is typically found in biological systems. Here, compound **1** is reduced completely. Compounds **2** and **5** showed a small reduction in the absorption intensity indicating that a small portion of the molecules reduced under these conditions. Compound **3** and **4** remained unchanged and indicated their stability towards 10 mM glutathione. The solution of compound **6** was observed to form a fine precipitate upon the addition of glutathione. Therefore, the reduction in absorption intensity in **6** can be presumed to be due to their reduction in solubility rather than the azo cleavage reaction.

To further examine sensitivity to glutathione, a much higher concentration of 100 mM was used in the next set of experiments. This study indicated that compound **2** could not withstand the higher concentration of glutathione and was reduced completely. Compounds **3**–**5** showed a small reduction in absorption intensity indicating some molecular decomposition. Compound **6** once again showed a much greater reduction in the absorption intensity. However, as mentioned before, this compound experienced poor solubility in the presence of glutathione. Compounds **3**–**4**, therefore, seem to be relatively robust towards biological reducing conditions created by glutathione. From these results, it appears that an *ortho* nitro substituent (as in compound **1**) helps in the reduction of the azo bond presumably by stabilizing the hydra-zobenzene intermediate (Fig. 3) with the oxygen atoms. To examine this hypothesis, a new compound, **7**, was prepared (Fig. 4 and Figs. S28-S29). Compound **7** cleaved with 10 mM glutathione concentration to a larger extent than **2** but to a lower extent than **1** (Fig. S30). This result indicated that the *ortho* nitro substituent does indeed help to cleave the azo bond, however, the second acceptor is also an important parameter. The best sensitivity is obtained when the molecule contains three donors and two acceptors and the *ortho* acceptor is a nitro substituent. A comparison with a previously known compound **8** which is observed to be less sensitive to **1**, **2**, and **7** and more sensitive to **3** and **4** reinforces this conclusion (Fig. S31) [16].
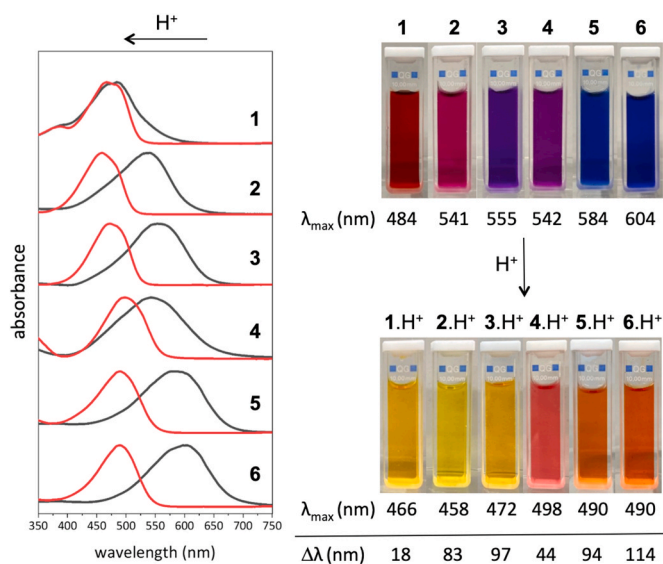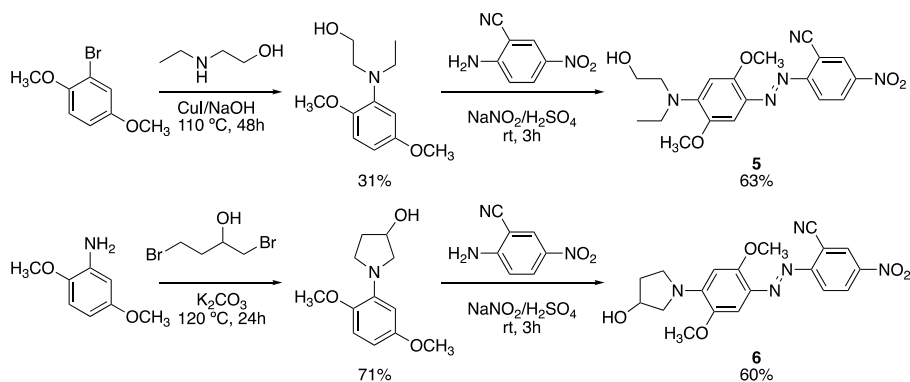

Fig. 1. UV–Vis spectra of compounds **1**–**4** in 2:8 DMSO:Water mixture and **5**–**6** in 3:7 DMSO:Water mixture before (black line) and after (red line) acidification with hydrochloric acid.

