Selected Paper

An Alkoxide-Bridged Dinuclear Zinc(II) Hexaazacryptate: A Novel Phosphate Capture Molecule in Aqueous Solution

Eiji Kinoshita, Kouhei Ishikawa, Emiko Kinoshita-Kikuta, Kazuhiro Ohtani, Motoo Shiro,[1] and Tohru Koike*

Department of Functional Molecular Science, Division of Medicinal Chemistry, Graduate School of Biomedical Sciences, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima 734-8551

[1]Rigaku Corporation X-ray Research Laboratory, Matsubaracho 3-9-12, Akishima, Tokyo 196-8666

Received July 14, 2004; E-mail: tkoike@hiroshima-u.ac.jp

A novel dinuclear zinc(II) hexaazacryptate (Zn_2L^{3+}) has been synthesized as a phosphate capture molecule in aqueous solution (L = an alkoxide form of 4,16-(2-hydroxypropano)-1,4,7,13,16,19-hexaazacyclotetracosane). Potentiometric pH titration study disclosed a 1:1 phenyl phosphate complexation with Zn_2L^{3+}. The dissociation constant, K_d (=$[Zn_2L^{3+}][PhOPO_3^{2-}]/[Zn_2L^{3+}–PhOPO_3^{2-}]$) is a small value of 6×10^{-7} M at 35 °C with $I = 0.10$ ($NaNO_3$). The X-ray crystal analysis of the *p*-nitrophenyl phosphate complex with Zn_2L^{3+} showed that the phosphate dianion binds as a bridging ligand to the two zinc(II) ions. The analogous 1:1 CH_3COO^--bound Zn_2L^{3+} complex was isolated and characterized by X-ray analysis.

Reversible protein phosphorylation is an essential mechanism for enzymatic and cellular regulation.[1] The significance of protein phosphorylation is becoming apparent from the ongoing genome projects, especially after the discovery that a few percent of the entire human genome may encode protein kinases.[2] A widely used method for defining a particular phosphorylation event is to label a target molecule with [^{32}P]orthophosphate followed by separation on two-dimensional gels and visualization using autoradiography.[3] Newer, non-radioactive methods using poly- and monoclonal antibodies (i.e., natural phosphate capture molecules) for the detection of phosphorylated residues have been reported.[4–6] The determination of phosphorylation sites on proteins is a vital part of phospho-proteomics.[7]

The chemical design of selective host molecules for various phosphate anions has attracted great interest. The majority of the hosts are organic molecules bearing acidic hydrogen atoms at complementary positions to formation of hydrogen bonds with phosphate O^- donors. The dissociation constants (K_d = [host][phosphate]/[phosphate-bound host]) are in the range of 10^{-2} to 10^{-6} M in non-aqueous solvents such as chloroform and dimethyl sulfoxide.[8–11] These hydrogen bonds, however, cannot compete against the hydration of phosphate anions in water, resulting in the dissociation of the phosphate-bound host complexes. To date, only a limited number of phosphate capture molecules with weak affinities (i.e., $K_d \gg \mu$M concentrations), which work in aqueous solution, have been reported.[12,13]

Previously, we found that macrocyclic polyamine zinc(II) complexes are useful as a family of phosphate capture molecules ($K_d = 10^{-3}$ to 10^{-6} M) at physiological conditions.[14–18] Their molecular design was originally conceived from the fact that phosphates act as substrates or inhibitors by reversible coordination to zinc(II) ion in zinc–enzymes (e.g., carbonic anhydrase, alkaline phosphatase, and carboxypeptidase).[19] In the zinc–enzyme model studies with macrocyclic polyamine zinc(II) complexes, we reached the generalized hypothesis that the selective association of phosphate dianions is feasible with two zinc(II) ions that are within a distance of 3–4 Å.[20]

Recently, we have found that a dinuclear zinc(II) complex (1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-olatodizinc(II) complex[21]) acts as a novel phosphate capture molecule at neutral pH.[22] This finding led to a simple, rapid, and sensitive procedure for a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of phosphorylated compounds.[22] The X-ray crystal structure of the dinuclear zinc(II) complex with *p*-nitrophenyl phosphate showed that each phosphate O^- donors binds to a zinc(II) at the fifth coordination site and that the two zinc(II) ions are separated by a distance of 3.55 Å.[23] Thus, the dinuclear zinc(II) complex having a vacancy on the two zinc(II) ions is suitable for the access of a phosphate monoester dianion ($ROPO_3^{2-}$) as a bridging ligand ($K_d = 25$ nM for phenyl phosphate at 25 °C).[23] Herein we report a novel phosphate capture molecule **1** (dinuclear zinc(II) hexaazacryptate, Zn_2L^{3+}), which has almost the same coordination environment as that for 1,3-bis[bis(pyridin-2-ylmethyl)-amino]propan-2-olatodizinc(II) complex (see Chart 1). The dinuclear zinc(II) hexaazacryptate forms a stable 1:1 complex with a phosphate monoester dianion (**2**) in aqueous solution.

Results and Discussion

Synthesis. The purpose of the synthesis of the dinuclear zinc(II) cryptate, **1** was to prepare a novel phosphate capture molecule, which has a similar zinc(II) coordination structure to that reported for 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-olatodizinc(II) complex.[23] The synthetic pathways for the ligand (HL, **6**) and its zinc(II) complexes (Zn_2L^{3+}–anion) are shown in Fig. 1. The tetraethyl ester **4** (1,3-diamino-2-

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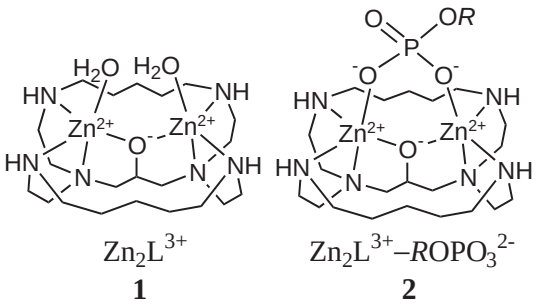
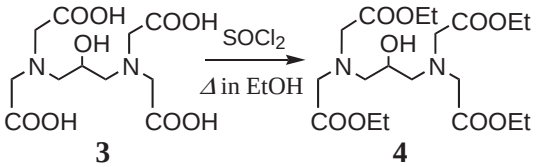
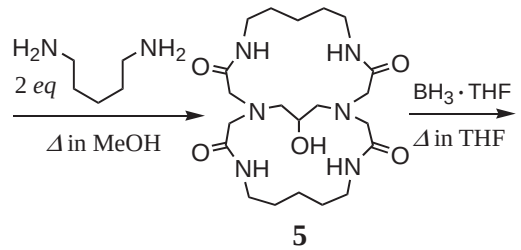


Chart 1.





