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Ligand modifications modulate the mechanism of binuclear phosphatase biomimetics

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ABSTRACT

Complexation of dimethyl-6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((2-hydroxyethyl) azanediyl)bis(methylene)dipicolinate (Me_2H_3**L4**) and 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(meth ylene)bis(((6-methylpyridin-2-yl)methyl)azanediyl)diethanol (H_3**L5**) with Zn(II) afforded the complexes $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)$ and $[Zn_2(H_2$**L5**$)(CH_3CO_2)(H_2O)](PF_6)_2{\cdot}2H_2O$, which were characterized by ^1H and ^{13}C NMR spectroscopy, mass spectrometry, microanalysis, and the former by X-ray crystallography. Functional studies of the zinc complexes with the substrate bis(2,4-dinitrophenyl)phosphate (BDNPP) showed the complexes to be competent catalysts with $k_{cat} = 3.52 \pm 0.03 \times 10^{-4}$ and $1.27 \pm 0.04 \times 10^{-3}$ s^{-1} ($K_m = 6.7 \pm 0.9$; 13.8 ± 1.5 mM), with catalytically relevant pK_as of 9.4 and 6.6, respectively. The pK_a values are discussed with respect to the potential nucleophilic species and the effect of the donor environment.

1. Introduction

Organophosphorous compounds (OPs) are commonly used as pesticides [1,2] and nerve agents [1,3,4], and act by inhibiting acetylcholinesterase, thus preventing proper functioning of nerve cells. OP-hydrolyzing enzymes [3–5] are thought to natively contain one or two Zn(II) ions in the active site, although other combinations have been suggested [6,7]. The active site of the metalloenzyme is typically composed of a nitrogen/oxygen coordination environment with metal ions coordinated by nitrogen donors located on histidine ligands, oxygen donors typically from an aspartate, and often including a metal ion-bridging hydroxide ion [8–12]. Our interest is in model systems capable of reproducing the electronic, structural and reactivity characteristics of OP-hydrolyzing metalloenzyme systems [13–16]. Appropriately placed substituents, such as hydrogen bonding groups (e.g. amines), have been shown to have pronounced effects on reactivity in model systems [17,18], and these also mimic the second coordination sphere effects seen in metalloenzyme systems [19–21]. In this work we have explored the role of a carboxylate substituent using the zinc(II) complex of the ligand 6,6′-((((2-hydroxy-5-methyl-1,3-phenylene)bis(methylene))bis((2-hydroxyethyl)azanediyl))bis(methylene))dipicolinic acid (H_5**L4**) ($[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4){\cdot}3H_2O$) and a methyl substituent, with 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene) bis(((6-methylpyridin-2-yl)methyl)azanediyl)diethanol (H_3**L5**) and $[Zn_2(H_2$**L5**$)(CH_3CO_2)(H_2O)](PF_6)_2{\cdot}2H_2O$. The catalytic potential of both complexes has been explored using the phosphodiester substrate bis(2,4-dinitrophenyl)phosphate (BDNPP).

2. Experimental

2.1. Materials and physical methods

Nuclear Magnetic Resonance (NMR) spectra were measured with Bruker AV300, AV400 and AV500 instruments. The spectra were recorded in $CDCl_3$, CD_3OD or $(CD_3)_2SO$. Chemical shifts were determined in ppm, relative to known residual solvent peak references. Coupling constants are given in Hz. Two-dimensional correlation spectroscopy (COSY), heterobinuclear single quantum correlation (HSQC), and heterobinuclear multiple bond connectivity (HMBC) experiments were used to assign each signal in the spectra of the final ligands. Low resolution mass spectral (LRMS) data were collected with a Bruker Esquire high capacity ion trap electrospray ionization mass spectrometer (HCT ESI-MS), in methanol (MeOH), acetonitrile (MeCN) or 50:50 MeCN:water, with a Bruker ES source. High resolution mass spectra were obtained using a Bruker microTOFQ ESI-MS in MeOH. The predicted isotopic

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splitting patterns of peaks were calculated using the program Molecular Weight Calculator [22]. UV–Vis absorption spectra were measured with a Hitachi U3000 Spectrophotometer with 10 mm glass or quartz cuvettes. FT-Infrared Spectroscopy was carried out with a Perkin Elmer FT-IR Spectrometer SPECTRUM 2000, with a Smiths DuraSamplIR II ATR diamond window. Silica gel 60 from Merck 0.040–0.063 mm, 230–400 mesh, was employed for column chromatography. Elemental analyses were performed using the microanalysis facilities at The University of Queensland.

2.2. Syntheses of ligands

Caution: *some of the compounds prepared below are perchlorate salts of organic-metal complexes and are potentially explosive. Even small amounts of material should be handled with caution.*

2,6-Bis(chloromethyl)-4-methylphenol, [23] 2-(pyridyl-2-ylmethylamino)ethanol [24], 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((pyridin-2-ylmethyl)azanediyl)diethanol (CH_3H_3**L1**) and the corresponding dizinc(II) complex [25] were prepared as described previously.

2.2.1. Synthesis of methyl-6-(hydroxymethyl)picolinate

This compound was synthesized after a published procedure [26]. Dimethyl pyridine-2,6-dicarboxylate (2.00 g, 10.2 mmol) was dissolved in a 7:3 mixture of dry methanol/chloroform (100 mL), then sodium borohydride (390 mg, 10.3 mmol) was added in small portions at 0 °C. After stirring for 3 h at room temperature, the solution was neutralized with saturated ammonium chloride solution, concentrated to 30 mL *in vacuo*, and extracted with dichloromethane (3 × 50 mL). The organic layers were combined, dried over sodium sulfate, and the solvent was removed *in vacuo*. The resulting oil was purified using column chromatography (hexane/ethyl acetate 1:1) to yield 950 mg (55.9%) of a colorless oil which crystallized upon standing. ^{1}H NMR ($CDCl_3$, 500.13 MHz); δ 3.98 (s, 3H, OC*H*$_3$); 4.85 (s, 2H, C*H*$_2$OH); 7.52 (d, 1H, py*H*, J = 7.8 Hz); 7.85 (t, 1H, py*H*, J = 7.8 Hz); 8.02 (d, 1H, py*H*, J = 7.7 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 52.9 (O*C*H$_3$); 64.2 (*C*H$_2$OH); 124.0 (py*C*H); 124.5 (py*C*H); 138.4 (py*C*H); 146.4 (py*C*); 160.2 (py*C*); 165.5 (*C*O$_2$CH$_3$). ESI mass spectrometry (methanol) m/z 168.24 $[C_8H_9NO_3+H]^+$. FT-IR spectroscopy (υ, cm^{-1}) 3284.8 (s, O–H str); 2956.1, 2908.8, 2849.3 (w, CH_2 str); 1741.3 (s, C=O str); 1290.4 (s, C–O str); 1145.5 (s, C–O str); 759.4 (m, Py-H def). Melting point 85.8–90.6 °C lit. m.p. 88 °C [27].

