



Fluorescent detection of phosphate anion by a highly selective chemosensor in water

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ABSTRACT

A macrocyclic dinuclear copper complex, $[\text{Cu}_2^{\text{II}}(\mathbf{1})\text{Br}_4] \cdot 2\text{H}_2\text{O}$ has been synthesized and characterized by X-ray crystallography, in which each metal ion is pentacoordinated in a square pyramidal environment and the macrocycle is folded to form a boat-shaped empty cavity. As studied by an indicator displacement assay, the dinuclear complex shows strong selectivity for phosphate over sulfate, nitrate, perchlorate and halides in water at physiological pH.

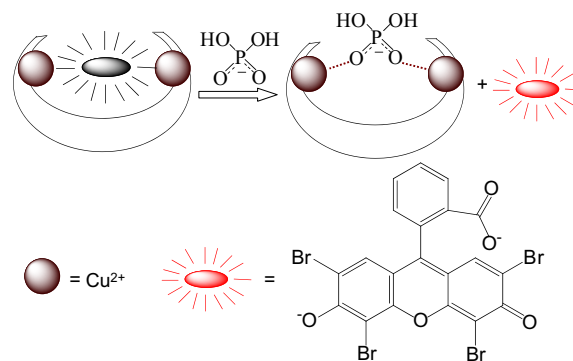
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While there has been an increasing interest in anion binding with a diverse variety of synthetic receptors, selective recognition of biologically relevant anions still remains a challenge in water particularly at physiological pH.¹ Phosphate is a fundamental building block of living systems, for example, nucleic acids, nucleotides and nucleosides, and plays a crucial role in many biochemical processes. It is known that phosphate exists as both H_2PO_4^- and HPO_4^{2-} at physiological pH.² However, the high free energy of hydration ($\Delta G^0 = -465 \text{ mol}^{-1}$) often makes it difficult to design a selective receptor for this anion in water.³ Therefore, the development of selective phosphate-binding receptors particularly in water at neutral pH is highly desirable. Neutral receptors, such as amides,⁴ thioamides,⁵ urea,⁶ thiourea,⁷ pyrroles,⁸ and indoles⁹ are well-documented systems for anion binding, however, their applications are limited to non-aqueous solvents due to the poor solubility in water. An obvious approach is to use water-soluble polyamines to bind phosphate, but their anion binding ability is largely dependent on electrostatic interactions which require low pH.¹⁰ Another strategy is to use metal complexes,¹¹ for example, cryptand-based dinuclear metal complexes that were employed by Nelson¹² and Fabbrizzi¹³ as selective receptors for different anions providing vacant axial sites to coordinate anions through electrostatic interactions. The distance as well as coordination geometry of the two metal centers indeed is important for the selectivity of an anion.

Monocycle-based polyamines have the potential to incorporate two metal ions, which can be applied for anion detection by using an indicator displacement assay originally introduced by Anslyn in the development of sensors.¹⁴ In this case, a sensor, formed by a synthetic receptor, and an indicator can display a detectable signal

due to the removal of the indicator upon the complexation with an anion.¹⁵ During the course of our work in anion binding studies, we obtained a crystal of a macrocycle-based synthetic receptor with two copper ions, providing a boat-shaped empty cavity, which we used for the detection of anions using fluorescent indicator displacement assay (Scheme 1). Our preliminary results indicate that the sensor shows very high affinity and selectivity for H_2PO_4^- in water at physiological pH.

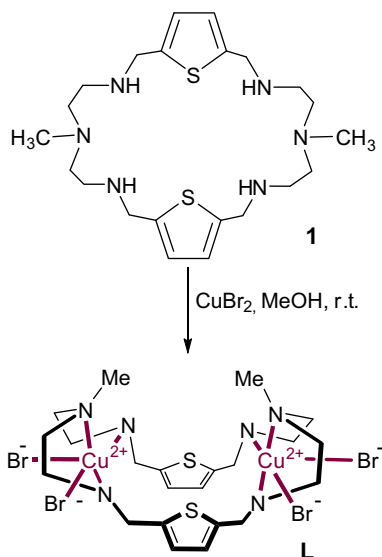
The ligand **1** was prepared from the high dilution condensation reactions of an equimolar methyl-2,2'-diaminodiethylamine and 2,5-thiophenedicarbaldehyde followed by NaBH_4 reduction in methanol.¹⁶ The dinuclear copper(II) complex (**L**) was prepared by adding 2 equiv of CuBr_2 to **1** in methanol (Scheme 2). The precipitate formed was collected and the crystals suitable for X-ray analysis were obtained by slow evaporation of a water solution of the complex.



