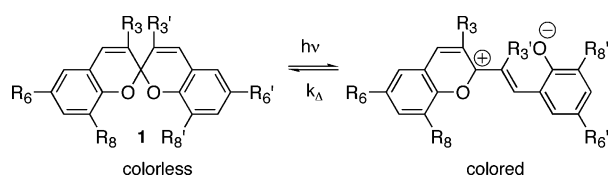## Substituent Effects on Thermal Decolorization Rates of Bisbenzospiropyrans

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A novel application of photochromic molecules is to mimic physiological oscillatory calcium signals by reversibly binding and releasing calcium ions in response to light. Substituent changes on the largely unexplored photochromic bisbenzospiropyran scaffold led to significant changes in thermal fading rates in several organic solvents. Excellent correlations have been found between fading rates and empirical Hammett constants as well as calculated ground-state energies. These correlations can be used to improve scaffold design.

Calcium (Ca^{2+}) is a second messenger in important signal transduction systems that translate extracellular signals into cellular response.[1] Often, in both excitable and nonexcitable cell types, the intracellular calcium signal is oscillatory.[2] Extensive experimental and theoretical work has been carried out to understand how the oscillations arise, but much less is known about the effects of Ca^{2+} oscillations on a molecular level. Understanding calcium signaling will lead to insights about both normal and disease states in humans. Photoreversible calcium-specific chelators appear to be an effective approach to this area of research. Photochromic compounds are well suited for controlled ion binding and release because they undergo reversible structural changes upon irradiation that can form or disrupt a binding cavity. For reversible ion binding, the photochromic molecule must interconvert between low affinity (B) and high affinity (A) binding states. (Figure 1). The use of light to control metal ion binding and release is well

FIGURE 1. Representation of a photochromic chelator. The square represents a metal ion.

FIGURE 2. Photochromic scaffold bisbenzospiropyran **1** as well as one of eight possible stereoisomeric open forms.

documented.[3–5] However, these systems lack specificity for calcium over other univalent and divalent cations, and are poorly soluble in water. To date, there are no calcium-selective binders that can be used to generate the wide spectrum of physiological oscillatory calcium signals in aqueous systems.

This novel application requires a compound with both suitable metal binding and appropriate photochemical properties. Compound **1**, a substituted 2,2′-spirobi[2*H*-1-benzopyran] where $R_{8,8'} = -N(CH_2CO_2^-)_2$ shown in Figure 2, was designed to serve as a photochromic calcium-selective chelator. The shape of this photochromic scaffold uniquely allows multiple convergent chelating carboxylate ligands to be appended to it. The spiropyran class of photochromic compounds undergoes heterolytic cleavage from closed to open forms, and photochromism is observable at a given temperature unless the rate of thermal closure (k_Δ) is too fast.[6,7] Prior work by this group on **1** ($R_{3,3'} = -CH_3$; $R_{8,8'} = -N(CH_2CO_2^-)_2$) showed moderately strong and selective binding of calcium over magnesium in aqueous buffered systems, but was not found to be photochromic under our experimental conditions.[8] Current efforts are directed toward

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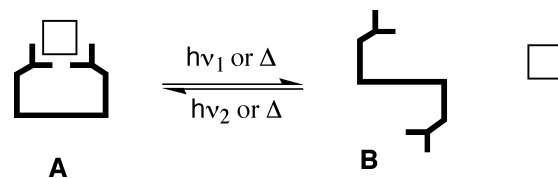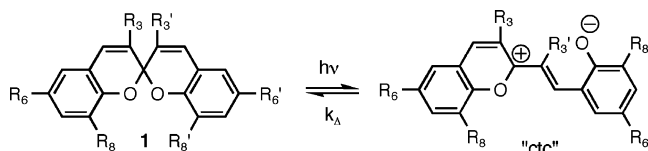
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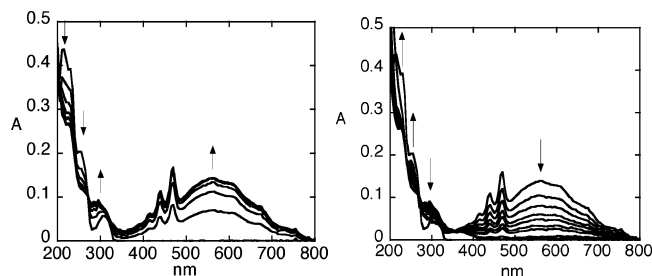
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TABLE 1. Substituted Bisbenzospiropyrans (1) Synthesized

