DOI: 10.1002/ejoc.200900570

Specific Ca^{2+} Fluorescent Sensor: Signaling by Conformationally Induced PET Suppression in a Bichromophoric Acridinedione

Pichandi Ashokkumar,[a] Vayalakkavoor T. Ramakrishnan,[a] and Perumal Ramamurthy*[a]

Keywords: Sensors / Calcium / Fluorescence / Electron transfer / Photophysics / Acyclic polyethers

A series of acridinedione-based bichromophoric podand systems **1a–c** were synthesized and characterized. Among these, bichromophore **1c** shows specific binding of Ca^{2+} in the presence of other biologically important metal ions like Na^+, K^+, Mg^{2+}, and Zn^{2+}. The selective complexation was proved by steady-state emission, time-resolved emission, and ^1H NMR titration. Signaling of the binding event was achieved by Ca^{2+}-induced folding of the bichromophore, resulting in PET suppression in the acridinedione chromophore. Involvement of a PET process in the optical signaling was confirmed by comparing bichromophores **1a–c** with non-PET compound **2** and monochromophore model compound **3**. Non-PET compound **2** failed to give optical response upon Ca^{2+} binding as a result of the absence of a PET process in the Ca^{2+}-bound complex. Monochromophore **3** shows a similar optical response, which is the same as that in **1c**. Titration of the metal-ion-bound complex of **1c** with EDTA released the metal ion from the complex, thereby regaining the original photophysical properties of the bichromophore.

Introduction

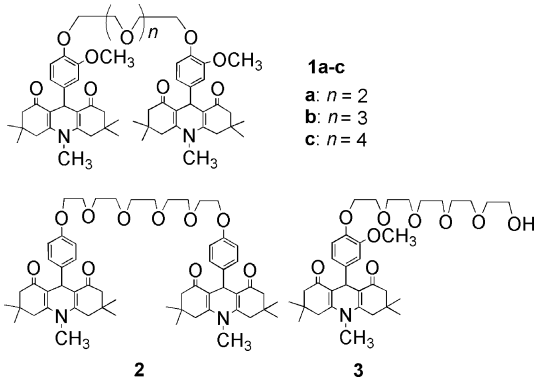
Fluorescent chemosensors capable of selectively recognizing metal ions have potential analytical applications in different fields including chemistry, biology, and medicine.[1] The design of chemosensors for the selective detection of biologically important metal ions such as Na^+, K^+, Mg^{2+}, Ca^{2+}, and Zn^{2+} is important,[2–6] because many physiological processes are controlled by these ions.[5b] Ca^{2+} is an intracellular divalent cation with the largest concentration variations, which plays a critical role as a signal transmitter.[7] Because of the importance of Ca^{2+} in biological processes, particular interest has been paid to the design of chemosensors for the specific detection of Ca^{2+} in the presence of other similar metal ions like Na^+, K^+, and Mg^{2+}. As a result of the similar chemical properties of Mg^{2+} and Ca^{2+}, selective detection between these two metal ions is a difficult task. Only a few examples of chemosensors for selective detection of Ca^{2+} over Mg^{2+} have been reported.[8] EDTA is a well known strong chelator of Mg^{2+} and Ca^{2+}, and it is commonly used to determine the concentration of these ions in solution. However, it does not show any selectivity for the two metal ions and forms two stable complexes. To evaluate the concentration of Ca^{2+}, Mg^{2+} must be removed from the system as $Mg(OH)_2$ in high pH solutions. If this evaluation is able to be achieved in one step, it will be of great advantage for fast and reliable analysis.

Even though a large number of cyclic crown ethers and related macrocyclic chemosensors have been reported, the corresponding acyclic polyether-based sensors are relatively rare.[9,10] Cyclic crown ethers have been found to be powerful extracting agents for alkali metal salts.[11] They form rigid and highly stable complexes with alkali and alkaline earth metal ions. The corresponding acyclic polyether shows only weak binding, but it changes its conformation from a linear to a pseudocyclic structure upon complex formation with metal ions. If this conformational change could be converted into physical signs, such as absorption and fluorescence, it would be possible to establish more sensitive metal ion sensors. The pseudocyclic structure increases the binding force and plays important roles not only in artificial ionophores but also in natural ionophores for the selective binding of metal ions.[12] Among the early reports on acyclic polyether-based chemosensors, reported by Nakamura et al. and Ajayaghosh et al., the signaling of the binding event was achieved by excimer formation,[10d] exciton interaction,[8b,8c,10e] twisted intramolecular charge transfer (TICT)[8a,8e] process, and electron[10a] or energy transfer[10b] process between two terminal chromophore units. None of the systems utilize the well-established photoinduced electron transfer (PET) process,[1c] which can operate in a single chromophore unit. Herein, we describe the concept of PET suppression resulting from the metal-ion-induced conformational folding of the acridinedione bichromophore.

[a] National Centre for Ultrafast Processes, University of Madras, Taramani Campus, Chennai 600113, India
Fax: +91-44-24546709
E-mail: prm60@hotmail.com

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.200900570.

Acridinedione (ADD) dyes have been reported as laser dyes with lasing efficiency comparable to that of coumarin-102, and these dyes have structural similarities with NADH.[13] Because both PET and intramolecular charge transfer (ICT) mechanisms can operate in acridinedione, it will be good to use it as a signaling unit in sensor molecules. We have already reported a few acridinedione-based anion and metal ion sensors, which operate through PET and ICT mechanisms.[14] In the present chemosensors **1a**–**c**, an oxyethylene moiety (ionophore) is linked with an acridinedione chromophore. In addition, an OCH_3 group having electron-donating ability is incorporated as an additional binding site as well as a signal transducing group based on PET process. It has already been reported that an electron-donating OCH_3 substituent increases the HOMO level of the donor, which makes the substituted phenyl ring a better electron donor in the PET process.[15] Involvement of a PET process in the optical signaling was confirmed by reference compound **2**, which does not have a PET process, and monochromophore model compound **3**, which has a PET process (Scheme 1).



Scheme 1. Structures of chemosensors **1**, **2**, and **3**.

