Carboxylic and Phosphate Ester Hydrolysis Catalysed by Bivalent Zinc and Copper Metallosurfactants

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The syntheses of three new lipophilic ligands are reported: N^{α}-(1,10-phenanthrolin-2-ylmethyl)-N^{τ}-dodecylhistamine (**1**), *N*-dodecyl-2-aminomethyl-1,10-phenanthroline (**2**), and N^{α}-(2-pyridylmethyl)-N^{τ}-dodecylhistamine (**3**). Mixed micellar systems made of complexes of these ligands with Zn^{II} and Cu^{II} in the presence of an inert cosurfactant (CTABr or Brij 35) are efficient catalysts in the hydrolysis of *p*-nitrophenyl picolinate (PNPP) and diphenyl-*p*-nitrophenyl phosphate (DPPNPP). Kinetic studies strongly indicate the formation of a reactive ternary complex composed of metal ion, ligand, and substrate. These synzymes operate *via* a metal–hydroxide-ion catalysed mechanism and exhibit turn-over behaviour while retaining their full catalytic activity.

Micelles of functionalized amphiphiles exhibit similar structure and kinetic properties to enzymes. Therefore they have been extensively studied as models for hydrolytic enzymes.[1,2a] However, for micellar models of hydrolytic metalloenzymes, only a few examples have been reported. These artificial enzymes are effective in promoting the cleavage of phosphoric,[3–5] and carboxylic esters.[2b–d,6] The functions of the transition metal ion in these metallomicelles are: (*i*) serving as a template between the substrate and the nucleophile, (*ii*) electrophilic activation of the carbonyl or phosphoryl bond of the substrate and (*iii*) activation of the nucleophile (a hydroxy group bound to the ligand or a metal-bound hydroxide ion).

Metallosurfactants must contain a chelating head group for metal-ion fixation. We have chosen 2-substituted 1,10-phenanthroline or pyridine derivatives to meet this requirement. Phenanthrolines are able to act as ligands with a variety of metal ions.[7] Non-micellar metal complexes of 2-substituted 1,10-phenanthrolines have been used in biomimetic studies of metalloenzyme reactions for carboxypeptidase A, NADH–alcohol dehydrogenase and metalloenzymes that catalyse phosphoryl group transfer or phosphate ester hydrolysis. Fife and his co-workers have studied the effect of metal ions on the hydrolysis of 1,10-phenanthroline ester,[8a] amide,[8b] phosphate ester,[8c] and acyl phosphate.[8d] Breslow *et al.* have reported the metal-ion catalysed hydration of 2-cyano-1,10-phenanthroline to the corresponding amide.[9] In these enzyme models the phenanthroline-bound metal ion is in close proximity to the reaction centre. The Zn^{II} promoted reaction of ATP and *p*-nitrophenyl acetate with 1,10-phenanthroline-2-carbinol,[10] and the reduction of pyridine-2-carboxaldehyde by 1,4-dihydronicotinamide which is covalently bond to 1,10-phenanthroline,[11] are examples of biomimetic model reactions that operate *via* the formation of a reactive ternary complex composed of metal ion, functionalized 1,10-phenanthroline and substrate. No studies of metal-ion catalysis of functionalized phenanthroline surfactant molecules have been published so far. In this paper we report on the syntheses of the strongly chelating 1,10-phenanthroline ligands **1** and **2** and the related, but moderately chelating, pyridine ligand **3** and present a study of their esterolytic reactivity towards PNPP and DPPNPP in mixed micelles in the presence of Zn^{II} and Cu^{II}. Ligands **1** and **3** possess, in addition to the metal ion binding site, an imidazole group in order to test the possibility of bifunctional catalysis, *i.e.* electrophilic activation of the substrate by the metal ion and nucleophilic or general-base catalysis by the imidazole group. Ligand **2** does not contain the imidazole group so the catalytic activity of this compound can be used for comparison. Similarly, the specific function of the phenanthroline nucleus can be identified by comparison of the catalytic activities of **1** and **3**.

