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Maleimide-Functionalized Photochromic Spirodihydroindolizines

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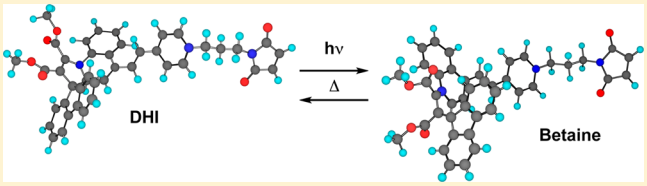
Supporting Information

ABSTRACT: Two photochromic spirodihydroindolizine/betaine systems for tethering to peptides and proteins via a maleimide function have been prepared. The absorption spectra of the betaines are in the red region of the visible spectrum and in the near-IR spectral domain, which are suitable energies of light for future in vivo applications. The half-times of cyclization have been determined for both DHI/betaine systems. The findings are consistent with a thermal barrier of varying size between the *transoid* and *cisoid* conformers of the betaines.



INTRODUCTION

Photochromic spirodihydroindolizines (DHIs) undergo significant changes in their shapes and dipole moments when switched from the closed dihydroindolizine form to the open betaine form. The photochemical ring-opening of the DHIs to the betaines is a conrotatory 1,5 electrocyclic reaction, whereas the thermal ring-closing occurs in the disrotatory mode.[1−3] The back-reaction from the betaine to the DHI can be achieved photochemically for some photochromic systems;[4] however, this reaction is mechanistically still not fully understood today. The DHI/betaine system has been used for the design of systems for information storage[4] and logical gates.[5−8] The next logical step is the application of photochromic substances as on- and off-switches for biological processes in model systems, followed by in vivo studies. Bayley and co-workers have investigated the photochemical isomerization of tethered azobenzene in the nanopore of the channel protein α-hemolysin.[9] However, azobenzene has a tendency toward inversion and is, therefore, only of limited value as a trigger for conformational changes.[10] The photochromic spiropyran/merocyanine system has been used as an optical gate of a cysteine-modified MscL-channel (mechanosensitive channel of large conductance).[11,12] Whereas azobenzenes show pronounced changes in geometry[13] but only minor changes in their dipole moments ($\Delta m \leq 3$ D), spiropyranes undergo drastic polarity changes but almost no changes in geometry ($\Delta m \leq 15$ D).[14] The ideal optical switch should combine maximal changes in geometry with significant changes in polarity.

The ultimate model system for this endeavor is the vision process, in which an organic molecule (11-*cis*-retinal) is attached to a protein from the opsin family via an imide-bond-forming rhodopsin[15,16] and exposure to light triggers the isomerization from 11-*cis*-retinal to *all-trans*-retinal.[17] The photochemically induced shape-shift causes rhodopsin to convert to metarhodopsin II, which then activates transducin.[11] The result is a series of biochemical events that eventually leads to the hyperpolarization of a membrane, which leads to an electrical impulse being sent to the brain.[11] The performance of proteins labeled with azobenzenes, spiropyrans, and fulgides has been investigated since the mid-1990s.[18,19] Because of its superior photochemical properties, it is anticipated that the photochromic spirodihydroindolizine/betaine system will lead to improved performance of labeled proteins and increased numbers of conversion cycles.

Here, we report the selection and subsequent synthesis of spirohydroindolizines for biological and in vivo applications. The DHIs will feature a maleimide group because it permits the linking of the photochromic molecule to peptides/proteins via cysteine mutants.[20] Maleimides exhibit Michael reaction-type addition to thiols under *in vivo* conditions without the necessity

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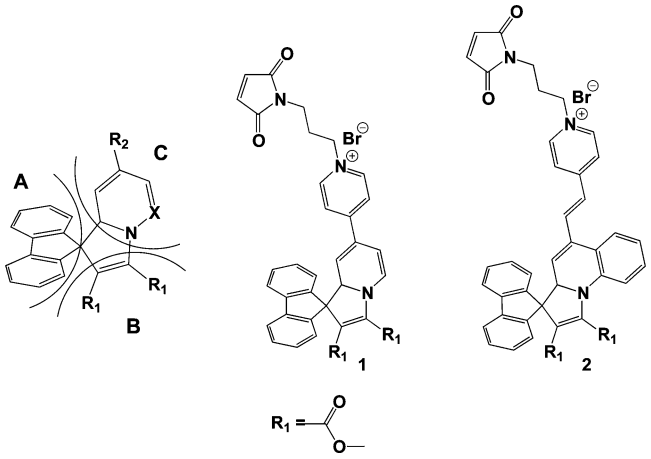
of adding other reagents to the system.[21] They are ideal for coupling reactions in the generally reducing environment of the cytoplasm. The photophysical properties of DHI can be tuned by chemical changes in their A (aromatic), B (double bond), and C (heterocyclic) regions (Scheme 1).[2] The 9*H*-fluorene unit in region A has been proven to yield DHIs that are superior in stability, and ester substituents in region B further increase the stability of the DHI/betaine toward thermal and photochemical decomposition reactions. The structure of the heterocyclic region C has the greatest influence on the color of the resulting betaine and the kinetics of the thermal back reaction. The important "regions" in spirodihydroindolizines and the two target DHIs, **1** and **2**, are shown in Scheme 1.

