Supplementary information for:

Photoswitching azo compounds *in vivo* with red light.

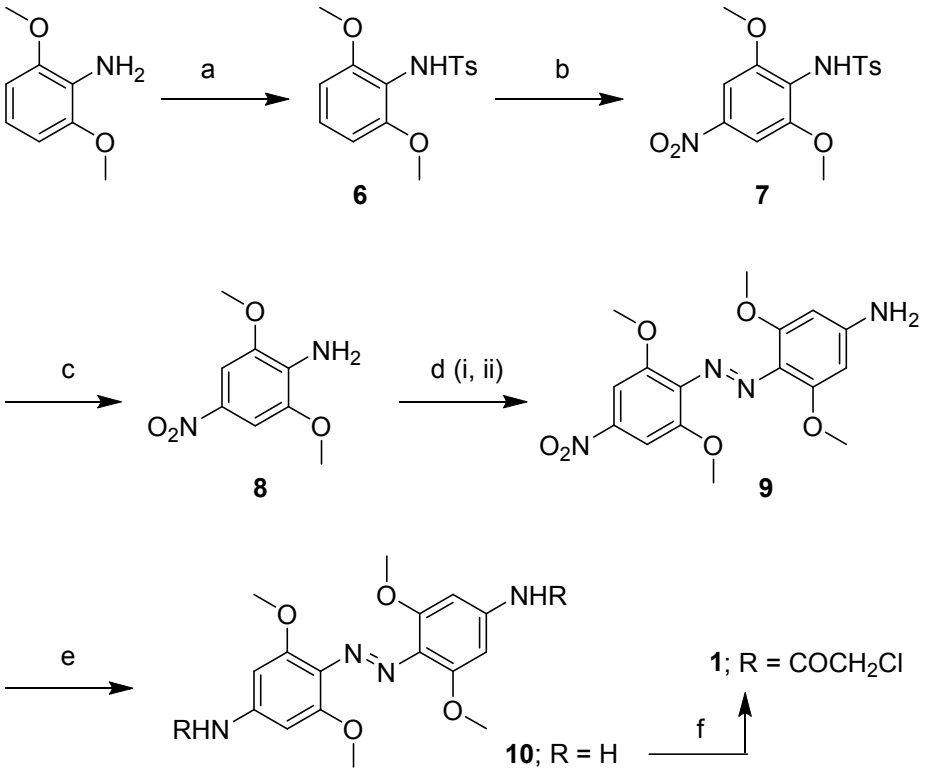
Subhas Samanta[‡], Andrew A. Beharry[‡], Oleg Sadovski, Theresa M. McCormick, Amirhossein Babalhavaeji,Vince Tropepe[#] & G. Andrew Woolley*

Department of Chemistry, University of Toronto, 80 St. George St., Toronto, ON, M5S 3H6, Canada and [#]Department of Cell & Systems Biology and Centre for the Analysis of Genome Evolution and Function, University of Toronto, 25 Harbord St., Toronto, ON, M5S 3G5, Canada.

Synthetic methods.

General aspects: All commercial materials (solvents, reagents and substrates) were used as received. The NMR spectra were recorded either on Varian Vnmr-S 400 MHz or Varian Mercury 400 MHz or Varian UnityPlus 500 MHz or Agilent DD2 500 MHz spectrometers. Silica gel of particle size 40-63 μm from Silicycle Chemical Division was used for all column chromatography.

The overall synthetic route to compound **1** is shown below:



SCHEME 1. Synthesis of **1**. (a) p-Toluenesulfonyl chloride, pyridine, 100^{o}C, 1 h, yield 80 %; (b) $NaNO_2$, HNO_3/AcOH, yield 60%; (c) Conc. H_2SO_4, 16 h, yield 70%; (d) i) $NaNO_2$, dil. HCl, 0-5^{o}C, 20 min ii) 3, 5-dimethoxyaniline, H_2O, sat. $NaHCO_3$ (pH 8-9), 5^{o}C to rt, 12 h, yield 31%; (e) $SnCl_2$, anh. DMSO, 48 h, yield 7%; (f) chloroacetyl chloride, triethylamine, $CHCl_3$, 5^{o}C to rt, 12 h, yield 62%.

(*N*-(3,5-dimethoxyphenyl)-4-methylbenzenesulfonamide (**6**) called *N*-tosyl-2,6-dimethoxyaniline in the original reference was synthesized exactly as described.[1] The starting material, 2,6-dimethoxyaniline was obtained from Alfa Aesar. ^{1}H NMR (399 MHz, chloroform-*d*) δ ppm 2.42 (s, 3 H) 3.61 (s, 6 H) 6.26 (s, 1 H) 6.50 (d, *J*=8.4 Hz, 2 H) 7.12 (t, *J*=8.4 Hz, 1 H) 7.25 (d, *J*=8.4 Hz, 2 H) 7.73 (d, *J*=8.4 Hz 2 H).

(*N*-(3,5-dimethoxy-4-nitrophenyl)-4-methylbenzenesulfonamide (**7**) called N-tosyl-2,6-dimethoxy-4-nitroaniline in the original reference was synthesized as described [1,2]. ^{1}H NMR (300 MHz, chloroform-*d*) δ ppm 2.45 (s, 3 H) 3.74 (s, 6 H) 6.73 (s, 1 H) 7.32 (d, *J*=8.50 Hz, 2 H) 7.44 (s, 2 H) 7.79 (d, *J*=8.50 Hz, 2 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 21.55, 56.21, 100.31, 121.49, 127.07, 129.06, 138.34, 143.46, 145.80, 153.03; MS^+ obs'd: 353.08031, calc'd for $C_{15}H_{16}N_2O_6S$ $[M+H]^+$: 353.080183

3,5-dimethoxy-4-nitroaniline (**8**)**:** ^{1}H NMR (399 MHz, chloroform-*d*) δ ppm 3.94 (s, 6 H) 4.59 (br. s., 1 H) 7.53 (s, 2 H); ^{13}C NMR (100 MHz, chloroform-*d*) δ ppm 56.13, 101.05, 133.39, 137.34, 144.83; MS^+ obs'd: 199.07241, calc'd for $C_8H_{10}N_2O_4$ $[M+H]^+$: 199.071333

