Improved Guanidinium Ion-selectivity by Novel Calix[4]arene and Calix[6]arene Receptor Molecules on CHEMFETs

Felix J. B. Kremer, Gabriela Chiosis, Johan F. J. Engbersen and David N. Reinhoudt*
Department of Chemical Technology, MESA Institute of University of Twente, PO Box 217, 7500 AE Enschede, The Netherlands

Poly(vinyl chloride) membrane CHEMFETs incorporating a novel calix[4]arene derivative (**1**), novel calix[6]arene derivatives (**3**, **4**, **5**, **6** and **7**), phosphate derivatives of *p-tert*-butylcalix[4]arene (**2**) or *p-tert*-butylcalix[6]arene (**8**) with variable amounts of hydrophobic anions [potassium tetrakis(4-chlorophenyl)borate (KTCTPB)] have been tested for their response to guanidinium ions in the presence of the interfering ions Na^+, K^+, NH_4^+ and Ca^{2+}.

The best guanidinium selectivity has been obtained for the CHEMFET based on the 1,3,5-triamide derivative of *p-tert*-butylcalix[6]arene (**4**), with dibutyl phthalate as solvent mediator, and 50 mol% KTCTPB with respect to the ionophore. The selectivity coefficients ($\log K^{\text{pot}}_{\text{Gu.j}}$) are the highest reported for guanidinium ions in a potentiometric sensor. The CHEMFETs show an almost ideal Nernstian response in the linear range (slope 58–60 mV decade^{-1}).

Functionalised *p-tert*-butylcalix[4]arenes are excellent neutral host molecules for the selective recognition of cations. *p-tert*-Butylcalix[4]arenes functionalised with acetic ester or amide ligands at the phenolic positions are selective for sodium.[1,2] Functionalisation with a tetraethyleneglycol bridge between two diametrical phenolic groups introduces selectivity for potassium,[3,4] and sulfide, thioamide and dithiocarbamoyl ligands introduce selectivity for heavy-metal ions.[5] However, for the guanidinium ion, no receptor molecules based on these building blocks were known until now. The guanidinium cation is interesting because of its relevance in biological systems. The guanidinium residue is present in arginine which plays an important role in the binding sites of enzymes[6] and antibodies.[7] Moreover, the guanidinium ion has been used extensively as a model in urea complexation studies, as it is isoelectronic with the uronium cation.[8]

Receptors for the selective recognition of the guanidinium cation require a different geometry than that for most inorganic ions because of the non-spherical shape of this ion. A good receptor for guanidinium should possess multiple hydrogen-bond acceptor sites located in one plane. We have used *p-tert*-butyl-calix[4]arene and -calix[6]arene building blocks which have been specifically derivatized to accommodate the guanidinium cation. The transduction of the molecular recognition into an electronic signal is achieved with an ISFET device.[9] In our group the ISFET device has been further developed to chemically modified field effect transistors (CHEMFETs)[10,11] and these devices have been used in this study.

The selective recognition of the guanidinium cation has been the subject of several investigations in host–guest chemistry.[12–14] It was soon discovered that dibenzo-27-crown-9 (DB27C9[4,5]) has an appropriate ring size for selective recognition and was the most suitable for use in ion selective electrodes. DB27C9[4,5] was studied intensively by Thomas and co-workers[15–17] and Bochenska *et al.*[18] who found that a PVC membrane with dibutyl phthalate as plasticizer and 1–2 wt% tetraphenylborate as anionic sites gave the highest selectivities ($\log K^{\text{pot}}_{\text{Gu.K}} = 0.14$; $\log K^{\text{pot}}_{\text{Gu.Na}} = -1.5$) and a Nerstian slope in the linear concentration range (0.1 to 100 mmol dm^{-3}). More recent studies by Assubaie *et al.*[17] showed that, with bis-*m*-phenylene-26-crown-8 (BMP26C8) as the ionophore, even better results could be obtained ($\log K^{\text{pot}}_{\text{Gu.K}} = -0.7$; $\log K^{\text{pot}}_{\text{Gu.Na}} = -2$). All these measurements were carried out with ion selective electrodes (ISEs); guanidinium selective CHEMFETs have not been reported.

