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A specific and ratiometric chemosensor for Hg^{2+} based on triazole coupled *ortho*-methoxyphenylazocalix[4]arene

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A B S T R A C T

Calix[4]arene **3**, which contains two distal triazole groups on the lower rim and two distal *o*-methoxyphenylazo groups on the upper rim, was synthesized and found to be a specific and ratiometric sensor for Hg^{2+} in a polar protic solvent. A series of *o*-methoxyphenylazo derivatives (**3**, **4**, **5**, **7**, and **9**) were synthesized, which proved that the lower-rim triazoles and the hydroxyl azophenol(s) were the major ligands for metal ion binding. Though analogues **4** and **10** showed some sensitivity for Hg^{2+}, compound **3** was the only ratiometric chemosensor for Hg^{2+} among the series of azocalix[4]arenes synthesized in this work. The formation of $\mathbf{3}\cdot Hg^{2+}$ complex was supported by UV/vis and NMR titration studies and Mass spectrometry. Based on the symmetrical features of NMR spectra of $\mathbf{3}\cdot Hg^{2+}$, the complex is believed to be symmetrical with respect to the calix[4]arene cavity. Furthermore, the complex was determined to be 1:1 binding stoichiometry by Job's plot, and the association constant was determined to be $4.02\times10^3\ M^{-1}$ using Benesi–Hildebrand plot.

1. Introduction

Mercury is one of the toxic heavy transition-metal ions and as reported by US Environmental Protection Agency (EPA), the annual total global mercury emissions from all sources, both natural and human-generated, reached nearly 7500 tons in 2005.[1] When inhaled by human body, mercury can trigger several serious disorders, including sensory, motor, and neurological;[2] therefore, many sophisticated analytical tools have been developed to detect mercury ions. To design a useful chemosensor for metal ions,[3] two main issues should be considered: (1) high selectivity, and (2) the effective conversion of recognition event into optical or electrochemical signals. In this aspect, we have been interested in developing naked eye sensors for metal ions due to its simplicity and convenience.

Azo compounds are widely used in chromoionophores because they could exhibit substantial color changes observable by the naked eyes upon complexation with guest molecules or ions. Vögtle and co-workers were one of the pioneers in coupling the 4-(4-nitrophenyl)azo group with a crown ether and found that it showed a large hypsochromic shift when bonded with Ba^{2+}.[4] The binding ability of azocalixarenes for metal ions was intensively investigated in recent years,[5,6] in which the azo groups mostly functioned as a chromophore; occasionally, they also functioned as a metal-chelating ligand. When the upper-rim arylazo groups in a calix[4]arene only play the role as a chromophore, the lower-rim modification of the azocalixarenes, by ester, carboxylic acid, heterocyclic ring, or crown ether groups,[5] is usually required to endow it with metal-chelating ability. We were one of the first to introduce triazoles as the metal ion binding ligands of the chromogenic azocalix[4]arenes, for example, *para*-methoxyphenylazocalix[4]arene **10** (Scheme 1) was reported[5a] to be a chromogenic sensor for Ca^{2+} and Pb^{2+}, while the *para*-nitrophenylazocalix[4]arene analogue of **10** was found to be a dual sensor for Ca^{2+} and F^-.[5c]

Upper-rim arylazo functionalized calix[4]arenes, which play not only as a chromophore but also as a metal ion binding site, were also intensively explored.[6] The main working principle for the chromogenic sensing of these upper-rim arylazo functionalized calix[4]arenes toward metal ions is that metal ion enhances the tautomerization of the azophenols to quinone–hydrazones.[7] Nomura and co-workers reported the first example of using the upper-rim azophenol substituted calix[6]arene as both chromophores and metal ion chelating ligands, which showed high selectivity toward Ag^+, Hg^+, and Hg^{2+}.[6f] We reported that 5,17-bisallyl-11,23-bis-(*p*-methoxyphenyl)azocalix-[4]arene, without lower-rim modification, was a highly sensitive chromogenic sensor for Hg^{2+}, in which both the upper-rim *p*-allyl- and *p*-methoxyphenylazo groups were involved in the recognition of Hg^{2+}.[6g,h] Recently, Kim and co-workers also reported that 5,11,17,23-tetra[(2-ethylacetoethoxyphenyl)azophenyl]calix[4]arene exhibited selectivity toward transition-metal ions over alkali and alkaline earth metal ions.[6c,d]

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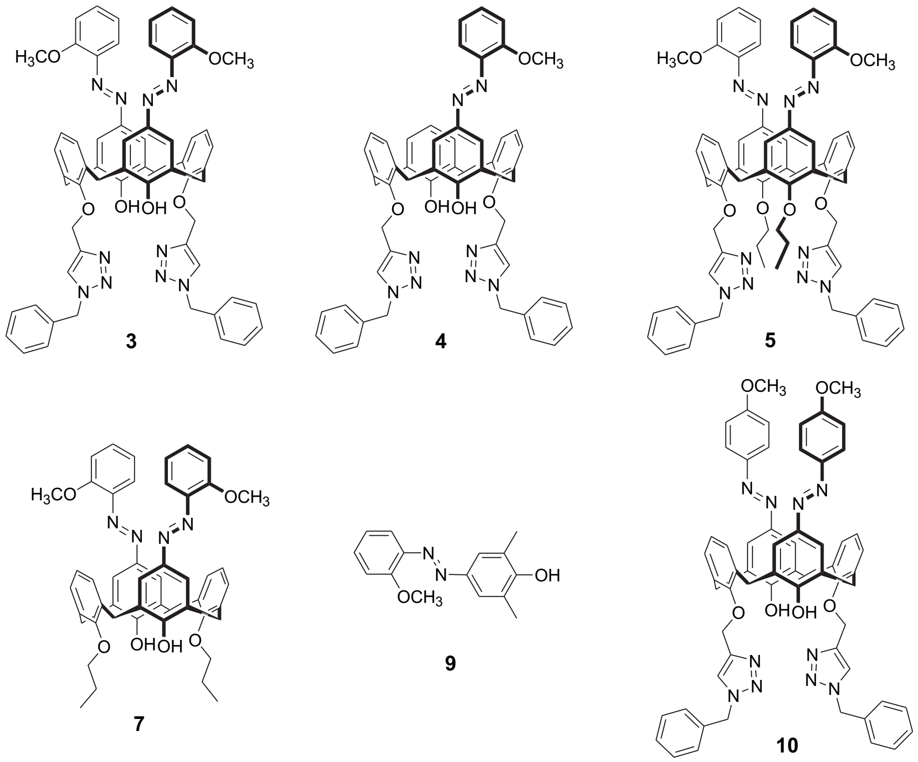


Scheme 1. The structures of target azo compounds.

