

# MOLECULAR IONICS OF ANION RECEPTOR MOLECULES

## A microcalorimetric study\*

*D. Grell*<sup>1,2</sup>, *E. Grell*<sup>3\*\*</sup>, *P. Bugnon*<sup>4</sup>, *B. Dietrich*<sup>1</sup> and *J.-M. Lehn*<sup>1</sup>

<sup>1</sup>ISIS-Université Louis Pasteur, F-67000 Strasbourg, France

<sup>2</sup>Institut für organische Chemie, Johann Wolfgang Goethe-Universität, D-60439 Frankfurt, Germany

<sup>3</sup>Max-Planck-Institut für Biophysik, D-60439 Frankfurt, Germany

<sup>4</sup>Institut de Chimie moléculaire et biologique, EPFL-BCH, CH-1015 Lausanne, Switzerland

### Abstract

The coordination of divalent and monovalent inorganic anions to synthetic polyammonium receptors is investigated in aqueous solution around neutral pH by titration calorimetry and NMR spectroscopy. High-affinity 1:1 complexes are formed by a pyrrole type cryptand (**1**) with sulfate and phosphate, characterized by association constants of almost  $10^7 \text{ M}^{-1}$ . Affinities close to  $10^5 \text{ M}^{-1}$  are found for polyazacryptands (**3** and **4**) exhibiting  $\text{F}^-/\text{Cl}^-$  selectivity. The binding affinities and the anion selectivities are mainly caused by the charges of ligands and anions, which is discussed on the basis of simple calculations of the electrostatic contribution to the anion/receptor interactions. The binding of all investigated anions is exothermic at 298.2 K. The contribution of the large negative  $\Delta H$  values to the free energy of anion binding of the pyrrole type ligand is partially compensated by marked negative  $\Delta S$  values. These unfavorable entropic contributions are attributed to the additional inclusion of water molecules in the anion/receptor complexes.

**Keywords:** anion binding, anion receptors, microcalorimetry, polyammonium cryptands, titration calorimetry

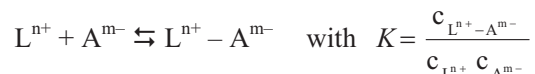
### Introduction

The selective interaction between inorganic anions and proteins is of great regulatory importance for many physiological processes. Biologically relevant anion selectivities concern, for example, the differentiation among tetrahedral anions such as sulfate and phosphate or planar ones like bicarbonate in relation to spherical chloride. Such differentiation requires diverse ligand topologies to provide suitable positions for the coordinating groups or atoms. In order to investigate the basic chemical nature of anion binding and selectivity, several type of synthetic molecules have been studied. They form quite stable and sometimes even selective anion complexes,

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\*\* Author for correspondence: E-mail: ernst.grell@mpibp-frankfurt.mpg.de

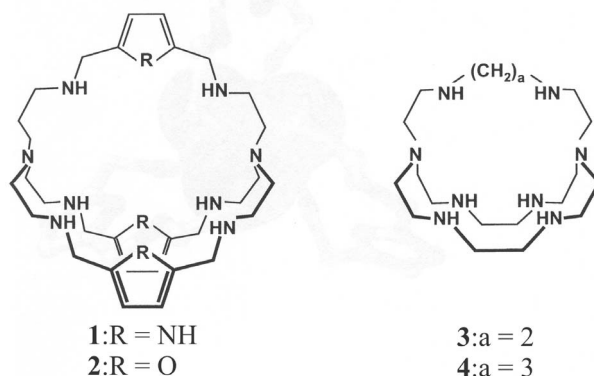
mostly due to electrostatic interactions and hydrogen bond formations [1–3]. The interaction between an anion  $A^{m-}$  and a ligand  $L^{n+}$  is usually characterized in terms of the stability or association constant  $K$  for a 1:1 binding equilibrium



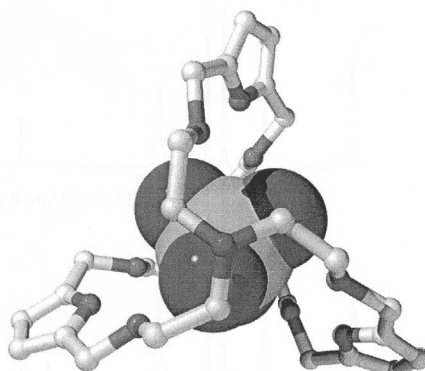
Up to now, no high affinity anion binding has been observed in aqueous solution of neutral pH. As coordinating groups of the synthetic ligands, positively charged functions have so far been chosen, although anion coordination by uncharged groups, for example by providing suitably oriented dipoles, appears to be of importance in the case of biological systems [4, 5]. Anion affinity is strongly dependent on the degree of protonation and varies thus markedly with pH if coordination occurs via protonated ammonium groups. For this reason, high affinities, characterized by stability constants around  $10^6 \text{ M}^{-1}$  have hitherto only been reported for the pH range between 4 and 5 [1–3, 6, 7] for ammonium type of ligands.

