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Synthesis and characterization of ortho bromo-methoxy aminoazobenzene derivatives

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Abstract

Aminoazobenzene derivatives with four *ortho* substituents with respect to the NN double bond are a relatively unexplored class of azo compounds that show promise for use as photoswitches in biology. Tetra-*ortho* methoxy aminoazobenzenes, in particular, can form azonium ions under physiological conditions and exhibit red-light photoswitching. Here, we report the synthesis and characterization of two *ortho* bromo-methoxy aminoazobenzene derivatives. These compounds form red light absorbing azonium ions but only under very acidic conditions (pH <1). While the low pKa makes the azonium form unsuitable, the neutral versions of these compounds undergo trans-to-cis photoisomerization with blue-green light and exhibit slow ($\tau_{1/2}$ ~10 min) thermal reversion and so may find applications under physiological conditions.

Keywords

azobenzene; azonium; photoisomerization; photoswitch; visible light

Introduction

Typically, the formation of azonium ions from aminoazobenzenes occurs at pH < 3.5. [1], however, the pKa of the trans azonium ion (**1**) is ~ 7.5 in aqueous solution [2, 3]. The elevated pKa of **1** has been attributed to resonance stabilization of the azonium cation together with intramolecular H-bonding between the azonium proton and methoxy groups *ortho* to the azo double bond [3]. Since the azonium ion **1** forms under physiological conditions, i.e. at neutral pH in an aqueous solution, it is useful as a photoswitch for the photo-control of biomolecules [4]. It absorbs red light, undergoes trans–cis photoisomerization and relaxes to the trans isomer in the dark on the timescale of seconds so that pulses of red light can be used to drive multiple isomerization cycles [3]. Efforts to apply **1** to photo-control protein-protein interactions have recently been reported [5].

Despite the usefulness of **1** as a photoswitch, compounds undergoing photoisomerization at longer wavelengths (>700 nm) would be valuable since the penetration of light through tissue is enhanced in the near-IR window [6]. Longer wavelength absorption is achieved by compounds **2** and **3** and related derivatives [2] (Fig. 1). However, the lifetime for thermal reversion of the cis isomer of **2** is only ~ 1 ms at neutral pH and the pKa for trans azonium ion formation is ~2.6. The low pKa was attributed to steric clash between the methoxy groups *meta* to the azo double bond and the six-member morpholine ring [2]. The rapid thermal reversion was attributed to the removal of two *ortho* methoxy groups leading to diminished steric strain in the transition state for reversion [2]. In compound **3**, all four *ortho* positions are substituted and the *meta* oxygen substituents are part of dioxane rings so that steric clash with the *para* amino substituents is reduced. Compound **3** and derivatives with pyrrolidino groups in the *para* positions were shown to be effective near-IR

switches undergoing isomerization with 720 nm light under physiological conditions [7]. While the photoswitching properties of **3** are suitable for use in biological systems, the overall size of **3** may limit possibilities for use as a component of photopharmaceutical agents. Therefore, we wished to explore other substitution patterns of these aminoazobenzene derivatives. To allow for the possibility of intramolecular H-bond formation we wished to retain at least one *ortho* methoxy group. To slow the rate of thermal reversion, only derivatives with substituents at all four *ortho* positions were considered. Time-dependent density functional theory (TD-DFT) calculations were used to predict the absorption wavelengths of possible derivatives. Based on these considerations, we carried out the synthesis and photochemical characterization of compounds **4** and **5**.