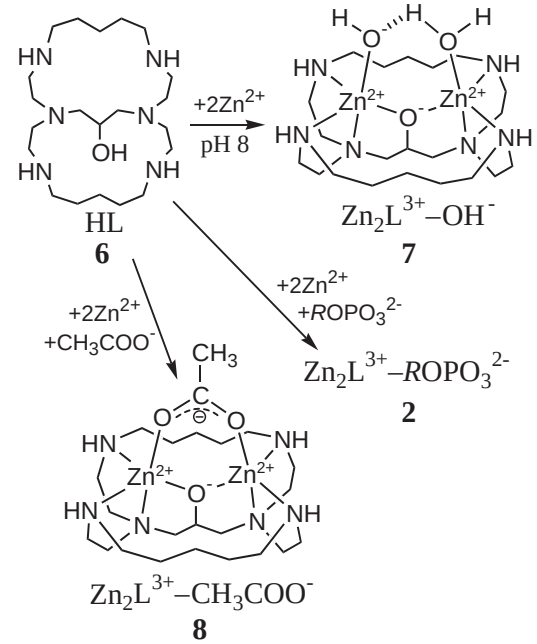


Fig. 1. Synthesis of hexaazacryptand **6** and dinuclear zinc(II) complexes **2**, **7**, and **8**.

propanol-*N*,*N*,*N*′,*N*′-tetraacetic acid tetraethyl ester) was prepared by esterification of corresponding carboxylic acid **3** using $SOCl_2$ in dry EtOH. The reaction of **4** with 2 equivalents of 1,5-diaminopentane (refluxing for 6 days in MeOH) gave a new bicyclic tetraoxo intermediate **5** in 27% yield. All the carbonyl groups were reduced with BH_3–THF complex in THF to give a propanol-bridged hexaazacryptand **6**, which was purified as its crystalline 4HCl salt in 42% yield.

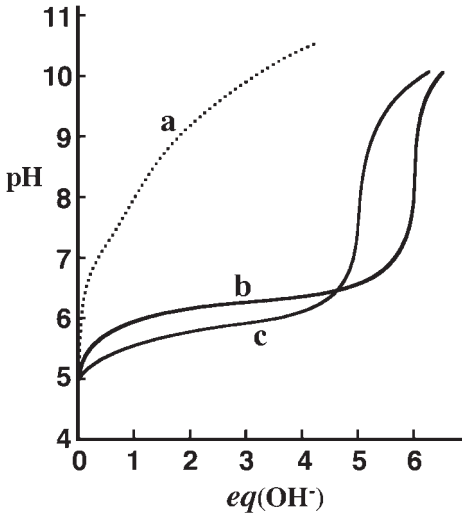


Fig. 2. Typical pH titration curves for HL·4HCl (**6**·4HCl) (1.00 mM) at 35 °C with $I = 0.10$ ($NaNO_3$) in aqueous solution: (a) in the absence of zinc(II) ion; (b) in the presence of 2.00 mM $Zn(NO_3)_2$; (c) in the presence of 2.00 mM $Zn(NO_3)_2$ and 1.0 mM phenyl phosphate disodium salt. *eq*(OH^-) is the number of equivalents of base added.

In an attempt to isolate the dinuclear zinc(II) cryptate **1** (a water-bound species), a hydroxide-bound Zn_2L^{3+} complex, **7**·$(ClO_4^-)_2$·$3H_2O$ was isolated as colorless fine crystals from an aqueous solution (pH 8) containing a 2:1 mixture of $Zn(ClO_4)_2$ and **6**. When $Zn(CH_3COO)_2$ was used instead of $Zn(ClO_4)_2$, the 1:1 CH_3COO^--bound Zn_2L^{3+} complex, **8**·$(ClO_4^-)_2$·H_2O was crystallized as colorless prisms from an aqueous solution containing excess amounts of $NaClO_4$. In the presence of an equivalent amount of phosphate monoester (i.e., phenyl phosphate and *p*-nitrophenyl phosphate), 1:1 phosphate-bound Zn_2L^{3+} complexes **2** were obtained. The elemental analysis, NMR, IR, and MALDI-TOF MS data of the zinc(II) cryptates (i.e., **2**, **7**, and **8**) suggested the formula for Zn_2L^{3+}–anion perchlorate salts (see Experimental).

Equilibria in Aqueous Solution. The potentiometric pH titration was performed with an aqueous solution of 1.00 mM ligand **6** (HL·4HCl) at 35 °C with $I = 0.10$ ($NaNO_3$). A typical pH titration curve for HL·4HCl is shown in Fig. 2a. The titration data were analyzed for four protonation equilibria (1). The four protonation constants ($\log K_{1-4}$) of 10.95 ± 0.03, 9.75 ± 0.03, 8.85 ± 0.03, and 7.60 ± 0.03 are assigned according to Fig. 3. Further deprotonation (e.g., an alkoxide (L) formation) was not observed below pH 11.

$$\mathrm{HL}\cdot(n-1)\mathrm{H}^+ + \mathrm{H}^+ = \mathrm{HL}\cdot n\mathrm{H}^+ \ (n = 1\text{–}4);$$
$$K_n = [\mathrm{HL}\cdot n\mathrm{H}^+]/[\mathrm{HL}\cdot(n-1)\mathrm{H}^+]a_{\mathrm{H}^+}. \quad (1)$$

The potentiometric pH titration curve of HL·4HCl (1.00 mM) in the presence of two equivalents of zinc(II) ion revealed the formation of stable zinc(II) complexes at pH > 5 with simultaneous deprotonation of the alcohol OH and water (i.e., Zn_2HL^{4+} to Zn_2L^{3+} and Zn_2L^{3+}–OH^-), a conclusion derived from the observation of the neutralization break at *eq*(OH^-) = 6 (see Fig. 2b). Further deprotonation (e.g., Zn_2L^{3+}–$(OH^-)_2$ formation) or precipitation of $Zn(OH)_2$ was

