



Allosteric effect of the first lead ion on stepwise extraction of the second lead ion with *p-t*-butylcalix[5]arene pentacarboxylic acid derivative

Birendra Babu Adhikari, Keisuke Ohto*, Manju Gurung, Hidetaka Kawakita

Department of Chemistry and Applied Chemistry, Faculty of Science and Engineering, Saga University, 1-Honjo, 840-8502, Saga, Japan

ARTICLE INFO

Article history:

Received 18 January 2010

Revised 18 March 2010

Accepted 19 March 2010

Available online 23 March 2010

Keywords:

Calix[5]arene
Allosteric effect
Conformation

ABSTRACT

A calix[5]arene-based solvent extraction reagent displaying very high selectivity for lead has been prepared, one molecule of which extracts two lead ions in a stepwise manner. The first Pb^{2+} ion is bound to five phenoxy oxygen atoms inside the calixarene cavity due to which the ligand undergoes conformational freezing in stable cone conformer. This causes positive allosteric effect for co-extraction of the second lead ion due to the aggregation of functional groups.

© 2010 Elsevier Ltd. All rights reserved.

In recent years, decontamination of heavy metal-bearing industrial effluents has become the topic of great interest. Among heavy metals, lead is one of the most abundant and certainly the most toxic metal ions causing adverse environmental and health problems including digestive, cardiac, kidney and especially neurological diseases. For global environmental problem, there is a need of renewed interest in processing of industrial effluents containing such toxic metal ions.

Calixarenes have been known to be a useful molecular scaffold in the development of unique supramolecular receptors and these macrocycles have proved their worth as a promising platform for the synthesis of selective ligands for solvent extraction separation of toxic metal ions. Though the ion discriminating supramolecular chemistry of crown ether type of macrocycles towards lead ion has been well addressed,^{1–4} however, only a few reports are documented with regard to calixarene. Previously, we had reported the solvent extraction behaviour of tetracarboxylic acid derivative of *p-t*-octylcalix[4]arene towards lead ion from weakly acidic media in chloroform and its application for the decontamination of real industrial samples.⁵ We found that one molecule of tetracarboxylic acid derivative of calix[4]arene extracts two lead ions in a stepwise manner and proposed the formation of 1:2 host–guest complex. Malkhede et al. reported the solvent extraction separation of lead with hexaacetato derivative of *p-t*-butylcalix[6]arene also from weakly acidic media in xylene.⁶ They reported the probable composition of extracted species as 2:1 host–guest complex. Thus, it became a matter of interest for us to explore what position the

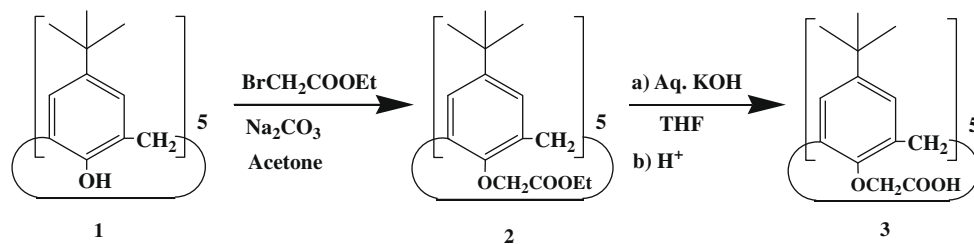
pentamer derivative would occupy in the spectrum of lead recognition in regard to stoichiometry and size effect of calixarene cavity.

While some examples of calixarene-based chromogenic⁷ and fluorogenic⁸ sensors have been reported for the recognition of Pb^{2+} ions with high sensitivity, their selective removal in the presence of other ions is, however, not well addressed by such analytical techniques and there is still a significant need to provide real time applicable reagents for the separation of trace amount of Pb^{2+} in drinking water, in polluted areas and industrial streams. In continuation of our interest in the design and synthesis of highly selective solvent extraction reagent for Pb^{2+} ions in the presence of various competing ions, we report the synthesis of *p-t*-butylcalix[5]arene carboxylic acid derivative (**3**) and its extraction behaviour towards some toxic divalent metal ions. Complexation behaviour of **3** with lead ion is studied in more detail to know the stoichiometry of extracted complex.

