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Calixarene-Based Fluorescent Molecular Sensors for Toxic Metals

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There is an increasing interest in the early detection of toxic metals in the environment. In this context, fluorescence is a very attractive detection method because of its intrinsic sensitivity, its response time and the possibility of imaging via fluorescence microscopy. Of the various complexing units available for the detection of toxic metal ions (chelators, open-chain structures, crown-ethers, cryptands), calixarenes offer distinct advantages in term of selectivity and the easy incorporation of a fluorophore into the structure. In this microreview, after recalling the main classes of fluorescent molecular sensors based on cation-induced changes in excited-state processes (photoinduced electron- or charge-transfer, excimer formation, energy transfer), we present various calixarene-based sensors designed for the detection of caesium, mercury, lead and cadmium.

Introduction

It is well known that heavy metals (such as mercury, cadmium and lead) and metals from nuclear waste (like caesium) are highly toxic, causing irreversible damage to the environment and deleterious effects on human health.[1] The wide variety of resulting symptoms, including digestive, cardiac, kidney and especially neurological diseases, suggests that they affect multiple targets in vivo. Thus, the detection of these detrimental ions is a topic that has attracted increasing interest over the past years because of the high demand in analytical chemistry, biology, biotoxicity and environmental chemistry. The permitted level of these toxic metal ions in the environment has been the subject of several regulations and is currently at the parts per billion level.[2] These ions can be detected by different sophisticated methods, such as atomic absorption, atomic emission and inductively coupled plasma spectroscopy or electrochemistry (potentiometric, amperometric or conductimetric methods). The use of fluorescence as a detection method offers a number of advantages in terms of sensitivity, selectivity and low cost. Considerable effort has therefore been devoted to the development of selective fluorescent molecular sensors for the detection of cations, especially toxic metal ions.[3,4]

Such fluorescent molecular sensors are composed of a recognition moiety (ionophore) linked to a signalling moiety (fluorophore). They are thus often called fluoroion-

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ophores. The choice of recognition moiety is of the utmost importance since it is responsible for the selectivity and the binding efficiency. The fluorophore acts as a signalling moiety since it converts the recognition event into an optical signal via the perturbation of various photoinduced processes such as electron transfer, charge transfer, excimer formation or energy transfer upon cation complexation.

Detection of a toxic metal ion is difficult because traces of this ion (typically submicro- to nanomolar concentration) must be detected in the presence of a large excess of interfering cations. For example, in the case of Pb^{2+} detection, the concentration of alkali or alkaline-earth cations is usually in the millimolar range whereas the concentration of lead to be detected is $50 \times 10^{-9}\,\text{mol}\,\text{L}^{-1}$. The stability constant of the complex with the target metal ion must therefore be at least three orders of magnitude higher than that of the complex with the interfering cations. Of the possible complexing units (open-chain structures, crown ethers, cryptands, etc.), calixarene derivatives have received much interest because of their intrinsic selectivity and the possibility of derivatising them with specific ligands and/or fluorophores.[5–9]

This microreview aims to highlight the great possibilities offered by the use of calixarenes in the design of fluorescent molecular sensors for the detection of toxic metal ions. After a brief summary of the various photoinduced processes involved in the cation-induced photophysical changes, different calixarene-based fluoroionophores for the detection of caesium, mercury, lead and cadmium will be presented. A classification of the fluoroionophores for each cation will be made according to the nature of the photoinduced process responsible for the photophysical changes.

1. Principles of Cation-Controlled Photoinduced Processes in Fluorescent Molecular Sensors

Only a brief account of cation-controlled photoinduced processes is given here. The interested reader is referred to other, more detailed reviews[4,10] and to ref.[11] for a further understanding of excited-state processes.

1.1. Photoinduced Electron Transfer (PET)

The principle behind PET-based fluorescent molecular sensors is shown in Schemes 1 and 2.

In the first case, an electron transfer from the lone pair of the ligand upon excitation of the fluorophore causes a strong quenching of the former due to the formation of non-fluorescent charge-transfer species ($D^{\cdot+}$). This effect can be predicted by using the Rehm–Weller equation [Equation (1)].

$$\Delta G^\circ = E^\circ_{D^{\cdot+}/D} - E^\circ_{A/A^{\cdot-}} - \Delta E_{00}(A) \qquad (1)$$

where $E^\circ_{D^{\cdot+}/D}$ is the oxidation potential of the ligand, $E^\circ_{A/A^{\cdot-}}$ is the reduction potential of the fluorophore and E_{00} is the excitation energy.

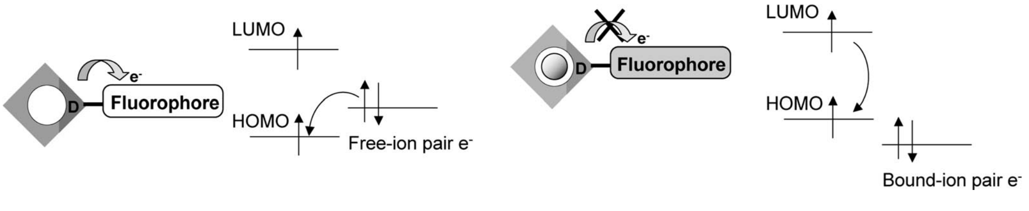
The inverse case can occur upon complexation with a transition metal ion: the sensor is fluorescent in the absence of a cation and cation binding induces an electron transfer from the fluorophore to the cation. This effect can be predicted by measuring the electrochemical potentials of both fluorophore and cation and inserting them in Equation (2).

$$\Delta G^\circ = E^\circ_{D^{\cdot+}/D} - E^\circ_{A/A^{\cdot-}} - \Delta E_{00}(D) \qquad (2)$$

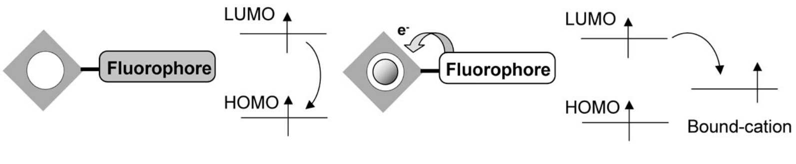
where $E^\circ_{D^{\cdot+}/D}$ is the oxidation potential of the fluorophore, $E^\circ_{A/A^{\cdot-}}$ is the reduction potential of the cation and $E_{00}(D)$ is the excitation energy.

1.2. Photoinduced Charge Transfer (PCT)

The photophysical properties of fluoroionophores can be modulated by the presence of cations in the case of systems based on push-pull fluorophores. The interaction of the cation with the electron-withdrawing group leads to a stabilization of the excited state and therefore to a red shift of the absorption and emission spectra (Scheme 3). Interaction with the donating group leads to the opposite effect because of destabilization of the excited state. It should be noted that only a slight blue shift of the emission spectra is observed in these cases, in contrast to the large blue shift of

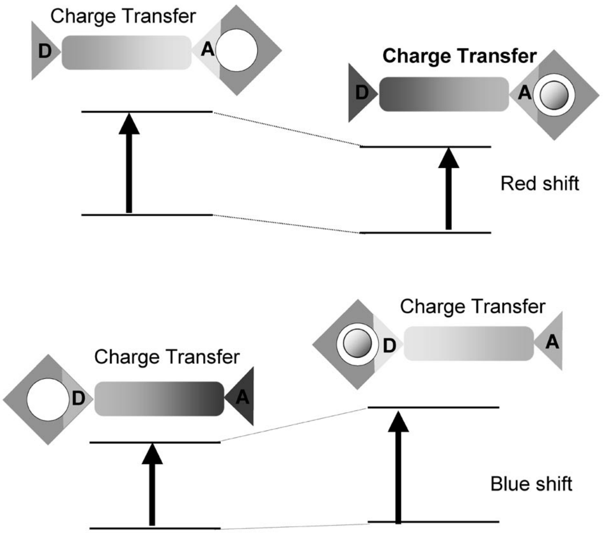


Scheme 1. Principle behind a PET-based fluoroionophore.



