

Dinuclear Zinc(II)–Iron(III) and Iron(II)–Iron(III) Complexes as Models for Purple Acid Phosphatases

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The heterodinuclear Zn^{II}Fe^{III} complex **1** and the isostructural Fe^{II}Fe^{III} complex **2** with the dinucleating ligand from 2,6-bis[*bis*(2-pyridylmethyl)amino]methyl-4-methoxyphenol (HBPMOP, **3**) were prepared and characterized by X-ray crystallography. Solution studies (UV/Vis spectroscopy; electrochemistry) are described. A pH-induced change in the coordination spheres of the metal centers is seen. These complexes serve as models for the mixed-valence oxidation state in purple acid phosphatases. The cleavage acceleration of the activated phosphodiester 2-hydroxypropyl *p*-nitrophenyl

phosphate (HPNP) was investigated in acetonitrile/water (1:1) in the presence of complexes of the ligand BPMOP and its methyl analogue BPMP with regards to its dependence on the pH value. At the optimum pH value (8.5 ± 0.2), the Zn^{II}-Fe^{III} complex from BPMOP shows a 2-fold higher rate acceleration compared with that of the complex containing BPMP. The diiron complex from BPMOP is 4-fold more reactive than the homologous complex from BPMP. The heterodinuclear Zn^{II}Fe^{III} catalysts are at least 10-fold more reactive than the homonuclear Fe^{II}Fe^{III} catalysts.

Introduction

Purple acid phosphatases (PAPs) catalyse the hydrolysis of activated phosphoric esters like *p*-nitrophenyl phosphate, naphthyl phosphate or ATP, at a pH range from 4 to 7.^[1] The active site of red kidney bean purple acid phosphatase (KBAP) harbours an Fe^{III} and a Zn^{II} ion, while those of the mammalian PAPs, isolated from bovine spleen (BSPAP) or from porcine uterus, contain a diiron site in an Fe^{II}Fe^{III} state for the active form. The inactive purple Fe^{III}Fe^{III} form, involves two high-spin iron ions which are antiferromagnetically coupled ($-J > 150 \text{ cm}^{-1}$). In the active Fe^{II}-Fe^{III} pink form, the two iron ions are weakly antiferromagnetically coupled ($-J = 19.8 \text{ cm}^{-1}$). The crystal structure of KBAP, firstly determined at a resolution of 2.9 Å^[2] and further at 2.65 Å,^[3] reveals a homodimeric 111 kDa enzyme, containing two domains in each subunit. In the active site, located in the carboxyl-terminal domain, the two metal ions are 3.26 Å apart and bridged by Asp164, each one involving octahedral coordination. The iron ion is further coordinated by Tyr167, His325, and Asp135 residues, and the zinc ion by His286, His323, and Asn201 residues. Three exogenous ligands are also included in the X-ray model to complete the coordination sphere: one terminal hydroxo ligand to the iron ion, one terminal water ligand to the zinc ion, and one μ -hydroxo group between the metal ions. The crystal structures of KBAP complexed with phosphate

(the product of the reaction) and with tungstate (a strong inhibitor), have also been determined^[3] allowing the proposal of a mechanism for the hydrolysis of phosphomonoesters (Figure 1).

In the proposed mechanism, the phosphate group of the substrate binds to the zinc ion by displacing the presumed water ligand. The terminal Fe^{III}-bound hydroxide ion attacks the phosphorus atom to form a pentacoordinated intermediate, and the P–O bond, opposite from the hydroxide ion attack, breaks to form the leaving group and phosphate which is consistent with the inversion at the phosphorus atom. Another kinetically indistinguishable mechanism has been suggested,^[4] involving a nucleophilic attack by a bridging oxide instead of iron-bound hydroxide.

In order to mimic the reactivity of PAPs, two isostructural dinuclear complexes, containing a Zn^{II}Fe^{III} and an Fe^{II}-Fe^{III} center, respectively, have been prepared and studied with the aim of comparing their activity towards phosphate ester cleavage and to allow comparisons between KBAP and BSPAP. The dinucleating ligand from HBPMOP, which is of the known HBPMOP type^[5,6] 2,6-bis[*bis*(2-pyridylmethyl)amino]methyl-4-methylphenol has been used. It bears a strong electron-donating methoxy group in the *para* position to the bridging phenoxo group: 2,6-bis[*bis*(2-pyridylmethyl)amino]methyl-4-methoxyphenol (**3**). Heterodinuclear Zn^{II}Fe^{III} complexes have been described as models for the active site of KBAP. The first heterodinuclear phosphate-bridged model compound, [FeZn(BPMP)]{(O₂P(OPh)₂)}₂, could be obtained by using HBPMOP.^[5a] The Fe–Zn distance (3.695 Å) in this complex, is significantly larger than in the carboxylate-bridged complex (3.428 Å)^[6] compared to the value of 3.33 Å measured on KBAP in the presence of phosphate. Some other heter-

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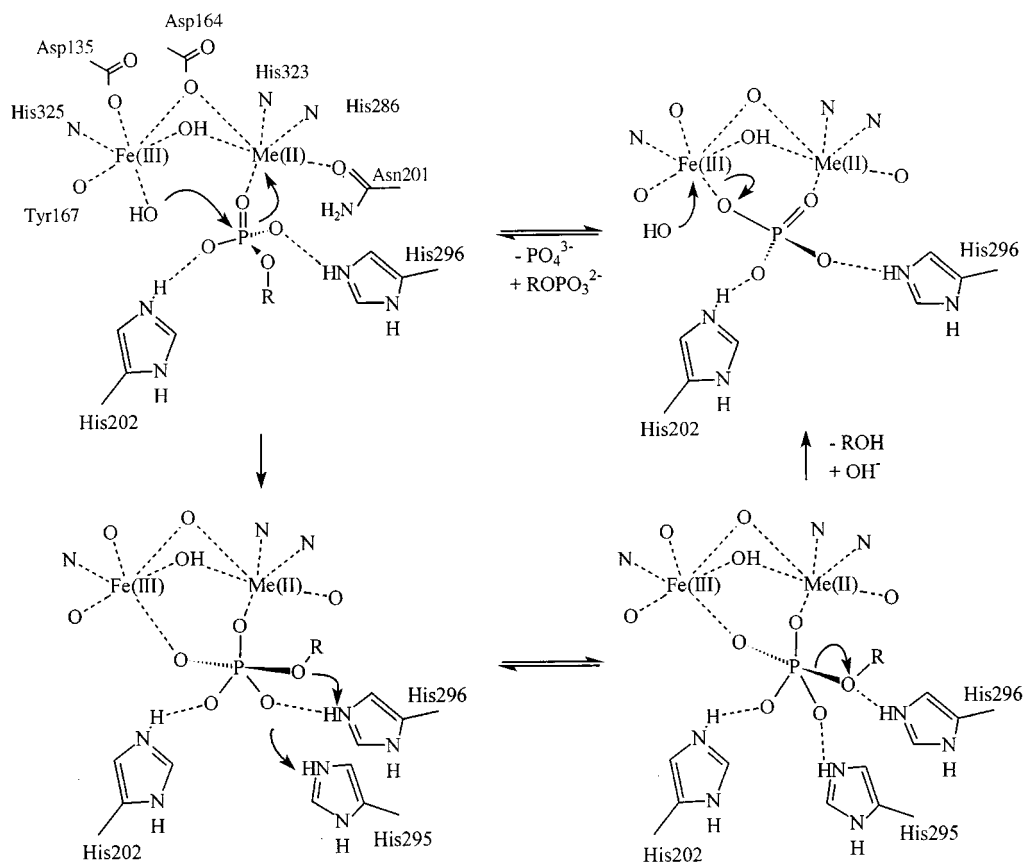