Synthesis of host **3** is illustrated in Scheme 1. Compound **1** was synthesized according to the procedure previously reported by Stewart and Gutsche.⁹ Synthesis of **2** has already been reported by Barrett et al. using potassium carbonate as a base.¹⁰ In our case, treatment of **1** with excess of ethyl bromoacetate in the presence of excess sodium carbonate in a manner similar to the Williamson synthesis of ether afforded the pentaester **2** in high yield.¹¹ Alkaline hydrolysis of pentaester followed by acidification of carboxylate salt afforded the corresponding pentacarboxylic acid derivative in high yield.¹² Structures of **2** and **3** were identified by ¹H NMR, ¹³C NMR and elemental analysis. The ¹H NMR and ¹³C NMR spectra revealed that **2** is in distorted cone conformation.¹⁰ Though **3** is conformationally more labile than **2**, the former also exists in cone-like conformation.

* Corresponding author.

E-mail address: ohatok@cc.saga-u.ac.jp (K. Ohto).

Scheme 1. Synthesis of **3**.

The solvent extraction of metal ions was carried out by conventional batch method. Metal ion solutions were prepared separately in 0.1 M HNO₃ and 0.1 M HEPES buffer solution and arbitrarily mixed to adjust the desired pH. Organic solution was prepared by diluting the extractant **3** with analytical grade chloroform to the desired concentration. The metal ion concentration before and after equilibrium was measured by atomic absorption spectrophotometer (AAS). The extraction ability of **3** towards different metal ions is expressed as distribution ratio (*D*) which is the ratio of concentration of metal ion in organic phase to the concentration of metal ion in aqueous phase at equilibrium condition. The experimental results showing the effect of equilibrium pH on logarithm of distribution ratio ($\log D$) of different metal ions by **3** are shown in Figure 1. Based on the reported values of formation constant for metal complexes of Pb²⁺, Cu²⁺, Zn²⁺ and Co²⁺ with carboxylic acid group,¹³ **3** should form stable complex with Cu²⁺ rather than with Pb²⁺ and hence must be selective for Cu²⁺. However, **3** is interestingly more selective for Pb²⁺ referring that it must have been extracted by the mechanism rather different than that of Cu²⁺ extraction. On the other hand, for all metals except Pb²⁺, the value of distribution ratio increases with a slope of about 2 in the plot of $\log D$ versus pH_e. This value is the same as expected for divalent metal ion indicating that the neutral complex (1:1) has been formed by the exchange of two protons from carboxyl groups (see Supplementary SE1 and SE2 for details). However, in the case of lead, a straight line with slope more than two is obtained which is not well-matched with the formation of 1:1 host–guest complex. Since lead is bivalent and if only the ion exchange mechanism takes place, the slope of line in the plot of $\log D$ versus pH_e is ex-

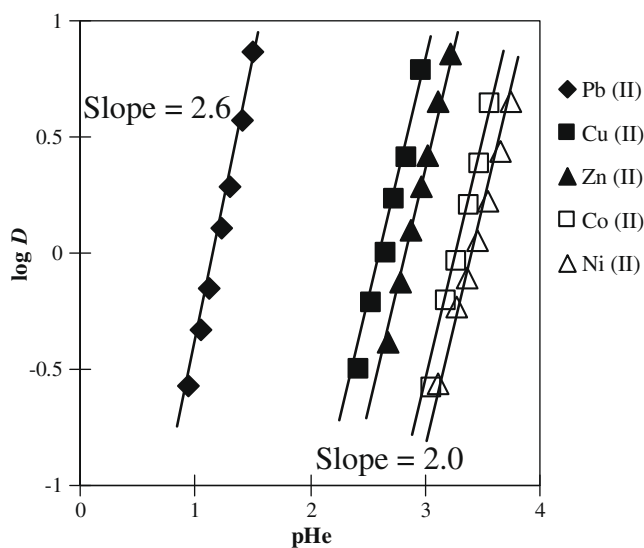


Figure 1. Effect of pH on distribution ratio of different metal ions on **3**. [Metal ion] = 0.1 mM, [**3**] = 2.0 mM, shaking time = 8 h at 303 K, organic/aqueous = 1 (v/v), adjustment of pH = 0.1 M HNO₃, 0.1 M HEPES buffer.

pected to be 2 for 1:1 and 4 for 1:2 (host–guest) complexes. In order to have further insight into the number of protons released into solution by ion exchange phenomenon with lead ion, we carried out the extraction experiment using 10-fold excess (1.0 mM) of respective Pb²⁺ salts in nitric acid as well as hydrochloric acid media keeping the concentration of **3** same (2.0 mM). In both the cases, straight line with slope 3 is obtained in the plot of $\log D$ versus pH_e (see Supplementary Fig. S6 for details).

