Highly Selective Optical-Sensing Membranes, Containing Calix[4]arene Chromoionophores, for Pb^{2+} Ions

Niels J. van der Veen,[a] Ewa Rozniecka,[b] Léon A. Woldering,[a] Michal Chudy,[b] Jurriaan Huskens,[a] Frank C. J. M. van Veggel,*[a] and David N. Reinhoudt*[a]

Abstract: Plasticized poly(vinyl chloride) (PVC) optode membranes containing novel calix[4]arene chromoionophores **1** or **2** and one equivalent of a lipophilic anion respond to Pb^{2+} ions with high selectivity over alkali, alkaline-earth, and other heavy metal ions. This selectivity stems from the combination of ligand specificity and a unique ion exchange scheme that employs both monovalent metal ions and protons as the exchanged ions. Complexation of Pb^{2+} ions inside the membrane is accompanied by deprotonation of the chromoionophores, which causes a bathochromic shift of the absorption maximum λ_{max}. The response to Pb^{2+} ions is modulated by pH and alkali metal ions in a fashion that is consistent with the proposed ion-exchange mechanism. Of all of the other metal ions tested, only Cs^+ and Ag^+ produce a color change. However, these monovalent metal ions cause hypsochromic shifts of λ_{max} instead of the bathochromic shift caused by Pb^{2+}, because the chromoionophores remain protonated upon complexation.

Keywords: calixarenes · chromoionophore · lead · optode membrane · sensors

Introduction

Optode membranes containing separate ionophores and proton-exchanging dyes are commonly used for optical sensing of metal ions. In these systems, pioneered by Charlton and co-workers[1] and further developed in the group of Simon,[2] the extraction of metal ions into the membrane by an ionophore leads to deprotonation of a lipophilized pH indicator resulting in a change in absorption or fluorescence. The ion-exchange mechanism, as well as other operating principles such as response time and durability have been extensively studied and have been reviewed.[3] These optodes are extrinsic sensors, as the optical signal is prompted by the change in bulk properties of the membrane as a result of the influx of ions. Sensors based on chromogenic ionophores are intrinsic, because the optical effect is directly coupled to recognition of the metal ion by the receptor molecule.

[a] Dr. Ir. F. C. J. M. van Veggel, Prof. Dr. Ir. D. N. Reinhoudt, Dr. N. J. van der Veen, Ing. L. A. Woldering, Dr. Ir. J. Huskens
Department of Supramolecular Chemistry and Technology and MESA+ Research Institute, University of Twente
PO Box 217, 7500 AE, Enschede (The Netherlands)
Fax: (+31) 53-4894645
E-mail: smct@ct.utwente.nl

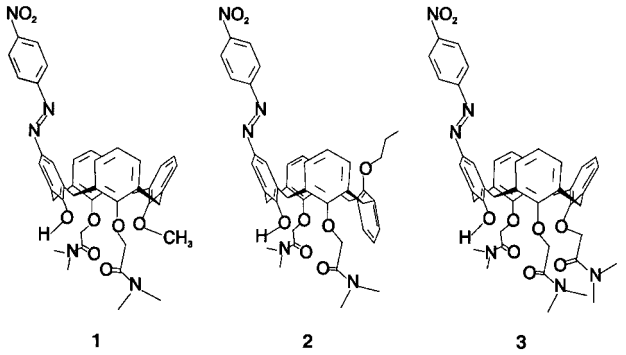
[b] E. Rozniecka, M. Chudy
Department of Analytical Chemistry
Warsaw University of Technology
Noakowskiego 3, 00-664, Warsaw (Poland)

In the last decade, numerous chromoionophores selective for different metal ions have been reported.[4] Many are based on calix[4]arene,[5] well known as an outstanding molecular platform for the synthesis of ionophores. The conformation of the calix[4]arene is an important factor in ligand selectivity.[6] A calix[4]arene ionophore in the 1,3-alternate conformation was shown to have higher K^+/Na^+ selectivity than valinomycin.[6b] Conversely, high Na^+/K^+ selectivity has been achieved with calix[4]arene ionophores in the cone conformation.[7] The ion radius of Pb^{2+} (1.19 Å)[8] is between those of Na^+ (1.02 Å) and K^+ (1.38 Å), and we have previously reported[9] that Pb^{2+} is preferentially bound in the partial-cone conformation by flexible calix[4]arene receptors.

Chromoionophores must be confined in order to be applicable in optical sensors. Several groups have reported immobilization on a solid substrate through electrostatic interactions[10] and covalent bonds.[11] Recently, we prepared monolayers of fluorescent chromoionophores on glass.[12] Nevertheless, incorporation in polymer membranes remains the most straightforward method of immobilization for screening new chromoionophores. Optode membranes containing metal-ion selective chromoionophores reported in the literature[13] cannot yet compete with the available extrinsic sensing membranes in terms of selectivity, sensitivity, and reversibility.

In this paper, we describe optode membranes that contain new Pb^{2+}-selective calix[4]arene chromoionophores (**1** and **2**) together with one equivalent of a lipophilic anion. The highly

  0947-6539/01/0722-4878 $ 17.50+.50/0

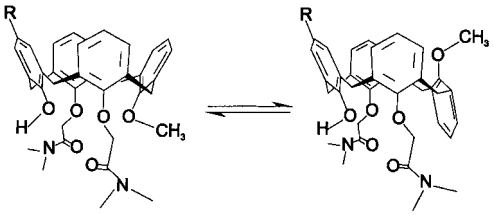


1 2 3

sensitive and selective response of these optodes clearly illustrates the advantages of intrinsic over extrinsic optical sensing. Their operation is explained by a novel ion-exchange mechanism, in which Pb^{2+} ions are exchanged for a proton and a monovalent metal ion. The influence on the sensitivity of the pH, Na^+ concentration, and the preorganization of the chromoionophore in the partial-cone conformation are studied. Membranes containing nonselective reference chromoionophore **3** are used to support the proposed ion-exchange mechanism and corroborate the peculiar effect that monovalent and divalent metal ions cause shifts of λ_{max} in opposite directions.

Results and Discussion

Chromoionophores: Chromoionophores **1**–**3** have chromophores that can be deprotonated upon complexation of a divalent metal ion and that differ by one substituent at the lower rim of the calix[4]arene. This substituent is used to control the conformation of the calix[4]arene moiety, which determines the size and geometry of the ion-binding site. Chromoionophore **1** has a methoxy substituent at the lower rim, allowing a ring-flip that interconverts the cone and partial-cone conformations,[14] as is depicted in Scheme 1. Chromoionophore **2** is locked in the partial-cone conformation by a propoxy substituent, whereas **3** has the cone conformation. In the neutral ligands, the calix[4]arene phenolic moiety is fixed by hydrogen bonding to the neighboring

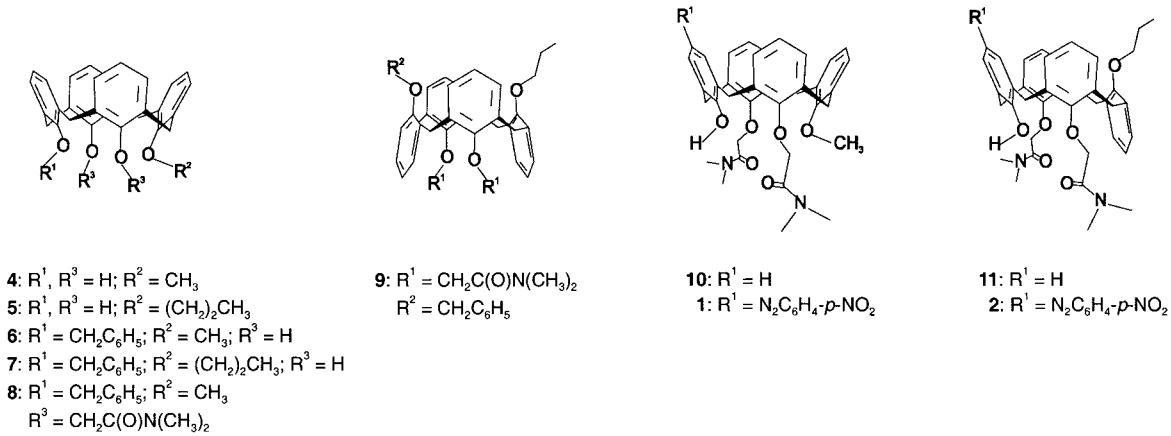


1, cone conformation **1**, partial-cone conformation

Scheme 1. Interconversion of the cone and partial-cone conformation. $R = N_2C_6H_4$-*p*-NO_2

ether oxygens.[14] In the complexes, the (deprotonated) oxygen atom is one of the ligating groups, binding the metal ion by electrostatic interactions. In partial-cone complexes of **1** and **2** with metal ions, metal–π interactions contribute to binding.[9, 15] As a result of the incompatibility of Na^+ and the partial-cone conformer, selectivity for Pb^{2+} over Na^+ is expected. Chromoionophore **3** has three tertiary amido functionalities available for complexation of metal ions in the cone conformation; this makes it a good ligand for both Na^+ and Ca^{2+} ions.[16]

Chromoionophores **1** and **2** were synthesized in four steps from the monoalkylated[17] calix[4]arenes **4** and **5**, respectively (Scheme 2). After benzylation at the diametric phenolic moiety, the *N*,*N*-dimethylaminocarbamoyl substituents were introduced at the remaining lower rim positions, yielding **8** and **9**. Calix[4]arene **9** was synthesized in the 1,3-alternate



Scheme 2. Synthesis of chromoionophores **1** and **2**. a) K_2CO_3, acetonitrile, reflux, 16 h, 34%; b) NaH, acetonitrile, reflux 16 h, 77%; c) Cs_2CO_3, DMF, 90 °C, 16 h, 31%; d) ethanol/THF, RT, over night, 33–49%; e) water/methanol, 0 °C, $NaClO_4$ or $Pb(ClO_4)_2$, 2–4 h, 40–51%.