Figure 1. The active site of KBPAP and proposed mechanism of phosphate ester hydrolysis^[3]

odinuclear $\text{Zn}^{\text{II}}\text{Fe}^{\text{III}}$ complexes have been described,^[7–9] all of them containing symmetrical ligands. A Zn–Fe complex involving a constitutionally unsymmetrical ligand, but with identical chemical environments (two N_3O coordination spheres), has also been described in solution.^[10] Mixed-valence phenoxo-bridged diiron complexes have also been reported as models for BSPAP by several authors.^[5a,6,10–17] A recent review has been published, describing structural and functional model compounds of PAPs.^[18]

Results and Discussion

Synthesis of the Complexes

The reaction of equimolecular amounts of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Fe}(\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$ with HBPMOP in methanol, yielded blue-green crystals of $[\text{Zn}^{\text{II}}\text{Fe}^{\text{III}}\text{BPMOP}\{\text{O}_2\text{P}(\text{OPh})_2\}_2](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ (**1**) after the addition of diphenyl phosphate. These were suitable for X-ray crystallography. Green crystals of $[\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}\text{BPMOP}\{\text{O}_2\text{P}(\text{OPh})_2\}_2](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ (**2**) were obtained by the analogous reaction with 2 equiv. of $\text{Fe}(\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$. The only metal species isolated was the mixed-valence diiron complex, indicating the participation of a redox process during complex formation. It is known that the diiron(III) species are strong oxidants, suggesting that the reductant is the methanol solvent.^[6]

Crystal Structures of **1** and **2**

Compounds **1** and **2** are isostructural complexes and are therefore described in comparison with each other. The triclinic elementary cells of **1** and **2** contain two formulas. The metal centers exhibit octahedral coordination. In the heterodinuclear complex **1** (Figure 2), the metal atoms are linked by a μ -phenoxo group from the ligand and by two μ -diphenylphosphato bridges with a distance of 3.7030(7) Å. The angle over $\text{Zn}(1)–\text{O}(1)–\text{Fe}(1)$ is 122.9(1)°. The two iron centers in the analogous complex **2** (Figure 3) have a distance of 3.6400(6) Å, which is slightly shorter than the corresponding distance in **1**. The angle $\text{Fe}(1)–\text{O}(1)–\text{Fe}(2)$ is 121.44(9)°. The nitrogen donor atoms from BPMOP complete the coordination sphere per facial coordination, resulting in an equivalent N_3O_3 donor set. Selected bond lengths and angles are given in Table 1.

The structure of **2** confirms the mixed valence state. The crystal structure of complex $[\text{Zn}^{\text{II}}\text{Fe}^{\text{III}}\text{BPMP}\{\text{O}_2\text{P}(\text{OPh})_2\}_2](\text{ClO}_4)_2 \cdot 1.5\text{CH}_3\text{OH} \cdot \text{H}_2\text{O}$ which involves the same coordination sphere as in **1** has been reported.^[5a] The Zn–Fe distance of 3.695(1) Å is in the range of the Zn–Fe distance in **1** [3.7030(7) Å]. This distance is significantly shorter in the carboxylate-bridged analogue [3.437(1) Å]^[6] and larger in the molybdato-bridged complex from 2,6-bis[$\{\text{bis}(2\text{-pyridylmethyl})\text{amino}\}\text{methyl}\}$ -4-*tert*-butylphenol (HBPPB) [3.819(4) Å].^[9] This can be due to the different bite distance of the phosphate group and therewith indi-

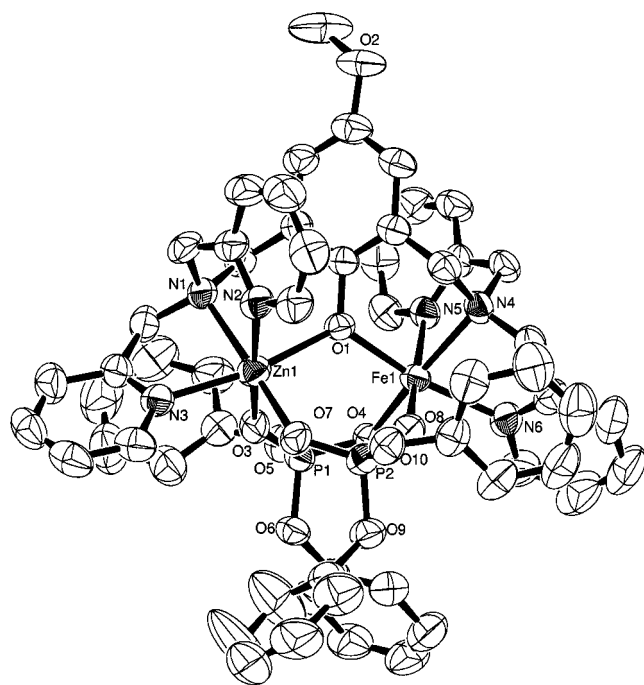


Figure 2. ORTEP molecular representation of complex **1** showing 50% probability displacement ellipsoids; H atoms, water molecule, and perchlorate ions have been omitted for clarity