Scheme 1. (Left) Spirodihydroindolizine (A, Aromatic Region; B, Double-Bond Region; C, Heterocyclic Region; R_1 = $-COOCH_3$, R_2 = Extension of the Aromatic System); (Middle) 4-[2′,3′-Bis(methoxycarbonyl)-8a′*H*-spiro(fluorene-9,1′-indolizin)-7′-yl]-1-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]pyridin-1-ium Bromide (1); (Right) (*E*)-4-[2-[1′,2′-Bis(methoxycarbonyl)-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinolin)-5′-yl]vinyl]-1-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]pyridin-1-ium Bromide (2)



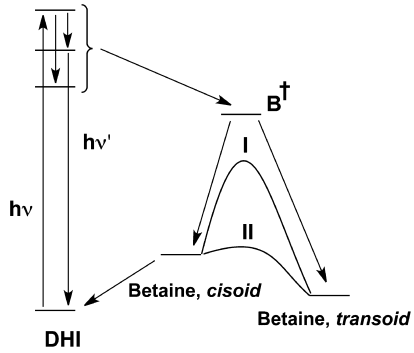
For the design of photochromic switches for biological applications, region R_2 of **1** and **2** (Scheme 1) should be large enough to permit a maximal "shape shifting" effect. Furthermore, this strategy enables the fine-tuning of the electronic density of the heterocyclic region C to achieve lifetimes of the betaine in the microsecond to millisecond range. This latter point is important because this range of lifetimes corresponds to biological processes such as neurostimulation.[22]

RESULTS AND DISCUSSION

1. Selection of the Photochromic Target Molecules. The selection of the two target DHIs **1** and **2** was based on previous studies[23,24] in which related systems, that do not feature quaternization at the aromatic nitrogen, have been synthesized and investigated. From these studies, it was expected that the betaine form of **1**, **1B**, will feature an absorption maximum in the red region of the visible electromagnetic spectrum, whereas the betaine derived from **2**, **2B**, will possess a maximum in the near-infrared region. Both wavelengths are suitable for in vivo studies, taking into account the absorption and scattering spectra of tissue and water.[25]

As already discussed, the photochemical ring-opening of DHIs to betaines is a conrotatory 1,5 electrocyclic reaction. The disrotatory thermal ring-closing occurs from the *cisoid* betaine. The lifetime of the cisoid betaine is usually of the order of 10−500 μs in typical organic solvents at room temperature, indicating that the activation energy for this reaction must be small. The lifetime of the *transoid* betaine can be of the order of seconds to hours, depending on the size of the kinetic barrier between the *transoid* and the *cisoid* betaine. In DHI/betaine systems that do not form stable betaines at room temperatures, the size of the thermal barrier between *transoid* and the *cisoid* betaine is small, thus permitting a rapid conversion from transoid to cisoid betaine and then disrotatory ring closure (Scheme 2).

Scheme 2. Simplified Generalized Photochemical Reaction Scheme for the DHI/Betaine System Showing the Generation of *Cisoid* and *Transoid* Betaine Following Excitation of DHI, Adapted from Ref 26[a]



[a]The thermal back-reaction is fast for *cisoid* betaine, whereas that for the *transoid* species is slower owing to a large activation barrier to isomerization. A thermal barrier exists between the *cisoid* and *transoid* betaine conformations. When the thermal barrier between both betaines is large (I), the betaine is sufficiently long-lived for detection by steady-state absorption spectroscopy. If the thermal barrier is small (II), only the cisoid betaine can be detected by time-resolved absorption spectroscopy.

2. Synthesis of 4-[2′,3′-Bis(methoxycarbonyl)-8a′*H*-spiro(fluorene-9,1′-indolizin)-7′-yl]-1-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]pyridin-1-ium Bromide (1). The first step of the reaction sequence summarized in Scheme 3 consisted of a Mitsunobu reaction of 1*H*-pyrrole-2,5-dione and 3-bromopropan-1-ol. We have optimized a procedure described in ref 27 obtaining 69% of 3-bromopropan-1-ol **3**. Compound **3** was then heated with 4,4′-bipyridine to yield 62% of the monoaddition product **4**. The last step of this sequence consisted of the electrocyclic reaction between the heterocyclic compound **4** and spirocyclopropene, which was prepared as described in ref 26. After recrystallization, 66% of target DHI **1** was obtained.

3. Synthesis of (*E*)-4-[2-[1′,2′-bis(methoxycarbonyl)-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinolin)-5′-yl]-vinyl]-1-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-propyl]pyridin-1-ium Bromide (2). (*E*)-4-[2-(Pyridin-4-yl)vinyl]quinolone **5** was synthesized via condensation in acetic anhydride, which was a modification of the procedure published in ref 24 where $ZnCl_2$ was used. Compound **5** was mixed with spirocyclopropene in a 1:1 ratio as shown in Scheme 4. Three principal reaction products have been

