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# Synthesis of azobenzenes with high reactivity towards reductive cleavage: Enhancing the repertoire of hypersensitive azobenzenes and examining their dissociation behavior

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Trifluoromethyl ($CF_3$), cyano (CN), and nitro ($NO_2$) groups were demonstrated to be effective acceptors in the molecular design of triple-donor/acceptor-based hypersensitive azobenzenes. The synthesis of these reactive scaffolds requires only two steps with overall yields ranging from 70 to 73%. UV–Vis absorption spectroscopy indicated that in all cases, a few seconds of exposure to mild reducing conditions is sufficient for complete cleavage of the azo bond. Liquid chromatography coupled with mass spectrometry (LC-MS) established the formation of two aniline fragments in the case of the $CF_3$ and CN-substituted azobenzenes. In the case of the $NO_2$-substituted compound, however, partial reduction of the nitro group results in the formation of three different anilines.

## Introduction

The reductive cleavage of the azo (N=N) bond serves as a basis for various release/activation mechanisms in chemical biology and materials science applications (Fig. 1) [1–7]. In most of these applications, a rapid dissociation of the azo bond is required. For example, drug delivery to the colon relies on fast rupture of a cross-linked azobenzene coat to release active therapeutics [3]. In sensing, bioimaging, and theranostics applications, cleavage of the azo linkage under mild hypoxic conditions translates to reliability of detection [4]. Thus, an easily obtained azobenzene scaffold with enhanced activity is an important research goal. In this context, we have recently shown that an increase in the number of donor substituents in a donor/acceptor system enhances the azo scission reaction to very high levels [8]. The most active system contained three donors and one acceptor and complete azo cleavage could be achieved with a 0.5 mM aqueous sodium dithionite solution in <10 s. A previous state-of-the-art system required 12-times higher concentration of the reductant to achieve a similar response [9]. In our previous study, only one molecule, with methyl benzoate ester as the acceptor, was identified as the true hyperactive scaffold. This prompted us to investigate the synthesis and cleavage properties of new triple-donor/acceptor systems to check the versatility of the concept.

## Result and discussion

The synthesis begins with copper-catalyzed amination of dimethoxyiodobenzene with ethylethanolamine to afford the triple-donor fragment (**1**) in 78% yield (Scheme 1). In the second step, **1** was combined with electron deficient anilines possessing trifluoromethyl ($CF_3$), cyano (CN), or nitro ($NO_2$) groups through a diazotization reaction [10]. The isolated yields for this step range from 90 to 95%. In this way, azobenzenes **2**–**4** can be prepared in two synthetic steps (Figs. S1–S6). The hydroxyl group in azobenzenes **2**–**4** can potentially be functionalized based upon a desired application [8]. For instance, in sensing applications, a dye can be attached through an esterification reaction.

In the $^1H$ NMR spectra, the aromatic resonances indicate that the $CF_3$ group is nearly as electron-withdrawing as the azo bond and the two sets of protons located on the aromatic ring containing the acceptor unit (Fig. S1) resonate very close to each other. In the case of the CN group, the protons on the adjacent carbons to the acceptor move downfield indicating that it is a stronger electron-withdrawing group than $CF_3$ (Fig. S3). In the case of the $NO_2$ group, this signal moves further downfield and indicates that it is the strongest acceptor in the present set of compounds (Fig. S5). The Hammett substituent constants for the three groups ($CF_3$ = +0.54, CN = +0.66, $NO_2$ = +0.77) support this hypothesis. In the UV–Vis spectra, the three compounds exhibit strong absorption bands in the range of 350–600 nm belonging to the $\pi$-$\pi^*$ electronic transition (Fig. 2 and Figs. S7–S9). The absorption maxima for