Due to the exceptional extraction behaviour of **3** towards lead ion, stoichiometry of complexation was determined by using continuous variation method in two-phase solvent extraction keeping the sum of concentration of host and guest species to be 1 mM. It is obvious from the results of typical Job's plot (Fig. 2) that the lead concentration in organic phase becomes maximum at mole fractions of **3** in between 0.3 and 0.4, which is compatible with the formation of 1:2 host–guest complex. Formation of 1:2 complex is also evident from loading test¹⁴ (see Supplementary Fig. S7 for details).

The results of Job's plot and loading test support the formation of 1:2 host–guest complexes with lead ion. However, the number of protons released into solution during ion exchange with lead ion is 3. Thus, it can be inferred that two lead ions are extracted with the release of three protons from the host **3** along with ion-pair formation mechanism. However, the results indicate that lead extraction with **3** is independent of nitrate concentration (see Supplementary Fig. S8). Hence, as in the case of other metal ions, solely ion exchange mechanism is proposed for lead extraction as well.¹⁵

In order to explore how two lead ions are extracted by the single molecule of host, we carried out the ¹H NMR titration experiment. Upon the addition of 0.5 equiv of lead, downfield shifted new peaks appeared for aromatic hydrogen and methylenoxy hydrogen along with original peaks of free host with 1:1 intensity ratio (Fig. 3). This indicated about 1:1 mixture of lead complexed

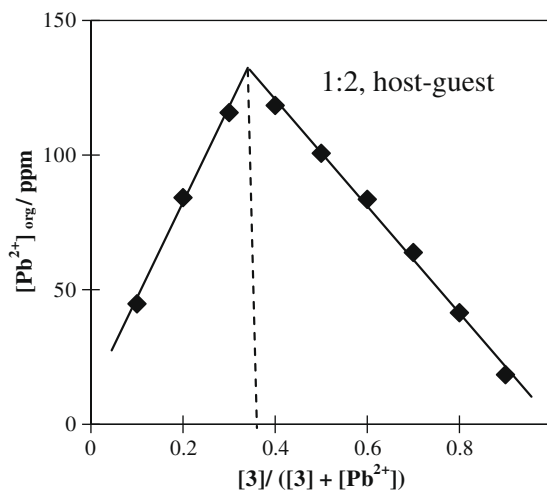


Figure 2. Typical Job's plot for the extraction of lead with **3**.

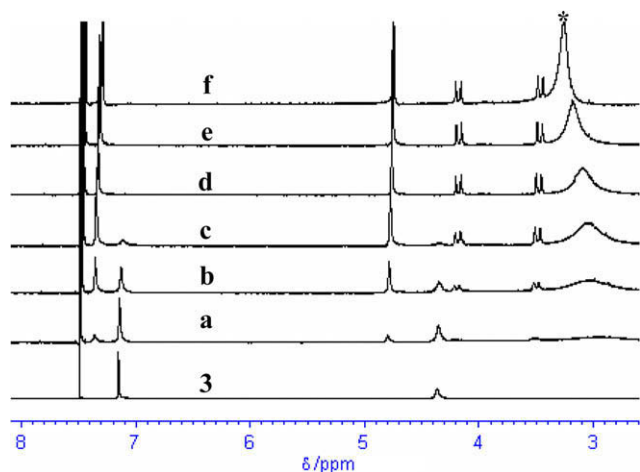


Figure 3. ^1H NMR titration of **3** (2.5 mM) with (a)–(f) = 0.25, 0.5, 0.75, 1.0, 2.0, 5.0 equiv of $\text{Pb}(\text{ClO}_4)_2$ in $\text{CDCl}_3/\text{CD}_3\text{CN} = 1/1$. * = new peak due to $\text{Pb}(\text{ClO}_4)_2 \cdot x\text{H}_2\text{O}$, residual solvent peak due to CDCl_3 at 7.49 ppm.