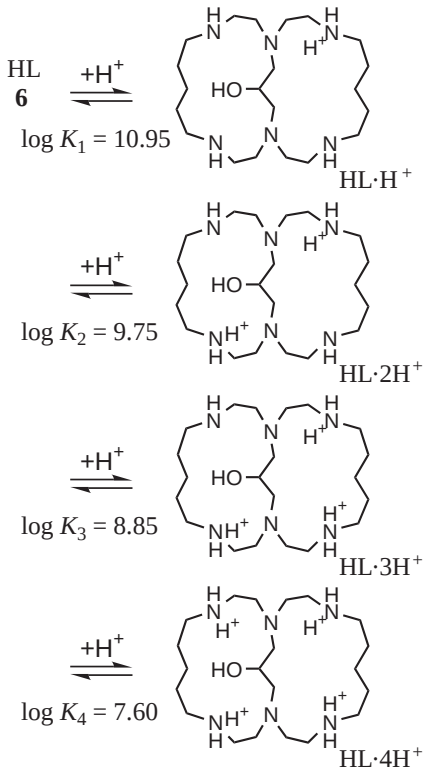


Fig. 3. Four protonation equilibria of **6** at 35 °C with $I = 0.10$ ($NaNO_3$) in aqueous solution.

not observed at $eq(OH^-) > 6$, indicating that the zinc(II) complex Zn_2L^{3+}–OH^- **7** remains stable up to pH 11. NMR signals for Zn_2L^{3+}–OH^- (i.e., 2:1 mixture of $Zn(ClO_4)_2$ and **6**) in D_2O solution at pD 10 were almost the same as shown those for the symmetric dizinc(II) complexes **8**, so that the proton exchange reaction between the Zn^{2+}–OH_2 and Zn^{2+}–OH^- in OH^--bound Zn_2L^{3+} (**7**) is sufficiently fast in aqueous solution. From the analysis of the pH titration data, the complex formation constant, $\log K_c$ of 12.2 ± 0.2 and deprotonation constants pK_a of 6.7 ± 0.1 were obtained (see equilibria (2) and (3)). The facile and simultaneous deprotonation of the alcoholic OH is probably due to a double coordination with two zinc(II) ions. A similar alkoxide-bridged dizinc(II) complex with a hydroxyoctaazacryptand was reported,[20] for which the pK_a value of the alcoholic OH is 4.0 at 25 °C. It should be noted that only the dinuclear species Zn_2L^{3+} (**1**) and Zn_2L^{3+}–OH^- (**7**) were confirmed under the experimental conditions employed. Therefore, as one zinc(II) ion binds to the ligand HL, the second zinc(II) ion simultaneously comes in the 1:1 Zn^{2+}–$HL{\cdot}nH^+$. The structural assignment for the alkoxide O^--bridged dinuclear zinc(II) complex comes from the X-ray crystal structure analyses of the *p*-nitrophenyl phosphate-bound complex **2** and CH_3COO^--bound complex **8** presented below. A typical diagram for species distribution as a function of pH at [total zinc(II)] = 2 mM and [total ligand] = 1 mM is displayed in Fig. 4.

$$HL + 2Zn^{2+} = Zn_2L^{3+}(\mathbf{1}) + H^+;$$
$$K_c = [Zn_2L^{3+}]a_{H^+}/[HL][Zn^{2+}]^2 \quad (2)$$

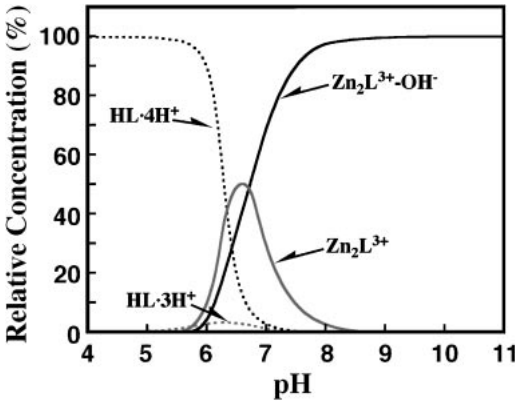


Fig. 4. Distribution diagram for **6** (1 mM) in the presence of zinc(II) ion (2 mM) as a function of pH ($-\log a_{H^+}$) at 35 °C with $I = 0.10$ ($NaNO_3$) in aqueous solution.

$$Zn_2L^{3+} = Zn_2L^{3+}\text{–}OH^-(\mathbf{7}) + H^+;$$
$$K_a = [Zn_2L^{3+}\text{–}OH^-]a_{H^+}/[Zn_2L^{3+}]. \quad (3)$$

Dissociation constants of the 1:1 anion-bound dizinc(II) complexes were determined by the potentiometric pH titrations in the presence of 2.0 mM CH_3COO^-, 1.0 mM phenyl phosphate dianion ($PhOPO_3^{2-}$), or 5.0 mM bis(phenyl) phosphate anion at 35 °C with $I = 0.10$ ($NaNO_3$). Because of less solubility (<0.2 mM) of the *p*-nitrophenyl phosphate complex with Zn_2L^{3+}, the pH titration with *p*-nitrophenyl phosphate was not conducted under the experimental conditions. A typical titration curve for 1.0 mM $PhOPO_3^{2-}$ in the presence of HL·4HCl (1.00 mM) and zinc(II) ion (2.0 mM) is presented in Fig. 2c. From the comparison of the titration curves Figs. 2b and 2c, we conclude that a stable dizinc(II) complex with $PhOPO_3^{2-}$ (Zn_2L^{3+}–$PhOPO_3^{2-}$) is formed at $eq(OH^-) < 5$, as shown by the curve c in the lower pH region, but at $eq(OH^-) > 5$ the Zn_2L^{3+}–OH^- complex coexists at pH > 8. Elaborate calculations of the titration data are consistent with the equilibrium (4) and the dissociation constants, K_d of the anion-bound complexes as follows: $(6.2 \pm 0.5) \times 10^{-7}$ M for phenyl phosphate dianion, $(4.7 \pm 0.5) \times 10^{-4}$ M for CH_3COO^-, $>1.0 \times 10^{-2}$ M for bis(phenyl) phosphate anion. From the order of the dissociation constants, one may say that the more basic anion has stronger affinity to the hexaazacryptate **1** (cf. $pK_a = 5.8$ for phenyl phophoric acid, 4.5 for CH_3COOH, and <2 for bis(phenyl) phosphoric acid[14]). Thus, the phenyl phosphate dianion is the most favorable bridging ligand to Zn_2L^{3+}. A typical diagram for species distribution as a function of pH at [total zinc(II)] = 2 mM, [total ligand] = 1 mM, and [phenyl phosphate] = 1 mM is displayed in Fig. 5, which shows a little dissociation (<5%) of Zn_2L^{3+}–$PhOPO_3^{2-}$ when **1** and phenyl phosphate dianion (both at 1 mM) are mixed at physiological pH. The final structural assignment for the phosphate-bridged dinuclear zinc(II) complex comes from the X-ray crystal structure analysis of *p*-nitrophenyl phosphate-bound complex presented below.

