

Chapter 2

Experimental

2.1 Introduction

This chapter will give an introduction of the experimental methods used in enzyme expression and purification and the synthesis of the compounds discussed in this thesis.¹ A brief introduction will be given about the concepts of ligand design and the nomenclature used throughout this thesis.

2.2 Ligand Syntheses

2.2.1 Phenolate Based Ligands

Phenolate based molecules as templates for dinucleating ligands have been around for more than 40 years [1–3]. The term di- or binucleating ligands was first used by Robson in 1970 who reported a Schiff-base copper complex with a phenol core [3]. While the numbers of applications are vast, the purpose of the phenolic

¹ Parts of this chapter appeared in: L.J. Daumann et al., “Immobilization of the enzyme GpdQ on magnetite nanoparticles for organophosphate pesticide bioremediation” *J Inorg Biochem.* **2014**, 131, 1–7; L.J. Daumann et al., “Asymmetric Zinc(II) Complexes as Functional and Structural Models for Phosphoesterases” *Dalton Trans.* **2013**; 42, 9574–9584; L.J. Daumann et al., “Dinuclear Cobalt(II) Complexes as Metallo- β -lactamase Mimics” *Eur. J. Inorg. Chem.* **2013**, 17, 3082–3089. L.J. Daumann et al. “Synthesis, Magnetic Properties and Phosphoesterase Activity of Binuclear Cobalt(II) Complexes” *Inorg. Chem.* **2013**, 52 (4), 2029–2043; L.J. Daumann et al., “Promiscuity Comes at a Price: Catalytic Versatility vs. Efficiency in Different Metal Ion Derivatives of the Potential Bioremediator GpdQ” *BBA—Proteins and Proteomics* **2013**, 1834, 1, 425–432; L.J. Daumann et al., “Cadmium(II) Complexes; Mimics of Organophosphate Pesticide Degrading Enzymes and Metallo- β -lactamases” *Inorg. Chem.* **2012**, 51, 7669–7681; L.J. Daumann et al., “The Role of Zn-OR and Zn-OH Nucleophiles and the Influence of *p*-Substituents in the Reactions of Binuclear Phosphatase Mimetics” *Dalton Trans.* **2012**, 41, 1695–1708. L.J. Daumann et al., “Spectroscopic and mechanistic studies of dinuclear metallohydrolases and their biomimetic complexes” *Dalton Trans.* **2014**, 43, 910–928.”.

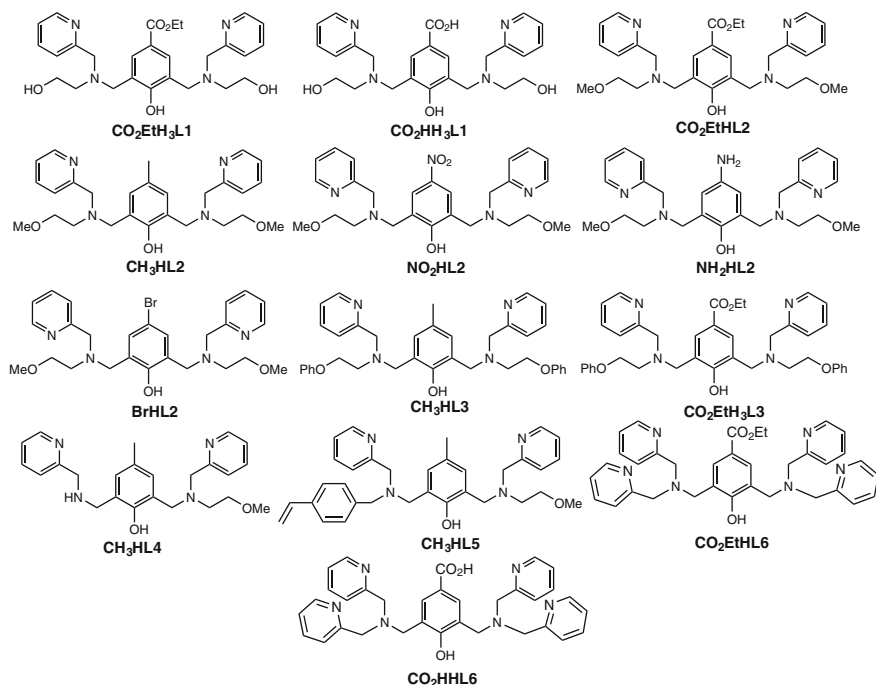


Fig. 2.1 Overview of the ligands synthesized in this work

framework that allows two metals in close proximity remains the same. The complexes of phenolate based ligands have served as biomimetic catalysts [4–12], spectroscopic models [13, 14], phosphate sensors [15], therapeutic agents [16], anionic receptors for supramolecular chemistry [17] or oxygen sensors [18] to name just a few applications. Complexes are known for almost all first row transition metals such as Co(II), Zn(II), Ni(II), Cu(II) Ti(I) V(IV)/(V) Mn(II)/(III) and Fe(II)/(III).

2.2.2 Overview of Ligands Synthesized in this Work

The new phenolate-based ligands synthesized in this work are shown in Fig. 2.1. These ligands were designed to study the influence of groups in the *para*-position to the bridging phenolic oxygen (CO₂EtHL2, CH₃HL2, NO₂HL2 and BrHL2) on magnetism (Chap. 6) and phosphoesterase activity (Chaps. 4 and 6) or to compare mechanistic implications of directly bound groups like methyl ether and alcohol (CO₂EtH₃L1 and CO₂EtHL2, Chaps. 5 and 6). Also a study of steric influences of bulky ligands arms is presented by comparing complexes of CH₃HL2 and CH₃HL3 (Chap. 4). Moreover, some ligands feature functional groups (CO₂HH₃L1,

NH₂HL**2**, CO₂HHL**5** and CH₃H₂L**4**) which allow attachment of the ligands to resins, inorganic supports such as silica and magnetite nanoparticles (Chap. 8). The pyridine residues are mimics for histidine, and the alcohol and ether arms are mimicking asparagine and aspartate residues, present in enzyme active sites. The bridging phenol mimics a bridging aspartate or hydroxide. The coordination sphere in the metal complexes will predominantly be completed by bridging acetato ligands.

2.2.3 Nomenclature

The nomenclature employed for the ligands is as follows: CO₂EtH₃L**1** denotes that the ligand has an ethyl ester (CO₂Et-) at the position *para* to the phenolic oxygen and that the ligand has potentially three sites for deprotonation, the phenol and two pendant alcohol donors. Two pyridines and two alcohol arms make up the L**1** donor atom set. The second ligand class L**2** reported in this thesis offers a direct comparison of methyl ether donors with the alkoxide donor in CO₂EtH₃L**1** and denotes symmetric ligands with two pendant pyridines and two methyl ethers. As only one proton can be potentially abstracted from the L**2** ligands upon deprotonation, the nomenclature was adjusted accordingly, e.g. CH₃HL**2**. L**3** ligands feature the more bulky phenyl ether instead of the methyl ether in L**2**. CH₃HL**4** is one of the two asymmetric ligands reported here. CH₃HL**5** is derived from the CH₃HL**4** ligand with an additional vinyl benzyl group. The class of L**6** ligands are lacking oxygen donor atoms but feature two pyridine moieties on either site of the dinucleating ligand instead.

2.2.3.1 Syntheses of the Precursors

The precursors for the phenolate-backbone of the ligands ethyl-4-hydroxy-3,5-bis(hydroxymethyl)benzoate and 4-bromo-2,6-bis(hydroxymethyl)phenol were readily derived by reacting the corresponding phenols with formaldehyde and base. Both syntheses have been reported in the literature, however, the procedure for the former was altered slightly to improve yield and purity of the precursor [19, 20]. Application of lower temperatures and longer reaction times in the formaldehyde reactions seemed superior over the higher temperatures in previously reported syntheses as those caused a significant amount of polymer by-products. Also the application of freshly opened formaldehyde solution seemed to have a positive influence of the outcome of reaction. The hydroxymethyl groups were subsequently converted into bromomethyl groups by stirring the compounds with concentrated hydrogen bromide in acetic acid, as reported previously [21]. The synthesis of 2,6-bis(chloromethyl)-4-methylphenol after Paine's method [22] was straightforward and the starting material 2,6-bis(hydroxymethyl)-4-methylphenol was commercially available. 2,6-bis(bromomethyl)-4-nitrophenol was obtained in low yield after a published multistep procedure [23]. Syntheses of the ligand arms 2-methoxy-N-(pyridin-2-ylmethyl)

aminoethanol, 2-phenoxy-N-(pyridin-2-ylmethyl)ethanamine and N-(2-pyridylmethyl)-2-aminoethanol followed a similar approach as previously reported [24, 25]. Typically pyridine-2-carboxaldehyde was reacted in methanol with methoxy-, phenoxy- or ethanolamine to give the corresponding Schiff base. The imine was then reduced with sodium borohydride to yield the crude precursors. Three things were crucial for a high yield: (i) Only a minimum amount of methanol had to be used (ii) freshly opened sodium borohydride was used, and (iii) the use of brine during the workup procedure was avoided for N-(2-pyridylmethyl)-2-aminoethanol as this decreased the yield of pure product significantly. The use of dry methanol or molecular sieves during the reaction had no positive effect on yield or purity of the products. The final ligands were usually synthesized by reacting two equivalents of ligand arm with the respective phenol-precursor in the presence of triethylamine as base in dichloromethane/tetrahydrofuran. After removal of the precipitated triethylamine hydrochloride/bromide by filtration and the solvent, the ligands were purified with flash column chromatography.

2.3 Materials and Methods: Model Complexes

^1H NMR spectra were recorded, unless otherwise stated, at room temperature with a 300, 400 or 500 MHz Bruker AV 300/400/500 spectrometer. Chemical shifts are reported in δ units relative to CHCl_3 ($\delta_{\text{H}} = 7.24$), $\text{d}^4\text{-MeOD}$ ($\delta_{\text{H}} = 3.30$), CD_3CN ($\delta_{\text{H}} = 1.93$), $\text{d}^6\text{-acetone}$ ($\delta_{\text{H}} = 3.31$) or $\text{d}^6\text{-DMSO}$ ($\delta_{\text{H}} = 2.50$). The following abbreviations were used: s = singlet, d = doublet, t = triplet, dd = doublet of doublet, dt = doublet of triplet, m = multiplet. The software used for data processing was TOPSPIN 2.1 from BRUKER [26].

^{13}C NMR spectra were recorded at room temperature with a 100 MHz Bruker AV 400/500 spectrometer. Chemical shifts are given in δ units relative to CDCl_3 (central line of triplet: $\delta_{\text{C}} = 77.0$), $\text{d}^4\text{-MeOD}$ ($\delta_{\text{C}} = 49.0$), CD_3CN ($\delta_{\text{C}} = 1.30$), D_2O with 5 % dioxane ($\delta_{\text{C}} = 67.2$). 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 [27]. ^{13}C -NMR of $[\text{Cd}_4(\text{CO}_2\text{EtH}_2\text{L1})_2(\text{CH}_3\text{COO})_{3.75}\text{Cl}_{0.25}(\text{H}_2\text{O})_2](\text{PF}_6)_2$ with penicillin: the solution was initially 0.075 M in penicillin G and 0.075 M of $[\text{Cd}_4(\text{CO}_2\text{EtH}_2\text{L1})_2(\text{CH}_3\text{COO})_{3.75}\text{Cl}_{0.25}(\text{H}_2\text{O})_2](\text{PF}_6)_2$ in deuterated DMSO/acetone 1:1. The spectrum was recorded at room temperature after 24 h of mixing of both components.

^{31}P NMR spectra were recorded at room temperature with 85 % phosphoric acid as external standard ($\delta_{\text{P}} = 0.00$). ^{31}P NMR spectra of the Cd(II) complexes were recorded with a 600 MHz Bruker AV600 spectrometer at room temperature in the digital acquisition mode (operating frequency 242 MHz) at the NMR facilities of the Anorganisch-Chemisches Institut, University of Heidelberg.

^{113}Cd NMR was measured with a Bruker AV400 instrument and an operating frequency of 89 MHz. Cd-shifts were referenced to $\text{Cd}(\text{OAc})_2 \cdot 2\text{H}_2\text{O}$ in D_2O (−46 ppm). The measurements for $[\text{Cd}_4(\text{CO}_2\text{EtH}_2\text{L1})_2(\text{CH}_3\text{COO})_{3.75}\text{Cl}_{0.25}(\text{H}_2\text{O})_2](\text{PF}_6)_2$ in presence of substrates were conducted using the following procedure: to a 0.01 mM solution of the complex in $\text{CD}_3\text{CN}/\text{D}_2\text{O}$ 1:1 were added 10 equivalents of diphenylphosphate and the solution was left to incubate for 6 h prior to spectra recording. The ^{13}C NMR spectrum of penicillin G in $\text{d}^6\text{-DMSO}/\text{d}^6\text{-acetone}$ (1:1) with one equivalent of $[\text{Cd}_2(\text{CO}_2\text{EtH}_2\text{L1})-(\text{CH}_3\text{COO})_2]^+$ was recorded 24 h after mixing.

^{18}O -labeling studies were conducted by recording the ^{31}P NMR spectra of complex/substrate mixtures with a 400 MHz Bruker AV400 spectrometer at room temperature in the digital acquisition mode (operating frequency 161.9 MHz). Chemical shifts are reported in δ units relative to 85 % H_3PO_4 in D_2O as external reference ($\delta_{\text{P}} = 0.00$). For the ^{18}O -labeled sample (50 ^{18}O ; 97 % purity) (Novachem, Victoria, Australia) the solution was made up from a solution of complex (0.01 mmol) in acetonitrile (0.3 mL), 100 mM HEPES buffer pH 8 (0.15 mL) and ^{18}O -water (97 %, 0.15 mL) (50:50 mixture of acetonitrile:buffer). To this, one equivalent BDNPP (5.1 mg) was added and the mixture left for one week at room temperature prior to recording the ^{31}P NMR spectra. For experiments with ^{16}O -water the solution was composed of complex (0.01 mmol) in acetonitrile (0.3 mL), 100 mM HEPES buffer pH 8 (0.15 mL) and distilled water (0.15 mL). To this, one equivalent BDNPP (5.1 mg) was added and the mixture left for one week at room temperature prior to recording the ^{31}P NMR spectra.

Magnetic Moments in Solution were determined with the Evans method [28] using

$$\chi_A^M = \frac{3M}{4\pi c} \left(\frac{\Delta\nu}{\nu} \right) \quad (2.1)$$

and

$$\mu = 2.828 \sqrt{\chi_A^M T} \quad (2.2)$$

and were measured for a solution of known concentration of each complex in deuterated methanol with non-deuterated methanol in the inner capillary [28]. No diamagnetic corrections were applied. The experiments were conducted on a superconducting magnet and appropriate corrections were considered in the equation [29].

Solid State Magnetic Susceptibility Measurements were conducted with a MPMS-XL 5T superconducting quantum interference device (SQUID) from Quantum Design at the University of Heidelberg in collaboration with Professor Peter Comba and recorded as a function of the applied field (0–5 T), and at temperatures ranging from 2 to 300 K (zero field cooled method). The powdered samples were pressed into a PTFE band to avoid field-induced orientation of the

powder and incorporated into two plastic straws as sample holder. The data were corrected for the diamagnetism of the PTFE band and sample holder; Pascal constant corrections for each sample were applied [30]. The program MagSaki used for the analysis of magnetic susceptibility data of the Co(II) complexes [31].

Magnetic Circular Dichroism studies were conducted at Middlebury College, Vermont, USA in collaboration with Professor Jim Larrabee. Complex samples were measured both as solid mulls (poly(dimethylsiloxane)) and as saturated ethanol solutions. Samples of air sensitive compounds were prepared in degassed ethanol. The MCD system used has a JASCO J815 spectropolarimeter and an Oxford Instruments SM4000 cryostat/magnet. Data were collected at increments of 0.5 Tesla (T) from 0 to 7.0 T and at temperatures of 1.4, 4.2, 11.3, 26 and 50 K. Each spectrum was corrected for any natural CD by subtracting the zero-field spectrum of the sample. Even when there is no sample present the instrument baseline exhibits a small deviation from zero that is both field- and wavelength-dependent. Therefore, each spectrum was also corrected by subtraction of a spectrum recorded at the same magnetic field but with no sample present. The resultant spectra were fitted to the minimum number of Gaussian peaks to achieve a satisfactory composite spectrum using the GRAMS AI software [32]. The software VTVH 2.1.1 was used to fit the data [33].

Angular Overlap Model Calculations Spectral simulations were made using the angular overlap model (AOM) using the program AOMX [34, 35]. Calculations were performed for $[\text{Co}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2](\text{PF}_6)$ and $[\text{Co}_2(\text{CO}_2\text{EtL2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$ based on the transitions obtained from the diffuse reflectance and MCD data. The coordinates were generated from the respective crystal structures, and each Co(II) metal site was treated separately. The Racah parameters C and B were fitted separately, with n in $C = nB$ varying from 4 to 4.7.

Crystallographic Data for the complexes were collected, unless otherwise stated, at room temperature with an Oxford Diffraction Gemini Ultra dual source (Mo and Cu) CCD diffractometer with Mo ($\lambda_{\text{K}\alpha} = 0.71073 \text{ \AA}$) or Cu ($\lambda_{\text{K}\alpha} = 1.5418 \text{ \AA}$) radiation. The structures were solved by direct methods (SIR-92) and refined (SHELXL 97) by full matrix least squares methods based on F^2 [36]. These programs were accessed through the WINGX 1.70.01 crystallographic collective package [37]. All non-hydrogen atoms were refined anisotropically unless they were disordered. Hydrogen atoms were fixed geometrically and were not refined. X-ray data of the published structures were deposited with the Cambridge Crystallographic Data Center.

Positive Ion Electrospray Mass Spectrometry was carried out with a Q-Star time of flight mass spectrometer and data were processed with Bruker Compass Data Analysis 4.0 software. Mass spectral studies of the organophosphate hydrolysis reaction by $[\text{Zn}_2(\text{CH}_3\text{L1})(\text{CH}_3\text{COO})_2]^+$ were conducted by mixing one equivalent of this complex with 25 equivalents of either bis(4-nitrophenyl) phosphate (BPNPP) or 4-nitrophenyl phosphate (PNPP), the latter a hydrolysis product of the former, in 1:1 acetonitrile:water, and recording the spectrum of the resulting mixture after 1 h. BPNPP and its hydrolysis product PNPP were used as they are slower substrates (due to the lack of one nitro-group) and permitted more

ready analysis than the substrate used for the kinetic studies (BDNPP). The complex $[\text{Cd}_4(\text{CO}_2\text{EtH}_2\text{L1})_2(\text{CH}_3\text{COO})_{3.75}\text{Cl}_{0.25}(\text{H}_2\text{O})_2](\text{PF}_6)_2$ was dissolved in acetonitrile: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 equivalents of PNPP (4-nitrophenyl phosphate), a product mimic, or 25 equivalents of DPP (diphenylphosphate), a substrate mimic lacking the activating nitro groups and is therefore not hydrolyzed by the model complex. The solutions were left to incubate at room temperature for one hour prior to spectra recording.

General FT-Infrared Spectroscopy was carried out with a Perkin Elmer FT-IR Spectrometer SPECTRUM 2000 with a Smiths DuraSamplIR II ATR diamond window.

Solution IR Measurements 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 spectrum of nitrocefin and in the presence of one equivalent $[\text{Cd}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2]^+$ was recorded in acetonitrile:water (50:50) at 293 K every 2 min for 2 h. The initial concentrations of complex and substrate were 5 mM each. In-solution IR spectra for the Co(II) complexes were also 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 penicillin G in the presence of one equivalent of $[\text{Co}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2]^+$ or $[\text{Co}_2(\text{CO}_2\text{EtL2})(\text{CH}_3\text{COO})_2]^+$ were recorded in dimethylsulfoxide(DMSO)/water (90:10) at 293 K every 2 min for one hour. The solvent system and the substrate penicillin, instead of nitrocefin, were chosen to directly compare the results with literature values. The initial concentrations of complex and substrate were 7 mM each.

UV-Vis Studies

Phosphatase Activity Measurements were conducted with bis(2,4-dinitrophenyl) phosphate (BDNPP) as substrate with a Varian Cary50 Bio UV/Visible spectrophotometer with a Peltier temperature controller and measured 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. Throughout the pH range studied (4.75–8.5), the extinction coefficient of this product at 400 nm varies from 7180 at pH 4.5, 10080 at 5.0; 11400 at pH 5.5 to 12000 at 6.0 and 12100 at pH 6.5–8.5 [38]. All assays were measured in 50:50 acetonitrile:buffer with the substrate and complex initially dissolved in acetonitrile. The aqueous multicomponent buffer solution was comprised of 50 mM 2-(N-morpholino)ethanesulfonic acid (MES, pH 5.50–6.70), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, pH 7.00–8.50), 2-(N-cyclohexylamino)ethane sulfonic acid (CHES, pH 9.00–10.00) and N-cyclohexyl-3-aminopropanesulfonic acid (CAPS, pH 10.5–11) with controlled ionic strength (LiClO_4) 250 mM. The pH values reported 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. Assays

for pH dependence were 40 μM complex and 5 mM BDNPP and for substrate dependence 40 μM in complex and 1–11.5 mM in BDNPP, respectively. pH-dependence data for monoprotic (Eq. 2.3) or diprotic (Eq. 2.4) events were fit to

$$v_0 = \frac{v_{\max}}{1 + \frac{[H^+]}{K_a}} \quad (2.3)$$

or

$$v_0 = \frac{v_{\max}}{\left(1 + \frac{[H^+]}{K_{a1}} + \frac{K_{a2}}{[H^+]}\right)} \quad (2.4)$$

respectively [39]. The data derived from substrate dependence were fit to the Michaelis-Menten equation (Eq. 2.5) [39]. Here, v is the initial rate, $v_{\max}$ is the maximum rate, K_m is the Michaelis constant, and $[S]$ is the substrate concentration.

$$v = \frac{v_{\max}[S]}{K_m + [S]} \quad (2.5)$$

[Complex] dependence of the catalytic rate was measured with a fixed substrate concentration at 5 mM and complex concentrations ranging from 20–120 μM . Background assays were conducted by measuring the autohydrolysis and hydrolysis by two equivalents of the free respective metal ($\text{M}(\text{OAc})_2$, $\text{M} = \text{Cd}, \text{Zn}, \text{Co}$, 2 mM) and were subtracted from the data. Where no solid could be obtained in complex synthesis a 1 mM stock solution of the respective Co(II), Cd(II) and Zn(II) complex was generated in acetonitrile in situ by refluxing one equivalent ligand and two equivalents metal acetate for 30 min and confirming the successful formation by mass spectrometry.

For the kinetic investigation of the immobilized Zn(II) complexes on Merrifield (MR) resin, an assay was established which allowed determination of the amount of BDNPP hydrolysed by the resins. In order to obtain initial rates, the absorbance of a solution of multicomponent buffer, acetonitrile and BDNPP was determined ($T = 0$), and then 0.5 mg of resin added. After 15 min 1 mL of the suspension was transferred to an Eppendorf tube and centrifuged for 10 s; the absorbance of the supernatant was measured again ($T = 15$) and the Abs/min calculated. For every value this was done in triplicate, however, due to swelling properties of the resin and inhomogeneous catalyst loading on the Merrifield beads the errors were larger than for the free complex.

