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Hydrogen-Bonding Ionophores for Inorganic Anions and Nucleotides and Their Application in Chemical Sensors

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Abstract. The potential and limits of hydrogen-bonding ionophores and their use in chemical sensors are discussed. Several hydrogen-bonding bis-thiourea ionophores have been found to complex inorganic anions, among them most strongly $H_2PO_4^-$. Using such ionophores, ion-selective electrodes for chloride and sulfate have been developed. Furthermore, hosts that bind nucleotides with up to five hydrogen bonds have been synthesized. They have been applied in nucleotide selective electrodes, optodes and voltammetric sensors mimicking ion channels.

Key words: ion-selective electrode, optode, voltammetric sensor, hydrogen bond, thiourea, nucleotide, anion.

1. Introduction

Many synthetic ionophores for inorganic cations have been developed and applied in chemical sensing, such as with ion-selective electrodes (ISEs) [1,2] or voltammetric and optical [1, 2] sensors. On the other hand, only relatively few hosts have been used in chemical sensing of inorganic and organic anions. Among the ionophores that have no metal center and that have been tested for such purposes are macrocyclic polyamines and guanidines, which bind anions primarily by charge–charge interactions, as well as trifluoroacetophenones and benzaldehydes, which bind carbonate and sulfite, respectively, by formation of a covalent bond [2]. However, only few examples of sensor applications of neutral hosts that bind anions primarily by hydrogen bonding have been reported so far. We describe here our recent work on hosts that bind anions by hydrogen bonding and their application for various types of chemical sensors.

Table I. Association constants (K_{11}, M^{-1}, in DMSO-d_6) of hosts **2** and **4** to **8** with various anions

Anion[a]	Host **2**	Host **4**	Host **5**	Host **6**	Host **7**	Host **8**
$H_2PO_4^-$	110	820	4600	1000	55000	195000
CH_3COO^-	47	470	2300	350	38000	[b]
Cl^-	4	9	10	5	840	1000
HSO_4^-	1	2	[b]	[b]	[b]	[b]
NO_3^-	<1	<1	[b]	[b]	[b]	[b]
ClO_4^-	nc[c]	nc[c]	[b]	[b]	[b]	[b]

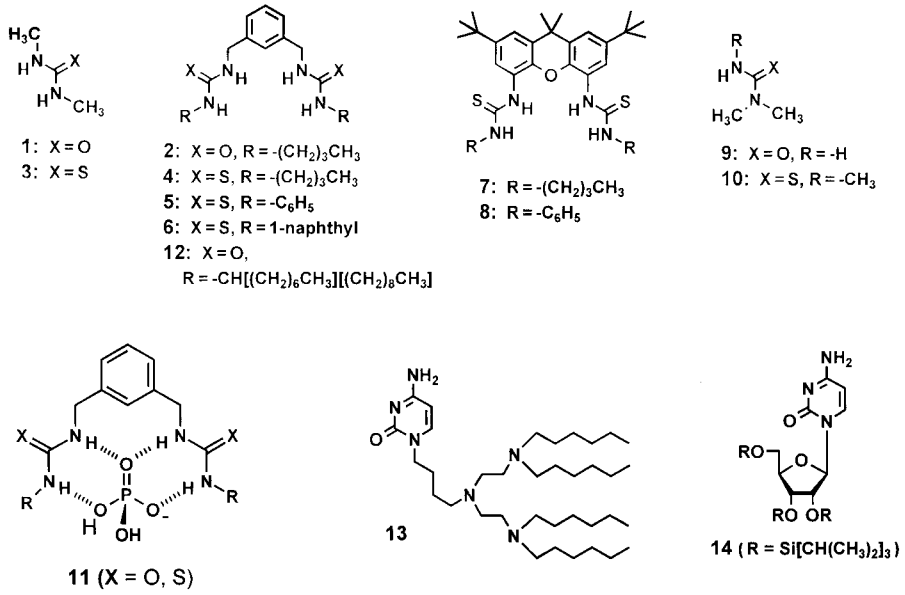
[a] Counter-ion: $N(C_4H_9)_4^+$.
[b] Not determined.
[c] Negligible complexation.

