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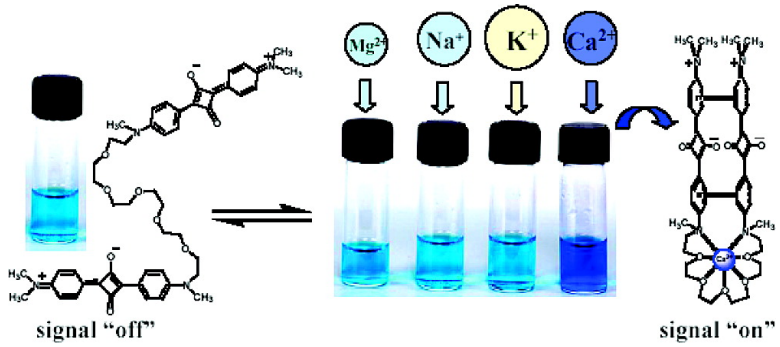

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Selective Calcium Ion Sensing with a Bichromophoric Squaraine Foldamer

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Abstract: Several squaraine tethered bichromophoric podand systems **1a**−**d** and a monochromophoric analogue **2** were prepared and characterized. Among these, the bichromophore, **1b**, containing five oxygen atoms in the flexible podand moiety was found to specifically bind Ca^{2+} in the presence of other metal ions such as K^+, Na^+, and Mg^{2+}. The selective binding of Ca^{2+} is clear from the absorption and emission spectral changes as well as by the visual color change of **1b** from light-blue to an intense purple-blue. Benesi−Hildebrand and Job plots confirmed a 1:1 binding between **1b** and Ca^{2+}. Signaling of the binding event is achieved by the cation-induced folding of the bichromophore and the resultant exciton coupling between the squaraine chromophores. The monochromophoric squaraine dye **2** failed to give optical signals upon Ca^{2+} binding, due to the absence of exciton interaction in the bound complex. Titration of the folded complex **9** with EDTA released the metal ion from the complex, thereby regaining the original absorption and emission properties of the bichromophore. The squaraine foldamer **1b** reported here is the first example of a selective chromogenic Ca^{2+} sensor, which works on the principle of exciton interaction in the folded Ca^{2+} complex of a bichromophore, the optical properties of which are similar to those of the "H"-type aggregates of analogous squaraine dyes.

Introduction

Design of chemosensors for the selective detection of a specific analyte is a topic of considerable interest, due to their wide ranging application in the broad areas of chemistry and biology.[1] Selective detection of biologically important cations such as Na^+, K^+, Mg^{2+}, and Ca^{2+}, particularly the detection of Ca^{2+} in the presence of Na^+ and K^+, is important because many physiological processes are triggered, regulated, or influenced by calcium ion.[2] Because of the importance of ionic calcium in biological processes, chemists have been interested in the design of chemosensors for the specific detection of calcium ions. The early work by Tsien and co-workers has contributed significantly to the design of Ca^{2+} sensors under biological conditions.[3] Later, de Silva et al. have reported the synthesis of a few Ca^{2+} specific sensors using the principle of photoinduced electron transfer (PET).[4] In addition, there are several reports in the literature which pertain to the use of various chromophore appended systems as Ca^{2+} sensors.[5] In several other reports, chromophore-linked pseudocyclic polyether systems (podands) have been shown to bind cations.[6] Among these, the early reports by Vögtle and co-workers and the subsequent work by Nakamura and co-workers are noteworthy.[7] In the majority of the chemosensors reported so far, the signaling of the binding event is achieved by electron transfer,[8] energy or charge-transfer processes,[9] and/or chromophore (excimer) interaction.[10]

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One of the important criteria for the designing of chemosensors is the selection of the signaling unit, which should produce a strong signal, preferably as a visual color change (chromogenic), upon an analyte binding. Organic dyes, particularly squaraine dyes whose optical properties are sensitive to the surrounding media, are ideal for this purpose. Although squaraine dyes have been used for the design of chemosensors,[11] the selectivity and sensitivity of most of these sensors are poor except in a very few cases.[11b,c] Earlier, we have shown that conjugated squaraine oligomers containing podand side chains have the ability to specifically bind Li^+ and K^+ with high sensitivity.[12] Recently, in a novel but simple approach, we have shown selective sensing of calcium and other alkaline earth metal ions using the principle of metal ion-induced folding of squaraine tethered podands.[13] In this approach, we introduced the concept of exciton interaction in squaraine "H"-aggregate as an alternate signaling mechanism for a cation binding process. The rationale behind the design of such chemosensors is based on the phenomena of "H" and "J" aggregation of organic dyes such as cyanines, merocyanines, and squaraines, which show distinctly different optical properties between aggregates and monomers.[14,15] Using our strategy of exciton coupled signaling through cation-induced conformational folding, Block and Hecht have recently reported the synthesis of a few polysquaraine-based sensors.[16] Our sustained interest in designing a selective chemosensor for Ca^{2+}, a biologically significant cation, which is difficult to detect in the presence of other similar ions, leads to the design of a new chromogenic probe based on a squaraine foldamer.[17] To our surprise, squaraines when tethered to a podand with five oxygen atoms, in a bichromophoric fashion, become insensitive to alkali metal ions but show remarkable selectivity and response toward Ca^{2+} as shown in Figure 1.

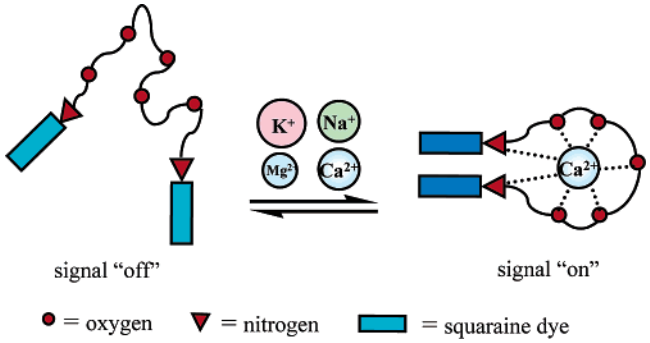

Figure 1. A chromogenic chemosensor based on a specific cation-driven, exciton interaction in a dye-linked podand.

Results and Discussion

Synthesis and Characterization of the Bichromophores 1a−d and the Monochromophore 2. Synthesis of **1a−d** was achieved as shown in Scheme 1. 3-(4-(*N*,*N*-Dimethylamino)-phenyl)-4-hydroxycyclobut-3-ene-1,2-dione (**8**) was prepared according to reported procedures.[18] The bisaniline derivatives **5a−d** were prepared by the reaction of the aniline derivative **3** with the corresponding ditosylates (**4a−d**) in 45−55% yields. Reaction of **8** with **5a−d** in 2-propanol/tributyl orthoformate under refluxing yielded the bichromophores **1a−d** in 20−25% yield. FT-IR spectra of **1a−d** showed a strong absorption at 1590 cm^{-1} characteristic of the resonance stabilized zwitterionic structure of squaraine dyes. ^1H NMR spectra of **1a−d** showed a set of peaks corresponding to the aromatic protons. Four of the aromatic protons together appeared as two doublets, which upon merging looked like a triplet at δ 6.8 ppm. The remaining four aromatic protons appeared as two doublets around δ 8.3 ppm. The ^{13}C NMR spectra, MALDI-TOF mass spectra, HRMS data, and elemental analysis data of **1a−d** were in agreement with the structures. Similarly, the monochromophore **2** was characterized using FT-IR, ^1H NMR, ^{13}C NMR, and elemental analyses.

