PERKIN 2

Selective complexation of soft Pb^{2+} and Hg^{2+} by a novel allyl functionalized thioamide calix[4]arene in 1,3-alternate conformation: a UV-visible and ^1H NMR spectroscopic investigation

Giuseppe Arena,* Annalinda Contino, Elisa Longo, Domenico Sciotto and Giuseppe Spoto

Dipartimento di Scienze Chimiche, Università di Catania, Viale Andrea Doria 6,
95125 Catania, Italy

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A new calix[4]arene, functionalized with two thioamide moieties and with two allylic groups at the lower rim and the upper rim, respectively, and fixed in the 1,3-alternate conformation has been synthesized and its complexing properties towards Pb^{2+}, Hg^{2+}, Cd^{2+} and Na^+ have been determined by UV–Vis titrations and by ^1H NMR spectroscopy in organic media.

The stoichiometries of lead and mercury complexes (1 : 1) were at first determined by the mole ratio method and by Job plots. The speciation of the two systems was obtained making use of two different multiwavelength and multivariate treatments of spectral data. The computer treatment of the data confirmed the 1 : 1 stoichiometry for the two complexes and gave reliable values for the corresponding stability constants ($\log K_{Pb} = 7.7$; $\log K_{Hg} = 7.8$). The ligand shows a high selectivity for lead and mercury over cadmium and sodium ions which are not complexed at all.

Structural and conformational properties of the two complex species were obtained by ^1H NMR spectroscopy. ^1H NMR data show that, although having the same stoichiometry, the lead and mercury interact with the ligand molecule in a different manner.

Introduction

In recent years, the importance of monitoring the levels of environmental pollutants in waters and soils and the need for the recycling of resources and for waste water treatment[1] has generated increasing interest in the designing of new extractants for toxic heavy metals. The ultimate goal is to obtain more and more selective and efficient receptors to be used in the extraction of toxic metals from real matrices containing alkali, alkaline earth and other metal ions that usually accompany lead and mercury.

Among macrocyclic ligands capable of complexing/sequestering heavy metal ions, calixarenes[2] occupy an important position thanks to the fact that they can be preorganized and tuned to complex metal ions.[3]

Several calixarenes with different donor atoms have been examined as heavy metal ionophores, in the presence of interfering ions.[4] Arnaud-Neu *et al.*[5] described how to switch from a preference for alkali and alkaline earth cations to a preference for heavy and transition metal cations by replacing *hard* oxygen-based binding groups with *softer* sulfur-based binders. Based on the affinity of Pb^{2+}, Hg^{2+} and Cd^{2+} for *soft* sulfur atoms, a few upper rim[6] or lower rim[7] sulfur derivatized calixarenes have been synthesized and their complexing properties have been tested by liquid–liquid extraction. Among these, lower rim thioamide derivatized calix[4]arenes, fixed in the *cone* conformation, have demonstrated good efficiency in the selective recognition of cadmium and lead cations.[8] More recently, Lugtenberg *et al.*[9] have synthesized two novel Pb^{2+} and Cd^{2+} selective calix[4]arenes, functionalized with two or four thioamide moieties and fixed in their 1,3-alternate conformation. These ligands are more selective towards Pb^{2+} and Cd^{2+} than the analogous cone conformational isomers. In fact, 1,3 alternate isomers can complex the target ions both by a direct interaction with the thioamide groups and by cation–π interactions between the metal ion and the aromatic rings of the calixarene cavity. The selectivity of these ligands towards the target ions has been evaluated in chemically modified field effect transistors (CHEMFETs), thus obtaining potentiometric selectivity coefficients for each ion, based on the results of competition experiments in the presence of different interfering salts.

As part of a more general project devoted to the realization of systems intended for the selective removal and/or determination of unwanted species,[10] we are designing new stationary phases for the chromatographic separation of heavy metals. To this end, we have modified the backbone of the 1,3-alternate dithioamide functionalized calix[4]arene reported by Lugtenberg *et al.*,[9] by introducing two allyl groups[10a] on two opposite aromatic rings (compound **4**, see Scheme 1) in order to obtain a receptor that can later on be covalently linked to silica gel[11] and thus be used for chromatographic separations.