Metallo- β -lactamase-like Activity Assays were conducted in the same aqueous multi-component buffer as mentioned above with the ionic strength controlled by 250 mM LiClO_4 . Assays were carried out at 37 $^\circ\text{C}$ in 50:50 acetonitrile:buffer, with nitrocefin as substrate initially dissolved in acetonitrile (10 mM) and the complex dissolved in acetonitrile: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) or cobalt(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. Nitrocefin and its hydrolysis product both absorb at 390 nm therefore a corrected extinction coefficient of $13415 \text{ M}^{-1} \text{ cm}^{-1}$ was used to calculate the rate of catalysis [40, 41].

Nitrocefin Hydrolysis Product Inhibition In order to elucidate how many molecules of nitrocefin one complex could hydrolyze a titration experiment was conducted where successively one to four equivalents of nitrocefin were added to the complex under the conditions used for kinetic experiments (acetonitrile/buffer pH 8, 37 °C, 300–700 nm). After each addition the solution was scanned at intervals of one minute for a total time of 2 h. Initial concentrations were in general 0.05 mM in each complex and substrate, respectively.

Synthesis of the Blue Species and UV-Vis Experiments were conducted by measuring the absorption spectrum of 0.05 mM nitrocefin in dry acetonitrile at 37 °C and then adding one equivalent of $[\text{Cd}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2]^+$ and recording the spectrum again after 2 min. The same conditions were applied after the addition of 10 μL water to the mixture. Mass Spectra were recorded immediately after mixing equimolar amounts of nitrocefin and $[\text{Cd}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2]^+$ in dry acetonitrile. Initial concentrations of complex and substrate were 0.01 mM.

Fluoride Inhibition studies were conducted by adding 2000 equivalents sodium fluoride to a stock solution of the complex $[\text{Cd}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2]^+$. The mixture was left at room temperature to incubate for at 24 h prior to activity measurements that were conducted as described in the kinetics section for nitrocefin and BDNPP hydrolysis.

Diffuse Reflectance Spectroscopy was conducted at the Middlebury College, Vermont, using a Varian Cary 6000i and a Purged Praying Mantis Diffuse Reflection Attachment (Harrick). Magnesium oxide was used as blank and sample carrier.

Cyclic Voltammetry was carried out with a BAS 100 W potentiostat coupled with an Ag/AgNO₃ reference electrode in acetonitrile, platinum or glassy carbon as working and platinum wire as standard electrode. 0.1 M tetraethyl ammonium perchlorate in dry acetonitrile served as electrolyte with ferrocene as standard. Data analysis was performed with the software BAS100 W version 2.3.

Elemental Microanalyses (C, H, N, S) were performed with a Carlo Erba Elemental Analyzer model NA1500 by Mr. George Blazak at the University of Queensland.

Transmission Electron Microscopy (TEM) was conducted at the Center of Microscopy and Microanalysis, University of Queensland together with Dr. Graeme Auchterlonie. Nanoparticles were taken up in water and sonicated for 10 min after which time the suspension was transferred onto a carbon grid. A JEOL JEM2100

LaB₆ STEM analytical transmission electron microscope with an EM-21010/21020 single tilt holder was used to analyse the samples after the water was evaporated.

X-ray Photoelectron spectroscopy (XPS) was conducted with Dr. Barry Wood at the Center for Microscopy and Microanalysis, University of Queensland, with a Kratos Axis Ultra photoelectron spectrometer which uses Al K α (1253.6 eV) X-rays. The software Casa XPS was used for data processing [42].

Hydrogenations were done with a H-cube hydrogen reactor equipped with 10 % Pd on charcoal cartridges as catalyst [43].

2.4 Materials and Methods: GpdQ

Atomic Absorption Spectroscopy was conducted for the half-apo GpdQ enzyme to confirm the presence of one equivalent of iron. Standards containing 20, 40, 60, 80, and 100 ppb Fe, Zn, Mn, and Co were prepared in 50 mM buffer (HEPES, pH 7) from analytical stock solutions. Protein samples were prepared using the same buffer.

Expression and Purification of wt-GpdQ and Mutant Forms were conducted following a modification of a previously published method [4]. In brief, DH5R *Escherichia coli* cells were transformed with the GpdQ/pCY76 plasmid. Cells were grown in 4 L of TB medium containing 50 μ g/mL ampicillin and 0.1 mM CoCl₂·6H₂O at 30 °C for 30 h. Furthermore, cobalt is added to the growth media as this has been shown in previous experiments that it enhances the yield of expressed protein. The plasmid used expresses GpdQ in a constitutive manner, and no induction was required. The following steps were performed at 4 °C. Cells were harvested by centrifugation and then resuspended in 40 mL of 20 mM TRIS-HCl buffer, pH 8.0. Following lysis using a French press at 10,000 psi, the cell debris was pelleted by centrifugation. The supernatant was filtered using a 0.22 μ m filter (Millipore) and loaded onto a HiPrep 16/10 DEAE FF column (GE Healthcare). A linear NaCl gradient (0 to 1 M) was applied to elute the protein over 10 column volumes, and the elute was collected in 8 mL fractions. A 5 μ L aliquot of each fraction was assayed with 2 mM BPNPP, and the fractions with phosphodiesterase activity were combined. Other proteins were precipitated by addition of 4 M (NH₄)₂SO₄ to a final (NH₄)₂SO₄ concentration of 1.3 M. The protein solution was loaded onto a HiLoad 26/10 Phenyl Sepharose HP column (GE Healthcare). The proteins were eluted using an (NH₄)₂SO₄ gradient (1.5 to 0 M) over 10 column volumes and collected again in 8 mL fractions. Fractions with phosphodiesterase activity were combined, and the proteins were concentrated to approximately 4 mL. Finally, the solution was loaded onto a HiPrep 16/10 Sephacryl S-200 HR gel filtration column and eluted with 20 mM HEPES, 0.15 M NaCl, pH 8.0. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of the purified protein generally shows a single band migrating at $\sim$ 31 kDa. The final yield was $\sim$ 70 mg/L. Protein concentrations were determined by measuring the absorption at the 280 nm (ϵ = 39,880 M⁻¹ cm⁻¹ for a monomer).

Expression of the 8-3 Mutant of GpdQ from competent *E. Coli* cells was conducted at the University of Queensland using same protocol as for wt-GpdQ. The plasmid was obtained from Prof. David Ollis, Research School of Chemistry, ANU, Canberra.

Purification of the GpdQ 8-3 Mutant involved two chromatographic steps using FPLC and was carried out at the Australian National University after a protocol obtained from Ms Sylvia Yip [44]: 5 g of the cells were suspended in 45 mL buffer (50 mM TRIS pH 8) and were lysed using a French Press (14,000 psi, two cycles). The cell debris was separated by centrifugation and the supernatant was kept at 4 °C at all times. Approximately 45 mL protein solution was loaded on a Q Sepharose (strong anion exchange) column using a peristaltic pump (Buffer A 50 mM TRIS pH 8; buffer B 1 M NaCl, 50 mM TRIS pH 8) and eluted with a linear NaCl gradient (0.0–0.6 M) over 10 column volumes (cv) (75 ml = cv, flow 2 mL/min). The 14 fractions containing GpdQ (8 mL, GpdQ eluted between 0.32 and 0.5 M NaCl) were collected. After SDS-page the most pure fractions were combined. From the combined fractions GpdQ was precipitated with $(\text{NH}_4)_2\text{SO}_4$ (final concentration 2.5 M). The suspension was stirred for 30 min at 4 °C and was subsequently centrifuged, the supernatant discarded and the precipitated protein resuspended in 2 mL buffer (50 mM TRIS pH 8). The suspension was subsequently loaded onto a HiPrep 16/10 Sephacryl S-200 HR gel filtration column (GE Healthcare) and the different oligomers of GpdQ eluted with 50 mM TRIS pH 8.

Site Directed Mutagenesis was conducted at the Australian National University together with Ms T. Murray. The F and R mutagenesis primers for the Y19F and S127A mutants were: GpdqY19FSDMFor—CG AGA AGC TGT TCG GCT TTA TCG ACG, GpdqY19FSDMRev—CGA TAA AGC CGA ACA GCT TCT CGC CGC; GpdqS127ASDMFor—CGC CGG CAC TGC AAA AGG CTG GCT GAC C and GpdqS127ASDMRev—GCC AGC CTT TTG CAG TGC CGG CGC GGC. The Polymerase Chain Reaction (PCR) protocol was as follows: 51 μL milliQ water; 7.5 μL 10 $\times$ pfu buffer; 3 μL dNTP (10 mM); 3 μL Fi primer (10 μM); 3 μL Ri primer (10 μM); 1.5 μL pfu. From this mixture 46 μL were transferred into a PCR tube and 4 μL DNA (PCY76 wt GpdQ 141 ng/ μL) were added, to the remaining blank 2 μL milliQ water were added. The PCR was conducted with a MJ Mini Personal Thermal Cycler from BioRad, PCR program: 95 °C 45 s; (95 °C 45 s, 55 °C 45 s, 72 °C 8 min) $\times$ 30; 72 °C 10 min; 4 °C hold. The purification was conducted with a kit from Promega (Wizard SV Gel + PCR cleanup kit). After digestion (50 μL purified PCR mix, 6.5 μL NEB IV, 6.5 μL 10 $\times$ BSA and 1 μL Dpn I (total of 65 μL) incubate mix for 3 h at 37 °C) the PCR cleanup was repeated. The subsequent transformation of DH5 α cells was as follows: 50 μL of electro competent DH5 α cells were thawed on ice. 5 μL of cleaned up digestion and 5 μL water were then added. After electroporation the cells were resuspended quickly in 1 mL YenB (ampicillin) and recovered for one hour at 37 °C. After plating 50 μL of solution on Agar/Ampicillin plates they were incubated overnight at 37 °C. Three colonies were chosen and incubated overnight in LB/Ampicillin (5 mL) each. From the spun down cells DNA was extracted using the Qiagen Miniprep kit. For sequencing 1 μL BDT, 3.5 μL 5 $\times$ buffer, 1 μL

primer i (3.2 pmol/ μ L), 6 μ L DNA and 8.5 μ L water were combined and subjected to the following program in a MJ Mini Personal Thermal Cycler: 94 °C 5 min (96 °C 10 s, 50 °C 5 s, 60 °C 4 min) $\times$ 35, 4 °C hold.

Preparation of half-apo GpdQ An Econo-Pac 10DG (Bio-Rad) gel filtration column was used to generate the half-apo enzyme. The column was treated with 5 mL of a chelating solution, which contained 5 mM tetrasodium salt of ethylenediaminetetraacetic acid, 5 mM 1,10-phenanthroline, 5 mM 2,6-pyridine dicarboxylic acid, 5 mM 8-hydroxyquinoline-5-sulfonic acid, 5 mM 2-mercaptoethanol, and 20 mM HEPES (pH 7.0) and was subsequently equilibrated by washing with 5 column volumes of Chelex-treated buffer (50 mM HEPES, pH 7.0) prior to use. Approximately 250 μ L of GpdQ ($\sim$ 0.35 mM) were diluted to 3 mL with the chelating solution and then loaded onto the column. The enzyme was collected in 4 mL of buffer (50 mM HEPES, pH 7.0). The concentration of the protein was calculated by measuring its absorbance at 280 nm ($\epsilon = 39,880 \text{ cm}^{-1} \text{ M}^{-1}$) [45, 46]. Atomic absorption spectroscopy was used to determine the metal ion content.

Phosphatase Activity of wt-GpdQ, and S127A and Y19F Mutants were measured spectrophotometrically with BPNPP as substrate by monitoring the formation of the product 4-nitrophenolate at 405 nm. All kinetic experiments were done in duplicate. In the assay solution the concentration of added metal ions was 20 μ M. Reactions were measured to less than 5 % of total substrate hydrolysis for 2 min and the assay mix was equilibrated for 1 min before the measurement. Rates were linear in the time frame studied. The extinction coefficient of 4-nitrophenolate was determined at each relevant pH by using a standard solution of 4-nitrophenol in the same buffer used for the enzymatic assays. All reagents (buffer, substrate, etc.) were prepared in water that had been treated with Chelex. The multicomponent buffers (50 mM of each of MES, HEPES, CHES, and CAPS) that ranged from pH 5.5 to 11.0 were also treated with Chelex for 24 h. Chelex was removed by filtration with 0.45 μ m Millex syringe-driven filter units. The pH-profiles of the catalytic parameters were derived from rate measurements that were conducted at various pH values between pH 5.5 and 11.0. The enzyme was stable over the entire pH range studied; autohydrolysis of the substrate was taken into account at each pH. In the substrate concentration dependence studies, the concentrations of the half-apoenzyme and M(II) remained constant at 200 nM and 20 μ M, respectively, while that of the substrate increased from 0 to 10 mM. Similar studies were also conducted in the presence of mixtures of first row divalent transition metal ions (Zn(II), Mn(II) and Co(II)) at pH 9. The data were fit to Eq. 2.5 (p.17) [39].

Kinetic Assays for the 8-3 Mutant were also performed with the substrate BPNPP and hydrolysis was followed by monitoring the formation of the reaction product 4-nitrophenol at a wavelength of 405 nm over 0.5 min at 25 °C. The assays were run in 1 mL plastic cuvettes with 10 mm pathlength using 929 μ L buffer (50 mM TRIS pH8), 70 μ L BPNPP (33.6 mM) and 1 μ L of the GpdQ fraction or with addition of metal: 919 μ L buffer, 1 μ L enzyme fraction (final concentrations ranged from 41–67 nM), 70 μ L BPNPP (33.6 mM, final concentration 2.3 mM) and 10 μ L of a 2.3 mM CoSO₄ or MnSO₄ solution (final concentration 23 μ M).

Magnetic Circular Dichroism Protein samples of Fe(II)Co(II) wt-GpdQ were prepared by adding one equivalent Co(II) and ten equivalents phosphate to the Fe(II) half-apo enzyme, or for the Ser127Ala mutant by adding two equivalents Co(II). The protein was dissolved in a 60 %/40 % (v/v) mixture of glycerol/buffer (50 mM HEPES, pH 7.0) and loaded in a 0.62 cm pathlength nickel-plated copper sample cell with quartz windows. The MCD system used has a JASCO J815 spectropolarimeter and an Oxford Instruments SM4000 cryostat/magnet. Data were collected at increments of 0.5 Tesla (T) from 0 to 7.0 T and at temperatures of 1.4, 4.2, 11.3, 26 and 50 K. Each spectrum was corrected for any natural CD by subtracting the zero-field spectrum of the sample. The resultant spectra were fitted to the minimum number of Gaussian peaks to achieve a satisfactory composite spectrum using the GRAMS AI software [32].

Metal Binding Affinity of Half-apo GpdQ was determined employing activity measurements by varying the amount of added M(II), where $M = \text{CoSO}_4 \cdot 6\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot \text{H}_2\text{O}$ or $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ to the activity assay mixture. The concentrations of the half-apoenzyme and substrate remained constant at 200 nM and 10 mM, respectively, while that of M(II) increased from 0–50 μM . The addition of M(II) to wild-type and mutant half-apo forms of GpdQ at a constant pH and substrate concentration demonstrated saturation behavior similar to that described by the Michaelis-Menten Equation (Eq. 2.5). The binding function (r), which is the molar ratio of the amount of metal ion bound to the total amount of enzyme active sites, was calculated using Eq. 2.6 (p. 24), where n is the number of sites associated with K_d , the dissociation constant, and $[M]_{\text{free}}$ is the free metal ion concentration [47]. The concentration of free metal ion was calculated from Eq. 2.7 (p. 24), where $[M]_{\text{total}}$ is the total concentration of metal ions added and $[E]$ is the concentration of the enzyme. Since the added metal ion concentration is in large excess over enzyme concentration, $[M]_{\text{free}}$ is assumed to equal $[M]_{\text{total}}$. This assumption reduces Eq. 2.6 to Eq. 2.8 [47]

$$r = \frac{n[M]_{\text{free}}}{K_d + [M]_{\text{free}}} \quad (2.6)$$

$$[M]_{\text{free}} = [M]_{\text{total}} - r[E] \quad (2.7)$$

$$r = \frac{n[M]_{\text{total}}}{K_d + [M]_{\text{total}}} \quad (2.8)$$

K_{Assoc} values, the inverse of the K_d values, were obtained from an equation similar to Eq. 2.5. The data of the pH-dependence of K_{Assoc} were fit to Eq. 2.9 in the case of Fe(II)Co(II), Fe(II)Mn(II) and Fe(II)Zn(II) wt-GpdQ and the Fe(II)Co(II) derivatives of Tyr19Phe and Ser127Ala mutants of GpdQ [47].

$$K_{\text{Assoc}} = \frac{K_{\text{Assoc}}(\text{max})}{\left(1 + \frac{[H^+]}{K_{A1}} + \frac{K_{A2}}{[H^+]}\right)} \quad (2.9)$$

Equation 2.10 was used to fit the data of the Fe(II)Cd(II) derivative of wt-GpdQ.

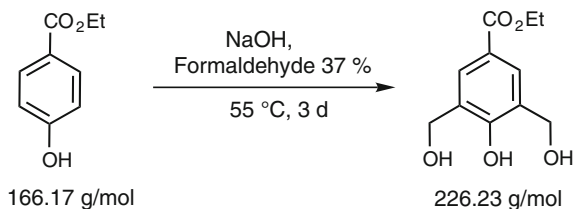
$$K_{Assoc} = \frac{K_{Assoc(max)}}{\left(1 + \frac{[H^+]}{K_{A1}}\right)} \quad (2.10)$$

The equation to fit the data the Fe(II)Zn(II)-substituted His217Ala mutant (117) of GpdQ is based on a triprotic model and was derived elsewhere (Eq. 2.11). [48]

$$K_{Assoc} = K_{assoc1} + \left(\frac{(K_{assoc4} - K_{assoc1})K_{A1}K_{A2}K_{A3} + (K_{assoc3} - K_{assoc1})K_{A1}K_{A2}[H^+] + (K_{assoc2} - K_{assoc1})K_{A1}[H^+]^2}{[H^+]^3 + K_{A1}[H^+]^2 + K_{A1}K_{A2}[H^+] + K_{A1}K_{A2}K_{A3}} \right) \quad (2.11)$$

2.5 Syntheses of the Ligands

2.5.1 Synthesis of Ethyl-4-hydroxy-3,5-bis(hydroxymethyl)benzoate

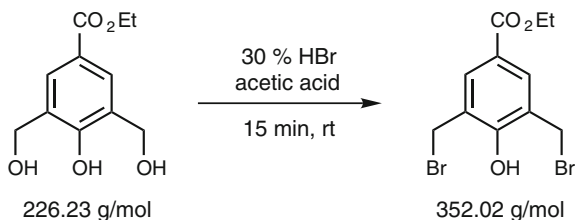


The compound was prepared by a modification of a previously published procedure [20]. To a cool 12 % aqueous solution of sodium hydroxide (70 mL, 0.21 mol), commercially available ethyl 4-hydroxybenzoate (15 g, 0.09 mol) was added at 0 °C. Then aqueous formaldehyde solution (37 %, 60 mL, 2.16 mol) was added. The reaction was stirred at 55 °C for 3 days. The red solution was allowed to reach room temperature and subsequently ethyl acetate (100 mL) added. The organic layer was discarded and to the remaining aqueous phase ethyl acetate (100 mL) and saturated ammonium chloride solution were added. The organic phase was collected and dried over sodium sulfate. Subsequent removal of the solvent in vacuo left an orange solid which was recrystallized from chloroform/methanol (1:1) to yield a pale yellow powder (11.7 g, 57 %).

¹H NMR (d⁴-MeOD, 400.13 MHz); δ 1.37 (t, 3H, CH₂CH₃, J = 7.1 Hz); 4.32 (q, 2H, CH₂CH₃, J = 7.1 Hz); 4.80 (s, 4H, CH₂OH); 7.71 (s, 2H, Ar-H). **¹³C NMR** (d⁴-MeOD, 100.62 MHz) δ 14.7 (CH₂CH₃); 61.6 (CH₂CH₃); 61.8 (CH₂OH); 122.4 (arCCO₂Et); 128.4 (arCH); 129.6 (arCCH₂OH); 159.4 (arCOH); 168.5 (CO₂Et). **ESI mass spectrometry** (methanol) m/z 153.10 [C₈H₈O₃ + H]⁺.

FT-IR spectroscopy (ν , cm^{-1}) 3428 (m, O–H str); 2985, 2942, 2871 (w, CH_2 str); 1689 (s, $\text{C}=\text{O}$ str); 1206 (s, $\text{C}-\text{O}$ str); 1010 (m, $\text{C}-\text{O}$ str). **Melting Point** 137.6–139.4 °C Lit: 139.0 °C (benzene/petroleum ether) [20], 137.0–138.0 °C (chloroform) [49].

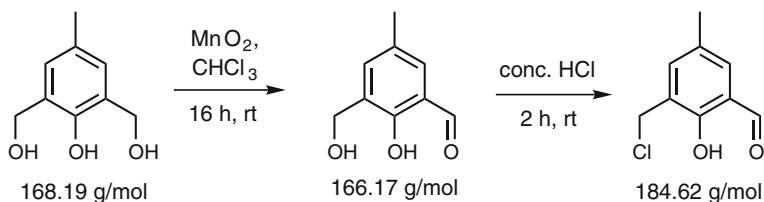
2.5.2 Synthesis of Ethyl 3,5-bis(bromomethyl)-4-hydroxybenzoate



The compound was prepared by a previously published procedure [21]. 2,6-bis(hydroxymethyl)-4-hydroxybenzoate (0.58 g, 16 mmol) was added whilst stirring to a solution of hydrogen bromide in acetic acid (30 % HBr, 2 mL, 50 mmol). After 5 min a clear solution formed, from which after 10 min an orange precipitate separated. The sticky mass was cooled, filtered and washed thoroughly with cold petroleum ether until an orange solid was obtained. The resulting solid was dried in vacuo. (0.83 g, 90.45 %).

^1H NMR (CDCl_3 , 300.13 MHz); δ 1.37 (t, 3H, CH_2CH_3 , $J = 7.1$ Hz); 4.34 (q, 2H, CH_2CH_3 , $J = 7.1$ Hz); 4.54 (s, 2H, CH_2Br); 6.19 (s, 1H, OH); 7.97 (s, 2H, arH). **^{13}C NMR** (CDCl_3 , 100.62 MHz); δ 14.3 (CH_3); 28.4 (CH_2Br); 61.2 (CH_2CH_3); 123.4 (ar CCO_2Et); 124.9 (ar CCH_2); 132.8 (arCH); 157.1 (arCOH); 165.4 (CO_2Et). **FT-IR spectroscopy** (ν , cm^{-1}) 3291 (m, O–H str); 2985 (w, C–H str); 1881 (s, $\text{C}=\text{O}$ str); 1235, 1191.6 (s, $\text{C}-\text{O}$ str); 585 (s, $\text{C}-\text{Br}$ str). **Melting point** 155.0–157.4 °C Lit: 155.0–157.0 °C (petroleum ether) [21].

