Tetrazoles and para-Substituted Phenylazo-Coupled Calix[4]arenes as Highly Sensitive Chromogenic Sensors for Ca2+

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Tetrazoles and *para*-Substituted Phenylazo-Coupled Calix[4]arenes as Highly Sensitive Chromogenic Sensors for Ca^{2+}

Yu-Jen Chen[a] and Wen-Sheng Chung*[a]

Keywords: Cycloaddition / Inclusion compounds / Nitrogen heterocycles / Calixarenes / Ionophores

Calix[4]arenes **3a** (R = OMe) and **3b** (R = NO_2) with 5,17-bis[4-(4-substituted-phenyl)azo] and 25,27-bisoxymethyltetrazole groups were synthesized by 1,3-dipolar cycloaddition of oxyacetonitrile azocalix[4]arenes **2a** and **2b** activated with trimethylsilyl azide. UV/Vis screening of **3a** and **3b** with 14 metal ions showed that **3a** (with *p*-methoxyphenylazo substituent) was a highly chromogenic sensor to Ca^{2+}, whereas **3b** (with *p*-nitrophenylazo substituent) showed color changes toward Ca^{2+}, Ba^{2+}, and Pb^{2+}. Job plot experiments revealed 1:1 binding stoichiometry for each of the complexes. The association constants for **3a**·Ca^{2+}, **3b**·Ca^{2+}, **3b**·Ba^{2+}, and **3b**·Pb^{2+} were determined by Benesi–Hildebrand plots. On the basis of ^{1}H NMR titration results, Ca^{2+} was bound to the two partially deprotonated hydroxy azophenol groups and one of the two tetrazole groups of **3a** and **3b**.

Introduction

In the design of artificial receptors for cations and anions, *para*-substituted phenyl azophenol groups are frequently used as the chromogenic units, because they give rise to intense color changes upon complexation with certain metal ions or anions.[1,2] Recently, we reported the syntheses of a series of upper-rim 4-allyl- and 4-methoxyphenylazo-coupled calix[4]arenes that were found to be sensitive chromogenic sensors for Hg^{2+} by azophenol to quinone–hydrazone tautomerism.[1a,1b] It was also found that upper-rim arylazo-coupled calix[4]arenes with lower-rim triazole groups showed selective color changes toward Ca^{2+} and Pb^{2+}.[1c] Besides cation sensing, these mono- or bisarylazo substituted calix[4]arenes were also found to be useful in anion sensing as reported by the research groups of Hong,[2a] Kim,[2b] and Chen.[2c] Using calix[4]arenes with and without a 4-(4-nitrophenyl)azo group, Kim and co-workers[2i] successfully demonstrated that cyclic voltammetry is a useful complement to UV/Vis absorption spectroscopy in the detection of Ca^{2+} ions.

Tetrazoles are heterocyclic compounds having similar pK_a values with those of carboxylic acids, and they are frequently swapped with carboxylic acids during the development of pharmaceuticals.[3,4] The cationic binding abilities of tetrazoles in their anionic and neutral forms have been independently studied by Hof[4a,4b] and Kraft.[4c–4e] Because tetrazoles are quite resistant to chemical and enzymatic degradation, they have been frequently used as robust linkers to construct 2D and 3D coordination polymers[5] and *C*-glycoclusters on a calix[4]arene scaffold.[6a,6b] Despite the high potential of tetrazoles and its derivatives in metal ion coordination, their use in conjunction with a calix[4]arene scaffold in metal-ion sensing is still scarce. One such rare application[6c] was the synthesis of calix[4]arenes bearing two or four tetrazole ligating groups at the upper rim, which formed a strong 2:2 complex with palladium dichloride.

The efficient and high-yielding synthesis of tetrazoles by Huisgen 1,3-dipolar cycloaddition reactions of nitriles with organic azides has become very popular and useful after the tremendous efforts by Sharpless and co-workers;[7] for instance, they have explored various possible electron-withdrawing cyanides or by adding $ZnBr_2$ in water to improve the overall yields of the reactions. Yamamoto and co-workers[8] have also reported a palladium-catalyzed three-component coupling reaction to synthesize tetrazoles with good to excellent yields. Accordingly, tetrazole groups can be readily synthesized by the reaction of nitrile with activated azide through the addition of either strong Lewis acids,[9] amine salts,[9b,9c] or dialkyltin oxides.[10,11] These reports prompted us to explore the syntheses of calix[4]arenes with bistetrazoles at the lower rim and *para*-substituted phenylazo moieties at the upper rim and their application in chromogenic sensing of metal ions, especially Ca^{2+}.