In this paper we report on the synthesis and characterization of compound **4**, as well as on its complexing properties towards Pb^{2+}, Hg^{2+}, Cd^{2+} and Na^+. In fact, despite the growing presence in the literature of new calixarene based receptors designed to efficiently complex heavy metal cations, only a few papers deal with the determination of their stability constants. Recently Navakun *et al.*[12] reported the synthesis and the complexation properties of some closed ring *p*-*tert*-butylcalix[4]arene derivatives containing both N- and S-donor atoms; the stability constants of these compounds with Hg^{2+}, Cd^{2+} and Zn^{2+} have been evaluated by potentiometric titrations in organic media.

In the present study, the complexation efficiency and selectivity of receptor **4** towards the above mentioned cations have been investigated by UV–Vis titration in CH_3CN to determine both the stoichiometries and the stability constant values of the complex species formed by **4** by using two different software packages that make use of a multiwavelength and multivariate

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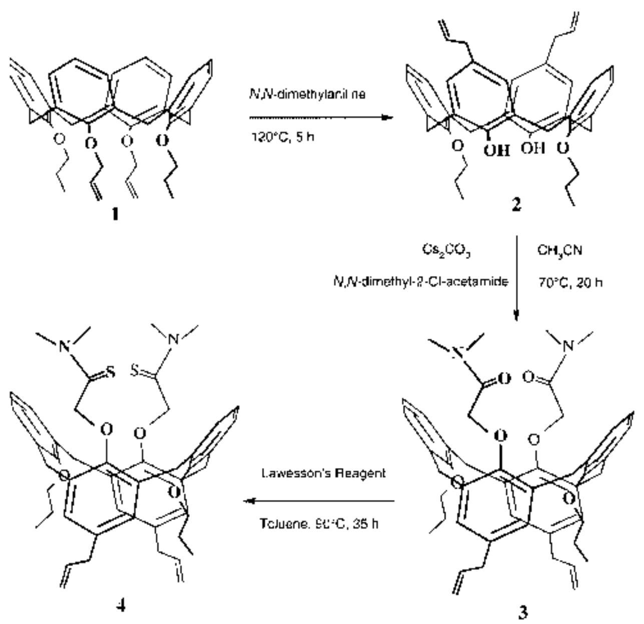

Scheme 1

treatment of spectral data. The determination of the stability constants of **4** with lead, mercury, cadmium and sodium ions was intended (i) to check the consequence, if any, of the introduction of the allylic moieties into the calixarene backbone; (ii) to assess its actual selectivity toward the target ions. The use of acetonitrile was dictated by the need to have a polar solvent that would reproduce to some extent the characteristics of the aqueous phase to be eventually treated chromatographically.

Compound **4** and its complexes were also investigated by ^{1}H NMR spectroscopy to have a more detailed picture of the conformational properties of the receptor as well as of its complex species in solution.

Results and discussion

Characterization

The ^{1}H NMR spectrum of **4** in CDCl$_3$ (Fig. 1) clearly shows that this new compound has a 1,3 alternate blocked conformation.[13] The pattern of the bridging methylene protons looks like a typical AB system, resulting from the slight anisochrony of the axial and equatorial protons ($\Delta\delta_{(\mathrm{Hax}\ -\ \mathrm{Heq})}$ = 0.116 ppm). According to the molecular architecture of the ligand, three different resonances are detected in the aromatic region of the spectrum: these concern the *meta*-protons of *para*-substituted aromatic rings (δ = 6.87), the *para* (δ = 6.70) and the *meta*-protons (δ = 6.91) of unsubstituted aromatic rings. In addition, four distinct signals are detected for the four different methylene groups of the molecule, *i.e.* –O–CH_2–C=S, O–CH_2–CH$_2$–CH$_3$, O–CH$_2$–CH_2–CH$_3$ e O–CH_2–CH=CH$_2$ (see Experimental section). It is noteworthy that the two *N*-methyl groups show a sizable chemical shift difference. This results from the fairly different chemical environment experienced by the methyl groups facing and those pointing out of the cavity.

UV–Vis titrations

Calix[4]arene **4** shows two absorption bands in the UV–Vis region centered at 200 and 278 nm, respectively, and due to the absorption of the phenyl and *N*,*N*-dimethyldithiocarbonyl groups.

Upon addition of sodium or cadmium, the ligand spectrum remains practically unchanged, thus showing that compound **4** complexes neither Cd^{2+} nor Na$^+$. In fact, the addition of the metal ion solution up to a metal : ligand ratio of 2000 : 1 and 8000 : 1 for cadmium and sodium, respectively, does not cause either a significant change in the shape of the absorption peaks or a shift in their maximum positions.

