Azocalix[4]arene-based chromogenic anion probes†

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Azocalix[4]arene derivatives **1–5**, with one or two azophenol chromophores and different binding sites, were found to allow not only for high selective and sensitive colorimetric detection but also easy colorimetric differentiation of F^-, AcO^- and $H_2PO_4^-$, of similar basicity, depending upon the azocalix[4]arene structure, guest basicity and conformational complementarity between the host and guest.

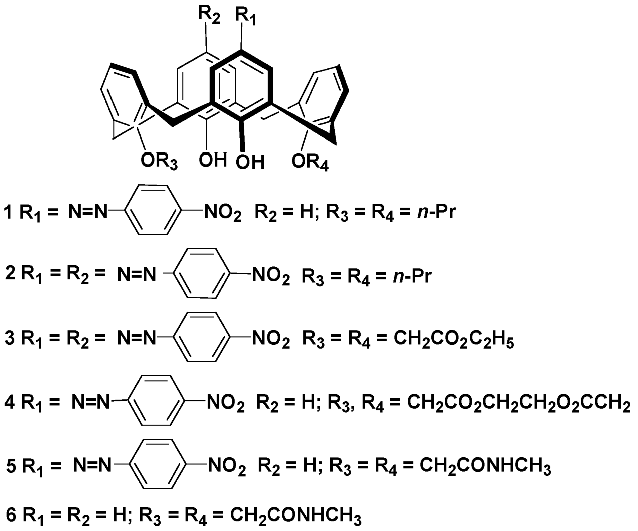
Introduction

In host–guest chemistry, the development of new receptors for anions has attracted a great interest in recent years due to the fact that anions play numerous fundamental roles in biological and chemical process.[1] One of particular interest in this regard involves the construction of colorimetric anion sensors, since they allow "naked-eye" detection.[1a,2] Consequently, some chromogenic anion sensors, including metal complexes[3] and non-metal hosts,[4,5] have been developed.

Calixarenes are a class of well defined macrocyclic oligomers of phenol bridged by methylene groups. Owing to their (1) convenient preparation in large quantities, (2) easy chemical modification on both their lower and upper rims, and (3) unique structural properties, they have been ideal platforms for the development of complexing agents, not only for cations and molecules, but also anions. In the past few years, some chromogenic sensors based on calix[4]arene[6] have been developed for cations[7] and molecules.[8] However, to the best of our knowledge, no calixarene-based chromogenic sensors for anions have been reported so far.[9] Considering that azophenol dyes can be used as chromogenic anion sensors,[3b,5] we deduced that the easily available azocalixarenes, containing one or more azophenol chromophores and different binding sites, would be ideal candidates for developing new chromogenic sensing systems for anions. Herein, we report a series of chromogenic azocalix[4]arene derivatives **1–5**, which allow not only for selective and sensitive colorimetric detection but also easy colorimetric differentiation of F^-, AcO^-, and $H_2PO_4^-$, depending upon the azocalix[4]arene structure, guest basicity and conformational complementarity between the host and guest.

Results and discussion

Azocalix[4]arene derivatives **1–4**[7b,10] were prepared according to literature procedures. Azocalix[4]arene **5** was easily synthesized by the reaction of **6** with *para*-nitrobenzenediazonium tetrafluoroborate in THF. The presence of two pairs of doublets at 4.18, 4.16, 3.62 and 3.59 ppm in the ^{1}H NMR spectrum of **5**, and two signals at 31.7 and 31.5 ppm in its ^{13}C NMR spectrum[11] indicated that **5** adopted the cone conformation.



