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Cadmium(II) Complexes: Mimics of Organophosphate Pesticide Degrading Enzymes and Metallo-β-lactamases

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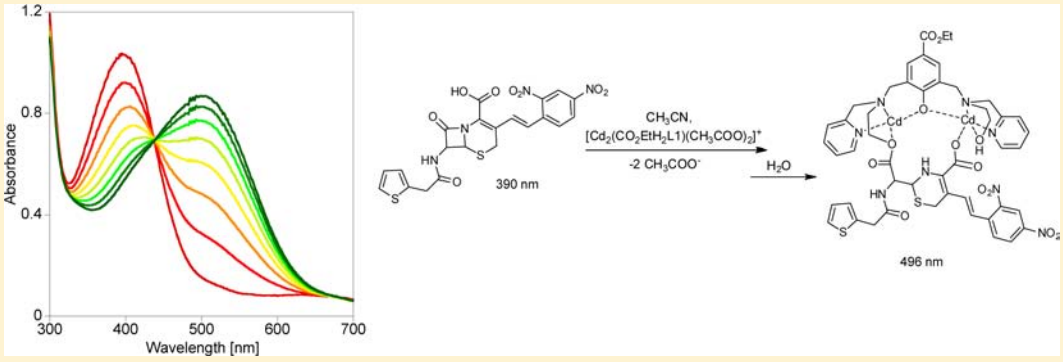
Supporting Information



ABSTRACT: Cadmium(II) complexes of ethyl 4-hydroxy-3,5-bis(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)methyl)-benzoate (CO_2EtH_3**L1**) and ethyl 4-hydroxy-3,5-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoate (CO_2EtH**L2**) are described. The two ligands possess an ethyl ester (CO_2Et-) at the position para to the phenolic −OH; CO_2EtH**L2**, with methyl ether donors in contrast to potentially nucleophilic alkoxide donors in CO_2EtH_3**L1**, offers a direct comparison of potential ligand-centered nucleophiles. The complex with CO_2EtH_3**L1** was characterized using ^1H and ^{13}C NMR spectroscopy, mass spectrometry and microanalysis; X-ray crystallography defined a tetranuclear structure $[Cd_4(CO_2EtH_2L1)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$. Functional studies of the cadmium(II) complexes were undertaken with the substrates bis(2,4-dinitrophenyl)phosphate (BDNPP), and nitrocefin to assess their phosphatase and β-lactamase activities, respectively. The complexes with CO_2EtH_3**L1** and CO_2EtH**L2** are competent phosphoesterase mimics with K_M = 9.4 ± 2.1 mM and 10.1 ± 3.4 mM, k_{cat} = 9.4 ± 0.2 × 10^{-3} s^{-1} and 9.7 ± 2.7 × 10^{-3} s^{-1}, respectively. Use of a solvent mixture containing $H_2{}^{18}O/H_2{}^{16}O$ in the reaction with BDNPP showed that for the complex with CO_2EtH_3**L1** the ^{18}O label was incorporated in the reaction product suggesting that the nucleophile involved is a Cd−OH moiety and not a metal bound alkoxide; for CO_2EtH**L2** the presence of the methyl-ether dictates that the active nucleophile must also be a hydroxide. The cadmium(II) complex with CO_2EtH_3**L1** was furthermore found to be a competent β-lactamase mimic with k_{cat} = 1.39 × 10^{-2} ± 3 × 10^{-3} s^{-1}, K_M = 0.11 ± 0.03 mM, and pK_a = 7.9 ± 0.1. Mass spectral evidence suggested that the active nucleophile in this reaction is the alkoxide; lack of β-lactamase activity of the complex with CO_2EtH**L2** supports this assignment. Similar to enzyme-catalyzed reactions, a blue reaction intermediate in the β-lactamase reaction of the CO_2EtH_3**L1** complex was also identified. It is proposed that the Cd(II) complexes of CO_2EtH_3**L1** and CO_2EtH**L2** react identically as phosphatases, with a terminal hydroxide as the nucleophile; the former exhibits β-lactamase activity with the alkoxide as a nucleophile, while the latter, without a potentially nucleophilic alkoxide, is inactive.

INTRODUCTION

The structurally diverse dinuclear metallohydrolases include ureases, the purple acid phosphatases (PAPs), phosphotriesterases, exonucleases and metallo-β-lactamases (Mβ Ls), among others.[1−4] These metalloenzymes employ a dinuclear metal ion center to catalyze the hydrolysis of amides and esters of carboxylic and phosphoric acids.[1] Interest in these systems arises from their potential as targets for drug design and application in bioremediation.[1,2,5,6] The metallohydrolases display similarities in the first coordination sphere across the entire family but exhibit variations in metal ion specificity and basic mechanism.[1] Thus, the identity of the metal ions can vary from the well characterized Fe(III)−Fe(II) and Fe(III)−Zn(II)/Mn(II) centers in PAPs and putative Fe(II)−Zn(II) centers in phosphotriesterases to the di-Zn(II) combination in Mβ Ls.[1,7−11] In some cases, the enzymes are promiscuous and activity can be reconstituted with a range of divalent metal

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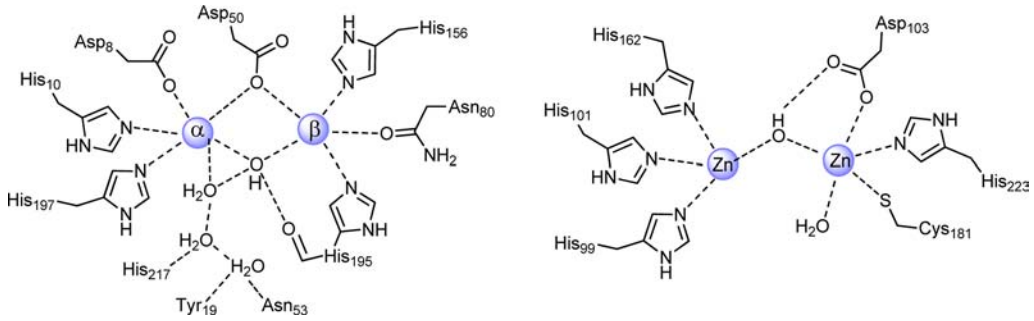
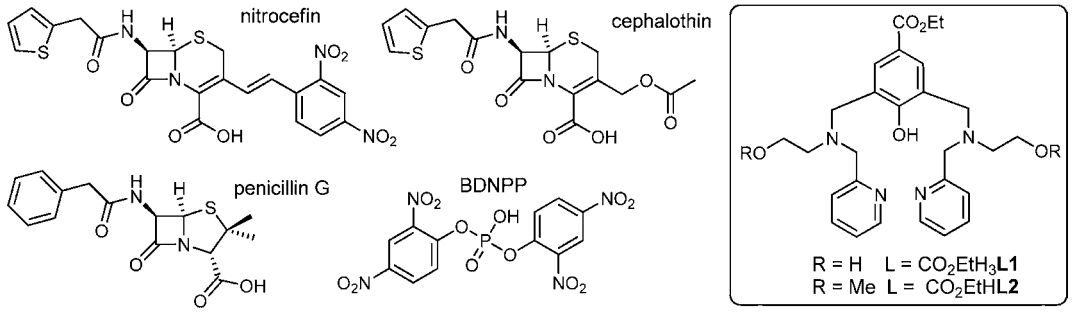


Figure 1. Active site of GpdQ and part of the hydrogen bond network (left) and the active site of the metallo-β-lactamase from *B. fragilis* (right).

Chart 1. Substrates Commonly Used in β-Lactamase and Phosphatase Assays and the Structures of Co_2EtH_3L1 and CO_2EtHL2



ions.[12,13] Of particular interest in these systems is the identity of the attacking nucleophile. Thus, for the PAPs proposals for the nucleophile include a μ-OH, a quasi-terminal OH, a terminal OH bound to the Fe(II) site and a second coordination sphere nucleophile.[1,14−16] For the phosphotriesterases pathways involving μ- and terminal-OH are proposed,[13,17] and a metal bound OH is proposed as the nucleophile in the MβLs.[11]

Our current interest is focused on organophosphate-degrading phosphotriesterases and antibiotics-inactivating MβLs. In the former case we are specifically interested in a glycerophosphodiesterase from *Enterobacter aerogenes* (GpdQ), a highly promiscuous phosphoesterase with activity toward mono-, di- and triphosphoesters including the toxic EA 2192, a product of VX degradation, and organophosphate pesticides.[5,18−22] The active site of GpdQ contains two metal ions with a buried α site and a more solvent exposed β site.[1,23,24] The metal ions are coordinated by four histidines, two aspartates, and an asparagine residue.[1,23,24] Enzymatic activity in GpdQ can be reconstituted in the presence of Zn(II), Co(II), Mn(II), and Cd(II).[18,20,25,26] MβLs catalyze the hydrolytic cleavage of the β-lactam ring of antibiotics, such as the penicillins.[27−29] The MβLs use zinc ions in vivo to gain full catalytic activity. The two metal ions are usually bridged by a hydroxide and have a 4-, 5-mixed coordination sphere, although there is discussion as to the absolute requirement for the second metal ion.[11,30,31] The active site structures of GpdQ and MβL, from *B. fragilis*, are shown in Figure 1.

To study these metallohydrolases effective use has been made of model systems. Model complexes are capable of reproducing electronic, structural and reactivity characteristics of metalloenzyme systems and although they generally exhibit less substrate specificity they are often more robust than the metalloenzymes themselves.[32−37] The nucleophilic agent in these models is typically a metal-bound hydroxide; there are examples where the nucleophilic agent varies from μ−OH to terminal OH for the same substrate but different model systems.[38,39] Furthermore, examples of model systems with either, or both, a metal−OH and a metal-OR implicated in the mechanism have been reported.[32,40−43]

While the rationale for the use of zinc(II) in model systems is obvious, cadmium(II) is not commonly considered to be a biological metal ion. The recent discovery of a carbonic anhydrase from the marine diatom *Thalassiosira weissflogii* with cadmium bound in the active site has prompted interest in this metal ion.[44,45] Recently, we showed that $(Cd^{2+})_2$-GpdQ and $[Cd_2((HP)_2B)(OAc)_2(OH_2)](PF_6)$ (((HP)$_2$B) = 2,6-bis([(2-pyridylmethyl)(2-hydroxyethyl)amino]methyl)-4-methylphenol[43]) are active catalysts toward the phosphodiester substrates bis-(4-nitrophenyl)phosphate (BNPP) and bis-(2,4-dinitrophenyl)phosphate (BDNPP), and although not approaching the efficiency of the metalloenzyme itself, our interest was aroused by the relative efficiency of the cadmium(II) model system.[25]

To expand the study of cadmium model complexes the synthesis and structural characterization of a cadmium containing system based on ethyl 4-hydroxy-3,5-bis(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoate (CO_2EtH_3**L1**) is reported. We discuss an investigation of the phosphoesterase and MβL activity of the complex using the substrates BDNPP and nitrocefin, a β-lactam compound, respectively. In addition, a second ligand, ethyl 4-hydroxy-3,5-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-benzoate (CO_2EtH**L2**) and its cadmium(II) complex were synthesized to investigate the mechanism in the absence of potential alkoxide nucleophiles. The structures of the substrates and ligands employed in this work are shown in Chart 1.