Scheme 2. Principle behind an inverse-PET-based fluoroionophore.

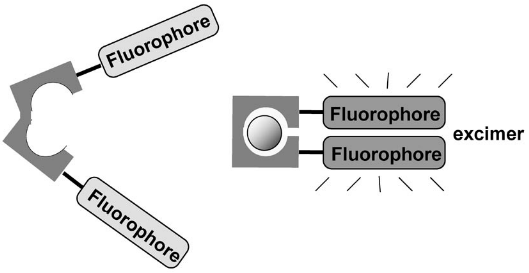
the absorption spectra. This effect is explained by a photodisruption of the interaction between the cation and the donating group.[12]



Scheme 3. Principle behind a PCT fluoroionophore.

1.3. Excimer Formation or Disappearance

Excimers are dimer in the excited state, and many fluorophores such as pyrene or anthracene can form excimers. The fluorescence band corresponding to the excimer is located at higher wavelengths than that of the monomer and does not show any vibronic structure. The principle behind this excimer-formation-based fluoroionophore is given on Scheme 4. When a fluoroionophore contains two fluorophores whose mutual separation is affected by cation complexation, cation binding can be monitored by measuring the monomer/excimer fluorescence-intensity ratio.



Scheme 4. Principle behind an excimer fluoroionophore.

1.4. Energy Transfer

Förster resonance energy transfer (FRET) occurs from a donor fluorophore to an acceptor fluorophore if the emission spectrum of the donor overlaps the absorption spectrum of the acceptor. The efficiency of this energy transfer is correlated to the distance between the donor and the acceptor, the spectral overlap and the relative orientation of the dipole moment.[13] The energy transfer efficiency (Φ_T) is given by Equation (3).

$$\Phi_T = \frac{1}{1 + (r/R_0)^6} \tag{3}$$

where r is the distance between the acceptor and the donor and R_0 is the Förster critical radius, defined by

$$R_0 = 0.2108[\kappa^2 \Phi^\circ_D n^{-4} \int_0^\infty I_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda]^{1/6}$$

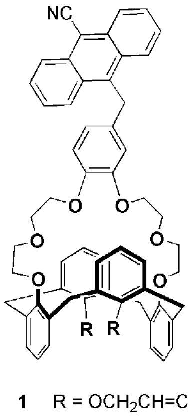
where κ^2 is the orientational factor, Φ°_D is the donor fluorescence quantum yield, n the refractive index of the medium in the wavelength range where spectral overlap is significant, $I_D(\lambda)$ the normalized fluorescence spectrum of the donor, $\varepsilon(\lambda)$ the molar absorption coefficient ($L\,mol^{-1}\,cm^{-1}$) of the acceptor, and λ the wavelength in nanometres. If the fluorophores are free to rotate, the orientation factor is equal to 2/3.

The energy transfer efficiency is affected when cation complexation is accompanied by changes in the spectral overlap or the distance between the donor and the acceptor.

2. Caesium Sensing

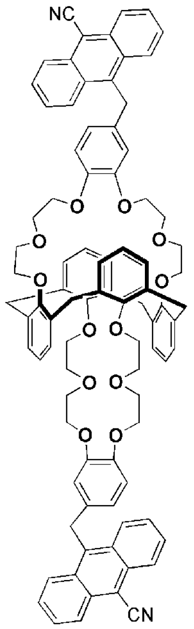
There is a significant need for the development of sensors able to selectively detect caesium ions. The main source of caesium is nuclear waste materials and its toxicity is due to its ability to replace potassium in muscles and red blood cells. In nuclear waste, caesium must be detected in a medium where sodium and potassium are present in a large excess. In order to achieve good selectivity, the choice of ionophore is of major importance, and in this respect 1,3-alternate calix[4]arene-bis(crown-6 ether) exhibits remarkable selectivity towards Cs^+ vs. Na^+ and to a lesser extent vs. K^+. It has therefore been extensively used for solvent extraction from nuclear waste solutions.[14,15] In the following paragraphs, we describe different examples of Cs^+ fluoroionophores.

Dabestani and co-workers have reported Cs^+ PET sensors **1**, **2** and **3** based on a calix[4]crown-6 ether in which a 9-cyanoanthracene fluorophore is linked to a phenyl ring inserted into the crown.[16] In the absence of cation, a photoinduced electron transfer occurs from the benzocrown to the cyanoanthracene fluorophore. Cs^+ binding to the crown in CH_2Cl_2/MeOH (1:1 v/v) inhibits this PET effect, which induces an enhancement of the fluorescence emission. A 1:1 stoichiometry is found for compounds **1** and **2**, which leads to stability constants of 4×10^6 and 1.2×10^7, respectively. Analysis of the evolution of the emission spectra for compound **3** reveals that two complexes, with 1:1 and 2:1 stoichiometries, are formed, which allows the equilibrium constants for the binding of a first cation (K_{11}) and a second one (K_{21}) to be determined. These stability constants are 10^6 and 10^4, respectively.[17] It should be noted that complexation of a second cation is made more difficult by the presence of a bound cation. This system can be considered to be anti-cooperative as the K_{21}/K_{11} ratio is much smaller



1 R = OCH$_2$CH=CH$_2$

2 R = H



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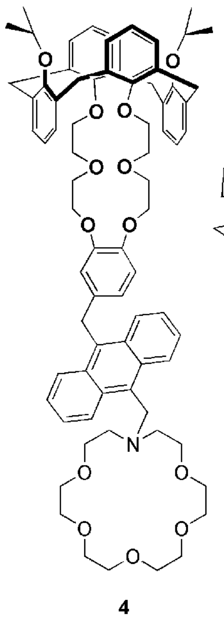

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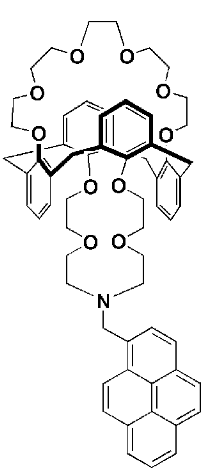

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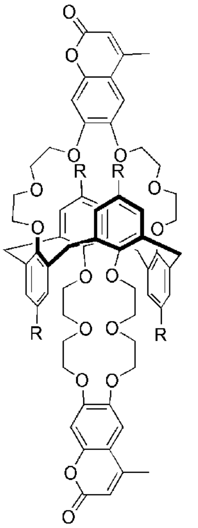
than the statistical value of 1:4 that would be observed if the two binding sites were identical and independent.[18] Such an anti-cooperative effect is most likely due to the electrostatic repulsion between the two cations and is commonly observed for calix[4]arene-bis(crown ether).[19]

In another approach, Dabestani et al. have synthesized compound **4**, which can be used either for the detection of K$^+$ or Cs$^+$.[20] Under basic conditions in methanol, fluorescence enhancement is observed upon K$^+$ complexation because of the PET process from the azacrown ether to the anthracene fluorophore, whereas in an acidic medium the nitrogen atom of the azacrown ether is protonated, which increases the fluorescence quantum yield. Under these conditions, complexation of Cs$^+$ results in a 3.8-fold enhancement of the fluorescence because of the PET process from the dialkoxybenzene to the fluorophore.

The calixarene **5**, which belongs to the PET class of fluoroionophores, was synthesized in order to show selectivity towards Cs$^+$.[21] This compound shows efficient Ag$^+$ binding to the azacrown, which enhances the fluorescence quantum yield due to the inhibition of the PET process. Complexation of Cs$^+$ to the crown induces Ag$^+$ ejection and fluorescence quenching is observed.