Scheme 1. Schematic representation of the chemosensor displacing a dye upon the complexation of dihydrogen phosphate.

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Scheme 2. Synthesis of $[\text{Cu}_2^{\text{II}}(\mathbf{1})\text{Br}_4]\cdot 2\text{H}_2\text{O}$.

The X-ray analysis¹⁷ of the complex reveals that the macrocycle contains two Cu(II) ions at both N_3 sites. Each Cu(II) is coordinated with three macrocyclic nitrogens and two bromides to provide a square pyramidal geometry. The macrocycle is folded to form a boat-like structure with an empty cavity (Fig. 1), with the Cu–Cu distance of 6.243 Å. The Cu–Br_{equatorial} distances are 2.3390(17) and 2.3764(16) Å for Cu1–Br1 and Cu2–Br2, respectively, while Cu–Br_{axial} distances are 2.9461(18) and 2.9714(18) Å for Cu1–Br3 and Cu2–Br3', respectively. The longer distances in Cu–Br_{axial} are due to the Jahn–Teller distortion.¹¹ The Cu–N distances, which range from 2.001(8) to 2.054(8) Å, are comparable to the corresponding Cu–N distances from (1.973(5) to 2.056(5) Å) observed in *m*-xylyl-based macrocycle.¹² One bromide (Br4) remains outside the cavity and does not interact with the macrocycle. As seen in

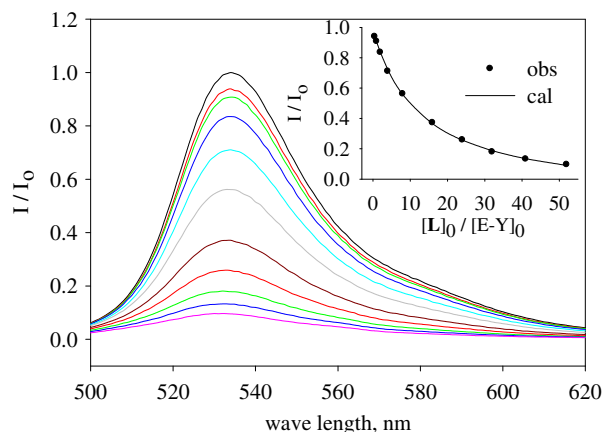


Figure 3. The decrease of fluorescence intensity of the indicator (EY) upon the addition of **L**. $[\text{EY}]_0 = 1 \times 10^{-7}$ M, $[\text{L}]_0 = 5.0 \times 10^{-5}$ M. Excitation = 470 nm. Inset shows the plot of I/I_0 against the equivalents of **L** at the wavelength of 537 nm. HEPES (0.05 M) in water buffered at pH 7.0 was used as a solvent.

Figure 1A, both copper centers are available to accommodate a suitable anion within the macrocyclic cavity. The macrocycles are connected through the axial bromides to form a linear chain along the *b* axis (Fig. 2). The Cu–Br–Cu angles are nearly linear [171.74°]. In this linear chain, the cavities are faced to the same side.

In order to evaluate the anion binding ability of the dinuclear ligand (**L**), an indicator displacement assay was performed using Eosine Y (EY) fluorescent dye. Upon increasing addition of **L** (5.0×10^{-5} M) to a solution of EY (1.0×10^{-7} M) buffered at pH 7.0 (0.05 M HEPES), the fluorescence intensity of EY gradually decreased, resulting in an almost complete quenching of the emission with 50 equiv of **L** (Fig. 3). Such quenching is due to the formation of an ion pair between **L** and the anionic dye, as expected.¹⁵ The change in the fluorescence intensity gave the best fit of a 1:1 binding model,¹⁸ yielding the binding constant $\log K = 5.9$.

