Anion Receptors

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A Bambusuril Macrocycle that Binds Anions in Water with High Affinity and Selectivity**

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Abstract: *Synthetic receptors that function in water are important for the qualitative and quantitative detection of anions, which may act as pollutants in the environment or play important roles in biological processes. Neutral receptors are particularly appealing because they are often more selective than positively charged receptors; however, their affinity towards anions in pure water is only in range of 1–10^3 L mol^{-1}. The anion-templated synthesis of a water-soluble bambusuril derivative is shown to be an outstanding receptor for various inorganic anions in pure water, with association constants of up to 10^7 L mol^{-1}. Furthermore, the macrocycle discriminates between anions with unprecedented selectivity (up to 500000-fold). We anticipate that the combination of remarkable affinity and selectivity of this macrocycle will enable the efficient detection and isolation of diverse anions in aqueous solutions, which is not possible with current supramolecular systems.*

Anions play key roles in biochemical processes and some of them are known to be essential for human health.[1] Receptors for both anions and cations have been investigated since the late 1960s.[2] However, the majority of these efforts have focused on the supramolecular chemistry of cations. The major difficulty in developing supramolecular anion receptors stems from their high free energy of solvation compared to cations of similar size, which means that anion receptors must compete more efficiently with the solvent. Water is the most strongly competitive solvent and can negate most of the stabilizing intermolecular forces that enable the binding of anions in host–guest systems. Synthetic receptors with high affinities for anions in nonpolar solvents are therefore usually nonfunctional in aqueous environments. To increase their binding efficiency in water, many anion receptors are cationic; multiply charged species such as polyammonium ions are common.[3] The disadvantage of these receptors is that they rely on non-directional electrostatic binding, which results in poor anion selectivity. Moreover, positively charged receptors are always accompanied by counteranions that compete with the anions of interest for the binding site. There is therefore interest in neutral receptors, which lack these two disadvantages. The supramolecular interactions that stabilize the complexes of neutral receptors and anions are often suppressed by the strong solvating effect of water. This is why no association constant in excess of 10^4 L mol^{-1} between an inorganic anion and a neutral receptor in 100% water has previously been reported.[4] Another drawback of neutral synthetic receptors is their low solubility in water, which significantly limits their applications.

The bambusurils are a family of neutral macrocyclic compounds that can act as anion receptors.[5] We have previously shown that bambusuril macrocycles containing six repeating constitutional units can bind anions with affinities of up to 3×10^9 L mol^{-1} in chloroform. However, binding studies in polar solvents were complicated by the low solubility of the bambusurils, particularly in the absence of anions. Herein, we describe the synthesis of a water-soluble bambusuril **5** and demonstrate its ability to behave as an outstanding receptor for inorganic anions in pure water.

The solubility of bambusurils can be tuned by varying the substituents on the nitrogen atoms of the macrocyclic portals. We therefore investigated the water solubility of the benzoate-substituted bambusuril **5** (Scheme 1). It was synthesized by transforming 4-methoxycarbonylbenzylamine **1** into dialkylurea **2** and then dialkylglycoluril **3**. The macrocycle was prepared by coupling glycoluril **3** with paraformaldehyde in toluene under *p*-toluenesulfonic acid catalysis. Tetrabutylammonium bromide was added to the macrocyclization mixture so that the bromide anions would serve as templates and drive the reaction towards the desired six-membered ring. In the absence of template, the only identifiable macrocyclic product was a four-membered ring. The six-membered macrocycle **4** was isolated as a 1:1 complex with bromide, which was subsequently treated with LiCl. The resulting macrocycle–chloride complex was then treated with KOH in methanol/dichloromethane mixture to convert their ester groups into potassium carboxylates. The anionic form of the macrocycle was neutralized by treating with CF_3COOH in water and washed with methanol and acetonitrile. The water-soluble macrocycle **5** free of anion was obtained from **1** in an overall yield of 15%. Anion impurity can be easily monitored by ^{1}H NMR spectroscopy as the macrocycle dissolved in [D$_6$]DMSO forms very strong complexes with all tested anions in this solvent arising to a new set of signals next to those of anion-free macrocycle. The products of all five