MATERIALS AND METHODS

General Methods. Nuclear magnetic resonance (NMR) spectra were measured with Bruker AV300, AV400, AV500, and AV600 instruments. The spectra were recorded in $CDCl_3$, $(CD_3)_2CO$, $(CD_3)_2SO$, CD_3OD, CD_3CN, or D_2O. Chemical shifts were

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determined in ppm, relative to known residual solvent peak references. ^{113}Cd NMR was measured with the Bruker AV400 instrument and an operating frequency of 89 MHz; Cd-shifts were referenced to cadmium(II) acetate dihydrate in D_2O (−46 ppm). Coupling constants are given in Hz. ^{31}P NMR spectra were recorded with a 600 MHz Bruker AV600 spectrometer in the digital acquisition mode (operating frequency 242 MHz) at room temperature at the NMR facilities of the Anorganisch-Chemisches Institut, University of Heidelberg. Chemical shifts are reported in δ units relative to 85% H_3PO_4 in D_2O as external reference (δ_P = 0.00). Two-dimensional correlation spectroscopy (COSY), heterobinuclear single quantum correlation (HSQC) and heterobinuclear multiple bond connectivity (HMBC) experiments were used to assign each signal in selected spectra. Low resolution mass spectral 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. Predicted isotopic splitting patterns of peaks were calculated using the program ChemCalc.[46] Elemental analyses were performed with a Carlo Erba NA 1500 Elemental Analyzer. UV−vis spectrophotometric experiments were performed with a Varian Cary50 Bio UV/Visible spectrophotometer with a Peltier temperature controller in 10 mm quartz cuvettes. General FT-Infrared Spectroscopy was carried out with a Perkin-Elmer FT-IR Spectrometer SPECTRUM 2000 with a Smiths DuraSamplIR II ATR diamond window.

Synthesis of Ethyl-4-hydroxy-3,5-bis(hydroxymethyl)-benzoate. The compound was prepared by a modification of a previously published procedure.[47] To a cool 12% aqueous solution of NaOH (70 mL, 0.21 mol), commercially available ethyl 4-hydroxybenzoate (15 g, 0.09 mol) was added at 0 °C. An aqueous formaldehyde solution (37%, 60 mL, 2.16 mol) was added and the reaction was stirred at 55 °C for 3 days. The red solution was allowed to reach room temperature and ethyl acetate (100 mL) was added. The organic layer was discarded and ethyl acetate (100 mL) and a saturated aqueous solution of NH_4Cl were added to the remaining aqueous phase. The organic phase was collected and dried over Na_2SO_4. Removal of the solvent in vacuo left an orange solid which was recrystallized from $CHCl_3$/MeOH (1:1) to yield a pale yellow powder (Yield 11.7 g, 51.7 mmol, 57%). ^{1}H- NMR (CD_3OD, 400.13 MHz); δ 1.37 (t, 3H, CH_2CH_3, J = 7.1 Hz); 4.32 (q, 2H, CH_2CH_3, J = 7.1 Hz); 4.80 (s, 4H, CH_2OH); 7.71 (s, 2H, Ar−H). ^{13}C NMR (CD_3OD, 100.62 MHz) δ 14.7 (CH_2CH_3); 61.6 (CH_2CH_3); 61.8 (CH_2OH); 122.4 (arCCO_2Et); 128.4 (arCH); 129.6 (arCCH_2OH); 159.4 (arCOH); 168.5 (CO_2Et). ESI mass spectrometry (methanol) m/z 153.10 $[C_8H_8O_3+H]^+$. FT-IR spectroscopy (ν, cm^{-1}) 3428 (m, O−H str); 2985, 2942, 2871 (w, CH_2 str); 1689 (s, C═O str); 1206 (s, C−O str); 1010 (m, C−O str). Melting Point 137.6−139.4 °C; 139.0 °C (benzene/petroleum ether);[47] 137.0−138.0 °C (chloroform).[48]

Synthesis of *N*-(2-Pyridylmethyl)-2-aminoethanol. The compound was prepared by a modification of previously published procedures.[49,50] Pyridine 2-carboxaldehyde (4.42 g, 0.04 mol) in methanol (10 mL) was added dropwise to ethanolamine (2.42 mL, 0.04 mol) in methanol (20 mL) at 0 °C. The yellow solution was brought to room temperature and stirred for two hours. Sodium borohydride (3.60 g, 0.1 mol) was added in small portions at 0 °C and the solution stirred for a further two hours. Water (30 mL) was added, the reaction mixture concentrated to approximately 30 mL and the remaining solution extracted with dichloromethane (3 × 20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent removed in vacuo to yield a yellow oil (Yield 2.50 g, 16.4 mmol, 41%). ^{1}H NMR ($CDCl_3$, 400.13 MHz); δ 2.14 (s, 1H, N*H*); 2.91 (t, 2H, NCH_2CH_2, J = 5.7 Hz); 3.79 (t, 2H, CH_2OH, J = 5.1 Hz); 5.27 (s, 2H, arCH_2N); 5.40 (bs, 1H, O*H*); 7.26 (m, 1H, py*H*, J = 6.2 Hz); 7.63 (d, 1H, py*H*, J = 7.7 Hz); 7.69 (tt, 1H, py*H*, J = 7.8, 1.8 Hz), 8.55 (dd, 1H, py*H*, J = 4.9 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 56.9 (NCH_2CH_2); 58.2 ($arCH_2N$); 59.7 (CH_2 OH); 123.2 (pyCH); 123.2 (pyCH); 137.3 (pyCH); 149.4 (pyCH); 153.8 ($pyCCH_2$). ESI mass spectrometry (methanol) m/z 175.07 $[C_8H_{12}N_2O+Na]^+$; 153.11 $[C_8H_{12}N_2O+H]^+$. FT-IR spectroscopy (ν, cm^{-1}) 3214 (m, O−H str); 2919 (m, C−H str); 2850 (m, C−H str); 1596 (m, C═C str); 1433 (m, O−H def); 832, 763 (s, py-H).

Synthesis of 2-Methoxy-*N*-(pyridin-2-ylmethyl)-aminoethanol. The compound was prepared according to a previously published procedure.[32]

Synthesis of Ethyl 3,5-Bis(bromomethyl)-4-hydroxybenzoate. The compound was prepared by a previously published procedure.[51]

Synthesis of Ethyl 4-Hydroxy-3,5-bis(((2-hydroxyethyl)-(pyridin-2ylmethyl)amino) methyl)benzoate (CO_2EtH_3L1). Ethyl 3,5-bis(bromomethyl)-4-hydroxybenzoate (0.50 g, 14 mmol) in dichloromethane (4 mL) was added dropwise to *N*-(2-pyridylmethyl)-2-aminoethanol (0.43 g, 28 mmol) and triethylamine (0.80 g) in tetrahydrofuran (4 mL) at 0 °C. The resulting yellow solution was stirred for 48 h, filtered to remove the white precipitate of triethylamine hydrobromide and the solvent removed under vacuum. The resulting brown oil was purified by flash column chromatography (EtOAc/MeOH, 8:2, I_2 stain, R_f = 0.44) giving a yellow oil (Yield 0.50 g, 1.0 mmol, 72%). ^{1}H NMR ($CDCl_3$, 500.13 MHz); δ 1.32 (t, 3H, CH_2CH_3, J = 7.1 Hz); 2.67 (t, 4H, NCH_2CH_2, J = 5.0 Hz); 3.67 (t, 4H, NCH_2CH_2, J = 5.0 Hz); 3.77 (s, 4H, arCH_2N); 3.83 (s, 4H, NCH_2py); 4.26 (q, 2H, CH_2CH_3, J = 7.1); 7.09 (m, 2H, py*H*, J = 5.8, 0.8 Hz); 7.20 (d, 2H, py*H*, J = 7.8 Hz); 7.54 (td, 2H, py*H*, J = 7.6, 1.7 Hz); 7.64 (s, 2H, ar*H*); 8.51 (dq, 2H, py*H*, J = 4.8, 0.8 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 14.4 (CH_2CH_3); 55.8 (arCCH_2), 56.5 (NCH_2CH_2); 58.5 (NCH_2CH_2); 58.8 (NCH_2py); 60.6 (CH_2CH_3); 122.5 (pyC); 123.1 (arCCO_2Et); 123.2 (arCCH_2); 123.4 (pyC); 131.8 (arCH); 137.0 (pyC); 149.1 (pyC); 157.3 ($pyCCH_2$); 160.9 (arCOH); 166.3 (CO_2Et). FT-IR spectroscopy (ν, cm^{-1}) 3296 (m, O−H str); 2826 (m, C−H str); 1704 (m, C═O str); 1595 (m, C═C str); 1571 (m, C═C str); 1304 (m, C−O sym def); 1204 (s, C−O asym def); 1026 (m, C−O str); 758 (s, py-H). ESI mass spectrometry (methanol) m/z 495.19 $[C_{27}H_{34}N_4O_5 + H]^+$.