Solvent-Induced Aggregation Behavior of 1a−d and 2. The bichromophores **1a−d** showed a strong absorption maximum

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(17) Foldamers are molecules designed to utilize noncovalent interactions to stabilize a well-defined conformation in solution. Although oligomers or polymers are generally referred to as foldamers, a dimer can be considered as a simplest foldamer unit. In the present context, a foldamer is a foldable bichromophore (dimer) attached to a flexible polyether, which undergoes a solvent- or cation-induced folding. (a) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173. (b) Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, *101*, 3893. (c) Schmuck, C. *Angew. Chem., Int. Ed.* **2003**, *42*, 2448. (d) Lokey, R. S.; Iverson, B. L. *Nature* **1995**, *375*, 303
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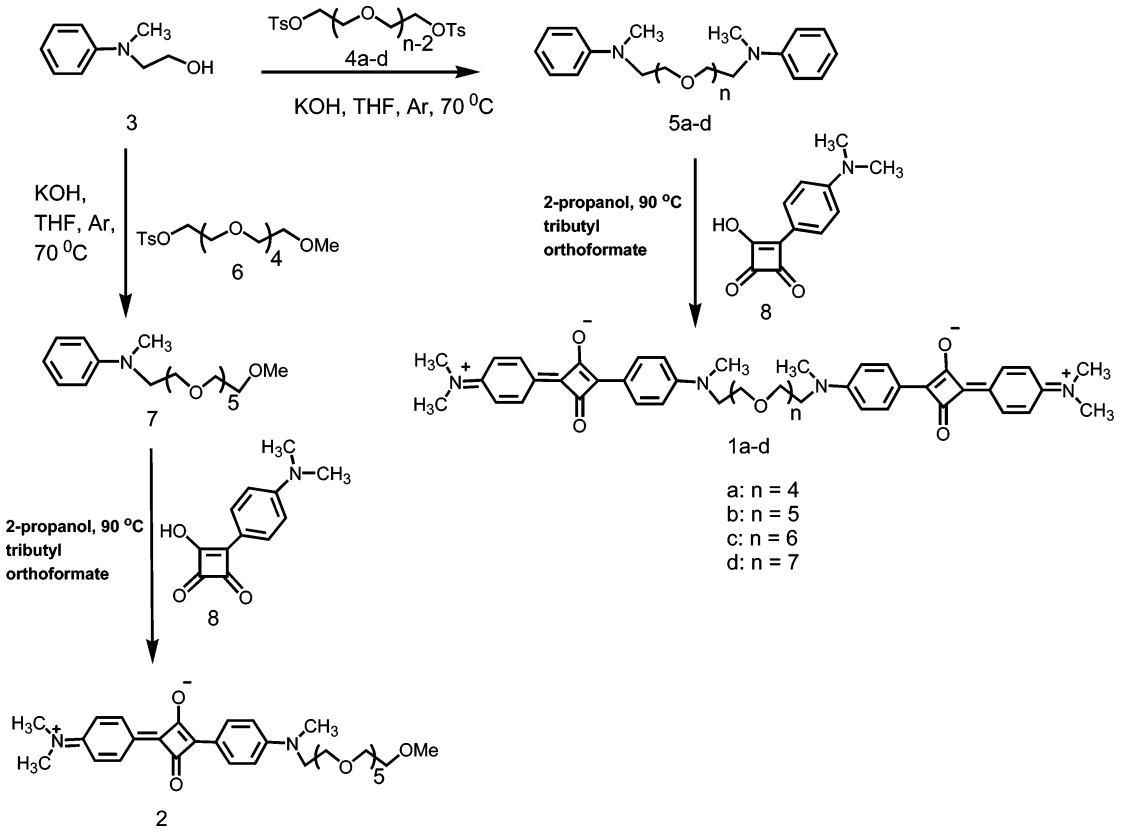
Scheme 1



around 630 nm in acetonitrile with an emission maximum around 655 nm. It is interesting to note that **1a**−**d** have low quantum yields of emission that gradually increased from 0.024 to 0.04 with an increase in the length of the podand chain. However, in acetonitrile, **2** showed a strong emission at 662 nm with a relatively high quantum yield of 0.21. This is in analogy to the report of Liang et al. who showed that squaraines, which are linked to a flexible alkyl chain in a bichromophoric fashion, show exciton coupled spectral changes, depending upon the length of the alkyl chain.[19]

Interestingly, in DMSO−H_2O mixtures of different compositions, **1a**−**d** showed a blue-shifted absorption band at 586 nm in addition to the monomer band at 649 nm as shown in the case of **1b** (Figure 2a). As the percentage of water in DMSO is increased, the intensity of the 649 nm band decreased with the concomitant increase in the intensity of the band at 586 nm through an isosbestic point at 623 nm. The monochromophore **2** showed similar but weak changes in the absorption spectrum in DMSO−H_2O as shown in Figure 2b. These observations are attributed to the "H"-aggregation, which are analogous to the report by Whitten et al.[20] In the case of **1a**−**d**, the detailed studies indicated that the aggregation is independent of concentration with an aggregation number of unity, characteristic of an intramolecular folding of the bichromophoric podands. However, the monochromophore **2** showed a concentration-dependent aggregation with an aggregation number of two, characteristic of an intermolecular interaction. Interestingly, in the case of **1b**, the solvent-induced aggregation resulted in a visual color change from light-blue to purple-blue (Figure 2a,

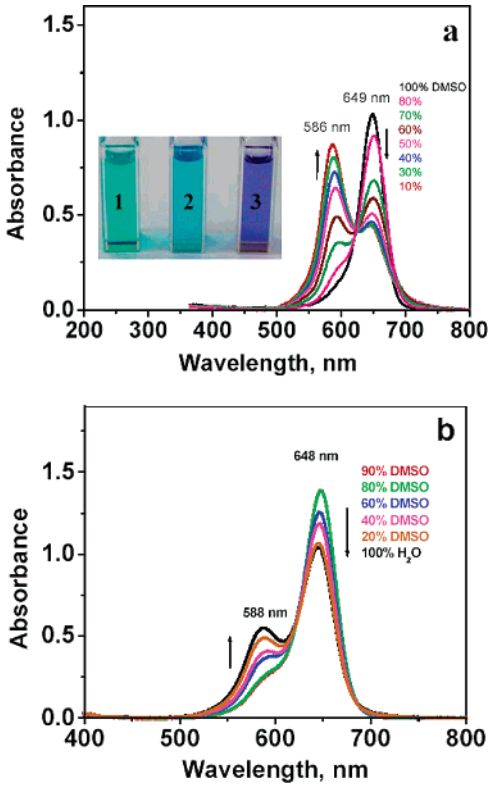


Figure 2. Absorption changes in different water−DMSO compositions of (a) **1b** (7.57 μM) and (b) **2** (5.9 μM). Inset picture shows **1b** in (1) 100% DMSO; (2) 50% DMSO/50% H_2O; and (3) 10% DMSO/90% H_2O.