In order to develop a high affinity ligand for the coordination of inorganic anions, the uncharged pyrrole residue containing macrobicyclic receptor **1** (Fig. 1), which exhibits  $C_3$  symmetry, has been synthesized according to Chen and Martell [8]. Previous studies by Morgan *et al.* [9] with the furan containing azacryptand **2** (Fig. 1) provided the first crystal structure of an oxoanion complex with perchlorate as encapsulated anion. In this complex, perchlorate oxygen atoms form three direct hydrogen bonds with protonated secondary amine nitrogens. In addition, this tetrahedral anion is coordinated inside the ligand's cavity via bridging hydrogen bond formation with six included water molecules. The furan oxygen atoms are not involved in the coordination. Some investigations of **2** in solution have been carried out very recently [10].

Another type of polyammonium receptor molecule, the polyaza cryptand **3** (Fig. 1) exhibits a pronounced  $F^-/Cl^-$  selectivity in solution [6], which is determined by the size of the corresponding cavity. In order to increase the affinity for  $Cl^-$ , cryptand **4** (Fig. 1), characterized by one elongated bridge ( $a = 3$ ) in comparison to **3**,



**Fig. 1** Chemical structures of cryptands



**Fig. 2** Representative ball-and-stick model of an energy minimized structure of the  $\text{PO}_4^{3-}$  complex of cryptand **1**

is included in this study. According to the crystal structure of the  $\text{F}^-$  complex of **3**, the anion is bound in its deprotonated state [6].

In order to study whether the structure of **1** is potentially capable to coordinate tetrahedral anions with high affinity and whether **4** is able to bind chloride more strongly than **3**, molecular modeling studies are carried out. In addition, anion coordination in solution at neutral pH is investigated by titration calorimetry and NMR spectroscopy. For the experimental studies reported here, the cryptands are investigated in form of their tosylate (TsO) salts because this anion is too large to be bound inside the central cavities of the ligands.

## Experimental

### *Modeling studies*

All molecular modeling calculations were done using the software Hyperchem 3.0 (Hypercube Inc., 1993). Atom charges have been determined with help of the semi-empirical AM1 method [11, 12], whereas molecular dynamics calculations were done using the MM+ force field [13] in vacuum without any cutoffs. Previous to MD simulations, structures were energy minimized using the implemented Polak-Ribiere algorithm until a RMS gradient  $< 0.04$  kJ/(Å mol) was reached. For efficient conformational space sampling, MD simulations were performed at 800 K for 25 ps.

### *Experimental and materials*

#### General chemicals

Chemicals were of analytical grade and supplied by Merck and Fluka. The buffer compounds [N-morpholino]ethane sulfonic acid (MES) and 3-[N-morpholino]-2-hydroxypropane sulfonic acid (MOPSO) were adjusted with sodium hydroxide. Tetrabutylammonium (TBA) hydrogensulfate was obtained from Sigma

(99% purity). Tetramethylammonium hydroxide of grade puriss. p.a. was supplied by Fluka. Ionic strength adjustments were made with tetramethylammonium tosylate (TMATsO) obtained from Acros (99% purity).

#### Cryptands

**1** has been synthesized according to a modified procedure of [8] with a yield of 34%. It was obtained as TsO salt (*Mp.* 408.2 K, decomp.) of the composition  $C_{30}H_{51}N_{11} \cdot 6.5TsOH \cdot 4H_2O$  and was analytically pure according to the  $^1H$ -NMR- in  $D_2O$  and DMSO- $d_6$ ,  $^{13}C$ -NMR- in  $D_2O$ , FAB-MS-spectra in thioglycerol containing 1% TsOH as well as to combustion analysis.

**3** and **4** have been synthesized similar to [6]. These ligands were used as the TsO salts and were analytically pure according to the  $^1H$ -NMR- in  $D_2O$ ,  $^{13}C$ -NMR- in  $D_2O$ , FAB-MS-spectra in 4-nitrobenzyl alcohol/ethanol with the composition  $C_{18}H_{42}N_8 \cdot 8TsOH \cdot 4H_2O$  (**3**) and  $C_{19}H_{44}N_8 \cdot 7TsOH \cdot 3H_2O$  (**4**) according to combustion analysis.