	1a	1b	1c	1d	1e	1f	1g	1h
$R_{3,3'}$	H	H	H	H	H	H	Me	Me
$R_{6,6'}$	OMe	Me	H	F	Cl	NO_2	OMe	Me
$R_{8,8'}$	H	H	H	H	H	H	H	H

	1i	1j	1k	1l	1m	1n	1o
$R_{3,3'}$	Me	Me	Ph	Ph	H	H	H
$R_{6,6'}$	H	NO_2	Me	H	H	H	H
$R_{8,8'}$	H	H	H	H	OMe	Me	F

TABLE 2. Thermal Fade Rate Constants at −70 °C for Selected Compounds 1 in Various Solvents

	k_Δ (min^{-1})			
	MeCy	toluene	THF	^{i}PrOH
6-OMe	7.75×10^{-3}	4.20×10^{-3}	2.19×10^{-3}	1.65×10^{-3}
6-Me	4.94×10^{-2}	1.37×10^{-2}	8.77×10^{-3}	2.09×10^{-3}
6-H	1.12×10^{-1}	1.32×10^{-2}	1.09×10^{-2}	1.50×10^{-3}
6-F	7.86×10^{-2}	1.64×10^{-2}	8.91×10^{-3}	2.65×10^{-3}
6-Cl	1.44×10^{-1}	1.61×10^{-2}	8.52×10^{-3}	7.24×10^{-3}
1f−l	N/A			
8-OMe	8.32×10^{-2}			
8-Me	1.88×10^{-2}			
8-F	9.27×10^{-2}			

FIGURE 3. UV−vis spectra showing the photochemical opening of **1b** in methylcyclohexane at −70 °C with UV light (left, during 6 min of irradiation) and thermal fading (right, during 200 min of darkness).

a systematic investigation of the tunability of photochromic properties of bisbenzospiropyran, given the limited structure−activity data published for this photochromic scaffold. In this report, the effect of changing substituents on k_Δ is examined. These efforts will aid in assessing the viability of this photochromic scaffold for use in a calcium-signaling mimic.

The compounds listed in Table 1 were prepared according to literature methods[9] as described in the Supporting Information.

Representative photochromic behavior is illustrated by Figure 3. After reaching a photostationary state upon UV irradiation at −70 °C, (thermal) fading was monitored in darkness at −70 °C. This temperature was chosen so that kinetics of the fastest reaction monitored were slow relative to the instrument scan rate. Thermal fading follows first-order kinetics at shorter, but not at longer times. This complex kinetic behavior is also characteristic of many indolino-spiropyrans.[7,10] Table 2 lists first-order rate constants analyzed at short times (first 10% of experiment). As discussed in more detail below, these data show both solvent dependence and substituent effects on thermal fading rates. Qualitatively, the more polar solvent and the more electron donating substituent slow the rate of thermal fading. Higher temperatures increase the rate of thermal closure (data not shown) so that photocoloration is not observed at room temperature for these compounds.