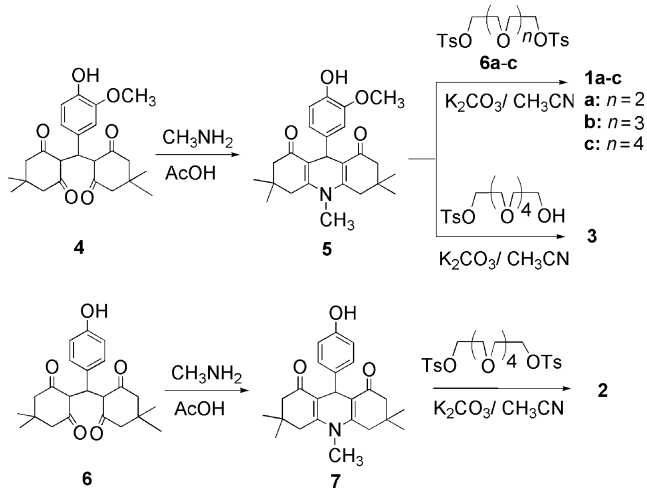
Results and Discussion

Synthesis

The synthesis of bichromophores **1a**–**c** and **2** and monochromophore **3** is outlined in Scheme 2. The acridinediones were obtained by refluxing methylamine with the respective tetraketones. Reaction of acridinedione with the corresponding ditosylate **6a**–**c** in the presence of K_2CO_3 yielded bis(acridinedione)s **1a**–**c** and **2**; the monotosylate yielded **3**. All the above compounds were characterized by spectral analyses.

Photophysical Studies

The absorption and emission spectra of all the compounds show a maximum at 371 and 436 nm, respectively, in acetonitrile, which are assigned to the ICT from the ring nitrogen atom to the ring carbonyl center within the ADD moiety. The fluorescence quantum yield and lifetime of all



Scheme 2. Synthesis routes to compounds **1**, **2**, and **3**.

the compounds are shown in Table 1. A relatively lower quantum yield and shorter fluorescence lifetime of **1a**–**c**, **3**, and **5** compared to those of **2** and **7** are attributed to the intramolecular PET process through space from the electron-rich OCH_3 group to the relatively electron-deficient excited state of the ADD fluorophore. Non-PET dyes **2** and **7** show a single exponential decay, whereas all other PET-based dyes show biexponential decay with relatively shorter fluorescence lifetimes. This kind of PET process through space from *p*-OCH_3[16] and *p*-$N(CH_3)_2$[14a] groups to the ADD moiety has already been reported. In this study, the PET process from the *m*-OCH_3 group is utilized as a signaling mechanism to monitor metal ion binding.

Table 1. Fluorescence quantum yields and lifetimes of **1a**–**c**, **2**, **3**, **5**, and **7** in CH_3CN.

	1a	1b	1c	2	3	5	7
Φ_f[a]	0.122	0.122	0.123	0.314	0.140	0.122	0.282
τ_f, ns	0.76	0.78	0.78	5.15	0.92	0.76	4.22
(B)[b]	(17.3)	(18.1)	(18.2)	(100)	(13.9)	(16.8)	(100)
	2.60	2.80	2.83	–	3.65	2.94	–
	(82.7)	(81.9)	(81.8)		(86.1)	(83.2)	

[a] Fluorescence quantum yields were determined by exciting the sample at 366 nm with the use of quinine sulfate as the standard (Φ_f = 0.546 in 0.1 N H_2SO_4); ± 5%. [b] Relative amplitude corresponding to the lifetime.

Metal Ion Binding Studies

Addition of Ca^{2+} to an acetonitrile solution of **1c** shows a marginal but reproducible (3 nm) redshifted absorption maximum with an isosbestic point at 381 nm. The corresponding fluorescence spectrum, when excited at its isosbestic point, shows a fluorescence enhancement without any spectral shift (Figure 1). Evidence for 1:1 complex formation is provided by the linear relationship obtained in the Benesi–Hildebrand plot[17] of $1/I_o - I$ against $[Ca^{2+}]^{-1}$ (Supporting Information, Figure S2). A stability constant ($\log K$) of 4.83 was estimated from this plot, which is reasonably high for an acyclic polyether. Addition of other

metal ions, such as Na^+, K^+, Mg^{2+}, and Zn^{2+}, did not show any significant change in the absorption and emission spectra, whereas the addition of Ca^{2+} to this solution caused a similar kind of fluorescence enhancement as that shown in Figure 1. These observations show the unique ability of **1c** to selectively detect Ca^{2+}, which is clear from the plot (Figure 2) of the variation of fluorescence intensity against the ratio of the metal ion and **1c**.

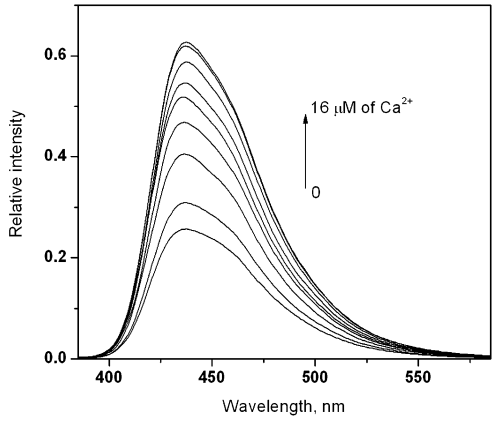


Figure 1. Fluorescence enhancement of **1c** (11 μM) upon addition of Ca^{2+} (0→16 μM) in acetonitrile; λ_{ex} = 381 nm.

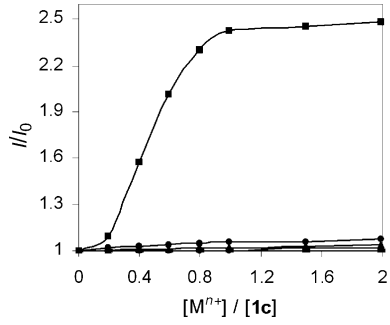


Figure 2. Plot of I/I_0 vs. the ratio between metal ion and **1c** in acetonitrile, which illustrates the selectivity for Ca^{2+} (■) over Na^+ (♦), K^+ (▲), Mg^{2+} (●), and Zn^{2+} (▬).

The complex formation between Ca^{2+} and **1c** was also investigated by time-resolved fluorescence. Bichromophore **1c** shows biexponential decay with a PET quenched lifetime of 0.78 ns (18.2%) and 2.83 ns (81.8%). Figure 3 presents the fluorescence decay of **1c** at different concentrations of Ca^{2+} in acetonitrile. During the addition of Ca^{2+} to **1c**, the shorter component disappears gradually, and the longer lifetime increases (2.83 to 5.68 ns) along with an increase in the amplitude. An increase in the lifetime of the longer component in the presence of Ca^{2+} and the disappearance of the short component indicates the suppression of PET process during the Ca^{2+} binding. We observe single exponential decay with longer lifetime component (5.68 ns) on complete complex formation between Ca^{2+} and **1c**. Addition of other metal ions did not show any significant change in the decay profile of **1c**.

