Transduction of Selective Recognition of Heavy Metal Ions by Chemically Modified Field Effect Transistors (CHEMFETs)

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Abstract: Specifically substituted calix[4]arenes that complex selectively silver (2), copper (13), cadmium (33), and lead (29) cations were synthesized via di- and tetraalkylation of p-tert-butylcalix[4]arene. Calix[4]arenes derivatized with thioether, thioamide, and dithiocarbamoyl functionalities could be obtained in high overall yields. Integrated on a chemically modified field effect transistor (CHEMFET) the selective complexation of heavy metal ions by these calix[4]arenes is transduced directly into an electrical signal. An architecture for CHEMFETs is used in which a hydrophilic polyHEMA hydrogel is covalently attached between the gate oxide surface of a modified ISFET and the hydrophobic sensing membrane that contains the receptor molecule. CHEMFETs based on the calix[4]arene (2) with two diametrically substituted thioether functionalities responded selectively (60 mV decade⁻¹) to a change in silver activity in the aqueous solution in the presence of potassium, calcium, cadmium, and copper ions (log $K_{f} = -4$). The selectivity toward mercury (log $K_{f} = -2.7$) is comparable to the best neutral carrier based SEF so far. The calix[4]arene (13), with four dithiocarbamoyl groups, incorporated in a CHEMFET responded Nernstian (30 mV decade⁻¹) to a change in copper activity. The calix[4]arene (33), which has four dimethylthiocarbamoylmethoxythioureido substituents, was selective in a CHEMFET (30 mV decade⁻¹) toward cadmium and potassium ions. Two classes of ionophores can be distinguished for the selective detection of lead(II) cations by CHEMFETs, oxazime and thioamide ionophores, respectively. Tetrasubstitution of the calix[4]arene with thioamide groups (29) is a prerequisite for the selective detection of lead and leads to a highly selective CHEMFET (log $K_{f} = -3.4$ to -5.2).

Introduction

In supramolecular chemistry the analogy between biological receptors and abiotic synthetic receptor molecules is limited to the first step in the cascade of events that occurs in biological receptors, viz., the selective recognition of a guest species. We are currently investigating a more active role of molecular recognition viz., the subsequent transduction of these molecular recognition processes into macroscopic properties. A system that has the potential to transfer this molecular recognition into an electrical signal is an ion sensitive field effect transistor (ISFET), an electronic device in a semiconductor chip.1 The ISFET (Figure 1a) consists of two n-type diffusion regions (source and drain) embedded in a p-type silicon substrate (bulk). The conductance in the channel between the source and the drain is a function of the electrical field perpendicular to the gate oxide surface. When the gate oxide contacts an aqueous solution, a change of pH will change the electronic signal as a function of the state of ionization of the amphoteric surface SI0H groups. Typical pH responses of SiO₂ ISFETs are 37-40 mV per decade.24 When an ion-selective membrane is attached to the gate oxide of the ISFET, it is a further development, in which the ISFET response.3 The extraction ability of the ionophore/membrane combination determines which ion governs the membrane potential. The selectivities with respect to other cations are given by the ionophore potential (retention) coefficient ($K_{e}$) in the (empirical) Nicolsky-Eisenman equation (Equation 1) and the (empirical) Nicolsky-Eisenman equation

$$\text{log } K_{f} = 0.67 + \left(4.47 - \right) \text{pH}$$

where $K_{f}$ is the partition coefficient between the free amounts of the ion and the ionophore, $z$ is the charge of the ion, and $a_{i}$ and $a_{j}$ are the activities of the primary and interfering ion, respectively.6,7

However, some serious problems have been recognized of such a modified ISFET (CHEMFET); the membrane is mechanically not stable, the response is influenced by CO₂ and most receptor molecules are not sufficiently hydrophobic to give chemically stable systems because they leak out of the membrane to the contacting aqueous solution.

We have designed a novel architecture for CHEMFETs (Figure 1b) in order to solve the problems mentioned above. Firstly, the attachment of the membrane was improved by mechanical2,8 anchoring to the surface of the gate oxide. Secondly, we have introduced a hydrogel layer (polyHEMA) between the gate oxide and the sensing membrane in which an aqueous buffered solution of salts can be absorbed.11 This eliminates the interference of CO₂ on the CHEMFET response, and it stabilizes the potential developed in the sensing membrane.12,13 Plasticized PVC membranes that contain an ionophore are widely used as the sensing membrane, but leakage of plasticizer to the contacting aqueous solution and weak adhesion of the membrane to the ISFET prompted us to develop other polymeric membranes.12,13 However, in order to study the selectivity of new ionophores CHEMFETs based on plasticized PVC membranes can be used. In this paper the selective recognition of heavy metal ions by specifically substituted calix[4]arenes integrated with chemically modified ion sensitive field effect transistors (CHEMFETs) is described. Previously, we reported highly selective CHEMFETs14,15 based on calix[4]arenes that are selective for hard

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(4) van den Berg, A.; Bergveld, P.; Reinholdt, D. N.; Sudhölter, E. J. R. Sens. Actuators 1985, 9, 129-146.
(10) Harrison, D. J.; Teelermans, A.; Cunningham, L. Anal. Chem. 1989, 61, 244-251.
1 R₁ = R₂ = R₃ = R₄ = OH, R₅ = t-Bu  
2 R₁ = R₂ = OCH₃CH₂CH₃, R₃ = R₄ = OH, R₅ = t-Bu  
3 R₁ = R₂ = R₃ = R₄ = OCH₃CH₂CH₃, R₅ = t-Bu  
4 R₁ = R₂ = On-Pr, R₃ = R₄ = OH, R₅ = t-Bu  
5 R₁ = R₂ = OCH₃CH₂CH₃, R₃ = R₄ = On-Pr, R₅ = t-Bu  
6 R₁ = R₂ = OH, R₃ = H  
7 R₁ = R₂ = OPO(O)OC₂H₅, R₃ = R₄ = OH, R₅ = H  
8 R₁ = R₂ = R₃ = H, R₄ = R₅ = OH  
9 R₁ = R₂ = OCH₃CH₂CH₃, R₃ = R₄ = H  
10 R₁ = R₂ = OCH₂C(O)OC₂H₅, R₅ = t-Bu  
11 R₁ = R₂ = OCH₃CH₂OH, R₅ = t-Bu  
12 R₁ = R₂ = OCH₃CH₂OH, t-Bu  
13 R₁ = R₂ = OCH₃CH₂(O)C(=O)CH₃, R₅ = t-Bu  
14 R₁ = R₂ = OCH₃CH₂OH, R₃ = R₄ = On-Pr, R₅ = t-Bu  
15 R₁ = R₂ = OCH₃CH₂OH, R₅ = t-Bu  
16 R₁ = R₂ = OCH₃CH₂OH, R₃ = R₄ = On-Pr, R₅ = t-Bu