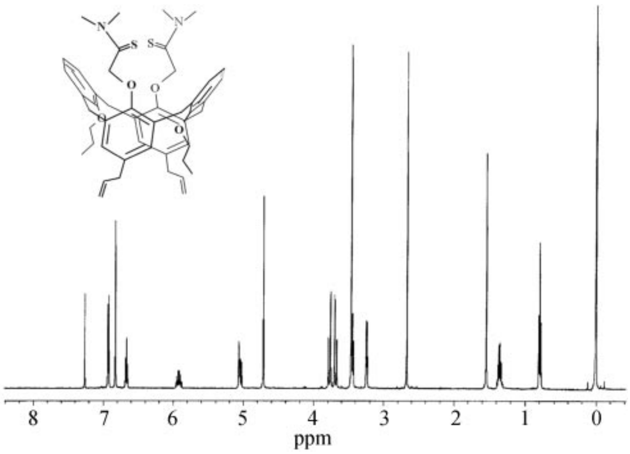

Fig. 1 ^{1}H NMR spectrum of ligand **4** in CDCl$_3$ (500 MHz). The resonances at 1.55 and 7.25 refer to water and chloroform respectively.

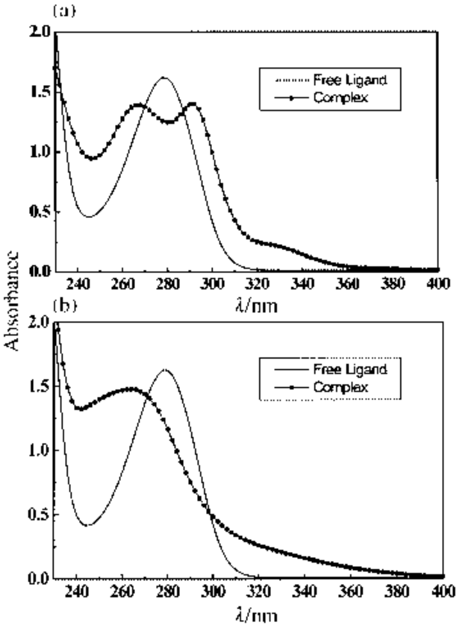

Fig. 2 a) UV–Vis absorption spectra of **4** and [Pb(**4**)]$^{2+}$ in CH$_3$CN. b) UV–Vis absorption spectra of **4** and [Hg(**4**)]$^{2+}$ in CH$_3$CN.

On the contrary, upon addition of lead or mercury solutions, the UV–Vis ligand spectrum undergoes clear changes that indicate the formation of at least one metal complex species. In particular, the addition of Pb^{2+} to compound **4** leads to a *splitting* of the ligand absorption band centered at 278 nm into two new bands centered at 266 and 290 nm, respectively (Fig. 2a). In addition a third band, which is likely due to charge transfer, appears at higher wavelength (330 nm). The addition of Hg^{2+} to **4** causes a hypsochromic shift (14 nm) of the band of the free ligand centered at 278 nm; moreover, this absorption peak becomes flatter and broader than that of the free ligand (Fig. 2b).

The stoichiometries of Pb–**4** and Hg–**4** main species were initially determined by both the mole ratio[14] and the Job plot[15] method. Both methods indicated a 1 : 1 stoichiometry (Fig. 3) for both Pb–**4** or Hg–**4** complexes.

However, since the above methods are not sensitive enough to reveal the presence of minor species that may accompany the formation of 1 : 1 complexes, the speciation of the systems was also carried out resorting to multiwavelength and multivariate treatments of the spectral data, by using two different computer programs, *i.e.* SPECFIT[16] and HYPERQUAD.[17] In addition to the 1 : 1 species, the latter program gave evidence for small

Table 1 log K values[a] for the complex formation of Na^+, Cd^{2+}, Pb^{2+} and Hg^{2+} with host **4** at 25 °C in CH_3CN

Cation	Ionic radius/Å	log K SPECFIT	log K HYPERQUAD	No. of independent runs	No. of points
Na^+	0.97	No complexation	No complexation	3	90
Cd^{2+}	0.97	No complexation	No complexation	3	90
Pb^{2+}	1.20	7.8(2)	7.7(2)	6	275
Hg^{2+}	1.10	7.5(4)	7.8(2)	9	444

[a] σ in parentheses.

