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Recognition of phosphate monoester dianion by an alkoxide-bridged dinuclear zinc(II) complex

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Recognition of phosphate monoester dianion by an alkoxide-bridged dinuclear zinc(II) complex (Zn_2L^{3+}) has been studied (L = alkoxide species of 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-ol). Potentiometric pH titration study disclosed a 1 : 1 phenyl phosphate complexation with Zn_2L^{3+} in aqueous solution. The dissociation constant (= $[Zn_2L^{3+}][PhOPO_3^{2-}]/[Zn_2L^{3+}–PhOPO_3^{2-}]$) is an extremely small value of 2.5×10^{-8} mol dm^{-3} at 25 °C with $I = 0.10$ ($NaNO_3$). The X-ray crystal analysis of the dizinc(II) complex with *p*-nitrophenyl phosphate showed that the phosphate dianion binds as a bridging ligand to the two zinc(II) ions.

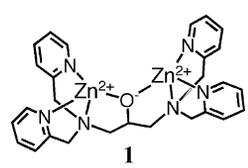
1 Introduction

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.[1] 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.[2] Newer, non-radioactive methods using poly- and monoclonal antibodies for the detection of phosphorylated residues (*i.e.*, phosphate monoester dianions) have been reported.[3–5] The determination of phosphorylation sites on proteins is a vital part of phospho-proteomics.[6]

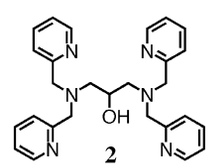
The chemical design of selective host molecules for various phosphate anions has attracted great interest. The majority of the hosts are organic molecules bearing acidic hydrogen atoms at complementary positions to formation of hydrogen bonds with phosphate anions. The dissociation constants (K_d = [host][phosphate]/[phosphate-bound host]) are in the range of 10^{-2} to 10^{-6} mol dm^{-3} in non-aqueous solvents such as chloroform and dimethyl sulfoxide.[7–10] These hydrogen bonds, however, can not compete against the hydration of phosphate anion 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 > micromolar concentrations), that work in aqueous solution, have been reported.[11–13]

Previously, we found that macrocyclic polyamine zinc(II) complexes are useful as a family of host molecules for phosphate monoester dianions ($K_d = 10^{-3}$ to 10^{-6} mol dm^{-3}) at physiological conditions.[13–15] 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.[16] 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.[17]

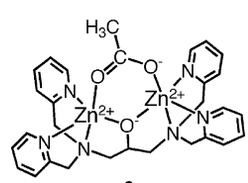
Recently, we have found that a dinuclear zinc(II) complex {1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-olato}dizinc(II), Zn_2L^{3+},[18] **1**: L = alkoxide species of 1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-ol **2**, HL) acts as a novel phosphate capture molecule at neutral pH. This finding was introduced 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.[19] The dinuclear zinc(II) complex (Zn–Zn distance = 3.6 Å) was reported as an CH_3COO^--bound species **3** (Zn_2L^{3+}–CH_3COO^-) by Fenton and co-workers,[18] in which each acetate oxygen atom binds to a zinc(II) at the fifth coordination site. Thus, the dinuclear zinc(II) complex has a vacancy on the two zinc(II) ions that is suitable for the access of a phosphate monoester dianion ($ROPO_3^{2-}$) as a bridging ligand (see structure of Zn_2L^{3+}–$ROPO_3^{2-}$, **4**). Herein we present the solution chemistry of **1** and the X-ray crystal structure of the 1 : 1 dizinc(II) complex with the *p*-nitrophenyl phosphate(2−) anion.



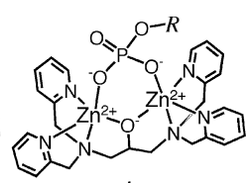
1
Zn_2L^{3+}



2
HL



3
Zn_2L^{3+}–CH_3COO^-



4
Zn_2L^{3+}–$ROPO_3^{2-}$

2 Results and discussion

2.1 Equilibria in aqueous solution

The potentiometric pH titration was performed with an aqueous solution of 1.00 mmol dm^{-3} ligand **2** ($HL·4HClO_4$) at 25 °C with I = 0.10 ($NaNO_3$). A typical pH titration curve for $HL·4HClO_4$ is shown in Fig. 1(a). The titration data were analyzed for four protonation equilibria (1). The four protonation constants ($\log K_{1–4}$) of 6.47 ± 0.02, 5.34 ± 0.02, 3.81 ±

$$HL·(n-1)H^+ + H^+ = HL·nH^+ \ (n = 1–4); \quad K_n = [HL·nH^+]/[HL·(n-1)H^+]a_{H^+} \quad (1)$$