and free ligand. Interestingly, two new doublets also appeared in δ 3.49 and 4.22 ppm region of NMR spectrum upon the addition 0.5 equiv lead. These new peaks, which appeared as double doublets in typical AB resolving pattern of cone calix[4]arene, are consistent with the peaks of bridged methylene protons of **2** indicating that lead complexed **3** is fixed in cone conformation. Upon the addition 1 equiv of lead, the peaks of free host **3** were vanished and only the new peaks were apparent. Further new peaks were not observed on adding up to 5 equiv of lead. The percentage shift of aryl peak resulted due to the percentage loading of lead ion on **3** was also evaluated as a function of number of equivalents of metal ion added. Both the values increase with increasing equivalents of added lead and coincide well with each other indicating that the loaded lead causes equivalent shift in peak position (Supplementary Fig. S9). If the two lead ions were simultaneously extracted, the percentage shift would be just half of the percentage loading. Metal ion-induced chemical shift changes in the ^1H NMR titration spectra support that the two lead ions are extracted independently and in a stepwise manner. The first lead is bound to five phenoxy oxygens which leads to freezing of molecule in stable cone conformation and causes positive allosteric effect for co-extraction of the second lead ion at aggregated carboxyl functional group site. However, it may be emphasized that extraction of the second lead ion at carboxyl functional group site takes place only in aqueous system through ion exchange by the release of carboxylic proton. This fact is supported by the result of ^1H NMR titration of Pb^{2+} ion with *p*-tert-butylphenoxyacetic acid, the acyclic ligand having structure related to cyclic ligand **3**. In $\text{CDCl}_3/\text{CD}_3\text{CN}$ system, the ^1H NMR titration of Pb^{2+} ions with the acyclic ligand does not show any peak shift (Supplementary Fig. S10). However, in the ^1H NMR spectrum of CDCl_3 fraction taken from two-phase extraction of lead ion at different pH, there is gradual shift in peak position towards upfield region with increasing amount of lead extracted at increasing pH (Supplementary Fig. S11). This confirms that lead uptake by ion exchange with carboxyl group is feasible only in two-phase extraction.

In contrary to the addition of lead, addition of copper to the solution of **3** did not form new peaks (Fig. 4) suggesting that neither the participation of phenoxy oxygen atoms nor the conformational change of **3** occurs during complexation. As the size of copper does not fit the cavity size of **3**, it is taken up by the carboxyl functional groups through ion exchange mechanism where the neutral complex has been formed by the release of two protons from carboxyl groups in aqueous system. However, in non aqueous

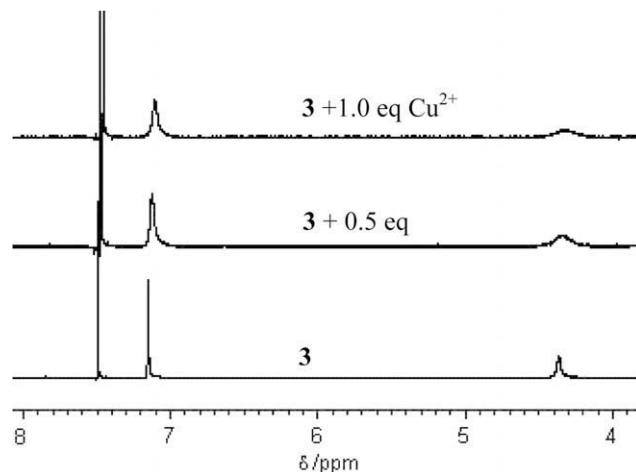


Figure 4. ^1H NMR titration of **3** (2.5 mM) with various equivalents of $\text{Cu}(\text{ClO}_4)_2$ in $\text{CDCl}_3/\text{CD}_3\text{CN} = 1/1$. Residual solvent peak due to CDCl_3 at 7.49 ppm.

system, proton exchange is quite suppressed and copper ion should form very weak complex with **3** through the participation of carbonyl group and ^1H NMR shows only the peak broadening. This may be attributed to the fact that the exchange rate between free ligand **3** and the weak complex is too fast for NMR time scale to observe the structural difference and an average peak is observed at slightly upfield region with peak broadening.