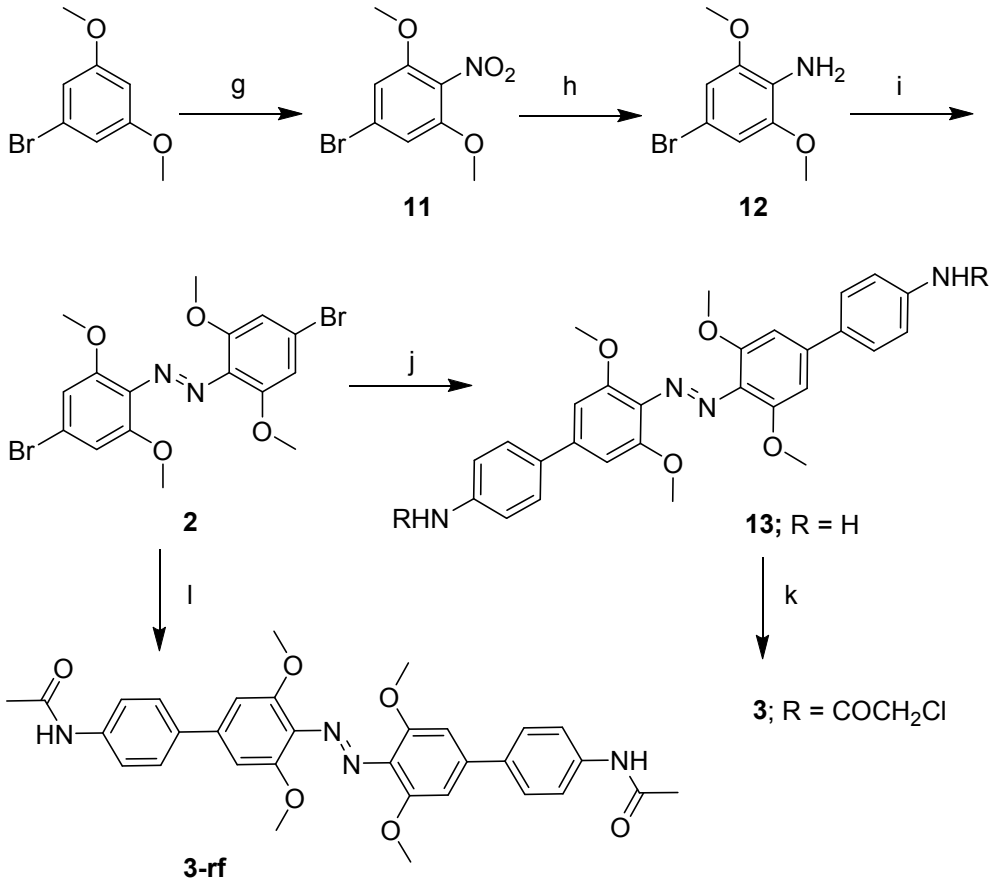
4-((2,6-dimethoxy-4-nitrophenyl)diazenyl)-3,5-dimethoxyaniline (9): 1.08 g (0.016 mol) of $NaNO_2$ in 6.3 mL of H_2O was added to a solution of 3,5-dimethoxy-4-nitroaniline (**8**) (3.1 g, 0.016 mol) in mixture of 3 mL H_2O and 3.86 mL of 12.1 N HCl at 0-5^{o}C for 20 min. This diazonium salt was slowly added to a suspension of 3,5-dimethoxyaniline (Aldrich)(2.39 g, 0.016 mol) in 100 mL of H_2O at 5^{o}C. The pH of the mixture was adjusted to 8-9 by adding saturated sodium bicarbonate and left stirring overnight. The solid was filtered and purified by chromatography on silica with chloroform/ methanol as eluant. Yield 1.57 g, 31%. ^{1}H NMR (399 MHz, methanol-d_4) δ ppm 3.81 (s, 6 H) 3.86 (s, 6 H) 6.02 (s, 2 H) 7.61 (s, 2 H); ^{13}C NMR (100 MHz, methanol-d_4) δ ppm 55.02, 55.92, 89.86, 100.72, 123.95, 135.01, 146.49, 151.94, 154.26, 157.86; MS^+ obs'd: 362.129, calc'd for $C_{16}H_{18}N_4O_6$ $[M]^+$: 362.122

4,4'-(diazene-1,2-diyl)bis(3,5-dimethoxyaniline) (10): To a solution of (**9**) (0.43 g, 1.2 mmol) in 10 mL of dry DMSO was added 2.13 g (9.4 mmol) of $SnCl_2$. The mixture was stirred in an inert atmosphere for 48h then diluted with 100 mL of saturated NaCl. The pH was adjusted to 11-12 by adding of 3N NaOH solution and the mixture was extracted with EtOAc (5X30 mL). The organic layer was dried with anhydrous Na_2SO_4, evaporated and the product was separated

by silica gel chromatography with EtOAc/MeOH/NEt_3=100/20/5. Yield: 0.027 g, 7%. ^{1}H NMR (399 MHz, methanol-d_4) δ ppm 3.80 (s, 12 H) 6.07 (s, 4 H)**;** ^{13}C NMR (126 MHz, methanol-d_4) δ ppm 55.74, 67.68, 90.78, 130.96, 154.25**;** MS^+ obs'd: 333.1549, calc'd for $C_{16}H_{20}N_4O_4$ $[M+H]^+$: 333.1557

4,4'-diazene-1,2-diylbis(3,5-dimethoxychloroacetanilide) (1): 4,4'-(*E*)-diazene-1,2-diylbis(3,5-dimethoxyaniline) **(10)** 0.027 g (0.08 mmol) was dissolved in 2 mL of $CHCl_3$ with 0.056 mL (0.4 mmol) of NEt_3. To this mixture was slowly added at 5^0C 0.019 mL (0.0.027g, 0.4 mmol) of chloroacetylchloride. After stirring overnight the mixture was washed with water and extracted with ethyl acetate (3x20mL). The organic phase was dried, evaporated and separated by silica gel chromatography using $CHCl_3$ / Hexane – 5/1. Yield 0.025g, 62%; ^{1}H NMR (399 MHz, chloroform-*d*): δ ppm 3.87 (s, 12 H) 4.20 (s, 4 H) 6.98 (s, 4 H) 8.33 (s, 2 H); ^{13}C NMR (126 MHz, chloroform-*d*) δ ppm 42.95, 56.62, 96.69, 131.25, 138.26, 153.27, 163.82; MS^+ obs'd: 485.09985, calc'd for $C_{20}H_{22}Cl_2N_4O_6$ $[M+H]^+$: 485.0989

The synthetic route to compound **3** and **3-rf** is outlined in the scheme below:



SCHEME 2. Synthesis of **3** and **3-rf**. (g) HNO_3, Ac_2O, 0°C to rt, 4h, yield 19%; (h) Fe, NH_4Cl, MeOH, reflux 4 h, yield 57%; (i) $KMnO_4$-$CuSO_4.5H_2O$, dichloromethane, reflux, 48 h, yield 20%; (j) 4-aminophenylboronic acid hydrochloride, $Pd(PPh_3)_4$, $NaHCO_3$, anh. 1,2-dimethoxyethane, 90°C, 24 h, yield 54%; (k) chloroacetyl chloride, K_2CO_3, anh. DMF, 0°C-rt, 3 h, yield 62%; (l) 4-acetamidophenylboronic acid, $Pd(PPh_3)_4$, K_2CO_3, anh. 1,4-dioxane, 110°C, 24 h, yield 33%.

Synthesis of 5-bromo-1,3-dimethoxy-2-nitrobenzene (11): To an ice-cold solution of 1-bromo-3,5-dimethoxybenzene (Sigma-Aldrich) (10.9 g, 27.8 mmol) in acetic anhydride (100.0 mL) was added drop wise conc. HNO_3 (6.4 mL, 71.0 mmol) over a period of 30 min. The resulting

reaction was stirred at 0°C for 1 hour and at room temperature for 3 hours. It was then poured into ice-water with vigorous stirring; the yellow solid obtained was filtered and washed with water. The crude product was subjected to a silica gel column chromatography to isolate 5-bromo-1,3-dimethoxy-2-nitrobenzene (**11**) (2.5 g, 19% yield) as a pale yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.87 (s, 6H), 7.15 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm 58.0, 109.6, 125.5, 152.2.

Synthesis of 4-bromo-2,6-dimethoxyaniline (12): To a solution of **11** (1.0 g, 3.6 mmol) in MeOH (80 mL) was added water (15 mL) followed by Fe powder (0.6 g) and ammonium chloride (2.5 g). The resulting reaction was heated to reflux at 80°C for 2h, then 2.5 g of ammonium chloride was added and it was refluxed for further 2h. The completion of the reaction was monitored by TLC analysis. After cooling, the reaction was filtered, washed with methanol and dried under high vacuum. The crude product was subjected to silica gel column chromatography to isolate **12** (0.5 g, 57% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.77 (br, NH), 3.83 (s, 6H), 6.65 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 56.3, 107.9, 108.9, 124.9, 147.9.