We now turn our attention to the Cs$^+$ photoinduced charge-transfer fluoroionophores. Compound **6** (Calix-COU2) is composed of calix[4]bis(crown-6 ether) in which a dioxycoumarin is incorporated into each crown.[22,23] This fluorophore is strongly fluorescent and an intramolecular charge transfer occurs between the oxygen atoms at the 7- and 6-positions of the coumarin to the lactone carbonyl group (acceptor). The coumarin is non-fluorescent in the absence of donor substituents. Complexation with alkali metal ions results in a fluorescence quenching and a blue shift of the fluorescence spectrum due to interaction of the oxygen atoms with the bound cation, which reduces the electron-donating character of these atoms and therefore reduces the intramolecular charge transfer.



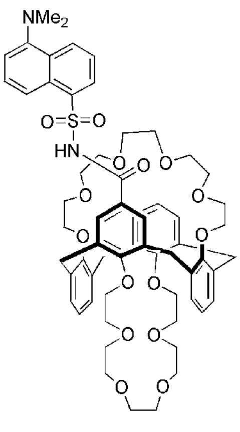
6 R = H

7 R = SO$_3^-$

Two complexes with 1:1 and 2:1 stoichiometries, with equilibrium constants $\log K_{11} = 6.68$ and $\log K_{21} = 3.81$, respectively, were found from the evolution of the fluorescence spectrum upon addition of caesium acetate in ethanol. The selectivity, expressed as the ratio of the stability constants of the 1:1 complexes in ethanol, was found to be 1.6×10^4 for Cs$^+$ vs. Na$^+$ and 75 for Cs$^+$ vs. K$^+$.

With the aim of practical applications, we have prepared an analogue of **6** (Calix-COU2) that is soluble in water. By using the method developed by Lemaire and co-workers for introducing sulfonate groups onto calixarene,[24] we synthesized compound **7**, which contains four sulfonyl groups.[25] This compound is highly fluorescent ($\Phi_F = 0.4$) and completely soluble in water. Moreover, its absorption and emission spectra are independent of pH (in the range pH 6–9).

Addition of Cs^+ induces a 10-nm bathochromic shift in the absorption spectra and an enhancement of the fluorescence intensity (with respect to Calix-COU2). These effects can be explained by the close interaction of the caesium ion with the two sulfonate groups in the complexes, which results in an overall negative charge (equal to that of an electron) in the vicinity of the oxygen atoms at the 6- and 7-positions of the coumarin fluorophore. This reinforces the electron-donating character of these atoms. A linear response of the fluorescence intensity as a function of caesium concentration was observed from 0 to 0.1 mM. The detection limit was calculated to be 0.3 µM on the basis of the ratio of the fluorescence intensities at 420 nm for two excitation wavelengths [I(360)/I(320)]. This limit is of the same order of magnitude as that observed with caesium fluoroionophores soluble in organic solvents but is unprecedented for a water-soluble fluoroionophore.



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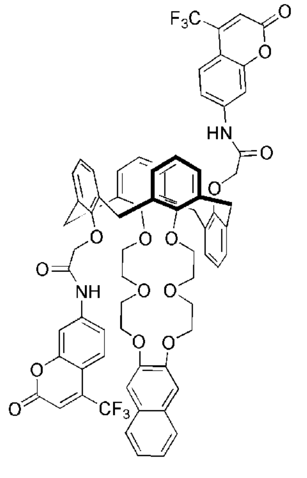


9

Bartsch's group has recently synthesised a new calixarene **8** containing a calix-crown ether linked to a dansyl fluorophore for the sensing of Cs^+ and Tl^+ in H_2O/CH_3CN (1:1 v/v).[26] The fluorophore in this sensing molecule is a carboxydansyl group in which there is an efficient photoinduced charge transfer. Cs^+ complexation induces a blue shift of the emission spectra of the dansyl fluorophore because of the NH deprotonation of the carboxysulfonyl group. Ratiometric measurements were possible thanks to this bathochromic shift. The stability constant of the complex was found to be 2300 and the detection limit 4×10^{-7} mol L^{-1} (53 µg/L). A good selectivity was observed as no effect was detected upon addition of a 100-fold excess of interfering cations.

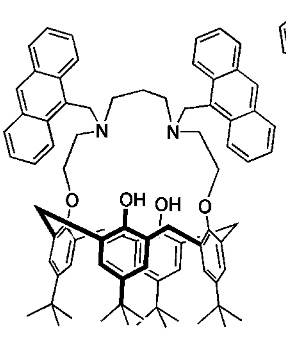
Compound **9** contains a calix[4]arene bearing one 2,3-naphthocrown-6 and two coumarin amide units at the lower rim in a *partial-cone* conformation. In this compound, modification of several photoinduced effects (charge transfer, electron transfer and energy transfer) occurs upon cation complexation.[27] An enhancement of the coumarin-based emission is observed upon complexation with the Cs^+ ion due to inhibition of the PET process from the oxygen atoms to the naphthalene group in acetonitrile. This enhancement increases the spectral overlap between donor emission (naphthalene) and acceptor absorption (coumarin), which increases the energy transfer efficiency. This system shows selectivity for Cs^+ ion over other metal cations, although similar effects are observed upon complexation with F^-.

2. Mercury Sensing

Hg^{2+} is a highly toxic heavy metal ion that causes environmental and health problems.[28] A wide variety of symptoms are observed upon exposure, including digestive, kidney and especially neurological disease. The level of this ion has therefore been the subject of strict regulation and should not currently exceed 1 µg L^{-1}.[2] Several fluorescent molecular sensors have been developed for the detection of mercury,[29] some of which are based on calixarene. Selective detection can be attained by using soft bases. In the following paragraphs, different calixarene-based fluorescent sensors are presented according to the different photoinduced processes involved.

Several PET-based systems have been developed in which mercury complexation can enhance or quench fluorescence. The observed changes can be interpreted by using the Rehm–Weller equation. Since the reduction potential of Hg^{2+} [Hg^{2+}/Hg_2^{2+} (E = 0.68 V/SCE)] is relatively high, oxidation of several fluorophores, which causes fluorescence quenching, is possible. If the fluoroionophore contains a nitrogen atom that is able to reduce the fluorophore, the inverse process can occur and an enhancement of the fluorescence is observed upon cation binding.

Compound **10**, which is based upon *p-tert*-butylcalix[4]arene–azacrown ethers containing anthrylmethyl moieties, can be classified in this category. Inclusion of mercury inside the cavity increases the fluorescence intensity by a factor of about 87-fold in MeOH/THF. This system shows a relatively high selectivity towards alkali and alkaline-earth cations, although the most important interference was observed upon complexation with transition metal cations, especially copper. The stability constant for Hg^{2+} was found to be 1.8×10^3 and the detection limit was estimated to be 6×10^{-5} mol L^{-1}.[30]



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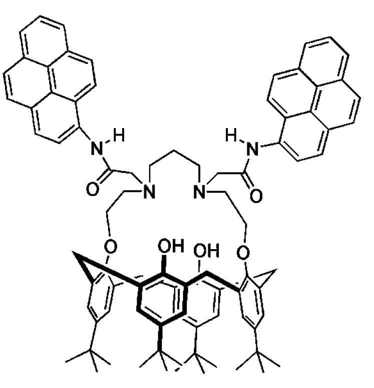

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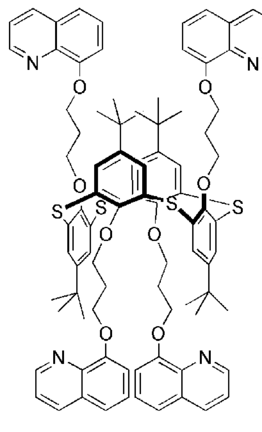
Calixarene **11**, which has almost the same recognition moiety but functionalized with two pyrenylacetamide moieties, was reported by Kim et al.[31] Photoinduced electron transfer from the fluorophore to Hg^{2+} ions in this fluoroionophore is responsible for the fluorescence quenching. The association constant was found to be 4.5×10^4 in methanol. In the presence of water ($MeOH/H_2O$), a reorganization of the fluoroionophore leads to the formation of excimers whose fluorescence is also efficiently quenched upon mercury complexation.