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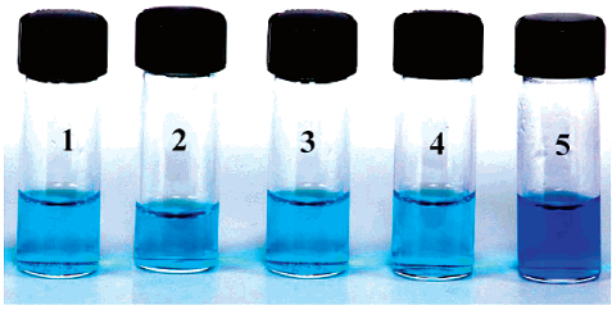

Figure 3. Visible color change of **1b** (5.1 μM) in acetonitrile upon addition of Ca^{2+}: (1) in the absence of metal salts, (2) Mg^{2+}, (3) Na^{+}, (4) K^{+}, and (5) Ca^{2+}.

inset), indicating a strong chromophore interaction, whereas in the case of **2**, the color change was weak. In light of these observations, we were keen to know the changes in the optical properties of **1a**−**d** and **2** in the presence of various added metal ions.

Metal Ion Binding Studies of 1a−**d.** To find out whether the bichromophores **1a**−**d** induce any visual color change, a series of experiments were conducted in the presence of different metal salts under various conditions. These studies revealed a visible color change from light-blue to an intense purple-blue in the presence of calcium perchlorate similar to the color change noticed in DMSO−H_2O mixtures. Surprisingly, in the case of **1b**, the color change was intense and specific toward Ca^{2+}, whereas addition of Na^{+}, K^{+}, and Mg^{2+} did not show any change at all (Figure 3). Interestingly, in acetonitrile (5.1 μM), significant changes in the absorption and emission spectra of **1b** were noticed with the addition of $Ca(ClO_4)_2$ as shown in Figure 4a and b, respectively. Upon gradual addition of $Ca(ClO_4)_2$, the intensity of the absorption maximum at 630 nm is decreased with the concomitant formation of a hypsochromically shifted band at 552 nm through an isosbestic point at 580 nm. Similarly, a fluorescence emission maximum of **1b** at 652 nm when excited at 580 nm (5.1 μM, CH_3CN) underwent considerable quenching upon gradual addition of $Ca(ClO_4)_2$ (Figure 4b). The fluorescence quantum yield of **1b**, which was 0.032 before the addition of $Ca(ClO_4)_2$, was decreased to a minimum value of 0.008. Singlet excited-state lifetime measurements of the bichromophore **1b** in acetonitrile showed monoexponential decay with a short lifetime of 200 ps. Upon addition of $Ca(ClO_4)_2$, the lifetime is enhanced to 240 ps with a monoexponential decay (see Supporting Information). Although the fluorescence quantum yield of **1b** in the presence of $Ca(ClO_4)_2$ is lower than that in the absence of $Ca(ClO_4)_2$, the observed excited-state lifetime is 40 ps higher in the former case. This result is in accordance with some of the previous reports pertaining to the excited-state dynamics of cyanine dyes.[14e,21]

Because of the importance of the selective detection of Ca^{2+} under aqueous physiological conditions, we attempted the titration of $Ca(ClO_4)_2$ against **1b** in acetonitrile−water (1:1) using *N*-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid (TES, 0.01 M) buffer at a pH of 7.2. However, in the buffer solution, two absorption maxima at 636 and 583 nm could be observed even before the addition of $Ca(ClO_4)_2$, indicating the aggregation of the chromophores. This observation reveals that

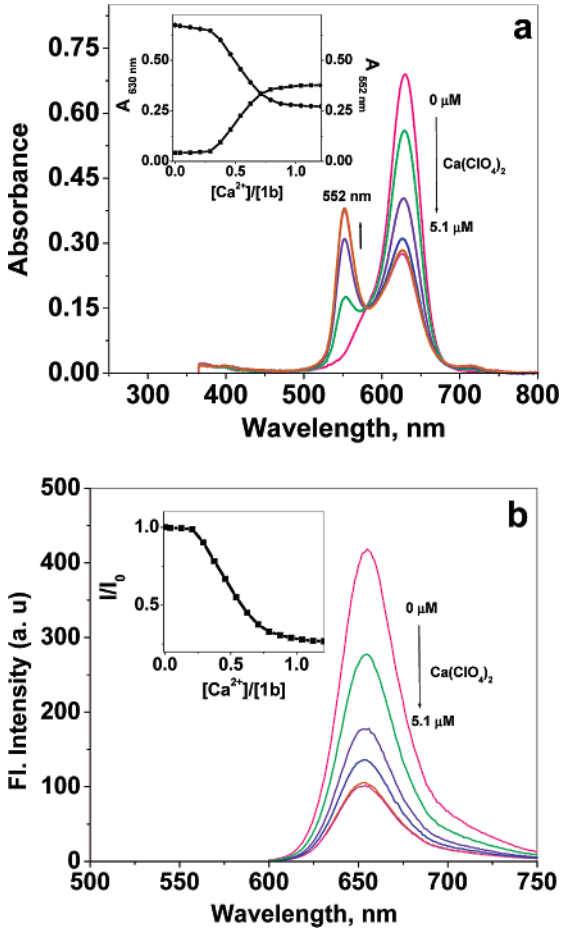

Figure 4. Changes in the (a) absorption and (b) emission spectra of **1b** (5.1 μM) in acetonitrile with the addition of $Ca(ClO_4)_2$. Insets show variation of (a) absorbance at 552 and 630 nm and (b) fluorescence intensity of **1b** with increasing concentration of $Ca(ClO_4)_2$.

the dye is folded to form the H-type dimer as in the case of DMSO−H_2O mixtures. Therefore, further changes in the absorption and emission spectra in buffer solutions were marginal upon addition of $Ca(ClO_4)_2$ (see Supporting Information).

