C_{3v} Symmetric Receptors Show High Selectivity and High Affinity for Phosphate

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The design of synthetic receptors for the purpose of binding anions in water with high selectivity and affinity is one of the goals of current molecular recognition pursuits.[1] Inorganic anions provide a variety of interesting targets due to their differences in size, shape, and charge. Examples of host/guest associations between synthetic receptors and anionic guests such as acetate, nitrate, chloride, fluoride, carbonate, sulfate, and phosphate have been reported.[2] Many of the receptors for phosphate display moderate to high affinity constants in organic media and low affinities in aqueous media. Lehn,[3] Bazzicalupi,[4] and Beer[5] have reported host/phosphate affinities in water as high as 10^5 M^{-1} at low pH, but the selectivity of these polyamine hosts for phosphate over other anions was either not reported or was low. Recently, Kim reported a sensor ensemble with high affinity and selectivity for phosphate in water.[6] However, the lack of a general strategy to create effective receptors for phosphate in water provides the impetus for the work described herein. We report a specific design principle to target tetrahedral oxyanions.

The cavities we designed are complementary to three faces of a tetrahedron (eq 1). Accordingly, the design of receptors **1** and **2**

$$\bigvee \quad \rightleftharpoons \quad \bigtriangledown \quad \tag{1}$$

features a C_{3v} symmetric cavity with a single Cu(II) along the C_3 axis for phosphate binding. Three additional functional groups bearing positive charges are positioned to provide the additional binding interactions commensurate with the general design strategy.

Receptor **1** is derived from a tris(2-ethylamino)amine unit with appended benzylamine groups, similar to designs exploited by Fabrizzi[7] and others.[8] Similarly, receptor **2** is derived from a tris-[(2-pyridyl)methyl]amine subunit functionalized with appended guanidinium groups, analogous to compounds studied by Canary,[9] Karlin,[10] and others.[11] A stoichiometric amount of copper(II) chloride preorganizes the ligands to yield the desired receptors. UV/vis spectroscopy was followed as aliquots of copper(II) chloride were added to solutions of the ligands (1.2 mM as chloride salts). The data was used to generate a mole ratio plot, showing a 1:1 binding stoichiometry of ligand to Cu(II) for both *apo*-**1** and *apo*-**2**.

A 98:2 H_2O/MeOH solution of **1** was prepared for titration purposes and buffered at pH 7.4 (5 mM HEPES). The change in the absorbance of **1** (0.71 mM) was monitored by UV/vis spectroscopy as aliquots of a phosphate solution (12.94 mM) were introduced. The resulting binding isotherm was fit[12] with a curve indicative of a 2.5×10^4 M^{-1} binding constant. This is one of the largest binding constants of phosphate to a synthetic receptor in water at neutral pH reported to date.

Similar methods were used to determine the binding affinities of several other anions to **1** in aqueous media (Table 1). The affinity of arsenate with **1** is the same as phosphate, within our error

Table 1. Binding Affinities Determined for a Series of Anions Using **1** and the Control Hosts **3** and **4**

receptor	anion[a]	stoichiometry anion:1	binding constants[b] (M^{-1})
1	HPO_4^{2-}	1:1	2.5×10^4 ($\pm 6 \times 10^2$)
1	$HAsO_4^{2-}$	1:1	2.5×10^4 ($\pm 6 \times 10^2$)
1	ReO_4^-	1:1	2.0×10^3 ($\pm 7 \times 10^2$)
1	AcO^-	1:1	<900
1	NO_3^-	1:1	<20
1	HCO_3^-	2:1	n.d.[c]
1	Cl^-	2:1	n.d.
1	SO_4^{-2}	n.d.	n.d.
3	HPO_4^{2-}	1:1	8.0×10^3 ($\pm 7 \times 10^2$)
3	$HAsO_4^{2-}$	1:1	9.0×10^3 ($\pm 7 \times 10^2$)
4	HPO_4^{2-}	1:1	9.0×10^2 ($\pm 3 \times 10^2$)

[a] All counterions to the anions are sodium. [b] K_a values were determined from a curve fit analysis to UV/vis data collected as aliquots of analyte solution were added to an aqueous solution of **1** at pH 7.4 at 25 °C. [c] n.d. = not able to determine.
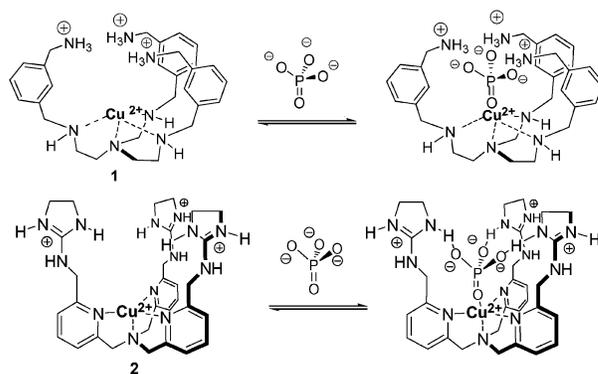
margins, due to the similarity in size, shape, and charge. Perrhenate, also tetrahedral in shape, has a binding constant on the order of 10^3, likely due both to the larger size and reduced charge relative to phosphate. Addition of sulfate to **1** gave so small a spectral change that the scatter in the data was too large to determine a reproducible binding constant. Anions of different geometries demonstrated much lower affinities to **1**, and in some cases the binding stoichiometry was 2:1 guest:host (Cl^- and HCO_3^-, see Table 1). Therefore, the shape, size, and charge of **1** lead to a high affinity and selectivity for phosphate relative to perrhenate, and spherical or trigonal bipyramidal anions.