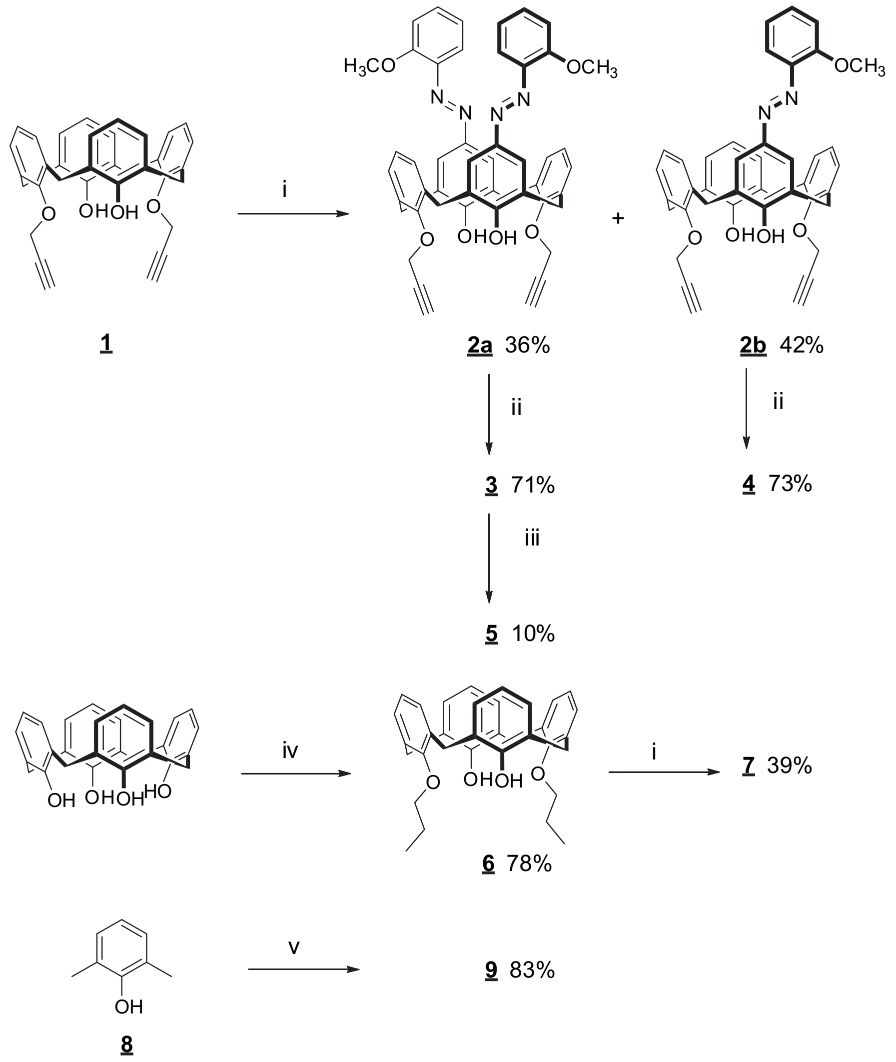
Initially we intended to design an allosteric calix[4]arene **3** by combining the lower-rim two distal triazole units as one metal ion binding site and the upper-rim two distal *ortho*-methoxyphenylazo units as the other; the idea came from the report that *ortho*-substituted-phenylazo groups might help to bind metal ions.[6i] However, in the end we found that **3** did not function as an allosteric sensor; it only exhibited a high selectivity toward Hg^{2+} in MeOH. To assess the relative binding abilities of the upper-rim *ortho*-methoxy-phenylazophenol and the lower-rim triazoles of **3**, we also synthesized compounds **4**, **5**, **7**, and **9** (Scheme 1) for comparison. UV/vis, NMR, MASS spectrometry, and molecular modeling (DMol3)[14] were used to identify the possible binding modes of **3** with Hg^{2+}.

2. Results and discussion

Five new *o*-methoxyphenylazo-coupled chemosensors (**3**–**5**, **7**, and **9**) were synthesized and their structures and synthetic pathways are shown in Schemes 1 and 2. Chemosensor **3** and its analogue **4** were obtained via azo-coupling reaction of **1** followed by click reaction.[8] The azotization of 25,27-bis(*O*-propargyl)calix[4]arene **1**[5a,9] using *o*-anisidine in concd HCl and $NaNO_2$ in acetone and pyridine gave the upper-rim distal bis-azo-coupled calix[4]arene **2a** and mono-azo-coupled calix[4]arene **2b** in 36% and 42% yield, respectively. The ratio of **2a**/**2b** increased with the reaction time and their structures can be distinguished by ^1H NMR spectra. Both **2a** and **2b** exhibited similar singlet peaks for the methoxy protons around δ 4.0 ppm, but their methylene bridge protons showed different splitting patterns. The methylene bridge protons of **2a** exhibited two doublet peaks at δ 4.2 and 3.4 ppm; whereas those of **2b** exhibited four doublet peaks around δ 4.2 and 3.3 ppm.

After building mono- and bis-azo groups on the upper rim of calix[4]arene, the lower-rim two distal triazoles groups were obtained by 1,3-dipolar cycloaddition reactions. 1,3-Dipolar cycloaddition reactions of **2a** and **2b** with 1-(azidomethyl)benzene under the Click condition[8] afforded the azocalix[4]arenes **3** and **4** in 71% and 73% yield, respectively. Further etherification of **3** with 1-iodopropane in the presence of K_2CO_3 was unsuccessful presumably due to the steric hindrance of the flanking triazole groups next to the hydroxyl phenols. When stronger base, such as sodium hydride, was used to react **3** with 1-iodopropane in DMF at 80 °C, it gave **5** only in 10% yield even after 18 h of reaction time and 87% of **3** was recovered. In contrast, 1,3-dipropylation of calix[4]arene was readily carried out with an excess of 1-iodopropane and K_2CO_3 in refluxing CH_3CN to afford **6** in 78% yield.[10] Control compounds **7** and **9**, using **6** and 2,6-dimethylphenol **8**, respectively, as the starting materials, were synthesized by the azo-coupling reaction similar to that used in the preparation of **2a** and **2b** and the yields were 39% and 83%, respectively (see Scheme 2). The lower yield for the azotization of **6** compared to that of **1** was due to the recovery of 50% of starting material. The structures of all azo-coupled calix[4]arenes were fully characterized by NMR (^1H and ^{13}C), MS, and HRMS (see Experimental section). In ^1H NMR spectra, the methylene bridge protons of these azocalix[4]arenes **3**–**5** and **7** exhibit one set of doublet signals around δ 4.4–4.1 ppm and δ 3.6–3.0 ppm, implying that they are more stable in cone conformations. ^{13}C NMR of the methylene bridge carbons of these azocalix[4]arenes **3**–**5** and **7** exhibited signals around δ 32 ppm further support that they were in cone conformations.[11]

The absorption maxima (λ_{max}) and molar extinction coefficients of the five target azo-coupled calix[4]arenes synthesized in this work are summarized in Table 1. As can be seen, the absorption maxima (λ_{max}) of **3**, **4**, **7**, and **9** with azophenol form are above 369 nm. Azocalix[4]arene **5**, whose two distal hydroxyl groups of azophenol were converted to propoxy groups, had a shorter λ_{max} (364 nm). Besides, the λ_{max} of **3**, which contains two azophenol units was almost the same as that of **4** with mono-azophenol. Furthermore, azo-coupled calix[4]arenes **3** and **7**, which contain



Scheme 2. Synthetic pathways for chromoionophores **3**–**5**, **7**, and **9**. Reagents and conditions: (i) *o*-anisidine/acetone, $NaNO_2$/concd HCl, pyridine, 0 °C, 3 days; (ii) 1-(azidomethyl)-benzene, CuI, THF/H_2O, 50 °C, 18 h; (iii) 1-iodopropane, NaH/DMF, 80 °C, 18 h; (iv) 1-iodopropane, K_2CO_3/CH_3CN, reflux 1 day; (v) *o*-anisidine/acetone, $NaNO_2$/concd HCl, pyridine, 0 °C, 12 h.

