Communication

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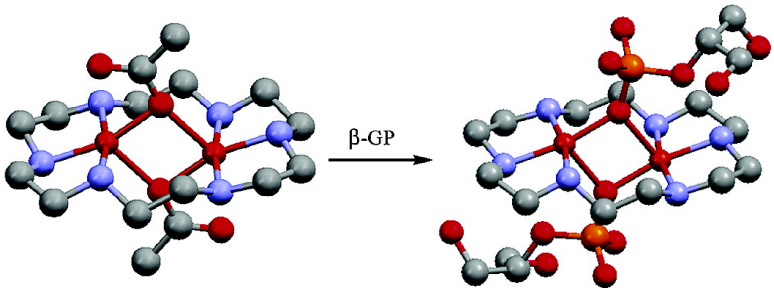

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Dicopper-[18]ane-N_6 Complex as the Platform for Phosphate Monoester Binding

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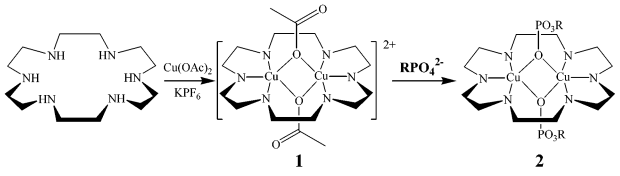
The synthesis of receptor molecules with high affinity and selectivity for phosphate and phosphate-containing metabolites is currently topical.[1] Intense efforts have been focused on organic receptors based on polyaza macrocycles, azocryptands, and polypyroles where high binding constants for phosphate were realized.[2,3] While many receptors exhibit high binding constants for phosphate anions in organic media, affinities tend to diminish in aqueous solution. Hence, a great challenge remains to create receptors with high affinity and selectivity in aqueous solution at physiological pH. Recently, the laboratories of Kim,[4] Anslyn,[5] and Hamachi[6] reported Zn^{2+}/Cu^{2+} complexes that bind HPO_4^{2-} and phosphorylated peptides with both high *affinity* and *selectivity* in aqueous solution, demonstrating the great promise of metallo receptors in phosphate recognition. In this communication, we report a novel dicopper motif based on a hexaaza macrocycle, [18]ane-N_6 (1,4,7,-10,13,16-hexaazacyclooctadecane, Scheme 1), that binds phosphate monoesters in aqueous solution with high affinity and selectivity.

Scheme 1. Synthesis of Dicopper-[18]ane-N_6 Complexes; R = H (**2a**), Ph (**2b**), and $-CH(CH_2OH)_2$ (**2c**)



Treating an aqueous solution of [18]ane-N_6 with $Cu(OAc)_2$ at room temperature resulted in a royal blue solution, and dark blue crystals were isolated with the addition of KPF_6 (Scheme 1).[7] Single-crystal X-ray diffraction analysis revealed the compound as $\{Cu_2(\mu\text{-}O\text{-}OAc)_2([18]ane\text{-}N_6)\}(PF_6)_2$ (**1**), and the structure of the cation is shown in Figure 1.[8] The bulk of the cation is a rugged rectangle consisting of the [18]ane-N_6 ring with two Cu(II) ions encircled, and the latter are bridged by two μ-*O*-acetates above and beneath the rectangle. The coordination sphere of the Cu center is best described as distorted square pyramidal, where the basal ligand centers are O1, N1, N2, and N3, and the apical ligand center is O1′. In the majority of [18]ane-N_6 complexes reported thus far, the macrocycle supports a single metal center (M) in the hexadentate mode with M as Co^{3+}, Cr^{3+}, Hg^{2+}, $(UO_2)^{2+}$, Nd^{3+}, and Er^{3+}.[9] In the only dinuclear example, the [18]ane-N_6 ligand supports two noninteracting Pd^{2+} centers.[10] Hence, cation **1** represents a novel coordination motif of the [18]ane-N_6 macrocycle.