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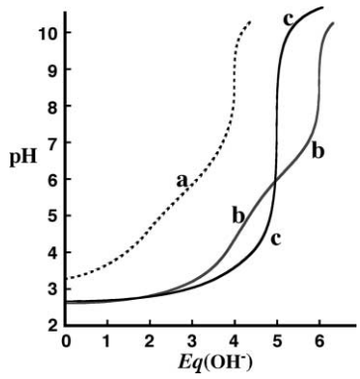
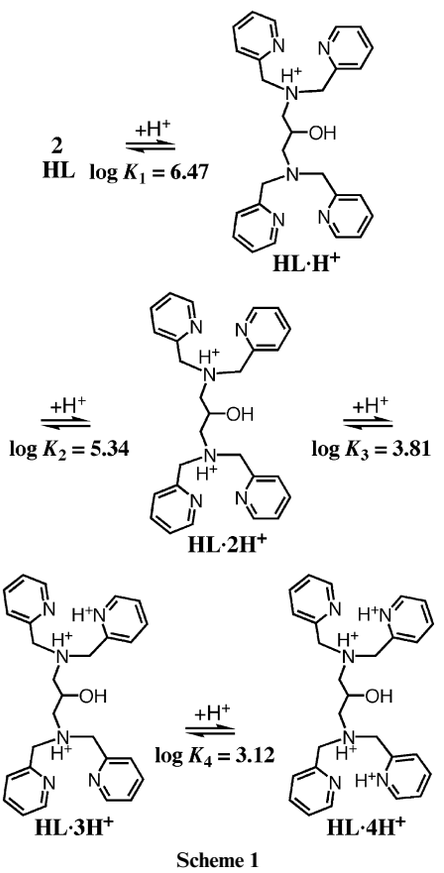


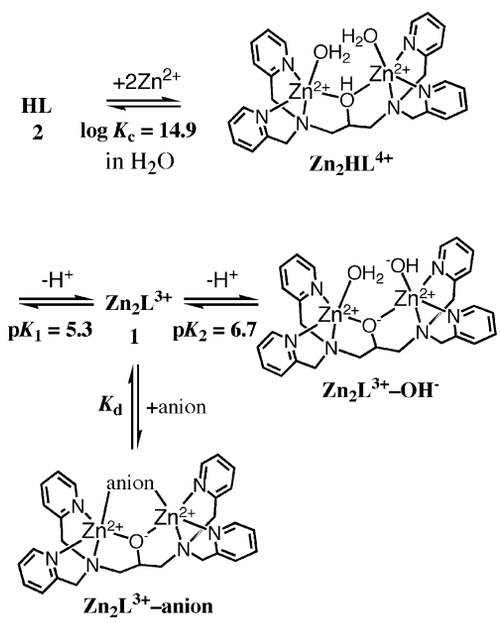
Fig. 1 Typical titration curves for HL·4HClO$_4$ (**2**·4HClO$_4$) (1.00 mmol dm^{-3}) at 25 °C with I = 0.10 (NaNO$_3$) in aqueous solution: (a) in the absence of zinc(II) ion; (b) in the presence of 2.00 mmol dm^{-3} Zn(NO$_3$)$_2$; (c) in the presence of 2.00 mmol dm^{-3} Zn(NO$_3$)$_2$ and 1.00 mmol dm^{-3} phenyl phosphate disodium salt. *eq*(OH$^-$) is the number of equivalents of base added.



Scheme 1

0.03, and 3.12 ± 0.05 are assigned according to Scheme 1. Further deprotonation (*e.g.*, an alkoxide (L) formation) was not observed below pH 11.