Table 1
λ_{max} and corresponding extinction coefficients of azo-coupled compounds **3**–**5**, **7**, and **9** in methanol–chloroform (v/v, 98:2) cosolvent at 25 °C

Compound	λ_{max} (nm)	ε (M^{-1} cm^{-1})
3	373	43,500 (±2500)
4	372	23,000 (±1000)
5	364	23,500 (±1500)
7	371	32,500 (±1500)
9	369	24,500 (±2500)

bis-*o*-methoxyphenly-azophenol groups had the larger molar extinction coefficients compared to those of **4** and **9**, which contain only mono-azophenol. Note that the molar extinction coefficient of bis-arylazo-coupled calix[4]arene **3** was almost twice as big as that of its mono-arylazo-coupled analogue **4**. Smaller extinction coefficient and λ_{max} of **7** versus that of **3** implies that there may be hydrogen bonding interaction between the lower-rim triazole and the hydroxyl groups of the azophenols in **3**, which slightly favors the tautomerization of the azophenol to quinone–hydrazones.[6g,h]

The binding properties of ligands **3**–**5**, **7**, and **9** were assessed by the addition of 10 equiv of metal perchlorates (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ag^+, Cu^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, and Cr^{3+}) in a MeOH/$CHCl_3$ (v/v, 98:2) cosolvent and the concentration of these ligands in the titration experiments were around 10–20 μM depending on their molar extinction coefficients. The results are shown in Figs. 1 and 2. The UV/vis spectra of the upper-rim distal *o*-methoxyphenylazo-coupled calix[4]arenes **3**, **5**, and **7** with and without 15 metal perchlorates were studied (Fig. 1a,c, and d). Ligand **7**, a control compound of **3** whose lower-rim distal triazole units were replaced by two propoxy groups, did not show any shift in its λ_{max} after adding 15 different metal perchlorates (Fig. 1d). The results imply that the triazole units are the major binding ligands for Hg^{2+}. Ligand **5**, another control compound of **3**, in which the lower-rim two triazoles units were intact but the two azophenol groups were protected by propoxy groups, exhibited much smaller affinities toward Hg^{2+}, Ag^+, and Cu^{2+} ions with some hypsochromic shifts (Fig. 1c). Compound **9**, *o*-methoxy-phenylazophenol, did not show any affinity toward the 15 metal ions (Fig. 1e), implying that not only the azophenol groups but also the triazole groups are necessary components for the chromogenic sensing of Hg^{2+}.

To further explore the relationship between the binding ability and the number of the azophenol units, UV/vis spectra of ligands **3** (with distal bis-azophenol units) and **4** (with mono-azophenol unit) were screened with various metal ions. Ligands **3** and **4** exhibited a specific selectivity toward Hg^{2+} ion over all other metal ions (Fig. 1a and b) but with different binding affinities. When

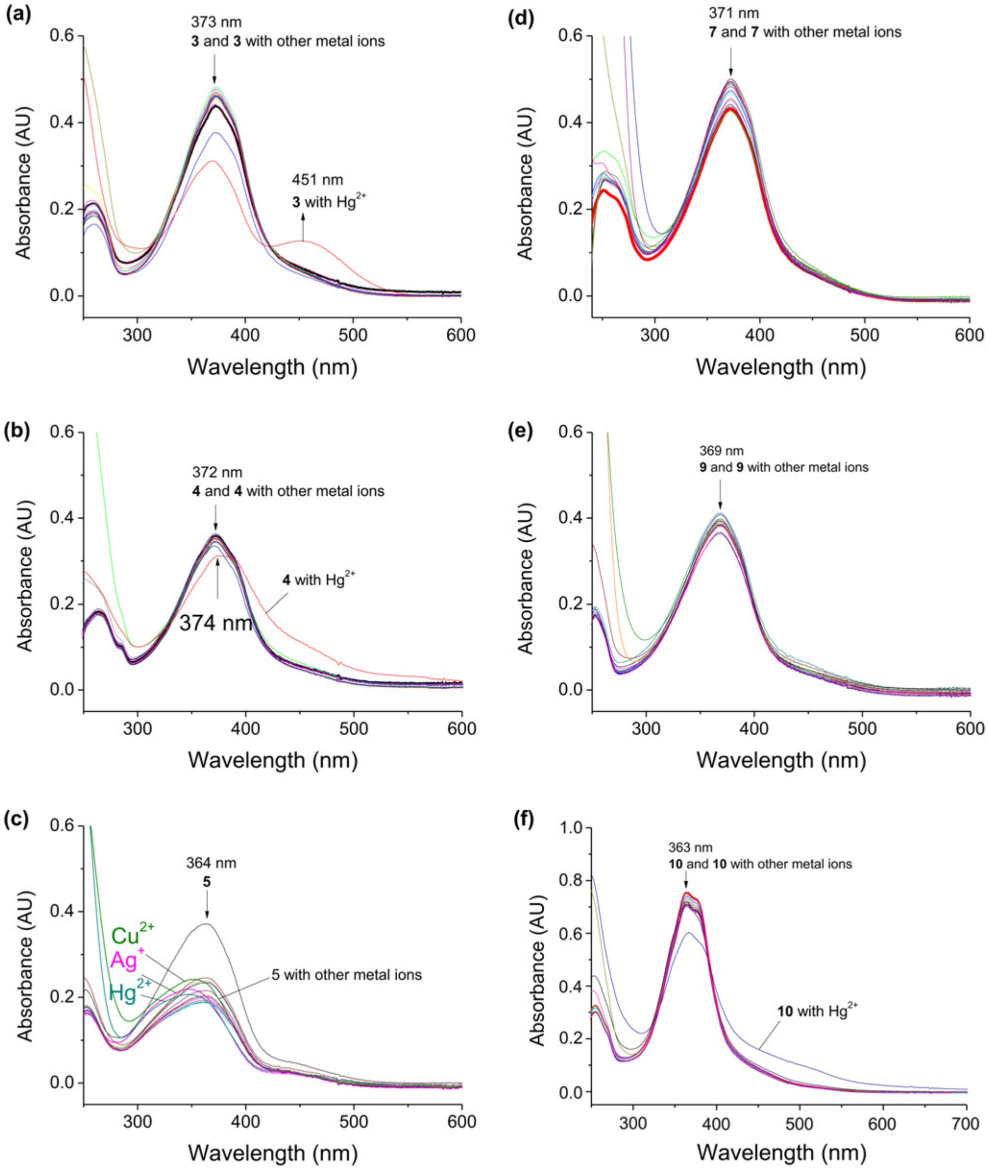


Fig. 1. UV/vis spectra of various ligands in the presence and absence of 10 equiv of 15 different metal perchlorate salts (Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Ag^+, Cu^{2+}, Ni^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+}, and Cr^{3+}) in MeOH/$CHCl_3$ (v/v, 98:2): (a) ligand **3**, 10 μM; (b) ligand **4**, 15 μM; (c) ligand **5**, 10 μM; (d) ligand **7**, 10 μM; (e) ligand **9**, 20 μM, and (f) ligand **10**, 10 μM.