  0947-6539/01/0722-4879 $ 17.50+.50/0

conformation by using Cs_2CO_3 as a base[18] in order to arrive at the partial-cone conformation of chromoionophore **2**. The 1,3-alternate conformation of **9** was confirmed by ^{13}C NMR spectroscopy; chemical shifts of $\delta = 35.14$ and 37.04 were found for the calix[4]arene methylene carbon atoms that bridge between the aromatic rings.[19] Reductive removal of the benzyl groups afforded compounds **10** and **11**, in which the ionophoric sites are completed. Diazonium coupling with *p*-nitroaniline gave chromoionophores **1** and **2**. Addition of $NaClO_4$ or $Pb(ClO_4)_2$ improved the yields of the diazonium coupling, possibly because of the lower pK_a of the phenol upon complexation of metal ions, since deprotonation enhances the reaction rate. The conformational flexibility of **1**[20] causes broad signals in the NMR spectra, but addition of excess $NaClO_4$ to the NMR samples led to the formation of the Na^+ complex, which exhibits sharp NMR signals. The ^{13}C NMR chemical shifts of $\delta = 30.53$ and 30.81 for the bridging methylene carbons of the calix[4]arene indicate that the Na^+ complex of **1** has the cone conformation.[19] The partial-cone conformation of **2** is confirmed by the chemical shifts of the calix[4]arene bridging methylene carbons, which are found at $\delta = 30.6$ and 37.4. Chromoionophore **3** was synthesized as we have reported previously.[9]

In dichloromethane, **2** and **3** have λ_{max} values at 408 and 410 nm, respectively. The absorption spectra of both compounds show a second maximum around 600 nm, corresponding to the deprotonated chromophores. Upon addition of a trace of acetic acid to the samples this band disappears. The absorption band of the neutral ligand also shifts for both compounds, but in the opposite direction, giving new maxima at 470 and 384 nm, respectively. The different response to the acidic medium should be a consequence of the different ion binding cavities. The blue shift observed for **3** can be explained by complexation of an H_3O^+ ion by the lower rim ether oxygen atoms or the amido moieties. The red shift of the λ_{max} of **2** suggests that in an acidic medium, the chromophore has a different conformation, in which the hydroxyl group of the chromophore is involved in (intramolecular) hydrogen bonding. The same λ_{max} value is found for **2** in the membrane matrix (vide infra). The absorption maxima of the solutions after extraction of solid perchlorate salts into dichloromethane are given in Table 1. Complexation of Li^+ and Ag^+ by **2** was accompanied by hypsochromic shifts, while Ca^{2+}, Cd^{2+}, and Pb^{2+} caused bathochromic shifts. Apparently, the monovalent metal ions are complexed by the neutral ligand, whereas the chromoionophore is deprotonated upon complexation of the divalent metal ions. The lack of a shift of λ_{max} for the Cu^{2+} and Ba^{2+} cases shows that these metal ions were not extracted. The extraction of Li^+, Ag^+, and all divalent metal ions by chromoionophore **3** was accompanied by a red shift.

Table 1. λ_{max} of (complexes of) chromoionophores **2** and **3** in dichloromethane. The samples were prepared by solid extraction of perchlorate salts by solutions of the receptors (2.75×10^{-5} and 2.70×10^{-5} M, respectively) containing a trace of acetic acid.

Cation	λ_{max} **2** [nm]	λ_{max} **3** [nm]
NEt_4^+	470	386
Li^+	378	486
Na^+	468	384
K^+	468	384
Cs^+	470	384
Ag^+	386	486
Ca^{2+}	486	490
Ba^{2+}	470	480
Cu^{2+}	470	486
Cd^{2+}	496	498
Pb^{2+}	492	486

Optode membranes: Chromoionophores **1**–**3** were incorporated in the membrane together with one equivalent of the lipophilic anion tetrakis[3,5-bis(trifluoromethyl)phenyl]borate. To achieve electro-neutrality, a counter ion for the anionic site is necessary. The measurements described here were conducted in the presence of Na^+. Upon exposure of the membranes to divalent metal ions, ion-exchange may occur in which the divalent metal ion replaces both a monovalent cation and the proton originating from the chromoionophore. Depending on whether the monovalent cation is complexed by the chromoionophore, there are two possibilities for ion-exchange (Figure 1).

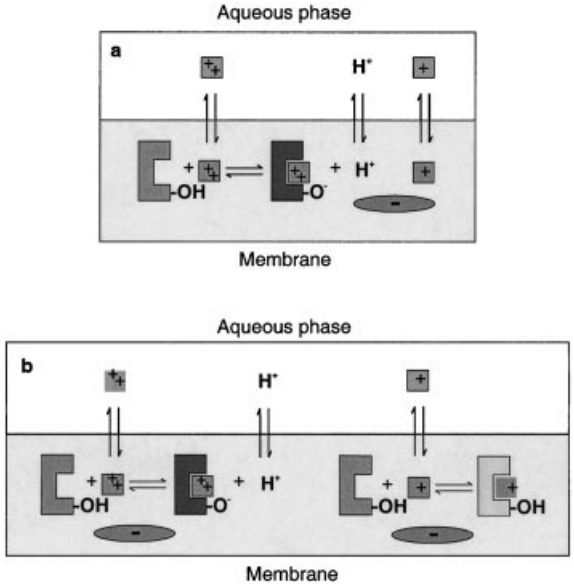


Figure 1. Response mechanism of optode membranes containing chromoionophores **1**–**3**. a) The monovalent metal counterion is not complexed by the ligand inside the membrane. Partitioning divalent metal ions are exchanged for a proton and a monovalent cation, accompanied by a red shift of λ_{max}. b) The monovalent metal ion is complexed by the ligand inside the membrane, giving rise to a blue shift of λ_{max}. Partitioning of the divalent metal ion results in a red shift of λ_{max} caused by the difference in color of the two different complexes.

The absorption spectra of optode membranes containing chromoionophores **1** or **2** are the same as those of the chromoionophores in solution. Variation of the Na^+ concentration did not affect the absorption spectra; this eliminates specific binding of Na^+ ions by these chromoionophores. Exposure to Pb^{2+} leads to a concentration dependent red shift of λ_{max}, as can be seen from the titration curve of a membrane containing **1** depicted in Figure 2. The isosbestic point at

0947-6539/01/0722-4880 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 22

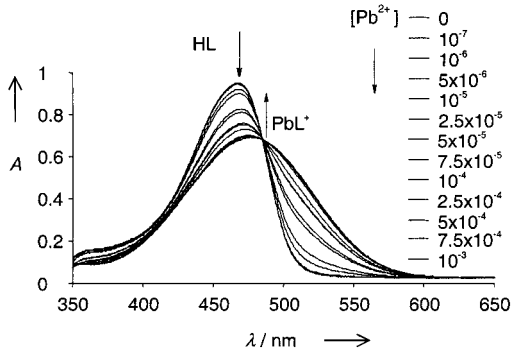


Figure 2. Response of optode membrane containing **1** to Pb^{2+} ions. Upon titration with Pb^{2+}, the absorption peak due to the free ligand (HL, $\lambda_{max} = 470$ nm) decreases in intensity, while the absorption peak of the deprotonated Pb^{2+} complex (PbL^+, $\lambda_{max} = 495$ nm) increases. [NaCl] = 10^{-3} M, pH = 6.5 (MES).

484 nm proves that there are two absorbing species involved in the exchange mechanism. The response should therefore be according to the ion-exchange mechanism depicted in Figure 1a. Membranes containing chromoionophore **2** show the same type of response.