2.2.2. Synthesis of methyl-6-formylpicolinate

This compound was synthesized after a modified published procedure [28]. Methyl-6-(hydroxymethyl)picolinate (1 g, 6 mmol) was dissolved in dichloromethane (25 mL), and manganese dioxide (5.2 g, 80 mmol) was added. The suspension was stirred at room temperature for 5 days and subsequently filtered through Celite. The filtrate was concentrated *in vacuo*, and the crude product was purified using flash column chromatography (dichloromethane/methanol 85:15, I_2 stain, R_f = 0.73) to yield 206 mg (21%) of a white solid (250 mg of starting material were re-isolated). ^{1}H NMR ($CDCl_3$, 500.13 MHz); δ 4.05 (s, 3H, OC*H*$_3$); 8.03 (td, 1H, py*H*, J = 7.7, 0.8 Hz); 8.12 (dd, 1H, py*H*, J = 7.8, 1.2 Hz); 8.33 (dd, 1H, py*H*, J = 7.7, 1.2 Hz); 10.17 (d, 1H, C*H*O, J = 0.8 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 53.3 (O*C*H$_3$); 124.4 (py*C*H); 129.0 (py*C*H); 138.4 (py*C*H); 148.6 (py*C*); 152.8 (py*C*); 164.9 (*C*O$_2$CH$_3$); 192.6 (*C*HO). ESI mass spectrometry (methanol) m/z 188.30 $[C_8H_7NO_3+Na]^+$. FT-IR spectroscopy (υ, cm^{-1}) 2860.8 (w, C–H str); 1719.4, 1708.2 (s, C=O str); 1140.9 (m, C–O str); 762.5 (m, py-H def). m.p. 82.4–84.2 °C.

2.2.3. Synthesis of methyl-6-(2-hydroxyethylamino)methyl picolinate

Methyl-6-formylpicolinate (1 g, 6.1 mmol) was dissolved in methanol (10 mL) and cooled to 0 °C. 2-Aminoethanol (0.37 mg, 6.1 mmol) in methanol (5 mL) was added dropwise, and the mixture was stirred for two hours at room temperature. The mixture was cooled to 0 °C, and $NaBH_4$ (0.22 g, 6.1 mmol) was added in small portions. After stirring for 10 min at 40 °C, water (20 mL) was added and the mixture was concentrated to 20 mL *in vacuo*. The aqueous phase was extracted with dichloromethane (2 × 20 mL), then the aqueous phase was acidified to pH 2 and further extracted with dichloromethane (2 × 20 mL). The combined organic layers were dried over sodium sulfate. After removal of the solvent, a yellow oil was obtained in 47 % yield (580 mg) ^{1}H NMR ($CDCl_3$, 400.13 MHz); δ 2.05 (s, 1H, N*H*); 2.95 (t, 2H, NC*H*$_2$CH$_2$, J = 4.9 Hz); 3.54 (bs, 1H, O*H*); 3.72 (t, 2H, NCH$_2$C*H*$_2$, J = 4.9 Hz); 3.98 (s, 3H, OC*H*$_3$); 4.11 (s, 2H, NC*H*$_2$py); 7.56 (m, 1H, py*H*, J = 4.1, 0.9 Hz); 7.84 (m, 1H, py*H*); 8.00 (dt, 1H, py*H*, J = 7.6, 0.6 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 51.0 (*C*H$_2$); 52.8 (O*C*H$_3$); 53.8 (*C*H$_2$); 60.4 (NCH$_2$*C*H$_2$); 123.8 (py*C*H); 125.9 (py*C*H); 137.7 (py*C*H); 147.4 (py*C*CO$_2$Me); 160.3 (py*C*CH$_2$); 165.5 (*C*O$_2$Me). ESI mass spectrometry (methanol) m/z 211.25 $[C_{10}H_{14}N_2O_3+H]^+$, 233.05 $[C_{10}H_{14}N_2O_3+Na]^+$ FT-IR spectroscopy (υ, cm^{-1}) 3325.6 (m, O–H str); 3117.9, 3083.4 (m, N–H sym/asym str); 2952.9 (w, C–H str); 1727.2 (s, C=O str); 1298.5, 1234.5 (s, CO_2Me sym/asym def); 1063.6 (m, C–O str); 758.6 (m, py-H def).

2.2.4. Dimethyl-6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis-(methylene)bis((2-hydroxyethyl) azanediyl)bis(methylene)-dipicolinate (Me_2H_3***L4***)

Methyl-6-(2-hydroxyethylamino)methyl picolinate (0.58 g, 2.7 mmol) was dissolved together with triethylamine (0.27 g) in tetrahydrofuran (2 mL), and 2,6-bis(dichloromethyl)cresol (0.28 g, 1.3 mmol) in dichloromethane (2 mL) was added dropwise at 0 °C. The reaction was allowed to warm to room temperature, and was stirred for 3 days. The yellow reaction mixture was filtered and concentrated *in vacuo*. The oily residue was taken up in dichloromethane (5 mL) and dried over sodium sulfate. After filtration and removal of the solvent *in vacuo*, the desired ligand was obtained as yellowish oil in 89% yield (650 mg). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ 2.15 (s, 3H, C*H*$_3$); 2.75 (t, 4H, NC*H*$_2$CH$_2$, J = 6.1 Hz); 3.69 (t, 4H, NCH$_2$C*H*$_2$, J = 6.0 Hz); 3.71 (s, 4H, arC*H*$_2$N); 3.73 (s, 4H, pyC*H*$_2$N); 3.85 (s, 6H, OC*H*$_3$); 4.37 (s, 2H, O*H*); 6.99 (s, 2H, ar*H*); 7.61 (dd, 2H, py*H*, J = 7.4, 1.4 Hz); 7.80 (m, 2H, py*H*, J = 7.7, 1.4 Hz); 7.91 (m, 2H, py*H*, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz) δ 20.2 (*C*H$_3$); 52.4 (O*C*H$_3$); 54.4 (ar*C*H$_2$N); 55.7 (N*C*H$_2$CH$_2$); 58.5 (NCH$_2$*C*H$_2$); 59.3 (py*C*H$_2$N); 123.2 (py*C*H); 123.4 (ar*C*CH$_2$); 126.4 (py*C*H); 126.7 (ar*C*CH$_3$); 129.2 (ar*C*H); 137.8 (py*C*H); 146.6 (py*C*CO$_2$CH$_3$); 153.1 (ar*C*OH); 159.6 (py*C*CH$_2$); 165.3 (*C*O$_2$CH$_3$). ESI mass spectrometry (methanol, neg) m/z 551.78 $[C_{29}H_{35}N_4O_7]^-$. FT-IR spectroscopy (υ, cm^{-1}) 3284.4 (b, O–H str); 2951.4 (m, C–H str); 1725.2 (m, C=O str); 1226.4, 1138.6 (m, C–O str); 728.8 (s, py-H).

2.2.5. Synthesis of 2-((6-methylpyridin-2-yl)amino)ethanol

6-Methylpicolin-aldehyde (1.54 g, 0.013 mol) was dissolved in 24 mL of MeOH and cooled to 0 °C. 2-Aminoethanol (0.77 mL, 0.013 mol) was added dropwise at 0 °C, after which the reaction was allowed to reach room temperature and then stirred for an additional 2 h. After this time, thin layer chromatography (TLC) analysis (100% MeOH) showed almost complete disappearance of the starting material. The reaction was once again cooled to 0 °C and, while monitoring by TLC, $NaBH_4$ (0.30 g, 0.008 mol) was added in small portions until disappearance of the intermediate imine. Water (24 mL) was then added in small portions at 0 °C. The volume of the solution was reduced to ~24 mL by rotary evaporation. This solution was extracted with 3 × ~20 mL DCM, the combined organic layers were washed with 2 × 20 mL brine and

dried over anhydrous Na_2SO_4, and the dichloromethane was removed by rotary evaporation. A viscous amber oil resulted (0.94 g, 44.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.52 (3H, s, py-CH_3), 2.85 (2H, t, J = 5.1 Hz, N–CH_2–CH$_2$–OH), 3.63 (2H, t, J = 5.1 Hz, CH$_2$–OH), 3.89 (2H, s, py-CH$_2$–N), 7.01 (1H, d, J = 7.6 Hz, py-H), 7.04 (1H, d, J = 7.6 Hz, py-H), 7.51 (1H, t, J = 7.6 Hz, 4′-py-H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 24.33 (ar-CH$_3$), 51.14 (CH$_2$), 54.29 (CH$_2$), 60.92 (CH$_2$–OH), 119.15 (py-CH), 121.62 (py-CH), 136.86 (py-CH), 158.03 (py-C), 159.10 (py-C). ESI mass spectrometry: m/z = 167.1179 (calculated for $[C_9H_{14}N_2O+H]^+$ = 167.1179).

