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Synthesis, Photochromic Properties, and Light-Controlled Metal Complexation of a Naphthopyran Derivative

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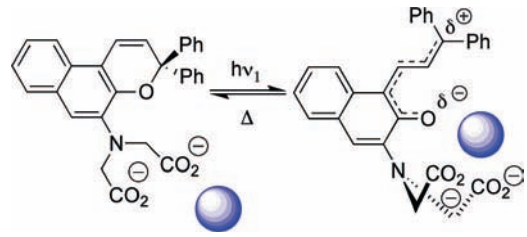
ABSTRACT



A light-controlled reversible binding switch based on photochromic 3*H*-naphtho[2,1-*b*]pyran is under development for studying cellular oscillatory calcium signals. The binding affinities of the closed and open forms of substituted naphthopyran 1 for Ca^{2+}, Mg^{2+}, and Sr^{2+} in buffer were determined. The photochemically ring-opened form of the receptor exhibited increased affinity compared to the thermally stable closed form of the receptor. The binding affinity difference for Ca^{2+} was ~77-fold at pH 7.6.

Calcium (Ca^{2+}) is a second messenger in many cell types, where it is used to translate extracellular signals into a wide variety of intracellular events.[1] Important cellular processes are controlled by Ca^{2+}, and many disease states are associated with defects in the calcium signalosome. Manipulation of Ca^{2+} concentration in cells through cage compounds is an important tool for learning about this widespread signaling system.[2] Caged Ca^{2+} compounds undergo irreversible photochemical reactions that either release or take up Ca^{2+} when triggered. However, cage compounds are less well suited to the examination of oscillatory calcium signals, which may encode information through both amplitude and frequency modulation. The origin of these oscillatory signals is known, but their effects at a molecular level are less well understood. There is a need to develop new methodologies to study the effects of spatiotemporal changes in Ca^{2+} concentration.

One approach to studying calcium oscillations is the development of a water-soluble reversible small molecule cage for Ca^{2+} triggered by light.[3] To date, such a cage has not been documented in the literature. In addition to satisfying the design criteria for classic caged Ca^{2+},[4] a reversible binding photoswitch must exist in two interconvertible forms with at least 10-fold difference in binding affinity. The photoswitch must resist degradative processes to mimic the wide variety of observed physiological repetitive and oscillatory calcium signals. Photochromic compounds, which can change structure dramatically and reversibly upon

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irradiation, are well suited to this purpose. Photochromic scaffolds are successfully incorporated into structures in which chelation may be switched on and/or off through irradiation.[5] Naphthopyrans are a class of photochromic compounds which show promise for this application.[6] Irradiation of the colorless form with 360 nm light leads to the cleavage of a C−O bond, resulting in colored isomeric ring-opened forms which revert to the original form predominantly through thermal processes. Crown-ether-substituted naphthopyran scaffolds are effective binding photoswitches for metal ions in organic solvents.[7]

In the present work, we report the synthesis and characterization of compound **1**, a new water-soluble 3*H*-naphtho[2,1-*b*]pyran with an iminodiacetic acid substituent at position 5 (Figure 1). This compound is designed so that

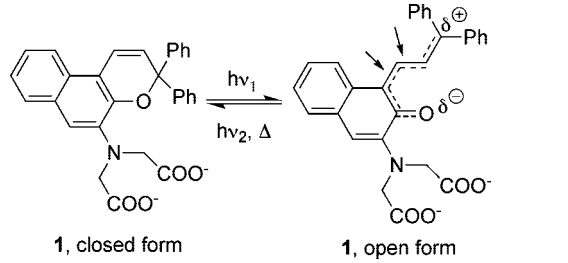

Figure 1. Two interconvertible forms of photochromic molecule **1**. Open forms may adopt a cisoid or transoid configuration at the indicated bonds.

the open form of **1** will exhibit a higher affinity for Ca^{2+} than the closed form. The binding affinities of closed and open forms of compound **1** were determined, and the effects of buffer composition and pH, on this reversible calcium binding photoswitch were also examined.

Naphthopyran chelator **1** was obtained in five steps (Scheme 1 and Supporting Information) with an overall yield of 11%. Compound **1** is soluble up to $\sim 5.7 \times 10^{-4}$ M in aqueous solutions buffered at pH 8.7.