The determination of k_Δ was not possible for certain compounds (**1f−l**). For example, substituents at $R_{3,3'}$ prohibited photocoloration under these conditions. In contrast, prior work by Appriou et al. with a flashlamp

showed related compounds substituted at $R_{3,3'}$ were in fact photochromic at room temperature in toluene.[11] The different intensity of the light sources may explain these different observations. Also, the presence of a nitro group (**1f**) caused an immediate change in the visible spectrum upon irradiation, suggesting very efficient photocoloration, but almost as quickly the signal disappeared, even while under irradiation. This is consistent with the observation that nitro groups improve quantum yields, but also can increase k_Δ and side reactions.[12]

More polar solvents lower the rate of thermal closure up to 100-fold (graphical representation of these data is in the Supporting Information). This effect is consistent with more polar solvents better stabilizing the more polar open form of the molecules. In addition, more polar solvents decrease the sensitivity of k_Δ to substituents. This trend has been observed for other photochromic spiropyrans,[13] with a notable exception. One group has reported that substituent effects are negligible in nonpolar solvents, but strongly present in polar solvents.[14] They cited a change in ground-state structure and mechanism due to solvation effects. In nonpolar solvents, a quinoid resonance form was dominant, following the substituent-insensitive electrocyclic ring closure pathway. In polar solvents, the zwitterionic resonance form was a major contributor, with large substituent effects on closure rates. The data in Table 2 do not suggest a mechanism change due to either substituent or solvent effects.

The symmetric substitution of the bisbenzospiropyran decreases rates of thermal closure up to 20-fold. These effects, while small, are significant. The same substituent at R_6 and R_6' should have both stabilizing and destabilizing effects on the zwitterionic open form, which might be expected to cancel out completely. Interestingly, they do not.

Substituent effects have been studied on related spirobenzopyrans with indoline and benzothiazoline heterocyclic systems.[7,11,15] In some cases, structural modifi-

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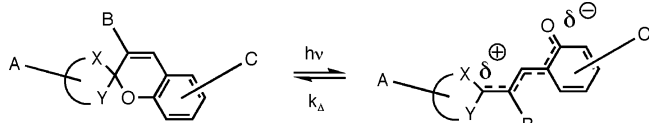
FIGURE 4. Substituent effects in positions A, B, and C on k_Δ. The heterocyclic ring is indoline or benzothiazoline.
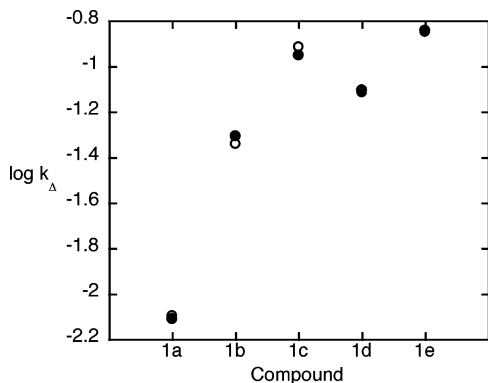
FIGURE 5. For compounds **1a**−**e**, experimental log k_Δ (closed circles) compared to predicted values (open circles) obtained from a dual parameter Hammett plot (multiple $R = 0.999$ when σ_{p+} and σ_{p-} parameters were used).

cations have resulted in dramatic changes of thermal fading rate constants of 3−4 orders of magnitude in both nonpolar and polar solvents. Generally, as the open form is stabilized electronically by substituents, k_Δ decreases. A summary of specific substituent effects on k_Δ is illustrated with Figure 4. Electron-donating substituents decrease k_Δ in positions A and B, while electron-withdrawing substituents decrease k_Δ in position C. These substituent effects were found to be additive. A dual parameter Hammett plot for kinetic data of a large number of indolinospirobenzopyrans yielded the following equation: log $k = (1.727) \times (\Sigma\sigma_{indoline}) - (1.703) \times (\Sigma\sigma_{pyran}) - 1.372$ ($R = 0.937$).[7]