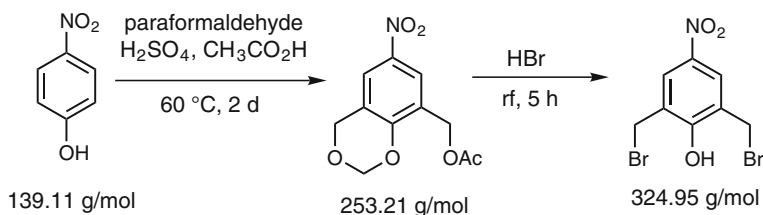
2.5.3 Synthesis of 3-(chloromethyl)-2-hydroxy-5-methylbenzaldehyde



The compound was prepared after a previously published procedure [50]. 2,6-bis(hydroxymethyl)-4-methylphenol (16 g, 0.12 mol) was stirred with activated MnO_2 (85 %, 80 g, 1.14 mol) in chloroform (250 mL) at room temperature for 16 h. The black slurry was then filtered through Celite and the yellow filtrate was concentrated to a brown oil in vacuo. The residue was taken up in hot toluene (40 mL) and filtered. A brown oil was obtained after removal of the solvent in vacuo which solidified upon standing. The solid was dissolved in ethanol (30 mL) and added dropwise to conc. hydrochloric acid (80 mL) at 40 °C. The mixture was subsequently stirred for 2 h at room temperature and the white powder obtained was filtered off and washed with water until the filtrate was pH neutral (3.94 g, 17.91 %).

^1H NMR (CDCl_3 , 500.13 MHz); δ 2.33 (s, 3H, CH_3); 4.64 (s, 2H, CH_2); 7.32 (d, 1H, arH, $J = 1.8$ Hz); 7.43 (d, 1H, arH, $J = 2.0$ Hz); 9.84 (s, 1H, CHO). **^{13}C NMR** (CDCl_3 , 100.62 MHz) δ 20.2 (CH_3); 39.8 (CH_2); 120.4 (arC); 125.7 (arC); 129.2 (arC); 133.9 (arC); 138.6 (arC); 157.3 (arCOH); 196.4 (CHO). **FT-IR spectroscopy** (ν , cm^{-1}) 3187 (s, O–H str); 2976, 2854 (w, C–H str); 1659 (s, C=O str). **Melting point** 92–94 °C Lit: 92–93 °C [50].

2.5.4 Synthesis of 2,6-bis(bromomethyl)-4-nitrophenol

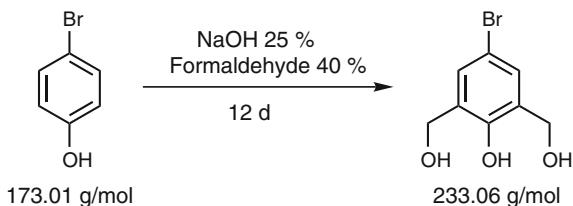


The compound was prepared after a previously published procedure [23]. In a round bottom flask paraformaldehyde (95 %, 12 g, 0.4 mol), acetic acid (50 mL) and sulfuric acid (21.9 mL) were heated to 55 °C and 4-nitrophenol (13.9 g, 0.1 mol) was added. The temperature was maintained at 55 °C and the reaction was stirred for 35 h. After cooling to room temperature water (60 mL) was added and the mixture was neutralized with solid potassium carbonate (90 g). The pale yellow precipitate which resulted was filtered, washed with copious amounts of water and dried in vacuo. After recrystallization from ethanol (3 $\times$) 2 g (0.008 mol) of the 8-acetoxymethyl-6-nitro-1,3-benzodioxene were refluxed with hydrobromic acid (48 %, 60 mL) for 5 h. After cooling the solid was filtered, washed with cold water and recrystallized from chloroform. The desired product was obtained as a yellow powder (2.02 g, 40 %).

^1H NMR (CDCl_3 , 300.13 MHz); δ 4.55 (s, 4H, CH_2Br); 6.58 (bs, 1H, OH); 8.19 (s, 2H, arH). **^{13}C NMR** (CDCl_3 , 100.62 MHz); δ 27.1 (CH_2Br); 126.6 (arCCH₂Br); 127.7 (arCH); 140.9 (arCNO₂); 158.4 (arCOH). **FT-IR spectroscopy**

(ν , cm^{-1}) 3407 (s, O–H str); 2921, 2851 (w, C–H str); 1510 (s, N=O asym. str); 1331 (s, N=O sym. str), 569 (s, C–Br str). **Melting point** 147.5–149.9 °C Lit: 149.0–150.0 °C (ethanol/hexane); [17] 146.0–147.0 °C (chloroform) [23].

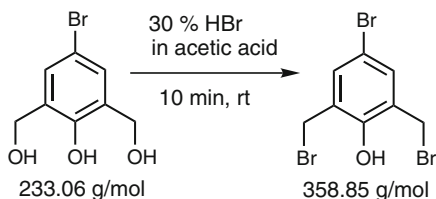
2.5.5 Synthesis of 4-bromo-2,6-bis(hydroxymethyl)phenol



The compound was synthesized after a previously published procedure [19]. 4-Bromophenol (17.3 g, 0.1 mol) in methanol (25 mL), aqueous sodium hydroxide solution (25 %, 50 mL) and formaldehyde were combined and left at room temperature for 12 d. Subsequently water (50 mL) and glacial acetic acid (15 mL) were added and after 2 h the solution was concentrated to 50 mL in vacuo and then cooled on ice. The solution was decanted from the orange oil/precipitate obtained on standing and the remaining oil was dissolved in aqueous sodium hydroxide solution (10 %, 50 mL). Activated charcoal was added and the suspension stirred overnight. After filtration the solution was acidified with 2 M HCl to pH 5. The resulting yellow precipitate was collected on a sintered glass funnel, recrystallized from water, dried in air and washed with cold chloroform (~20 mL) to yield the desired product as a white powder (7 g, 23 %).

^1H NMR (CD_3COCD_3 , 300.13 MHz); δ 4.73 (s, 4H, CH_2); 7.28 (s, 2H, arCH) **^{13}C NMR** (CD_3COCD_3 , 100.62 MHz); 61.4 (CH_2); 111.6 (arCBr); 129.5 (arCH); 130.5 (arCCH₂); 153.4 (arCOH). **FT-IR spectroscopy** (ν , cm^{-1}) 3401, 3300 (s, O–H str); 2966, 2912, 2887 (w, CH_2 str); 1649, 1610, 1587 (w, C=C str); 1454 (s, CH_2 def); 1331 (s, O–H def); 1065 (s, C–OH def); 870 (m, Ar–H def). **Melting point** 161.7 °C Lit: 149.0–151.0 °C; [51] 164.0–168.0 °C; [52] 162.0–164.0 °C [19].

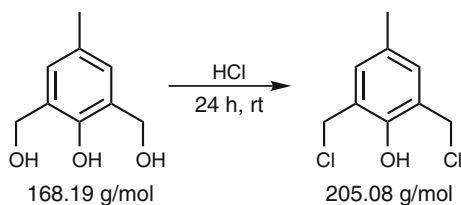
2.5.6 Synthesis of 4-bromo-2,6-bis(bromomethyl)phenol



4-Bromo-2,6-bis(hydroxymethyl)phenol (3 g, 13 mmol) was added to hydrogen bromide in acetic acid (30 %, 15 mL) and stirred for 10 min until all starting material had dissolved. Upon addition of water the product precipitated from the solution and which was collected on a filter and washed thoroughly with cold petroleum spirit. The desired product was obtained as yellow powder (3.3 g, 70.5 %).

¹H NMR (CDCl₃, 300.13 MHz); δ 4.49 (s, 4H, CH₂); 5.66 (bs, 1H, OH); 7.39 (s, 2H, arCH) **¹³C NMR** (CDCl₃, 100.62 MHz) 27.9 (CH₂); 112.7 (arCBr); 127.2 (arCCH₂); 133.7 (arCH); 152.3 (arCOH). **FT-IR spectroscopy** (ν , cm⁻¹) 3537 (s, O–H str); 3050, 2979 (w, CH₂ str); 1648, 1604 (w, C=C str); 1467 (s, CH₂ def); 869 (m, Ar–H def). **Melting point** 79.3–88.2 °C.

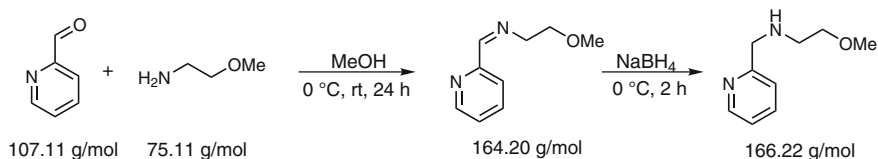
2.5.7 Synthesis of 2,6-bis(chloromethyl)-4-methylphenol



The compound was synthesized after a previously published procedure [22]. 2,6-bis(hydroxymethyl)-4-methylphenol (5 g, 29 mmol) was suspended in concentrated hydrochloric acid (75 mL) at room temperature and stirred overnight. The suspension was extracted with dichloromethane (3 × 50 mL) and the combined organic layers dried over sodium sulfate. After removal of the solvent in vacuo the product was obtained as white solid (5.5 g, 93.2 %).

¹H NMR (CDCl₃, 300.13 MHz); δ 2.26 (s, 3H, CH₃); 4.64 (s, 4H, CH₂Cl); 5.50 (s, 1H, OH); 7.07 (s, 2H, arH). **¹³C NMR** (CDCl₃, 100.62 MHz) 20.3 (CH₃); 42.5 (CH₂Cl); 124.6 (arCCH₂); 130.5 (arCCH₃); 131.6 (arCH); 150.9 (arCOH). **FT-IR spectroscopy** (ν , cm⁻¹) 3532 (m, O–H str); 2978, 2921 (w, CH₂ str); 1608 (w, C=C str); 1141 (m, C–O str); 869 (m, Ar–H def); 658 (m, C–Cl, skeletal vibr.). **Melting point** 81.9–84.1 °C Lit: 82.0–84.0 °C [53].

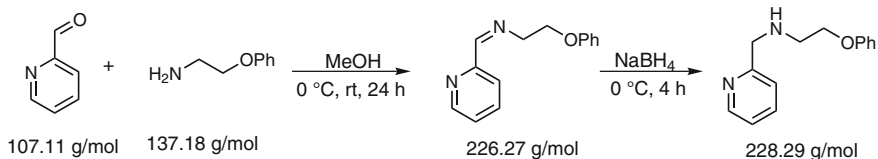
2.5.8 Synthesis of 2-methoxy-N-(pyridin-2-ylmethyl)aminoethanol



Pyridine 2-carboxaldehyde (2.25 g, 0.02 mol) in methanol (5 mL) was added dropwise to 2-methoxyethanolamine (1.58 g, 0.02 mol) in methanol (10 mL) at 0 °C. The solution was brought to room temperature on a thawing ice bath and stirred overnight. Sodium borohydride (0.83 g, 0.02 mol) was added in small portions and the solution was subsequently stirred for a further 2 h. Water (30 mL) was added and the reaction mixture was concentrated to about 30 mL in vacuo. The remaining solution was extracted with dichloromethane (3 × 20 mL) and the combined organic extracts were washed three times with brine, the solution dried over sodium sulfate and the solvent removed under vacuum. The desired product was obtained as a yellow oil (2.85 g, 90.6 %).

¹H NMR (CDCl₃, 300.13 MHz); δ 2.26 (s, 1H, NH); 2.72 (t, 2H, NCH₂CH₂, J = 5.4 Hz); 3.20 (s, 3H, OCH₃); 3.43 (t, 2H, CH₂OCH₃, J = 5.4 Hz); 3.79 (s, 2H, arCH₂N); 7.02 (dq, 1H, pyH, J = 7.5, 4.9 Hz); 7.19 (dt, 1H, pyH, J = 7.7, 1.0 Hz); 7.48 (td, 1H, pyH, J = 7.6, 1.8 Hz); 8.40 (dq, 1H, pyH, J = 4.9, 1.73, 0.9 Hz). **¹³C NMR** (CDCl₃, 100.62 MHz); δ 48.6 (NCH₂CH₂); 54.9 (OCH₃); 58.8 (arCH₂N); 71.8 (NCH₂CH₂); 121.7 (pyCH); 121.9 (pyCH); 136.2 (pyCH); 149.1 (pyCH); 159.6 (pyCCH₂). **ESI mass spectrometry** (methanol) m/z 167.10 [C₉H₁₄N₂O + H]⁺. **FT-IR spectroscopy** (ν, cm⁻¹) 3319 (m, N–H str); 2889, 2830 (m, C–H str); 1592 (m, C=C str); 1435 (m, C–H def); 1357 (w, C–H sym. def); 1110 (s, C–O–C str); 757 (s, py–H).

2.5.9 Synthesis of 2-phenoxy-N-(pyridin-2-ylmethyl)ethanamine

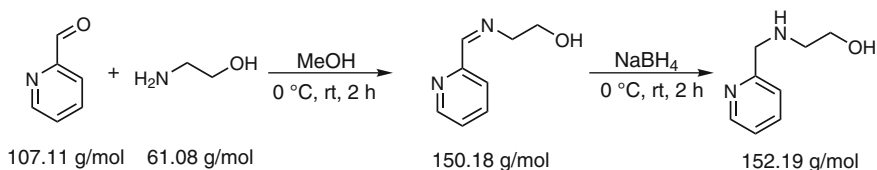


Pyridine 2-carboxaldehyde (0.39 g, 0.004 mol) in methanol (2 mL) was added dropwise to 2-phenoxyethanolamine (0.5 g, 0.004 mol) in methanol (3 mL) at 0 °C. The solution was brought to room temperature and stirred overnight. Subsequently sodium borohydride (0.2 g, 0.005 mol) was added in small portions and the solution stirred for a further 4 h. Water (6 mL) was added and the reaction mixture concentrated to about 6 mL in vacuo. The remaining solution was extracted with dichloromethane (3 × 10 mL) and the combined organic extracts dried over sodium sulfate and the solvent removed under vacuum to yield a yellow oil (0.75 g, 91.2 %).

¹H NMR (CDCl₃, 500.13 MHz); δ 2.14 (s, 1H, NH); 3.04 (t, 2H, NCH₂CH₂, J = 5.3 Hz); 3.98 (s, 2H, arCH₂N); 4.09 (t, 2H, NCH₂CH₂, J = 5.3 Hz); 6.89 (m, 2H, arH, 6-H); 6.91 (m, 1H, arH); 7.13 (m, 1H, pyH, J = 7.3, 4.9, 1.0 Hz); 7.24 (m, 2H, arH, J = 7.4 Hz); 7.31 (d, 1H, pyH, J = 7.8 Hz); 7.61 (td, 1H, pyH, J = 7.7, 1.8 Hz); 8.54 (dq, 1H, pyH, J = 4.9, 1.7, 0.9 Hz). **¹³C NMR** (CDCl₃,

100.62 MHz); δ 48.4 (NCH₂CH₂); 55.1 (arCH₂N); 67.3 (CH₂O); 114.5 (arCH); 120.7 (arCH); 121.9 (pyCH); 122.1 (pyCH); 129.3 (arCH); 136.4 (pyCH); 144.3 (pyCH); 158.8 (arCO); 159.6 (pyCCH₂). **ESI mass spectrometry** (methanol) m/z 229.10 [C₁₄H₁₆N₂O + H]⁺. **FT-IR spectroscopy** (ν , cm⁻¹) 3405 (m, N–H str); 2924 (m, C–H str); 1594 (m, C=C str); 1510 (s, N–H def); 1448 (m, C–H def); 1331 (w, C–H sym. def); 1212 (s, C–O–C str); 777 (w, Ar–H); 746 (s, py–H); 692 (w, Ar–H).

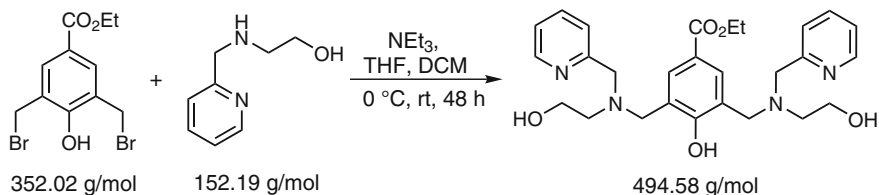
2.5.10 Synthesis of *N*-(2-Pyridylmethyl)-2-aminoethanol



The compound was prepared by a slight modification of previously published procedures [24, 25]. 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 2 h. Sodium borohydride (3.60 g, 0.1 mol) was added in small portions at 0 °C and the solution was stirred for a further 2 h. Water (30 mL) was added and the reaction mixture was concentrated to about 30 mL in vacuo. The remaining solution was extracted with dichloromethane (3 × 20 mL) and the combined organic phases were dried over sodium sulfate and the solvent removed under vacuum to yield a yellow oil. (2.50 g, 40.58 %).

¹H NMR (CDCl₃, 400.13 MHz); δ 2.14 (s, 1H, NH); 2.91 (t, 2H, NCH₂CH₂, J = 5.7 Hz); 3.79 (t, 2H, CH₂OH, J = 5.1 Hz); 5.27 (s, 2H, arCH₂N); 5.40 (bs, 1H, OH); 7.26 (m, 1H, pyH, J = 6.2 Hz); 7.63 (d, 1H, pyH, J = 7.7 Hz); 7.69 (tt, 1H, pyH, J = 7.8, 1.8 Hz); 8.55 (dd, 1H, pyH, J = 4.9 Hz). **¹³C NMR** (CDCl₃, 100.62 MHz); δ 56.9 (NCH₂CH₂); 58.2 (arCH₂N); 59.7 (CH₂ OH); 123.2 (pyCH); 123.2 (pyCH); 137.3 (pyCH); 149.4 (pyCH); 153.8 (pyCCH₂). **ESI mass spectrometry** (methanol) m/z 175.07 [C₈H₁₂N₂O + Na]⁺; 153.11 [C₈H₁₂N₂O + H]⁺. **FT-IR spectroscopy** (ν , cm⁻¹) 3214 (m, O–H str); 2919 (m, C–H str); 2849 (m, C–H str); 1596 (m, C=C str); 1433 (m, O–H def); 832, 763 (s, py–H).

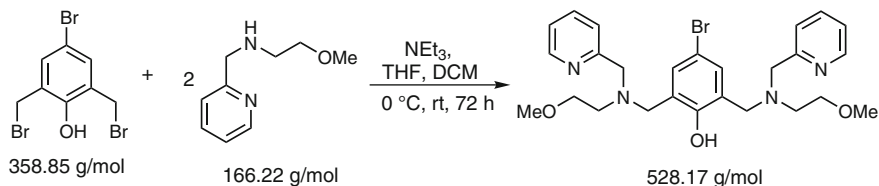
2.5.11 Synthesis of Ethyl 4-hydroxy-3,5-bis(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoate ($\text{CO}_2\text{EtH}_3\text{L1}$)



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 and monitored with TLC, 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 (ethyl acetate/methanol, 8:2, I_2 stain, $R_f = 0.44$) to yield a yellow oil (0.50 g, 72.2 %).

^1H 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, pyH , $J = 5.8$, 0.8 Hz); 7.20 (d, 2H, pyH , $J = 7.8$ Hz); 7.54 (td, 2H, pyH , $J = 7.6$, 1.7 Hz); 7.64 (s, 2H, arH); 8.51 (dq, 2H, pyH , $J = 4.8$, 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); 2827 (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 [$\text{C}_{27}\text{H}_{34}\text{N}_4\text{O}_5 + \text{H}$] $^+$.

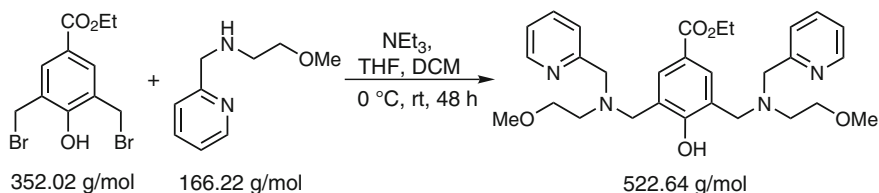
2.5.12 Synthesis of 4-bromo-2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)phenol (BrHL2)



To a solution of 2-methoxy-N-(pyridin-2-ylmethyl)aminoethanol (0.925 g, 5.6 mmol) and triethylamine (1.25 g) in tetrahydrofuran (10 mL) was added dropwise a solution of 4-bromo-2,6-bis(bromomethyl)phenol (1 g, 2.7 mmol) in dichloromethane (10 mL) at 0 °C. The reaction mixture was stirred for 72 h and filtered to remove the precipitate of triethylamine hydrobromide. Removal of the solvent resulted in a yellow oil which was further purified by flash column chromatography (1 g crude ligand, length = 30 cm, diameter = 1.5 cm, ethyl acetate (until first band is eluted) then methanol/ethyl acetate 1:5, FeCl₃ stain, R_f = 0.55 in ethyl acetate). The ligand was obtained as a yellow oil (930 mg, 63 %).

¹H NMR (CDCl₃, 300.13 MHz) δ 2.79 (t, 4H, NCH₂CH₂); 3.26 (s, 6H, OCH₃); 3.52 (t, 4H, CH₂OCH₃); 3.79 (s, 4H, CH₂N); 3.96 (s, 4H, NCH₂py); 5.17 (s, 1H, OH); 7.08 (m, 2H, pyCH); 7.19 (s, 2H, arCH); 7.44 (m, 2H, pyCH), 7.64 (m, 2H, pyCH); 8.50 (m, 2H, pyCH). **¹³C NMR** (CDCl₃, 100.62 MHz); δ 52.8 (arCH₂N); 54.6 (NCH₂); 58.7 (OCH₃); 59.8 (NCH₂py); 69.9 (CH₂OCH₃); 110.7 (arCBr); 122.45 (pyCH); 123.6 (pyCH); 123.8 (arCCH₂); 131.9 (arCH); 136.8 (pyCH); 148.9 (pyCH); 156.0 (arCOH); 156.8 (pyCCH₂). **FT-IR spectroscopy** (ν, cm⁻¹) 3231 (m, O–H str); 2927, 2879 (m, CH₂ str); 1592, 1572 (m, C=C str); 1435 (s, CH₂ def); 1112 (s, C–O str); 832, 756 (s, Ar–H). **ESI mass spectrometry** (methanol) m/z 529.10, 531.07 [C₂₆H₃₃BrN₄O₃ + H]⁺; 551.16, 553.16 [C₂₆H₃₃BrN₄O₃ + Na]⁺.