Synthesis of Ethyl 4-Hydroxy-3,5-bis(((2-methoxyethyl)-(pyridin-2-ylmethyl)amino) Methyl)Benzoate (CO_2EtHL2). To a solution of 2-methoxy-*N*-(pyridin-2-ylmethyl)aminoethanol (2.8 g, 16.8 mmol) and triethylamine (3.96 g) in tetrahydrofuran (25 mL) a solution of ethyl-3,5-bis(bromomethyl)-4-hydroxybenzoate (2.5 g, 7.1 mmol) in dichloromethane (25 mL) at 0 °C was added dropwise. The reaction mixture was stirred for 48 h, concentrated to 4 mL in vacuo and filtered to remove the precipitated triethylamine hydrobromide. Removal of the solvent resulted in a brown oil, which was further purified by flash column chromatography (2.5 g crude ligand, l=30 cm, Ø=1.5 cm, MeOH/EtOAc 2:8 (1 L), MeOH/EtOAc 2:1 (300 mL), MeOH (300 mL), fraction volume 10 mL, 50 fractions collected. $FeCl_3$ stain, R_f = 0.46 in MeOH/EtOAc 2:8). The ligand was obtained as a yellow oil (Yield 2.19 g, 4.2 mmol, 60%). ^{1}H NMR ($CDCl_3$, 300.13 MHz); δ 1.34 (t, 3H, CH_2CH_3, J = 7.1 Hz); 2.73 (t, 2H, NCH_2CH_2, J = 5.7 Hz); 3.24 (s, 6H, OCH_3); 3.49 (t, 2H, NCH_2CH_2, J = 5.7 Hz); 3.81 (s, 2H, arCH_2N); 3.85 (s, 2H, NCH_2py); 4.30 (q, 2H, CH_2CH_3, J = 7.1 Hz); 7.10 (ddd, 2H, py*H*, J = 7.4, 4.9, 1.1 Hz); 7.42 (d, 2H, py*H*, J = 7.8 Hz); 7.57 (td, 4H, py*H*, J = 7.7, 1.3 Hz); 7.83 (s, 2H, ar*H*); 8.48 (dq, 2H, py*H*, J = 4.9, 1.7, 0.8 Hz). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 14.2 (CH_2CH_3); 53.1 (NCH_2CH_2), 55.0 ($arCCH_2$); 58.5 (OCH_3); 60.4, 60.5 (CH_2CH_3/NCH_2py); 70.7 (NCH_2CH_2); 120.4 (pyC); 121.5 ($arCCO_2Et$); 122.9 ($arCCH_2$); 123.6 (pyC); 130.4 (arCH); 136.4 (pyC); 148.6 (pyC); 158.5 ($pyCCH_2$); 160.3 (arCOH); 166.5 (CO_2Et). FT-IR spectroscopy (ν, cm^{-1}) 2927 (m, C−H str); 1705 (m, C═O str); 1602 (m, C═C str); 1197 (m, C−O−C str): 1109 (m, C−O str); 832 (m, Ar−H); 762 (s, py-H). ESI mass spectrometry (methanol) m/z 523.21 $[C_{29}H_{38}N_4O_5 + H]^+$.

Synthesis of $[Cd_4(CO_2EtH_2L1)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$. CO_2EtH_3**L1** (50 mg, 0.1 mmol) was dissolved in MeOH (2 mL) and a methanol solution (2 mL) of cadmium(II) acetate dihydrate (50 mg, 0.2 mmol) with sodium acetate (8 mg, 0.1 mmol) was added dropwise. Sodium hexafluorophosphate (50 mg, 0.3 mmol) was added and the resulting yellow solution left to evaporate at room temperature. A white product was obtained after 5 days. Recrystallization from methanol resulted in white crystals of the complex (Yield 40 mg, 0.02

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mmol, 66%). Microanalysis $C_{61.5}H_{81.25}Cd_4Cl_{0.25}N_8O_{19.5}P_2F_{12}$: calc. C: 37.06, H: 4.11, N: 5.62; found: C 37.43, H 4.28, N 5.63%. ESI mass spectrometry (methanol) *m/z*: 837.0 Calc for $[C_{31}H_{39}Cd_2N_4O_9]^+$, *m/z* 837.08. FT-IR spectroscopy (ν, cm^{-1}): 3173 (b, O−H str in water) 2906 (w, C−H str), 1670 (m, C═O str), 1604 (m, asym str OAc); 1546 (m, asym str OAc); 1413 (m, sym str OAc); 1373 (m, sym str OAc). 1015 (w, C−OH str), 831 (s P−F str), 766 (m, py C−H def), 556 (s, P−F). ^{1}H NMR (CD_3CN/D_2O 1:1, 500.13 MHz, referenced to D_2O); δ 1.70 (t, 3H, CH_2CH_3, J = 7.12 Hz); 2.39 (s, 6H, acetateCH_3); 3.28 (t, 4H, NCH_2CH_2, J = 8.40 Hz); 3.43 (m, 2H, CH_2, J = 11.60 Hz); 4.13 (m, 4H, CH_2CH_2OH); 4.15−4.23 (m, 2H, CH_2); 4.45 (m, 2H, CH_2, J = 5.85 Hz); 4.59−4.66 (m, 4H, CH_2/CH_2CH_3), 7.39 (d, 2H, py*CH*, J = 7.85 Hz); 7.58 (t, 2H, py*CH*, J = 6.10 Hz); 7.77 (s, 2H, ar*CH*); 8.00 (t, 2H, py*CH*, J = 7.72 Hz); 8.70 (d, 2H, py*CH*, J = 4.70 Hz). ^{13}C NMR (CD_3CN, $/D_2O$ 1:1, 100.62 MHz, referenced to CD_3CN); δ 14.5 (CH_2CH_3); 22.7 (acetateCH_3); 56.9 (CH_2); 57.7 (CH_2); 58.3 (CH_2); 58.7 (CH_2); 61.6 (CH_2); 124.4 (pyCH); 124.5 (pyCH); 125.5 (ar*C*); 134.4 (ar*CH*); 140.1 (pyCH); 148.8 (pyCH); 156.1(py*C*); 161.1 (ar*C*OH); 166.2 (CO_2Et); 181.8 (CO_2^-); (the arCCO_2Et signal was not assigned due to overlapping with the solvent signal).^{113}Cd NMR (CD_3CN/D_2O 1:1, 89 MHz) δ 36.4.

Synthesis of $[Cd_2(CO_2EtL2)(CH_3COO)_2](PF_6)$. Et$CO_2$H**L2** (180 mg, 0.34 mmol) was dissolved in methanol and cadmium(II) acetate dihydrate (183 mg, 0.68 mmol) and sodium acetate (56 mg, 0.68 mmol) added. The yellow solution was subsequently refluxed for 30 min and then allowed to cool to room temperature and sodium hexafluorophosphate (115 mg, 0.68 mmol) added. After filtration, the yellow solution was left to evaporate at room temperature to leave a yellow oil. Attempts to crystallize the complex from a range of solvents were unsuccessful. After repeated (5x) evaporations of the methanolic complex solution a white powder was obtained. FT-IR spectroscopy (ν, cm^{-1}): 3385 (b, O−H str in water) 2927 (w, C−H str); 1702 (m, C═O str); 1603 (m, asym str OAc); 1574 (m, asym str OAc); 1441 (m, sym str OAc); 1368 (m, sym str OAc). 833 (s, P−F str). ESI mass spectrometry (methanol) found *m/z* 864.0 (100%), 863.0 (90%) $[C_{33}H_{43}Cd_2N_4O_9]^+$ calc *m/z* 865.11 (100.0%), 863.11 (88.3%), 864.11 (86.7%).^{1}H NMR (CD_3CN/D_2O 1:1, 500.13 MHz, referenced to D_2O); δ 1.84 (t, 3H, CH_2CH_3, J = 7.12 Hz); 2.46 (s, 6H, acetateCH_3); 3.33 (m, 2H, CH_2); 3.43 (m, 1H, CH_2); 3.63−3.77 (m, 7H, CH_2); 3.79 (s, 6H, OCH_3); 3.93−4.49 (m, 6H, CH_2); 4.75 (q, 2H, CH_2CH_3, J = 7.16 Hz); 7.57 (m, 1H, py*CH*); 7.76 (m, 2H, py*CH*); 7.87 (t, 1H, py*CH*, J = 6.79 Hz); 7.97, 8.12 (s, 2H, ar*CH*); 8.19 (m, 1H, py*CH*); 8.32 (m, 1H, py*CH*); 8.83 (m, 1H, py*CH*); 8.94 (m, 1H, py*CH*). ^{13}C NMR (CD_3CN, $/D_2O$ 1:1, 100.62 MHz, referenced to CD_3CN); δ 14.6 (CH_2CH_3); 22.8 (acetateCH_3); 56.3 (CH_2); 59.9 (OCH_3); 60.5 (CH_2); 60.7 (CH_2); 61.8 (CH_2); 68.2 (CH_2); 68.5 (CH_2); 124.6 (pyCH); 124.9 (pyCH); 125.5 (ar*C*); 125.7 (ar*C*); 134.4 (ar*CH*); 134.8 (ar*CH*); 140.3 (pyCH); 140.6 (pyCH); 148.8 (pyCH); 149.3 (pyCH); 155.9 (py*C*); 168.5 (ar*C*OH); 171.0 (CO_2Et); 181.1 (CO_2^-); ^{113}Cd NMR (CD_3CN/D_2O 1:1, 89 MHz) δ 23.4.

Crystallographic Measurements. X-ray diffraction data for a crystal of $[Cd_4(CO_2EtH_2\mathbf{L1})_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ were collected at room temperature with an Oxford Diffraction Gemini Ultra dual source (Mo and Cu) CCD diffractometer with Cu ($\lambda_{K\alpha}$ = 1.5418 Å) radiation. The structures were solved by direct methods (SIR-92) and refined (SHELXL 97) by full matrix least-squares methods based on F^2.[52,53] These programs were accessed through the WINGX 1.70.01 crystallographic collective package.[53] All non-hydrogen atoms were refined anisotropically unless they were disordered. Hydrogen atoms were fixed geometrically and were not refined. Selected crystal data and some details of refinements are given in Supporting Information Table S1; selected bond distances and angles are presented in Table S2.