#### Nuclear magnetic resonance (NMR)

$^1H$ -NMR spectra have been obtained with a Bruker AC 200 instrument. The chemical shift in  $d_6$ -dimethyl sulfoxide (DMSO) is given in ppm, relative to the content of non-deuterated solvent at 2.50 ppm (ambient temperature).  $^1H$  broadband decoupled  $^{31}P$ -NMR spectra were measured with a Bruker AC 300 instrument at 298.2 K in  $H_2O/D_2O$  (4:1, v/v). The chemical shift is given relative to that of phosphoric acid ( $\delta = 0$ ).

#### Potentiometric titrations

Titration have been performed with TMAOH at 298.2 K in the absence of  $CO_2$  ( $N_2$  atmosphere) employing the titrator 716 DMS Titrino (Mettler) in combination with a pHG 200 pH glass electrode (Radiometer) and a separate Calomel reference electrode, equipped with an external mantle (Möller, Zurich), filled with 0.5 M TMATsO to avoid leaking and subsequent binding of  $Cl^-$  during titrations.

#### Microcalorimetry

Microcalorimetric titrations have been performed at 298.2 K with a MicroCal MCS ITC instrument. To prepare buffer solutions nominally free of  $Cl^-$ , the contact with the combined pH electrode for the pH adjustment was omitted. High affinity binding of anions has been measured at a mean ionic strength around 0.05 M. In the case of low affinity anion binding, TMATsO was added to all solutions to achieve a total ionic strength of 0.1 M. All solutions have been vacuum degassed. For the evaluation of the calorimetric titrations, the manufacturer's program (single site model [14]) has been applied. The equilibrium constant  $K$  is defined according to Eq.(4) in [15]. The determined thermodynamic parameters represent apparent values because no detailed pH dependence has been investigated.

## Results

### *Molecular modeling*

Based on the crystal structure of the perchlorate complex of cryptand **2** [9], molecular modeling studies are carried out to obtain information whether ligand **1** is likely to coordinate anions such as the fully deprotonated phosphate ion. Formally, the three furan oxygens are exchanged against pyrrole NH groups; otherwise the coordinates of the complex are maintained. The perchlorate ion is substituted by a model, consisting of a spherical anion with relevant features of phosphate (van-der-Waals radius: 3.3 Å; charge:  $-3$ ). The results of high temperature molecular dynamics simulations ( $T = 800$  K) indicate that structure, cavity size and flexibility of cryptand **1** appear to be suitable for a strong coordination of the large tetrahedral oxoanions phosphate and sulfate. In all generated structures, the bound anion remains in the cavity of the cryptand whereas the pyrrole ring is turned out of the originally assumed, furan-like position to a new location. In this position, the NH groups of **1** are capable to form stable hydrogen bonds with the anion. Moreover, the shape and size of the ligand's cavity appear to be controlled by the conformational properties of the cryptand. Similar studies, carried out with the  $\text{Cl}^-$  complex of **4**, showed that size and geometry of the ligand cavity should be sufficiently large to allow the complexation of this spherical anion.

### *Estimation of association constants*

Based on electrostatic interactions, the model of Fouss [16] allows the calculation of the equilibrium constant  $K_{\text{el}}$  according to

$$K_{\text{el}} = \frac{4\pi}{3000} r^3 N_A e^{-\phi} \quad \phi = \frac{z_+ z_- e_0^2}{4\pi \epsilon_r \epsilon_0 r k_B T}$$

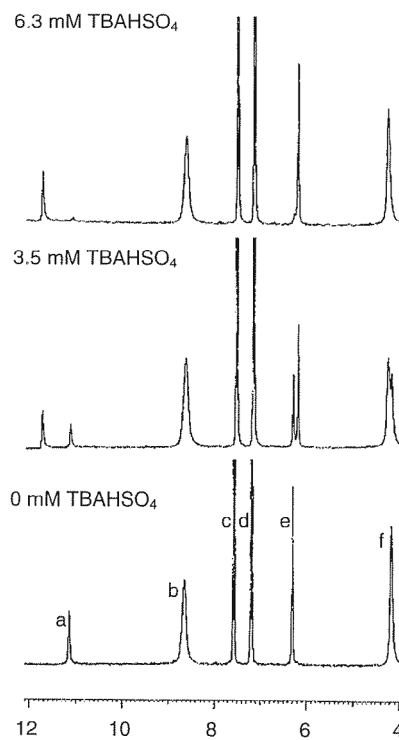
with  $z_+$  and  $z_-$  as charges of cationic ligand and anion, respectively,  $e_0$  as electron charge,  $N_A$  as Avogadro's constant, the dielectric constants  $\epsilon_r$  and  $\epsilon_0$  of medium and vacuum, respectively, and the Boltzmann constant,  $k_B$ .