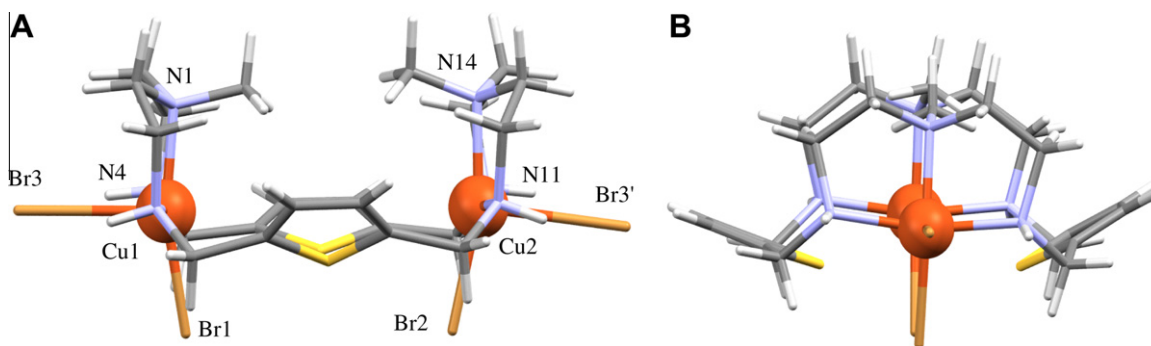


Figure 1. Crystal structure of $[\text{Cu}_2^{\text{II}}(\mathbf{1})\text{Br}_4]\cdot 2\text{H}_2\text{O}$: side view (A) and view down the Cu–Cu axis (B). Water molecules are not shown for clarity.

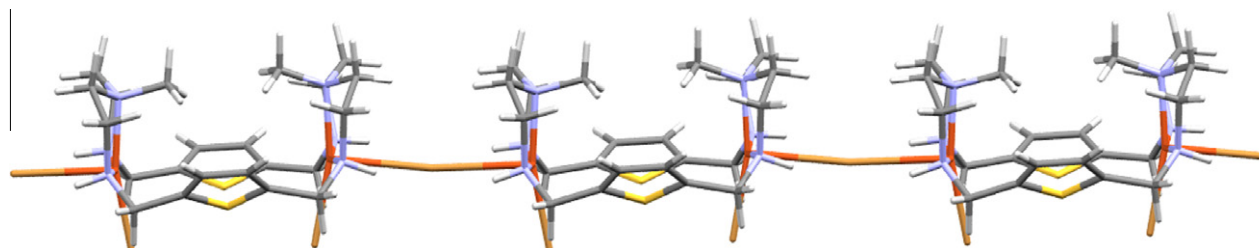


Figure 2. View down the *b* axis showing a linear chain of macrocycles.

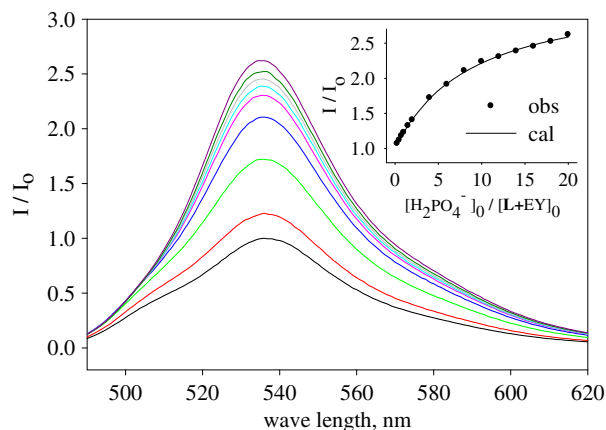


Figure 4. The enhancement of fluorescence intensity of the indicator EY-receptor ion pair upon the addition of phosphate. EY-receptor solution (dye/complex = 1:5) was prepared mixing EY (5×10^{-6} M) and L (2.5×10^{-5} M). $[\text{H}_2\text{PO}_4^-]_0 = 2.5 \times 10^{-3}$ M. Excitation = 470 nm. Inset shows the plot of I/I_0 against the equivalents of H_2PO_4^- at 537 nm. HEPES (0.05 M) in water buffered at pH 7.0 was used as a solvent.

