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 log $K = 1.8$ ($\text{Cl}^-$) and log $K = 4.5$ ($\text{SCN}^-$). Initially we investigated a wide range of potential guest molecules and ions for their binding properties 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).\textsuperscript{14}

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.\textsuperscript{14}

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 log $K = 1.8$ ($\text{Cl}^-$) and log $K = 4.5$ ($\text{SCN}^-$). 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-diaminoheptane, 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$_3$CO$_2^-$ and the anions in Table 1) and a series of neutral guests (propionitrile, CO$_2$, CS$_2$ and propargylalcohol) 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 (ESI†) where no changes were observed as a function of concentration. 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.

**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

<table>
<thead>
<tr>
<th>Anion</th>
<th>log($K_a$)</th>
<th>log($K_a$)</th>
<th>$\Delta H^b$ (kJ mol$^{-1}$)</th>
<th>$T\Delta S^b$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>1.8 (1.7$^a$)</td>
<td>1.5 (1.0$^a$)</td>
<td>$-$30.7</td>
<td>$-$21.8</td>
</tr>
<tr>
<td>NaBr</td>
<td>3.0</td>
<td>2.7</td>
<td>$-$37.5</td>
<td>$-$21.6</td>
</tr>
<tr>
<td>NaI</td>
<td>3.7</td>
<td>3.4</td>
<td>$-$42.8</td>
<td>$-$23.0</td>
</tr>
<tr>
<td>KI$^a$</td>
<td>3.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CsI</td>
<td>3.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>NaNO$_3$</td>
<td>1.9</td>
<td>1.7</td>
<td>$-$32.3</td>
<td>$-$22.2</td>
</tr>
<tr>
<td>NaN$_3$</td>
<td>2.9</td>
<td>2.6</td>
<td>$-$31.1</td>
<td>$-$16.1</td>
</tr>
<tr>
<td>NaClO$_4$</td>
<td>2.7</td>
<td>2.4</td>
<td>$-$33.3</td>
<td>$-$19.5</td>
</tr>
<tr>
<td>KCNO</td>
<td>2.0</td>
<td>1.8</td>
<td>$-$29.9</td>
<td>$-$19.6</td>
</tr>
<tr>
<td>KSeCN</td>
<td>4.3</td>
<td>4.0</td>
<td>$-$37.7</td>
<td>$-$14.5</td>
</tr>
<tr>
<td>NaSCN</td>
<td>4.5</td>
<td>4.1</td>
<td>$-$41.5</td>
<td>$-$11.2</td>
</tr>
</tbody>
</table>

$^a$ Data was obtained at pH 10.8 in carbonate buffer.$^{14}$ $^b$ $\Delta H$ and $\Delta S$ are from the ITC data at 30 °C. All data obtained had less than 6% error.
constants (were obtained. By applying non-linear curve fitting the binding anion concentration the characteristic graphs shown in Fig. 2d pointing into the cavity were monitored (Fig. 2c). When plotting the change in chemical shift value as a function of the values for the protons on the convex side of each biotin unit monitor the binding event, the change in chemical shift achieved (Fig. 2d, left) than thiocyanate and selenocyanate needs to be added to the Biotin[6]uril before saturation is strongly than cyanate does.

An interesting observation was that there is no binding to the ClO₃⁻ anion even though the receptor binds the ClO₄⁻ anion, which might be because of the trigonal pyramidal structure of the ClO₃⁻ anion which has a smaller thermodynamic radii than the ClO₄⁻ anion (0.171 nm vs. 0.240 nm). We did not observe any oxidative degradation of the Biotin[6]uril when exposing it to ClO₃⁻ or ClO₄⁻. We speculate that the ClO₃⁻ anion binds more weakly due to the smaller size – and therefore its fit to the cavity within the Biotin[6]uril is simply less favourable. 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 ClO₃⁻ anion is only slightly larger than the SCN⁻ anion (0.213 nm) and the I⁻ (0.210 nm) anion its binding is much weaker. We hypothesise that this is due to the larger size that makes the ClO₄⁻ anion fit in the cavity less well.

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. 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 in the Gaussian 09 program. 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. The electrostatic potential is very similar to that reported for Bambus[6]urils, 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.

In our previous work we have reported two single crystal X-ray structures of Biotin[6]uril. 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...
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 within the 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 to 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 ESI† 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 molecular ions for the 1 : 1 complexes are observed.
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). 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.

Acknowledgements

We acknowledge financial support from the Danish Research council (FNU) for a Steno Fellowship (MP) and the Lundbeck Foundation for a Young Group Leader Fellowship (MP) and a PhD scholarship (BMO). The support and sponsorship provided by COST Action CM1005 and MP1002 are acknowledged. We thank Dr Sophie R. Beerens for insightful discussions.

Notes and references