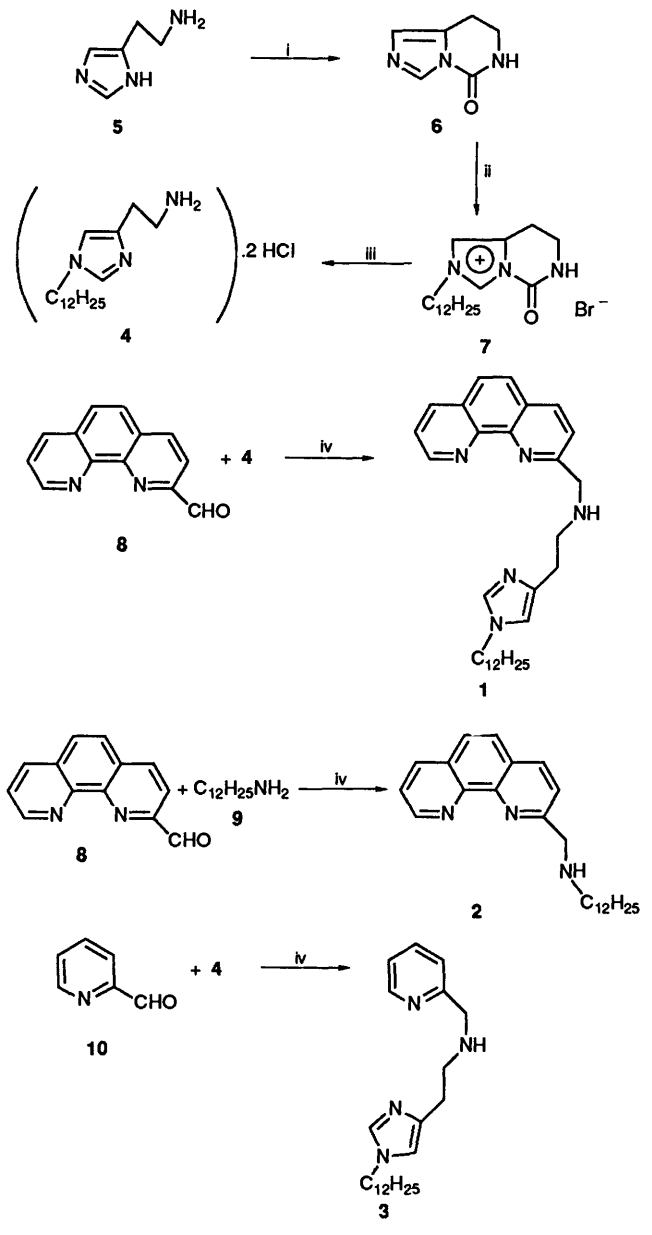
Results and Discussion

The lipophilic ligands **1**, **2** and **3** were prepared following the synthetic pathways shown in Scheme 1. The regiospecific alkylation of histamine (**5**) at the N^{τ} position was accomplished by temporarily protecting the two other nitrogen atoms by reaction with 1,1′-carbonyldiimidazole which yields **6**.[12a,b] Reaction of **6** with 1-bromododecane (**9**) to give **7**, followed by hydrolysis yields N^{τ}-dodecylhistamine (**4**). 1,10-Phenanthroline-2-carboxaldehyde was obtained according to a modified literature procedure from 1,10-phenanthroline. After cyanation of phenanthroline *via* the Reissert reaction,[10,13,14] 2-carbomethoxy-1,10-phenanthroline could be obtained directly in excellent yield by methanolysis. Subsequently this compound was reduced to the carbinol with $NaBH_4$, followed by SeO_2 oxidation to the aldehyde **8**. Attempts to convert 2-cyano-1,10-phenanthroline into **8** in a one-step reaction with diisobutyl aluminium hydride[15] were not successful. Reductive coupling of the aldehydes **8** and **10** with the appropriate amine (**4** and **9**) afforded the lipophilic ligands **1**, **2** and **3**.

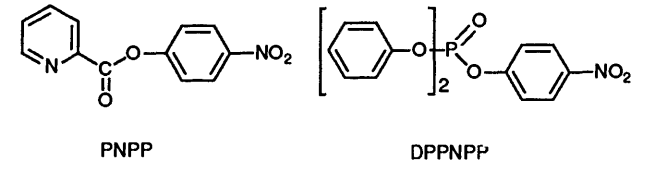
Addition of Zn^{II} to the 1,10-phenanthroline derivatives **1** and **2** gave rise to a characteristic absorbance change in the 270–300 nm region as has been previously found for binding of bivalent metal ions to 1,10-phenanthroline.[16] For **1** and **2** the absorbance maximum shifted from 267 to 273.5 nm and a shoulder at 295 nm appeared. From these changes in the UV spectra it can be concluded that complexation of **1** and **2** is complete in the presence of one equivalent of Zn^{II}. Addition of Zn^{II} to **3** did not induce significant changes in the absorbance spectrum. Therefore the binding of Zn^{II} to **3** could not be quantitatively determined from the spectrum.

The esterolytic activity of the Zn^{II} complexes of the lipophilic ligands **1**, **2** and **3** was tested with PNPP (Scheme 2) as substrate in the absence of cosurfactant, as well as in micellar systems admixed with the chemically inert cationogenic CTABr and neutral Brij 35 surfactants. The hydrolysis of the ester was followed by observing the release of *p*-nitrophenolate spectrophotometrically (400 nm) at pH 7.00 and 25 °C. Pseudo-first-order rate constants, determined under conditions of excess ligand over substrate at constant pH, are shown in Table 1.