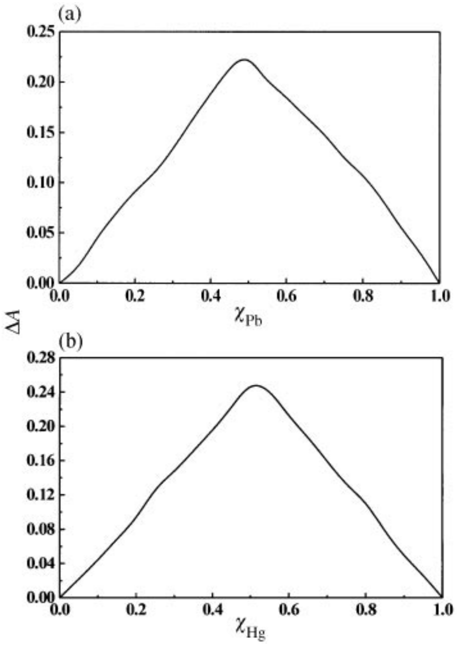

Fig. 3 a) Job plot for mixtures of **4** and $Pb(ClO_4)_2$ in CH_3CN. The ΔA at 292 nm is plotted against the molar fraction χ_{Pb}. b) Job plot for mixtures of **4** and $Hg(ClO_4)_2$ in CH_3CN. The ΔA at 246 nm is plotted against the molar fraction χ_{Hg}.

amounts (up to 5%) of the 1 : 2 (metal : ligand) species for both Pb^{2+}–**4** and Hg^{2+}–**4** when high ligand : metal ratios were employed. This explains the small, but still detectable and reproducible, curvature detected in the initial portion of each titration curve (ligand : metal > 10).

log K values show that the complexation of Pb^{2+} and Hg^{2+} by **4** is highly favoured, thus indicating the good efficiency of the ligand toward the target ions. Moreover the calix[4]dithioamide shows a marked selectivity towards lead and mercury over sodium and cadmium that are not complexed at all. These differences in stability data cannot be ascribed to size effects only (see Table 1). In fact, even if Pb^{2+} and Hg^{2+} are larger than Cd^{2+} and Na^+, the ionic radius difference between mercury and cadmium (1.10 *vs.* 0.97 Å) is not so high as to justify such a dramatic selectivity difference. The size effect is probably enhanced by the different degree of softness of the two metal ions; this explains why the somewhat *softer* Hg^{2+} is efficiently complexed by receptor **4**, whereas the *harder* Cd^{2+} is not complexed at all.

^{1}H NMR studies

In order to better understand the structural and conformational properties of the complex species, we performed a ^{1}H NMR study of both Hg^{2+} and Pb^{2+} complexes at different metal to ligand ratios (Fig. 4).

The ^{1}H NMR spectrum carried out at $[Pb^{2+}]/[ligand] = 0.8$ (Fig. 4b) shows distinct sets of signals for the free and the complexed species, thus indicating that the exchange between the complexed and the uncomplexed ligand is slow on the NMR time scale. The signal intensities of the free and complexed ligand are in the ratio 1 : 4. Since the log K value indicates that the reaction may be considered quantitative, a 1 : 4 ratio of the intensities of the NMR signals implies that 20% and 80% of the ligand is present as the free and complexed forms, respectively. Obviously the ^{1}H NMR spectrum obtained at $[Pb^{2+}]/[ligand] = 3.0$ (Fig. 4c) shows the signals of the complexed species only.