### *Protolysis*

In order to characterize the charge of the investigated ligands in dependence of pH, potentiometric titrations are carried out. The  $\text{pK}_a$  values of **3** have been determined recently [6], those of **4** are 10.6, 9.7, 8.7, 7.5, 6.35 and 4.1. Although the structure of **4** is very close to that of **3**, the  $\text{pK}_a$  values are rather different. The bridgehead nitrogen atoms of the polyammonium cryptands do not contribute to the charge at neutral pH because the corresponding  $\text{pK}_a$  values are assumed to be below 2. A precise determination of the  $\text{pK}_a$  values of **1** failed, which is attributed to a pH dependent binding of bicarbonate, remaining even in a  $\text{N}_2$  atmosphere, essentially devoid of  $\text{CO}_2$ .

*Qualitative aspects of anion binding by NMR*

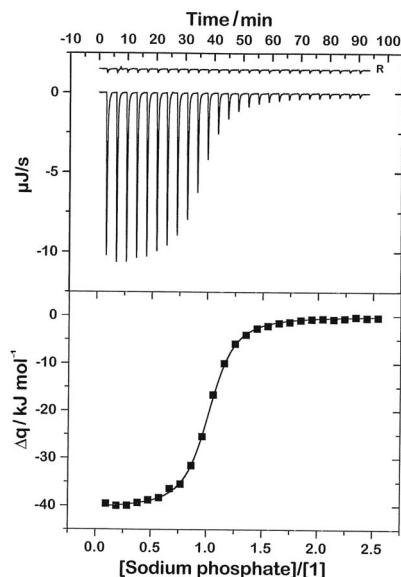
$^{31}\text{P}$ -NMR phosphate binding studies in  $\text{H}_2\text{O}/\text{D}_2\text{O}$  at pH 6.5 are carried out with cryptand **1**. The observed 3.7 ppm down-field shift of phosphate in the presence of saturating concentrations of the ligand is indicative of phosphate coordination, predominantly by its protolytic state  $\text{HPO}_4^{2-}$ .  $^1\text{H}$ -NMR studies of **1** in  $\text{D}_2\text{O}$  with sulfate and of **4** with tetramethylammonium chloride as well as of **1** in  $\text{DMSO}-d_6$  with TBA hydrogensulfate (Fig. 3) are consistent with the concept of 1:1 anion complex formation. Upon the coordination of the anion, the pyrrole NH signal ('a' in Fig. 3) is shifted downfield by 1.6 ppm, due to deshielding as a consequence of the interaction with the anion. On the other hand, the pyrrole CH signal ('e' in Fig. 3) is shifted upfield by 0.2 ppm, which is assigned to a change in the ring current effect of the heterocycle. Thus, the pyrrole ring of **1** changes its orientation as a consequence of anion binding so that pyrrole NH groups can participate in the coordination of the anion. These results confirm the predictions of the modeling studies (cf. Fig. 2). The fact that azacryptand **3** is capable to bind spherical anions has been demonstrated previously [6] and needs not to be emphasized again.



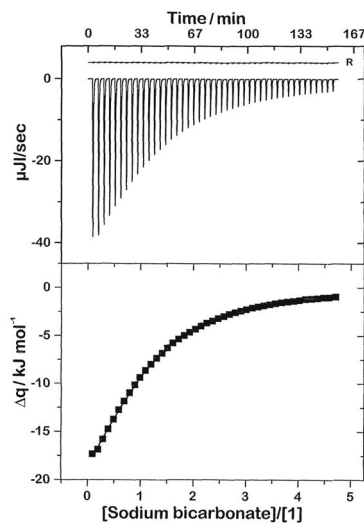
**Fig. 3**  $^1\text{H}$ -NMR spectra of 5.7 mM cryptand **1** in  $\text{DMSO}-d_6$  as well as in the presence of different concentrations of tetrabutylammonium hydrogensulfate ( $\text{TBAHSO}_4$ ). Assignments are given in the text