Plots of the decrease in the absorbance at 630 nm and the increase in absorbance at 552 nm reached the saturation point when the ratio of $Ca(ClO_4)_2$ concentration reached the equivalent dye concentration, indicating a 1:1 complexation (inset, Figure 4a). The inset of Figure 4b shows the variation of fluorescence intensity of **1b** with increasing $Ca(ClO_4)_2$ concentration, where I_o and I are the fluorescence intensities before and after the addition of the metal perchlorates. The fluorescence intensity at 652 nm is gradually decreased until the Ca^{2+} concentration reached the dye concentration, thereby supporting a 1:1 complexation. The Job plot showed a maximum value for the absorbance at 552 nm when the mole fraction of **1b** reached 0.5, which is a signature of a 1:1 binding between the dye and Ca^{2+} (Figure 5).[22] The stability constants K_s were determined from the Benesi−Hildebrand plot of $A_o/(A_o - A)$ against $[M]^{-1}$, which showed a linear relationship, characteristic of a 1:1

(21) (a) Khairutdinov, R. F.; Serpone, N. *J. Phys. Chem. B* **1997**, *101*, 2602. (b) Seifert, J. L.; Connor, R. E.; Kushon, S. A.; Wang, M.; Armitage, B. A. *J. Am. Chem. Soc.* **1999**, *121*, 2987.

(22) (a) Job, P. *Ann. Chim.* **1928**, *9*, 113. (b) Gil, V. M. S.; Oliveira, N. C. *J. Chem. Educ.* **1990**, *67*, 473. (c) Specht, A.; Bernard, P.; Goeldner, M.; Peng, L. *Angew. Chem., Int. Ed.* **2002**, *41*, 4706. (d) Huang, F.; Gibson, H. W.; Bryant, W. S.; Nagvekar, D. S.; Fronczek, F. R. *J. Am. Chem. Soc.* **2003**, *125*, 9367. (e) Huang, F.; Fronczek, F. R.; Gibson, H. W. *Chem. Commun.* **2003**, 1480.

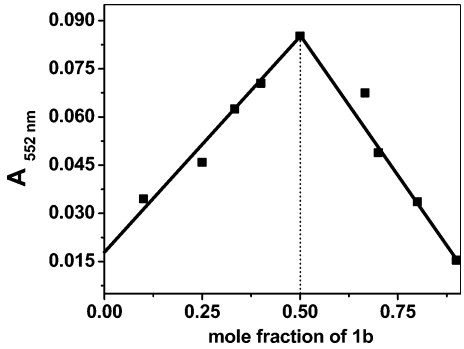

Figure 5. Job plot obtained for a 1:1 complexation of Ca^{2+} and **1b**.

Table 1. Binding Constants[a] (log *K*) of **1a**−**d** with Various Metal Ions in Acetonitrile

compound	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}	Na^{+}	K^{+}
1a	3.86	3.95	3.80	3.62	c	c
1b	b	4.29	b	b	c	c
1c	3.61	3.98	b	b	c	c
1d	3.75	3.93	b	b	c	c

[a] Reported binding constants are based on the assumption that the concentration of the free ions is equal to the total salt concentration. [b] Changes were too small to calculate the binding constant. [c] No response.

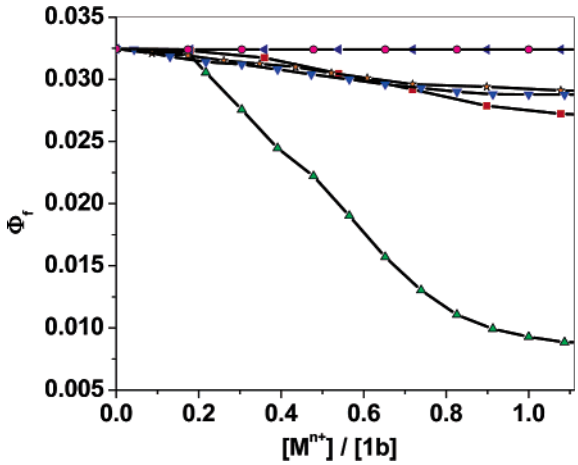

Figure 6. Plot of Φ_f versus the ratio between metal ion and **1b**, which illustrates the selectivity for Ca^{2+} (▲) over Na^{+} (△), K^{+} (●), Mg^{2+} (■), Sr^{2+} (★), and Ba^{2+} (▼).

binding.[23] The stability constants were calculated from the ratio of intercept/slope, and their values are shown in Table 1.

Addition of other alkaline earth metal salts such as $Mg(ClO_4)_2$, $Sr(ClO_4)_2$, and $Ba(ClO_4)_2$ showed relatively weak changes in the absorption and emission spectra (see Supporting Information). On the other hand, addition of a 3-fold excess each of Na^{+}, K^{+}, and Li^{+} to a solution of **1b** in CH_3CN could not induce any change in the absorption or emission properties. These observations show the unique ability of **1b** to selectively bind Ca^{2+}, which is clear from the plot of the variation of Φ_f against the ratio of the metal ion and **1b** (Figure 6). The maximum decrease in Φ_f is obtained for the binding of Ca^{2+}, revealing the high sensitivity and selectivity of **1b** to Ca^{2+}.

Cation binding studies of other bichromophores **1a**, **1c**, and **1d** with Mg^{2+}, Ca^{2+}, Sr^{2+}, and Ba^{2+} showed relatively weak response when compared to those of **1b**. However, similar to **1b**, Ca^{2+} showed maximum response toward **1a**, **1c**, and **1d**

(23) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703.

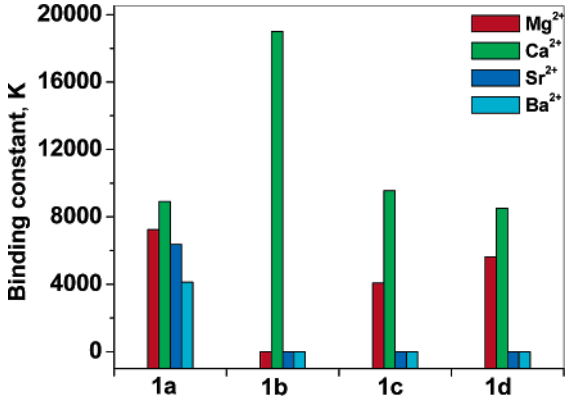

Figure 7. Plot of binding constants of **1a**−**d** with various metal cations.

(see Supporting Information). Addition of alkali metal ions such as Li^{+}, Na^{+}, and K^{+} did not show any measurable response with **1a**, **1c**, and **1d**. Binding constants of **1a**−**d** with various metal cations are calculated from the changes in the absorption spectra, and these values are shown in Table 1. The highest binding constant is obtained for the binding of Ca^{2+} to the bichromophore **1b**. **1a** showed weak binding constants with all of the cations added, whereas **1c** and **1d** showed weak binding toward Mg^{2+} and Ca^{2+}. The binding constants of other metal cations with **1b** could not be determined because the corresponding changes in the absorption or emission properties were too weak. Figure 7 shows the bar diagram of the binding constants of each bichromophore against various alkaline earth metal ions.