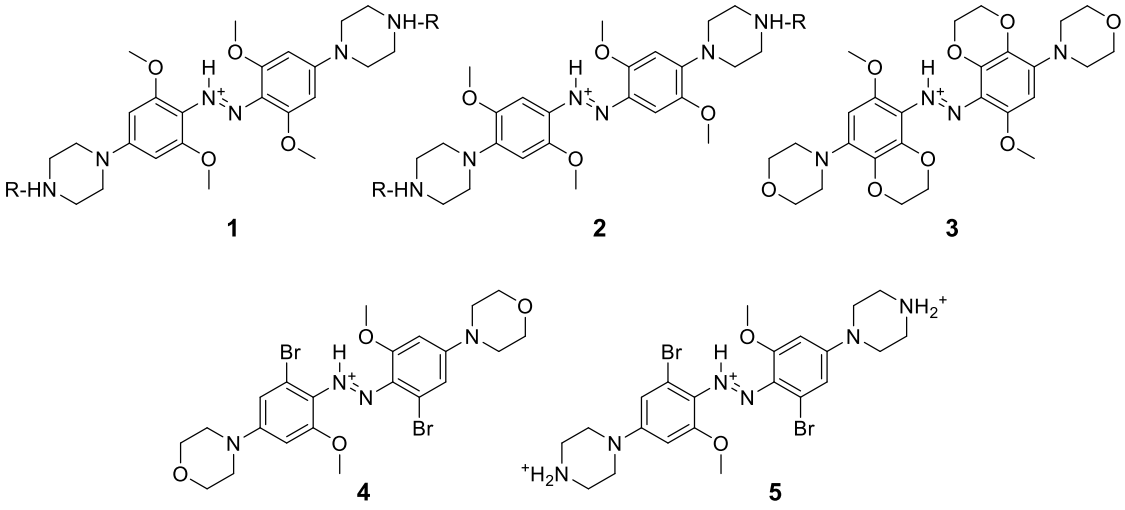


Figure 1: Structures of azonium ions studied.

Results and Discussion

Computational chemistry

Calculations were performed using density functional methods (B3LYP/6-31+G**) to optimize geometry, and TD-DFT to calculate absorption wavelength maxima. These computational methods have been used successfully with related compounds [8, 9]. The relative stability of different conformations of the molecule were calculated, i.e. with the methoxy substituents on the same side or on opposite sides of the NN double bond. The conformation with methoxy groups on opposite sides of the NN double bond was found to be the most stable. Calculating effects of the substitution patterns on pK_as is problematic [10] and was not attempted here. Figure 2 shows calculated structures and spectra for the neutral forms of simplified models of **4/5** with either a pyrollidino or piperidino *para* substituent as well as the corresponding azonium ions. Calculations indicated that the nature of the amino substituent did not have a large effect on the positions of wavelength maxima. These models predict that compounds **4**, and **5** should absorb at longer wavelengths than **1** [3]. Figure 2 also shows experimental spectra, to be discussed below.