### Quantitative binding studies by titration calorimetry

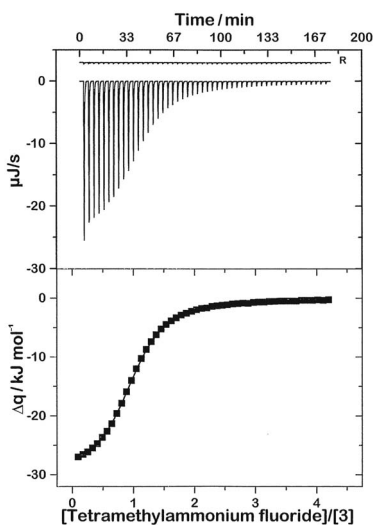
There is nearly no thermodynamic data available for the binding of inorganic anions by synthetic receptor molecules in aqueous solution. Previous attempts to determine stability constants were mostly applying potentiometric titrations in the presence and absence of the coordinated anion [1–3, 6, 10]. Since ligand **1** does not exhibit suitable absorption or fluorescence properties, which are often applied for the determination of association constants by spectrometric titrations, we have performed microcalorimetric titrations in aqueous solution under conditions of almost constant ionic strength at pH 6.1 (50 mM MES/NaOH) and pH 7.0 (50 mM MOPSO/NaOH). Having determined  $K$  and  $\Delta H$ , the entropy change  $\Delta S$  can be calculated [15]. For ligand **1**, the binding of phosphate, sulfate, bicarbonate, perchlorate and chloride (as sodium salts) and for azacryptands **3** and **4** that of fluoride and chloride (as TMA salts) is investigated. As typical examples, a high affinity titration of **1** with phosphate and a low affinity titration with bicarbonate are shown in Figs 4 and 5, respectively. With regard to the azacryptands, Fig. 6 shows a titration of **3** with  $F^-$ . The obtained results are summarized in Tables 1 and 2. Receptor **1** exhibits affinities for sulfate and phosphate nearly up to  $10^7$ , for bicarbonate around  $10^3$  and for the spherical chloride  $<10^2 M^{-1}$ . The binding of all investigated anions is exothermic. All performed fits are based on stoichiometric coefficients ranging between 0.96 and 1.0,



**Fig. 4** Calorimetric titration of 0.047 mM cryptand **1** (cf. Fig. 1) in 50 mM MOPSO/NaOH pH 7.5 with  $26 \times 3 \mu\text{L}$  2 mM sodium phosphate in the same buffer at 298.2 K together with the corresponding reference titration ( $R$ , shifted by an offset of  $1.5 \mu\text{J s}^{-1}$ ) in the absence of **1** (top). Difference heat changes  $\Delta q$  are shown as a function of the concentration ratio between phosphate and cryptand **1** (bottom). The resulting stoichiometric coefficient is 0.99; the thermodynamic parameters are given in Table 1



**Fig. 5** Calorimetric titration of 0.47 mM cryptand **1** (cf. Fig. 1) in 50 mM MOPSO/NaOH pH 7.0 with  $44 \times 6 \mu\text{L}$  10 mM sodium bicarbonate in the same buffer at 298.2 K together with the corresponding reference titration (*R*, shifted by an offset of  $4 \mu\text{J s}^{-1}$ ) in the absence of **1** (top). Difference heat changes  $\Delta q$  are shown as a function of the concentration ratio between bicarbonate and cryptand **1** (bottom). The resulting stoichiometric coefficient is 0.98; the thermodynamic parameters are given in Table 1



**Fig. 6** Calorimetric titration of 0.2 mM cryptand **3** (cf. Fig. 1) in 50 mM MES/TMAOH pH 6.1 with  $1 \times 5 \mu\text{L}$  and  $49 \times 4 \mu\text{L}$  5.2 mM tetramethylammonium fluoride in the same buffer at 298.2 K together with the corresponding reference titration (*R*, shifted by an offset of  $3 \mu\text{J s}^{-1}$ ) in the absence of **3** (top). Difference heat changes  $\Delta q$  are shown as a function of the concentration ratio between fluoride and cryptand **3** (bottom). The resulting stoichiometric coefficient is 0.99; the thermodynamic parameters are given in Table 2