Further evidence that Na^+ is not bound by chromoionophores **1** and **2** in the membrane comes from control experiments with membranes containing chromoionophore **3**. In these membranes, binding of Na^+ by the neutral chromoionophore does occur and a blue shift of λ_{max} is observed (Figure 3a). Upon subsequent exposure to increasing concentrations of Ca^{2+} at a constant Na^+ concentration these monovalent ions are replaced and a deprotonated complex with Ca^{2+} is formed, as indicated by the concomitant red shift (Figure 3b). The absence of isosbestic points indicates that more than two species are involved in the ion-exchange equilibria of membranes containing chromoionophore **3**.

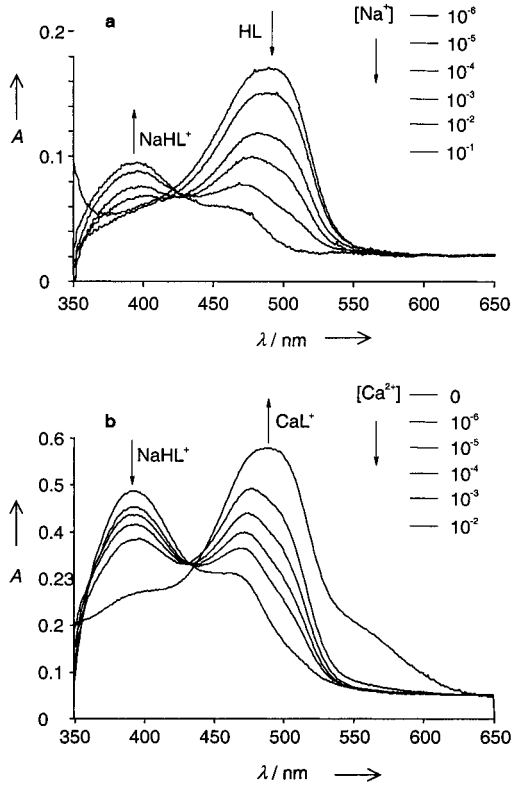


Figure 3. Responses of optode membrane containing **3** to Na^+ and Ca^{2+} ions. a) As the concentration of Na^+ is raised, the intensity of the peak due to free ligand decreases and the $NaHL^+$ complex is formed. b) When the membrane is exposed to Ca^{2+}, formation of the deprotonated complex CaL^+ again leads to a red shift.

The ion-exchange mechanism: The overall ion-exchange equilibrium in membranes containing **1** or **2** is expressed by Equation (1), in which HL and PbL^+ represent the chromoionophore and the deprotonated complex with Pb^{2+}, respectively.

The corresponding exchange constant $K_{exch}^{PbL^+}$ of the system for Pb^{2+} ions is expressed in Equation (2), in which it is assumed that the activities of species in the membrane are proportional to their concentrations. Because the measurements were conducted at constant Na^+ concentration, and the measured Pb^{2+} concentrations are low, the same approximation was made for the species in the aqueous phase.

$$Pb^{2+}_{aq} + HL_{mem} + Na^+_{mem} \rightleftharpoons PbL^+_{mem} + H^+_{aq} + Na^+_{aq} \quad (1)$$

$$K_{exch}^{PbL^+} = \frac{[PbL^+]_{mem}[H^+]_{aq}[Na^+]_{aq}}{[Pb^{2+}]_{aq}[HL]_{mem}[Na^+]_{mem}} = \frac{k_{Pb^{2+}} K_{PbL^+}}{k_{H^+} k_{Na^+} K_{HL}} \quad (2)$$

In this expression, $k_{Pb^{2+}}$, k_{H^+}, and k_{Na^+} are the relative lipophilicities (partition coefficients) of Pb^{2+}, H^+, and Na^+, respectively. The protonation constant K_{HL}[21] of the chromoionophore and the complexation constant K_{PbL^+}[22] are defined in the membrane. The response curves of optical sensors are usually depicted as the normalized absorption α, as a function of the metal-ion concentration. This makes it possible to directly compare membranes of different thickness. The measured absorption is converted into α by Equation (3), in which A_{HL} is the absorption of the free ligand, A_{PbL^+} the absorption of the complex, and A the measured absorption. At 530 nm, the only absorbing species is the complex PbL^+, so that α is equal to the molar fraction of the complex f_{PbL^+}.

$$\alpha = \frac{A_{HL} - A}{A_{HL} - A_{PbL^+}} \quad (3a)$$

$$\alpha_{530} = \frac{[PbL^+]_{mem}}{[L_T]_{mem}} = f_{PbL^+} \quad (3b)$$

In Equation (3b), $[L_T]_{mem}$ is the total concentration of chromoionophore. In these membranes, $[L_T]_{mem}$ is equal to the concentration of lipophilic anionic sites $[R^-]_{mem}$. When Equation (2) is combined with the mass balance for the chromoionophore[23] and the electro-neutrality condition,[24] the response function in Equation (4) can be derived, which is used to fit the observed absorption and to calculate $K_{exch}^{PbL^+}$.[25]

$$\frac{K_{exch}^{PbL^+}[L_T]_{mem}}{[H^+]_{aq}[Na^+]_{aq}} = \frac{f_{PbL^+}}{f^2_{HL}[Pb^{2+}]_{aq}} \quad (4)$$

Equation (2) shows that the sensitivity to Pb^{2+} depends on both the pH and the Na^+ concentration. This implies that the binding curves for Pb^{2+} should be measured with pH-buffered solutions with a constant $[Na^+]$. Measuring the response

curves at different pH revealed the dependence of the sensitivity on the pH. Figure 4 shows the response curves at 530 nm for **2** at different pH values. The membrane clearly becomes more sensitive to Pb^{2+} as the pH increases. Fitting of

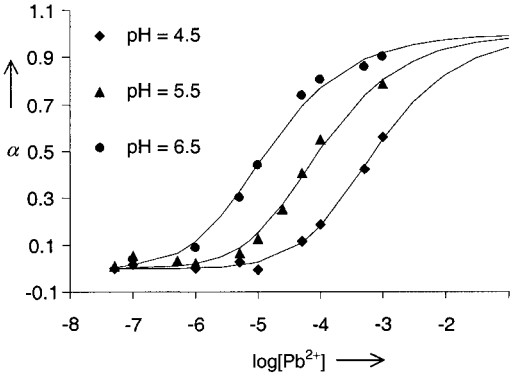


Figure 4. Normalized response curves to Pb^{2+} of membranes containing **2**, measured at pH = 4.5, 5.5, and 6.5. [NaCl] = 10^{-3} M, λ = 530 nm. The solid lines are calculated with the $K_{exch}^{PbL^+}$ obtained from the fit: 2.75×10^3, 1.96×10^4, and 1.45×10^5, respectively.

the response at pH 4.5, 5.5, and 6.5 leads to values for $K_{exch}^{PbL^+}$ of 2.75×10^3, 1.96×10^{-4}, and 1.45×10^{-5}, respectively. By varying the pH, the sensitivity of the optode can therefore be tuned as required.

Optode membranes containing chromoionophore **1** showed the same response behavior as membranes containing **2**. The sensitivity toward Pb^{2+} also depends on the Na^+ concentration. The $K_{exch}^{PbL^+}$ values obtained from fitting the response curves measured at different pH and [Na^+] are given in Figure 5. For both chromoionophores **1** and **2**, plotting

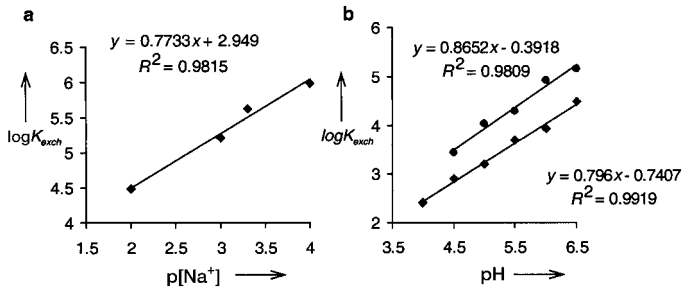


Figure 5. Dependence of the sensitivity on [Na^+] and pH. a) Dependence of $K_{exch}^{PbL^+}$ of membranes containing compound **1** on [Na^+]. b) Dependence of $K_{exch}^{PbL^+}$ of membranes containing compound **1** (◆) and **2** (●) on the pH. The lines were obtained from a least square fit.

$\log K_{exch}^{PbL^+}$ against the pH or log[Na^+] gives straight lines, in agreement with the proposed ion-exchange model. This is a clear mechanistic divergence from the response to divalent metal ions of optode membranes operating by transduction schemes with pH indicators. In those systems, two protons need to be exchanged for a divalent metal ion; this increases the pH dependency of the sensitivity as well as the selectivity of the response.[2] The data currently available do not allow interpretation of the deviation of the slopes from one. Membranes with chromoionophore **2** have the highest $K_{exch}^{PbL^+}$ over the whole pH range of the measurements, probably as a result of the preorganization of this ligand in the correct conformation for binding Pb^{2+} ions.