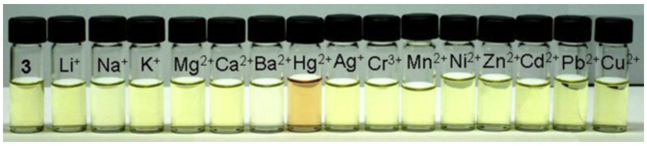


Fig. 2. Colors of ligand **3** (10 μM) in the absence and presence of 10 equiv of 15 different metal perchlorate salts in MeOH/$CHCl_3$ (v/v, 98:2). From left to right: free ligand **3**, and ligand **3** with 10 equiv of Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Hg^{2+}, Ag^+, Cr^{3+}, Mn^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, respectively.

complexed with Hg^{2+}, the λ_{max} of **3** showed not only a bathochromic shift ($\Delta\lambda_{max}$=78 nm) but also a hyperchromic effect (increase in intensity). It was showing a much less effect on λ_{max} and absorption intensity when ligand **4** was treated with Hg^{2+}. Therefore, the color of solution **3** changed from pale yellow to beige upon adding Hg^{2+}, which can be easily observed by the naked eyes, whereas, the color of solution **4** did not show any discernable change upon adding Hg^{2+} (Fig. 1b). Thus, compound **3**, which contains two upper-rim arylazo phenols, is a better sensor for Hg^{2+} than compound **4**, which contains only 'one' upper-rim arylazophenol unit.

In order to evaluate the role of the position of the methoxy substituent on the arylazo group, the UV/vis spectra of ligand **3** and its structural isomer **10**[5a] were screened over 15 metal ions (Fig. 1a and f). The only difference between **3** and **10** is the position of the methoxy substituent on the upper-rim arylazo units (Scheme 1) where **3** contains two distal *ortho*-methoxy-phenylazophenol units while **10** contains two distal *para*-methoxypenylazophenol units. It is important to note that ligand **10** was previously reported[5a] to be a chromogenic sensor for Ca^{2+} and Pb^{2+} in acetonitrile. In this work, ligand **10** also showed some UV/vis response toward Hg^{2+} in MeOH/$CHCl_3$ (v/v, 98:2) protic cosolvent and its spectra changes are similar to those of **4** (cf. Fig. 1b and f) but to a much less extent compared to that of ligand **3**. Upon adding Hg^{2+}, ligand **3** showed a more distinct bathochromic shift ($\Delta\lambda_{max}$=78 nm) compared to that of **10** in MeOH/$CHCl_3$ (v/v, 98:2) cosolvent (cf. Fig. 1a and f). The results indicated that **3**, which contained two *ortho*-

methoxyphenyl-azophenol groups, was a more effective chromogenic sensor for Hg^{2+} than **10** that contained two *para*-methoxyphenyl-azophenol groups.

Upon adding $Hg(ClO_4)_2$ to the solution of **3** in MeOH/$CHCl_3$ (v/v, 98:2) cosolvent, it exhibited a marked bathochromic shift in its λ_{max} as shown in Fig. 3. The intensity of the absorption maximum at 373 nm gradually decreased with the formation of a new absorption band at 451 nm ($\Delta\lambda_{max}$=78 nm). Two isosbestic points at 311 and 420 nm were observed in the titration spectra of **3** with $Hg(ClO_4)_2$ and the spectral feature was consistent with a 1:1 binding ratio between **3** and Hg^{2+}. Further support of the 1:1 binding ratio came from Job plot experiments,[12] where absorption of the complex at 451 nm was plotted against the molar fraction of **3** under the condition of an invariant total concentration. As a result, the concentration of the complex $\mathbf{3}\cdot Hg^{2+}$ approached a maximum when the molar fraction of [**3**]/([**3**]+[Hg^{2+}]) was about 0.5 (Fig. 4a).[12] The 1:1 association constant of the bis-*o*-methoxyphenylazocalix[4]arene **3** with Hg^{2+} in MeOH/$CHCl_3$ cosolvent was determined to be 4.02×10^3 M^{-1} on the basis of Benesi–Hildebrand plot (Fig. 4b).[13] The UV/vis spectral change between **3** and complex $\mathbf{3}\cdot Hg^{2+}$ was fully reversible, namely, the addition of H_2O to the solution of $\mathbf{3}\cdot Hg^{2+}$ reversed its color to free ligand **3** (Fig. S19, Supplementary data).

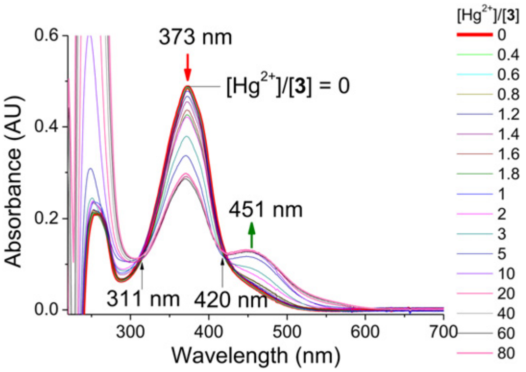


Fig. 3. UV/vis spectra of **3** (10 μM) upon adding various amounts of $Hg(ClO_4)_2$ in MeOH/$CHCl_3$ (v/v, 98:2) cosolvent at 25 °C.

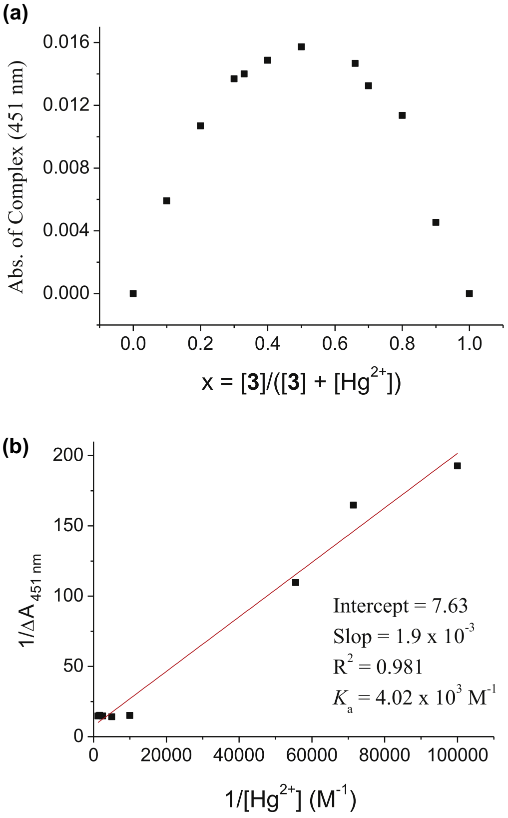


Fig. 4. (a) Job's plot of a 1:1 complex of **3** and Hg^{2+}, where the absorption of complex at 451 nm was plotted against the mole fraction of **3** at an invariant total concentration of 10 μM; (b) Benesi–Hildebrand plot of **3** with $Hg(ClO_4)_2$.

A similar Job plot experiment on compound **4** with Hg^{2+} was carried out, which showed a 1:1 binding ratio of $\mathbf{4}\cdot Hg^{2+}$ (Fig. S1, Supplementary data). Note that the absorption change of the complex $\mathbf{4}\cdot Hg^{2+}$ was too small to allow an accurate estimation of the binding constant (Fig. S2, Supplementary data). An electrospray mass spectrometry of complex $\mathbf{3}\cdot Hg^{2+}$ showed a peak at m/z=1235.4 corresponding to the mass of $[\mathbf{3}-H+Hg]^+$ (Fig. S3, Supplementary data).

