Functionalised 1,10-Phenanthroline Metallocatalysts as Models for Hydrolytic Metalloenzymes

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The synthesis of three new 1,10-phenanthroline derivatives is reported: 2-(*N*-methyldodecylamino)-methyl-9-(hydroxymethyl)-1,10-phenanthroline (**1**), 2-(*N*,*N*-dimethylamino)methyl-9-(hydroxymethyl)-1,10-phenanthroline (**2**), and 2-(*N*-methyldodecylamino)methyl-1,10-phenanthroline (**3**). The esterolytic activities of the complexes **1**–M^{II} and **3**–M^{II} in the presence of cationogenic micelles and of **2**–M^{II} in pure buffer toward the substrates *p*-nitrophenyl picolinate (PNPP), *p*-nitrophenyl octanoate (PNPO), and *p*-nitrophenyl dodecanoate (PNPD) have been investigated. The lipophilic ligand **1** in mixed micelles forms only 1:1 complexes with bivalent metal ions and the order of ligand activation is $Zn^{II} > Co^{II} > Cd^{II} > Ni^{II}$, while its water soluble analogue **2** forms both 1:1 complexes and 2:1 complexes. The order of ligand activation for **2** is also different: $Co^{II} > Ni^{II} \approx Zn^{II} > Cd^{II}$. The metallosurfactant **3**, lacking the nucleophilic hydroxy group, is 25 times less active than **1**–Zn^{II}. For the hydrolysis of PNPP catalysed by **1**–Zn^{II}, a detailed kinetic analysis is given.

Among the enzymes that have been mimicked in enzyme model studies, the Zn^{II}-containing metalloprotein carboxypeptidase A (CPA) has been particularly well studied.[1,2] The metal ion in this enzyme is considered to activate H_2O as a nucleophile at neutral pH. Additional roles attributed to the metal ion are electrophilic activation of the carbonyl group of the substrate and functioning as a template to bring catalytic groups and substrate together.[3] The geometry of metal-ion coordination is considered to be an important factor for the catalytic activity of CPA. Replacement of the Zn^{II} ion of CPA by Ni^{II} and Mn^{II} results in maintenance of the peptidase activity, while substitution with Co^{II} leads to an even more active enzyme.[4]

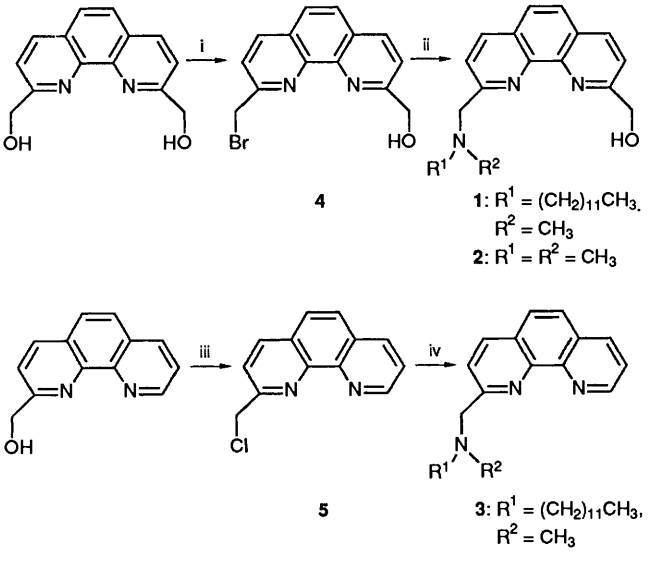
In biomimetic models the effect of metal ions has been studied in substrates in which the metal-ion binding site and scissile groups are covalently linked together.[3,4,5] These systems exhibit intramolecular metal-ion catalysis, but there is no turn-over. Biomimetic models of hydrolytic metalloenzymes which show turn-over behaviour operate *via* the formation of a reactive ternary complex composed of metal ion, ligand, and substrate.[6] In order to improve the catalytic efficiency, systems have been designed to enhance the binding of substrate to the metal centre, *e.g.*, cyclodextrins,[7,8] paracyclophanes,[9] and polymers,[10,11] all provided with metal-ion chelating groups. Moreover, aggregates of functionalised surfactants as biomimetic hydrolytic metallocatalysts have attracted considerable attention, homo and mixed metallomicelles are effective in promoting the cleavage of phosphoric,[12–14] and carboxylic esters.[15–19] Recently reversed mixed micelles,[20] and functionalised vesicles[21–23] have also been investigated as metalloenzyme models.

In a previous study, we have demonstrated that lipophilic ligands containing the 1,10-phenanthroline moiety are strong metal-ion chelating agents, forming metallomicelles. These Zn^{II}- and Cu^{II}-containing metallosurfactants are effective in the hydrolysis of carboxylic and phosphoric esters in homo and mixed micelles.[19]

In this paper we report on the synthesis of three new 1,10-phenanthroline ligands and present a study of their esterolytic activity in the presence of various bivalent metal ions. The lipophilic ligand **1** and its water-soluble counterpart **2** possess a hydroxy function in addition to the metal-ion binding site. According to CPK models, **1** and **2** are constructed in such a way that coordination of metal ions to the metallocleft of **1** and **2** activates the hydroxy group for nucleophilic attack. Sigman *et al.* showed that the hydroxymethyl group at the α-position of the phenanthroline ring is the active nucleophile in the Zn^{II}-catalysed reaction of ATP and *p*-nitrophenyl acetate with 2-hydroxymethyl-1,10-phenanthroline.[6b] Ligand **3** lacks the hydroxymethyl group so that the catalytic activity of this compound can be used for comparison.