2.2.6. Synthesis of 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis(((6-methylpyridin-2-yl)methyl)azanediyl)diethanol (H_3**L5**)

Triethylamine (0.78 mL, 6 mmol) was added dropwise to a solution of 2-((6-methylpyridin-2-yl)amino)ethanol (0.94 g, 5.6 mmol) in 20 mL of dry tetrahydrofuran at 0 °C. To this was added a solution of 2,6-bis(chloromethyl)-p-cresol (0.57 g, 2.8 mmol) in 20 mL of dichloromethane at 0 °C. The resulting pale yellow solution was allowed to reach room temperature and then stirred overnight. The mixture was filtered to remove the white precipitate of triethylamine hydrochloride, and the solvent was removed under vacuum, yielding the product as a pale yellow wax (1.28 g, 99.1% yield). ^{1}H NMR (500 MHz, CD_3OD) δ: 2.18 (3H, s, py-CH_3), 2.50 (6H, s, py-CH_3), 2.68 (4H, t, J = 5.0 Hz, N–CH_2–CH$_2$–OH), 3.66 (4H, t, J = 5.0 Hz, CH_2–OH), 3.70 (4H, s, ar-CH_2–N), 3.77 (4H, s, py-CH_2–N), 6.86 (2H, s, ar-H), 7.10 (2H, d, J = 7.7 Hz, 5′-py-H), 7.25 (2H, d, J = 7.7 Hz, 3′-py-H), 7.60 (2H, t, J = 7.7 Hz, 4′-py-H). ^{13}C NMR (125 MHz, CD_3OD, 328K) δ: 20.49 (ar-CH$_3$), 23.82 (py-CH$_3$), 56.64 (ar-CH$_2$–N), 57.34 (N–CH$_2$–CH$_2$–OH), 60.56 (N–CH$_2$–CH$_2$–OH), 60.87 (py-CH$_2$–N), 121.79 (3′-py–CH), 123.04 (5′-py-CH), 124.95 (ar-C–CH$_2$), 129.04 (ar-C–CH$_3$), 131.01 (ar-CH), 138.56 (4′-py-CH), 154.72 (ar-C–OH), 158.86 (6′-py-C–CH$_3$), 159.69 (2′-py-C–CH$_2$). ESI mass spectrometry: m/z = 465.2872 (calculated for $[C_{27}H_{36}N_4O_3+H]^+$ = 465.2860).

2.2.7. Synthesis of 6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((2-hydroxyethyl)azanediyl) bis(methylene)dipicolinamide (H_3**L6**)

The synthesis was adapted from a previously published procedure [29]. Me_2H_3**L4** (1.04 g, 1 mmol) was suspended in ~15 mL of concentrated NH_4OH and stirred overnight in a sealed reaction vessel, resulting in the formation of a yellow waxy solid. The suspension was sonicated for four hours, then the solvent was removed, affording the product as a yellow wax (0.97 g, 98.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.17 (3H, s, ar-CH_3), 2.75 (4H, t, J = 5.3 Hz, N–CH_2–CH$_2$–OH), 3.66 (4H, t, J = 5.3 Hz, N–CH$_2$–CH_2–OH), 3.75 (4H, s, ar-CH_2–N), 3.88 (4H, s, py-CH_2–N), 6.78 (2H, s, ar-H), 7.37 (2H, dd, J = 7.7 Hz, 0.9 Hz, py-H), 7.74 (2H, t, J = 7.7 Hz, 4′-py-H), 8.03 (2H, dd, J = 7.7 Hz, 0.9 Hz, py-H), 8.22 (2H, broad, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.39 (ar-CH$_3$), 55.85 (CH$_2$), 56.24 (CH$_2$), 59.26 (CH$_2$), 59.46 (CH$_2$), 121.10 (ar/py-C), 123.31 (ar/py-C), 125.58 (ar/py-C), 128.10 (ar/py-C), 130.21 (ar/py-C), 137.73 (ar/py-C), 149.43 (ar/py-C), 153.57 (ar/py-C), 157.56 (ar/py-C), 166.82 (CONH$_2$). ESI mass spectrometry: m/z = 523.26 (calculated for $[C_{27}H_{34}N_6O_5+H]^+$ = 523.27), m/z = 545.25 (calculated for $[C_{27}H_{34}N_6O_5+Na]^+$ = 545.25).

2.3. Synthesis of metal complexes

2.3.1. Synthesis of $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)\cdot 3H_2O$

Me_2H_3**L4** (0.35 g, 0.6 mmol) was dissolved in MeOH (20 mL), to which was added zinc acetate dihydrate (0.60 g, 1.3 mmol). The resulting pale yellow solution was refluxed for 0.5 h. The total solvent volume was reduced to ~6 mL, Bu_4NClO_4 (0.33 g, 1 mmol) was added, and the mixture was heated to ~60 °C in a water bath for ~10 min and then allowed to concentrate by slow evaporation at room temperature, resulting in a colorless solid (0.060 g, 12% yield). *Anal.* calc. for $C_{27}H_{39}ClN_4O_{16}Zn_2$: C, 38.5; H, 4.67; N, 6.65: Found: C, 38.9; H, 4.66; N, 6.66%. ^{1}H NMR ($(CD_3)_2SO$) δ: 1.97 (3H, s, ar-CH_3), 2.80 (2H, br m, N–HCH–CH$_2$–OH), 3.04 (2H, br m, N–HCH–CH$_2$–OH), 3.23 (2H, d, J = 11.3 Hz, ar/py-HCH–N), 3.66 (2H, br m, HCH-OH), 3.72 (2H, d, J = 17.7, ar/py-HCH–N), 3.84 (2H, d, J = 11.5 Hz, ar/py-HCH–N), 3.90 (2H, d, J = 17.7 Hz, ar/py-HCH–N), 4.00 (2H, br m, HCH–OH), 6.65 (2H, s), 7.30 (2H, d, J = 7.7 Hz, py-H), 7.82 (2H, d, J = 7.6 Hz, py-H), 7.94 (2H, t, J = 7.7 Hz, 4′-py-H). ^{13}C NMR ($(CD_3)_2SO$) δ: 19.87 (ar-CH_3), 54.47 (CH$_2$), 56.76 (CH$_2$), 56.88 (CH$_2$), 57.44 (CH$_2$-OH), 121.08 (py-CH), 123.92 (py-CH$_2$), 125.00 (ar-C), 125.83 (ar-C), 131.46 (ar-CH), 141.33 (4′-py-CH), 148.29 (py-C), 153.65 (py-C), 158.79 (ar-C–OH), 165.04 (CO_2^-). ESI mass spectrometry (CH_3OH): m/z 649.1 (70%); 651.1 (99%); 653.1 (100%) 653.02. (Calculated for $[C_{27}H_{29}N_4O_7Zn_2]^+$ 649.1 (82%); 651.1 (99%); 653.1 (100%)). Crystals suitable for X-ray structure determination, but which desiccated readily on removal from solvent, were grown on prolonged standing of the reaction mixture in an ethanol/methanol solvent mixture. These crystals were subsequently characterized crystallographically as $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)$ $2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$.