For purposes of comparison, an additive empirical Hammett-type plot of the form $a \times \sigma + b \times \sigma + c$ was made here for bisbenzospiropyran. The slope a should be positive for the electron-poor ring and the slope b should be negative for the electron-rich ring. By using σ_{p+} and σ_{p-}, an excellent correlation ($R = 0.999$) was obtained with the equation log $k = (1.73) \times (\sigma_{p+}) - (0.653) \times (\sigma_{p-}) - (0.914)$. Figure 5 shows predicted and observed values for each compound tested. Additional plots of predicted and observed log k_Δ versus each independent variable (σ_{para+} and σ_{para-}) are provided in the Supporting Information. A comparison of the slopes of the dual parameter Hammett plots reveals that the bisbenzospiropyran system studied here appears to have a larger difference in sensitivity to substituents between the two rings than the indolinospirobenzopyran system has. For **1a**−**e**, the substituent effect on the electron-deficient ring is significantly more important than that for the electron-

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TABLE 3. Relative Energies (kcal/mole) Calculated for Closed and Open Forms of Bisbenzospiropyrans

	1a	1b	1c	1d	1e
closed	0	0	0	0	0
ctc	4.85	6.09	7.16	7.27	7.37
ctt	5.93	7.51	8.68	8.35	8.82
ttt	6.41	8.08	9.08	8.89	9.37
ttc	7.18	8.15	9.24	9.37	9.48
cct	12.63	14.45	15.51	14.89	15.22
tct	13.58	15.38	16.54	16.37	16.79
tcc	14.64	15.71	16.66	16.81	16.80
ccc	14.47	15.74	16.70	16.51	16.57

rich ring. This is consistent with the observation that even symmetric substitution influences k_Δ. When substituents at $R_{8,8'}$ are included (**1m**−**o**), the best fit requires the E_S (Taft) substituent parameter for position $R_{8,8'}$, and the overall fit decreases ($R = 0.968$). The best equation is log $k = (2.155) \times (\sigma_{p+}$ or $E_S) - (1.452) \times (\sigma_{p-}$ or $E_S) - (0.812)$.

A correlation between initial rates of thermal closure of **1a**−**e** in methylcyclohexane and calculated quantities was also attempted. Density functional calculations (B3LYP/6-31G*) were performed to obtain ground-state energies of both closed spiro and open planar, conjugated forms (Table 3). Closed forms were found to be more stable than open forms by approximately 5−7 kcal/mol. The open forms exist as eight possible stereoisomers. Each of the three bonds of the chain linking the aromatic rings may be cisoid ("c") or transoid ("t"). For compounds **1a**−**e**, the lowest energy open stereoisomer is "ctc", shown in Figure 2. Illustrations of all eight stereoisomers including stereochemical designations are included in the Supporting Information.

Unlike other reports,[15b,c] no correlation was found here between log k_Δ and any calculated bond length, bond length alternation in the open form, HOMO−LUMO gap of closed or open forms, or dipole change. However, there was excellent correlation between ($E_{clsosed} - E_{open}$) and log k_Δ ($R = 0.996-0.953$ for the four most stable transoid isomers of each compound). These plots are included in the Supporting Information. As substituents stabilize the open form relative to the less polar transition state, the kinetic barrier to thermal closure increases.

This work demonstrates that both solvent and substituents can be used to tune the photochromism of 2,2′-spirobi[1*H*-1-benzopyran]. To mimic biological calcium signals at *physiological* temperatures, the original designed chelator **1** where $R_{3,3'} = CH_3$; $R_{6,6'} = H$; $R_{8,8'} = N(CH_2CO_2-)_2$ must be modified significantly. The present study also demonstrates that the synthetically simple symmetric substitutions used here are not sufficient. However, we have shown that either computational or empirical Hammett correlations can be used reliably to design a more appropriate scaffold. For example, the Hammett equation predicts that if $R_6 = NMe_2$ and $R_{6'} = CN$, the molecule's thermal closure rates will be approximately 500-fold slower than the slowest rate reported here. If water solvation does not overwhelm substituent effects and if photocoloration remains efficient, these new scaffolds should allow the observation of photochromism under physiological conditions. The

optimized photochromic chelator will be used to gain insight into complex calcium signaling on a molecular level.