Results and Discussion

The 1,10-phenanthroline derivatives **1**, **2** and **3** were prepared according to the synthetic pathways outlined in Scheme 1. The lipophilic ligand **1** and the water soluble analogue **2**, were



Scheme 1 Synthesis of the ligands. *Reagents and conditions:* i, 47% HBr, 120 °C; ii, R^1R^2NH, $CHCl_3$, 25 °C; iii, $SOCl_2$, 0 °C; iv, R^1R^2NH, $CHCl_3$, 50 °C.

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Table 1 Pseudo-first-order rate constants ($k_{obs}/10^{-5}$ s^{-1}) for the cleavage of PNPP in mixed micellar systems[a]

Ligand	Metal ion	$k_{obs}/10^{-5}$ s^{-1}	k_{obs}/k_0
none	none	2.3	1
none	Zn^{II}	15	6.5
none	Co^{II}	16	7.0
none	Cd^{II}	—[b]	—
none	Ni^{II}	38	16.5
1	none	12	5.2
1	Zn^{II}	1686	733
1	Co^{II}	740	322
1	Cd^{II}	353	153
1	Ni^{II}	205	89
3	none	5.1	2.2
3	Zn^{II}	66	29

[a] Conditions: 25 °C, pH = 7.00 (0.01 mol dm^{-3} *N*-ethylmorpholine–HBr buffer), [CTABr] = 4×10^{-3} mol dm^{-3}, [ligand] = 5×10^{-4} mol dm^{-3}, [M^{II}] = 5×10^{-4} mol dm^{-3} and [PNPP] = 5×10^{-5} mol dm^{-3}. [b] In the absence of 1,10-phenanthroline ligand $CdBr_2$ is insoluble in a CTABr micellar solution.

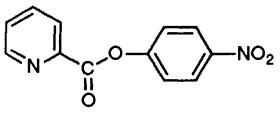
Table 2 Pseudo-first-order rate constants ($k_{obs}/10^{-5}$ s^{-1}) for the cleavage of PNPP under non-micellar conditions[a]

Ligand	Metal ion	$k_{obs}/10^{-5}$ s^{-1}	k_{obs}/k_0
none	none	1.0	1
none	Zn^{II}	20	20
none	Co^{II}	19	19
none	Cd^{II}	6.8	6.8
none	Ni^{II}	67	67
2	none	2.5	2.5
2	Zn^{II}	508	508
2	Co^{II}	964	964
2	Cd^{II}	65	65
2	Ni^{II}	510	510

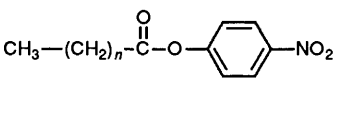
[a] Conditions: 25 °C, pH = 7.00 (0.01 mol dm^{-3} *N*-ethylmorpholine–HBr buffer), [**2**] = 5×10^{-4} mol dm^{-3}, [M^{II}] = 5×10^{-4} mol dm^{-3} and [PNPP] = 5×10^{-5} mol dm^{-3}.

obtained by treatment of 2,9-bis(hydroxymethyl)-1,10-phenanthroline[24] with aqueous HBr to give the bromoalcohol **4**, followed by coupling of **4** with *N*-methyldodecylamine[25] or dimethylamine. Except for the earlier reported synthesis of 9-formyl-1,10-phenanthroline-2-carboxylic acid,[26] which could not be reproduced by Chandler *et al.*[24] or by us, these are the first examples of the synthesis of asymmetrically substituted 1,10-phenanthrolines. For the synthesis of **3**, 2-hydroxymethyl-1,10-phenanthroline[5b,19] was converted to the chloromethyl derivative **5** with $SOCl_2$, and then reacted with *N*-methyldodecylamine.

The lipophilic ligands **1** and **3** are only slightly soluble in water, even in the presence of metal ions. However, solubilisation of **1** and **3** in chemically inert CTABr micelles results in clear and stable solutions. Ligand **2** is water soluble.



PNPP



PNPO: $n = 6$
PNPD: $n = 10$

The esterolytic activity of bivalent metal ions alone, non-metallated ligands, and complexes of ligands and metal ions toward the metallophilic substrate *p*-nitrophenyl picolinate (PNPP), was studied in mixed micellar systems for **1** and **3** and in non-micellar media for **2**. The hydrolysis of the ester was followed by observing the release of *p*-nitrophenolate (PNP) spectrophotometrically (400 nm) at pH = 7.00 and 25 °C. Pseudo-first-order rate constants, determined under conditions of excess of metallocatalyst over substrate, in buffered micelles and in pure buffer solutions are shown in Tables 1 and 2, respectively.

These tables indicate that both in the presence and in the absence of the cosurfactant CTABr, addition of Zn^{II}, Co^{II}, Cd^{II} and Ni^{II} causes only a slight rate enhancement.* The non-metallated ligands also have only a slight effect. However, in the presence of equimolar amounts of metal ion and ligand, the rate enhancement is much larger than the summation of the separate effects. Clearly, metal ion and ligand catalyse the reaction synergistically. For the lipophilic ligand **1**, comicellised in CTABr, the activation is in the order $Zn^{II} > Co^{II} > Cd^{II} > Ni^{II}$. Comparison of **1**–$Zn^{II}$ with **3**–Zn^{II}, demonstrates that the hydroxymethyl group is essential for the high catalytic activity. The metal-ion activation of the water-soluble ligand **2** is in the order $Co^{II} > Ni^{II} \approx Zn^{II} > Cd^{II}$, which is different from that of the micellar analogue **1**. Using the same concentrations of metal ion and ligand, **1**–Zn^{II} is 3.3 times more active than the non-micellar **2**–Zn^{II}. In contrast, **2**–Co^{II} is 1.3 times more active than **1**–Co^{II}. This result is somewhat surprising as it was expected that the micellar reaction would be faster than the non-micellar reaction, as is observed for Zn^{II} complexes of **1** and **2** since in the case of **1**–Zn^{II} both substrate and catalyst are concentrated in the micellar pseudo-phase. At the present stage of investigation, it is not completely clear why the water soluble Co^{II} complex is more active than the micellar Co^{II} complex. Compared to the micellar medium, the greater availability of water in the non-micellar system may result in a greater hydration of the activated complex and this effect may dominate for the Co^{II}-complex-catalysed hydrolysis. It has been suggested,[16d] that for Cu^{II} ternary complexes the nucleophilic activity changes from the coordinated hydroxymethyl group to metal-ion-bound H_2O (or OH^-) on going from micelles to pure water. However, in view of the high rates of hydrolysis and biphasic turn-over behaviour it is likely that for both **1** and **2** the hydroxymethyl group is the nucleophilic species.