Results and Discussion

The synthetic pathways for the syntheses of azocalix[4]-arenes with two distal oxyacetonitrile groups **2a** and **2b**, two distal oxymethyltetrazole groups **3a** and **3b**, and control compounds **4** and **6** are depicted in Scheme 1. 5,17-Bis[4-

[a] Department of Applied Chemistry, National Chiao Tung University, Hsinchu,
30050 Taiwan, Republic of China
E-mail: wschung@cc.nctu.edu.tw

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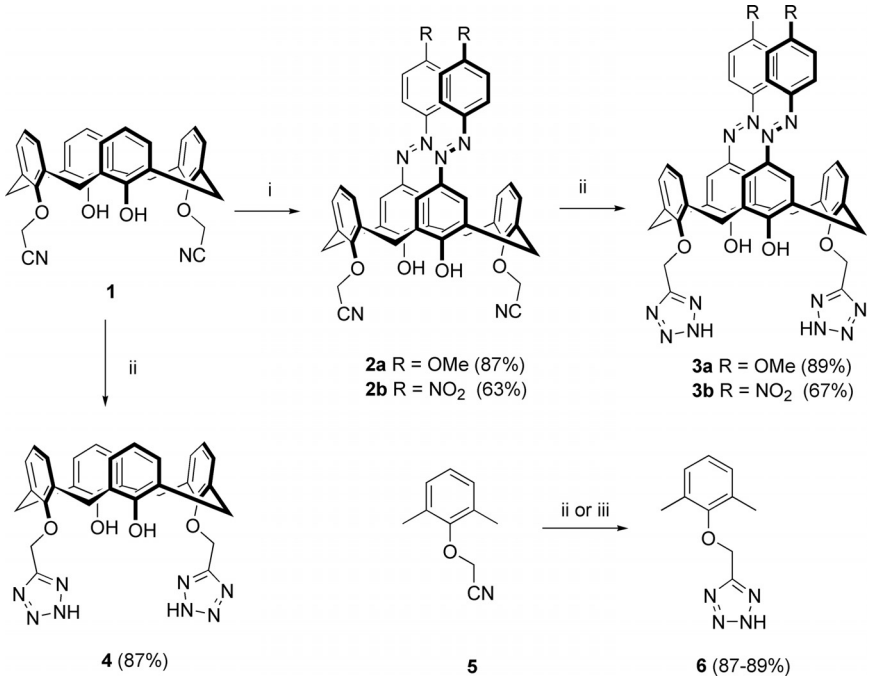


Scheme 1. Synthesis of chromogenic calix[4]arenes **2**, **3**, and control compounds **4** and **6**. Reagents and conditions: (i) *para*-substituted aniline/acetone, $NaNO_2$/4 N HCl, pyridine, 0 °C, 4 h; (ii) $TMSN_3$, Bu_2SnO, toluene, 100 °C, 16–36 h; (iii) $TMSN_3$, TBAF, 120 °C, 22 h.

(4-substituted-phenyl)azo]-25,27-dioxyacetonitrilecalix[4]-arenes **2a** (R = OMe) and **2b** (R = NO_2) were synthesized starting from 25,27-dioxyacetonitrilecalix[4]arene **1**[12] by a diazo coupling reaction with the use of *para*-substituted aniline[1] in 87 and 63% yield, respectively. The di-*n*-butyltin oxide mediated addition of trimethylsilyl azide ($TMSN_3$) with oxyacetonitrile calix[4]arenes **2a** and **2b** in toluene afforded the very polar 5,17-bis[4-(4-substituted-phenyl)azo]-25,27-bis[(oxymethyl)-2*H*-tetrazole]calix[4]arenes **3a** and **3b** in 89 and 67% yield, respectively. This method of forming tetrazole rings is adapted from the work of Lukyanov;[10] however, traditional heating instead of microwave irradiation was used instead.

The formation of calix[4]arene **3a**, with lower-rim oxymethyltetrazoles, from **2a** can be monitored by variation of the ^1H NMR signal of the lower-rim oxymethylene protons, which are downfield shifted from ca. 4.9 ppm in **2a** to 6.3 ppm in **3a** (Supporting Information); furthermore, the signals of the carbon atoms of the corresponding oxymethylenes are also downfield shifted from ca. 62 ppm in oxyacetonitrile **2a** to ca. 67 ppm in oxymethyltetrazole **3a**. Further support of the tetrazole-modified azocalix[4]arenes **3a** and **3b** came from their HRMS spectra. Control compound **4**, without the azo substituents, was synthesized in 87% yield from compound **1** by using similar reaction conditions to those used in the syntheses of **3a** and **3b**. Calix[4]arenes **2a**, **2b**, **3a**, **3b**, and **4** are all in cone conformation, as could be recognized from their respective methylene bridge signals appearing around 31 ppm in the ^{13}C NMR spectra.[13] Control compound **6** can be prepared by using tetrabutylammonium fluoride (TBAF) and trimethylsilyl azide under neat conditions,[14] or by treating **5** with sodium azide (NaN_3) in the presence of a Lewis acid in tetrahydrofuran.[9] Compound **6** has the characteristic downfield and broad signal of the tetrazole proton at 14.2 ppm in $CDCl_3$ (Supporting Information, Figure S11). The proton signals of the tetrazole units in calix[4]arenes **3a**, **3b**, and **4** were not observed in CD_3CN, perhaps as a result of its participation in intramolecular hydrogen bonding.[4b,4f]

The UV/Vis absorption maxima (λ_{max}) and molar extinction coefficients of the chromogenic calix[4]arenes **2a**, **2b**, **3a**, and **3b**, and control compounds **4** and **6** are summarized in Table 1. In acetonitrile solutions, the molar extinction coefficients of **4** and **6** are smaller than those of **2a**, **2b**, **3a**, and **3b** as a result of their lack of conjugated arylazo groups in the skeleton. The 4-(4-methoxyphenyl)azocalix[4]arenes **2a** and **3a** have shorter λ_{max} values (ca. 362 nm) than those of 4-(4-nitrophenyl)azocalix[4]arenes **2b** and **3b** (ca. 385 nm).