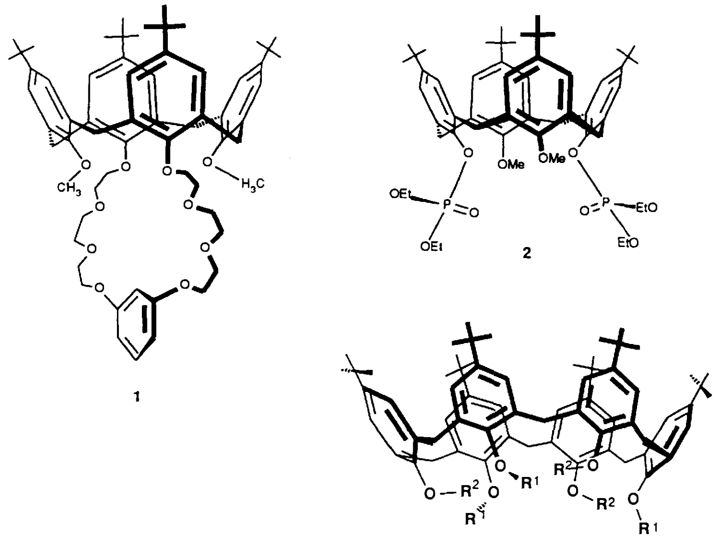
This paper describes the synthesis of novel guanidinium receptors based on *p-tert*-butyl-calix[4]arene and -calix[6]-arene derivatives (Fig. 1), which can be used in a PVC membrane as a guanidinium selective CHEMFET sensor. The PVC membrane composition has been optimized with respect to the type of plasticizer and the concentration of anionic sites, in order to achieve the maximum selectivity and sensitivity.

Results and discussion

The novel guanidinium ionophores **1** and **5**–**8** were synthesized according to general procedures developed for *p-tert*-butyl-calix[4]arene derivatives[19] and calix[6]arene derivatives. The calix[4]arenes were selectively 1,3-functionalized with two methoxy groups and subsequently with a crown ether bridge for **1** and two phosphate groups for **2**.[20] The calix[6]arene ionophores were specifically 1,3,5-functionalized with three methoxy groups and subsequently with three amide **4**,[21] ketone **5**, ester **6**, tosylate **7** or diethylphosphate **8**[22] groups at the remaining positions. Together with the hexamide derivative **3**[21] these guanidinium receptors have been incorporated into PVC membranes and studied with respect to response on changing guanidinium concentrations. The influence of different plasticizers and the influence of the concentration of anionic sites have been investigated as a function of the sensor response.

Influence of the Plasticizer on the Response of CHEMFETs with PVC Membranes.—Assubaie and co-workers have shown in their study on guanidinium selective electrodes with DB27C9[4,5][17] that the electrode composition has an important influence on the electrode performance. Different plasticizers induce differences in ion selectivity, as is given for DB27C9[4,5] in Table 1. This behaviour may be caused partly by differences in the relative permittivity of the plasticizers which will change the partition of the ions between the membrane and the aqueous phase. The effect of differences in ion partition will be the strongest for membranes possessing ionophores of relatively low selectivity, because in this case the sensor selectivity is strongly dominated by the nature of the plasticizer.

We have examined the influence of the plasticizer on the



R^1	R^2	
$CH_2CON(C_2H_5)_2$	$CH_2CON(C_2H_5)_2$	3
$CH_2CON(C_2H_5)_2$	Me	4
CH_2COCH_3	Me	5
$CH_2COOC_2H_5$	Me	6
TOSYL	Me	7
$PO(OEt)_2$	Me	8

Fig. 1 Guanidinium ionophores on calix[4]arene derivatives (**1** and **2**) and calix[6]arene derivatives (**3**, **4**, **5**, **6**, **7** and **8**)

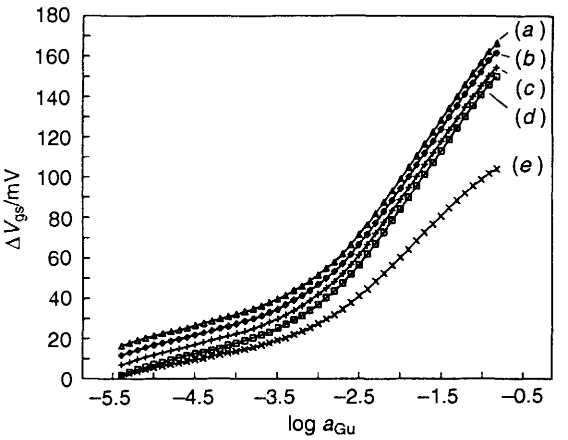


Fig. 2 Guanidinium response of CHEMFETs containing ionophore **4** (1 wt%) in PVC membranes with different plasticizers: (*a*) DBP; (*b*) DOS; (*c*) DOA; (*d*) TOP; (*e*) *o*-NPOE. The interfering salt is 0.1 mol dm^{-3} NaCl.

