Triazole- and azo-coupled calix[4]arene as a highly sensitive chromogenic sensor for Ca$^{2+}$ and Pb$^{2+}$ ions

Kai-Chi Chang, a In-Hao Su, a Gene-Hsiang Lee b and Wen-Sheng Chung a, *

a Department of Applied Chemistry, National Chiao-Tung University, Hsinchu 30050, Taiwan, ROC
b Instrumentation Center, National Taiwan University, Taipei 106, Taiwan, ROC

Received 8 July 2007; revised 13 August 2007; accepted 14 August 2007
Available online 16 August 2007

Abstract—A novel chromogenic calix[4]arene 3, which has within a molecule both the triazoles and the hydroxyl azophenols as the metal-binding sites and the azophenol moiety as a coloration sites was designed and synthesized. Calix[4]arene 3 is highly sensitive to Ca$^{2+}$ and Pb$^{2+}$ ions, which can be detected by the naked eye. Furthermore, the association constants for the 1:1 complexes of 3 Ca$^{2+}$ and 3 Pb$^{2+}$ were determined to be $7.06 \times 10^4$ M$^{-1}$ and $8.57 \times 10^3$ M$^{-1}$, respectively.

© 2007 Elsevier Ltd. All rights reserved.

The design and synthesis of new chemosensors for metal ions is an important subject in the field of supramolecular chemistry due to their fundamental role in biological, environmental, and chemical processes. Chromogenic ionophores have been intensively investigated as a specific metal ion indicator since Vögtle reported the use of 4-(4-nitrophenyl)azo-coupled crowns and azacrowns as chromoionophores, which showed large UV/vis band shifts when cations were added. Calix[4]arenes have been shown to be useful molecular scaffold in the development of chromoionophores, especially for metal ion recognition. Shinkai and co-workers reported that calix[4]arene having a 4-(4-nitrophenyl)azophenol unit with three ethyl ester groups showed a perfect lithium ion selectivity with respect to the UV/vis band shift. Chang et al. reported a bathochromic shift of p-tert-butylcalix[4]arene bearing a 1,3-diazophenol unit upon calcium ion complexation. Reinoudt et al. also reported that a calix[4]arene with monoalkylated azophenol unit and trimides on the lower rim is a highly selective Pb$^{2+}$ sensor, in which the direction of the shift was dependent on the conformation of the calix[4]arenes.

In continuation of our interests in the design and synthesis of chromogenic and fluorogenic chemosensors, we report here the synthesis of a novel chromogenic calix[4]arene using the Click chemistry of an azide and an alkyne to form a triazole cationic binding site.

The synthesis of host 3 is illustrated in Scheme 1. Our synthesis began with 25,27-bis(O-propargyl)calix[4]arene 1 followed by diazo coupling reaction using p-anisidine in HCl and NaNO$_2$ in acetone and pyridine gave the desired product 2 in 62% yield. Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction of 2 with 1-(azido-methyl)-benzene in the Click condition afforded the 5,17-bis(p-methoxy-phenyl)azo-25,27-bis(1,2,3-triazole)-calix[4]arene 3 in 71% yield.

Control compound 4 was synthesized in 79% yield using a method similar to that used in the preparation of 2. Besides traditional organic spectroscopic identification ($^1$H and $^{13}$C NMR, MS, and HRMS spectra) of all these calix[4]arenes, single-crystal X-ray analysis of 2 and 3 confirmed the structures to be in cone conformations (Fig. 1).

The absorption maxima ($\lambda_{max}$) and molar extinction coefficients of the chromogenic calix[4]arenes and control compound synthesized in this work are summarized in Table 1. Next, we then investigated the affinities of these azo-compounds for a series of groups 1A, 2A, and transition-metal ions in MeCN/CHCl$_3$ (v/v = 1000:4).