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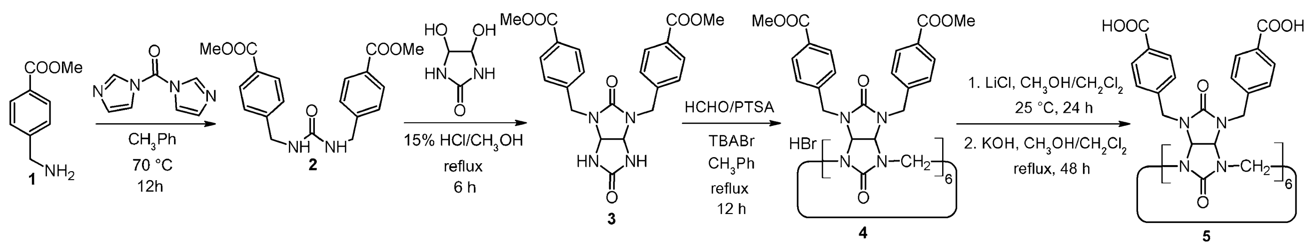
Supporting information for this article (including detailed experimental conditions and procedures, syntheses and compound characterizations, ^{1}H and ^{13}C NMR spectroscopic analyses, mass spectrometry data, titration data and analyses, and ITC data) is available on the WWW under http://dx.doi.org/10.1002/anie.201409895.



Scheme 1. Synthesis of the water-soluble bambusuril macrocycle **5**. TBA = tetra-*n*-butylammonium, PTSA = *p*-toluenesulfonic acid.

synthetic steps were isolated in pure form as precipitates with no need for any chromatographic purification. This synthesis enabled the gram-scale preparation of the macrocycle.

The supramolecular properties of **5** were studied using ^{1}H NMR spectroscopy in D_2O at 30 °C. The solution of the macrocycle at the typical concentration of 1 mM in 20 mM K_2DPO_4 was prepared resulting in a pD of 7.1. These conditions ensured that the macrocycle was soluble at milimolar level for NMR experiments. The inclusion of an anion in the macrocycle cavity was accompanied by changes in the chemical shifts of the methine and methylene protons of the macrocycle (Figure 1 A,B). Rate of chemical exchange between free and bound macrocycle depends on the overall stability of resulting complexes. Anions with lower affinity (F^-, Cl^-, CN^-, IO_4^-, ReO_4^-) interact with the macrocycle in fast exchange regime on the NMR timescale. In these cases the chemical shifts of the host signals fitted 1:1 binding isotherms very well when plotted against anion concentration, yielding the calculated equilibrium association constants (K_a) shown in Table 1.

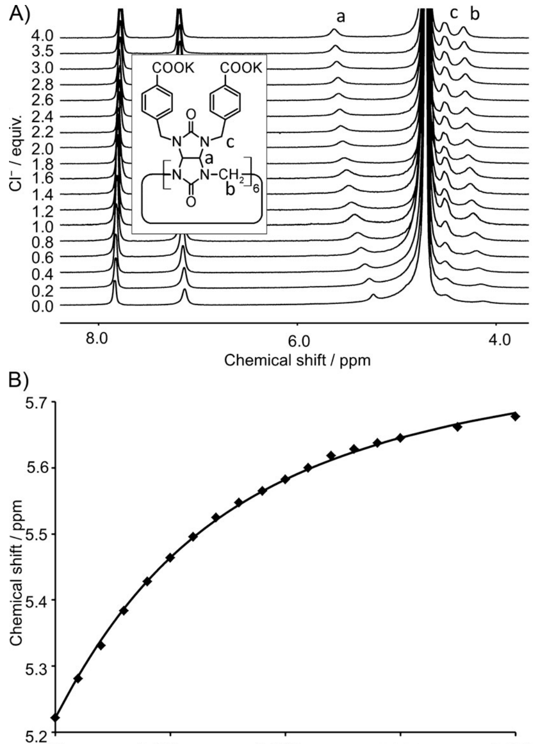


Figure 1. A) NMR titration of **5** with Cl^- as its sodium salt (D_2O, 20 mM K_2DPO_4, pD 7.1, 500 MHz, 303 K). B) Plot of the chemical shift of the methine proton (a) of **5** (1 mM) in the presence of increasing Cl^- concentrations (0–4 mM). The curve shows the best fit of the experimental data to a 1:1 binding model.