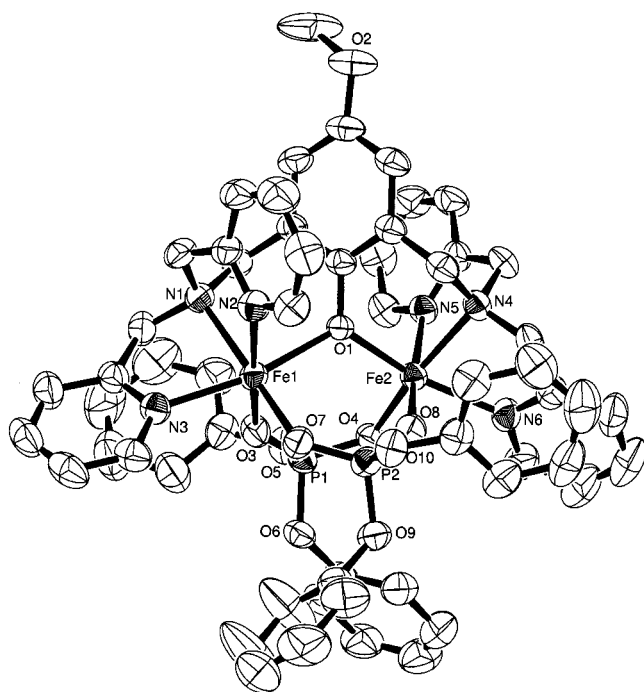


Figure 3. ORTEP molecular representation of complex **2** showing 50% probability displacement ellipsoids; H atoms, water molecule, and perchlorate ions have been omitted for clarity

cates a remarkable structural flexibility of the phenoxo-bridged intermetal(II,III) distance which may be important for the catalytic activity. The Zn–Fe distance in the native KBPAP is 3.33 Å in the presence of phosphate. The Fe^{II}–Fe^{III} distance in **2** [3.6400(6) Å] is also significantly larger than in the carboxylate-bridged Fe^{II}Fe^{III} complex

Table 1. Selected bond lengths [Å] and angles [°] in **1** and **2**

1		2	
Zn(1)–Fe(1)	3.7030(7)	Fe(1)–Fe(2)	3.6400(6)
Zn(1)–O(1)	2.168(2)	Fe(1)–O(1)	2.215(2)
Zn(1)–O(3)	2.042(2)	Fe(1)–O(3)	2.096(2)
Zn(1)–O(7)	2.028(2)	Fe(1)–O(7)	2.060(2)
Zn(1)–N(1)	2.183(3)	Fe(1)–N(1)	2.212(3)
Zn(1)–N(2)	2.122(3)	Fe(1)–N(2)	2.166(3)
Zn(1)–N(3)	2.124(3)	Fe(1)–N(3)	2.160(3)
Fe(1)–O(1)	2.047(2)	Fe(2)–O(1)	1.958(2)
Fe(1)–O(4)	1.974(2)	Fe(2)–O(4)	1.972(2)
Fe(1)–O(8)	1.984(2)	Fe(2)–O(8)	1.956(2)
Fe(1)–N(4)	2.168(3)	Fe(2)–N(4)	2.179(3)
Fe(1)–N(5)	2.128(3)	Fe(2)–N(5)	2.147(3)
Fe(1)–N(6)	2.125(3)	Fe(2)–N(6)	2.131(3)
O(1)–Zn(1)–O(3)	86.52(9)	O(1)–Fe(1)–O(3)	86.13(7)
O(1)–Zn(1)–O(7)	97.33(9)	O(1)–Fe(1)–O(7)	97.77(7)
O(1)–Zn(1)–N(1)	87.0(1)	O(1)–Fe(1)–N(1)	86.17(8)
O(1)–Zn(1)–N(2)	87.3(1)	O(1)–Fe(1)–N(2)	87.33(8)
O(1)–Zn(1)–N(3)	162.4(1)	O(1)–Fe(1)–N(3)	160.29(8)
O(3)–Zn(1)–O(7)	94.5(1)	O(3)–Fe(1)–O(7)	97.40(8)
O(3)–Zn(1)–N(1)	93.5(1)	O(3)–Fe(1)–N(1)	92.88(9)
O(3)–Zn(1)–N(2)	171.8(1)	O(3)–Fe(1)–N(2)	170.37(9)
O(3)–Zn(1)–N(3)	86.5(1)	O(3)–Fe(1)–N(3)	85.03(8)
O(7)–Zn(1)–N(1)	171.2(1)	O(7)–Fe(1)–N(1)	170.13(9)
O(7)–Zn(1)–N(2)	91.7(1)	O(7)–Fe(1)–N(2)	91.48(9)
O(7)–Zn(1)–N(3)	99.3(1)	O(7)–Fe(1)–N(3)	100.70(9)
N(1)–Zn(1)–N(2)	80.8(1)	N(1)–Fe(1)–N(2)	79.62(9)
N(1)–Zn(1)–N(3)	77.3(1)	N(1)–Fe(1)–N(3)	76.72(9)
N(2)–Zn(1)–N(3)	97.9(1)	N(2)–Fe(1)–N(3)	98.97(9)
O(1)–Fe(1)–O(4)	97.17(9)	O(1)–Fe(2)–O(4)	99.41(8)
O(1)–Fe(1)–O(8)	88.3(1)	O(1)–Fe(2)–O(8)	91.24(8)
O(1)–Fe(1)–N(4)	89.7(1)	O(1)–Fe(2)–N(4)	90.44(8)
O(1)–Fe(1)–N(5)	88.6(1)	O(1)–Fe(2)–N(5)	88.36(8)
O(1)–Fe(1)–N(6)	164.7(1)	O(1)–Fe(2)–N(6)	165.42(9)
O(4)–Fe(1)–O(8)	95.2(1)	O(4)–Fe(2)–O(8)	96.41(9)
O(4)–Fe(1)–N(4)	168.0(1)	O(4)–Fe(2)–N(4)	165.30(8)
O(4)–Fe(1)–N(5)	90.9(1)	O(4)–Fe(2)–N(5)	90.30(9)
O(4)–Fe(1)–N(6)	97.5(1)	O(4)–Fe(2)–N(6)	95.08(9)
O(8)–Fe(1)–N(4)	94.8(1)	O(8)–Fe(2)–N(4)	94.25(9)
O(8)–Fe(1)–N(5)	173.4(1)	O(8)–Fe(2)–N(5)	173.26(9)
O(8)–Fe(1)–N(6)	86.1(1)	O(8)–Fe(2)–N(6)	85.54(9)
N(4)–Fe(1)–N(5)	79.4(1)	N(4)–Fe(2)–N(5)	79.03(9)
N(4)–Fe(1)–N(6)	76.6(1)	N(4)–Fe(2)–N(6)	75.66(9)
N(5)–Fe(1)–N(6)	95.5(1)	N(5)–Fe(2)–N(6)	93.17(9)
Zn(1)–O(1)–Fe(1)	122.9(1)	Fe(1)–O(1)–Fe(2)	121.44(9)

from BPMP [3.365(1) Å]^[6] and slightly shorter than in the non-bridged Fe^{II}Fe^{III} complex of the ligand BPBP [3.726(2) Å].^[9]