Table 1 Selectivity data (log $K^{\mathrm{pot}}_{\mathrm{Gu,Na}}$) and slopes (mV $decade^{-1}$) for guanidinium with different plasticizers in PVC membranes and sodium chloride as the interfering salt

			log $K^{\mathrm{pot}}_{\mathrm{Gu,Na}}$ (slope)[a]	
Entry	Plasticizer	ε_r	DB27C9[4,5][b]	**4**[c]
1	DOS	3.88	−0.6 (55 mV)	−1.85 (59 mV)
2	DOA	4.13	−1.2 (55 mV)	−1.85 (59 mV)
3	TOP	4.90	—	−1.85 (59 mV)
4	DBP	6.42	−1.5 (58 mV)	−1.85 (59 mV)
5	*o*-NPOE	24	−1.2 (52 mV)	−1.5 (52 mV)

[a] Interfering salt concentration is 10 mmol dm^{-3} NaCl. [b] Data from ref. 17, measured with ISEs. [c] Data from this work, measured with CHEMFETs.

selectivity of ionophore **4** in a membrane consisting of 33 wt% PVC, 66 wt% plasticizer, 1 wt% ionophore and 50 mol% potassium tetrakis(4-chlorophenyl)borate (KTCPB) relative to the ionophore. In this study five plasticizers of different polarity were used, *viz.*, bis(2-ethylhexyl) sebacate (DOS), bis(2-ethylhexyl) adipate (DOA), tris(2-ethylhexyl) phosphate (TOP), dibutylphthalate (DBP) and 2-nitrophenyl octyl ether (*o*-NPOE). The results are given in Table 1 and in Fig. 2.

The data show that for ionophore **4**, the nature of the plasticizers, within the group of plasticizers of low relative permittivity (DOS, DOA, TOS and DBP), does not influence its behaviour. However, the plasticizer with the highest relative permittivity (*o*-NPOE) gives both a decrease in Nernstian behaviour (slope 52 mV $decade^{-1}$) and in selectivity (log $K^{\mathrm{pot}}_{\mathrm{Gu,Na}}$ −1.5). It is interesting to note that for the more selective ionophore **4** the dependence on the plasticizer is indeed considerably lower than for DB27C9[4,5].

Influence of the Anionic Site on the Response.—From the literature it is known that a guanidinium-sensitive electrode (ISE) can be produced using a membrane without an ionophore, containing only KTCPB as ionic sites.[16] These sensors show Nernstian behaviour (slope 60 mV) but are not very selective (log $K^{\mathrm{pot}}_{\mathrm{Gu,Na}} = -1.6$). The reason for guanidinium selectivity is that KTCPB acts as a cation exchanger which preferentially extracts the relatively lipophilic guanidinium cation into the membrane. Preference of interactions of the dispersed charges of guanidinium cation and tetraphenylborate anion may contribute to some additional selectivity.

The effect of anionic sites in the membrane has been studied by Simon *et al.*[23] They showed that there is an optimal concentration of the KTCPB in the membrane which gives the highest sensitivity and selectivity. For monovalent (alkali-metal) cations the optimal number of anionic sites is usually 50 mol% with respect to the ionophore. In that case 50 mol% of the ionophores will be complexed with the primary ion, in order to maintain electroneutrality. An equal concentration of complexed and free ionophore in the membrane generally ensures the lowest variation of the concentration of free primary ion in the membrane phase and therefore the highest selectivity.[24]

In order to investigate the influence of the amount of anionic sites in the membrane system with DBP as plasticizer and calix[6]arene hexamide derivative **3** as the ionophore, the selectivities of these membranes were measured with three

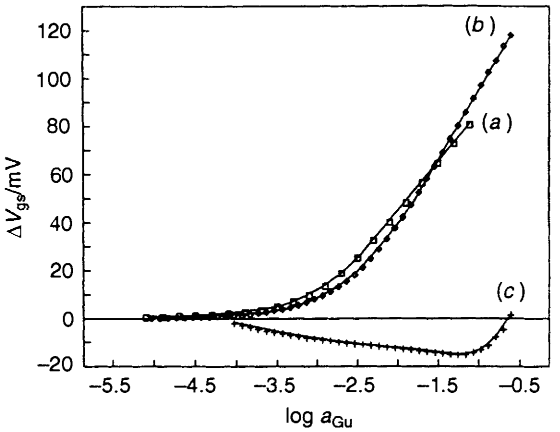


Fig. 3 Guanidinium ion response in the presence of 0.1 mol dm^{-3} sodium chloride of CHEMFETs containing ionophore the calix[6]-arene hexamide **3** and DBP as plasticizer with different numbers of anionic sites: (*a*) 0 mol%; (*b*) 50 mol%; (*c*) 100 mol% with respect to the ionophore