Experimental Section

Photochemistry: Light from a 200-W Mercury Xenon lamp, filtered by water and a 320 nm band-pass filter, was directed via a 3-ft quartz fiber optic bundle into the top of a reaction vessel. The irradiated, stirred samples were cooled, using a thermostated cryobath, to various temperatures. UV−vis observations were made with a Hellma 10-mm immersion probe.

Computation: The program Spartan '04 was used. Closed forms were subjected to geometry optimization (B3LYP/6-31G*) and the resulting geometry was used to determine energy. For open forms, two isomers ("ttt" and "tcc") were used as starting points to generate a conformer distribution (four isomers in each distribution). A figure defining the structures of the open forms is included in the Supporting Information. For compounds with methoxy substituents, AM1 was used to generate the conformer distribution. The conformers were subjected to geometry opimization (B3LYP/6-31G*) and then single point energy determination. Cartesian coordinates and energies are listed after synthetic and spectral data of the Supporting Information.

6,6′-Dimethoxy-2,2′-spirobi[2*H*-1-benzopyran] (1a). The following are representative procedures for syntheses of the molecules studied here.

Base-catalyzed route: 5-Methoxysalicyladehyde (1.22 g, 8.02 mmol), acetone (0.3 mL, 4.04 mmol), 4 N NaOH (4 mL, 16 mmol), and ethanol (5 mL) were stirred in a 25-mL round-bottom flask at room temperature for 12 h. The resulting reaction mixture was added to water and neutralized with 5% HCl (25 mL). The precipitate was extracted into ethyl acetate, which was dried over anhydrous MgSO$_4$ and evaporated under reduced pressure. The residue was dried under vacuum and added to ethoxyethyl acetate (10 mL) in a 25-mL round-bottom flask. The resulting solution was refluxed for 1 h. The reaction was monitored by TLC, which indicated completion of reaction after 1 h. The reaction mixture was cooled and poured in water and extracted into diethyl ether. The organic layer was washed twice with 1 M NaOH. The organic layer was dried over MgSO$_4$ and evapo-

rated under reduced pressure. The resulting crude product was subjected to flash column chromatography on silica gel. The product was eluted with 15% diethyl ether in hexane. Yield 17.10%.

Acid-catalyzed route: 5-Methoxysalicyladehyde (0.5 mL, 4.0 mmol), acetone (0.30 mL, 4.04 mmol), and a stir bar were added to a round-bottom flask containing anhydrous ethanol (10 mL). The reaction mixture was cooled with the help of an ice bath and hydrochloric acid gas was bubbled through the reaction for 2 h. The reaction mixture was allowed to come to room temperature and stirred for 24 h. After 24 h, 5-methoxysalicyladehyde (0.5 mL, 4.0 mmol) was added to the reaction mixture, cooled with an ice bath, and HCl (g) was bubbled through the reaction for another 2 h. The following day, the reaction was extracted with methylene chloride several times. The organic layer was dried over MgSO$_4$. The crude material was subjected to flash column chromatography on silica gel. The product was eluted with 10% ethyl acetate in hexane. Yield 31%.

^{1}H NMR (300 MHz, CDCl$_3$): δ 6.83−6.75 (m, 8H), 6.01−5.98 (d, $J = 9$ Hz, 2H), 3.79 (s, 6H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 154.6, 145.0, 126.8, 122.6, 120.4, 117.6, 115.9, 111.9, 95.3, 56.0. HRMS (DEI) (*m/z*): calcd M$^+$ 308.1150; found 308.1043 (M$^+$).

Supporting Information Available: General information, analytical and spectral characterization data, graph of log k_Δ vs E_T, calculated ($E_{spiro} − E_{open}$) versus log k_Δ in methylcyclohexane at −70 °C for compounds **1a−e**, alternate representations of dual parameter Hammett plots, nomenclature guide for open forms of bisbenzospiropyrans, Cartesian coordinates of calculated lowest energy geometries of all open forms of bisbenzospiropyrans **1a−e**, proton NMR spectra for compounds whose rate data are reported in this work. This material is available free of charge via the Internet at http://pubs.acs.org.

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