Table 1: Equilibrium association constants K_a[a] for 1:1 complexes of the bambusuril macrocycle **5** with various anions determined by ^{1}H NMR spectroscopy (D_2O, 20 mM K_2DPO_4, pD 7.1, 303.15 K).

Anion	K_a [L mol^{-1}]
F^-	1.1×10^2
Cl^-	9.1×10^2
CN^-[b]	1.1×10^3
IO_4^-	6.5×10^3
ReO_4^-	3.0×10^4
Br^-	1.4×10^5
NO_3^-	4.8×10^5
PF_6^-	2.2×10^6
BF_4^-	4.3×10^6
I^-	1.0×10^7
ClO_4^-	5.5×10^7

[a] Standard deviations calculated from two independent measurements are lower than 10%. [b] CN^- is largely protonated under experimental conditions.

On the other hand, slow exchange regime and quantitative complex formation at the submilimolar concentrations were recorded for strongly bound anions (Br^-, NO_3^-, PF_6^-, BF_4^-, I^-, ClO_4^-; Supporting Information, Figures S20 and S21). Therefore, the K_a values for the strongly bound anions were determined by competition experiments using another anion, usually Cl^- (see the Supporting Information for a detailed description of this method). The formation of equimolar host–guest complexes was further confirmed by the observation of signals corresponding to the expected *m*/*z* values of these complexes in MALDI-TOF mass spectra (see the Supporting Information).

The most intriguing property of **5** is its high affinity towards all tested inorganic anions in pure water. Anion receptors are usually potent in organic solvents but exhibit significantly reduced binding abilities in the presence of water. For example, the affinity of Jeong's foldamer for Br^- decreases from 1350 to 19 L mol^{-1} on going from a 4:1 (v/v) DMSO/MeOH mixture to water.[6] The most stable previously known host–guest system involving a neutral receptor and an inorganic anion in pure water is that formed by Schmidtchen's zwitterionic host and I^-, which has an association constant of 6480 L mol^{-1}.[3] Bambusuril **5** is able to capture I^- three orders of magnitude more strongly than this, with an association constant of 1.0×10^7 L mol^{-1} (Table 1).

Particularly interesting is the unprecedentedly strong binding of **5** to the weakly coordinating anions BF_4^-, PF_6^-, and ClO_4^-: the association constants for the formation of 1:1 complexes of these ions with **5** in water were all above $2.2 \times 10^6\,L\,mol^{-1}$. The highest binding affinity among all tested anions was achieved for ClO_4^- ($5.5 \times 10^7\,L\,mol^{-1}$). Moreover, **5** is selective for ClO_4^- in preference to anions that occur naturally in the environment, such as Cl^- ($K_a(ClO_4^-)/K_a(Cl^-) = 60440$). ClO_4^- is considered to be toxic because it interferes with iodine uptake into the thyroid gland. The high affinity and selectivity of **5** for ClO_4^- may be useful in detecting and remediating this pollutant.

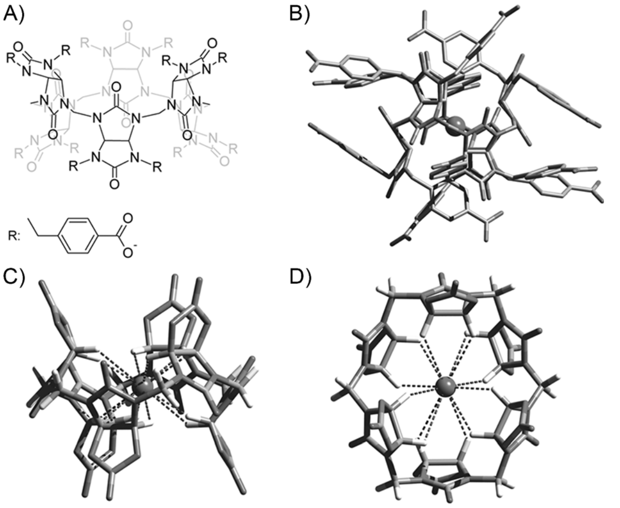


Figure 2. A) Chemical formula of **5**. B) Pictorial representation of the **5**·Cl^- complex with hydrogen atoms omitted for clarity; C) side and D) top views of the **5**·Cl^- complex with benzyl substituents omitted for clarity. Weak hydrogen bonding interactions C—H···Cl^- are showed as dashed lines.