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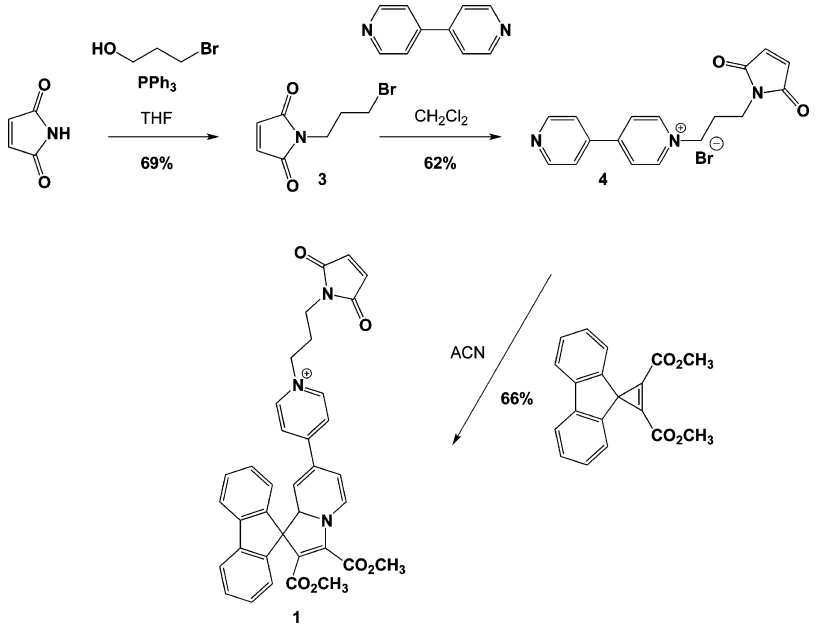
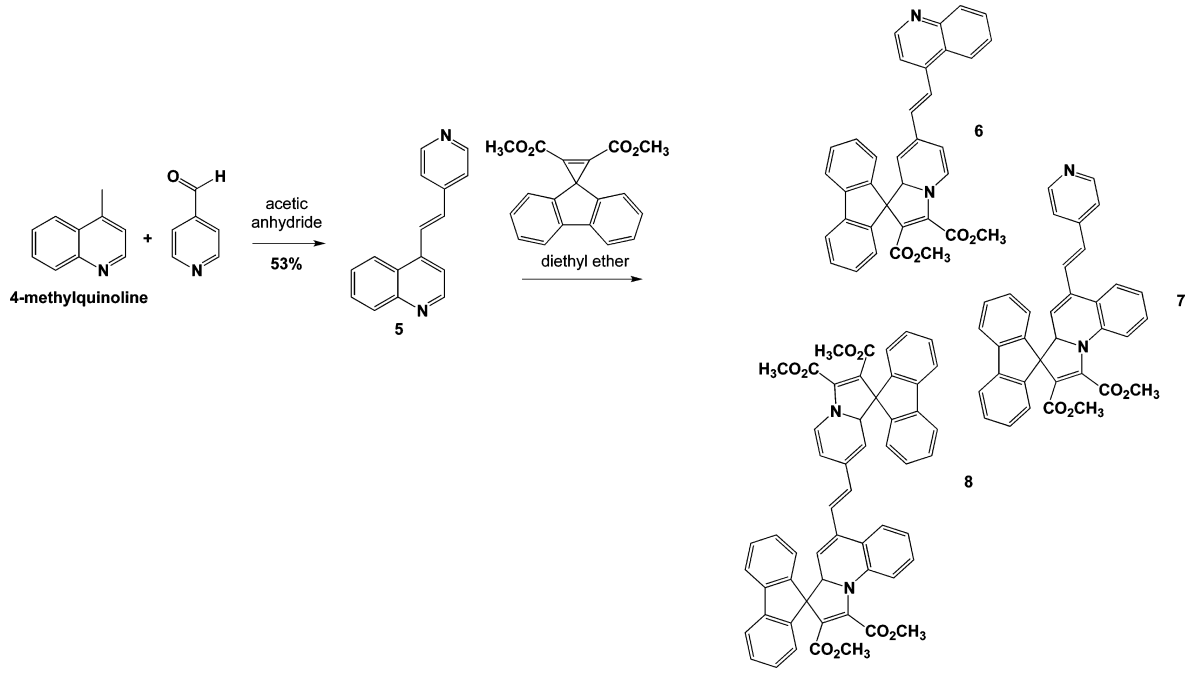
Scheme 3. Synthesis of Target DHI 1



Scheme 4. Pathway 1 to Nonquaternized DHIs 6–8



isolated: (*E*)-dimethyl 7′-[2-(quinolin-4-yl)vinyl]-8a′*H*-spiro-(fluorene-9,1′-indolizine)-2′,3′-dicarboxylate **6** (66%), (*E*)-dimethyl 5′-[2-(pyridin-4-yl)vinyl]-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinoline)-1′,2′-dicarboxylate **7** (3%), and (*E*)-dimethyl 5′-[2-[2′,3′-bis(methoxycarbonyl)-8a′*H*-spiro-(fluorene-9,1′-indolizin)-7′-yl]vinyl]-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinoline)-1′,2′-dicarboxylate **8** (10%), as depicted in Scheme 4. However, the reaction of compound **7** with compound **3** did not lead to the desired maleimide-functionalized DHI **2** but to a polymeric product of undefined structure. Quaternization attempt of DHI **6** proved unsuccessful as well. The DHI **7** is virtually identical with (*E*)-diisopropyl 5′-[2-(pyridin-4-yl)vinyl]-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]-quinoline)-1′,2′-dicarboxylate reported in ref 24.

The first step in the addition mechanism of a heterocylic aromatic base to spirocyclopropene is the nucleophilic attack on the electron-deficient double bond. Since the product distribution from the addition of compound **5** to spirocyclopropene indicated that the pyridyl nitrogen is more nucleophilic than the quinolyl nitrogen, we have quaternized the former before reaction with spirocyclopropene (Scheme 5). Indeed, we were unable to isolate the isomer having the maleimide group attached via the quinolyl nitrogen. Subsequent reaction with spirocyclopropene yielded target DHI **2**. The low