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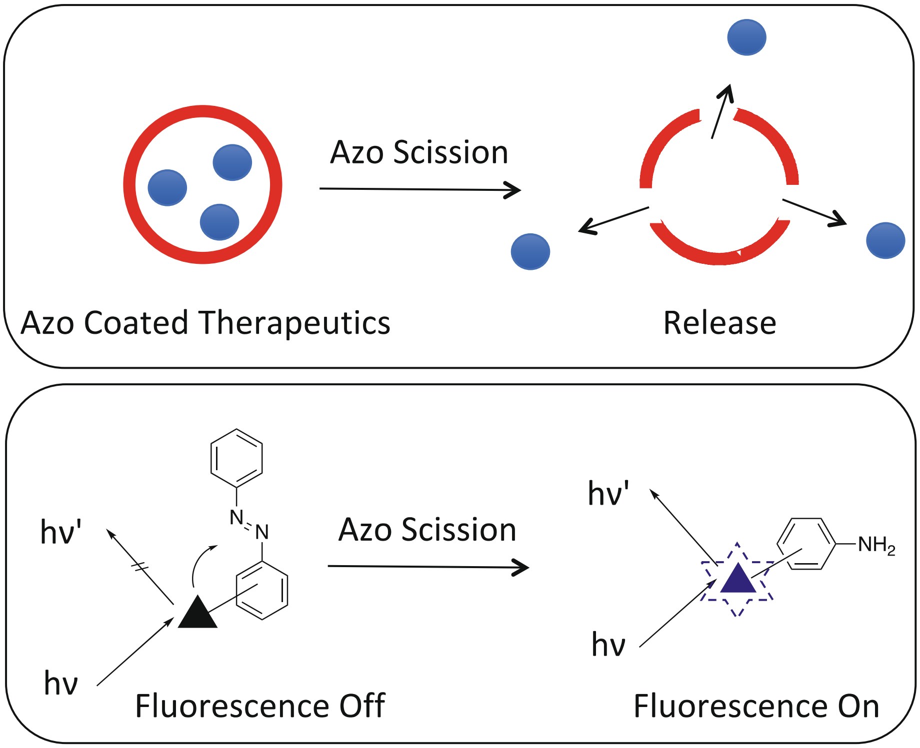
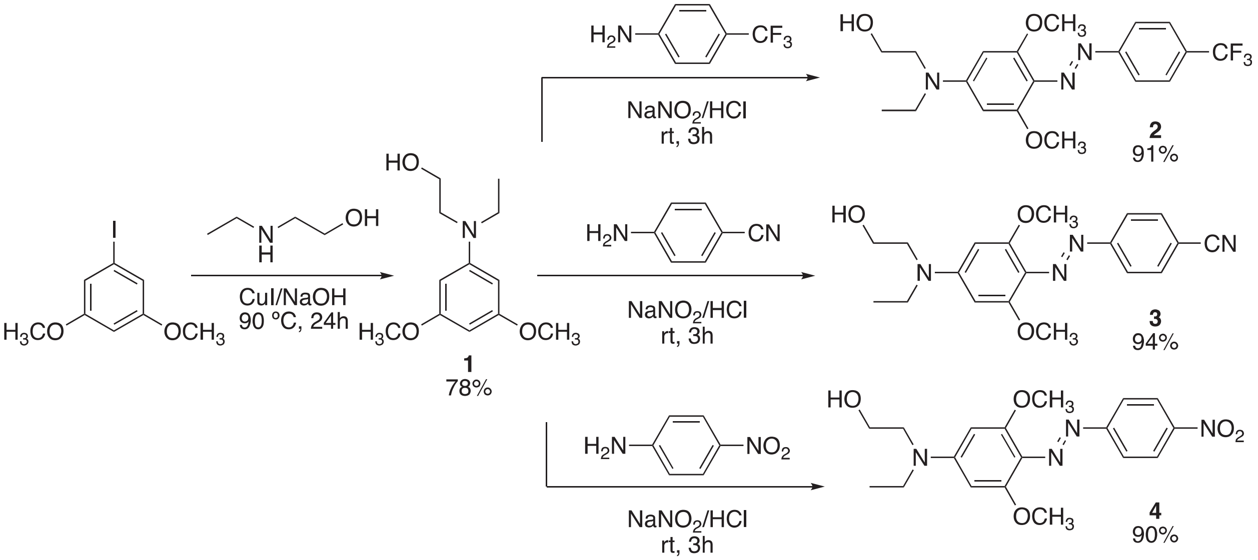


**Fig. 1.** Drawing of release (top) and activation (bottom) concepts utilizing the scission of the azo bond. In release applications, an active therapeutic is encapsulated in azo-based cross-linked coatings and delivered in areas of high reductive stress such as tumors with hypoxic conditions. In sensing applications, the azobenzene isomerization is utilized as an energy sink until the azo bond ruptures under reductive stress in diseased tissue and the attached fluorophore becomes a reporter.



