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Calix[4]arenes Bearing Two Distal Azophenol Moieties: Highly Selective Chromogenic Ionophores for the Recognition of Ca^{2+} Ion

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There has been much recent interest in the design of new stimuli responsive host systems. Of these, the developments of chromogenic or fluorogenic ionophores have been a very active research area in supramolecular chemistry, since these can be applied to many useful and important applications in chemical sensor technologies.[1] Calixarenes are ideal frameworks for the development of chromogenic ionophores in the molecular recognition of ionic species of chemical and biological interest[2] since the incorporation of a suitable sensory group into the calixarene having a preorganized guest binding site results in a tailored chromogenic receptor. A variety of compounds based upon calixarenes having nitrophenylazophenol,[3−6] nitrophenol,[7] indoaniline,[8] indophenol,[9] and azophenol[10] functional groups have been successfully designed and exhibit a pronounced chromogenic behavior toward Na^+, K^+, Cs^+, Ca^{2+}, $UO_2{}^{2+}$, and even chiral amines. Shinkai et al. have synthesized calix[4]arene with a 4-(4-nitrophenyl)azophenol unit and three ethyl ester residues on the lower rim and observed the lithium sensitivity of the ionophore.[3] Tóth et al. also reported the sodium selectivity of related azophenol derivatives.[11] However, there have been no reports on the bis(azophenol) type chromoionophores based upon 1,3-distal dialkylated calix[4]arenes, which are easily available and used as key precursors for the development of many supramolecular systems having interesting functions.[12] We report in this paper the synthesis and the chromogenic behavior toward alkali and alkaline earth metal cations of bis(dinitrophenylazophenol) type chromoionophores based upon distal 1,3-diamide and 1,3-diester derivatives of *p-tert*-butylcalix[4]arene.

The chromogenic hosts based upon *p-tert*-butylcalix[4]arene diamide or diester bearing two distal azophenol units **3a** and **3b** were prepared from their corresponding diquinones as shown in Scheme 1. The diquinone derivatives **2**, which were prepared by the oxidation of diphenols **1** with $Tl(CF_3CO_2)_3$ following the known procedure,[13,14] were treated with 2,4-dinitrophenylhydrazine in $CHCl_3$ and EtOH mixture with a trace of H_2SO_4 to yield bis(dinitrophenylazophenol) derivatives **3a** and **3b** in 61% and 72% yield, respectively.

^1H NMR spectra of the bis(dinitrophenylazophenol) compounds showed the usual AB type splitting pattern of the methylene protons consisting of a pair of doublets at δ 4.69 and 3.32 for **3a** and δ 4.53 and 3.53 for **3b** in $CDCl_3$, which is characteristic of the syn-oriented conformation of the resulting products. All the derivatives seem to interconvert between cone and partial cone or 1,3-alternate conformations relatively freely by flipping the azophenol moiety. The evidence for the cone conformation of the free ionophores in solution state ($CDCl_3$) is that the observation of the ^{13}C NMR peaks for the bridging methylene carbons at δ 32.33 for **3a** and δ 31.87 for **3b** which are close to the diagnostic value for the syn orientation of the adjacent phenyl rings of a calixarene framework.[15]

The chromogenic behavior of the azophenol ionophores **3a** and **3b** was investigated by a two phase liquid−liquid extraction technique employing chloroform and an appropriate aqueous buffer solution. The aqueous metal chloride solution (0.5 mL of buffered solution) was extracted with a chloroform solution which contains the chromogenic host (1.0 mL, 3.0×10^{-5} M) in a quartz UV cell and, after the complete phase separation with brief centrifugation, the UV spectra of the chloroform phase were obtained.

The diamide-azophenol type host **3a** having ligating groups of *N*,*N*-diethylamide function is found to exhibit remarkable selectivity toward Ca^{2+} ion among the alkaline earth metal as well as alkali metal ions at the basic region of pH 7 to 11. The selectivity pattern is as follows: $Ca^{2+} \gg Sr^{2+} > Ba^{2+} > Na^+ > Mg^{2+}$. Upon interaction with aqueous $CaCl_2$ solution, the amide-based chromoionophore **3a** experienced a marked bathochromic shift in its λ_{max} from 437 to 605 nm ($\Delta\lambda = 168$ nm). The yellowish solution turned into a greenish blue color and exhibited an isosbestic point at 488 nm. The two *N*,*N*-diethylamide moieties provide an efficient binding site by utilizing strongly ligating amide carbonyl functions having a high dipole moment toward metal ions. According to the Corey−Pauling−Koltun space filling mo-