Scheme 2. Synthesis of **5**–**6**.
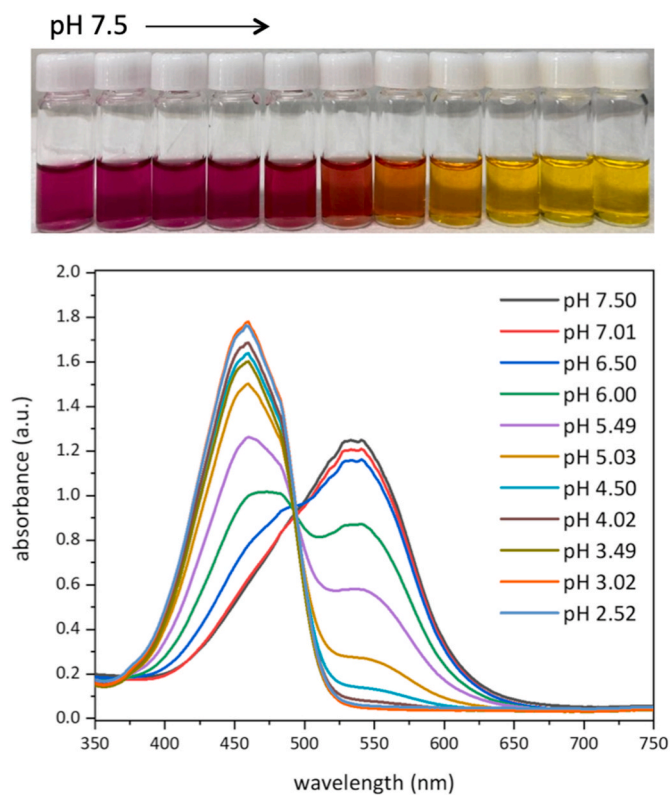

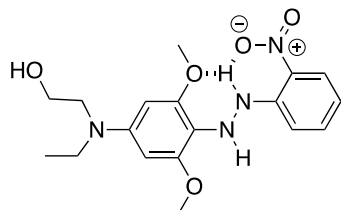**Fig. 2.** Changes in the UV–Vis spectrum of compound **2** as a function of solution pH.

Fig. 3. Chemical structure of the hypothetical hydrazobenzene intermediate.
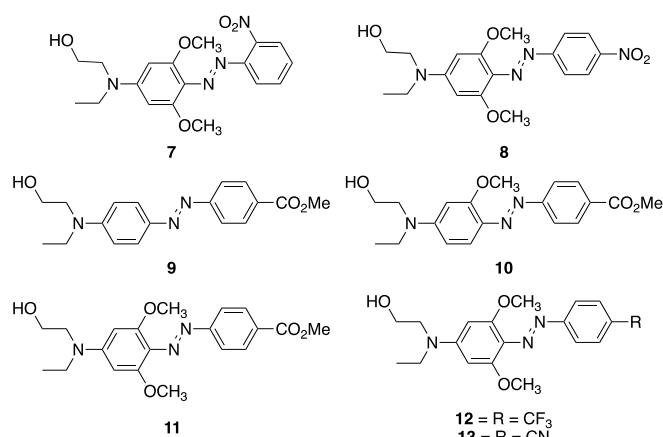
Fig. 4. Chemical structures of compounds **7**–**13**.

design of halochromatic azobenzene compounds. To further study this aspect, known compounds **8**–**13** were acidified (Figs. S33-S38). However, no compounds were observed to undergo a significant blue-shift in their absorption spectrum. This meant that 2–3 donors and 2 acceptors were necessary to observe a strong blue-shift. Furthermore, the arrangement of the acceptors should be such that the stronger acceptor should be *ortho* to the azo bond.

Finally, the reduction of the azo bond under chemically induced reducing condition by using sodium dithionite was investigated (Figs. S39-S44). Here, compounds **1**–**3** were reduced in a few seconds of exposure to 0.5 M sodium dithionite and indicated that these compounds belong to the family of hypersensitive azobenzenes. Compounds **4**–**6**, however, behaved in a less predictable fashion and incomplete cleavage of the azo bond and competing nitro group reduction could be observed in these cases. Therefore, if azobenzene scaffolds are required for cleavage applications in a chemical environment, compounds **1**–**3** are the most appropriate molecules as they cleanly break at the azo bond within a few seconds and in relatively less concentrated sodium dithionite solutions (0.5 mM).

3. Conclusions

In summary, simple monoazobenzene scaffolds with multiple donor and acceptor groups are capable of absorbing in the red region of the electromagnetic spectrum. They can be synthesized in two simple synthetic steps from commercially available starting materials. In an acidic environment, contrary to the expected bathochromic shift, they display a negative halochromism. Some of these compounds are highly sensitive towards cleavage of the azo bond under biological and chemical reducing conditions. Their sensitivities can be tuned by the arrangement

Having compound **7** which carries three donors, like compound **2**, but only one acceptor allowed for examining whether two acceptors were necessary for the design of negative halochromic compounds. Under acidic conditions, a slight bathochromic shift was observed for **7** (Fig. S32). This meant that having two acceptors was critical in the

of the donor and acceptors on the aromatic scaffold. Due to these characteristics, the described series of push-pull azo chromophores are of potential use in sensing applications [6].

Author statement

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2021.109197.

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