2.3.2. Synthesis of $[Zn_2(H_2$**L5**$)(CH_3CO_2)(H_2O)](PF_6)_2\cdot 2H_2O$

H_3**L5** (0.50 g, 0.001 mol) was dissolved in 30 mL of MeOH, to which was added zinc acetate dihydrate (0.60 g, 3 mmol). The resulting pale yellow solution was then refluxed for 0.5 h. After this time, the total solvent volume was reduced to ~15 mL by rotary evaporation and to the solution was added $NaPF_6$ (0.53 g, 0.003 mol). The solution was heated to ~60 °C in a water bath for ~10 min, gravity filtered, and stored at 0 °C. Colorless crystals (0.52 g, 52% yield), which desiccated in open air, were collected. All attempts to produce diffraction quality crystals were unsuccessful. Elemental *Anal.* Calc. for $C_{29}H_{44}F_{12}N_4O_8P_2Zn_2$: C, 34.92; H, 4.45; N, 5.62; Zn, 13.12. Found: C, 34.87; H, 4.25; N, 5.55; Zn, 12.97%. ^{1}H NMR (500 MHz, (CD_3OD) δ: 2.03 (3H, s, ar-CH_3), 2.16 (3H, s, CH_3–CO_2^-), 2.77 (6H, s, py-CH_3), 2.99 (2H, dt, J = 12.3 Hz, 3.4 Hz, CH_2), 3.08–3.23 (4H, br m, CH_2), 3.77–3.86 (6H, s overlapping with br m, CH_2), 3.98 (2H, br m, CH_2), 4.14 (2H, d, J = 12.0 Hz, CH_2), 6.64 (2H, s, ar-CH), 6.80 (2H, d, J = 6.8 Hz, 3′-py-H), 7.19 (2H, d, J = 7.7 Hz, 5′-py-H), 7.58 (2H, t, J = 7.7 Hz, 4′-py-H). ^{13}C NMR (100 MHz, $(CD_3)_2SO$) δ: 19.93 (ar-CH_3), 23.80 ($CH_3CO_2^-$), 23.99 (CH_3-py), 56.79 (CH$_2$), 58.65 (2xCH$_2$), 59.57 (CH$_2$), 121.27 (3′-py-CH), 124.06 (ar-C), 125.43 (5′-ar-CH), 128.29 (ar-C), 133.64 (ar-CH), 141.38 (4′-py-CH), 156.21 (py-C), 159.17 (ar-C-OH, py-C), 182.00 ($CH_3CO_2^-$). ESI mass spectrometry: m/z = 589.08 (calculated for $[C_{27}H_{33}N_4O_3Zn_2]^+$ = 589.11).

2.3.3. Attempted synthesis of $[Zn_2(H_2$**L6**$)(H_2O)_2](BF_4)$

H_3**L6** (0.97 g, 2 mmol) was dissolved in 60 mL of MeOH, to which was added zinc acetate dihydrate (0.70 g, 4 mmol). The resulting pale yellow solution was then refluxed for 0.5 h. After this time, the total solvent volume was reduced to ~15 mL by rotary evaporation, and $NaBF_4$ was added, resulting in colorless product in low yield. ESI mass spectrometry and microanalysis indicated that the complex was $[Zn_2(H_2$**L4**$)(H_2O)_2](BF_4)\cdot 3H_2O$. Elemental *Anal.* calc. for $C_{27}H_{35}BF_4N_4O_{10}Zn_2$: C, 40.88; H, 4.45; N, 7.06. Found: C, 40.43; H, 4.24; N, 6.97%. LRMS (CH_3OH): m/z 649.1 (70%); 651.1 (99%); 653.1 (100%) 653.02. (Calculated for $[C_{27}H_{29}N_4O_7Zn_2]^+$ 649.1 (82%); 651.1 (99%); 653.1 (100%)).

2.4. Crystallography

X-ray diffraction data for a crystal of $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)\cdot 2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$ were collected on a Bruker APEX II CCD diffractometer, with graphite monochromated Mo Kα (λ = 0.71069 Å) radiation at 90 K, in a nitrogen gas stream. Intensities were corrected for Lorentz polarization effects, and a multiscan

Table 1
Summary of crystallographic data for $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)\cdot 2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$.

Empirical formula	$C_{31}\ H_{47}\ Cl\ N_4\ O_{17.50}\ Zn_2$
Formula weight	921.92
Temperature	90(2) K
Wavelength	0.71069 Å
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	
a (Å)	12.703(3)
b (Å)	12.852(2)
c (Å)	13.240(3)
α (°)	75.919(5)
β (°)	69.716(9)
γ (°)	68.503(9)
V (Å^3)	1869.7(7)
Z	2
D_{calc}	1.638 Mg/m^3
Reflections collected	16106
Independent reflections	6909 [R(int) = 0.0170]
Refinement method	Full-matrix least-squares on F^2
Goodness-of-fit (GOF) on F^2	1.048
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0334, wR2 = 0.0910
R indices (all data)	R1 = 0.0363, wR2 = 0.0932

Table 2
Selected bond lengths (Å) and angles (°) for $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)\cdot 2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$.

Zn(1)–O(1)	2.0597(17)	Zn(1)–O(5)	2.3528(19)
Zn(1)–O(8)	2.1448(18)	Zn(1)–O(10)	1.9808(19)
Zn(1)–N(3)	2.168(2)	Zn(1)–N(4)	2.053(2)
Zn(2)–O(1)	2.0405(18)	Zn(2)–O(4)	2.287(2)
Zn(2)–O(7)	2.1087(18)	Zn(2)–O(11)	2.015(2)
Zn(2)–N(1)	2.194(2)	Zn(2)–N(2)	2.058(2)
Zn(1)...Zn(2)	3.60		
Zn(1)–O(1)–Zn(2)	122.94(8)	O(1)–Zn(1)–N(3)	93.22(8)
N(3)–Zn(1)–N(4)	78.68(8)	O(1)–Zn(2)–N(1)	93.48(7)
N(1)–Zn(2)–N(2)	77.93(8)		

absorption correction was applied. The structure was solved by direct methods using SIR97 and refined on F^2 using SHELXL97 running within the WinGX interface [30]. Plots were drawn using ORTEP3 [31]. All non-hydrogen atoms were refined with anisotropic thermal parameters. The crystal structure contained disordered components. The ClO_4^- counter ion showed translational disorder over two sites, with site occupancy factors of 0.69 and 0.31. Both of the methanol solvent molecules showed translational disorder over two sites, with site occupancy factors of 0.70 and 0.30 for C33 and O15, and 0.57 and 0.43 for C32 and O16. Four H_2O molecules were found to only partially occupy sites, with two molecules having site occupancy factors of 0.50, and two of 0.25. Selected crystal data and some details of refinements are given in Table 1. Selected bond distances and angles are presented in Table 2. X-ray data were deposited with the Cambridge Crystallographic Data Center CCDC-793400. These data can be obtained free of charge from the Cambridge Crystallographic Data Center via http://www.ccdc.cam.ac.uk/data_request/cif.

