Anion Binding by Biotin[6]uril in Water

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Anion Binding by Biotin[6]uril in Water

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In this contribution we show that the newly discovered 6+6 biotin-formaldehyde macrocycle (Biotin[6]uril) binds a variety of anionic guest molecules in water. We discuss how and why the anions are bound based on data obtained using NMR spectroscopy, mass spectrometry, isothermal titrations calorimetry (ITC), computational calculations and single crystal X-ray crystallography.

Molecular recognition in water is particularly difficult to achieve due to the competitive nature of water as a hydrogen bond donor and acceptor.[1] One may argue that medicinal chemistry rely heavily on supramolecular chemistry in water, which highlights the importance of pursuing new fundamental understanding within this particular area.[2] In this paper we address the particular challenge of recognising anions in water.[3] A large number of elegant and complex receptors have been prepared and studied for their molecular recognition properties. In supramolecular chemistry the most utilized receptors are those that are easily prepared, naturally occurring or commercially available. Among these are symmetrical macrocyclic structures such as calix[*n*]arenes,[4] calix[*n*]pyrroles,[5] and calix[*n*]resorcinarenes.[6] Naturally occurring receptors such as the cyclodextrins[7] and linear amyloses have also been studied.[8] Recently a number of urea-based macrocycles,[9] such as cucurbit[*n*]urils,[10] hemicucurbit[*n*]urils[11] and bambus[*n*]urils[12] have been introduced and especially the family of cucurbit[*n*]urils have become popular due to the rich supramolecular chemistry offered by these structures in water.[13] Common to many of the popular macrocycles is that they are prepared by simple condensation reactions using aldehydes as a condensation partner. To introduce attractive features such as chirality and enhanced water-solubility into these structures subsequent and sometimes elaborate synthetic efforts are necessary. We recently introduced a new type of receptor molecule that is easily prepared in a single synthetic step, is chiral, is water soluble and is capable of binding anions in water: the Biotin[6]uril (fig. 1).[14]

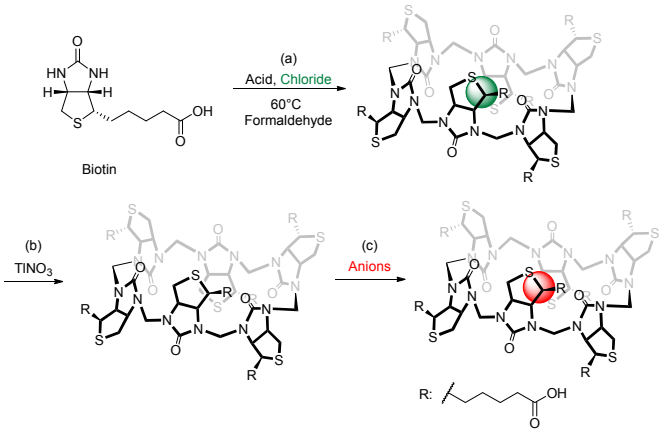

Fig. 1. (*a*) Synthetic route to Biotin[6]uril (with chloride or bromide encapsulated, green), (*b*) synthesis of anion-free Biotin[6]uril and (*c*) the reincorporation of other anions (red) studied in this work.

The synthesis of this regiochemically well-defined macrocyclic structure was achieved in multigram quantities in one synthetic step, and the product precipitated directly from the reaction mixture, which made the purification of the product simple. The six biotin units of the Biotin[6]uril have twelve protons from the convex side of each biotin unit pointing into the cavity of the Biotin[6]uril. This makes the cavity distinctly hydrophobic (fig. 1). In previous work we described how this hydrophobic pocket is the binding site for the halide anions in water at pH 10.8 in carbonate buffer.[14]

