Synthetically accessible, high-affinity phosphate anion receptors†

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We report synthetically accessible receptors with protonated amines attached to a conformationally constrained backbone including amide units, and demonstrate that these receptors have high affinities (log $K > 5$) for phosphate anions in aqueous media at pH 7 in the presence of high concentrations of competitive chloride anions (*ca.* 100-fold excess).

Phosphate anions are ubiquitous in Nature and constitute an important target for supramolecular chemists.[1,2] If binding is to be biologically relevant, it should be established in water, which is highly competitive and attenuates anion binding interactions. Receptors studied in this context include polyamines,[3] which mimic one of Nature's own phosphate binders spermine,[4] and bind phosphate anions under physiological conditions *via* a combination of electrostatic interactions and hydrogen bonds. Macrocyclic polyamine receptors have been developed,[5] but generally require multi-step syntheses. Phosphate binding proteins typically employ a mixture of N–H^+ groups and amide (N–H) and alcohol (O–H) groups to achieve binding.[6] In synthetic receptors, amides have the potential to enhance binding, by providing additional interaction sites, or pre-organising the receptor.[7] Kubik *et al.* have used cyclic peptides to bind anions, including phosphate, in 80% H_2O–MeOH,[8] while Bowman-James and co-workers have combined amides with amines for anion binding in polar organic solvents.[9]

There is still an urgent need for synthetically accessible compounds, constructed from cheap, readily available starting materials, which bind phosphates with high affinity in competitive aqueous media – such systems have potential biomedical applications. Here, we present open-chain (**1**, **2**) and macrocyclic (**3**) receptors (Fig. 1), which contain amides and amines, and describe the high-affinity of **2** and **3** for phosphate anions.

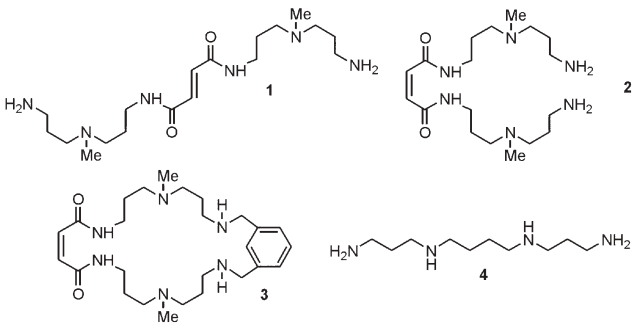

Fig. 1 Receptors investigated in this paper (shown in free base form).

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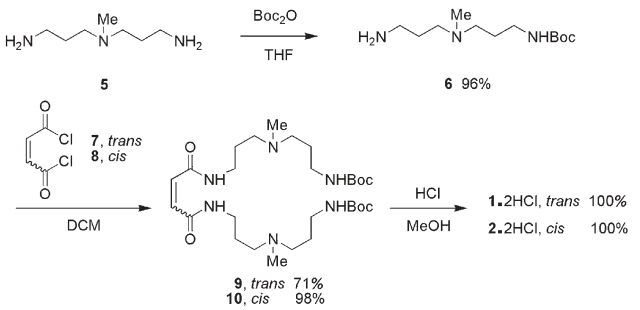
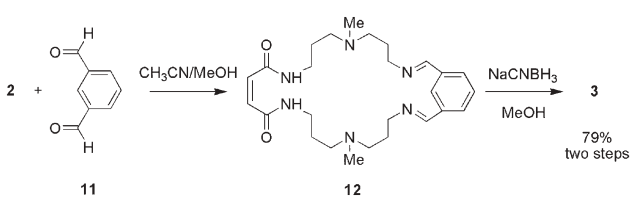
† Electronic supplementary information (ESI) available: Potentiometric titration data for phosphate anion binding and characterisation data for receptors **1**–**3**. See DOI: 10.1039/b706227c