**Scheme 1.** Synthesis of azobenzenes **2**–**4**.

azobenzenes **3** and **4** shifts by 5 and 25 nm, respectively, towards higher wavelengths and once again indicates that CN and $NO_2$ are stronger acceptors than the $CF_3$ group.

Scission of the azo bond in azobenzenes **2**–**4** was carried out using 2, 1, 0.75, and 0.5 mM aqueous sodium dithionite solution. The azo scission reaction breaks the electronic conjugation between the two aromatic rings and the colored solutions become colorless (Fig. 2 and Fig. S7–S9). This transition can be observed using UV–Vis spectroscopy. As the cleavage reaction proceeds, the $\pi$-$\pi^*$ absorption band decreases in intensity. From this data (Table S1 and Fig. S10), it is clear that although the comparatively weaker $CF_3$ acceptor causes a relatively sluggish reaction in azobenzene **2**, overall, all systems (**2**–**4**) show half-lives within one second.

The identity of the cleaved products and purity of the scission reaction was investigated using liquid chromatography coupled with mass spectrometry (LC-MS) (Scheme 2, Figs. S11–S13). In the case of azobenzenes **2** and **3**, the azo cleavage produced only the expected aniline fragments **5**/**6** and **5**/**7**, respectively. In the case of azobenzene **4**, however, the nitro group was also reduced

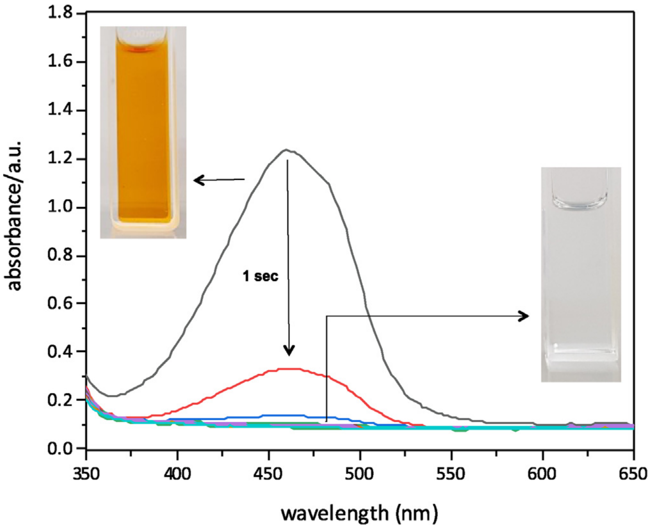


**Fig. 2.** UV–Vis spectra of compound **3** (50 µm) upon exposure to 1 mM sodium dithionite in 2:8 DMSO:Tris-HCl buffer solution. The time lapse between each trace is 1 s. The digital picture shows a change in color of the azo compound **3** (left) upon reduction (right).

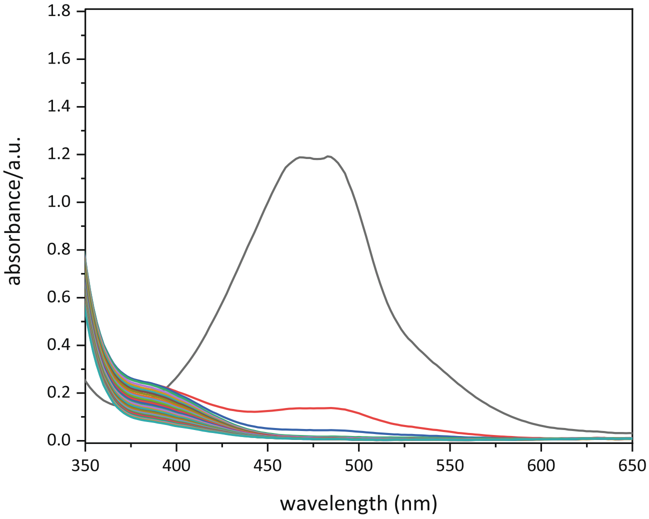


**Fig. 3.** UV–Vis spectra of compound **4** (50 µm) upon exposure to 1 mM sodium dithionite in 2:8 DMSO:Tris-HCl buffer solution.

to the amine and the reaction produced three anilines **5**, **8**, and **9**. In the absorption spectra, the formation of **8** and its transformation into **9** was evident through formation of a new absorption band at 400 nm and its subsequent decrease in intensity as the reduction reaction proceeded (Fig. 3) [11].