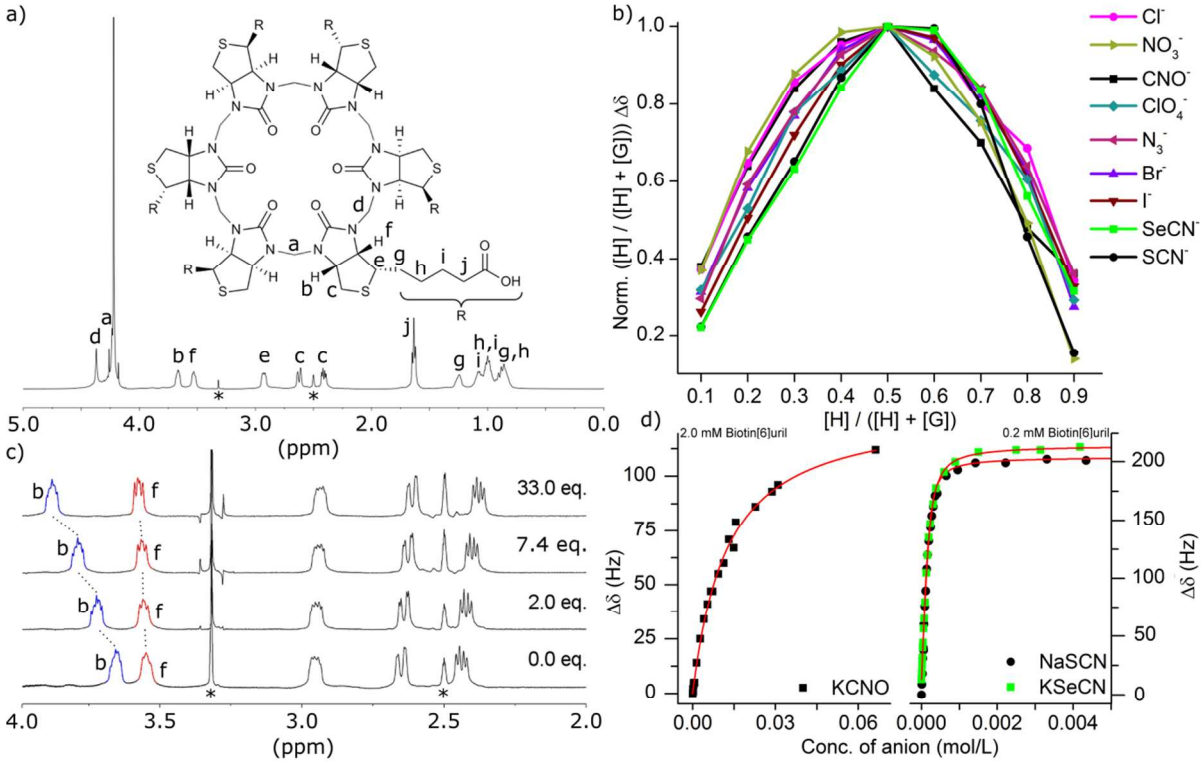

Fig. 2. a) ^{1}H NMR spectrum of Biotin[6]uril. b) Job plots for a series of anions with Biotin[6]uril. Plots are constructed using ^{1}H NMR data performed in 100 mM phosphate buffer pH 7.5 in D_2O. The normalized data are shown. No binding was observed for phosphate anions, thus making the phosphate buffer suitable for the binding studies. K^+ was used as counter ion for CNO^- and $SeCN^-$ whereas Na^+ was used for all other anions. c) ^{1}H NMR spectra illustrating the change in chemical shift values when determining binding to Biotin[6]uril. All spectra were recorded with an internal standard of d_6-DMSO*. Spectra were recorded in 100 mM phosphate buffer pH 7.5 in D_2O. As binding occurs in the central cavity, the signals for the bridgehead proton are monitored. If binding occurs then these signals shift chemical shift values. The data shown here are for cyanate. d) ^{1}H-NMR titration of KSeCN, NaSCN and KCNO in 100 mM phosphate buffer at pH 7.5. NaSCN and KSeCN were titrated into 0.2 mM Biotin[6]uril and KCNO with 2 mM of Biotin[6]uril. All NMR data was recorded at 500 MHz.