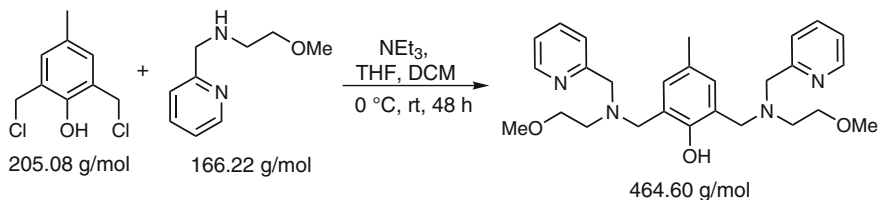
2.5.13 Synthesis of Ethyl 4-hydroxy-3,5-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoate (CO₂EtHL2)



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) was added dropwise a solution of ethyl-3,5-bis(bromomethyl)-4-hydroxybenzoate (2.5 g, 7.1 mmol) in dichloromethane (25 mL) at 0 °C. A white precipitate, identified as triethylamine hydrobromide, formed immediately. The reaction mixture was stirred for 48 h, concentrated to 4 mL in vacuo and then filtered to remove 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, length = 30 cm, diameter = 1.5 cm, methanol/ethyl acetate 2:8 (1 L), methanol/ethyl acetate 2:1 (300 mL), methanol (300 mL), fraction volume 10 mL, 50 fractions collected. FeCl₃ stain, R_f = 0.46 in methanol/ethyl acetate 2:8). The ligand was obtained as a yellow oil (2.19 g, 60 %).

^1H 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, pyH , $J = 7.4$, 4.9, 1.1 Hz); 7.42 (d, 2H, pyH , $J = 7.8$ Hz); 7.57 (td, 4H, pyH , $J = 7.7$, 1.3 Hz); 7.83 (s, 2H, arH); 8.48 (dq, 2H, pyH , $J = 4.9$, 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 ($\text{CH}_2\text{CH}_3/\text{NCH}_2\text{py}$); 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 [$\text{C}_{29}\text{H}_{38}\text{N}_4\text{O}_5 + \text{H}$] $^+$.

2.5.14 Synthesis of 2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-4-methylphenol ($\text{CH}_3\text{HL2}$)

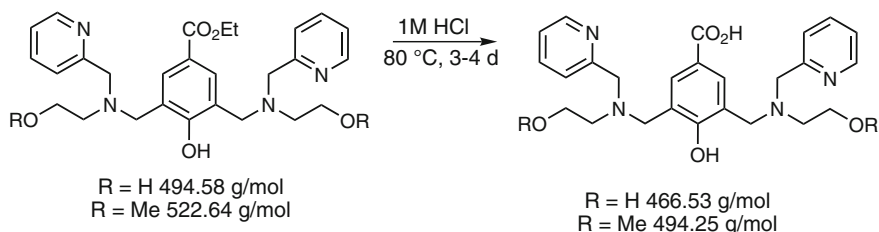


To a solution of 2-methoxy-N-(pyridin-2-ylmethyl)aminoethanol (1.50 g, 9 mmol) and triethylamine (0.91 g, 9 mmol) in tetrahydrofuran (4.5 mL) was added dropwise a solution of 2,6-bis(chloromethyl)-*p*-cresol (0.94 g, 5 mmol) in dichloromethane (4 mL) at $0\text{ }^\circ\text{C}$. A white precipitate formed immediately. The reaction mixture was stirred for 48 h and then filtered to remove precipitated triethylamine hydrochloride. Removal of the solvents left a yellow oil which was further purified by flash column chromatography (2.17 g crude ligand, ethyl acetate/methanol 8:2 (900 mL), ethyl acetate/methanol 1:1 (400 mL), I_2 stain, $R_f = 0.66$ in ethyl acetate/methanol 8:2). The ligand was obtained as a yellow oil (1.35 g, 65.4 %).

^1H NMR (CDCl_3 , 500.13 MHz); δ 2.21 (s, 3H, CH_3); 2.75 (t, 2H, N-CH_2 -, $J = 5.8$ Hz); 3.25 (s, 6H, OCH_3); 3.50 (t, 4H, CH_2OCH_3 , $J = 5.8$ Hz); 3.77 (s, 4H, arCH_2N); 3.84 (s, 4H, NCH_2ar); 6.92 (s, 2H, arCH); 7.10 (ddd, 2H, pyCH , $J = 7.4$, 4.9, 1.1 Hz); 7.46 (d, 2H, pyCH , $J = 7.8$ Hz); 7.60 (td, 2H, pyCH , $J = 7.6$, 1.8 Hz); 8.47 (dq, 2H, pyCH , $J = 4.9$, 1.7 Hz). **^{13}C NMR** (CDCl_3 , 100.62 MHz); δ 20.6 (arCH_3); 53.8 (NCH_2CH_2), 55.6 (arCH_2N); 58.7 (OCH_3); 60.9 (NCH_2py); 71.0 (CH_2OCH_3); 123.0 (pyCH); 124.0 (pyCH); 124.7 (arCCH_2); 128.2 (arCCH_3); 130.3 (arCH); 137.4 (pyCH); 149.8 (pyCH); 154.6 (arCOH); 159.8 (pyCCH_2). **FT-IR spectroscopy** (ν , cm^{-1}) 2922, 2875, 2817 (m, C–H str); 1591 (m, C=C str);

1434 (m, O–H def); 1365 (m, C–O–C str); 1109 (m, C–O str); 844 (m, Ar–H); 756 (s, py–H). **ESI mass spectrometry** (methanol) m/z 487.18 [$C_{27}H_{36}N_4O_3 + Na$] $^+$, 465.22 [$C_{27}H_{36}N_4O_3 + H$] $^+$.

2.5.15 Synthesis of 4-hydroxy-3,5-bis(((2-hydroxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoic acid (CO_2HHL1) and 4-hydroxy-3,5-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)benzoic acid (CO_2HHL2)



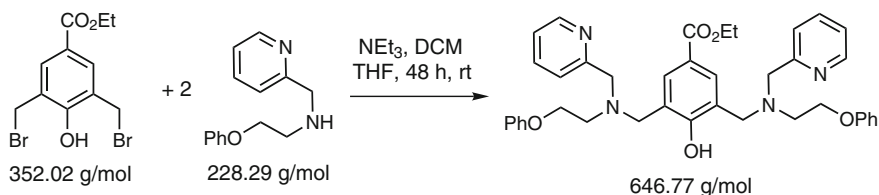
The ethyl ester ligand (0.1 g, 0.2 mmol) was dissolved in 1 M HCl (5 mL, 5 mmol) and stirred at 80 °C for 4 days. The solvent was removed from the reaction mixture in vacuo leaving an orange solid which was redissolved twice in 20 % DCl in D_2O and repeatedly concentrated. NMR analysis showed that the compound was obtained as hydrochloride with all nitrogens protonated (60 mg, 50 %).

CO_2HHL1 : 1H NMR ($CDCl_3$, 300.13 MHz); δ 3.47 (t, 4H, NCH_2CH_2); 4.04 (t, 4H, NCH_2CH_2); 4.75 (s, 4H, $arCH_2$); 4.98 (s, 4H, NCH_2py); 7.96 (t, 2H, pyH); 8.13 (s, 2H, arH); 8.15 (d, 2H, pyH); 8.45 (t, 2H, pyH); 8.70 (d, 2H, pyH). ^{13}C NMR ($CDCl_3$, 100.62 MHz); δ 55.3 ($arCCH_2$); 55.7 (NCH_2CH_2), 57.1 (NCH_2CH_2 , NCH_2py); 119.8 ($arCCH_2$); 124.7 ($arCCO_2H$); 128.3 ($pyCH$); 129.9 ($pyCH$); 138.1 ($arCH$); 145.7 ($pyCH$); 146.2 ($pyCH$); 147.7 (pyC); 161.6 ($arCOH$); 167.9 (CO_2H). **ESI mass spectrometry** (methanol) m/z 489.29 [$C_{24}H_{30}N_4O_5Na$] $^+$; 457.20 [$C_{25}H_{31}N_4O_5$] $^+$; 315.15 [$C_{17}H_{19}N_2O_4$] $^+$; 271.17 [$C_{16}H_{18}N_2O_2$] $^+$. **FT-IR spectroscopy** (ν , cm^{-1}) 3362 (m, O–H str); 2954, 2851 (m, C–H str); 2499 (s, N–H str); 1720 (s, C=O str); 1435 (m, O–H def); 1236 (m, C–O–C str); 771 (s, py–H).

CO_2HHL2 : The 1H and ^{13}C peaks of the methyl ether group were not observed in some spectra as this group has been cleaved under the conditions. 1H NMR ($CDCl_3$, 500.13 MHz); δ 3.03 (s, 6H, OCH_3); 3.39 (t, 4H, NCH_2CH_2 , $J = 4.5$ Hz); 3.77 (t, 4H, NCH_2CH_2 , $J = 4.5$ Hz); 4.42 (s, 4H, $arCH_2N$); 4.61 (s, 4H, NCH_2py); 7.82 (t, 2H, pyH , $J = 6.1$ Hz); 7.87 (s, 2H, arH); 7.91 (d, 2H, pyH , $J = 7.7$ Hz); 8.32 (t, 2H, pyH , $J = 7.6$ Hz); 8.66 (d, 2H, pyH , $J = 5.0$ Hz). ^{13}C NMR (d^4 -MeOD, 100.62 MHz) 55.2 ($arCH_2N$); 56.7 (NCH_2CH_2); 59.4 (NCH_2CH_2); 119.9 ($arCCH_2$); 123.2 ($arCCO_2H$); 127.2 ($pyCH$); 127.9 ($pyCH$); 136.6 ($arCH$); 143.8

(pyCH); 146.7 (pyCH); 149.2 (pyC); 161.4 (arCOH); 169.1 (CO₂H). **FT-IR spectroscopy** (ν , cm⁻¹) 3365 (m, O–H str); 2936, 2822 (m, C–H str); 2496 (s, N–H str); 1704 (s, C=O str); 1441 (m, O–H def); 1394 (m, C–O–C str); 1104 (m, C–O str); 767 (s, py–H). **ESI mass spectrometry** (methanol) m/z 495.19 [C₂₆H₃₄N₄O₅ + H]⁺

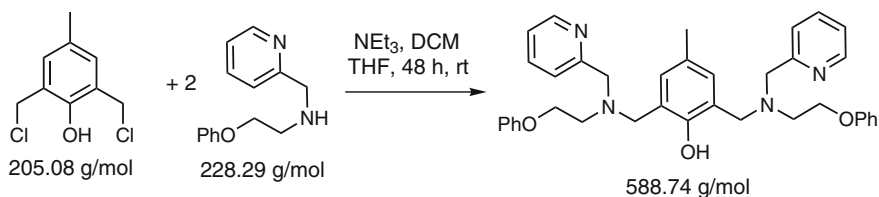
2.5.16 Synthesis of ethyl 4-hydroxy-3,5-bis(((2-phenoxyethyl)(pyridin-2-ylmethyl)amino)methyl)Benzoate (CO₂EtHL3)



Ethyl 3,5-bis(bromomethyl)-4-hydroxybenzoate (0.19 g, 0.55 mmol) in dichloromethane (2 mL) was added dropwise to a mixture of 2-phenoxy-N-(pyridin-2-ylmethyl)ethanamine (0.25 g, 1.09 mmol) and triethylamine (0.30 g) in tetrahydrofuran (2 mL) at 0 °C. The resulting yellow solution was stirred for 48 h at room temperature and monitored with TLC. The solution was then filtered to remove the white precipitated triethylamine hydrobromide and the solvent removed under vacuum. The brown oil was purified by flash column chromatography (ethyl acetate/methanol, 1:1, FeCl₃ stain) to yield a yellow oil (0.18 g, 50 %).

¹H NMR (CDCl₃, 500.13 MHz) δ 1.39 (t, 3H, CH₂CH₃, J = 7.2); 3.04 (t, 4H, NCH₂CH₂, J = 5.7); 3.94 (s, 4H, arCH₂N); 3.99 (s, 4H, NCH₂py); 4.11 (t, 4H, NCH₂CH₂, J = 5.7); 4.35 (q, 2H, CH₂CH₃, J = 7.2); 6.86 (m, 4H, arH, J = 8.7, 1.0); 6.92 (tt, 2H, arH, J = 7.4 1.0); 7.16 (ddd, 2H, pyH, J = 7.5, 4.9, 1.0); 7.25 (m, 4H, arH, J = 7.3); 7.58 (d, 2H, pyH, J = 7.8); 7.63 (td, 2H, pyH, J = 7.6, 1.7); 7.90 (s, 2H, arH); 8.49 (dq, 2H, pyH, J = 4.9, 1.7, 0.8). **¹³C NMR** (CDCl₃, 100.62 MHz) δ 14.4 (CH₂CH₃); 52.4 (NCH₂CH₂), 54.9 (arCCH₂N); 60.3 (NCH₂py/CH₂CH₃); 65.5 (NCH₂CH₂); 114.4 (arCH); 120.7 (arCH); 122.1 (pyCH); 122.2 (arCCH₂); 122.7 (pyCH); 123.1 (arCH); 123.7 (arCCO₂Et); 129.3 (arCH); 130.8 (arCH); 136.6 (pyCH); 148.8 (pyCH); 158.5 (pyC); 160.6 (arCOH); 166.7 (CO₂Et). **ESI mass spectrometry** (methanol) m/z 647.26 [C₃₉H₄₂N₄O₅ + H]⁺. **FT-IR spectroscopy** (ν , cm⁻¹) 2924, 2850 (m, C–H str); 1704 (m, C=O str); 1597 (m, C=C str); 1241 (m, C–O–C str); 1199 (m, C–O str); 752 (s, py–H).

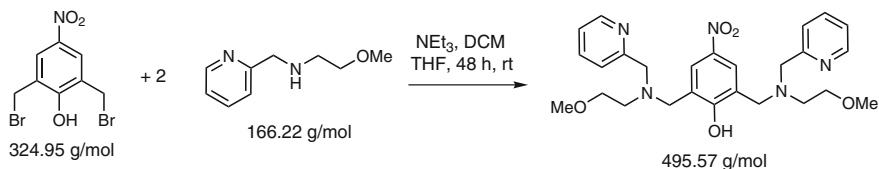
2.5.17 Synthesis of 4-methyl-2,6-bis(((2-phenoxyethyl)(pyridin-2-ylmethyl)amino)methyl)phenol ($\text{CH}_3\text{HL3}$)



2,6-bis(chloromethyl)-*p*-cresol (0.16 g, 0.55 mmol) in dichloromethane (2 mL) was added dropwise to a mixture of 2-phenoxy-*N*-(pyridin-2-ylmethyl)ethanamine (0.25 g, 1.09 mmol) and triethylamine (0.30 g) in tetrahydrofuran (2 mL) at 0 °C. The resulting yellow solution was stirred for 48 h at room temperature. It was then filtered to remove the white precipitate of triethylamine hydrobromide and the solvent removed under vacuum. The yellow oil was purified by flash column chromatography (ethyl acetate/methanol, 9:1, FeCl_3 stain) to yield the ligand as yellow oil (0.17 g, 52 %).

^1H NMR (CDCl_3 , 500.13 MHz) δ 2.26 (s, 3H, CH_3); 3.03 (t, 4H, CH_2O , $J = 5.8$ Hz); 3.88 (s, 4H, arCH_2N); 3.98 (s, 4H, NCH_2py); 4.10 (t, 4H, NCH_2CH_2 , $J = 5.8$ Hz); 6.85 (m, 4H, arCH , $J = 8.7, 1.0$ Hz); 6.92 (tt, 2H, arCH , $J = 7.4, 1.0$ Hz); 6.99 (s, 2H, arCH); 7.13 (ddd, 2H, pyCH , $J = 7.4, 4.9, 1.1$ Hz); 7.24 (m, 4H, CH , $J = 7.4$ Hz); 7.49 (d, 2H, pyCH , $J = 7.8$ Hz); 7.60 (td, 2H, pyCH , $J = 7.6, 1.8$ Hz); 8.53 (dq, 2H, pyCH , 4.8, 1.7, 0.8 Hz). **^{13}C NMR** (CDCl_3 , 100.62 MHz); δ 20.5 (CH_3); 52.3 (CH_2OPh), 55.2 (arCH_2N); 60.4 (NCH_2py); 65.5 (NCH_2); 114.4 (arCH); 120.6 (arCH); 122.0 (pyCH); 123.2 (pyCH); 123.3 (arC); 127.6 (arCCH_3); 129.3 (arCH); 136.5 (pyCH); 148.8 (pyCH); 153.6 (arCOH); 158.6 (pyC). **FT-IR spectroscopy** (ν , cm^{-1}) 2922, 2831 (m, C–H str); 1597 (m, C=C str); 1475 (m, C–H def); 1242 (m, C–O–Ph str); 752 (s, py–H); 729, 691 (s, Ar–H). **ESI mass spectrometry** (methanol) m/z 611.23 [$\text{C}_{37}\text{H}_{40}\text{N}_4\text{O}_3 + \text{Na}$] $^+$; 589.25 [$\text{C}_{37}\text{H}_{40}\text{N}_4\text{O}_3 + \text{H}$] $^+$.

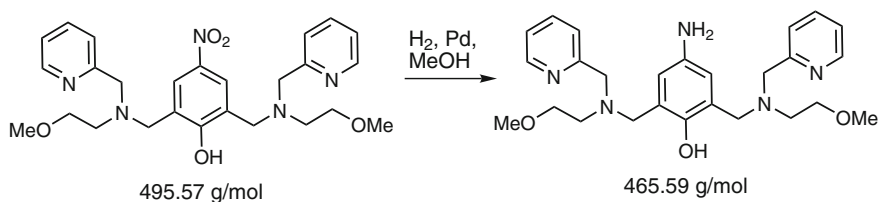
2.5.18 Synthesis of 2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-4-nitrophenol ($\text{NO}_2\text{HL2}$)



2-methoxy-N-(pyridin-2-ylmethyl)aminoethanol (0.51 g, 31 mmol) was dissolved together with triethylamine (0.7 g) in tetrahydrofuran (5 mL) and cooled to 0 °C. Upon dropwise addition of 2,6-bis(bromomethyl)-4-nitrophenol (0.5 g, 15 mmol) in dichloromethane (7 mL) the solution turned bright yellow and a precipitate emerged immediately. The mixture was stirred for 3 days at room temperature, filtered and concentrated in vacuo. After further purification with column chromatography (ethyl acetate/methanol, 8:1, FeCl₃ stain, R_f = 0.76) a yellow oil was obtained (0.5 g, 68 %).

¹H NMR (CDCl₃, 500.13 MHz) δ 2.80 (t, 3H, NCH₂CH₂, J = 5.6 Hz); 3.29 (s, 6H, OCH₃); 3.52 (t, 4H, NCH₂CH₂, J = 5.6 Hz); 3.85 (s, 4H, arCH₂N); 3.91 (s, 4H, NCH₂py); 7.16 (dd, 2H, pyCH, J = 6.9 Hz); 7.42 (d, 2H, pyCH, J = 7.8 Hz); 7.65 (td, 2H, pyCH, J = 7.8, 1.7 Hz); 8.14 (s, 2H, arCH); 8.52 (d, 2H, pyCH, J = 4.4 Hz). **¹³C NMR** (CDCl₃, 100.62 MHz) δ 53.2 (NCH₂); 54.6 (arCH₂N); 58.8 (OCH₃); 60.3 (NCH₂py); 70.6 (CH₂OCH₃); 122.2 (pyCH), 123.0 (pyCH); 124.5 (pyCH); 125.0 (arCH); 136.7 (pyCH); 139.7 (CNO₂); 148.9 (pyCH); 158.5 (pyC); 162.5 (COH). **FT-IR spectroscopy** (ν, cm⁻¹) 3372 (m, O–H str); 2927, 2878, 2821 (m, C–H str); 1592 (m, C=C str); 1514 (m, N=O asym. str); 1471 (m, C–H def); 1329 (s, N=O sym. str); 1095 (m, C–O str); 845 (w, ar–H); 752 (s, py–H). **ESI mass spectrometry** (methanol) m/z 496.25 [C₂₆H₃₃N₅O₅ + H]⁺.

2.5.19 Synthesis of 4-amino-2,6-bis(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)phenol (NH₂HL2)

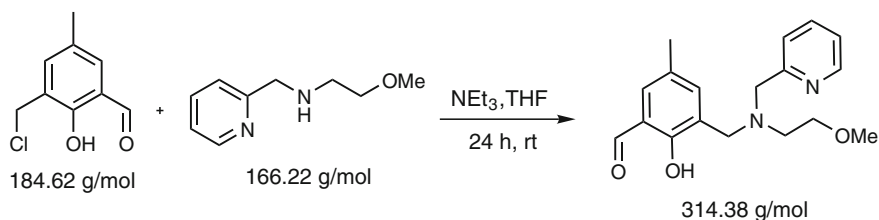


A 0.05 M solution of NO₂HL2 (130 mg) in methanol was cycled twice in an H-cube Hydrogen reactor equipped with a palladium cartridge (full H₂ mode, 1 mL/min flow) at room temperature and atmospheric pressure. After one cycle the orange color of the solution had faded to yellow. After removal of the solvent the desired product was obtained as yellow oil (105 mg, 85 %).

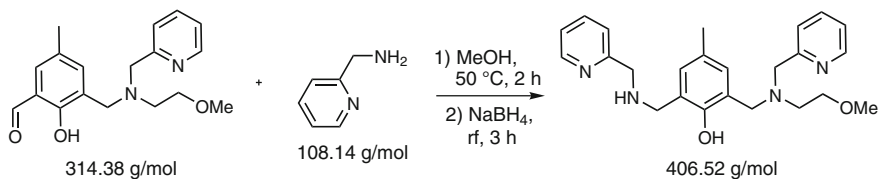
¹H NMR (CDCl₃, 500.13 MHz) δ 2.82 (t, 3H, NCH₂CH₂, J = 5.5 Hz); 3.24 (s, 6H, OCH₃); 3.50 (t, 4H, NCH₂CH₂, J = 5.5 Hz); 3.79 (s, 4H, arCH₂N); 3.91 (s, 4H, NCH₂py); 6.56 (s, 2H, arCH); 7.13 (t, 2H, pyCH, J = 5.7 Hz); 7.42 (d, 2H, pyCH, J = 7.8 Hz); 7.60 (t, 2H, pyCH, J = 3.8 Hz); 8.47 (d, 2H, pyCH, J = 3.9 Hz). **¹³C NMR** (CDCl₃, 100.62 MHz) δ 52.9 (NCH₂); 55.2 (arCH₂N); 58.6 (OCH₃); 59.9 (NCH₂py); 70.0 (CH₂OCH₃); 116.63 (arCH); 122.2 (pyCH);

123.4 (pyCH); 123.6 (pyCH); 136.6 (pyCH); 138.1 (CNH₂); 148.6 (COH); 148.8 (pyCH); 158.0 (pyC). **FT-IR spectroscopy** (ν , cm⁻¹) 3349 (m, N-H/O-H str); 2928, 2881, 2824 (m, C-H str); 1622 (m, N-H def); 1572 (m, C=C str); 1476 (m, C-H def); 1100 (m, C-O str); 760 (s, py-H). **ESI mass spectrometry** (methanol) m/z 466.15 [C₂₆H₃₅N₅O₃ + H]⁺.