Selectivity: In the presence of Na^+ ions, membranes with chromoionophores **1** and **2** are remarkably selective for Pb^{2+} ions. The only other metal ions that caused responses were Cs^+ or Ag^+. These monovalent metal ions exchange with Na^+ in the membrane without deprotonation of the chromoionophore, resulting in hypsochromic shifts. Although in this case a blue shift is also observed, the response of a membrane containing **2** to Ag^+ (Figure 6) is different from the response

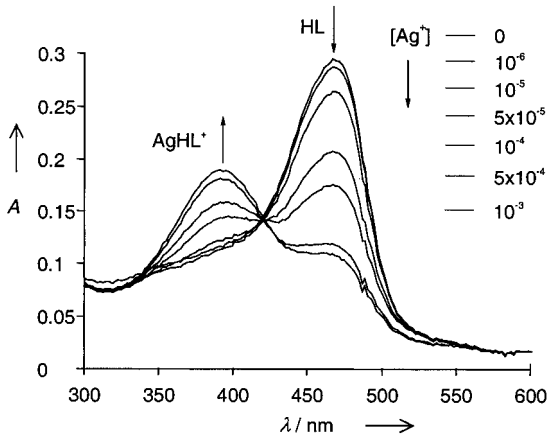


Figure 6. Response of membrane containing compound **2** to Ag^+. Upon exposure to Ag^+, the absorption shifts from the spectrum of the free ligand (HL, λ_{max} = 470), to that of the protonated Ag^+ complex ($AgHL^+$, λ_{max} = 395). [$NaNO_3$] = 10^{-3} M, pH = 6.5 (MES).

of membranes containing chromoionophore **3** to Na^+ (Figure 3a). The isosbestic point observed in the response to Ag^+ shows that in this case, only two absorbing species are involved in the equilibria, the free ligand and the Ag^+ complex.

Other alkali and alkaline-earth metal ions did not provoke a color change. Even Cu^{2+} and Cd^{2+}, metal ions that usually interfere severely with sensing of Pb^{2+}, did not cause any response up to concentrations of 10^{-3} M. This compares favorably with the extrinsic Pb^{2+}-sensitive optodes reported by Simon and co-workers[26] and Pretsch and co-workers,[27] which were more sensitive to Cd^{2+} and Ag^+ than to Pb^{2+}, while Cu^{2+} caused irreversible effects. As Pb^{2+} partitions only slightly better into bis(2-ethylhexyl)sebacate/poly(vinyl chloride) (DOS/PVC) than Ca^{2+}, Cd^{2+}, and Cu^{2+},[28] the selectivity of membranes containing **1** or **2** for Pb^{2+} is mainly due to the specificity of the ligand. In absence of monovalent metal ions, the ion-exchange mechanism does not function correctly and other divalent metal ions are extracted into the membrane, producing red shifts of λ_{max}. The selectivity K_{sel} of the optode membrane for Pb^{2+} over other divalent metal ions is defined by Equation (5), in which k is the partition coefficient for the metal ion, and K_{ML^+} the complexation constant of the metal ion in the membrane.

$$K_{sel} = \frac{K_{exch}^{PbL^+}}{K_{exch}^{ML^+}} = \frac{k_{Pb^{2+}} K_{PbL^+}}{k_{M^{2+}} K_{ML^+}} \quad (5)$$

0947-6539/01/0722-4882 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 22

In Figure 7 the selectivity of the optode membranes is illustrated by a plot of the response to Pb^{2+} in the absence and presence of a group of interfering metal ions. The small

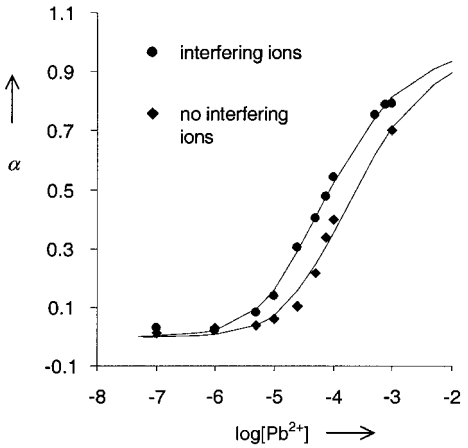


Figure 7. Response of membrane containing compound **2** to Pb^{2+} in presence of interfering ions. Normalized response to Pb^{2+} ions in absence (◆) and presence (●) of interfering ions: K^+, Cd^{2+}, Cu^{2+}, and Ca^{2+} (all 10^{-4} M) and Hg^{2+} (10^{-5} M). $[NaNO_3] = 10^{-3}$ M, pH = 6.5 (MES). The solid lines are calculated with a fitted $K_{exch}^{PbL^+}$ of 8.6×10^3 and 2.4×10^4, respectively.

increase in sensitivity to Pb^{2+} in the presence of interfering ions might be related to the increased ionic strength, which influences the water content of the membrane.[29]

The high selectivity over alkali metal ions, which have higher partition coefficients,[28] is also related to the ion-exchange mechanism. The lipophilic anions in the membrane prevent deprotonation of the chromoionophores upon complexation of monovalent metal ions. Therefore, the electrostatic contribution to the binding energy due to the negative charge on the ligand is only present with divalent cations. The importance of this effect becomes especially clear when the response to metal ions of membranes containing nonselective chromoionophore **3** is considered. The three amide groups render **3** a good ligand for Na^+, which is complexed in the membrane leading to a blue shift of λ_{max} (Figure 3a). However, even in the presence of a 100-fold excess of Na^+, the membrane with chromoionophore **3** was sensitive to Ca^{2+} and even Pb^{2+}, even though Na^+ partitions much better into the membrane than these divalent metal ions. This leads to the conclusion that $K_{CaL^+} > K_{PbL^+} \gg K_{NaHL^+}$. Membranes containing **3** also respond to other metal ions; however, the complex responses could not be fitted with the same accuracy as the response of membranes with **1** and **2**.

Reversibility and response time: The response curve shown in Figure 8 shows that the membrane response is completely reversible. The data depicted represent part of a three-day session of full-time measurements with a flow cell. At the end of that durability test, the total response had decreased to about 76% of the original. This is probably due to leaching of the "lipophilic" anion, which is more hydrophilic than the calix[4]arene chromoionophore. Loss of anionic sites reduces the fraction of receptors that complex ions at full saturation.

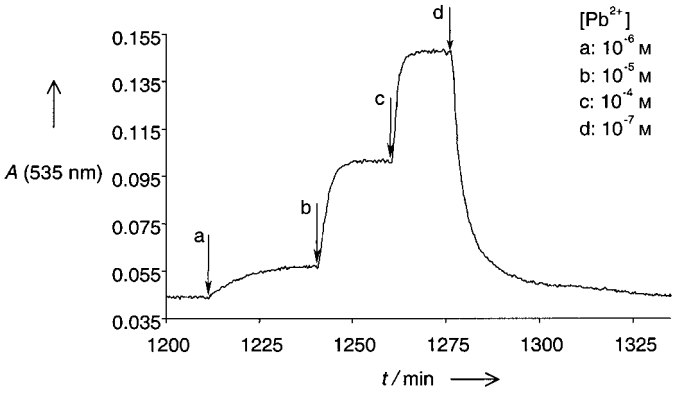


Figure 8. Time dependence of the response of a membrane containing compound **1** to Pb^{2+} ions. Response to $Pb(NO_3)_2$ at pH 6.5 (MES) and 10^{-3} M $NaNO_3$, as measured in time during a three-day continuous measurement. Arrows indicate the switch to solutions with the respective Pb^{2+} concentrations.

The response time of this type of optode membrane depends on a number of variables such as the membrane thickness, receptor concentration, flow-rate, and the concentration of the analyte ion. The optode membranes described here have not been optimized with respect to these variables. The cast membranes with an estimated thickness of about 3 μm needed about 20 minutes to develop 95% of the equilibrium signal upon exposure to a Pb^{2+} concentration of 10^{-6} M, whereas it took only four minutes to respond to a Pb^{2+} concentration of 10^{-4} M. The extraction rate is higher than the wash-out rate. The slow response to low concentrations of Pb^{2+} is a practical problem in determining the detection limit. However, the response time may be improved with spin-coated membranes and optimized component concentrations, making it possible to measure in conditions (neutral pH, lower $[Na^+]$) with detection limits below 10^{-7} M.

