

A self-assembled receptor for the recognition of phosphate and acetate anions in neutral aqueous solution†

Céline Olivier, Zacharias Grote, Euro Solari, Rosario Scopelliti and Kay Severin*

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The reaction of $[(\text{benzene})\text{RuCl}_2]_2$ with a piperazine-bridged bis(dihydroxypyridine) ligand and LiOH leads to the formation of an expanded helicate, which is able to bind phosphate and acetate in aqueous solution at neutral pH.

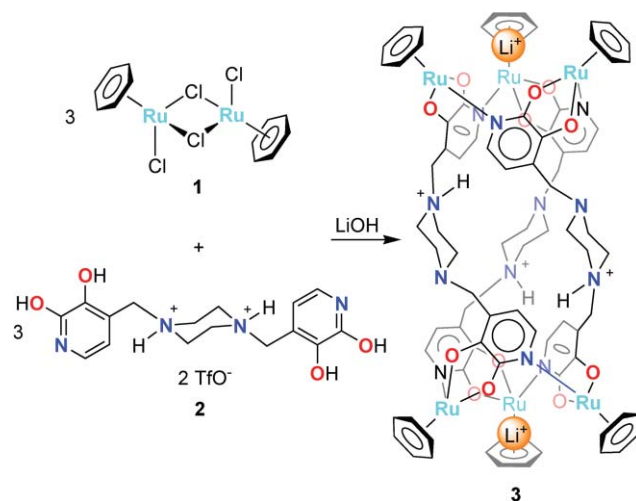
Anions are ubiquitous in the natural systems. The diversity of their functions has stimulated considerable interest in studying anion recognition.^{1–8} Synthetic receptors that efficiently bind small anions have been used as tools to understand the principles and mechanisms of anion recognition and also to develop chemosensors.² The most common binding motifs in artificial receptors are convergent hydrogen bond donor groups (*e.g.* amides³), precisely positioned positively charged groups (*e.g.* ammonium,⁴ imidazolium⁵ or guanidinium⁶ groups), and metal ions with available binding sites.⁷ Due to the potentially very strong interaction between metal ions and anions, metal-based receptors have shown considerable success in binding anions in water.⁷ However, the development of synthetic hosts that can function in water and that solely rely on hydrogen bonds and electrostatic interactions still remains a formidable challenge.⁸ In the following, we describe a self-assembled receptor which is able to bind phosphate and acetate with mM affinities in neutral aqueous solution.

In biological systems, phosphate is among the most important inorganic anion, since it plays a key role in energy storage and signal transduction.⁹ Carboxylates, on the other hand, are a common functional group in biomolecules. It is therefore not surprising that significant efforts have been devoted to the development of synthetic receptors for phosphate and acetate.^{1–8} Whereas excellent receptors are known for organic solvents, there are only a few hosts with metal-free binding sites which are able to recognize phosphate and/or acetate in neutral aqueous solution.^{10,11}

The design of our receptor was based on a structural motif which can be described as an expanded helicate. In these structures, the chiral metal complexes of helicates are replaced by likewise chiral metallamacrocycles. We have recently reported first examples of such assemblies.¹² They were obtained by reaction of $(\eta^6\text{-toluene})\text{Ru}^{\text{II}}$ or $(\eta^5\text{-C}_5\text{Me}_4\text{H})\text{Rh}^{\text{III}}$ complexes with bis(dihydroxypyridine) ligands with an excess of base. Crystallographic analyses revealed that the helicates have a cylindrical shape with two 12-metallacrown-3 complexes¹³ at the