Phosphoesterase-like Activity. The phosphoesterase activity of the complexes was determined by measuring hydrolysis of the substrate BDNPP, which was synthesized after a previously published method.[54] 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 this product at 400 nm, throughout the pH range studied, is 12,100 M^{-1} cm^{-1}.[55−57] An aqueous multicomponent buffer (50 mM in each of 2-(*N*-morpholino)ethanesulfonic acid (MES; pH 3.50−6.50), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; pH 7.00−8.50) and 2-(*N*-cyclohexylamino)ethane sulfonic acid (CHES; pH 9.00−10.00) and *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS; pH 10.5−11.0), 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. The pH values refer to the aqueous component, it should however be noted that the pH of a solution of the buffer was the same within error as a 1:1 mixture of buffer and acetonitrile. Solid $[Cd_4(CO_2EtH_2\mathbf{L1})_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ was dissolved in MeCN to give a 1 mM stock solution. A 1 mM stock solution of $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ was generated in MeCN in situ by refluxing one equivalent ligand and two equivalents cadmium acetate for 30 min and confirming the successful formation by mass spectrometry. Assays conducted to investigate the pH dependence of catalytic properties were 40 μM in complex and 5 mM in BDNPP and showed no significant buffer effects. [Substrate] dependence assays were 40 μM in complex and 1−11.5 mM in BDNPP, and [complex] dependence assays were 20−120 μM in complex and 5 mM in BDNPP. Background assays of autohydrolysis in the presence of two equivalents of cadmium(II) acetate were subtracted from the data. All data were fitted by nonlinear least-squares regression analysis.

Metallo-β-lactamase-like Activity. MβL assays were conducted in the same aqueous multicomponent buffer as mentioned above with the ionic strength controlled by 250 mM $LiClO_4$. Nitrocefin was purchased from Merck, Darmstadt, Germany (Cambiochem, ≥ 95% purity). Assays were carried out at 37 °C in 50:50 MeCN:buffer, with nitrocefin initially dissolved in MeCN (10 mM) and the complex dissolved in MeCN:water (1 mM). Assays conducted to investigate pH dependence were 5 μM in complex and 50 μM in nitrocefin. [Substrate] dependence assays were 5 μM in complex and 10−50 μM in nitrocefin, and [complex] dependence assays were 20−120 μM in complex and 5 mM in nitrocefin. Background assays of autohydrolysis in the presence of two equivalents of cadmium(II) acetate were subtracted from the data. The assays were monitored for the hydrolysis of the substrate at 390 nm for 5 min and left to equilibrate for 2 min before each measurement. Since nitrocefin and its hydrolysis product both absorb at 390 nm a corrected extinction coefficient of 13 415 M^{-1} cm^{-1} was used to calculate the rate of catalysis.[38,39]

Fluoride Inhibition Studies. Two thousand equivalents NaF were added to a stock solution of $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$. The mixture was incubated at room temperature for 24 h prior to activity measurements conducted as described for nitrocefin and BDNPP hydrolysis.

^{18}O-Labeling Studies. ^{18}O-labeled water was purchased from Novachem, Victoria, Australia (97% purity). The substrate bis(2,4-dinitrophenyl)phosphate (BDNPP) was hydrolyzed in a 50:50 mixture of MeCN:buffer (50 mM, CHES, pH 9.8) in the presence of one equivalent complex. In the isotopic labeling studies the buffer solution contained 50% ^{18}O-labeled water (97% purity). For the unlabeled experiment the solution was made up of 0.005 mmol of complex in MeCN (0.3 mL), 100 mM CHES buffer pH 9.8 (0.15 mL) and distilled water (0.15 mL). To this, one equivalent BDNPP (2.5 mg) was added and the mixture was left for three days at room temperature prior to spectra recording. For the ^{18}O-labeled sample the solution was made up from a solution of 0.005 mmol $[Cd_4(CO_2EtH_2\mathbf{L1})_2CH_3$-$OO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ complex in MeCN (0.3 mL), 100 mM CHES buffer pH 9.8 (0.15 mL) and ^{18}O-water (97%, 0.15 mL). To this one equivalent BDNPP (2.5 mg) was added and the mixture was again left for three days at room temperature prior to ^{31}P NMR spectra recording.

Binding of Phosphoesters in Solution. Two methods were employed: (i) mass spectrometry and (ii) ^{113}Cd NMR. Using the solvent system employed for the kinetic studies the mass spectrum was measured in order to monitor species forming during phosphate ester hydrolysis. The complex $[Cd_4(CO_2EtH_2\mathbf{L1})_2(CH_3COO)_{3.75}Cl_{0.25}$-

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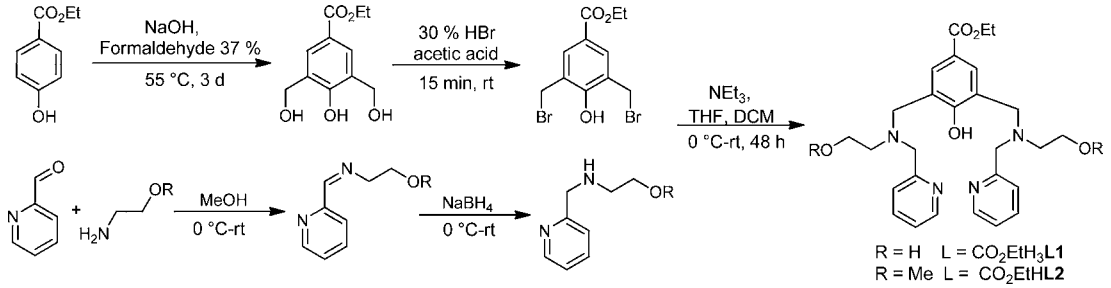

Figure 2. Synthesis of the ligands CO_2EtH_3**L1** and CO_2EtH**L2**.

$(H_2O)_2](PF_6)_2$ was dissolved in MeCN/water 1:1 and the mass spectra recorded with complex concentrations ranging from 0.1 mM to 10 μM. With the same solvent conditions the mass spectra were recorded in the presence of either 20 equiv of PNPP (4-nitrophenyl phosphate), a product mimic, or 25 equiv of DPP (diphenylphosphate), a substrate mimic lacking the activating nitro groups and which is therefore not hydrolyzed by the model complex. The solutions were left to incubate at room temperature for one hour prior to recording of spectra. A ^{113}Cd NMR spectrum of a 0.01 mM solution of $[Cd_4(CO_2EtH_2$**L1**$)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ in CD_3CN/D_2O 1:1 was recorded after the addition of 10 equiv of DPP and incubation for 6 h.

Binding of β-Lactams in Solution. Three methods were employed: (i) solution IR measurements, (ii) ^{13}C NMR, and (iii) UV−vis studies. IR spectra were recorded at the University of Heidelberg (Organisch-Chemisches Institut) with a ReactIR 45 m from Mettler Toledo between 650 and 2800 cm^{-1}. The spectra of nitrocefin alone and in the presence of 1 equiv of cadmium(II) complex were recorded in MeCN/water (50:50) at 293 K every 2 min for two hours. The initial concentrations of complex and substrate were 5 mM each. In the ^{13}C NMR experiments the solution was initially 0.075 M in penicillin G and 0.075 M of $[Cd_4(CO_2EtH_2$**L1**$)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ in $(CD_3)_2CO/(CD_3)_2SO$, 1:1. This solvent system and substrate were employed in order to directly compare the chemical shifts obtained to literature values. The spectrum was recorded at room temperature after 24 h of mixing both components. To elucidate how many molecules of nitrocefin could be hydrolyzed by each complex, a UV−vis titration experiment was conducted where successively one to four equivalents of nitrocefin were added to the complex under conditions used for kinetic experiments (MeCN/buffer pH 8, 37 °C, 300−700 nm). After each addition the solution was scanned in intervals of one minute for a total time of one hour. Initial concentrations of nitrocefin and complex were 0.05 mM.

Intermediate Species formed during the Hydrolysis of Nitrocefin. UV−vis experiments were conducted by measuring the absorption spectrum of 0.05 mM nitrocefin in dry MeCN at 37 °C and then adding one equivalent of cadmium(II) complex and recording the spectrum again after 2 min. The same conditions were applied after the addition of 10 uL water to the mixture. Mass spectra were also recorded immediately after mixing equimolar amounts of nitrocefin and cadmium(II) comlex in dry acetonitrile. Initial concentrations of complex and substrate were 0.01 mM.

RESULTS AND DISCUSSION

Ligand and Complex Syntheses. Reaction between ethyl 3,5-bis(bromomethyl)-4-hydroxybenzoate and two equivalents of *N*-(2-pyridylmethyl)-2-aminoethanol in dichloromethane/tetrahydrofuran in the presence of triethylamine, and subsequent column chromatography, resulted in the desired ligand, ethyl 4-hydroxy-3,5-bis(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoate (CO_2EtH_3**L1**). Reaction of two equivalents of 2-methoxy-N-(pyridin-2-ylmethyl)-aminoethanol with ethyl-3,5-bis(bromomethyl)-4-hydroxybenzoate yielded ethyl 4-hydroxy-3,5-bis(((2-methoxyethyl) (pyridin-2-ylmethyl)amino)methyl)benzoate (CO_2EtH**L2**). An overview of the syntheses is shown in Figure 2. The nomenclature employed follows that used previously for these types of ligands.[32] Thus, CO_2EtH_3**L1** indicates that the ligand has an ethyl ester (CO_2Et-) para to the phenolic −OH and has potentially three sites for deprotonation, the phenol and the two pendant alcohol donors. The second ligand, CO_2EtH**L2**, offers a direct comparison of methyl ether donors with the alkoxide donor in CO_2EtH_3**L1** (Figure 2). As only one proton can be potentially subtracted from the former ligand upon deprotonation, the nomenclature is adjusted accordingly. Upon crystallization, the complex with CO_2EtH_3**L1** was characterized as $[Cd_4(CO_2EtH_2$**L1**$)_2(CH_3COO)_{3.75}$ $Cl_{0.25}(H_2O)_2](PF_6)_2$, the nomenclature indicating a single deprotonation had occurred. Based on mass spectral characterization the complex with CO_2EtH**L2** was formulated as $[Cd_2(CO_2Et$**L2**$)(CH_3COO)_2](PF_6)$, where a single deprotonation of the ligand had occurred. The crystallization of this complex proved to be difficult. However after several recrystallization attempts the complex could be obtained in low yield and was analyzed further with IR and NMR spectroscopy.

Crystal Structure of $[Cd_4(CO_2EtH_2L1)_2(CH_3COO)_{3.75}$-$Cl_{0.25}(H_2O)_2](PF_6)_2$. X-ray quality crystals of a complex subsequently identified as $[Cd_4(CO_2EtH_2$**L1**$)_2(CH_3COO)_{3.75}$-$Cl_{0.25}(H_2O)_2](PF_6)_2$ were isolated after addition of methanol solution of cadmium(II) acetate in the presence of sodium acetate and sodium hexafluorophosphate and subsequent evaporation of the solvent. The chloride ion is believed to arise from contamination of the cadmium(II) starting material. The structure and atomic numbering scheme are illustrated in Figure 3.