The difference in the binding affinity of **1a**−**d** with various cations under investigation can be attributed to the length of the podand chains. Unlike in macrocyclic crown ethers, the ionic size of the cation is not the only parameter that determines the binding affinity of pseudocyclic polyether systems. The number of oxygen atoms, the size of the pseudo crown cavity, the charge density, and the coordination number of the cation all play a considerable role in the binding interactions.[24] Surprisingly, the polyether chain length of **1b** with five oxygen atoms is found to be ideal for the binding of Ca^{2+}. This observation is clear from a comparison of the plots of the fluorescence quantum yields of **1a**−**d** against Ca^{2+} binding (Figure 8). The maximum quenching of fluorescence is obtained for Ca^{2+} binding when the number of oxygen atoms becomes five in the podand chain as observed with **1b**. Our finding is in analogy to the previous reports in the literature on selective binding of Ca^{2+} to similar podand chains.[6d,7a−c]

Cyclic Voltammetric Measurements. The cyclic voltammogram (CV) of **1b** in acetonitrile using tetrabutylammonium hexafluorophosphate as the supporting electrolyte at a scan speed of 100 mV/s shows two redox waves (Figure 9). The first wave at 270 mV was reversible and the second one at 586 mV was irreversible at slow scan rate under thin layer electrochemical conditions. The first and the second formal redox potentials were obtained by averaging the anodic and cathodic peak potentials of the CV. It was earlier reported by Law et al.[25] that substituents, both at the nitrogen atom and in the phenyl ring, have a strong influence on the oxidation potential, which

(24) Watanabe, S.; Ikishima, S.; Matsuo, T.; Yoshida, K. *J. Am. Chem. Soc.* **2001**, *123*, 8402.
(25) Law, K.-Y.; Facci, J. S.; Balley, F. C.; Yanus, J. F. *J. Imaging Sci.* **1990**, *34*, 31.

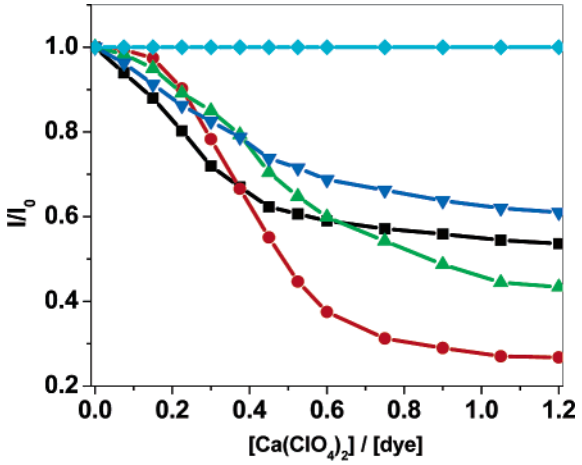

Figure 8. Plots of I/I_0 versus the ratio of $[Ca(ClO_4)_2]$ to [dye], illustrating the fluorescence response of various bichromophores, **1a** (■), **1b** (●), **1c** (▲), **1d** (▼), and **2** (◆), with increasing concentration of $Ca(ClO_4)_2$.

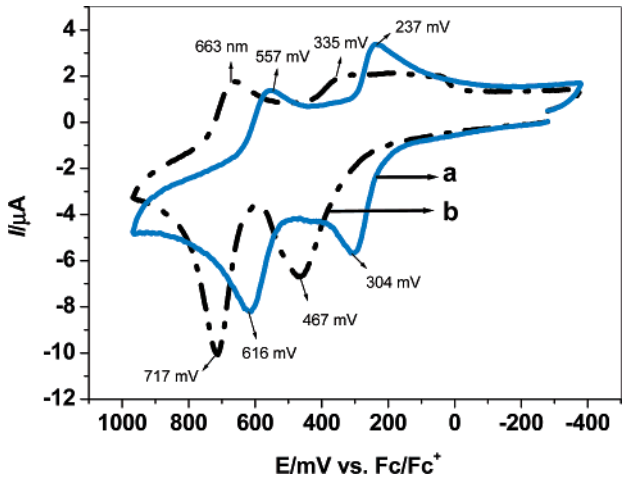

Figure 9. Cyclic voltammogram of **1b** in CH_3CN using 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte at a scan rate of 100 mV/s: (a) before and (b) after the addition of $Ca(ClO_4)_2$.

decreases as the electron-donating ability of the substituent increases. Addition of $Ca(ClO_4)_2$ into a solution of **1b** caused the shift of the redox potential to more positive values, that is, from 270 to 401 mV and from 586 to 690 mV. This result is attributed to the coordination of the nitrogen lone pair of electrons with Ca^{2+} that decreases the charge-transfer character of the chromophore. This finding is in accordance with the report of Loutfy and Law, who observed that the oxidation potential of D−A molecules generally increases as the CT character of the chromophore decreases.[26] Therefore, the observed increase in the redox potential of **1b** is attributed to the binding of Ca^{2+}.

^{1}H NMR Binding Studies. Changes in the ^{1}H NMR spectrum of **1b** before and after the addition of $Ca(ClO_4)_2$ in $CDCl_3/CD_3CN$ (8:2) at 27 °C are shown in Figure 10. The resonance signal due to the $-NCH_3$ protons, which appeared at δ 3.18 ppm, is shifted upfield upon addition of 1 equiv of Ca^{2+}. Similarly, the signals due to the $-OCH_2-$ and $-NCH_2-$ protons appearing at δ 3.59−3.69 ppm changed to a broad multiplet. Aromatic signals, which appeared at δ 6.73−6.79 ppm and δ 8.33−8.38 ppm, became weak and broad upon addition of Ca^{2+} (Figure 10c). These changes are indications of chromophore interaction

(26) Loutfy, R. O.; Law, K.-Y. *J. Phys. Chem.* **1980**, *84*, 2803.

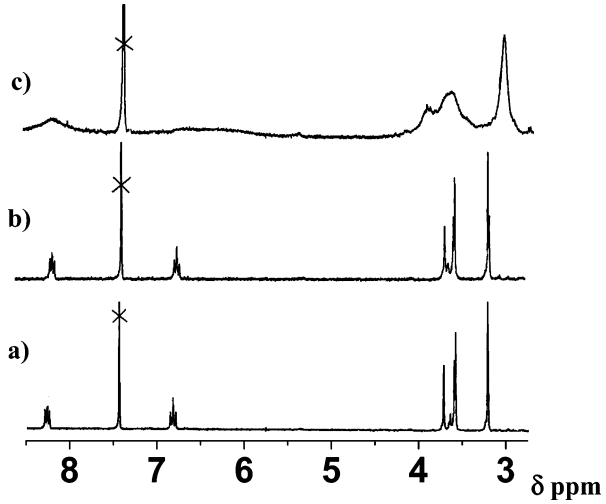

Figure 10. ^{1}H NMR spectra in 80% $CDCl_3$−20% CD_3CN of **1b** (3.1 mM) (a) before addition of metal perchlorates, (b) with 3.1 mM $NaClO_4$, and (c) with 3.1 mM $Ca(ClO_4)_2$.

as a result of the Ca^{2+}-induced folding and stacking of **1b**, which is in equilibrium between the folded and the unfolded conformations. On the other hand, addition of Na^+ or K^+ did not cause any considerable change in the ^{1}H NMR spectrum of **1b** (Figure 10b). Any influence of the perchlorate counteranion on the NMR peak broadening is ruled out because the addition of $NaClO_4$ or $KClO_4$ did not show any change in the NMR spectrum of **1b**. Similarly, any role of a permanent damage of the dye in the presence of $Ca(ClO_4)_2$ and the associated NMR signal broadening is ruled out by adding EDTA to the solution of **9** which brought back the original optical properties of the dye.