Electronic Spectra

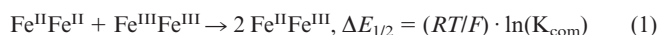
The main feature of the electronic spectra of **1** and **2** in acetonitrile is the LMCT transition from phenolate to iron(III) which is observed at $\lambda_{\text{max}} = 654$ nm ($\epsilon \approx 900$) in **1** and 673 nm ($\epsilon \approx 900$) in **2**, respectively. In addition, a shoulder is observed in **2**, at $\lambda_{\text{max}} = 385$ nm ($\epsilon \approx 3000$) which can be assigned to a charge-transfer transition from Fe^{II} to pyridine. The analogous complexes from HBPMP^[5a] show absorption maxima in methanol at $\lambda_{\text{max}} = 569$ nm ($\epsilon \approx 800$) for the Zn^{II}Fe^{III} complex and at $\lambda_{\text{max}} = 610$ nm ($\epsilon \approx 950$) for the Fe^{II}Fe^{III} complex. A shoulder in the spec-

trum of the latter is observed at $\lambda_{\text{max}} = 365 \text{ nm}$ ($\epsilon \approx 3480$).^[6]

Electrochemistry

Although PAPs catalyse non-redox-active hydrolytic reactions, the catalytic activity is dependent on the oxidation state of the iron active site, the mixed-valence oxidation state being active towards the hydrolysis of the phosphate esters while the oxidized form is inactive. The determination of the electrochemical stability domain of synthetic models thus appears of prime importance. Electrochemical investigations by cyclic voltammetry (CV) of **1** in CH_3CN (Figure 4, A, curve 1) show two reversible one-electron waves at $E_{1/2} = 0.13 \text{ V}$ and 1.36 V (vs. SCE). This is confirmed by rotating disc electrode (RDE) experiments. The RDE curve recorded under the same experimental conditions (Figure 4, A, curve 2) displays two well-behaved waves of very close intensity, without contamination of the $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}$ complex **2** electrochemical response (see below). The cathodic wave at $E_{1/2} = 0.13 \text{ V}$ is attributed to the $\text{Fe}^{\text{III}}\text{Zn}^{\text{II}}/\text{Fe}^{\text{II}}\text{Zn}^{\text{II}}$ redox couple while the anodic wave corresponds to the ligand-based oxidation. The CV curve in CH_3CN of the BPMP analog of **1**, $[\text{Zn}^{\text{II}}\text{Fe}^{\text{III}} \text{BPMP}\{\text{O}_2\text{P}(\text{OPh})_2\}_2](\text{ClO}_4)_2 \cdot 1.5 \cdot \text{CH}_3\text{OH} \cdot \text{H}_2\text{O}$, shows a reversible one-electron transfer at a potential of 143 mV ($\text{Fe}^{\text{III}}\text{Zn}^{\text{II}}/\text{Fe}^{\text{II}}\text{Zn}^{\text{II}}$ redox couple) and a weak signal at $+765 \text{ mV}$, which has been attributed to a slight contamination with Fe^{II} .^[5a] The ligand-based oxidation is not observed in the accessible potential range. The electron-donating effect of the methoxy group in *para* position of the bridging phenolic moiety causes a stabilization of the oxidized form of the BPMP ligand compared to the non-substituted BPMP ligand. In the case of **2**, two reversible one-electron waves are observed on the CV curve (Figure 4, B, curve 1) at $E_{1/2} = 0.12 \text{ V}$ and 0.74 V (vs. SCE) which are assigned to the redox couples $\text{Fe}^{\text{II}}\text{Fe}^{\text{II}}/\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}$ and $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}/\text{Fe}^{\text{III}}\text{Fe}^{\text{III}}$, respectively. The RDE curve (Figure 4, B, curve 2) is characterized by one cathodic and one anodic wave confirming the mixed-valence character of **2**.

Similar results have been found in the CV behavior of $[\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}\text{BPMP}\{\text{O}_2\text{P}(\text{OPh})_2\}_2](\text{ClO}_4)_2 \cdot \text{CH}_3\text{OH} \cdot \text{H}_2\text{O}$ ($E_{1/2} = +135 \text{ mV}$ and $+755 \text{ mV}$, respectively^[5a]). Comparing the electrochemical features of **1** or **2** with those of their BPMP analogs, only a weak cathodic shift, around 20 mV , in the metal-centered electron transfers, are observed, i.e. the substitution by the methoxy group in the *para* position on the bridging phenolic moiety in **1** and **2** did not markedly influence the metal-centered electron transfer potential. From the separation of the redox potentials in **2**, we have determined the stability of the mixed-valence form of **2** by evaluating the comproportionation constant, K_{com} , for the equilibrium according to Equation (1).



Using $\Delta E_{1/2} = 0.62 \text{ V}$, a value of $3.0 \cdot 10^{10}$ ($\Delta G = -59 \text{ kJ mol}^{-1}$) is found, which is close to the value of $3.2 \cdot 10^{10}$ determined for the BPMP analog of **2**. Electron delocalization, electrostatic interaction and steric interaction are the