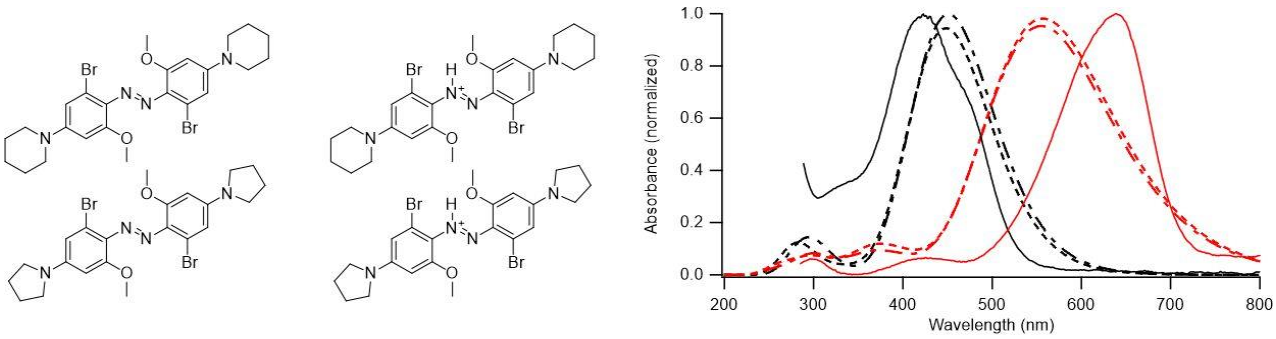
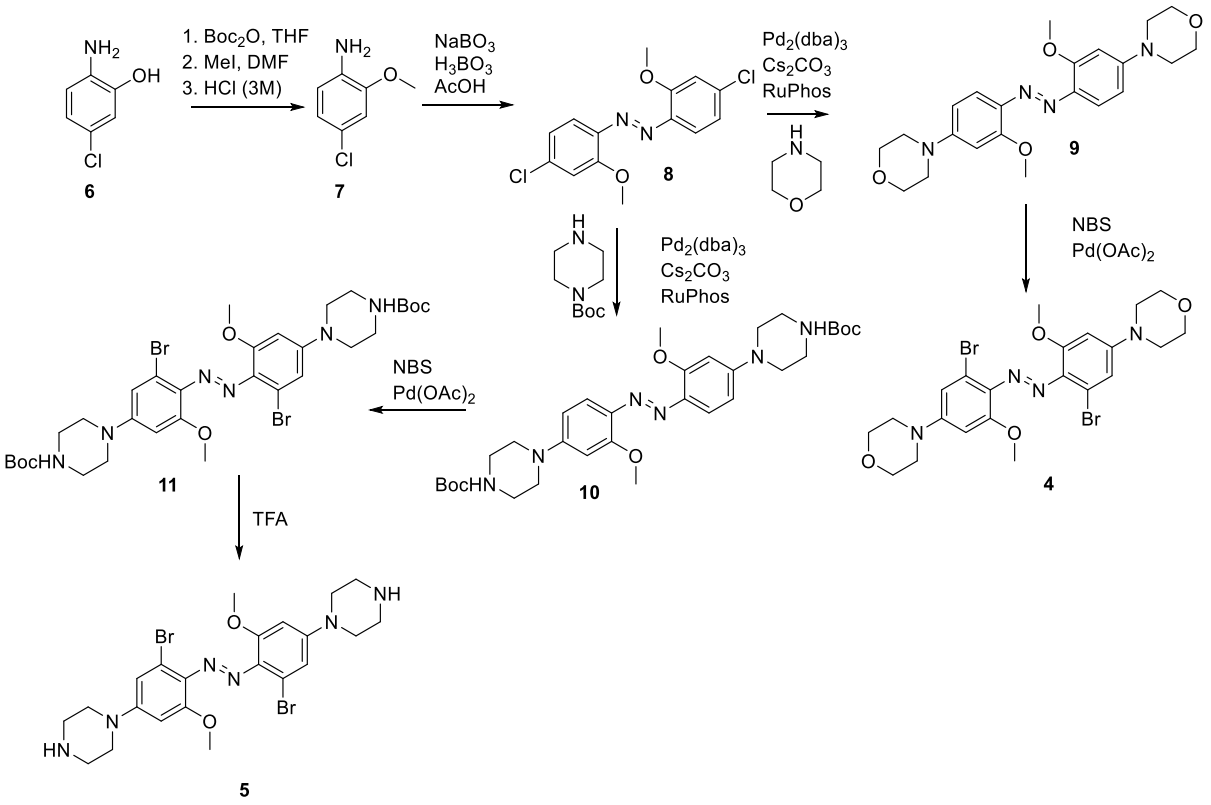


Figure 2: Structures of model compounds used for computations (see Experimental section). Neutral forms (left structures, black spectra) and azonium forms (right structures, red spectra) were calculated with either piperidino (top row, dashed lines) or pyrollidino (bottom row, dash-dot lines)) *para* substituents. Solid lines show experimental spectra for **4** in DCM without (black) or with (red) added TFA.

Synthesis

The overall synthetic route taken is shown in Scheme I. The azo compound **8** with two *ortho* methoxy groups was prepared from **7** using an oxidative coupling approach [11]. *Para*-chloro substituents were then replaced with amino substituents using a Buchwald-Hartwig coupling [12]. Since calculations predicted that 5- and 6-membered rings would have similar effects on the position of the absorption maxima we opted to use 6-membered rings, specifically a morpholino substituent and a piperazino substituent in an attempt to enhance aqueous solubility.



Scheme 1: Synthesis of *ortho* halo methoxy aminoazobenzenes.

Photochemical characterization

Despite the morpholino substituent, compound **4** was found to be insoluble in water. We therefore dissolved **4** in dichloromethane (DCM) to obtain the UV-Vis spectrum of the neutral form. Addition of trifluoroacetic acid to this solution produced the azonium ion. The spectra of the neutral and azonium forms of **1** are shown as solid lines in Figure 1. Observed maxima were at 426 (neutral form) and 640 nm (azonium form). While the observed wavelength of maximum absorption was close to the predicted wavelength for the neutral form, the observed wavelength of maximum absorption of the azonium ion was significantly longer than predicted, although the long wavelength tail was less pronounced. The wavelength of maximum absorption of the azonium ion was also longer than that observed for compound **1** [3], as predicted.