Excess perchlorate salts (10 equiv) of Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, Cr$^{3+}$, Pb$^{2+}$, Cd$^{2+}$, Ag$^+$, Ni$^{2+}$, Mn$^{2+}$, and Zn$^{2+}$ ions were tested to evaluate the metal...
ion binding properties of 2–4. Ligand concentration in all titration experiments was fixed at 10⁻⁵ M in MeCN/CHCl₃ (v/v = 1000:4). Free hosts 2, 3, and 4 exhibited absorption bands at 364, 365, and 360 nm in MeCN/CHCl₃ (v/v = 1000:4), respectively. The triazole–azophenol host 3, having triazoles as the metal ligating groups, is found to exhibit remarkable selectivity toward Ca²⁺ and Pb²⁺ ions over all other metal ions. For example, the addition of 10 equiv of Ca²⁺ and Pb²⁺ ions induced a bathochromic shift of triazole ionophore 3 from kₘₐₓ 365 nm to 527 and 541 nm, respectively (Fig. 2). However, the UV/vis spectra of control compounds 2 and 4 showed a weak bathochromic shift to Cr³⁺ ion only, and the rest of the metal ions did not show any change (see Figs. S7 and S8).

The two triazole moieties of 3 are proven to form an efficient metal ion binding site, whereas compounds 2 and 4 are in lack of such an efficient metal ion binding site. Furthermore, the geometry of the binding site of the host, comprising the two nitrogen atoms of triazole units and two hydroxyls of the azophenol units, seems to be ideal in terms of size and arrangement for 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 of metal ions with the triazole unit.

Upon interaction with Ca(ClO₄)₂, the chromogenic sensor 3 in MeCN/CHCl₃ (v/v = 1000:4) solution experienced a marked bathochromic shift in its kₘₐₓ as shown in Figure 3. The absorption maximum at 365 nm gradually decreased in intensity with the formation of a new absorption band at ca. 527 nm (Δkₘₐₓ = 162 nm). Three isosbestic points are 270, 305, 300, and 400 nm. A plot of absorbance at each wavelength is given in Figure 4.

Table 1. kₘₐₓ and corresponding extinction coefficients of azo-compounds 2–4 in MeCN/CHCl₃ (1000:4, v/v)

<table>
<thead>
<tr>
<th>Compound</th>
<th>kₘₐₓ (nm)</th>
<th>e (M⁻¹ cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>364</td>
<td>61,000</td>
</tr>
<tr>
<td>3</td>
<td>365</td>
<td>55,000</td>
</tr>
<tr>
<td>4</td>
<td>360</td>
<td>88,000</td>
</tr>
</tbody>
</table>

Figure 1. X-ray single-crystal structures of 2 and 3.

Figure 2. UV/vis spectra of 3 (10 μM) before and after adding 100 μM concentration of various metal perchlorates in MeCN/CHCl₃ (1000:4, v/v).
and 422 nm for the titration spectra of 3 by Ca(ClO₄)₂. The spectral features in Figure 3 are consistent with a 1:1 binding ratio between calix[4]arene 3 with Ca²⁺ ion. Further support of the 1:1 binding ratio comes from a Job plot experiment, where the absorptions of the complex at 527 nm were plotted against molar fractions of 3 under the conditions of an invariant total concentration. As a result, the concentration of 3ÆCa²⁺ complex approached a maximum when the molar fraction of [3]/([3] + [Ca²⁺]) was about 0.5 (see Fig. 4).

Electrospray mass spectrometry also supports the formation of complex 3ÆCa²⁺ and 3ÆPb²⁺, where a peak at m/z = 1173.6 corresponding to the mass of [3 + Ca + ClO₄]⁺ and a double charged peak at m/z = 537.4 which corresponds to [3 + Ca]²⁺ were observed. Furthermore, a peak at m/z = 1241.6 corresponding to the mass of [3 + Pb – H]⁺ and a double charged peak at m/z = 621.2 which corresponds to [3 + Pb]²⁺ were also observed (see Figs. S9 and S10 for detail).