2.5.20 Synthesis of 2-(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-4-methyl-6-(((pyridin-2-ylmethyl)amino)methyl)phenol (CH₃HL4)



Triethylamine (2.6 mL) was added dropwise to a mixture of 3-(chloromethyl)-2-hydroxy-5-methylbenzaldehyde (1.0 g, 6.0 mmol) and 2-methoxy-N-(pyridin-2-ylmethyl)ethanamine (1.0 g, 6.0 mmol) in tetrahydrofuran (45 mL) at room temperature and the mixture stirred for 24 h. After filtration and concentration in vacuo the residue was taken up in water (30 mL) and extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over sodium sulfate and concentrated in vacuo to yield 1.69 g (89 %) of crude 2-hydroxy-3-(((2-methoxyethyl)(pyridin-2-ylmethyl)amino)methyl)-5-methylbenzaldehyde as an orange oil which was used in the next step without further purification.

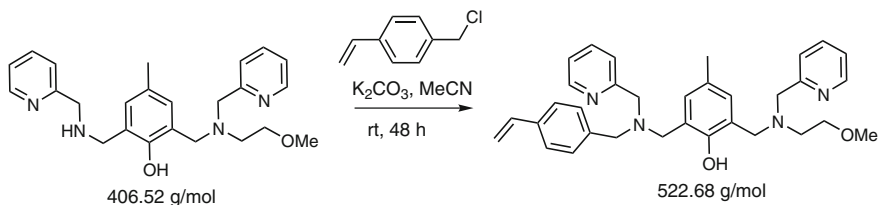


2-hydroxy-3-(((2-methoxyethyl) (pyridin-2-ylmethyl)amino)methyl)-5-methylbenzaldehyde (1.69 g, 5.37 mmol) was dissolved in methanol (50 mL) and 2-aminomethylpyridine (0.58 g, 5.37 mmol) in methanol (25 mL) was added dropwise at room temperature. The resulting mixture was stirred at 50 °C for 2 h. The mixture was subsequently cooled to 0 °C and sodium borohydride (0.72 g, 19.0 mmol) was added in small portions. After heating to reflux for 3 h the crude ligand solution was concentrated in vacuo, taken up in acidified water (100 mL, pH 2) and extracted with dichloromethane (3 × 35 mL). The combined organic

layers were washed with saturated sodium bicarbonate solution (3×50 mL) and dried over sodium sulfate. After removal of the solvent in vacuo the crude ligand (1.53 g, 70 %) was purified with flash column chromatography (ethyl acetate until first band is eluted, methanol/ethyl acetate 9:1, FeCl_3 stain, $R_f = 0.48$ in ethyl acetate) to yield the ligand as a yellow oil (900 mg, 41 %).

^1H NMR (CDCl_3 , 500.13 MHz) δ 2.20 (s, 3H, arCCH_3); 2.76 (t, 2H, NCH_2CH_2 , $J = 5.5$ Hz); 3.26 (s, 3H, OCH_3); 3.50 (t, 2H, NCH_2CH_2 , $J = 5.5$ Hz); 3.77 (s, 2H, arCCH_2N); 3.85 (s, 2H, arCCH_2NH); 3.92 (s, 2H, NCH_2py); 3.96 (s, 2H, NHCH_2py); 6.78 (d, 1H, arCH , $J = 1.8$ Hz); 6.92 (d, 1H, arCH , $J = 1.8$ Hz); 7.13 (qd, 2H, pyH , $J = 7.4, 1.2$ Hz); 7.34 (dd, 2H, pyH , $J = 5.1, 1.5$ Hz); 7.62 (tt, 2H, pyH , $J = 7.6, 1.9$ Hz); 8.51 (ddt, 2H, pyH , $J = 6.6, 1.7, 0.9$ Hz). **^{13}C NMR** (CDCl_3 , 100.62 MHz) δ 20.4 (arCCH_3); 49.2 (NCH_2py); 52.6 (NCH_2CH_2); 53.7 (NHCH_2py); 56.9 (arCCH_2N); 58.6 (OCH_3); 60.0 (arCCH_2NH); 70.1 (NCH_2CH_2); 122.0 (pyCH); 122.2 (pyCH); 122.3 (pyCH); 122.4 (arC); 123.4 (pyCH); 124.4 (arC); 127.8 (arCCH_3); 129.2 (arCH); 129.7 (arCH); 136.5 (pyCH); 136.6 (pyCH); 148.9 (pyCH); 149.0 (pyCH); 153.5 (arCOH); 158.0 (pyCCH_2); 158.6 (pyCCH_2). **FT-IR spectroscopy** (ν , cm^{-1}) 3402 (m, O–H str); 2917, 2819 (m, C–H str); 1108 (m, C–O str); 863 (w, ar–H); 755 (s, py–H). **ESI mass spectrometry** (methanol) m/z 407.23 [$\text{C}_{24}\text{H}_{30}\text{N}_4\text{O}_2 + \text{H}$] $^+$.

2.5.21 Synthesis of 2-(((2-methoxyethyl)(pyridine-2-ylmethyl)amino)methyl)-4-methyl-6-(((pyridin-2-ylmethyl)(4-vinylbenzyl)amino)methyl)phenol ($\text{CH}_3\text{HL5}$)

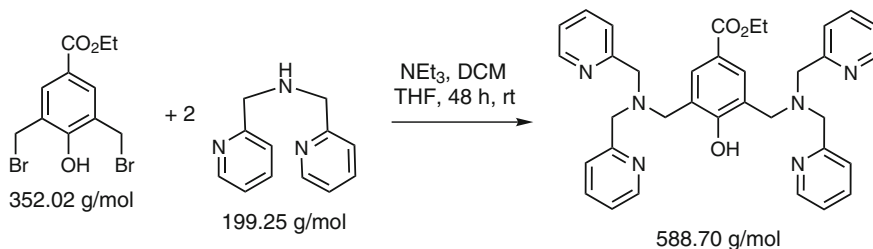


$\text{CH}_3\text{HL4}$ (500 mg) and vinylbenzylchloride (0.174 mL) were stirred with dry powdered potassium carbonate (0.256 g) in acetonitrile (5 mL) at room temperature for two days. After this time the mixture was filtered and concentrated in vacuo. The crude ligand was purified by flash column chromatography (ethyl acetate/methanol 5:2) and obtained as orange oil (Yield: 38 %, 0.24 g).

^1H NMR (CDCl_3 , 500.13 MHz) δ 2.24 (s, 3H, arCCH_3); 2.77 (t, 2H, NCH_2CH_2 , $J = 5.7$ Hz); 3.27 (s, 3H, OCH_3); 3.52 (t, 2H, NCH_2CH_2 , $J = 5.7$ Hz); 3.65 (s, 2H, CH_2); 3.71 (s, 2H, CH_2); 3.77 (s, 2H, CH_2); 3.79 (s, 2H, CH_2); 3.86 (s, 2H, CH_2); 5.19 (dd, 1H, $\text{CH}=\text{CH}_2$ cis, $J = 10.9, 0.6$ Hz); 5.69 (dd, 1H, $\text{CH}=\text{CH}_2$ trans, $J = 17.6, 0.8$ Hz); 6.67 (dd, 1H, $\text{CH}=\text{CH}_2$, $J = 17.6, 10.9$ Hz); 6.89 (d, 1H, arCH , $J = 1.7$ Hz); 7.03 (d, 1H, arCH , $J = 1.8$ Hz); 7.13 (m, 2H, pyCH , $J = 6.4, 0.9$ Hz);

7.34 (s, 4H, vinylbenzylH); 7.43 (d, 1H, pyCH, $J = 7.8$ Hz); 7.51 (d, 1H, pyCH, $J = 7.8$ Hz); 7.62 (m, 2H, pyCH, $J = 7.7, 1.8$ Hz); 8.51 (m, 2H, pyCH, $J = 0.9$ Hz). ^{13}C NMR (CDCl_3 , 100.62 MHz) δ 20.6 (arCCH_3); 52.9 (NCH_2CH_2); 53.8 (CH_2); 55.7 (CH_2); 57.9 (CH_2); 58.6 (OCH_3); 59.6 (CH_2); 60.4 (CH_2); 70.4 (NCH_2CH_2); 113.4 ($\text{CH}=\text{CH}_2$); 121.8 (pyCH); 122.0 (pyCH); 122.9 (pyCH); 123.2 (pyCH); 123.9 ($\text{arC}-\text{CH}=\text{CH}_2$); 126.1 (vinylbenzyl arCH); 127.5 (arCCH_3); 129.0 (arCH); 129.1 (vinylbenzyl arCH); 129.3 (arCH); 136.4 (pyCH); 136.5 (pyCH); 136.6 ($\text{CH}=\text{CH}_2$); 138.5 (arC); 148.8 (pyCH); 148.9 (pyCH); 153.5 (arCOH); 158.0 (pyCCH₂); 158.6 (pyCCH₂). **FT-IR spectroscopy** (ν , cm^{-1}) 2918, 2816 (m, C–H str); 1109 (m, C–O str); 861 (w, ar–H); 755 (s, py–H). **ESI mass spectrometry** (methanol) m/z 523.20 [$\text{C}_{33}\text{H}_{38}\text{N}_4\text{O}_2 + \text{H}$]⁺.

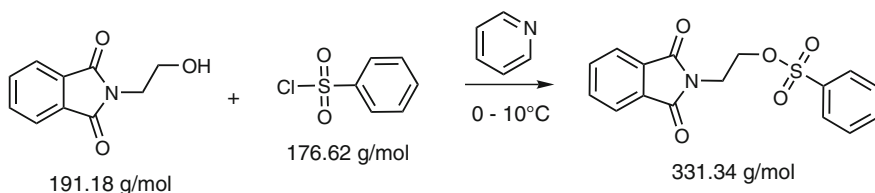
2.5.22 Synthesis of Ethyl 4-hydroxy-3,5-bis((bis(pyridine-2-ylmethyl)amino)methyl)benzoate ($\text{CO}_2\text{EtHL6}$)



Bis(pyridine-2-ylmethyl)amine (0.57 g, 28 mmol) was dissolved in tetrahydrofuran (4 mL) together with triethylamine (0.8 g) and cooled to 0 °C. Upon addition of ethyl 3,5-bis(bromomethyl)-4-hydroxybenzoate (0.50 g, 14 mmol) in dichloromethane (4 mL) a precipitate emerged immediately. The mixture was stirred for 48 h at room temperature, filtered and concentrated in vacuo. After further purification with column chromatography (ethyl acetate/methanol, 8:1, FeCl_3 stain, $R_f = 0.55$) a brown oil was obtained (0.5 g, 61 %).

^1H NMR (CDCl_3 , 500.13 MHz) δ 1.31 (t, 3H, CH_2CH_3 , $J = 7.1$ Hz); 3.77 (s, 4H, arCH_2N); 3.82 (s, 8H, NCH_2py); 4.25 (q, 2H, CH_2CH_3 , $J = 7.1$ Hz); 7.06 (ddd, 4H, pyH, $J = 7.4, 4.9, 1.0$ Hz); 7.42 (d, 4H, pyH, $J = 7.8$ Hz); 7.54 (td, 4H, pyH, $J = 7.7, 1.8$ Hz); 7.88 (s, 2H, arH); 8.45 (d, 4H, pyH, $J = 4.8$ Hz). ^{13}C NMR (CDCl_3 , 100.62 MHz) δ 14.3 (CH_3); 50.1 (arCCH_2N); 60.2 (pyCH); 60.3 (CH_2CH_3); 120.3 (arCCO_2Et); 121.9 (pyCH); 122.8 (pyCH); 124.1 (arCCH_2); 130.9 (arCH); 136.5 (pyCH); 148.6 (pyCH); 158.8 (pyC); 160.4 (arCOH); 166.6 (CO_2Et). **ESI mass spectrometry** (methanol) m/z 589.25 [$\text{C}_{35}\text{H}_{36}\text{N}_6\text{O}_3 + \text{H}$]⁺. **FT-IR spectroscopy** (ν , cm^{-1}) 2982, 2928, 2822 (m, C–H str); 1704 (m, C=O str); 1592 (m, C=C str); 1199 (m, C–O str); 758 (m, py–H); 728 (s, py–H).

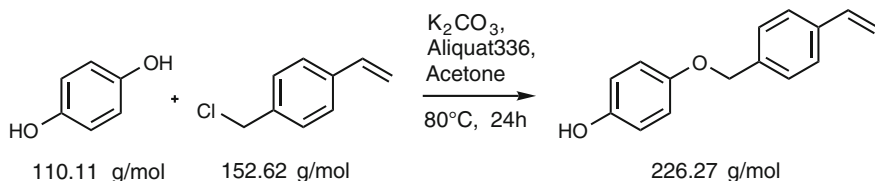
2.5.23 Synthesis of 2-(1,3-dioxoisindolin-2-yl)ethyl Benzenesulfonate



N-(2-hydroxyethyl)-phthalimide (5 g, 26 mmol) was dissolved in pyridine (30 mL) in a dry 3-necked flask equipped with a condenser, pressure equalizing dropping funnel, thermometer and nitrogen inlet and cooled to 0 °C. Benzenesulfonylchloride (10.5 mL, 79 mmol) was added dropwise such the temperature did not rise above 10 °C. Upon completion of the addition, the slurry was stirred at 50 °C for 30 min, cooled to room temperature and poured into a mixture of hydrochloric acid (40 mL), water (50 mL), methanol (100 mL) and ice (25 g). The white precipitate was dried under vacuum, washed twice with cold methanol (50 mL) and recrystallized from ethanol (~140 mL) to yield the desired product as white needles (7 g, 80 %).

¹H NMR (CDCl₃, 500.13 MHz) δ 3.91 (t, 2H, J = 5.4 Hz, CH₂OSO₂); 4.32 (t, 2H, J = 5.3 Hz, NCH₂CH₂); 7.36 (tt, 2H, J = 8.0, 1.7 Hz, arH); 7.46 (tt, 1H, J = 7.6, 1.2 Hz, arH); 7.68–7.72 (m, 2H, arH); 7.76–7.81 (m, 4H, arH). **¹³C NMR** (CDCl₃, 100.62 MHz) δ 36.8 (NCH₂); 66.5 (CH₂OSO₂); 123.4 (arCH); 127.7 (arH); 129.0 (arH); 131.7 (arCCO); 133.6 (arH); 134.1 (arH); 135.6 (arCSO₃); 167.6 (CON). **ESI mass spectrometry** (methanol) m/z 353.96 [C₁₆H₁₃NO₅S + Na]⁺. **FT-IR spectroscopy** (ν, cm⁻¹) 2949 (w, C–H str); 1773 (m, C=O_{Imid}); 1714 (s, C=O_{Imid}); 1390, 1356 (s, CH₂OSO₂–Ph); 1185 (s, SO₂–Ph); 1045 (m, S=O); 748, 722, 679 (m, ar–H). **Melting point** 138.2 °C.

2.5.24 Synthesis of 4-((4-vinylbenzyl)oxy)phenol

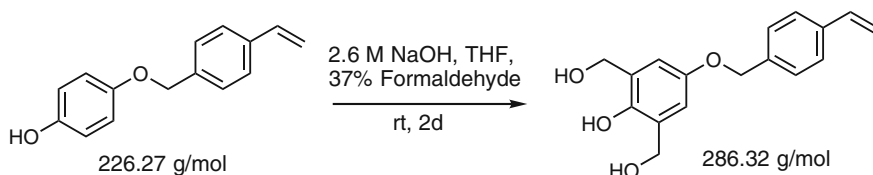


The compound was prepared after a slight modification of a previously published protocol [54]. 1,4-hydroquinone (8.8 g, 80 mmol), 4-vinylbenzyl chloride (3.0 g, 20 mmol), dried, powdered potassium carbonate (2.8 g, 20 mmol) and Aliquat336 (0.8 g, 2 mmol) were dissolved in acetone (75 mL) and the mixture heated to

80 °C for 24 h. After cooling to room temperature the mixture was concentrated in vacuo, chloroform (30 ml) added and the mixture filtered through Celite. The crude product was purified with flash column chromatography (ethyl acetate/hexane 9:1, R_f = 0.76, I_2 stain) to yield an orange solid (1.66 g, 36 %).

^1H NMR (CDCl_3 , 500.13 MHz) δ 5.01 (s, 2H, CH_2); 5.26 (dd, 1H, $\text{CH}=\text{CH}_2$, J = 10.8 Hz); 5.79 (dd, 1H, $\text{CH}=\text{CH}_2$, J = 17.6, 1.5 Hz); 6.73–6.88 (m, 5H, arH, $\text{CH}=\text{CH}_2$); 7.37–7.43 (q, 4H, arH, J = 10.8 Hz). **^{13}C NMR** (CDCl_3 , 100.62 MHz) δ 70.5 (OCH_2); 114.0 ($\text{CH}=\text{CH}_2$); 116.0 (arCH); 116.1 (arCH); 126.4 (arCH); 127.7 (arCH); 136.5 ($\text{CH}=\text{CH}_2$); 136.8 (arC); 137.3 (arC); 149.7 (arCOH); 153.0 (arCOCH₂). **ESI mass spectrometry** (methanol) m/z 249.12 [$\text{C}_{15}\text{H}_{14}\text{O}_2 + \text{Na}$]⁺. **FT-IR spectroscopy** (ν , cm^{-1}) 3422 (m, O–H str); 3032 (w, C=C str); 2942, 2864 (w, CH_2 str); 1236 (s, C–O str); 1013 (s, C–O–C str); 991 (m, $\text{CH}=\text{CH}_2$ def); 818 (s, ar–H). **Melting point** 127.6–130.5 °C Lit: 143.0–144.0 °C [54].

2.5.25 Synthesis of 2,6-bis(hydroxymethyl)-4-[(4-vinylbenzyl)oxy]phenol

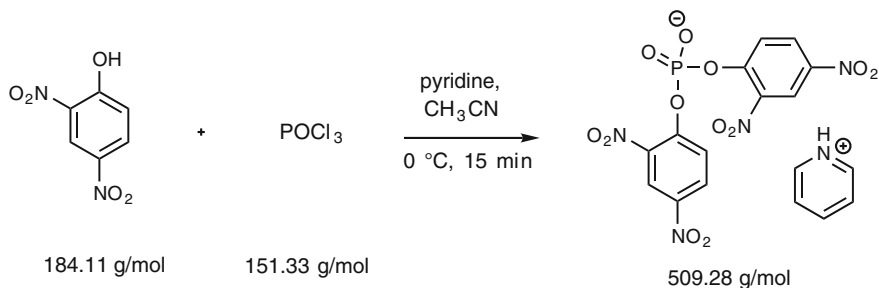


This compound was prepared after a previously published procedure [55]. The 4-((4-vinylbenzyl)oxy)phenol (1 g, 4.4 mmol) was dissolved in tetrahydrofuran (2 mL) and formaldehyde (37 %, 1 mL) and aqueous sodium hydroxide solution (2.6 M, 2.5 mL) were added. The brown suspension was stirred for two days at room temperature, subsequently hydrochloric acid (0.1 M, 5 mL) were added and the aqueous phase extracted with ethyl acetate (2 × 15 mL). The combined organic layers were dried over sodium sulfate and the solvent removed in vacuo. After purification with column chromatography (hexane/ethyl acetate 1:1, FeCl_3 stain, R_f = 0.3) a pale yellow powder was obtained (372 mg, 59 %).

^1H NMR (CDCl_3 , 500.13 MHz) δ 4.75 (s, 4H, CH_2OH); 4.96 (s, 2H, arCH₂O); 5.22 (dd, 1H, $\text{CH}=\text{CH}_2$, J = 10.8 Hz); 5.75 (dd, 1H, $\text{CH}=\text{CH}_2$, J = 17.6, 0.6 Hz); 6.67–6.76 (m, 3H, arH, $\text{CH}=\text{CH}_2$, J = 10.9 Hz); 7.32–7.40 (q, 4H, arH, J = 12.4 Hz). **^{13}C NMR** (CDCl_3 , 100.62 MHz) δ 63.7 (CH_2OH); 70.6 (OCH_2); 114.2, 114.4 ($\text{CH}=\text{CH}_2$); 126.5 (arCH); 127.1 (arCCH₂OH); 127.7 (arCH); 136.5 (arCH); 136.7 (arC); 137.4 (arCCH=CH₂); 148.7 (arCOH); 151.8 (arCOCH₂). **ESI mass spectrometry** (methanol) m/z 309.20 [$\text{C}_{17}\text{H}_{18}\text{O}_4 + \text{Na}$]⁺. **FT-IR spectroscopy** (ν , cm^{-1}) 3423 (m, O–H str); 3032 (w, C=C str); 2942, 2864 (w, CH_2 str); 1236 (s, C–O str); 1013 (s, C–O–C str); 991 (m, $\text{CH}=\text{CH}_2$ def); 818 (s, ar–H). **Melting point** 100.2–105.5 °C.

2.6 Substrate Synthesis

2.6.1 Synthesis of bis(2,4-dinitrophenyl)Phosphate (BDNPP)

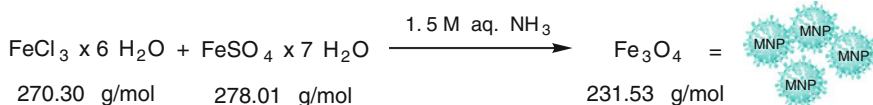


This compound was prepared with slight modifications of a previously published procedure [56]. 2,4-dinitrophenol was recrystallized from ethanol 3 times and stored under ethanol prior to use. Acetonitrile was distilled and dried with activated 3 Å molecular sieves. Pyridine was distilled and dried over 4 Å molecular sieves. 2,4-Dinitrophenol (2.76 g, 15 mmol) was dried and transferred into a Schlenk flask which was carefully evacuated and flushed with nitrogen twice. Acetonitrile (30 mL) and pyridine (2.5 mL) were added via a syringe/septum. Freshly distilled POCl₃ (0.5 mL, 5 mmol) was added after the mixture was cooled to 0 °C on an ice bath. The mixture was stirred for 15 min and subsequently poured on ice water (200 mL). A yellow oil released from the reaction mixture which crystallized overnight in the fridge. The precipitate was dried in vacuo and recrystallized twice from acetone/diethyl ether to yield a white powder (1.6 g, 60 %).

¹H NMR (d⁴-MeOD, 500.13 MHz); δ 7.85 (d, 2H, arH, J = 9.1 Hz); 8.04 (t, 2H, pyH, J = 6.8 Hz); 8.40 (dd, 2H, arH, J = 9.2, 2.8 Hz); 8.59 (tt, 1H, pyH, J = 7.8, 2.9 Hz); 8.66 (dd, 2H, arH, J = 1.3, 0.5 Hz); 8.79 (d, 2H, pyH, J = 5.2 Hz). **¹³C NMR** (d⁴-MeOD, 100.62 MHz); δ 122.1 (arCH); 124.4 (arCH); 128.7 (arCH); 129.5 (arCNO₂); 143.1 (arCNO₂); 144.2 (arCNO₂); 148.2 (arCNO₂); 151.2 (arCO); 151.3 (arCO). **³¹P NMR** (d⁴-MeOD, 161.9 MHz) δ -14.19 **FT-IR spectroscopy** (ν, cm⁻¹): 2569 (b, P–O str); 2160 (w, py, N⁺–H str); 1602 (m, C=C str), 1526 (s, C–NO₂ asym str); 1341 (s, C–NO₂ sym str); 1258 (s, P = O str); 1239 (m, P–O–Ar str); 874, 758, 739, 683 (m, Ar–H str). **Microanalysis** C₁₇H₁₂N₅O₁₂P: calc. C: 40.09, H: 2.37, N: 13.75; found: C 40.08, H 2.24, N 13.62 %. **Melting Point** 166.5–170.9 °C Lit: 157.0–160.0 °C [56].