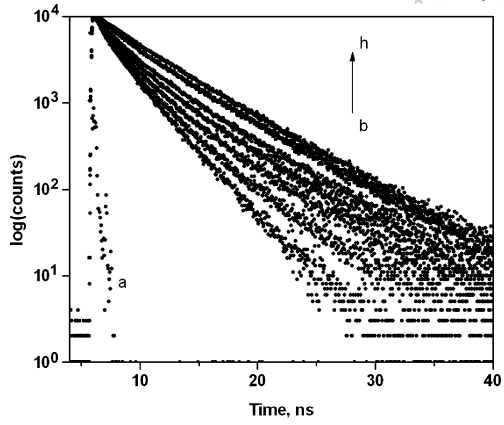


Figure 3. Fluorescence decay profiles of **1c** (11 μM) at different concentrations of Ca^{2+} in acetonitrile; λ_{ex} = 375 nm and λ_{em} = 436 nm: (a) laser profile, (b) **1c** alone, (c) 4.46, (d) 6.70, (e) 8.93, (f) 11.62, (g) 15.62, (h) 22.32 μM of Ca^{2+}.

Metal ion binding studies of bichromophores **1a** and **1b** showed similar behavior with weak response when compared to those of **1c**. However, both the bichromophores showed maximum response towards Ca^{2+}, which is the same as in the case of **1c** (see Supporting Information). Addition of Mg^{2+} showed weak response, and Na^+, K^+, and Zn^{2+} did not show any measurable response. Binding constants of **1a**–**c** with various metal ions were calculated from the changes in the fluorescence spectra, and these values are shown in Table 2.

Table 2. Binding constants (log *K*) of **1a**–**c** with various metal ions in acetonitrile at 22 °C.

	Mg^{2+}	Ca^{2+}	Na^+	K^+	Zn^{2+}
1a	[a]	3.69	[b]	[b]	[b]
1b	[a]	3.83	[b]	[b]	[b]
1c	[a]	4.83	[b]	[b]	[b]

[a] Changes were too small to calculate the binding constant. [b] No response.

The highest binding constant was obtained for the binding of Ca^{2+} to bichromophore **1c**. Compounds **1a** and **1b** showed relatively weak binding towards Ca^{2+}. The binding constants of other metal ions could not be determined because of the insignificant changes in the corresponding fluorescence spectra. The difference in the binding ability of **1a**–**c** with Ca^{2+} can be attributed to the number of oxygen atoms and the size of the pseudo-crown cavity. Binding affinity of macrocyclic crown ethers mainly depends on the ionic size of the cation, whereas in pseudocyclic systems, combined effects of the size of the pseudo-crown ether cavity, number of oxygen atoms, the charge density, and the coordination number of the cations play a considerable role. This may be the reason for the specific binding of Ca^{2+} over other cations. This observation is in analogy to the previous reports on selective binding of Ca^{2+} to similar podand chains.[8b,9b]

^{1}H NMR Binding Studies

To unravel the structure of the complex in detail, a ^{1}H NMR spectroscopic study was carried out in [D_3]acetonitrile at 22 °C. The spectra of **1c** before and after addition of Mg^{2+} and Ca^{2+} are shown in Figure 4.

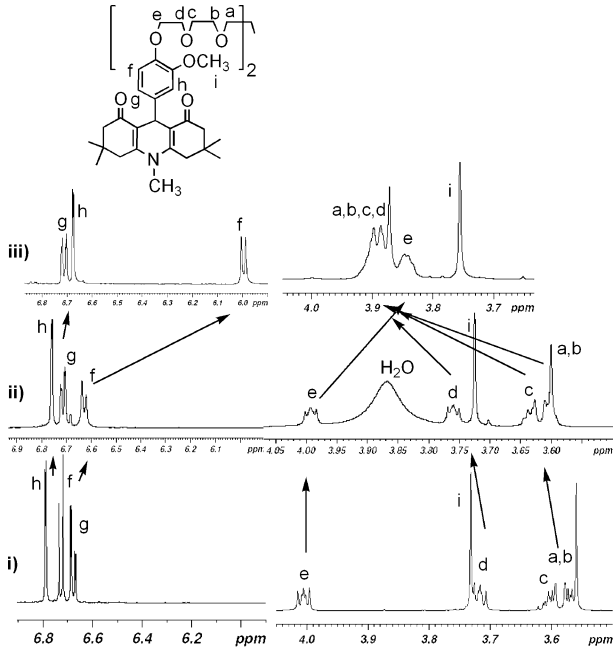


Figure 4. Partial ^{1}H NMR spectra of **1c** in CD_3CN: (i) **1c** alone, (ii) **1c**-Mg^{2+}, and (iii) **1c**-Ca^{2+}.

After the addition of Ca^{2+}, the well-resolved oxyethylene proton peaks (a–d) became broad and shifted to lower magnetic field ($\Delta\delta$ = 0.32–0.17 ppm) due to the reduction in the electron density on the oxygen atom by the coordinated metal ion. In contrast, the oxyethylene proton peak e ($\Delta\delta$ = 0.16 ppm) nearest to the benzene ring and aromatic proton f ($\Delta\delta$ = 0.73 ppm) shifted to higher magnetic field as a result of shielding by the uncoordinated oxygen atom. A similar high magnetic field shift of aromatic proton f was also observed in complex **1b**-Ca^{2+} with a relatively lower $\Delta\delta$ value of 0.45 ppm, whereas in complex**1a**-Ca^{2+} all the protons appeared at lower magnetic field. This confirms that the two oxygen atoms attached to the benzene rings move away and effectively shield the adjacent protons in **1c** and **1b**-Ca^{2+}, whereas in **1a** all the oxygen atoms are involved in Ca^{2+} binding. Addition of Mg^{2+} showed a smaller change, whereas the other metal ions did not show any change in the entire proton spectrum. This clearly reveals that there is no binding between these ions and bichromophores **1a**–**c**.