The high binding affinities observed in this work are presumably due to the isolation of the bound anion from water molecules by its inclusion in the receptor's hydrophobic pocket, as discussed by Kubik, You, Jeong, and Flood.[7] In the previously reported cases, the pocket was formed by the inclusion of one anion between two rather flat-shaped receptor molecules or by the receptor folding around the anion. The latter principle is typical for natural systems, where anion binding often occurs in a cavity formed by a folded peptide chain. Host **5** consists of glycoluril units connected by a row of methylene bridges along the equator of the macrocycle (Figure 2A). The alternating glycoluril units form a deep electropositive cavity in which one anion is included and isolated from water molecules. This binding mode is shown in Figure 2B–D and derived from the structures of several bambusuril-based complexes that have been determined previously by crystal diffractometry.[5c] We believe that also in water the complex is stabilized by multiple weak C—H···A^- hydrogen bonding interactions between the methine hydrogen atoms of the macrocycle and the anion. The internal volume of the host cavity can adapt to the size of the anion by tilting the glycoluril units.As a result, the receptor can accommodate anions of various shapes and radii, ranging from F^- (1.33 Å)[8] to PF_6^- (2.76 Å).[9] Desolvation plays an important role during the complexation process.[10] F^- is solvated much more strongly than ions such as I^- and ClO_4^-, and the increase in binding affinity from F^- to ClO_4^- is therefore attributed to the decreasing solvation energy in this anion series. This is consistent with thermodynamic parameters for the binding of halides (Figure 3) that were obtained using isothermal titration calorimetry (ITC; Table 2).These measurements show that the inclusion of anions in the bambusuril **5** cavity is driven by enthalpy. Fluoride pays the highest enthalpic penalty for desolvation, resulting in the lowest enthalpy of host–guest association compared to lager halides.[11] Entropy of binding is negative for all of the halides. The negative entropy of binding is surprising particularly for fluoride. This strongly coordinating anion is known to be solvated by well-ordered water molecules. Release of these water molecules from the solvation shell prior to anion inclusion was expected to result in a large gain in entropy. The reason for the observed negative entropy of binding is not

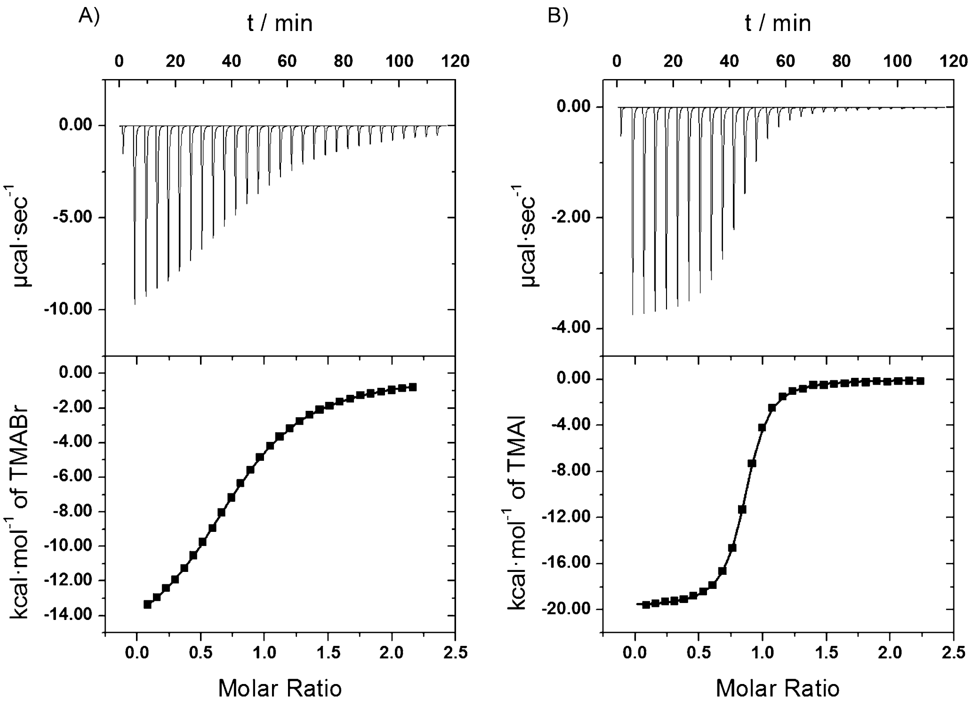


Figure 3. Raw heats of binding obtained by ITC when A) Br^- and B) I^- as its tetramethylammonium (TMA) salts where mixed with **5** (H_2O, pH 7.1, 303.15 K). Binding isotherms fitted to the raw data using 1:1 binding model as indicated.