2. Recognition of Inorganic Anions by Bis-Urea and Bis-Thiourea Hosts

A number of neutral hosts that bind inorganic and organic phosphates by hydrogen bonds or a group with Lewis acidity have been reported [3], but fully satisfactory compounds for use in sensors are very few. We were interested in relatively easily synthesizable hosts with a good solubility in organic solvents and a high chemical stability. Given reports on hosts that bind dicarboxylates, disulfonates and diphosphonates by hydrogen bonds to urea groups [4–6] and a dicarboxylate host with two thiourea groups [5], we wondered whether appropriate preorganization of hosts with two or more urea or thiourea groups would result in phosphate-selective hosts. We have synthesized a number of such compounds, characterized their complexes with anions in dimethyl sulfoxide (DMSO) and determined the selectivity of ISEs based on membranes containing them.

The strengths of the complexes between $H_2PO_4^-$ and urea **1**, bis-urea **2**, thiourea **3**, or one of the bis-thioureas **4** to **8** have been determined by ^1H NMR spectroscopy with DMSO-d_6 as the solvent [7, 8]. Stability constants, K_{11}, for 1 : 1 complexes are given in Table I. The increases in stability of the $H_2PO_4^-$ complexes from mono-urea **1** (28 M^{-1}) to bis-urea **2** (110 M^{-1}) or from thiourea **3** (120 M^{-1}) to bis-thiourea **4** (820 M^{-1}) show the effectiveness of multitopic hydrogen bonding. The hydrogen bond acceptor in these complexes seems to be only $H_2PO_4^-$ and neither the carbonyl nor the thiocarbonyl groups, as evidenced by the extremely weak binding of $H_2PO_4^-$ by *N*, *N*-dimethylurea (**9**) and *N*, *N*, *N*′-trimethylthiourea (**10**). The structure of the $H_2PO_4^-$ complexes of the bis-urea and bis-thiourea hosts (**11**) seems to strongly resemble the structure that has been suggested for the complex of $H_2PO_4^-$ and a bisguanidine [9].

The stabilities of the $H_2PO_4^-$ complex increase significantly when the ureas are replaced by thiourea groups [7], which is the result of an increase in the hydrogen bond acidity of the latter. Similarly, the replacement of butyl by phenyl substituents



Scheme 1–14.

increases complex stabilities, the phenyl groups enhancing the strength of the hydrogen bond donors [10]. Further increases in complex stabilities could be obtained by using xanthene as the linking element between the two thiourea groups [8]. This spacer has been introduced by Rebek et al. as a convenient platform for the synthesis of highly preorganized hosts [4]. Its rigidity seems to be the primary reason for the high stabilities of the $H_2PO_4^-$ complexes of **7** and **8**. To the best of our knowledge, no published neutral host forms $H_2PO_4^-$ complexes that are stronger than those of these two bis-thioureas.

The selectivity of synthetic hosts is of primary interest for the development of a chemical sensor. The strengths of the 1 : 1 complexes of bis-urea and bis-thiourea hosts have, therefore, been determined by ^{1}H NMR (Table I) [7,8]. In DMSO, $H_2PO_4^-$ is bound most strongly and CH_3COO^- is the only other anion bound substantially. The guest basicity and structure seem to explain this selectivity to a large extent, but the solvent in which the complexation takes place cannot be neglected [8]. The free energies of hydration, $\Delta G^\circ_{n-1,n}$, for the hydration equilibria $X^-(H_2O)_{n-1} + H_2O \rightleftarrows X^-(H_2O)_n$ in the gas phase, which have recently become available by electrospray mass spectroscopy, provide a good measure of hydrogen bond acceptor strength. The free energies of hydration $\Delta G^\circ_{0,1}$ (in kcal/mol) in the gas phase for CH_3COO^-, Cl^-, $H_2PO_4^-$, NO_3^-, HSO_4^- and ClO_4^- (9.4, 8.2, 7.6, 7.1, 5.9 and 4.8, respectively) and K_{11} (M^{-1}) for the 1 : 1 complexation of host **4** with these anions (470, 9, 820, <1, 2 and no binding, respectively) show that the weak binding of NO_3^-, HSO_4^- and ClO_4^- can be explained by the relatively low hydrogen bond acceptor strength of these anions. The fairly weak binding of Cl^-

to the hosts **2** and **4** seems, however, to be partly a solvation effect. Indeed, strong ion-dipole interactions between Cl^- and DMSO are well known. It is therefore not too surprising that an ISE based on host **7** gives a good Cl^- selectivity (vide infra).