Complexation of the closed form of **1** with metal ions was examined spectroscopically. The UV−vis spectra of **1** show minimal changes upon addition of excess Mg^{2+}, Ca^{2+}, and Sr^{2+} (Figure S1, Supporting Information). The addition of metal ions to the closed form of **1** did not result in any detectable thermal ring opening induced by metal complexation, unlike that observed for some photochromic chelators.[5d,7d,8] Small but significant and reproducible ^{1}H NMR shifts were observed upon addition of metal ions (Table S2, Supporting Information). One or more aromatic protons moved downfield upon addition of all metals to the closed form of **1**. The methylene protons only shifted when calcium was added, and they moved upfield. The same pattern of complexation-induced shifting, but with smaller magnitudes, was also observed upon the addition of the same three metals to phenyliminodiacetic acid in buffer (Table S3, Supporting Information). The binding titration data were fit to the two simultaneous binding equilibria shown below. An additional equilibrium (1 chelator:2 metal ions) was considered but did not improve the fit to the data. The best fit binding constants at two different pH's are tabulated in Table 1 below. The pH of 7.6 was chosen to simulate physiological environments as well as to completely deprotonate **1**, closed. A pH of 8.7 was also used in case the open form of **1** had higher pK_a's. Clearly, each metal ion binds very weakly as a 1:1 complex to the closed form of **1**. The effect of pH on the binding affinities is negligible. For Sr^{2+} at pH 7.6, the binding affinities and the theoretical maximum upfield shifts of **1** predicted by the binding model are inaccurate. This inaccuracy is due to the difficulty determining a very small binding affinity and the very small observable complexation induced shifting. The relatively large binding constant K_{21} for all metals with **1** suggests that a second chelator binds strongly with the 1:1 complex. This observation is consistent with an incomplete coordination of the cations by the iminodiacetic acid group and/or additional hydrophobic association of the planar portion of the tricyclic ring systems in the aqueous buffered solution.

Complexation of the open form of **1** with metal ions was also examined spectroscopically. When **1** was irradiated with UV light for 2 min in buffer, a visible absorption band appeared (434 nm), which faded after irradiation was halted (Figure 2). This long wave absorption corresponds to the more conjugated open form of **1**. The UV−vis spectra of irradiated **1** in the presence of excess alkaline earth metal ions were also recorded (Figure S2, Supporting Information). A red shift (20 nm) of the long wave absorption band to 454 nm when irradiation of **1** occurred in the presence of an excess of Ca^{2+} and a smaller red shift (7 nm) to 441 nm occurred in the presence of an excess of Sr^{2+}. The red shifting of the long wave absorption of the open form of a naphthopyran derivative upon addition of metal ions in acetonitrile is also documented by other groups.[7a,c] The shifting is thought to be indicative of stabilizing interactions

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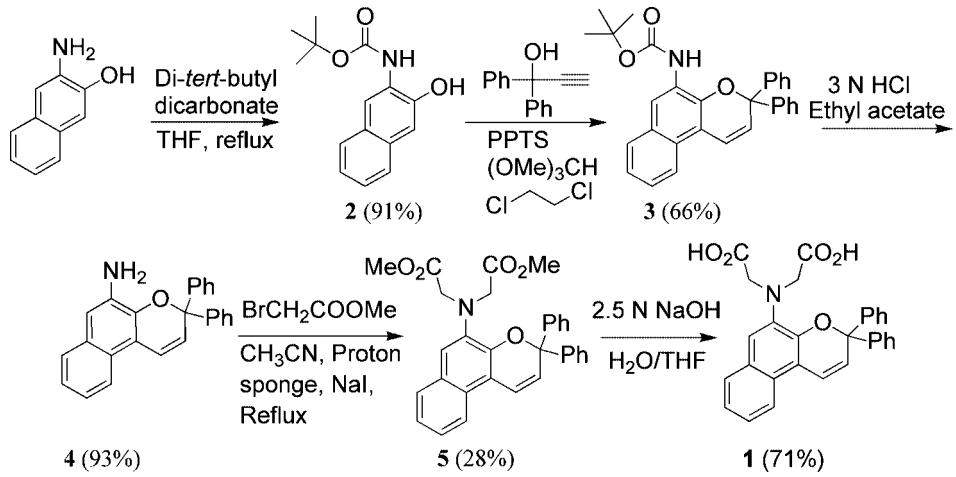
Scheme 1. Synthesis of **1**