In this contribution we show how Biotin[6]uril is capable of binding a series of monovalent anions at pH 7.5 in phosphate buffer with binding constants ranging from logK = 1.8 (Cl^-) and logK = 4.5 (^-SCN). Initially we investigated a wide range of potential guest molecules and ions for their binding properties towards the Biotin[6]uril using ^{1}H NMR spectroscopy. Binding was indicated by a change in the chemical shifts of the protons on the convex side of each biotin unit pointing into the cavity (protons b and f in fig. 2c). We tested a series of aliphatic amines (e.g. 1,6-diaminohexane, 1,7-diamonoheptane, ethanolamine, and propargylamine), a series of cations (e.g. Na^+, K^+, and Cs^+), a series of anions (e.g. CN^-, ClO_4^-, PF_6^-, $PhCO_2^-$ and $CH_3CO_2^-$ and the anions in table 1) and a series of neutral guests (propionitrile, CO_2, CS_2 and propargyl-alcohol) for binding by Biotin[6]uril in water at pH 7.5 in 100 mM phosphate buffer.

We were pleased to find that Biotin[6]uril interacts with a range of singly charged anions (table 1). No binding to divalent anions was observed, as exemplified by experiments with SO_4^{2-}, WO_4^{2-}, CO_3^{2-} and HPO_4^{2-}.

For the series of singly charged anions we measured the binding stoichiometries using the continuous variation method of Job by means of ^{1}H NMR spectroscopy in water at pH 7.5 in 100 mM phosphate buffer (fig. 2b). All the anions presented here showed a 1:1 binding stoichiometry. To confirm that the chemical shift changes were not due to aggregation events we measured the ^{1}H NMR spectra at different concentrations (supporting material). To further evaluate the binding interactions of Biotin[6]uril with the anions we proceeded to measure the binding affinities using both ^{1}H NMR titrations (fig. 2c) and Isothermal Calorimetric Titration (ITC, fig. 3). The binding affinity data are summarised in Table 1.

In the titration experiments using ^{1}H NMR spectroscopy to monitor the binding event, the change in chemical shift values for the protons on the convex side of each biotin unit pointing into the cavity were monitored (fig. 2c). When plotting the change in chemical shift value as a function of the anion concentration the characteristic graphs shown in fig. 2d were obtained. By applying non-linear curve fitting the binding constants (K_a) and therefore binding energy (ΔG) can be found (table 1 and supporting material). In fig. 2d three such plots are shown for the three anions cyanate (OCN^-), thiocyanate (SCN^-) and selenocyanate ($SeCN^-$). It can be seen that a lot more cyanate needs to be added to the Biotin[6]uril before saturation is achieved (fig. 2d, left) than thiocyanate and selenocyanate (fig. 2c, right). This immediately indicates that thiocyante and selenocyanate bind Biotin[6]uril significantly more strongly than cyanate does.

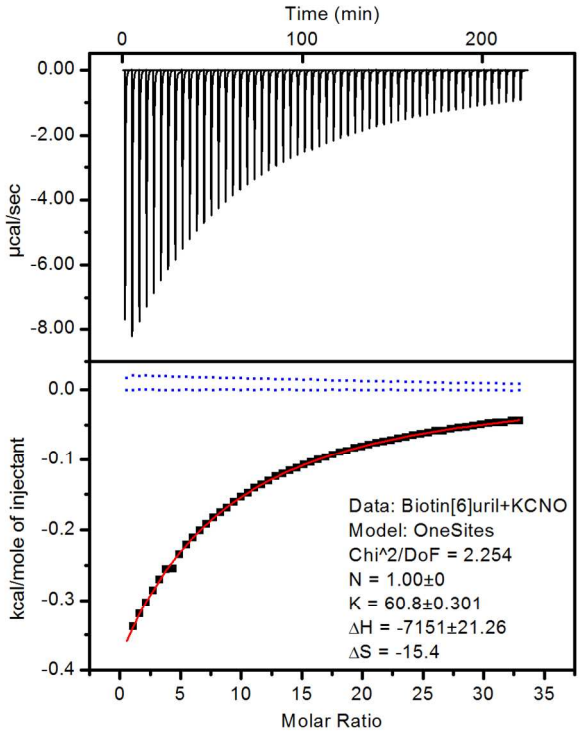

Fig. 3. ITC of Biotin[6]uril with KCNO in 100 mM phosphate buffer at pH 7.5 at 30°C. Black: observed data, Red: fitted data, Blue: dilution contribution. K (M^{-1}), ΔH (cal mol^{-1}) og ΔS (cal mol^{-1} deg^{-1}).