3. Ion-Selective Electrodes Based on Bis-Thiourea Hosts

The bis-urea host **12** and bis-thiourea hosts **4**, **5** and **7** have been incorporated into solvent polymeric membranes as conventionally used in ion-selective potentiometry [11, 12]. The potentiometric response of electrode membranes containing bis-urea **12** and bis-thiourea **4** did not differ much from that of membranes without ionophore. To interpret this, it is necessary to know that membranes containing a neutral carrier but no highly lipophilic or immobilized cationic components (often called 'cationic sites') cannot respond to changes in the anion concentration with a lasting change in the measured potential. The neutral ionophore promotes salt extraction in the absence of such ionic sites but a lasting EMF response does not result [13, 14]. Therefore, the membrane of a neutral ionophore-based electrode for anions necessarily contains ionophore, cationic sites, and as counterions to the cationic sites the anion of interest. Variation of the mole ratio of the anion ionophore and the cationic sites results in selectivity changes, which provides an extremely useful means for optimizing the potentiometric selectivity [1]. When a membrane contains either no ionophore or an inefficient ionophore, the potentiometric selectivity is determined by the hydrophilicity of the investigated anions and their solvation in the membrane. Anions with a high hydrophilicity give only small potentiometric responses. The resulting sequence of response is called the Hofmeister series:

$$ClO_4^- > SCN^- > I^- > \text{salicylate}^- > NO_3^- > Br^- > Cl^- > HCO_3^-$$

$$> OAc^- > SO_4^{2-} > HPO_4^{2-}$$

An efficient ionophore, however, modifies the response to various anions, as can be seen in Figure 1 for an electrode based on the better ionophore **5**. Such hosts enhance the transfer of specific anions from the sample solution into the membrane by selective complexation. The higher the anion hydrophilicity (i.e. the further to the right the anion of interest stands in the Hofmeister series), the more difficult it becomes, however, to construct an electrode that is truly selective for that anion. This explains why the present electrodes based on hosts for $H_2PO_4^-$ respond more strongly to other anions than phosphate even though the complexation in homogeneous solution is selective. A truly phosphate selective electrode must be based on a host that binds phosphate considerably more strongly than any other anion in the Hofmeister series because the free complexation energy of the phosphate complex must exceed the free energy of complexation of the other anions by more than the difference in the free energies for the ionophore-unassisted transfer

of phosphate and those for other anions into the membrane. Similar to our bis-thiourea ionophores, uranyl salophen derivatives have been demonstrated to bind $H_2PO_4^-$ selectively. Their use does not give phosphate- but rather nitrite-selective electrodes [15].

Striking is however the strong response and surprising selectivity of electrodes based on host **5** for the dianion sulfate (Figure 1a) [12]. A response down to 1 μM sulfate is obtained, which is a very big improvement as compared to the response of an ionophore-free anion exchanger ISE (Figure 1b). To the best of our knowledge, the electrode based on **5** is the first host-based electrode with an appreciable selectivity for sulfate (for selectivity coefficients see ref. [12]). The development of sulfate selective electrodes is of particular interest for drinking and waste water as well as for soil analysis.

On the other hand, electrodes based on ionophore **7** have shown a good selectivity for chloride [11]. In contrast to electrodes based on ionophore **5** (Figure1a), electrodes based on **7** (Figure 1c) discriminate sulfate fairly well (for selectivity coefficients see ref. [11]). Furthermore, the discrimination of other anions is large enough to allow determinations of the chloride concentration in serum samples, as has been demonstrated with control horse serum. The interference from the clinically relevant salicylate [2] as observed with this electrode is much smaller than for ionophore-free ion-exchanger electrodes (Figure 1b), which is surprising when considering that ionophore **7** forms 1 : 1 complexes of considerable strength with acetate in DMSO. An explanation for this finding could be that at relatively low concentrations of the cationic site a 2 : 1 complex between ionophore and Cl^- is formed.