Metal Ion Decomplexation Studies

Because of the high stability constant of the EDTA-Ca^{2+} complex, it was anticipated that addition of EDTA would induce decomplexation of Ca^{2+}, thereby restoring the original photophysical properties of **1c**. The changes in the emission spectrum of **1c**-Ca^{2+} upon addition of a solution of EDTA are shown in Figure 5. Addition of EDTA shows gradual quenching of emission intensity without any spectral shift. When 1 equivalent of EDTA was added, the emission spectrum restored its original intensity of **1c**. Time-resolved studies also support the similar kind of decomplexation. These results clearly indicate that the observed changes in the photophysical properties of **1c** with Ca^{2+} are due to reversible complexation.

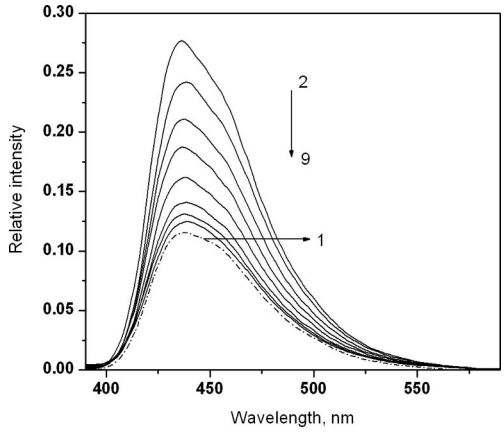


Figure 5. Changes in the emission spectra of **1c**-Ca^{2+} (11 μM: 11 μM) in acetonitrile with the addition of EDTA. λ_{ex} = 381 nm: (1) **1c** alone, (2) **1c** + Ca^{2+}, (3–9) **1c** + Ca^{2+} + EDTA (1.8 μM →11 μM).

To confirm the involvement of a PET process from the OCH_3 group in the optical signaling of bichromophores **1a**–**c** upon Ca^{2+} binding, reference compound **2** without a PET group (OCH_3) and monochromophore compound **3** with a PET group were synthesized and treated with all four metal ions. No significant change in the absorption, emission, or fluorescence lifetime was observed in **2**. It is interesting to see that **2** is optically silent towards Ca^{2+} despite their binding to **2**. This is proved by the ^{1}H NMR spectroscopic studies of **2** in CD_3CN in the presence of Ca^{2+} (Supporting Information, Figure S17). Addition of Ca^{2+} results in a considerable downfield shift of the oxyethylene proton peaks. Thus, whereas the NMR spectroscopy experiment clearly supports the binding of Ca^{2+} to **2**, the optical silence strongly supports the proposed mechanism where there is no possibility for a PET process. In contrast, the addition of Ca^{2+} to monochromophore model compound **3** shows a change similar to that observed for **1c** (Supporting Information, Figure S13). This clearly proved that the optical output did not arise from the interaction of two terminal chromophore units; instead, it arises within the single chromophore through a PET suppression mechanism. Addition of Ca^{2+} induced folding of the bichromophore, and the Ca^{2+} ions become nestled within the oxyethylene moiety and the OCH_3 groups. This binding suppresses the PET process and also results in the rigidification of the host molecular framework. This, in turn, results in the observed fluorescence intensity and lifetime enhancement.

Even though the simple monochromophoric system **3** is equally suitable for Ca^{2+} sensing, the selectivity ratio of Ca^{2+} over Mg^{2+} is comparatively lower in the monochromo-

phore. The selectivity plot of monochromophore **3** towards different metal ions is shown in Figure 6. Because of the greater flexibility of the oxyethylene chain and steric freedom of the monochromophore, Mg^{2+} also shows a slight enhancement in fluorescence intensity. So, we prefer the metal ion binding studies with a bichromophore instead of a monochromophore.

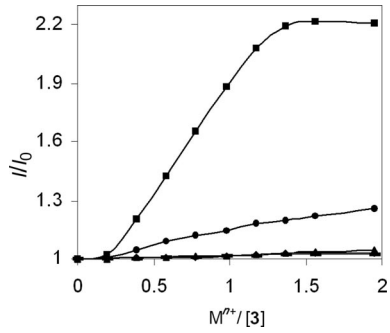


Figure 6. Plot of I/I_0 vs. the ratio between metal ion and **3** in acetonitrile, which shows the response for Ca^{2+} (■) and Mg^{2+} (●) over Na^+ (♦), K^+ (▲), and Zn^{2+} (▬).

To clarify the structural change of monochromophore **3** in the presence of Ca^{2+}, a ^1H NMR spectroscopy study was carried out in [D_3]acetonitrile (Supporting Information, Figure S18). During the addition of Ca^{2+}, all the oxyethylene protons showed a lower magnetic field shift ($\Delta\delta$ = 0.21–0.08 ppm). This confirms that all of the oxygen atoms are involved in Ca^{2+} binding, whereas in **1c** and **1b**, the two oxygen atoms attached to the benzene rings were not involved in Ca^{2+} binding as a result of steric crowdedness and the flexibility of the oxyethylene moiety.

On the basis of ^1H NMR and fluorescence spectral changes of all the compounds, an expected structural change in **1c** in the presence of Ca^{2+} is depicted in Figure 7. Before the formation of the complex, **1c** shows weak fluorescence due to the PET process from the OCH_3 group. After the addition of Ca^{2+}, the PET process is suppressed and results in a fluorescence enhancement. Because of the practical application of the selective detection of Ca^{2+} in aqueous media, we attempted the titration of Ca^{2+} with **1c** in various acetonitrile/water mixtures. As the percentage of water was increased, the sensitivity for Ca^{2+} decreased dramatically and the sensor molecule eventually did not respond at all in 20% water. Even though the present system works well in acetonitrile medium, the concept of PET suppression from the additional binding site incorporated into the spacer unit is the first of its kind.

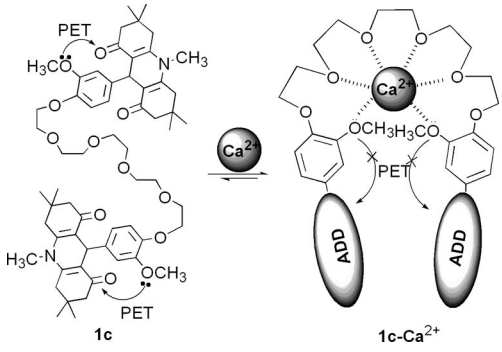


Figure 7. Schematic representation of Ca^{2+}-induced folding of bichromophore **1c** in acetonitrile.