Conclusion

Bulk optodes with high sensitivity and selectivity for Pb^{2+} ions have been prepared by the incorporation of calix[4]arene chromoionophores in plasticized PVC membranes. The results demonstrate that intrinsic optical sensors which use chromoionophores have advantages over extrinsic sensors in terms of sensitivity and selectivity. Because of the integration of the chromophore with the receptor unit, the optode membranes containing **1** and **2** respond only to ions that are complexed, not to those that partition into the membrane without binding to the receptor. It was therefore possible to introduce a novel ion-exchange mechanism for optode membranes, which makes use of monovalent metal ions and protons as the exchanged ions. The responses of membranes containing chromoionophore **3** illustrate that enhanced selectivity for divalent metal ions is already achieved with this ion-exchange scheme alone, because of the extra electrostatic interaction when the chromoionophores are deprotonated in complexes with divalent metal ions. This is important, since monovalent metal ions have higher partition coefficients. The sensitivity of the resulting membrane is comparable to that of

the best extrinsic optode membranes,[26] whereas different characteristics are obtained in terms of modulation of the pH and the presence of other ions. An advantage of the intrinsic transduction mechanism is that interfering monovalent metal ions give a blue shift of the absorption maximum instead of a red shift. This should make it possible to eliminate their interference during data analysis.[30]

Experimental Section

General: All chemicals used for synthesis were of reagent grade quality, obtained from Acros, Merck, or Aldrich, and were used without further purification. Solvents were dried and purified by standard laboratory methods. THF was freshly distilled from Na. Silica gel 60 was used for all column chromatography and preparative TLC. NMR spectra were recorded on a Varian Unity INOVA (300 MHz) spectrometer. FAB-MS spectra were measured on a Finnigan MAT 90 spectrometer with *m*-nitrobenzylalcohol (NBA) as matrix. Melting points are uncorrected. All reactions were conducted under argon atmosphere unless otherwise noted. In the systematic name of the prepared compounds, calix[4]arene is used in stead of the official chemical abstract name: pentacyclo-[19,3,1,1^{3,7},1^{0,13},1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-25,26,27,28-tetrol. The optode membranes were prepared with high molecular weight poly(vinyl chloride) (PVC), bis(2-ethylhexyl)sebacate (DOS), and potassium tetrakis[1,3-bis(trifluoromethyl)phenyl]borate from Fluka. The optode membrane measurements were conducted at 21 °C, with either a flow cell or an optical fiber probe. The Helma flow cell was used in combination with a peristaltic pump (Gilson model M313) and a Hewlett Packard 8452 diode array spectrophotometer; the Helma optical fiber probe was used in combination with a Cary 3E UV-visible spectrophotometer, in the spectral region of 350 – 650 nm, as the switching between the Vis and UV lamps below 350 nm gave offsets in the spectra. During purification by column chromatography, compounds containing amido groups were usually eluted from silica by using a 15:1 mixture of ethyl acetate and methanol, in which $NaClO_4$ (0.5 g per 500 mL eluent) was dissolved. After evaporation of the solvents under reduced pressure, the product was therefore isolated as the Na^+ complex. The salts were removed by adding dichloromethane to the residue followed by extensive washing with dilute HCl (0.01 M) and water. The salt-free product was obtained either by precipitation, or by evaporation of the dichloromethane. This frequently led to inclusion of dichloromethane in the solid, as shown by the elemental analysis and ^{1}H NMR spectra.

25,27-Dihydroxy-26-methoxy-28-phenylmethoxycalix[4]arene (6): Monomethoxy calix[4]arene **4** (1.50 g, 3.5 mmol), K_2CO_3 (0.73 g, 4.8 mmol), and benzyl bromide (0.88 g, 4.8 mmol) were added to acetonitrile (250 mL), after which the mixture was heated under reflux at 90 °C for 48 h. The solvent was then evaporated under reduced pressure and dichloromethane (100 mL) was added to the residue. This was followed by washing with dilute HCl (3 × 50 mL, 0.01 M) and water (50 mL). The product was purified by column chromatography (SiO_2, dichloromethane/hexane 1:1) followed by precipitation from dichloromethane/hexane to give 0.64 g (34 %) of compound **6** as a white powder. M.p. 253 – 258 °C; ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 3.31 and 4.30 (ABq, ^2J(H,H) = 13.2 Hz, 4H; Ar-CH$_2$-Ar), 3.41 and 4.34 (ABq, ^2J(H,H) = 13.2 Hz, 4H; Ar-CH$_2$-Ar), 4.01 (s, 3H; O-CH$_3$), 5.12 (s, 2H; O-CH$_2$-Ar), 6.68 (t, ^3J(H,H) = 7.2 Hz, 2H; *p*ArH), 6.73 – 6.78 (m, 2H; *p*ArH), 6.90 (br d, ^3J(H,H) = 7.2 Hz, 4H; *m*ArH), 7.06 and 7.09 (2 dd, ^3J(H,H) = 4.2 Hz, ^4J(H,H) = 1.8 Hz, 4H; *m*ArH), 7.41 – 7.50 (m, 3H; ArH), 7.65 (dd, ^3J(H,H) = 7.5 Hz, ^4J(H,H) = 1.8 Hz, 2H; *o*ArH), 7.77 (s, 2H; OH); MS (FAB): *m*/*z* (%): 529.2 (100) [*M*+H]$^+$, 551.2 (10) [*M*+Na]$^+$; elemental analysis calcd (%) for $C_{36}H_{32}O_4 \cdot 0.05\ CH_2Cl_2$ (528.65): C 81.25, H 6.07; found: C 81.24, H 6.06.

25,27-Dihydroxy-26-phenylmethoxy-28-propoxycalix[4]arene (7): Monopropoxy calix[4]arene **5** (3.10 g, 6.6 mmol), K_2CO_3 (0.96 g, 6.6 mmol), and benzyl bromide (5.94 g, 33 mmol) were added to acetonitrile (300 mL). The mixture was heated under reflux overnight at 90 °C. After evaporation of the solvent, dichloromethane (250 mL) was added to the crude product, followed by washing with aqueous HCl (3 × 100 mL, 0.01 M) and water (100 mL). The product was purified by column chromatography (SiO_2, dichloromethane/hexane 1:1), followed by precipitation (methanol/dichloromethane) to yield 1.26 g (34 %) of **7** as a white powder. M.p. 231 – 236 °C; ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 1.28 (t, ^3J(H,H) = 7.5 Hz, 3H; CH$_2$CH$_2$-C*H*$_3$), 2.09 (m, ^3J(H,H) = 7.5 Hz, 2H; CH$_2$-C*H*$_2$-CH$_3$), 3.41 and 4.34 (ABq, ^2J(H,H) = 12.9 Hz, 4H; Ar-CH$_2$-Ar), 3.42 and 4.40 (ABq, ^2J(H,H) = 13.2 Hz, 4H; Ar-CH$_2$-Ar), 3.99 (t, ^3J(H,H) = 6.6 Hz, 2H; O-C*H*$_2$-CH$_2$CH$_3$), 5.11 (s, 2H; O-CH$_2$-Ph), 6.69 (t, ^3J(H,H) = 7.5 Hz, 2H; *p*ArH), 6.74 – 6.83 (m, 2H; *p*ArH) , 6.95 (d, ^3J(H,H) = 7.5 Hz, 2H; *m*ArH), 6.97 (d, ^3J(H,H) = 7.8 Hz, 2H; *m*ArH), 7.11 (br d, ^3J(H,H) = 7.8 Hz, 4H; *m*ArH), 7.42 – 7.52 (m, 3H; *m*,*p*ArH), 7.79 (dd, ^3J(H,H) = 6.6 Hz, ^4J(H,H) = 1.5 Hz, 2H; *o*ArH), 8.18 (s, 2H; OH); MS (FAB): *m*/*z* (%): 556.3 (100) [*M*]$^+$, 557.3 (90) [*M*+H]$^+$, 579.3 (6) [*M*+Na]$^+$; elemental analysis calcd (%) for $C_{38}H_{36}O_4 \cdot 0.75\ H_2O$ (557.71): C 80.04, H 6.63; found: C 80.07, H 6.21.