4. Hydrogen Bond-Based Recognition of Nucleotides

Whereas a large number of elaborate synthetic hosts with both hydrogen bond acceptor and donor sites have been described in supramolecular chemistry, so far only very few of them have been used in potentiometry. Our interest has been the use of such hosts for nucleotide sensing. Early potentiometric sensors for these analytes have been based on macrocyclic polyamines [16, 17], which seem to bind nucleotides upon protonation and primarily interact with the phosphate groups of these analytes. More recently, electrodes based either on a cytosine derivative with a pendant triamine (**13**), and electrodes based on a macrocyclic polyamine and a lipophilic cytidine derivative (**14**) have been reported (double host approach) [18, 19]. To the best of our knowledge, these electrodes were the first examples of ISEs based on ionophores with hydrogen bond acceptor and donor sites that interact simultaneously with the analyte ion.

Electrodes based on the cytosine-pendant triamine **13** responded to guanosine-5′-monophosphate (5′-GMP) and guanosine-5′-triphosphate (5′-GTP) while adenosine-5′-monophosphate (5′-AMP) and adenosine-5′-triphosphate (5′-ATP) did not yield any responses. This good selectivity was explained by ditopic recog-

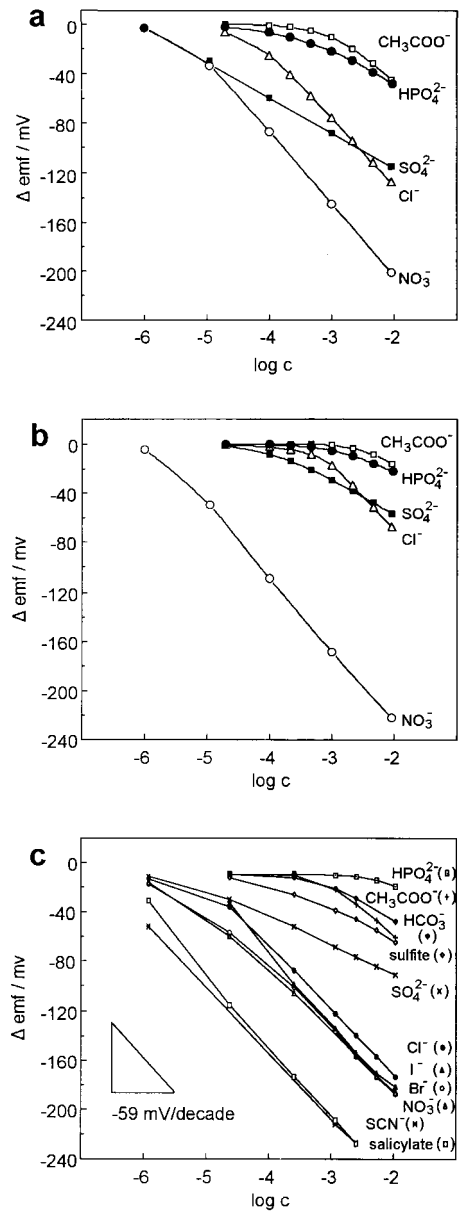
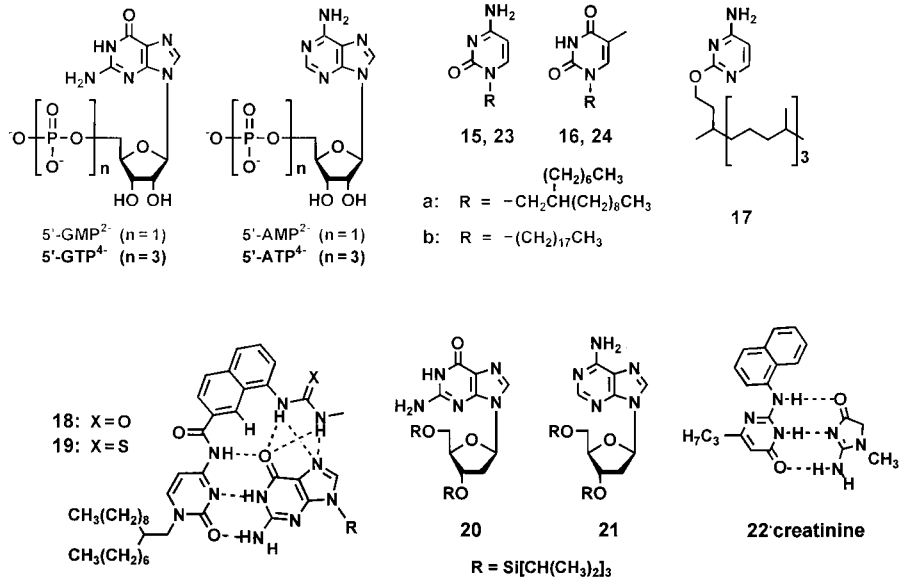


