Metal-Ion Complexes of Functionalised 1,10-Phenanthrolines as Hydrolytic Synzymes

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Metal-Ion Complexes of Functionalised 1,10-Phenanthrolines as Hydrolytic Synzymes

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Stellingen

1. De conclusie van Tagaki *et al*, dat de hydroxymethyl-groep op de 2-positie van natrium 2-[[[1-dodecyl-2,5-bis(hydroxymethyl)-1*H*-imidazo-4-yl]methyl]thio]ethaansulfonaat geacyleerd wordt, wordt niet door hun ^{1}H-NMR-gegevens onderbouwd.

   W. Tagaki, K. Ogino, T. Fujita, T. Yoshida, K. Nishi en Y. Inaba, *Bull. Chem. Soc. Jpn.*, 1993, **66**, 140.

2. De bewering van Ho dat d4d-systemen "disjoint" zijn en daarom niet fragmenteerbaar, gaat voorbij aan de door Grob gevonden homofragmentatie waarbij een cyclopropaanstructuur wordt gevormd.

   T.-L. Ho, *Heterolytic Fragmentation of Organic Molecules*, Wiley, New York, 1993, p. 258.

   W. Fischer, C. A. Grob, R. Hanreich, G. von Sprecher en A. Waldner, *Helv. Chim.* Acta, 1981, **64**, 2298.

3. Appendino *et al.* bewijzen niet overtuigend dat de door hen geïsoleerde 5-cinnamoylacetyltaxicines echte bestanddelen van *Taxus baccata* L. zijn en geen, tijdens de extractie uit de overeenkomstige esters van Wintersteinzuur gevormde, artefacten.

   G. Appendino, P. Gariboldi, A. Pisetta, E. Bombardelli en B. Gabetta, *Phytochemistry*, 1992, **31**, 4253.

4. De bespreking van het massaspectrum van 5-(octyloxy-3,3,4,4-t_4)pentaanzuur door Devadas *et al.* is gezien de mate van ingebouwde radioactiviteit niet volledig.

   B. Devadas, S. P. Adams en J. I. Gordon, *J. Label. Compd. Radiopharm.*, 1991, **29**, 157.

5. De door Danda *et al.* geteste mate van racemisatie van (S)-4-acetoxy-3-methyl-2-(2-propynyl)-2-cyclopenteen-1-on onder zure omstandigheden, bewijst onvoldoende dat racemisatie niet optreedt onder de door hen gebruikte (basische) reactieomstandigheden.

   H. Danda, A. Maehara en T. Umemura, *Tetrahedron Lett.*, 1991, **32**, 5119;

   H. Danda, T. Nagatomi, A. Maehara en T. Umemura, *Tetrahedron*, 1991, **41**, 8701.

6. De methode waarop Moss *et al.* de 2^e-orde snelheidsconstante , k_2, bepalen is voor ernstige kritiek vatbaar.

   R. A. Moss, P. Scrimin, S. Bhattacharya en S. Swarup, *J. Am. Chem. Soc.*, 1987, **109**, 6209.

7. Omdat Chung *et al.* in de inleiding van hun artikel binucleaire Co^{III}-complexen vanwege hun coöperatieve werking aanprijzen als potentieel waardevolle katalysatoren voor de hydrolyse van o.a. niet-geactiveerde fosfaat mono-esters, is het des te merkwaardiger dat zij zelf deze substraten niet testen.

   Y. Chung, E. U. Akkaya, T. K. Venkatachalam en A. W. Czarnik, *Tetrahedron Lett.*, 1990, **31**, 5413.

8. Er zijn goede alternatieve syntheseroutes voor O-gefosforyleerd threonine bevattende peptides bekend, die door Larsson *et al.* toegepast hadden kunnen worden om het door hen geconstateerde probleem van β-eliminate te vermijden.

   E. Larsson, B. Lüning en D. Heinegård, *Acta Chem. Scand.*, 1993, **47**, 565;
   H. B. A. de Bont, J. H. van Boom en R. J. M. Liskamp, *Tetrahedron Lett.*, 1990, **31**, 2497.