25,27-Bis(dimethylaminocarbonylmethoxy)-26-methoxy-28-phenylmethoxycalix[4]arene (8): Calix[4]arene **6** (0.490 g, 0.93 mmol), NaH (0.051 g, 1.86 mmol), and *N*,*N*-dimethylchloroacetamide (0.67 g, 5.58 mmol) were mixed in acetonitrile (125 mL). This mixture was heated under reflux overnight at 90 °C. After the solvent was removed under reduced pressure, the residue was taken up in dichloromethane (60 mL) and washed with aqueous HCl (3 × 30 mL, 0.01 M) and water (30 mL). The crude product was purified by column chromatography (SiO_2, ethyl acetate/methanol/Na-ClO_4). Most impurities were eluted off the column with 5 % methanol in ethyl acetate, after which $NaClO_4$ was added to the eluent (0.5 g in 500 mL) and **6** was collected, yielding 0.50 g (77 %). ^{1}H NMR (300 MHz, [D$_3$]acetonitrile, $NaClO_4$, 25 °C): δ = 2.82 (s, 6H; N-CH$_3$), 3.19 (s, 6H; N-CH$_3$), 3.23 and 3.78, 3.47 and 4.22 (2 ABq, ^2J(H,H) = 12.5 Hz, 8H; Ar-CH$_2$-Ar), 3.84 (s, 3H; O-CH$_3$), 4.48 and 4.57 (ABq, ^2J(H,H) = 14.7 Hz, 4H; O-CH$_2$-C(O)), 5.01 (s, 2H; O-CH$_2$-Ph), 6.89 (t, ^3J(H,H) = 7.8 Hz, 2H; *p*ArH), 6.91 (t, ^3J(H,H) = 7.8 Hz, 1H; *p*ArH), 6.92 (t, ^3J(H,H) = 7.8 Hz, 1H; *p*ArH), 7.19 – 7.24 (m, 6H; ArH) 7.26 (d, ^3J(H,H) = 7.8 Hz, 2H; *m*ArH), 7.28 (d, ^3J(H,H) = 7.8 Hz, 2H; *m*ArH), 7.35 (dd, ^3J(H,H) = 9.9 Hz, ^4J(H,H) = 2.1 Hz, 2H; ArH), 7.49 (dd, ^3J(H,H) = 7.5 Hz, ^4J(H,H) = 2.7 Hz, 1H; ArH); ^{13}C NMR (300 MHz, [D$_3$]acetonitrile, $NaClO_4$, 25 °C): δ = 29.08, 29.39, 34.94, 63.38, 73.67, 79.75, 125.74, 126.25, 128.53, 129.10, 129.21, 129.37, 130.51, 135.12, 135.47, 135.53, 135.81, 136.16, 150.96, 151.86, 153.96, 168.89; MS (FAB): *m*/*z* (%): 721.3 (100) [*M*+Na]$^+$.

25,27-Bis(dimethylaminocarbonylmethoxy)-26-phenylmethoxy-28-propoxycalix[4]arene (1,3-alternate) (9): Calix[4]arene **7** (0.150 g, 0.27 mmol), Cs_2CO_3 (0.507 g, 1.6 mmol), and *N*,*N*-dimethylchloroacetamide were dissolved in DMF (50 mL). The mixture was heated overnight at 90 °C. After removal of the solvent under reduced pressure, the crude product was taken up in dichloromethane (60 mL) and washed with aqueous HCl (3 × 30 mL, 0.01 M) and water (30 mL). The product was twice purified by preparative TLC (SiO_2, first: methanol/ethyl acetate 1:10, second: methanol/ethyl acetate 1:7). Recrystallization from dichloromethane/hexane afforded 50 mg (31 %) of compound **9**. M.p. 192 – 198 °C; ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 0.97 (t, ^3J(H,H) = 7.5 Hz, 3H; CH$_2$CH$_2$-C*H*$_3$), 1.70 (m, ^3J(H,H) = 7.5 Hz, 2H; CH$_2$-C*H*$_2$-CH$_3$), 2.55 (s, 6H; N-CH$_3$), 2.92 (s, 6H; N-CH$_3$), 3.57 – 3.82 (m, 10H; Ar-CH$_2$-Ar and -C*H*$_2$-CH$_2$CH$_3$), 4.15 (s, 4H; O-CH$_2$-C(O)), 4.77 (s, 2H; O-CH$_2$-Ph), 6.45 (t, ^3J(H,H) = 7.8 Hz, 2H; *p*ArH), 6.63 (bd, ^3J(H,H) = 7.2 Hz, 2H; *m*ArH), 6.72 (t, ^3J(H,H) = 7.5 Hz, 1H; *p*ArH), 6.78 (t, ^3J(H,H) = 7.2 Hz, 1H; *p*ArH), 6.93 (dd, ^3J(H,H) = 6.9 Hz, ^4J(H,H) = 1.5 Hz, 2H; *o*ArH), 7.02 – 7.07 (m, 6H; *m*ArH), 7.23 – 7.29 (m, 3H; *m*,*p*ArH); ^{13}C NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 9.94, 23.47, 35.14, 37.04, 37.29, 37.39, 71.72, 72.50, 73.07, 117.82, 122.22, 122.75, 122.82, 127.27, 127.84, 127.97, 130.40, 130.92, 131.05, 134.12, 135.00, 135.25, 138.33, 157.08, 157.82, 161.04, 168.87; MS (FAB): *m*/*z* (%): 727.4 (53) [*M*+H]$^+$, 749.4 (100) [*M*+Na]$^+$.

25,27-Bis(dimethylaminocarbonylmethoxy)-26-hydroxy-28-methoxycalix[4]arene (10): Calix[4]arene **8** (0.40 g, 0.58 mmol) was dissolved in 1:1 ethanol/THF (50 mL), after which the mixture was deoxygenated by bubbling through argon. A catalytic amount of 5 % Pd on activated carbon was added, and the flask was then flushed with hydrogen. The reaction mixture was stirred overnight under a hydrogen atmosphere at room temperature. The catalyst was removed by filtration through paper. The solvents were removed under reduced pressure, and the product was purified by preparative TLC (SiO_2, methanol/ethyl acetate 1:10), yielding 170 mg (49 %) of compound **10** as a white powder. M.p. 102 – 109 °C; ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 3.05 (s, 6H; N-CH$_3$), 3.21 (s, 6H; N-CH$_3$), 3.31, 3.37, 4.39, and 4.48 (2 ABq, ^2J(H,H) = 13.5 Hz, 8H;

0947-6539/01/0722-4884 $ 17.50+.50/0 *Chem. Eur. J.* **2001**, *7*, No. 22

Ar-CH_2-Ar), 4.00 (s, 3H; O-CH_3), 4.49 and 4.66 (ABq, ^2J(H,H) = 12.9 Hz, 4H; O-CH_2-C(O)), 6.47–6.56 (m, 6H; ArH), 6.80 (t, ^3J(H,H) = 7.2 Hz, 1H; *p*ArH), 6.97 (t, ^3J(H,H) = 7.2 Hz, 1H; *p*ArH), 7.13 (d, ^3J(H,H) = 7.8 Hz, 2H; *m*ArH), 7.18 (d, ^3J(H,H) = 7.8 Hz, 2H; *m*ArH), 8.10 (s, 1H; OH); ^{13}C NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 30.59, 35.03, 36.22, 60.39, 73.50, 119.06, 122.83, 123.47, 127.62, 127.72, 127.93, 128.64, 130.32, 131.80, 133.03, 136.04, 152.76, 153.99, 157.44, 167.42; MS (FAB): *m*/*z* (%): 608.4 (31) $[M]^+$, 609.4 (94) ($[M+H]^+$, 631.5 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{37}H_{40}N_2O_6$ (608.74): C 73.01, H 6.62, N 4.60; found: C 72.93, H 6.65, N 4.53.

25,27-Bis(dimethylaminocarbonylmethoxy)-26-hydroxy-28-propoxycalix-[4]arene (partial-cone) (11): 1,3 Alternate calix[4]arene **9** (0.70 g, 0.96 mmol) was dissolved in 1:1 ethanol/THF (100 mL). This was saturated with argon for half an hour. A catalytic amount of 5% Pd on activated carbon was added, and the flask flushed with hydrogen. The reaction mixture was stirred overnight at room temperature under a hydrogen atmosphere. The mixture was then filtered through paper twice, and the solvents were evaporated. The product was purified by preparative TLC (SiO_2, methanol/dichloromethane 1:1, $NaClO_4$). This yielded 200 mg (33%) of pure **11** as a white powder. M.p. 212–215 °C; ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 0.62 (t, ^3J(H,H) = 7.5 Hz, 3H; CH_2CH_2-CH_3), 1.27 (sx, ^3J(H,H) = 7.5 Hz, 2H; CH_2-CH_2-CH_3), 3.02 (s, 6H; N-CH_3), 3.04 (s, 6H; N-CH_3), 3.24 (t, ^3J(H,H) = 7.5 Hz, 2H; O-CH_2-CH_2CH_3), 3.28 and 4.2 (ABq, ^2J(H,H) = 13.2 Hz, 4H; Ar-CH_2-Ar), 3.85 and 3.91 (ABq, ^2J(H,H) = 15.6 Hz, 4H; Ar-CH_2-Ar), 4.41 and 4.62 (ABq, ^2J(H,H) = 12.6 Hz, 4H; O-CH_2-C(O)), 4.73 (s, 1H; OH), 6.67 (t, ^3J(H,H) = 7.2 Hz, 1H; *p*ArH), 6.76 (t, ^3J(H,H) = 7.5 Hz, 2H; *p*ArH), 6.88 (dd, ^3J(H,H) = 7.8 Hz, ^4J(H,H) = 1.7 Hz, 2H; *m*ArH), 6.90 (t, ^3J(H,H) = 7.2 Hz, 1H; *p*ArH), 7.00 (dd, ^3J(H,H) = 7.2 Hz, ^4J(H,H) = 1.5 Hz, 2H; *m*ArH), 7.05 (d, ^3J(H,H) = 7.5 Hz, 2H; *m*ArH), 7.22 (d, ^3J(H,H) = 7.8 Hz, 2H; *m*ArH); ^{13}C NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 9.25, 22.39, 30.66, 35.13, 36.41, 37.31, 72.05, 72.23, 117.93, 121.20, 123.53, 127.33, 127.64, 128.92, 129.75, 132.58, 133.06, 133.66, 152.75, 154.29, 156.43, 167.38; MS (FAB): *m*/*z* (%): 636.1 (27) $[M]^+$, 637.2 (80) $[M+H]^+$, 659.2 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{39}H_{44}N_2O_6$ (636.79): C 73.56, H 6.96, N 4.40; found: C 73.30, H 7.02, N 4.56.