opposite ends. The mantle of the cylinder is comprised of linkers with six amine groups, which appeared to be ideally suited to bind small guests. Of particular interest to us was the question whether these helicates could be used to bind anions in water. Unfortunately, the solubility of the neutral helicates in aqueous solution was very low. To address this problem, we have studied the reaction of the Ru complex $[(\eta^6\text{-benzene})\text{RuCl}_2]_2$ (**1**)—a $(\pi\text{-ligand})\text{M}$ complex which lacks lipophilic CH_3 groups—with the bis(dihydroxypyridine) ligand **2** and controlled amounts of CsOH in D_2O with ^1H NMR spectroscopy. The addition of increasing amounts of CsOH induced the formation of the helicate but the completion of the reaction was accompanied by the start of precipitation. In order to increase the solubility, we then used LiOH instead of CsOH as the base. The lithium ions were expected to bind to the 12-metallacrown-3 complexes,¹⁴ thereby increasing the charge of the assembly. This strategy proved to be successful: when five equivalents of LiOH were added to a solution of complex **1** and ligand **2** in D_2O , the helicate **3** was formed with a yield of >90% as evidenced from *in situ* NMR spectroscopy (see ESI†) (Scheme 1).

The ^1H NMR spectrum of complex **3** showed only one set of signals for the six π -ligands as well as for the three bridging oxopyridine ligands. The aromatic protons of the latter gave rise to doublets at 6.03 and 6.88 ppm, which is very characteristic for 12-metallacrown-3 complexes coordinated to Li^+ .¹⁴ Very broad signals were observed for the diastereotopic CH_2 protons adjacent to the piperazine N-atoms, plausibly due to restricted conformational freedom.



Scheme 1 Synthesis of the expanded helicate **3**.

Institut des Sciences et Ingénierie Chimiques, École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland.
E-mail: kay.severin@epfl.ch; Fax: +41(0)21 6939305;
Tel: +41(0)21 6939302

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Single crystals of the helicate without Li^+ cations were obtained by slow diffusion of NEt_3 into a solution of **1** and **2** in water. As the amount of base cannot be controlled with this method, it afforded a neutral complex (**4**) (Fig. 1).† The complex displays pseudo C_3 symmetry with all six Ru centres having the same relative configuration. The length of the cylindrical molecule is 2.1 nm (maximum H-to-H distance). Significant amounts of water were observed in the crystal (39.25 H_2O per helicate). None of the water molecules were found in the interior of the helicate although numerous H-bond interactions with heteroatoms of the ligands were observed. Attempts to crystallize the cationic version of the helicate with different counter anions have remained unsuccessful so far.

The host–guest chemistry of complex **3** was investigated using NMR spectroscopy. For that purpose, D_2O solutions of **3** (2.7 mM) were prepared *in situ* by adding appropriate amounts of LiOH to a solution of complex **1** and ligand **2**. The resulting mixture showed a pD of 6.6. NMR titration experiments were then performed with D_2O stock solutions of KCl , KBr , KI , KNO_3 , K_2SO_4 , KH_2PO_4 , and KOAc . The pD of the titrants was likewise adjusted to 6.6 using DCl or K_2HPO_4 , respectively. Only minor changes in the pD were observed during the titration.

Of all anions investigated, only phosphate and acetate gave rise to changes in the NMR spectra. In both cases, a significant sharpening in the signals of the NCH_2 protons was observed, whereas the signals of the pyridine protons and of the π -ligands were hardly affected. Parts of the NMR spectra obtained for titration experiments with phosphate are shown in Fig. 2.

The selective sharpening of the NCH_2 signals is evidence that the interaction of complex **3** with H_2PO_4^- and acetate is mediated *via* the piperazine amine groups (Scheme 2). Since five equivalents of LiOH were used to generate **3**, it is expected that approximately half of the six amine groups are protonated. Receptor **3** can thus provide hydrogen donor *and* acceptor groups for the binding of H_2PO_4^- or acetate. The complexation of the anion should result in a stabilization of the ‘boat’ conformation of the piperazine ring, which could explain the sharpening of the signals.

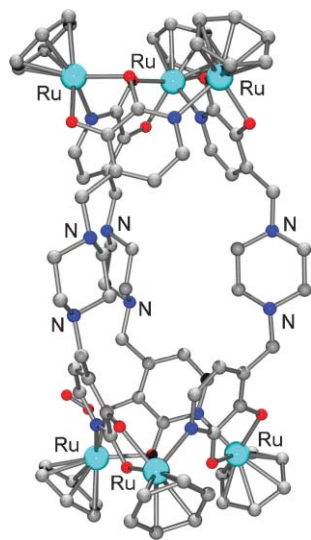


Fig. 1 Graphic representation of the molecular structure of complex **4** in the crystal. The co-crystallized water molecules and the hydrogen atoms are not shown for clarity.