The synthesis of the open-chain receptors (Scheme 1) started with straightforward mono-Boc protection of *N*-methyl-*N*,*N*-bis(3-aminopropyl)amine (**5**) to generate **6** – excess amine could be recovered by distillation. Two equivalents of compound **6** were then coupled with the diacid chloride of either fumaric (**7**) or maleic (**8**) acid to yield compounds **9** and **10**, respectively. These Boc-protected intermediates were obtained in excellent yields and subsequently deprotected using gaseous HCl to provide receptors **1** and **2**, which were isolated as their hydrochloride salts. The degree of protonation was established by potentiometric titration (see below). Macrocyclic receptor **3** was synthesised by condensation of **2** with isophthalaldehyde, using dilute conditions (Scheme 2). Schiff base intermediate **12** was not isolated, being immediately reduced to amine **3** using sodium cyanoborohydride. Reduction with the more reactive sodium borohydride resulted in reduction of the alkene and was avoided. Macrocycle **3** was purified by preparative gel permeation chromatography and isolated as the free base. The macrocyclisation occurred in surprisingly high yield, perhaps due to the pre-organisation of the primary amines on cis-oriented compound **2** (see below).

All compounds were characterised using standard methods, and data were in agreement with the proposed structures (see ESI†). The difference between the open-chain receptors is their configuration around the double bond, which surprisingly leads to significant differences in ^{1}H NMR spectra. In d_6-DMSO, the Boc-protected *trans* compound **9** had the expected spectrum for a C_2 symmetric molecule (*i.e.* equivalent alkene protons). However, *cis* compound **10** exhibited a doublet of doublets for the alkene



Scheme 1 Synthesis of acyclic receptors **1** and **2**.



Scheme 2 Synthesis of macrocyclic receptor **3**.

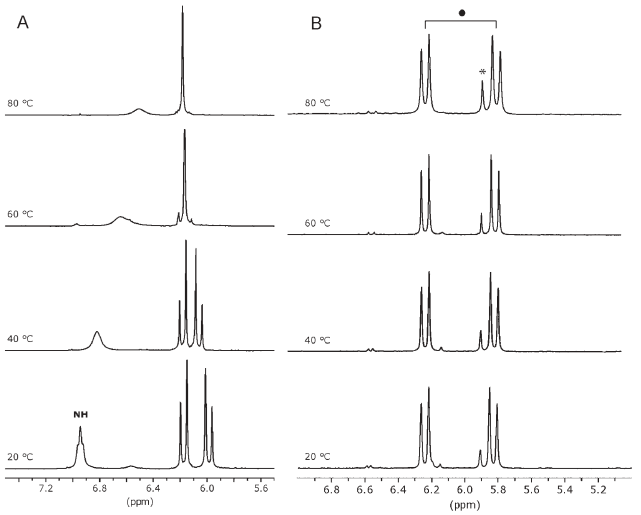


Fig. 2 Variable temperature NMR spectra of: (A) compound **10** in d_6-DMSO, (B) compound **2** in D_2O showing both the asymmetric (●) and symmetric (*) conformations.

protons in d_6-DMSO, indicating they are inequivalent (Fig. 2(A)). Similarly, in the ^{13}C NMR spectrum of compound **10**, two signals were observed for the double bond carbons and there were also two discrete carbonyl resonances. Apparently therefore, compound **10** adopts a conformation which breaks its symmetry. When the temperature was increased from room temperature to 80 °C, the doublets evolved into the expected singlet (Fig. 2(A)). Coalescence occurred at 75 °C and from this temperature, the barrier to conformational change was determined as 49 ± 3 kJ mol^{-1}. On heating, the peak at 6.92 ppm corresponding to an amide proton changed from a relatively well-resolved triplet into an upfield-shifted broad singlet. This indicates that a hydrogen bond is broken on heating, and it can be postulated that a hydrogen bond between the carbonyl of one amide and the NH of the other, forming a seven-membered ring, is responsible for the asymmetric conformation. Similar restricted conformations have been previously proposed for related compounds containing cis-oriented amide groups.[10]

Acyclic receptor **2** (and macrocyclic **3**) exhibited the same phenomenon of asymmetry, even in D_2O. In this case, both conformations were observed in slow exchange at room temperature (Fig. 2(B)). When the temperature was raised, the resonances remained in slow exchange, but their ratio changed slightly in favour of the symmetric conformation. These receptors therefore have significant conformational constraint, which we reason may act to preorganise the binding site.