Synthesis of 1,2-bis(4-bromo-2,6-dimethoxyphenyl)diazene (2): To a solution of 4-bromo-2,6-dimethoxyaniline **12** (0.19 g, 0.82 mmol) in dichloromethane (15 mL) was added a pre-ground mixture of $CuSO_4$ x 5 H_2O (0.45 g) and $KMnO_4$ (0.45 g) and the resulting heterogeneous reaction mixture was heated to reflux for 48h with vigorous stirring. After cooling to room temperature, the brown suspension was filtered through celite and washed with CH_2Cl_2. The crude product was subjected to silica gel column chromatography to obtain **2** (0.77 g, 20 %) as a red solid. ^{1}H NMR for a mixture of cis and trans in 1:1.2 ratio (400 MHz, $CDCl_3$) δ ppm 3.66 (s, 12H)(cis), 3.84 (s, 12H)(trans), 6.60 (s, 4H)(cis), 6.82 (s, 4H)(trans); ^{1}H NMR after heating at 50-55°C (400 MHz, $CDCl_3$) δ ppm 3.84 (s, 12H), 6.82 (s, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 56.8, 108.8, 122.8, 133.1, 152.7; ESI-HRMS: m/z calc'd for $C_{16}H_{17}N_2O_4Br_2$: 458.9549 $[M+H]^+$; found: 458.9547.

X-ray crystal structure of (2) with $CHCl_3$.

Crystals of **2** were grown by slow evaporation of a solution in MeOH-$CHCl_3$ (95:5). Crystal data for **2** (type I with $CHCl_3$): Monoclinic, P21/c, C_{18} H_{18} Br_2 Cl_6 N_2 O_4, a = 10.992(2) Å, b = 14.485(3) Å, c = 8.1333(15) Å, α=90°; β=100.71(4)°, γ=90°, V=1272.4(4) $Å^3$, Z=2, T=150(1) K, M=698.86, ρ=1.824 Mg/m3, $Mo_{K\alpha}$ radiation (λ=0.71073 Å). R_1=0.0265 for 147 parameters and 2969 unique reflections with (I>2σ(I)) and ωR_2=0.0596 for all 10236 reflections with GOF=1.030. Data have been deposited with the Cambridge Crystallographic Data Centre under accession number CCDC 908218. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

X-ray crystal structure of (2).

Crystals of **2** were grown by slow evaporation of a solution in MeOH-$CHCl_3$ (95:5). Crystal data for **2** (type II without solvent): Monoclinic, P21/c, C_{16} H_{16} Br_2 N_2 O_4, a = 12.2797(13) Å, b = 16.1354(18) Å, c = 8.5089(3) Å, α=90°; β=90.149(3)°, γ=90°, V=1685.9(3) $Å^3$, Z=4, T=250(1) K, M=460.13, ρ=1.813 Mg/m3, $Mo_{K\alpha}$ radiation (λ=0.71073 Å). R_1=0.0394 for 221 parameters and 3853 unique reflections with (I>2σ(I)) and ωR_2=0.0981 for all 8065 reflections with GOF=1.048. Data have been deposited with the Cambridge Crystallographic Data Centre under accession number CCDC 908219. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Synthesis of 4',4'''-(diazene-1,2-diyl)bis(3',5'-dimethoxy-[1,1'-biphenyl]-4-amine) (13): A mixture of 1,2-dimethoxy ethane (DME) (6 mL) and 1M aq. $NaHCO_3$ (1 mL) in an oven-dried pressure tube, pre-cooled under nitrogen gas, was thoroughly purged with nitrogen gas. To this solvent mixture were added **2** (0.1 g, 0.22 mmol), 4-aminophenylboronic acid hydrochloride (0.15 g, 0.87 mmol) (Sigma-Aldrich) and $Pd(PPh_3)_4$ (0.03 g, 0.025 mmol) under a nitrogen gas atmosphere and the tube was finally teflon stoppered and heated at 90°C for 24-36 hours. After cooling, the reaction was extracted with ethyl acetate, the combined organic layer was washed with brine, dried over anhyd Na_2SO_4 and concentrated under vacuum. The residue obtained was subjected to silica gel column chromatography to obtain **13** (0.057 g, 54%) as a mixture of trans and cis isomers in the ratio 2.6:1. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.60 (s, 12H)(cis), 3.79 (s, 12H)(trans), 5.22 (br, NH)(cis), 5.32 (br, NH)(trans), 6.57 (d, 4H, *J* = 8.8 Hz)(cis), 6.65 (d, 4H, *J* = 8.4 Hz)(trans), 6.66 (s, 4H)(cis), 6.89 (s, 4H)(trans), 7.37 (d, 4H, *J* = 8.8 Hz)(cis), 7.49 (d, 4H, *J* = 8.4 Hz)(trans); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm (trans) 56.9, 103.1, 114.7, 127.6,

128.2, 132.4, 142.8, 149.7, 152.9; ESI-HRMS: m/z calc'd for $C_{28}H_{29}N_4O_4$: 485.2183 $[M+H]^+$; found: 485.2187.

N,N'-(diazene-1,2-diylbis(3',5'-dimethoxy-[1,1'-biphenyl]-4',4-diyl))bis-2-chloroacetamide (3): To a mixture of **13** (10 mg, 0.021 mmol) and K_2CO_3 (8.3 mg, 0.06 mmol) in anhydrous DMF (1.5 mL) was added dropwise a solution of α-chloroacetyl chloride (6.7 mg, 0.06 mmol) in DMF (0.2 mL) at ice-cold condition. The reaction was stirred at room temperature for 3h and the DMF was removed under high vacuum. The residue was suspended in water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified with silica gel column chromatography to give **3** (8.0 mg, 62% yield) as red solid consisting of 85% trans and 15% cis isomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 3.64 (s, 12H)(cis), 3.82 (s, 12H)(trans), 4.24 (s, 4H)(cis), 4.28 (s, 4H)(trans), 6.80 (s, 4H)(cis), 7.03 (s, 4H)(trans), 7.62 (d, 4H, *J* = 8.8 Hz) (cis), 7.67 (d, 4H, *J* = 8.8 Hz)(cis), 7.71 (d, 4H, *J* = 8.8 Hz)(trans), 7.80 (d, 4H, *J* = 8.8 Hz)(trans), 10.36 (br, NH)(cis), 10.43 (brs, NH)(trans); ESI-HRMS: m/z calc'd for $C_{32}H_{31}N_4O_6Cl_2$: 637.1615 $[M+H]^+$; found: 637.1624.