2.5. Catalytic studies

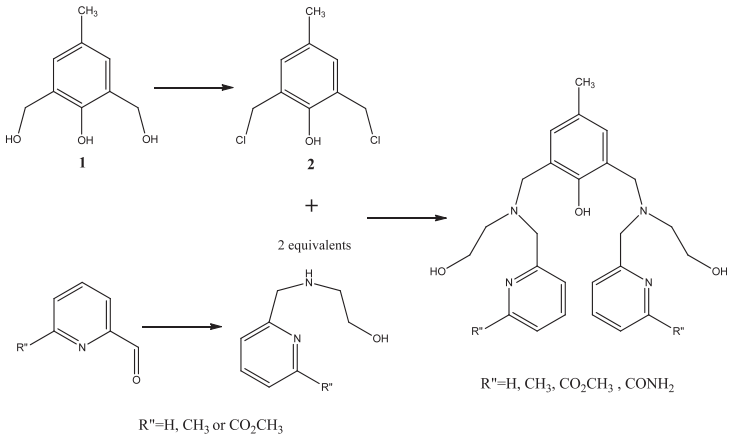
The phosphoesterase-like activity of the complexes was determined by measuring hydrolysis of the substrate BDNPP. A Varian Cary50 Bio UV/Visible spectrophotometer with a Peltier temperature controller was used to measure changes in absorbance values in 10 mm quartz cuvettes. The initial rate method was employed and assays were measured such that the initial linear portion of the data was used for analysis. Product formation was determined at 25 °C by monitoring the formation of 2,4-dinitrophenol. The extinction coefficient for 2,4-dinitrophenolate at 400 nm, throughout the pH range studied, is 12,100 M^{-1} cm^{-1} [32,33]. For each assay, corrections for the rate of autohydrolysis were applied. An aqueous multi-component buffer (50 mM in each of 2-(N-morpholino)ethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) and 2-(N-cyclohexylamino)ethane sulfonic acid (CHES), with the ionic strength controlled by 250 mM $LiClO_4$) was used. Assays were carried out in 50:50 MeCN:buffer, with substrate and complex initially dissolved in MeCN. Assays conducted to investigate pH dependence were 40 μM in complex and 5 mM in BDNPP and showed no significant buffer effects. Assays used to assess the substrate and complex dependence were carried out at pH 10.5 for the complex. [Substrate] dependence assays were 40 μM in complex and 1–9 mM in BDNPP, and [complex] dependence assays were 20–160 μM in complex and 5 mM in BDNPP. The change in absorbance produced by hydrolysis of BDNPP by free Zn(II) (from $Zn(ClO_4)_2$), in the same concentration as the complex, did not differ significantly from autohydrolysis values. All data were fitted by non-linear least squares regression analysis [34].

3. Results and discussion

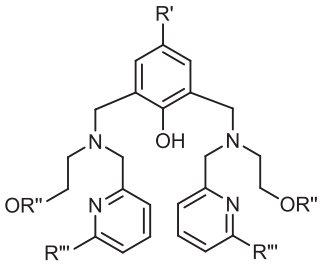
3.1. Synthesis

The ligand dimethyl-6,6′-(2-hydroxy-5-methyl-1,3-phenylene) bis(methylene)bis((2-hydroxyethyl)azanediyl)bis(methylene)di-picolinate ($H_3Me_2\mathbf{L4}$) has been synthesized through a substitution reaction between 2,6-bis(chloromethyl)-4-methylphenol and methyl-6-((2-hydroxyethylamino)methyl)picolinate; the ligand was isolated as the methyl ester. Methyl 6-formylpicolinate was prepared by reduction of dimethyl pyridine-2,6-dicarboxylate to the corresponding mono-alcohol, and then reaction with MnO_2 to prepare the aldehyde. $H_3\mathbf{L5}$ was prepared by the reaction of 2-((6-methylpyridin-2-yl)amino)ethanol with 2,6-bis(chloromethyl)-4-methylphenol. The amide ligand 6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((2-hydroxyethyl)azanediyl)bis(methylene)dipicolinamide ($H_3\mathbf{L6}$) was prepared by the reaction of $H_3Me_2\mathbf{L4}$ with aqueous ammonia. The reaction scheme employed is shown in Scheme 1. The complexes were prepared after reaction with zinc(II) acetate; in both cases no attempts were made to optimize yields, and the complexes proved to be difficult to obtain as X-ray quality crystals. Reaction of the methyl ester $H_3Me_2\mathbf{L4}$ with the metal salt results in hydrolysis of the ester, an effect observed previously with similar ligands [35]. Crystals of $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$ were obtained in low yield, although they contained mixed solvent molecules in the lattice, and they desiccated on removal from the parent solvent. The kinetic studies reported herein employed the crystallized sample. On reaction of $H_3\mathbf{L6}$ with zinc(II) acetate hydrolysis of the amide occurred resulting in the isolation of the dizinc(II) complex of $H_2\mathbf{L4}^{3-}$.

The nomenclature employed for the ligands follows that described previously and denotes the number of removable protons upon complexation (Chart 1) [36]. The free acid ligand, 6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((2-hydroxyethyl) azanediyl)bis(methylene)dipicolinic acid was not isolated, hence the nomenclature $Me_2H_3\mathbf{L4}$ denotes the methyl ester and potentially three sites for deprotonation, the phenyl and the two pendant alcohols. Upon reaction with zinc acetate and after hydrolysis of the methyl ester groups, the resulting complex is designated as $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$ ($H_2\mathbf{L4}^{3-}$), with two carboxylate donors and the phenoxide deprotonated; the two pendant alcohols are



Scheme 1. Synthesis of 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene) bis((pyridin-2-ylmethyl)azanediyl)diethanol (CH_3H_3**L1**), dimethyl-6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((2-hydroxyethyl) azanediyl)bis(methylene) dipicolinate (Me_2H_3**L4**), 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis (((6-methylpyridin-2-yl)methyl) azanediyl) diethanol (H_3**L5**) and 6,6′-(2-hydroxy-5-methyl-1,3-phenylene)bis (methylene)bis((2-hydroxyethyl)azanediyl) bis (methylene)dipicolinamide (H_3**L6**).



R' = CH_3,	R" = H	R''' = H	CH_3H_3**L1**
R' = CH_3,	R" = CH_3	R''' = H	CH_3H**L2**
R' = Br,	R" = CH_3	R''' = H	Br**HL2**
R' = NO_2,	R" = CH_3	R''' = H	NO_2H**L2**
R' = CH_3,	R" = Ph	R''' = H	CH_3H**L3**
R' = CH_3	R" = H	R''' = COOH	H_5**L4**
R' = CH_3	R" = H	R''' = CH_3	H_3**L5**
R' = CH_3	R" = H	R''' = $CONH_2$	H_3**L6**

Chart 1. Ligand abbreviations. CH_3H_3**L1** = 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((pyridin-2-ylmethyl)azanediyl)diethanol;[25] CH_3H**L2** = 2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-4-methylphenol; Br**HL2** = 4-bromo-2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl) phenol; NO_2H**L2** = 2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-4-nitrophenol; CH_3H**L3** = 4-methyl-2,6-bis(((2-phenoxyethyl)(pyridin-2-ylmethyl) amino)methyl)phenol [36]; H_5**L4** = 6,6′-((((2-hydroxy-5-methyl-1,3-phenylene)bis (methylene))bis((2-hydroxyethyl)azanediyl))bis(methylene)) dipicolinic acid; H_3**L5** = 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis(((6-methylpyridin-2-yl) methyl)azanediyl) diethanol; (H_3**L6**) = 6,6′-(2-hydroxy-5-methyl-1,3-phenylene) bis(methylene) bis((2-hydroxyethyl)azanediyl) bis (methylene)dipicolinamide.

protonated. Similarly, for H_3**L5** the ligand has potentially three sites for deprotonation, the phenyl and the two pendant alcohols; characterization of the complex as $[Zn_2(H_2$**L5**$)(CH_3CO_2)(H_2O)](PF_6)_2\cdot 2H_2O$ implies that the phenol is deprotonated.