**Table 1** Thermodynamic parameters of anion binding to **1** (water; 298.2 K)

Anion <sup>[i]</sup>	pH	$K/M^{-1}$	$-RT \ln K/$ kJ mol <sup>-1</sup>	$\Delta H/$ kJ mol <sup>-1</sup>	$\Delta S/$ J mol <sup>-1</sup> K <sup>-1</sup>	$I^{[ii]}/$ mol l <sup>-1</sup>
Phosphate	6.1 <sup>[iii]</sup>	$(4.7 \pm 0.5) \times 10^6$	-38.1	$-50.2 \pm 2.5$	-41.0	0.051
Sulfate	6.1 <sup>[iii]</sup>	$(8.3 \pm 0.5) \times 10^6$	-39.5	$-52.8 \pm 2.5$	-44.5	0.050
Phosphate	7.0 <sup>[iv]</sup>	$(1.7 \pm 0.2) \times 10^6$	-35.6	$-40.0 \pm 2.0$	-14.9	0.050
Sulfate	7.0 <sup>[iv]</sup>	$(9.7 \pm 0.5) \times 10^4$	-28.5	$-29.9 \pm 2.0$	-5.0	0.053
Bicarbonate	7.0 <sup>[iv]</sup>	$(2.5 \pm 0.2) \times 10^3$	-19.4	$-36.0 \pm 3.5$	-55.8	0.055
Perchlorate	7.0 <sup>[iv]</sup>	<100		< 0		0.1 <sup>[v]</sup>
Chloride	7.0 <sup>[iv]</sup>	<100		< 0		0.1 <sup>[v]</sup>

<sup>[i]</sup> Na<sup>+</sup> as cation, <sup>[ii]</sup> average ionic strength, <sup>[iii]</sup> 50 mM MES/NaOH, <sup>[iv]</sup> 50 mM MOPSO/NaOH, <sup>[v]</sup> adjusted with NaTsO

**Table 2** Thermodynamic parameters of anion binding to **3** and **4** (water; 298.2 K)

Rec.	Anion <sup>[i]</sup>	pH	$K/M^{-1}$	$-RT \ln K/$ kJ mol <sup>-1</sup>	$\Delta H/$ kJ mol <sup>-1</sup>	$\Delta S/$ J mol <sup>-1</sup> K <sup>-1</sup>	$I^{[ii]}/$ mol l <sup>-1</sup>
3	Fluoride	6.1 <sup>[iii]</sup>	$(5.5 \pm 0.7) \times 10^4$	-27.0	$-28.9 \pm 2.5$	-6.2	0.055
3	Chloride	6.1 <sup>[iii]</sup>	<500				~0.1 <sup>[v]</sup>
4	Fluoride	6.1 <sup>[iv]</sup>	$(1.4 \pm 0.2) \times 10^4$	-23.7	$-23.6 \pm 2.5$	0	0.056
4	Chloride	6.1 <sup>[iv]</sup>	<1000				~0.1 <sup>[v]</sup>

<sup>[i]</sup> TMA as cation, <sup>[ii]</sup> average ionic strength, <sup>[iii]</sup> 50 mM MES/TMAOH, <sup>[v]</sup> adjusted with TMATsO

which indicates 1:1 stoichiometry. For ligand **1** at pH 6.1, the free energy of interaction with phosphate and sulfate is dominated by a large enthalpic contribution, up to  $-53 \text{ kJ mol}^{-1}$  at 298.2 K and an unfavorable entropic term, as high as  $-45 \text{ J mol}^{-1} \text{ K}^{-1}$ . Cryptands **3** and **4**, which can adopt nearly spherical cavities, are characterized by fairly high affinities for the monovalent anion F<sup>-</sup>, ranging between  $10^4$  and  $10^5 \text{ M}^{-1}$ , and  $\Delta H$  values around  $-25 \text{ kJ mol}^{-1}$ . The corresponding entropic contributions are low. Based on potentiometric studies [6], a higher value has been reported for the association constant of the fluoride complex of **3**. The affinity for the larger Cl<sup>-</sup> is considerably lower than for F<sup>-</sup>. The F<sup>-</sup> affinity decreases upon increasing the cavity size from **3** to **4**, whereas that of Cl<sup>-</sup> increases (cf. Table 2), as expected. All investigated ligands exhibit marked anion selectivities.