We confirmed that the neutral form of **4** underwent photoisomerization. Exposure of a solution of **4** in DCM to 440 nm light led to a photostationary state in which the absorbance at 440 nm was diminished and the absorbance at 330 nm was slightly enhanced (Fig. 3). Thermal relaxation from the photostationary state was monitored by UV-Vis spectroscopy, recording the spectrum every minute. As shown in Figure 3, a half-life of 6.5 min was observed at room temperature.

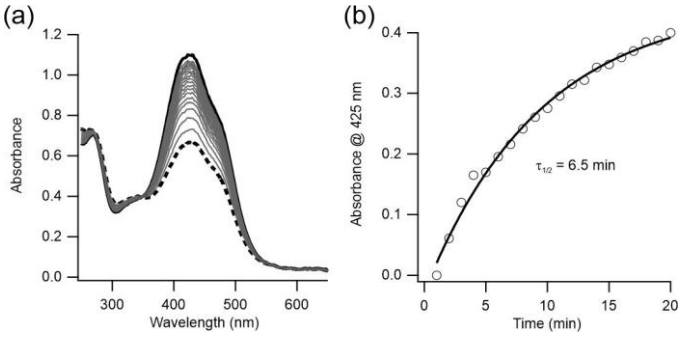


Figure 3: (a) UV-Vis absorbance spectra of **4** in DCM at the photostationary state with 440 nm irradiation (dashed line) and during thermal reversion to the dark-adapted state (solid black line). (b) Time course of thermal reversion at 22°C.

To enhance water solubility, the morpholino substituents were interchanged for piperazino groups. The secondary amino groups on the piperazino units are expected to have pK_as near 10 [13], and so should be protonated at neutral pH, creating a doubly charged species. As anticipated, compound **5** was found to be significantly more water soluble that **4**.

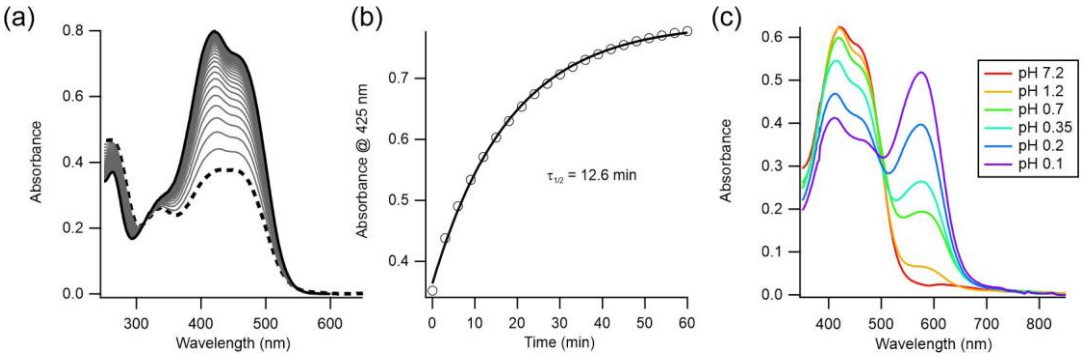


Figure 4: (a) UV-Vis absorbance spectra of **5** in aqueous solution (pH 7)(5% methanol) at the photostationary state with 440 nm irradiation (dashed line) and during thermal reversion to the dark-adapted state (solid black line). (b) Time course of thermal reversion at 22°C. (c) Spectra of an aqueous solution of **5** with addition of HCl to the pHs indicated. The wavelength of maximum absorption of the azonium ion is 575 nm.

The UV-Vis spectrum of **5** at neutral pH is shown in Figure 4. Irradiation with blue light (440 nm) produced the photostationary state shown (dashed line). Thermal reversion from the cis isomer was monitored by UV-Vis spectroscopy, measuring the UV-Vis spectra every 3 minutes, and a half-life of 12.6 min was obtained.