To rapidly assess the affinity of our receptors for phosphate we studied the binding of fluorescent phosphate derivative **13** (Fig. 3) On titration of a receptor into a solution of this probe in buffered water (0.1 M Tris, pH 7.0), the emission was quenched indicating binding (Fig. 3). These data were fitted to a 1 : 1 binding model giving binding constants (log *K*) of 5.15 and 5.30 for **2** and **3**, respectively. Addition of trans receptor **1** did not result in significant quenching of the emission and a binding constant could not be determined. The log *K* values for **2** and **3** are the highest reported to date for a synthetic receptor of this type binding an anionic phosphate derivative under physiological conditions, and indicate that our compounds are very promising receptors.

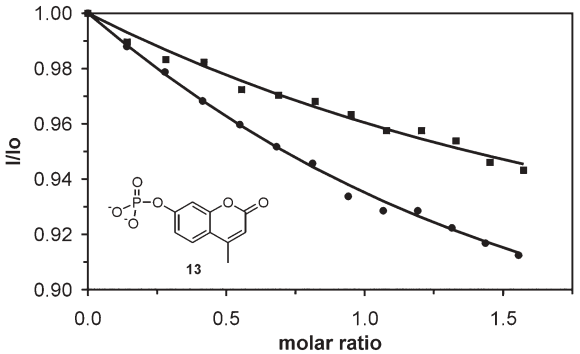


Fig. 3 Fluorescent derivative **13** and titration curves for its binding (5.0×10^{-6} M) to **2** (■) and 3 (●) in a 0.1 M TRIS buffer of pH 7.0 at 20 °C.

NMR investigations of receptor **2** with and without phosphate anions did not lead to significant shifts in the NMR spectra (N–H protons are not observed in D_2O). However, there was no noticeable perturbation of the alkene peaks, indicating that receptor **2** remained mainly in the asymmetric, H-bonded conformation on phosphate binding. This implies that the amide groups cannot themselves form hydrogen bonds to the phosphate anion, but instead play a structural role in the pre-organisation of the receptor.

To study the binding in more detail we turned to potentiometry. We first investigated the protonation constants of our receptors (Table 1). We calibrated our system with spermine (**4**) – the data agreed with literature values.[5h] The *trans* receptor **1** behaves like a flexible open-chain polyamine (*e.g.* spermine) – consecutive protonation steps being only mildly affected by the presence of nearby protonated amines. The *cis* receptor, **2**, on the other hand, although acyclic, has pK_a values more typical of a macrocycle. The third and fourth protonation steps for **2** are significantly more difficult than for **1**. This can be assigned to the cis configuration of the double bond and the rigid amide groups, which constrain the receptor, concentrating the protonated amines into a smaller area, much like a macrocycle. The second, third and fourth pK_a values for macrocycle **3** are a little lower than the corresponding values for pseudo-macrocycle **2**, reflecting an extra effect on covalent closure of the macrocyclic ring.

We then investigated the binding of inorganic phosphate anions to the receptor using potentiometry (Table 1) in the presence of excess chloride anions (0.1 M, *ca.* 100 eq.). Compound **1** displays moderate affinity for phosphate, typical of an open-chain receptor (log K = 2.9–3.4, depending on protonation states). Pseudo-macrocycle **2** displays much stronger binding (log K = 4.4–8.6). Macrocycle **3** has even higher affinity for phosphate anions (log K = 7.5–14.2). For each receptor, the data follow the expected trend that the largest binding constants are observed when the charges are highest, demonstrating the importance of electrostatic interactions. These receptors show a higher affinity for inorganic phosphate than organic phosphate **13**, reflecting the fact that inorganic phosphate has a higher charge density and is significantly smaller, decreasing steric repulsion. The affinity of pseudo-macrocycle compound **2** for inorganic phosphate under competitive conditions is remarkably high – higher than for most macrocycles reported in the literature.[5h] We propose that this may be due to the enhanced conformational pre-organisation of our