The ^{1}H NMR titrations revealed that Biotin[6]uril binds the halide anions in phosphate buffer at pH 7.5 in the order Cl^- > Br^- > I^- which is the same as previously reported at pH 10.8 in carbonate buffer with comparable binding affinities at the two sets of conditions. To evaluate whether the cation plays a role in the binding of the anions we tested the Na^+, K^+ and Cs^+ salts of iodide (table 1). We found that the binding affinities were comparable. This indicates that any cation interaction is insignificant for the binding event in solution. This can also be seen by analysing the crystal structure of Biotin[6]uril with NaI that shows an interaction from a urea-carbonyl carbon to a Na^+, but not to the anion binding cavity.

The binding of the halides show a trend where the larger anions fit better in the cavity. The same trend is observed for the cyanate anion family (fig. 2 and 3). When increasing the thermodynamic radii of the anion going from CNO^- (0.195 nm) to $SeCN^-$ and SCN^- (0.213 nm) the binding gets significantly stronger.[15] The larger binding affinities for the anions with larger thermodynamic radii can also be viewed as a consequence of the increased softness of the larger ions. Biotin[6]uril favours larger softer anions over smaller harder anions, until a certain size, as the anion needs to fit in the cavity of the receptor. The ITC data shows that all the binding interactions are enthalpically favorable and entropically unfavorable.

An interesting observation was that there is no binding to the ClO_3^- anion even though it binds the ClO_4^- anion, which might be because of the trigonal pyramidal structure of the ClO_3^- anion has a smaller thermodynamic radii than the ClO_4^- anion (0.171 nm vs 0.240 nm).[15] We did not observe any oxidative degradation of the Biotin[6]uril when exposing it to ClO_3^- and ClO_4^-. We speculate that the ClO_3^- anion binds more weakly due to its smaller size – and therefore its fit to the cavity size of Biotin[6]uril is simply less well. We have also observed that the F^- anion binds very weakly which is possibly also due to the small size of this anion. Even though the ClO_4^- anion is only slightly bigger than the SCN^- anion (0.213 nm) and the I^- (0.210 nm) anion its binding is much lower. We hypothesise that this is due to the larger size that makes the ClO_4^- anion fit in the cavity less well.

	^{1}H-NMR log(K_a)	ITC log(K_a)	ΔH[b] (kJ/mol)	TΔS[b] (kJ/mol)
NaCl	1.8 (1.7[a])	1.5 (1.0[a])	-30.7	-21.8
NaBr	3.0	2.7	-37.5	-21.6
NaI	3.7	3.4	-42.8	-23.0
KI[a]	3.3	-	-	-
CsI	3.7	-	-	-
$NaNO_3$	1.9	1.7	-32.3	-22.2
NaN_3	2.9	2.6	-31.1	-16.1
$NaClO_4$	2.7	2.4	-33.3	-19.5
KCNO	2.0	1.8	-29.9	-19.6
KSeCN	4.3	4.0	-37.7	-14.5
NaSCN	4.5	4.1	-35.0	-11.2

Table 1: ^{1}H NMR Titrations were performed in 100 mM phosphate buffer at pH 7.5 at 25°C. ITC data were collected at 30°C. [a] Data was obtained at pH 10.8 in carbonate buffer.[14] [b] ΔH and ΔS are from the ITC data at 30°C. All data obtained had less than 6% error.