Metal Ion Decomplexation Studies. EDTA is known to be a strong chelator of Ca^{2+} and Mg^{2+}. Because of the high stability constant of the EDTA−Ca^{2+} complex, it was anticipated that addition of EDTA will induce the decomplexation of the Ca^{2+} from the complex **9**, thereby restoring the original photophysical properties of **1b**. With this intention, 1 equiv of EDTA solution is added to the Ca^{2+} complex of **1b**, which visibly turned the intense purple-blue color of the **1b**−Ca^{2+} complex into the initial pale-blue color of **1b**. The change in the absorption spectrum of **1b**−Ca^{2+} complex upon addition of EDTA solution is shown in Figure 11a, which is compared to the original spectrum of **1b**. The short wavelength absorption band of the **1b**−Ca^{2+} complex at 552 nm completely disappeared, and the spectrum matched that of **1b** where the original intensity of the band at 630 nm is regained. The fluorescence emission intensity is also restored upon addition of the EDTA to the Ca^{2+} complexed dye (Figure 11b). These results clearly indicate that the observed change in the absorption and emission properties of **1b** with Ca^{2+} is due to the formation of a reversible complex between the two.

Figure 12 shows the effect of temperature on the absorption properties of the **1b**−Ca^{2+} complex. As the temperature is increased from 25 to 70 °C, the intensity of the peak at 630 nm gradually increased, whereas the intensity of the blue-shifted band at 552 nm gradually decreased. This can be attributed to the decomplexation of Ca^{2+} from the bound metal complex of **1b** at higher temperatures. However, a complete change in the absorption spectrum could not be obtained at 70 °C. A further increase in temperature was not possible because the complexation was carried out in acetonitrile.

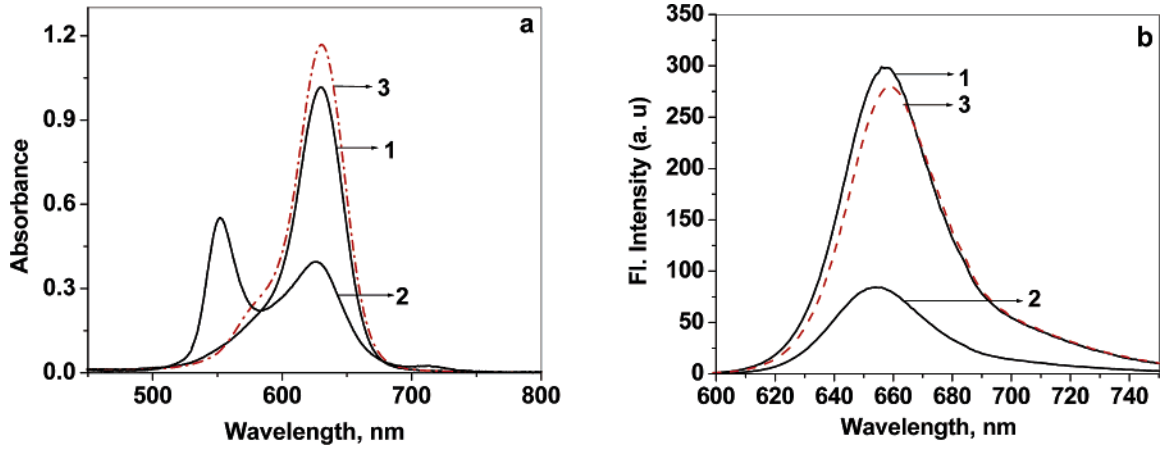

Figure 11. Change in (a) absorption and (b) emission spectra of **9** (7.3 μM) with the addition of 7.3 μM EDTA. Spectra 1, 2, and 3 show the absorption and emission of **1b**, **1b** + Ca^{2+}, and **1b** + Ca^{2+} + EDTA, respectively.

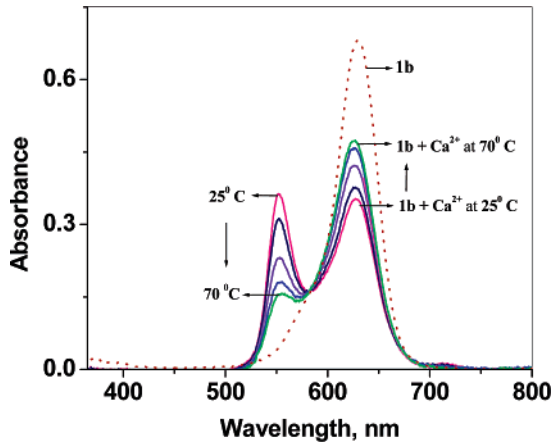

Figure 12. Effect of temperature on the absorption spectrum of **1b** after adding 1 equiv of $Ca(ClO_4)_2$.

Cation-Driven Folding and the Signal Transduction Process. The changes observed in the absorption, emission, ^1H NMR, and redox properties upon the addition of $Ca(ClO_4)_2$ can be rationalized on the basis of the Ca^{2+}-induced folding of the bichromophore to a folded complex **9** (Figure 13). The optical properties of **1b**−Ca^{2+} are similar to those of the solvent-induced "H"-aggregate of **1b**. These observations reveal that the Ca^{2+}-induced changes in the absorption and emission are due to the exciton interaction of the folded complex.[27] In the case of the folded complex **9**, the fluorescence is weaker when compared to that of the unfolded **1b**, because the internal conversion from an upper excited state to a lower one occurs immediately and the emission from a lower excited state is theoretically forbidden (inset, Figure 13). The 1:1 complexation mode obtained by the Job plots and the changes noted in the absorption and emission spectra strongly support the proposed cation-driven folding of the bichromophores. This conclusion is further confirmed by the Ca^{2+} titration studies of the monochromophore **2**, which contains only one squaraine chromophore. In this case, although a 1:1 binding of Ca^{2+} is evident from the ^1H NMR spectral data (see Supporting Information), no change either in the absorption or in the emission was observed. These results support the proposed signaling mechanism of the bichromophores.