3.2. Discussion of X-ray structure

The complex $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)\cdot 2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$ crystallized in the triclinic $P\bar{1}$ space group. The structure is composed of the complex cation, a perchlorate anion and solvent molecules completing the structure. Selected crystallographic data are shown in Table 1, selected bond lengths and angles are displayed in Table 2. An ORTEP [31] plot of the complex cation is shown in Fig. 1. For $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)\cdot 2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$, the ligand has the two carboxylic acid groups and the bridging phenol moiety deprotonated; the pendant alcohol moieties are protonated. Both of the zinc(II) centers display a distorted six coordinate geometry. In both cases the N_2O_4 coordination environment of the metal ions is composed of the tertiary amine (Zn(1)–N(3), 2.168(2) Å; Zn(2)–N(1), 2.194(2) Å), the pyridine nitrogen (Zn(1)–N(4), 2.053(2) Å; Zn(2)–N(2), 2.058(2) Å), the alcohol (Zn(1)–O(5), 2.3528(19) Å; Zn(2)–O(4), 2.287(2) Å), the deprotonated carboxylate (Zn(1)–O(8), 2.1448(18) Å; Zn(2)–O(7), 2.1087(18) Å), and a water molecule (Zn(1)–O(10), 1.9808(19) Å; Zn(2)–O(11), 2.015(2) Å). The six coordinate geometry is completed by the bridging oxygen from the phenoxide (Zn(1)–O(1), 2.0597(17) Å; Zn(2)–O(1), 2.0405(18) Å) with Zn(1)–O(1)–Zn(2), 122.94(8)°; the Zn(1)–Zn(2) distance is 3.608 Å. The

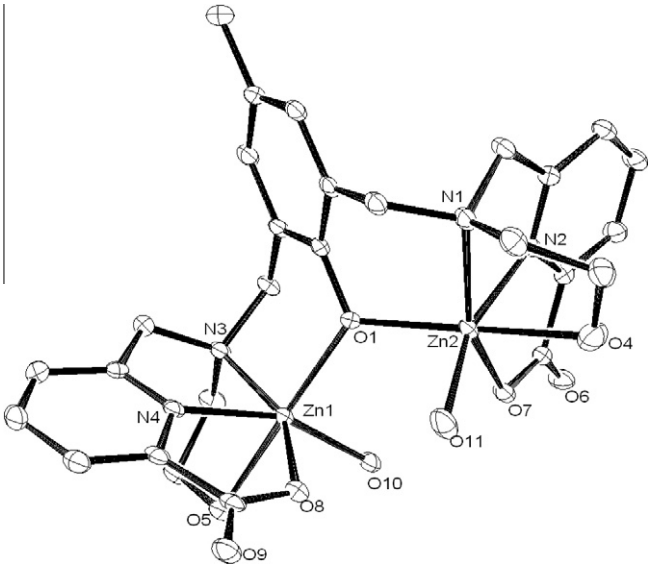


Fig. 1. ORTEP [31] plot of $[Zn_2(H_2$**L4**$)(H_2O)_2](ClO_4)\cdot 2CH_3OH\cdot 1.5H_2O\cdot CH_3CH_2OH$ with 50% probability level of thermal ellipsoids. Hydrogen atoms, counter ions and non-coordinated solvent molecules have been omitted for clarity, as have carbon atom labels.

Zn–O and Zn–N distances appear typical of those reported for the di-zinc(II) complex of 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((pyridin-2-ylmethyl)azanediyl)diethanol (CH_3H_3**L1**; Chart 1) [25], particularly the alcohol Zn–O distances (2.3528(19), 2.287(2) Å) which appear typical for a protonated alcohol bound to zinc(II) [25].

3.3. Mass spectrometry

Mass spectral analysis, determined with solvent conditions employed in the hydrolytic studies, H_2O:CH_3CN (1:1), shows that the complexes exist as dinuclear zinc species with the isotopic patterns for Zn_2 species being distinctly different from that observed for a mono-zinc species. Thus, species indicative of $[Zn_2(H_2\mathbf{L4})]^+$ (*m/z* 649.1 (82.5%; 651.1 (99.2%); 653.1 (100%)) (Fig. 2) and $[Zn_2(\mathbf{L5})]^+$ (m/z 589.1 (83.6%); 591.1 (99.3%); 593.1 (100%)) were observed. The mass spectral evidence suggests that under the solvent conditions employed in the kinetic studies the acetate ligands are lost [36].

3.4. Phosphodiesterase-like activity

Phosphatase-like activity was measured at various pH values ranging from 5 to 10.5 using the activated substrate BDNPP (Fig. 3(a)). For both complexes the data were consistent with the presence of one protonation equilibrium and were fitted to an equation derived for a monoprotic system (Eq. (1)) [37].

$$V_0 = \frac{V_{max}}{\left(1 + \frac{[H^+]}{K_{es}}\right)} \quad (1)$$

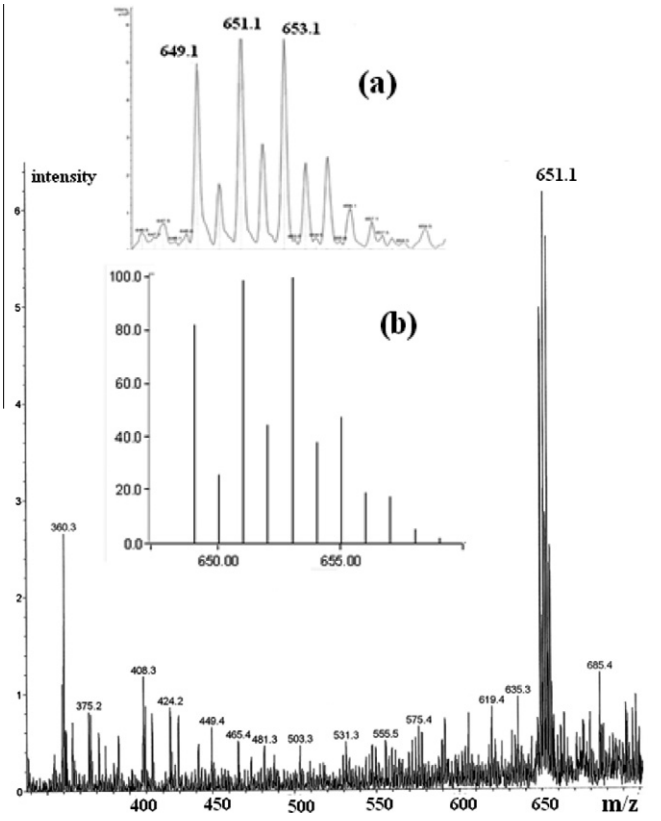


Fig. 2. ES-mass spectrum of the complex $[Zn_2(H_2\mathbf{L4})]^+$ recorded in H_2O:CH_3CN (1:1); inset (a) the isotopic pattern for the envelope at *m/z* 651.1; (b) calculated isotopic pattern indicative of a dizinc(II) complex.