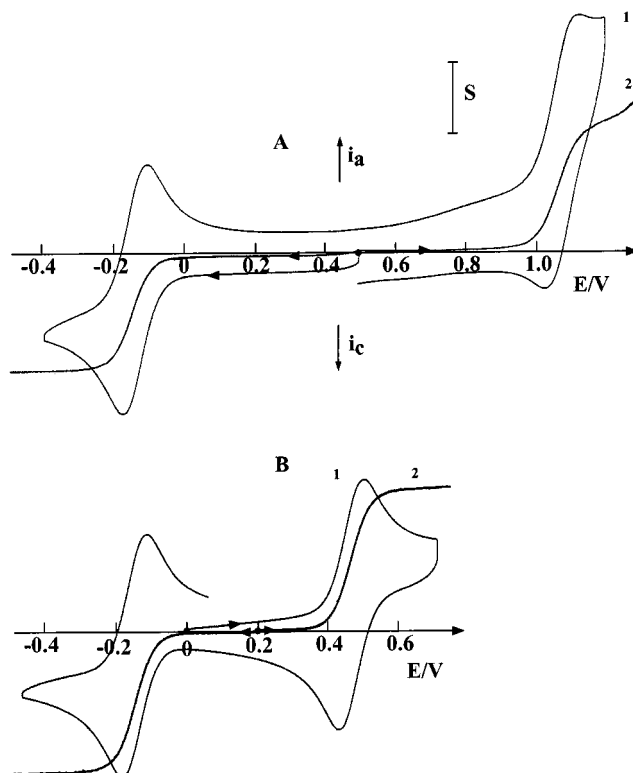


Figure 4. Voltammograms of **1** (A) and **2** (B), 0.9 mM , in $\text{CH}_3\text{CN} + \text{TBAP}$ (0.1 M); E vs. Ag/AgNO_3 (10 mM) ($+339 \text{ mV}$ vs. SCE); curve 1: CV at a vitreous carbon disc electrode (5 mm diameter), $\nu = 0.1 \text{ Vs}^{-1}$, S (scale) = $20 \mu\text{A}$; curve 2: voltammetry at the RDE (3 mm diameter), 600 rpm , $S = 10 \mu\text{A}$

main factors involved in the magnitude of K_{com} although, considering related complexes,^[5b] the electron delocalization energy has been found to be too small to contribute significantly to the mixed-valence state stability. Electrostatic interactions may strongly influence the stability domain. Taking into account the close proximity of the two metal ions, the II,II form of the complex will be more readily oxidized than the II,III form leading to an increase in the stability domain of **2**. On the other hand, by comparing other related synthetic models of PAPs,^[11,13] it is shown that the replacement of pyridyl groups in phenoxo-bridged di-iron complexes by one and two phenolate terminal groups leads to a cathodic shift in the redox potentials by ca. -0.4 V and -1.0 V , respectively, the stability domain ranging between 0.5 and 1.0 V according to the substitution on the phenoxo-bridging ligand and to the exogenous ligands. It must also be noticed that porcine purple acid phosphatase (uteroferrin) has a reduction potential ($\text{Fe}^{\text{III}}\text{Fe}^{\text{III}}/\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}$ redox couple) of $367 \text{ mV}^{[19]}$ vs. NHE (i.e. 122 mV vs. SCE). This is close to the value of 0.12 V found for **2**.

pH-Induced Changes in the Coordination Sphere

A UV/Vis titration (monitoring of the LMCT band) of a solution of HBPMOP and equimolar amounts of zinc(II) perchlorate and iron(III) perchlorate has been performed in acetonitrile/water ($1:9$) upon addition of concentrated aqueous solution of NaOH or HClO_4 (Figure 5). In the pH range $1.95\text{--}3.35$, an isosbestic point is observed at 575 nm

indicating the presence of only two absorbing species (Figure 5, A). The spectrophotometric data were fitted and the calculations afforded a pK_1 value of 2.8 ± 0.05 . Another equilibrium occurs at higher pH values. At $pH \approx 5$, the colour of the solution turns from blue to brown and a new isosbestic point is detected at 525 nm in pH range 4.5–6.1 (Figure 5, B).

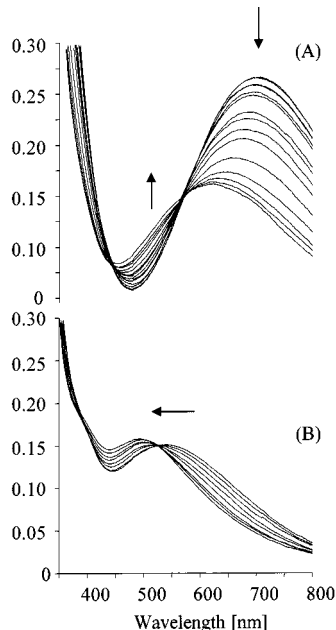


Figure 5. UV/Vis titration curves in acetonitrile/water of **1** as a function of the pH value: (A) in the range of $1.95 < pH < 3.35$ and (B) in the range of $4.5 < pH < 6.1$; arrows indicate the spectral trends upon increasing the pH value

The fitting of the values of absorbance vs. pH leads to a $pK_2 = 4.9 \pm 0.26$. The species distribution curves are shown in Figure 6, providing evidence for three pH-dependent species. It must be emphasized that the pH-driven conversion between these species is reversible. Same results were obtained by decreasing or increasing the pH value of the solution.

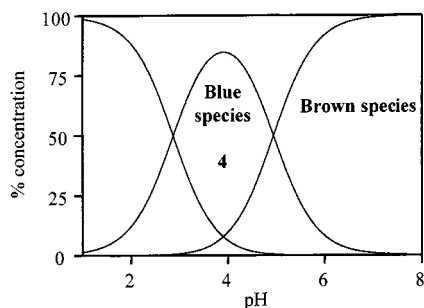


Figure 6. Species distribution curves of **1** as a function of the pH value

Attempts to isolate the pure products involved in this titration have not yet proved successful. Nevertheless by independent preparations, a blue product (**4**) was obtained by addition of equimolecular amounts of $Zn(ClO_4)_2 \cdot 6H_2O$ and $Fe(ClO_4)_3 \cdot H_2O$, dissolved in water, to a solution of HBPMOP in acetone and precipitation as a powder after addition of diethyl ether. The positive ion FAB mass spec-

trum of **4** exhibits a parent peak corresponding to the respective heterodinuclear core (no peak corresponding to the homodinuclear $Zn-Zn$ or $Fe-Fe$ species was observed). The molecular ion peak is $m/z = 681$ and corresponds to $[ZnFeBPMOP(OH)]^+$. The electronic spectrum of **4** in acetonitrile shows one absorption maximum at $\lambda_{max} = 661$ nm ($\epsilon = 2200$); this band is shifted to 585 nm ($\epsilon = 900$) in a solution of acetonitrile/water (1:9). In addition, the UV/Vis spectra of the titration described above, in the intermediate pH range 3–5 (Figure 6), exhibit the same features as the spectrum of the isolated complex **4** for which we propose a μ -OH-bridged heterodinuclear compound. At higher pH values (> 6), the brown species is formed following progressive and reversible deprotonation of **4**. This species will prove to be the active species towards the cleavage of phosphate diesters (vide infra). One possibility is that this complex has at least one pendant hydroxo ligand. Another possibility is that the bridging μ -OH (**4**) was deprotonated to form a μ -oxo ligand between the two metallic ions.^[4]