The ability to accommodate two μ-O ligands by the Cu_2-{[18]-ane-N_6} core raises the possibility of binding other oxo anions, such as phosphate. Addition of $(NH_4)_2HPO_4$ to a light-blue solution of **1** resulted in an immediate darkening of the solution, indicating a possible displacement of acetate by HPO_4^{2-}. Similar changes also occurred with the addition of $PhOPO_3^{2-}$ or glycerol 2-phosphate ($(HOCH_2)_2CHOPO_3^{2-}$, GP) to the solution of **1**. Furthermore,

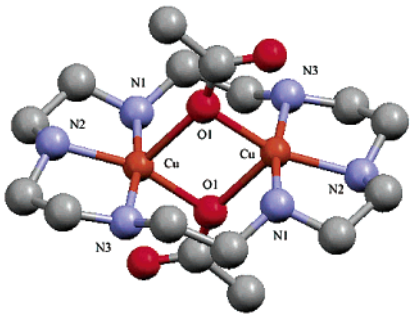


Figure 1. Ball-and-stick representation of **1**; Selected bond lengths (Å): Cu−N1, 2.068(9); Cu−N2, 1.990(3); Cu−N3, 1.985(10); Cu−O1, 2.009(3), Cu−O1′, 2.249(3).

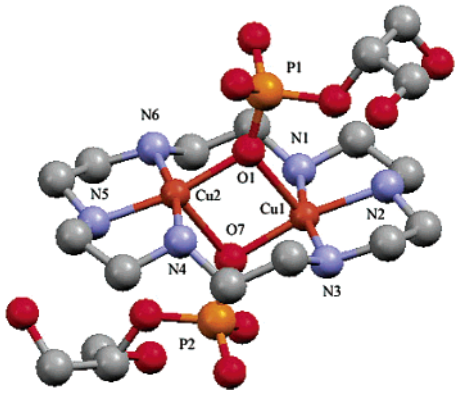


Figure 2. Ball-and-stick representation of **2c**; Selected bond lengths (Å): Cu1−N1, 2.062(8); Cu1−N2, 2.004(8); Cu1−N3, 2.051(7); Cu2−N4, 2.049(8); Cu2−N5, 1.982(9); Cu2−N6, 2.034(8); Cu1−O1, 2.253(6); Cu1−O7, 1.966(6); Cu2−O1, 1.961(6); Cu2−O7, 2.233(6).

addition of three molar equivalents of phosphate monoester to aqueous **1** resulted in the isolation of compounds **2** as crystalline materials (Scheme 1),[11] and each was identified through single-crystal structure analysis. The molecular structure of **2c**,[12] {[18]-ane-N_6}$Cu_2(\mu\text{-}O\text{-GP})_2$, is shown in Figure 2 and represents the first structurally characterized transition metal complex of glycerol 2-phosphate.[13] On the basis of the comparison of Figures 1 and 2, the overall features of **2c** are very similar to those of **1** with the coordination sphere of the Cu center being square pyramidal. The notable distinction is that the $Cu-O_{eq}$ bonds (Cu1−O7 and Cu2−O1) are significantly shortened in **2c**, reflecting the strong donor nature of phosphoate monoester. It is noteworthy that the structure of **2c** is reminiscent of 1:2 complexes between protonated sapphyrin and phenyl phosphate.[14]

Structural information about compounds **2** is highly relevant to the mechanistic understanding of bi/polymetallic phosphate esterases, for which a two-metal-ion mechanism (Scheme 2) suggested by Steitz and Steitz has gained wide acceptance in recent years.[15]

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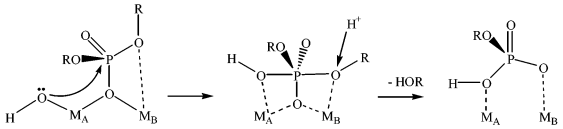
Scheme 2. Two-Metal-Ion Mechanism of Activation of Phosphate Diester



I **II** **III**

Since the single oxo-bridging mode depicted for **I** has not been identified in biological systems, structural insight for phosphate ester activation by metal centers has been extrapolated from a limited number of model studies, notably those of Cu^{2+} and Ni^{2+} complexes of phosphate *diesters* by Lippard.[16] Since compounds **2** are all based on phosphate *monoesters*, their topological features complement those of Lippard's compounds and may be significant in the understanding of enzymatic structures.