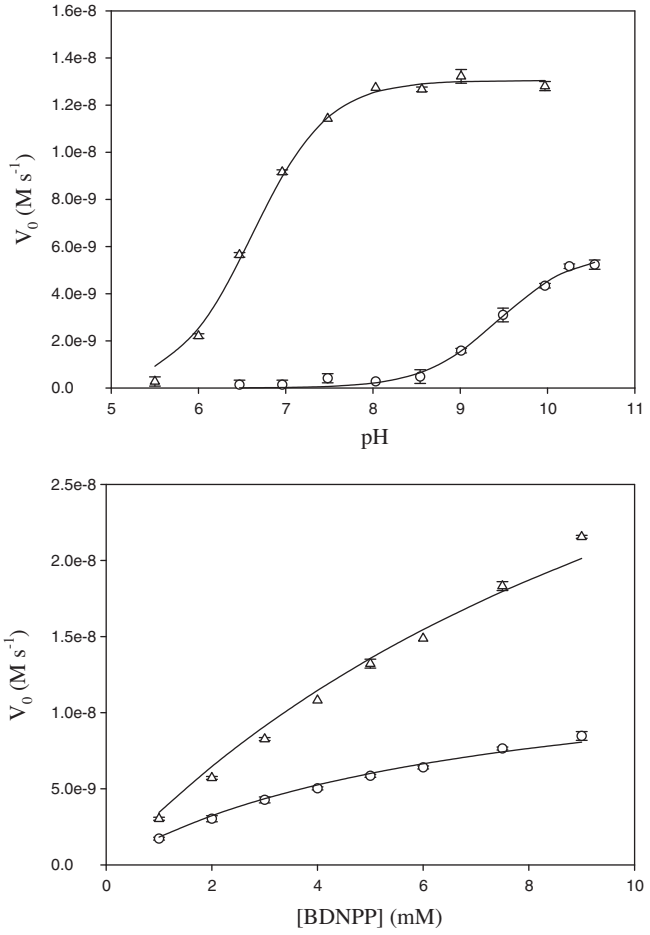


Fig. 3. pH dependence and substrate concentration dependence of rate of BDNPP cleavage by $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$ (o) and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)](PF_6)_2$ (Δ) at 25 °C; aqueous multi-component buffer (50 mM each of MES, HEPES and CHES), ionic strength controlled by 250 mM $LiClO_4$; 50:50 MeCN:buffer).

Here, V_0 is the initial reaction rate, V_{max} is the maximum rate with BDNPP as substrate (5 mM), and K_{es} (=K_a) is the protonation equilibrium constant relevant to catalysis. The catalytically relevant species derived from $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)]^{2+}$ exhibited pK_as of 9.4 ± 0.05 and 6.6 ± 0.05, respectively. The substrate concentration dependence of catalysis was also measured at pH 10.5 and 8.0 for $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)]^{2+}$, respectively. Michaelis–Menten type behavior was observed (Fig. 3(b)) and the data fitted using non-linear least square analysis (Eq. (2)) gave the Michaelis constants, V_{max} and k_{cat} (V_{max}/[complex]) [37].

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \quad (2)$$

Here, K_m is the Michaelis constant and [S] the substrate concentration. $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)]^{2+}$ exhibited $k_{cat} = 3.52 \pm 0.03 \times 10^{-4}$ and $1.27 \pm 0.04 \times 10^{-3}$ s^{-1} (K_m = 6.7 ± 0.9; 13.8 ± 1.5 mM), respectively. The rate of hydrolysis of BDNPP (5 mM) was linear for complex concentrations from 20 to 160 μM.

3.5. Mechanism of reaction

Data for the rate of hydrolysis of BDNPP with a range of comparable complexes are shown in Table 3. The effect of electron withdrawing groups *para*- to a phenolic oxygen donor on the

Table 3
Data for the rate of hydrolysis of BDNPP with dizinc(II) complexes. IPCPMP = 2-(*N*-isopropyl-*N*-((2-pyridyl)methyl)aminomethyl)-6-(*N*-(carboxylmethyl)-*N*-((2-pyridyl)methyl)amino methyl)-4-methylphenol [56].

Complex	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m ($M^{-1}\,s^{-1}$)	pK_a
$[Zn_2(CH_3H\mathbf{L1})(CH_3COO)(H_2O)](PF_6)$	$1.07 \pm 0.04 \times 10^{-3}$	12.5 ± 7.0	0.086	6.6
$[Zn_2(CH_3\mathbf{L2})(CH_3COO)_2](PF_6)$ [36]	$5.70 \pm 0.04 \times 10^{-3}$	20.8 ± 5.0	0.274	6.7
$[Zn_2(Br\mathbf{L2})(CH_3COO)_2](PF_6)$ [36]	$1.90 \pm 0.04 \times 10^{-3}$	7.1 ± 4.0	0.268	6.5
$[Zn_2(NO_2\mathbf{L2})(CH_3COO)_2](PF_6)$ [36]	$0.76 \pm 0.04 \times 10^{-3}$	6.5 ± 1.4	0.117	6.5
$[Zn_2(CH_3\mathbf{L3})(CH_3COO)_2](PF_6)$ [36]	$3.60 \pm 0.04 \times 10^{-3}$	18.9 ± 3.5	0.190	7.7
$[(Zn_2(IPCPMP)(CH_3COO))_2](PF_6)_2$ [56]	6.4×10^{-4}	16 ± 5	0.040	6.6
$[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$	$3.52 \pm 0.03 \times 10^{-4}$	6.7 ± 0.9	0.053	9.4
$[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)](PF_6)_2$	$1.27 \pm 0.04 \times 10^{-3}$	13.8 ± 1.5	0.092	6.6

magnitude of k_{cat} has shown that, generally, the more electron-withdrawing the substituent the lower the k_{cat} value [36,38]. For the complexes $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)]^{2+}$ and $[Zn_2(CH_3H\mathbf{L1})(CH_3CO_2)(H_2O)]^+$ with COO^-, CH_3 and H substituents *meta*- to the pyridine nitrogen, a Hammett style plot shows a linear relationship for the inductive influence of the substituent on both the rate of the BDNPP hydrolysis reaction (k_{cat}) and with K_m (Fig. 4) [39,40]. The sequence of k_{cat} with *m*-CH_3 > *m*-H > *m*-COO^- is in accord with previous studies with Fe(III) complexes, and is ascribed to the Lewis acidity of the metal center being directly affected by the donor/acceptor properties of the substituent [38]. The negative value of Hammett ρ parameter for the k_{cat} plot (−1.3) reflects both the distance between the substituent and the nucleophile, and the decreasing charge on the aromatic ring [38,41].