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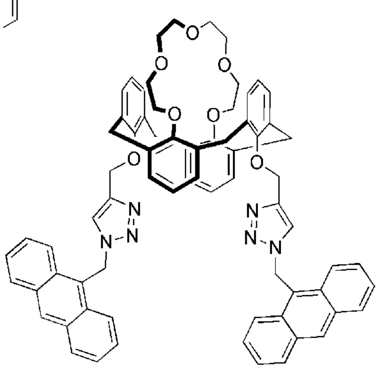

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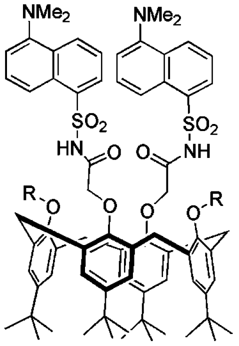
Compound **12** is a thiacalix[4]arene in a 1,3-alternate conformation bearing four quinolinoloxy groups linked through propyl chains.[32] Hg^{2+} complexation induces a fluorescence quenching according to the same mechanism. Additionally, a bathochromic shift of the absorption spectrum occurs due to the interaction between the mercury ion and the oxyquinoline fluorophore. This fluorescent molecular sensor can operate in both THF and 10% H_2O/THF systems. A high selectivity was observed since addition of 10 equivalents of interfering cation had almost no effect on the fluorescence. The detection limit for Hg^{2+} was found to be $2.0 \times 10^{-6}\ mol\,L^{-1}$ in H_2O/THF. The structure of the complex was investigated by ^1H NMR spectroscopy and MALDI-TOF mass spectrometry, and revealed the formation of a tetramercury complex with the ligand in which each quinoline fluorophore is involved in mercury complexation. Compound **13** is a triazole-modified calix[4]crown whose fluorescence is quenched upon heavy metal ion complexation due to a photoinduced electron-transfer process. The fluorescence is also quenched upon addition of Cu^{2+}, Cr^{3+} and Pb^{2+}.[33]



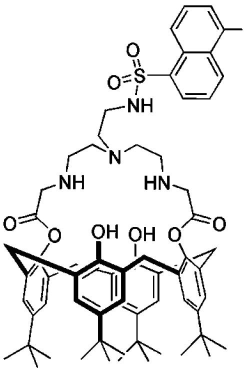
Calix-DANS2 **14** R = CH_3

Calix-DANS$_2$Si(OEt)$_3$ **15** R = $(CH_2)_{11}Si(OEt)_3$

Bartsch and co-workers have shown that a calix[4]arene bearing an N(X)sulfonylcarboxamide exhibits a good extraction selectivity for Hg^{2+} and Pb^{2+} over alkali and transition-metal ions.[34] They also demonstrated that a calix[4]arene (**14**) with two dansyl groups as part of the N(X)sulfonylcarboxamide (Calix-DANS2) can be used for selective Hg^{2+} extraction in acidic solutions in the millimolar concentration range.[35] We have further examined the photophysical properties of this compound and have synthesized a model fluorophore (5-dimethylamino-1-naphthalenecarboxysulfonamido) and calix-DANS4, which bears four carboxydansyl fluorophores (see the section devoted to Pb^{2+} complexation). The photophysical properties of these ligands are exceptionally rich in terms of photoinduced effects (charge-, proton- and energy-transfer).[36] Binding of mercury ion to Calix-DANS2 results in a strong fluorescence quenching due to an electron-transfer process from the excited dansyl fluorophore to the mercury ion, which is consistent with the Rehm–Weller equation. This strong quenching is associated with the appearance of a very short lifetime (50 ps), as measured by time-resolved fluorometry. The nature of the quenching mechanism was confirmed by performing fluorometric investigations at low temperature (100 K). Calix-DANS2 shows a high selectivity towards Hg^{2+} over interfering cations (Na^+, K^+, Ca^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+} and Pb^{2+}) and a sensitivity in the $10^{-7}\ mol\,L^{-1}$ concentration range. Analysis of the whole emission spectra upon mercury binding reveals the formation of a complex with a 1:1 stoichiometry and an apparent stability constant of 1.5×10^7. The selectivity towards Hg^{2+}, expressed as the ratio of the apparent stability constants, was found to be higher than around 500 with respect to other interfering cations.

In order to detect mercury in water, Calix-DANS2 was grafted onto a large-pore mesoporous silica material (SBA-15) via two long alkyl chains containing triethoxysilane groups.[37] An analogue of calix-DANS2, the fluoroionophore **15**, was also synthesized and grafted onto mesoporous silica. Characterization of the obtained material showed that the organized structure was preserved after the post-grafting procedure. As previously observed for Calix-DANS2, addition of mercury to the resulting solid induces a strong fluorescence quenching because of the electron-transfer process. The functionalized material is able to reversibly detect Hg^{2+} with a response time of a few seconds and a detection limit of $0.33 \times 10^{-6}\ mol\,L^{-1}$ in water. Furthermore, this system offers a high selectivity over several interfering cations (Na^+, K^+, Ca^{2+}, Cu^{2+}, Cd^{2+}, Pb^{2+}).

Compound **16**, which is based on a dansyl amide-containing calix[4]-aza-crown, has also been reported to be a fluorescent molecular sensor for mercury.[38] It exhibits high sensitivity and selectivity toward Hg^{2+} over a wide range of metal ions in $MeCN/H_2O$ (4:1, v/v). The association con-



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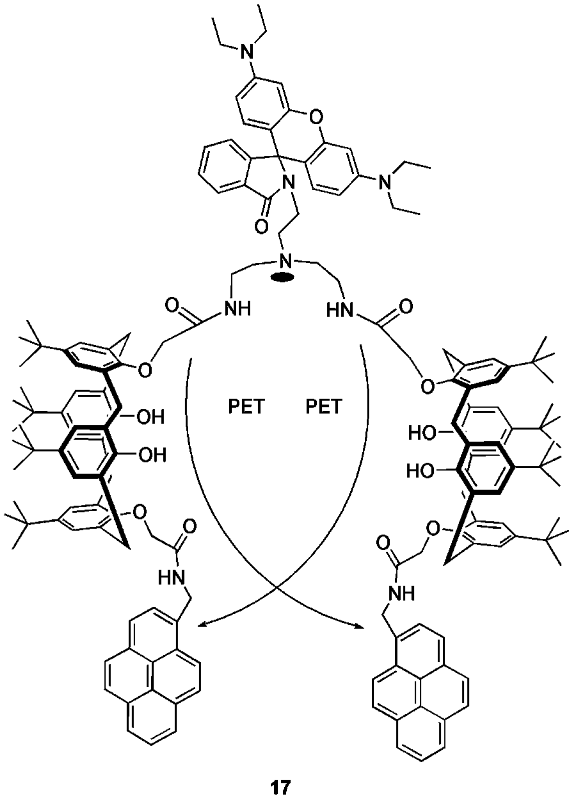
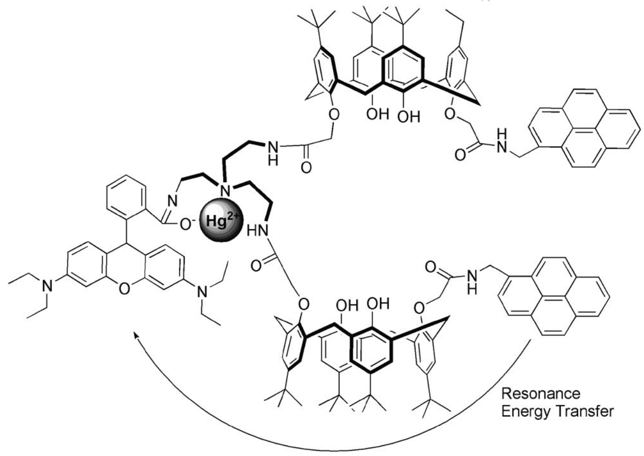
stant of the 1:1 complex formation for 2-Hg^{2+} was calculated to be 1.31×10^5, and the detection limit for Hg^{2+} was found to be 4.1×10^{-6} mol L^{-1}.