Table 1. The absorption maxima (λ_{max}) and corresponding extinction coefficients of arylazocalix[4]arenes **2** and **3** and control compounds **4** and **6** in CH_3CN at 298 K.

Compound	λ_{max} (nm)	ε ($M^{-1}\,cm^{-1}$)
2a	362	88000
2b	385	73000
3a	363	86000
3b	386	95000
4	277	6700
6	262	290

The affinities of these 4-(4-substituted-phenyl)azocalix-[4]arenes for metal ions with or without lower-rim oxymethyltetrazole groups were investigated next. The UV/Vis

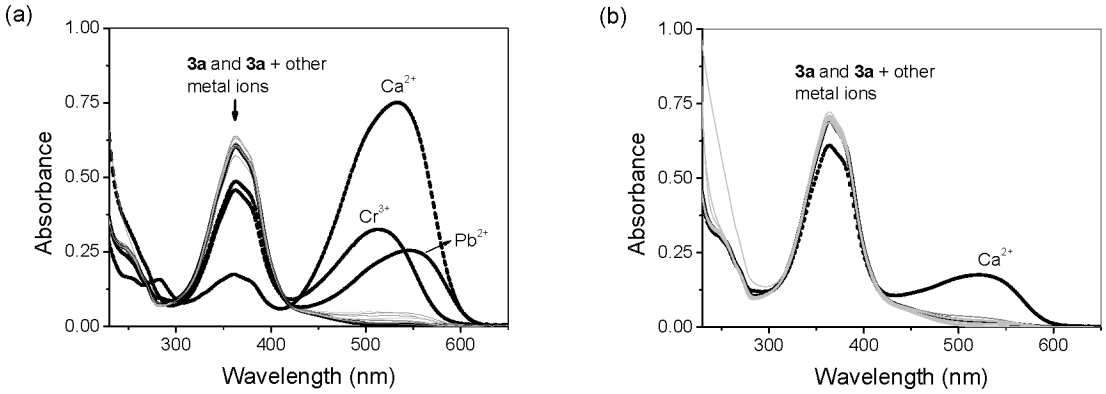


Figure 1. UV/Vis spectra of host **3a** (10 μM) in the absence and presence of 14 different metal perchlorates (10 equiv.) in (a) CH_3CN and (b) CH_3CN/MeOH (9:1) at 298 K.

spectra of **3a** (10 μM) in the presence of perchlorate salts of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Pb^{2+}, Cr^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, and Hg^{2+} (10 equiv.) in CH_3CN are shown in Figure 1a. Tetrazole–azophenol host **3a** exhibited a remarkable bathochromic shift (from 363 to 533 nm) and hyperchromic effect toward Ca^{2+}. Medium responses to Cr^{3+} and Pb^{2+} were also found for **3a**; however, no change in UV/Vis was found for the rest of the metal ions examined. In contrast, control compound **7**, without a calix[4]-arene scaffold, showed only a small bathochromic shift toward Cr^{3+}.[1c] When a mixed solvent of CH_3CN/MeOH (9:1) was used, compound **3a** showed a chromogenic response toward Ca^{2+} only, although with a smaller extinction coefficient. We did not use the mix solvent system for metal-ion screening because of its lower sensitivity relative to that in CH_3CN.

The results from UV/Vis titration of **3a** with metal ions in CH_3CN support that oxymethyltetrazole moieties are strong ligands for metal-ion complexation; furthermore, the electrostatic interactions between the phenols and metal cations bring about the bathochromic shift of **3a**. Although **2a** also showed apparent bathochromic shifts toward Cr^{3+} and Ca^{2+} (Supporting Information, Figure S13), its association constant was too small to be measured for Ca^{2+} or smaller than that with **3a**. Accordingly, the oxymethyltetrazole units on **3a** are superior ligands than the oxyacetonitrile units on **2a** in chelating Ca^{2+} ions. In contrast, the screening of 4-(4-nitrophenyl)azo analogues **3b** and **2b** toward metal ions showed strong bathochromic shifts toward Ca^{2+}, Pb^{2+}, and Ba^{2+} (with decreasing K_a values; Supporting Information, Figures S14 and S15; Table 2). Calix[4]arenes **3b** and **2b**, using the more-popular 4-(4-nitrophenyl)-azo groups, showed poorer metal-ion selectivity relative to that of their 4-(4-methoxyphenyl)azo analogues, **3a** and **2a**.

The UV/Vis spectra of **3a** upon titration with various concentrations of Ca^{2+} in CH_3CN are show in Figure 2. As the concentration of Ca^{2+} increased, the absorption maximum of free host **3a** at 363 nm gradually decreased in intensity with a concomitant increase in the absorption band at 533 nm with three isosbestic points at 268, 299, and 420 nm. The bathochromic shift of **3b**·Ca^{2+} and **3b**·Pb^{2+} was about 115 nm but was about 101 nm for **3b**·Ba^{2+}. By increasing the amounts of Ca^{2+}, the color of **3a** changed from colorless to magenta; thus, **3a** can be used as a naked-eye sensor for Ca^{2+} (Figure 3).