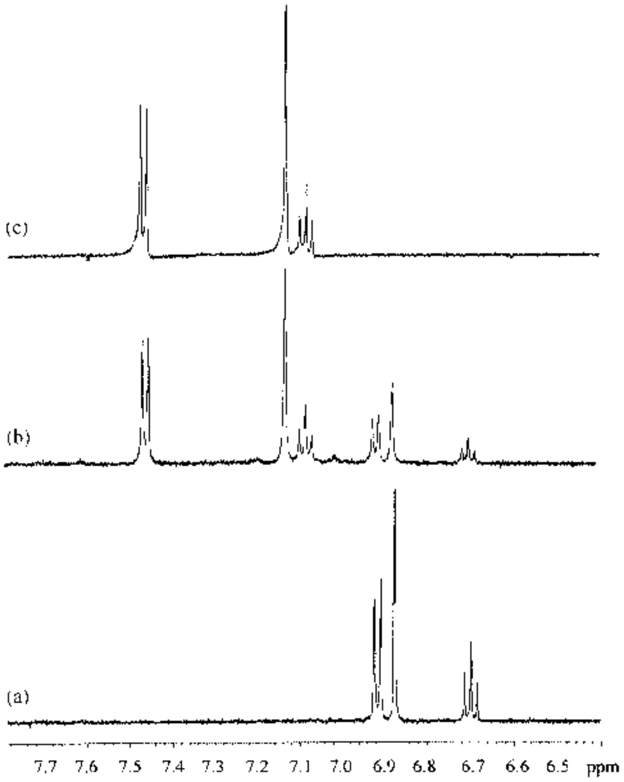

Fig. 4 ^{1}H NMR spectra in CD_3CN of (a) compound **4**; (b) compound **4** and $Pb(ClO_4)_2$, $[Pb^{2+}]/[\mathbf{4}] = 0.8$; (c) compound **4** and $Pb(ClO_4)_2$, $[Pb^{2+}]/[\mathbf{4}] = 3.0$.

These data show that upon complexation with Pb^{2+} nearly all proton resonances of **4** undergo a clear paramagnetic effect, which is significantly different for the various protons of the molecule. The largest downfield shifts are detected for the protons of the $-CH_2C{=}S$ residue and for the protons of one of the two *N*-methyl groups. The upper rim aromatic protons are more deshielded than the lower rim ones, thus indicating that the metal ion is complexed in the region of the dimethylaminothiocarbonyl groups. It is noteworthy that upon Pb^{2+} complexation, the chemical shift difference between the axial and equatorial $ArCH_2Ar$ protons becomes smaller ($\Delta\delta_{(Hax\ -\ Heq)} = 0.027$ ppm), which indicates that the conformation of the upper rim cavity of the complexed species is more flattened than that of the free ligand. Very likely the host cavity widens in order to accommodate the bulky metal ion.

In contrast, ^{1}H NMR data for the Hg^{2+}–**4** system show an evident broadening of ligand resonances, which indicates a moderate rate of exchange, in the NMR time scale, between free and complexed species. The kinetics of this system turn out to be moderately faster than those of Pb^{2+}–**4**, even though the complex formation process is not fast enough (on the NMR time scale) to produce signals that are the "average" between the free and the complexed species. It is noteworthy that upon Hg^{2+} complexation both the proton signals of the upper part as well as those of the lower part of the molecule undergo a significant downfield shift. Unlike what observed for Pb^{2+}, all the signals of the aromatic protons shift downfield. This clearly indicates that in [Hg–**4**]$^{2+}$ Hg^{2+} exchanges between the two coordination regions, although still formally maintaining a 1 : 1 stoichiometry.

Conclusions

We have shown that binding constants of systems (*viz.* Hg–**4** and Pb–**4**), having values at the edge of determinability, may be determined with a relatively high degree of accuracy, provided appropriate concentrations of reactants and proper experimental conditions (very sensitive dispenser, appropriate selection of wavelengths) are chosen. This work also shows that the newly synthesised ligand is able to discriminate mercury and lead from cadmium and sodium. This is of particular interest in view of the use of this ligand as a chromatographic selector. In fact the ligand has recently been covalently linked to silica gel and is being tested for the separation of the title cations. It may be of interest to underline that Pb^{2+} and Hg^{2+}, though forming species with the calixarene having the same stoichiometry and stability, do interact with different regions of the molecule, as shown by ^{1}H NMR results. This leaves us some confidence that the chromatographic experiments, which are currently under way, will lead to the separation of these cations.

Experimental

Materials

All reagents and solvents were used as purchased unless otherwise stated. Toluene was distilled over sodium and stored with molecular sieves.