17

Compound **17**, developed by Vicens et al.,[39] belongs to the class of energy-transfer sensors and undergoes a complex process upon mercury complexation. Thus, addition of Hg^{2+} to a CH_3CN solution of **17** results in a highly enhanced fluorescence at 575 nm due to energy transfer from the pyrenyl excimer to a ring-opened rhodamine moiety. A possible structure according to this mechanism is shown in Scheme 5. Furthermore, the stability constant was found to be 3.9×10^4. Only a small effect was observed upon addition of a large excess of interfering cation (Na^+, Cs^+, Ag^+, Mg^{2+}; 100 equiv.).

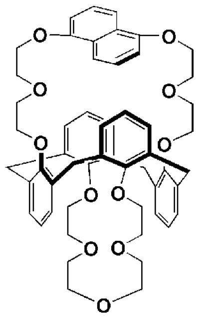


Scheme 5. Structure of the complex $Hg^{2+} \subset$**17**.

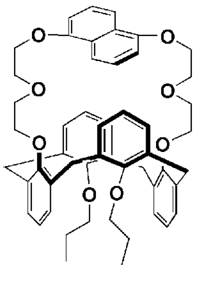
3. Lead Sensing

The detection of lead is very important as this cation causes mental retardation, especially in children. The level of this toxic ion, which is present in tap water because of dissolution from household plumbing systems, is therefore the subject of several official regulations. The World Health Organisation established a guideline for drinking-water quality in 2004 which includes a maximal lead value of 10 µg L^{-1}.[40] Several examples of lead detection are presented here according to the photoinduced processes involved.

Compound **18** is an unsymmetrical 1,3-calix[4]biscrown containing one crown-5 and one crown-6 with a 1,5-naphthylene unit in the middle.[40] Lead complexation causes fluorescence quenching due to inverse PET process in CH_3CN. Complexation studies showed that a 1:1 complex with a stability constant of 6.0×10^4 is formed. The stability constant for **19** is lower because of the lack of a second crown.

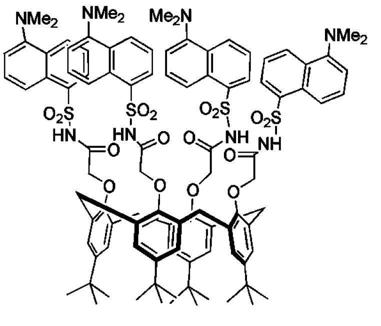


18

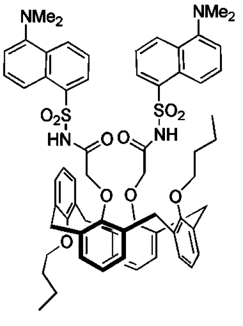


19

We now turn our attention to PCT lead sensors. The fluorescent molecular sensor Calix-DANS4 (**20**) contains a calix[4]arene bearing four carboxydansyl groups. The photophysical characteristics of this compound are very rich in terms of photoinduced processes.[41] The carboxydansyl fluorophore belongs to the class of charge-transfer fluorophores, and deprotonation is accompanied by strong changes in the photophysical properties of this compound. Efficient nonradiative energy transfer has been shown to occur from a dansyl fluorophore in the basic form to another one in the neutral form in this multichromophoric compound. As regards the complexing properties of Calix-DANS4 in CH_3CN/H_2O (60:40 v/v), this compound exhibits an extremely high affinity for lead ion:[42] a noticeable blue shift of the fluorescence spectrum and an increase of the fluorescence quantum yield are observed upon addition of lead. These effects can be explained by deprotonation of the sulfoxycarboxamide functions upon cation binding. It should be noted that two complexes, with 2:3 and 1:1 (metal:ligand) stoichiometries and stability constants $\log K_{23} = 33.5$ and $\log K_{11} = 10$, respectively, are successively formed upon addition of lead. The number of fluorophores involved in the complexation was determined from time-resolved fluorescence experiments.[43] The selectivity with respect to various competing ions is excellent and the unprecedented detection limit ($4\ \mu g\,L^{-1} = 20 \times 10^{-9}\ mol\,L^{-1}$) is fully compatible with the level defined by the World Health Organization. A further development was achieved upon incorporation of this fluorescent molecular sensor into a microfluidic system – a very low detection limit was attained by this method, which is very promising.[44]



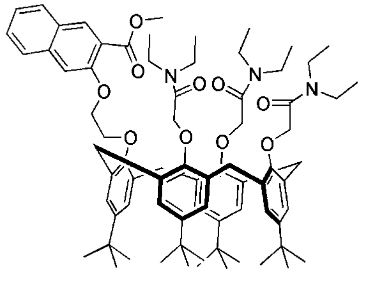
20



21

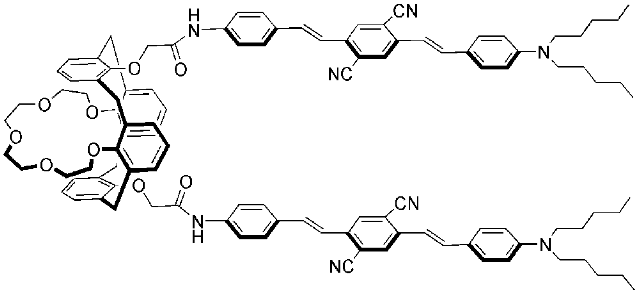
Talanova et al.[45] have synthesized the new analogue **21** that belongs to this series of fluoroionophores, which contains two dansylcarboxamide groups with a calixarene in the partial cone conformation. In acidic $MeCN/H_2O$ (1:1 v/v) solution, the complexation of Pb^{2+} also induces a blue shift of the emission spectra as a result of deprotonation of the carboxydansyl fluorophore. The detection limit was found to be $1.2 \times 10^{-8}\ mol\,L^{-1}$, which corresponds to 2.5 ppb. A high selectivity for Pb^{2+} is observed since the only interfering cation is Hg^{2+}. Addition of more than 100 equivalents of this cation induces significant effects, thus suggesting that preorganization of the calix[4]arene moiety of the fluoroionophore in the *paco* conformation promotes Pb^{2+} complexation.