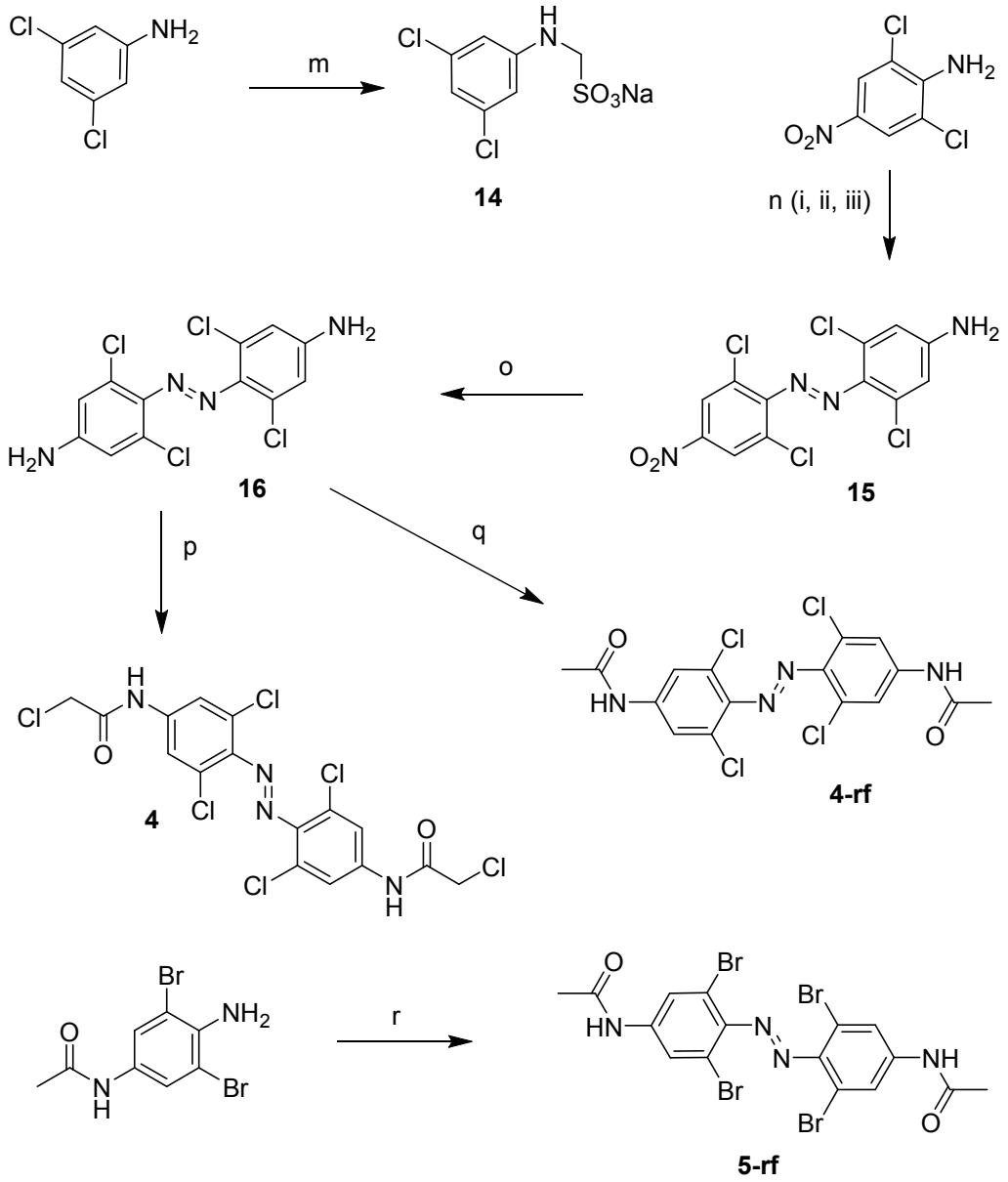
Synthesis of N, N'-(diazene-1,2-diylbis(3',5'-dimethoxy-[1,1'-biphenyl]-4',4-diyl))diacetamide (3-rf): A mixture of 1,4-dioxane (6 mL) and 0.6M aq. K_2CO_3 (2 mL) in an oven-dried pressure tube, pre-cooled under nitrogen gas, was thoroughly purged with nitrogen gas. To this solvent mixture were added **2** (77.0 mg, 0.17 mmol), 4-acetamidophenylboronic acid (90 mg, 0.50 mmol) (Sigma-Aldrich) and $Pd(PPh_3)_4$ (17.0 mg, 0.014 mmol) under nitrogen gas atmosphere and the tube was finally teflon stoppered and heated at 110^{o}C for 24 hours. After cooling, 1,4 dioxane was removed using a rotary evaporator, and the reaction was extracted with ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue obtained was subjected to silica gel column chromatography to obtain **3-rf** as reddish brown solid (31.0 mg, yield 33%) containing 85% trans and 15% cis isomers. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 2.03 (s, 6H)(cis), 2.07 (s, 6H)(trans), 3.64 (s, 12H)(cis), 3.82 (s, 12H)(trans), 6.79 (s, 4H)(cis), 7.01 (s, 4H)(trans), 7.62 (brs, 4H), 7.70 (d, 4H, *J* = 8.8 Hz), 7.75 (d, 4H, *J* = 8.8 Hz), 9.99 (s, NH)(cis), 10.06 (s, NH)(trans); ^{13}C NMR (100 MHz, DMSO-d_6) *δ* ppm 24.8, 56.0, 57.0, 79.9, 104.1, 119.7, 119.8,

127.6, 127.9, 133.1, 134.9, 140.0, 141.8, 150.3, 152.8, 169.1; MS-DART @450C: m/z calc'd for $C_{32}H_{32}N_4O_6$: 569.2400 $[M+H]^+$; found: 569.2384.

X-ray crystal structure of (3-rf).

Crystals of **3-rf** were grown by slow evaporation of a solution in MeOH-dichloromethane (95:5). Crystal data for **3-rf**: Monoclinic, C2/c, C_{32} H_{34} N_4 O_7, a = 36.643(5) Å, b = 8.7979(12) Å, c = 19.260(3) Å, α=90°; β=111.473(3)°, γ=90°, V=1685.9(3) $Å^3$, Z=8, T=150(1) K, M=586.63, ρ=1.349 Mg/m^3, $Mo_{K\alpha}$ radiation (λ=0.71073 Å). R_1=0.0485 for 405 parameters and 6697 unique reflections with (I>2σ(I)) and ωR_2=0.1335 for all 25302 reflections with GOF=1.006. Data have been deposited with the Cambridge Crystallographic Data Centre under accession number CCDC 908220. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

Syntheses of the tetra-*ortho*-halo derivatives (**4**, **4-rf**, and **5-rf**) are outlined in the scheme below:



SCHEME 3. Synthesis of **4, 4-**and **5-rf**. (m) HCHO, $NaHSO_3$, EtOH/H_2O, 70°C, 24 h, yield 84%; (n) i) $NaNO_2$, H_2SO_4, AcOH/DMF, 0° C, 2 h, ii) **14**, DMF, 0°C to rt, 72 h, iii) 20% NaOH, 70°C, 2 h, overall yield 26%; (o) Na_2S, 1,4-dioxane/EtOH/H_2O, 90°C, 24 h, yield 42%; (p) chloroacetyl chloride, pyridine/diethyl ether, 0°C to rt, 1 h, yield 67%; (q) acetic anhydride, pyridine, 0°C to rt, 12 h, yield 70%; (r) AgO, acetone, rt, 72h, yield 10%.

Synthesis of sodium 3,5-dichloroanilinomethanesulfonate (14): Synthesis of sodium 3,5-dichloroanilinomethanesulfonate was adopted from our earlier paper[3]. Formaldehyde (0.28 g, 9.26 mmol) was added to a solution of sodium hydrogen sulfite (0.96 g, 9.26 mmol) in water (1

mL). After stirring at room temperature for 1h, the reaction was diluted with water (9 mL) and a solution of 3,5-dichloroaniline (Sigma-Aldrich) (1.5 g, 9.26 mmol) in EtOH (10 mL) was added. It was stirred at 70°C for 24h. After cooling it to room temperature, EtOH was removed by rotary evaporation. The resulting reaction mixture was cooled in an ice bath and the precipitate obtained was filtered, washed with cold dichloromethane and dried in vacuo. The product **14** (2.161 g, 84 %) was pure enough to use for the next step. ^{1}H NMR (400 MHz, DMSO-d_6/D_2O): δ ppm 3.87 (br, NH), 3.87 (br, NH), 3.88 (s, 2H), 6.56 (s, 1H), 6.70 (d, J = 2 Hz, 2H); ^{13}C NMR (400 MHz, DMSO-d_6) δ ppm 96.8, 110.8, 114.1, 133.8, 150.6.

Synthesis of 3,5-dichloro-4-[(2,6-dichloro-4-nitrophenyl)diazenyl]aniline (15): Sodium nitrite (0.20 g, 2.88 mmol) was added to a stirred solution of concentrated H_2SO_4 (2 mL). The mixture was heated at 70°C and then cooled to 0°C in an ice bath. To this ice cold mixture was added a solution of 2,6-dichloro-4-nitroaniline (TCI America) (0.60 g, 2.88 mmol) in a mixture of acetic acid/DMF (3.5 mL:9 mL). The reaction was stirred at 0°C for 2 hours. To this diazotized solution, a solution of sodium 3,5-dichloroanilinomethanesulfonate **14** (0.4 g, 1.44 mmol) in DMF (6 mL) was added drop by drop. The reaction mixture was stirred at 0°C for 1h, then warmed to room temperature and stirred for 72h. The mixture was poured on ice, and the precipitate obtained was filtered and washed with H_2O. It was further basified with 20% aqueous sodium hydroxide (15 mL) and heated to 70°C for 2 hours. After cooling to room temperature, the reaction mixture was extracted with EtOAc. The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The crude product was purified with a silica gel column chromatography to yield **15** (0.136 g, 26 %) as a reddish brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 4.28 (br, NH), 6.73 (s, 2H), 8.27 (s, 2H); ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm 104.3, 115.0, 124.2, 127.5, 132.9, 149.46; ESI-HRMS: *m/z* calc'd for $C_{12}H_6Cl_4N_4O_2$: 378.9333 $[M+H]^+$; found: 778.9317.