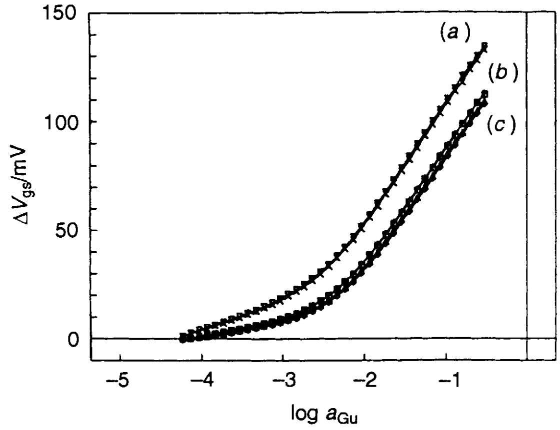


Fig. 4 Guanidinium response of CHEMFETs containing ionophores: (*a*) **4**; (*b*) **5**; (*c*) **1**, in the presence of 0.1 mol dm^{-3} NaCl

different concentrations of KTCPB, *i.e.*, 0, 50 and 100 mol% with respect to the ionophore **3**. Fig. 3 gives the response curves upon increasing the concentration of guanidinium chloride in the presence of 0.1 mol dm^{-3} sodium chloride. As can be seen from the figure there is not a large difference between the response curves contining 0 mol% and 50 mol% of anionic sites. Slightly better Nernstian behaviour is found for the CHEMFET with 50 mol% KTCPB (59 mV decade^{-1}) than for that without KTCPB (57 mV decade^{-1}). An additional advantage of the presence of the KTCPB in the membrane is that the CHEMFETs based on the calix[6]arene hexamide derivative without KTCPB need a very long (longer than 3 days) of conditioning time before they give a stable signal and Nernstian behaviour, probably due to a slower establishment of the guanidinium partition equilibrium. The conditioning time can be reduced to one night when KTCPB is present in the membrane. Membranes with calix[4]arene ionophore **1** and the 1,3,5-triamide calix[6]arene **4** did not require such long conditioning times.

In the case where the concentration of KTCPB is increased to 100 mol% with respect to the ionophore (L), a large effect on both the response and the selectivity is observed. At low guanidinium concentrations this sensor gives an anion response. Since ionophore and anionic sites are present in equal concentrations in the membrane after conditioning with guanidinium ions, they will be present as an LGu$^+$A$^-$ complex. Owing to the high lipophilicity of the guanidinium ion a slight excess of guanidinium ions will be present in the membrane phase which will act as cationic sites. Consequently, the sensor responds to a change in concentration of chloride ions in a Nernstian way. The concentration of Cl$^-$ increases from 10^{-1} mol dm^{-3} at the start of the titration (log a_{Gu} = −5.5) to 2 × 10^{-1} mol dm^{-3} at log a_{Gu} = −1. Therefore Δ log a_{Cl} = 0.3, which corresponds to an expected Nerstian ΔV_{gs} of 0.3 × 59 = 17.7 mV, which coincides with curve (*c*).

Guanidinium Selective Membranes.—From the above results it can be concluded that the optimal membrane for a guanidinium-selective CHEMFET should contain the DBP plasticizer and 50 mol% KTCPB. The membrane typically used is composed of: 33 wt% PVC, 66 wt% DBP, 1 wt% ionophore and 50 mol% (relative to ionophore) KTCPB. Eight different ionophores for guanidinium, *viz*. **1**, **2**, **3**, **4**, **5**, **6**, **7** and **8**, were studied in membranes deposited on CHEMFETs.

All ionophores have been evaluated for their selectivity towards guanidinium ions in the presence of various interfering ions. The results are depicted in Fig. 4 and Table 2. For comparison, a membrane containing only KTCPB was also studied.