To gain understanding of why Biotin[6]uril, which is a hexa-carboxylic acid, and therefore presumably an anionic species at pH 7.5, binds anions we calculated a plot of the electrostatic potential for Biotin[6]uril using density functional theory (DFT) calculations.[16] The calculations were performed directly for the crystal structures employing the long-range corrected CAM-B3LYP functional in combination with the 6-31G(d) basis set and the Gaussian 09 program.[17] From the electrostatic potential plot it is clear that the macrocycle has a positive electrostatic potential in the cavity (blue, fig. 4), whereas around the oxygen atoms of the urea-carbonyl groups the electrostatic potential is more negative (red, fig. 4). The sulphur atoms lead also to a weakly negative electrostatic potential.

Biotin[6]uril has an alternating orientation of the negatively charged urea carbonyls relative to the equator of the macrocyclic receptor. Therefore the macrocycle does not bind cations at the peripheries like the cucurbit[*n*]urils do.[13] The electrostatic potential is very similar to that reported for Bambus[6]urils, which also show a significant positive electrostatic potential in the cavity.[12]

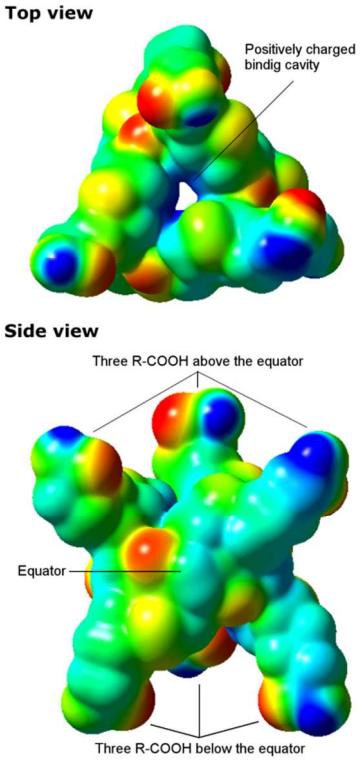

Fig. 4. Electrostatic surface potential of Biotin[6]uril at the CAM-B3LYP/6-31G(d) level of theory. Top view: showing the central binding cavity with a positive electrostatic potential. Side view: showing that the macrocycle has three carboxylic acids pointing in either direction of the equator of the macrocycle (defined as the area surrounding the binding cavity. Red: -31 kcal/mol blue: +31 kcal/mol.

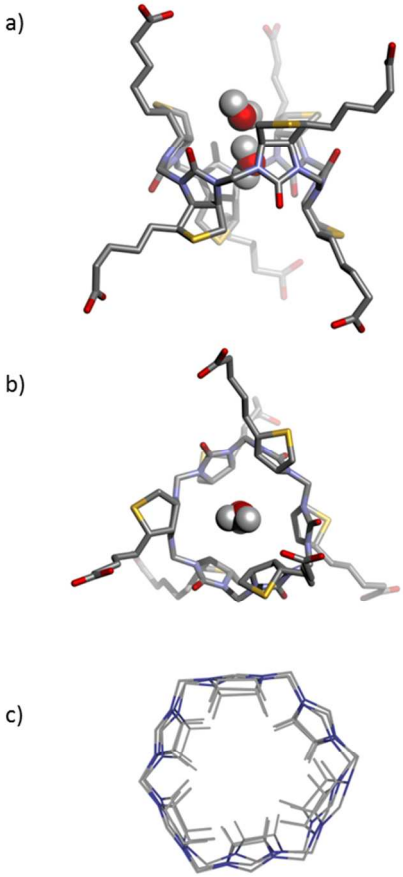

Fig. 5. (*a*) top view of the single crystal X-ray structure of Biotin[6]uril containing two molecules of water in the cavity. (*b*) Side view of the crystal structure. (*c*) Overlay of the urea-part of the three crystal structures of Biotin[6]uril showing that the diameter of the binding cavity only changes marginally with the three different guest molecules (iodide, EtOH and water).