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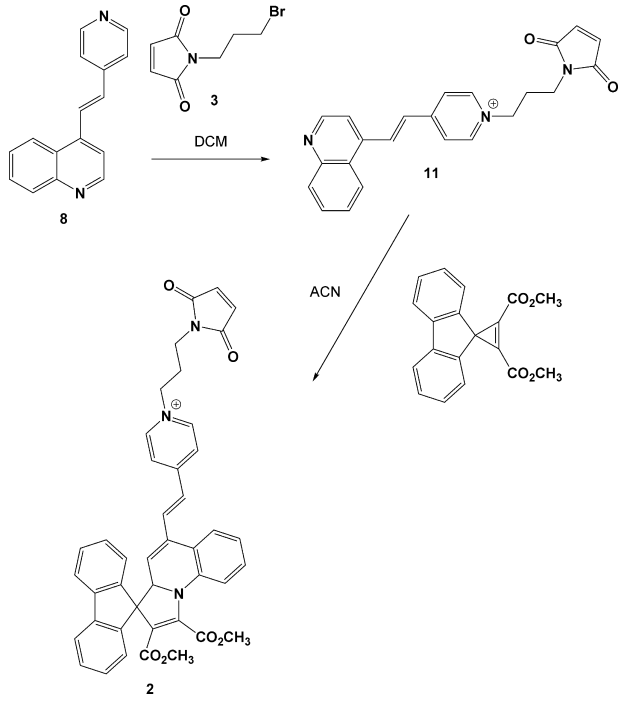
Scheme 5. Synthesis of Target DHI 2



yield of this step can be attributed to the required recrystallization procedure.

4. Photophysical Properties of DHI 1 and 2. *4.1. Steady-State Absorption Studies.* The photochromic properties of DHI **1** and **2** have been established by irradiating DHI solutions in acetonitrile by means of a 150 W Xe arc lamp (Figure 1). At 293 K, DHI/betaine **1**/**1B** showed a broad betaine maximum at λ_{max} = 663 nm. The half-life of the *transoid* betaine is $\tau_{1/2}$ = 171 s ($k = 4.05 \times 10^{-3}$ s^{-1}) at 293 K. We were unable to generate the betaine absorption for DHI/betaine **2**/**2B** at 293 K. However, after decreasing the temperature to 253 K, a broad betaine absorption peak was recorded, which is virtually stable at this temperature ($\tau_{1/2}$ > 1200 s). In agreement with our experimental planning, the betaine absorption of DHI (λ_{max} = 820 nm) extends into the near-infrared region, which is suitable for in vivo measurements. The observed difference in photochemical reactivity of DHI **1** and **2** is an indication that the thermal barriers between the *cisoid* and *transoid* betaines are of a different magnitude. Due to a larger energy barrier, *transoid* betaine **1B** can be accumulated at room temperature, whereas *transoid* betaine **2B**, having the smaller energy barrier, can only be seen at lower temperature.

4.2. Time-Resolved Absorption Studies. The transient absorption decays measured following excitation of 0.10 mM DHI in acetonitrile with a laser pulse (355 nm, fwhm ~8 ns) are shown in Figure 2. They indicate that both cisoid betaines

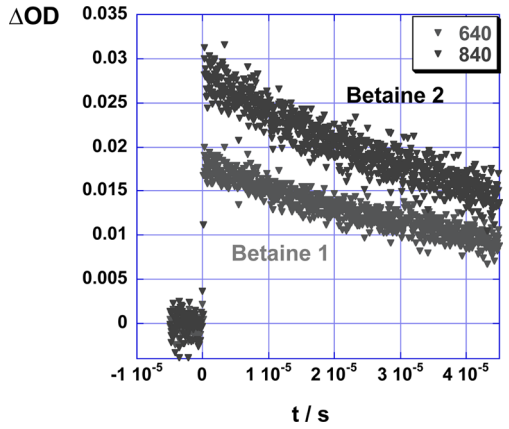


Figure 2. Transient absorption decays of the betaines **1B** (measured at λ_{max} = 640 nm) and **2B** (measured at λ_{max} = 820 nm) at 293 K in acetonitrile.

feature half-times of ring closure in the 50 μs range (betaine **1B** to DHI **1**: $\tau_{1/2} = 4.69 \times 10^{-5}$ s, $k = 1.478 \times 10^{4}$ s^{-1}; betaine **2B** to DHI **2**: $\tau_{1/2} = 4.68 \times 10^{-5}$ s, $k = 1.481 \times 10^{4}$ s^{-1}), which is again very suitable for biological applications, such as channel gating (Scheme 6). Both decays were fitted to monoexponential decays (see the Supporting Information). The photophysical data for DHI/betaine **1B** and **2B** are summarized in Table 1.

It is noteworthy that the changes in dipole moment between the spirodihydroindolizine and the betaine forms are significant, as AM1 calculations indicate. For the DHI/betaine **1**/**1B** pair, we have estimated 17 D for the DHI and 13 D for the betaine. For DHI/betaine **2**/**2B**, the dipole moment changes from 16 to 13 D during the ring-opening from the DHI to the transoid betaine. This makes the spirodihydroindolizine/betaine system the only photochromic system that is characterized by a significant reduction of the dipole moment after the photo-driven isomerization has occurred.[28] According to the same

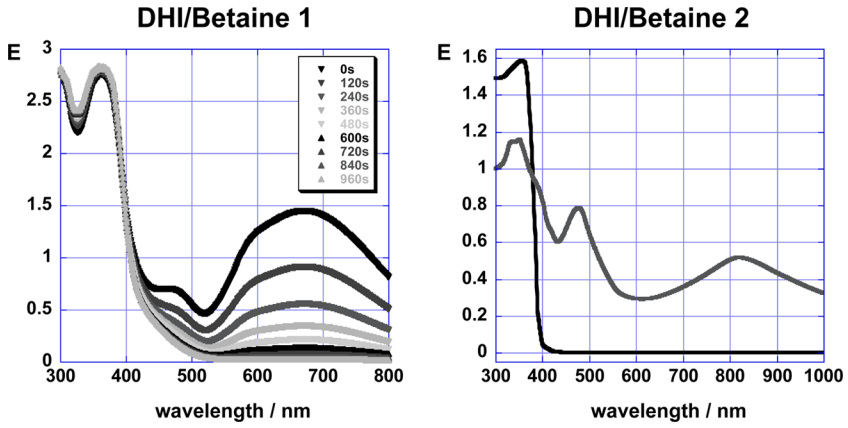


Figure 1. UV/vis/near-IR absorption spectra of DHI/betaine **1**/**1B** (293 K) as a function of time and DHI/betaine **2**/**2B** (253 K) in acetonitrile.