Conclusions

In conclusion, a chemosensor for the selective detection of Ca^{2+} in the presence of other similar metal ions such as Na^+, K^+, Mg^{2+}, and Zn^{2+} involving an acridinedione-derived foldamer, which works on the principle of a PET suppression, was described. The optical silence of non-PET reference compound **2** and a similar response of monochromophore compound **3** with Ca^{2+} support the suggested signaling mechanism. The reversible complex formation between the bichromophore and Ca^{2+} was proved by restoring the initial photophysical properties of **1c** with the addition of EDTA to the **1c**-Ca^{2+} complex. However, for practical applications, it is necessary that the molecular probe described here is able to detect Ca^{2+} under biological conditions. The challenge here is the synthesis of highly water soluble acridinedione derivatives that can act as a good fluorophore under aqueous physiological conditions.

Experimental Section

General Procedures and Materials: Dimedone was purchased from Lancaster (India), Ltd. Pentaethylene glycol, vanillin, and all metal perchlorates were purchased from Sigma Aldrich Chemicals Pvt. Ltd. and were used as received. Acetonitrile used in this investigation was of HPLC grade, purchased from Qualigens India, Ltd. Pentaethylene glycol ditosylate and pentaethylene glycol monotosylate were synthesized according to a reported procedure.[18] Absorption spectra were recorded with an Agilent 8453 diode array spectrophotometer. Emission spectra were recorded with a Perkin–Elmer MPF-44B fluorescence spectrophotometer interfaced with a PC through Rishcom-100 multimeter and a HORIBA JOBIN YVON Fluoromax 4P spectrophotometer. Fluorescence decays were recorded by using an IBH time-correlated single-photon counting technique as reported elsewhere. NMR spectra were recorded with Bruker Avance III 500 MHz and Bruker 300 MHz instruments in deuterated solvents as indicated; TMS or the residual solvent peaks were used as internal standards. NMR peak assignments were made possible by using HSQC and HMBC 2D NMR spectral studies. Chemical shifts are reported in ppm and coupling constants ($J_{X-X'}$) are reported in Hz. In the case of ^{1}HNMR measurements of metal complexes, a 3.1×10^{-2} M concentration of chromophore were taken in [D_3]acetonitrile. Metal ions were added in excess amount to ensure complete formation of the complex. MS (ESI) were performed with an ECA LCQ Thermo system with ion-trap detection in positive and negative mode. Elemental C, H, and N analyses were performed with a Euro EA Elemental analyzer.

Synthesis of BisADD-1c

4: To a solution of dimedone (5.0 g, 36 mmol) in aqueous methanol (25 mL) was added vanillin (2.72 g, 18 mmol), and the mixture was warmed until the solution became cloudy. (4-Hydroxy-3-methoxybenzylidene)bis(dimedone) (**4**; 6.95 g, 94%) started to separate out.

The reaction mixture was diluted with water and allowed to stand overnight in the refrigerator; the tetraketone was collected by filtration, dried, and recrystallized from methanol.

5: A mixture of tetraketone **4** (2 g, 4.83 mmol) and methylamine (40% solution, 0.42 mL, 4.83 mmol) was kept under reflux in acetic acid (20 mL) for 6 h. After completion of the reaction as indicated by TLC, the reaction mixture was cooled and poured into crushed ice. The solid obtained was purified by recrystallization from $CHCl_3$/MeOH (8:2) to isolate acridinedione **5** (1.52 g, 77%) as a brown solid. M.p. 223–225 °C. FTIR (KBr): $\tilde{\nu}$ = 3170 [br. (OH)], 1640 [vs. (conj. CO)], 1367 [s (–C=C–)] cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, [D_6]DMSO, TMS): δ = 1.00 and 1.03 (2 s, 12 H, *gem*-dimethyl), 2.14 (s, 4 H, C^2,C^7 CH_2), 2.41 and 2.75 (2 d, J = 17.4 Hz, 4 H, C^4,C^5 CH_2), 3.27 (s, 3 H, NCH_3), 3.68 (s, 3 H, OCH_3), 4.96 (s, 1 H, C^9-H), 6.42–6.45 (dd, J = 1.8, 8.1 Hz, 1 H, ArH), 6.54 (d, J = 8.1 Hz, 1 H, ArH), 6.67 (d, J = 1.8 Hz, 1 H, ArH), 8.49 (s, 1 H, OH) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, [D_6]DMSO, TMS): δ = 27.8, 28.4, 29.8, 32.1, 33.1, 40.4, 49.6, 55.3, 111.5, 113.3, 114.8, 118.9, 137.0, 144.3, 146.8, 152.0, 194.7 ppm. MS (ESI): m/z = 410.37 $[M + 1]^+$. $C_{25}H_{31}N_1O_4$ (409.52): calcd. C 73.32, H 7.63, N 3.42; found C 73.53, H 7.68, N 3.39.

1c: To a solution of **5** (1.5 g, 3.66 mmol) in acetonitrile (20 mL) was added anhydrous K_2CO_3 (1.52 g, 10.98 mmol). The reaction mixture was stirred for 2 h at room temperature under an atmosphere of nitrogen, to which pentaethylene glycol ditosylate (0.80 mL, 1.83 mmol) in acetonitrile (10 mL) was added dropwise with stirring. The whole mixture was then kept under reflux for 16 h. The hot reaction mixture was filtered, and the resultant solution was evaporated under reduced pressure. The residue was dissolved in dichloromethane, washed with distilled water, and dried with $MgSO_4$, and the solvents were evaporated. The product was purified by column chromatography over silica gel ($CHCl_3$/MeOH, 98:2) to isolate pure **1c** (1.06 g, 57%) as a yellow powder. M.p. 80–82 °C. FTIR (KBr): $\tilde{\nu}$ = 1631 [vs. (conj. CO)], 1369 [s (–C=C–)] cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ = 1.04 and 1.08 (2 s, 24 H, *gem*-dimethyl), 2.23 (s, 8 H, C^2 and C^7 CH_2), 2.36 and 2.60 (2 d, J = 16.8 Hz, 8 H, C^4 and C^5 CH_2), 3.26 (s, 6 H, NCH_3), 3.63 (s, 4 H, OCH_2), 3.64–3.68 (m, 8 H, OCH_2), 3.78–3.81 (m, 10 H, OCH_2 and OCH_3), 4.07 (t, 4 H, OCH_2), 5.22 (s, 2 H, C^9-H), 6.54–6.57 (dd, J = 1.8, 8.2 Hz, 2 H, ArH), 6.68 (d, J = 8.4 Hz, 2 H, ArH), 6.95 (d, J = 1.8 Hz, 2 H, ArH) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ = 28.7, 31.0, 32.7, 33.4, 40.6, 50.0, 55.9, 68.5, 69.6, 70.6, 70.7, 112.6, 113.7, 115.0, 118.7, 139.3, 146.4, 149.2, 151.0, 195.5 ppm. MS (ESI): m/z = 1022.23 $[M + 1]^+$. $C_{60}H_{80}N_2O_{12}$ (1021.28): calcd. C 70.56, H 7.90, N 2.74; found C 70.43, H 7.92, N 2.72.