1 R_1 = N=N–C$_6$H$_4$–NO$_2$ R_2 = H; $R_3 = R_4$ = *n*-Pr
2 $R_1 = R_2$ = N=N–C$_6$H$_4$–NO$_2$ $R_3 = R_4$ = *n*-Pr
3 $R_1 = R_2$ = N=N–C$_6$H$_4$–NO$_2$ $R_3 = R_4 = CH_2CO_2C_2H_5$
4 R_1 = N=N–C$_6$H$_4$–NO$_2$ R_2 = H; R_3, $R_4 = CH_2CO_2CH_2CH_2O_2CCH_2$
5 R_1 = N=N–C$_6$H$_4$–NO$_2$ R_2 = H; $R_3 = R_4 = CH_2CONHCH_3$
6 $R_1 = R_2$ = H; $R_3 = R_4 = CH_2CONHCH_3$

The binding abilities of azocalix[4]arenes **1–5** for anions were investigated by the UV-vis absorption method. Because **1–5** don't dissolve in water or even aqueous acetonitrile, all titration experiments were performed in MeCN only and the anions added as their tetrabutylammonium salts. To obtain a quantitative insight into the anionic affinity of the chromogenic azocalix[4]arenes, we determined the wavelength changes upon complexation of the various anions. The wavelength changes for **1–5** are listed in Table 1. From the UV-vis titration data, the association constants[12,13] for the complexation between hosts **1–5** and the anions were further determined by a linear curve-fitting procedure and summarized in Table 2.

Initially, we paid attention to azocalix[4]arene **1** with a *para*-nitrophenylazo group on the upper rim. In the absence of anions, the spectrum of **1** is characterized by the presence of one absorption maximum at 394 nm. Upon addition of F^-, the peak at 394 nm decreased while a new peak at 628 nm appeared (Fig. 1 (a)) and the solution of **1** showed a significant color change from light yellow to blue (Fig. 1 (b)). Under the

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Table 1 The optical response of **1–5** (10^{-5} M) to different anions in MeCN[ab]

			Anion-induced wavelength changes ($\Delta\lambda_{max}$/nm)								
Compound	λ_{max}/nm	ε/cm^{-1} M^{-1}	F^-	Cl^-	Br^-	I^-	AcO^-	$H_2PO_4^-$	HSO_4^-	NO_3^-	CN^-
1	394	2.77×10^4	234	n	n	n	n	n	n	n	n
2	392	5.13×10^4	234	n	n	n	216	n	n	n	n
3	393	5.03×10^4	245	n	n	n	219	217	n	n	n
4	393	3.59×10^4	230	n	n	n	199	222	n	n	n
5	385	2.48×10^4	219	n	n	n	219	219	n	n	n

[a] The 'n' denotes no obvious spectral changes. [b] Because dyes **1–5** don't respond to the anions in aqueous solution, we didn't carry out experiments on the effect of pH.

same conditions, no obvious absorption spectral or color changes were observed upon addition of Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^- or CN^-. This high selectivity of **1** toward F^- may be due to both the strong basicity of F^- and a steric hindrance effect from the host.[5c]

Compared to **1**, compound **2**, containing two chromophores, allows for not only selective colorimetric detection but also easy colorimetric differentiation of F^- and AcO^- of similar basicity. Consequently, addition of fluorides into the solution of **2** gave a significant bathochromic shift from 392 to 626 nm ($\Delta\lambda$ = 234 nm), as shown in Table 1 and Fig. 2 (a). Meanwhile, AcO^- induced a similar but smaller wavelength change in **2** ($\Delta\lambda$ = 216 nm). In the cases of the other examined anions, no obvious absorption spectrum changes were observed. As expected from the UV-vis data, **2** showed a noticeable color change from light yellow to blue upon the addition of F^-, and to violet-blue for the same amount of AcO^- (Fig. 2 (b)). The results presented here indicate not only that the charge density and size of the F^- and AcO^- anions influence their binding selectivity, but that the azocalix[4]arene skeleton also does so greatly.

Different from **2**, azocalix[4]arene **3** contains two carboxylate groups at its lower rim, resulting in significant absorption spectrum and color changes (Fig. 3) not only for F^- and AcO^-, but also for $H_2PO_4^-$, bearing OH groups. UV-vis titrations further displayed that the selectivity trend of the binding affinity of **3** for the anions was $F^- > AcO^- > H_2PO_4^- \gg Cl^-, Br^-, I^-, HSO_4^-, NO_3^-$ and CN^-. This result also suggested that a change of binding site attached to a chromogenic azocalix[4]arene could enable it to show different colorimetric characteristics, which would provide many opportunities to develop various azocalix[4]arene-based chromogenic systems for selected anions.