Figure 1. Potentiometric responses of electrodes with membranes prepared with (a) 1 wt% ionophore **5** and 55 mol% of tridodecylmethylammonium chloride (TDDMACl, relative to the ionophore; pH 6.8); (b) 6 wt% TDDMACl (no ionophore; pH 6.8); (c) 1 wt% ionophore **7** and 50 mol% of TDDMACl (pH 7.0). The membrane matrix and plasticizer were poly(vinyl chloride) and *o*-nitrophenyl octyl ether (1 : 2), respectively. The sample solutions were buffered with 0.1 M HEPES-NaOH. Figure 1c adapted from [11] with permission.



Scheme 15–22.

nition based on complementary base pairing and electrostatic interactions between the guanosine nucleotides and the di-protonated cytosine-pendant triamine. The slopes of the emf responses of this electrode were, however, smaller than expected from the charge of the guanosine nucleotides, reducing the sensor sensitivity. Also, the response slope of the electrodes containing the lipophilic cytidine derivative and macrocyclic amines could not be easily interpreted. It seems that multiple protonation equilibria must be considered for a thorough discussion of the very complex response mechanism of these electrodes.

To analyze the influence of ionophore solvation and self-association and the complex stoichiometry on the potentiometric selectivities, we recently investigated simpler systems of membranes containing either only sterically crowded, cationic sites, or the neutral nucleobase derivatives **15** or **16**, and sterically crowded, cationic sites [20]. 5′-Monophosphates of guanosine and adenosine, which are both expected to form Watson–Crick-analogous base-pairs with ionophores **15** and **16**, respectively, were used as analyte ions.

As reference systems, electrode membranes prepared from the chloride salt of the lipophilic tridodecylmethylammonium ion (TDDMACl; 3.0 wt%) but no ionophore, and therefore no selectivity inducing components, were used. During conditioning of the membrane in nucleotide solutions prior to measurements, the chloride ions leave the membrane bulk and are replaced by nucleotide anions. These electrodes showed similar potentiometric responses to 5′-GMP and 5′-AMP (Figure 2), and the slopes of the emf responses (–29 mV/decade, pH 6.8) were as expected for an equilibrium response to a dianion (Nernstian slope). This shows that the response of this electrode can be interpreted on the basis of thermodynamic