The CHEMFET with the 1,3,5-triamide calix[6]arene derivative **4** as the ionophore gave the best response. A Nernstian slope was found in the presence of all interfering ions. Potassium, sodium and ammonium ions are the strongest interfering ions, but even in the presence of these ions the ionophore shows very good selectivity for guanidinium (log $K^{pot}_{Gu,K}$ = −1.8, log $K^{pot}_{Gu,Na}$ = −1.85, log K^{pot}_{Gu,NH_4} = −1.8). Compared with BMP26C8 and DB27C9[4,5], ionophore **4** is 50 to 650 times more selective, depending on the interfering ion. It is also important to note that there is a significant difference in selectivity between the CHEMFET with ionophore **4** and the CHEMFET containing only KTCPB. This confirms that the selectivity measured has a significant contribution from the complexation of guanidinium by the ionophore and is not simply due to cation exchange in the membrane.

The ionophores **1**, **2**, **3**, **5**, **6**, **7** and **8** are slightly less effective but all give a Nernstian response (58–60 mV). The potentiometric selectivity coefficients (log K_{ij}) relative to calcium, potassium, sodium and ammonium vary from −1.6 to −2.8, values which are better than for the receptors used in ISE measurements reported in the literature to date. This study shows for the first time that *charged organic* molecules can be measured selectively with a CHEMFET provided an appropriate membrane composition and ionophore are used. The presence of anionic sites in the membrane is important for the stability of the sensor.

Experimental

Reagents and Materials.—The 1,3,5-triamide and the hexamide derivative of calix[6]arene (compounds **3** and **4**)[21] were kindly supplied by Professor R. Ungaro of the University of Parma. The phosphate-modified ionophores **2** and **8** have been described previously by our group.[20,22]

^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC250F (250 MHz) spectrometer in CDCl$_3$ unless stated otherwise. FAB-mass spectra were obtained with a Finnigan MAT90 mass spectrometer using *m*-nitrobenzyl alcohol (NBA) as a matrix. Melting points were determined with a Reichert melting-point apparatus and are uncorrected. Tetrahydrofuran was freshly distilled from sodium–benzophenone; pyridine was dried and freshly distilled from KOH pellets; DMF was stored over molecular sieves (4 Å). All other solvents and chemicals were of reagent grade and were used without purification. Chromatographic separations were performed on silica gel 60 (SiO$_2$, Merck, Germany, particle size 0.040–0.063 mm, 230–240 mesh). Commercial 3-methacryloxypropyl(tri-

Table 2 Selectivity data (log $K^{pot}_{Gu,j}$) and slopes (mV decade^{-1}) for guanidinium-selective CHEMFETs containing different ionophores in the presence of 0.1 mol dm^{-3} different interfering ions

Ionophore	Interfering ions KCl	NaCl	$CaCl_2$	NH_4Cl
1	−1.1 (54 mV)	−1.5 (57 mV)	−2.75 (59 mV)	−1.6 (59 mV)
2	−1.2 (59 mV)	−1.55 (57 mV)	−2.8 (59 mV)	−1.6 (58 mV)
3	nd^a	−1.7 (57 mV)	nd^a	−1.6 (58 mV)
4	−1.8 (59 mV)	−1.85 (59 mV)	−2.85 (59 mV)	−1.8 (59 mV)
5	−1.15 (53 mV)	−1.6 (58 mV)	−2.8 (59 mV)	−1.5 (58 mV)
6	−0.95 (48 mV)	−1.6 (58 mV)	−2.75 (59 mV)	−1.55 (58 mV)
7	−1.4 (57 mV)	−1.75 (58 mV)	−2.8 (59 mV)	−1.6 (58 mV)
8	−1.4 (59 mV)	−1.8 (58 mV)	−2.4 (59 mV)	−1.6 (58 mV)
KTCPB	−1.3 (61 mV)	−1.6 (60 mV)	−2.6 (61 mV)	−1.4 (60 mV)

^{a} nd = not determined.

methoxy)silane (MEMO, Janssen Chemica), ethylene glycol dimethacrylate (EGDMA, Merck-Schuchardt, Germany), 2-hydroxyethyl methacrylate (HEMA, Merck-Schuchardt, Germany) and 2,2-dimethoxy-2-phenylacetophenone (photoinitiator, Janssen Chimica, Belgium) were used without further purification. Poly(vinylpyrrolidone) (PVP), Janssen Chimica, Belgium) with an average weight of 360.000 Dalton was used. The plasticizer di-*N*-butyl phthalate (DBP) was obtained from BDH (England) and bis(2-ethylhexyl) sebacate (DOS), bis(2-ethylhexyl) adipate (DOA), tris(2-ethylhexyl) phosphate (TOP), and 2-nitrophenyl octyl ether (*o*-NPOE) were purchased from Fluka (Switzerland), as was potassium tetrakis(4-chlorophenyl) borate (KTCPB). Guanidinium hydrochloride was obtained from Janssen Chimica (Belgium). The chloride salts of calcium, ammonium, potassium and sodium were of analytical reagent grade (Merck-Schuchardt, Germany). All solutions were prepared with deionized, doubly distilled CO_2-free water.