The calix[4]arene building block is easily accessible, and it gives a high hydrophobicity to a receptor molecule. Calix[4]arenes are cyclic tetramers of p-tert-butylphenol and formaldehyde. Calix[4]arenes exist in four different extreme conformations (cone, partial cone, 1,2-alternate, and 1,3-alternate), but for the research described in this paper the cone conformation is the most important conformer.

In receptor molecules for the softer heavy metal cations or nitrogen donor atoms are preferred, but for our purpose nitrogen donor atoms have to be excluded, because these might induce an unfavorable pH sensitivity of the ultimate sensor. Several sulfur containing functionalities, like thioethers (silver selective), thiaoxadimides (cadmium selective), and thiocarbonyl groups (copper selective), have been used for receptor molecules in ion-selective electrodes. However, the hydrophobicity of these compounds is rather low for the application in CEMFETs.

Calix[4]arene based receptor molecules for heavy metal ions offer the possibility of an increased hydrophobicity and a synthetic building block for the positioning of the coordination sites in one molecule.

Results and Discussion

Synthesis of Receptor Molecules. For the complexation of silver, calix[4]arene was derivatized in various ways with a thioether functionality. Reaction of p-tert-butylcalix[4]arene 1 with 3 equiv of 2-chloroethyl methyl sulfoxide in the presence of 1 equiv of K₂CO₃ in refluxing acetonitrile afforded the diametrically substituted bis[2-(methylthio)ethoxy]calix[4]arene 2 in 67% yield. The formation of 2 clearly followed from its symmetrical 1H NMR spectrum showing among others a typical AB pattern at δ 4.31 and 3.32 for the methylene bridge protons (J = 13.1 Hz). The tetratrasubstituted calix[4]arene 3 could be obtained by reaction of disubstituted calix[4]arene 2 with excess 2-chloroethyl methyl sulfoxide in NaH/DMF in 61% yield. The characteristic AB pattern at δ 3.43 and 3.15 (J = 12.7 Hz) for the methylene bridge protons and the typical value of δ 3.11 for the corresponding carbon absorption indicate the cone conformation of 3. The synthesis of a receptor molecule with two pairs of diametrically located different substituents was performed starting from the previously described 26,28-dipropoxyxyl[4]arene 4. Analogously to the formation of 3, reaction of 4 with excess 2-chloroethyl methyl sulfoxide in NaH/DMF gave 25,27-bis[2-(methylthio)ethoxy]-26,28-dipropoxyxyl[4]arene 5 in the cone conformation in 61% yield. In order to prepare an ionophore with increased flexibility the selectively dehalogenated calix[4]arene 8 was reacted with excess 2-chloroethyl methyl sulfoxide in NaH/DMF to give the...
Transduction of Selective Recognition of Heavy Metal Ions

Figure 1. Schematic representation of (a) an ISFET and (b) a CHEM-FET.


The first target molecule for the complexation of copper (11) contains four thiocarbamoyl groups at one face of the calix[4]arene moiety (i.e., the calix[4]arene is in the cone conformation). The synthesis starts from the known tetraesters 10 which upon reduction with LiAlH₄ in Et₂O at room temperature gave 11 in 86% yield. Reaction of 11 with p-toluene sulfonyl chloride in pyridine at 0 °C gave the tetraesterate 12 which was reacted with the sodium salt of N,N-diethylthiobarbituric acid in acetonitrile to give the target tetrasulfato(calix)arene 13 in 72% yield. Throughout the whole reaction sequence the original cone conformation of 10 was retained as followed from the characteristic AB pattern at δ 4.53 and 3.38 (J = 12.7 Hz) for the methylene bridge protons in the ¹H NMR spectrum of 13 and the typical value of δ 3.14 for the corresponding carbon atoms in the ¹³C NMR spectrum. In addition to a satisfactory FAB mass spectrum and elemental analysis the structure of 13 was confirmed by its NMR spectra. The ¹H NMR spectrum exhibits among others a singlet at δ 6.77 for the aromatic hydrogens, a triplet at δ 4.26 (J = 6.7 Hz) for the ArOCH₃ protons, a quartet at δ 3.76 (8 H) for part of the NCH₃ groups (the other eight H's coincide with a multiplet of other CH₃ signals), and a singlet at δ 1.07 for the tert-butylic groups. Characteristic absorptions in the ¹³C NMR spectrum are δ 195.3 (C=O), δ 73.4 (ArOCH₃), two signals due to hindered rotation, at δ 40.3 and 46.8 for the NCH₃ moieties, and δ 36.6 (CH₃). In the second target molecule 17 the calix[4]arene skeleton is, in addition to two propoxy groups, only functionalized with two thiocarbamoyl groups. In this case the synthesis starts from the diametrically substituted 26,28-dipropoxycalix[4]arene 24 which was reacted with excess ethyl bromocarbonate in NaH/DMF at 60 °C to give 14 in 84% yield. The cone conformation of 14 followed from the typical absorptions (vide supra) in both the ¹H NMR and ¹³C NMR spectrum. In a similar way as described for 13, receptor molecule 17 could be prepared starting from 14.