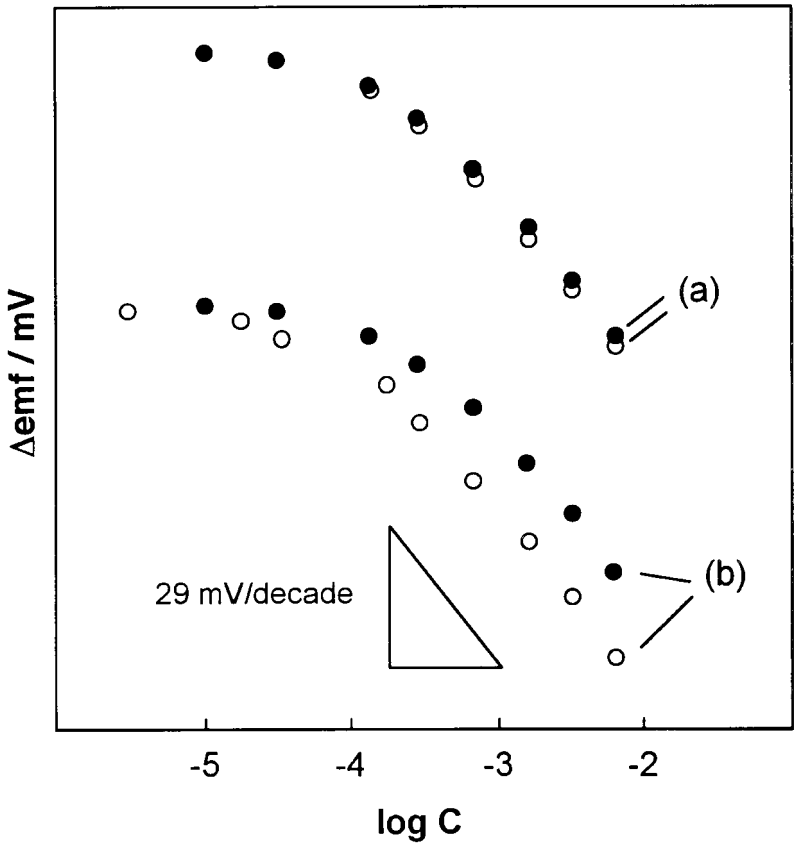


Figure 2. Potentiometric responses to 5′-GMP (○) and 5′-AMP (●) of membranes prepared with (a) the lipophilic salt tridodecylmethylammonium chloride (TDDMACl; 3.0 wt%) and (b) TDDMACl (3.0 wt%) and the neutral cytosine derivative **15** (1.3 wt%) as ionophore (150 mol% cationic sites relative to **15**). The membrane matrix and plasticizer were poly(vinyl chloride) and bis(2-ethylhexyl) phthalate (1 : 2), respectively. Measured at pH 6.8 (0.1 M HEPES-NaOH buffer). From [20] with permission.

equilibria at the phase boundary of the ISE membrane and the sample solution. Kinetic effects on the selectivites need not to be considered. Also electrodes based on the cytosine derivative **15** (1.3 wt%) as ionophore and 150 mol% cationic sites (Figure 2) gave Nernstian responses, the potentiometric responses being selective for 5′-GMP over 5′-AMP. This may seem easily explainable by the number of hydrogen bonds formed between ionophore **15** and the base moiety of these nucleotides in the corresponding 1 : 1 complexes, cytosine forming three hydrogen bonds with the guanine base but only two with adenine base. The difference in the emf of 12 mV corresponds, however, only to a free energy, ΔG, of 2.0 kJ/mol (as obtained from $\Delta G = zF\Delta\phi$, z being the charge of the analytes and $\Delta\phi$ the difference in the emf), while the free energy of formation of a typical hydrogen bond in $CDCl_3$ is about –5.0 ± 1.0 kJ/mol. This suggests that the experimentally

observed potentiometric selectivity is not determined by the stabilities of the 1 : 1 complexes alone.

For the similar alkoxy host **17**, which can also form 1 : 1 complexes with guanosine nucleotides, the change of the selectivity with the mole ratio of the ionophore and cationic sites showed a maximum in potentiometric selectivity in the presence of 150 mol% cationic sites. This would not be expected if 5′-GMP and 5′-AMP formed only 1 : 1 complexes with the host, but it can be explained by formation of complexes with a higher stoichiometry, or by the occurrence of multiple complex stoichiometries. ^{13}C NMR spectra of ISE membranes showed that hydrogen bonds between the ionophore and the membrane plasticizer are formed, which is expected to decrease the potentiometric selectivity.

It can be summarized that the potentiometric selectivities were not as large as expected only from simple consideration of 1 : 1 host-guest complexation. Self-association of the ionophore, hydrogen bond formation between ionophore and membrane plasticizer and multiple complexation equilibria are possible explanations for this finding. While similar effects probably also played a role in earlier nucleotide ISEs, the selectivity of those electrodes could not be analyzed further because of their non-Nernstian responses. Possible measures that are expected to improve the potentiometric selectivity seem to be (i) use of ionophores that form stronger complexes, (ii) modification of the ionophore to avoid ionophore self-association and (iii) a use of membrane plasticizers that do not interact appreciably with the ionophores. While workers in supramolecular chemistry are often primarily interested in the properties of 1 : 1 host-guest complexes, this work once more shows that use of hosts for sensing purposes must be based on a host design that goes beyond this viewpoint.