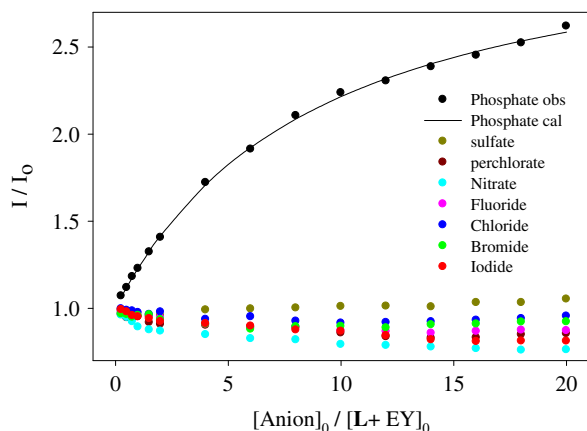


Figure 5. The plot of I/I_0 against the equivalents of different inorganic anions at 537 nm.

The receptor-dye complex was then examined for different inorganic anions, such as halides, nitrate, sulfate, phosphate, and perchlorate. In a typical titration, aliquots of the corresponding sodium salt in water (2.5×10^{-3} M) were added to a solution (2 mL) containing 2.5×10^{-5} M of L and 5×10^{-6} M of EY (dye/L = 1:5). All the solutions were buffered at pH 7.0 using 0.05 M HEPES in water. Upon the addition of H_2PO_4^- to L-EY solution, the fluorescence intensity was found to increase, indicating the displacement of the dye (EY) from the receptor-dye ion pair to the solution. The addition of 20 equiv of the anion resulted in a significant enhancement of fluorescence intensity (Fig. 4). The titration profile again gave the best fit of a 1:1 binding model.¹⁸ The calculated binding constant $\log K$ was found to be 4.1.

It is remarkable that under the identical condition, the addition of other anions including fluoride, chloride, bromide, iodide, sulfate, nitrate and perchlorate to the receptor-dye complex did not result in any enhancement of the fluorescent intensity (Fig. 5). This observation suggests that the dye was not displaced due to the weak or negligible affinity of the receptor-dye complex for these anions. A slight decrease in intensity in some cases, such as fluoride, nitrate and iodide, can be attributed to the simple dilution effect with the added anionic solution.

In summary, we have synthesized and structurally characterized a new macrocycle-based dinuclear complex, providing a boat-shaped empty cavity which has been successfully employed in an indicator displacement approach to detect phosphate in water at physiological pH. The strong selectivity of the receptor for phosphate could be due to the interaction of the charged phosphate ion with the pentacoordinated metal ions within the cavity which is well shaped to wrap the anion. The system described herein does not show any affinity for other common inorganic anions, but is able to selectively detect dihydrogen phosphate in water.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.07.078.

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17. Crystal data for L: $C_{22}H_{38}Br_4Cu_2N_6S_2 \cdot 2(H_2O)$, $M = 933.46$, triclinic, $a = 7.988(2)$ Å, $b = 9.116(3)$ Å, $c = 11.625(3)$ Å, $\alpha = 86.690(6)$, $\beta = 73.837(14)$, $\gamma = 80.279(12)$, $V = 801.3(4)$ Å³, $T = 100(2)$ K, space group $P1$, $Z = 1$, 25,560 reflections measured, 3941 independent reflections ($R_{int} = 0.0679$). The final R_1 values were 0.0474 ($I > 2\sigma(I)$). The final $wR(F^2)$ value was 0.1008 ($I > 2\sigma(I)$). The final R_1 value was 0.0644 (all data). The final $wR(F^2)$ value was 0.1060 (all data). CCDC 782240.
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