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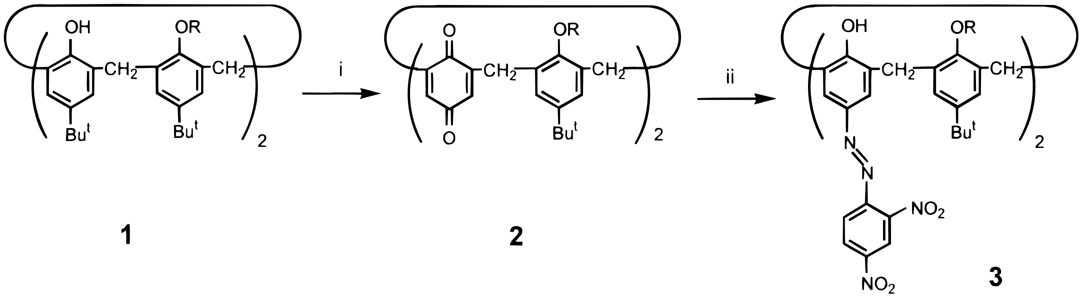
Scheme 1



a R = CH_2CONEt_2
b R = CH_2CO_2Et

Reagents and conditions: (i) $Tl(CF_3CO_2)_3$, TFA, in dark, rt;
(ii) 2,4-Dinitrophenylhydrazine, H_2SO_4, EtOH, $CHCl_3$, 2 h, rt.

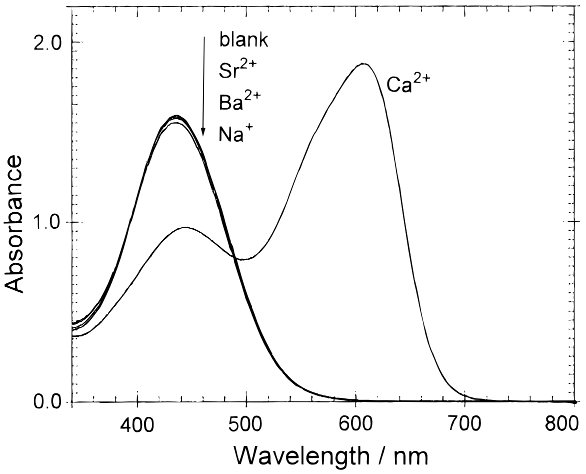


Figure 1. UV absorption spectra of **3a** (3.0×10^{-5} M, $CHCl_3$) upon extraction with aqueous metal chloride solution (buffered at pH 7). $[Ca^{2+}] = 0.05$ M, $[Sr^{2+}] = [Ba^{2+}] = [Na^{+}] = 0.1$ M.

lecular model, the geometry of the binding site of the host, comprising the two amide phenol ethers and two azophenol units, seems to be ideal in terms of size and arrangement for the recognition of doubly charged metal cations. Of primary importance is the electrostatic interaction of metal cations with two azophenol moieties as well as the ion–dipole interaction with the binding site lined with two pairs of ether-amide carbonyl moieties. At acidic solution below pH 6, however, the ionophore exhibits much reduced response upon extraction even with a higher concentration of metal chloride solutions (a 2.0 M solution of Ca^{2+} ion induced an absorbance value of only 0.60 at pH 6).

A remarkable thing to note is the fact that at pH 7, the Ca^{2+} ion exclusively induced a bathochromic shift of amide ionophore **3a** into λ_{max} of 605 nm as shown in Figure 1. In this condition, the ionophore **3a** showed almost no response to the other tested metal ions, such as Sr^{2+}, Ba^{2+}, and Mg^{2+} as well as Na^{+} and K^{+}. This observation might be due to the subtle balance between the deprotonation of the azophenol and complexation induced release of protons from the phenol units at pH 7.[16] Above this pH value, selectivity of **3a** toward Ca^{2+} ion is somewhat leveled off among the alkaline earth metal cations by responding moderately also to Sr^{2+}, Ba^{2+}, and even to Na^{+} ion.

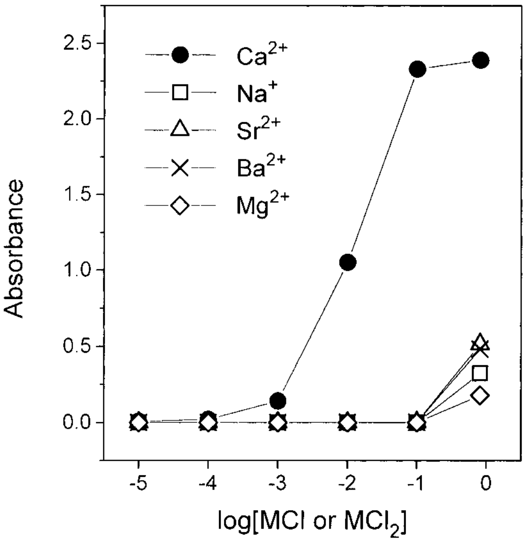


Figure 2. Variations in absorbance of **3a** (3.0×10^{-5} M, $CHCl_3$) at 605 nm as a function of metal chloride concentration in aqueous phase at pH 7.