To decipher the roles of the various binding sites on **1**, pH titrations on **1** were performed, and hosts **3** and **4** were examined. The pK_as of the ammonium groups in **1** are above 8.0 and therefore are predominantly fully protonated at the pH that the studies were performed (the protonation state of phosphate when bound is not known). While the affinity of phosphate with **3** is comparable to **1** (three times smaller), it serves to demonstrate that the cavity of **1** appears to be slightly better suited for small tetrahedral oxyanions. The K_a value of 900 M^{-1} for the binding of phosphate to **4** suggests that a large portion of the binding of phosphate to **1** is due to the metal center, a key feature in the host design. However, it also

Table 2. Binding Affinities Determined for a Series of Anions Using **2** and the Control Host **5**

receptor	anion[a]	stoichiometry anion:2	binding constants[b] (M^{-1})
2	HPO_4^{2-}	1:1	$1.5 \times 10^4 (\pm 6 \times 10^2)$
2	$HAsO_4^{2-}$	1:1	$1.7 \times 10^4 (\pm 6 \times 10^2)$
2	ReO_4^-		<100
2	AcO^-		<100
2	NO_3^-		<100
2	HCO_3^-		<100
2	Cl^-		<100
2	SO_4^{-2}		<100
5	HPO_4^{2-}	1:1	$4.0 \times 10^3 (\pm 7 \times 10^2)^c$

[a] All counterions to the anions are sodium. [b] K_a values were determined from a curve fit analysis to UV/vis data collected as aliquots of analyte solution were added to an aqueous solution of **1** at pH 7.4 at 25 °C. [c] Determined using 15% MeOH/water solution due to low solubility.

3   **4**
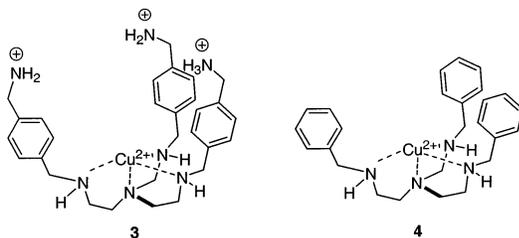
indicates that the ammonium groups are contributing significantly to the observed affinity constant for phosphate.

To further test the utility of C_{3v} symmetric receptors, an analogue to **1** was studied which was predicted to be even more complementary to phosphate due to increased preorganization. Hence, titrations of solutions containing receptor **2** (1.16 mM) with anions were performed. Modulations in the UV/vis absorption of **2** were monitored as 5 μL aliquots of a phosphate solution (19.8 mM) were added at pH 7.4 (10 mM TRIS). The resulting binding isotherm was fit with a 1:1 algorithm to yield an affinity of 1.5×10^4 M^{-1}. The titration with arsenate resulted in a similar binding affinity (Table 2), much as in the case of **1**. Although the K_a for phosphate and **2** is slightly smaller than that of the **1**:phosphate complex, this is still one of the highest reported in the literature to date. Titrations with other anions were performed using identical conditions, and no binding was observed. Even sulfate showed little to no affinity. Host **2** therefore has both a high affinity and excellent selectivity in water at neutral pH for phosphate and arsenate.

To decipher the roles of the various binding sites in **2**, host **5** was examined. For solubility reasons, a 15% MeOH/water solvent

5
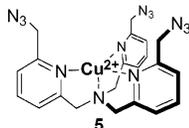
system was used. Titration of a phosphate solution (29.3 mM) into a solution of receptor **5** (1.5 mM), which lacks the guanidinium groups, demonstrates a binding affinity of 4.0×10^3 M^{-1}. This binding constant is higher than would be expected in the 2% MeOH/water system used with **2**, yet the reduced magnitude relative to **2** is sufficient to verify the cooperative effect of the guanidiniums and the Cu(II) center. Apparently, the guanidinium groups in **2** improve the binding, but more importantly they create a cavity that is highly complimentary to tetrahedral oxyanions.

In conclusion, two C_{3v} Cu(II) receptors having high affinity and selectivity for phosphate in aqueous media at neutral pH are reported. The selectivity for phosphate is ascribed to the design of the cavities, which provides excellent shape, size, and charge complimentarity to the anion. The high affinities reported for phosphate to both **1** and **2** are attributed to the combined charge-pairing interactions of the ammoniums/guanidiniums and the Cu(II) center with the oxygens of the tetrahedral anion. The inherent flexibility of **1** compared to that of **2** decreases its selectivity for phosphate. In contrast, the rigidity of **2** leads to a decrease in affinity for phosphate while increasing its selectivity. Such high selectivity is not generally observed with receptor designs based on polyaza macrocycles or clefts which compliment oxyanions in charge but not in size or in shape.

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