In order to gain insight into the structure of complex $\mathbf{3}\cdot Hg^{2+}$, we carried out ^1H NMR titration experiments on **3** with different amounts of Hg^{2+}. ^1H NMR spectra of **3** (2.5 mM) in the presence of different equivalent of $Hg(ClO_4)_2$ were measured in a methanol-d_4/$CDCl_3$ (v/v, 1:1) cosolvent (Fig. 5). When 1.5 mM (0.6 equiv) of Hg^{2+} was added to **3**, solid precipitates started to occur, which gradually caused the signal to decrease as the concentration of Hg^{2+} increased (Fig. 5b). Upon the complexation of **3** with Hg^{2+}, the chemical shifts of protons H_a–H_f on the azophenol units of ligand **3** were little influenced. Proton H_f initially moved downfield by 0.03 ppm in the presence of 0.6 equiv of Hg^{2+} (Fig. 5b), however, it moved back to its original location (at δ 8.03 ppm) in the presence of 2 mM (0.8 equiv) of Hg^{2+} (Fig. 5c), and then buried under the chloroform peak in the presence of 1 equiv of Hg^{2+} (Fig. 5d), the signal became weaker because more precipitates formed. During the addition of 0–0.8 equiv Hg^{2+}, the peak of the proton H_f on the azophenol unit of **3** remained singlet until it disappeared (Figs. 5 and S4, Supplementary data).These spectral features imply that the two distal hydroxyl azophenol units of ligand **3** formed a weak but symmetrical complex with Hg^{2+}. Protons H_h and H_i on the calix[4]arene skeleton of ligand **3** were downfield shifted by only 0.04 ppm in the presence of 1 equiv of Hg^{2+}, whereas the methylene bridge protons H_j had more noticeable changes in chemical shifts. When 1 equiv of Hg^{2+} was added to **3**, one set of the methylene bridge protons, H_{j1}, was downfield shifted by about 0.13 ppm and then partly merged into the peak of MeOH; the other set of methylene bridge protons, H_{j2}, was upfield shifted by about 0.04 ppm and the peaks eventually merged partly into the methoxy group protons (H_a).

The chemical shift difference ($\Delta\delta$), between the two sets of methylene bridge protons, was about 0.85 ppm in free ligand **3** (Fig. 5a) but was reduced to 0.68 ppm in the presence of 1 equiv of Hg^{2+} (Fig. 5d), which implied that the complex $\mathbf{3}\cdot Hg^{2+}$ was in a flattened cone conformation.[5b,11c] The protons H_k, H_m, and the nearby phenyl group were little influenced when the concentration of Hg^{2+} increased, but the triazole proton H_l was downfield shifted by 0.16 ppm in the presence of 1 equiv of Hg^{2+}, which showed the most significant shift among all the protons of ligand **3**. Accordingly, we proposed that Hg^{2+} was bonded to the two nitrogen atoms of the lower-rim distal triazole units of ligand **3** and formed a symmetrical flattened cone conformation, in which the two distal hydroxyl azophenol also assisted in the binding of Hg^{2+} by electrostatic interaction (Chart 1). The upper-rim two distal arylazo units only functioned as the signal transduction units in ligand **3**. It should be noted that the symmetrical complexation of Hg^{2+} in the protic cosolvent system used in this work is quite different from those reported in other azo-coupled calix[4]arenes studied in

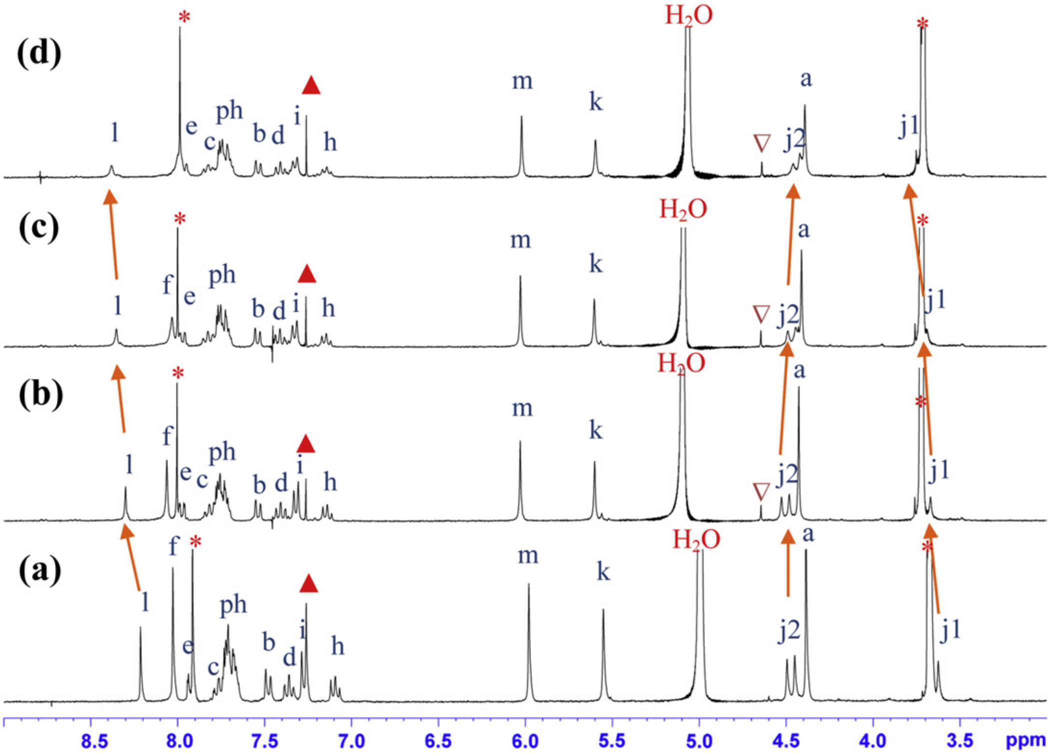


Fig. 5. ^{1}H NMR spectra of **3** (2.5 mM) in a methanol-d_4/$CDCl_3$ (v/v, 1:1) cosolvent in the presence of different amount of $Hg(ClO_4)_2$: (a) 0 mM; (b) 1.5 mM (0.6 equiv); (c) 2 mM (0.8 equiv), and (d) 2.5 mM (1 equiv), where * denotes signals from solvents MeOH and $CHCl_3$, ▲ denotes an external standard $CHCl_3$, and ▽ denotes the sweeping noise from the instrument.

acetonitrile,[5a,b] in which the metal complexation was mostly unsymmetrical with respect to the calix[4]arene skeleton.