Formation of 1:2 host guest complex with Pb^{2+} and 1:1 complex with Cu^{2+} by **3** is also supported by the FT-IR spectra of $3 \cdot \text{Cu}^{2+}$ and $3 \cdot (\text{Pb}^{2+})_2$ complexes (Supplementary Fig. S12). In the IR spectrum of $3 \cdot \text{Cu}^{2+}$ complex, the $\text{C}=\text{O}$ stretching band at 1741 cm^{-1} for free ligand **3** is decreased in intensity with the appearance of new band at 1603 cm^{-1} which may be attributed to $\text{C}=\text{O} \cdots \text{Cu}^{2+}$ stretching. Similarly, the band due to $\text{C}-\text{O}-\text{H}$ bending vibration at 1479 cm^{-1} of free ligand **3** is also decreased in intensity with the appearance of another band at 1415 cm^{-1} presumably due to $\text{C}-\text{O} \cdots \text{Cu}^{2+}$ bending. On the other hand, the IR spectrum of $3 \cdot (\text{Pb}^{2+})_2$ complex is remarkable in the sense that the band due to free $\text{C}=\text{O}$ stretching at 1741 cm^{-1} is completely lost and a new band is observed at 1618 cm^{-1} which may be attributed to $\text{C}=\text{O} \cdots \text{Pb}^{2+}$ stretching. In addition, the band at 1479 cm^{-1} for $\text{C}-\text{O}-\text{H}$ bending is further decreased in intensity with the appearance of another band at 1417 cm^{-1} which may be attributed to $\text{C}-\text{O} \cdots \text{Pb}^{2+}$ bending vibration. By contrary, the FT-IR spectrum of $3 \cdot \text{Pb}^{2+}$ complex obtained by the complexation of 1 equiv lead with **3** consists of free $\text{C}=\text{O}$ stretching band at 1734 cm^{-1} along with the appearance of new band at 1606 cm^{-1} for $\text{C}=\text{O} \cdots \text{Pb}^{2+}$ stretching (Supplementary Fig. S13). From the results of IR spectra of the complexes, it can be inferred that copper forms only 1:1 complex whereas lead forms both 1:1 and 1:2 complexes with **3**. In the case of 1:1 complex, all the carbonyl groups are not engaged in complexation. However, in the case of 1:2 complex formation, all the carbonyl groups are engaged in complexation. The results further support the stepwise extraction of two lead ions.¹⁶

The complexation behaviour of **3** towards Pb^{2+} and Cu^{2+} ions in non aqueous system ($1 \times 10^{-5} \text{ M}$ in $\text{CH}_3\text{CN}/\text{CHCl}_3 = 10/1$) was also evaluated by UV/vis spectroscopy. The absorption spectra of **3** before and after the addition of organosoluble metal perchlorates are illustrated in Figure 5. The free host **3** exhibited an absorption band with two absorption maxima at 227 nm and 278 nm which is attributed to $\pi-\pi^*$ and $n-\pi^*$ electronic transitions, respectively. The presence of 10 equiv of Pb^{2+} enhanced the absorption intensity both at 227 nm and 278 nm with nominal hypsochromic shift of the later by 4 nm. The presence of 10 equiv of Cu^{2+} also produced

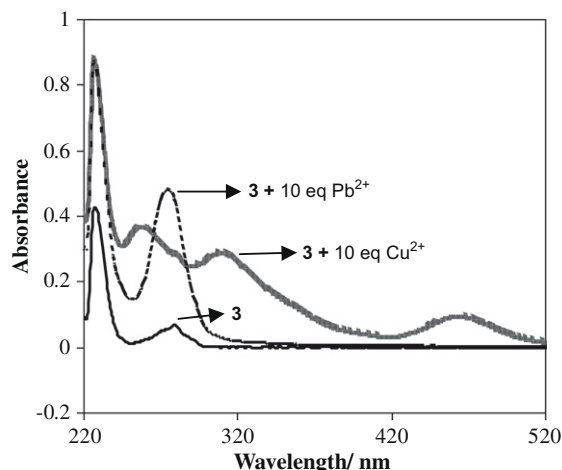


Figure 5. UV/vis spectra of **3** (10 μ M) upon addition of 10 equiv of metal perchlorates in MeCN/CHCl₃ (10:1).

the increase in absorption intensity both at 227 nm and 278 nm but with notable bathochromic shift of the later by 31 nm. Addition of copper perchlorate also led to the formation of other new absorption peaks at λ_{max} 257 nm and 463 nm. From the results, the following plausible complexation behaviour is proposed. As the complexation is carried out in non aqueous media, proton exchange from carboxyl groups is markedly diminished so that only the first lead ion is extracted inside the calixarene cavity. Consequently, strong interaction of lead ion with carbonyl chromophoric group is not feasible and only nominal shift in λ_{max} value is observed. In the case of copper, as it does not fit the calixarene cavity, it forms a weak complex with oxygen-rich carbonyl function. Due to the formation of new species, new absorption peaks at different λ_{max} value than that of free host have appeared. Also, on complexation, the electron density on carbonyl group slightly flows towards copper and there is considerable bathochromic shift in λ_{max} value at 278 nm. These results also support the fact that binding sites of **3** for lead and other ions studied, for example, copper, are different.