5. Optical Sensing of Organic Analytes Based on Multitopic Hydrogen Bonding

The hosts **18** and **19**, which can form five hydrogen bonds to nucleotides, were synthesized in an attempt to obtain higher complexation selectivity [21]. To the best of our knowledge, these are only the second and third synthetic receptors that form more than three hydrogen bonds to the guanine base. The UV/visible spectra of **18** and **19** in a mixed solvent of $CHCl_3$ and DMSO (4 : 1, v/v) undergo no significant changes upon complexation of **20**, but the fluorescence emission of both hosts is quenched by this guest. The lipophilic adenosine derivative **21** on the other hand did not influence the fluorescence spectra, as shown for host **18** in Figure 3. The fluorescent response of an optical sensor based on a membrane containing host **18** and cationic sites was examined by using 5′-triphosphates of guanosine and adenosine as analytes. The response mechanism of this optode is based on ion-exchange: nucleotides enter the membrane where they form complexes with the host while, in exchange, chloride ions leave the membrane, thus maintaining

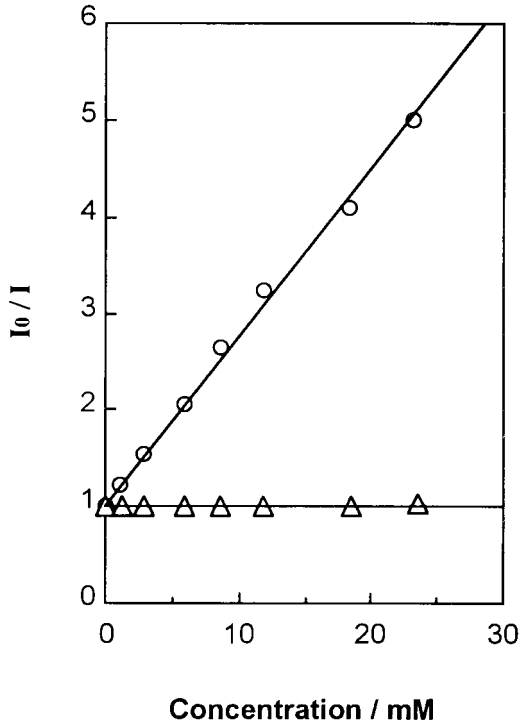


Figure 3. Plot of the ratios of fluorescence intensities of solutions of host **18** (0.11 mM) in $CHCl_3$-DMSO (4 : 1 v/v) in absence (I_0) and in presence (I) of guest **20** (○) or **21** (△) . Excitation at 373 nm, emission at 488 nm. From [21] with permission.

electroneutrality in the bulk phases [1]. This optode showed a selectivity that was smaller than the DMSO/$CHCl_3$ system but it was still selective for 5′-GTP.

A UV and not fluorescence transduction has also been found for the host **22** derived from 2-amino-4(3*H*)pyrimidone, which forms three hydrogen bonds to creatinine, allowing for extraction of this guest into an organic solvent [22, 23]. The origin of the optical response in this case is based on a shift in the tautomerization equilibrium of the host due to stabilization of one of the tautomers in the creatinine complex. More recently, Bell and co-workers showed in a similar approach that a more highly preorganized host forming four hydrogen bonds leads to a decrease in the detectable concentration of creatinine [24].