25,27-Bis(dimethylaminocarbonylmethoxy)-26-hydroxy-28-methoxy-11-*p*-nitrophenyldiazacalix[4]arene (1): *p*-Nitroaniline (0.25 g, 1 mmol) and aqueous HCl (0.2 mL 12 M, 2.4 mmol) were added to a mixture of methanol (50 mL) and water (25 mL), and the mixture was cooled to 0 °C. $NaNO_2$ (0.069 g, 0.99 mmol) was added, after which the mixture was stirred for 30 minutes at 0 °C before it was added dropwise to a solution of calix[4]arene **10** (0.20 g, 0.33 mmol) in THF (250 mL) at 0 °C. After stirring for 30 minutes, $NaClO_4$ (100 mg, mmol) was added, and the reaction mixture was carefully titrated to neutral pH with an aqueous solution of KOH (1 M). After 3 h the reaction temperature was allowed to rise to room temperature, and the mixture was acidified with dilute HCl (0.1 M). The organic solvents were removed under reduced pressure, and the remaining mixture was extracted with dichloromethane (3 × 80 mL). The organic phase was washed with dilute HCl (3 × 20 mL, 0.01 M) and water (20 mL). Removal of the solvent afforded the crude product, which was purified by preparative TLC (SiO_2, methanol/dichloromethane, methanol/ethyl acetate), followed by precipitation from dichloromethane/hexane to yield 100 mg of compound **1** (40%) as a dark orange solid. M.p. 140 °C (decomp); ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 2.98 (s, 6H; N-CH_3), 3.10 (s, 6H; N-CH_3), 3.22 and 3.42, 4.35 and 4.38 (2 ABq, ^2J(H,H) = 13.2 Hz, 8H; Ar-CH_2-Ar), 3.91 (br s, 3H; O-CH_3), 4.53 (br s, 4H; O-CH_2-C(O)), 6.38–6.50 (m, 4H; *p*ArH), 6.57 (dd, ^3J(H,H) = 6.9 Hz, ^4J(H,H) = 2.1 Hz, 1H; *p*ArH), 6.83–7.14 (m, 6H; ArH), 7.74 (s, 2H; *m*ArH), 7.91 (dd, ^3J(H,H) = 8.3 Hz, ^4J(H,H) = 1.8 Hz, 2H; C(N$_2$)-CH), 8.29 (dd, ^3J(H,H) = 9.0 Hz, ^4J(H,H) = 2.1 Hz, 2H; C(NO$_2$)-CH); ^{13}C NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 30.53, 30.81, 35.06, 36.05, 60.07, 73.24, 122.36, 122.77, 123.63, 124.10, 124.20, 127.68, 128.16, 128.56, 129.61, 130.88, 133.13, 135.79, 144.97, 147.40, 153.94, 155.95, 157.58, 158.24, 167.24; UV/Vis (dichloromethane): λ_{max} (ε) = 450 nm (50000 mol^{-1} dm^3 cm^{-1}); MS (FAB): *m*/*z* (%): 758.4 (33) $[M+H]^+$, 780.5 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{43}H_{43}N_5O_8 \cdot 0.5\,H_2O$ (757.85): C 67.35, H 5.78, N 9.13; found: C 67.15, H 5.83, N 9.03.

25,27-Bis(dimethylaminocarbonylmethoxy)-26-hydroxy-28-propoxy-11-*p*-nitrophenyldiazacalix[4]arene (partial-cone) (2): *p*-Nitroaniline (0.104 g, 2.7 mmol) and aqueous HCl (0.25 mL 12 M, 3 mmol) were added to a mixture of methanol (25 mL) and water (125 mL) and cooled to 0 °C. $NaNO_2$ (0.057 g, 0.83 mmol) was added, after which the mixture was stirred for 30 minutes at 0 °C before it was added dropwise to a solution of calix[4]arene **11** (0.160 g, 0.25 mmol) and potassium *tert*-butoxide (0.28 g, 0.25 mmol) in THF (125 mL) at 0 °C. After 1 h stirring, $Pb(ClO_4)_2$ (50 mg, mmol) was added, and the reaction mixture stirred 1 h. The reaction mixture was then poured into water (800 mL), and the precipitate was filtrated off. The water layer was extracted with dichloromethane (3 × 80 mL) to remove the last traces of product, after which the organic layer was combined with the residue of the filtration. Washing of the dichloromethane solution with aqueous HCl (3 × 50 mL, 0.01 M and water (50 mL) and subsequent removal of the solvent afforded the crude product, which was purified by preparative TLC (SiO_2, methanol/dichloromethane 1:10), followed by precipitation from dichloromethane/hexane to yield 100 mg of compound **2** (51%) as a red/orange solid. M.p. 236–246 °C; ^{1}H NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 0.63 (t, ^3J(H,H) = 7.8 Hz, 3H; -$CH_2CH_2CH_3$), 1.28 (m, ^3J(H,H) = 7.8 Hz, 2H; -$CH_2CH_2CH_3$), 3.04 (s, 6H; N-CH_3), 3.07 (s, 6H; N-CH_3), 3.32 (t, ^3J(H,H) = 7.5 Hz, 2H; -$CH_2CH_2CH_3$), 3.42 and 4.26 (ABq, ^2J(H,H) = 13.2 Hz, 4H; Ar-CH_2-Ar), 3.87 and 3.93 (ABq, ^2J(H,H) = 16.6 Hz, 4H; Ar-CH_2-Ar), 4.43 and 4.69 (ABq, ^2J(H,H) = 13.2 Hz, 4H; O-CH_2-C(O)), 6.80 (t, ^3J(H,H) = 7.8 Hz, 2H; *p*ArH), 6.93 (t, ^3J(H,H) = 7.2 Hz, 1H; *p*ArH), 6.99 (d, ^3J(H,H) = 8.1 Hz, 2H; *m*ArH), 7.03 (d, ^3J(H,H) = 9.3 Hz, 2H; *m*ArH), 7.22 (d, ^3J(H,H) = 7.2 Hz, 2H; *m*ArH), 7.80 (s, 2H; *m*ArH), 7.99 (d, ^2J(H,H) = 9.0 Hz, 4H; C(N$_2$)-CH), 8.38 (d, ^2J(H,H) = 8.7 Hz, 2H; C(NO$_2$)-CH), 8.52 (s, 1H; OH); ^{13}C NMR (300 MHz, [D$_1$]chloroform, 25 °C): δ = 9.26, 22.56, 30.65, 35.21, 36.26, 37.44, 52.91, 71.82, 121.40, 122.29, 123.69, 123.92, 124.19, 128.35, 128.95, 129.16, 129.52, 131.83, 133.28, 133.61, 144.91, 147.28, 154.32, 156.05, 156.37, 158.04, 167.36; UV/Vis (dichloromethane): λ_{max} (ε) = 450 nm (50000 mol^{-1} dm^3 cm^{-1}); MS (FAB): *m*/*z* (%): 808.7 (100) $[M+Na]^+$; elemental analysis calcd (%) for $C_{45}H_{47}N_5O_8$ (785.90): C 68.77, H 6.03, N 8.91; found: C 68.53, H 6.00, N 8.85.

Membrane preparation: In THF (1 mL), chromoionophore (1 mg: 1.6 μmol **1**, 1.3 μmol **2**), 1 equivalent of potassium tetrakis[3,5-bis(trifluoro- methyl)]borate (1.4 mg, 1.6 μmol and 1.1 mg 1.3 μmol, respectively), high molecular weight PVC (26 mg), poly(urethane) (7 mg), and DOS (65 mg) were dissolved. 50 μL of the membrane solutions were cast directly onto the flow cell or the fiber optic probe, after which the THF was allowed to evaporate. Before using the membranes they were conditioned in a pH-buffered (10^{-3} M 2-(*N*-morpholinyl)ethanesulfonic acid (MES) buffer) solution of NaCl until a stable signal was obtained. The sample flow in the flow cell was 6 mL min^{-1}. The sample solutions measured with the fiber optic probe were stirred magnetically.

Acknowledgements

This research was supported by the Technology Foundation STW, applied science division of NWO and the technology program of the Ministry of Economic Affairs.