To have an insight into the selectivity pattern of **3a** toward Ca^{2+} ion, the changes in absorbance at 605 nm in chloroform were measured as a function of metal concentration in aqueous buffered solution of pH 7. As can be seen from Figure 2, the chromogenic response of **3a** is almost exclusive toward Ca^{2+} ion from the concentration range of around 10^{-3} M to 0.1 M. From this plot, we tried to calculate the selectivity ratio for the Ca^{2+} ion over other interfering metal cations, but the small changes in absorbance induced by the interfering ions precluded the precise estimation of the selectivities. So, the selectivity ratio was estimated indirectly from the concentration of metal ions which is required to induce somewhat arbitrarily chosen absorbance change[6] of 0.2 unit at 605 nm. The selectivity of **3a** toward Ca^{2+} over Sr^{2+}, which is the most interfering ion among the tested metal ions, and biologically important Mg^{2+} and Na^{+} ions is estimated to be 195, 725, and 680, respectively, at pH 7.

The extraction experiments revealed that the ester **3b** also showed significant chromogenic behavior. Upon extraction with aqueous $CaCl_2$ solution, the yellowish solution of **3b** also experienced a marked bathochromic shift in its λ_{max} from 425 to 596 nm ($\Delta\lambda = 171$ nm). In this case, the Ca^{2+} ion again induced the most sensitive

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chromogenic behavior, but the selectivity is moderate toward Ca^{2+} ion over Ba^{2+} and Na^{+} ions and requires somewhat more alkaline condition of pH 10. The selectivity of the ester derivative **3b** toward Ca^{2+} over Ba^{2+} and Na^{+} at pH 10 was estimated to be 61 and 110, respectively. At solutions of lower pH, chromoionophore **3b** exhibited much lower responses toward most of the metal ions tested. The reduced chromogenic response of **3b** might be due to the much weaker ligating interaction of the ester functions compared with the *N*,*N*-diethylamide carbonyl group. That is the two ester functions are not strong enough to accommodate doubly charged alkaline earth cations to interact efficiently with the chromogenic moiety of the host. The detection limit, which is arbitrarily defined as a concentration of Ca^{2+} ion which gives rise to a discernible change in absorbance of 0.02 unit in UV spectra at their bathochromic shifted λ_{max}, is around 1×10^{-4} M for both **3a** (at pH 7) and **3b** (at pH 10).

In conclusion, the amide-based ionophore **3a** exhibits a remarkable selectivity toward Ca^{2+} ion at physiological pH region that suggests possible applications as a new chromogenic ionophore for a sensing material useful for the biologically important Ca^{2+} ion.

Experimental Section

General Procedure. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a 300 MHz spectrometer in CDCl$_3$. IR spectra were recorded on neat films. UV spectra were recorded in CHCl$_3$. Fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS AX505WA spectrometer. Solvents were of reagent grade and purified prior to use. Analytical TLC was performed on precoated silica gel plates (Merck silica gel 60 F$_{254}$) and column chromatography was done with silica gel 60 (Merck, 230−340 mesh, ASTM). *p-tert*-Butylcalix[4]arene *N*,*N*-diethylamide diquinone **2a**[13] and *p-tert*-butylcalix[4]arene diethyl ester diquinone **2b**[14] were prepared according to literature procedures.