The rate data in Table 1 shows that low concentration (0.4



Scheme 1 *Reagent and solvents:* i, $(Im)_2CO$; ii, $C_{12}H_{25}Br$, anhydrous DMF; iii, HCl (5 mol dm^{-3}); iv, H_2/Pd–C, EtOH



PNPP DPPNPP

Scheme 2

mmol dm^{-3}) of the metallosurfactants induces a rate enhancement of a factor *ca.* 50. Clear and stable solutions were obtained at the concentrations used, so it is likely that the Zn^{II}–ligand complexes form micellar aggregates. Remarkably, addition of 10 mole equivalents of cosurfactant significantly increased the catalytic activity of the metallosurfactants. It should be noted that solutions containing only the cosurfactant exhibited almost no rate enhancing effect. A possible explanation for the lower catalytic activity of the metallosurfactants in the absence of cosurfactant might be the formation of less active cylindrical micelles with a larger aggregation number in these solutions, while in the presence of a cosurfactant globular micelles with a smaller aggregation number are

Table 1 Pseudo-first-order rate constants ($k_{obs}/10^{-3}$ s^{-1}) for the hydrolysis of PNPP with various cosurfactants[a]

	Cosurfactant		
Catalyst	None	CTABr	Brij 35
None	0.010	0.029	0.012
1-Zn^{II}[b]	0.54	2.37	1.24
2-Zn^{II}[b]	0.58	2.13	1.43
3-Zn^{II}[c]	0.49	3.65	2.28

[a] Conditions: 25 °C, pH 7.00 (0.01 mol dm^{-3} *N*-ethylmorpholine–HBr buffer), [PNPP] = 4×10^{-5} mol dm^{-3}, [ligand] = 4×10^{-4} mol dm^{-3}, $CH_3(CH_2)_{15}N(CH_3)_3Br$, [CTABr] = 4×10^{-3} mol dm^{-3}, $CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH$, [Brij 35] = 4×10^{-3} mol dm^{-3}. [b] [Zn^{II}] = 4×10^{-4} mol dm^{-3}. [c] [Zn^{II}] = 1.2×10^{-3} mol dm^{-3}.

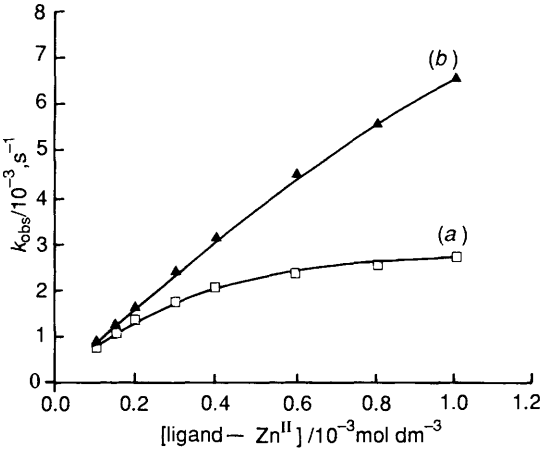


Fig. 1 Plots of pseudo-first-order rate constants for the hydrolysis of PNPP as a function of ligand–Zn^{II} concentration at pH 7.00 and 25 °C, [PNPP] = 4×10^{-5} mol dm^{-3}, [CTABr] = 4×10^{-3} mol dm^{-3}, (*a*) [**2**]:[Zn^{II}] = 1:1, (*b*) [**3**]:[Zn^{II}] = 1:3

formed. A similar decrease in micellar reactivity, ascribed to a change from globular to cylindrical micelles, was previously found by Melhado and Gutsche.[5a] However, the formation of ligand–metal ion complexes with a stoichiometry different from 1:1 might also occur in micelles without cosurfactant, thereby affecting the reactivity.[17]

In the cationogenic micelles (CTABr as the cosurfactant) the rate of cleavage of PNPP was somewhat higher than in neutral mixed micelles (Brij 35 as the cosurfactant). This is in accordance with the observation that the hydroxide-ion concentration in the solvent–micelle interface of cationogenic micelles is larger than in the case of neutral micelles.[18] Since almost no difference was observed in esterolytic activity between **1**-Zn^{II} and **2**-Zn^{II} it can be concluded that the role of the imidazole group in the catalytic activity of **1** is not very large, if any.

In order to establish the affinity of PNPP for the metallosurfactants we measured the rate of hydrolysis as a function of the **2**-Zn^{II} and **3**-Zn^{II} concentration (Fig. 1). For **2**-Zn^{II} rapid saturation kinetics were observed indicating strong affinity of PNPP for this ligand, whereas the slope of the curve for **3**-Zn^{II} indicates a much weaker binding affinity. However, the larger k_{obs} values for **3**-Zn^{II} point to a faster turn-over of the catalytic complex of **3**-Zn^{II}–PNPP.