Conclusions

A chromogenic sensor for the selective detection of Ca^{2+} in the presence of other cations such as Na^+, K^+, and Mg^{2+}, based on a squaraine foldamer, which works on the principle of exciton coupled signal transduction is described. The specific binding of Ca^{2+} is clear from the visual color change as well as the change in the absorption and emission spectra, thereby allowing a three-way signaling. The Job plot and Benesi−Hildebrand plot satisfy a 1:1 stoichiometry for the complexation of **1b** with Ca^{2+}. The cation-steered folding of the bichromophore and the consequent exciton coupling in the folded complex allowed the signaling of the binding event. The optical silence of the analogous monochromophore **2** with Ca^{2+} supports the suggested signaling mechanism. Regeneration of the initial absorption and emission property of the **1b**−Ca^{2+} complex **9** upon addition of EDTA shows the reversibility of the suggested complexation mode. In conclusion, we have shown that aggregation, which is a drawback in the case of several of the organic dyes, becomes a useful phenomenon in the design of ion specific chemosensors. However, for a practical application, it is necessary that the molecular probe described here should be able to detect calcium ions under biological conditions. The challenge here is the synthesis of highly water-soluble squaraine foldamers that are stable and should not self-aggregate under aqueous physiological conditions.

Experimental Section

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified and dried by standard methods prior to use. Melting points were determined with a Mel-Temp-II melting point apparatus and are uncorrected. High-resolution mass spectra were recorded in a Finnigan MAT 95 instrument using Xenon as ionization gas. ^1H and ^{13}C NMR spectra were measured on a 300 MHz Bruker Avance DPX spectrometer. FT-IR spectra were recorded on a Nicolet Impact 400D infrared

(27) The allowed and the forbidden transitions to the excitons are governed by the tilt angle "α" of the transition moments with respect to the line of centers. When "α" is less than 54°, transition to the lowest excited level is allowed, resulting in a bathochromically shifted absorption due to head-to-tail "J"-aggregates. If "α" is greater than 54°, transition to the highest excited level is favored leading to hypsochromically shifted absorption which corresponds to the head-to-head "H"-aggregates. (a) Kasha, M.; Rawls, H. R.; EI-Bayoumi, M. A. *Pure Appl. Chem.* **1965**, *11*, 371. (b) Davydov, A. S. *Theory of Molecular Excitons*; Plenum Press: New York, 1971. (c) McRae, E. G.; Kasha, M. *Physical Process in Radiation Biology*; Academic Press: New York, 1964; p 17. (d) Hochstrasser, R. M.; Kasha, M. *Photochem. Photobiol.* **1964**, *3*, 317. (e) Czikkely, V.; Forsterling, H.; Kuhn, H. *Chem. Phys. Lett.* **1970**, *6*, 11. (f) Bucher, H.; Kuhn, H. *Chem. Phys. Lett.* **1970**, *6*, 183.

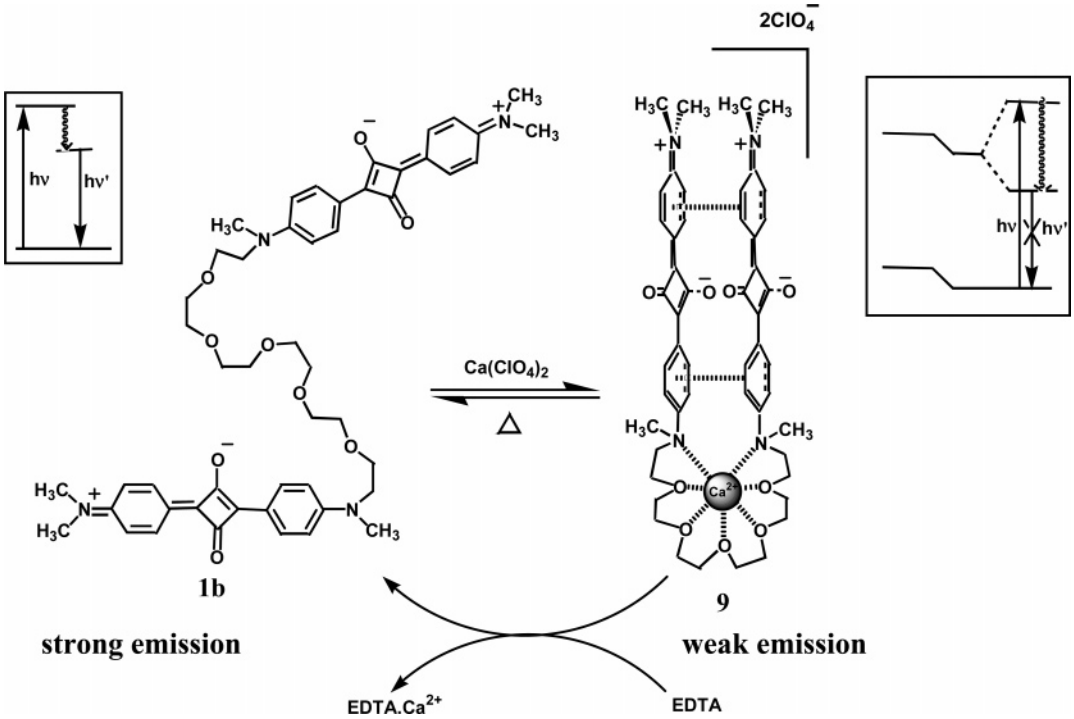

Figure 13. Ca^{2+}-induced folding of **1b** leading to an intramolecular "H"-dimer **9**. Insets show the corresponding allowed and forbidden transitions in the two conformations.

spectrophotometer. Elemental analyses were done using a Perkin-Elmer series-II 2400 CHN analyzer. Cyclic voltammetry was performed in a conventional undivided electrochemical cell with a three electrode arrangement using a computer-controlled Amel 5000 or EG&G 283 system.[28] The emission spectra were measured on a Spex-Fluorolog F112X spectrofluorimeter. Fluorescence quantum yields were determined in spectroscopic grade CH_3CN using optically matching solutions of 4,4-[bis-(*N*,*N*-dimethylamino)phenyl]squaraine dye ($\Phi_f = 0.70$ in chloroform) as standard at an excitation wavelength of 580 nm.[29] Fluorescence lifetimes were measured using a Tsunami Spectra Physics single photon counting system. The stability constant K_s was determined from absorption spectral changes using eq 1,

$$\frac{A_0}{A_0 - A} = \frac{\epsilon_L}{\epsilon_L - \epsilon_{ML}}\left(\frac{1}{K_s[M]} + 1\right)$$

where ϵ_L and ϵ_{ML} are the molar extinction coefficients of the ligand and the complex, respectively. The quantity $A_0/(A_0 - A)$ is plotted versus $[M]^{-1}$, and the stability constant is then given by the ratio of intercept/slope.

General Procedure for the Metal Ion Binding Titrations. Metal perchlorate solutions were prepared in spectroscopic grade acetonitrile. Bichromophores were completely dissolved in acetonitrile with sonication and slight warming in a water bath. Metal ion titrations were carried out by adding small volumes (1−5 μL) of the metal solutions (10^{-6} M in CH_3CN, 4 mL) in quartz cuvette. After the addition of metal salt solution to the cuvette, using a microliter syringe, the solution was shaken well and kept for 1 min before recording the absorption and emission spectra of the metal complexed dye.