Finally, the optimized geometries of **3** and **10** were calculated by the molecular modeling DMol3[14] and simulated in MeOH environment (Fig. 6 and Tables S1–4, Supplementary data). The DMol3 method from Material Studio 5.0 is developed by Accelrys Inc. in which the wavefunctions are expanded in terms of accurate numerical basis set. We used a double-numeric quality basis set with polarization functions (DNP). The size of the DNP basis set is comparable to Gaussian 6-31G**, but DNP is more accurate than a Gaussian basis set of the same size.[15a] During geometry optimization, displacements were set at 10^{-3} Bohr radius (0.529177 Å), gradient at 10^{-3} Hartree/Bohr radius, and energy at 10^{-5} Hartree.[15b] In general, the calculated optimized geometries of **3** and **10** posses the cone formation in MeOH, where the two distal phenoxy groups of the triazolylmethoxy benzenes are almost parallel to each other while the other two distal azophenol groups are oriented outward to give a 'pinched cone' (or so-called 'flattered cone') conformation.

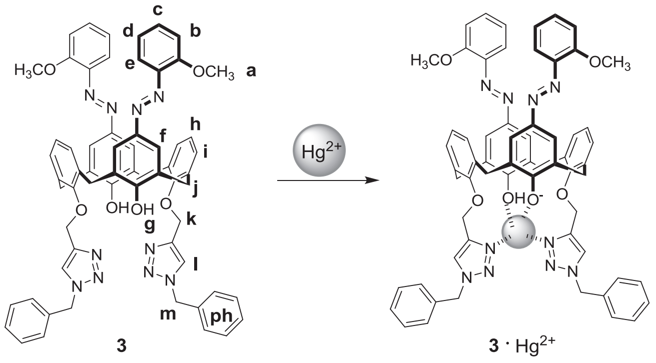


Chart 1. A possible binding mode of **3**·Hg^{2+}.

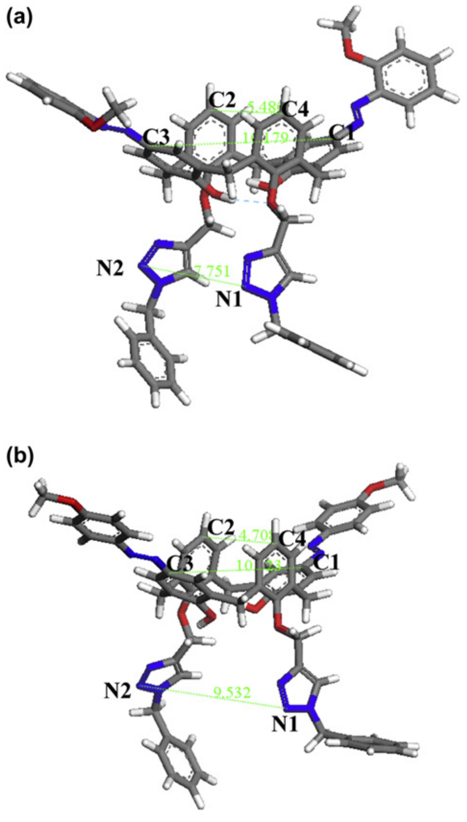


Fig. 6. Optimized geometries for ligands (a) **3** and (b) **10** in MeOH by DMol3 calculation.

Scrutiny of the optimized geometries of **3** and **10** in MeOH (Fig. 6) showed some important features: (1) the C2–C4 distance between two distal phenolic ethers is 5.480 Å in **3** but is 4.708 Å in **10**; (2) the C1–C3 distance between two distal upper-rim azophenyl groups is 10.179 Å in **3** but is 10.323 Å in **10**; (3) in particular, the N1–N2 distance of the lower-rim two distal triazole units is 7.751 Å in **3** but is 10.323 Å in **10**. The features indicate that in order to decrease the repulsion between *ortho*-methoxyphenylazo units and its neighboring phenolic units, the two distal phenolic units on the calix[4]arene **3** were more flattened than that of **10** and thus resulted in the closer distance between the two distal triazole units on the lower rim of **3**. We speculate that the smaller lower-rim cavity of ligand **3** compared to that of **10**, might have some effect on its more effective complexation with Hg^{2+}.

3. Conclusions

A series of *ortho*-methoxyphenylazocalix[4]arenes (**3**, **4**, **5**, and **7**) with different lower-rim substituents or different number of the upper-rim azo unit were synthesized to probe the ion sensing abilities of the triazole groups and the *o*-methoxy-phenylazophenol units. Ligand **3**, which has two distal *ortho*-methoxyphenylazo groups on the upper-rim and bis-benzyl-1,2,3-triazolylmethoxy groups at the lower rim, was proven to be a ratiometric and specific chromogenic sensor for Hg^{2+} in polar protic solvent MeOH/$CHCl_3$ (v/v, 98:2). All other analogues synthesized (**4**, **5**, and **7**) and compound **10**[5a] reported previously were not as good as that of **3** even though their skeletons and attached functional groups were quite similar. The complex **3**$\cdot Hg^{2+}$ in protic solvent studied here has a flattened cone conformation and is symmetrical with respect to the calix[4]arene skeleton. Our studies in the past[5a,c,6g,h] and this work showed that *solvent effect* plays a crucial role in determining the selectivity and sensitivity of chromogenic sensors for metal ions based on upper-rim arylazo-coupled calix[4]arenes.

4. Experimental section

4.1. general

All reported yields were isolated yields. Flash column chromatography was performed using silica gel (70–230 mesh). ^1H NMR spectra were recorded in a 300 MHz NMR spectrometer, using the $CDCl_3$ solvent peak as an internal standard. ^{13}C NMR spectra were recorded at 75.4 MHz. The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m). High resolution Mass spectra were measured at FAB mode or Electron Impact by JMS-700 HRMS. Melting points were measured with a Yanaco MP500D apparatus and were uncorrected. UV/vis spectra were measured with an HP-8453 spectrophotometer and solvents were of HPLC grades.

25,27-Bis(*O*-propargyl)calix[4]arene **1**[5a,8] and 25,27-bis(*O*-propyl)calix[4]arene **6**[10] were synthesized according to the literature procedures.

4.2. General procedures for the synthesis of 2a, 2b, 7, and 9

To an ice cold solution of *o*-anisidine 0.18 g (1.50 mmol) in 4 N HCl (4 mL) and acetone (5 mL) was added a solution of $NaNO_2$ (0.20 g, 3.00 mmol) in H_2O (5 mL), and the mixture was stirred for 3 h in ice-water bath. The combined solution was added, respectively to another ice cold solution of **1**, **6**, or **8** (0.60 mmol) in pyridine (10 mL) to produce a colored solution. The reaction mixture was stirred for 1–3 days at 0 °C and then treated with 4 N HCl (50 mL) to give a colored precipitate. The solid residue was purified by column chromatography with ethyl acetate/hexane as an eluent and gave the corresponding products in 20–83% yield.