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Scheme 6. DHI/Betaine Reaction Scheme

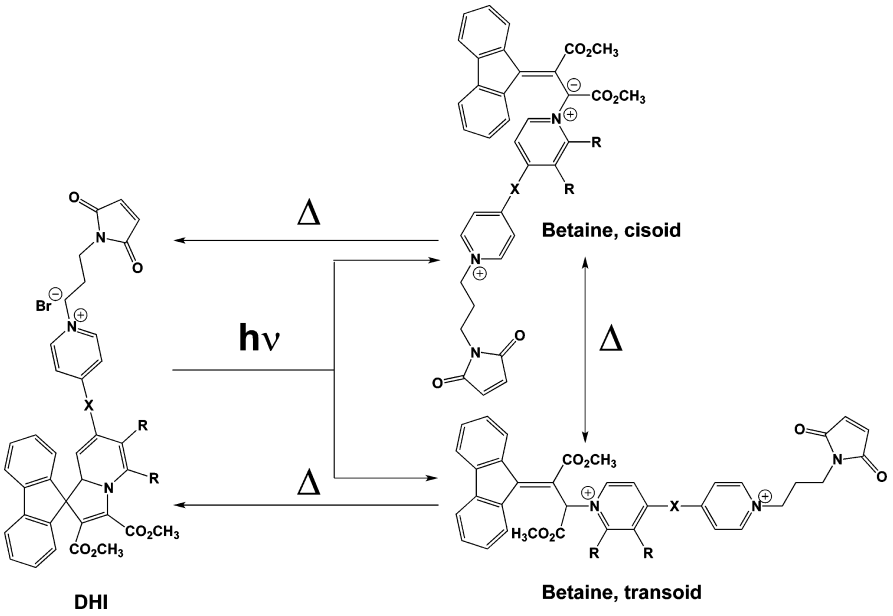

Table 1. Photophysical Parameters of DHI/Betaine 1/1B and 2/2B in Acetonitrile at 293 K

	λ_{max} (nm)	$\tau_{1/2}$ (s)	$k_{ring\ closure}$ (s^{-1})	X =	R =
DHI **1**	376				
Betaine **1B**	663	4.69×10^{-5}	1.478×10^{4}		
DHI **2**	362			−CH═CH−	−CH═
betaine **2B**	485, 820	4.68×10^{-5}	1.481×10^{4}	−CH═CH−	−CH═

calculations, the difference in length between DHI **1** and betaine **1B** is 0.42 nm (approximately 20%). It is 0.29 nm (approximately 13%) for DHI **2** and betaine **2B**.

CONCLUSIONS

We have synthesized two novel photochromic spirodihydroindolizines (DHI) that feature maleimide groups for tethering to cysteine sites in peptides and proteins. Both DHI have corresponding betaines that show light absorption in the red region of the visible spectrum (betaine 1) or extend significantly to the near-infrared spectral domain (betaine 2). Both wavelengths are suitable for in vivo applications. The *cisoid* conformations of both betaines **1B** and **2B** show half lives in the 50 μs range for their disrotatory ring-closure. Interestingly, only betaine **1B** has a significant lifetime of its transoid conformer at 293 K due to the occurrence of a significant energy barrier between the *transoid* and *cisoid* conformers. Betaine **2B** does not exhibit a similar barrier in spite of the greater sterical hindrance. This is an indication for the dominance of electronic factors, what was expected for the disrotatory ring closure, but not necessarily for a hindered rotation process.

EXPERIMENTAL SECTION

Steady-State Absorption. Absorption spectra were recorded in acetonitrile. The DHIs (5×10^{-5} M) have been irradiated by means of a 150 W Xe arc lamp for 5 min prior to recording the spectra.

Laser Flash Photolysis. Transient absorption spectra and lifetimes were measured on a home-built instrument pumped by a frequency tripled (355 nm) Nd:YAG laser (fwhm 8 ns, 5 mJ per pulse). The output from a 150 W Xe arc lamp was focused onto the sample at 90° with respect to the laser beam. The white light transmitted by the sample was collimated and focused onto the entrance slit of a monochromator (1200 gr/mm) and was detected utilizing a photomultiplier tube and processed by a 400 MHz oscilloscope.[26,29,30]

Chemicals and Instrumentation. All chemicals were used as purchased unless noted otherwise. The compounds were characterized by using 400 and 200 MHz NMR spectrometers, FT-IR, and a triple quadrupole mass spectrometer with electrospray and APCI sources.