General Procedure for the Preparation of the Bichromophores 1a−d and the Monochromophore 2. To a 100 mL round-bottomed flask containing 50 mL of 2-propanol were added 0.22 mmol of **5a-d**, 117 mg (0.54 mmol) of 3-(4-(*N*,*N*-Dimethylamino)phenyl)-4-hydroxycyclobut-3-ene-1,2-dione (**8**), and 1 mL of tributyl orthoformate. The reaction mixture was then refluxed for 20 h. The hot reaction mixture was filtered, and the solid was washed with 2-propanol until the filtrate was almost colorless. Column chromatography (chloroform/methanol, 9/1) of the crude product over silica gel (100−200 mesh) gave the pure product. The monochromophore **2** was prepared by the reaction of **7** and **8** as described above.

1a. Yield: 22%. mp 261−263 °C. FT-IR (KBr): ν = 2362, 1585, 1487, 1363, 1171, 1117, 933, 836, 791 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ = 3.17 (s, 18H, $-NCH_3$), 3.55−3.68 (m, 20H, $-NCH_2$ and $-OCH_2$), 6.73−6.78 (dd, J = 1.38, 9.37 Hz, 8H, aromatic), 8.32−8.37 (dd, J = 2.74, 9.32 Hz, 8H, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.6, 40.34, 52.35, 68.5, 70.58, 70.63, 70.89, 112.35, 112.53, 119.86, 120.2, 133.52, 154.46, 154.95, 183.32. Anal. Calcd for $C_{48}H_{54}N_4O_8$: C, 70.74; H, 6.68; N, 6.87. Found: C, 70.36; H, 6.78; N, 6.96. HRMS-FAB: $[M + H]^+$ calcd for $C_{48}H_{54}N_4O_8$, 815.9761; found 815.975.

1b. Yield: 24%. mp 225−227 °C. FT-IR (KBr): ν = 2361, 1593, 1401, 1367, 1182, 1122, 937, 830, 784.5 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ = 3.18 (s, 18H, $-NCH_3$), 3.59−3.69 (m, 24H, $-NCH_2$ and $-OCH_2$), 6.74−6.79 (dd, J = 1.2, 9.2 Hz, 8H, aromatic), 8.33−8.38 (dd, J = 2.8, 9.1 Hz, 8H, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.5, 40.64, 52.75, 68.7, 70.48, 70.33, 70.99, 112.15, 112.33, 119.75, 120.6, 133.12, 154.28, 154.98, 183.92. Anal. Calcd for $C_{50}H_{58}N_4O_9$: C, 69.91; H, 6.81; N, 6.52. Found: C, 69.61; H, 6.41; N, 6.65. HRMS-FAB: $[M + H]^+$ calcd for $C_{50}H_{58}N_4O_9$, 859.4282; found 859.4272.

1c. Yield: 20%. mp 235−237 °C. FT-IR (KBr): ν = 2355, 1588, 1480, 1366, 1174, 1118, 831, 791 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ = 3.18 (s, 18H, $-NCH_3$), 3.59−3.69 (m, 28H, $-NCH_2$ and $-OCH_2$), 6.73−6.79 (dd, J = 1.28, 9.5 Hz, 8H, aromatic), 8.34−8.39 (dd, J = 2.43, 9.2 Hz, 8H, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.3, 40.48, 52.53, 68.62, 70.42, 70.54, 70.78, 112.2, 112.53, 119.86, 120.2, 133.52, 154.46, 154.95, 183.32. Anal. Calcd for $C_{52}H_{62}N_4O_{10}$: C, 69.16; H, 6.92; N, 6.2. Found: C, 68.97; H, 6.78; N, 6.35. HRMS-FAB: $[M + H]^+$ calcd for $C_{52}H_{62}N_4O_{10}$, 904.0653; found 904.0647.

1d. Yield: 22%. mp 218−220 °C. FT-IR (KBr): ν = 2359, 1595, 1372, 1183, 1120, 833, 784 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, TMS):

(28) Bueschel, M.; Stadler, C.; Lambert, C.; Beck, M.; Daub, J. *J. Electroanal. Chem.* **2000**, *484*, 24 and references therein.
(29) Cornelissen-Gude, C.; Rettig, W.; Lapouyade, R. *J. Phys. Chem. A* **1997**, *101*, 9673.

δ = 3.18 (s, 18H, $-NCH_3$), 3.59−3.7 (m, 32H, $-NCH_2$ and $-OCH_2$), 6.73−6.79 (dd, J = 1.27, 9.28 Hz 8H, aromatic), 8.34−8.39 (dd, J = 2.46, 9.26 Hz, 8H, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.71, 40.33, 52.32, 68.56, 70.58, 70.8, 112.3, 112.4, 119.8, 119.9, 154.4, 154.9, 183.4. Anal. Calcd for $C_{54}H_{66}N_4O_{11}$: C, 68.48; H, 7.02; N, 5.92. Found: C, 68.25; H, 6.88; N, 5.63. HRMS-FAB: $[M + H]^+$ calcd for $C_{54}H_{66}N_4O_{11}$, 948.1733; found 948.1726.

2. Yield: 32%. mp 48−49 °C. FT-IR (KBr): ν = 2874, 2355, 1588, 1370, 1339, 1179, 1096, 838, 784 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.2 (s, 3H, $-NCH_3$), 3.21 (s, 6H, $-NCH_3$), 3.37 (s, 3H, $-OCH_3$), 3.55−3.72 (m, 24H, $-NCH_2$ and $-OCH_2$), 6.75−6.8 (m, 4H, aromatic), 8.36−8.41 (m, 4H, aromatic). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 39.7, 40.33, 52.32, 58.99, 68.56, 70.46, 70.51, 70.58, 70.61, 70.86, 71.88, 112.33, 112.45, 119.85, 119.93, 133.2, 154.43, 154.98, 183.38 ppm. Anal. Calcd for $C_{32}H_{44}N_2O_8$: C, 65.73; H, 7.58, N, 4.79. Found: C, 65.57; H, 7.89; N, 4.99. HRMS-FAB: $[M + H]^+$ calcd for $C_{32}H_{44}N_2O_8$, 585.8849; found 585.884.

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Supporting Information Available: Fluorescence lifetime profiles of **1b**. Changes in the absorption and emission properties of **1b** under aqueous buffer solution, with $Mg(ClO_4)_2$, $Sr(ClO_4)_2$, $Ba(ClO_4)_2$, $LiClO_4$, $NaClO_4$, and $KClO_4$. Changes in the optical properties of **1a**, **1c**, and **1d** with $Ca(ClO_4)_2$. ^{1}H NMR spectral changes of **2** with the addition of $Ca(ClO_4)_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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