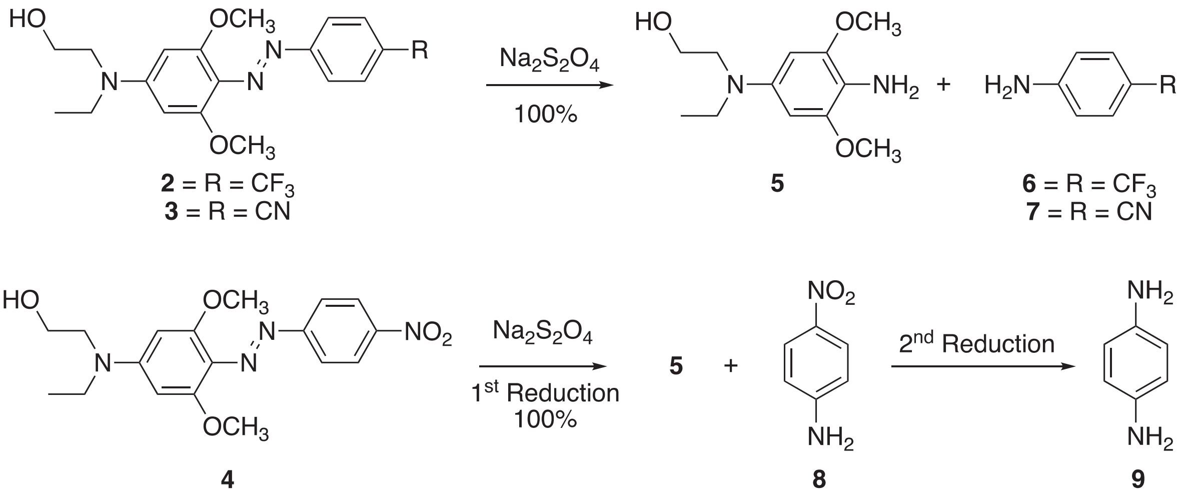
Finally, azobenzene derivative (**10**) which did not contain an electron-withdrawing group was prepared (Scheme 3, Figs. S14–15, and Table S1). Under mild conditions (0.5 mM aq. $Na_2S_2O_4$), **10** remained partially intact (Figs. S16–17). This data, along with literature reports [5e,9], indicate that a donor/acceptor arrangement of functional groups on the azobenzene nucleus is necessary for enhancing its sensitivity towards azo (N═N) cleavage.

## Conclusion

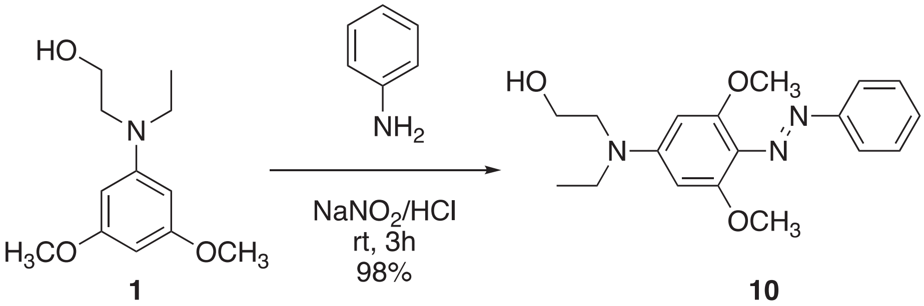
Triple-donor azobenzene nuclei with trifluoromethyl (**2**), cyano (**3**), or nitro (**4**) acceptors can be easily synthesized (70–73% yield over two steps) and exhibit a very fast (<10 s) azo bond dissociation under mild (0.5 mM $Na_2S_2O_4$) reducing conditions. In the case of trifluoromethyl (**2**) and cyano (**3**) groups, the cleavage reaction produces only the two expected aniline fragments, **5**/**6** and **5**/**7**, respectively. In the case of the nitro group (**4**), however, the reduction reaction also produces *para*-diamino aniline (**8**). Through UV–Vis spectroscopy, it can be concluded that the nitro group reduces after reduction of the azo bond. In all cases, the azo cleavage reaction is quantitative. This study extends the range of hypersensitive azobenzenes by including three new molecular structures and examining their dissociation behavior. The results presented here are anticipated to be useful in the design of new responsive materials for release/sensing applications.

## 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.



**Scheme 2.** Azobenzene dissociation products in compounds **2**, **3**, and **4**.



**Scheme 3.** Synthesis of azobenzene **10**.

## Acknowledgement

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2020.152018.

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