1-(3-Bromopropyl)-1*H*-pyrrole-2,5-dione (3). PPh_3 (1.35g, 5.15 mmol) was charged in a 100 mL round-bottom flask and dissolved in 20 mL of dry THF. The temperature of the reaction was cooled to −78 °C by using dry ice/acetone. Diisopropyl azodicarboxylate (1.01 mL, 5.15 mmol) was added dropwise to the reaction mixture during 2 min resulting in a yellow solution. The reaction was stirred for 10 min, followed by addition of 3-bromo propanol (0.77 mL, 8.5 mmol) during 2 min. After 5 min of stirring, maleimide (0.5g, 5.15 mmol) was added in solid form at −78 °C, which dissolved within 10 min. The reaction was then warmed to room temperature and allowed to stir for 10 h. A dark gray solution resulted; TLC (2:1 hexane/EtOAc) indicated formation of product. The reaction mixture was concentrated by using a rotary evaporator and adsorbed onto silica gel. Column chromatography was performed using silica (75 g) with 2:1 hexane/EtOAc as eluent. After removal of the solvent, 0.78 g (69.64%) pale yellow crystals of 1-(3-bromopropyl)-1*H*-pyrrole-2,5-dione (**3**) were obtained: IR (dropcast on KBr) ν cm^{-1} 1235, 1409, 1701, 2925, 2960, 3088; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 2.18 (q, J = 6.6 Hz, 2H), 3.37 (t, J = 6.6, 2H), 3.68 (t, J = 6.8 Hz, 2H), 6.72 (s, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm) 29.8, 31.7, 36.8, 134.4, 170.8; mp 45−46 °C.

1-[3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl](4,4′-bipyridin)-1-ium Bromide (4). 4,4′-Bipyridine (0.10 g, 0.641 mmol) and 0.14 g (0.641 mmol) of compound **3** were heated in 8.0 mL of anhydrous dichloromethane at 40 °C for 24 h. This resulted in monoquaternization of 4,4′-bipyridine. The light brown salt was filtered and dried in vacuum to obtain 0.15g (62.5%): IR (dropcast on KBr) ν cm^{-1} 697, 815, 1178, 1363, 1409, 1465, 1639, 1701, 2849, 2920, 2950; ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 2.16 (q, J = 6.8 Hz), 3.5 (t, J = 6.4 Hz, 2H), 4.6 (t, J = 8 Hz, 2H), 7.05 (s, 2H), 8.0 (d, J = 6.24 Hz, 2H), 8.62 (d, J = 6.8 Hz), 9.19 (d, J = 6.8 Hz, 2H), 8.84 (d, J = 6.24 Hz, 2H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm) 30.0, 34.0, 58.0, 121.8, 125.3, 134.7, 140.8, 145.4, 151.0, 152.3, 171.1; mp 312−315 °C.

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4-[2′,3′-Bis(methoxycarbonyl)-8a′*H*-spiro(fluorene-9,1′-indolizin)-7′-yl]-1-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]pyridin-1-ium Bromide (1). Compound **4** (20 mg, 0.05 mmol) and 20 mg (0.064 mmol) of spirocyclopropene (dimethyl spiro(cycloprop[2]ene-1,9′-fluorene)-2,3-dicarboxylate)[24] was dissolved in acetonitrile and stirred overnight (15 h) in the dark at room temperature. The reaction mixture was filtered, and the solvent was removed in vacuum. The reaction product was recrystallized from acetonitrile yielding 66% of the light brown product **1**: ^{1}H NMR (CD_3CN, 400 MHz) δ (ppm) 2.2 (quintet, 2H invisible in CD_3CN, but visible in DMSO) 3.25 (s, 3H), 3.5 (t, J = 6 Hz, 2H), 4.1 (s, 3H), 4.8 (t, J = 6.5 Hz, 2H), 4.9 (s, 1H), 5.43 (d, J = 7.7 Hz, 1H), 5.6 (d, J = 3.6 Hz, 1H), 6.69 (s, 2H), 6.74(d, J = 7.7 Hz, 1H), 7.2−7.8 (m, 10 H), 9.2 (d, J = 7 Hz, 2H); ^{13}C NMR (CD_3CN, 200 MHz) δ (ppm) 31.3, 35.2, 52.1, 54.7, 59.6, 65.8, 70.5, 102.4, 118.7, 121.3, 121.8, 122.5, 125.1, 125.3, 127.3, 128.6, 129.2, 129.3, 129.8, 130.2,132.9, 135.8, 142.1, 143.2, 145.7, 147.7, 154.9, 164.3, 172.5; mass $C_{36}H_{30}BrN_3O_6$ m/z calcd 679.13, found 680.4 (M + 1) and 600.4 (M − Br); mp 255−257 °C dec. Anal. Calcd for CHN: C, 63.54; H, 4.44; N, 6.17. Found: C, 63.50; H, 4.48; N, 6.14.

(*E*)-4-(2-(Pyridin-4-yl)vinyl)quinolone (5). Lipidine (4-methylquinoline, 0.5 mL, 3.82 mmol) and 4-carboxyaldehydepyridine (0.359 mL, 3.82 mmol) were dissolved in 10 mL of acetic anhydride in a 50 mL round bottom flask. The reaction mixture was refluxed under nitrogen. The reaction progress was monitored by TLC (silica/CH_2Cl_2/MeOH 98:2 v/v). After 2 days, the starting materials were consumed and the reaction mixture was cooled to rt. The dark brown reaction mixture was concentrated to dryness via rotary evaporation. Compound **5** was purified by SiO_2 column chromatography. Ethyl acetate was used followed by 5% methanol in ethyl acetate to elute product **5**. Single spotted fractions were mixed, evaporated, and recrystallized in ethyl acetate in hexane to yield brown crystals of **5** (0.470 g, 2.01 mmol 53% yield): ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 8.16−8.209 (t, J = 7.8 Hz, 2H), 8.01−8.05 (d, J = 16.2 Hz, 1H), 7.76−7.80 (t, J = 6.8 Hz, 1H), 7.64−7.66 (t, J = 6.8 Hz, 1H), 7.61−7.62 (d, J = 4.5 Hz, 1H), 7.49−7.50 (d, J = 4.5 Hz, 2H),7.26−7.28 (d, J = 16.2 Hz, 1H), 8.68−8.69 (d, J = 4.5 Hz, 2H), 8.95 - 8.96(d, J = 4.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm) 117.8, 121.4, 123.4, 126.4, 127.2, 127.8, 129.8, 130.4, 132.7, 142.0, 143.9, 148.8, 150.3, 150.6; MS $C_{16}H_{12}N_2$ m/z [M + 1] 233.