Synthesis of 3,5-dichloro-4-[(2,6-dichloro-4-aminophenyl)diazenyl]aniline (16): To a solution of **15** (0.12 g, 0.31 mmol) in a mixture of dioxane/EtOH/H_2O (8 mL:2 mL:0.5 mL) was added Na_2S (0.073 g, 0.93 mmol) and the reaction mixture was heated at 90°C for 24h. Since reaction did not complete as judged by TLC analysis, an additional Na_2S (0.11 g, 1.41 mmol) was added and it was stirred at 90°C for further 5h. After cooling, the reaction was concentrated by rotary evaporation and extracted with ethyl acetate. The organic layer was washed with brine.

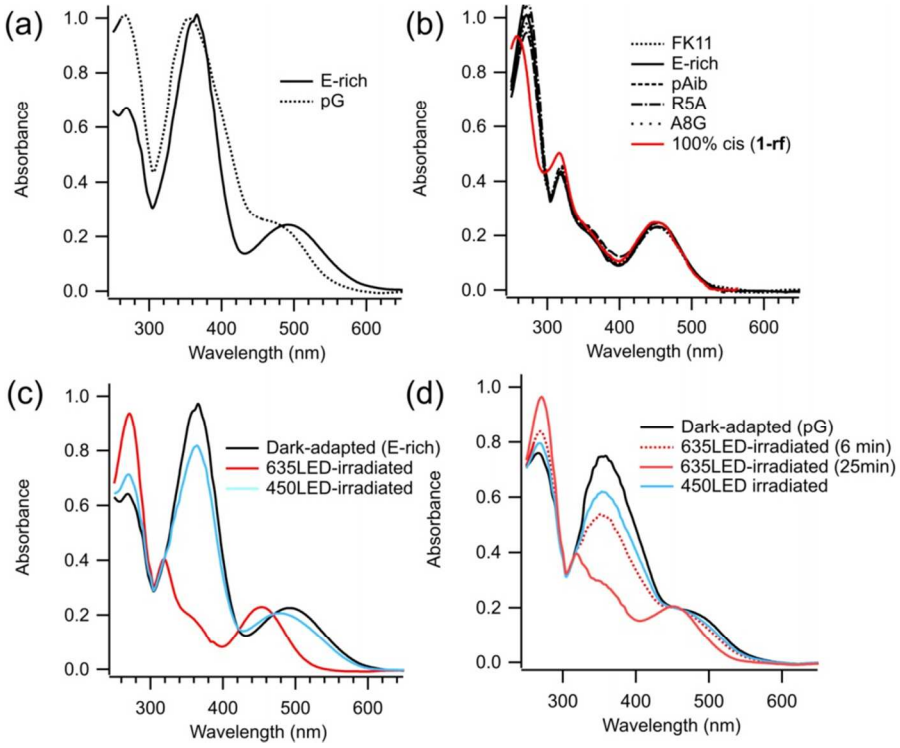
The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo. Purification by silica gel column chromatography gave **16** (0.046 g; 42 %) as a dark red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 6.52 (br, NH), 6.69 (s, 4H); ^{13}C NMR (400 MHz, $CDCl_3$): δ ppm 115.4, 128.4, 144.9, 149.7; ESI-HRMS: *m/z* calc'd for $C_{12}H_8Cl_4N_4$: 348.95813 $[M+H]^+$; found: 348.95840.

Synthesis of diazene-1,2-diylbis(3,5-dichloro-4,1-phenylene)bis(2-chloroacetamide) (4): To an ice cold solution of **16** (32 mg, 0.09 mmol) in an ether/pyridine mixture (3:1.5 mL) was added dropwise a solution of chloroacetic anhydride (47 mg, 0.27 mmol) in ether (3 mL). The resulting reaction was warmed to room temperature and allowed to stir for 1h. The solvent was removed under vacuum. The residue obtained was subjected to a silica gel column chromatography to obtain **4** (30 mg, yield 67%) as a orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 4.32 (s, 4H), 7.87 (s, 4H), 10.84 (br, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 43.9, 120.0, 128.0, 140.8, 142.1, 166.2; ESI-HRMS: m/z calc'd for $C_{16}H_{11}N_4O_2Cl_6$: 500.9007 $[M+H]^+$, found: 500.8991.

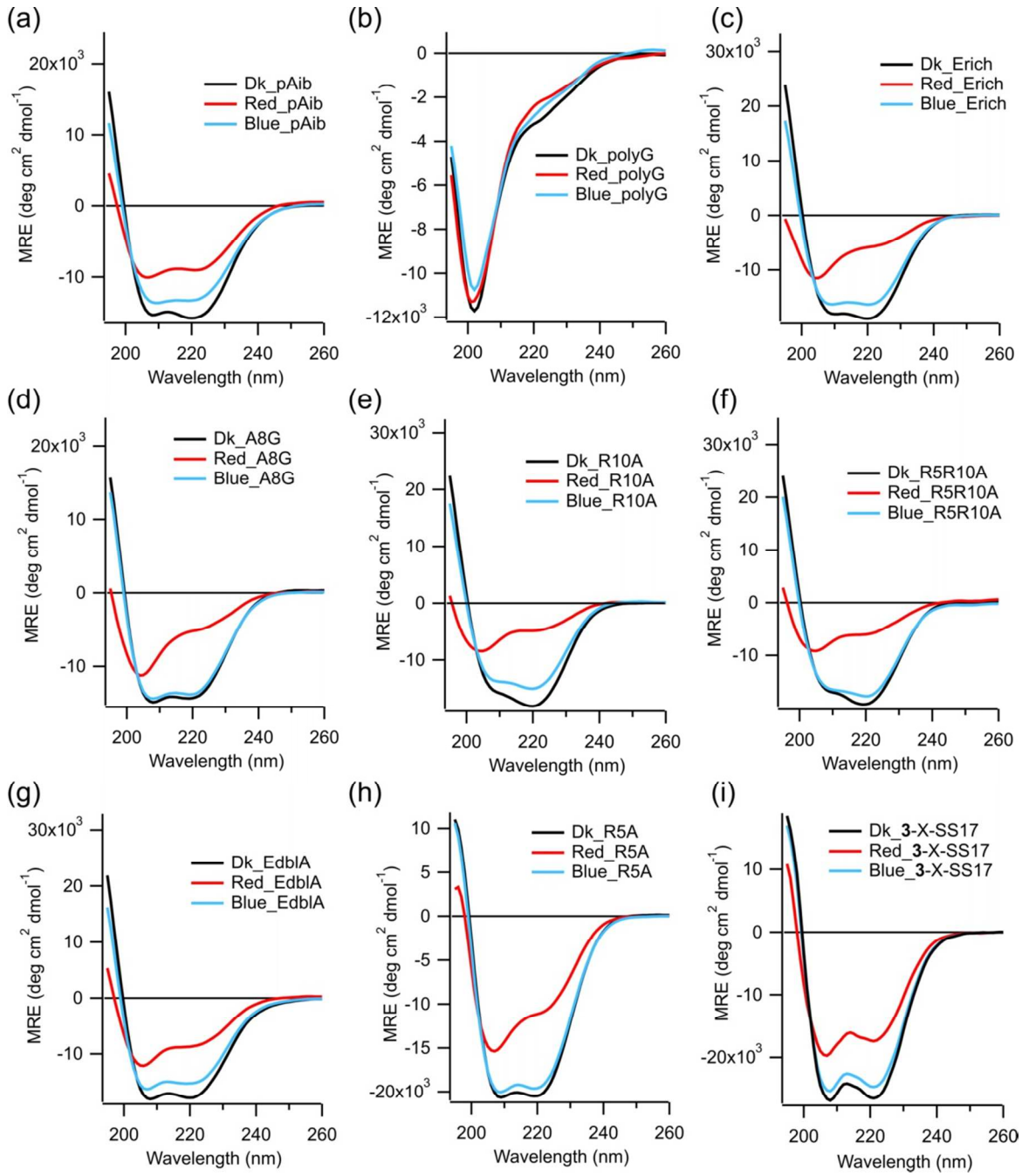
Synthesis of 3,5-dichloro-4-[(2,6-dichloro-4-acetanilido)diazenyl]acetanilide (4-rf): To a ice-cold solution of **16** (0.046 g, 0.13 mmol) in pyridine (2 mL) was added acetic anhydride (50 µL, 0.054 g, 0.53 mmol) and the mixture was stirred at room temperature for 12h. Then the solvent was removed by rotary evaporation and further dried in vacuo. The residue was recrystallized in DMF/H_2O to yield **4-rf** (0.039 g, 70 %) as a orange solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.08 (s, 6H), 7.85 (s, 4H), 10.47 (s, NH); ^{13}C NMR (400 MHz, DMSO-d_6): δ ppm 24.9, 119.7, 128.3, 141.9, 142.9, 170.1; ESI-HRMS: *m/z* calcd for $C_{16}H_{12}Cl_4N_4O_2$: 432.9792 $[M+H]^+$; found: 432.9784.