Table 1 Protonation constants (log *K*) of receptors **1–4** and phosphate anion binding constants (log *K*) of receptors **1–3**[a]

		1	**2**	**3**	**4**
Protonation data	$H^+ + L \leftrightarrows HL^+$	10.45(2)	10.04(3)	10.32(1)	10.64(1)
	$2H^+ + L \leftrightarrows H_2L^{2+}$	20.05(2)	19.06(3)	18.82(1)	20.63(1)
	$3H^+ + L \leftrightarrows H_3L^{3+}$	28.42(2)	26.52(3)	25.14(1)	29.50(1)
	$4H^+ + L \leftrightarrows H_4L^{4+}$	35.50(2)	30.27(4)	28.11(2)	37.58(1)
	$H^+ + L \leftrightarrows HL^+$	10.45	10.04	10.32	10.64
	$H^+ + HL^+ \leftrightarrows H_2L^{2+}$	9.60	9.02	8.50	9.99
	$H^+ + H_2L^{2+} \leftrightarrows H_3L^{3+}$	8.37	7.46	6.32	8.87
	$H^+ + H_3L^{3+} \leftrightarrows H_4L^{4+}$	7.08	3.75	2.97	8.08
Phosphate anion binding	$3H^+ + L + A^{3-} \leftrightarrows H_3LA$	[b]	34.94(4)	37.83(4)	
	$4H^+ + L + A^{3-} \leftrightarrows H_4LA^+$	42.87(4)	43.27(7)	46.85(4)	
	$5H^+ + L + A^{3-} \leftrightarrows H_5LA^{2+}$	50.44(3)	50.30(7)	53.76(5)	
	$6H^+ + L + A^{3-} \leftrightarrows H_6LA^{3+}$	[b]	56.33(9)	57.40(5)	
	$H_2L^{2+} + HA^{2-} \leftrightarrows H_3LA$	[b]	4.40	7.53	
	$H_3L^{3+} + HA^{2-} \leftrightarrows H_4LA^+$	2.90	5.27	10.24	
	$H_4L^{4+} + HA^{2-} \leftrightarrows H_5LA^{2+}$	3.39	8.55	14.17	
	$H_4L^{4+} + H_2A^- \leftrightarrows H_6LA^{3+}$	[b]	7.67	10.90	

[a] Determined by potentiometry in 0.1 M KCl solution at 25 °C. Values in parentheses are standard deviations of the last decimal. Protonation constants (log *K*) for phosphate were determined independently as 2.42, 6.91 and 11.48, respectively, in agreement with literature.[3g] [b] Equilibrium does not occur significantly.

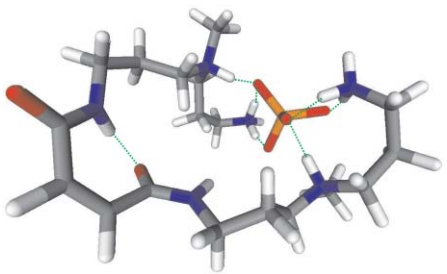


Fig. 4 Molecular modelling of complex between $\mathbf{2}.4H^+$ and PO_4^{3-} generated in the gas phase using PCSpartanPro (MMFF94).

receptors, demonstrated by the NMR studies. Interestingly, many proteins achieve phosphate binding by employing acyclic loops of binding residues, which exist in pseudo-macrocyclic conformations within the protein structure.[6]

In order to gain further insight, we modelled the binding of fully protonated compound **2** to fully deprotonated phosphate anions using PCSpartanPro and the MMFF94 force field. The resulting minimised structure (Fig. 4) demonstrates the intramolecular hydrogen bond which controls receptor conformation. There are electrostatic/hydrogen bond interactions between all the protonated amines on the receptor and the bound phosphate anion.

With high phosphate affinities and relatively trivial synthetic procedures, these receptors have genuine potential to find biomedical applications.[11] Work in this direction is currently in progress, and further studies to synthesise related receptors, investigate the origin of the remarkable affinities for phosphate, and determine anion selectivities are currently underway.

Notes and references

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