11,23-Diprop-2-enyl-26,28-dipropoxycalix[4]arene (2)

11,23-Diprop-2-enyl-26,28-dipropoxycalix[4]arene (**2**) was prepared as described earlier.[11a] The full procedure followed for its synthesis is described in details below. 25,27-Diprop-2-enyl-26,28-dipropoxycalix[4]arene (**1**) (0.25 g, 4.24 mmol) was refluxed (T = 200 °C) in *N*,*N* dimethylaniline (15 ml) under nitrogen atmosphere for 5 hours. The solution was then reacted (T = 4 °C) with 6 M HCl (2 × 100 ml) and washed with EtOAc (3 × 100 ml). The organic phase was removed under reduced pressure and the crude product was purified by recrystallization from dichloromethane–hexane to give compound **2** as a white powder (0.19 g, 65%). Mp 198–200 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.23 (s, 2H, Ar-OH), 6.97 (d, 4H, Ar 4,6,16,18-H, J = 7.5 Hz), 6.92 (s, 4H, Ar 10,12,22,24-H), 6.82 (t, 2H, Ar 5,17-H), 5.90 (m, 2H, $CH_2CH{=}CH_2$), 5.06 (s, 2H, CH_2-$CH{=}CH_{2a}$), 5.00 (d, 2H, CH_2-$CH{=}CH_{2b}$), 4.32 (d, 4H, $ArCH_2Ar$ ax, J = 12.9 Hz), 3.98 (t, 4H, $OCH_2CH_2CH_3$, J = 6.3 Hz), 3.35 (d, 4H, $ArCH_2Ar$ eq, J = 12.9 Hz), 3.26 (d, 4H, Ar-CH_2-$CH{=}CH_2$), 2.08 (m, 4H, $OCH_2CH_2CH_3$), 1.32 (t, 6H, $OCH_2CH_2CH_3$, J = 7.4 Hz). MS-FAB$^+$ *m*/*z* 589 (M + 1)$^+$. Anal. Calcd. for $C_{40}H_{44}O_4$: 81.60% C, 7.53% H. Found: 80.01% C, 7.44% H.

11,23-Diprop-2-enyl-25,27-bis(dimethylaminocarbonylmethoxy)-26,28-dipropoxycalix[4]arene, 1,3-alternate (3)

11,23-Diprop-2-enyl-26,28-dipropoxycalix[4]arene 1,3 alternate (**2**) (0.30 g, 0.51 mmol), Cs_2CO_3 (0.66 g, 2.04 mmol) and *N*,*N′*-dimethyl-2-chloroacetamide (0.25 g, 2.04mmol) were refluxed in anhydrous CH_3CN (30 ml) for 20 hours. The solvent was removed under reduced pressure and the remaining product was dissolved in CH_2Cl_2 (100 ml) and washed with 0.5 M HCl (60 ml) and water (3 × 100 ml). Finally, the solvent was removed under reduced pressure and the crude product was purified by recrystallization from CH_3CN to give compound **3** as a white powder (0.23 g, 60%). Mp 198–200 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.99 (d, 4H, Ar 4,6,16,18-H, J = 7.0 Hz), 6.84 (s, 4H, Ar 10,12,22,24-H), 6.69 (t, 2H, Ar 5,17-H, J = 7.0 Hz), 5.92 (m, 2H, $CH_2CH{=}CH_2$), 5.07 (s, 2H, CH_2-$CH{=}CH_{2a}$), 5.04 (d, 2H, CH_2-$CH{=}CH_{2b}$), 4.16 (s, 4H, OCH_2CO), 3.77 (*A*B, 4H$_{ax}$, $ArCH_2Ar$, J = 15.5 Hz), 3.70 (A*B*, 4H$_{eq}$, $ArCH_2Ar$, J eq 15.5 Hz), 3.47 (t, 4H, $OCH_2CH_2CH_3$, J = 7.5), 3.24 (d, 4H, Ar-CH_2-$CH{=}CH_2$, J = 6.5), 2.95 (s, 6H, N-CH_{3a}), 2.44 (s, 6H, N-CH_{3b}), 1.39 (m, 4H, $OCH_2CH_2CH_3$, J = 7.5), 0.82 (t, 6H, OCH_2-CH_2CH_3, J = 7.5). MS-FAB *m*/*z* 759 (M+1)$^+$. Anal. Calcd. for $C_{48}H_{58}O_6N_2$: 75.96% C, 7.70% H, 3.69% N. Found: 74.57% C, 7.52% H, 3.69% N.