For the complexation of cadmium, calix[4]arene was used as a molecular platform to organize two or four thiocarbamoyl moieties in the same molecule. Reaction of p-tert-butylcalix[4]arene 1 with 2.2 equiv of N,N-dimethyl 2-chloroacetamide using 1 equiv of KH₂PO₄ in CH₂CN afforded the diametrically dialkylated calix[4]arene 18 in 87% yield which, upon treatment with Lawesson's reagent in toluene, gave the corresponding 26,28-bis(calix)[4]arene 19 in 73% yield. The 26,28-bis(calix)[4]arene 19 could be obtained by reaction of 1 with 2.2 equiv of N,N-dimethyl 2-chloroacetamide in the presence of 4.2 equiv of NaH in DMF at 60 °C to afford the proximal difunctionalized calix[4]arene 20 in 44% yield and subsequent treatment with Lawesson's reagent in toluene to give 21 in 90% yield. Very characteristic for proximal functionalization are in the ¹H NMR spectrum of 21 the three AX signals for the methylene bridge protons at δ 4.25 and 3.35, and 4.26 (1:2:1 H₃) at δ 3.37, 3.38, and 3.33 (1:1:2 H₃). Subsequently, attention was focused on calix[4]arenes having OCH₃, OCH₂CH₃, OCH₂(OH)(CH₃)₂ substituents, the unit also present in the cadmium ionophore N,N,N,N'-tetraethyl-3,6-dioxoacetonitrilium. Reaction of tetrakis(hydroxyethoxy)calix[4]arene 14 (cone conformation) with N,N-dimethyl 2-chloroacetamide as NaH as a base in DMF gave the tetrasubstituted compound 22 in 86% yield which was converted into the corresponding tetrakis(thio)calix[4]arene 23 in 48% yield upon treatment with Lawesson's reagent. The original cone conformation was retained in 23 as was clearly indicated in the ¹H NMR spectrum of the typical AB pattern of δ 4.39 and 3.11 (J = 12.6 Hz) for the methylene bridge protons and the characteristic value of δ 31.2 for the corresponding carbon absorption in the ¹³C NMR spectrum. In an analogous way ionophore 28 containing two OCH₃, OCH₂(OH)(CH₃)₂(NH₂) and two propoxy groups was prepared in 52% yield starting from 25,27-bis(hydroxyethoxy)-26,28-dipropoxycalix[4]arene 15 (cone conformation).

Finally, functionalized calix[4]arenes for the selective complexation of lead were prepared. 26,28-Dipropoxycalix[4]arene 24 was reacted with 4 equiv of N,N-dimethyl 2-chloroacetamide using NaH/DMF to give the ionophore 26 in 56% yield. In the ¹H NMR spectrum the characteristic AB pattern at δ 4.53 and 3.17 (J = 12.7 Hz) for the methylene bridge protons and in the ¹³C NMR spectrum the value of δ 31.4 for the corresponding carbon absorption prove the core conformation of 26. Treatment of 26 with Lawesson's reagent in toluene at 85-90 °C afforded the corresponding thioamid 27 in 52% yield. In a similar way calix[4]arene 29 was synthesized in 83% yield starting from compound 28.

Silver Selective CHEMFETS. In order to compare the selectivity coefficients of silver selective CHEMFETS based on the different synthesized ionophores (2, 3, 5, and 9), PVC/DOS (=bis(2-ethylhexyl)silane) membranes were used in which, besides the ionophore, a small amount of potassium tetrakis(4-chloro-


(23) The tetrasubstituted calix[4]arene 3 could also be obtained by direct reaction of p-tert-butyldioxysilane with excess 2-chloroethyl methyl sulfide, but purification was more difficult and the yield lower.


Table I. Selectivity Data of Ag⁺ Selective CHEMFETs Containing Different Callix[4]arene Ionomophores (2, 3, 5, or 9) in the Presence of Different Interfering Nitrate Salts

<table>
<thead>
<tr>
<th>entry</th>
<th>ionophore</th>
<th>( \text{KNO}_3 ) (1 M)</th>
<th>( \text{Ca(NO}_3)_2 ) (0.1 M)</th>
<th>( \text{Cu(NO}_3)_2 ) (0.1 M)</th>
<th>( \text{HNO}_3 ) pH = 2.5</th>
<th>( \text{Hg(NO}_3)_2 ) 1 ppm (pH = 2.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none</td>
<td>c</td>
<td>-2.7e (49 mV)</td>
<td>-2.7e (44 mV)</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>-4.6</td>
<td>-4.1</td>
<td>-4.2</td>
<td>-4.3</td>
<td>-2.4</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>-4.6</td>
<td>-4.2</td>
<td>-3.9</td>
<td>-4.0</td>
<td>-2.2</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>-4.7</td>
<td>-4.2</td>
<td>-4.3</td>
<td>-4.4</td>
<td>-2.5</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>-4.7</td>
<td>-4.2</td>
<td>-4.2</td>
<td>-2.3</td>
<td>-2.1</td>
</tr>
</tbody>
</table>

*PVC/DOS \( \approx 1:2 \) 0.6-0.8 wt% KTCPB 2 (3, 5, and 9) or 1 (9) wt% ionophore. *Not present. *No cation response. *0.01 M interfering salt concentration.

Table II. Selectivity Data of Cu⁺ Selective CHEMFETs Containing Different Ionomophores (13 or 17) in the Presence of Different Interfering Chloride Salts

<table>
<thead>
<tr>
<th>entry</th>
<th>ionophore</th>
<th>membrane</th>
<th>( \text{CaCl}_2 ) (0.01 M)</th>
<th>( \text{KCl} ) (0.1 M)</th>
<th>( \text{CdCl}_2 ) (0.01 M)</th>
<th>( \text{PbCl}_2 ) (0.01 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>PVC/DOP (1:2)</td>
<td>-1.7</td>
<td>-2.0</td>
<td>-1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>KTCPB (1.4 wt%)</td>
<td>(31 mV)</td>
<td>(31 mV)</td>
<td>(31 mV)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>PVC/NPOE (1:1)</td>
<td>-1.9</td>
<td>-2.1</td>
<td>-1.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>KTCPB (0.9 wt%)</td>
<td>(59 mV)</td>
<td>(58 mV)</td>
<td>(54 mV)</td>
<td></td>
</tr>
</tbody>
</table>

*No cation response.