The potentiometric pH titration curve of HL·4HClO$_4$ (1.00 mmol dm^{-3}) in the presence of two equivalents of zinc(II) ion revealed the formation of stable zinc(II) complexes at pH > 4 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. 1(b)). Further deprotonation (*e.g.*, Zn_2L^{3+}–$(OH^-)_2$ formation) or precipitation of Zn(OH)$_2$ was not observed at *eq*(OH$^-$) > 6, indicating that the zinc(II) complex Zn_2L^{3+}–OH^- remains stable up to pH 11. ^{1}H NMR signals for Zn_2L^{3+} in D$_2$O solution at pD 10 were almost the same as shown those for the symmetric dizinc(II) complexes **3** and **4** (R = *p*-nitrophenyl), so that the proton exchange reaction between the Zn^{2+}–OH_2 and Zn(II)–OH^- in OH^-–bound Zn_2L^{3+} is sufficiently fast in aqueous solution. From the analysis of the pH titration data, the complex formation constant, log K_c of 14.9 ± 0.1 and deprotonation constants pK_1 of 5.3 ± 0.1 and pK_2 of 6.7 ± 0.1 were obtained (see equilibria (2)–(4)). The extremely small pK_1 value indicates the facile deprotonation of the alcohol involved in a double coordination with two zinc(II) ions. A similar alkoxide-bridged dizinc(II) complex with a hydroxyl-octaazacryptand was reported,[17] with a pK_a value of the alcoholic OH of 4.0 at 25 °C. It should be noted that only the dinuclear species Zn_2HL^{4+}, Zn_2L^{3+} and Zn_2L^{3+}–OH^- (see Scheme 2), was confirmed under the experimental conditions employed. Therefore, as one zinc(II) ion binds to the ligand HL, the second zinc(II) ion simultaneously enters in the 1 : 1 Zn^{2+}–HL·nH$^+$ species. The structural assignment for the alkoxide O^--bridged dinuclear zinc(II) complex comes from the X-ray crystal structure analyses of the CH_3COO^--bound complex **3**[18] and the *p*-nitrophenyl phosphate(2−)-bound complex presented below. A typical diagram for species distribution as a function of pH at [total zinc(II)] = 2 mmol dm^{-3} and [total ligand] = 1 mmol dm^{-3} is displayed in Fig. 2.



Scheme 2

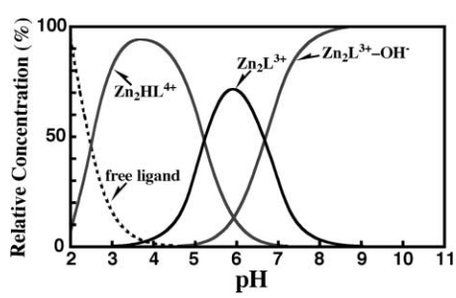


Fig. 2 Distribution diagram for **2** (1 mmol dm^{-3}) in the presence of zinc(II) ion (2 mmol dm^{-3}) as a function of pH ($-\log a_{H^+}$) in aqueous solution at 25 °C with I = 0.10. Free ligand is a total of metal-free ligand species (HL·nH$^+$).

$$HL + 2Zn^{2+} = Zn_2HL^{4+};\ K_c = [Zn_2HL^{4+}]/[HL][Zn^{2+}]^2 \quad (2)$$

$$Zn_2HL^{4+} = Zn_2L^{3+} + H^+;\ K_1 = [Zn_2L^{3+}]a_{H^+}/[Zn_2HL^{4+}] \quad (3)$$

$$Zn_2L^{3+} = Zn_2L^{3+}\text{–}OH^- + H^+;\ K_2 = [Zn_2L^{3+}\text{–}OH^-]a_{H^+}/[Zn_2L^{3+}] \quad (4)$$

Dissociation constants of the 1 : 1 anion-bound dizinc(II) complexes were determined by the potentiometric pH titrations in the presence of various anions (*i.e.*, Cl^-, SO_4^{2-}, CH_3COO^-, *p*-nitrophenyl phosphate(2−), phenyl phos-

phate(2−), and bis(phenyl) phosphate(−)) at 25 °C with I = 0.10 ($NaNO_3$). A typical titration curve for 1.00 mmol dm^{-3} phenyl phosphate in the presence of $HL{\cdot}4HClO_4$ (1.00 mmol dm^{-3}) and zinc(II) ion (2.00 mmol dm^{-3}) is presented in Fig. 1(c). From the comparison of the titration curves Fig. 1(b) and (c), a stable dizinc(II) complex with phenyl phosphate(2−) (Zn_2L^{3+}–phenyl phosphate(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 as shown in Scheme 2 and the dissociation constants, K_d (see eqn. (5)) of the anion-bound complexes as follows: (2.0 ± 0.4) ×

$$Zn_2L^{3+}\text{–anion} = Zn_2L^{3+} + \text{anion};$$
$$K_d = [Zn_2L^{3+}][\text{anion}]/[Zn_2L^{3+}\text{–anion}] \quad (5)$$