The association constant for 3ÆCa²⁺ in MeCN/CHCl₃ (1000:4, v/v) was determined to be 7.06 x 10⁴ M⁻¹ by Benesi–Hilderbrand plot (Fig. 5). Similar UV/vis titration behavior and 1:1 binding stoichiometry was also observed in the case of 3 with Pb²⁺ (see Figs. S11–S13 for details); and its association constant was estimated to be 8.57 x 10³ M⁻¹.

Metal ion-induced chemical shift changes in the ¹H NMR (in CD₃CN) spectra support that Ca²⁺ is bound to the two nitrogen atoms of the triazole units and the two hydroxyl azophenol groups of 3 (see Fig. 6). In the presence of 10.0 equiv of Ca²⁺, chemical shifts of protons H₅–H₇ on the azophenol unit of 3 changed significantly; the peaks of H₅–H₇ were upfield shifted by 0.18, 0.17, and 0.42 ppm, respectively, but the peak of H₈ was downfield shifted by 2.74 ppm. In particular, the peak of H₈ was split into two peaks, one was upfield shifted by 0.23 ppm and the other was downfield shifted by 0.03 ppm. However, the peaks of H₉ and H₁₀ were each split into two sets of signals and upfield shifted.

---

Figure 3. UV/vis spectra of 3 (10 µM) upon titration by various equivalents of Ca(ClO₄)₂ in MeCN/CHCl₃ (1000:4, v/v).

Figure 4. Job plot of a 1:1 complex of 3 and Ca²⁺ ion, where the absorption at 527 nm was plotted against the mole fraction of 3 at invariant total concentration of 10 µM in MeCN/CHCl₃ (1000:4, v/v).

Figure 5. Benesi–Hilderbrand plot of 3 with Ca(ClO₄)₂.

Figure 6. ¹H NMR spectra of 3 (2.5 mM) in CD₃CN (a) and in the presence of 25 mM (10.0 equiv) of Ca(ClO₄)₂ (b), where * denotes an external standard CHCl₃.
The protons H₆, H₁, and H₉ were little influenced. Furthermore, ¹³C NMR spectroscopy proved that ionophore 3 forms a complex with Ca²⁺ in a cone conformation. The methylene carbon atoms bridging the aromatic rings appear at δ 31.2 and 32.1, which are typical resonances for a cone conformation of calix[4]arenes (Fig. S14). These results suggest that Ca²⁺ ion not only is bound by triazole–azophenol host 3, but also breaks the symmetry of the host molecule after complexation.

Due to the poor solubility of complex 3 Pb²⁺ in CD₂CN, the following titration was in CDCl₃/CD₂CN (v/v = 3:1) co-solvent system. Upon adding 10.0 equiv of Pb²⁺ to the solution of 3 (Fig. S15 for detail), the peak of H₉ was downfield shifted by 0.21 ppm, but did not split. The peaks of H₂ and H₈ stayed intact. The peaks of H₇, H₈, and H₉ were also downfield shifted by 0.08 and 0.12 ppm. Interestingly, the peak of H₆ was upfield shifted by 3.58 ppm. These results suggest that Pb²⁺ ion can also be bound by host 3, but was forming a symmetrical metal ion complex.

In conclusion, we have developed a new calix[4]arene sensor with bistriazoles and azophenols as the metal ion binding sites and azo groups as the signal transducer unit, which showed selective coloration of Ca²⁺ and Pb²⁺ ions. The Ca²⁺ and Pb²⁺ ion detection gives rise to a large bathochromic shift in the absorption spectrum (from light yellow to red), which is clearly visible to the naked eye.

Acknowledgements
We thank the National Science Council (NSC) and MOE ATU program of the Ministry of Education of Taiwan, ROC for financial support.

Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.08.045.

References and notes


7. (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Angew. Chem., Int. Ed. 2001, 40, 2004; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew. Chem., Int. Ed. 2002, 41, 2596.