Phenylborate was incorporated. The polyHEMA hydrogel of the CHEMFET was conditioned with 0.01 molar silver nitrate \( \text{HNO}_3 \) pH = 4 prior to membrane deposition. First a CHEMFET \( \text{Ag}^+ \) was tested without any ionophore present. Such a tetrakis(4-chlorophenyl)borate containing membrane is already silver selective in the case of ditovalent interfering cations, etc., calcium, cadmium, and copper \( \log K_{i,j} = 2.7 \), Table I, entry 1. The slope of these responses is less than Nemstian (ca. 45 mV decade \(^{-1} \)). In the case of the monovalent interfering ions no response could be measured at all (Table I, entry 1). This measurement indicates that without ionophore the selectivity is related to the hydration energy.

All the CHEMFETs based on the callix[4]arene ionophores (2, 3, 5, and 9) showed a good response toward silver. In general the slopes are Nemstian (55-60 mV decade \(^{-1} \)) Figure 2 except for the callix[4]arene 3 which exhibits at the high concentrations (0.1 M) of the interfering ions (calcium, cadmium, and copper), a somewhat lower slope of around 50 mV decade \(^{-1} \). The slope is even lower (4 mV decade \(^{-1} \)) with (1 M) potassium nitrate (Table I, entry 3) as the interfering salt. The selectivities toward calcium, potassium, calcium, cadmium, and copper for these callix[4]arenes are between 8 X 10\(^{2}\) and 50 X 10\(^{2}\). The selectivity toward silver versus potassium is the highest \( \log K_{\text{AgK}} = \pm 4.7 \) and nearly identical for all compounds of Table I.

The dehydroxylated callix[4]arene 9 shows a higher selectivity toward calcium (log \( K_{i,j} = -8.5 \)) than the callix[4]arene 2, 3, and 5 with four (calcium coordinating) oxygen atoms \( \log K_{i,j} = +4.2 \). A similar result was found with the heavy metal ions cadmium and copper as the interfering ions, although the effect is less pronounced. The callix[4]arene 3 with the four soft sulfur donor sites showed for both interfering ions the highest interference \( \log K_{i,j} = -3.9 \) The other three callix[4]arenes (2, 5, and 9) are more selective to silver. The selectivity coefficients \( \log K_{i,j} \) in nitric acid (pH = 2.5) are between -2.1 and -2.5. The interference of mercury is most severe, but still a selective response was found. The selectivities of the two disubstituted callix-

Figure 2. Ag⁺ responses of CHEMFETs containing callix[4]arene ionophore 2 in the presence of 0.1 M Ca(NO\(_3\)).

(32) Interfering cations are chosen as representative for a group of alkali (potassium) or alkaline earth metal (calcium) cations. Other heavy metal interfering cations are taken into account because of their specific interference for a particular ion-selective electrode.
copper (0.01 M, HCl, pH = 4) solution.

The calix[4]arene ionophore 13 (2.6 wt%) with four thio-
 carbamoyl groups was incorporated in a PVC/DOP (bis[2-
 ethylhexyl]phthalate) membrane containing potassium tetrakis-
 (4-chlorophenyl) borate (1.4 wt%). The CHEMFETs with such a
 membrane respond Nernstian (31 mV decade⁻¹) to copper(II)
 ions (Figure 3).

CHEMFETs with a PVC/DOP membrane and only potassium
 tetrakis(4-chlorophenyl) borate, without ionophore 13, did not
 respond at all to copper(II) ions. The PVC/DOP system with
 ionophore 13 was selective for copper(II) ions in the presence of
 calcium (log K_{13} = -1.7), cadmium (log K_{13} = -2.0), and lead (log
 K_{13} = -1.6). Potassium chloride interferes with the electrode
 response (Table II, entry 1), which might be due to the higher
 partition of potassium to the membrane compared to copper(II)
 ions. A more polar membrane plasticizer (e.g., octylphenyl octyl
 ether (o-NPOE)) can increase the selectivity toward copper versus
 potassium cations, but PVC/o-NPOE based CHEMFETs with
 the same ionophore respond not reproducible to copper(II) ions.

The calix[4]arene ionophore 17 with two thio carbamoyl groups,
 incorporated in a PVC/DOP membrane containing potassium
 tetrakis(4-chlorophenyl) borate, responded irreproducibly to copper
 (II) ions. Better results were obtained in a PVC/o-NPOE
 membrane, although the amount of ionophore had to be raised to
 9.2 wt% in order to obtain a stable sensor. These CHEMFETs
 responded selectively toward copper(II) ions with a slope of 54–59
 mV decade⁻¹ (Table II, entry 2). An increased slope of 60 mV
 decade⁻¹ for a divalent cation was described earlier by Lindner
 et al.²⁴ using a lead-selective electrode. Lead is detected as the
 monovalent species of the type PbX²⁻ (X = OH, Cl, CH₃COO⁻).
 Thus, the slope of 60 mV decade⁻¹ is obtained. It might also be
 possible that sample anions associate in the sensing membrane
 with the cation-ionophore complex, which leads to an increased
 slope of this copper response. For copper in the presence of
 potassium chloride no selective response was found. The poten-
tiometric selectivity coefficients of copper versus calcium, cad-
mium, and lead are -1.9, -2.1, and -1.7, respectively. Membranes
 without ionophore 17 present showed no intrinsic selectivity toward
 copper(II) ions.

Cadmium. The thiolamide functionality of the cadmium iono-
 phore N,N,N',N'-tetrabutyl-3,6-dioxoacetanilide-thiolamide²⁵ is also
 present in the four synthesized novel calix[4]arenes 19, 21, 23,
 and 25. These calix[4]arenes were used in PVC plasticized PVC
 membranes in a CHEMFET. The polyHEMA hydrogel of the
 CHEMFET was conditioned with 0.01 M Cd(NO₃)₂ (HNO₃, pH =
 4). The results of the measurements²⁶ of the CHEMFETs are
 summarized in Table III.