10^{-2} mol dm^{-3} for Cl^-, (4.0 ± 0.8) × 10^{-4} mol dm^{-3} for CH_3COO^-, (1.3 ± 0.2) × 10^{-4} mol dm^{-3} for SO_4^{2-}, (5.0 ± 0.3) × 10^{-8} mol dm^{-3} for *p*-nitrophenyl phosphate(2−), (2.5 ± 0.5) × 10^{-8} mol dm^{-3} for phenyl phosphate(2−), >5.0 × 10^{-2} mol dm^{-3} for bis(phenyl) phosphate(−). The dissociation constant of OH^--bound complex, Zn_2L^{3+}–OH^- is calculated to be 6.3 × 10^{-8} mol dm^{-3} from the pK_2 value. Thus, the phenyl phosphate(2−) is the most favorable ligand for Zn_2L^{3+}. A typical diagram for species distribution as a function of pH at [total zinc(II)] = 2 mmol dm^{-3}, [total ligand] = 1 mmol dm^{-3} and [phenyl phosphate] = 1 mmol dm^{-3} is displayed in Fig. 3. As expected by the previous MALDI-TOF MS study of phosphate(2−)-bound Zn_2L^{3+},[19] we see very little dissociation (<1%) of Zn_2L^{3+}–phenyl phosphate(2−) when **1** and phenyl phosphate(2−) anion (both at 1 mmol dm^{-3}) 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 the *p*-nitrophenyl phosphate(2−)-bound complex presented below.

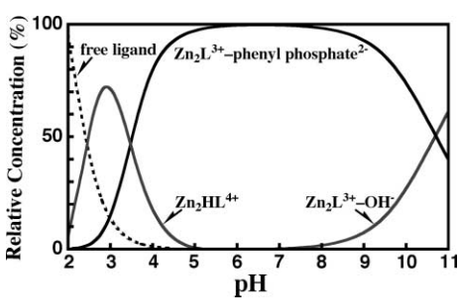


Fig. 3 Distribution diagram for **2** (1 mmol dm^{-3}) in the presence of zinc(II) ion (2 mmol dm^{-3}) and phenyl phosphate(2−) (1 mmol dm^{-3}) as a function of pH ($-\log a_{H^+}$) in aqueous solution at 25 °C with I = 0.10. Free ligand is a total of metal-free ligand species ($HL{\cdot}nH^+$). Relative concentration of **1** (ZnL^{3+}) is below 1%.

2.2 Structure of the *p*-nitrophenyl phosphate(2−)-bound Zn_2L^{3+} complex

In an attempt to isolate a phosphate(2−)-bound Zn_2L^{3+} (**4**), 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+}–acetate or 2 : 1 mixture of Zn^{2+} and HL) in aqueous solution at pH *ca.* 7. Among the phosphate monoester ligands, *p*-nitrophenyl phosphate gave fine yellowish crystals of the 1 : 1 anion complex in 80% yield. The elemental analysis, NMR, and IR data of the product suggested the formula [Zn_2L^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O. A yellow crystal of [Zn_2L^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O·2EtOH of size 0.20 × 0.10 × 0.05 mm suitable for X-ray diffraction studies was obtained by slow recrystallization from EtOH. 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. 4 together with selected bond distances and bond angles around zinc(II).