In addition to establishing the binding mode of phosphate monoesters via X-ray study, it is essential to gauge its binding strength in solution. Compounds **1** and **2** absorb around 620 and 635 nm, respectively, and the latter is about twice more intense than the former (see Supporting Information). The substantial difference in intensity permits an estimation of phosphate monoester *apparent association constants* relative to acetate ($K_{rel} = K_{phosphate}/K_{acetate}$) through spectroscopic titrations[14] performed under both unbuffered and buffered conditions,[17] and subsequent fitting according to the established formalism.[18] As shown in Table 1, the association constants (K_{rel}) are on the order of 10^4 for all phosphate monoesters under the unbuffered conditions. Phosphate binding by compound **1** is also clearly selective: the association constants for common anions such as F^-, NO_3^-, HCO_3^-, and PhO^- are at least *2 orders* of magnitude lower than those of phosphate monoesters.

Table 1. Anion Binding Constants of **1**

	K_{rel}	
anions	unbuffered	buffered
HPO_4^{2-}	$2.4 \pm 0.7 \times 10^4$	$4.2 \pm 0.6 \times 10^3$
$(PhO)PO_3^{2-}$	$1.1 \pm 0.1 \times 10^4$	$1.34 \pm 0.06 \times 10^3$
$(HOCH_2)_2CHOPO_3^{2-}$	$3.3 \pm 0.2 \times 10^4$	$2.8 \pm 0.2 \times 10^3$
F^-	160 ± 25	n.d.[a]
NO_3^-	20 ± 3	n.d.[a]
HCO_3^-	160 ± 70	n.d.[a]
PhO^-	n.d.[a]	n.d.[a]
HEPES	44 (±4)	−

[a] Not detected within the experimental errors

All above-mentioned titrations occurred in the pH range of 6.0−8.0. To gauge the binding ability of **1** under the physiological conditions, a second set of phosphate monoester titrations was performed using HEPES buffer to maintain pH at 7.4. We were surprised to note that the association constants (Table 1) have decreased by ca. 1 order of magnitude in comparison with those obtained from unbuffered titrations. It was subsequently determined that the HEPES buffer binds **1** weakly ($K_{rel} \approx 50$), presumably through the sulfonate group. Clearly, the competitive binding nature of HEPES buffer reduced the phosphate ester binding to **1**. In fact, the competition between phosphate and sulfonate for the same recognition site has long been recognized in structural biology.[19]

In summary, the novel Cu_2-{[18]ane-N_6} core displays high affinities toward a broad spectrum of phosphate monoesters and low affinities toward other common anions. The structural information about the complexes with phosphate monoesters is also highly relevant to metallophosphatases. Further improvements of the selectivity by introducing functionalized side chains as *C*-substituents of the Cu_2-{[18]ane-N_6} core are being pursued in our laboratory.