The rate–concentration profiles can be analysed quantitatively by assuming the rapid reversible formation of a reactive ternary complex composed of ligand–Zn^{II} and PNPP,[19] followed by a rate-determining hydrolysis [eqns. (1) and (2)].

$$Zn^{II}\text{–L} + \text{PNPP} \overset{K}{\rightleftharpoons} Zn^{II}\text{–L–PNPP} \xrightarrow{k_c} Zn^{II}\text{–L} + \text{PNP} + \text{picolinate} \quad (1)$$

Table 2 Association constants (K) and catalytic rate constants (k_c) for the hydrolysis of PNPP in the presence of 2-Zn^{II} and 3-Zn^{II} [a]

Catalyst	K/dm^3 mol^{-1}	$k_c/10^{-3}$ s^{-1}
2-Zn^{II} [b]	2060 ± 20	4.36 ± 0.05
3-Zn^{II} [c]	224 ± 10	34.7 ± 1.1

[a] Conditions: 25 °C, pH 7.00 (0.01 mol dm^{-3} N-ethylmorpholine–HBr buffer), [CTABr] = 4 × 10^{-3} mol dm^{-3}. [b] [2]:[Zn^{II}] = 1:1. [c] [3]:[Zn^{II}] = 1:3.

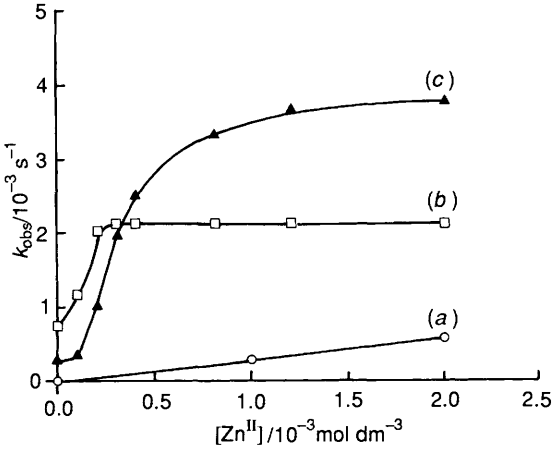


Fig. 2 Plots of pseudo-first-order rate constants for the hydrolysis of PNPP as a function of [Zn^{II}] under fixed concentration of ligand at pH 7.00 and 25 °C, [CTABr] = 4 × 10^{-3} mol dm^{-3}, [PNPP] = 4 × 10^{-5} mol dm^{-3}, (*a*) no ligand, (*b*) [**2**] = 4 × 10^{-4} mol dm^{-3} and (*c*) [**3**] = 4 × 10^{-4} mol dm^{-3}

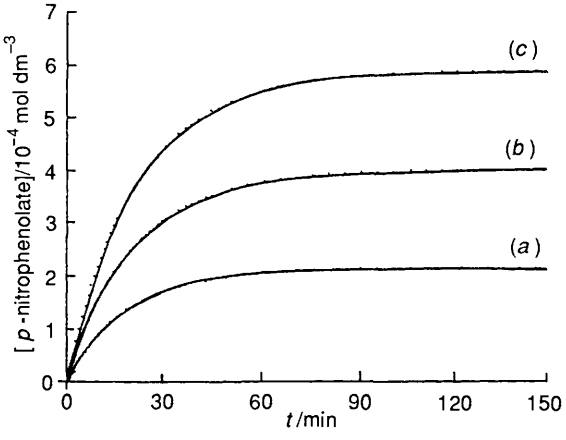


Fig. 3 Time-courses for p-nitrophenolate release from PNPP [(*a*) 2 × 10^{-4} mol dm^{-3}, (*b*) 4 × 10^{-4} mol dm^{-3} and (*c*) 6 × 10^{-4} mol dm^{-3}] as catalysed by **2** ([**2**] = 2 × 10^{-4} mol dm^{-3}) in the presence of 6 × 10^{-4} mol dm^{-3} $ZnBr_2$ (0.05 mol dm^{-3} N-ethylmorpholine–HBr buffer, pH 7.00 and 25 °C). Ligands **1** and **3** show similar behaviour.

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

$$k_{obs} = k_0 + \frac{k_c K[Zn^{II}\text{–L}]}{1 + K[Zn^{II}\text{–L}]} \quad (3)$$

$$\frac{1}{k_{obs} - k_0} = \frac{1}{k_c K[Zn^{II}\text{–L}]} + \frac{1}{k_c} \quad (4)$$

Kinetic parameters for this reaction scheme are given in eqn. (3), where K is the association constant between ligand–Zn^{II} complex and substrate and k_c is the catalytic rate constant. From the double reciprocal plot given in eqn. (4) the association constant, K, and the catalytic rate constant, k_c, can be obtained. These values for 2-Zn^{II} and 3-Zn^{II} are given in Table 2.

The stability constant of PNPP to 2-Zn^{II} is 9 times larger than that to 3-Zn^{II}. However, the catalytic rate constant for 3-Zn^{II} is 8 times higher which results in a more efficient overall catalytic activity of 3-Zn^{II}.