BisADD-1b: By using the above procedure, addition of tetraethylene glycol ditosylate (0.50 mL, 1.23 mmol) to **5** (1.0 g, 2.44 mmol) yielded **1b** (0.66 g, 55%) as a yellow powder. M.p. 87–89 °C. FTIR (KBr): $\tilde{\nu}$ = 1631 [vs. (conj. CO)], 1370 [s (–C=C–)] cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, TMS): δ = 1.04 and 1.08 (2 s, 24 H, *gem*-dimethyl), 2.23 (s, 8 H, C^2 and C^7 CH_2), 2.36 and 2.59 (2 d, J = 16.8 Hz, 8 H, C^4 and C^5 CH_2), 3.26 (s, 6 H, NCH_3), 3.65 (t, 4 H, OCH_2), 3.79–3.82 (m, 11 H, OCH_2 and OCH_3), 4.06 (t, 4 H, OCH_2), 5.21 (s, 2 H, C^9-H), 6.55–6.58 (dd, J = 1.8, 8.2 Hz, 2 H, ArH), 6.68 (d, J = 8.4 Hz, 2 H, ArH), 6.95 (d, J = 1.8 Hz, 2 H, ArH) ppm. ^{13}C NMR (300 MHz, $CDCl_3$, TMS): δ = 28.7, 31.0, 32.7, 33.4, 40.6, 50.0, 55.9, 68.5, 70.2, 70.7, 112.5, 113.7, 115.0, 118.7, 139.3, 146.4, 149.2, 151.0, 195.5 ppm. MS (ESI): m/z = 978.18 $[M + 1]^+$. $C_{58}H_{76}N_2O_{11}$ (977.23): calcd. C 71.29, H 7.84, N 2.87; found C 71.18, H 7.87, N 2.84.

BisADD-1a: By using the above procedure, addition of triethylene glycol ditosylate (0.84 g, 1.83 mmol) to **5** (1.5 g, 3.66 mmol) yielded **1a** (1.06 g, 62%) as a yellow powder. M.p. 94–96 °C. FTIR (KBr): $\tilde{\nu}$ = 1631 [vs. (conj. CO)], 1369 [s (–C=C–) cm–1] cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, TMS): δ = 1.00 and 1.03 (2 s, 24 H, *gem*-dimethyl), 2.22 (s, 8 H, C^2 and C^7 CH_2), 2.34 and 2.61 (2 d, J = 16.5, 17 Hz, 8 H, C^4 and C^5 CH_2), 3.25 (s, 6 H, NCH_3), 3.67 (s, 4 H, OCH_2), 3.79 (t, 10 H, OCH_2 and OCH_3), 4.07 (t, 4 H, OCH_2), 5.21 (s, 2 H, C^9-H), 6.55–6.57 (dd, J = 2, 8.5 Hz, 2 H, ArH), 6.69 (d, J = 8.5 Hz, 2 H, ArH), 6.94 (d, J = 2 Hz, 2 H, ArH) ppm. ^{13}C NMR (300 MHz $CDCl_3$, TMS): δ = 28.7, 31.0, 32.7, 33.4, 40.6, 50.0, 55.9, 68.5, 69.7, 70.7, 112.5, 113.8, 115.0, 118.7, 139.3, 146.4, 149.2, 151.1, 195.5 ppm. MS (ESI): m/z = 934.26 $[M + 1]^+$. $C_{56}H_{72}N_2O_{10}$ (933.18): calcd. C 72.08, H 7.78, N 3.00; found C 72.16, H 7.75, N 2.98.

Synthesis of BisADD-2

6: Treatment of 4-hydroxybenzaldehyde (2.20 g, 18 mmol) with dimedone (5.0 g, 36 mmol) afforded (4-hydroxybenzylidene)bis(dimedone) (**6**; 5.62 g, 82%).

7: Refluxing a mixture of tetraketone **6** (2.0 g, 5.2 mmol) with methylamine (0.45 mL, 5.2 mmol) yielded acridinedione **7**. The crude compound was purified by recrystallization from $CHCl_3$/MeOH (6:4) to isolate **7** (1.48 g, 75%) as a bright yellow crystalline solid. M.p. >260 °C. FTIR (KBr): $\tilde{\nu}$ = 3268 [br. (OH)], 1629 [vs. (conj. CO)], 1369 [s (–C=C–)] cm^{-1}. ^1H NMR (500 MHz, [D_6]DMSO, TMS): δ = 0.95 and 1.00 (2 s, 12 H, *gem*-dimethyl), 2.11 (d, J = 6.5 Hz, 4 H, C^2 and C^7 CH_2), 2.41 and 2.76 (2 d, J = 17 Hz, 4 H, C^4 and C^5 CH_2), 3.26 (s, 3 H, NCH_3), 4.92 (s, 1 H, C^9-H), 6.52 (d, J = 8.5 Hz, 2 H, ArH), 6.87 (d, J = 8.5 Hz, 2 H, ArH), 9.00 (s, 1 H, -OH) ppm. ^{13}C NMR (500 MHz, [D_6]DMSO, TMS): δ = 28.2, 28.8, 30.2, 32.6, 33.7, 40.5, 50.0, 113.7, 115.0, 128.4, 137.1, 152.7, 155.6, 195.3 ppm. MS (ESI): m/z = 380.43 $[M + 1]^+$. $C_{24}H_{29}N_1O_3$ (379.49): calcd. C 75.96, H 7.70, N 3.69; found C 75.85, H 7.66, N 3.64.