Table 2 Association constants (K_a/M^{-n}) for the complexes of azocalix[4]arenes **1–5** with anions in MeCN[a]

	K_a (Host : Guest ratio)		
	F^-	AcO^-	$H_2PO_4^-$
1	2.6×10^4 (1 : 1)	—	—
2	1.2×10^4 (1 : 1)	3.0×10^3 (1 : 1)	—
3	3.3×10^9 (1 : 2)	7.4×10^4 (1 : 1)	3.5×10^4 (1 : 1)
4	4.9×10^9 (1 : 2)	2.0×10^4 (1 : 1)	1.3×10^4 (1 : 1)
5	3.7×10^9 (1 : 2)	4.0×10^7 (2 : 3)	4.2×10^4 (1 : 1)

[a] Anions were added as their tetrabutylammonium salts, all errors are $<\pm 10\%$. n = 1, 1.5 or 2, depending on the host : guest ratio.

In anionic sensing, it is still a challenge to develop new sensors for easy colorimetric differentiation of F^-, AcO^-, and $H_2PO_4^-$ of similar basicity.[4b,5a] Although two azophenol chromophores and two carboxylates with a free chain structure in **3** contributed to its selective sensing of F^-, AcO^- and $H_2PO_4^-$, **3** cannot allow for colorimetric differentiation of AcO^- and $H_2PO_4^-$, in which almost the same wavelength changes were observed (Table 1). To develop new selective anion sensors, the binding properties of azocalix[4]crown **4**, with two carboxylate groups, toward anions were further tested. The results showed that F^-, AcO^- and $H_2PO_4^-$ induced significantly different red shifts in **4**, while no obvious spectral changes were observed in the cases of the other anions. The new peaks were found to be at 622 nm for F^-, 592 nm for AcO^- and 615 nm for H_2PO_4, respectively, corresponding to a maximum absorption intensity trend of $F^- > AcO^- > H_2PO_4^{2-}$ (Fig. 4 (a)). As expected from the UV-vis data, different color changes occurred upon addition of the different anions to solutions of **4** (Fig. 4 (b)). Thus **4** proved to be a potential chromogenic anion sensor, not only

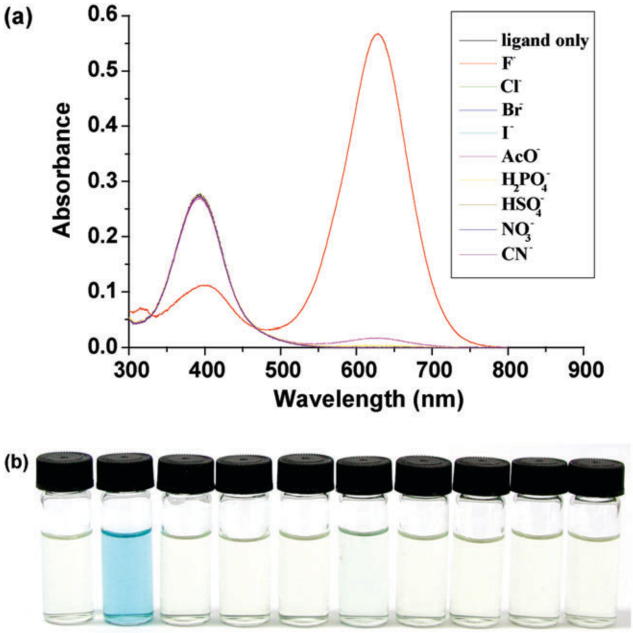

Fig. 1 (a) UV-vis and (b) color changes of **1** in MeCN (10^{-5} M) before and after the addition of 8 equivalents of each anion. From left to right: no anion, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^- and CN^-.