As PNPP is a substrate with the potential to bind metal ions, we also investigated the effect of free Zn^{II} ion in solution on the rate of hydrolysis. Fig. 2 shows that addition of Zn^{II} in the absence of ligand has only a weak enhancing effect on the rate of hydrolysis. No saturation kinetics are observed up to 6 mmol dm^{-3} of Zn^{II}, suggesting only weak binding affinity of Zn^{II} to PNPP. Thus the presence of ligand is essential for catalysis. The effect of variation of the Zn^{II} concentration in the presence of a fixed concentration (0.4 mmol dm^{-3}) of **2** and **3** is depicted in Fig. 2. At [Zn^{II}] = 0 the addition of EDTA does not change the rate of hydrolysis, indicating that the k_{obs} values at this concentration represent the catalysis by **2** and **3** essentially free of metal ion. Addition of Zn^{II} to **2** causes a rapid increase in reaction rate until the ratio of **2** and Zn^{II} reaches unity. Further increase of the Zn^{II} concentration has no effect. This is further evidence for the strong binding of Zn^{II} to **2**. Moreover, the absence of any catalytic effect due to excess of free Zn^{II} in solution indicates that PNPP has a large binding affinity for 2-Zn^{II} and is hydrolysed relatively fast within the 2-Zn^{II}–PNPP complex.

For ligand **3** the rate increases more gradually upon addition of Zn^{II} until a maximum is reached at *ca.* three equivalents of Zn^{II}. This is in accordance with the previously observed lower binding affinity of Zn^{II} for **3** as compared with **2**.

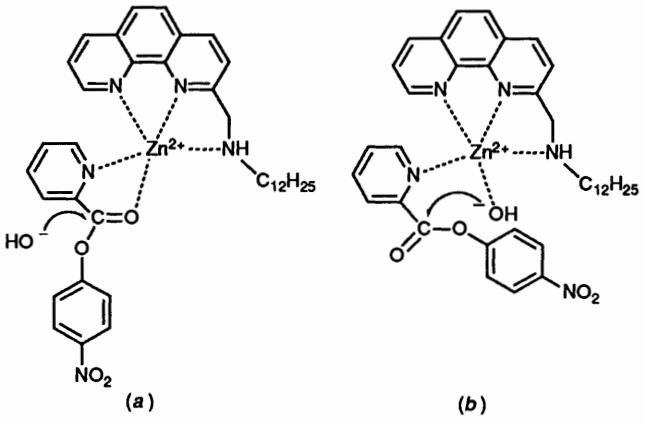
In order to test the turn-over behaviour of the catalysts we investigated the catalytic activity of the metallosurfactants under conditions of [PNPP] > [ligand–Zn^{II}]. For all three ligand–Zn^{II} complexes, p-nitrophenolate was produced in quantitative yield (Fig. 3). The release of p-nitrophenolate shows no biphasic behaviour, indicating that the catalyst is rapidly regenerated during hydrolysis of PNPP. Thus, the ligand–Zn^{II} complexes exhibit good turn-over behaviour, a requisite for a true catalyst.

At large excess of PNPP over ligand–Zn^{II}, the rate of hydrolysis is gradually retarded due to the formation of picolinate (product inhibition). Picolinate is a strong chelating agent,[20] which competitively binds to the catalyst and is able to remove Zn^{II} from the metallocleft of the ligand. However, for 2-Zn^{II} product inhibition by picolinate could be completely suppressed by addition of two equivalents of Zn^{II} in excess. For ligand **3**, having a lower binding affinity for Zn^{II}, picolinate inhibition is only partly suppressed in the presence of excess of two equivalents of Zn^{II}.

The rate of hydrolysis of PNPP catalysed by Zn^{II} complexes of **1**, **2** and **3** shows a pH-dependent behaviour. Over the pH range 6–8.5, k_{obs} in buffered solutions is proportional to the hydroxide-ion concentration. The bimolecular rate constants k_{OH}, obtained by fitting the straight lines, are 5.76 × 10^3, 1.77 × 10^3 and 6.12 × 10^3 dm^3 mol^{-1} s^{-1} for the catalysts 1-Zn^{II}, 2-Zn^{II} and 3-Zn^{II}, respectively. In the absence of ligand, k_{OH} is much lower: 4.53 × 10^2 dm^3 mol^{-1} s^{-1}. This points to the involvement of a hydroxide ion in the mechanism, whose action is catalysed by the presence of ligated metal ion.

The absence of burst kinetics,[21] or any other form of biphasic behaviour in the rate profile points to a hydrolysis mechanism without the intermediacy of an acylated ligand. Acylated intermediates have been found in catalysis by active hydroxy[2,6] and imidazole groups.[22,23] From the pH profiles and the turn-over behaviour two kinetically equivalent possibilities for the mechanism can be postulated, which involve a different role of the hydroxide ion, as is shown in Scheme 3.