9. Bij het voorstel van de universiteiten om aio' s een beurs te geven in plaats van een salaris, is niet gedacht aan het Engelse gezegde: "If you pay them peanuts, you get monkeys."

10. Het gelijktijdig uitreiken van een donorcodicil met het rijbewijs geeft weinig blijk van vertrouwen in de vaardigheid van de nieuwe chauffeur.

Stellingen behorende bij het proefschrift: "Metal-Ion Complexes of Functionalised 1,10-Phenanthrolines as Hydrolytic Synzymes"

Wageningen, 29 november 1993 J. G. J. Weijnen

Aan mijn ouders
Voor Ria

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Chapter 1

Introduction and Objectives

Enzymes are highly specific and extremely efficient catalysts of chemical reactions in biological systems. These biocatalysts enhance reaction rates 10^5-10^{10}-fold in relatively dilute aqueous solutions around neutral pH and at ambient temperatures.[1] The outstanding characteristic of enzymes is that they are highly specific, both in the reaction catalysed and in the choice of the substrates. This specificity makes it possible that in biological systems one single substrate molecule or a set of closely related substrate molecules out of a complex mixture are efficiently transformed into the desired products.

The catalysis takes place in a specific region of the enzyme referred to as the active site. This is a three-dimensional cavity which can accommodate the substrate and is furnished with all the amino acid residues which participate in the binding of the substrate and in the catalytic process. The mechanisms by which enzymes operate are very complicated processes. Many techniques have been employed to elucidate enzyme mechanisms including kinetic, spectroscopic, and mutagenetic methods. Detailed structural information about several enzymes, their active sites, and substrate interactions have been obtained by X-ray crystallographic studies. From this knowledge and with the tools of synthetic chemistry, it has become possible to design active site models which contain reactive chemical groups oriented in the geometry dictated by the enzyme but lacking the macromolecular peptide backbone.[2]

The main purpose of model studies of an enzyme is to reproduce the major characteristics of the enzyme, such as the ability to recognise substrate structures and to accelerate the conversion of substrates into products.[3] Model studies which mimic a key parameter of an enzyme function on a much simpler level can provide valuable information for a better understanding of the chemistry involved in the action of the target enzyme.[2,4] Another goal of biomimetic chemistry is the development of artificial enzymes based on the catalytic principles employed by native enzymes.[3,5]

Besides catalysis, molecular recognition is the most important aspect of enzyme action. Enzymes (hosts) recognise substrates (guests) primarily by means of non-covalent interactions.[6] In model studies, synthetic host-guest systems mimic the organisational ability of enzymes by bringing reactants together in highly structured and specific environments. This is accomplished by the non-covalent binding of the

guests to the sites provided by the hosts. The chemical challenge is to construct host molecules that are easy to synthesise and stable, but at the same time bind appropriate guests selectively and reversibly, and provide the catalytic groups at the proper site.

Studies of the role of functionalised metal-ion complexes in hydrolytic reactions, for example, can give us information about features of the catalytic activity of hydrolytic metallo-enzymes. Moreover, catalysis by micelles is considered as a, although primitive, mimic for enzyme reactions exhibiting Michaelis-Menten kinetics. The objectives of this study were the design and synthesis of functionalised amphiphilic ligands which contain the strongly chelating 1,10-phenanthroline group as metal-ion binding site and the investigation of the catalytic activity of metal-ion complexes of these ligands in hydrolytic reactions in micellar and vesicular assemblies. In the designed systems catalytically active groups in hydrolysis and binding sites for non-covalent substrate binding are combined.

In chapter 2 the catalytic role that metal ions play in hydrolytic reactions is discussed and an overview is given of the function of micellar aggregates as models for hydrolytic (metallo)-enzymes.

In chapter 3 the synthesis of two amphiphilic 1,10-phenanthroline ligands and one pyridine ligand, substituted at the 2 position is described. The catalytic activity of these ligands in micellar aggregates in the presence of Zn^{II} and Cu^{II} was investigated in the hydrolysis of the carboxylic ester *p*-nitrophenyl picolinate (PNPP) and the phosphate triester diphenyl *p*-nitrophenyl phosphate (DPPNPP).