In order to determine the stoichiometry of the metal-ion–ligand complexes, we have studied the effect of variation of the M^{II} concentration on the rate of hydrolysis in the presence of a fixed concentration of **1** and **2**. As is illustrated in Fig. 1, addition of Zn^{II} or Co^{II} to **1** causes a rapid increase in the rate of hydrolysis until equimolar amounts of M^{II} and **1** are present. Addition of more than one equivalent of Zn^{II} has no effect on the rate of hydrolysis, while addition of excess Co^{II} increases the rate of hydrolysis only slightly. This demonstrates the high affinity of the metal ions for the 1,10-phenanthroline ligands. The slope of the first part of the graph is very steep. It is remarkable that under the condition of [**1**]:[Zn^{II}]:[PNPP] = 10:1:1 the pseudo-first-order rate constant is already one-third of the maximum value of k_{obs}. Moreover, under these conditions, the hydrolysis of PNPP still proceeds by a first-order reaction. This shows that after the reaction of the substrate with the metal-ion–ligand complex, the metal ion is able to move from the acylated phenanthroline ligand (*vide infra*) to a free phenanthroline ligand and activate catalysis at this moiety. By this metal-ion hopping mechanism the hydrolysis is truly catalytic in terms of the metal ion.

Fig. 2 presents the rate profiles observed for increasing Zn^{II} and Co^{II} concentrations at a fixed concentration of **2**. Both curves have sigmoid shapes, suggesting a less active 2:1 com-

* Free Cu^{II}, in the absence of ligand, enhances the rate of PNPP more than 5700 fold. On addition of the lipophilic ligand **1** the rate of cleavage is only 2.5 times further increased.

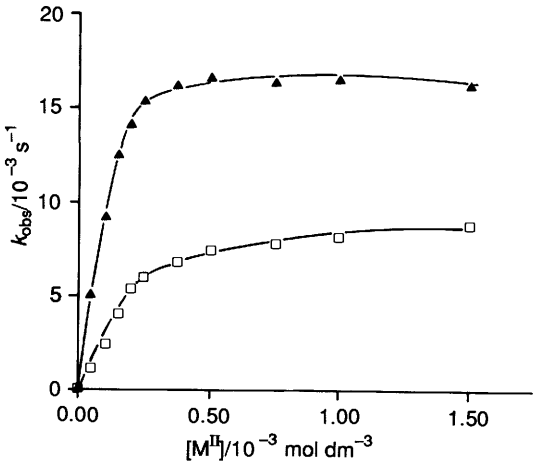


Fig. 1 Plots of pseudo-first-order rate constants for the cleavage of PNPP in a mixed micellar system as a function of [Zn^{II}] (▲) and [Co^{II}] (□) under fixed concentration of **1** at pH = 7.00 and 25 °C, [CTABr] = 4×10^{-3} mol dm^{-3}, [**1**] = 5×10^{-4} mol dm^{-3} and [PNPP] = 5×10^{-5} mol dm^{-3}

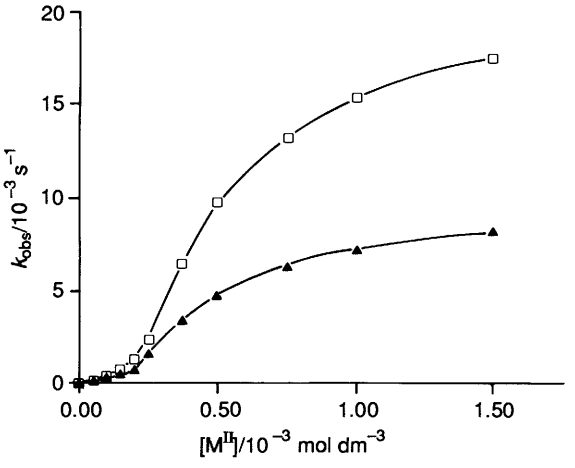


Fig. 2 Plots of pseudo-first-order rate constants for the cleavage of PNPP as a function of [Zn^{II}] (▲) and [Co^{II}] (□) under fixed concentration of **2** at pH = 7.00 and 25 °C, [**2**] = 5×10^{-4} mol dm^{-3} and [PNPP] = 5×10^{-5} mol dm^{-3}

plex (ligand:metal ion) at low metal-ion concentration, and a more active 1:1 complex at high metal-ion concentration. In the 2:1 complex, all available coordination positions around the metal ion are occupied by the phenanthroline nucleus, the dimethylamine moieties and the hydroxy groups. Therefore binding of the metallophilic substrate PNPP to the metallocatalyst to form the reactive ternary complex is hindered. The micellar analogue **1** is less able to form 2:1 complexes in mixed micellar systems. Although formation of 2:1 complexes in micelles may be entropically more favourable than in non-micellar media, the preferential orientation of the hydrocarbon chains of the ligands towards the micellar core makes the formation of such complexes sterically difficult.