Synthesis of 3,5-dibromo-4-[(2,6-dibromo-4-acetanilido)diazenyl]acetanilide (5-rf): A heterogeneous mixture of 4-acetamido-2,6-dibromoaniline (Princeton Biomolecular Research Inc. Monmouth Junction, New Jersey, USA) (0.300 g) and freshly prepared silver (II) oxide (1.0 g) in dry acetone (45 mL) was vigorously stirred at room temperature under dark condition. After 2 days of stirring, more silver (II) oxide (1.0 g) was added and stirred for further 3 days. The completion of the reaction was judged by TLC. The reaction mixture was filtered through celite pad and the solvent was removed by rotary evaporation. The crude product was subjected to a

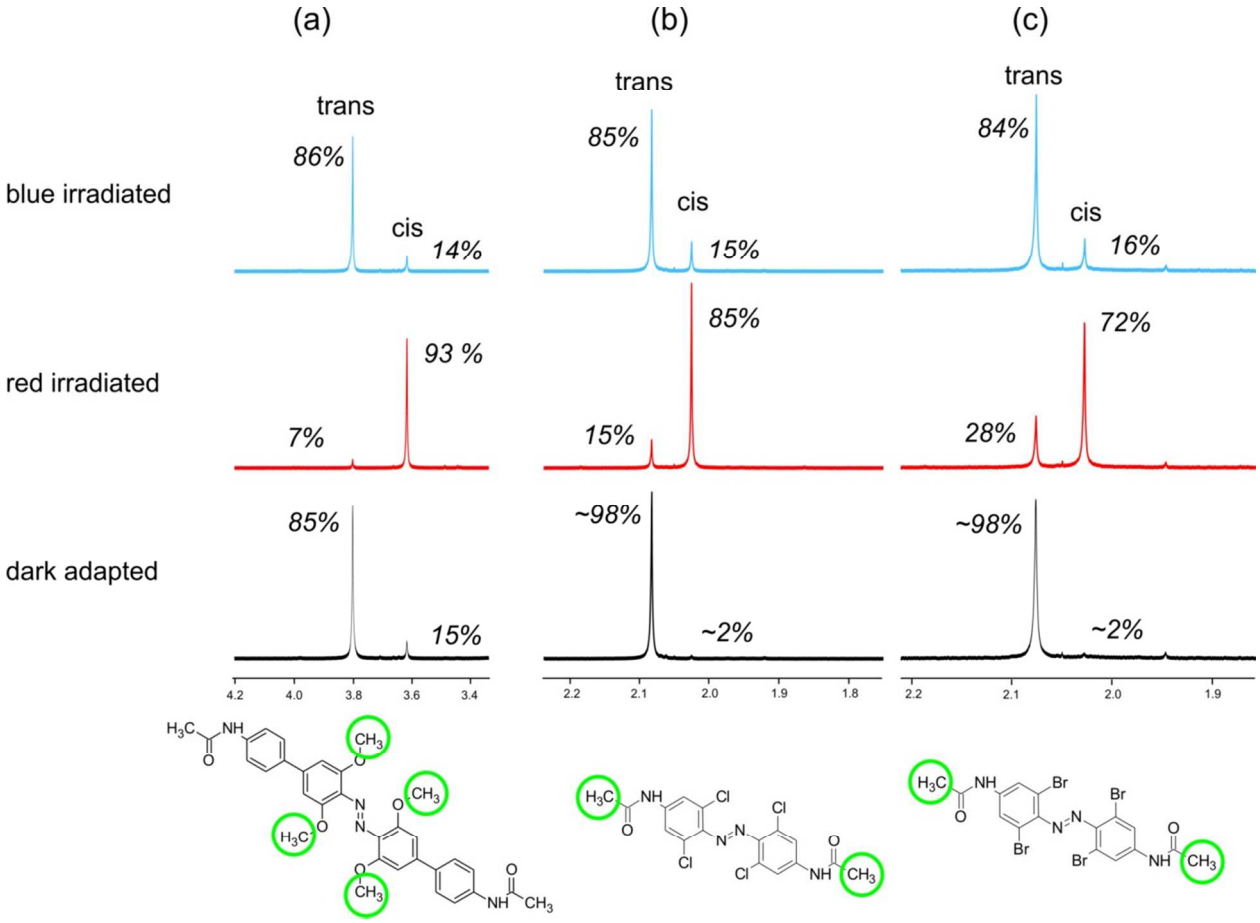
silica gel column chromatography to obtain **5-rf** (0.06 g, yield 10%) as dark brown solid. ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 2.08 (s, 6H), 8.06 (s, 4H), 10.42 (br, NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ ppm 24.7, 116.8, 123.1, 141.9, 143.0, 169.8; ESI-HRMS: m/z calc'd for $C_{16}H_{13}N_4O_2Br_4$: 608.7766 $[M+H]^+$, found: 608.7761.



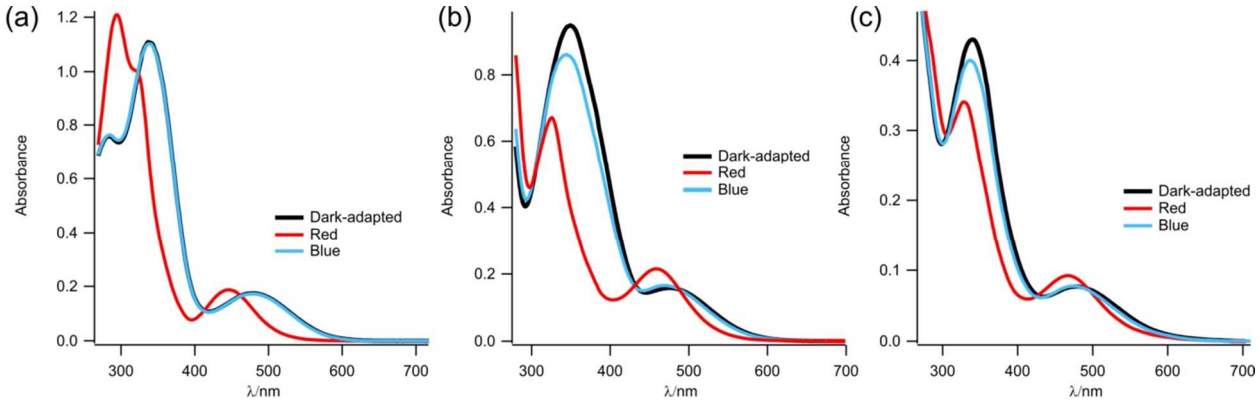
Supplementary Figure 1: The dark-adapted trans forms of peptides cross-linked with **1** exhibited a range of spectra with varying degrees of red-shifting of the n- π* bands consistent with a dependence of the planarity and HOMO/LUMO energy on detailed solvation patterns. (a) dark-adapted (*trans*) spectra of two peptides (See Table 1 for sequences) with very different n-π* transitions. (b) Irradiated (red light 635 nm; 90 mW/cm^2) spectra of all peptides are nearly identical and correspond to ~98% cis. (c) Reversion to the *trans* isomer can be accomplished with blue light (450 nm). (d) For peptides such as pG (See Table 1 for sequences) that have relatively less red shifted n-π* transitions, longer irradiation times are required for *trans* to *cis* conversion.