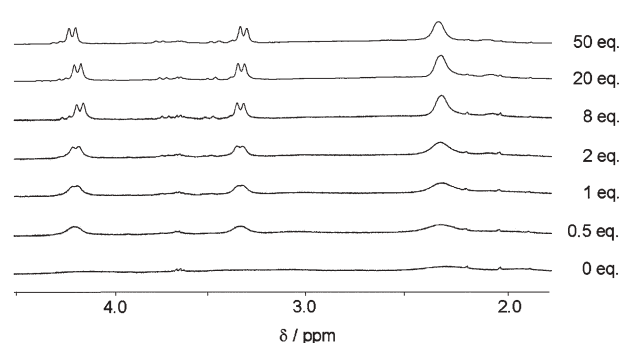
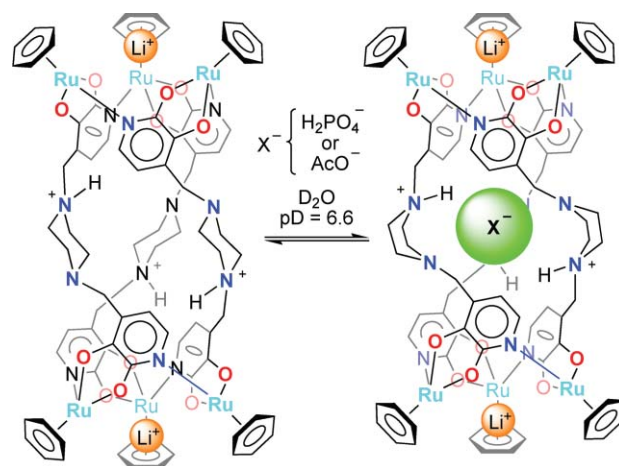


Fig. 2 Parts of the ^1H NMR spectra obtained upon addition of increasing amounts of H_2PO_4^- to a solution of receptor **3** (2.7 mM) in D_2O .



Scheme 2 Phosphate or acetate recognition by receptor **3** in D_2O .

Measuring the linewidth at half height of the peaks corresponding to the NCH_2 groups of **3** allowed us to calculate a binding constant with help of the non-linear least square fitting program WinEQNMR.^{15,16} A good fit was obtained assuming a 1 : 1 anion–receptor stoichiometry (Fig. 3) and a Job’s plot experiment provided additional evidence for a 1 : 1 association process (see ESI†). Following this model, a value of $K_a = 950 \text{ M}^{-1}$ was obtained for the binding constant for phosphate. Titration

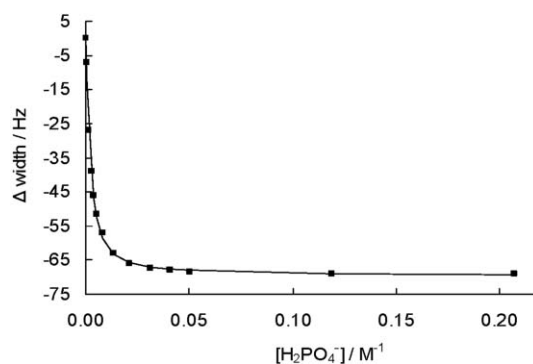


Fig. 3 Binding isotherm obtained from a ^1H NMR titration experiment with receptor **3** (2.7 mM) and KH_2PO_4 (680 μM –207 mM) in D_2O at pD 6.6. The linewidth corresponds to the signal at 2.3 ppm. The solid line represents the curve fitting.

experiments with acetate resulted in a similar sharpening of the signals of the NCH₂ protons (see ESI†). A linewidth analysis gave an association constant of $K_a = 270 \text{ M}^{-1}$. This value is among the highest observed so far for acetate binding in pure water by synthetic receptors with metal-free binding sites.^{6,11}