Metallocomplexes of **1**, comicellised in CTABr, and of **2** in pure buffer, are both catalytically active in the cleavage of PNPP. Besides the different order of metal-ion activation in **1** and **2**, there are also differences in the location of the complexes in the reaction medium. Table 3 shows that the **2**–Zn^{II}-catalysed hydrolysis of PNPP is retarded by the addition of CTABr. This may be explained by the partition of PNPP between the bulk solvent and the micellar pseudo-phase, while **2**-M^{II} remains largely in the bulk solvent due to the electrostatic repulsion between **2**–M^{II} and the cationic head groups of the CTABr micelle.

Table 3 Pseudo-first-order rate constants ($k_{obs}/10^{-5}$ s^{-1}) for the cleavage of PNPP, PNPO and PNPD by Zn^{II} complexes of **1** and **2**[a]

Metallocatalyst	Comicellar additive	Substrate	$k_{obs}/10^{-5}$ s^{-1}
1–Zn^{II}	CTABr	PNPP	1686
2–Zn^{II}	none	PNPP	508
2–Zn^{II}	CTABr	PNPP	300
none	CTABr	PNPO	0.6
none	CTABr	PNPD	0.6
1–Zn^{II}	CTABr	PNPO	42
1–Zn^{II}	CTABr	PNPD	44
2–Zn^{II}	CTABr	PNPO	0.6
2–Zn^{II}	CTABr	PNPD	0.5

[a] Conditions: 25 °C, pH = 7.00 (0.01 mol dm^{-3} *N*-ethylmorpholine–HBr buffer), [CTABr] = 4×10^{-3} mol dm^{-3}, [**1**] = 5×10^{-4} mol dm^{-3}, [**2**] = 5×10^{-4} mol dm^{-3}, [M^{II}] = 5×10^{-4} mol dm^{-3}, [PNPP] = 5×10^{-5} mol dm^{-3}, [PNPO] = 5×10^{-5} mol dm^{-3} and [PNPD] = 5×10^{-5} mol dm^{-3}.

Next, we investigated the substrate specificity (Table 3). The hydrolysis of the lipophilic esters *p*-nitrophenyl octanoate (PNPO) and *p*-nitrophenyl dodecanoate (PNPD) is catalysed by **1**–Zn^{II}, although the observed rate constant is 37 times slower than that of PNPP. This demonstrates that substrate coordination to the metal-ion ligand complex plays an important role for a large rate enhancement. The hydrophilic complex **2**–Zn^{II} has no rate accelerating effect on the cleavage of PNPO and PNPD in the presence of CTABr, indicating that these substrates are completely incorporated into the micellar pseudo-phase whereas **2**–Zn^{II} is not. (PNPO and PNPD are insoluble in buffer in the absence of CTABr.)

In order to allow a non-complicated, full kinetic analysis of the metallocomplex-catalysed hydrolysis of PNPP, it is important that there is only one kinetically active species present in the reaction mixture. The hydrolysis of PNPP, catalysed by **1**–Zn^{II} comicellised in CTABr, is a good example of such a reaction. Since **1** has a high affinity for Zn^{II}, the 1:1 complex is essentially completely formed and consequently contributions from free Zn^{II}, non-metallated **1**, and the 2:1 complex of Zn^{II} and **1** are negligible. From previous work of others,[5,9,15,16] and ourselves,[19] and the present results, it is likely that the reaction exhibits a kinetic feature consistent with a mechanism which involves pre-equilibrium complexation of the metallosurfactant with PNPP (association constant *K*), followed by pseudo-intramolecular acyl transfer (rate constant k_a) and subsequent hydrolysis of the acylated ligand (rate constant k_d) [eqns. (1) and (2)]. It is clear that the system cannot be termed catalytic if

$$\text{1–Zn}^{II} + \text{PNPP} \underset{}{\overset{K}{\rightleftharpoons}} \text{1–Zn}^{II}\text{—PNPP} \xrightarrow{k_a} \text{P–1–Zn}^{II} \xrightarrow{-\text{PNP}} \xrightarrow{k_d} \text{1–Zn}^{II} + \text{P} \quad (1)$$

$$\text{PNPP} \xrightarrow{k_0} \text{PNP} + \text{P} \quad (2)$$

only one stoichiometric equivalent of PNP is released and the hydroxy group of the catalyst is regenerated in a relatively slow deacylation step. In Fig. 3 the observed rate of release of PNP is plotted against increasing concentration of **1**–Zn^{II}. At higher concentrations, saturation kinetics are observed. The association constant and the acylation rate constant, evaluated from the usual double reciprocal plot of $(k_{obs} - k_0)^{-1}$ *vs.* [**1**–Zn^{II}]$^{-1}$ are: $K = (1.08 \pm 0.05) \times 10^3$ dm^3 mol^{-1} and $k_a = (3.77 \pm 0.03) \times 10^{-2}$ s^{-1}.

Experiments under conditions of excess of substrate over **1**–Zn^{II} and **3**–Zn^{II} were performed in order to test the turnover

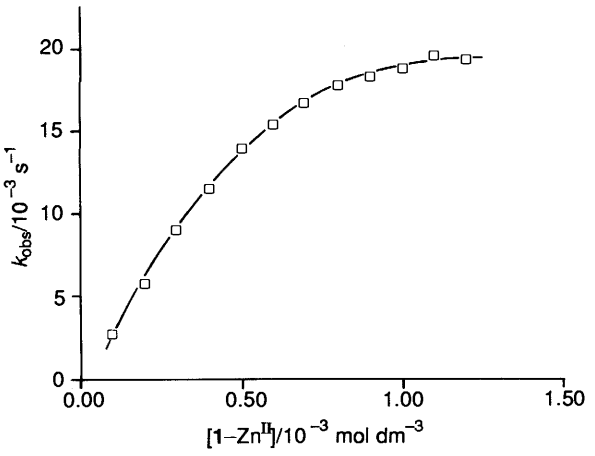


Fig. 3 Pseudo-first-order rate constants for the cleavage of PNPP as a function of **1**–Zn^{II} concentration at pH = 7.00 and 25 °C, [CTABr] = 4×10^{-3} mol dm^{-3}, [PNPP] = 5×10^{-5} mol dm^{-3} and [**1**]:[Zn^{II}] = 1