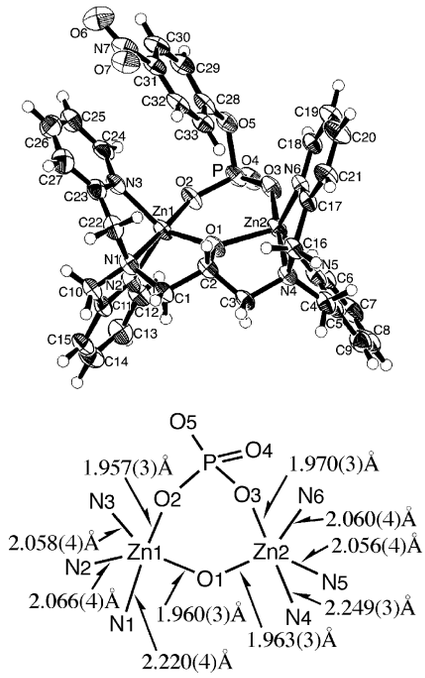


Fig. 4 Crystal structure of *p*-nitrophenyl phosphate(2−)-bound dizinc(II) complex, [ZnL^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O·2EtOH. Perchlorate ion, water and two ethanol molecules are omitted for clarity. Selected angles (°): O1–Zn1–O2, 99.0(1); O1–Zn1–N1, 81.4(1); O1–Zn1–N2, 116.2(1); O1–Zn1–N3, 125.5(1); O2–Zn1–N1, 177.9(2); O2–Zn1–N2, 102.4(1); O2–Zn1–N3, 100.6(1); N1–Zn1–N2, 79.2(1); N1–Zn1–N3, 77.6(1); N2–Zn1–N3, 108.3(2); O1–Zn2–O3, 100.1(1); O1–Zn2–N4, 82.8(1); O1–Zn2–N5, 113.8(2); O1–Zn2–N6, 114.8(1); O3–Zn2–N4, 177.2(1); O3–Zn2–N5, 99.5(1); O3–Zn2–N6, 99.7(1); N4–Zn2–N5, 78.9(1); N4–Zn2–N6, 79.3(1); N5–Zn2–N6, 122.9(1); O2–P–O3, 114.2(2); Zn1–O1–Zn2, 129.8(1).

In the *p*-nitrophenyl phosphate(2−)-bound Zn_2L^{3+} complex, both zinc(II) ions are almost equivalent in a distorted trigonal-bipyramidal environment: Zn1 (or Zn2) is coordinated by the two pyridyl amines N2 (or N5) and N3 (or N6) and an alkoxide O^- anion (O1) as equatorial donors and the ternary amine N1 (or N4) and one of the phosphate anionic oxygens O2 (or O3) as apical donors. The apical Zn–O^- bond distances (1.96 and 1.97 Å) are as short as those for the equatorial Zn–O^- bonds (1.96 Å). The coordination bond distance between phosphate O^- and zinc(II) (1.96 and 1.97 Å) are shorter than those of Zn–O^-(acetate) in the analogous acetate-bound complex **3** (1.99 and 2.00 Å),[18] which indicates the stronger interaction between the phosphate and the zinc(II) ions. Each zinc(II) ion lies almost in each basal plane defined by N2, N3 and alkoxide O1, and N5, N6 and O1, where the total angles around the zinc(II) atoms are 350.0° (O1–Zn1–N2, N3–Zn1–N2 and N3–Zn1–O1) and 351.5° (O1–Zn2–N5, N5–Zn2–N6 and N6–Zn2–O1). The apical angles are almost linear at 177.9° for N1–Zn1–O2 and 177.2° for N4–Zn2–O3. The two zinc(II) ions are separated by a distance of 3.55 Å, which is an appropriate distance to accept two oxygen atoms of the phosphate ester as part of the bridging ligand.[17]

3 Conclusions

The phosphate capture molecule, **1** was characterized by potentiometric pH titration and X-ray crystal analysis. In the crystal of the *p*-nitrophenyl phosphate complex with **1**, both 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 phosphate oxygen anions bind as

apical donors. In aqueous solution, the dizinc(II) complex **1** strongly binds to phenyl phosphate(2−) ($K_d = 2.5 \times 10^{-8}$ mol dm^{-3}) under physiological conditions. The anion selectivity indexes of phenyl phosphate(2−) against SO_4^{2-}, CH_3COO^-, Cl^- and bis(phenyl) phosphate(−) are 5.2×10^3, 1.6×10^4, 8.0×10^5, and $>2 \times 10^6$, respectively. The present new findings may serve to rationalize the essence of dinuclear zinc(II) phosphatases such as selective recognition of phosphate monoesters, inhibition by inorganic phosphate, or stabilization of the phosphoryl-transferred intermediates.[16] 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.

4 Experimental

4.1 General

All reagents and solvents used were of analytical quality and used without further purification. All aqueous solutions were prepared using deionized and distilled water. An aqueous solution of 0.100 mol dm^{-3} NaOH for potentiometric pH titration was made by dilution of 10.0 mol dm^{-3} NaOH (Merck No. 6495) with decarbonated water. The 10.0 mol dm^{-3} NaOH solution is kept in a refrigerator below 5 °C, where Na_2CO_3 is less soluble (<1%), and taken out before raising the solution temperature. An aqueous solution of 0.100 mol dm^{-3} $Zn(NO_3)_2$ was purchased from Kishida Chemical Co. and used with pH adjustment at 6. IR spectra with KBr pellets were recorded on a Horiba FT-710 infrared spectrometer at 25 ± 2 °C. UV spectra were obtained with a Hitachi U-3500 spectrometer at 25.0 ± 0.1 °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 DMSO-d_6) and 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (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^{-1}) 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$^+$).