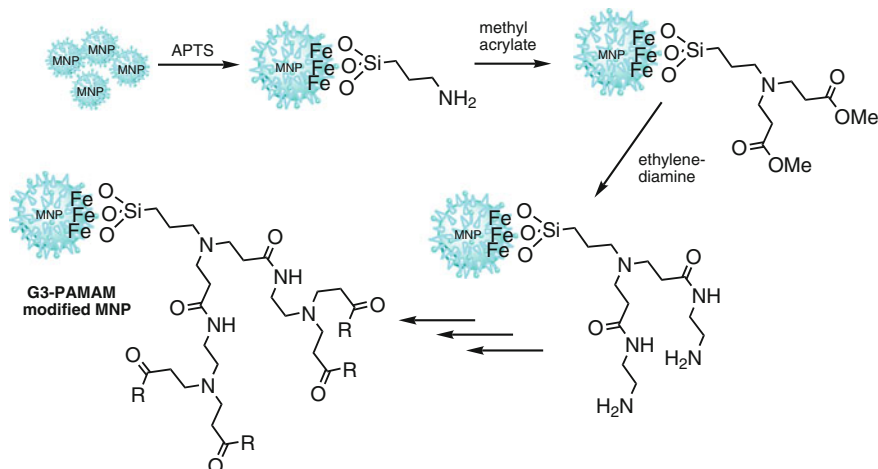
2.7 Nanoparticle Synthesis and Immobilization Procedures

2.7.1 Synthesis of Functionalized Magnetite Nanoparticles



The magnetite nanoparticles were synthesized after a previously published procedure [57, 58]. Solutions of ferric trichloride (1.15 g in 50 mL degassed water) and ferrous sulfate (0.69 g, in 50 mL degassed water) were combined under nitrogen and 1.5 M ammonia solution was added dropwise with vigorous stirring. As the pH reached 9, the black precipitated magnetite was collected with a magnet and washed with water (5 × 50 mL) and ethanol (2 × 50 mL). The black powder was dispersed in ethanol to give a 5 wt % suspension.

FT-IR spectroscopy (ν , cm^{-1}) 560 (m, Fe–O str). **Microanalysis** Fe_3O_4 : found C 0.24, H 0.35, N 0.05, S 0.75 % (slight sulfur contamination due to sulfate residues)



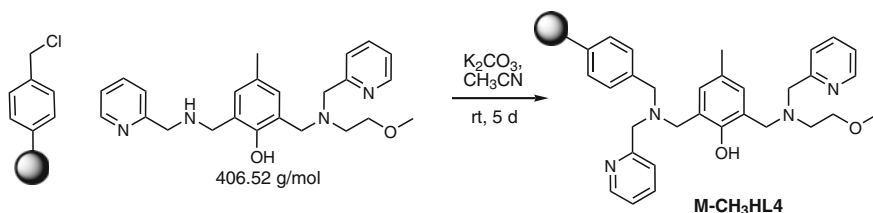
Magnetite stock solution (100 mL) was sonicated for 30 min and diluted with ethanol (300 mL) in a two-necked flask equipped with a reflux condenser and a pressure equalized dropping funnel. 3-aminopropyltrimethoxysilane (APTS, 20 mL) was added over 7 h at 60 °C and the nanoparticles were subsequently washed with methanol (3 × 50 mL) by magnetic separation and dried in vacuo.

FT-IR spectroscopy (ν , cm^{-1}) 2949, 2866 (w, C–H str); 1015 (m, Si–O str); 569 (m, Fe–O str). **Microanalysis** found C 1.69, H 0.64, N 0.47, S 0.69 % (slight sulfur contamination due to sulfate residues).

1.16 g of APTS modified magnetite nanoparticles were dispersed in methanol (100 mL) and methyl acrylate (40 mL) was added. After sonication for 5 min the mixture was stirred overnight at room temperature and washed with methanol (5×20 mL). Ethylenediamine (8 mL) and methanol (10 mL) were added and stirred at 50 °C for 5 h. This step was repeated twice with increased amounts of methyl acrylate (60 and 80 mL) and ethylenediamine (12 and 20 mL) and the final product was washed with methanol (3×50 mL) and water (3×50 mL).

FT-IR spectroscopy (ν , cm^{-1}) 3314 (b, N–H); 2668 (w, C–H str); 1625, 1551 (m, C=O_{Amide}); 1017 (m, Si–O str); 715 (w, CH₂); 560 (m, Fe–O str). **Microanalysis** found C 3.53, H 0.75, N 1.44, S 0.00 %.

2.7.2 Immobilization of CH₃HL4 on Merrifield Resin



To Merrifield resin (1 % cross-linked, 3.5 mmol/g Cl, 500 mg) in acetonitrile (10 mL) was added CH₃HL4 (320 mg, 0.78 mmol) and dried potassium carbonate (250 mg) and the mixture was stirred for 10 days. After this time the orange beads of the resin were suction dried on a sintered glass funnel (the filtrate was collected to recover any unbound ligand) and washed with water (20 mL) and methanol (20 mL). The resin was further dried under high vacuum to yield 646 mg of M-CH₃HL4. 26 mg of ligand were recovered from the filtrate.

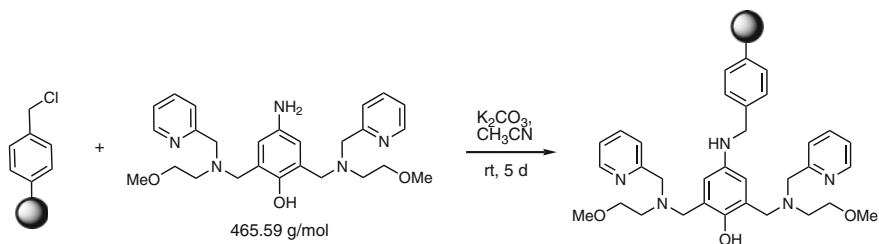
FT-IR spectroscopy (ν , cm^{-1}) 3352 (b, O–H str); 3025, 2922 (w, C–H str); 1110 (m, C–O–C str); 814, 756, 699 (m, Ar–H/Py–H str). **Microanalysis** found C 77.39, H 7.22, N 4.86, S 0.00 %.

2.7.2.1 Zinc Complex of MR-CH₃HL4

To MR-CH₃HL4 (400 mg) in methanol (10 mL) was added zinc(II) acetate dihydrate (400 mg) and the mixture was refluxed for 30 min and then stirred for 24 h. Subsequently the resin was suction dried and washed with water (10 mL) and methanol (20 mL) to yield 505 mg of the immobilized complex MR-[Zn₂(CH₃L4)(CH₃COO)₂].

FT-IR spectroscopy (ν , cm^{-1}) 3410 (b, O–H str); 3025, 2924 (w, C–H str); 1596 (s, bridging acetate asym. str); 1421 (s, bridging acetate sym. str); 809, 761, 700, 664 (m, Ar–H/Py–H str). **Microanalysis** found C 64.42, H 6.18, N 3.58, S 0.00 %.

2.7.3 Immobilization of $\text{NH}_2\text{HL2}$ on Merrifield Resin



To Merrifield resin (1 % cross-linked, 3.5 mmol/g Cl, 150 mg) in acetonitrile (5 mL) was added $\text{NH}_2\text{HL2}$ (100 mg, 0.21 mmol) and dried potassium carbonate (75 mg) and the mixture was stirred for 10 days. After this time the brown beads were suction dried on a sintered glass funnel (the filtrate was collected to recover any unbound ligand) and washed with water (10 mL) and methanol (10 mL). The resin was further dried under high vacuum to yield 80 mg of MR-NHHL2. 36 mg of ligand were recovered from the filtrate.

FT-IR spectroscopy (ν , cm^{-1}) 3359 (b, O–H str); 3025, 2921 (w, C–H str); 1111 (m, C–O–C str); 818, 757, 699 (s, Ar–H/Py–H str). **Microanalysis** found C 78.41, H 7.35, N 5.23, S 0.00 %.

2.7.3.1 Zinc Complex of MR-NHHL2

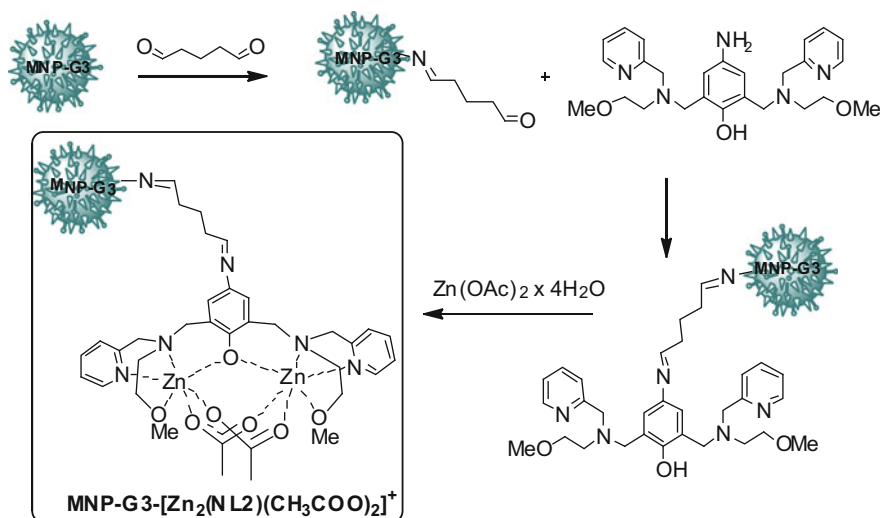
To MR-NHHL2 (58 mg) in methanol (3 mL) was added zinc(II) acetate dihydrate (58.8 mg) and the mixture was refluxed for 30 min. Subsequently the resin was washed with methanol (20 mL) and suction dried. After removal of residual solvent in high vacuum 69 mg of the immobilized complex MR-[$\text{Zn}_2(\text{NHL2})(\text{CH}_3\text{COO})_2$] were obtained.

FT-IR spectroscopy (ν , cm^{-1}) 3417 (b, O–H str); 3027, 2921 (w, C–H str); 1599 (s, bridging acetate antisym. str); 1414 (s, bridging acetate sym. str); 704 (m, Ar–H/Py–H str). **Microanalysis** found C 64.56, H 6.43, N 3.84, S 0.00 %.

2.7.4 Functionalizing G3-MNP with Glutaraldehyde

G3-MNP (150 mg) were suspended in glutaraldehyde (25 %, 20 mL) and sonicated for one hour. After 12 h standing the nanoparticles were separated from glutaraldehyde and washed with TRIS buffer pH 8 (5×15 mL). G3-MNP-glutaraldehyde were used in the next step without further purification for the GpdQ immobilization and for the ligand immobilization they were washed with methanol (1 mL).

2.7.4.1 Immobilization of $[\text{Zn}_2(\text{NH}_2\text{HL2})(\text{CH}_3\text{COO})_2]$ on G3-MNP-glutaraldehyde



To G3-MNP-glutaraldehyde (75 mg) in methanol (1 mL) was added $\text{NH}_2\text{HL2}$ (50 mg) and dried potassium carbonate (75 mg) and the mixture was left standing at room temperature for five days. After this time the nanoparticles were washed by magnetic separation until the solution was clear. The nanoparticles were then resuspended in methanol (1.5 mL) and zinc acetate dihydrate (50.2 mg) was added. After stirring for 30 min the MNP were washed with methanol (5×1.5 mL) and dried in vacuo.

FT-IR spectroscopy (ν , cm^{-1}) 3359 (b, O–H str); 3025, 2921 (w, C–H str); 1111 (m, C–O–C str); 818, 757, 699 (s, Ar–H/Py–H str). **Microanalysis** found C 78.41, H 7.35, N 5.23, S 0.00 %.

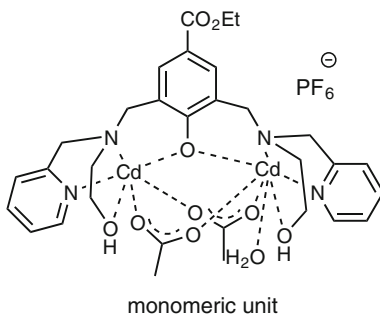
2.7.4.2 Immobilization of Ser127Ala Mutant GpdQ on PAMAM MNP

The G3-MNP (50 mg) that had been functionalized with glutaraldehyde were added to a GpdQ stock solution (0.17 mM, 2 mL) and the mixture incubated 24 h at 4 °C. After this time a sufficiently high amount of enzyme has bound to the nanoparticles via amide formation between the lysine residues of the enzyme and the pendant aldehydes on the particles (Initially the enzyme solution was incubated for five days with the nanoparticles but analysis showed that the amount of enzyme immobilized did not increase significantly after 24 h). This was confirmed by the decrease of absorbance at 280 nm of the supernatant. After a thorough washing process (5×1.5 mL 50 mM TRIS pH 8) the GpdQ-nanoparticles were suspended in 0.5 mL buffer (50 mM TRIS pH 8) and stored at 4 °C until further use. The amount of GpdQ that was immobilized with this procedure was 1.488 μmol per g MNP.

2.8 Syntheses of the Metal Complexes

2.8.1 Synthesis of $[Cd_4(CO_2EtHL1)_2(CH_3COO)_{3.75}Cl_{0.25}(H_2O)_2](PF_6)_2$

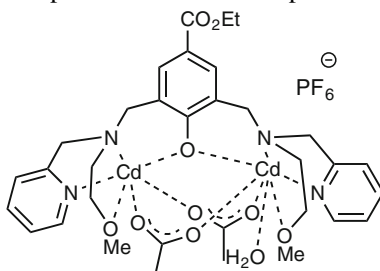
Methanol solutions of CO_2EtHL1 (0.1 M, 1 mL), cadmium acetate (0.1 M, 2 mL), sodium acetate (0.1 M, 1 mL) and sodium hexafluorophosphate (0.1 M, 3 mL) were combined with methanol (2 mL) and the yellow solution was left to evaporate slowly at room temperature. White crystals were obtained after 5 days. After recrystallization from a minimum of hot methanol the complex was obtained in a 66 % yield (40 mg).



ESI mass spectrometry (methanol) m/z : 837.0 $[C_{31}H_{29}Cd_2N_4O_9]^+$, 777.0 $[C_{29}H_{35}Cd_2N_4O_7]^+$, 606.1 $[C_{54}H_{66}Cd_2N_4O_{10}]^{2+}$. **Microanalysis** Anal calc. for $C_{61.5}H_{81.25}Cd_4N_8Cl_{0.25}O_{19.5}P_2F_{12}$ C 37.06, H 4.11, N 5.62 % Found: C 37.43, H 4.28, N 5.63 %. **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); 1547 (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). **1H 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, acetate CH_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 The ar CCO_2Et signal was not assigned due to overlapping with the solvent signal. 14.5 (CH_2CH_3); 22.7 (acetate CH_3); 56.9 (CH_2); 57.7 (CH_2); 58.3 (CH_2); 58.7 (CH_2); 61.6 (CH_2); 124.4 (py CH); 124.5 (py CH); 125.5 (ar C); 134.4 (ar CH); 140.1 (py CH); 148.8 (py CH); 156.1 (py C); 161.1 (ar COH); 181.8 (CO_2^-). **^{113}Cd NMR** (CD_3CN/D_2O 1:1, 89 MHz, referenced to $Cd(OAc)_2 \times 2H_2O$ in D_2O) δ 36.4.

2.8.2 Synthesis of $[Cd_2(CO_2EtL2)(CH_3COO)_2H_2O](PF_6)$

CO_2EtHL2 (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. The complex was, however, readily formed as confirmed by mass spectroscopic measurements. After repeated (5×) evaporation of the methanolic complex solution a white powder was obtained.



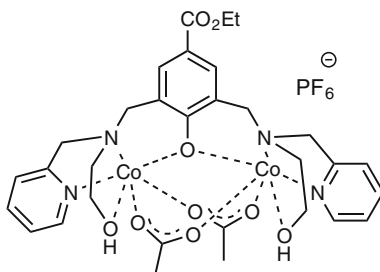
FT-IR spectroscopy (ν , cm^{-1}): 3385 (b, O–H str in water) 2927 (w, C–H str); 1702 (m, C=O str); 1603 (m, asym str OAc); 1574 (m, asym str OAc); 1441 (m, sym str OAc); 1368 (m, sym str OAc). 834 (s, P–F str). **ESI mass spectrometry** (methanol) found m/z 864.0 (100 %), 863.0 (90 %) $[C_{33}H_{43}Cd_2N_4O_9]^+$ calc. m/z 865.11 (100.0 %), 863.11 (88.3 %), 864.11 (86.7 %). **1H 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, acetate CH_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, $pyCH$); 7.76 (m, 2H, $pyCH$); 7.87 (t, 1H, $pyCH$, $J = 6.79$ Hz); 7.97, 8.12 (s, 2H, $arCH$); 8.19 (m, 1H, $pyCH$); 8.32 (m, 1H, $pyCH$); 8.83 (m, 1H, $pyCH$); 8.94 (m, 1H, $pyCH$). **^{13}C NMR** (CD_3CN/D_2O 1:1, 100.62 MHz, referenced to CD_3CN); δ 14.6 (CH_2CH_3); 22.8 (acetate CH_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 (arC); 125.7 (arC); 134.4 ($arCH$); 134.8 ($arCH$); 140.3 ($pyCH$); 140.6 ($pyCH$); 148.8 ($pyCH$); 149.3 ($pyCH$); 155.9 (pyC); 168.5 ($arCOH$); 171.0 (CO_2Et); 181.1 (CO_2^-); **^{113}Cd NMR** (CD_3CN/D_2O 1:1, 89 MHz) δ 23.4.

2.8.3 Synthesis of $[Co_2(CO_2EtL1)(CH_3COO)_2](PF_6)$

Method 1 The methanol ligand solution of CO_2EtHL1 (0.1 M, 1 mL) was combined with a solution of cobalt(II) acetate tetrahydrate (0.1 M, 2 mL) in methanol. Subsequently sodium hexafluorophosphate (0.1 M, 3 mL) was added. The solution

was left at room temperature to evaporate. Pink clusters of crystals were obtained after two days in 88 % yield (310 mg).

Method 2 (X-ray suitable crystals were obtained with this procedure) All solutions were made up in degassed methanol and combined under nitrogen in a Schlenk flask. A flask containing diethyl ether (10 mL) was attached to the Schlenk flask. When the diffusion was complete, the pink solution was poured in a beaker and left to evaporate for 3 days to yield pink needles which were subsequently analyzed by X-ray crystallography. The mass spectra of the crystals obtained by methods 1 and 2 were identical.

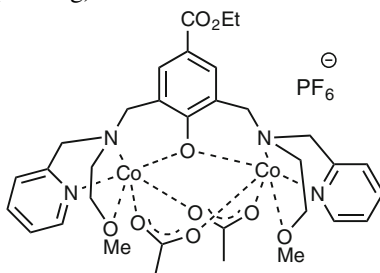


ESI mass spectrometry (methanol) m/z : 729.2 $[\text{C}_{31}\text{H}_{29}\text{Co}_2\text{N}_4\text{O}_9]^+$, 669.1 $[\text{C}_{29}\text{H}_{35}\text{Co}_2\text{N}_4\text{O}_7]^+$, 609.1 $[\text{C}_{27}\text{H}_{31}\text{Co}_2\text{N}_4\text{O}_5]^+$, 305.1 $[\text{C}_{27}\text{H}_{31}\text{Co}_2\text{N}_4\text{O}_5]^{2+}$.

Microanalysis Anal calc. for $\text{C}_{31}\text{H}_{39}\text{Co}_2\text{N}_4\text{O}_9\text{PF}_6$ C 42.58, H 4.50, N 6.41; found: C 42.16, H 4.40, N 6.39 %. **FT-IR spectroscopy** (ν , cm^{-1}) 1605 (m, asym str acetate); 1442 (s, sym str acetate) 1022 (m, O-H); 834 (s, P-F str); 767 (m, Py-H def); 555 (m, P-F). **Magnetic moment** in solution by Evans method [28]. 6.14 BM. **UV-Vis spectroscopy** Acetonitrile, 0.02 M, $\lambda_1 = 493 \text{ nm}$ ($\epsilon = 63.2 \text{ L mol}^{-1} \text{ cm}^{-1}$).

2.8.4 Synthesis of $[\text{Co}_2(\text{CO}_2\text{EtL2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$

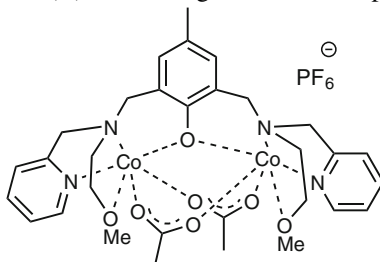
$\text{CO}_2\text{EtHL2}$ (100 mg, 0.19 mmol) was dissolved in methanol (4 mL) and was combined with a solution of cobalt(II) acetate tetrahydrate (95 mg, 0.38 mmol) in methanol (6 mL). Subsequently solid sodium hexafluorophosphate (95 mg, 0.57 mmol) was added. The solution was left in a beaker at room temperature to evaporate. Pink crystals which were suitable for X-ray crystallography were obtained in 70 % yield (120 mg).



ESI mass spectrometry (methanol) m/z : 757.36 $[\text{C}_{33}\text{H}_{43}\text{Co}_2\text{N}_4\text{O}_9]^+$, 729.37 $[\text{C}_{32}\text{H}_{43}\text{Co}_2\text{N}_4\text{O}_8]^+$. **Microanalysis** Anal calc. for $\text{C}_{33}\text{H}_{43}\text{Co}_2\text{N}_4\text{O}_9\text{PF}_6$ C 43.91, H 4.80, N 6.21; found: C 43.21, H 4.61, N 6.10 %. **FT-IR spectroscopy** (ν , cm^{-1}) 1601 (s, asym str acetate); 1421 (s, sym str acetate); 1022 (m, O–H); 830 (s, P–F str); 762 (m, Py–H def); 556 (m, P–F). **Magnetic moment** (d^4 -MeOD) in solution by Evans method [28]. 6.18 BM. **UV-Vis spectroscopy** Acetonitrile, 0.02 M, $\lambda_1 = 508 \text{ nm}$ ($\epsilon = 73.5 \text{ L mol}^{-1} \text{ cm}^{-1}$).