9. Compound 2: To an ice cold solution of 0.07 g (1.00 mmol) of NaNO₂ in 6 mL of 4 N HCl was added a solution of p-anisidine in 3 mL of acetone, and the mixture was stirred for 30 s. The combined solution was then added to another ice cold solution of 0.10 g (0.20 mmol) of 25,27-dipropargyloxy-26,28-dihydroxy-calix[4]arene 1 in 12 mL of pyridine to produce a colored solution. The reaction mixture was stirred for 18 h at 0°C and then treated with 50 mL of 4 N HCl to give a colored precipitate. The solid residue was recrystallized from CH₃Cl/methanol mixture to give 0.095 g (62%) of 2 as a red solid; mp 226–228°C; Rₚ = 0.5 (hexane/EtOAc = 3:1); ¹H NMR (CDCl₃, 300 MHz) δ 7.87 (d, J = 8.9 Hz, 4H), 7.73 (s, 4H), 7.62 (s, 2H), 7.00 (d, J = 8.9 Hz, 4H), 6.96 (d, J = 7.6 Hz, 4H), 6.76 (t, J = 7.6 Hz, 2H), 4.84 (d, J = 2.3 Hz, 4H), 4.46 (d, J = 13.5 Hz, 4H), 3.87 (s, 6H), 3.57 (d, J = 13.5 Hz, 4H), 2.63 (t, J = 2.3 Hz, 2H); ¹³C NMR (CDCl₃, 75.4 MHz) δ 161.3 (Cq), 158.5 (Cq), 151.3 (Cq), 147.2 (Cq), 145.7 (Cq), 132.7 (Cq), 129.4 (CH), 128.5 (Cq), 125.9 (CH), 124.1 (CH), 123.4 (CH), 114.1 (CH), 78.0 (Cq), 63.6 (CH₂), 55.5 (CH₃), 31.8 (CH₂); FABMS m/z 770 (M⁺+1.2), 769 (M⁺, 2), 460 (6), 307 (100); HR FABMS Calcd for C₄₈H₄₀N₄O₆ 768.2948. Found 768.2936.

10. Compound 3: To a mixture of 2 (0.20 g, 0.26 mmol) and 9-(azidomethyl)benzene (0.084 g, 0.63 mmol) in THF and water (v/v = 2:1, 30.0 mL) was added CuI (about 1 mg, 0.005 mmol). The heterogeneous mixture was stirred vigorously at 50°C for 24 h. The mixture was extracted thrice with chloroform. The chloroform solution was dried over MgSO₄ and evaporated to give the solid crude product. Chromatography on silica gel eluting with hexane/ethyl acetate (v/v = 1:1) gave 0.19 g (71%) of 3 as a beige solid; mp 192–194°C; Rₚ = 0.3 (hexane/EtOAc = 3:1); ¹H NMR (CDCl₃, 300 MHz) δ 8.04 (s, 2H), 7.87 (d, J = 9.0 Hz, 4H), 7.73 (s, 2H), 7.67 (s, 4H), 7.35–7.27 (m, 10H), 6.98 (d, J = 9.0 Hz, 4H), 6.93 (d, J = 7.6 Hz, 4H), 6.70 (t, J = 7.6 Hz, 2H), 5.58 (s, 4H), 5.17 (s, 4H), 4.22 (d, J = 13.2 Hz, 4H), 3.83 (s, 6H), 3.37
(d, J = 13.2 Hz, 4H); 13C NMR (CDCl₃, 75.4 MHz) δ 61.2 (Cq), 155.7 (Cq), 151.3 (Cq), 147.1 (Cq), 145.7 (Cq), 143.6 (Cq), 134.9 (Cq), 129.4 (CH), 129.0 (CH), 128.7 (CH), 129.2 (Cq), 127.9 (CH), 125.8 (CH), 124.1 (CH), 123.5 (CH), 123.4 (CH), 114.1 (CH), 69.6 (CH₂), 55.4 (CH₃), 54.1 (CH₂), 31.4 (CH₂); FABMS m/z 1035 (M+, 9), 91 (100); HR FABMS Calcd for C₁₆₃H₁₄₄N₁₀O₆ 1034.4228. Found 1034.4221.