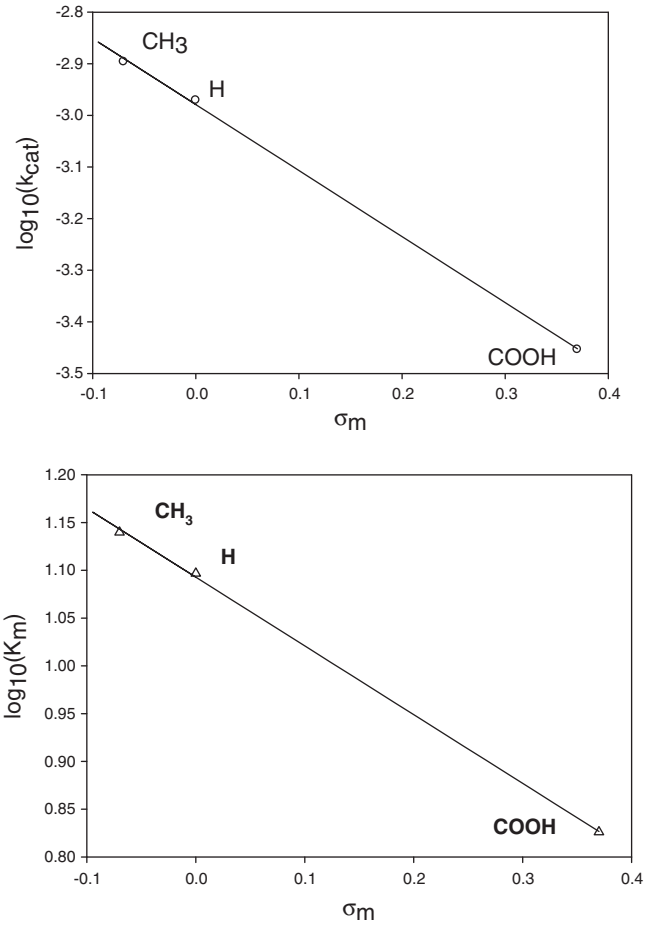


Fig. 4. Linear correlation for the Hammett parameter (σ_m) and (a) $\log_{10}(k_{cat})$-(o) (b) $\log_{10}(K_m)$ (Δ) for $[Zn_2(CH_3H\mathbf{L1})(CH_3COO)_2](PF_6)$ (*m*-H), $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$ (*m*-COO^-) and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)](PF_6)_2$ (*m*-CH_3).

As observed previously, the trend is less well defined for the respective pK_a values [36]. What is evident is the difference in the kinetically relevant pK_a between $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$ (pK_a 9.4) and that exhibited by $[Zn_2(\mathbf{L5})]^+$ (pK_a 6.6) and other similar dizinc(II) complexes (Table 3). Complexes with -**L1**, -**L4** and -**L5** type ligands (Table 3) possess two potentially nucleophilic entities for the hydrolysis of the substrate BDNPP, Zn–OH and Zn–OR (alkoxide), whereas those with -**L2** type ligands (Chart 1) have the alkoxide replaced by an ether moiety and the nucleophile is a Zn–OH species [36]. Theoretical studies suggest that a zinc(II)-coordinated alcohol has a lower pK_a than a zinc(II)-coordinated water [42] and is more reactive [42–46]. The pK_a value of 9.4, determined for the catalytically relevant pK_a for $[Zn_2(H_2\mathbf{L4})]^+$, is at the high end of the range observed for terminally bound H_2O ligands [25,36,42,45–56], but not typical for a zinc(II) bound alcohol. The pK_a is similar to those reported for the aminopeptidase mimics $[Zn_2(bocp)(CH_3COO)_2]BPh_4$ and $[Zn_2(bomp)(CH_3COO)_2]BPh_4$ (bocp = 4-chloro-2,6-bis(((2-methoxyethyl)(methoxymethyl)amino)methyl) phenol; bomp = 2,6-bis(((2-methoxyethyl) (methoxymethyl)amino) methyl)-4-methylphenol, (9.1 and 9.4, respectively) [57]. As well, substitution of a neutral donor with an anionic ligand in the primary coordination sphere reduces the Lewis acidity of the zinc(II) center, raising the pK_a of the Zn–OH_2 [51,58,59]. For example, the zinc(II) complexes of the ligand 2-(bis(pyridin-2-ylmethyl) amino)acetic acid, and similar ligands, display a pK_as typically around 9 [58,60–63]. In addition, the higher coordination number of the Zn(II) ions will also contribute to the higher pK_a [64].

Comparison of the efficiency (k_{cat}/K_m) for $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)]^{2+}$, 0.053 $M^{-1}\,s^{-1}$ and 0.092 $M^{-1}\,s^{-1}$, respectively (Table 3), shows that the latter is similar to that for $[Zn_2(CH_3H\mathbf{L1})(CH_3COO)_2](PF_6)$ and within the range reported for the hydrolysis reactions of BDNPP for a number of heterodinuclear complexes [32,56,65–68]. $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ displays a lower k_{cat} and binds the substrate relatively more strongly than, for example, $[(Zn_2(IPCPMP)(CH_3COO))_2](PF_6)_2$ which displays a k_{cat} of similar magnitude and binds the substrate more weakly (k_{cat}/K_m = 0.040 $M^{-1}\,s^{-1}$) [56].

Structural and kinetic similarities between $[Zn_2(CH_3H\mathbf{L1})(CH_3COO)(H_2O)](PF_6)$ [25] and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)](PF_6)_2$, coupled with the results of previous studies [36], suggest that the active nucleophile for these complexes is a Zn(II)–OH species. Structurally the situation for $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ is different. This complex has one exchangeable binding site on each of the metal ions and, consequently, a more crowded active site. Mechanistic proposals based on monodentate coordination of the substrate include the terminal hydroxide on the second zinc(II) site acting as the nucleophile, or acting as a general base, to deprotonate a neighboring water molecule which then attacks the phosphate ester [36,56,69,70]. If, however, the substrate BDNPP binds in a bi-dentate manner, as suggested for bis(*p*-nitrophenyl)phosphate (BNPP) [25,71], then proposals for the hydrolysis could include (i) an additional binding site for a potentially nucleophilic aqua/hydroxo moiety, necessitating a seven coordinate zinc(II) site,

(ii) exchange of the coordinated alcohol for a potentially nucleophilic water molecule, (iii) the coordinated alcohol itself is the active nucleophile, or (iv) the coordinated alcohol acts as a general base to activate a neighboring water molecule. The enhanced coordination environment appears unlikely, and previous studies have questioned the mechanistic steps required for regeneration of the active site if the alkoxide is the primary nucleophile [36]. Mechanistic pathways involving monodentate coordination of the BDNPP followed by attack of the nucleophile coordinated to the second Zn(II) site [56], or a general base acting on a bidentate substrate, appear possible [36,69,70]. The kinetically relevant pK_a (9.4) associated with the electronic and steric influences on the zinc(II) site suggest that the former pathway is more probable.

4. Conclusion

The complexes $[Zn_2(H_2\mathbf{L4})(H_2O)_2](ClO_4)$ and $[Zn_2(H_2\mathbf{L5})(CH_3CO_2)(H_2O)](PF_6)_2$possess properties analogous to hydrolase metalloenzymes [19]. The latter complex has kinetic properties and a kinetically relevant pK_a (6.6) similar to those reported for a number of related complexes and it is proposed that the active nucleophile is a Zn(II)–OH moiety [36]. Structurally $[Zn_2(H_2\mathbf{L4})(H_2O)_2]^+$ presents a crowded active site motif and a more basic kinetically relevant pK_a (9.4). For this complex monodentate coordination of the substrate followed by attack of the nucleophile coordinated to the second metal ion is proposed.

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