In Scheme 3(*a*), binding of the pyridine moiety and carbonyl group of the substrate to Zn^{II} results in electrophilic activation of the carbonyl bond and a consequent enhancement of external



Scheme 3

Table 3 Pseudo-first-order rate constants for the hydrolysis of DPPNPP in CTABr[a]

Catalyst	$k_{obs}/10^{-3}$ s^{-1}
None	0.005
Zn^{II}[b]	0.005
Cu^{II}[b]	0.006
1-Zn^{II}[b]	0.138
1-Cu^{II}[b]	0.094
2-Zn^{II}[b]	0.084
2-Cu^{II}[b]	0.132
3-Zn^{II}[c]	1.17
3-Cu^{II}[c]	0.190

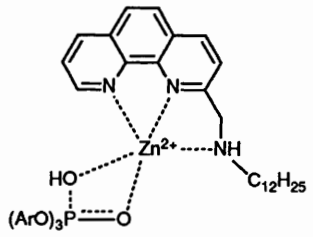
[a] Conditions: 25 °C, pH 7.00 (0.01 mol dm^{-3} *N*-ethylmorpholine–HBr buffer), [CTABr] = 4×10^{-3} mol dm^{-3}, [ligand] = 4×10^{-4} mol dm^{-3} and [DPPNPP] = 4×10^{-5} mol dm^{-3}. [b] [M^{II}] = 4×10^{-4} mol dm^{-3}. [c] [M^{II}] = 1.2×10^{-3} mol dm^{-3}.

attack of hydroxide ion. In Scheme 3(*b*), substrate binding to the metal ion brings the ester group into close proximity with a Zn^{II}-bound hydroxide ion, enabling intramolecular nucleophilic attack of this Zn^{II}-bound hydroxide ion to the carbonyl group.

We also tested the catalytic activity of the metallosurfactants in the hydrolysis of the phosphate ester DPPNPP (Scheme 2). Phosphate esters are less sensitive to hydrolysis than carboxylic esters. It has been found that hydrolysis can be catalysed by micellar and non-micellar metal complexes.[3,4] Table 3 gives the observed pseudo-first-order rate constants for DPPNPP catalysed by Zn^{II} or Cu^{II} complexes of the ligands **1**, **2** and **3** under micellar conditions at pH 7.00 and 25 °C.

The data show that addition of Zn^{II} and Cu^{II} in the absence of ligand has no effect on the rate of hydrolysis. In the presence of ligand however, complexes of **1** and **2** with Zn^{II} and Cu^{II}, and of **3** with Cu^{II} moderately increase the rate of hydrolysis. For the **3**-Zn^{II} complex a large rate enhancement is observed. The difference in catalytic activity of **3**-Zn^{II} and **3**-Cu^{II} may be a consequence of differences in the geometry of the metallosurfactant–substrate complexes. The geometry of metal-ion coordination is an important factor in the catalytic activity of metalloenzyme models,[2d,6b] and it is likely that ternary complexes of **3**-Zn^{II}-DPPNPP adopt a tetrahedral geometry,[24] whereas **3**-Cu^{II}-DPPNPP has a planar geometry.[25] The more efficient catalysis of the tetrahedral intermediate may then be due to the 'push–pull' mechanism as suggested by Breslow and his co-workers.[3] In this hybrid mechanism the metal ion delivers a coordinated hydroxide ion to a DPPNPP molecule and simultaneously, the Zn^{II} centre polarizes the P=O bond (Scheme 4).

In conclusion, the present study clearly demonstrates that metallomicelles made of **1**-Zn^{II}, **2**-Zn^{II} and **3**-Zn^{II}, in the presence



Scheme 4

of an inert cosurfactant, can function as efficient synzymes for the hydrolysis of PNPP and DPPNPP. These mixed micelles exhibit turn-over behaviour, an important characteristic of a truly catalytic system. The phenanthroline ligands **1** and **2** bind Zn^{II} more tightly than the pyridine ligand **3**. Moreover, the PNPP substrate binds more strongly to the phenanthroline metallosurfactant **2**-Zn^{II} than to the pyridine analogue **3**-Zn^{II}. This is an advantage for turn-over catalysis, in the cases where products having a large affinity for the metal ion are formed, as in the hydrolysis of PNPP. In the presence of an equivalent amount of Zn^{II} to bind picolinate, the metallosurfactant **2**-Zn^{II} retains its full catalytic activity. Moreover, the presence of an imidazole function in **1** does not have a marked effect on the catalysis relative to the activity of **2**.

Experimental

Materials.—$ZnBr_2$ (Janssen Chimica), $CuBr_2$ (Baker), *N*-ethylmorpholine (Janssen Chimica), CTABr (Merck) and Brij 35 (Aldrich) were used without further purification. *p*-Nitrophenyl picolinate (PNPP), m.p. 148–156 °C (decomp.) [lit.[19] 144–146 °C] and diphenyl *p*-nitrophenyl phosphate (DPPNPP), m.p. 48–49 °C [lit.[26] 49–51 °C] were prepared according to the literature. Acetonitrile and ethanol used in the kinetic experiments were of spectrophotometric grade. ^{1}H NMR spectra were recorded on Bruker AC 200-E or Varian EM390 spectrometers. Coupling constants are in Hz. Absorbance spectra and kinetic measurements were run on a Beckman DU-7 spectrophotometer with thermostatted cell compartment and kinetic device.