Cleavage of Phosphate Diesters

Model studies have shown that simple dinuclear metal complexes can provide rate accelerations for hydrolyzing phosphate esters, shedding light on how metal ions may promote these reactions.^[20–28] A heterodinuclear $Zn^{II}Fe^{III}$ complex has been reported by Komiyama et al. which hydrolyses RNA.^[8]

In order to test the activity of complexes towards phosphate ester hydrolysis or cleavage, activated phosphoric acid esters are used. In this work 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP) was applied. In the absence of the metallocatalysts, HPNP is very stable.^[24] The pH dependence of the HPNP cleavage acceleration, in the presence of $Zn^{II}Fe^{III}$ or $Fe^{II}Fe^{III}$ [29] complexes from the ligands BPMOP and BPMP respectively, has been studied. The rate increases corresponding to the pH and optimum values were obtained at $pH = 8.5 \pm 0.2$. The rate constant (k_{uncat}) for background hydrolysis was similarly obtained. Some observed rate constants are listed in Table 2. The rate acceleration was also measured in dependence of the substrate and complexes concentration, showing first order dependence in each case.

Table 2. Observed pseudo-first-order rate constants for transesterification of HPNP hydrolysis in CH_3CN /aqueous buffer CHES (1:1) at $pH = 8.5 (\pm 0.2)$ and $25^\circ C$ with $[catalyst] = 0.5$ mM and $[HPNP] = 0.82$ mM

Catalyst	k_{obs} [$10^{-5}s^{-1}$]	k_{obs}/k_{uncat}
$[ZnFe(BPMOP)]^{4+}$	2.39	112
$[ZnFe(BPMP)]^{4+}$	1.14	54
$[Fe_2(BPMOP)]^{4+}$	0.227 ^[a]	11
$[Fe_2(BPMP)]^{4+}$	0.065 ^[a]	3
spontaneous	0.0213	1

^[a] 10% of DMSO has been added to avoid precipitate.

The $Zn^{II}Fe^{III}$ complex of the ligand BPMOP shows a 2-fold higher rate acceleration compared with the complex

containing the ligand BPMP. The diiron complex from HBPMOP is 4-fold more reactive than the homologous complex from BPMP. The heterodinuclear $\text{Zn}^{\text{II}}\text{Fe}^{\text{III}}$ complexes from BPMOP and BPMP are 10-fold and 18-fold, respectively, more reactive than the corresponding homodinuclear $\text{Fe}^{\text{II}}\text{Fe}^{\text{III}}$ complexes. These differences in reactivities may be ascribed to the greater ability of the zinc center compared with the iron center, to coordinate the substrate. The optimum pH value for the hydrolysis of HPNP is notably higher than that for the enzymes. This observation confirms the necessity of a deprotonation process as underlined with the enzymes.^[1b] The different activities of the complexes of BPMOP and BPMP can be attributed to the stronger electron donating effect of the methoxy group compared with that of the methyl group in the *para* position to the bridging oxygen atom.

Experimental Section

All chemicals were obtained from commercial sources and used as received. Solvents were purified by standard methods before use. **Caution:** Although no problems were encountered during the preparation of perchlorate salts, suitable care should be taken when handling such potentially hazardous compounds.

Spectrometry: Fast-atom bombardment (FAB) mass spectra in the positive mode were recorded with a Nermag R 1010C apparatus equipped with an M scan (Wallis) atom gun (8 kV, 20 mA). – UV/

Vis spectra were obtained using a UVIKON 933 spectrometer of Kontron instrument equipped with 1.0-cm matched quartz cells and operating in the range 200–900 nm; ϵ values are given in $\text{M}^{-1}\text{cm}^{-1}$. – ^1H NMR and ^{13}C NMR spectra were recorded with a BRUKER AC 200 spectrometer at 25 °C, in CDCl_3 or $[\text{D}_6]\text{DMSO}$ as solvent. Chemical shifts (ppm) were referenced to residual solvent peaks.

Electrochemistry: Electrochemical experiments were carried out using a PAR model 273 potentiostat equipped with a Kipp-Zonen x-y recorder. All experiments were run at room temperature under argon. A standard three-electrode cell was used. For the experiments performed in acetonitrile as solvent, potentials are referenced to an Ag/10 mM AgNO_3 reference electrode with 0.1 M tetra-*n*-butylammonium perchlorate (TBAP) as supporting electrolyte. Vitreous carbon disc electrodes for cyclic voltammetry (CV) (5 mm diameter) and rotating disc electrode (RDE) (3 mm diameter) were polished with 1 μm diamond paste.

X-ray Data Collection and Crystal Structure Determinations: The main details dealing with the diffraction data collections and the strategy used for the structure determinations are gathered in Table 3. The diffraction data were processed through the texSan software^[30] for complex **1** and through the Collect Software Nonius BV for complex **2**.^[31] Crystallographic data (excluding structure factors) for the two structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-140082 (**1**) and -140083 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: (internat.) + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].

Table 3. Crystallographic data for **1** and **2**

Complexes	1	2
Formula	$\text{C}_{57}\text{H}_{53}\text{Fe}_1\text{N}_6\text{O}_{10}\text{P}_2\text{Zn}_1 \cdot 2\text{ClO}_4 \cdot \text{H}_2\text{O}$	$\text{C}_{57}\text{H}_{53}\text{Fe}_2\text{N}_6\text{O}_{10}\text{P}_2 \cdot 2\text{ClO}_4 \cdot \text{H}_2\text{O}$
Molecular mass	1382.18	1372.65
Cell dimensions: <i>a</i> , <i>b</i> , <i>c</i> [Å];	10.237(5), 13.381(7), 23.899(10);	10.269(5), 13.396(5), 23.991(9);
α β γ [°]	106.01(2), 94.08(2), 98.47(2)	105.74(1), 94.26(1), 98.31(1)
Volume [Å ³]	3091(3)	3121(1)
<i>Z</i>	2	2
<i>d</i> _{calcd.}	1.483	1.458
System, space group	triclinic/ <i>P</i> $\bar{1}$	triclinic/ <i>P</i> $\bar{1}$
Diffractometer	Nonius-CAD4	Nonius Kappa CCD
Wavelength [Å]	Mo- <i>K</i> α = 0.7107	Ag- <i>K</i> α = 0.5608
Monochromator	graphite plate	graphite plate
Crystal size [mm]	0.27 × 0.31 × 0.32	0.30 × 0.35 × 0.35
Temperature	293 K	293 K
Scan mode	omega	—
Theta range [°]	2–27	2–30
Reflections measured	12107	21780
No of independent rfls.	7625	7713
<i>I</i> / σ (<i>I</i>)	2.2	3
μ [cm ^{−1}]	8.31	3.57
Absorption correction	none	none
Solution	direct methods (SIR92) ^[32]	direct methods (SIR92) ^[32]
LS refinements	on <i>F</i> /full matrix	on <i>F</i> /full matrix
H-atoms location	geometry and Fourier methods	geometry and Fourier methods
Thermal parameters	anisotropic for non-H atoms isotropic for H atoms	anisotropic for non-H atoms isotropic for H atoms
Number of parameters/number of reflections	9.2	9.5
Final <i>R</i> ^[a] and <i>R</i> (<i>w</i>) ^[b]	0.055/0.062	0.053/0.055
Program used	texSan software ^[30]	texSan software ^[30]