$1^5,23^5,25^5,28^5$-*Tetra*-tert-*butyl*-$25^2,28^2$-*dimethoxy*-2,5,8,11,-13,16,19,22-*octaoxa*-1(2,1,3),12(1,3),23(2,1,3),25(1,3),28(1,3)-*pentabenzenabicyclo*[21.3.3]*nonacosaphane* (**1**).—A suspension of 0.2 g (0.295 mmol) 1,3-dimethoxy-*p*-*tert*-butylcalix[4]-arene[21] and 0.14 g (5.9 mmol) NaH in 250 cm^3 THF–DMF (v:v = 5:1) was stirred for 1 h at room temperature. Subsequently 0.29 g (0.295 mmol) of *m*-phenylenedioxybis(3,6-dioxaoctyl toluene-*p*-sulfonate) in 5 cm^3 of the same solvent were slowly added over 1 h at 40 °C with vigorous stirring. The reaction mixture was refluxed for 24 h after which a small amount of NaI and triethylamine were added to remove the unchanged tosylate. The reaction mixture was refluxed for another hour. After cooling, the solvent was removed by evaporation and the remaining residue was dissolved in dichloromethane. The organic layer was washed twice with a saturated solution of NH_4Cl in water and the organic solvent was removed by evaporation. The product was crystallized from methanol–acetone to yield white crystals of pure **1** (85 mg, 29%); m.p. 160–162 °C (methanol–acetone) (Found: C, 75.45; H, 8.5. $C_{64}H_{86}O_{10}$ requires C, 75.71; H, 8.54%); δ_H(250 MHz; CD_2Cl_2) 0.92 [18 H, s, $C(CH_3)_3$], 1.33 [18 H, s, $C(CH_3)_3$], 3.19 (4 H_{eq}, d, *J* 17, $ArCH_2Ar$), 3.54 (6 H, s, $ArOCH_3$), 3.55–3.71 (8 H, m), 3.72–3.83 (8 H, m), 3.84–3.90 (4 H, m), 3.97–4.07 (4 H, s, OCH_2CH_2O), 4.16 (4 H_{ax}, d, *J* 17, $ArCH_2Ar$), 6.38 (1 H_{ar}, m), 6.43 (2 H_{ar}, s), 6.55 (4 H_{ar}, s, calix), 6.95–7.04 (1 H_{ar}, m) and 7.1 (H_{ar}, s, calix); δ_C(250 MHz; CD_2Cl_2) 31.32, 31.78 [q, $C(CH_3)_3$], 33.58, 33.99 [s, $C(CH_3)_3$], 59.49 (q, OCH_3), 68.25, 69.94, 70.85, 70.90, 71.09, 73.27 (t, OCH_2), 102.02, 108.15 (d, C_{ar}), 125.02, 125.97 (d, C_{ar} calix), 129.75, 132.24 (s, C_{ar} calix), 135.19 (d, C_{ar}), 144.19, 144.55 (s, C_{ar} calix), 153.77, 155.98 (s, C_{ar} calix) and 160.29 (s, C_{ar}); *m*/*z* (FAB; NBA) 1014.6 (M^+, calc. 1014.6).