Compound **22** is based on a *p-tert*-butylcalix[4]arene with three *N*,*N*-diethylacetamide groups and one methyl 3-ethoxynaphthalene-2-carboxylate. Lead complexation to this calixarene results in a strong quenching of the fluorescence and a slight red-shift of the emission. This effect can be rationalized by an interaction of lead with the carbonyl group of the naphthalene derivative leading to a change of charge transfer. Complexation studies in CH_3CN/H_2O (3:1 v/v) showed that a 1:1 complex with a stability constant of 6.5×10^4 is formed.[46]



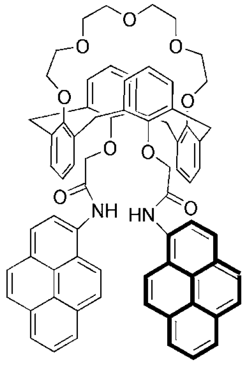
22

Calixarene **23** was the first calix[4]arene fluoroionophore to be excited by two-photon absorption.[47] It is based on a 1,3-alternate calix[4]arene functionalized with two-photon absorbing chromophores. The maximum of the absorption band for this compound is located at 461 nm and the emission is weak, with a maximum at 600 nm, which is attributed to self-quenching between the two adjacent fluorophores; no two-photon-excited fluorescence is observed upon excitation at 780 nm. Lead complexation in acetonitrile causes a blue-shifted absorption and an enhancement of the emission spectra due to inhibition of the coupling between the two fluorophores. The emission spectra can be measured upon complexation by excitation at 780 nm by a two-photon process. Complexation of K^+ to the crown induces an allosteric effect and the emission enhancement is larger. Al^{3+} is a strong interferent ion as very large effects are observed upon complexation with this cation.

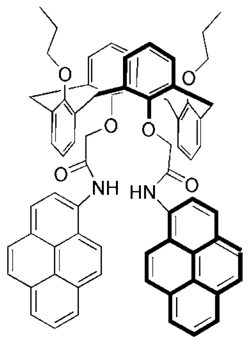


23

The calixarene-based fluorescent sensors **24** and **25** described by Kim et al. are based on changes in excimer formation upon cation complexation.[48] A strong excimer band is observed in the absence of cation because of the close proximity of the two pyrene fluorophores. This excimer fluorescence is quenched upon lead complexation in acetonitrile because of Pb^{2+} complexation with the carbonyl group. The stability constants for these sensors were found to be 4.4×10^5 and 5.2×10^5, respectively. Addition of K^+ to the complex $Pb^{2+}\subset$**24** revives the excimer emission, thus indicating that K^+ complexation leads to release of the lead ion. Cu^{2+} complexation was examined for the same compound[49] and strong differences were observed. Thus, the excitation and emission spectra are strongly red-shifted because, in contrast to Pb^{2+}, Cu^{2+} interacts with the nitrogen atoms of the amide groups, which results in a PCT mechanism.

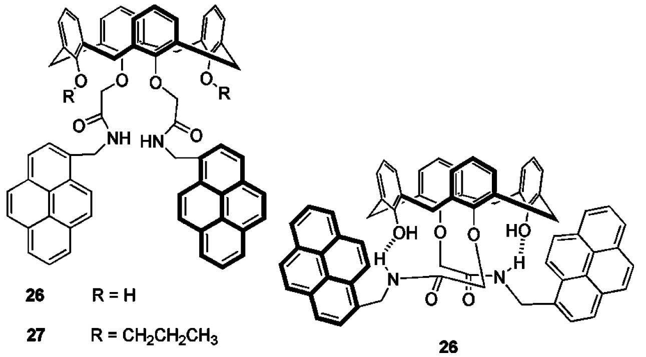


24



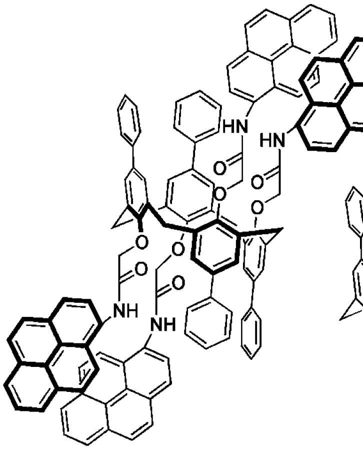
25

Calixarenes **26** and **27** also contain two pyrene groups and it should be noted that there is no conjugation between the recognition unit and the fluorophore. In the excited state, the two pyrene units of **26** form a strong intramolecular excimer with an emission at 472 nm and a relatively weak monomer emission at 395 nm. In contrast, **26** exhibits only a monomer emission at 398 nm because intramolecular hydrogen bonding between the phenolic OH oxygens and the amide hydrogen prevents stacking of the two pyrene groups (Scheme 6). Lead complexation in acetonitrile induces strong quenching of the emission of **26** and **27** because of a heavy metal ion effect. The emissions of the monomer and excimer are quenched for **27**. The fluorescence changes resulting from addition of Pb^{2+} show that a 1:1 complex is formed, and the stability constants are 540 for **26** and 1.2×10^4 for **27**.[50] The selectivity was examined and Ca^{2+} was found to be the major interferent. In^{3+} complexation has strong effects on the fluorescence of **26**.

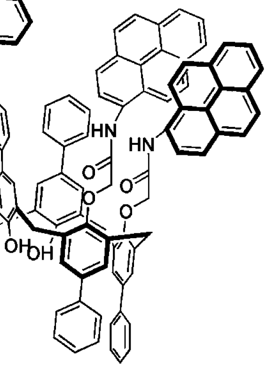


Scheme 6. Reorganization of compound **27** by hydrogen bonding.

Fluoroionophores **28** and **29** contain a C-1,2-alternate tetrahomodioxacalix[4]arene functionalized with pyrene-amides.[51] The emission spectra of these compounds exhibit both monomer and excimer bands. Pb^{2+} complexation in $CHCl_3/CH_3CN$ (1:3, v/v) has a quenching effect on the monomer and the excimer band, whereas the receptor provides an enhanced excimer and declined monomer emission with ratiometric response upon Ca^{2+} complexation. The observed changes were rationalized by molecular calculations.

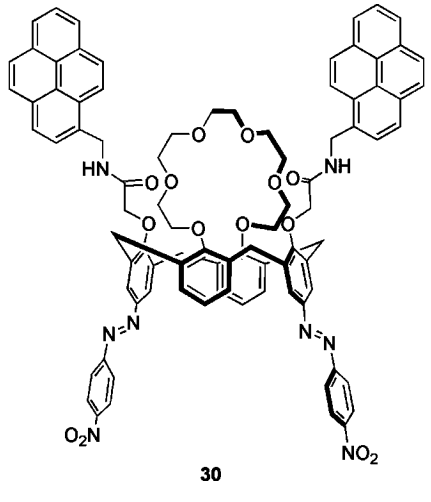


28



29

Compound **30** contains two pyrene groups coupled with an azo phenyl unit.[52] The fluorescence of **30** is low because of an efficient energy transfer from the pyrene group to the diazo compound. Lead complexation in CH_3CN results in an enhancement of the pyrene fluorescence because of the reduction of the energy transfer efficiency between the pyrene and the diazo compound. Indeed, complexation of the lead with the oxygen of the phenyl of the diazo compound results in a blue shift of the absorption spectra of this subunit. The spectral overlap between the emission spectrum of pyrene and the absorption spectrum of the diazo compound is smaller, which causes a decrease in the energy transfer efficiency. The stability constant was found to be 4.0×10^6.

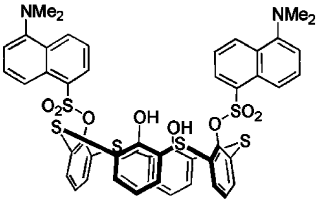


30

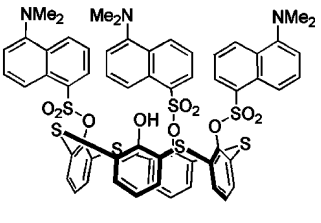
4. Cadmium Sensing

Cadmium is a heavy metal widely used in industrial processes such as electroplating of metals, colouring agents and rechargeable Cd-Ni batteries. Cadmium is therefore found in air, water, or in plants as a result of anthropogenic pollution mainly from the smelting and refining of nonferrous metals, fossil fuel combustion and municipal waste incineration. Unfortunately, cadmium is highly toxic for all living things as it accumulates in cells, the liver and kidneys with subsequent physiological disorders or carcinogenic effects.[53] For this reason, the level of cadmium in drinking water should not exceed 3 $\mu g\,L^{-1}$ according to the World Health Organization.[2] There are only a few examples of calixarene-based fluoroionophores developed for the detection of Cd^{2+}. These examples are described below.