In chapter 4 the synthesis of asymmetrically disubstituted 1,10-phenanthroline derivatives is reported. The catalytic role of a hydroxyl group, covalently attached to the ligand in close proximity to the metal ion is discussed and a comparison is made between the catalytic activities of a metallo-amphiphile in the micellar phase and a water-soluble analogue.

Chapter 5 deals with the enantioselective hydrolysis of *p*-nitrophenyl esters of N-protected amino acids catalysed by chiral 1,10-phenanthroline ligands. The effect of the nature of the metal ions, the structure of the ligand, the micellar environment, and the hydrophobicity of the substrate on the direction and magnitude of the enantioselectivity is discussed.

Chapter 6 describes the catalytic activity and stereoselectivity of metal-ion complexes of amphiphilic 1,10-phenanthroline derivatives in vesicular aggregates.

Chapter 7 describes the catalytic activity of metal-ion complexes of 1,10-phenanthroline derivatives in water and in micelles toward phosphate triesters, diesters, and monoesters. The effect of a metal-ion chelating moiety in the leaving group of the substrate is discussed.

Chapter 8 deals with the synthesis and enzymatic resolution of alanine and bis-alanine derivatives of pyridine, benzene and 1,10-phenanthroline.

In Chapter 9 some concluding remarks on the study described in this thesis are made.

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Chapter 2

Enzyme Models

2.1 Introduction

Enzymes are large flexible macromolecules composed of amino acids, whose molecular weights are in the tens of thousands. The large size of enzymes is needed for specific interactions in living cells and to create active sites with the required micro-environment, rigidity, stereochemistry, and ability to undergo conformational changes upon substrate binding.[1] The binding of substrates to enzymes stems from non-covalent interactions, such as hydrophobic, electrostatic, hydrogen-bonding, metal-coordination and charge-transfer phenomena. These interactions are studied in supramolecular chemistry. Some insight into the importance of these interactions in the catalytic process can be obtained by enzyme models. In order to bring the catalytic groups and the substrate together in an enzyme model, the catalytic subunits have to be bound to a matrix. The function of the matrix is to hold and to orientate the substrate towards the catalytic groups and to provide a micro-environment at the catalytic site, which is quite different from that in bulk water. According to Lehn,[2] bio-organic models can be divided into two categories of supramolecular systems, depending on the kind of matrix applied: supermolecules and polymolecular assemblies. In supermolecules the catalytic subunits are covalently bound to the matrix, which can be *e.g.* cyclodextrins,[3] paracyclophanes,[4] or a polymeric network.[5] In polymolecular assemblies the catalytic units are non-covalently bound into micelles or vesicles (see 2.3 and 2.4). These aggregated host systems provide hydrophobic binding sites for the substrates in aqueous media.

2.2 Catalysis by Metal Ions

2.2.1 Catalytic Roles of Metal Ions

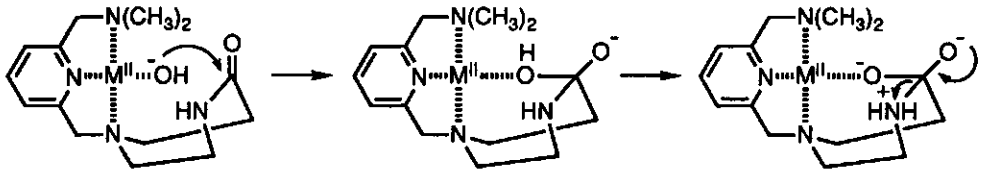
A catalyst increases the rate of a reaction by providing a new reaction pathway for reducing the Gibbs free energy of activation. Metal-ion catalysts are no exception to this general rule and catalysis originates from the ability of metal ions to coordinate to the

reactants and thereby stabilising the transition state to a greater extent than the initial state. Catalysis by metal ions can be divided into (i) redox catalysis in which the metal ion serves as a carrier of electrons, and (ii) super acid catalysis in which the role of the metal ion is that of an electrophile or general acid. In super acid catalysis, the function of the metal ion is similar to that of a proton. However, a metal ion is superior to a proton because multiple positive charge can be involved, exerting greater electrostatic effects compared to protons. In addition, metal ions can coordinate to several donor atoms, whereas a proton can coordinate to only one. Furthermore, the metal-ion concentration may be high in neutral solutions.[6]