$$Zn_2L^{3+}\text{–anion} = Zn_2L^{3+}(\mathbf{1}) + \text{anion};$$
$$K_d = [Zn_2L^{3+}][\text{anion}]/[Zn_2L^{3+}\text{–anion}]. \quad (4)$$

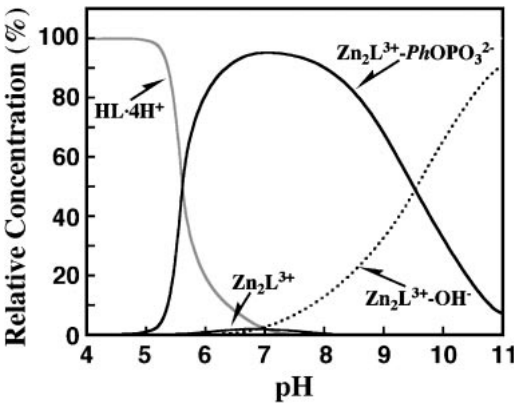


Fig. 5. Distribution diagram for **6** (1 mM) in the presence of zinc(II) ion (2 mM) and phenyl phosphate (1 mM) as a function of pH ($-\log a_{H^+}$) at 35 °C with $I = 0.10$ ($NaNO_3$) in aqueous solution.

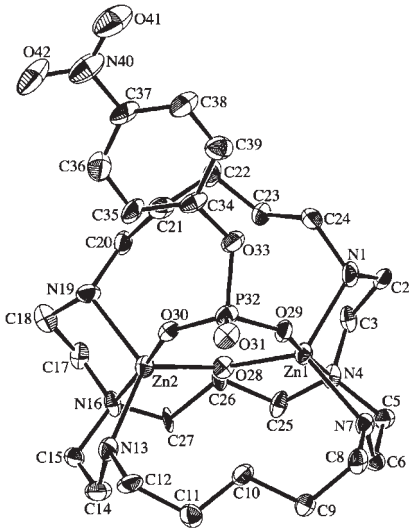


Fig. 6. One of the crystallographically independent molecules of **2** ($R = p$-nitrophenyl) with 50% probability ellipsoids. Hydrogen atoms, perchlorate ion, and water are omitted for clarity.

Molecular Structures of $ROPO_3^{2-}$-Bound and CH_3COO^--Bound Complexes. In an attempt to isolate a $ROPO_3^{2-}$-bound Zn_2L^{3+} (**2**), a phosphate monoester (i.e., phenyl phosphate, *p*-nitrophenyl phosphate, *O*-phosphoryl serine, *O*-phosphoryl tyrosine, and adenosine monophosphate) was mixed with an equivalent amount of Zn_2L^{3+} (Zn_2L^{3+}–OH^- or 2:1 mixture of $Zn(ClO_4)_2$ and HL) in aqueous solution at pH ca. 7. Among the phosphate monoester ligands, *p*-nitrophenyl phosphate gave a yellowish fine crystal of [Zn_2L^{3+}–*p*-nitrophenyl phosphate](ClO_4^-)$\cdot 2H_2O$ by slow recrystallization from water. The crystal was subjected to X-ray crystal analysis. The crystal structure provided unequivocal evidence for the alkoxide-bridged dinuclear zinc(II) complex, which is shown in the ORTEP drawing with 50% probability thermal ellipsoids in Fig. 6. The selected bond distances and bond angles around zinc(II) are listed in Table 1.

In the *p*-nitrophenyl phosphate-bound Zn_2L^{3+} complex, **2** ($R = p$-nitrophenyl), both zinc(II) ions are almost equivalent in a distorted trigonal-bipyramidal environment: Zn1 (or Zn2) is coordinated by the two secondary amines N1 (or N13) and N7 (or N19) and an alkoxide O^- anion (O28) as equatorial donors and the ternary amine N4 (or N16) and one of the phosphate anionic oxygen O29 (or O30) as apical donors. The apical Zn–O^- bond distances (1.98 and 2.01 Å) are as short as those for the equatorial Zn–O^- bonds (1.98 and 1.99 Å). The coordination bond distance between phosphate O^- and zinc(II) (1.98 and 2.01 Å) are shorter than those of Zn–O^-(acetate) in the analogous acetate-bound complex **8** (2.02 and 2.03 Å, see below). These facts indicate the stronger interaction between the phosphate and the zinc(II) ions. Each zinc(II) ion lies almost in each basal plane defined by N1, N7, and alkoxide O28, and N13, N19, and O28, where the total angles around the zinc(II) atoms are 352.7° (N1–Zn1–N7, N7–Zn1–O28, and O28–Zn1–N1) and 352.8° (N13–Zn1–N19, N19–Zn1–O28, and O28–Zn1–N13). The apical angles are almost linear at 176.7° for N4–Zn1–O29 and 176.9° for N16–Zn2–O30. The two zinc(II) ions are separated by a distance of 3.54 Å, which is an appropriate distance to accept two oxygen atoms of the phosphate ester dianion as part of the bridging ligand.[20]

Table 1. Selective Bond Distances (Å), Zn1···Zn2 Distance (Å), and Selective Angles (deg) of **2** ($R = p$-nitrophenyl) and **8**