$1^5,3^5,5^5,7^5,9^5,11^5$-*Hexa*-tert-*butyl*-$1^2,5^2,9^2$-*trimethoxy*-3^2,-$7^2,11^2$-*tri(methoxycarbonylmethoxy)*-1(1,3),3(1,3),5(1,3),7(1,3), 9(1,3),11(1,3)-*hexabenzenacyclododecaphane* (**5**).—To a stirred solution of 111 mg (0.74 mmol) of NaI and 68.5 mg (0.74 mmol) of chloroacetone in 2 cm^3 THF–DMF (v:v = 5:1) were added, after 15 min, 241 mg (0.74 mmol) of Cs_2CO_3, 100 mg (0.098 mmol) of 1,3,6-trimethoxycalix[6]arene[21] and 10 cm^3 of solvent. The mixture was refluxed for 24 h and after cooling the solvent was removed under reduced pressure. The residue was taken up in water and heated for 1 h at 60 °C. After cooling, the product was extracted into dichloromethane. The solvent was evaporated and the crude product was recrystallized from methanol to give pure **5** as white crystals (77 mg, 66%); m.p. 175–178 °C (methanol) (Found: C, 79.75; H, 8.6. $C_{78}H_{102}O_9$ requires C, 79.15; H, 8.69%); δ_H(250 MHz; $CDCl_3$) 1.07 [27 H, s, $C(CH_3)_3$], 1.22 [27 H, s, $C(CH_3)_3$], 1.82 (9 H, s, $COCH_3$), 3.11 (9 H, s, OCH_3), 3.7–3.89 (12 H, br s, $ArCH_2Ar$), 4.01 (6 H, s, OCH_2CO), 6.9 (6 H_{ar}, s) and 7.12 (6 H_{ar}, s); δ_C(250 MHz; $CDCl_3$) 25.89 (q, CH_3), 30.17 (t, $ArCH_2Ar$), 31.30, 31.46 [q, $C(CH_3)_3$], 34.11, 34.18 [s, $C(CH_3)_3$], 60.24 (q, OCH_3), 77.52 (t, OCH_2CO), 125.58, 126.68 (d, Ar *meta*), 132.98, 133.02, 146.02, 146.44, 151.51, 153.81 (d, C Ar) and 205.33 (s, CO); *m*/*z* (FAB; NBA) 1184.3 ($M + H^+$, calc. 1182.8).

$1^5,3^5,5^5,7^5,9^5,11^5$-*Hexa*-tert-*butyl*-$1^2,5^2,9^2$-*trimethoxy*-$3^2,7^2$,-11^2-*tri(ethoxycarbonylmethoxy)*-1(1,3),3(1,3),5(1,3),7(1,3),-9(1,3),11(1,3)-*hexabenzenacyclododecaphane* (**6**).—To a stirred mixture of 100 mg (0.598 mmol) of 2-bromoethyl acetate and 90 mg (0.6 mmol) of NaI in 2 cm^3 THF–DMF (v: = 5:1) were added, after 20 min, 100 mg (0.098 mmol) of 1,3,6-trimethoxycalix[6]arene[21] and 10 mg (0.42 mmol) of NaH in 10 cm^3 of THF–DMF. The mixture was refluxed for 3 days at 70 °C and subsequently cooled to room temperature. The mixture was filtered over Celite and the solvent was evaporated off at reduced pressure. The remaining iodoacetate was removed under high vacuum. The crude product was crystallized from methanol–acetone to yield a slightly yellow solid. Recrystallization from methanol gave white crystals of pure **6** (105 mg, 84%); m.p. 192–193 °C (Found: C, 77.0; H, 8.2. $C_{81}H_{108}O_{12}$ requires C, 77.65; H, 8.09%); δ_H(250 MHz; $CDCl_3$) 0.78 [27 H, s, $C(CH_3)_3$], 1.25 (9 H, t, *J* 7.2, CH_2CH_3), 1.3 [27 H, s, $C(CH_3)_3$], 2.25 (6 H, s, OCH_3), 3.18–3.58 (6 H_{eq}, br s, $ArCH_2Ar$), 4.20 (6 H, q, *J* 7, $COCH_2CH_3$), 4.12–4.53 (6 H_{ax}, br s, $ArCH_2Ar$), 6.63 (6 H_{ar}, s) and 7.18 (6 H_{ar}, s); δ_C(250 MHz; $CDCl_3$) 14.24 (q, CH_3), 30.11 (t, $ArCH_2Ar$), 31.16, 31.59 [q, $C(CH_3)_3$], 34.02, 34.21 [s, $C(CH_3)_3$], 60.07 (q, OCH_3), 61.05 (t, OCH_2CH_3), 69.65 (t, OCH_2CO), 123.98, 128.06 (d, Ar *meta*), 132.89, 133.53, 145.84, 146.28, 154.66, 169.25 (s, C Ar) and 151.54 (s, CO); *m*/*z* (FAB; NBA) 1272.6 (M^+, calc. 1272.8).