(*E*)-Dimethyl 7′-[2-(Quinolin-4-yl)vinyl]-8a′*H*-spiro(fluorene-9,1′-indolizine)-2′,3′-dicarboxylate (6). (*E*)-4-[2-(Pyridin-4-yl)-vinyl]quinolone (**5**) (100 mg, 0.43 mmol) and spirocyclopropene (131.8 mg, 0.43 mmol) were dissolved in 25 mL of diethyl ether in a 50 mL round bottom flask, and the reaction mixture was protected by argon gas. The reaction mixture was stirred overnight at room temperature in the dark. The progress of the reaction was monitored by TLC. TLC indicated that there are two regioisomers formed (DHIs **6** and **7**), as well as the bis-adduct DHI **8**. The three DHIs were separated by descending silica gel column. First, 4:1 hexane/ethyl acetate was used as an eluent to remove unreacted spirene, and then 1:1 hexane/ethyl acetate v/v was used as eluent to separate DHI **6** light brown powder (155 mg, 0.287 mmol, 66%) and DHI **8** light brown powder (37 mg, 0.043 mmol, 10%). Compound **8** appeared first. Compound **7** eluted from the column after switching to pure ethyl acetate. After removal of the solvent in a rotatvap, the resulting crude mixture was recrystallized from diethyl ether. The first fraction of crystals was discarded and compound **7** was obtained as second fraction (7 mg, 0.013 mmol, 3%).

Compound **6**: ^{1}H NMR ($CDCl_3$) δ 8.76−8.77 (d, J = 4.6 Hz, 1H), 8.05−8.09 (t, J = 8.7 Hz, 2H), 7.75−7.77 (d, J = 7.6 Hz, 2H), 7.68−7.72 (t, J = 6.8 Hz, 1H), 7.61−7.63 (d, J = 7.4 Hz, 1H), 7.52−7.57 (t, J = 6.8 Hz, 1H), 7.50−7−52 (d, J = 7.4 Hz, 1H), 7.34−7.45 (m, 3H), 7.30−7.31 (d, J = 4.6 Hz, 1H), 7.22−7.26 (m, 3H), 6.65−6.67, (d, J = 7.6 Hz, 1H), 6.51−6.55 (d, J = 16.0 Hz, 1H),5.71−5.73 (d, J = 7.6 Hz, 1H), 5.63 (s, 1H), 4.69 (s, 1H), 4.04 (s, 3H), 3.30 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.8, 162.3, 150.2, 147.2, 146.6, 142.4, 141.9, 140.6, 133.2, 133.0, 130.3, 129.5, 128.7, 128.5, 128.0, 127.5, 126.7, 126.4, 126.0, 124.8, 123.8, 123.5, 122.4, 120.3, 120.0, 119.7, 117.1, 101.7, 70.1, 64.33, 53.61, 51.33; MS $C_{35}H_{26}N_2O_4$ [M + H] calcd 538.2, found 539.4; mp 250−252 °C dec. Anal. Calcd for CHN: C, 78.05; H, 4.87; N, 5.20. Found: C, 78.11; H, 4.88; N, 5.15.

(*E*)-Dimethyl 5′-[2-(pyridin-4-yl)vinyl]-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinoline)-1′,2′-dicarboxylate (7): ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.68−7.71 (d, J = 3.44, 5.4 Hz, 4H), 7.58−7.61 (t, J = 6.88 Hz, 2H,), 7.31−7.40 (m, 6H), 7.13−7.18 (m, 4H), 7.06−7.08 (d, J = 7.40 Hz, 2H), 6.13−6.15 (d, J = 5.40 Hz, 1H), 4.10 (s, 1H), 3.58 (s, 3H), 3.77 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ (ppm) 163.0, 162.8, 148.8, 148.6, 144.7, 142.0, 140.6, 128.6, 128.4, 127.8, 127.5, 127.3, 127.0, 125.2, 122.9, 122.7, 119.6, 119.4, 115.8, 109.6, 68.90, 66.06, 53.1, 51.8 ppm; mp 150 °C dec; mass $C_{35}H_{26}N_2O_4$ m/z calcd 539.19, found 540.2 (M +1).