2.8.5 Synthesis of $[\text{Co}_2(\text{CH}_3\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$

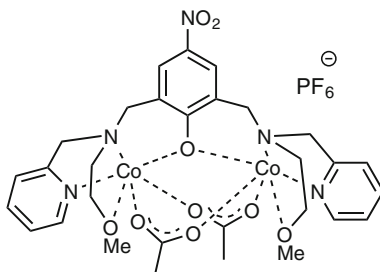
$\text{CH}_3\text{HL2}$ (125 mg, 0.25 mmol) was dissolved in methanol (2 mL) and a methanol (3 mL) cobalt(II) acetate tetrahydrate (125 mg, 0.5 mmol) solution was added dropwise at room temperature. Subsequently sodium hexafluorophosphate (140 mg, 0.8 mmol) dissolved in methanol (3 mL) was added. The pink crystals which emerged upon standing after a couple of were filtered and dried in air (150 mg, 71 %). Crystals were obtained suitable for X-ray crystallography after 2 days by using only one equivalent Co(II) and adding the hexafluorophosphate as solid.



ESI mass spectrometry (methanol) m/z : 699.2 $[\text{C}_{31}\text{H}_{41}\text{Co}_2\text{N}_4\text{O}_7]^+$. **FT-IR spectroscopy** (ν , cm^{-1}): 2925 (w, C–H str), 1596 (m, C=O asym str, acetate); 1475 (m, C=O sym str, acetate); 1423 (m, C–H def); 1085 (w, C–O str), 829 (s P–F str), 555 (s, P–F str). **Microanalysis** Anal calc. for $\text{C}_{31}\text{H}_{41}\text{Co}_2\text{N}_4\text{O}_7\text{PF}_6$ C 44.09, H 4.89, N 6.63; found: C 44.09, H 4.96, N 6.58 %. **Magnetic moment** (d^4 -MeOD) in solution by Evans method [28]. 6.09 BM. **UV-Vis spectroscopy** Acetonitrile, 0.02 M, $\lambda_1 = 471 \text{ nm}$ ($\epsilon = 46.2 \text{ L mol}^{-1} \text{ cm}^{-1}$), $\lambda_2 = 519 \text{ nm}$ ($\epsilon = 50.5 \text{ L mol}^{-1} \text{ cm}^{-1}$).

2.8.6 Synthesis of $[\text{Co}_2(\text{NO}_2\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$

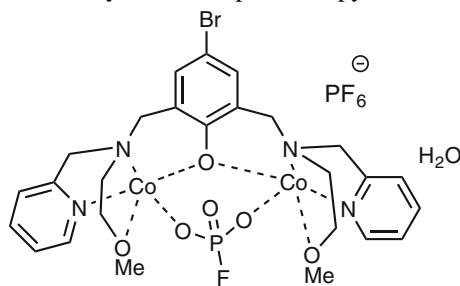
$\text{NO}_2\text{HL2}$ (50 mg, 0.1 mmol) was dissolved in methanol (2 mL) and cobalt(II) acetate tetrahydrate (49 mg, 0.1 mmol) in methanol (3 mL) was added dropwise at room temperature. Subsequently sodium hexafluorophosphate (50 mg, 0.3 mmol) was added. The orange crystals which emerged upon standing were filtered and dried in air (60 mg, 66 %).



ESI mass spectrometry (methanol) m/z 784.1 $[\text{C}_{30}\text{H}_{44}\text{Co}_2\text{N}_5\text{O}_{12}]^+$. **FT-IR spectroscopy** (ν , cm^{-1}) 2933 (w, C–H str); 1595 (s, acetate asym str); 1506 (w, NO_2 asym str); 1429 (s, acetate sym str); 1317 (s, NO_2 sym str); 1086 (m, C–O str); 829 (s, P–F str); 752 (m, py C–H def); 658 (w, Ar–H def); 556 (s, P–F). **Microanalysis** Anal calc. for $\text{C}_{30}\text{H}_{40}\text{Co}_2\text{N}_5\text{O}_{10}\text{PF}_6$ C 40.33, H 4.51, N 7.84; found: C 40.41, H 4.42, N 7.92 %. **Magnetic moment** (d^4 -MeOD) in solution by Evans method [28]. 7.31 BM. **UV-Vis spectroscopy** Acetonitrile, 0.02 M, $\lambda_1 = 466$ nm ($\varepsilon = 77.7$ L mol^{−1} cm^{−1}).

2.8.7 Synthesis of $[\text{Co}_2(\text{BrL2})(\text{PO}_3\text{F})](\text{PF}_6)$

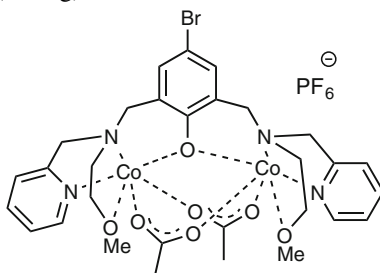
BrHL2 (74 mg, 0.14 mmol) was dissolved in methanol (3 mL) and combined with a solution of cobalt(II) acetate tetrahydrate (65 mg, 0.28 mmol) in methanol (3 mL). Sodium hexafluorophosphate (70 mg, 0.4 mmol) was added and, after filtration, the solution was left in a beaker to evaporate at room temperature. Pink crystals were obtained in low yield (25 mg) after 2 days. As the sodium hexafluorophosphate employed was wet, the complex crystallized with PO_3F^{2-} instead of acetates as suggested by mass spectrometry, infrared spectroscopy and microanalysis.



ESI mass spectrometry (methanol) m/z : 679.08 $[\text{C}_{26}\text{H}_{34}\text{BrN}_4\text{O}_5\text{Co}_2]^+$, 779.10 $[\text{C}_{26}\text{H}_{36}\text{BrN}_4\text{O}_5\text{Co}_2\text{FO}_3\text{P}]^+$. **Microanalysis** Anal calc. for $\text{C}_{26}\text{H}_{36}\text{BrN}_4\text{O}_8\text{Co}_2\text{P}_2\text{F}_7$ C 33.75, H 3.92, N 6.06; found: C 33.70, H 3.68, N 6.05 %. **FT-IR spectroscopy** (ν , cm^{-1}) 2932, 2855 (w, CH_2 str); 1435 (m, CH_2 def); 1186 (m, P = O, str); 1117 (s, C–O–C str); 830 (s, P–F str); 774 (m, Py–H def); 555 (m, P–F).

2.8.8 Synthesis of $[Co_2(BrL2)(CH_3COO)_2](PF_6)$

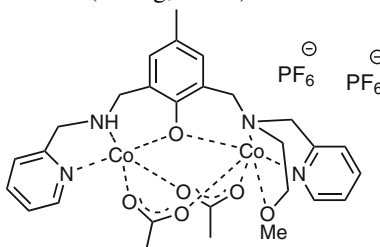
BrHL2 (110 mg, 0.2 mmol) was dissolved in methanol (5 mL) and was combined with a solution of cobalt(II) acetate tetrahydrate (103 mg, 0.4 mmol) in methanol (5 mL). Solid sodium hexafluorophosphate (104 mg, 0.6 mmol) was added and after filtration the solution was left in a beaker to evaporate at room temperature. Pink crystals were obtained after two days that were suitable for X-ray crystallography in 32 % yield (62 mg).



ESI mass spectrometry (methanol) m/z 734.99 $[C_{29}H_{38}BrCo_2N_4O_6]^+$. **FT-IR spectroscopy** (ν , cm^{-1}) 2931 (m, C–H); 1599 (m, C=O asym str, acetate); 1421 (s, C=O sym str, acetate); 831 (s, P–F str); 759 (m, Py–H def); 556 (m, P–F). **Microanalysis** Anal calc. for $C_{30}H_{38}BrCo_2N_4O_7PF_6$ C 39.62, H 4.21, N 6.16; found: C 39.35, H 4.20, N 6.18 %. **Magnetic moment** (d^4 -MeOD) in solution by Evans method [28]. 6.09 BM. **UV-Vis spectroscopy** Acetonitrile, 0.01 M, $\lambda_1 = 460$ nm ($\epsilon = 4.0$ L mol $^{-1}$ cm $^{-1}$), $\lambda_2 = 509$ nm ($\epsilon = 4.1$ L mol $^{-1}$ cm $^{-1}$).

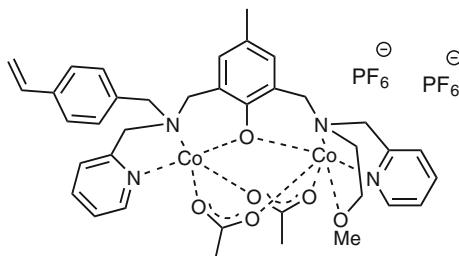
2.8.9 Attempted Synthesis of $[Co(II)Co(III)(CH_3L4)(CH_3CO_2)_2](PF_6)_2 \cdot MeOH$

CH₃HL4 (32 mg, 0.079 mmol) was dissolved in degassed methanol and solid cobalt(II) acetate tetrahydrate (39 mg, 0.15 mmol) was added under a nitrogen stream. N₂ was continuously bubbled through the solution until all starting material had dissolved and subsequently solid sodium hexafluorophosphate (39 mg, 0.24 mmol) was added and after filtration the solution was left in a beaker to evaporate at room temperature in air. After two days the solution had undergone a color change from pink to brown and after a further two days a brown solid was obtained which was dried in air (42 mg, 58 %).



FT-IR spectroscopy (ν , cm^{-1}) 2929 (m, C–H); 1601 (m, C=O asym str, acetate); 1415 (s, C=O sym str, acetate); 830 (s, P–F str); 765, 739 (m, Py–H def); 555 (m, P–F). **Microanalysis** Anal calc. for $\text{C}_{29}\text{H}_{39}\text{Co}_2\text{N}_4\text{O}_7\text{P}_2\text{F}_{12}$ C 36.15, H 4.08, N 5.82; found: C 36.70, H 4.30, N 5.92 %.

2.8.10 Attempted Synthesis of $[\text{Co(II)Co(III)}(\text{CH}_3\text{L5})(\text{CH}_3\text{COO})_2](\text{PF}_6)_2$

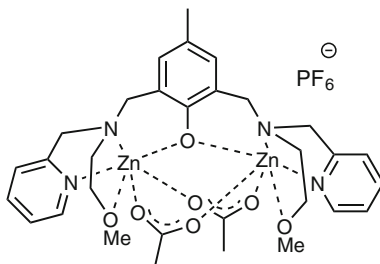


$\text{CH}_3\text{HL5}$ (22 mg, 0.041 mmol) was dissolved in degassed methanol and solid cobalt(II) acetate tetrahydrate (21 mg, 0.082 mmol) was added under a nitrogen stream. N_2 was continuously bubbled through the solution until all starting material had dissolved and subsequently solid sodium hexafluorophosphate (20 mg, 0.12 mmol) was added and after filtration the solution was left in a beaker to evaporate at room temperature in air. After two days the solution had undergone a color change from light pink to dark-pink/red and after a further two days a pink solid was obtained which was dried in air (15 mg, 37 %).

FT-IR spectroscopy (ν , cm^{-1}) 2925 (m, C–H); 1602 (m, C=O asym str, acetate); 1419 (s, C=O sym str, acetate); 833 (s, P–F str); 762 (m, Py–H def); 556 (m, P–F). **Microanalysis** Anal calc. for $\text{C}_{37}\text{H}_{43}\text{Co}_2\text{F}_{12}\text{N}_4\text{O}_6\text{P}_2$ C 42.42, N 5.35, H 4.14 %, found C 42.40, N 5.34, H 4.13 %.

2.8.11 Synthesis of $[\text{Zn}_2(\text{CH}_3\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)_2$

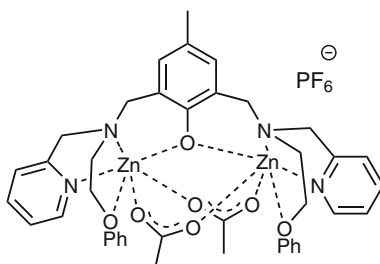
$\text{CH}_3\text{HL2}$ (0.250 g, 0.5 mmol) was dissolved in methanol (20 mL), and a methanol solution (10 mL) of zinc(II) acetate dihydrate (0.240 g, 0.9 mmol) was added dropwise. The resulting pale yellow solution was then heated under reflux for 0.5 h. The solution was permitted to cool to room temperature and sodium hexafluorophosphate (0.140 g, 0.8 mmol) was added. Upon standing colorless crystals were deposited and these were collected by filtration (0.31 g, 71 %).



ESI mass spectrometry (methanol) m/z : 711.2 $[\text{C}_{31}\text{H}_{41}\text{N}_4\text{O}_7\text{Zn}_2]^+$. **Microanalysis**
 Anal. Calc. for $\text{C}_{31}\text{H}_{41}\text{Zn}_2\text{N}_4\text{O}_7 \text{PF}_6$ C 43.42, H 4.82, N 6.53 % Found: C 43.25, H 4.79, N 6.53 %. **FT-IR spectroscopy** (ν , cm^{-1}): 2924, 2851 (w, C–H str), 1594 (m, C=O asym str, acetate); 1476 (m, C=O sym str, acetate); 1428 (m, C–H def); 1093 (w, C–O str), 831 (s P–F str), 555 (s, P–F str). **^1H NMR** (CD_3CN , 500.13 MHz); δ 1.90 (s, 6H, acetate CH_3); 2.22 (s, 3H, CH_3); 2.52 (m, 4H, NCH_2 , $J = 5.7$ Hz); 2.71 (bm, 4H, $\text{CH}_2\text{CH}_2\text{O}$); 2.98 (s, 6H, OCH_3); 3.63 (d, 2H, arCH_2N , $J = 11.9$ Hz); 3.85 (d, 2H, NCH_2py , $J = 14.7$ Hz); 4.39 (d, 2H, NCH_2py , $J = 13.8$ Hz); 4.48 (d, 2H, arCH_2N , $J = 9.3$ Hz); 7.03 (s, 2H, arCH); 7.49–7.51 (m, 4H, pyCH); 7.99 (t, 2H, pyCH , $J = 7.4$ Hz); 8.74 (d, 2H, pyCH , $J = 4.6$ Hz). **^{13}C NMR** (CD_3CN , 100.62 MHz); 20.4 (CH_3); 24.6 (acetate CH_3); 55.1 (NCH_2); 59.0 (OCH_3); 61.3 (arCH_2N); 61.6 (NCH_2py); 69.7 (CH_2O); 125.2 (pyCH); 125.8 (arC); 126.8 (CCH_3); 132.8 (arCH); 140.8 (pyCH); 148.6 (pyCH); 155.8 (pyC); 161.1 (COH); 177.7 (CO_2^-). Second set of low intensity (25 %) signals: **^1H NMR** (CD_3CN , 500.13 MHz) H,H-COSY; δ 2.07 (s, 6H); 3.12 (s, 6H); 3.27 (d, 2H, $J = 5.4$ Hz); 3.74 (m, 4H); 3.74 (s, 2H); 3.86 (d, 2H, $J = 15.8$ Hz); 4.01 (s, 2H); 6.77 (s, 2H); 7.21 (d, 2H, $J = 6.1$ Hz); 7.37 (t, 2H, $J = 5.8$ Hz); 7.81 (t, 2H, $J = 7.3$ Hz); 8.51 (bs, 2H).

2.8.12 Synthesis of $[\text{Zn}_2(\text{CH}_3\text{L3})(\text{CH}_3\text{COO})_2](\text{PF}_6) \cdot \text{CH}_3\text{OH}$

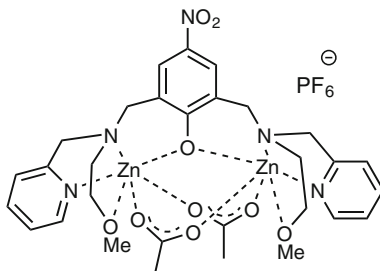
$\text{CH}_3\text{HL3}$ (50 mg, 0.085 mmol) was dissolved in methanol (2 mL) and zinc(II) acetate dihydrate (0.37 mg, 0.17 mmol) in methanol (3 mL) was added dropwise at room temperature. The pale yellow solution was refluxed for 30 min, cooled slowly to room temperature and subsequently sodium hexafluorophosphate (43 mg, 0.26 mmol) was added. Colorless crystals emerged upon standing after 2 h which were filtered and dried in air (49 %, 41 mg).



ESI mass spectrometry (methanol) m/z : 855.7 $[\text{C}_{41}\text{H}_{47}\text{N}_4\text{O}_8\text{Zn}_2]^+$; 825.7 $[\text{C}_{40}\text{H}_{47}\text{N}_4\text{O}_7\text{Zn}_2]^+$. **Microanalysis** Anal. Calc. for $\text{C}_{42}\text{H}_{48}\text{Zn}_2\text{N}_4\text{O}_8\text{PF}_6$ C 49.77, H 4.87, N 5.53 % found: C 49.50, H 4.60, N 5.55 %. **FT-IR spectroscopy** (ν , cm^{-1}): 2927 (w, C–H str), 1594 (m, C=O asym str, acetate); 1483 (m, C=O sym str, acetate); 1434 (m, C–H def); 1225 (w, C–O str), 833 (s P–F str), 760 (s, Ph–H); 694 (s, Ph–H); 555 (s, P–F str). **^1H NMR** (CD_3CN , 400.13 MHz); δ 1.72 (s, 6H, acetate CH_3); 2.24 (s, 3H, CH_3); 2.77 (m, 3H); 3.04 (m, 3H); 3.37 (d, 4H); 3.54 (m, 3H); 3.65 (d, 2H, $J = 12.3$ Hz); 3.77 (d, 2H, $J = 11.8$ Hz); 3.94 (m, 5H); 4.18 (m, 5H); 7.21 (m, 4H, arCH); 6.94 (m, 2H, arCH); 6.62 (d, 4H, $J = 8.1$ Hz, arCH); 7.41–7.50 (m, 4H, pyCH); 7.97 (td, 2H, $J = 7.7, 1.4$, pyCH); 8.71 (d, 2H, $J = 5.1$, pyCH). Second set of low intensity aromatic proton signals: 7.21 (m, 4H); 6.94 (m, 2H); 6.73 (d, 4H, $J = 8.6$ Hz); 7.41–7.50 (m, 4H); 7.93 (td, 2H, $J = 7.7, 1.6$ Hz); 8.75 (d, 2H, $J = 5.3$ Hz).

2.8.13 Synthesis of $[\text{Zn}_2(\text{NO}_2\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$

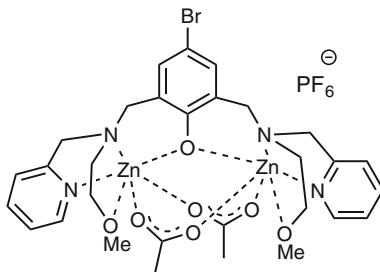
$\text{NO}_2\text{HL2}$ (50 mg, 0.1 mmol) was dissolved in methanol (2 mL) and was combined with zinc(II) acetate dihydrate (44 mg, 0.2 mmol) in methanol (2 mL) at room temperature. The pale yellow solution was refluxed for 30 min, cooled slowly to room temperature and subsequently sodium hexafluorophosphate (50 mg, 0.3 mmol) was added. Colorless needles emerged upon standing immediately which were filtered and dried in air (30 mg, 33 %).



ESI mass spectrometry (methanol) m/z : 744.0 $[\text{C}_{30}\text{H}_{38}\text{N}_5\text{O}_9\text{Zn}_2]^+$. **Microanalysis** Anal. calc. for $\text{C}_{31}\text{H}_{41}\text{Zn}_2\text{N}_5\text{O}_{10}\text{PF}_6$ C 40.45, H 4.60, N 7.61 % found: C 39.36, H 4.27, N 7.67 %. **FT-IR spectroscopy** (ν , cm^{-1}) 2931 (w, C–H str); 1596 (m, C=O asym str, acetate); 1506 (w, NO_2 asym str); 1426 (m, C=O sym str, acetate); 1320 (m, NO_2 sym str); 1092 (m, C–O str); 833 (s P–F str); 755 (m, py C–H def); 659 (w, Ar–H def); 556 (s, P–F). **^1H NMR** (CD_3CN , 500.13 MHz); δ 1.91 (s, 6H, acetate CH_3); 2.57 (m, 4H, NCH_2); 2.74 (bm, 4H, $\text{CH}_2\text{CH}_2\text{O}$); 2.99 (s, 6H, OCH_3); 3.82 (d, 2H, ar CH_2N , $J = 12.3$ Hz); 3.90 (d, 2H, NCH_2py , $J = 14.8$ Hz); 4.42 (d, 2H, NCH_2py , $J = 15.1$ Hz); 4.48 (d, 2H, ar CH_2N , $J = 12.7$ Hz); 7.52 (m, 4H, pyCH); 8.01 (t, 2H, pyCH, $J = 6.9$ Hz); 8.2 (s, 2H, arCH); 8.72 (m, 2H, pyCH).

2.8.14 Synthesis of $[Zn_2(BrL2)(CH_3COO)_2](PF_6)$

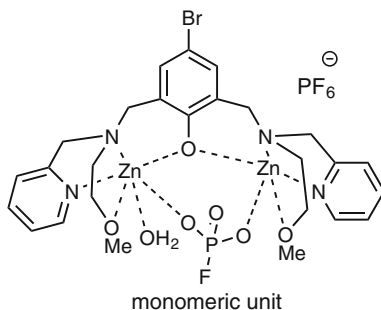
BrHL2 (100 mg, 0.2 mmol) was dissolved in methanol (8 mL) with zinc(II) acetate dihydrate (82 mg 0.4 mmol). The mixture was heated under reflux for 30 min, permitted to cool to room temperature and dry sodium hexafluorophosphate (95 mg, 0.6 mmol) added. After filtration the solution was left to stand at room temperature. Colorless crystals, suitable for X-ray crystallography, emerged after 2 h (62 mg, 33.6 %).



ESI mass spectrometry (methanol) m/z : 749.0 $[C_{29}H_{38}BrN_4O_6Zn_2]^+$, 707.3 $[C_{27}H_{36}BrN_4O_5Zn]^+$, 593.1 $[C_{26}H_{32}BrN_4O_3Zn]^+$. **Microanalysis** Anal calc. for $C_{30}H_{38}BrN_4O_7Zn_2PF_6$ C 39.07, H 4.15, N 6.07 % Found: C 39.33, H 4.13, N 6.14 % **FT-IR spectroscopy** (ν , cm^{-1}) 2930 (w, CH_2 str); 1597 (s, bridging acetate antisym. str); 1424 (s, bridging acetate sym. str); 829 (s, P–F str); 760, 658 (m, Ar–H def); 555 (m, P–F). **1H NMR** (CD_3CN , 500.13 MHz); δ 1.99 (s, 6H, acetate CH_3); 2.54 (m, 4H, NCH_2); 2.72 (bm, 4H, CH_2CH_2O); 2.99 (s, 6H, OCH_3); 3.64 (d, 2H, $arCH_2N$, $J = 12.1$ Hz); 3.84 (d, 2H, NCH_2py , $J = 14.7$ Hz); 4.38 (d, 2H, NCH_2py , $J = 14.4$ Hz); 4.44 (d, 2H, $arCH_2N$, $J = 11.3$ Hz); 7.38 (s, 2H, $arCH$); 7.48–7.50 (m, 4H, $pyCH$, $J = 5.4$ Hz); 7.99 (t, 2H, $pyCH$, $J = 7.1$ Hz); 8.72 (m, 2H, $pyCH$).