The complex crystallized as a dimer of dimer structure with disorder apparent around the chloride and acetate ligands. The dimers are connected through the carbonyl oxygen donor of the ethyl ester bonded to one of the two cadmium(II) ions bridged by the CO_2EtH_2**L1**$^-$ ligand. The dimers are therefore connected intermolecularly "head to tail" through the carbonyl oxygen donors. The dimeric unit has two cadmium ions coordinated by one ligand molecule. The seven-coordinate Cd(1) has a distorted pentagonal bipyramidyl geometry while Cd(2) has a distorted six-coordinate geometry. One formally bidentate acetate ligand is coordinated to Cd(1) and Cd(2) is coordinated by disordered chloride and acetate ligands. The coordination sphere of Cd(1) is composed of two nitrogen donors (Cd(1)−N(1), 2.412(4) Å and Cd(1)−N(2), 2.318(4) Å), the protonated alcohol (Cd(1)−O(2), 2.475(4) Å), a water molecule (Cd(1)−O(8), 2.273(4) Å), a bidentate acetate ligand (Cd(1)−O(4), 2.384(4) Å and Cd(1)−O(5), 2.428(4)Å), the phenoxide oxygen that bridges both cadmium ions with (Cd(1)−O(1), 2.368(3) Å, Cd(2)−O(1), 2.251(3)

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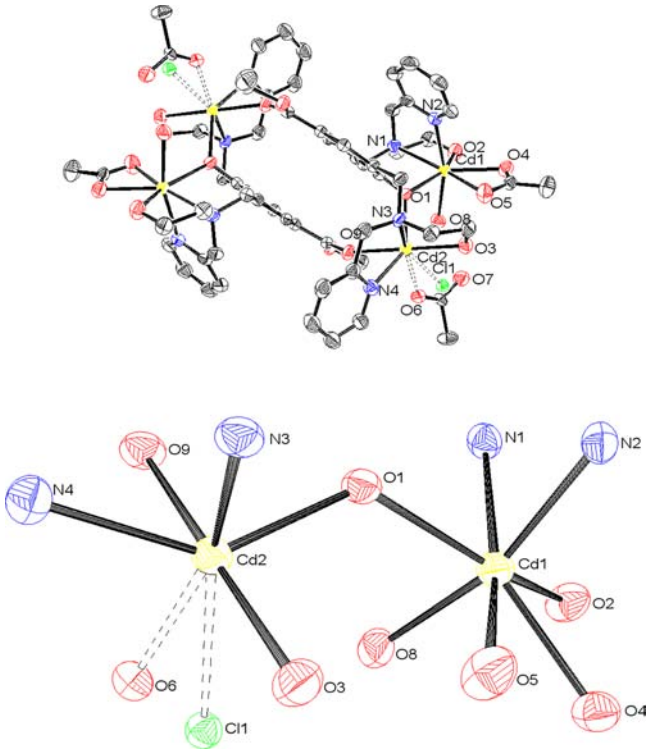

Figure 3. Structure of the tetranuclear complex and a view of the first coordination sphere. The disordered coordinating acetate oxygen and the chloride are shown with dashed bonds. Counter ions and hydrogen atoms have been omitted for clarity (25% ellipsoid probability in all ORTEP plots).

Å), Cd(1)−O(1)−Cd(2) 128.59(14)°, and Cd(1)···Cd(2) 4.162 Å. For the second metal ion, the coordination sphere is composed of two nitrogen donors (Cd(2)−N(3), 2.365(4) Å and Cd(2)−N(4), 2.374(4) Å), the protonated alcohol (Cd(2)−O(3), 2.367(4) Å) and a disordered chloride (Cd(2)−Cl(1), 2.635(13) Å; 0.18) and monodentate acetate (Cd(2)−O(6) 2.181(4) Å; 0.82). The coordination sphere of Cd(2) is completed by an interaction with the carbonyl oxygen atom of the neighboring dimer (Cd(2)−O(9), 2.413(4) Å), resulting in the dimer of dimer configuration. For both Cd(1) and Cd(2), the Cd−O bond lengths from the pendant alcohol appear typical as do the Cd−N bond lengths.[25,58−61] The alcohol arm of the ligand is protonated and the charge is balanced by two PF_6^- anions.

The structure of the cadmium(II) complex with CO_2EtH**L2** has been inferred based on spectroscopic techniques. A similar zinc(II) complex exhibited long Zn(II)-OR interactions,[32] and it is likely that in solution the ether donors are not coordinated to the cadmium(II) sites, making them more accessible to substrate (vide infra) and the complex more difficult to crystallize.

Infrared Spectrum of $[Cd_4(CO_2EtH_2L1)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$. The IR spectrum of a solid sample of the complex displays, in addition to typical ligand and hexafluorophosphate bands, distinct carbonyl stretches from the ethyl ester of the ligand and the acetate groups. The ester carbonyl stretch at 1670 cm^{-1} is at lower frequency than observed for the free ligand (1704 cm^{-1}), attributed to the weakening of the C═O bond upon coordination to cadmium(II). The asymmetric and symmetric bands from unidentate acetato ligand were observed at 1604 and 1373 cm^{-1}, respectively, with the $\Delta\nu_{asym\text{-}sym}$ = 231 cm^{-1}, while infrared bands from the bidentate nonbridging acetate ligands were assigned to 1547 and 1413 cm^{-1}, respectively, with $\Delta\nu_{asym\text{-}sym}$ = 134 cm^{-1}.[62]

Infrared Spectrum of $[Cd_2(CO_2EtL2)(CH_3COO)_2(H_2O)](PF_6)$. After repeated dissolution in hot MeOH and evaporation a white powder was obtained and the IR spectrum recorded. In addition to typical ligand and hexafluorophosphate bands, the carbonyl stretch from the ethyl ester of the ligand was observed at 1702 cm^{-1}, not very different from the free ligand (1704 cm^{-1}), suggesting that the complex exists as a single dinuclear complex and that the ester carbonyl of another unit is not coordinated to either of the metal ions. The asymmetric and symmetric bands from the acetato ligands were, however, similar to those observed for $[Cd_4(CO_2EtH_2L1)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ and are found at 1603, 1574, 1368, and 1441 cm^{-1}, respectively. Here $\Delta\nu_{asym}$ and $\Delta\nu_{sym}$ are 235 and 133 cm^{-1}, respectively, which suggest the presence of bidentate nonbridging, and unidentate acetate. Moreover, the broad band at 3385 cm^{-1} also suggests the presence of water.

Mass Spectral Characterization. The mass spectrum of the complex with CO_2EtH_3**L1** in methanol displays an isotope pattern typical of a $Cd(II)_2$ species,[63] the most prominent pattern being $[C_{31}H_{39}Cd_2N_4O_9]^+$ (*m*/*z* (100%) found 837.0, calc. *m*/*z* 837.08) assigned as $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$(Calculated and observed isotope patterns can be found in Supporting Information Figure S1). Loss of an acetate results in $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)\text{-}H]^+$ (found *m*/*z* (100%) 777.0, calc. *m*/*z* 777.06 $[C_{29}H_{35}Cd_2N_4O_7]^+$), while coordination of methanol results in $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2(CH_3OH)]^+$ (found *m*/*z* (100%) 867.0, calc. *m*/*z* 868.11 (100%), 867.1 (66%) $[C_{32}H_{42}Cd_2N_4O_{10}]^+$). A half mass peak at 605.14 (calc. *m*/*z* 606.15 (100%), 605.15 (83%)) is attributed to $([Cd_2(CO_2EtH_2$**L1**$)_2]^{2+}$; $[C_{54}H_{66}Cd_2N_8O_{10}]^{2+}$). The base peak of the spectrum measured in MeCN/water (1:1) again corresponds to $([Cd_2(CO_2EtH_2$**L1**$)_2]^{2+}$; found *m*/*z* 605.14, calc. *m*/*z* 606.15 (100%) and 605.15 (82.8%)). The mass spectrum of the complex with CO_2EtH**L2** in methanol showed the typical Cd_2 pattern, the peak at *m*/*z* 864.0 (100%), 863.0 (90%) $[C_{33}H_{43}Cd_2N_4O_9]^+$ calc. *m*/*z* 865.11 (100.0%), 863.11 (88.3%), 864.11 (86.7%) attributed to $[Cd_2(CO_2Et$**L2**$)(CH_3COO)_2]^+$ (Supporting Information Figure S2). The base peak of the spectrum in MeCN/water 1:1 corresponds to a doubly positive charged complex formed by two ligands and two cadmium ions $([Cd_2(CO_2Et$**L2**$)_2]^{2+}$; found *m*/*z* 634.2, calc. *m*/*z* 634.18 (100.0%), 633.68 (91.5%), $[C_{58}H_{74}Cd_2N_8O_{10}]^{2+}$).

NMR Spectra. The ^1H and ^{13}C NMR spectra were recorded for both complexes in a mixture of D_2O/CD_3CN 1:1. Only one resonance for the acetate methyl group was found in the ^1H NMR of $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$ and $[Cd_2(CO_2Et$**L2**$)(CH_3COO)_2]^+$ at 2.39 and 2.46 ppm, respectively. Also the ^{13}C resonances support the presence of two equivalent acetate ligands in both complexes. A single resonance at 22.7 ppm and 22.8 ppm, for the acetate-CH_3, and single acetate-carbonyl resonances at 181.8 ppm and 181.1 ppm were observed for $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$ and $[Cd_2(CO_2Et$**L2**$)(CH_3COO)_2]^+$, respectively. The spectra can be found in the Supporting Information (Figures S3 and S4). Analysis of the ^{113}Cd NMR spectrum of $([Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$ suggests a similar coordination environment for the two Cd(II) ions; only one resonance is found at 36.4 ppm. For $[Cd_2(CO_2Et$**L2**$)(CH_3COO)_2]^+$ the observation of only

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one cadmium resonance at 23.4 ppm also suggests two Cd(II) ions in a similar environment.

Binding of Phosphoester and β-Lactam Substrates to the Cadmium(II) Complex of (CO_2EtH_3L1) in Solution. To characterize the substrate binding and species present during catalysis, mass spectrometry, in-solution infrared measurements UV−vis, ^{13}C- and ^{113}Cd NMR spectroscopy were carried out with mixtures of the complex with substrates, substrate mimics and products.