*4.2.1. Data for 5,17-bis(o-methoxyphenyl)azo-25,27-dipropargyloxy-26,28-dihydroxycalix[4]arene (**2a**).* The solid was eluted with ethyl acetate/hexane (v/v, 1:1) and gave 0.17 g (36%) of an orange powder; mp 213–214 °C; R_f=0.65 (EtOAc/hexane, 1:1); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.77 (4H, s, Ph–*H*), 7.63 (2H, s, O*H*), 7.60 (2H, dd, J=8.0, 1.6 Hz, Ph–*H*), 7.43–7.37 (2H, m, Ph–*H*), 7.09–6.94 (8H, m, Ph–*H*), 6.76 (2H, t, J=7.5 Hz, Ph–*H*), 4.83 (4H, d, J=2.4 Hz, OCH_2), 4.44 (4H, d, J=13.2 Hz, Ph–CH_2–Ph), 4.03 (6H, s, OCH_3), 3.58 (4H, d, J=13.2 Hz, Ph–CH_2–Ph), 2.63 (2H, t, J=2.4 Hz, OCH_2CC*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 156.7 (Cq), 156.6 (Cq), 151.7 (Cq), 146.7 (Cq), 143.1 (Cq), 133.0 (Cq), 131.8 (CH), 129.9 (Cq), 129.6 (CH), 128.9 (Cq), 126.3 (CH), 124.4 (CH), 121.3 (CH), 117.6 (CH), 112.8 (CH), 78.4 (Cq), 77.6 (CH), 64.0 (CH_2), 56.7 (CH_3), 32.1 (CH_2); MS (FAB, m/z) 769 [$M+H^+$]; HRMS m/z calcd for $C_{48}H_{41}N_4O_6$ 769.3026, found 769.3028.

*4.2.2. Data for 5-(o-methoxyphenyl)azo-25,27-dipropargyloxy-26,28-dihydroxycalix[4]arene (**2b**).* The solid was eluted with ethyl acetate/hexane (v/v, 1:2) and gave 0.16 g (42%) of an orange powder; mp 207–209 °C; R_f=0.73 (EtOAc/hexane, 1:1); ^1H NMR ($CDCl_3$, 300 MHz) δ 7.76 (2H, s, Ph–*H*), 7.63 (1H, s, O*H*), 7.62–7.58 (1H, m, O*H*), 7.42–7.37 (1H, m, Ph–*H*), 7.11–6.99 (5H, m, Ph–*H*), 6.93 (2H, dd, J=7.5, 1.5 Hz, Ph–*H*), 6.86 (2H, dd, J=7.7, 1.3 Hz, Ph–*H*), 6.76–6.69 (3H, m, Ph–*H*), 4.81 (4H, d, J=2.4 Hz, OCH_2), 4.44 (2H, d, J=13.2 Hz, Ph–CH_2–Ph), 4.41 (2H, d, J=13.2 Hz, Ph–CH_2–Ph), 4.03 (3H, s, OCH_3), 3.56 (2H, d, J=13.2 Hz, Ph–CH_2–Ph), 3.43 (2H, d, J=13.2 Hz, Ph–CH_2–Ph), 2.60 (2H, t, J=2.4 Hz, OCH_2CC*H*); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 156.8(Cq), 156.6 (Cq), 153.4 (Cq), 151.7 (Cq), 146.6 (Cq), 143.1 (Cq), 133.7 (Cq), 133.1 (Cq), 131.7 (CH), 129.7 (CH), 129.6 (Cq), 129.0 (CH), 128.9 (CH), 128.7 (Cq), 126.2 (CH), 124.4 (CH), 121.3 (CH), 119.6 (CH), 117.6 (CH), 112.8 (CH), 78.6 (Cq), 77.6 (CH), 63.9 (CH_2), 56.7 (CH_3), 32.2 (CH_2), 32.1 (CH_2); MS (FAB, m/z) 635 [$M+H^+$]; HRMS m/z calcd for $C_{41}H_{35}N_2O_5$ 635.2546, found 635.2532.

*4.2.3. 5,17-Bis(o-methoxyphenyl)azo-25,27-bis(1-benzyl-1H-1,2,3-triazolylmethyloxy)-26,28-dihydroxycalix[4]arene (**3**).* To a mixture of **2a** 0.20 g (0.26 mmol) and 1-(azidomethyl)benzene 0.12 g (0.92 mmol) in THF/H_2O 30 mL (v/v, 6:1) was added CuI 2.50 mg (0.01 mmol) and then reflux for 18 h. After evaporation of solvent, the residue was dissolved in CH_2Cl_2 and washed thrice with H_2O. The organic layer was dried over $MgSO_4$ and the filtrate was concentrated under reduce pressure. The crude product was purified by column chromatography with ethyl acetate/hexane (v/v, 3:1) to afford pure compound **3** 0.19 g (71%); mp 256–257 °C; R_f=0.18 (EtOAc/hexane, 1:1); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.08 (2H, s, O*H*), 7.78 (2H, s, CC*H*N), 7.70 (4H, s, Ph–*H*), 7.60 (2H, dd, J=8.0, 1.62 Hz, Ph–*H*), 7.43–7.25 (12H, m, Ph–*H*), 7.09–6.99 (4H, m, Ph–*H*), 6.94 (4H, d, J=7.6 Hz, Ph–*H*), 6.75 (2H, t, J=7.6 Hz, Ph–*H*), 5.62 (4H, s, NCH_2Ph), 5.21 (4H, s, OCH_2), 4.17 (4H, d, J=13.2 Hz, PhCH_2Ph), 4.14 (6H, s, OCH_3), 3.37 (4H, d, J=13.2 Hz, PhCH_2Ph); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 156.8 (Cq), 156.6 (Cq), 151.7 (Cq), 133.0 (Cq), 131.8 (CH), 130.0 (Cq), 129.5 (CH), 129.3 (CH), 128.6 (Cq), 128.5 (CH), 126.3 (CH), 124.4 (CH), 124.0 (CH), 121.3 (CH), 117.6 (CH), 112.8 (CH), 70.0 (CH_2), 56.7 (CH_3), 54.8 (CH_2), 31.8 (CH_2); MS (FAB, m/z) 1036 [$M+H^+$]; HRMS m/z calcd for $C_{62}H_{54}N_{10}O_6$ 1034.4228, found 1034.4235.

*4.2.4. 5-(o-Methoxyphenyl)azo-25,27-bis(1-benzyl-1H-1,2,3-triazolylmethyloxy)-26,28-dihydroxycalix[4]arene (**4**).* To a mixture of **2b** 0.20 g (0.32 mmol) and 1-(azidomethyl)benzene 0.15 g (1.10 mmol) in THF/H_2O 30 mL (v/v, 6:1) was added CuI 6.00 mg (0.03 mmol) and then reflux for 18 h. After evaporation of solvent, the residue was dissolved in CH_2Cl_2 and washed thrice with H_2O.

The organic layer was dried over $MgSO_4$ and the filtrate was concentrated under reduce pressure. The crude product was purified by column chromatography with ethyl acetate/hexane (v/v, 3:1) to afford pure compound **4** 0.21 g (73%); mp 146–147 °C; R_f=0.25 (EtOAc/hexane, 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.16 (1H, s, O*H*), 7.77 (2H, s, CC*H*N), 7.68 (2H, s, Ph–*H*), 7.60 (1H, dd, *J*=8.0, 1.7 Hz, Ph–*H*), 7.55 (1H, s, O*H*), 7.41–7.26 (10H, m, Ph–*H*), 7.09–6.98 (4H, m, Ph–*H*), 6.91 (2H, dd, *J*=7.5, 1.4 Hz, Ph–*H*), 6.84 (2H, dd, *J*=7.7, 1.5 Hz, Ph–*H*), 6.73–6.64 (3H, m, Ph–*H*), 5.58 (4H, s, NCH_2Ph), 5.17 (4H, s, OCH_2C), 4.17 (2H, d, *J*=13.1 Hz, PhCH_2Ph), 4.16 (2H, d, *J*=13.2 Hz, PhCH_2Ph), 4.03 (3H, s, OCH_3), 3.35 (2H, d, *J*=13.2 Hz, PhCH_2Ph), 3.26 (2H, d, *J*=13.1 Hz, PhCH_2Ph); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 156.8 (Cq), 156.6 (Cq), 153.4 (Cq), 151.8 (Cq), 146.6 (Cq), 144.3 (Cq), 143.1 (Cq), 135.3 (Cq), 133.6 (Cq), 133.0 (Cq), 131.7 (CH), 129.7 (CH), 129.6 (CH), 129.5 (CH), 129.1 (Cq), 128.9 (CH), 128.7 (CH), 128.4 (CH), 128.2 (CH), 126.2 (CH), 124.3 (CH), 123.9 (CH), 121.3 (CH), 119.6 (CH), 117.5 (CH), 112.8 (CH), 70.2 (CH_2), 56.7 (CH_3), 54.6 (CH_2), 31.8 (CH_2), 31.7 (CH_2); MS (FAB, *m*/*z*) 901 [M+H$^+$]; HRMS *m*/*z* calcd for $C_{55}H_{48}N_8O_5$ 900.3748, found 900.3737.