2: The reaction of **7** (1.5 g, 3.96 mmol) with pentaethylene glycol ditosylate (0.86 mL, 1.98 mmol) in the presence of K_2CO_3 (1.52 g, 10.98 mmol) afforded bis(acridinedione) **2**, which was purified by column chromatography over silica gel ($CHCl_3$/MeOH, 98:2) to isolate **2** (0.83 g, 44%) as a pale yellow powder. M.p. 140–142 °C. FTIR (KBr): $\tilde{\nu}$ = 1631 [vs. (conj. CO)], 1368 [s (–C=C–)] cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.98, 1.02 and 1.07, 1.09 (4 s, 24 H, *gem*-dimethyl), 2.16 and 2.22 (2 d, J = 16.5, 16 Hz, 8 H C^2 and C^7 CH_2), 2.35 and 2.58 (2 d, J = 17 Hz, 8 H, C^4 and C^5 CH_2), 3.26 (s, 6 H, NCH_3), 3.63 (s, 4 H, OCH_2), 3.65–3.70 (m, 8 H, OCH_2), 3.76–3.80 (m, 4 H, OCH_2), 4.02 (t, 4 H, OCH_2), 4.69 (s, 1 H, C^9-H); 5.19 (s, 1 H, C_9'-H), 6.70 (d, J = 8.5 Hz, 2 H, ArH), 6.75 (d, J = 8.5 Hz, 2 H, ArH), 7.11 (d, J = 8.5 Hz, 2 H, ArH); 7.18 (d, J = 8.5 Hz, 2 H, ArH) ppm. ^{13}C NMR (500 MHz, $CDCl_3$, TMS): δ = 27.3, 28.6, 28.7, 29.3, 30.9, 31.0, 32.2, 32.7, 33.4, 40.6, 40.9, 50.0, 50.8, 67.2, 69.7, 69.8, 70.6, 70.8, 114.2, 115.2, 115.8, 128.2, 128.5, 129.0, 129.3, 136.7, 138.4, 150.8, 156.9, 157.2, 162.1, 195.4, 196.4 ppm. MS (ESI): m/z = 962.17 $[M + 1]^+$. $C_{58}H_{76}N_2O_{10}$ (961.23): calcd. C 72.47, H 7.97, N 2.91; found C 72.29, H 7.93, N 2.88.

Monochromophore 3: The reaction of **5** (0.75 g, 1.83 mmol) with pentaethylene glycol monotosylate (0.60 mL, 1.82 mmol) in the presence of K_2CO_3 (0.76 g, 5.50 mmol) afforded monochromophore **3**, which was purified by column chromatography over silica gel ($CHCl_3$/MeOH, 97:3) to isolate **3** (0.60 g, 52%) as a pale yellow semisolid. FTIR (KBr): $\tilde{\nu}$ = 3300 [br. (OH)], 1634 [vs. (conj. CO)], 1367 [s (–C=C–)] cm^{-1}. ^1H NMR (500 MHz, CD_3CN): δ = 1.03 and 1.07 (2 s, 12 H, *gem*-dimethyl), 2.18 (s, 4 H, C^2 and C^7 CH_2), 2.45 and 2.72 (2 d, J = 17.5, 17 Hz, 4 H, C^4 and C^5 CH_2), 2.93 (t, 1 H, -OH), 3.26 (s, 3 H, NCH_3), 3.49 (t, 2 H, OCH_2), 3.57–3.63

(m, 14 H, OCH_2), 3.73–3.74 (m, 5 H, OCH_2 and OCH_3), 4.02 (t, 2 H, OCH_2), 5.05 (s, 1 H, C^9-H), 6.67–6.69 (dd, J = 2, 8.5 Hz, 1 H, ArH), 6.74 (d, J = 8.0 Hz, 1 H, ArH), 6.79 (d, J = 2.0 Hz, 1 H, ArH) ppm. ^{13}C NMR (500 MHz, CD_3CN): δ = 27.5, 27.7, 30.7, 32.2, 33.1, 39.7, 49.7, 55.2, 61.0, 68.2, 69.4, 70.1, 70.2, 70.3, 72.3, 111.6, 112.9, 113.6, 119.3, 139.5, 146.5, 149.0, 152.7, 195.5 ppm. MS (ESI): m/z = 630.70 $[M + 1]^+$. $C_{35}H_{51}N_1O_9$ (629.78): calcd. C 66.75, H 8.16, N 2.22; found C 66.68, H 8.19, N 2.18.

Supporting Information (see also the footnote on the first page of this article): HSQC and HMBC 2D NMR spectra of **1c**; NMR spectra of **1a**, **2**, and **3**; absorption spectra of **1c** in the presence of Ca^{2+}; Benesi–Hildebrand plot of $1/(I_0 - I)$ vs. $1/Ca^{2+}$ for **1c**; fluorescence spectra of **1a**–**c**, **2**, and **3** in the presence of various metal ions; fluorescence emission colors; ^1H NMR studies of **1b**, **1c**, **2**, and **3** with Ca^{2+}; schematic representation of the proposed structural change of **3** after the addition of Ca^{2+}.

Acknowledgments

The authors thank the Department of Science and Technology (DST), Government of India for financial support through SERC scheme project number DST/SR/S1/PC-31/2005. Financial support by DST-IRHPA is also gratefully acknowledged. NMR facilities provided at Sophisticated Analytical Instruments Facility (SAIF), IIT Madras by DST is thankfully acknowledged.

[1] a) L. Fabbrizzi, A. Poggi, *Chem. Soc. Rev.* **1995**, *24*, 197–202; b) J. P. Desvergne, A. W. Czarnik, *Chemosensors of Ion and Molecule Recognition*, Kluwer, Dordrecht, **1997**; c) A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566; d) B. Valeur, I. Leray, *Coord. Chem. Rev.* **2000**, *205*, 3–40.

[2] a) M. Dolman, I. O. Sutherland, *J. Chem. Soc., Chem. Commun.* **1993**, 1793–1795; b) I. Leray, F. O'Reilly, J.-L. H. Jiwan, J.-Ph. Soumillion, B. Valeur, *Chem. Commun.* **1999**, 795–796.

[3] a) W.-S. Xia, R. H. Schmehl, C.-J. Li, *Eur. J. Org. Chem.* **2000**, 387–389; b) H. He, M. A. Mortellaro, J. P. Leiner, R. J. Fraatz, J. K. Tusa, *J. Am. Chem. Soc.* **2003**, *125*, 1468–1469; c) J. Lee, H.-J. Kim, J. Kim, *J. Am. Chem. Soc.* **2008**, *130*, 5010–5011.

