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Synthesis and metal complexation properties of bisbenzospiropyran chelators in water

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ABSTRACT

As a step towards developing a light-controlled reversible binding switch based on photochromic bisbenzospiropyran for investigating intracellular calcium signaling, substituted bisbenzospiropyran and phenolic chelators were synthesized and examined for metal binding strength. The complexation of these compounds with alkaline earth and zinc ions in methanol and buffer was characterized using NMR and luminescence spectroscopies. An increased length of convergent ligands on the rigid scaffold maintained binding affinity for Ca^{2+} but decreased selectivity for Ca^{2+} over Mg^{2+}. Results indicate that at least three carboxylate ligands are required for significant binding, and increased length of the ligands will result in a fully water-soluble photoswitch that exhibits two states with approximately 300-fold difference in binding affinity for Ca^{2+}.

1. Introduction

Calcium ion (Ca^{2+}) is an essential signaling molecule for a number of vital cell processes, including fertilization and development.[1] This signal may act locally in small compartments of the cell or globally throughout the cell. Additionally, the concentration of Ca^{2+} may oscillate at a variety of frequencies and different durations. However, little is known about the effect of these oscillations at molecular level. A photochromic molecule that binds and releases Ca^{2+} in response to light would provide an excellent tool for understanding the effect of these oscillations on a single protein by imposing oscillations of defined frequency. Since magnesium ion (Mg^{2+}) is also present at significant concentrations in cells, the photochromic system should be selective for Ca^{2+} over Mg^{2+}. Cage compounds have been developed and reported in the literature for binding or releasing Ca^{2+} under the influence of light.[2] The main drawback in using caged calcium for mimicking an oscillatory signal is that the technique leads to irreversible decomposition of the cage, limiting the number of oscillations possible.

A number of research groups have reported photochromic compounds for binding and releasing divalent cations.[3] While these compounds show promise as photoswitchable systems, they are poorly soluble in water and are therefore less suitable for biochemical applications. To date, no fully water-soluble reversible photoswitch for binding and releasing Ca^{2+} has been demonstrated.

A photochromic bisbenzospiropyran derivative **1a** has been proposed previously as a reversible calcium cage (Fig. 1). The substituent and solvent effects on the photochromic properties of bisbenzospiropyran have been reported.[4] The metal binding affinities of three metal ions with the water-soluble compound **1a** were determined by NMR and reported in a preliminary letter.[5] Compound **1a** was found to be fairly selective for Ca^{2+} over Mg^{2+}, but the binding affinity for Ca^{2+} was not adequate for use in cellular systems, and the ability of compound **1a** to release Ca^{2+} upon irradiation was not established. Here, we report a significant extension of the preliminary study. The results of increasing the length of the ligating arm on this rigid scaffold (**1b**) as well as the effect of varying the number of chelating groups (**10a** and **10b**) on the binding affinity for Ca^{2+} and Mg^{2+} as well as other cations are presented. The use of cations with a range of atomic radii (from Mg^{2+} to Sr^{2+} or even Ba^{2+}) better allows the identification of trends

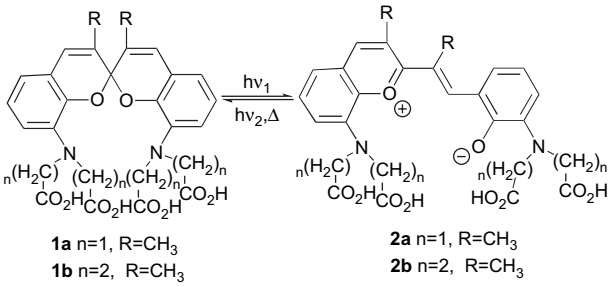

Figure 1. Forms **1** and **2** (interconvertible and photochromic if R=H).

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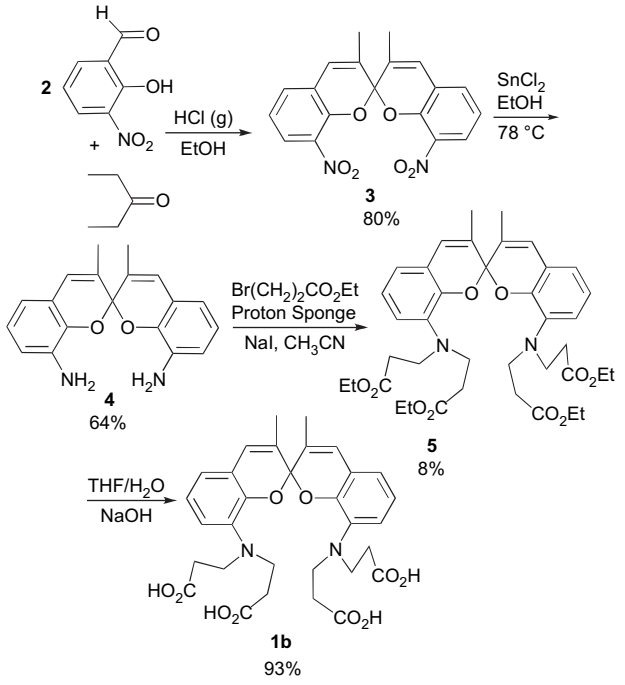
in binding affinity and selectivity. Additionally, to estimate the binding affinity of the short-lived open form **2** with metal ions, two control molecules possessing iminodiacetic acid and iminodipropionic acid groups (**12a** and **12b**) were also investigated. Taken together, these results establish the suitability of rigid bisbenzospiropyran derivatives as binding switches for Ca^{2+}.