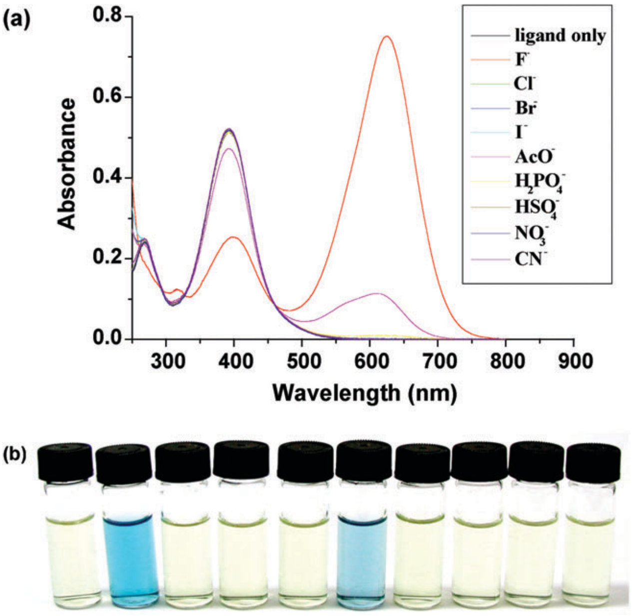

Fig. 2 (a) UV-vis and (b) color changes of **2** in MeCN (10^{-5} M) before and after the addition of 6 equivalents of each anion. From left to right: no anion, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^- and CN^-.

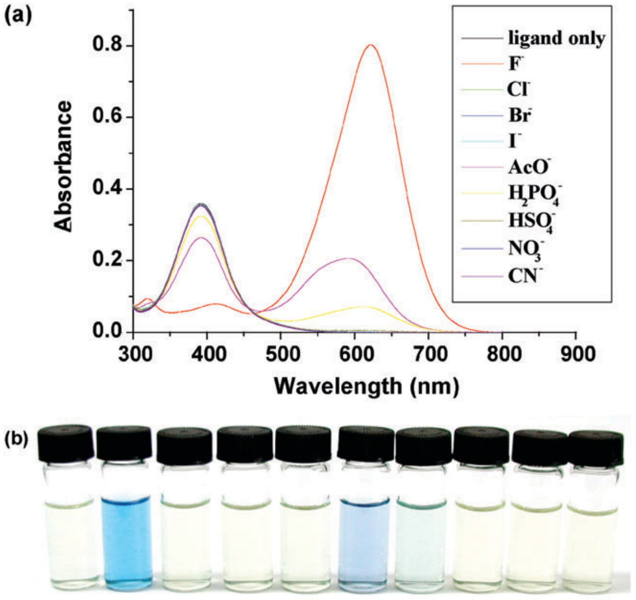

Fig. 4 (a) UV-vis and (b) color changes of **4** in MeCN (10^{-5} M) before and after the addition of 3 equivalents of each anion. From left to right: no anion, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^- and CN^-.

for selective colorimetric detection but, at the same time, easy colorimetric differentiation of F^-, AcO^- and $H_2PO_4^-$.

Moreover, we also examined the binding properties of azocalix[4]arene **5**, with two amide groups, toward anions, and found that it showed increased binding abilities toward F^-, and especially AcO^- and $H_2PO_4^-$. However, no obvious binding selectivities between F^-, AcO^- and $H_2PO_4^-$ for **5** were observed. These results were supported by the UV-vis and color changes (Fig. 5) of **5** upon the addition of the various anions. Consequently, F^-, $H_2PO_4^-$ and AcO^- induced the same wavelength changes in **5** ($\Delta\lambda$ = 219 nm), while the color of the solution of **5** changed from violet-blue for F^- and