The formation of the azonium ion of **5** in aqueous solutions was then explored. Addition of HCl to a neutral solution of **5** was carried out. Spectra as a function of pH are shown in Figure 4. As shown in Figure 4, formation of the azonium ion of compound **5** required very acidic conditions. Even at pH ~0.1, a substantial fraction of the neutral species was still present implying the pK_a for the azonium ion is <~0.2. This pK_a is at least 7 pH units lower than for the tetra-*ortho*-methoxy compound **1**. As noted above, the presence of the piperazino amino groups makes compound **5** doubly positively charged at neutral pH. This feature is expected to suppress the pK_a

of the azonium ion through electrostatic effects. The first pK_a of substituted piperazines falls in the range ~4-6 (vs. 9-10 for the second pK_a) [13]. In addition, the electron withdrawing Br atoms are also expected to lower the azonium ion pK_a (the pKa of 2-bromobenzoic acid is 2.85, vs. 4.2 for unsubstituted benzoic acid [14]). While electrostatic effects on the pK_a could be ameliorated (i.e. by adding negatively charged groups), this would place additional constraints on the generally applicability of the compounds. In addition, unlike compound **4**, the wavelength of maximum absorption of the azonium ion of **5** was not red shifted relative to that of **1**. Conceivably, the wavelength of maximum absorption is affected by the charged piperazino groups; calculations were done with uncharged piperidino substituents shown in Fig. 2. Nevertheless, the lack of a red shift, combined with the significantly lowered pK_a, makes **5** unsuitable as azonium photoswitch under physiological conditions.

Despite this undesired effect on the pK_a, the steric bulk afforded by the Br substituents does appear to slow thermal relaxation of the neutral (unprotonated azo) forms of these compounds. Compound **5** can be switched with blue and green light under physiological conditions and thermally relaxes with a half-life of 12 min. This relaxation rate is substantially slower than other blue-green absorbing azo compounds without substituents in all four positions *ortho* to the azo unit, which show half-lives ranging from 50 ms [15] to a few seconds [16]. Instead compounds **4** and **5** exhibit photoswitching properties similar to those reported for tetra *ortho* thiol substituted azobenzenes [9].

Experimental

Synthesis

***tert*-butyl (4-chloro-2-hydroxyphenyl)carbamate:** 2-amino-5-chlorophenol (3.5 mmol, 500 mg) was dissolved in THF at room temperature. Then, Boc_2O (7 mmol, 1.52 g) was added and the resulting mixture was stirred for 18 h at room temperature. The reaction was monitored by TLC. When the reaction was finished, the solvent was removed under reduced pressure resulting in a yellowish oil. The final compound was purified by column chromatography using a mixture of hexane/ethyl acetate (4:1) as eluent. The product was used directly in the next step.

***tert*-butyl (4-chloro-2-methoxyphenyl)carbamate:** The phenol tert-butyl (4-chloro-2-hydroxyphenyl)carbamate (2 mmol, 500 mg) was dissolved in DMF. Then, an aqueous solution of K_2CO_3 (6 mmol, 828 mg) was added together with methyl iodide (6 mmol, 851 mg). The final mixture was stirred for 18 h until the starting reagent was consumed. Then, the solvent was removed under reduced pressure resulting in a brown oil. The reaction mixture was poured into water and extracted three times with dichloromethane. The organic fractions were collected and evaporated under reduced pressure. The product was used directly in the next step.

4-chloro-2-methoxyaniline (7): The compound *tert*-butyl (4-chloro-2-methoxyphenyl)carbamate (400 mg, 1.16 mmol) was dissolved in 3 mL of ethyl acetate. Then, 9 mL of fuming HCl were added dropwise to the reaction mixture with vigorous stirring. The resulting mixture was stirred for 1 hour. Then, the solvent was evaporated under reduced pressure giving a brown oil. The mixture was added to 30

mL of hexane in an ice bath, precipitating the final compound. The product was isolated by filtration.

^{1}H NMR (400 MHz, Methanol-d_4) δ 7.36 (d, *J* = 8.4 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 7.11 (dd, *J* = 8.4, 2.1 Hz, 1H), 3.99 (s, 3H).

(*E*)-1,2-bis(4-chloro-2-methoxyphenyl)diazene (8): The formation of the azo bond took place under oxidative conditions. For this purpose, 4-chloro-2-methoxyaniline (2.5 mmol, 400 mg) was dissolved in acetic acid. Boric acid (2.11 mmol, 135 mg) was added to the reaction mixture. Then, sodium perborate (2.5 mmol, 680 mg) was added, in three portions each, over 15 minutes. Then, the reaction was heated at 70ºC for 18 hours. The reaction was monitored by TLC, then when it was finished, the solvent was removed under reduced pressure. The resulting oil was poured into water and extracted with dichloromethane three times. The organic fractions were collected, and the solvent was removed. The final oil was purified by column chromatography using as eluent a mixture of hexane/ethyl acetate (4:1).