Table 2. Association constants K_a (M^{-1}) and bathochromic shifts for 1:1 complexes of azocalix[4]arenes **2a**, **2b**, **3a**, and **3b** with metal perchlorates in CH_3CN at 298 K.

Compound	Metal ion	λ_{max}, $\Delta\lambda$ (nm)[a]	K_a (M^{-1})[b]
2a	Ca^{2+}	531, 169	–[c]
2a	Cr^{3+}	510, 148	8.1×10^4
2b	Ca^{2+}	503, 118	4.2×10^4
2b	Ba^{2+}	492, 107	3.1×10^4
3a	Ca^{2+}	533, 170	9.1×10^4
3a	Cr^{3+}	512, 149	1.1×10^4
3a	Pb^{2+}	544, 181	8.4×10^3
3b	Ca^{2+}	500, 114	3.1×10^5
3b	Ba^{2+}	487, 101	1.1×10^5
3b	Pb^{2+}	501, 115	1.6×10^5

[a] $\Delta\lambda_{max} = \lambda_{complex} - \lambda_{host}$. [b] Calculated by Benesi–Hildebrand plots. [c] K_a was not determined, because the absorbance difference at low metal ion concentration was too small to be evaluated.

The Job plot of **3a** with Ca^{2+} revealed a 1:1 stoichiometry for the complex (Figure 4).[15] The association constant of **3a**·Ca^{2+} in CH_3CN was determined to be 9.1×10^4 M^{-1} by Benesi–Hildebrand plot[16] (Figure 5). The association constants of hosts **2a**, **2b**, **3a**, and **3b** with metal ions that showed color changes are summarized in Table 2. The binding affinities of **3b** toward Ca^{2+}, Ba^{2+}, and Pb^{2+} were 10–30 times larger than those of **3a** with corresponding metal ions; nevertheless, **3b** showed a poorer selectivity compared to that of **3a** (which is selective to Ca^{2+}). Furthermore, the affinities of **2b** toward Ca^{2+}, Ba^{2+}, and Pb^{2+} ions are in general smaller than those of **3b**, implying that the oxymethyltetrazoles are superior to oxyacetonitriles as metal coordination ligands in the lower rim of the bisarylazocalix[4]arenes. We also noted that tetrazole receptor **3a**, like its triazole analogue **8**,[1c] had a very similar binding behavior and association constants toward Ca^{2+} and Pb^{2+}. The K_a of **8**·Ca^{2+} and **8**·Pb^{2+} in CH_3CN was reported to be 7.06×10^4 and 8.57×10^3 M^{-1}, respectively.[1c] For comparison, the K_a of **3**·Ca^{2+} and **3**·Pb^{2+} was 9.1×10^4 and

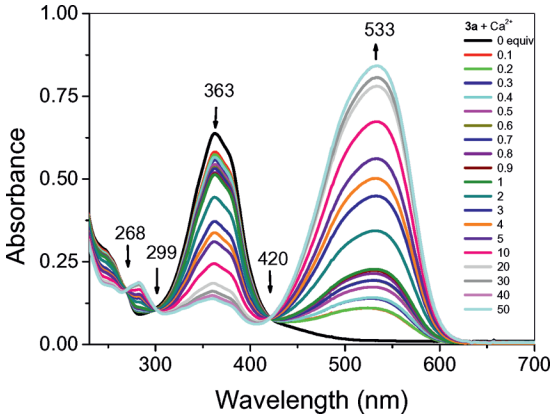


Figure 2. UV/Vis spectra of **3a** (10 μM) upon titration with various equivalents of $Ca(ClO_4)_2$ in CH_3CN at 298 K.

Figure 3. (a) The color of host **3a** (10 μM in acetonitrile) and (b) in the presence of Ca^{2+} (50 μM). Solutions that did not show color changes are omitted.

8.4×10^3 M^{-1}, respectively. The only noticeable difference between the two receptors may be on their association constant K_a toward Cr^{3+}, as it is two orders of magnitude stronger in tetrazole receptor **3a** than in triazole analogue **8**.

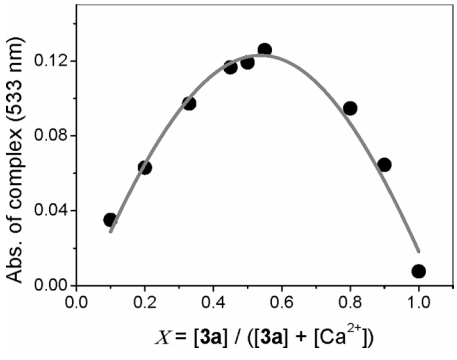


Figure 4. Job plot of a 1:1 complex of **3a** with Ca^{2+}, where the absorbance at 533 nm was plotted against the molar fraction of **3a** at an invariant total concentration of 10 μM in CH_3CN at 298 K.

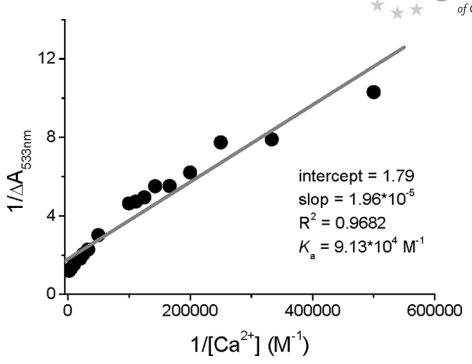


Figure 5. Benesi–Hildebrand plot of **3a** with $Ca(ClO_4)_2$ in CH_3CN at 298 K.