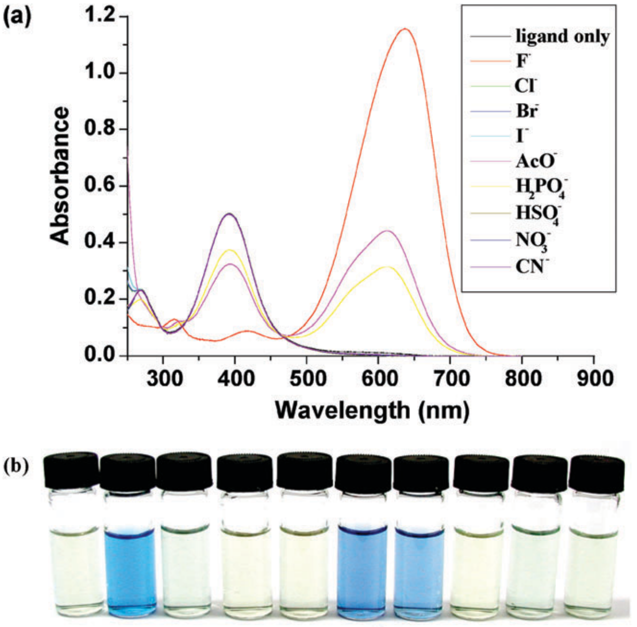

Fig. 3 (a) UV-vis and (b) color changes of **3** in MeCN (10^{-5} M) before and after the addition of 8 equivalents of each anion. From left to right: no anion, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^- and CN^-.

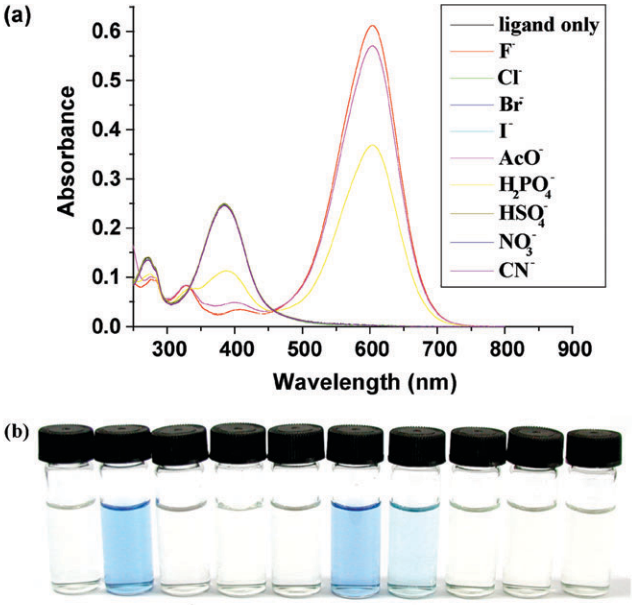

Fig. 5 (a) UV-vis and (b) color changes of **5** in MeCN (10^{-5} M) before and after the addition of 3 equivalents of each anion. From left to right: no anion, F^-, Cl^-, Br^-, I^-, AcO^-, $H_2PO_4^-$, HSO_4^-, NO_3^- and CN^-.

AcO^-, and light violet-blue for $H_2PO_4^-$ in the presence of 2.4 equivalents of the anions.

It is known[14] that the electronic excitation of an azophenol chromophore generally occurs through a charge transfer from the donor oxygen of the phenol to the acceptor substituent ($–NO_2$) of the chromophore. Upon complex formation between the azocalix[4]arene and an anion, the excited state would be more strongly stabilized by anion binding, resulting in a bathochromic shift in the absorption maxima as well as in color changes. Thus, it turns out that the hydrogen bonding ability and basicity of the anions play an important role in anion binding to azocalix[4]arenes.[5c] As expected from their anion basicities, F^-, AcO^- and $H_2PO_4^-$ give the stronger complexes and thus show noticeable UV-vis and color changes in azocalix[4]arenes **1–5** compared to other anions. Because of the differences in their size and structure,[1c] F^-, $H_2PO_4^-$ and AcO^-, of similar basicity, can also be differentiated by azocalix[4]arene-based hosts with rationally designed binding sites. As a result, azocalix[4]arene **2** can discriminate F^- from AcO^- while **4** can discriminate F^-, AcO^- and $H_2PO_4^-$ from each other.