^[a] $R = \Sigma ||F_o| - |F_c|| / \Sigma |F_o|$. – ^[b] $R(w) = [\Sigma (|F_o| - |F_c|)^2 / w F_o^2]^{1/2}$ with $w = 1/[\sigma^2(F_o) + 3.4 \cdot 10^{-4} |F_o|^2]$.

Syntheses

HBPMP: 2,6-Bis[*bis*(2-pyridylmethyl)amino]methyl]-4-methoxyphenol has been synthesized in three steps from *p*-methoxyphenol.

2,6-Bis(hydroxymethyl)-4-methoxyphenol (5): To 4-methoxyphenol (10 g, 80.6 mmol) in 25 mL of methanol was added drop by drop a solution of sodium hydroxide (60 mL, 25%) under nitrogen at 0 °C. After 30 min of stirring, 120 mL of formaldehyde (37% in methanol) was added dropwise. The resulting mixture was refluxed during 12 h. Then the reaction mixture was neutralised at 0 °C to pH = 5 with 15 mL of pure acetic acid in 30 mL of water. Evaporation of the solvent yielded a solid. After addition of 200 mL of water, the product was extracted with ethyl acetate (3 × 200 mL). The combined organic layers were dried with sodium sulfate and concentrated to yield the crude product which was purified by flash chromatography (silica gel, ethyl acetate/cyclohexane, 1:1) and afforded **5** as a white powder (11.2 g, 75% yield); m.p. 114 °C. – ¹H NMR (200 MHz, [D₆]DMSO): δ = 3.66 (s, 3 H, OCH₃), 4.51 (s, 4 H, CH₂), 5.19 (s, 2 H, OH), 6.73 (s, 2 H, CH). – ¹³C NMR (50 MHz, [D₆]DMSO): δ = 55.8, 59.9, 111.6, 130.4, 145.6, 153.0. – C₉H₁₂O₄ (184.19): calcd. C 58.69, H 6.57; found C 58.75, H 6.60. – MS (DCI, NH₃, isobutane): *m/z* = 202 [M⁺ + NH₄⁺], 184 [M⁺].

2,6-Bis(chloromethyl)-4-methoxyphenol (6): 2,6-Bis(hydroxymethyl)-4-methoxyphenol (2.43 g, 13.2 mmol), suspended in 20 mL of dry dichloromethane under nitrogen atmosphere, was cooled with an ice bath. A solution of freshly distilled thionyl chloride (16 g, 132 mmol) in dry dichloromethane (20 mL) was added dropwise. The reaction was complete after 30 min. The excess thionyl chloride was removed in a rotary evaporator. The remaining solid was washed several times with dry pentane and 2.85 g (12.9 mmol, 98%) of **6** as a white powder was obtained. The product was used without any purification. – ¹H NMR (200 MHz, CDCl₃): δ = 3.77 (s, 3 H, OCH₃), 4.66 (s, 4 H, CH₂), 6.84 (s, 2 H, CH). – ¹³C NMR (50 MHz, CDCl₃): δ = 42.4, 55.8, 116.1, 126.0, 146.7, 152.9.

2,6-Bis[bis(2-pyridylmethyl)aminomethyl]-4-methoxyphenol (3): 2,6-Bis(chloromethyl)-4-methoxyphenol (5.0 g, 22.6 mmol) and dry tetrahydrofuran (50 mL) were placed in a two-necked round-bottomed flask under nitrogen and the mixture was cooled with an ice bath. A solution containing dipicolylamine (9.0 g, 45.2 mmol), distilled triethylamine (15 mL, 90.4 mmol) and dry tetrahydrofuran (20 mL) was added dropwise. The reaction was controlled by thin-layer chromatography (ethyl acetate/dichloromethane, 1:1). After 2.5 d of stirring at room temperature, the reaction mixture was filtered, washed with tetrahydrofuran and the filtrate was concentrated to dryness. The resulting oil was dissolved in dichloromethane (150 mL) and water was added (100 mL). The organic phase was separated and the aqueous phase was extracted three times with dichloromethane (100 mL). The organic phases were combined and dried with sodium sulfate. Evaporation of the solvent yielded a yellow oil, which was further purified by flash chromatography (silica gel, acetone). Removal of the solvent afforded 8.9 g (16.3 mmol, 72%) of **3** as a pale yellow powder, m.p. 76 °C. – ¹H NMR (300 MHz, CDCl₃): δ = 3.75 (s, 3 H, OCH₃), 3.80 (s, 4 H, CH₂), 3.88 (s, 8 H, CH₂), 6.85 (s, 2 H, CH), 7.08–7.14 (m, 4 H, Py-*H*), 7.47–7.51 (m, 4 H, Py-*H*), 7.55–7.64 (m, 4 H, Py-*H*), 8.52 (d, *J* = 4.8 Hz, 4 H, Py-*H*), 10.9 (s, 1 H, OH). – ¹³C NMR (75 MHz, CDCl₃): δ = 54.6, 55.5, 59.6, 114.1, 121.8, 122.7, 124.7, 136.4, 148.7, 149.5, 151.8, 159.0. – C₃₃H₃₄N₆O₂ (546.68): calcd. C 72.50, H 6.27, N 15.37; found C 72.55, H 6.25, N 15.41. – MS (DCI, NH₃, isobutane): *m/z* = 547 [M⁺ + H⁺].