In order to gain insight into the complexation modes of **3a** with Ca^{2+} ion, we took a series of ^1H NMR spectra of **3a** (1.25 mM in CD_3CN) in the presence of different amounts of Ca^{2+}. Upon complexation of **3a** with Ca^{2+}, the signal of the methoxy protons (H^a) was upfield shifted by about 0.04 ppm, whereas the hydroxy azophenol proton H^e was downfield shifted to 11.9 ppm (with integration of 2 H). Protons on the 4-methoxyphenylazo groups (H^b and H^c) exhibited significant upfield shifts by ca. 0.11–0.28 ppm. Furthermore, the singlet peak on the azophenol H^d was split into two peaks: one was downfield shifted by 0.20 ppm and the other upfield shifted by 0.08 ppm. Moreover, the two doublets of the methylene bridge protons H^h were also upfield shifted by ca. 0.19–0.33 ppm. Note that the ^1H NMR signals from **3a** and complex **3a**·Ca^{2+} coexisted under the experimental conditions (Figure 6). Similar chemical shift changes were found in the titrations of **3b** with Ca^{2+}, Ba^{2+}, and Pb^{2+} ions (Supporting Information, Figures S34–S36), indicating that the metal-ion binding modes of **3a** and **3b** were similar. The chemical shift differences ($\Delta\delta$) between the two methylene bridge protons of receptors **2a**,**b** and **3a**,**b** fall in the range of 0.635 ± 0.015 ppm in CD_3CN, which implies that they are of flattened cone conformation. In the presence of Ca^{2+}, the phenylazo groups of ligand **3a** converged to facilitate the cation binding by the OH groups; therefore, the $\Delta\delta$ between the two methylene bridge protons became 0.77 ppm. The move toward cone conformation upon complexation with metal ion is corroborated with the upfield shift of the aromatic protons H^b and H^c (vide supra), which experience a mutual diamagnetic shielding from the opposite aryl ring.[17a] Accordingly, we propose that Ca^{2+} was bound to the 4-(4-substituted-phenyl)azocalix[4]arenes **3a** and **3b** by the two hydroxy azophenol groups and one of the two oxymethyltetrazole groups to form an asymmetric complex (see structure in Figure 6).

All proton signals were downfield shifted when **2a** or **3a** was titrated with Cr^{3+}; however, with no change in any of the splitting patterns (Supporting Information, Figures S31 and S33). The results imply that **2a** or **3a** formed a symmetrical complex with Cr^{3+}.[17b] On the basis of UV/Vis titration results, **2a** ($K_a = 8.1 \times 10^4$ M^{-1}) has a larger binding affinity than **3a** ($K_a = 1.1 \times 10^4$ M^{-1}) toward Cr^{3+}. Com-

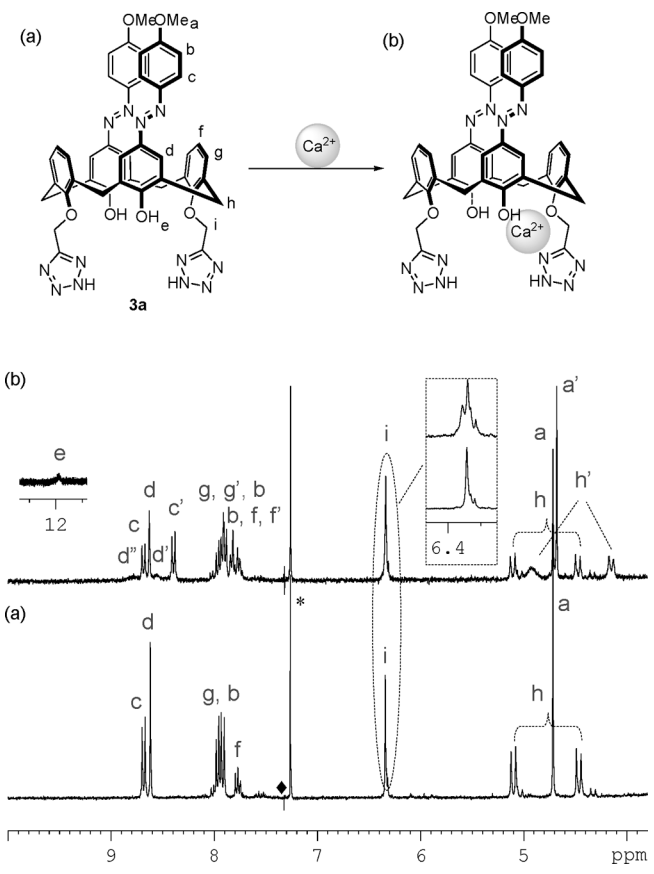
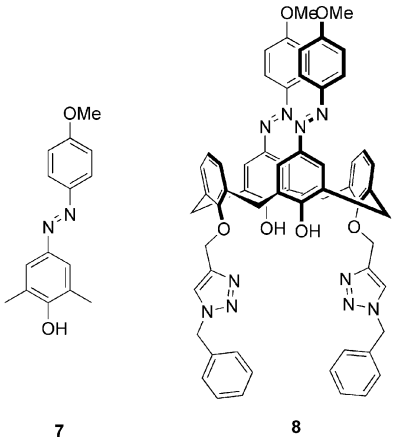