[4] a) S. Watanabe, S. Ikishima, T. Matsuo, K. Yoshida, *J. Am. Chem. Soc.* **2001**, *123*, 8402–8403; b) Q.-Z. Yang, L.-Z. Wu, H. Zhang, Z.-X. Wu, L.-P. Zhang, C.-H. Tung, *Inorg. Chem.* **2004**, *43*, 5195–5197; c) D. Ray, P. K. Bharadwaj, *Inorg. Chem.* **2008**, *47*, 2252–2254.

[5] a) R. Y. Tsein, *Biochemistry* **1980**, *19*, 2396–2404; b) L. Stryer, *Biochemistry*, 3rd ed., Freeman, New York, **1988**; c) A. P. de Silva, H. Q. N. Gunaratne, *J. Chem. Soc., Chem. Commun.* **1990**, 186–188.

[6] a) E. M. Nolan, J. Jaworski, K. Okamoto, Y. Hayashi, M. Sheng, S. J. Lippard, *J. Am. Chem. Soc.* **2005**, *127*, 16812–16823; b) H.-H. Wang, Q. Gan, X.-J. Wang, L. Xue, S.-H. Liu, H. Jiang, *Org. Lett.* **2007**, *9*, 4995–4998; c) F. Qian, C. Zang, Y. Zhang, W. He, X. Gao, P. Hu, Z. Guo, *J. Am. Chem. Soc.* **2009**, *131*, 1460–1468.

[7] a) D. E. Clapham, *Cell* **1995**, *80*, 259–268; b) E. Carafoli, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 1115–1122.

[8] a) T. Morozumi, T. Anada, H. Nakamura, *J. Phys. Chem. B* **2001**, *105*, 2923–2931; b) A. Ajayaghosh, E. Arunkumar, J. Daub, *Angew. Chem. Int. Ed.* **2002**, *41*, 1766–1769; c) E. Arunkumar, A. Ajayaghosh, J. Daub, *J. Am. Chem. Soc.* **2005**, *127*, 3156–3164; d) C.-F. Lin, Y.-H. Liu, C.-C. Lai, S.-M. Peng, S.-H. Chiu, *Chem. Eur. J.* **2006**, *12*, 4594–4599; e) J. Kim, T. Morozumi, H. Nakamura, *Org. Lett.* **2007**, *9*, 4419–4422.

[9] a) B. Tummler, G. Maass, E. Weber, W. Wehner, F. Vogtle, *J. Am. Chem. Soc.* **1977**, *99*, 4683–4690; b) F. Vogtle, E. Weber, *Angew. Chem.* **1979**, *91*, 813–837; *Angew. Chem. Int. Ed. Engl.* **1979**, *18*, 753–776; c) H.-G. Lohr, F. Vogtle, *Acc. Chem. Res.* **1985**, *18*, 65–72; d) B. Valeur, J. Pouget, J. Bourson, M. Kaschke, N. P. Ernsting, *J. Phys. Chem.* **1992**, *96*, 6545–6549.

[10] a) R. Tahara, K. Hasebe, H. Nakamura, *Chem. Lett.* **1995**, *24*, 753–754; b) Y. Suzuki, T. Morozumi, Y. Kakizawa, R. A. Bartsch, T. Hayashita, H. Nakamura, *Chem. Lett.* **1996**, *25*, 547–548; c) R. Tahara, T. Morozumi, Y. Suzuki, Y. Kakizawa, T. Akita, H. Nakamura, *J. Inclusion Phenom. Macrocyclic Chem.* **1998**, *32*, 283–294; d) Y. Suzuki, T. Morozumi, H. Nakamura, M. Shimomura, T. Hayashita, R. Bartsch, *J. Phys. Chem. B* **1998**, *102*, 7910–7917; e) E. Arunkumar, P. Chitra, A. Ajayaghosh, *J. Am. Chem. Soc.* **2004**, *126*, 6590–6598; f) Y. Liu, Z.-Y. Duan, H.-Y. Zhang, X.-L. Jiang, J.-R. Han, *J. Org. Chem.* **2005**, *70*, 1450–1455.

[11] C. J. Pedersen, *J. Am. Chem. Soc.* **1967**, *89*, 7017–7036.

[12] a) E. M. Choy, D. F. Evans, E. L. Cussler, *J. Am. Chem. Soc.* **1974**, *96*, 7085–7090; b) W. L. Duax, G. D. Smith, P. D. Strong, *J. Am. Chem. Soc.* **1980**, *102*, 6725–6729.

[13] a) P. Shanmugasundaram, P. Murugan, V. T. Ramakrishnan, N. Srividya, P. Ramamurthy, *Heteroat. Chem.* **1996**, *7*, 17–22; b) P. Murugan, P. Shanmugasundaram, V. T. Ramakrishnan, B. Venkatachalapathy, N. Srividya, P. Ramamurthy, K. Gunasekaran, D. Velmurugan, *J. Chem. Soc. Perkin Trans. 2* **1998**, 999–1004; c) N. Srividya, P. Ramamurthy, P. Shanmugasundaram, V. T. Ramakrishnan, *J. Org. Chem.* **1996**, *61*, 5083–5089.

[14] a) V. Thiagarajan, C. Selvaraju, E. J. Padmamalar, P. Ramamurthy, *ChemPhysChem* **2004**, *5*, 1200–1209; b) V. Thiagarajan, P. Ramamurthy, D. Thirumalai, V. T. Ramakrishnan, *Org. Lett.* **2005**, *7*, 657–660; c) V. Thiagarajan, P. Ramamurthy, *J. Lumin.* **2007**, *126*, 886–892.

[15] a) T. Ueno, Y. Urano, K. Setsukinai, H. Takakusa, H. Kojima, K. Kikuchi, K. Ohkubo, S. Fukuzumi, T. Nagano, *J. Am. Chem. Soc.* **2004**, *126*, 14079–14085; b) Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, T. Nagano, *J. Am. Chem. Soc.* **2005**, *127*, 4888–4894.

[16] a) V. Thiagarajan, V. K. Indirapriyadharsini, P. Ramamurthy, *J. Inclusion Phenom.* **2006**, *56*, 309–313; b) R. Kumaran, P. Ramamurthy, *J. Phys. Chem. B* **2006**, *110*, 23783–23789.

[17] a) H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707; b) V. K. Indirapriyadharshini, P. Karunanitihi, P. Ramamurthy, *Langmuir* **2001**, *17*, 4056–4060.

[18] M. Ouchi, Y. Inoue, T. Kanzaki, T. J. Hakushi, *J. Org. Chem.* **1984**, *49*, 1408–1412.

Received: May 23, 2009
Published Online: October 14, 2009