In our previous work we have reported two single crystal X-ray structures of Biotin[6]uril.[14] In one of those structures an iodide anion was bound in the binding cavity, and in the other structure a molecule of ethanol was situated in the cavity. Herein we report a new single crystal X-ray structure of Biotin[6]uril (fig. 5a,b). In this structure the cavity contains two molecules of water that are hydrogen bonded to each. On the outside of the binding cavity we observe further molecules of water (disordered) to one side, and a carboxylic acid moiety from one of the side arms of the Biotin[6]uril to the other side. The two water molecules that reside in this cavity must be replaced by the anions upon binding of the anion to the cavity.

While the cavities of the Biotin[6]uril containing water, EtOH and iodide appear similar the cavities are actually subtly different. In fig. 5c the urea-containing five membered rings of the each Biotin unit (including the H's that point into the binding cavity) and the connecting formaldehyde derived CH_2-groups are shown (overlaid). This shows that the radius of the binding pocket is relatively unaffected by the different guest molecules. It is, however, possible for the biotin units the rotate slightly within the macrocyclic structure, thus changing slightly the directionality of the C-H bonds with respect to the centre of the cavity, and also the length of the binding cavity. The cavity size is similar to that of Bambus[6]uril reported by Sindelar and co-workers.[12]

One can view the centre of Biotin[6]uril as a cylinder shaped binding cavity defined by the 12 hydrogen atoms originating from the six C-H bonds from the biotin units. The 12 H–atoms define two offset circles with 6 H-atoms at the rim of each circle. By measuring the radius of each of these circles and the distance between them we get a cylinder shaped cavity with a volume of 86-102 $Å^3$ (see supporting material for details) for the three X-ray structures. This internal volume is not constant for the three crystal structures, indicating that the binding cavity does have some flexibility. This iodide containing structure has a volume of 93 $Å^3$, the EtOH containing structure a volume of 102 $Å^3$ and the water containing cavity a volume of 86 $Å^3$. This difference in cavity sizes are mainly due to the six biotin units of the macrocycle tilting slightly, giving a longer more narrow binding pocket.

Finally we studied the Biotin[6]uril-anion complexes using electrospray ionisation mass spectrometry. Solutions of Biotin[6]uril and an excess of the various anions were prepared in water, and these were analysed by direct injection ESI mass spectrometry. The spectra convincingly indicate the formation of complexes of the Biotin[6]uril and the anions. For the halide

series of anions the mass spectra are shown in fig. 6. In all three cases (Cl^-, Br^- and I^-) clear moleculare ions for the 1:1 complexes are observed.

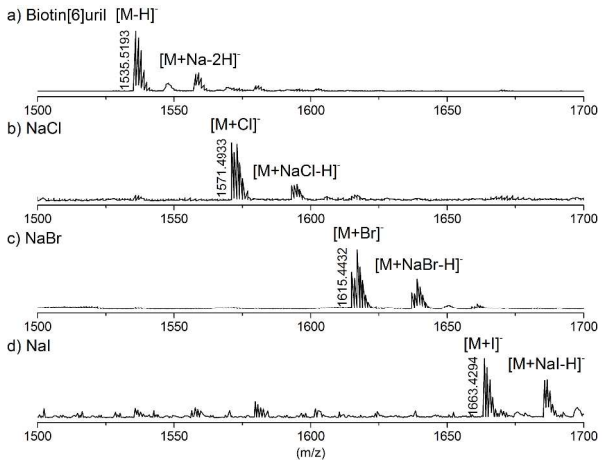

Fig. 6. High resolution mass of the Biotin[6]uril without guest (a), with NaCl (b), NaBr (c), and NaI (d).

Conclusions

In this communication we show how it is possible to bind a series of simple mono-charged anions to our recently discovered anion receptor Biotin[6]uril in water. We notice that the cavity of the Biotin[6]uril contains two water molecules, which upon release could contribute favourably to the enthalpically driven binding (non-classical hydrophobic effect).[18] The enthalpically driven binding event is evident from the ITC data. The binding of anions, we speculate, is governed by a delicate balance between the anions size in order to fit in the cavity, and the hardness/softness of the anion.

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Electronic Supplementary Information (ESI) available: Binding studies, X-ray crystal structure and Job Plots. See DOI: 10.1039/c000000x/

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