6. Ion Channel Sensors for Guanosine 5′-Monophosphate Based on Multitopic Hydrogen Bonding

Kunitake and co-workers have reported that thymidine and adenine bind to monolayers with diaminotriazine and orotate head groups, respectively [25], suggesting that multitopic hydrogen bonding interactions could also be used for nucleotide recognition at monolayer-modified electrodes. We have previously reported on a

Table II. Nucleotide-induced permeabilities of monolayers of the receptors **23**, **24** or **18**: Decrease in the oxidation current for a 1 mM $[Fe(CN)_6]^{4-}$ subphase in presence of nucleotides, as observed with cyclic voltammetry

Receptor	Nucleotide	Concentration of nucleotide	Decrease in oxidation current[a]
23	5′-GMP	3.00 mM	47.4%[b]
23	5′-AMP	3.00 mM	31.6%[b]
24	5′-GMP	3.00 mM	35.2%[c]
24	5′-AMP	3.00 mM	50.7%[c]
18	5′-GMP	1.00 mM	15.3%[d]
18	5′-AMP	1.00 mM	3.3% [d]
18	5′-GMP	3.00 mM	36.9%[d]
18	5′-AMP	3.00 mM	19.9%[d]

[a] Current decreases are given relative to the corresponding current in the absence of a nucleotide at the oxidation peak potential for the respective monolayer in the absence of a nucleotide; CV sweep rate 100 mV/s for the scan -0.5 V $\rightarrow$ +0.8 V $\rightarrow$ -0.5 V; all potentials are vs. Ag/AgCl.
[b] At +150 mV.
[c] At +438 mV.
[d] At +382 mV.

number of such sensors resembling the ion-channels of biomembranes (see [26] and cited references therein). For this purpose, we formed monolayers of hosts for nucleotides at the water/air interface of $[Fe(CN)_6]^{4-}$ solutions [26]. A lateral pressure was externally applied to minimize the number of membrane imperfections. Planar electrodes were then brought in parallel position above these monolayers and lowered to touch the monolayers, taking care to cause minimum damage on impact. Upon application of an external potential, electrical currents due to oxidation of the marker $[Fe(CN)_6]^{4-}$ can be observed. In solutions containing the negatively charged guests 5′-AMP or 5′-GMP, binding nucleotides to the host-based monolayers on the electrode surface results in a negatively charged surface layer of complexes. Electrostatic repulsion between these complexes and the $[Fe(CN)_6]^{4-}$ marker results in a decrease of the oxidation currents. To quantifiy this effect, current decreases were measured at the oxidation peak potential for the respective monolayer as observed in the absence of a nucleotide. This method was chosen because even in the absence of a nucleotide the position of the oxidation peak potential depended on the type of monolayer, which seems to be mainly the result of variations in the tightness of the investigated monolayers.

An electrode with a monolayer based on the cytosine derivative **23** exhibited larger decreases in the oxidation currents for 5′-GMP than for 5′-AMP solutions (Table II). This can be explained by the complementarity of this host and the guanine base of 5′-GMP, which allows for formation of three hydrogen bonds in the complex. On the other hand, an electrode modified with a monolayer containing

the thymine derivative **24** responded more strongly to 5′-AMP. Here too, the selectivity is analogous to the base pairing selectivity of DNA, and can be explained by formation of two hydrogen bonds between the thymine derivative **24** and 5′-AMP. For monolayers with host **18**, which can form five hydrogen bonds to 5′-GMP, a somewhat smaller influence of 5′-GMP on the absolute currents than for monolayers of cytosine derivative **23** was observed. The monolayers of **18** are tighter than those of **23**, as also indicated by the higher peak potential for $[Fe(CN)_6]^{4-}$ oxidation even in the absence of guests. However, in accordance with the expected formation of five hydrogen bonds in complexes of host **18** and 5′-GMP, the highest voltammetric selectivity was observed for monolayers with host **18**.

7. Conclusions

Hydrogen bonds have been recently used for the design of many synthetic hosts. It seems that anion ionophores with hydrogen bond donor groups may become just as useful as cation ionophores with ether oxygens or amide groups, as demonstrated here for a chloride and a sulfate ISE. Unless particular consideration is given to prevent self-association and higher aggregates, the full selectivity of ionophores with both hydrogen bond donor and acceptor groups can, however, not be taken advantage of. Besides use of stronger ionophores and use of membrane solvents that do not interact with the ionophore, an ionophore design that prevents self-association and guest recognition at ordered monolayers, where the hosts cannot readily self-associate, seem to be promising approaches to circumvent such problems.

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