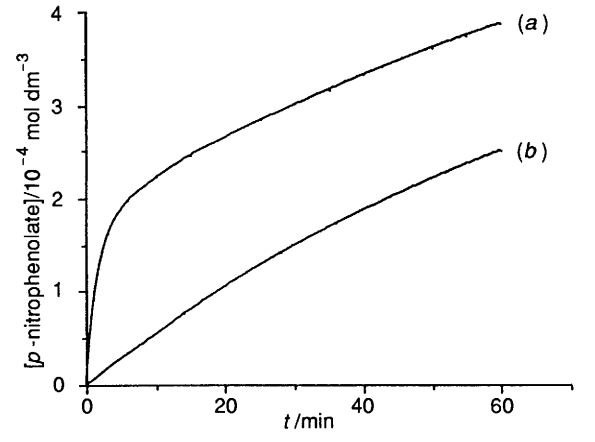


Fig. 4 Time courses for *p*-nitrophenolate release from PNPP as catalysed by **1**–Zn^{II} (*a*) and **3**–Zn^{II} (*b*) at pH = 7.00 and 25 °C, [CTABr] = 4×10^{-3} mol dm^{-3}, [**1**] = 2×10^{-4} mol dm^{-3}, [**3**] = 2×10^{-4} mol dm^{-3}, [Zn^{II}] = 2×10^{-4} mol dm^{-3} and [PNPP] = 6×10^{-4} mol dm^{-3}

behaviour of the mixed micellar systems. As shown in Fig. 4, both reactions proceed beyond the stoichiometric conversion range. A striking difference between the systems is that **1**–Zn^{II} displays distinct biphasic kinetics while **3**–Zn^{II}, lacking the hydroxymethyl group, does not. In the case of **1**–Zn^{II}, after an initial burst release of PNPP stoichiometrically equivalent to the amount of metallosurfactant, the deacylation of the intermediate to regenerate the catalyst is the rate-determining step. The same biphasic behaviour was also observed for **1**–Co^{II}, solubilised in CTABr micelles and for **2**–Zn^{II} and **2**–Co^{II} in pure buffer. The burst kinetic profiles are analysed by a modification of the kinetic method of Murakami *et al.*[9] The initial rate for PNP release is given by eqn. (3) in which [**1**–Zn^{II}–PNPP]$_0$ is

$$v_0 = d[PNP]_0/dt = k_a[\mathbf{1}\text{–}Zn^{II}\text{–}PNPP]_0 \quad (3)$$

the concentration of the ternary complex at $t = 0$. From the initial slope of the burst release of PNP, k_a can be evaluated; $k_a = (2.98 \pm 0.25) \times 10^{-2}$ s^{-1}. This k_a value agrees reasonably well with that obtained from the double reciprocal plot of $(k_{obs} - k_0)^{-1}$ *vs.* [**1**–Zn^{II}]$^{-1}$. The rate after the stationary phase has been attained is given by eqn. (4) in which [**1**–Zn^{II}–PNPP]$_s$

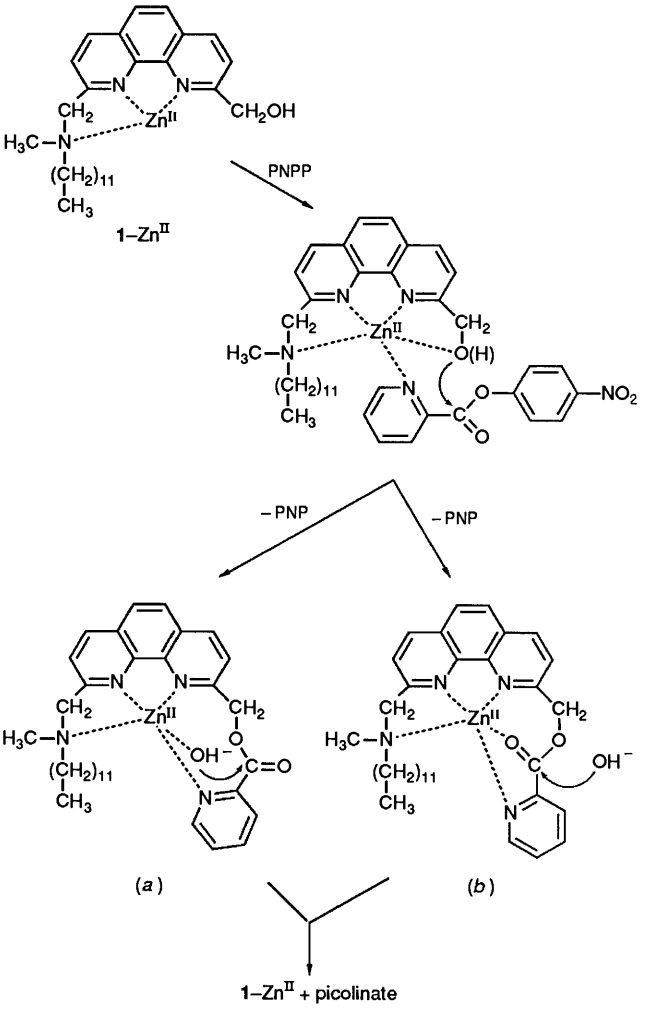
$$v_s = d[PNP]_s/dt = k_a[\mathbf{1}\text{–}Zn^{II}\text{–}PNPP]_s + k_0[PNPP]_s \quad (4)$$

represents the concentration of the ternary complex in the stationary phase. The concentration of the acylated intermediate in the stationary phase, [P–**1**–Zn^{II}]$_s$, is constant as shown in eqn. (5).