(*E*)-Dimethyl 5′-[2-[2′,3′-Bis(methoxycarbonyl)-8a′*H*-spiro-(fluorene-9,1′-indolizin)-7′-yl]vinyl]-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinoline)-1′,2′-dicarboxylate (8). Compound **8**: ^{1}H NMR ($CDCl_3$) δ 7.64−7.76 (m, 5H), 7.52−7.57 (m, 2H), 7.28−7.42 (m, 7H), 7.22−7.25 (m,1H), 7.20−7.12 (m, 4H), 7.02−7.08 (m, 2H), 6.96−6.98 (t, J = 7.6 Hz, 6.48−6.51 (t, J = 7.2 Hz, 1H), 6.18−6.22 (t, J = 14.6 Hz, 1H), 5.67−5.71 (d, J = 15.8 Hz, 1H), 5.52−5.56 (d, J = 15.8 Hz, 1H), 5.39−5.44 (m, 2H), 5.30−5.32(d, J = 8 Hz, 1H), 5.39−5.44 (m, 2H), 5.31 (d, J = 8 Hz, 1H), 4.79−4.81 (d, J = 7.6 Hz, 1H), 4.29−4.31 (d, J = 9.4 Hz, 1H), 4.02 (s,3H), 4.0 (s, 3H), 3.26−3.27 (d, J = 2.5 Hz, 3H), 3.21−3.22 (d, J = 2.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 164.3, 164.0, 163.8, 162.3, 148.3, 146.7, 143.8, 141.9, 141,7, 140.5, 136.2, 135.9, 133.6, 132.7, 130.7, 130.6, 129.1, 129.0, 128.6, 128.6, 128.5, 128.3, 128.2, 128.0, 127.9, 127.4, 125.9, 125.4, 125.4, 125.3, 125.1, 124.8, 124.7, 124.0, 123.9, 123.7, 123.7, 123.3, 123.2, 121.7, 121.3, 120.2, 119.9, 117.9, 117.7, 115.5, 115.4, 109.5, 109.1, 102.3, 102.1, 70.0, 69.9, 69.6, 64.1, 63.1, 53.7, 53.5, 51.3, 51.14; mp 250−252 °C dec. Anal. Calcd for $C_{54}H_{40}N_2O_8$: C, 76.76; H, 4.77; N, 3.32. Found: C, 76.77; H, 4.75; N, 3.34.

(*E*)-1-[3-(2,5-Dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]-4-[2-(quinolin-4-yl)vinyl]pyridin-1-ium Bromide (9). 1-(3-Bromopropyl)-1*H*-pyrrole-2,5-dione **3** (40 mg, 0.172 mmol) and (*E*)-4-[2-(pyridin-4-yl)vinyl]quinolone **5** (38 mg, 0.174 mmol) were dissolved in 3 mL of dichloromethane in a 10 mL round bottom flask. The reaction mixture was refluxed overnight. The light brown precipitate, formed after 24 h, was filtered and washed with cold dichloromethane to give compound **9** as brown solid (40 mg, 0.088 mmol, 51% yield): ^{1}H NMR (DMSO, 400 MHz) δ (ppm) 2.21 (t, J = 7.4 Hz, 2H), 3.51 (t, J = 6.4 Hz, 2H), 4.57 (t, J = 7.6 Hz, 2H), 7.08 (s, 2H), 7.76 (t, J = 7.02 Hz, 2H), 7.83−7.88 (m, 3H), 7.97−7.98 (d, J = 4.6 Hz, 1H), 8.02−8.02 (d, J = 4.5 Hz, 1H), 8.11 (d, J = 8.2 Hz, 1H), 8.54 (d, J = 6.6 Hz, 2H),), 8.63 (d, J = 8.3 Hz, 1H), 8.80 (d, J = 16.2 Hz, 1H), 9.08 (d, J = 6.6 Hz, 2H); ^{13}C NMR (DMSO, 400 MHz) δ (ppm) 30.6, 34.7, 58.34, 118.6, 124.8, 125.7, 126.2, 127.9, 130.2, 130.6, 135.2, 135.3, 135.4, 140.8, 145.4, 148.9, 151.0, 152.6, 171.8; MS $C_{23}H_{20}N_3O_2Br$ calcd 450.33, found 370.300 (M − Br); mp 140−142 °C dec. Anal. Calcd for $C_{23}H_{20}BrN_3O_2$: C, 61.34; H, 4.48; N, 9.33. Found: C, 61.40; H, 4.50; N, 9.37.

4-[1′,2′-Bis(methoxycarbonyl)-3a′*H*-spiro(fluorene-9,3′-pyrrolo[1,2-*a*]quinolin)-5′-yl]-1-[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]pyridin-1-ium Bromide (2). Compound **9** (23 mg, 0.049 mmol) and spirocyclopropene (15 mg, 0.049 mmol) were dissolved in 5 mL of DMSO and stirred overnight (24 h) in the dark at room temperature. After completion of the reaction, DMSO was removed in high vacuum at 50 °C yielding a light brown precipitate (16 mg, 0.021 mmol, 41% yield). The product was recrystallized from acetonitrile: ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 2.11 (quintet, J = 7.6, 2H), 3.18 (s, 3 H), 3.5 (t, imbedded in water peak), 4.01 (s, 3H), 4.45 (t, J = 7.4 Hz, 2H) 5.24 (s,1 H), 5.707 (s, 1H), 6.88 (d, 12.5 Hz, 1H), 7.01−7.16 (m, 4 H), 7.22−7.54 (m, 9 H), 7.74 (m, 2H, 7.90 (d, J = 7.7 Hz, 2H), 8.12 (d, J = 6.2, 2H), 8.87 (d, J = 6.2, 2H); ^{13}C NMR (DMSO, 400 MHz) δ (ppm) 10.8, 16.2, 33.7, 49.5, 54.9, 62.4, 81.6, 97.6, 108.7, 119.4, 120.8, 123.8, 124.3, 125.7, 126.4, 127.5, 128.6, 129.8, 132.9, 134.3, 135.4, 140.5, 141.3, 143.4, 144.2, 147.3, 148.2, 152.3, 162.9, 163.8; MS $C_{42}H_{34}BrN_3O_6$ calcd 756.60, found 676.300 (M − Br); mp 250−253 °C dec. Anal. Calcd: C, 66.67; H, 4.53; N, 5.55. Found: C, 66.71; H, 4.50; N, 5.51.

■ ASSOCIATED CONTENT

Supporting Information

Analysis of the time-resolved absorption decays and the NMR spectra of compounds **1**, **2**, **6**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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