Table III. Selectivity Data of Cd²⁺ Selective CHEMFETs Containing
Different Calix[4]arene Ionophores (19, 21, 23, or 25) in the Presence of
Different Interfering Nitrate Salts

<table>
<thead>
<tr>
<th>Interfering ions</th>
<th>KTCPB (μM)</th>
<th>KNO₃ (0.1 M)</th>
<th>Ca(NO₃)₂ (0.1 M)</th>
<th>Pb(NO₃)₂ (0.01 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>entry</td>
<td>(wt%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>19 (2.1)</td>
<td>1.7</td>
<td>-3.2 (30 mV)</td>
<td>-0.6</td>
</tr>
<tr>
<td>2</td>
<td>21 (2.3)</td>
<td>1.7</td>
<td>-2.1 (25 mV)</td>
<td>-2.1</td>
</tr>
<tr>
<td>3</td>
<td>23 (2.8)</td>
<td>0.8</td>
<td>-3.0 (30 mV)</td>
<td>-2.0</td>
</tr>
<tr>
<td>4</td>
<td>23 (3)</td>
<td>2.0</td>
<td>-2.8 (30 mV)</td>
<td>-2.8</td>
</tr>
<tr>
<td>5</td>
<td>25 (2.1)</td>
<td>0.8</td>
<td>-2.2 (29 mV)</td>
<td>-2.2</td>
</tr>
</tbody>
</table>

*No cation response.

The 26,28-difunctionalized calix[4]arene 19 exhibits a
Nernstian response (22–30 mV decade⁻¹) toward cadmium in the
presence of calcium (log K_{19} = -3.2) and of copper(II) ions (log
K_{19} = -0.6, Table III, entry 1). Variation of the disubstitution
pattern from 26,28 (compound 19) to 27,28 (compound 21) re-
vealed that the 26,28-disubstituted 19 is more selective than the
21 showed, compared to compound 19, a decreased selectivity for
cadmium(II) ions versus calcium (log K_{19} = -3.3) and copper(II)
(log K_{19} > 0) (Table III, entries 1 and 2).

Two calix[4]arene based ionophores (23 and 25) with an
extended cavity, were synthesized in order to study the effect of the
flexibility of the coordinating groups which are attached to the
polyHEMA building block. The tetraalkylated 23 shows the
best results. Incorporated in the membrane of the CHEMFET,
this ionophore introduces selectivity for cadmium ions (Figure
4) over calcium (log K_{ca} = -3.9) and potassium (log K_{ca} = -2.6)
ions (Table III, entry 1). A CHEMFET with compound 23
exhibits no selectivity for cadmium in the presence of lead(II)
ions or copper(II) ions. The introduction of a larger amount of po-
tassium tetrakis(4-chlorophenyl) borate (Table III, entry 4) did
not change the selectivity coefficients.

The tetraalkylation proved to be necessary because the
26,28-disubstituted 25 showed no selectivity versus potassium,
while the selectivity coefficient versus calcium decreased to log
K_{ca} = -3.2.

Lead Selective CHEMFETs. CHEMFETs Based on Oxamide
Ionophores. Several dioximates have been reported by Lindner
et al.²⁴ to induce selectivity toward lead(II) ions in an ionic selec-
tive electrode.

The novel calix[4]arene ionophores 24 and 26 were also in-
corporated in these PVC/BFPA (bis[bis(4-pentyl)adi]pate) based
CHEMFETs.²² The polyHEMA hydrogel was conditioned in a
lead(II) chloride solution (0.01 M, 10⁻⁶ M Mg(OAc)₂/HCl pH

Table IV. Selectivity Data of Pb\(^{2+}\) Selective CHEMFETs Containing Different Iophones (24 or 26) in the Presence of Different Interfering Chloride Salts

<table>
<thead>
<tr>
<th>Entry</th>
<th>Iophone</th>
<th>KTCPB (wt%)</th>
<th>KCl (0.01 M)</th>
<th>CaCl(_2) (0.01 M)</th>
<th>CdCl(_2) (0.01 M)</th>
<th>CuCl(_2) (0.01 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26 (2)</td>
<td>(0.8)</td>
<td>-2.2</td>
<td>-3.3</td>
<td>-5.2</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>24 (2.1)</td>
<td>(0.8)</td>
<td>-2.4</td>
<td>-2.7</td>
<td>-2.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>none(^{a})</td>
<td>(0.8)</td>
<td>-1.1</td>
<td>-1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\) PVC:BBPA = 1:2. \(^{b}\) Not present. \(^{c}\) No callion response. \(^{d}\) Corrected for drift.

Figure 5. Pb\(^{2+}\) response of CHEMFETs containing calix[4]arene ionophore 26 in the presence of 0.01 M Cd(NO\(_3\))\(_2\).

Figure 6. Pb\(^{2+}\) response of CHEMFETs containing calix[4]arene ionophore 28 in the presence of 1 M KCl.

Table V. Selectivity Data of Pb\(^{2+}\) Selective CHEMFETs Containing Different Calix[4]arene Ionophores (19, 27 or 29) in the Presence of Different Interfering Chloride Salts

<table>
<thead>
<tr>
<th>Entry</th>
<th>Iophone</th>
<th>KTCPB (wt%)</th>
<th>KCl (0.1 M)</th>
<th>CaCl(_2) (0.1 M)</th>
<th>CdCl(_2) (0.01 M)</th>
<th>CuCl(_2) (0.01 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29 (2)</td>
<td>(0.8)</td>
<td>-5.2</td>
<td>-4.3</td>
<td>-4.2</td>
<td>-3.4</td>
</tr>
<tr>
<td>2</td>
<td>19 (2)</td>
<td>(0.7)</td>
<td>-2.4</td>
<td>-3.7</td>
<td>-3.1</td>
<td>-1.9</td>
</tr>
<tr>
<td>3</td>
<td>27 (2)</td>
<td>(0.9)</td>
<td>-2.8</td>
<td>-4.2</td>
<td>-1.7</td>
<td>-2.7</td>
</tr>
</tbody>
</table>

\(^{a}\) PVC-NPOE = 1:2. \(^{b}\) 1 M KCl. \(^{c}\) 0.1 M.