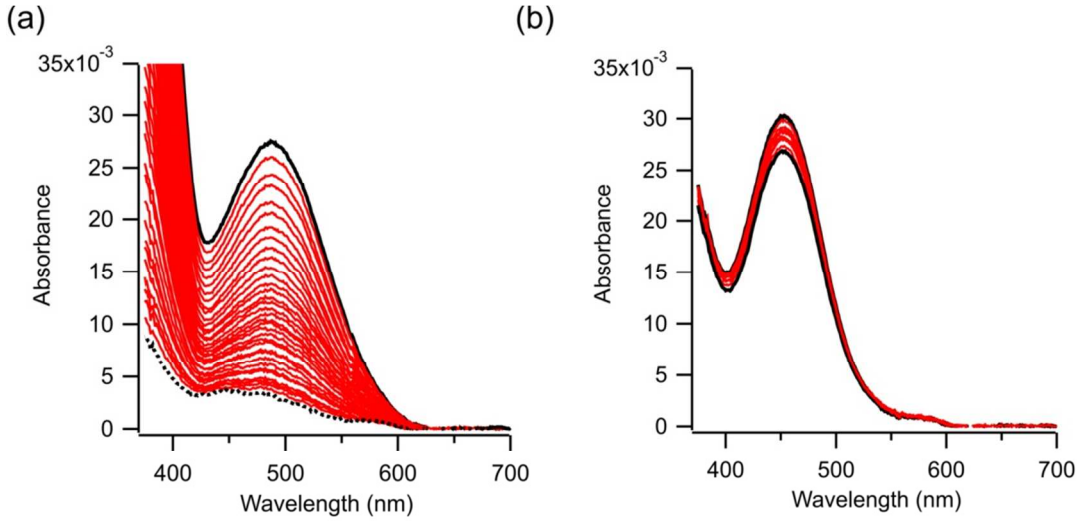
Supplementary Figure 2: Photo-control of peptide structure by red light. Circular dichroism spectra of peptides (see Supplementary Table 1 for sequences) cross-linked with **1** (a-h) or **3** (i) measured at 20°C, 10 mM sodium phosphate buffer pH 7.0. Black lines are fully trans spectra, red lines are after irradiation with 635 nm light (>98% cis). Blue lines are after irradiation at 450 nm (80-85% trans). The polyG peptide is unstructured under all irradiation conditions.



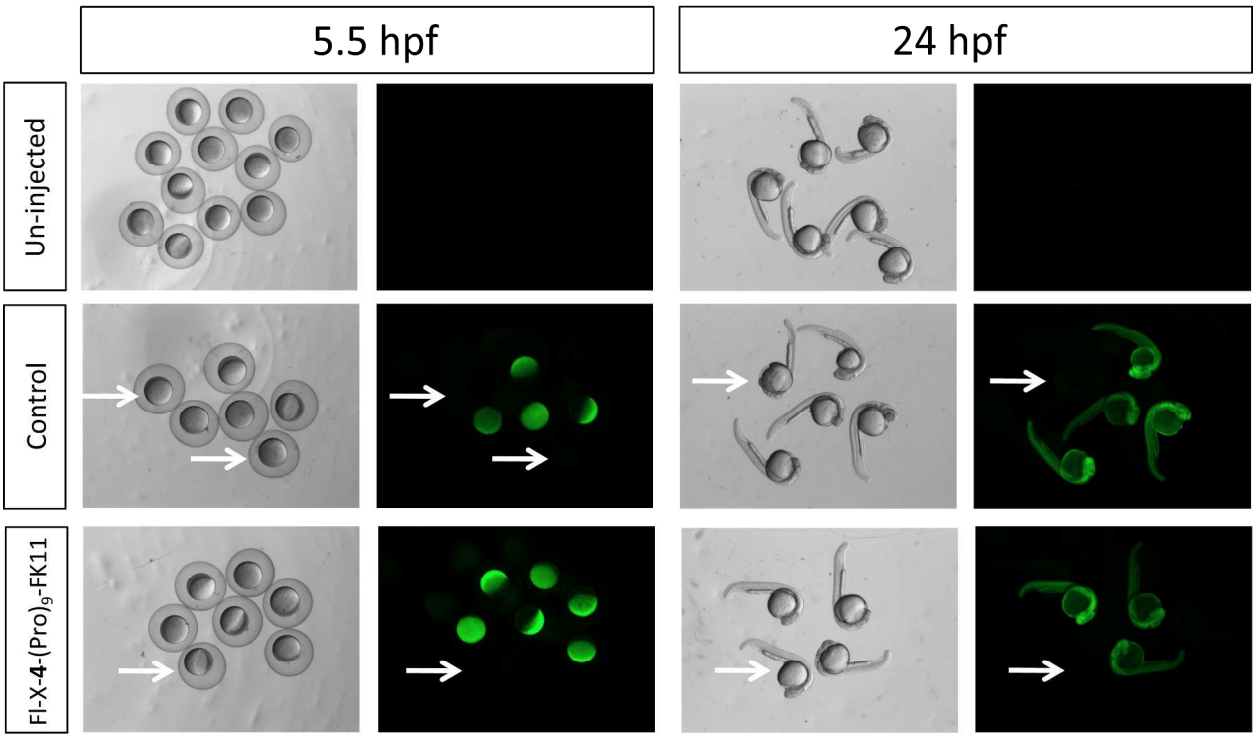
Supplementary Figure 3: ^{1}H NMR spectra for (a) **3-rf**, an expansion of the methoxy region, and for (b) **4-rf** and (c) **5-rf**, an expansion of the acetyl region. The protons responsible for the signals are indicated with green circles on the structures. A 3.5-4.5 mM solution in DMSO-d_6 in the NMR tube was irradiated while rotating the tube. After irradiation with a red LED (635 nm, 90 mW/cm^2) for 30 min or 15 min with a blue LED (450 nm, 40 mW/cm^2), the spectra were acquired immediately at 25^{o}C. For recording the dark-adapted spectra, the samples were pre-heated at 65-70^{o}C for 4-5h protected from light. For the tetramethoxy compound (**3-rf**) this is not long enough for complete conversion to the trans form.



Supplementary Figure 4: Red/blue light switching (indicated with respective colors) of **3-rf** (a), **4-rf** (b) and **5-rf** (c) in DMSO at 25°C **.**



Supplementary Figure 5: Test for photoswitch reduction by glutathione *in vitro*. (a) The trans isomer of photoswitch **1** cross-linked to FK11 was incubated in 10 mM reduced glutathione for 4 h at 25°C. The solid black curve is the initial scan and the dotted black curve is the final scan. The red curves are intermediate scans. A half-life of ~1h is observed. (b) The cis isomer of photoswitch **1** cross-linked to FK11 was incubated in 10 mM reduced glutathione for 4 h at 25°C. Very little change is observed and photoswitching was intact after incubation. Photoswitch **3** cross-linked to FK11 exhibited similar behavior.