Isothermal titration calorimetry (ITC) was used as an additional technique to investigate the interaction of receptor **3** with phosphate and acetate. Fitting the titration data obtained with phosphate to a 1 : 1 binding model gave a binding constant of $K_a = 130 \text{ M}^{-1}$ with $\Delta H^\circ = +4.4 \text{ kcal mol}^{-1}$ and $T\Delta S^\circ = +7.3 \text{ kcal mol}^{-1}$. An endothermic binding event with a strong entropy compensation was also observed for titrations with acetate ($K_a = 45 \text{ M}^{-1}$, $\Delta H^\circ = +3.2 \text{ kcal mol}^{-1}$, $T\Delta S^\circ = +5.5 \text{ kcal mol}^{-1}$). In both cases, the binding of the anion is thus an entropy-driven process. The substantial gain in entropy is likely a result of the desolvation of the receptor and the anion. Our findings are in line with the fact that entropy is often decisive for anion binding by artificial hosts.^{17,18} Presently, we have no explanation why the binding constants obtained with ITC are slightly lower than those obtained from NMR spectroscopy, but one should note that small discrepancies between NMR- and ITC-derived binding constants are quite common.¹⁹

In summary, we have described a new receptor which is able to bind orthophosphate and acetate with mM affinities in neutral aqueous solution. The receptor was obtained from self-assembly of a (benzene)Ru^{II} complex with a bis(dihydropyridine) ligand in the presence of LiOH. The experimental data suggest that the Ru complexes are not directly involved in anion binding but serve rather as a structure-directing element.²⁰ The work is further evidence that metal-based self-assembly processes can be used to create in a very efficient way the complex structures required for anion recognition in highly competitive solvents such as water.