between metal cation and oxygen that are enhanced in the presumably more polar excited state. Here, the absence of a red shift of the open form of **1** in the presence of Mg^{2+} suggests there is little interaction between this metal and the oxygen. The different absorbance values at the λ_{max}, despite constant chelator concentration, may be attributed to one or more of the following: a change in thermal fade rate constant in the presence of metal ions (see below), a difference in the extinction coefficients of metal-bound **1**, a change in the distribution of geometric isomers of **1** in the presence of different metals, and variable irradiation intensities.

Rates of thermal closure (k_Δ) of open forms of **1** to the closed form were determined in the presence of metal ions (Tables S4−S6, Supporting Information). Ca^{2+} addition decreased the observed rates of thermal closure of **1** at two different pH values. Sr^{2+} had this effect at pH 8.7 as well, although less pronounced than for Ca^{2+}. By contrast, the addition of Mg^{2+} increased the rate of thermal closure at pH 8.7 and had little effect on rates at pH 7.6. The increase of thermal fading rates of the open form of a crown-substituted naphthopyran by Mg^{2+} in acetonitrile has been documented previously,[9] but in that case, a red shift upon addition of Mg^{2+} was observed, in contrast to no observable shift of **1** here. To ascertain the effect of ionic strength changes on thermal fading kinetics, KCl was added to irradiated solutions of **1** and did not result in any changes in fading rates (Table S7, Supporting Information). Therefore, specific interactions between the divalent metal ions and the open forms of **1** must account for the observed rate changes. The observed changes in rates of thermal closure may be interpreted most simply as a result of stabilization (slowing thermal closure) or destabilization (increasing thermal closure rates) of the open forms by metals. Alternatively, the addition of metals may affect the distribution of geometric isomers formed upon irradiation, each isomer having a different fading rate.

The open form of **1** is short-lived, which prevents the use of a binding titration to determine metal binding affinities. However, the kinetics of thermal closure in the absence and presence of metal ions may be monitored to determine the binding equilibrium constant of the open form of a photo-

Table 1. Best Fit Binding Constants (K_{11}, K_{21}) and Maximum Shift ($\Delta\delta_{max}$) for Chelator **1** in 10 mM Tris by ^{1}H NMR[a]

$$\text{Chelator} + M^{2+} = \text{Chelator}\,M^{2+} \quad K_{11}$$
$$2\text{Chelator} + M^{2+} = \text{Chelator}_2\,M^{2+} \quad K_{21}$$

	K_{11} (M^{-1})	K_{21} (M^{-1})	$\Delta\delta_{max}$ (Hz)[d]
	pH 8.7		
Mg^{2+}	$1.20 \pm 0.12 \times 10^1$	$2.24 \pm 0.44 \times 10^5$	9.3 ± 1.8[b]
Ca^{2+}	$4.07 \pm 0.73 \times 10^1$	$7.77 \pm 1.05 \times 10^4$	18.0 ± 2.2[b]
Sr^{2+}	$3.16 \pm 0.51 \times 10$ °	$3.23 \pm 0.62 \times 10^4$	8.5 ± 1.0[b]
	pH 7.6		
Mg^{2+}	$7.57 \pm 0.60 \times 10$ °	$3.45 \pm 0.34 \times 10^5$	7.9 ± 0.6[c]
Ca^{2+}	$3.10 \pm 0.35 \times 10^1$	$5.10 \pm 0.20 \times 10^4$	13.8 ± 0.2[c]
Sr^{2+}	$6.55 \pm 0.54 \times 10^{-2}$	$6.77 \pm 1.31 \times 10^4$	389.5 ± 188.9[c]

[a] Average of 3 trials. $[\mathbf{1}]_0$: 1.97×10^{-4} M; $[M^{2+}] = 0 \sim 0.17$ M. [b] Both 400 and 600 MHz instruments were used. [c] Obtained on a 600 MHz instrument. [d] $\Delta\delta_{max}$ values are reported for the aromatic proton "b" (Table S2). The $\Delta\delta_{max}$ obtained by the 600 MHz instrument were divided by 1.5.