$$d[P\text{–}\mathbf{1}\text{–}Zn^{II}]_s/dt = k_a[\mathbf{1}\text{–}Zn^{II}\text{–}PNPP]_s - k_d[P\text{–}\mathbf{1}\text{–}Zn^{II}]_s = 0 \quad (5)$$

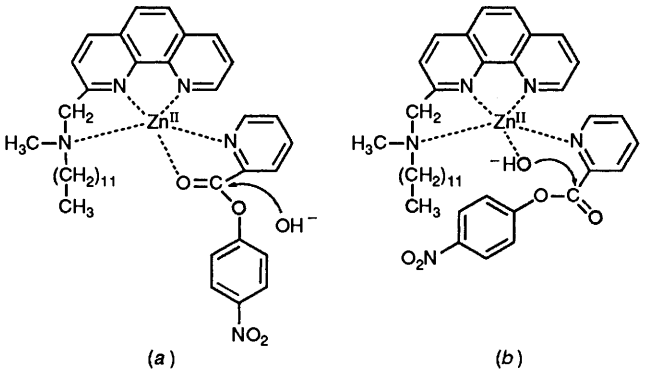
From the slope of the steady state section of the kinetic plot the k_d value can be calculated; $k_d = (2.24 \pm 0.12) \times 10^{-4}$ s^{-1}.

The schematic representations of the mechanism of the hydrolysis of PNPP, catalysed by **1**–Zn^{II} and **3**–Zn^{II} are shown in Scheme 2 and Scheme 3, respectively. In Scheme 2 the Zn^{II}



Scheme 2 Mechanism of the cleavage of PNPP catalysed by the metallosurfactant 1–Zn^{II}

ion serves as a template upon which **1** and PNPP are able to coordinate simultaneously. The geometry of the resulting ternary complex permits facile pseudo-intramolecular attack of the hydroxy function. Another catalytic function of the Zn^{II} ion in the metallosurfactant is to lower the pK_a value of the hydroxymethy group, providing a high concentration of the effective nucleophile at neutral pH. The rate of hydrolysis of PNPP catalysed by **1**–Zn^{II} is pH dependent and log k_{obs} in buffered solutions is proportional to pH, over the pH range 6.5–8.5. From this result, it can be concluded that the pK_a value of the hydroxymethyl group in the presence of Zn^{II} must be higher than 8.5. In the ternary complex, a pseudo-intramolecular attack of the hydroxy group on the carbonyl function of the activated ester,[7b,27] yields the intermediate transacylation product with simultaneous liberation of the



Scheme 3 Mechanism of the hydrolysis of PNPP catalysed by the metallosurfactant **3**–Zn^{II}

good leaving group PNP. Finally, the acylated intermediate is hydrolysed to regenerate the catalyst in a relatively slow step which determines the overall rate of the catalytic process. Two kinetically equivalent possibilities for the deacylation mechanism can be postulated: (*a*) a pseudo-intramolecular nucleophilic attack of metal-ion-coordinated OH^- onto the ester group, or (*b*) attack of free OH^- onto the ester group which is activated by coordination to the metal ion. The catalytic rate constant for this metal-ion-assisted deacylation step is 133 times lower than that of the transacylation step. The relatively high rate of hydrolysis of the unactivated ester is due to the strong binding of Zn^{II} to the acylated intermediate.[5a,c]

The metallosurfactant **3**–Zn^{II} operates *via* the zinc–hydroxide mechanism (Scheme 3) as was seen previously for the hydrolysis of PNPP catalysed by 2(-*N*-dodecylamino)methyl-1,10-phenanthroline.[19]

In summary, the present study demonstrates that metallocomplexes of **1** solubilised in an inert CTABr micellar matrix are efficient synzymes for the hydrolysis of PNPP, PNPO and PNPD. Ligand **1** has a high affinity for metal ions, and in CTABr-micelles 1:1 complexes of **1** and M^{II} are essentially completely formed. The water-soluble analogues **2**–M^{II} are good catalysts for the hydrolysis of PNPP, but not for the lipophilic substrates PNPO and PNPD. The metallosurfactant **3**–Zn^{II}, which lacks the nucleophilic hydroxy group, is 25 times less active in mixed micelles than **1**–Zn^{II}. In contrast to **3**–Zn^{II}, for **1**–Zn^{II} typical burst kinetics are observed in the presence of excess of PNPP. The ligand is rapidly acylated, releasing a stoichiometrically equivalent amount of PNP, followed by a rate-determining deacylation step which regenerates the catalyst.

Experimental

General Methods.—Melting points are uncorrected. ^1H NMR spectra were recorded on a Bruker AC 200-E spectrometer operating at 200.1 MHz. The chemical shifts are reported relative to internal $(CH_3)_4Si$ and all coupling constant values *J* are given in Hz. ^{13}C NMR spectra were recorded on the same spectrometer operating at 50.3 MHz and ^{13}C NMR shifts were measured relative to $CDCl_3$. Mass spectral data were recorded on a AEI MS 902 spectrometer equipped with a VG ZAB console using field desorption ionisation technique. Kinetic runs were recorded on a Beckman DU-7 spectrophotometer with a thermostatted cell compartment and kinetic device or on a Hewlett-Packard 8452 A Diode Array spectrophotometer. The temperature was controlled at 25 ± 0.1 °C.