	2 ($R = p$-nitrophenyl)	**8**
Zn1–N1	2.099(7)	2.115(4)
Zn1–N4	2.242(6)	2.208(4)
Zn1–N7	2.117(7)	2.116(4)
Zn1–O28	1.982(5)	1.962(3)
Zn1–O29	1.978(5)	2.024(4)
Zn2–N13	2.116(7)	2.105(3)
Zn2–N16	2.228(7)	2.227(3)
Zn2–N19	2.152(7)	2.095(4)
Zn2–O28	1.989(5)	1.964(3)
Zn2–O30	2.005(5)	2.034(3)
Zn1···Zn2	3.540(5)	3.500(3)
N1–Zn1–N7	110.8(3)	114.0(1)
N7–Zn1–O28	123.1(2)	122.4(1)
O28–Zn1–N1	118.8(2)	118.0(1)
N4–Zn1–O29	176.4(2)	176.3(1)
N13–Zn2–N19	121.9(3)	118.1(1)
N19–Zn2–O28	112.1(2)	115.5(1)
O28–Zn2–N13	118.8(2)	120.7(1)
N16–Zn2–O30	176.9(2)	178.2(2)
Zn1–O28–Zn2	126.1(2)	126.1(1)

The analogous 1:1 CH_3COO^--bound Zn_2L^{3+} complex **8** for X-ray analysis was prepared as [Zn_2L^{3+}–CH_3COO^-]-(ClO_4^-)$_2\cdot H_2O$ by slow crystallization from water. The crystal structure is shown in the ORTEP drawing with 50% probability thermal ellipsoids in Fig. 7. The selected bond distances and bond angles around zinc(II) are listed in Table 1. The zinc(II) coordination environment (i.e., a distorted trigonal-bipyramidal) is almost the same as that for *p*-nitrophenyl phosphate^{2-}-bound Zn_2L^{3+} complex. The apical Zn–O^- bond distances (2.02 and 2.03 Å) are longer than those for the equatorial Zn–O^- bonds (1.96 Å), which indicates that the interaction

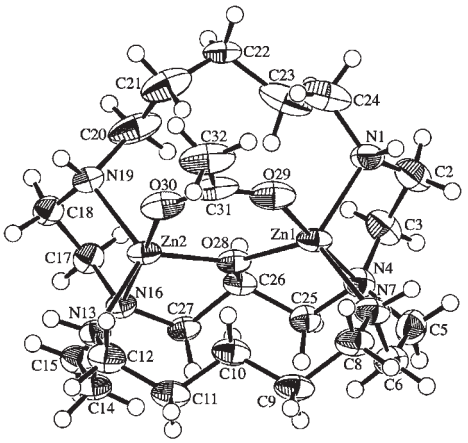


Fig. 7. Molecular structure of **8** with 50% probability ellipsoids. Perchlorate ions and water are omitted for clarity.

between the acetate and the zinc(II) ions is weaker than that for *p*-nitrophenyl phosphate. Each zinc(II) ion lies almost in each basal plane defined by N1, N7, and alkoxide O28, and N13, N19, and O28, where the total angles around the zinc(II) atoms are 354.4° (N1–Zn1–N7, N7–Zn1–O28, and O28–Zn1–N1) and 354.3° (N13–Zn1–N19, N19–Zn1–O28, and O28–Zn1–N13). The apical angles are almost linear at 176.3° for N4–Zn1–O29 and 178.2° for N16–Zn2–O30. The two zinc(II) ions are separated by a distance of 3.50 Å, which may be an appropriate distance to accept two oxygen atoms of the carboxylate anion.

Conclusion

The phosphate capture molecule, the alkoxide-bridged dinuclear zinc(II) hexaazacryptate (**1**) was characterized by potentiometric pH titration and X-ray crystal analysis. In the crystal of *p*-nitrophenyl phosphate complex with **1**, the two zinc(II) ions are almost equivalent and have distorted trigonal-bipyramidal structures; an alkoxide binds both zinc(II) ions as a shared equatorial donor and with phosphate oxygen anions as apical donors. In aqueous solution, the dizinc(II) complex **1** strongly binds to phenyl phosphate dianion ($K_d = 6 \times 10^{-7}$ M) under physiological condition. The anion selectivity indexes of phenyl phosphate dianion against CH_3COO^- and bis(phenyl) phosphate anion are 8×10^2 and $>2 \times 10^4$, respectively. The present new findings may serve to explain the key functions of dinuclear zinc(II) phosphatases, such as selective recognition of phosphate monoesters, inhibition by inorganic phosphate, or stabilization of the phosphoryl-transferred intermediates.[19] Suitable modification (e.g., binding with polymers or fluorophores) of the present phosphate capture molecule **1** may led to real and practical tools in phospho-proteomics.

Experimental

General Procedures. All reagents and solvents used were of the analytical quality and were used without further purification. All aqueous solutions were prepared using deionized and distilled water. An aqueous solution of 0.100 M NaOH for potentiometric pH titration was made by dilution of 10 M NaOH (Merck No. 6495) with decarbonated water and standardized with an aqueous solution of 0.100 M HCl. The 10 M NaOH solution is kept in a refrigerator below 5 °C, where Na_2CO_3 is less soluble (<1%), and is taken out before raising the solution temperature. An aqueous solution of 0.100 M $Zn(NO_3)_2$ was purchased from Kishida Chemical Co. and was used with pH adjustment at 6 prior to the potentiometric pH titration. IR spectra with KBr pellets were recorded on a Horiba FT-710 infrared spectrometer at 25 ± 2 °C. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra at 35.0 ± 0.1 °C were recorded on a JEOL LA500 spectrometer. Tetramethylsilane (in CD_3OD or d_6-DMSO) and sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4 (in D_2O) were used as internal references for ^{1}H and ^{13}C NMR measurements. Elemental analysis (CHN) was performed on a Perkin Elmer CHN Analyzer 2400. Thin-layer and column chromatographies were performed using Merck silica gel TLC plate (No. 5567) and Merck silica gel 60 (No. 5009), respectively.

MALDI-TOF mass spectra (positive reflector mode) were obtained on a Voyager RP-3 BioSpectrometry Workstation (PerSeptive Biosystems) equipped with a nitrogen laser (337 nm, 3 ns pulse). The accelerating voltage in the ion source was 20 kV. A matrix solution of 2,4,6-trihydroxyacetophenone (THAP, 20 mg/mL) in CH_3CN was used. Time-to-mass conversion was achieved by external calibrations using peaks for α-cyano-4-hydroxycinnamic acid (m/z 190.05 for $M + H^+$ and 212.03 for $M + Na^+$) and a peptide, Ac-Ile-Tyr-Gly-Glu-Phe-NH_2 (m/z 691.31 for $M + Na^+$).