Therefore, several thioamide containing calix[4]arenes (19, 27, and 29) were synthesized.

All the calix[4]arenes were incorporated in PVC/nPOE membranes containing potassium tetrafluoroborate. Without any ionophore no selectivity toward lead(II) ions was observed. The receptor molecule 29 introduced in the CHEMFET showed (Figure 6) a high selectivity toward lead with a Nernstian slope of 30 mV decade\(^{-1}\) (Table V, entry 1). Copper interfered most (log \(K_{Cu} = -3.4\)). The interference of calcium and cadmium was less (log \(K_{Ca} = -4.2\), and \(K_{Cd} = -1.9\), and potassium showed hardly any interference (log \(K_{K} = -5.2\)).

When the number of thioamide groups are reduced from four to two this lowers the selectivity toward lead cations (Table V, entries 2 and 3). If calcium was the interfering ion the deviation from compound 29 was the smallest (-0.6 (compound 19) or -0.1 (compound 27)). But toward the other interfering ions (potassium, calcium, and copper) this difference is more pronounced (compare in Table V entries 2 and 3 to entry 1). This leads to the conclusion that four thioamide groups are a prerequisite in a calix[4]arene based ionophore for the selective complexation of lead(II) ions.

Conclusions

In this paper the first sensors for heavy metal ions based on ion-selective field effect transistors (ISFETs) are described. Several new calix[4]arenes are designed and synthesized for the selective recognition of silver, copper, cadmium, and lead cations.

\(^{33}\) Barrilotti et al. have found a selective response of a CHEMFET toward Pb\(^{2+}\) species \((X = CO\text{-OH})\) of 60 mV decade\(^{-1}\) with the ligand \(N,N,N'-N'-\text{tetraoxo-1,6-dioxoacetane}^\text{amide} \). The selectivity coefficients cannot be compared because of the different experimental conditions.


\(^{37}\) Perrin, R.; Harris, S. Industrial Applications of Calixarenes in ref 18.


Transduction of Selective Recognition of Heavy Metal Ions

Experimental

Synthesis. General Methods. 1H and 13C NMR spectra were recorded on a Brucker AM 270 MHz spectrometer with CDCl3 unless stated otherwise with TMS as an internal standard. FAB mass spectra were obtained with a Finnigan MAT 900 mass spectrometer using m-nitrobenzyl alcohol (NBA) or polyethylene glycol (PEG) as a matrix. Melting points were determined with a Kofler melting point apparatus and are uncorrected. Tetrahydrofuran was freshly distilled from sodium/benzophenone ketyl; acetonitrile was dried over molecular sieves (4 Å); DMF was stored over molecular sieves (4 Å); pyridine was dried over KOH pellets prior to use. Petroleum ether refers to the fraction boiling at 60–80 °C, unless stated otherwise. All other solvents and chemicals were of reagent grade and were used without purification. Chromatographic separations were performed on silica gel (SiO2, E. Merck, particle size 0.040–0.063 mm, 230–400 mesh), whereas preparative TLC was performed on precoated 250–600 mesh silica plates (E. Merck, thickness 0.2 mm). Compounds 1, 4A, 2, 9B, 10A, and 28 were prepared according to the literature.


Reduction of Tetraselar 1,4,5,6-tetra-tert-butyl-tetrazine (14) and Diester 1,4,5,6-tetra-tert-butyl-tetrazine (14) with LiAlH4. Formation of 11 and 15. To a solution of compound 10 and 11 (5.0 mmol) was added LiAlH4 (2.4 g, 111.5 mmol) in small portions at 10 °C and the mixture was stirred until no more solid was visible. The reaction mixture was cooled to room temperature. HCl (2.0 N) was added dropwise to pH 7. The mixture was extracted with EtOAc. The extract was washed with saturated Na2S2O3 solution and brine. The organic layer was dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure.

25.26.27.28.29-Tetraselar (diethylidithiocarbamoyl)ethoxy)-5,11,17,23-tetraselar (1,4-dimethylthyl)-1,1,1-triazacyclooctadec-15-one (16): Yield 75%; mp 232–236 °C; IR (KBr) νmax = 1747, 1727, 1670 cm⁻¹. NMR (DMSO-d6) δH 3.30 (s, 6H, CH2), FAB mass spectrum (M+Na+= 682) (M+H+= 618). Anal. C27H31N11O2S2; C, 77.89; H, 8.22. Found: C, 77.88; H, 8.50.

Toylation of Tetraselar (3-hydroxyethoxy)-p-tert-butyl-tetrazine (11) and Bis(3-hydroxyethoxy)-p-tert-butyl-tetrazine (15) Formation of 12 and 16. To a solution of the carboxylic acid 11 and 15 (1.2 mmol) in pyridine (25 mL), was added p-toluenesulfonyl chloride (14.5 mmol in the case of 11; 6.0 mmol in the case of 15) at 0 °C, whereupon the homogenous solution was stirred under 1 atm of N2 at 4 °C. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure to give 12 and 16, which were pure enough for further reactions.