*4.2.5. 5,17-Bis(o-methoxyphenyl)azo-25,27-bis(1-benzyl-1H-1,2,3-triazolylmethyloxy)-26,28-dipropyloxycalix[4]arene (**5**).* To a well stirred solution of **3** 0.20 g (0.19 mmol) and NaH 0.14 g (5.78 mmol) in dry DMF (20 mL) was added 1-iodopropane 0.66 g (3.86 mmol) and then stirred at 80 °C for 18 h. After quenching the NaH by adding MeOH in an ice-water bath, the reaction solution was extracted thrice with CH_2Cl_2 (20 mL). The organic layer was dried over $MgSO_4$ and the filtrate was evaporated to give the crude product. The crude product was purified by column chromatography with acetone/hexane (v/v, 1:1) to afford pure compound **5** 0.03 g (14%); mp 82–84 °C; R_f=0.28 (EtOAc/hexane, 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.57–7.55 (2H, m, Ph–*H*), 7.51 (4H, s, C=C*H*N), 7.42–7.36 (10H, m, Ph), 7.30–7.28 (3H, m, Ph–*H*), 7.07 (3H, d, *J*=8.2 Hz, Ph–*H*), 6.96 (2H, t, *J*=15.2 Hz, Ph–*H*), 6.32 (6H, d, *J*=7.6 Hz, Ph–*H*), 5.52 (4H, s, NCH_2Ph), 5.01 (4H, s, OCH_2), 4.29 (4H, d, *J*=13.2 Hz, PhCH_2Ph), 4.01 (6H, s, OCH_3), 3.85 (4H, t, *J*=7.9 Hz, OCH_2CH$_2$CH$_3$), 3.00 (4H, d, *J*=13.5 Hz, PhCH_2Ph), 1.75–1.67 (4H, m, OCH$_2$CH_2CH$_3$), 0.69 (6H, t, *J*=7.41 Hz, OCH$_2$CH$_2$CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 161.0 (Cq), 156.9 (Cq), 154.3 (Cq), 148.6 (Cq), 144.9 (Cq), 143.0(Cq), 137.2 (Cq), 135.1 (Cq), 133.8 (Cq), 132.0 (CH), 129.7 (CH), 129.6 (CH), 129.5 (CH), 129.4 (CH), 128.5 (CH), 128.4 (CH), 124.0 (CH), 123.4 (CH), 123.3 (CH), 121.3 (CH), 117.4 (CH), 113.0(CH), 77.2 (CH_2), 67.7 (CH_2), 56.8 (CH_3), 54.6 (CH_2), 31.5 (CH_2), 23.4 (CH_2), 10.4 (CH_3); MS (FAB, *m*/*z*) 1121 [M+H$^+$]; HRMS *m*/*z* calcd for $C_{68}H_{66}N_{10}O_6$ 1118.5167, found 1118.5168.

*4.2.6. Data for 5,17-bis(o-methoxyphenyl)azo-25,27-dipropyloxy-26,28-dihydroxycalix[4]arene (**7**).* The solid was eluted with ethyl acetate/hexane (v/v, 1:3) and gave 0.09 g (20%) of an orange powder; mp 332–334 °C; R_f=0.9 (EtOAc/hexane, 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.84 (2H, s, OH), 7.75 (4H, s, Ph–*H*), 7.59 (2H, dd, *J*=8.0, 1.68 Hz, Ph–*H*), 7.41–7.36 (2H, m, Ph–*H*), 7.08–6.98 (8H, m, Ph–*H*), 6.78 (2H, t, *J*=7.5 Hz, Ph–*H*), 4.34 (4H, d, *J*=13.1 Hz, PhCH_2Ph), 4.05–4.01 (10H, m, 2OCH_3 and 2OCH_2CH$_2$CH$_3$), 3.55 (4H, d, *J*=13.1 Hz, PhCH_2Ph), 2.13–2.07 (4H, m, OCH$_2$CH_2CH$_3$), 1.35 (6H, t, *J*=7.4 Hz, OCH$_2$CH$_2$CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 157.3 (Cq), 156.6 (Cq), 152.2 (Cq), 146.5 (Cq), 143.2 (Cq), 133.1 (Cq), 131.7 (CH), 129.8 (CH), 128.7 (Cq), 125.8 (CH), 124.4 (CH), 121.7 (CH), 117.6 (CH), (CH), 78.8 (CH_2), 56.7 (CH_3), 31.7 (CH_2), 32.9 (CH_2), 11.3 (CH_3); MS (FAB, *m*/*z*) 778 [M+H$^+$]; HRMS *m*/*z* calcd for $C_{48}H_{48}N_4O_6$ 776.3574, found 776.3576.

*4.2.7. Data for 4-(o-methoxyphenyl)azo-2,6-dimethylphenol (**9**).* The solid was eluted with ethyl acetate/hexane (v/v, 1:1) and gave 0.52 g (83%) of an orange powder, mp 47–48 °C; R_f=0.15 (CH_2Cl_2/hexane, 1:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.65–7.61 (3H, m, Ph–H), 7.40–7.35 (1H, m, Ph–H), 7.08–6.98 (2H, m, Ph–H), 4.01 (3H, s, OCH_3), 2.31 (6H, s, 2CH_3); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 156.2 (Cq), 146.4 (Cq), 142.0 (Cq), 131.4 (CH), 124.9 (CH), 124.5 (CH), 121.3 (CH), 117.2 (CH), 112.7 (CH), 56.7 (CH_3), 16.4 (CH_3); MS (EI, *m*/*z*) 256 [M$^+$]; HRMS *m*/*z* calcd for $C_{15}H_{16}N_2O_2$ 256.1212, found 256.1207.

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Supplementary data

UV/vis titration spectra and Job's plot of **4** with $Hg(ClO_4)_2$; the mass spectrum of complex **3**·Hg^{2+} and the part expansion of ^{1}H NMR titration spectra of **3** with $Hg(ClO_4)_2$; ^{1}H and ^{13}C spectra of new compounds **2a**–**b**, **3**–**5**, **7** and **9**. Data calculated by molecular modeling DMol3 for the optimized geometries of **3** and **10**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.08.052. These data include MOL files and InChIKeys of the most important compounds described in this article.

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