[1] a) S. C. Charlton, R. L. Fleming, A. Zipp, *Clin. Chem.* **1982**, *28*, 1857; b) S. C. Charlton, R. L. Fleming, P. Hemmes, A. L. Y. Lau, USA 4645744, **1987** [*Chem. Abstr.* **1987**, *107*, 3789c].

[2] a) W. E. Morf, K. Seiler, B. Lehmann, C. Behringer, K. Hartmann, W. Simon, *Pure Appl. Chem.* **1989**, *61*, 1613–1618; b) W. E. Morf, K. Seiler, B. Rusterholz, W. Simon, *Anal. Chem.* **1990**, *62*, 738–742.

[3] E. Bakker, P. Bühlmann, E. Pretsch, *Chem. Rev.* **1997**, *97*, 3083–3132.

[4] P. Bühlmann, E. Pretsch, E. Bakker, *Chem. Rev.* **1998**, *98*, 1593–1687.

[5] a) D. Diamond, M. A. McKervey, *Chem. Soc. Rev.* **1996**, 15–24; b) A. Ikeda, S. Shinkai, *Chem. Rev.* **1997**, 97, 1713–1734; c) Y. Kubo, S. Tokita, Y. Kojima, Y. T. Osano, T. Matsuzaki, *J. Org. Chem.* **1996**, *61*, 3758–3765; d) H. Mohindra Chawla, K. Srinivas, *J. Org. Chem.* **1996**, *61*, 8464–8467; e) J. L. M. Gordon, V. Böhmer, W. Vogt, *Tetrahedron Lett.* **1995**, *36*, 2445–2448; f) M. McCarrick, S. J. Harris, D. Diamond, *J. Mater. Chem.* **1994**, *4*, 217–221; g) H. Shimizu, K. Iwamoto, K. Fujimoto, S. Shinkai, *Chem. Lett.* **1991**, 2147–2150.

[6] 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*, 2767–2777; b) A. Casnati, A. Pochini, R.

Ungaro, C. Bocchi, F. Ugozzoli, R. J. M. Egberink, H. Struijk, R. Lugtenberg, F. de Jong, D. N. Reinhoudt, *Chem. Eur. J.* **1996**, *2*, 436 – 445.
[7] a) A. Arduini, A. Pochini, S. Reverbi, R. Ungaro, *J. Chem. Soc. Chem. Commun.* **1984**, 981 – 982. b) J. A. J. Brunink, J. G. Bomer, J. F. J. Engbersen, W. Verboom, D. N. Reinhoudt, *Sens. Actuators B* **1993**, *15 – 16*, 195 – 198.
[8] Y. Marcus, *Ion Properties*, Dekker, New York, **1997**.
[9] N. J. van der Veen, R. J. M. Egberink, J. F. J. Engbersen, F. C. J. M. van Veggel, D. N. Reinhoudt, *Chem. Commun.* **1999**, 681 – 682.
[10] a) J. F. Alder, D. C. Ashworth, R. Narayanaswamy, R. E. Moss, I. O. Sutherland, *Analyst* **1987**, *112*, 1191 – 1192; b) F. V. Bright, G. E. Poirier, G. M. Hieftje, *Talanta* **1988**, *35*, 113 – 118; c) I. Klimant, M. Otto, *Microchim. Acta* **1992**, *108*, 11 – 17; d) A. Morales-Bahnik, R. Czolk, J. Reichert, H. J. Ache, *Sens. Actuators B* **1993**, *13 – 14*, 424 – 426; e) Z. Gong, Z. Zhang, *Anal. Chim. Acta* **1996**, *325*, 201 – 204; f) N. Malcik, O. Oktar, M. E. Ozser, P. Caglar, L. Bushby, A. Vaughan, B. Kuswandi, R. Narayanaswamy, *Sens. Actuators B* **1998**, *53*, 211 – 221.
[11] a) R. Czolk, J. Reichert, H. J. Ache, *Sens. Actuators B* **1992**, *7*, 540 – 543; b) D. Radloff, C. Matern, M. Plaschke, D. Simon, J. Reichert, H. J. Ache, *Sens. Actuators B* **1996**, *35 – 36*, 207 – 211.
[12] N. J. van der Veen, S. Flink, M. A. Deij, R. J. M. Egberink, F. C. J. M. van Veggel, D. N. Reinhoudt, *J. Am. Chem. Soc.* **2000**, *121*, 6112 – 6113.
[13] a) K. Suzuki, K. Tohda, Y. Tanda, H. Ohzora, S. Nishihama, H. Inoue, T. Shirai, *Anal. Chem.* **1989**, *61*, 382 – 384; b) K. Wang, K. Seiler, B. Rusterholz, W. Simon, *Analyst* **1992**, *117*, 57 – 60; c) K. Tóth, B. T. T. Lan, J. Jeney, M. Horváth, I. Bitter, A. Grün, B. Ágai, L. Töke, *Talanta* **1994**, *41*, 1041 – 1049; d) T. Hayashita, M. Kumazawa, J. C. Lee, R. A. Bartsch, *Chem. Lett.* **1995**, 711 – 712; e) I. Oehme, B. Prokes, I. Murkovic, T. Werner, I. Klimant, O. S. Wolfbeis, *Fresenius' J. Anal. Chem.* **1994**, *350*, 563 – 567; f) I. Murkovic, I. Oehme, G. J. Mohr, T. Ferber, O. S. Wolfbeis, *Mikrochim. Acta* **1995**, *121*, 249 – 258; g) C. Krause, T. Werner, C. Huber, O. S. Wolfbeis, *Fresenius' J. Anal. Chem.* **2000**, *367*, 426 – 428.
[14] C. D. Gutsche, B. Dhawan, J. A. Levine, K. H. No, L. J. Bauer, *Tetrahedron* **1983**, *39*, 409 – 426.
[15] a) F. Inokuchi, Y. Miyahara, T. Inazu, S. Shinkai, *Angew. Chem.* **1995**, *104*, 1459 – 1462; *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1364 – 1366; b) L. Prodi, F. Bolletta, M. Montalti, N. Zaccheroni, A. Casnati, F. Sansone, R. Ungaro, *New J. Chem.* **2000**, *24*, 155 – 158.
[16] F. Arnaud-Neu, M.-J. Schwing-Weil, K. Ziat, S. Cremin, S. J. Harris, M. A. McKervey, *New J. Chem.* **1991**, *15*, 33 – 37.
[17] L. C. Groenen, B. H. M. Ruel, A. Casnati, W. Verboom, A. Pochini, R. Ungaro, D. N. Reinhoudt, *Tetrahedron* **1991**, *47*, 8379 – 8384.
[18] W. Verboom, S. Datta, Z. Asfari, S. Harkema, D. N. Reinhoudt, *J. Org. Chem.* **1992**, *57*, 5394 – 5398.
[19] C. Jaime, J. de Mendoza, P. Prados, P. M. Nieto, C. Sánchez, *J. Org. Chem.* **1991**, *56*, 3372 – 3376.
[20] This is also observed for other calix[4]arenes containing a methoxy substituent.
[21] $K_{HL} = [HL]_{mem}/[H^+]_{mem}[L^-]_{mem}$
[22] $K_{PbL^+} = [PbL^+]_{mem}/[Pb^{2+}]_{mem}[L^-]_{mem}$
[23] $[L_T]_{mem} = [HL]_{mem} + [PbL^+]_{me}$: Here, it is assumed that at the operational pH the concentration of deprotonated, free ligand in the membrane is negligible. This assumption is validated by the lack of a UV/Vis absorption band at 600 nm.
[24] $[R^-]_{mem} = [PbL^+]_{mem} + [Na^+]_{mem}$: Here, $[L_T]_{mem} = [R^-]_{mem}$, so that $[Na^+]_{mem} = [HL]_{mem}$.
[25] The theoretical membrane absorption of $A = f_{PbL^+}A_{PbL^+} + f_{HL}A_{HL}$ is calculated from $K_{exch}^{PbL_2^+}$ and $[Pb^{2+}]$. Least-squares fitting of the calculated absorption to the experimental absorption allows the evaluation of $K_{exch}^{PbL_2^+}$.
[26] M. Lerchi, E. Bakker, B. Rusterholz, W. Simon, *Anal. Chem.* **1992**, *64*, 1534 – 1540.
[27] E. Anticó, M. Lerchi, B. Rusterholz, N. Achermann, M. Badertscher, M. Valiente, E. Pretsch, *Anal. Chim. Acta* **1999**, *388*, 327 – 338.
[28] A. Ceresa, E. Pretsch, *Anal. Chim. Acta* **1999**, *395*, 41 – 52.
[29] At higher ionic strength, the water content of the membrane is lower. The membrane becomes less polar and the binding constant for Pb^{2+} increases.
[30] J. Janata, M. Josowicz, D. M. DeVaney, *Anal. Chem.* **1994**, *66*, 207R – 228R.

Received: January 31, 2001
Revised: July 2, 2001 [F3043]

  0947-6539/01/0722-4886 $ 17.50+.50/0