## Discussion

The observed affinities of cryptand **1** for divalent inorganic anions are the highest ones that have so far been reported in aqueous solutions around neutral pH. Recently, for a twice positively charged, fluorinated diguanidinium ligand, affinities have also been determined by calorimetry in aqueous solution but were four orders of magni-

tude lower [18]. The effect of ligand charge on the binding affinity can be estimated on the basis of the simple electrostatic interaction model introduced by Fuoss for point charges [16]. This model allows the calculation of association constants  $K_{el}$ . If the charges are located on different atoms of a binding site such as in the case of the cryptands considered here, the model can still be applied but allows only to achieve estimations. Cryptand **1** is assumed to exhibit preferentially charge +4 around neutral pH and ligands **3** and **4** charge +5 at about pH 6. In the following we assume also that the ligands bind the coordinating form of the anion only via their protolytic states which predominate in aqueous solution at the given pH. Thus no marked change of the degree of ligand ionization upon binding is assumed to occur. In reality, however, several protolytic states of the anion complexes, characterized by different equilibrium constants, may coexist. As binding distance between anion and ligand site, we choose for central inner sphere coordination of sulfate or phosphate 4.5 Å and of fluoride or chloride 2.85 [6] and 3.3 Å, respectively. Under these circumstances, we calculate for divalent anion binding to **1** a  $K_{el}$  value of  $7 \cdot 10^4 \text{ M}^{-1}$ , which is only about an order of magnitude lower than the experimentally determined values (cf. Table 1). For a monovalent anion with the same reaction distance, the binding constant  $K_{el}$  would only be  $120 \text{ M}^{-1}$ . For simple ion pair binding of the divalent anion to the surface region of **1** (assumed local charge is +2), for example connected by hydrogen bonds forming water molecules, we assume a reaction distance of 7.1 Å. This leads to an expected value for  $K_{el}$  of  $50 \text{ M}^{-1}$ , which is rather low. This result indicates that anion coordination inside the central cavity of **1** is much more likely than surface binding. Again assuming a reaction distance of 4.5 Å, we calculate for sulfate binding to the fluorinated diguanidinium ligand of Berger and Schmidtchen [18] a  $K_{el}$  value of  $9 \cdot 10^5 \text{ M}^{-1}$  for methanol and  $120 \text{ M}^{-1}$  for water. Both calculated values are very close to the experimentally observed ones. This confirms that reasonable estimations can be achieved on the basis of the Fuoss model for ligands with multiple charges localized on different atoms. Thus, the comparisons discussed above clearly indicate that the electrostatic interactions strongly contribute to the observed affinities and selectivities between di- and monovalent inorganic anions. The calculated  $K_{el}$  for divalent anion binding to **1** is lower than the experimentally observed values. This can be considered to be consistent with the hypothesis mentioned above, that additional interactions such as hydrogen bond formation between the pyrrole NH group and the anion will also contribute markedly to the stability of the anion complex.

While ligand **1** is very flexible and can adapt its cavity dimensions to the size of the anion, the cavity size of cryptands **3** and **4** is sterically much more limited. Similar estimations of an electrostatic binding contribution under the conditions mentioned above leads to  $K_{el}$  values of  $2 \cdot 10^4$  and  $5 \cdot 10^3 \text{ M}^{-1}$  for  $\text{F}^-$  and  $\text{Cl}^-$  binding, respectively. Although these estimations are very coarse, the value for  $\text{F}^-$  is very close to the experimentally determined one (Table 2). With regard to the larger  $\text{Cl}^-$ , however, the determined value is much lower than the calculated one. As a plausible explanation for this difference, we suggest that the cavity of **3** has to expand in order to provide similar structural binding conditions for  $\text{Cl}^-$  as for the smaller  $\text{F}^-$ . To achieve such an expansion, a corresponding conformational rearrangement is required. Such a transition

would be energetically unfavorable and thus lead to a diminished affinity. The structural enlargement of a bridge and consequently of the cryptand cavity of **4**, compared to **3**, may explain the experimentally observed reduction of the stability constant of the  $F^-$  complex by the factor of four, but not necessarily the qualitative change of the  $F^-/Cl^-$  selectivity. Of course, the very strong hydrogen bond formation between  $F^-$  and the  $NH^+$  group, compared to  $Cl^-$ , will always markedly favor the binding of the smaller anion. Thus, the size of the cavity and the strength of hydrogen bond formation predominantly control the  $F^-/Cl^-$  selectivity.