4.2 Ligand synthesis

The ligand **2** (HL) was synthesized by a literature method.[20,21] The oily product was purified by silica gel column chromatography (eluent; CH_2Cl_2–CH_3OH–28% aqueous NH_3 = 10 : 1 : 0 to 15 : 1 : 0.1) to obtain **2** as a yellowish oil in 87% yield: TLC (eluent; CH_2Cl_2–MeOH–28% aqueous NH_3 = 10 : 1 : 0.1) $R_f = 0.35$. Aqueous 60% $HClO_4$ (2.7 ml) was added dropwise to an aqueous solution (10 ml) of HL (3.3 g, 7.3 mmol) at 60 °C. After cooling the solution to room temperature, 1,3-bis[bis-(pyridin-2-ylmethyl)amino]propan-2-ol tetrahydroperchloric acid salt (HL·4$HClO_4$·2.5H_2O, pale yellow crystals) was obtained in 78% yield (5.1 g, 5.7 mmol). IR (cm^{-1}): 3407, 1621, 1531, 1466, 1146, 1114, 1087, 768, 630. ^{1}H NMR (D_2O): δ 2.66 (2H, dd, J = 14.2 and 9.4 Hz, NCHC), 2.87 (2H, dd, J = 14.2 and 2.5 Hz, NCHC), 4.13 (1H, tt, J = 9.4 and 2.5 Hz, CHO), 4.29 (4H, d, J = 16.5 Hz, NC*H*Py), 4.38 (4H, d, J = 16.5 Hz, NC*H*Py), 7.94 (4H, dd, J = 6.0 and 8.0 Hz, 5-PyH), 8.02 (4H, d, J = 8.0 Hz, 3-PyH), 8.50 (4H, dd, J = 8.0 and 8.0 Hz, 4-PyH), 8.70 (4H, d, J = 6.0 Hz, 6-PyH). ^{13}C NMR (D_2O): δ 58.9, 61.5, 69.5, 129.1, 129.8, 144.4, 149.9, 155.6. Found: C, 35.7; H, 4.5; N, 9.3%. Calc. for $C_{27}H_{39}Cl_4N_6O_{19.5}$: C, 36.0; H, 4.4; N, 9.3%. UV absorption of 50 μmol dm^{-3} **2** in an aqueous solution of 25 mmol dm^{-3} NaH_2PO_4–NaOH (pH 6.8): λ_{max}/nm 261 (ε/dm^3 mol^{-1} cm^{-1} 1.4×10^4).

4.3 Synthesis of dizinc(II) complexes

4.3.1 [Zn_2L^{3+}–CH_3COO^-](ClO_4)$_2$·H_2O. The CH_3COO^--bound {1,3-bis[bis(pyridin-2-ylmethyl)amino]propan-2-olato}-dizinc(II) complex **3** (Zn_2L^{3+}–CH_3COO^-) was prepared as its diperchlorate salt monohydrate by a literature method.[18] IR (cm^{-1}): 3420, 1609, 1577, 1556, 1486, 1440, 1143, 1121, 1090, 1045, 1027, 770, 626. ^{1}H NMR (DMSO-d_6): δ 2.04 (2H, dd, J = 12.2 and 10.0 Hz, NCHC), 2.53 (3H, s, CH_3), 3.07 (2H, dd, J = 12.2 and 2.4 Hz, NCHC), 3.74 (1H, tt, J = 10.0 and 2.4 Hz, CHO^-), 4.02 (2H, d, J = 17.0 Hz, NC*H*Py), 4.23–4.35 (6H, m, NC*H*Py), 7.53–7.65 (8H, m, PyH), 8.05–8.12 (4H, m, PyH), 8.58 (4H, dd, J = 15.9 and 4.7 Hz, PyH). ^{13}C NMR (DMSO-d_6): δ 24.3, 55.7, 58.5, 59.5, 62.3, 124.5, 124.7, 124.78, 124.81, 141.0, 141.1, 147.2, 147.4, 155.6, 156.3, 181.3. Found: C, 40.4; H, 3.9; N, 9.9%. Calc. for $C_{29}H_{34}Cl_2N_6O_{12}Zn_2$: C, 40.5; H, 4.0; N, 9.8%. The water molecule in the crystal, which was identified by elemental analysis and ^{1}H NMR, is easily removed at 50 °C and 1 mmHg pressure for 5 h (as gradual color change to white). The acetate ligand in the crystal is replaced with an inorganic phosphate ($HOPO_3^{2-}$) in an aqueous solution of 10 mmol dm^{-3} 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* 677.1.[19] UV absorption of 50 μmol dm^{-3} **3** in an aqueous solution of 25 mmol dm^{-3} NaH_2PO_4–NaOH (pH 6.8): λ_{max}/nm 261 (ε/dm^3 mol^{-1} cm^{-1} 1.4×10^4).