1,10-Phenanthroline-2-carboxaldehyde (**8**), was prepared according to a modified literature procedure,[10,13,14] m.p. 145–150 °C [lit.[14] 152–153 °C (decomp.)]. In this modification, 2-cyano-1,10-phenanthroline was converted directly into methyl 1,10-phenanthroline-2-carboxylate as follows: a solution of 2-cyano-1,10-phenanthroline (3.5 g, 17.1 mmol) and a catalytic amount of sodium (25 mg, 1.1 mmol) in MeOH (150 cm^3) was refluxed for 0.5 h. The solution was cooled to 0–5 °C and made slightly acidic with 2% HCl (100 cm^3). After 0.5 h, the solution was neutralized with $NaHCO_3$. MeOH was removed under reduced pressure and the aqueous layer was extracted three times with $CHCl_3$. The organic layers were collected and dried (Na_2SO_4). After removal of the solvent under reduced pressure methyl 1,10-phenanthroline-2-carboxylate (3.3 g, 81%) was obtained, m.p. 110–112 °C [lit.[10] 112–114 °C]. $\delta_H(CDCl_3)$ 4.10 (3 H, s, CH_3), 7.66 (1 H, dd, 8-H), 7.85 (2 H, s, 5-H and 6-H), 8.25 (1 H, dd, 7-H), 8.40 (2 H, d, 3-H and 4-H) and 9.26 (1 H, dd, 9-H).

5-Oxo-5,6,7,8-tetrahydroimidazo[1,5-*c*]pyrimidine (**6**), was prepared as reported,[12] m.p. 216–219 °C [lit.[12a] 221–222 °C].

2-Dodecyl-5-oxo-5,6,7,8-tetrahydroimidazo[1,5-c]*pyridinium bromide* (**7**).—A solution of **6** (1.07 g, 7.75 mmol) and 1-bromododecane (9.66 g, 38.7 mmol) was heated at 90 °C overnight in 100 cm^3 of dry dimethylformamide (DMF). After cooling and addition of Et_2O the product was filtered off and washed with Et_2O to give **7** (2.87 g, 96%). $\delta_H(D_2O)$ 0.86 (3 H, t, CH_3), 1.25

[18 H, s, $(CH_2)_9CH_3$], 2.01 [2 H, br s, $CH_2CH_2(CH_2)_9$], 3.22 (2 H, t, CH_2-Im), 3.74 (2 H, t, CH_2NHCO), 4.48 [2 H, t, $NCH_2(CH_2)_{10}$], 7.72 (1 H, s, 1-H) and 9.58 (1 H, s, 3-H). This product was used without further purification for the synthesis of **4**.

N^{τ}-*Dodecylhistamine dihydrochloride* (**4**).—A solution of **7** (2.87 g, 7.44 mmol) in 5 mol dm^{-3} HCl was heated under reflux overnight. After evaporation **4** was obtained as a white solid (2.50 g, 96%) m.p. 110 °C. $\delta_H(D_2O)$ 0.85 (3 H, t, *J* 5.8, CH_3) 1.26 [18 H, s, $(CH_2)_9CH_3$], 1.87 [2 H, br s, $CH_2(CH_2)_9CH_3$], 3.14 (2 H, t, *J* 6.4, CH_2-Im), 3.36 (2 H, t, *J* 6.4, CH_2NH_2), 4.19 [2 H, t, $NCH_2(CH_2)_{10}$], 7.47 (1 H, s, 5-H) and 8.58 (2 H, s, 2-H); *m/z* (%): 279 (8), 278 (8), 250 (100), 249 (25), 235 (9), 221 (20), 207 (18), 193 (15), 179 (13), 165 (13) and 151 (13).

N^{α}-(1,10-*Phenanthrolin*-2-*ylmethyl*)-N^{τ}-*dodecylhistamine* (**1**).—A solution of **4** (0.66 g, 1.88 mmol), **8** (0.39 g, 1.88 mmol) and Et_3N (0.76 g, 7.5 mmol) in abs. EtOH was hydrogenated (0.05 g, 10% Pd–C) in a Parr apparatus until no more H_2 was absorbed. The catalyst was separated on a sintered-glass funnel, washed with EtOH and the combined filtrates were concentrated under reduced pressure. The residue was purified by column chromatography (Al_2O_3, 0–10% MeOH/$CHCl_3$). Ligand **1** (0.26 g, 29%) was obtained as a thick oil. $\delta_H(CDCl_3)$ 0.84 (3 H, t, *J* 6.4 CH_3), 1.20 [18 H, s, $(CH_2)_9CH_3$], 1.68 [3 H, t, $CH_2(CH_2)_9CH_3$], 2.86 (2 H, t, *J* 6.6, CH_2-Im), 3.07 (2 H, t, *J* 6.6, CH_2CH_2NH), 3.15 (1 H, br s, NH), 3.78 [2 H, t, *J* 7.2, $NCH_2(CH_2)_{10}$], 4.36 (2 H, s, CH_2-Ph), 6.68 (1 H, s, Im-H5), 7.31 (1 H, d, *J* 1.1, Im-H2), 7.60 (1 H, dd, *J* 4.4, 8.1, 8-H), 7.75 (2 H, d, *J* 1.2, 5-H and 6-H), 7.79 (1 H, d, *J* 8.1, 3-H), 8.19 (1 H, d, *J* 8.1, 4-H), 8.23 (1 H, dd, *J* 1.7, 8.1, 7-H) and 9.15 (1 H, dd, *J* 1.7, 4.4, 9-H); *m/z* 471.