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Notes and references

- (a) E. A. Katayev, Y. A. Ustynyuk and J. L. Sessler, *Coord. Chem. Rev.*, 2006, **250**, 3004; (b) J. W. Steed, *Chem. Commun.*, 2006, 2637; (c) P. A. Gale, *Acc. Chem. Res.*, 2006, **39**, 465; (d) P. D. Beer and P. A. Gale, *Angew. Chem., Int. Ed.*, 2001, **40**, 486; (e) F. P. Schmidtchen and M. Berger, *Chem. Rev.*, 1997, **97**, 1609.
- (a) T. Gunnlaugsson, M. Glynn, G. M. Tocci, P. E. Krüger and F. M. Pfeffer, *Coord. Chem. Rev.*, 2006, **250**, 3094; (b) B. T. Nguyen and E. V. Anslyn, *Coord. Chem. Rev.*, 2006, **250**, 3118; (c) R. Martínez-Mañez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419.
- (a) S. O. Kang, R. A. Begum and K. Bowman-James, *Angew. Chem., Int. Ed.*, 2006, **45**, 7882; (b) C. R. Bondy and S. J. Loeb, *Coord. Chem. Rev.*, 2003, **240**, 77.
- (a) E. García-España, P. Díaz, J. M. Llinares and A. Bianchi, *Coord. Chem. Rev.*, 2006, **250**, 2952; (b) J. M. Llinares, D. Powell and K. Bowman-James, *Coord. Chem. Rev.*, 2003, **240**, 57.
- J. Yoon, S. K. Kim, N. J. Singh and K. S. Kim, *Chem. Soc. Rev.*, 2006, **35**, 355.
- (a) P. Blondeau, M. Segura, R. Pérez-Fernández and J. de Mendoza, *Chem. Soc. Rev.*, 2007, **36**, 198; (b) C. Schmuck, *Coord. Chem. Rev.*, 2006, **250**, 3053; (c) M. D. Best, S. L. Tobey and E. V. Anslyn, *Coord. Chem. Rev.*, 2003, **240**, 3.
- (a) E. J. O'Neil and B. D. Smith, *Coord. Chem. Rev.*, 2006, **250**, 3068; (b) P. D. Beer and E. J. Hayes, *Coord. Chem. Rev.*, 2003, **240**, 167.
- S. Kubik, C. Reyheller and S. Stüwe, *J. Inclusion Phenom. Macrocyclic Chem.*, 2005, **52**, 137.
- A. K. H. Hirsch, F. R. Fischer and F. Diederich, *Angew. Chem., Int. Ed.*, 2007, **46**, 338.
- (a) S. Develay, R. Tripiet, N. Bernier, M. Le Baccon, V. Patinec, G. Serratrice and H. Handel, *Dalton Trans.*, 2007, 1038; (b) S. Develay, R. Tripiet, M. Le Baccon, V. Patinec, G. Serratrice and H. Handel, *Dalton Trans.*, 2006, 3418; (c) J. L. J. Blanco, P. Bootello, J. M. Benito, C. O. Mellet and J. M. G. Fernández, *J. Org. Chem.*, 2006, **71**, 5136; (d) D. Aldakov and P. Anzenbacher, Jr., *J. Am. Chem. Soc.*, 2004, **126**, 4752; (e) V. Král, H. Furuta, K. Shreder, V. Lynch and J. L. Sessler, *J. Am. Chem. Soc.*, 1996, **118**, 1595; (f) D. H. Vance and A. W. Czarnik, *J. Am. Chem. Soc.*, 1994, **116**, 9397; (g) M. E. Huston, E. U. Akkaya and A. W. Czarnik, *J. Am. Chem. Soc.*, 1989, **111**, 8735; (h) B. Dietrich, T. M. Fyles, J.-M. Lehn, L. G. Pease and D. L. Fyles, *J. Chem. Soc., Chem. Commun.*, 1978, 934.
- (a) C. Schmuck and L. Geiger, *J. Am. Chem. Soc.*, 2005, **127**, 10486; (b) C. Schmuck and L. Geiger, *J. Am. Chem. Soc.*, 2004, **126**, 8898; (c) C. Schmuck and V. Bickert, *Org. Lett.*, 2003, **5**, 4579; (d) C. Schmuck, *Chem.-Eur. J.*, 2000, **6**, 709.
- Z. Grote, S. Bonazzi, R. Scopelliti and K. Severin, *J. Am. Chem. Soc.*, 2006, **128**, 10382.
- (a) K. Severin, *Chem. Commun.*, 2006, 3859; (b) K. Severin, *Coord. Chem. Rev.*, 2003, **245**, 3.
- Organometallic 12-metallacrown-3 complexes bind Li⁺ in water with an association constant of $K_a \approx 10^3\text{--}10^4 \text{ M}^{-1}$: (a) Z. Grote, R. Scopelliti and K. Severin, *J. Am. Chem. Soc.*, 2004, **126**, 16959; (b) Z. Grote, M.-L. Lehaire, R. Scopelliti and K. Severin, *J. Am. Chem. Soc.*, 2003, **125**, 13638.
- M. J. Hynes, *J. Chem. Soc., Dalton Trans.*, 1993, 311.
- For the determination of protein-ligand binding affinities with signal linewidths, see: L. Fielding, S. Rutherford and D. Fletcher, *Magn. Reson. Chem.*, 2005, **43**, 463.
- F. P. Schmidtchen, *Coord. Chem. Rev.*, 2006, **250**, 2918.
- For entropy-driven phosphate and acetate binding by a transition metal-based receptor, see: S. L. Tobey and E. V. Anslyn, *J. Am. Chem. Soc.*, 2003, **125**, 14807.
- (a) P. Plitt, D. E. Gross, V. M. Lynch and J. L. Sessler, *Chem.-Eur. J.*, 2007, **13**, 1374; (b) J. L. Sessler, D. E. Gross, W.-S. Cho, V. M. Lynch, F. P. Schmidtchen, G. W. Bates, M. E. Light and P. A. Gale, *J. Am. Chem. Soc.*, 2006, **128**, 12281.
- C. R. Rice, *Coord. Chem. Rev.*, 2006, **250**, 2190.