Materials.—$ZnBr_2$ (Janssen Chimica), $CoBr_2$, $NiBr_2$ and $CdBr_2 \cdot 4H_2O$ (Alfa products), *N*-ethylmorpholine (Janssen Chimica), CTABr (Merck), PNPO and PNPD (Sigma) were used without further purification. PNPP, m.p. 148–156 °C (decomp.) (lit.[6a] 144–146 °C), and *N*-methyldodecylamine,[25] b.p. 96–98 °C/2 mmHg, were prepared according to literature methods. Acetonitrile and ethanol used in the kinetic experiments were of spectrophotometric grade.

2-*Bromomethyl*-9-*hydroxymethyl*-1,10-*phenanthroline* (**4**). A solution of 2,9-bis(hydroxymethyl)-1,10-phenanthroline[24] (2.0 g, 8.3 mmol) in 47% aqueous HBr (40 cm^3) was heated at 120 °C for 5 h. After cooling on ice the solution was neutralised by slow addition of 10% aqueous Na_2CO_3 and extracted with $CHCl_3$ (5 × 100 cm^3). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, 1% (v/v) $CH_3OH/CHCl_3$]. Analytically pure **4** (0.93 g, 37%) was obtained as a white solid, m.p. 125–130 °C (decomp.); $\delta_H(CDCl_3)$ 4.92 (2 H, s, CH_2Br), 5.10 (2 H, s, CH_2OH), 7.64 and 7.86 (2 H, 2 d, *J* 8.3, Phen 3-H and 8-H), 7.76 and 7.82 (2 H, 2 d, *J* 8.9, Phen 5-H and 6-H), 8.24 and 8.26 (2 H, 2 d, *J* 8.3, Phen 4-H and 7-H); $\delta_C(CDCl_3)$ 32.78 (CH_2Br), 64.70 (CH_2OH), 120.49 and 123.35 (Phen C-3 and C-8), 125.38 and 126.64 (Phen C-5 and C-6), 127.61 and 127.79 (Phen C-4a and C-7a), 136.81 and 137.57 (Phen C-4 and C-7), 143.68 and 143.97 (Phen C-1b and C-10b), 156.71 (Phen C-2) and 160.23 (Phen C-9); *m/z* 303/305 (MH^+).

2-(N-*Methyldodecylamino*)*methyl*-9-(*hydroxymethyl*)-1,10-*phenanthroline* (**1**). A solution of **4** (606 mg, 2.0 mmol), *N*-methyldodecylamine (450 mg, 2.26 mmol) and $(C_2H_5)_3N$ (250 mg, 2.47 mmol) in $CHCl_3$ (30 cm^3) was stirred under a N_2 atmosphere at room temperature for 16 h. The reaction mixture was washed with water containing 5% (w/v) $NaHCO_3$ and 2% (w/v) EDTA. Evaporation of the dried (Na_2SO_4) $CHCl_3$ layer yielded the crude product which was purified by column chromatography [Al_2O_3, 1% (v/v) $CH_3OH/CHCl_3$]. Pure **1** (0.80 g, 95%) was obtained as a white waxy solid which hardened at −20 °C. $\delta_H(CDCl_3)$ 0.84 [3 H, t, *J* 6.4, $(CH_2)_{11}CH_3$], 1.21 [18 H, s, $(CH_2)_9CH_3$], 1.53 [2 H, m, $CH_2(CH_2)_9CH_3$], 2.28 (3 H, s, CH_3N), 2.45 (2 H, t, *J* 7.4, CH_2CH_2N), 3.98 (2 H, s, $PhenCH_2N$), 5.09 (2 H, s, CH_2OH), 7.59 and 7.83 (2 H, 2 d, *J* 8.3, Phen 3-H and 8-H), 7.69 and 7.73 (2 H, 2 d, *J* 9.1, Phen 5-H and 6-H), 8.14 and 8.18 (2 H, 2 d, *J* 8.3, Phen 4-H and 7-H); $\delta_C(CDCl_3)$ 13.87 [$(CH_2)_{11}CH_3$], 22.41, 27.09, 27.18, 29.08, 29.38, 31.64 [$(CH_2)_{10}CH_3$], 42.31 (CH_3N), 57.93 [$NCH_2(CH_2)_{10}$], 64.03 and 65.25 (CH_2OH and $PhenCH_2N$), 120.19 and 122.27 (Phen C-3 and C-8), 125.41 and 125.51 (Phen C-5 and C-6), 127.34 (Phen C-4a and C-7a), 136.19 (Phen C-4 and C-7), 144.30 and 144.65 (Phen C-1b and C-10b), 160.17 and 160.93 (Phen C-2 and C-9); *m/z* 422 (MH^+).

2-(N,N-*Dimethylamino*)*methyl*-9-(*hydroxymethyl*)-1,10-*phenanthroline* (**2**). To a solution of **4** (500 mg, 1.65 mmol) in $CHCl_3$ (25 cm^3), was added dimethylamine (650 mg, 14.4 mmol) dissolved in $CHCl_3$ (5 cm^3). The reaction mixture was kept for 3 h, under stirring, at room temperature under a N_2 atmosphere. The solution was washed with water containing 5% (w/v) $NaHCO_3$ and 2% (w/v) EDTA, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified by column chromatography [Al_2O_3, 1% (v/v) $CH_3OH/CHCl_3$]. Ligand **2** (400 mg, 91%) was obtained as a white powder, m.p. 135–138 °C; $\delta_H(CDCl_3)$ 2.33 (6 H, s, CH_3), 3.94 (2 H, s, $PhenCH_2N$), 5.09 (2 H, s, CH_2OH), 5.25 (1 H, br s, OH), 7.59 and 7.79 (2 H, 2 d, *J* 8.3, Phen 3-H and 8-H), 7.66 and 7.71 (2 H, 2 d, *J* 9.1, Phen 5-H and 6-H), 8.11 and 8.17 (2 H, 2 d, *J* 8.3, Phen 4-H and 7-H); $\delta_C(CDCl_3)$ 45.55 (CH_3), 65.25 and 65.87 (CH_2OH and $PhenCH_2N$), 120.19 and 122.24 (Phen C-3 and C-8), 125.55 and 125.63 (Phen C-5 and C-6), 127.46 (Phen C-4a and C-7a), 136.33 (Phen C-4 and C-7), 144.36 and 144.69 (Phen C-1b and C-10b), 159.73 and 160.69 (Phen C-2 and C-9); *m/z* 268 (MH^+).