N-*Dodecyl*-2-*aminomethyl*-1,10-*phenanthroline* (**2**).—A solution of dodecylamine (0.86 g, 4.66 mmol), **8** (0.97 g, 4.66 mmol) and Et_3N (0.94 g, 9.33 mmol) in abs. EtOH was shaken with 10% Pd–C (0.2 g) under H_2 atmosphere in a Parr apparatus. After the theoretical amount of H_2 had been consumed, the catalyst was removed by filtration. EtOH was evaporated and the residue was dissolved in $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, 10–20% MeOH/$CHCl_3$). Ligand **2** (1.0 g, 57%) was obtained as an oil. $\delta_H(CDCl_3)$ 0.84 (3 H, t, *J* 6.3, CH_3), 1.22 [18 H, s, $(CH_2)_9CH_3$], 1.54 [2 H, t, *J* 7.1, $CH_2(CH_2)_9CH_3$], 2.01 (1 H, br s, NH), 2.71 [2 H, t, *J* 7.1, $NHCH_2(CH_2)_{10}$], 4.29 (2 H, s, CH_2-Ph), 7.60 (1 H, dd, *J* 4.4, 8.0, 8-H), 7.75 (2 H, d, *J* 1.8, 5-H and 6-H), 7.78 (1 H, d, *J* 8.4, 3-H), 8.20 (1 H, d, *J* 8.4, 4-H), 8.22 (1 H, dd, *J* 1.7, 8.0, 7-H) and 9.18 (1 H, dd, *J* 1.7, 4.4, 9-H); *m/z* 377.

N^{α}-(2-*Pyridylmethyl*)-N^{τ}-*dodecylhistamine* (**3**).—A solution of **4** (1.0 g, 2.84 mmol), pyridine-2-carboxaldehyde (0.3 g, 2.84 mmol) and Et_3N (1.15 g, 11.4 mmol) in abs. EtOH was hydrogenated (0.2 g, 10% Pd–C) in a Parr apparatus until no more H_2 was absorbed. After removal of the catalyst by filtration EtOH was evaporated and the residue was dissolved in $CHCl_3$. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 4–8% MeOH/$CHCl_3$). Ligand **3** (0.53 g, 50%) was obtained as an oil. $\delta_H(CDCl_3)$ 0.82 (3 H, t, *J* 6.5, CH_3), 1.19 [18 H, s, $(CH_2)_9CH_3$], 1.68 [2 H, t, *J* 7.0, $CH_2(CH_2)_9CH_3$], 2.55 (1 H, br s, NH), 2.74 (2 H, t, *J* 6.5, CH_2-Im), 2.91 (2 H, t, *J* 6.5, CH_2CH_2NH), 3.78 [2 H, t, *J* 7.1, $CH_2(CH_2)_{10}$], 3.89 (2 H, s, CH_2-Ph), 6.62 (1 H, s, Im-H5), 7.09 (1 H, m, 5-H) 7.27 (2 H, m, Im-H2 and 3-H), 7.57 (1 H, m, 4-H) and 8.84 (1 H, dd, *J* 1.8, 4.6, 6-H); *m/z* (%) 370 (43), 278 (100), 250 (60), 235 (7), 221 (11), 207 (10), 193 (9), 179 (8), 165 (8), 151 (8), 137 (9) and 121 (48). (Found: M^+, 370.3099. $C_{23}H_{48}N_4$ requires *M*, 370.3096).

Kinetic Studies.—Solutions were prepared in *N*-ethylmorpholine–HBr buffer pH 7.00. Absorbance spectra were recorded at 25 °C for micellar buffer solutions containing 2×10^{-5} mol dm^{-3} of **1** or **2** or 2×10^{-4} mol dm^{-3} of **3** in the presence or absence of 1 or 10 equivalents of $ZnBr_2$. The blank cell contained a micellar buffer solution.

Each kinetic run was initiated by injecting a 4 mm^3 portion of PNPP or DPPNPP in CH_3CN into a 1 cm cuvette containing 2 cm^3 of the buffer solution [containing 1% (v/v) EtOH and 0.6–1.4% (v/v) CH_3CN], the appropriate surfactant (4×10^{-3} mol dm^{-3}), the ligand and the metal ion. Pseudo-first-order rate constants for the hydrolysis of PNPP or DPPNPP were determined by monitoring the release of *p*-nitrophenolate at 400 nm in the conditions of excess catalyst over substrate. The cell holder was kept at 25 °C for all reactions. Reactions were generally followed for at least 8 half-lives. Linear first order plots of $\log(A_\infty - A_t)$ *vs.* time were always obtained for at least 3 half-lives. Kinetic runs, carried out in triplicate, gave rate constants with uncertainty of less than ±3%.

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