2.8.15 Synthesis of $[Zn_4(BrL2)_2(PO_3F)_2(H_2O)_2](PF_6)_2$

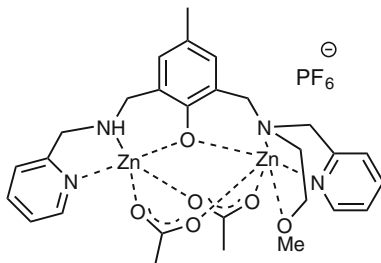
BrHL2 (60 mg, 0.1 mmol) was dissolved in methanol (6 mL) and was combined with zinc(II) acetate dihydrate (49 mg, 0.2 mmol). The complex solution was subsequently refluxed for 30 min, cooled to room temperature and sodium hexafluorophosphate (57 mg, 0.3 mmol) was added. After filtration the solution was left in a beaker to evaporate at room temperature. Colorless crystals were obtained after 2 days which were suitable for X-ray crystallography (40 mg, 22 %). As the sodium hexafluorophosphate employed was wet, the complex crystallized with PO_3F^{2-} instead of acetates as suggested by mass spectrometry, infrared spectroscopy and microanalysis.



ESI mass spectrometry (methanol) m/z : 754.99 $[\text{C}_{26}\text{H}_{32}\text{BrN}_4\text{O}_3\text{ZnPFO}_3]^+$, 593.12 $[\text{C}_{26}\text{H}_{32}\text{BrN}_4\text{O}_3\text{Zn}]^+$. **Microanalysis** Anal. calc. for $\text{C}_{52}\text{H}_{68}\text{Br}_2\text{N}_8\text{O}_{14}\text{Zn}_4\text{P}_4\text{F}_{14}$ C 33.94, H 3.72, N 6.09; found: C 33.35, H 3.61, N 5.99 %. **FT-IR spectroscopy** (ν , cm^{-1}) 2854, 2929, 2893 (w, CH_2 str); 1460, 1445 (m, CH_2 def); 1183 (m, P = O, str); 1121 (s, C–O–C str); 831 (s, P–F str); 774 (m, Py–H def); 555 (m, P–F).

2.8.16 Synthesis of $[\text{Zn}_2(\text{CH}_3\text{L4})(\text{CH}_3\text{CO}_2)_2](\text{PF}_6)$

$\text{CH}_3\text{HL4}$ (80 mg, 0.2 mmol) and zinc(II) acetate dihydrate (86 mg, 0.4 mmol) were combined in methanol (5 mL) and refluxed for 30 min, after cooling to room temperature sodium hexafluorophosphate (65 mg, 0.4 mmol) was added and the pale yellow solution filtered and left on the bench to evaporate slowly. White crystals formed after 3 days that were dried in air (85 mg, 54.8 %).



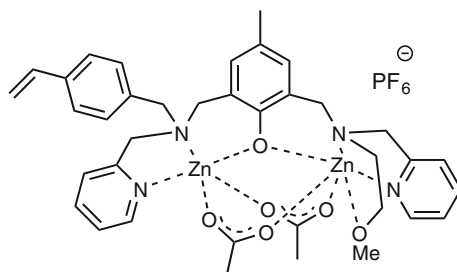
ESI mass spectrometry (methanol) m/z : 655.11 $[\text{C}_{28}\text{H}_{35}\text{N}_4\text{O}_6\text{Zn}_2]^+$, 595.06 $[\text{C}_{26}\text{H}_{31}\text{N}_4\text{O}_4\text{Zn}_2]^+$; (acetonitrile) m/z = 749.1, calc. m/z 749.17 (100 %), 748.17 (79.2 %), $[\text{C}_{60}\text{H}_{88}\text{N}_{10}\text{O}_{18}\text{Zn}_4]^{2+}$. **FT-IR spectroscopy** (ν , cm^{-1}) 3307 (w, N–H str); 2925 (w, C–H str); 1596 (s, bridging acetate antisym. str); 1422 (s, bridging acetate sym. str); 832 (s, P–F str); 768, 663 (m, pyH def); 556 (m, P–F). **Microanalysis** Anal. calc. for $\text{C}_{28}\text{H}_{38}\text{N}_4\text{O}_6\text{Zn}_2\text{PF}_6$ C 41.29, H 4.24, N 7.13 %; found: C 41.21, H 4.25, N 6.72 %. **^1H NMR** (CD_3CN , 500.13 MHz); δ 1.97 (s, 6H, acetate CH_3); 2.55 (m, 2H, NCH_2CH_2); 2.75 (m, 1H, NCH_2CH_2); 2.82 (d, 1H NCH_2CH_2 , J = 11.1 Hz); 3.04 (s, 3H, OCH_3); 3.65 (d, 1H, ar CH_2N , J = 12.2 Hz); 3.79 (d, 1H, NCH_2py , J = 11.4 Hz); 3.85 (d, 1H, NCH_2py , J = 15.0 Hz); 3.99 (t, 1H, ar CH_2N ,

$J = 12.8$ Hz); 4.13 (t, 1H, NCH_2py , $J = 11.6$ Hz); 4.21 (dd, 1H, $arCH_2N$, $J = 12.1$, 5.1 Hz); 4.31 (d, 1H, NCH_2py , $J = 15.2$ Hz); 4.35 (d, 1H, $arCH_2N$, $J = 12.3$ Hz); 6.94 (s, 1H, arH); 6.97 (s, 1H, arH); 7.40–7.35 (m, 4H, pyH); 7.98 (mc, 2H, pyH , $J = 7.7$, 0.8 Hz); 8.60 (d, 1H, pyH , $J = 4.8$ Hz); 8.77 (d, 1H, pyH , $J = 4.7$ Hz). ^{13}C NMR (CD_3CN , 100.62 MHz); 20.3 (CH_3); 24.3 (acetate CH_3); 51.5 ($arCH_2N$); 52.8 (NCH_2py); 55.1 (CH_2CH_2); 59.1 (OCH_3); 61.9 ($arCH_2N$); 69.6 (CH_2CH_2); 124.2 ($pyCH$); 125.0 ($pyCH$); 125.1 ($pyCH$); 125.3 ($pyCH$); 125.8 (arC); 126.4 (arC); 126.4 ($arCCH_3$); 132.2 ($arCH$); 132.8 ($arCH$); 140.8 ($pyCH$); 141.0 ($pyCH$); 148.6 ($pyCH$); 148.7 ($pyCH$); 155.8 (pyC); 161.1 ($arCO$); 168.4 (CO_2^-).

2.8.17 Synthesis of $[Zn_2(CH_3L5)(CH_3CO_2)_2](PF_6) \cdot MeOH$

Work reported in this thesis regarding the CH_3HL5 ligand and its Zn(II) complex was undertaken together with Miss Laurene Marty.

CH_3HL5 (50 mg, 0.09 mmol) and zinc(II) acetate dihydrate (42 mg, 0.19 mmol) were combined in methanol (5 mL) and refluxed for 30 min, after cooling to room temperature sodium hexafluorophosphate (48 mg, 0.29 mmol) was added and the pale yellow solution filtered and left on the bench to evaporate slowly. A white powder was obtained after 3 days which was dried in air (29 mg, 37 %). Crystals suitable for X-ray structure analysis were obtained with the following method: CH_3HL5 (30 mg, 0.05 mmol) and zinc(II) acetate dihydrate (25 mg, 0.11 mmol) were combined in methanol (3 mL) and refluxed for 30 min, after cooling to room temperature sodium tetraphenylborate (59 mg, 0.17 mmol) was added and the pale yellow solution filtered and left on the bench to evaporate slowly. The white powder was taken up in acetone/isopropanol and layered with hexane which yielded colorless crystals which desiccated readily upon removal from the solvent. X-ray structure analysis was thus conducted with copper radiation at 150 K. Further analysis was done with the hexafluorophosphate derivative.



ESI mass spectrometry (methanol) $m/z = 751.1$ (100 %), 749.1 (95 %) calc. for $[C_{35}H_{47}N_4O_6Zn_2]^+$ $m/z = 749.2$ (100 %), 751.2 (96.1 %); (acetonitrile) $m/z = 851.3$, calc. 851.23 (100 %), 849.23 (85.5 %) $[C_{41}H_{49}N_6O_6Zn_2]^+$; $m/z = 769.1$, calc. 769.17 (100 %), 767.18 (87 %), $[C_{37}H_{43}N_4O_6Zn_2]^+$; $m/z = 723.1$,

calc. 721.17 (100 %), 732.17 (96.6 %), $[\text{C}_{33}\text{H}_{43}\text{N}_4\text{O}_6\text{Zn}_2]^+$, $m/z = 653.1$, calc. m/z 653.11 (100 %), 655.11 (96.2 %), $[\text{C}_{28}\text{H}_{35}\text{N}_4\text{O}_6\text{Zn}_2]^+$. **FT-IR spectroscopy** (ν , cm^{-1}) 2927, 2856 (w, C–H str); 1598 (s, bridging acetate antisym. str); 1431 (s, bridging acetate sym. str); 832 (s, P–F str); 765, 729 (m, pyH def); 555 (m, P–F). **Microanalysis** Anal calc. for $\text{C}_{38}\text{H}_{48}\text{N}_4\text{O}_7\text{Zn}_2\text{PF}_6$ C 48.12, H 5.10, N 5.91 %; found: C 48.19, H 4.71, N 5.36 % **^1H NMR** (CD_3CN , 500.13 MHz) δ 1.94 (s, 6H, CH_3CO_2^-); 2.14 (s, 3H, ar CH_3); 2.50–2.56 (m, 2H, NCH_2CH_2); 2.84–2.90 (m, 2H, NCH_2CH_2); 3.03 (s, 3H, OCH_3); 3.57 (d, 1H, ar CH_2N , $J = 12.6$ Hz); 3.64 (d, 1H, NCH_2py , $J = 12.1$ Hz); 3.71–3.91 (m, 5H, CH_2); 4.15 (d, 1H, ar CH_2N , $J = 12.5$); 4.21–4.27 (m, 2H, CH_2); 5.20 (d, 1H, $\text{CH}=\text{CH}_2$, $J_{\text{cis}} = 10.9$ Hz); 5.25 (dd, 1H, $\text{CH}=\text{CH}_2$, $J_{\text{cis}} = 11.0$, 0.5 Hz, second isomer); 5.69 (d, 1H, $\text{CH}=\text{CH}_2$, $J_{\text{trans}} = 17.6$ Hz); 5.81 (d, 1H, $\text{CH}=\text{CH}_2$, $J_{\text{trans}} = 17.6$ Hz, second isomer); 6.62 (dd, 1H, $\text{CH}=\text{CH}_2$, $J = 17.7$, 10.9, 0.8 Hz); 6.71 (dd, 1H, $\text{CH}=\text{CH}_2$, $J = 17.7$, 10.9 Hz, second isomer); 6.81 (d, 2H, ar CH , $J = 1.8$ Hz); 6.89 (d, 2H, ar CH , $J = 1.8$ Hz); 6.95 (s, 2H, ar CH); 7.10–7.53 (m, 4H, py CH); 7.91 (m, 2H, py CH , $J = 7.8$, 1.6 Hz); 8.70 (m, 2H, py CH). **^{13}C NMR** (CD_3CN , 100.62 MHz) δ 20.2 (ar CCH_3); 23.1 (CH_3CO_2^-); 54.7 (CH_2); 58.6 (OCH_3); 59.1, 59.5, 60.8, 61.2, 61.3, 69.3 (CH_2); 114.9 ($\text{CH}=\text{CH}_2$); 115.2 ($\text{CH}=\text{CH}_2$, second isomer); 124.0, 124.3, 124.7, 125.0, 125.1 (py CH); 125.2, 125.3 (C_{quart}); 126.8, 126.9 (CH); 127.0, 127.3, 131.6 (C_{quart}), 132.8, 133.0 (CH); 133.2 (CH); 133.6 (CH); 133.7 (C_{quart}); 137.1 ($\text{CH}=\text{CH}_2$); 138.5 (ar C); 140.8, 141.3, 141.5, 148.2, 148.5, 149.8 (CH); 155.8, 156.5, 160.8, 161.4 (C_{quart}), 179.0 (CH_3CO_2).

2.9 Crystal Structures Included in this Thesis

Complex	Chapter
$[\text{Zn}_2(\text{CH}_3\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	4
$[\text{Zn}_2(\text{CH}_3\text{L3})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	4
$[\text{Zn}_2(\text{BrL2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	4
$[\text{Zn}_2(\text{NO}_2\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	4
$[\text{Zn}_4(\text{BrL2})_2(\text{PO}_3\text{F})_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$	4
$[\text{Zn}_4(\text{CH}_3\text{L2})_2(\text{NO}_2\text{C}_6\text{H}_5\text{OPO}_3)_2(\text{H}_2\text{O})_2](\text{PF}_6)_2$	4
$[\text{Zn}_2(\text{CH}_3\text{L4})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	8
$[\text{Zn}_2(\text{CH}_3\text{L5})(\text{CH}_3\text{COO})_2](\text{BPh}_4)$	8
$[\text{Co}_2(\text{CH}_3\text{L2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	6
$[\text{Co}_2(\text{BrL2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	6
$[\text{Co}_2(\text{CO}_2\text{EtL2})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	6
$[\text{Co}_2(\text{CO}_2\text{EtH}_2\text{L1})(\text{CH}_3\text{COO})_2](\text{PF}_6)$	6
$[\text{Cd}_4(\text{CO}_2\text{EtH}_2\text{L1})_2(\text{CH}_3\text{COO})_{3.75}\text{Cl}_{0.25}(\text{H}_2\text{O})_2](\text{PF}_6)$	5

References

1. G. Ambrosi, M. Formica, V. Fusi, L. Giorgi, M. Micheloni, *Coord. Chem. Rev.* **252**, 1121–1152 (2008)
2. R. Robson, *Aust. J. Chem.* **23**, 2217–2224 (1970)
3. R. Robson, *Inorg. Nucl. Chem. Lett.* **6**, 125–128 (1970)
4. R.E. Mirams, S.J. Smith, K.S. Hadler, D.L. Ollis, G. Schenk, L.R. Gahan, *J. Biol. Inorg. Chem.* **13**, 1065–1072 (2008)
5. R.R. Buchholz, M.E. Etienne, A. Dorgelo, R.E. Mirams, S.J. Smith, S.Y. Chow, L.R. Hanton, G.B. Jameson, G. Schenk, L.R. Gahan, *J. Chem. Soc., Dalton Trans.* 6045–6054 (2008)
6. L.R. Gahan, S.J. Smith, A. Neves, G. Schenk, *Eur. J. Inorg. Chem.* **19**, 2745–2758 (2009)
7. S.J. Smith, A. Casellato, K.S. Hadler, N. Mitić, M.J. Riley, A.J. Bortoluzzi, B. Szpoganicz, G. Schenk, A. Neves, L.R. Gahan, *J. Biol. Inorg. Chem.* **12**, 1207–1220 (2007)
8. H. Carlsson, M. Haukka, E. Nordlander, *Inorg. Chem.* **43**, 5681–5687 (2004)
9. H. Carlsson, E. Nordlander, M. Jarenmark, *C. R. Chim.* **10**, 433–462 (2007)
10. M. Jarenmark, E. Csapo, J. Singh, S. Wockel, E. Farkas, F. Meyer, M. Haukka, E. Nordlander, *J. Chem. Soc., Dalton Trans.* **39**, 8183–8194 (2010)
11. M. Jarenmark, S. Kappen, M. Haukka, E. Nordlander, *J. Chem. Soc., Dalton Trans.* 993–996 (2008)
12. S. Albedyhl, Marie T. Averbuch-Pouchot, C. Belle, B. Krebs, Jean L. Pierre, E. Saint-Aman, S. Torelli, *Eur. J. Inorg. Chem.* **2001**, 1457–1464 (2001)
13. J.A. Larrabee, W.R. Johnson, A.S. Volwiler, *Inorg. Chem.* **48**, 8822–8829 (2009)
14. J.A. Larrabee, S.A. Chyun, A.S. Volwiler, *Inorg. Chem.* **47**, 10499–10508 (2008)
15. A. Mangalum, R.C. Smith, *Tetrahedron* **65**, 4298–4303 (2009)
16. X. Dong, X. Wang, Y. He, Z. Yu, M. Lin, C. Zhang, J. Wang, Y. Song, Y. Zhang, Z. Liu, Y. Li, Z. Guo, *Chem. Eur. J.* **16**, 14181–14189 (2010)
17. T. Routasalo, J. Helaja, J. Kavakka, A.M.P. Koskinen, *Eur. J. Org. Chem.* **2008**, 3190–3199 (2008)
18. T. Inomata, K. Shinozaki, Y. Hayashi, H. Arii, Y. Funahashi, T. Ozawa, H. Masuda, *Chem. Commun. (Camb.)* 392–394 (2008)
19. H. Sharghi, M.A. Nasser, K. Niknam, *J. Org. Chem.* **66**, 7287 (2001)
20. A. Zinke, R. Ott, E. Leggewie, A. Hassanein, G. Zankl, *Monatshefte für Chemie / Chemical Monthly* **87**, 552–559 (1956)
21. V. Boehmer, F. Marscholke, L. Zetta, *J. Org. Chem.* **52**, 3200–3205 (1987)
22. R.T. Paine, Y.C. Tan, X.M. Gan, *Inorg. Chem.* **40**, 7009–7013 (2001)
23. J.d. Mendoza, P.M. Nieto, P. Prados and C. Sánchez, *Tetrahedron*, **46**, 671–682 (1990)
24. S. Striegler, M. Dittel, *Inorg. Chem.* **44**, 2728–2733 (2005)
25. E.F. Elslager, E.L. Benton, F.W. Short, F.H. Tendick, *J. Am. Chem. Soc.* **78**, 3453–3457 (1956)
26. Bruker, TopSpin 2.0 Software for NMR data processing
27. M. Hesse, H. Meier, B. Zeeh, *Spectroscopic Methods in Organic Chemistry* (G. Thieme, NY, 1997)
28. D.F. Evans, *J. Chem. Soc.*, 1959 (2003)
29. E.M. Schubert, *J. Chem. Educ.* **69**, 62 (1992)
30. G.A. Bain, J.F. Berry, *J. Chem. Educ.* **85**, 532–536 (2008)
31. H. Sakiyama, *Inorg. Chim. Acta* **360**, 715–716 (2007)
32. Thermo-Scientific, Grams/AI 9.0 Software
33. M. J. Riley, VTVH 2.1.1 Program for the simulation and fitting variable temperature—variable field MCD spectra (2008)
34. H. Adamsky, AOMX Program (1996)
35. F. Ely, K.S. Hadler, N. Mitić, L.R. Gahan, D.L. Ollis, N.M. Plugis, M.T. Russo, J.A. Larrabee, G. Schenk, *J. Biol. Inorg. Chem.* **16**, 777–787 (2011)
36. G.M. Sheldrick, SHELXL97: Program for the refinement of crystal structures (1997)

37. L.J. Farrugia, J. Appl. Crystallogr. **32**, 837–838 (1999)
38. R.A. Peralta, A.J. Bortoluzzi, B. de Souza, R. Jovito, F.R. Xavier, R.A. A. Couto, A. Casellato, F. Nome, A. Dick, L.R. Gahan, G. Schenk, G.R. Hanson, F.C.S. de Paula, E.C. Pereira-Maia, S.d.P. Machado, P.C. Severino, C. Pich, T. Bortolotto, H. Terenzi, E.E. Castellano, A. Neves, M.J. Riley, Inorg. Chem. **49**, 11421–11438 (2010)
39. I.H. Segel, *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems* (Wiley, NY, 1975)
40. N.V. Kaminskaia, B. Spingler, S.J. Lippard, J. Am. Chem. Soc. **122**, 6411–6422 (2000)
41. N.V. Kaminskaia, C. He, S.J. Lippard, Inorg. Chem. **39**, 3365–3373 (2000)
42. C.S. Ltd, CasaXPS: Processing Software for XPS, AES, SIMS and More (2009)
43. R.V. Jones, L. Godorhazy, N. Varga, D. Szalay, L. Urge, F. Darvas, J. Comb. Chem. **8**, 110–116 (2006)
44. K.S. Hadler, N. Mitic, S.H. Yip, L.R. Gahan, D.L. Ollis, G. Schenk, J.A. Larrabee, Inorg. Chem. **49**, 2727–2734 (2010)
45. C.J. Jackson, K.S. Hadler, P.D. Carr, A.J. Oakley, S. Yip, G. Schenk, D.L. Ollis, Acta crystallogr. F **64**, 681–685 (2008)
46. J.M. Walker, *The Proteomics Protocols Handbook* (Humana Press, Totowa, 2005)
47. V.M. D'Souza, B. Bennett, A.J. Copik, R.C. Holz, Biochemistry **39**, 3817–3826 (2000)
48. K.S. Hadler, E.A. Tanifum, S.H. Yip, N. Mitić, L.W. Guddat, C.J. Jackson, L.R. Gahan, K. Nguyen, P.D. Carr, D.L. Ollis, A.C. Hengge, J.A. Larrabee, G. Schenk, J. Am. Chem. Soc. **130**, 14129–14138 (2008)
49. H. Tsue, K. Enyo, K.-I. Hirao, Org. Lett. **2**, 3071–3074 (2000)
50. G.-C. Sun, Z.-H. He, Z.-J. Li, X.-D. Yuan, Z.-J. Yang, G.-X. Wang, L.-F. Wang, C.-R. Liu, Molecules **6**, 1001–1005 (2001)
51. T. Aoki, T. Kaneko, N. Maruyama, A. Sumi, M. Takahashi, T. Sato, M. Teraguchi, J. Am. Chem. Soc. **125**, 6346–6347 (2003)
52. W. Müller, P. Kipfer, D.A. Lowe, S. Urwyler, Helv. Chim. Acta **78**, 2026–2035 (1995)
53. A.S. Borovik, V. Papaefthymiou, L.F. Taylor, O.P. Anderson, L. Que, J. Am. Chem. Soc. **111**, 6183–6195 (1989)
54. E. Díez-Barra, J.M. Fraile, J.I. García, E. García-Verdugo, C.I. Herrerías, S.V. Luis, J.A. Mayoral, P. Sánchez-Verdú, J. Tolosa, Tetrahedron Asymmetry **14**, 773–778 (2003)
55. S. Carloni, V. Borzatta, L. Moroni, G. Tanzi, G. Sartori, R. Maggi, Catalysts Based on Metal Complexes for the Synthesis of Optically Active Chrysanthemic Acid (2005), p. 55 (Patent WO2005123254A1)
56. C.A. Bunton, S.J. Farber, J. Org. Chem. **34**, 767–772 (1969)
57. R.S. Molday, Canadian Patents and Development Limited (CA) Magnetic Iron-Dextran Microspheres (1984) (Patent 4452773)
58. B.-F. Pan, F. Gao, H.-C. Gu, J. Colloid Interface Sci. **284**, 1–6 (2005)

Spectroscopic and Mechanistic Studies of Dinuclear
Metallohydrolases and Their Biomimetic Complexes

Daumann, L.

2014, XXI, 239 p. 200 illus., 22 illus. in color., Hardcover

ISBN: 978-3-319-06628-8