Compounds **31** and **32** are based on thiacalix[4]arene derivatives modified with di- and tri-dansyl moieties.[54] Their complexing properties were investigated in 10% aqueous dmf solution. Cd^{2+} complexation leads to a fluorescence quenching, and the stability constant was found to be 3420. The use of similar compounds containing *tert*-butyl derivatives was also investigated and the sensing ability towards cadmium found to be lower.[55]



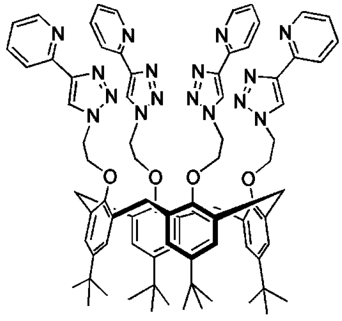
31



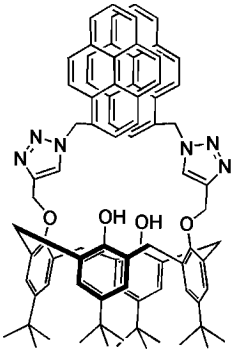
32

Calix[4]arene **33**, which is substituted at the lower rim with four pyridin-2′-yl-1,2,3-triazole chromophores, exhibits interesting photophysical and complexing properties towards Cd^{2+} in methanol.[56] Spectroscopic experiments and molecular modelling provided evidence for a photoinduced charge transfer from the triazole group to the pyridine ring. A broadening of the emission spectrum was observed with respect to the model fluorophore. A satisfactory fit of the fluorescence decays was obtained by considering the decay-time distributions successfully recovered by using the compressed hyperbola (or Becquerel) function.[57] Such distributions result from the heterogeneity of the microenvironments experienced by the chromophores and/or their mutual interactions in the excited state. The fluorescence intensity decreased upon complexation with cadmium and a new band centred at 480 nm, which can be assigned to the formation of excimers, appeared. Analysis of the whole emission spectra showed that two complexes are formed upon Cd^{2+} complexation (stability constants of 10^6 and 5×10^8 for the 1:1 and the 2:1 complexes, respectively). The effect of an interfering cation such as Zn^{2+} was investigated and the affinity found to be lower for this cation.

Vicens et al. have recently synthesized two pyrenyl-appended calix[4]arenes **34** and **35** featuring 1,2,3-triazole linkers.[58] Compound **34** exhibits an intense excimer band due to an intramolecular interaction between the pyrene fluorophores. The fluorescent spectral changes observed upon addition of various metal ions in acetonitrile show that **34** is highly selective for Cd^{2+} and Zn^{2+} over other metal ions, whereas **35** is selective for Hg^{2+} and Cu^{2+}. Addition of Cd^{2+} and Zn^{2+} to a solution of **34** results in ratiometric measurements. The association constants of **34** were thus determined to be 5.18×10^4 and 1.7×10^4 for Cd^{2+} and Zn^{2+}, respectively.



Calix-PT4 33



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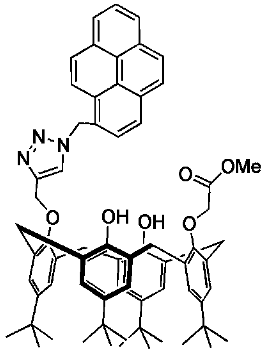

35

Conclusions

Calixarene-based fluorescent molecular sensors are very attractive for the detection of heavy metal ions. For caesium detection, a calixarene-crown moiety offers a very selective complexing unit and several fluorescent molecular sensors have been synthesized for caesium detection in the submicromolar range. It should be recalled that these systems can operate in an aqueous medium.

Different systems in which the complexing unit contains soft sulfur or nitrogen atoms have been designed for mercury detection. The synthesized compounds offer a submicromolar detection limit. It should be noted that this detection limit is significantly higher than that obtained with sulfur-based chelators.[29]

Very selective systems have also been designed for the detection of lead. In particular, calixarene systems functionalized with carboxydansyl groups offer a very low detection limit (20 nM) which is fully compatible with the official limits defined by the Word Health Organization.

Few systems have been designed for cadmium complexation. The use of triazole systems seems to be very promising but progress is still to be made regarding their selectivity and sensitivity. In particular, the possibility of complexing cadmium in an aqueous or organo-aqueous medium must be evaluated.

Acknowledgments

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[1] a) T. C. Hutchinson, K. M. Meema, in: *Lead, Mercury, Cadmium and Arsenic in the Environment*, John Wiley & Sons, New York, **1987**; b) G. H. Scoullos, M. J. Vonkeman, L. Thorton, Z. Makuch, in: *Mercury, Cadmium, Lead: Handbook for Sustainable Heavy Metals Policy and Regulation (Environment & Policy*, vol. 31), Kluwer Academic, **2001**; c) C. J. Coester, *Anal. Chem.* **2005**, *77*, 3737–3754; d) S. D. Richardson, T. A. Temes, *Anal. Chem.* **2005**, *77*, 3807–3838.

[2] World Health Organization, *Guidelines for drinking-water quality*, 3rd ed., vol. 1, Geneva, **2004**, 188.

[3] A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, T. E. Rice, *Chem. Rev.* **1997**, *97*, 1515–1566.

[4] B. Valeur, I. Leray, *Coord. Chem. Rev.* **2000**, *205*, 3–40.

[5] C. D. Gutsche, *Monographs in Supramolecular Chemistry*, vol. 6, *Calixarenes Revisited*, The Royal Society of Chemistry, Cambridge, U.K., **1998**.

[6] J. S. Kim, D. T. Quang, *Chem. Rev.* **2007**, *107*, 3780–3799.

[7] A. Ikeda, S. Shinkai, *Chem. Rev.* **1997**, *97*, 1713–1734.

[8] V. Böhmer, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 713–745.

[9] B. Valeur, I. Leray, *Inorg. Chim. Acta* **2007**, *360*, 765–774.

[10] B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, **2002**, chapter 10.

[11] B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, **2002**, chapter 4.

[12] a) P. Plaza, I. Leray, P. Changenet-Barret, M. M. Martin, B. Valeur, *ChemPhysChem.* **2002**, *3*, 668–674; C. Ley, F. Lacombat, P. Plaza, M. M. Martin, I. Leray, B. Valeur, *ChemPhysChem.* **2009**, *10*, 276–281.

[13] B. Valeur, *Molecular Fluorescence: Principles and Applications*, Wiley-VCH, Weinheim, **2002**, chapter 9.

[14] a) A. Casnati, A. Pochini, R. Ungaro, F. Ugozzoli, F. Arnaud, S. Fanni, M.-J. Schwing, R. J. M. Egberink, F. De Jong, D. N. Reinhoudt, *J. Am. Chem. Soc.* **1995**, *117*, 2767; b) F. Arnaud-Neu, Z. Asfari, B. Souley, J. Vicens, *New J. Chem.* **1996**, *20*, 453; c) V. Lamare, J. F. Dozol, S. Fuangswasdi, F. Arnaud-Neu, P. Thuery, M. Nierlich, Z. Asfari, J. Vicens, *J. Chem. Soc. Perkin Trans. 2* **1999**, 271–284.

[15] a) Z. Asfari, C. Bressot, J. Vicens, C. Hill, J. F. Dozol, H. Rouquette, S. Eymard, V. Lamare, B. Tournois, *Anal. Chem.* **1995**, *67*, 3133–3139; b) J. S. Kim, J. H. Pang, I. Y. Yu, W. K. Lee, I. H. Suh, J. K. Kim, M. H. Cho, E. T. Kim, D. Y. Ra, *J. Chem. Soc. Perkin Trans. 2* **1999**, 837–846.