In order to have a further insight into the complexation behaviour of **3** with lead and copper ions, the absorption profile as a function of metal ion concentration was obtained by means of UV/vis titration experiment. Figure 6 shows the characteristic absorption spectra of **3** in the spectrophotometric titration with

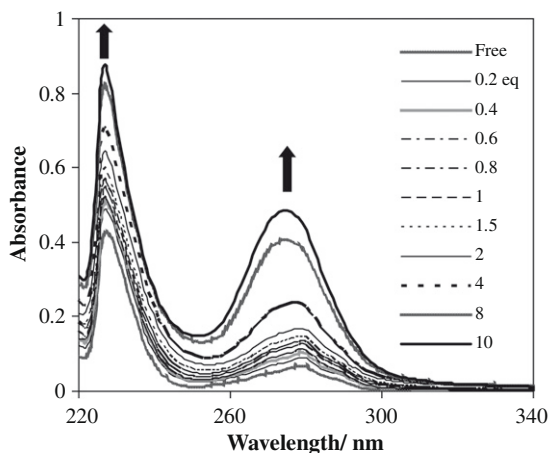


Figure 6. UV/vis spectra of **3** (10 μ M) upon titration by various equivalents of Pb(ClO₄)₂ in MeCN/CHCl₃ (10:1).

Pb(ClO₄)₂. The host **3** exhibited a gradual increase in absorption intensity at λ_{max} 227 nm and 278 nm upon addition of incremental lead ions. However, no clear isobestic points were observed up to the addition of 10 equiv of lead. The results are attributable to the fact that in non aqueous media, only one lead ion is extracted by **3** which is bound to phenoxy oxygen atoms and no new species are formed involving complexation of lead with chromophoric group. Nevertheless, the association constant for **3**·Pb²⁺ in CH₃CN/CHCl₃ (10:1) was determined to be $3.64 \times 10^4 \text{ M}^{-1}$ from modified Benesi–Hildebrand plot¹⁷ (see Supplementary Fig. S14).

The results of spectrophotometric titration of **3** with Cu(ClO₄)₂ are depicted in Figure 7. In this case also, host **3** exhibited a gradual increase in absorption intensity at λ_{max} 227 nm upon addition of incremental copper ions. However, a remarkable change occurs at λ_{max} 278 nm upon the addition of 4 equiv of copper. Formation of new absorption bands takes place at λ_{max} 257 nm, 309 nm and 463 nm which are also intensified upon adding incremental copper ions indicating the formation of new species at equilibrium condition. Association constant for **3**·Cu²⁺ in CH₃CN/CHCl₃ (10:1) was determined to be $9.06 \times 10^3 \text{ M}^{-1}$ from modified Benesi–Hildebrand plot (see Supplementary Fig. S15).

We then investigated the complexation behaviour of **3** with Pb²⁺ in aqueous system ($1 \times 10^{-4} \text{ M}$ in MeCN/H₂O = 3/2, pH 3.5 maintained by HClO₄) by UV/vis titration. In this case, free host **3** exhibited two absorption maxima at 272 nm and 279 nm. Up to the addition of 1.0 equiv of lead perchlorate, the peaks at both the absorption maxima were intensified. However, on further addition of lead perchlorate, only slight change in absorbance took place with the formation of slight isobestic points at 269 nm and 282 nm indicating the formation of new species in equilibrium condition (for detailed information, see Supplementary Fig. S16).

To conclude, we have synthesized a new solvent extraction reagent which can selectively extract Pb²⁺ ions in the presence of various other ions implying that it is useful for analytical purposes. The results of ¹H NMR titration, Job's plot, the slope of lines in the plot of log *D* versus pH_e and the IR spectra of extracted complexes support that one molecule of **3** extracts two lead ions in a stepwise manner and the fascinating selectivity of **3** for lead ion comes from the size fit effect of lead ion in calixarene cavity. The first lead ion is extracted into the cavity of calix[5]arene composed of phenoxy oxygen atoms. After complete loading of the first lead ion, another lead ion is taken up at oxygen-rich coordinating site composed of carboxyl groups. The association constant values for lead and