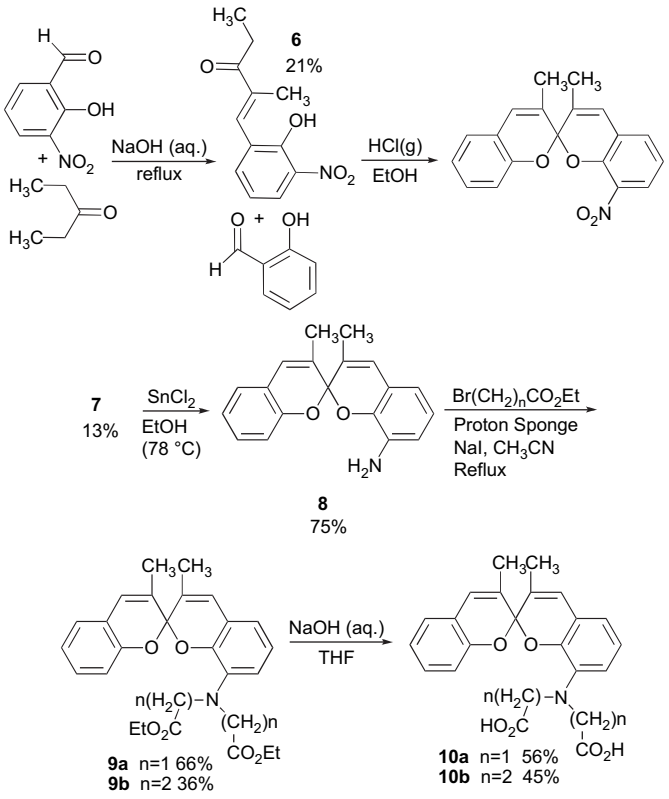
2. Results and discussion

2.1. Synthesis

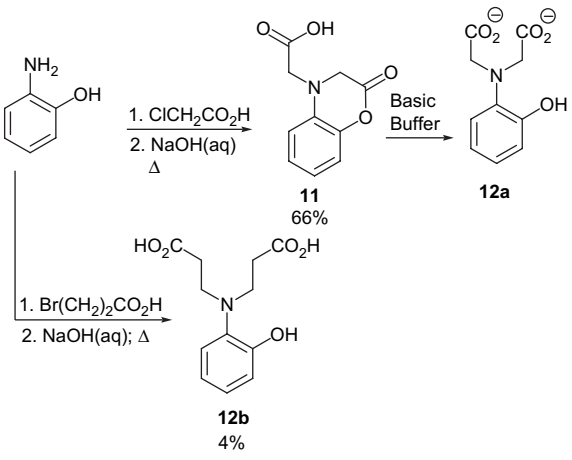
Chelator **1b** was obtained in four steps as shown in Scheme 1. The reaction of 3-nitrosalicylaldehyde with 3-pentanone in the presence of HCl (g) in ethanol provided 8,8′-dinitrobisbenzospiropyran **3** in excellent yield. Compound **3** was reduced with tin(II) chloride to give **4** in a good yield. Compound **4**, on reaction with ethyl bromopropionate, resulted in a workable yield of **5**. The yield of compound **5** is low, presumably due to the competing elimination reaction as well as to unfavorable sterics. Hydrolysis of **5** with aqueous NaOH in tetrahydrofuran provided target compound **1b** in good yield. Compound **1b** is soluble up to $\sim 2.5\times10^{-3}$ M in aqueous solutions buffered at pH 8.7–9.8.



Scheme 1. Synthesis of **1b**.



Scheme 2. Synthesis of **10a** and **10b**.



Scheme 3. Synthesis of **12a** and **12b**.

Chelators **10a** and **10b** were synthesized in five steps as shown in Scheme 2. The reaction of 3-nitrosalicylaldehyde with 3-pentanone under basic conditions provided compound **6** in moderate yield, which on reaction with salicylaldehyde in ethanol in the presence of HCl gave asymmetric 8-nitrobisbenzospiropyran (**7**). Compound **7** was converted to chelators **10a** and **10b** through reduction, addition of acetate or propionate groups, and hydrolysis.

Chelator **12a** was synthesized as a lactone **11** by reaction of chloroacetic acid with *o*-aminophenol in the presence of aqueous sodium hydroxide in good yield as described in the literature.[6] Compound **11** converts quantitatively to **12a** in basic buffer, confirmed by ^{1}H NMR and ^{13}C NMR. Chelator **12b** was synthesized by reacting bromopropionic acid with 2-aminophenol in the presence of aqueous base (Scheme 3). The yield was low due to the competing elimination reaction.

2.2. Metal complexation studies

2.2.1. Determination of binding affinity by NMR

NMR, UV, and luminescence spectroscopies have been used extensively to determine binding affinities.[7] Due to the very small changes in the UV absorption spectra on addition of metal ions to **1b**, **10a**, and **10b**, UV–vis spectroscopy was not used for binding constant determinations here. ^{1}H NMR titrations were performed to obtain binding affinities (K_a) and maximum complexation induced shifts ($\Delta\delta_{max}$) for chelators **1b**, **10a**, **10b**, **12a**, and **12b** with metal ions, as has been reported earlier for **1a**.[5] All the equilibria considered in fitting the data are shown in Figure 2. Figure 3 shows an example of the experimental values and the calculated values of the best fit binding model (1:1 binding only) for titration of Ca^{2+} with chelator **12a**.