Figure 6. (a) ^{1}H NMR spectra of **3a** (1.25 mM) in CD_3CN and (b) in the presence of 1.25 mM (1.00 equiv.) of $Ca(ClO_4)_2$, where * denotes an external standard $CHCl_3$ and ♦ denotes a sweeping noise from instrument.

pound **2b** with lower-rim oxyacetonitrile groups, showed relatively smaller change in chemical shifts when compared to the lower-rim oxymethyltetrazole **3b** in complexation with Ca^{2+}, Ba^{2+}, or Pb^{2+} ions (Supporting Information, Figure S32).[18] Surprisingly, lower-rim bisoxymethyltetrazole-modified calix[4]arene **4** and control compound **6** did not show any complexation-induced chemical shift change with Ca^{2+} (Supporting Information, Figures S37 and S38). The results suggest that both azophenols and oxymethyltetrazoles/or oxyacetonitrile groups are required in the chromogenic sensing with metal ions. Furthermore, it is interesting to note that calix[4]arenes **3a** and **3b**, with two *p*-substituted phenylazo groups at the upper rim and two oxymethyltetrazole groups at the lower rim, showed very similar chromogenic sensing abilities toward metal ions compared to their lower-rim bisoxymethyltriazole analogue **8**[1c] reported previously.

Summary and Conclusions

We report here the synthesis of calix[4]arenes **3a** (R = OMe) and **3b** (R = NO_2) with two distal tetrazole groups at the lower rim and two 4-R-phenylazo groups at the upper rim for metal-ion sensing studies. The bisoxymethyltetrazole-modified calix[4]arenes **3a** and **3b** were synthesized by



using 1,3-dipolar cycloaddition of oxyacetonitrile azocalix[4]arenes **2a** and **2b** activated with trimethylsilyl azide. UV/Vis screening showed that calix[4]arene **3a** (R = OMe) showed a large bathochromic shift toward Ca^{2+} with good selectivity, whereas **3b** (R = NO_2) showed color changes toward Ca^{2+}, Ba^{2+}, and Pb^{2+}. Calix[4]arenes **3a** and **3b** with two *p*-substituted phenylazo groups at the upper rim and two oxymethyltetrazole groups at the lower rim showed very similar chromogenic sensing abilities toward metal ions; furthermore, they showed stronger association constants toward Ca^{2+} than did **2a**, **2b**, or bistriazole analogue **8**[1c] reported previously. On the basis of ^{1}H NMR titration results, Ca^{2+} was bound to the two partially deprotonated hydroxy azophenol groups and one of the two tetrazole groups of **3a** and **3b**.

Experimental Section

All reported yields were isolated yields. Flash column chromatography was performed by using silica gel (70–230 mesh). Melting points were determined with a Yanaco MP500D apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz with the solvent peak (usually $CDCl_3$) as an internal standard, and ^{13}C NMR spectra were recorded at 75.4 MHz or 125 MHz. Mass spectra were recorded at electron ionization, Q-TOF LC–MS–MS or at FAB mode using *m*-nitrobenzyl alcohol (NBA) as the matrix. UV/Vis spectra were recorded with an HP-8453 spectrophotometer and solvents were of HPLC grade. 25,27-Dioxyacetonitrile-26,28-dihydroxycalix[4]arene (**1**) was synthesized according to a literature procedure.[12]

General Procedure for the Synthesis of 2a and 2b: To an ice-cold solution of $NaNO_2$ (7.50 mmol) in 4 N HCl (6.00 mL) was added a solution of *para*-substituted aniline (4.00 mmol) in acetone (8.00 mL), and the mixture was stirred for 1 min. The combined solution was added dropwise to another ice-cold solution of 25,27-dioxyacetonitrile-26,28-dihydroxycalix[4]arene (**1**; 1.00 mmol) in pyridine (12.00 mL) to produce an ox-blood-red solution. The reaction mixture was stirred at 0 °C for 4 h and treated with 4 N HCl (60 mL) to give a colored precipitate. The separated precipitate was dissolved in CH_2Cl_2 (20 mL) and added H_2O (40 mL) then extracted with CH_2Cl_2 (2 × 10 mL). The organic layers were combined and concentrated, and the residue was recrystallized with CH_2Cl_2/MeOH to give the corresponding products **2a** (2.68 g, 87%) and **2b** (2.02 g, 63%).

5,17-Bis[4-(4-methoxyphenyl)azo]-25,27-dioxyacetonitrile-26,28-dihydroxycalix[4]arene (2a): Yellow solid. M.p. 217–219 °C. R_f = 0.42 (hexane/ethyl acetate, 3:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.67 (d, J = 13.8 Hz, 4 H, H^h), 3.90 (s, 6 H, H^a), 4.32 (d, J = 13.8 Hz, 4 H, H^h), 4.91 (s, 4 H, H^i), 6.57 (s, 2 H, H^e), 6.81 (t, J = 7.5 Hz, 2 H, H^f), 6.96 (d, J = 7.5 Hz, 4 H, H^g), 7.02 (d, J = 9.0 Hz, 4 H, H^b), 7.76 (s, 4 H, H^d), 7.89 (d, J = 9.0 Hz, 4 H, H^c) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 31.8 (CH_2), 56.5 (CH_3), 61.6 (CH_2), 115.5 (CH), 117.4 (C_q), 124.3 (CH), 124.9 (CH), 127.1 (CH), 129.5 (CH), 130.6 (C_q), 133.7 (C_q), 146.2 (C_q), 147.4 (C_q), 152.4 (C_q), 156.3 (C_q), 162.2 (C_q) ppm. MS (FAB): m/z = 771 [M + H$^+$]. HRMS (FAB): calcd. for $C_{46}H_{39}N_6O_6$ [M + H$^+$] 771.2926; found 771.2927.