Synthesis of 4,16-(2-Hydroxypropano)-1,4,7,13,16,19-hexaazacyclotetracosane, 6 (HL). Thionyl chloride (23 mL, 0.32 mol) was added dropwise to a dry EtOH solution (150 mL) of 1,3-diamino-2-propanol-*N*,*N*,*N*′,*N*′-tetraacetic acid **3** (25 g, 78 mmol) over 1 h at 0 °C. After the reaction mixture was heated at reflux for 12 h, the solvent was evaporated. The oily residue was dissolved in CH_2Cl_2 (100 mL) and then an aqueous solution (100 mL) of saturated Na_2CO_3 was added. The mixed solution was extracted with CH_2Cl_2 (200 mL × 4) and then combined organic layers were dried over anhydrous Na_2SO_4. After the solvent was evaporated, the yellowish oily product, 1,3-diamino-2-propanol-*N*,*N*,*N*′,*N*′-tetraacetic acid tetraethyl ester (**4**) was obtained in 73% yield (24.8 g): TLC (eluent: AcOEt/hexane = 1:1) R_f = 0.58. ^{1}H NMR (CD_3OD) δ 1.26 (12H, t, J = 7.1 Hz, $OCCH_3$), 2.64 (2H, dd, J = 8.5, 13.5 Hz, NCHCC), 2.89 (2H, dd, J = 3.4, 13.5 Hz, NCHCC), 3.55 (4H, s, NCHCOO), 3.61 (4H, s, NCHCOO), 3.74 (1H, m, CCHC), 4.16 (8H, q, J = 7.1 Hz, $COOCH_2$).

A solution of **4** (13 g, 30 mmol) and 1,5-diaminopentane (6.1 g, 60 mmol) in MeOH (2 L) was heated at reflux for 6 days. After evaporation of the solvent, the residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/MeOH/28% NH_{3aq} = 15:1:0.1) followed by crystallization from H_2O/CH_3CN to obtain 4,16-(2-hydroxypropano)-2,6,14,18-tetraoxo-1,4,7,13,16,19-hexaazacyclotetracosane (**5**·3H_2O, 4.1 g, 8.1 mmol, 27% yield). TLC (eluent: CH_2Cl_2/MeOH/28% NH_{3aq} = 5:1:0.2) R_f = 0.65. IR (KBr pellet): 3356, 3265, 3087, 2929, 2852, 1670, 1643, 1552, 1444, 1356, 1325, 1303, 1280, 1267, 1213, 1173, 1155, 1136, 1101, 1053, 966, 744, 586 cm^{-1}. ^{1}H NMR (CD_3OD) δ 1.38–1.50 (4H, m, CCCHCC), 1.50–1.64 (8H, m, CCHCCHC), 2.33 (2H, dd, J = 10.7, 13.5 Hz, NCHCCN), 2.68 (2H, dd, J = 2.3, 13.5 Hz, NCHCCN), 3.29 (4H, s, NCHCO), 3.44 (4H, s, NCHCO), 3.35 (8H, t, J = 4.9 Hz, CHCCCCH), 3.63 (1H, dd, J = 2.3, 10.7, NCCHCN). ^{13}C NMR (CD_3OD) δ 25.5, 30.3, 40.0, 61.6, 62.0, 67.6, 174.3. Anal. Found: C, 50.0; H, 8.8; N, 16.4%. Calcd for $C_{21}H_{44}N_6O_8$: C, 49.6; H, 8.7; N, 16.5%.

To a suspended solution of the tetraoxocryptand, **5**·3H_2O (4.5

g, 8.9 mmol) in dry THF (30 mL) was added slowly a THF solution of 1 M BH_3–THF (0.21 L) at 0 °C. The mixture was stirred at room temperature for 1 h and heated at 60 °C for 5 days. After decomposition of the various borane complexes with water at 0 °C, the solvent was evaporated. The residue was dissolved in 50 mL water and washed with CH_2Cl_2 (60 mL × 2). After purification by anion exchange column (Amberlite IRA-400) with water, the obtained acid-free residue was crystallized from H_2O at pH 5.0 (adjusted with 6 M HCl) to obtain 4,16-(2-hydroxypropano)-1,4,7,13,16,19-hexaazacyclotetracosane, **6**·4HCl·0.5H_2O as colorless needles in 42% yield (2.1 g). TLC (eluent: CH_2Cl_2/MeOH/28% NH_{3aq} = 2:2:1) R_f = 0.33. IR (KBr pellet): 3201, 2947, 2837, 2783, 2405, 1577, 1468, 1423, 1330, 1273, 1159, 1092, 1064, 1051, 843, 806, 756, 601, 580 cm^{-1}. ^1H NMR (D_2O) δ 1.48–1.72 (4H, m, CCCHCC), 1.76–1.92 (8H, m, CCHCCHC), 2.56 (2H, dd, J = 10.7, 14.8 Hz, NCHCCN), 2.65 (2H, dd, J = 2.4, 14.8 Hz, NCHCCN), 2.89 (4H, dt, J = 4.4, 14.7 Hz, NCCHN), 3.04 (4H, br, NCCHN), 3.17 (4H, br, NCHCN), 3.22 (8H, br, NCHCCCCHN), 3.33 (4H, br, NCHCN), 3.80 (1H, dd, J = 2.4, 10.7 Hz, NCCHCN). ^{13}C NMR (D_2O) δ 25.3, 26.7, 47.4, 49.2, 54.8, 59.5, 72.7. Anal. Found: C, 45.4; H, 9.5; N, 15.3%. Calcd for $C_{21}H_{51}N_6Cl_4O_{1.5}$: C, 45.6; H, 9.3; N, 15.2%.