After addition of $[Cd_4(CO_2EtH_2\mathbf{L1})_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$ to a solution of the product mimic 4-nitrophenol phosphate (PNPP) in acetonitrile, the mass spectrum of the mixture shows free ligand and a peak at m/z 936.03 arising from $[Cd_2(CO_2EtH_2\mathbf{L1})(PNPP)]^+$ (calc. m/z (100%) 936.03; $[C_{33}H_{37}Cd_2N_5O_{11}P]^+$), in addition to the m/z 605 peak attributed to $[Cd_2(CO_2EtH_2\mathbf{L1})_2]^{2+}$, suggesting that the latter peak is not representative of the catalytically active species but a species produced under the conditions of the mass spectrometer (Supporting Information Figure S5). Under the same experimental conditions using diphenyl phosphate (DPP), a substrate mimic that lacks the activating nitro groups and is therefore not hydrolyzed by the model complex, resulted in a peak at m/z 1215.9 assigned to $[Cd_2(CO_2EtH_2\mathbf{L1})(DPP)_2]^+$ (Supporting Information Figure S6, calc. m/z (100%) 1216.11, (88.6%) 1215.11, $[C_{51}H_{52}Cd_2N_4O_{13}P_2]^+$).

The ^{113}Cd NMR of the cadmium(II) complex with $CO_2EtH_3\mathbf{L1}$ in CD_3CN/D_2O (1:1) displayed one resonance at +36.4 ppm, assigned to the $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ species and suggesting that the two Cd(II) ions have the same coordination environment in solution (Supporting Information Figure S7). Upon addition of ten equivalents of the substrate analogue DPP, the resonance shifted to −22.6 ppm; the observation of only one resonance indicates that the substrate, or substrates, are bound symmetrically (Supporting Information Figure S7).

β-Lactams can bind to the metal complexes through their carboxylate moiety, the β-lactam amide, a sulfur atom or terminal amides.[37,39,64] A study employing the dizinc(II) complex of a pyrazolate-based bioinspired ligand and a number of β-lactam antiobiotics, as well as inhibitors including sulbactam and 2-azeidinone, has shown that while the latter was deprotonated and bound by the two zinc(II) ions the other substrates bound preferentially by the carboxylate group.[37] The binding of nitrocefin to the cadmium(II) complex was investigated by in situ FT-IR spectroscopy in MeCN/water (50:50) solution. No change of the β-lactam carbonyl absorption was observed on addition of $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$; typically a shift of the carbonyl absorption of 20−50 cm^{-1} to lower wavenumbers would be expected upon coordination to the metal ion.[64−66] Hydrolysis of nitrocefin by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ was apparent through the characteristic color change from yellow to orange and the disappearance in the IR band at 1673 cm^{-1}, attributed to the lactam carbonyl group. Changes in the IR-spectrum, however, could only be visualized in the difference spectra as the amide and carboxylate bands overlap. Similar results have been reported for the reaction of a dizinc(II) complex upon reaction with penicillin G in DMSO/water.[67] A separate investigation of the hydrolysis of cephalothin, a more "nitrocefin-like" substrate, in pure DMSO showed no significant change of the β-lactam carbonyl IR absorptions, suggesting that this substrate is binding solely via its carboxylate group to transition metals.[38]

In an attempt to monitor the binding of the carboxylate group of penicillin to $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ equimolar amounts of penicillin and the complex were mixed in $(CD_3)_2CO$:$(CD_3)_2SO$ 1:1, and after one day the ^{13}C NMR spectrum was recorded (Supporting Information Figure S8). The lactam carbonyl resonance of penicillin (173.5 ppm) had disappeared suggesting that the complex cleaved the lactam bond under these (nonbuffered, water-free) conditions. Furthermore, an additional poorly resolved, broad (169.1−171.7 ppm) resonance appeared, indicative of a newly formed ester moiety, rather than a carboxylate (Supporting Information Figure S8).

Stopped-flow measurements of nitrocefin hydrolysis by MβLs have revealed that an open-ring intermediate forms during the course of hydrolysis.[68,69] This intermediate, "the blue species",[68,70,71] has been shown in model studies with a dizinc(II) complex and nitrocefin to be composed of a species with a hydrolyzed β-lactam ring and the carboxylate thus formed bound to the zinc(II) complex; the deprotonated secondary amine of the open-ring substrate is also bound to a zinc(II) ion.[70] The blue color, with an intense absorbance at 665 nm in the MβL from *Bacteroides fragilis*[68] and 640 nm in the model system,[70] has been suggested to arise from the deprotonation of the amine nitrogen and the resulting extended conjugation.

An intermediate with an absorbance at 640 nm was observed in the UV−vis spectrum when $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ was added to a solution of nitrocefin in dry acetonitrile. Upon addition of water the blue intermediate was protonated and a characteristic absorption at 485 nm from hydrolyzed nitrocefin was apparent (Supporting Information Figure S9). The solution was further analyzed by mass spectrometry. A peak at m/z 1313.8 is attributed to a species comprised of one ligand, two cadmium ions, two acetonitrile solvent molecules and the intermediate covalently bound to the complex through the alcohol arm of the ligand (calc. m/z 1315.13 (100.0%), 1314.13 (83.5%) $[C_{48}H_{47}Cd_2N_8O_{13}S_2(CH_3CN)_2]^+$ (Figure 4). As the color change suggested, hydrolysis of the substrate occurred, but no additional hydroxide was found in the mass spectrum, so it is concluded that the substrate was hydrolyzed by the alcohol arm of the ligand and thus covalently bound after hydrolysis. A peak at m/z 1748.7 was assigned to a similar species as above but with an additional unhydrolyzed nitrocefin bound and no solvent molecules coordinated (calc. m/z 1749.12 (100.0%), 1748.12 (80.0%), $[C_{48}H_{47}Cd_2N_8O_{13}S_2(C_{21}H_{15}N_4O_8S_2)]^+$). Further addition of nitrocefin to the mass spectrum sample caused the latter peak to become the major peak in the spectrum. A blue species was not observed when $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ was added to a solution of nitrocefin in dry acetonitrile; however, the complex with one or two intact nitrocefin molecules bound could be detected in the mass spectrum (found m/z 1320.9 and 1776.7).

Phosphatase Activity. BDNPP is an activated substrate employed in studies of phosphoesterase models. The hydrolytic reaction is followed by monitoring the formation of 2,4-dinitrophenol which has an intense chromophore at 400 nm.[55,56] The phosphatase-like activity promoted by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ in 50:50 MeCN/water was measured at pH 10.4 with BDNPP (5 mM). The [complex] dependence was linear in the region 0.005−0.05 mM. The initial rate of BDNPP cleavage, determined at pH 10.4 as a function of substrate concentration, reveals typical saturation

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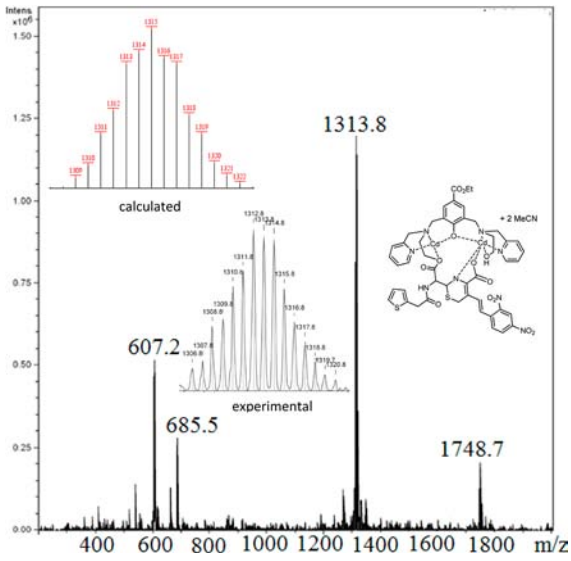

Figure 4. Mass spectrum of the blue intermediate (absorbance at 640 nm) when $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ was added to a solution of nitrocefin in dry MeCN. (peak at m/z 1313.8 is attributed to a species comprised of one ligand, two cadmium ions, two acetonitrile solvent molecules and the intermediate covalently bound to the complex through the alcohol arm of the ligand (calc. m/z 1315.13 (100.0%), 1314.13 (83.5%); peak at m/z 1748.7 assigned to a similar species as above but with an additional not hydrolyzed nitrocefin bound and no solvent molecules coordinated (calc. m/z 1749.12 (100.0%), 1748.12 (80.0%)).

behavior (Figure 5a). The data were fitted by nonlinear regression using the Michaelis–Menten equation,[72]

$$V = \frac{V_{max}[S]}{[S] + K_m} \quad (1)$$

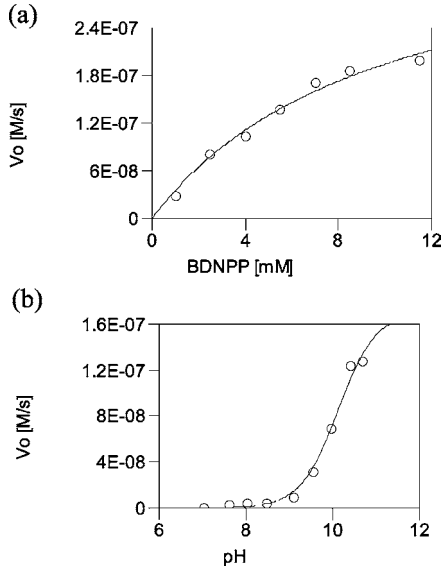

Figure 5. (a) Substrate concentration dependence for $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$, and (b) pH dependence of rate of BDNPP cleavage by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$, 25 °C, aqueous multicomponent buffer (50 mM each of MES, HEPES, and CHES), ionic strength controlled by 250 mM $LiClO_4$, 50:50 MeCN/buffer, 40 μM complex.

where $V_{max} = k_{cat}([complex])$, [S] is the substrate concentration, and K_m is the Michaelis constant. Here, $K_M = 9.4 \pm 2.1$ mM, $V_{max} = 3.76 \times 10^{-7}$ M s^{-1}, with k_{cat} ($=V_{max}/[complex]$) = $9.4 \pm 0.2 \times 10^{-3}$ s^{-1}. The dependence of catalysis on pH was measured from pH 7.0–0.7 resulting in a typical monoprotic curve exhibiting a limiting rate at high pH (Figure 5b). The data were fit to eq 2,[72]

$$V = \frac{V_{max}}{1 + \frac{[H^+]}{K_a}} \quad (2)$$

where V is the initial rate, V_{max} the limiting rate, and K_a the kinetically relevant ionization constant. The fit resulted in $pK_a = 10.1 \pm 0.6$. For $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ $pK_a = 8.7 \pm 0.6$, with $k_{cat} = 9.7 \pm 2.7 \times 10^{-3}$ s^{-1} and $K_m = 10.1 \pm 3.4$ mM (Figure S10).