4.3.2 [Zn_2L^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O. An aqueous solution (10 ml) of HL·4$HClO_4$·2.5H_2O (90 mg, 0.10 mmol) was added to $Zn(ClO_4)_2$·6H_2O (81 mg, 0.22 mmol). An aqueous solution (5.0 ml) of 0.10 mol dm^{-3} NaOH was added to the reaction mixture and then the solution was stirred for 1 h at 45 °C. The solution was filtered with a cellulose nitrate filter (0.45 μm) and then an aqueous solution (1.0 ml) of 0.10 mol dm^{-3} *p*-nitrophenyl phosphate disodium salt was added dropwise to the filtrate. After cooling the solution to room temperature, *p*-nitrophenyl phosphate(2−)-bound {1,3-bis-[bis(pyridin-2-ylmethyl)amino]propan-2-olato}dizinc(II) complex as its perchlorate salt monohydrate, [Zn_2L^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O was obtained as fine yellowish crystals in 80% yield (74 mg). IR (cm^{-1}): 3420, 1608, 1590, 1490, 1440, 1264, 1146, 1114, 1089, 1024, 1004, 886, 770, 735, 649. ^{1}H NMR (DMSO-d_6): δ 1.98 (2H, dd, J = 10.8 and 12.1 Hz, NCHC), 3.00 (2H, dd, J = 2.2 and 12.1 Hz, NCHC), 3.91 (2H, d, J = 17.2 Hz, NC*H*Py), 3.98 (1H, tt, J = 2.2 and 10.8 Hz, CHO^-), 4.21–4.32 (6H, m, NC*H*Py), 7.46–7.61 (10H, m, PyH and PhH), 8.00–8.06 (4H, m, PyH), 8.19 (2H, d, J = 9.2 Hz, PhH), 8.60 (2H, d, J = 5.0 Hz, PyH), 9.11 (2H, d, J = 4.8 Hz, PyH). ^{13}C NMR (DMSO-d_6): δ 55.0, 58.2, 59.2, 63.0, 119.88, 119.93, 124.4, 124.45, 125.49, 125.2, 140.8, 140.9, 141.0, 147.9, 148.3, 155.4, 156.4. Found: C, 43.1; H, 4.1; N, 10.3%. Calc. for $C_{33}H_{35}ClN_7O_{12}PZn_2$: C, 43.1; H, 3.8; N, 10.7%. MALDI-TOF MS: *m*/*z* 798.1. The same crystals were obtained from a 1 : 1 mixture of **3**(ClO_4)$_2$·H_2O and *p*-nitrophenyl phosphate disodium salt in aqueous solution in an almost quantitative yield. Crystals suitable for X-ray analysis was prepared as [Zn_2L^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O·2EtOH from ethanol. An ethanol molecule in the crystal is easily removed in the air (color change to yellowish white). Found: C, 43.5; H, 4.7; N, 9.7%. Calc. for $C_{36}H_{44}ClN_7O_{13.5}PZn_2$ ([Zn_2L^{3+}–*p*-nitrophenyl phosphate(2−)](ClO_4)·H_2O·1.5EtOH): C, 43.8; H, 4.5; N, 9.9%.

4.4 Potentiometric pH titrations

The electrode system (DKK Corporation Multi Channel Ion Meter IOL-40 with a Ross Combination pH Electrode 8102 BN) was calibrated as follows: an aqueous solution (50.0 ml) containing 4.00 mmol dm^{-3} HCl and 96 mmol dm^{-3} $NaNO_3$ (I = 0.10) was prepared under nitrogen atmosphere (>99.999% purity) at 25.0 ± 0.1 °C and then the first pH value (pH_1) was read. After 0.100 mol dm^{-3} 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 $pH_1' = 2.481$ and $pH_2' = 11.447$, respectively, using $\log K_w$ (= $\log a_{H^+} + \log a_{OH^-}$) = −14.00, $\log K'_w$ (= $\log[H^+][OH^-]$) = −13.79, and f_{H^+} (= a_{H^+}/H^+) = 0.825). The correct pH values ($pH = -\log a_{H^+}$) can be obtained using the following equations: $a = (pH_2' - pH_1')/(pH_2 - pH_1)$; $b = pH_2' - a \cdot pH_2$; pH = $a \times$ (pH-meter reading) + b.