11. Compound 4: To an ice cold solution of 1.40 g (20.3 mmol) of NaNO₂ in 10 mL of 4 N HCl was added a solution of 1.50 g (12.2 mmol) of p-anisidine in 15 mL of acetone, and the mixture was stirred for 30 s. The combined solution was then added to another ice cold solution of 0.50 g (4.1 mmol) of 2,6-dimethylphenol in 20 mL of pyridine to produce a colored solution. The reaction mixture was stirred for 18 h at 0°C and then treated with 100 mL of 4 N HCl to give a colored precipitate. The solid residue was purified by column chromatography with hexane/ethyl acetate (v/v = 3:1) to give 0.83 g (79 %) of 4 as an orange solid; mp 120–122°C; Rf = 0.6 (hexane/EtOAc = 3:1); 1H NMR (CDCl₃, 300 MHz) δ 7.86 (d, J = 9 Hz, 2H), 7.59 (s, 2H), 7.00 (d, J = 9 Hz, 2H), 4.98 (s, 1H), 3.88 (s, 3H), 2.33 (s, 6H); 13C NMR (CD₃CN, 75.4 MHz) δ 162.6 (Cq), 156.6 (Cq), 147.7 (Cq), 146.9 (Cq), 125.4 (Cq), 124.9 (CH), 123.9 (CH), 115.3 (CH), 56.3 (CH₃), 16.6 (CH₃); FABMS m/z 257 (M++1, 100), 256 (M+, 85), 136 (55), 121 (45); HR FABMS Calcd for C₁₅H₁₆N₂O₂ 256.1212. Found 256.1206.

12. Crystal structure data for 2: C₁₅₀H₁₄₄Cl₆N₁₀O₁₀, M = 1005.56, Triclinic, a = 13.339(6) Å, b = 14.2081(7) Å, c = 14.2081(7) Å, a = 74.752(1)°, b = 65.904(1)°, c = 14.2658(7) Å, γ = 72.891(1)°, V = 2361.89(19) Å³, T = 150(1) K, space group P-1, Z = 2, μ = 0.418 mm⁻¹, 25,311 reflections collected (R₁ = 0.0918, wR₂ = 0.2296), 8311 independent reflections (R(int) = 0.0433, R₁ = 0.1094, wR₂ = 0.2435). Crystal structure data for 3: C₁₅₀H₁₄₄Cl₁₄N₁₀O₁₀, M = 1156.54, Monoclinic, a = 25.3068(11) Å, b = 10.5670(4) Å, c = 21.5706(13) Å, α = 90°, β = 101.372(3)°, γ = 90°, V = 5655.1(5) Å³, T = 150(2) K, space group P2₁/c, Z = 4, μ = 0.225 mm⁻¹, 23,719 reflections collected (R₁ = 0.1076, wR₂ = 0.2617), 9868 independent reflections (R(int) = 0.0822, R₁ = 0.2174, wR₂ = 0.3089). Crystallographic data for the two structures in this letter have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication. Their CCDC numbers are 2 (CCDC 652909), 3 (CCDC 652910), respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44 1223 336033, e-mail: data_request@ccdc.cam.ac.uk.


16. Based on the results of different metal ion-induced chemical shift changes in the 1H NMR of ionophore 3, one may infer that the binding modes for 3Ca²⁺ and 3Pb²⁺ are different; that is, the Ca²⁺ is bound to one of the two triazole units and the two hydroxyl azophenol groups, whereas Pb²⁺ is bound to both the two triazole units and the two hydroxyl azophenol groups of 3.