Synthesis of OH^--Bound Dinuclear Zinc(II) Hexaazacryptate. The tetrahydrochloric acid salt **6**·4HCl·0.5H_2O (1.0 g, 1.8 mmol) was passed through an anion exchange column (Amberlite IRA-400) with water to obtain acid-free ligand **6** as a colorless oil. After dissolution of **6** in EtOH (30 mL), an aqueous solution (3.6 mL) of 1 M $Zn(ClO_4)_2$ was added. The solution pH was adjusted to 8.0 with an appropriate amount of 10 M NaOH. After the solvent was evaporated, the residue was crystallized from water to obtain OH^--bound cryptate **7**·$(ClO_4^-)_2$·3H_2O as colorless fine prisms in 55% yield (0.79 g). IR (KBr pellet): 3450, 3280, 3140, 2916, 2868, 2359, 1630, 1468, 1354, 1307, 1261, 1202, 1144, 1119, 1089, 1016, 989, 932, 879, 824, 668, 625, 590 cm^{-1}. ^1H NMR (d_6-DMSO) δ 0.95–2.10 (12H, m, NCCHCHCHCN), 2.30–3.10 (24H, br, NCHCHN and NCHCHCCCHN), 3.51 (2H, br, NCHCCHN), 3.77 (2H, br, NCHCCHN), 4.18–4.26 (1H, m, NCCHCN). ^{13}C NMR (d_6-DMSO) δ 22.2, 22.8, 25.6, 25.8, 40.6, 44.3, 46.6, 48.8, 49.4, 50.4, 57.1, 61.3. Anal. Found: C, 31.3; H, 6.7; N, 10.2%. Calcd for $C_{21}H_{52}N_6Cl_2O_{13}Zn_2$: C, 31.6; H, 6.6; N, 10.5%. The OH^- ligand and water in the crystal is replaced with an inorganic phosphate ($HOPO_3^{2-}$) in an aqueous solution of 10 mM NaH_2PO_4–NaOH (pH 6.9), which was shown by a MALDI-TOF MS signal of [$HOPO_3^{2-}$-bound Zn_2L^{3+}]$^+$ complex at m/z 621.2.

Synthesis of CH_3COO^--Bound Dinuclear Zinc(II) Hexaazacryptate. The tetrahydrochloric acid salt **6**·4HCl·0.5H_2O (1.0 g, 1.8 mmol) was passed through an anion exchange column (Amberlite IRA-400) with water to obtain acid-free ligand **6** as a colorless oil. After dissolution of **6** in EtOH (30 mL), an aqueous solution (3.6 mL) of 1 M $Zn(CH_3COO)_2$ was added. The solution pH was adjusted to 6.0 with an appropriate amount of 10 M NaOH. After addition of 1 M $NaClO_4$ (5.7 mL), CH_3COO^--bound cryptate **8**·$(ClO_4^-)_2$·H_2O was crystallized as colorless prisms in 90% yield (1.4 g). IR (KBr pellet): 3435, 3265, 3124, 2949, 2910, 2871, 2819, 1577, 1460, 1429, 1373, 1308, 1144, 1120, 1099, 1016, 991, 931, 881, 823, 681, 625, 544, 519 cm^{-1}. ^1H NMR (D_2O) δ 0.80–2.10 (15H, m, NCCHCHCHCN and CH_3COO^-), 2.38–3.12 (28H, m, NCH), 3.92–4.00 (1H, m, NCCHCN). ^{13}C NMR (D_2O) δ 24.4, 27.8, 28.0, 29.1, 44.9, 48.4, 51.1, 52.2, 52.4, 53.4, 60.3, 65.1. Anal. Found: C, 34.5; H, 6.4; N, 10.4%. Calcd for $C_{23}H_{50}N_6Cl_2O_{12}Zn_2$: C, 34.3; H, 6.3; N, 10.5%. The acetate ligand in the crystal is replaced with an inorganic phosphate ($HOPO_3^{2-}$) in an aqueous solution of 10 mM NaH_2PO_4–NaOH (pH 6.9), which was shown by a MALDI-TOF MS signal of [$HOPO_3^{2-}$–bound Zn_2L^{3+}]$^+$ complex at m/z 621.2.

Synthesis of $ROPO_3^{2-}$-Bound Dinuclear Zinc(II) Hexaazacryptates. An aqueous solution of 0.10 M $Zn(ClO_4)_2$ (2.8 mL) was added to an aqueous solution (3.6 mL) of **6**·4HCl·0.5H_2O (75 mg, 0.14 mmol). To the solution was added dropwise 5 equivalents of NaOH (0.10 M) at 45 °C for 1 h. The solution was filtered with a cellulose nitrate filter (0.45 μm) and then phenyl (or *p*-nitrophenyl) disodium phosphate (0.15 mmol) was added. After the solution was cooled to room temperature, aromatic phosphate-bound dinuclear zinc(II) cryptate was obtained in 72% yield for **2** (R = phenyl) ([Zn_2L^{3+}–*Ph*OPO_3^{2-}]·ClO_4^-·4H_2O, 85 mg) and 69% for **2** (R = *p*-nitrophenyl) ([Zn_2L^{3+}–(*p*-NO_2-*Ph*OPO_3^{2-})]·ClO_4^-·2H_2O, 82 mg). IR (KBr pellet) for **2** (R = phenyl): 3461, 3305, 3278, 3246, 3113, 2916, 2864, 2812, 1676, 1595, 1489, 1464, 1375, 1354, 1306, 1288, 1234, 1157, 1105, 1030, 997, 931, 877, 825, 762, 731, 692, 673, 625, 552 cm^{-1}. ^1H NMR (D_2O) for **2** (R = phenyl) δ 1.12–2.10 (12H, m, NCCHCHCHCN), 2.40–3.12 (28H, m, NCH), 3.97 (1H, t, J = 10.5 Hz, NCCHCN), 7.11 (1H, t, J = 7.4 Hz, *p*-ArH), 7.21 (2H, d, J = 8.3 Hz, *o*-ArH), 7.36 (2H, dd, J = 7.4, 8.3 Hz, *m*-ArH). Anal. Found: C, 37.5; H, 6.7; N, 9.7%. Calcd for $C_{27}H_{58}N_6ClO_{13}PZn_2$ for **2** (R = phenyl): C, 37.2; H, 6.7; N, 9.6%. MALDI-TOF MS for [Zn_2L^{3+}–*Ph*OPO_3^{2-}]$^+$: m/z 697.2. IR (KBr pellet) for **2** (R = *p*-nitrophenyl): 3500, 3404, 3305, 3253, 3109, 2922, 2864, 1603, 1589, 1512, 1493, 1464, 1340, 1265, 1173, 1111, 1030, 1001, 876, 825, 756, 733, 675, 650, 623, 567 cm^{-1}. ^1H NMR (DMSO-d_6) for **2** (R = *p*-nitrophenyl) δ 1.00–2.12 (12H, m, NCCHCHCHCN), 2.23–3.10 (28H, m, NCH), 3.62 (1H, br, NCCHCN), 7.34 (2H, d, J = 4.6 Hz, ArH), 8.11 (2H, d, J = 4.6 Hz, ArH). Anal. Found: C, 37.1; H, 6.1; N, 11.2%. Calcd for $C_{27}H_{53}N_7ClO_{13}PZn_2$ for **2** (R = *p*-nitrophenyl): C, 36.8; H, 6.1; N, 11.1%. MALDI-TOF MS for [Zn_2L^{3+}–(*p*-NO_2-*Ph*OPO_3^{2-})]$^+$: m/z 742.2.