$1^5,3^5,5^5,7^5,9^5,11^5$-*Hexa*-tert-*butyl*-$1^2,5^2,9^2$-*trimethoxy*-3^2,-$7^2,11^2$-*tri(toluene*-p-*sulfonyl)*-1(1,3),3(1,3),5(1,3),7(1,3),9(1,3),-11(1,3)-*hexabenzenacyclododecaphane* (**7**).—A solution of 38 mg (0.038 mmol) 1,3,5-trimethoxycalix[6]arene[21] and 120 mg (0.63 mmol) of tosyl chloride in 2 cm^3 of dry pyridine was stirred in an ice-bath overnight. Another 120 mg of tosylate in 2 cm^3 pyridine was added and the stirring was continued for another 12 h at 0 °C. The reaction was quenched by adding small pieces of ice and concentrated HCl (slowly) until the mixture was neutralized. The product was extracted with dichloromethane which yielded the crude product after evaporation. Crystallization from methanol afforded pure **7** (50 mg, 90%) as white crystals; m.p. 205–206 °C (MeOH) (Found: C, 73.1; H, 7.1. $C_{90}H_{102}O_{12}S_3$ requires C, 73.44; H, 6.98%); δ_H(250 MHz; $CDCl_3$) 0.70 [27 H, s, $C(CH_3)_3$], 1.29 [27 H, s, $C(CH_3)_3$], 1.98 (9 H, s, CH_3), 2.39 (9 H, s, OCH_3), 3.33 (6 H_{eq}, d, *J* 16.4, $ArCH_2Ar$), 4.19 (6 H_{ax}, d, *J* 16.4, $ArCH_2Ar$), 6.58 (6 H_{ar}, s), 7.15 (6 H_{ar}, s), 7.3 (6 H, d, *J* 8.3, OSO_2Ar) and 7.85 (6 H, d, *J* 8.3, OSO_2Ar); δ_C(250 MHz; $CDCl_3$) 21.74 (q, CH_3), 30.75 (t, $ArCH_2Ar$), 30.97, 31.57 [q, $C(CH_3)_3$], 34.11, 34.25 [s, $C(CH_3)_3$], 59.85 (q, OCH_3), 124.13 (d, $CHOSO_2Ar$), 128.25, 130.00 (d, Ar *meta*), 132.51, 133.83 (s, Ar), 134.82 (s, $ArCH_3$), 142.83, 145.30, 146.18, 148.73 (s, Ar) and 154.27 (s, OSO_2Ar); *m/z* (FAB; NBA) 1471.9 (M^+, calc. 1472.0).

CHEMFET Assembly and Measurements.—ISFETs were fabricated as described elsewhere.[11] The surface silylation of the wafer was performed as described in the literature.[25] On waferscale the gate oxide of the ISFET was covered by a polyHEMA hydrogel. This is applied by photopolymerization with a mask-aligner of a mixture of HEMA, water, PVP, photoinitiator and EGDMA in a w/w ratio of 100:40:10:4:0.4. The dimensions of the resulting polyHEMA layer were 800 × 800 × 15 μm. The ISFETs were prepared for use by mounting them on a printed circuit board, wire bonding and encapsulation with an epoxy resin (Hysol H-W796/C8W795) to prevent leakage currents to the bulk solution. The polyHEMA layer on the sensors was soaked for 1 h in a solution of 0.1 mol dm^{-3} guanidinium hydrochloride in buffer pH 4.0 (Merck) before applying the guanidinium-sensitive membrane onto the gate.

The ion-sensitive membranes were prepared by casting 10 μl of a freshly prepared solution of PVC–plasticizer–ionophore–KTCPB (if used) in freshly distilled tetrahydrofuran (THF) on the gate region. The response of the CHEMFETS was measured with an ISFET amplifier (Electro Medical Instrumentation, The Netherlands) source and drain follower type in a constant drain-current mode (I_d = 100 μA), with a constant drain-source potential of 0.5 V (V_{ds}).[11] All measurements were performed against a calomel reference electrode. The signal of the ISFET amplifier was recorded with an Apple IIgs computer.

The experimental set-up to measure the guanidinium sensitive sensors is described elsewhere and was used without modification.[26] The sensors could be used for measurement after conditioning for 1 night in a Merck buffer pH 4.0 solution to which was added 0.1 mol dm^{-3} of guanidinium hydrochloride and 10 mmol dm^{-3} of the chloride salt of one of the interfering ions (Na^+, K^+, NH_4^+ and Ca^{2+}). Longer conditioning times were necessary, up to 3 days, when calix[6]arene-based ionophores were used in membranes without anionic sites. The selectivity coefficients were obtained by the fixed interference method (FIM). The activity of the guanidinium ions in solution was increased in steps of 0.1 (log a_{Gu}). During addition the mixture was stirred in order to obtain a homogenous solution. The data acquisition started 15 s after the stirring had stopped. The burettes (Metrohm) and stirrer were controlled with an Apple IIgs computer which also collected and processed the data. The accuracy of the data obtained in this study are ±0.02 for log $K^{pot}_{i,j}$ and ±0.5 mV $decade^{-1}$ for the slope in the linear part of the response curves.

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