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 8.6 Hz, 2H), 7.07 (d, *J* = 2.1 Hz, 2H), 6.98 (dd, *J* = 8.6, 2.1 Hz, 2H), 4.01 (s, 6H).

^{13}C NMR (75 MHz, Chloroform-*d*) δ 157.4, 141.4, 138.3, 121.2, 118.5, 113.3, 56.7.

EM-ES (+): calc'd for $C_{14}H_{15}N_2O_2Cl_2$ $[M+H]^+$ 311.0349, found: 311.0341.

(*E*)-1,2-bis(2-methoxy-4-morpholinophenyl)diazene (9): (*E*)-1,2-bis(4-chloro-2-methoxyphenyl)diazene (100 mg, 0.32 mmol) was dissolved in toluene in a pressure tube. Then, morpholine (84 mg, 0.96 mmol), tris(dibenzylideneacetone)dipalladium (0) (29.3 mg, 0.032 mmol), RuPhos (29.8 mg, 0.064 mmol) and cesium carbonate (302.4 mg, 0.96 mmol) were added. The mixture was heated at 100ºC in the pressure tube for 24 hours. The reaction was monitored by TLC until the starting

reagent was consumed. The solvent was evaporated under reduced pressure, and the resulting oil was extracted with dichloromethane three times. The final mixture was purified by column chromatography using a mixture of hexane/ethyl acetate (1:4) as eluent.

Chemical Formula: $C_{22}H_{28}N_4O_4$; Molecular Weight: 412.4900

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.71 (s, 2H), 6.48 (m, 4H), 4.01 (s, 6H), 3.87 (m, 8H), 3.28 (m, 8H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 158.1, 154.0, 136.7, 118.5, 107.7, 98.9, 66.9, 56.7, 48.6.

EM-ES (+): calc'd for $C_{22}H_{29}N_4O_4$ $[M+H]^+$ 413.2189, found: 413.2186.

(*E*)-1,2-bis(2-bromo-6-methoxy-4-morpholinophenyl)diazene (4): (*E*)-1,2-bis(2-methoxy-4-morpholinophenyl)diazene (30 mg, 0.07 mmol) was dissolved in dichloromethane. To this solution, palladium acetate (1.6 mg, 0.007 mmol) was added, and the resulting mixture was stirred for 15 minutes. Then, N-bromosuccinimide (28.6 mg, 0.16 mmol) was added to the reaction. The reaction was stirred for an additional 30 minutes until completed. The solvent was evaporated under reduced pressure and the resulting oil was purified by column chromatography using a mixture of hexane/ethyl acetate (1:2) as eluent.

Chemical Formula: $C_{22}H_{26}Br_2N_4O_4$; Molecular Weight: 570.2820

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.91 (s, 2H), 6.67 (s, 2H), 4.03 (s, 6H), 3.89 (m, 8H), 3.17 (s, 8H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.3, 153.7, 138.8, 122.6, 110.8, 104.9, 67.0, 58.6, 51.9.

EM-ES (+): calc'd for $C_{22}H_{27}Br_2N_4O_4$ $[M+H]^+$ 569.0393, found: 569.0393.

di-tert-butyl 4,4'-(diazene-1,2-diylbis(3-methoxy-4,1-phenylene))(E)-bis(1l4-piperazine-1-carboxylate)(10): (*E*)-1,2-bis(4-chloro-2-methoxyphenyl)diazene (100 mg, 0.32 mmol) was dissolved in toluene in a pressure tube. Then, 1-Boc-piperazine (180 mg, 0.96 mmol), tris(dibenzylideneacetone)dipalladium (0) (29.3 mg, 0.032 mmol), RuPhos (29.8 mg, 0.064 mmol) and cesium carbonate (302.4 mg, 0.96 mmol) were added. The mixture was heated to 100ºC in a pressure tube for 36 hours. The reaction was monitored by TLC until the starting reagent was consumed. The solvent was evaporated under reduced pressure, and the resulting oil was extracted with dichloromethane three times. The final mixture was purified by column chromatography using a mixture of hexane/ethyl acetate (1:3) as eluent.