The potentiometric pH titrations of 1.00 mmol dm^{-3} HL·$4HClO_4$ were carried out in the presence or absence of 2.00 mmol dm^{-3} $Zn(NO_3)_2$ at 25.0 ± 0.1 °C with I = 0.10 ($NaNO_3$), where three independent titrations were performed. The four protonation constants (K_n = $[HL \cdot nH^+]/[HL \cdot (n-1)H^+]a_{H^+}$ mol^{-1} dm^3) of HL, the zinc(II) complexation constant (K_c = $[Zn_2HL^{4+}]/[Zn^{2+}]^2[HL]$ mol^{-2} dm^6), and two deprotonation constants of Zn_2HL^{4+} (K_1 = $[Zn_2L^{3+}]a_{H^+}/[Zn_2HL^{4+}]$ mol dm^{-3} and K_2 = $[Zn_2L^{3+}\text{–}OH^-]a_{H^+}/[Zn_2L^{3+}]$ mol dm^{-3}) were determined by means of the pH-titration program BEST.[22] The pH fit values (σ) defined in the program are smaller than 0.01 for K_n and 0.05 for K_c, K_1 and K_2. Under the same conditions, the protonation constants $\log K$ (K = $[H^+\text{–anion}]/[anion]a_{H^+}$ mol^{-1} dm^3) for CH_3COO^- (2.0 mmol dm^{-3}, 50 ml), p-nitrophenyl phosphate(2−) (1.0 mmol dm^{-3}, 50 ml) and phenyl phosphate(2−) (1.0 mmol dm^{-3}, 50 ml) were determined to be 4.64 ± 0.03, 5.19 ± 0.03, and 5.90 ± 0.02, respectively. To determine dissociation constants K_d of anion complexes with Zn_2L^{3+} (K_d = $[Zn_2L^{3+}][anion]/[Zn_2L^{3+}\text{–anion}]$ mol dm^{-3}), similar pH titrations with 1.00 mmol dm^{-3} HL and 2.00 mmol dm^{-3} $Zn(NO_3)_2$ were carried out in the presence of an anion (*i.e.*, 1.0 and 2.0 mmol dm^{-3} CH_3COONa, 1.0 and 2.0 mmol dm^{-3} Na_2SO_4, 25 and 50 mmol dm^{-3} NaCl, 1.0 mmol dm^{-3} p-nitrophenyl phosphate disodium salt, 1.0 mmol dm^{-3} phenyl phosphate disodium salt, and 10 mmol dm^{-3} bis(phenyl) phosphate sodium salt). The pH fit values (σ) for K_d values are smaller than 0.05. Relative species concentrations (%) at various pH values ($pH = -\log a_{H^+} = -\log[H^+] + 0.084$) were calculated using the program SPE.[22]

4.5 Crystal structure determination

Crystal data for [Zn_2L^{3+}–p-nitrophenyl phosphate(2−)](ClO_4)·H_2O·2EtOH: $C_{37}H_{47}ClN_7O_{14}PZn_2$, M = 1011.0, monoclinic, space group $C2/c$ (no. 15), a = 25.240(2), b = 16.441(1), c = 21.033(2) Å, β = 102.282(3)°, V = 8528(1) Å^3, T = 93(1) K, Z = 8, D_c = 1.575 g cm^{-3}, μ(Mo-Kα) = 13.0 cm^{-1}, 32195 reflections measured, 7932 independent reflections (R_{int} = 0.039), no. of observations ($I > 3.00\sigma(I)$) = 7931, R = 0.092 (= $\Sigma(F_o^2 - F_c^2)/\Sigma F_o^2$), R_w = 0.213 (= $[\Sigma w(F_o^2 - F_c^2)^2/\Sigma w(F_o^2)^2]^{0.5}$), $R1$ = 0.065 (= $\Sigma||F_o| - |F_c||/\Sigma|F_o|$ for $I > 2.0\sigma(I)$ data). Measurements were made on a Rigaku RAXIS-RAPID imaging plate diffractometer. The structure was solved by direct methods (SIR97) and expanded using Fourier techniques (DIRDIF94). An ethanol molecule and perchlorate are disordered at two locations. The non-hydrogen atoms, excluding those of ClO_4^- and two carbons of an EtOH, were refined anisotropically, while the remainder were refined isotropically. Hydrogen atoms, excluding those of two EtOH and H_2O, were included but not refined.

CCDC reference numbers 228304.

See http://www.rsc.org/suppdata/dt/b4/b400269e/ for crystallographic data in CIF or other electronic format.

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