11,23-Diprop-2-enyl-25,27-bis(dimethylaminothiocarbonylmethoxy)-26,28-dipropoxycalix[4]arene, 1,3-alternate (4)

Treatment of **3** (0.30 g, 0.40 mmol) with Lawesson's reagent[18] (0.18 g, 0.44 mmol) in anhydrous toluene at 90 °C for 35 hours, followed by purification by column chromatography (SiO_2, CH_2Cl_2–EtOAc 95 : 5) gave **4** as a white powder (0.25 g, 80%). Mp 235–237 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.92 (d, 4H, Ar 4,6,16,18-H, J = 7.5 Hz), 6.83 (s, 4H, Ar 10,12,22,24-H), 6.67 (t, 2H, Ar 5,17-H, J = 7.5 Hz), 5.92 (m, 2H, $CH_2CH{=}CH_2$), 5.06 (s, 2H, CH_2-$CH{=}CH_{2a}$) 5.03 (d, 2H, CH_2-$CH{=}CH_{2b}$), 4.71 (s, 4H, OCH_2CO), 3.77 (*A*B, 4H$_{ax}$, $ArCH_2Ar$, J = 15.5 Hz), 3.69 (A*B*, 4H$_{eq}$, $ArCH_2Ar$, J = 15.5 Hz), 3.46 (s, 6H, N-CH_{3a}), 3.44 (t, 4H, $OCH_2CH_2CH_3$, J = 7.5 Hz), 3.24 (d, 4H, Ar-CH_2-$CH{=}CH_2$, J = 6.5), 2.67 (s, 6H, N-CH_{3b}), 1.35 (m, 4H, $OCH_2CH_2CH_3$, J = 7.5), 0.80 (t, 6H, $OCH_2CH_2CH_3$, J = 7.5). Anal. Calcd for $C_{48}H_{58}O_4N_2S_2$: 72.87% C, 7.39% H, 3.54% N, 8.11% S. Found: 68.01% C, 7.12% H, 3.56% N, 8.23% S. This compound was also characterized by FAB spectrometry (*m*/*z* 791 (M+1)$^+$), UV–Vis measurements (λ_{max} (CH_3CN)/nm 278; ε/dm^3 mol^{-1} cm^{-1} 23600) and thermogravimetric analysis. This latter technique indicates that the compound is of the desired degree of purity.

Spectroscopic measurements

^{1}H NMR spectra were carried out in CD_3CN at 25 °C using a VARIAN INOVA 500 MHz spectrometer. Chemical shifts (δ, ppm) were internally referenced to $Si(CH_3)_4$ for all the systems reported here, with the exception of Hg^{2+}, for which the chemical shifts were referenced to CD_2HCN (1.938 δ), to avoid interactions of this cation with TMS. The host concentration was usually in the range 5.0×10^{-4} to 6.6×10^{-4} mol dm^{-3}. The spectra were recorded over a large metal/ligand ratio (0.8 < [M]/[**4**] < 3.0), which allowed the determination of the complexation induced shifts (CIS).

Stock solutions of the metal perchlorates were obtained by dissolving weighed amounts of the corresponding salts in CH_3CN. The analytical concentrations of lead and cadmium solutions were determined by complexometric titrations with EDTA,[19] whereas mercury solutions were obtained by dissolving known amounts of HgO with the stoichiometric amount of $HClO_4$ in CH_3CN.

The UV–Vis titrations were carried out at 25 °C in acetonitrile using a Hewlett–Packard 8542 diode array spectrophotometer. Titrations were performed in a spectrophotometric cell equipped with a magnetic stirrer; typically, to record a spectrum, the reagent was delivered by a precision burette (Hamilton, 1 ml) and the solution allowed to equilibrate for two minutes. Usually a 1.4×10^{-4}–0.16 mol dm^{-3} solution of the metal cation was added to a 2.0×10^{-5} to 6.5×10^{-5} mol dm^{-3} solution of the host, recording 60–70 points for each independent titration. The spectrophotometric data were collected over the range 230–400 nm for all the cations investigated. These data were analysed by the computer program SPECFIT,[16] which makes use of a multiwavelength and multivariate treatment of spectral data, combining a chemometric analysis (*Factor Analysis* and *Evolving Factor Analysis*)[20] with a non linear least squares minimization procedure.[21] However, the data compression procedure used by SPECFIT might introduce some distortion into the data. For this reason, the data were double checked by using the program HYPERQUAD,[17] which follows a different strategy to deduce the number of light-absorbing species adopting a procedure that eliminates the accumulation of errors inherent in the matrix compression procedure. Furthermore, HYPERQUAD is able to refine simultaneously the data from different experimental measurements.

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