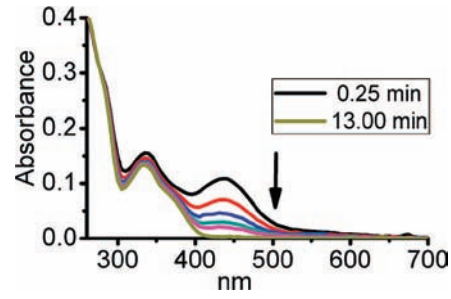


Figure 2. Thermal fading of **1** in the dark over time. $[\mathbf{1}] = 1.70 \times 10^{-5}$ M in 10 mM Tris, pH 8.7, at 24 °C.

chromic compound.[10] Using this procedure, a graphical method was used to obtain the binding affinity of the open form of **1** with metal cations (Figures S4−S9, Supporting Information). The resulting binding affinities between Ca^{2+} and $Sr^{2+}$and the open form of **1** at different pH are shown in Table 2. The effect of pH on the affinities was negligible.

Table 2. Metal Binding Affinities of the Open Form of **1** in 10 mM Tris at 24 °C

metal ion	pH	K_{eq} (M^{-1})
Ca^{2+}	8.7	$2.53 \pm 0.24 \times 10^3$
	7.6	$2.39 \pm 0.26 \times 10^3$
Sr^{2+}	8.7	$5.94 \pm 0.48 \times 10^2$

The faster rates observed in the presence of Mg^{2+} are incompatible with using this kinetic model for binding constant determination. As anticipated, the binding affinities for both Ca^{2+} and Sr^{2+} were significantly different for the closed versus open forms of **1**. For Ca^{2+}, there was a 77-fold enhancement in affinity upon irradiation at pH 7.6 and a 62-fold enhancement at pH 8.7.

The effects of pH and buffer salt composition on photochromism were determined for five cycles of irradiation and thermal closure. An example of photoswitching in 10 mM Tris, pH 8.7, is shown in Figure 3 below. Changing pH (7.6−9.8) and buffer salt identity (phosphate, HEPES) did not significantly affect photoswitching (Figures S13−S16, Supporting Information). Addition of 4 mM Ca^{2+} to the solution had little effect on the absorbance, except for a slight diminishment of the absorbance at the photostationary state (Figure S17, Supporting Information). Addition of Mg^{2+} resulted in a significant decrease of the absorbance at the photostationary state due to the increased rate of thermal closure (Figure S18 and S19, Supporting Information).

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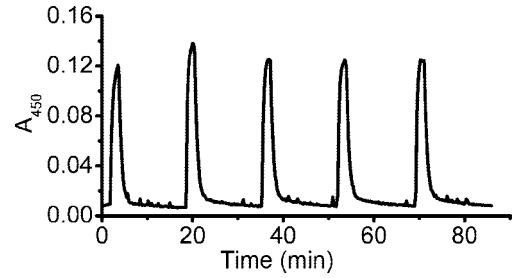

Figure 3. Five cycles of 2 min of UV irradiation of **1** ([**1**] = 1.70×10^{-5} M) followed by thermal (dark) closure in 10 mM Tris, pH 8.7, and 24 °C.

In summary, a water-soluble photoswitch for Ca^{2+} has been synthesized and characterized. Compound **1** exhibited a 77-fold affinity difference between closed and open forms, which is promising for a metal binding photoswitch. To the best of our knowledge, this is the first demonstration of a water-soluble, small molecule reversible photoswtich for Ca^{2+} binding. To achieve the binding affinity and oscillation periods more appropriate for intracellular conditions, further tuning of binding site geometry and switching kinetics through structural modifications of **1** is in progress. The work reported here represents a significant step in the development of a practical reversible cage for Ca^{2+} that will find use in investigations of intracellular calcium signaling.

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Supporting Information Available: Experimental procedures and spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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