***p-tert*-Butylcalix[4]arene Diethylamide Diazophenol (3a).** To a solution of diethylamide diquinone **2a** (50 mg, 6.3×10^{-2} mmol) in CHCl$_3$ (10 mL) was added absolute EtOH (6 mL), 2,4-dinitrophenylhydrazine (45.6 mg, 0.16 mmol), and concentrated H$_2$SO$_4$ (6 drops) in sequence. The reaction mixture was stirred for 2 h at room temperature and treated with cold H$_2$O and extracted with CHCl$_3$. The organic phase was separated and dried with MgSO$_4$ and evaporated to give a residue which was purified by the recrystallization (CHCl$_3$/MeOH) to yield 44 mg (61%) of orange colored crystals: mp 195−197 °C dec; ^{1}H NMR δ 10.34 (s, 2H), 8.67 (s, 2H), 8.39 (d, J = 9.0 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H), 7.62 (s, 4H), 7.16 (s, 4H), 5.04 (s, 4H), 4.69 (d, J = 12.9 Hz, 4H), 3.49 (q, J = 7.2 Hz, 8H), 3.32 (d, J = 12.9 Hz, 4H), 1.29 (t, J = 7.2 Hz, 12H), 1.22 (s, 18H); ^{13}C NMR δ 168.93, 159.63, 153.04, 149.36, 148.60, 146.96, 146.80, 146.50, 133.51, 129.79, 127.58, 126.65, 126.29, 120.13, 119.82, 72.48, 40.94, 40.47, 34.37, 32.33, 31.41, 14.19, 13.05; IR (film on KBr) 3280 (ν_{O-H}), 1647 ($\nu_{C=O}$), 1530 ($\nu_{N=O}$), 1344 ($\nu_{N=O}$) cm^{-1}; MS (FAB): *m*/*z* 1151 ([M + H]$^{+}$, calcd 1151.4). Anal. Calcd for C$_{60}$H$_{66}$N$_{10}$O$_{14}$: C, 62.60; H, 5.78; N, 12.17. Found: C, 62.34; H, 5.53; N, 12.39.

***p-tert*-Butylcalix[4]arene Diethyl Ester Diazophenol (3b).** Diethyl ester diquinone **2b** (50 mg, 6.8×10^{-2} mmol) and 2,4-dinitrophenylhydrazine (47 mg, 0.17 mmol) were combined following a procedure similar to that for **3a**. The crude reaction mixture was purified by the column chromatography (CH$_2$Cl$_2$/MeOH = 95/5) to yield 54 mg (72%) of an orange colored solid: mp 253−255 °C dec; ^{1}H NMR δ 8.99 (s, 2H), 8.74 (s, 2H), 8.45 (d, J = 9.0 Hz, 2H), 7.77 (d, J = 9.0 Hz, 2H), 7.74 (s, 2H), 7.08 (s, 4H), 4.84 (s, 4H), 4.53 (d, J = 13.2 Hz, 4H), 4.37 (q, J = 7.2 Hz, 4H), 3.53 (d, J = 13.2 Hz, 4H), 1.38 (t, J = 7.2 Hz, 6H), 1.13 (s, 18H); ^{13}C NMR δ 169.96, 159.34, 150.80, 149.44, 149.15, 146.99, 146.88, 146.69, 132.26, 129.86, 127.75, 126.76, 125.97, 120.24, 120.03, 72.32, 61.73, 34.36, 31.87, 31.25, 14.25; IR (film on KBr) 3400 (ν_{O-H}), 1741 ($\nu_{C=O}$), 1540 ($\nu_{N=O}$), 1344 ($\nu_{N=O}$) cm^{-1}; MS (FAB) *m*/*z* 1097 ([M + H]$^{+}$, calcd 1097.4). Anal. Calcd for C$_{56}$H$_{56}$N$_{8}$O$_{16}$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.29; H, 4.93; N, 10.41.

Preparation of Host and Guest Solutions. The stock solutions of the chromogenic ionophores were prepared by dissolution in CHCl$_3$ (HPLC grade, 3.0×10^{-5} M). They were stored in polyethylene bottles to avoid the possible contamination of metallic impurities, such as Na^{+} and Ca^{2+} ions, from the glassware. The guest solutions of alkali or alkaline earth metal chloride salts were prepared in a buffered solution (pH = 6−11)[17] as 0.1 or 0.05 M, respectively. Buffer solutions used are succinate buffer (succinic acid−NMe$_4$OH) for pH = 6, Tris-HCl buffer for pH = 7−9, and 3-(cyclohexylamino)propanesulfonic acid−NMe$_4$OH buffer for pH = 10−11.

Liquid−Liquid Extractions. The two phase liquid−liquid extraction experiments were performed by shaking the organic stock solution of the ionophore (1.0 mL in CHCl$_3$) and the aqueous guest solution (0.5 mL) in a tightly stoppered UV cell (quartz) for 30 s in a thermostated bath (25 ± 1 °C). The mixture was briefly centrifuged to ensure the complete phase separation, and the UV spectra of the organic phase were recorded. The titration of host solution (1.0 mL, CHCl$_3$) with aqueous guest solutions was performed by adding incremental aliquots of 20−50 μL of metal ionic guest solution into a quartz UV cell (equipped with a magnetic stirrer and thermostated at 25 °C) and following the changes in UV spectra of the organic phase.

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