Metallo-β-lactamase Activity. Nitrocefin is commonly employed in studies of β-lactamase mimics; the hydrolytic cleavage of the β-lactam ring results in a color change and the reaction is followed at 390 nm.[38,39] The pH-dependence data of the reaction of $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ with nitrocefin was monitored and the data fitted to eq 1 (Figure 6a).

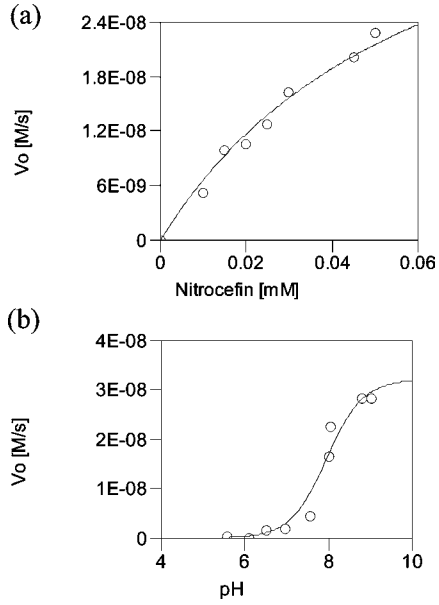

Figure 6. (a) Substrate concentration dependence for $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ and (b) pH dependence of rate of nitrocefin cleavage by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$, 25°C, aqueous multicomponent buffer (50 mM each of MES, HEPES, and CHES), ionic strength controlled by 250 mM $LiClO_4$, 50:50 MeCN/buffer, and 40 μM complex.

The pK_a of 7.9 ± 0.1, important for catalysis, is lower than that determined for the BDNPP hydrolysis. The substrate dependence was conducted at pH 8 and at 37 °C and resulted in $V_{max} = 6.97 \times 10^{-8}$ M s^{-1}, with $k_{cat} = 1.39 \times 10^{-2} \pm 3 \times 10^{-3}$ s^{-1} and $K_M = 0.11 \pm 0.03$ mM (Figure 6b). Substrate concentrations above 0.06 mM resulted in significantly decreased activity, suggesting substrate inhibition.

UV–vis titration experiments with $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ and successively one, two, three, and four equivalents of nitrocefin were undertaken (Figure 7). Reaction between the complex and one equivalent of nitrocefin was followed for approximately 60 min after which time spectral

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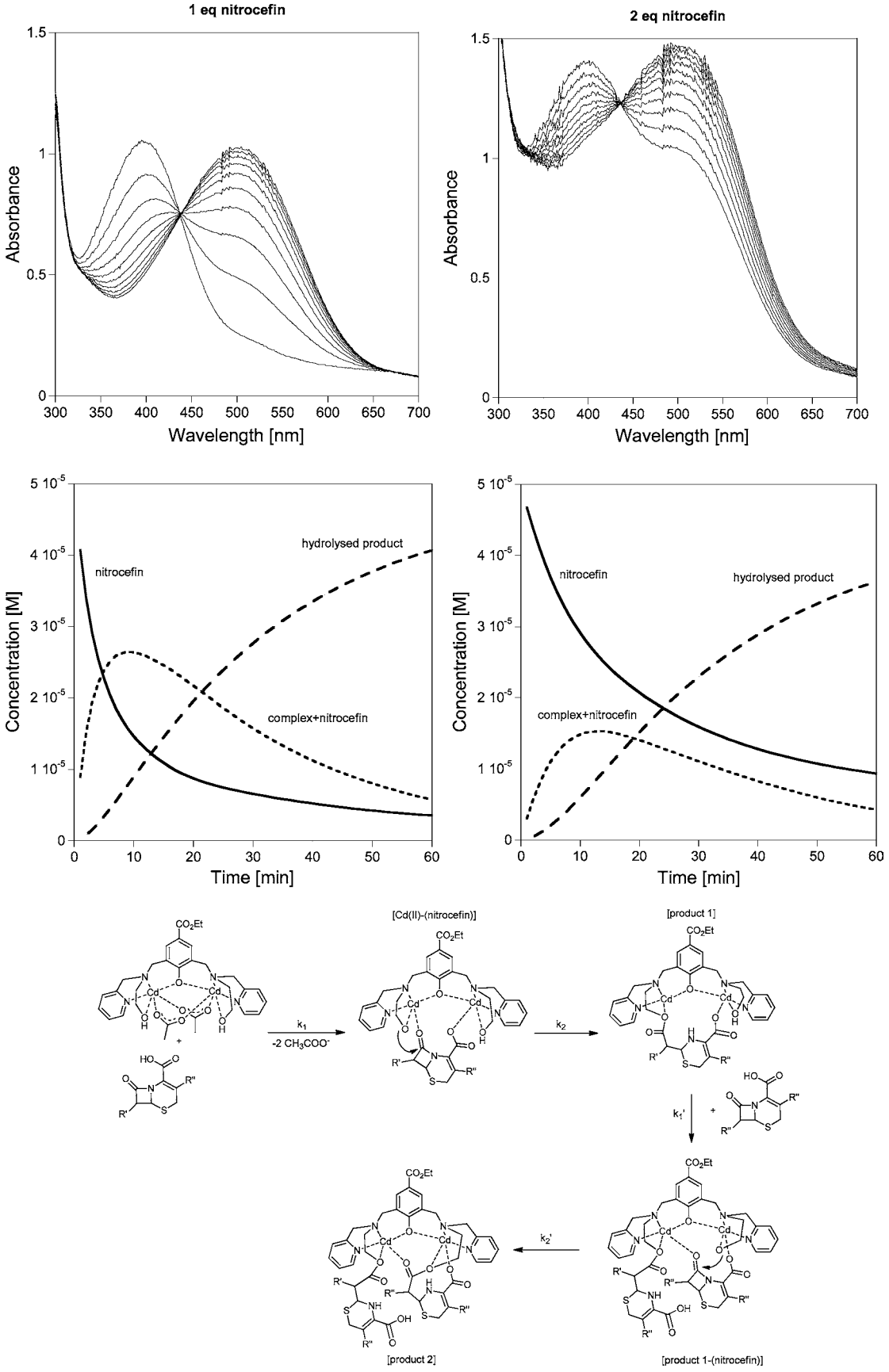

Figure 7. UV−vis spectra of the hydrolysis of successively one and two equivalents of nitrocefin by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$, pH 8, buffer:MeCN 1:1, (first ten minutes of the reaction are shown, initial concentration of complex and nitrocefin 0.05 mM; spectra recorded at 1 min intervals), 37 °C. Middle: Species distribution. Bottom: Proposed species.

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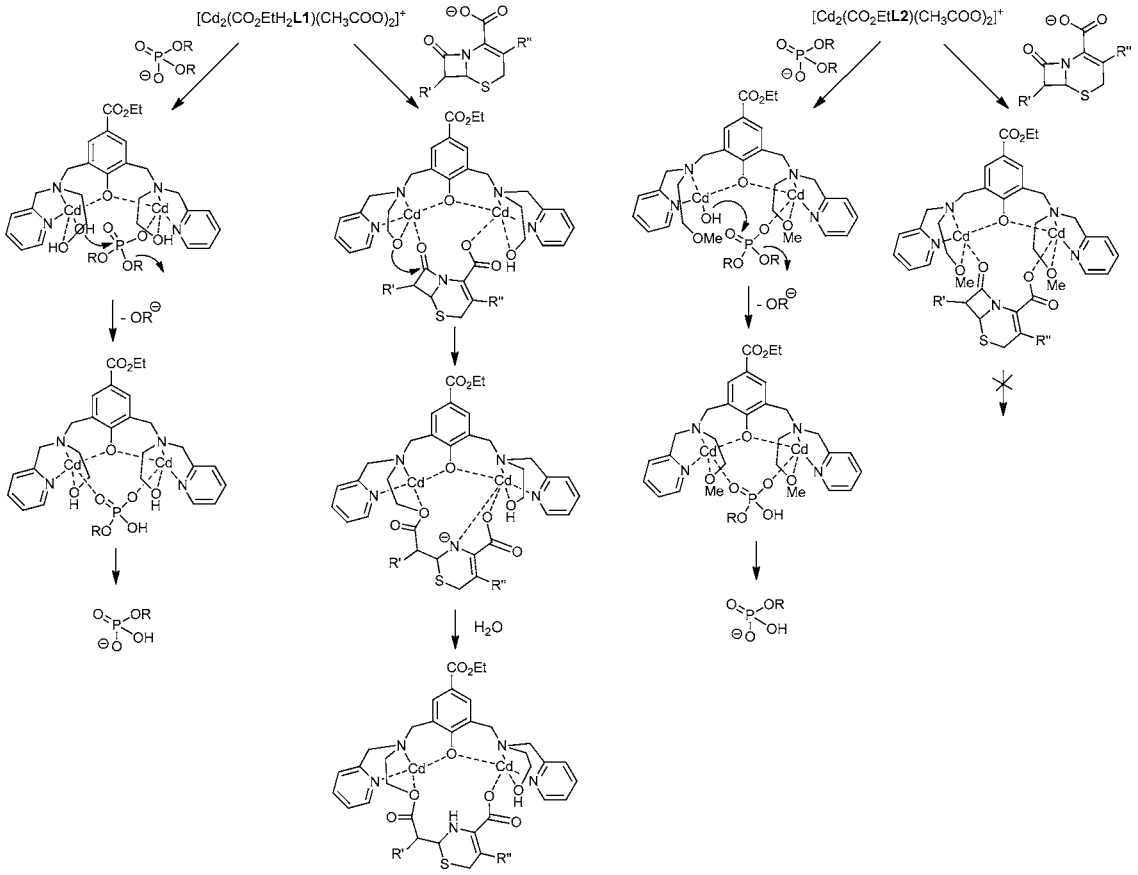