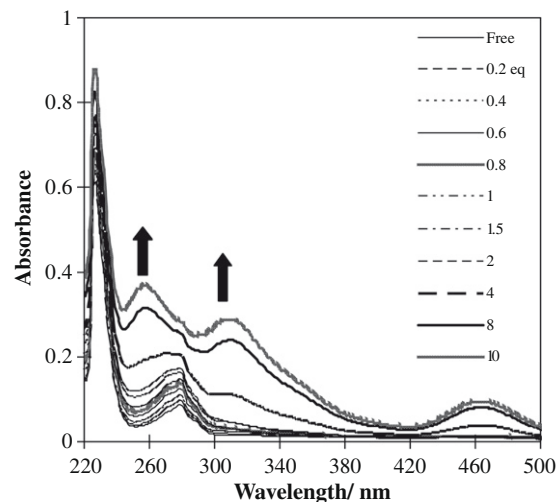


Figure 7. UV/vis spectra of **3** (10 μ M) upon titration by various equivalents of Cu(ClO₄)₂ in MeCN/CHCl₃ (10:1).

copper ions indicate that the former forms stronger complex with **3** than the latter. Taking into account the high lead selectivity of **3**, we aim to fabricate the highly sensing chromogenic and fluorogenic materials from **3** that are useful in aqueous media for the recognition of trace amount of lead ions present in polluted water.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.078.