Table 2: Equilibrium association constants K_a[a] and standard free energy ΔG, enthalpy ΔH, and entropy changes $T\Delta S$ for the formation of 1:1 complexes between the bambusuril macrocycle **5** and Br^- and I^- anions as determined by isothermal titration calorimetry (H_2O, pH 7.1, 303.15 K).

Anion[a]	K_a [$L\,mol^{-1}$]	ΔH [$kJ\,mol^{-1}$]	$T\Delta S$ [$kJ\,mol^{-1}$]	ΔG [$kJ\,mol^{-1}$]
F^-	13	−18.5	−12.0	−6.5
Cl^-	4.0×10^2	−45.4	−30.3	−15.1
Br^-	3.3×10^4	−68.0	−41.8	−26.2
I^-	2.0×10^6	−84.1	−47.5	−36.6

[a] Standard deviations calculated from two independent measurements are lower than 10% for Cl^-, Br^-, and I^-, and lower than 50% for F^-.

fully understood at the present time. One possible explanation relies on two entropy contributions that overcompensate anion desolvation effect. The first contribution is represented by configurational entropy resulting from the loss of mobility of the receptor and anion after the complex formation. The second, negative entropy contribution is connected with $H_2PO_4^-$. This anion is present in solutions under titration conditions and it is expected to undergo complexation with the macrocycle. During titration, $H_2PO_4^-$ positioned in the host cavity is replaced by anion of interest, for example, fluoride. Thus positive entropy of fluoride desolvation upon its complexation is compensated by the solvation of released $H_2PO_4^-$ anion. The association constants determined by NMR and ITC are in a good agreement given the different principles of the two techniques and slightly different experimental conditions (differences in pH and buffer concentration).

Finally, we decided to test possible influence of countercations, ionic strength, and pH on the anion interaction with **5**. We did not find any countercation effects on the macrocycle binding affinities. The association constant for the formation of the complex between **5** and Cl^- in the form of its sodium salt was identical (within experimental error) to that for the TMACl and CsCl (Supporting Information, Figure S7 and S8). We also determined K_a of the **5**$\cdot ClO_4^-$ complex by three independent competition experiments differing with the concentration of competitive Cl^- anion (0.2, 0.4, and 0.8 M NaCl; Supporting Information, Figure S14). K_a values differing by less than 15 % were obtained (Supporting Information, Table S1), which demonstrates a minor influence of ionic strength on the stability of complex inside this concentration window. Recently it was reported that strength of binding between an octa-acid host and ClO_4^- in water significantly increases with increasing content of NaCl.[12] We did not observe similar trend, presumably because attractive forces between **5** and ClO_4^- predominate over the salting out effect of NaCl. All of the NMR experiments presented in this study were performed at pD 7.1. To investigate the influence of pD on the binding we performed the titration of **5** with Cl^- in 20 mM Na_3PO_4 buffer of pD 11.3. The titration yielded the K_a of $1.4\times10^3\,L\,mol^{-1}$ which is about 45 % higher compared to that one determined at pD 7.1. Thus, we did not observed significant influence of pD on the binding ability of **5**. The insolubility of the macrocycle upon the protonation of its carboxylate groups precluded the binding study at low pD.

In conclusion, the bambusuril macrocycle **5** binds to inorganic anions with affinities and selectivities that are unprecedented for a neutral receptor in water. Its association constants range from $110\,L\,mol^{-1}$ for the most strongly solvated anion, F^-, to $5.5\times10^7\,L\,mol^{-1}$ for ClO_4^-. The macrocycle was prepared on a gram scale and exhibits good solubility in neutral and basic water. There is considerable interest in the development of effective anion receptors that function in water; we have demonstrated that the bambusuril **5** is such a receptor and as such may find diverse applications in biology as well as medicinal and analytical chemistry.

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