Figure 8. Proposed mechanisms for the BDNPP and nitrocefin hydrolysis by $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ and $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$. For the nitrocefin hydrolysis only the steps involving the first substrate molecule are included for clarity.

changes effectively ceased. Addition of a second equivalent of substrate to the solution again initiated changes in the spectra, which were followed until the reaction ceased. The spectral changes for the first and second equivalent of substrate were fitted separately to a model involving two consecutive reactions (Figure 7), where the [Cd(II)-(nitrocefin)] species represent a bound substrate complex and [product 1] represents a complex with the substrate hydrolyzed but still coordinated to the metal ion (product inhibition). Analysis of the data (300−700 nm; [complex] 0.05 mM, [substrate] 0.05 mM; pH 8) based on this model resulted in $k_1 = 4.8 \times 10^3 \pm 8.7\ M^{-1}\ min^{-1}$, $k_2 = 4.3 \times 10^{-2} \pm 1.4 \times 10^{-4}\ min^{-1}$, and $k_1' = 1.5 \times 10^3 \pm 9.5\ M^{-1}\ min^{-1}$, $k_2' = 6.1 \times 10^{-2} \pm 3 \times 10^{-4}\ min^{-1}$.[73] The data predict that the addition of the second substrate molecule is less facile and k_2 and k_2' are of the same order as expected if the reaction represents the hydrolysis of the lactam ring by the coordinated nucleophile. Addition of, successively, a third and fourth equivalent of nitrocefin resulted in very slow spectral changes indistinguishable from those observed for the autohydrolysis reaction. $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ showed no activity in the hydrolysis of nitrocefin over the pH range studied (pH 4.5−10).

Mechanism of Phosphodiester Hydrolysis. The absence of a metal bound alcohol in $[Cd_2(CO_2Et\mathbf{L2})(CH_3COO)_2]^+$ allows the assignment of the kinetically relevant pK_a (8.7) to a metal-bound terminal water molecule. This pK_a is similar to that reported previously for a cadmium(II) complex with pendant alcohol arms (pK_a = 8.9).[25] For $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ the pK_a of 10.1 is within the range reported for cadmium(II) complexes with either pendant hydroxyl groups or Cd(II)−OH_2 species.[25,74−79] Interestingly the complex $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ displays a pK_a similar to that reported for the Cd_2-substituted enzyme GpdQ (pK_a = 9.4).[25] For the enzyme a terminal water ligand is the proposed nucleophile.[25] To further probe the identity of the nucleophile in the phosphoester hydrolysis, an ^{18}O labeling experiment was conducted with $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$. ^{31}P NMR can be used to determine the extent of incorporation of ^{18}O-labeled water/hydroxide into the hydrolysis product of BDNPP as phosphorus signals display a high sensitivity to isotopic shifts from an ^{18}O instead of ^{16}O bound to the phosphorus.[80−82] The phosphorus resonance arising from $^{18}O-^{31}P$ is shifted to lower frequency, the shift dependent on the operating frequency.[32,81] A solvent mixture of H_2O^{16}/H_2O^{18} (50% vol) in MeCN was employed in the reaction of $[Cd_2(CO_2EtH_2\mathbf{L1})(CH_3COO)_2]^+$ with one equivalent BDNPP. After standing for three days at room temperature a resonance attributed to DNPP, the hydrolysis product of BDNPP, was observed at −3.15 ppm in the ^{31}P NMR spectrum. If the nucleophile is a cadmium(II) bound water/hydroxide, or a free water molecule from the solvent, DNPP with 50% ^{18}O incorporated is expected. This was observed in the ^{31}P-spectrum with the resonance for DNPP split into a doublet with a peak separation of approximately 16.9 Hz.[32,80−82] The result suggests that the active nucleophile in BDNPP hydrolysis is a terminally bound Cd(II)−OH. A bridging water/hydroxide as nucleophile was not considered as previous studies have shown that for a Cd(II)−(μ-H_2O)−Cd(II) complex the pK_a is <6.6.[59] Further, in the present study, a fluoride inhibition experiment did not alter the activity of the complexes; fluoride is likely to assume a bridging rather than a

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terminal position when added to the complexes.[83,84] Given that the complex with CO_2EtH**L2** can only exhibit hydrolytic activity through a coordinated hydroxide nucleophile it is proposed that in both complexes the active nucleophile is a terminal hydroxo group (Figure 8).

Other mechanistic scenarios are possible.[32,38] One would be where the alcohol arm of the ligand is actively involved in the mechanism. In this case it could be envisaged that the alcohol arm is deprotonated at pH 10.1 and acts as the primary nucleophile to hydrolyze the substrate BDNPP. The ligand is then recovered by an external water molecule and the substrate is released. A second scenario is possible where, upon deprotonation, the alkoxide acts as a general base to activate an external water molecule which then hydrolyzes the substrate. Both scenarios are consistent with the finding of a 50% ^{18}O labeled DNPP (Supporting Information Figure S11).

The differences in the pK_a observed for the complexes with CO_2EtH**L2** and CO_2EtH_3**L1**, although both are assigned to a Cd−OH_2 moiety, can be ascribed to the effect of ligand and substrate on the magnitude of the kinetically relevant pK_a. This has been illustrated previously in studies of the dizinc(II) complexes of the ligands 2,2′-(2-hydroxy-5-methyl-1,3-phenylene)bis(methylene)bis((pyridin-2-ylmethyl)azanediyl)-diethanol (CH_3H_3**L1**), 2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-4-methylphenol (CH_3H**L2**) and 4-methyl-2,6-bis(((2-phenoxyethyl)(pyridin-2-ylmethyl)amino)-methyl) phenol (CH_3H**L3**).[32,43] For $[Zn_2(CH_3H$**L1**$)(CH_3COO)(H_2O)](PF_6)$ with the substrate bis(4-nitrophenyl)phosphate (BNPP) the pK_a was reported as 7.13;[43] with substrate BDNPP the pK_a was reported to be 6.6.[32] Upon replacement of the alkoxide ligand in $[Zn_2(CH_3H$**L1**$)(CH_3COO)(H_2O)](PF_6)$ with a methyl- or phenyl-ether ($[Zn_2(CH_3$**L2**$)(CH_3COO)_2](PF_6)$ and $[Zn_2(CH_3$**L3**$)(CH_3COO)_2](PF_6)$, respectively) and with BDNPP as substrate, the kinetically relevant pK_a values were 6.7 and 7.7, respectively.[32]

Mechanism of β-Lactam Hydrolysis. The active nucleophile for the β-lactam hydrolysis by the complex formed with the ligand CO_2EtH_3**L1** could be a bridging or terminal Cd−OH_2/OH or a Cd-alkoxide, given the similarities in the pK_a values for these species.[25,74−79] The μ-OH was considered less likely given the lack of inhibitory effect of added fluoride (vide supra) and the Cd(II)···Cd(II) separation (4.162 Å). A number of findings suggest that the nucleophile is a metal bound alkoxide rather than a terminal Cd(II)−OH. First, $[Cd_2(CO_2Et$**L2**$)(CH_3COO)_2]^+$ was inactive toward lactam substrates. Second, in the mass spectrum a peak at m/z 1313.8, is assigned to a species comprised of CO_2EtH_2**L1**$^-$, two cadmium ions, two acetonitrile solvent molecules, and hydrolyzed nitrocefin, the latter proposed to be bound through the alkoxide arm of the ligand. The formation of an ester rather than a carboxylic acid was also found in the ^{13}C NMR of penicillin with the complex $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$ under water-free conditions. Alkoxide nucleophiles have been reported previously in the methanolysis of β-lactams,[85,86] and in an infrared study of the reaction of a dinuclear zinc complex with penicillin G in methanol it was observed that ester formation due to alcoholysis of zinc bound methoxide had occurred.[67] In addition, the addition of two equivalents of nitrocefin to $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$ and subsequent incubation at 37 °C for one hour resulted in a significant decrease in the ability of the complex to degrade the phosphate ester substrate BDNPP, suggesting that the bound and hydrolyzed nitrocefin sterically hinders coordination of phosphoester substrate; it is possible as well that hydrolyzed nitrocefin binds more tightly than phosphodiester substrates. Finally, the rapid decrease in activity after the complete hydrolysis of one equivalent of nitrocefin suggests the hydrolyzed substrate is bound irreversibly to the complex. A proposed mechanism for the hydrolysis of nitrocefin is shown in Figure 8.

CONCLUSIONS

The cadmium(II) complexes of two new ligands, ethyl 4-hydroxy-3,5-bis(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)-methyl)benzoate (CO_2EtH_3**L1**) and ethyl 4-hydroxy-3,5-bis-(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-benzoate (CO_2EtH**L2**) have been prepared. These complexes possess functional properties analogous to dinuclear phosphoesterases and β-lactamases. The results obtained for the hydrolysis of the activated substrate BDNPP suggest a terminal water is the active nucleophile, a conclusion based on an analysis of the incorporation of ^{18}O into the hydrolysis product and on the fact that the pK_a of a Cd(II) bound water molecule can range from 7.6−10.3.[87] Participation of the ligand alkoxide can, however, not be ruled out. The cadmium(II) complex with CO_2EtH_3**L1** also exhibits β-lactamase activity toward the substrate nitrocefin, mass spectral evidence suggesting that in this case the active nucleophile is the alkoxide group. An intermediate with an absorbance at 640 nm was observed in the UV−vis spectrum when $[Cd_2(CO_2EtH_2$**L1**$)(CH_3COO)_2]^+$ was added to a solution of nitrocefin in dry acetonitrile. A similar intermediate has been observed previously in zinc(II) model systems and in the reaction of the Mβ**L** enzyme. The cadmium(II) complex with CO_2EtH**L2**, without an alkoxide nucleophile is inactive toward this substrate under identical conditions.

ASSOCIATED CONTENT

Supporting Information

Tables S1 and S2, crystallographic data, and Figures S1−S11. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data (without structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 873960. Copies of the data can be obtained free of charge from the CCDC {12 Union Road, Cambridge CB2 1EZ, U.K.; phone (+44) 223−336−408; fax (+44) 1223−336−003; email deposit@ccdc.cam.ac.uk; Web site www.ccdc.cam.ac.uk].

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Notes

The authors declare no competing financial interest.

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