[Zn^{II}Fe^{III}BPMOP{O₂P(OPh)₂]₂(ClO₄)₂·H₂O (1): To a solution of HBPMP (23.5 mg, 0.043 mmol) in methanol (2 mL), a solution of Zn(ClO₄)₂·6H₂O (16.0 mg, 0.043 mmol) in methanol (1 mL) was added dropwise. Upon the subsequent addition of Fe(ClO₄)₃·H₂O (15.2 mg, 0.043 mmol), dissolved in methanol (1 mL), the solution turned blue-green. Diphenyl phosphate (21.6 mg, 0.086 mmol), dissolved in methanol (1 mL), was added and the solvent was allowed to evaporate slowly at room temperature. Blue-green crystals (13 mg, 0.010 mmol, 23%) suitable for X-ray crystallography were obtained after 2 d. – UV/Vis (acetonitrile): λ_{max} = 654 (ε ≈ 900) nm. – FAB-MS (Matrix NBA): *m/z* = 1264 [Zn^{II}Fe^{III}BPMOP{O₂P(OPh)₂]₂ClO₄]⁺. – C₅₇H₅₃FeN₆O₁₀P₂Zn·2ClO₄·H₂O (1382.18): calcd. C 48.53, H 4.01, N 6.08; found C 48.59, H 3.98, N 5.85.

[Fe^{II}Fe^{III}BPMOP{O₂P(OPh)₂]₂(ClO₄)₂·H₂O (2): Complex **2** was prepared in a similar manner to complex **1**. To a solution of HBPMP (23.5 mg, 0.043 mmol) in methanol (2 mL) a solution of Fe(ClO₄)₃·H₂O (32 mg, 0.086 mmol) in methanol (2 mL) was added dropwise. Upon the addition, the solution turned dark green. Diphenyl phosphate (21.6 mg, 0.086 mmol), dissolved in methanol (1 mL), was added and the solvent was allowed to evaporate slowly at room temperature. Green crystals suitable for X-ray crystallography were obtained after 1 d. – UV/Vis (acetonitrile): λ_{max} = 385 (ε ≈ 3000), 673 (ε ≈ 900) nm. – FAB-MS (Matrix NBA): *m/z* = 1155 [Fe^{II}Fe^{III}BPMOP{O₂P(OPh)₂]₂ClO₄]⁺. – C₅₇H₅₃Fe₂N₆O₁₀P₂·2ClO₄·H₂O (1372.65): calcd. C 49.88, H 4.04, N 6.12; found C 49.63, H 3.92, N 6.16.

Blue Powder (4): To a solution of HBPMP (150 mg, 0.275 mmol) in acetone (2 mL) a solution of Zn(ClO₄)₂·6H₂O (100.5 mg, 0.275 mmol) in water (1 mL) was added dropwise. Upon the addition of Fe(ClO₄)₃·H₂O (95.7 mg, 0.275 mmol), dissolved in water (1 mL), the solution turned blue-green and was stirred for 1 h. The acetone was removed in a rotary evaporator and diethyl ether (100 mL) was added in small portions. The solution was placed in a refrigerator and after a few days a blue powder (134.0 mg, 0.120 mmol, 31%) precipitated which was filtered and washed with diethyl ether. – UV/Vis (acetonitrile): λ_{max} = 661 nm (ε ≈ 2200). – FAB-MS (matrix NBA): *m/z* = 681 [Zn^{II}Fe^{III}BPMOP(OH)]⁺.

The Deprotonation Constants by UV/Vis Spectroscopy: UV/Vis titration (monitoring of the LMCT band) of a solution of the ligand HBPMP (2.5·10^{−4} M) and equimolar amounts of zinc(II) perchlorate and iron(III) perchlorate in acetonitrile/water (1:9) with an aqueous solution of sodium hydroxide leads to a formation of new species. The ionic strength was fixed at 0.1 M with sodium perchlorate. The reaction is reversible by addition of an aqueous solution of perchloric acid. The spectrophotometric data were fitted with the LETAGROP-SPEFO program.^[33] The program uses a non linear least-squares method and calculates the thermodynamic constants of the absorbing species and their corresponding electronic spectra. The calculations were done from absorbance data of seven wavelengths (between 400 and 800 nm) for 15 solutions in the pH range of 1.95 < pH < 3.35 and for eight solutions in the pH range of 4.5 < pH < 6.1.

HPNP (2-Hydroxypropyl *p*-Nitrophenyl Phosphate) was prepared according to a known procedure.^[34]

Kinetic Investigations of the HPNP Cleavage by UV/Vis Spectroscopy: The kinetic investigations were made at 25 °C in buffered solutions of acetonitrile/water (1:1). HEPES {2-[4-(2-hydroxyethyl)-piperazin-1-yl]ethanesulfonic acid} and CHES [2-(cyclohexylamino)ethanesulfonic acid] were used as buffers. The ionic strength was fixed at 0.1 M with sodium perchlorate. The complexes were

synthesized in situ by adding an aqueous solution of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ and $\text{Fe}(\text{ClO}_4)_3 \cdot \text{H}_2\text{O}$ to a solution of the ligand in acetonitrile. The spontaneous hydrolysis under these conditions was measured in a buffered acetonitrile/water (1:1) solution containing the substrate and 0.1 M sodium perchlorate. The pH value varied less than 0.1 unit during the experiments. In a typical experiment, an aqueous solution (40 μL , 0.82 mM) of HPNP was injected into a cuvette which contained an aqueous solution of the buffer (400 μL , 0.01 M), an aqueous solution of perchlorate (200 μL , 0.1 M), a solution of the complex in acetonitrile/water (10:2) (120 μL , 8.25 mmol), acetonitrile (990 μL), and water (340 μL). In order to determine the activity towards phosphate ester cleavage, the increase of the concentration of *p*-nitrophenolate (PNPat), was followed by UV/Vis spectroscopy at 400 nm, ($\epsilon = 18500 \text{ M}^{-1}\text{cm}^{-1}$). All kinetic experiments were run twice and the obtained values were merged. In respect to a pH-dependent equilibrium between *p*-nitrophenol (PNP) and *p*-nitrophenolate (PNPat), the increase of the concentration of the reaction product can be calculated ($\text{pH} = \text{p}K_{\text{S}} + \log[\text{PNPat}]/[\text{PNP}]$). The k_{obs} values were obtained by Equation (2).

$$k_{\text{obs}} \cdot dt = d([\text{PNP}]_{\text{total}})/[\text{HPNP}]_0 \quad (2)$$

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