Supplementary Figure 6: Zebrafish embryos (n>25 for each group) were injected at the 1-2 cell stage with a fluorescently tagged control peptide Fl-[D](Pro)$_9$-FK11 uncross-linked, or crosslinked with **4** (Fl-X-4-[D](Pro)$_9$-FK11) and subsequently imaged at 5.5 hpf or 24 hpf. Left panels show DIC images and right panels show fluorescent images of the same cohort for each time point. Each group of injected embryos were exposed to red LED light for ~ 5 min post-injection and then again after ~ 24 hrs. In each injected cohort, several embryos were left un-injected, but still exposed to red LED light as the others (arrows) to serve as controls. A separate cohort of completely untreated and uninjected embryos also served as a control. In all cases, there was no evidence of gross morphological changes or delays in the rate of development indicating no apparent peptide toxicity.

UV and CD spectroscopy

Ultraviolet absorbance spectra were obtained using either a Perkin-Elmer Lambda 25 or 35 spectrophotometer or a diode array UV–Vis spectrophotometer (Ocean Optics Inc., USB4000)

coupled to temperature controlled cuvette holders (Quantum Northwest, Inc.). Measurements of thermal relaxation rates were done be carrying out wavelength scans with a narrow slit width at specific time intervals. In this manner any effects of the measuring beam on the rate of isomerization were minimized. Irradiation of the sample (at 90^{o} to the light source and detector used for the absorbance measurements) was carried out using a high intensity LEDs of various wavelengths (~200 mW, Opto Technology, Inc., Wheeling, IL, Model OTLH-0480-UV (365nm)); Luxeon III Star LED Royal Blue (455 nm) Lambertian operating at approximately 40 mW/cm^2 (700 mA); red LEDs (Cree XP-E 1-Up Indus Star, 700 mA, 80 mW/cm^2 (660 nm), (LedEngin LZ4-40R200-0000, 700 mA, 635 nm, 90 mW/cm^2).

A molar extinction coefficient of ~4000 $M^{-1}cm^{-1}$ at 455 nm for the tetra-*ortho*-methoxy cross-linker (**1** and **3**) (cis isomer) was used to calculate concentrations of cross-linked peptides. For linker **4**, molar extinction coefficient of 3300 $M^{-1}cm^{-}1$ (465 nm) in DMSO for trans form was used to calculate the concentrations of cross-linked peptides.

CD spectra were obtained on an OLIS RSM instrument coupled with a Quantum Northwest peltier temperature control unit for both dark-adapted and irradiated peptides. Each spectrum was acquired from 260 nm to 190 nm (1 nm step) with an integration time of 2 seconds at each wavelength. Scans were smoothed and baseline subtracted. Measurements were carried out in a 0.1 cm cuvette with peptide concentrations of 5-50 μM in 5-10 mM sodium phosphate buffer at pH 7.0. For spectra of irradiated peptides, samples were irradiated using LEDs directly in the CD instrument.

Tests for stability to glutathione reduction

Cross-linked peptides (~10 μM) in 0.1 M pH 7.0 sodium phosphate buffer at 25°C were either dark adapted or fully isomerized (red light) to cis isomers. A stock solution of 0.125 M reduced glutathione (prepared in 0.5 M sodium phosphate buffer pH 7.0) was then added to give a final glutathione concentration of 10 mM. The final pH of the test solution was 7.0. Samples were incubated at $25^{o}C$ and absorbance scans recorded periodically. Isomerization was checked after 1 and 2 days at $25^{o}C$.

Design and synthesis of fluorescent reporter peptides:

The fluorescent reporter sequence (Fluorescein-[D]-PPPPPPPPPEACAREAAAREAACRQ; Fl-$(Pro)_9$-FK11) was synthesized by solid phase peptide synthesis as described above. Fluorophore labeling was performed during solid phase synthesis using 5(6)-carboxyfluorescein. The fluoresceinated peptide was purified by reverse-phase HPLC as described above. Cross-linking was performed as follows: 2 mM of Fl-$(Pro)_9$-FK11) and 2 mM of tris(carboxyethyl)phosphine (TCEP) in 50 mM sodium phosphate buffer (pH 8) were incubated for 1 h at room temperature to ensure cysteine residues were in their reduced state. An equivalent volume of dimethylformamide (DMF) was added to make a 50:50 (v/v) DMF: buffer mixture, giving a final concentration of 1 mM peptide. A final concentration of 2 mM of the linker was then added and heated to 40°C with stirring for 24 h. The cross-linked peptides was purified by reverse-phase HPLC and compositions identified by ESI-MS.

Fluorescence spectra and time courses *in vitro*.

Fluorescence spectra and time courses were measured on a Perkin-Elmer LS55B spectrofluorometer, in a 0.15 cm pathlength cuvette at 23^{o}C in phosphate buffered saline, pH 7.3 (PBS). The excitation beam was set to 460 nm to trigger cis-to-trans photoisomerization as well as to cause excitation of the fluorophore. Red light (635 nm) was used to form the cis state. The change in intensity was monitored at the fluorophore emission maximum (520 nm) as a function of time. The excitation slit width was varied to achieve different cis-to-trans switching rates.

Computational Methods

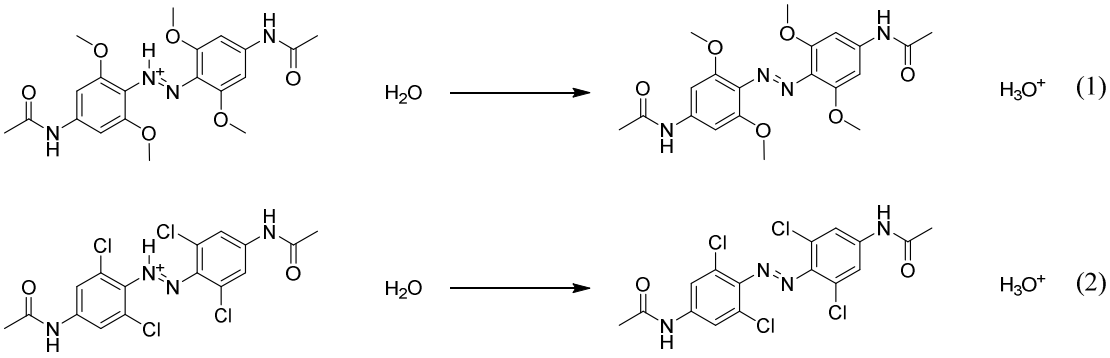
The first six singlet excited-states of molecules described in Figure 2 were calculated with TD-DFT using the Gaussian 09 suite of programs[4] and the nonlocal hybrid Becke three-parameter Lee-Yang-Parr (B3LYP) functional[5] with the 6-311++G** basis set for both the X-ray geometries and optimized geometries. The X-ray geometries of **2** were taken from the crystal structures with co-crystallized chloroform and one without co-crystallized solvent. The non-optimized geometry with water was generated starting from the crystal structure with co-crystallized chloroform and using Gaussview 5.0 [6] to remove the CCl_3 from chloroform and add

OH to make a water molecule. The geometries of these three configurations were optimized with DFT at the same level of theory and basis set used for the TD-DFT calculations.[7] The calculated UV-Vis spectra shown were generated from the TD-DFT data with Gaussview by applying a Gaussian function with 0.2 eV peak half width at half height placed on each transition.

The optimized geometry of **2** was employed to generate non-optimized structure of 1-rf by replacing bromine atoms with acetamide moieties. Methoxy groups in **1-rf** were replaced by chlorine atoms to construct **4-rf**. These structures were then optimized. All calculations were performed at the B3LYP/6-311++G** level of theory using Gaussian 09. Gaussview 5.0 was utilized to manipulate the structures.

Initial geometries for azonium ions were generated by adding one proton to a nitrogen atom of the azo group in either of optimized **1-rf** or **4-rf**. Energy minimization was performed on these species along with water molecules and hydronium ions. Harmonic vibrational frequency calculations were performed on all optimized structures to obtain zero point energies and thermal correction terms, which provided enthalpy values at 298.15K. No imaginary frequencies were found confirming that the predicted geometries represent the local minima on the potential energy surface.

The results were used to calculate the enthalpies of reactions shown below where protons are transferred from the azonium species to a water molecule:



ΔH (1)	+97.1 kcal mol^{-1}
ΔH (2)	+67.0 kcal mol^{-1}

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