Dyes **1–5**, containing azophenol chromophores, recognize anions by means of hydrogen bonding interactions, so protic solvents (such as water or methanol) will seriously influence the recognition process and selection. Actually, the hydrogen bond-based chromogenic anion sensor systems reported so far almost perform in aprotic solvents such as MeCN, DMSO, and even $CHCl_3$ and CH_2Cl_2. Of course, for practical application purposes, useful chromogenic anion sensor systems should be those that can recognize and sense anions in aqueous solution; however, few examples of such systems[3b] have been reported until now. Thus, we wish in our future work to develop chromogenic anion sensor systems based on water soluble calixarenes, although this may be a challenge.

Conclusion

In conclusion, we have demonstrated that the easily available azocalix[4]arenes can be used as potential chromogenic anion probes. Consequently, azocalix[4]arenes **1–5** allowed not only for highly selective and sensitive colorimetric detection but also easy colorimetric differentiation of F^-, $H_2PO_4^-$ and AcO^-, of similar basicity, depending upon the azocalix[4]arene structure, guest basicity and conformational complementarity between host and guest. Further studies on the development of azocalix[4]arene-based bifunctional chromogenic probes for anions and cations[15] are now in process.

Experimental

Syntheses

Compounds **1**,[7b] **2**,[7b] **3**,[10a] **4**[10b] and **6**[16] were prepared according to published procedures.

Compound 5. A mixture of **6** (200 mg, 0.353 mmol) and *para*-nitrobenzenediazonium tetrafluoroborate (176 mg, 0.741 mmol) in THF (12 mL) was stirred for 30 min at 0 °C and then pyridine (4 mL) added dropwise. After being stirred for another 48 h at 0 °C, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ (15 mL), the organic layer washed with 10% aqueous HCl solution (3 × 15 mL) and then dried over anhydrous $MgSO_4$. Removal of the organic solvent under reduced pressure afforded the crude product, which was further recrystallized from $CHCl_3$/MeOH to give **5** (198 mg, yield: 65%) as a reddish-colored solid (Found: C, 67.21; H, 5.21; N, 9.74. $C_{40}H_{37}N_5O_8$ requires C, 67.12; H, 5.21; N, 9.78%); mp: 256–258 °C; δ_H (300 MHz, $CDCl_3$) 8.91 (2 H, brs), 8.38 (2 H, d, J = 8.9 Hz), 8.19 (2 H, s), 7.99 (2 H, d, J = 8.9 Hz), 7.85–7.83 (2 H, m), 7.12–7.08 (4 H, m), 7.03 (2 H, t, J = 7.6 Hz), 6.90 (1 H, t, J = 7.6 Hz), 4.65 (4 H, s), 4.18 (2 H, d, J = 13.2 Hz), 4.16 (2 H, d, J = 13.4 Hz), 3.62 (2 H, d, J = 13.2 Hz), 3.59 (2 H, d, J = 13.4 Hz) and 3.04 (6 H, d, J = 5.7 Hz); δ_C (300 MHz, $CDCl_3$) 168.2, 156.2, 151.8, 150.5, 148.3, 146.5, 132.9, 130.2, 129.8, 129.2, 128.5, 127.5, 127.0, 124.8, 123.1, 120.9, 75.0, 31.7, 31.5 and 25.9; m/z (MALDI-TOF) 716.8 $[M + H]^+$.

General method for UV-vis titration and coloration experiments

The solvents for the UV-vis measurements and coloration experiments were all of spectroscopic grade. Stock solutions of the chromogenic hosts **1–5** were prepared by dissolution in acetonitrile (1.0×10^{-5} M). Anions used in this study were all in the form of their tetrabutylammonium salts, and their stock solutions were also prepared by dissolution in acetonitrile (1.0×10^{-2} M). UV-vis spectra were recorded on a Shimazu 2401 spectrophotometer. UV-vis titrations were performed with a series of solutions of every host compound, and anions were added by microsyringe. In coloration experiments, the appropriate amounts of the anions were added according to that used in the corresponding UV-vis titration.

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