25.11.17.23-Tetraselar (1,4-dimethylthyl)-25.26.27.28.29-Tetraselar (14-methylphenyl)-sulfonfonyl)oxetanyloxy)-1,1,1-triazacyclooctadec-15-one (12): Yield 85%; IR (KBr) νmax = 1747, 1727, 1670 cm⁻¹. NMR (DMSO-d6) δH 7.73 (s, 2H, NH), 4.65 (s, 3H, CH3), 3.00 (ABq, 8 H, J = 12.8 Hz, ArCH2), 4.15–4.05 (m, 8 H, ArCH2-CH2), 2.44 (s, 12 H, OSO2Ar), 1.05 (s, 6 H, CH3) 13°C NMR δ 152.1 (s, Ar 25.26,27,28,29-C), 142.5, 142.4 (s, Ar 5,11,17,23- and Ar-SO2-C), 133.1, 133.0 (Ar 1,3,7,9,13,15,19,21-C and O-SO2-CH2), 129.9, 128.0 (d, ArO2-C), 125.2 (d, Ar 4,6,10,12,16,18,22-CH), 71.6, 69.5 (ArCH2-CH2), 33.8 (s, CH3), 31.4 [s, (CH3)2], 31.0 [s, CH2 (CH3)], 14.0 [s, CH2 (CH3)]. FAB mass spectrum (M+) 682 (M+Na+= 704). Anal. C52H64N11O15S (M+Na+= 843.2) C, 54.5; H, 5.9; N, 15.6; S, 12.7. FAB mass spectrum (M+) 618 (M+Na+= 640). Anal. C37H44N11O2S (M+Na+= 542). C, 54.6; H, 5.4; N, 17.3; S, 12.9.

Reduction of Tetraselar (3-hydroxyethoxy)oxetanyloxy)-1,1,1-triazacyclooctadec-15-one (12) with LiAlH4. Formation of 13. The mixture was stirred until no more solid was visible. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The reaction mixture was stirred under 1 atm of N2 at 4 °C, whereupon the homogenous solution was stirred under 1 atm of N2 at 4 °C. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The reaction mixture was stirred under 1 atm of N2 at 4 °C, whereupon the homogenous solution was stirred under 1 atm of N2 at 4 °C. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The reaction mixture was stirred under 1 atm of N2 at 4 °C, whereupon the homogenous solution was stirred under 1 atm of N2 at 4 °C. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The reaction mixture was stirred under 1 atm of N2 at 4 °C, whereupon the homogenous solution was stirred under 1 atm of N2 at 4 °C. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure.

Toylation of Tetraselar (3-hydroxyethoxy)oxetanyloxy)-1,1,1-triazacyclooctadec-15-one (12) with LiAlH4. Formation of 13. The mixture was stirred until no more solid was visible. The reaction mixture was poured into ice-cold N H2O (250 mL), and the precipitate was collected and filtered. The solid was dissolved in CH2Cl2 (200 mL) and washed successively with HCl (200 mL) and brine (100 mL), and then dried over MgSO4. The solvent was removed under reduced pressure. The residue was taken up in CH2Cl2 (200 mL) and washed with water (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was removed under reduced pressure.
Alkaloids of Tetrasyphon lycopodioides: Bioassay of the Lycospo


Received for publication March 15, 1971.

ABSTRACT. The bioassay of the alkaloids of Tetrasyphon lycopodioides (L.) is discussed. The lycospermine, lycosperminine, and related compounds are found to be active against the nematode Haemonchus contortus. The activity of the lycosperminine is comparable to that of the related compounds. The lycosperminine is also active against the plant pathogen Phytophthora parasitica.

1. Introduction.

The alkaloids of Tetrasyphon lycopodioides (L.) have been shown to be active against the nematode Haemonchus contortus (1). The activity of the lycospermine, lycosperminine, and related compounds is comparable to that of the related compounds. The lycosperminine is also active against the plant pathogen Phytophthora parasitica.

2. Experimental.

The lycospermine, lycosperminine, and related compounds were isolated from the plant material using column chromatography on silica gel and preparative TLC. The purity of the compounds was checked by UV and HPLC analysis. The TLC plates were developed with an equal volume of chloroform:methanol (9:1). The compounds were identified by their Rf values and by comparison with authentic samples.

3. Results and Discussion.

The results of the bioassays are shown in Table 1. The lycospermine and lycosperminine are active against the nematode Haemonchus contortus with 

4. Conclusion.

The alkaloids of Tetrasyphon lycopodioides have shown promise as potential antihelminthic agents. Further studies are needed to determine the mechanism of action of the compounds and to evaluate their therapeutic potential.

References:


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C. 72.34; H. 8.88; N. 7.22; S. 6.23. Found: C. 72.23; H. 8.97; N. 2.44; S. 6.01. Karl Fisher titration calcd for 0.33H₂O. 0.38. Found: 0.57.

**Cobben et al.**

5.11.17,23-Tetrakis(1-(dimethylsilyl)-25-(1H-benzotriazol-1-yl))-26,28-diproplyoxylic acid(26). A suspension of 25.27-
dipropyl-26,28-diproplyoxylic acid(24) (1.5 g, 2.6 mmol) and tetrabutylammonium fluoride(25) (1.0 g, 0.1 M) in THF (10 mL) was stirred at room temperature for 1 h. Subsequently, N,N-dimethyl-2-chloroaniline(27) (1.5 g, 12.2 mmol) was added, and the mixture was stirred overnight at 55–60°C. After removal of the solvents under reduced pressure the residue was dried in CH₂Cl₂ (250 mL) and washed with saturated NH₄Cl solution (4 × 200 mL), followed by brine (1 × 100 mL). The organic layer was dried with MgSO₄, and the solvent was evaporated. The crude product was crystallized from diisopropanol ether to give 24 as a white powder (4.60 g, 56%); mp 222–223 °C (CH₂Cl₂/Methanol); 1H NMR δ 6.93, 6.61 (s, 4 H, ArH), 4.80 (s, 4 H, OCH₂C(O)N(CH₃)₂), 4.53 and 3.17 (ABQ, 8 H, J = 12.7 Hz, ArCH₂Ar), 3.92 (t, 4 H, J = 5.0 Hz, OCH₂CH₂), 3.09, 2.99 (s, 6 H, NCH₃), 2.1–1.95 (m, 4 H, OCH₂CH₂), 1.19, 0.95 (s, 18 H, C(CH₃)₃); 13C NMR δ 168.9 (s, C=O), 154.2, 152.3 (s, Ar 25.26,27,28,29), 146.7, 144.5 (s, Ar 7,11,17,23,24), 134.5, 132.2 (s, Ar 1,3,7,9,13,15,19,21,25,27,28,30), 125.3, 120.0 (d, all ArCH₂), 76.7 (t, ArOCH₂CH₂), 72.8 (t, ArOCH₂C(O)NMe₂), 37.1, 35.4 (q, NCH₃), 33.9, 33.7 (t, C(CH₃)₂), 31.6, 31.3 (t, C(CH₃)₃), 31.4 (t, ArCH₂Ar), 23.2 (t, ArOCH₂CH₂), 10.3 (q, OCH₂CH₂); FAB mass spectrum (NBA), m/z 903.8 (M⁺ + H), calcd 903.6. Anal. Calcd for C₄₂H₅₈N₆O₄: C, 75.39; H, 8.96; N, 3.02. Found: C, 75.51; H, 9.16; N, 2.93.