References and notes

- Hayasita, T.; Fujimoto, T.; Morita, Y.; Bartsch, R. A. *Chem. Lett.* **1994**, 2385–2386.
- Hiratani, K.; Sugihara, H.; Kasuga, K.; Fujiwara, K.; Hayasita, T.; Bartsch, R. A. *J. Chem. Soc., Chem. Commun.* **1994**, 319–320.
- Hiratani, K.; Takahashi, T.; Sugihara, H.; Kasuga, K.; Fujiwara, K.; Hayashita, T.; Bartsch, R. A. *Anal. Chem.* **1997**, 69, 3002–3007.
- Rogers, R. D.; Bond, A. H. *Inorg. Chim. Acta* **1992**, 192, 163–171.
- Ohto, K.; Fujimoto, Y.; Inoue, K. *Anal. Chim. Acta* **1999**, 387, 61–69.
- Malkhede, D. D.; Phadke, M.; Dhadke, P. M.; Khopkar, S. M. *Can. J. Anal. Sci. Spectros.* **1998**, 43, 144–148.
- Chang, K.-C.; Su, I.-H.; Lee, G.-H.; Chung, W.-S. *Tetrahedron Lett.* **2007**, 48, 7274–7278.
- For example: (a) Kim, S. K.; Lee, S. H.; Lee, J. Y.; Lee, J. Y.; Bartsch, R. A.; Kim, J. S. *J. Am. Chem. Soc.* **2004**, 126, 16499–16506; (b) Kim, S. H.; Choi, J. K.; Kim, S. K.; Sim, W.; Kim, J. S. *Tetrahedron Lett.* **2006**, 47, 3737–3741; (c) Liu, J.-M.; Bu, J.-H.; Zheng, Q.-Y.; Chen, C.-F.; Huang, Z.-T. *Tetrahedron Lett.* **2006**, 47, 1905–1908; (d) Meitviev, R.; Leray, I.; Valeur, B. *Chem. Commun.* **2003**, 996–997.
- Stewart, D. R.; Gutsche, C. D. *Org. Prep. Proced. Int.* **1993**, 25, 137.
- Barrett, G.; McKervey, M. A.; Malone, J. F.; Walker, A.; Arnaud-Neu, F.; Guerra, L.; Schwing-Weill, M.-J. *J. Chem. Soc., Perkin Trans. 2* **1993**, 1475–1479.
- Compound 2**: The slurry of 7.0 g (8.6 mmol) **1** and 7.3 g (69 mmol) Na₂CO₃ in 200 cm³ dry acetone was stirred at 50 °C for 1 h which was followed by the addition of 11.5 g (69 mmol) ethyl bromoacetate. The reaction mixture was refluxed for 4 days under nitrogen flow. After completion of the reaction (monitored by TLC), the cooled reaction mixture was filtered and the residue was washed with acetone followed by chloroform. The organic solutions were combined and the solvent was evaporated under pressure. The oily residue thus obtained was dissolved in chloroform and washed with 1 M HCl followed by several washings with water. Removal of solvent from dried (anhyd MgSO₄) organic layer in vacuo afforded the white solid (8.7 g, 82%) ¹H NMR (300 MHz, CDCl₃, TMS) δ 1.05 (s, 45H, ^tBu), 1.32 (t, J = 7.5 Hz, 15H, CH₃ of ethyl), 3.33 (d, J = 13.8 Hz, 5H, Ar–CH₂–Ar, *exo*), 4.24 (q, J = 7.2 Hz, 10H, –CH₂– of ethyl), 4.34 (d, J = 13.8 Hz, 5H, Ar–CH₂–Ar, *endo*), 4.50 (s, 10H, O–CH₂–), 6.97 (s, 10H, Ar–H). As reported by Barrett et al. and indicated by its ¹H NMR, the compound was presumably the Na⁺ complex of pentaester. The solid was dissolved in chloroform and stirred with warm water overnight. The chloroform layer was separated, dried over anhyd MgSO₄ and the solvent was removed under pressure. The desired compound was precipitated by adding hexane and recrystallized from chloroform/hexane (8.5 g, 80%). ¹H NMR (300 MHz, CDCl₃, TMS) δ 1.05 (s, 45H, ^tBu), 1.28 (t, J = 7.6 Hz, 15H, CH₃ of ethyl), 3.32 (d, J = 13.8 Hz, 5H, Ar–CH₂–Ar, *exo*), 4.18 (q, 10H, J = 7.0 Hz, –CH₂– of ethyl), 4.62 (s, 10H, O–CH₂–) 4.78 (d, 5H, J = 15.6 Hz, Ar–CH₂–Ar, *endo*), 6.86 (s, 10H, Ar–H). ¹³C NMR (300 MHz, CDCl₃) δ 14.15 (CH₃ of ethyl), 30.35 (Ar–CH₂–Ar), 31.31 (CH₃)₃, 33.94 (C(q) of *t*-butyl), 60.56 (–CH₂– of ethyl), 70.85 (–O–CH₂–), 125.83 (C(2)Ar), 133.34 (C(3)Ar), 145.66 (C(4)Ar), 152.09 (C(1)Ar), 170.08 (C=O of ester). Anal. Calcd for C₇₅H₁₀₀O₁₅ (1241.4): C, 72.55; H, 8.12. Found: C, 72.35; H, 8.12.
- Compound 3**: The suspension of 4.0 g (3.22 mmol) **2** and 2.25 g (40 mmol) KOH in 100 cm³ THF/H₂O (70:30) was refluxed for 48 h under nitrogen flow. After completion of the reaction (monitored by TLC), THF was evaporated and the remaining suspension was diluted by adding water. The residue collected after filtration was suspended with 50% HCl (v/v) for several hours to affect the protonation of the carboxylate salt. It was then filtered and washed several times with water. Recrystallization from THF/water yielded compound **3** (2.9 g, 82%). ¹H NMR (300 MHz, CDCl₃, TMS) δ 1.06 (s, 45H, ^tBu), 3.41 (br s, 5H, Ar–CH₂–Ar, *exo*), 4.59 (br s, 15H (5H + 10H), Ar–CH₂–Ar, *endo*, +–O–CH₂–), 6.96 (s, 10H, Ar–H). ¹³C NMR (300 MHz, CDCl₃) δ 30.15 (rudimentary peak) (Ar–CH₂–Ar), 31.28 (CH₃)₃, 34.06 (C(q) of *t*-butyl), 69.99 (–O–CH₂–), 126.17 (C(2)Ar), 133.02 (C(3)Ar), 146.45 (C(4)Ar), 151.31 (C(1)Ar), 174.54 (rudimentary peak) (C=O of carboxylic acid). Anal. Calcd for C₆₅H₈₀O₁₅ (1101.14): C, 70.89; H, 7.32. Found: C, 70.60; H, 7.31.
- Stability constants supplement No. 1, Special publication 25, The chemical society London SBN: 851860192, pp 114–116.
- In loading test, the ratio of initial concentration of extractant to the lead concentration in organic phase is plotted against the increasing initial concentration of lead. As indicated by the Supplementary Figure S7, the ratio of initial concentration of extractant to the loaded lead in organic phase gradually drops and becomes constant at 0.5. This means that the ratio of initial concentration of extractant to the extracted lead is 1:2 which also supports the formation of 1:2 host–guest complex.
- In Job's plot and loading test, the concentration of lead ions varies and becomes more than twice the concentration of **3** so that all the host molecules are completely loaded with lead ions and 1:2 complex is quite obvious. However, at low concentrations of lead, complete loading of host molecules is not achieved but two extraction equilibria may exist, one for 1:1 complex and another for 1:2 host–guest complex.
- As lead is extracted in a stepwise manner, it is reasonable that equilibrium is also achieved in a stepwise manner and both (1:1 and 1:2) complexes are formed at low concentration of lead.
- Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, 71, 2703.