5,17-Bis[4-(4-nitrophenyl)azo]-25,27-dioxyacetonitrile-26,28-dihydroxycalix[4]arene (2b): Red solid. M.p. 218–219 °C. R_f = 0.51 (CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.73 (d, J = 13.5 Hz, 4 H, H^g), 4.36 (d, J = 13.5 Hz, 4 H, H^g), 4.96 (s, 4 H, H^h), 6.89 (t, J = 7.5 Hz, 2 H, H^e), 7.01 (d, J = 7.5 Hz, 6 H, H^d + H^f), 7.89 (s, 4 H, H^c), 8.02 (d, J = 8.9 Hz, 4 H, H^b), 8.40 (d, J = 8.9 Hz, 4 H, H^a) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 31.8 (CH_2), 61.4 (CH_2), 117.5 (C_q), 123.9 (CH), 125.6 (CH), 126.0 (CH), 127.0 (CH), 130.0 (C_q), 130.7 (CH), 133.6 (C_q), 146.3 (C_q), 148.7 (C_q), 152.7 (C_q), 156.6 (C_q), 158.4 (C_q) ppm. MS (ESI): m/z = 801.4 [M + H$^+$]. HRMS (FAB): calcd. for $C_{44}H_{33}N_8O_8$ [M + H$^+$] 801.2416; found 801.2417.

General Procedure for the Synthesis of 3a and 3b: Dibutyltin oxide (0.18 mmol) and trimethylsilyl azide (3.68 mmol) were added to a solution of **2a** or **2b** (0.92 mmol) in anhydrous toluene (15 mL). The reaction mixture was stirred at 100 °C for 36 h and then cooled to room temperature. The solvent was removed, and the residue was then treated with 10% aqueous HCl (30 mL) and CH_2Cl_2 (3 × 10 mL). The extract was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to flash chromatography (CH_2Cl_2/ethyl acetate, 2:1; then ethyl acetate/methanol, 1:0–1:1 gradient) to give the corresponding products **3a** (0.70 g, 89%) and **3b** (0.55 g, 67%).

5,17-Bis[4-(4-methoxyphenyl)azo]-25,27-bis[(oxymethyl)-2*H*-tetrazole]-26,28-dihydroxycalix[4]arene (3a): Yellow solid. M.p. 237–239 °C. R_f = 0.34 (ethyl acetate/methanol, 1:1). ^{1}H NMR (300 MHz, CD_3CN): δ = 4.47 (d, J = 13.4 Hz, 4 H, H^h), 4.72 (s, 6 H, H^a), 5.10 (d, J = 13.4 Hz, 4 H, H^h), 6.34 (s, 4 H, H^i), 7.77 (t, J = 7.6 Hz, 2 H, H^f), 7.92 (d, J = 9.0 Hz, 4 H, H^b), 7.97 (d, J = 7.6 Hz, 4 H, H^g), 8.62 (s, 4 H, H^d), 8.69 (d, J = 9.0 Hz, 4 H, H^c) ppm. ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ = 31.4 (CH_2), 56.7 (CH_3), 67.0 (CH_2), 115.6 (CH), 124.4 (CH), 125.1 (CH), 126.9 (CH), 129.5 (C_q), 130.5 (CH), 130.7 (C_q), 146.2 (C_q), 147.4 (C_q), 152.8 (C_q), 154.4 (C_q), 156.5 (C_q), 162.3 (C_q) ppm. MS (FAB): m/z = 857 [M + H$^+$]. HRMS (FAB): calcd. for $C_{46}H_{41}N_{12}O_6$ [M + H$^+$] 857.3267; found 857.3276.

5,17-Bis[4-(4-nitrophenyl)azo]-25,27-bis[(oxymethyl)-2*H*-tetrazole]-26,28-dihydroxycalix[4]arene (3b): Yellow solid. M.p. 236–237 °C. R_f = 0.33 (ethyl acetate/methanol, 1:1). ^{1}H NMR (300 MHz, CD_3CN): δ = 4.50 (d, J = 13.5 Hz, 4 H, H^g), 5.12 (d, J = 13.5 Hz, 4 H, H^g), 6.35 (s, 4 H, H^h), 7.78 (t, J = 7.5 Hz, 2 H, H^e), 7.98 (d, J = 7.5 Hz, 4 H, H^f), 8.76 (s, 4 H, H^c), 8.82 (d, J = 9.0 Hz, 4 H, H^b), 9.22 (d, J = 9.0 Hz, 4 H, H^a) ppm. ^{13}C NMR (125 MHz, [D$_6$]-DMSO): δ = 31.2 (CH_2), 66.8 (CH_2), 123.9 (CH), 125.5 (CH), 126.0 (CH), 126.5 (CH), 129.9 (C_q), 130.4 (CH), 133.2 (C_q), 146.2 (C_q), 148.6 (C_q), 153.0 (C_q), 154.4 (C_q), 156.6 (C_q), 158.5 (C_q) ppm. MS (FAB): m/z = 887 [M + H$^+$]. HRMS (FAB): calcd. for $C_{44}H_{35}N_{14}O_8$ [M + H$^+$] 887.2757; found 887.2753.