[16] H. F. Ji, R. Dabestani, G. M. Brown, R. A. Sachleben, *Chem. Commun.* **2000**, 833–834.

[17] a) H. F. Ji, G. M. Brown, R. Dabestani, *Chem. Commun.* **1999**, 609–610; b) H. F. Ji, R. Dabestani, G. M. Brown, R. L. Hettich, *J. Chem. Soc. Perkin Trans. 2* **2001**, 585–591.

[18] K. A. Connors, *Binding Constants. The Measurement of Complex Stability*, Wiley, New York, **1987**.

[19] *Calixarenes 2001* (Eds.: Z. Asfari, V. Böhmer, J. Harrowfield, J. Vicens), Kluwer Academic Publishers, Dordrecht, The Netherlands, **2001**.

[20] H. F. Ji, R. Dabestani, G. M. Brown, *J. Am. Chem. Soc.* **2000**, *122*, 9306–9307.

[21] J. S. Kim, O. J. Shon, J. A. Rim, S. K. Kim, J. Yoon, *J. Org. Chem.* **2002**, *67*, 2348–2351.

[22] I. Leray, Z. Asfari, J. Vicens, B. Valeur, *J. Fluoresc.* **2004**, *14*, 451–458.

[23] I. Leray, Z. Asfari, J. Vicens, B. Valeur, *J. Chem. Soc. Perkin Trans. 2* **2002**, 1429–1434.

[24] L. Nicod, S. Pellet-Rostaing, F. Chitry, M. Lemaire, *Tetrahedron Lett.* **1998**, *39*, 9443–9446.

[25] V. Souchon, I. Leray, B. Valeur, *Chem. Commun.* **2006**, 4224–4226.

[26] a) G. G. Talanova, E. D. Roper, N. M. Buie, M. G. Gorbunova, R. A. Bartsch, V. S. Talanov, *Chem. Commun.* **2005**, 5673–5675; b) E. D. Roper, V. S. Talanov, M. G. Gorbunova, R. A. Bartsch, G. G. Talanova, *Anal. Chem.* **2007**, *79*, 1983–1989.

[27] M. H. Lee, D. T. Quang, H. S. Jung, J. Yoon, C. H. Lee, J. S. Kim, *J. Org. Chem.* **2007**, *72*, 4242–4245.

[28] M. Nendza, T. Herbst, C. Kussatz, A. Gies, *Chemosphere* **1997**, *35*, 1875–1885.

[29] E. M. Nolan, S. J. Lippard, *Chem. Rev.* **2008**, *108*, 3443–3480.

[30] N. R. Cha, M. Y. Kim, Y. H. Kim, J. I. Choe, S. K. Chang, *J. Chem. Soc. Perkin Trans. 2* **2002**, 1193–1196.

[31] J. H. Kim, A. R. Hwang, S. K. Chang, *Tetrahedron Lett.* **2004**, *45*, 7557–7561.

[32] L. Praveen, V. B. Ganga, R. Thirumalai, T. Sreeja, M. L. P. Reddy, R. L. Varma, *Inorg. Chem.* **2007**, *46*, 6277–6282.

[33] K. C. Chang, I. H. Su, A. Senthilvelan, W. S. Chung, *Org. Lett.* **2007**, *9*, 3363–3366.

[34] G. G. Talanova, H. S. Hwang, V. S. Talanov, R. A. Bartsch, *Chem. Commun.* **1998**, 419–420.

[35] G. G. Talanova, N. S. A. Elkarim, V. S. Talanov, R. A. Bartsch, *Anal. Chem.* **1999**, *71*, 3106–3109.

[36] R. Metivier, I. Leray, B. Valeur, *Photochem. Photobiol. Sci.* **2004**, *3*, 374–380.

[37] R. Metivier, I. Leray, B. Lebeau, B. Valeur, *J. Mater. Chem.* **2005**, *15*, 2965–2973.

[38] Q.-Y. Chen, C.-F. Chen, *Tetrahedron Lett.* **2005**, *46*, 165–168.

[39] A. B. Othman, J. W. Lee, J.-S. Wu, J. S. Kim, R. Abidi, P. Thuery, J. M. Strub, A. Van Dorsselaer, J. Vicens, *J. Org. Chem.* **2007**, *72*, 7634–7640.

[40] J. H. Bok, H. J. Kim, J. W. Lee, S. K. Kim, J. K. Choi, J. Vicens, J. S. Kim, *Tetrahedron Lett.* **2006**, *47*, 1237–1240.

[41] R. Metivier, I. Leray, B. Valeur, *Photochem. Photobiol. Sci.* **2004**, *3*, 374–380.

[42] R. Metivier, I. Leray, B. Valeur, *Chem. Commun.* **2003**, 996–997.

[43] R. Metivier, I. Leray, B. Valeur, *Chem. Eur. J.* **2004**, *10*, 4480–4490.

[44] French Patent Application, no. 0806954, 11/12/08.

[45] N. M. Buie, V. S. Talanov, R. J. Butcher, G. G. Talanova, *Inorg. Chem.* **2008**, *47*, 3549–3558.

[46] J. M. Liu, J. H. Bu, Q. Y. Zheng, C. F. Chen, Z. T. Huang, *Tetrahedron Lett.* **2006**, *47*, 1905.

[47] J. S. Kim, H. J. Kim, H. M. Kim, S. H. Kim, J. W. Lee, S. K. Kim, B. R. Cho, *J. Org. Chem.* **2006**, *71*, 8016–8022.

[48] S. K. Kim, S. H. Lee, J. Y. Lee, R. A. Bartsch, J. S. Kim, *J. Am. Chem. Soc.* **2004**, *126*, 16499–16506.

[49] J. K. Choi, S. H. Kim, J. Yoon, K.-H. Lee, R. A. Bartsch, J. S. Kim, *J. Org. Chem.* **2006**, *71*, 8011–8015.

[50] S. K. Kim, S. H. Kim, H. J. Kim, S. H. Lee, S. W. Lee, J. Ko, R. A. Bartsch, J. S. Kim, *Inorg. Chem.* **2005**, *44*, 7866–7875.

[51] J. K. Choi, A. Lee, S. Kim, S. Ham, K. No, J. S. Kim, *Org. Lett.* **2006**, *8*, 1601–1604.

[52] S. H. Lee, S. K. Kim, J. H. Bok, S. H. Lee, J. Yoon, K. Lee, J. S. Kim, *Tetrahedron Lett.* **2005**, *46*, 8163–8167.

[53] S. Dobson, *Cadmium−Environmental Aspects*, World Health Organization, Geneva, **1992**.

[54] Y. Higuchi, M. Narita, T. Niimi, N. Ogawa, F. Hamada, H. Kumagai, N. Iki, S. Miyano, C. Kabuto, *Tetrahedron* **2000**, *56*, 4659–4666.

[55] M. Narita, Y. Higuchi, F. Hamada, H. Kumagai, *Tetrahedron Lett.* **1998**, *39*, 8687–8690.

[56] V. Souchon, S. Maisonneuve, O. David, I. Leray, J. Xie, B. Valeur, *Photochem. Photobiol. Sci.* **2008**, *7*, 1323–1331.

[57] V. Souchon, I. Leray, M. N. Berberan-Santos, B. Valeur, *Dalton Trans.* **2009**, 3988–3992.

[58] S. Y. Park, J. H. Yoon, C. S. Hong, R. Souane, J. S. Kim, S. E. Mathews, J. Vicens, *J. Org. Chem.* **2008**, *73*, 8212–8218.

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