2-*Chloromethyl*-1,10-*phenanthroline* (**5**). A mixture of 2-hydroxymethyl-1,10-phenanthroline[5b,19] (5.0 g, 23.8 mmol) and

$SOCl_2$ (40 cm^3) was stirred for 2 h at 0 °C with the exclusion of moisture. Light petroleum (40–60 °C, 150 cm^3) was added to the pale orange reaction mixture to precipitate the product as an oil. After decantation of light petroleum, cold diethyl ether (150 cm^3) was added and the oil solidified. The suspension was stirred for 20 min at 0 °C and the crystalline solid was filtered off by suction and washed with diethyl ether. The monohydrochloride salt of **5** (6.0 g, 95%) was obtained as a pale yellow powder, m.p. 185 °C (decomp.); $\delta_H(CDCl_3)$ 5.09 (2 H, s, CH_2), 7.64 (1 H, dd, *J* 4.4, 8.1, Phen 8-H), 7.80 (2 H, s, Phen 5-H and 6-H), 7.91 (1 H, d, *J* 8.3, Phen 3-H), 8.26 (1 H, dd, *J* 1.7, 8.1, Phen 7-H), 8.30 (1 H, d, *J* 8.3, Phen 4-H) and 9.22 (1 H, dd, *J* 1.7, 4.4, Phen 9-H); $\delta_C(CDCl_3)$ 47.26 (CH_2), 122.16 and 122.88 (Phen C-3 and C-8), 125.98 and 126.67 (Phen C-5 and C-6), 127.66 and 128.70 (Phen C-4a and C-7a), 135.92 and 137.05 (Phen C-4 and C-7), 144.91 and 145.59 (Phen C-1b and C-10b), 150.25 (Phen C-9) and 156.92 (Phen C-2); *m/z* 228/230 (M^+) and 229/231 (MH^+).

2-(N-*Methyldodecylamino*)*methyl*-1,10-*phenanthroline* (**3**). A stirred mixture of the free base of **5** (1.14 g, 5.0 mmol) (obtained by treatment of the monohydrochloride of **5** with a mixture of aqueous $NaHCO_3$ and $CHCl_3$), *N*-methyldodecylamine (1.19 g, 6.0 mmol) and $(C_2H_5)_3N$ (0.76 g, 7.5 mmol) in $CHCl_3$ (50 cm^3), was heated at 50 °C under a N_2 atmosphere for 16 h. After the reaction mixture had been washed with water containing 5% (w/v) $NaHCO_3$ and 2% (w/v) EDTA, the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (Al_2O_3, $CHCl_3$). The lipophilic ligand **3** (1.41 g, 72%) was obtained as a yellow oil which solidified at −20 °C; $\delta_H(CDCl_3)$ 0.84 [3 H, t, *J* 6.4, $(CH_2)_{11}CH_3$], 1.22 [18 H, s, $(CH_2)_9CH_3$], 1.54 [2 H, m, $CH_2(CH_2)_9CH_3$], 2.30 (3 H, s, CH_3N), 2.49 (2 H, t, *J* 7.4, CH_2CH_2N), 4.06 (2 H, s, PhenCH_2N), 7.58 (1 H, dd, *J* 4.4, 8.1, Phen 8-H), 7.71 and 7.77 (2 H, 2 d, *J* 9.1, Phen 5-H and 6-H), 7.91 (1 H, d, *J* 8.3, Phen 3-H), 8.19 (1 H, d, *J* 8.3, Phen 4-H), 8.21 (1 H, dd, *J* 1.8, 8.1, Phen 7-H) and 9.18 (1 H, dd, *J* 1.8, 4.4, Phen 9-H); $\delta_C(CDCl_3)$ 13.80 [$(CH_2)_{11}CH_3$], 22.33, 27.09, 29.00, 29.29, 31.56 [$(CH_2)_{10}CH_3$], 42.25 (CH_3N), 57.87 [$NCH_2(CH_2)_{10}$], 64.37 (PhenCH_2N), 122.21 and 122.38 (Phen C-3 and C-8), 125.45 and 126.07 (Phen C-5 and C-6), 127.18 and 128.36 (Phen C-4a and C-7a), 135.67 and 135.99 (Phen C-4 and C-7), 145.02 and 145.59 (Phen C-1b and C-10b), 149.76 (Phen C-9) and 160.61 (Phen C-2); *m/z* 392 (MH^+).

Kinetic Studies.—Solutions were prepared in *N*-ethylmorpholine–HBr buffer, pH = 7.00. Each kinetic run was initiated by injecting a 0.02 mol dm^3 solution of ester substrate into a 1 cm cuvette containing 2 cm^3 of the buffer solution [containing 1% (v/v) C_2H_5OH and 0.25–0.75% (v/v) CH_3CN] and the desired reagents. The release of PNP was monitored at 400 nm for at least 10 half-lives. Observed pseudo-first-order rate constants were obtained by fitting the data with Marquardt's algorithm or according to the Guggenheim method, under the conditions of excess of catalyst over substrate. Kinetic runs, carried out at least in triplicate, gave rate constants with an uncertainty of less than ±3%.

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