From the data given in Tables 1 and 2, we can conclude that the binding affinities of all investigated anions are dominated by the enthalpic contribution. The experimentally obtained exothermic enthalpy changes can consist of contributions from the binding processes of the interacting species, their degree of ionization as well as buffer ionization. The buffer compounds used in this study for different pH values are of very similar chemical nature and are therefore expected to exhibit similar ionization enthalpies, provided this aspect will turn out to be of importance. Since there appears to be a correlation between the values of the association constants and the enthalpy changes, we assume that the  $\Delta H$  values are mainly dominated by the ligand/anion binding enthalpy. Therefore, the conclusions derived from the electrostatic interactions discussed above are also of importance for the interpretation of the  $\Delta H$  values, although this can only be done here in qualitative manner. The  $\Delta H$  values for the binding of phosphate and sulfate to **1** at pH 6.1 are nearly identical, which can be indicative of similar electrostatic interactions. This would be consistent with a conclusion of the NMR studies discussed before, namely that phosphate is bound in its protolytic state  $HPO_4^{2-}$ , thus exhibiting the same charge as sulfate. The positive enthalpic contribution of the deprotonation of the remaining fraction of  $H_2PO_4^-$  present at pH 6.1, leading to the complete formation of  $HPO_4^{2-}$ , is small [19]. The pH dependent decrease of the  $\Delta H$  values of **1** upon increasing the pH from 6.1 to 7.0 is possibly attributed to the decrease of the ligand's positive charge, which leads to a weaker electrostatic interaction. Although the electrostatic contribution to the affinity, resulting from the interaction between the monovalent bicarbonate ion and the receptor, must be smaller than that for a divalent oxoanion, the  $\Delta H$  value for bicarbonate binding is still rather high. Thus, other interactions, for example between ligand  $NH^+$  groups and  $O=C$  group dipoles of the anion are very likely to contribute here significantly to the resulting enthalpy change. For **3** and **4**, the  $\Delta H$  values, characterizing  $F^-$  binding at pH 6.1, are smaller than those of **1** for the divalent anions. This may be attributed to the fact that the contribution of the higher charges of **3** and **4** to  $K_{cl}$  with regard to **1** is not sufficient to compensate the diminished electrostatic contribution of the monovalent anions, compared to that of the divalent oxoanions. This charge reduction of the anion will thus lead to a decreased electrostatic interaction according to the estimations given above. The smaller  $\Delta H$  value observed for the binding of  $F^-$  to **4** than to **3** may also be due to the result of a lower electrostatic interaction as a consequence of the too large cavity in the case of **4**.

The multidentate coordination between a solvated anion and a strongly solvated ligand is expected to be accompanied by the release of solvent molecules and thus to lead to a positive entropy change. However, for anion binding of **1** a large negative entropy change is found, which is largest for bicarbonate (Table 1). These negative  $\Delta S$  values

provide a positive  $T\Delta S$  term, which compensates partially the negative enthalpic term and thus lead to a lowering of the equilibrium constants. Such negative  $\Delta S$  values can result as a consequence of solvent binding and/or of a conformational transition. Indeed, the crystal structure determination of the perchlorate complex of **2** provided strong evidence that this anion is not directly coordinated to the ligand but via hydrogen bond formation involving six bound water molecules. Thus anion coordination to **1** with participation, respectively binding of water molecules, is likely to represent an important reason for the observed negative  $\Delta S$  values. The reduction of the number of charged  $\text{NH}^+$  groups upon the pH increase from 6.1 to 7.0 may lead to a lower number of bound water molecules and thus to a lower negative  $\Delta S$  value, as seen in Table 1. With regard to bicarbonate, it is plausible to assume that the ligand has to adopt a more rigid conformation in order to bind the planar anion, compared to binding conditions suitable for the coordination of tetrahedral oxoanions. Consequently, this can contribute to an even larger negative  $\Delta S$  term. The entropy change characterizing the binding of  $\text{F}^-$  to **3** is positive, as expected for the release of solvate molecules upon coordination, which corresponds to the observations obtained for cation binding to negatively charged ligands [20]. A  $\Delta S$  value close to zero, as found for the binding of  $\text{F}^-$  to **4**, may imply that the anion, bound to an actually too large cavity, may remain partially solvated and/or that the ligand has to adopt an entropically unfavorable conformation. MD simulations of halogenide anion/azacryptand interactions in  $\text{H}_2\text{O}$  provide evidence for a preferential interaction of the anion with only two of the three cryptand bridges [21]. This differs markedly from the structural features in the crystalline state. Thus, the partial solvation hypothesis mentioned before can't be ruled out.

In summary, the thermodynamic parameters of anion binding by positively charged, multidentate cryptand type of ligands can be understood in molecular terms on the basis of the electrostatic interaction between the charges of ligand and anion, charge/dipole interactions, anion structures and ligand conformational properties as well as hydrogen bonding involving also water binding to the formed coordination complexes. The obtained results clearly demonstrate that titration calorimetry is a very valuable technique for the quantitative investigation of this type of interaction.

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