General Procedure for the Synthesis of 25,27-Bis[(oxymethyl)-2*H*-tetrazole]-26,28-dihydroxycalix[4]arene (4): To a solution of **1** (0.45 g, 0.90 mmol) in anhydrous toluene (20 mL) was added dibutyltin oxide (0.09 g, 0.36 mmol) and trimethylsilyl azide (0.52 g, 4.50 mmol). The reaction mixture was stirred at 100 °C for 16 h and then cooled to room temperature. The solvent was removed, and the residue was then treated with 10% aqueous HCl (30 mL) and CH_2Cl_2 (3 × 10 mL). The extract was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to flash chromatography (hexane/ethyl acetate, 2:1; then ethyl acetate/methanol, 1:0–5:1 gradient) to give **4** (0.46 g, 87%) as a white solid. M.p. 239–241 °C. R_f = 0.20 (ethyl acetate/methanol, 5:1). ^{1}H NMR (300 MHz, CD_3CN): δ = 3.50 (d, J = 13.5 Hz, 4 H, H^f), 4.21 (d, J = 13.5 Hz, 4 H, H^f), 5.48 (s, 4 H, H^g), 6.75 (t, J = 7.5 Hz, 2 H, H^a), 6.91 (t, J = 7.5 Hz, 2 H, H^d), 7.07 (d, J = 7.5 Hz, 4 H, H^b), 7.19 (d, J = 7.5 Hz, 4 H, H^e) ppm. ^{13}C NMR (75 MHz, CD_3CN): δ = 32.2 (CH_2), 68.6 (CH_2), 121.8 (CH), 127.8 (CH), 129.2 (C_q), 130.4 (CH), 130.8 (CH), 135.2 (C_q), 152.7 (C_q), 153.5 (C_q), 155.4 (C_q) ppm. MS (FAB): m/z = 889 [M + H$^+$]. HRMS (FAB): calcd. for $C_{32}H_{28}N_8O_4$ [M$^+$] 588.2234; found 588.2238.

General Procedure for the Synthesis of 5-[(2,6-Dimethylphenoxy)-methyl]-2*H*-tetrazole (6): A mixture of 2-(2,6-dimethylphenoxy)acetonitrile (**5**; 0.65 g, 4.04 mmol), tetrabutylammonium fluoride (0.50 g, 2.02 mmol), and trimethylsilyl azide (0.70 g, 6.06 mmol) was stirred at 120 °C for 22 h and then cooled to room temperature. The crude products were treated with 10% aqueous HCl (30 mL) and CH_2Cl_2 (3 × 10 mL). The extract was dried with Na_2SO_4 and concentrated under reduced pressure. The residue subjected to column chromatography (CH_2Cl_2/ethyl acetate, gradient) to give **6** (0.72 g, 87%) as a white solid. M.p. 107–109 °C. R_f = 0.31 (hexane/ethyl acetate, 1:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.21 (s, 6 H, H^c), 5.24 (s, 2 H, H^d), 6.90–7.03 (m, 3 H, ArH), 14.21 (s, 1 H, H^e) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 16.1 (CH_3), 63.5 (CH_2), 125.2 (CH), 129.2 (CH), 130.5 (C_q), 154.3 (C_q) ppm. MS (EI): m/z (%) = 122 (100), 121 (92), 204 (55) [M$^+$], 77 (54), 91 (53). HRMS (EI): calcd. for $C_{10}H_{12}N_4O$ [M$^+$] 204.1011; found 204.1013.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR spectra of compounds **2**–**6**; UV/Vis titration spectra; Job plots; Benesi–Hildebrand plots of **2a**, **2b**, and **3b**; ^{1}H NMR spectra of **2**–**6** with metal perchlorate salts.

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[17] a) We thank one of the reviewers for this strong insight into the conformational change of receptors **2a**,**b** and **3a**,**b** from flattened cone to cone upon complexation with metal ions; b) A low-temperature (253 K) experiment was carried out; however, the spectrum of **3a** and Cr^{3+} did not show any new splitting.

[18] (i) The K_a obtained from ^{1}H NMR titration ($2.5–4.2 \times 10^3$ M^{-1}) was about 20–36 times smaller than that obtained by UV/Vis techniques (9.1×10^4 M^{-1}). A possible reason for this discrepancy was that in ^{1}H NMR titration some precipitates might have formed at mM concentration, whereas there was no such possibility at the μM concentration range used in the UV/Vis technique. A similar discrepancy in the determination of K_a by fluorescence spectroscopy (in THF) and by ^{1}H NMR titration (in [D$_6$]DMSO) was reported by Fang, (ii) the K_a was two orders of magnitude smaller when determined by ^{1}H NMR compared to that by fluorescence titration. See: J.-H. Liao, C.-T. Chen, J.-M. Fang, *Org. Lett.* **2002**, *4*, 561–564.

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