^{1}H NMR (400 MHz, Methylene Chloride-d_2) δ 7.57 (m, 2H), 6.50 (m, 4H), 3.99 (s, 6H), 3.58 (m, 8H), 3.29 (m, 8H), 1.47 (s, 18H).

di-tert-butyl 4,4'-(diazene-1,2-diylbis(3-bromo-5-methoxy-4,1-phenylene))(E)-bis(1λ^4-piperazine-1-carboxylate)(11): Di-tert-butyl 4,4'-(diazene-1,2-diylbis(3-methoxy-4,1-phenylene))(*E*)-bis(1λ^4-piperazine-1-carboxylate) (30 mg, 0.04 mmol) was dissolved in dichloromethane, palladium acetate (1 mg, 0.004 mmol) was added, and the resulting mixture was stirred for 15 minutes. Then, N-bromosuccinimide (22 mg, 0.1 mmol) was added to the reaction. The reaction was stirred for an additional 30 minutes until complete. The solvent was evaporated under reduced pressure and the resulting oil was purified by column chromatography using a mixture of hexane/ethyl acetate (1:1) as eluent.

Chemical Formula: $C_{32}H_{45}Br_2N_6O_6$; Molecular Weight: 768.5480

^{1}H NMR: (400 MHz, methylene chloride-d_2) δ 7.74 (s, 2H), 6.61 (s, 2H), 3.93 (s, 6H), 3.53 (m, 8H), 3.00 (m, 8H), 1.39 (s, 18H).

^{13}C NMR (126 MHz, Methylene Chloride-d_2) δ 152.9, 150.2, 149.4, 134.1, 117.5, 106.2, 75.1, 52.1, 47.0, 23.7.

EM-ES (+): calc'd for $C_{32}H_{45}Br_2N_6O_6$ $[M+H]^+$ 767.1767, found: 767.1762.

(E)-1,2-bis(2-bromo-6-methoxy-4-(piperazin-1-yl)phenyl)diazene (5): Di-tert-butyl 4,4'-(diazene-1,2-diylbis(3-bromo-5-methoxy-4,1-phenylene))(E)-bis(1λ^4-piperazine-1-carboxylate) (15 mg, 0.02 mmol) was dissolved in 5 mL of dichloromethane, and 0.25 mL of TFA was added. The resulting mixture was stirred for 16 hours. Then, the solvent was evaporated, and the resulting mixture did not need further purification. Chemical Formula: $C_{22}H_{28}Br_2N_6O_2$; Molecular Weight: 568.3140

^{1}H NMR: (400 MHz, Methylene Chloride-d_2) δ 7.92 (s, 2H), 6.97 (s, 2H), 4.11 (s, 6H), 3.45 (m, 8H), 3.00 (m, 8H).

^{13}C NMR (126 MHz, Methylene Chloride-d_2) δ 158.9, 154.0, 140.2, 123.0, 111.5, 107.2, 66.9, 57.3, 45.1.

EM-ES (+): calc'd for $C_{22}H_{29}Br_2N_6O_2$ $[M+H]^+$ 567.0719, found 567.0713.

Computational Methods

DFT calculations were performed using the Gaussian 09 suite of programs at the B3LYP level of theory using the 6-31+G(d,p) basis set [17]. Optimizations were followed by harmonic oscillator frequency calculations at the same level of theory to verify the absence of imaginary frequencies. Though an exhaustive conformational search was not performed for any of the species, the following calculations were performed: To ensure the conformation shown in Fig. 2 is the thermodynamically most stable arrangement around the azo moiety, free energies for two alternative arrangements - conformers 2 with Br atoms on the same side of the NN double bond, and conformer 3 with a Br on the position of the methoxy group that makes an H-

bond with the azonium proton, - were calculated for each compound. Both alternate conformers were predicted to have higher energies in vacuo (conformer 2, 1.1 kJ/mol for the 6-membered ring; 2.2 kJ/mol for the 5-membered ring; conformer 3 (20 kJ/mol for the 6-membered ring; 19 kJ/mol for the 5-membered ring). Second, for neutral species, the N=N-C-C dihedral angles in the optimized structures were manually set to 20 degrees to generate new input files. Re-optimization yielded the same structures and free energies. The optimized geometries of all structures were subjected to TD-SCF calculations using the same functionals and basis set, assuming the first 15 singlet excitations and by applying the SMD solvation model to implicitly approximate the effect of solvent [18]. TD-SCF data were used to generate the simulated UV-Vis spectra by applying a Gaussian function with 0.333 eV peak half-width at half-height to each transition.

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