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Supporting Information Available: Experimental details of spectral titration of compound **1** by both phosphate monoesters and other anions, ORTEP plots of compounds **2a** and **2b** (PDF), and crystallographic data (CIF) of compounds **1**, **2a**, **2b** and **2c**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Coord. Chem. Rev.* **2003**, *240*.
(2) Lehn, J.-M. *Supramolecular Chemistry*; VCH: Weinheim, 1995.
(3) (a) Sessler, J. L.; Camiolo, S.; Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 17. (b) Beer, P. D.; Gale, P. A. *Angew. Chem., Int. Ed.* **2001**, *40*, 486.
(4) Han, M. S.; Kim, D. H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3809.
(5) (a) Tobey, S. L.; Jones, B. D.; Anslyn, E. V. *J. Am. Chem. Soc.* **2003**, *125*, 4026. (b) Tobey, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5*, 2029.
(6) Ojida, A.; Inoue, M.-a.; Mito-oka, Y.; Hamachi, I. *J. Am. Chem. Soc.* **2003**, *125*, 10184.
(7) In 2 mL of H_2O was suspended [18]ane-N_6·$3H_2SO_4$ (200 mg), and 1.0 M KOH was added until the solution became clear. $Cu(OAc)_2$·H_2O (150 mg) and KPF_6 (270 mg) were then added to the stirring mixture to yield a solution of deep blue color. Dark blue crystals of **1** were isolated after the solution was evaporated in air for a week (170 mg, 59%). Anal. Calcd (Found) for **1** ($C_{16}H_{36}Cu_2F_{12}N_6O_4P_2$): C, 24.22(23.98); H, 4.57(4.35); N, 10.59(10.32).
(8) *Crystal data* for **1**: $C_{16}H_{36}Cu_2F_{12}N_6O_4P_2$, *C*2, $a = 16.313(2)$ Å, $b = 9.823(1)$ Å, $c = 9.306(1)$ Å, $\beta = 97.010(2)°$, $V = 1480.0(2)$ Å^3, $Z = 2$; R1 = 0.048.
(9) (a) Royer, D. J.; Grant, G. J.; Derveer, D. G. V.; Castillo, M. J. *Inorg. Chem.* **1982**, *21*, 1902. (b) Chandrasekhar, S.; Fortier, D. G.; McAuley, A. *Inorg. Chem.* **1993**, *32*, 1424. (c) Carrondo, M. A. A. F. de C. T.; Félix, V.; Duarte, M. T.; Santos, M. A. *Polyhedron* **1993**, *12*, 931. (d) Nierlich, M.; Sabattie, J.-M.; Keller, N.; Lance, M.; Vigner, J.-D. *Acta Crystallogr., Sect. C* **1994**, *50*, 52. (e) Bu, X. H.; Lu, S. L.; Zhang, R. H.; Wang, H. G.; Yao, X. K. *Polyhedron* **1997**, *16*, 3247. (f) Wang, R. Y.; Zhao, J. J.; Jin, T. Z.; Xu, G. X.; Zhou, Z. Y.; Zhou, X. G. *Polyhedron* **1998**, *17*, 43.
(10) (a) Bencini, A.; Bianchi, A.; Dapporto, P.; Garcia-Espana, E.; Micheloni, M.; Paoletti, P.; Paoli, P. *Chem. Commun.* **1990**, 1382. (b) McAuley, A.; Whitcombe, T. W.; Zaworotko, M. J. *Inorg. Chem.* **1991**, *30*, 3513.
(11) To a solution of **1** (20 mg) in 5 mL H_2O was added $(NH_4)_2HPO_4$ (10 mg) with vigorous stirring. The resultant solution was evaporated in air for a week to yield **2a** as blue needles (15 mg, 83%). Anal. Calcd (Found) for **2a**·$6H_2O$ ($C_{12}H_{44}N_6O_{14}P_2Cu_2$): C, 21.02(21.08); H, 6.47(6.34); N, 12.26-(12.25); Syntheses of **2b** (45%) and **2c** (60%) were similar to that of **2a** with $(NH_4)_2HPO_4$ being replaced by Na_2PO_4Ph and $Na_2(PO_6C_3H_7)$, respectively.
(12) *Crystal data* for **2c**: $C_{12}H_{32}Cu_2N_6O_{16}P_2$, *Cc*, $a = 23.187(8)$ Å, $b = 8.135(2)$ Å, $c = 18.158(7)$ Å, $\beta = 106.72(2)°$, $V = 3280(2)$ Å^3, $Z = 4$; $R1 = 0.063$.
(13) A search of CSD revealed that the only known crystal structure of glycerol-2-phosphate is its sodium salt (CSD codes: NAGLYP01 and NAGLYP10); *Cambridge Structural Database System*; Conquest 1.5, October 2002 release.
(14) Kral, V.; Furuta, H.; Shreder, K.; Lynch, V.; Sessler, J. L. *J. Am. Chem. Soc.* **1996**, *118*, 1595.
(15) Steitz, T.; Steitz, J. *Proc. Natl. Acad. Sci., U.S.A.* **1993**, *90*, 6498.
(16) (a) He, C.; Lippard, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 184. (b) He, C.; Gomez, V.; Spingler, B.; Lippard, S. J. *Inorg. Chem.* **2000**, *39*, 4188.
(17) In a typical experiment, 3.0 mL of a 1.0 mM solution of **1** (aq) was either unbuffered or buffered with 5 mM HEPES at pH = 7.40, and titrated with a 0.10 M aqueous solution of appropriate phosphate monoester. For buffered experiments, phosphate ester solutions were adjusted to a pH of 7.40. All titrations were performed at 23 °C.
(18) Apparent association constants were determined as described by Sessler et al. (ref 14). We recognized the necessity of quantifying the solution equilibria associated with the formation of a 1:2 complex as did Sessler et al. However, both the paramagnetic nature of Cu complex and the lack of fine structures in visible spectra hamper such efforts in the present case.
(19) Kanyo, Z. F.; Christianson, D. W. *J. Biol. Chem.* **1991**, *266*, 4264.

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