Potentiometric pH Titrations. The electrode system (DKK Corporation Multi Channel Ion Meter IOL-40 with a Ross Combination pH Electrode 8102 BN and Hiranuma Auto Buret UCB-900) was calibrated as follows: An aqueous solution (50.0 mL) containing 4.00 mM HCl and 96 mM $NaNO_3$ (I = 0.10) was prepared under nitrogen atmosphere (>99.999% purity) at 35.0 ± 0.1 °C and then the first pH value (pH_1) was read. After 0.100 M NaOH (4.00 mL) was added to the acidic solution, the second pH value (pH_2) was read. The theoretical pH values corresponding to pH_1 and pH_2 are calculated to be $pH_1' = 2.483$ and $pH_2' = 11.128$, respectively, using $\log K_W$ ($= \log a_{H^+} \cdot a_{OH^-}$) $= -13.68$, $\log K'_W$ ($= \log[H^+][OH^-]$) $= -13.48$, and f_{H^+}($= a_{H^+}/H^+$) $= 0.823$).[24] The correct pH values ($pH = -\log a_{H^+}$) can be obtained using the following equations: $a = (pH_2' - pH_1')/(pH_2 - pH_1)$; $b = pH_2' - a \times pH_2$; $pH = a \times$ (pH-meter reading) + b.

The potentiometric pH titrations of 1.00 mM HL·4HCl were carried out in the presence or absence of 2.00 mM $Zn(NO_3)_2$ at 35.0 ± 0.1 °C with I = 0.10 ($NaNO_3$), where three independent titrations were performed. The four protonation constants ($K_n = [HL \cdot nH^+]/[HL \cdot (n-1)H^+]a_{H^+}$ M^{-1}) of HL, the zinc(II) complexation constant ($K_c = [Zn_2L^{3+}]a_{H^+}/[Zn^{2+}]^2[HL]$ M^{-1}), and a deprotonation constant of Zn_2L^{3+} (K_a = [HO^-–Zn_2L^{3+}]a_{H^+}/[Zn_2L^{3+}] M) were determined by means of the pH-titration program BEST.[25] The pH fit values (σ) defined in the

program are smaller than 0.01 for K_n and smaller than 0.05 for K_c and K_a. To determine dissociation constants K_d of anion complexes with Zn_2L^{3+} ($K_d = [Zn_2L^{3+}][anion]/[Zn_2L^{3+}\text{–anion}]$ M), similar pH titrations with 1.00 mM HL and 2.00 mM $Zn(NO_3)_2$ were carried out in the presence of an anion (i.e., 1.0 and 2.0 mM CH_3COONa and 1.0 mM phenyl disodium phosphate). The pH fit values (σ) for K_d values are smaller than 0.05. Relative species concentrations (%) at various pH values ($pH = -\log a_{H^+} = -\log[H^+] + 0.085$) were calculated using the program SPE.[25]

X-ray Crystallography. All measurements were made on a Rigaku RAXIS-RAPID imaging plate diffractometer with graphite monochromated Mo Kα radiation. The structure was solved by heavy-atom Patterson methods (PATTY) and expanded using Fourier techniques (DIRDIF94).[26] All calculations were performed using the teXsan crystallographic software packages (1985 and 1999) of Molecular Structure Corporation. Crystallographic data have been deposited with Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 252387 and CCDC 252388. Copies of the data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44 1223 336033; ordeposit@ccdc.cam.ac.uk).

Crystal data for $[Zn_2L^{3+}\text{–}CH_3COO^-](ClO_4^-)_2\cdot H_2O$: $C_{23}H_{50}Cl_2N_6O_{12}Zn_2$, $M = 804.35$, crystal dimensions $= 0.15 \times 0.10 \times 0.02$ mm, monoclinic, $a = 16.540(5)$, $b = 15.307(4)$, $c = 27.446(6)$ Å, $\beta = 104.37(3)^\circ$, $V = 6731(3)$ Å^3, $T = -130$ °C, space group $C2/c$ (No. 15), $Z = 8$, $D_{calcd} = 1.587$ g cm^{-3}, μ(Mo Kα) $= 16.50$ cm^{-1}, no. of reflections measured $= 36218$, no. of independent reflections $= 9808$ ($R_{int} = 0.047$), no. of observations $= 9808$ ($I > -3.00\sigma(I)$), $R = 0.110$ ($= \Sigma(F_o^2 - F_c^2)/\Sigma F_o^2$), $R_w = 0.181$ ($= [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{0.5}$), $R1 = 0.065$($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for $I > 2.0\sigma(I)$ data). Some non-hydrogen atoms were refined anisotropically, while the rest (some oxygen atoms of ClO_4^-) were refined isotropically. Hydrogen atoms were included but not refined.

Crystal data for $[Zn_2L^{3+}\text{–}(p\text{-}NO_2\text{-}PhOPO_3^{2-})](ClO_4^-)\cdot 2H_2O$: $C_{27}H_{53}ClN_7O_{13}PZn_2$, $M = 871.93$, crystal dimensions $= 0.15 \times 0.10 \times 0.05$ mm, orhorhombic, $a = 11.440(3)$, $b = 57.0789$, $c = 11.202(3)$ Å, $V = 7314(2)$ Å^3, $T = -180$ °C, space group $Pna2_1$ (No. 33), $Z = 8$, $D_{calcd} = 1.583$ g cm^{-3}, μ(Mo Kα) $= 14.98$ cm^{-1}, no. of reflections measured $= 84323$, no. of independent reflections $= 8925$ ($R_{int} = 0.068$), no. of observations $= 16616$ ($I > -3.00\sigma(I)$), $R = 0.130$ ($= \Sigma(F_o^2 - F_c^2)/\Sigma F_o^2$), $R_w = 0.154$($= [\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{0.5}$), $R1 = 0.083$ ($= \Sigma||F_o| - |F_c||/\Sigma|F_o|$ for $I > 2.0\sigma(I)$ data). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms, except those of waters, were included but not refined. Two crystallographically independent molecules exist in an asymmetric unit; they adopt almost the same conformation, as shown in Fig. 6.

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