5.11.17,23-Tetrakis(1-(dimethylsilyl)-25-(1H-benzotriazol-1-yl))-26,28-bis(diisopropylcarbamoylmethyl)oxetane(27) was synthesized starting from 26 (4.10 g, 4.54 mmol) in analogy with the procedure given for the preparation of 23. The mixture was stirred at room temperature for 24 h with slow addition of 28 (6.05 g, 6.09 mmol) in analogy with the procedure given for the preparation of 23. The mixture was crystallized from MeOH and purified by column chromatography (SiO₂, CH₂Cl₂/EtOAc 8:2) to yield 29 as an off-white powder (5.23 g, 81%); mp 267–287 °C (CH₂Cl₂/Methanol); 1H NMR δ 6.80 (s, 8 H, ArH), 5.04 (s, 8 H, ArOCH₂), 4.69 and 3.14 (ABQ, 8 H, J = 12.8 Hz, ArCH₂Ar), 3.48, 3.28 (t, 12 H, NCH₃), 1.07 (t, 36 H, C(CH₃)₃); 13C NMR δ 197.3 (s, C=O), 152.8 (s, Ar 25.26,27,28,29), 142.2 (s, Ar 7,11,17,23,24), 133.3 (s, Ar 1,3,7,9,13,15,19,21,25,27,28,30), 125.4 (d, all ArC-H), 80.1 (t, ArOCH₂), 44.7, 42.6 (q, NCH₃), 35.9 (t, C(CH₃)₂), 31.4 (t, C(CH₃)₃), 31.3 (t, ArCH₂Ar); FAB mass spectrum (NBA), m/z 1054.1 (M⁺ + H), calcd 1053.5. Anal. Calcd for C₇₂H₇₄N₁₂O₁₂: C, 68.40; H, 6.04; N, 5.32; S, 12.17. Found: C, 68.33; H, 6.03; N, 5.22; S, 12.10.

**CHEMFEET.** Chemicals. Commercial (&-methoxypropyl)trimethoxysilane (SiMCM) (Janssen Chimica) was used. Poly(vinylpyrrolidone) (PVP) with an average molecular weight of 360,000 g/mol (Janssen Chimica) was used. Potassium tetrakis(4-chlorophenyl)borate (KTCPB), bis(2-ethylhexyl)selenoborate (DBSB), bis(2-ethylhexyl)phosphate (DOP), and o-nitrophenyl octyl ether (o-NPOE) were obtained from Fluka. Tetrahydrofuran (THF) was freshly distilled from benzophenone/sodium before use. Cadmium chloride was obtained from Janssen Chimica. Lead chloride was purchased from BDH Chemicals Ltd. All other chloride or nitrate salts or solids were used as reaction-grade reagents (Merck-Schuchardt), and solutions were prepared with doubly distilled, deionized, CO₂-free water.

**CHEMFEET.** The ISFETs were fabricated as described previously, but the detailed structure of the ISFET has been changed compared to the previous design. The external dimensions were enlarged from 1.2 × 3.0 to 3.0 × 5.0 mm, and the number of contact pads were reduced from three to two by connecting the source and the bulk internally. The size of the contact pads are 1.0 × 1.25 mm. Surface silylation of a wafer with MEMO was performed as described before. A hydrogel was applied on wafer scale on the gate oxide of the ISFET by photopolymerization with a mask aligner of a mixture of EGDMA, HEMA, PVP, water, and photoinitiator in a w/w ratio of 0.4:1:0:10:40:40, respectively. The resulting poly HEMA layer had dimensions of 800 μm × 800 μm × 15 μm. The encapsulated sensors were prepared by mounting the ISFET on a printed circuit board. After wire bonding and encapsulation with an epoxy resin (Vysol H-W796/CS W795) the sensor was soaked in a solution of 10 mM NaCl ready for use in the body. The dimen-
sionless membranes were prepared in a glass tube by dissolving a 10% mixture of ionophore, KTCPB, plasticizer, and PVC in THF. Fifteen micrometers of this solution was applied to the encapsulated ISFETs. These solutions were only used once. The response of the CHEMFEETs was measured with a source and drain follower type ISFET amplifier in a constant drain-current mode (Id = 100 μA), with a constant drain-source voltage (Vds = 0.5 V). The reference electrode was a calcium electrode. The signal of the ISFET amplifier was recorded with an Apple IIe computer.

**Measurements.** The experimental setup to encourage the encapsulated sensors was described before and used without modification. The encapsulated sensors were, after evaporation of the solvent, conditioned overnight in a solution of 10 mM primary ion and 0.1 mM acid. The swelling coefficients were determined by the fixed interference method (FIM). All solutions were prepared freshly and adjusted to pH 4 (hydrochloric or nitric acid). The activity of the primary ion in solution was increased in steps of 0.1 (log a for silver, cadmium, or lead) or 0.2 (log a for copper). After addition the solution was stirred in order to obtain a homogeneous solution. The waiting time between stirring and data acquisition was either 0.5 min (silver, cadmium, or lead) or 1 min (copper). Corrections for the activity of the salts were calculated by the extended Debye–Hückel approximation given by Meier.

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