Super acid catalysis occurs in many nucleophilic reactions of organic compounds, among them hydrolysis of phosphoric and carboxylic acid derivatives. Strong complexation of the metal ion in close proximity to the reacting group in the substrate is a requirement of metal-ion catalysis. This implies that the substrate must contain one or more donor atoms near the reaction centre to which the metal ion can coordinate.[7] Since metal ions are able to bind to several donor atoms, it is possible to form mixed ligand complexes in which substrate and nucleophilic agent are coordinated simultaneously to the metal ion. This directional or template effect of the metal ion converts intermolecular reactions into intracomplex processes, which makes the entropy of activation less negative. Moreover, in the case of anionic substrates metal ions screen the negative charge, rendering the substrates more susceptible to attack by nucleophiles.[8] Other catalytic roles of metal ions acting as Lewis acid in hydrolytic reactions will be discussed by means of the examples presented in Figures 2.1-2.3.

In metal-ion catalysed hydrolysis of esters, amides, and phosphate esters the catalytically active species may be a metal-bound hydroxide ion, a metal-bound water molecule, the metal ion itself, or a combination of these.[9] In the hydrolysis of the amide **a** in Figure 2.1, the metal-bound hydroxide ion acts as an intracomplex nucleophile, and after formation of the tetrahedral intermediate, it serves as the proton source needed for the expulsion of the amine moiety. The ionisation of water is facilitated by coordination to a metal ion, and high concentrations of metal-bound hydroxide can be obtained at neutral or acidic pH. For example, the pK_a value of a Cu^{II}-bound water molecule in the complex of Figure 2.1 is 7.2, which means that the acidity of the coordinated water molecule is about $10^{8.5}$ times higher than the acidity of a free water molecule.[10]

When the metal ion itself acts as a catalytic group, it may enhance the electrophilicity of the carbonyl group by binding to the carbonyl oxygen, as is illustrated by the metal-ion promoted hydrolysis of the activated ester in Figure 2.2a. Under acidic conditions a metal-bound water molecule is the nucleophilic agent. Since a

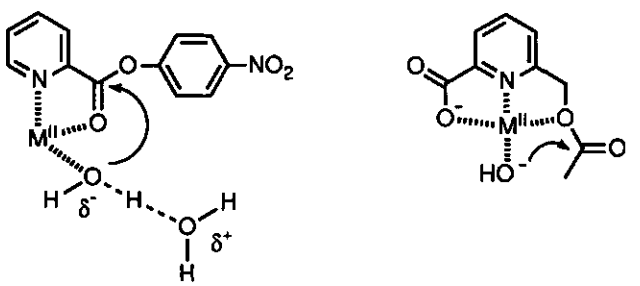


a b c

Figure 2.1 Intracomplex attack by a metal-bound hydroxide ion on an amide functionality, followed by proton shift of this metal-bound group to the amine leaving group.

water molecule coordinated to a metal ion is a poor nucleophile, attack by the metal-bound water molecule on the carbonyl group is enhanced by the template effect and by assistance of general bases.[11]

Another catalytic feature of metal ions is the enhancement of the leaving group ability by lowering the basicity of the leaving group. This is illustrated for 2-(hydroxymethyl)pyridine esters in Figure 2.2b.[12]



a b

Figure 2.2 Metal-ion catalysed ester hydrolysis by carbonyl group activation (**a**) and by leaving group activation (**b**).

Of special interest is the metal-ion catalysis observed for the hydrolysis of phosphate diesters, due to the relevance of this chemistry to biological systems. Phosphate diesters are very stable in aqueous solutions, since nucleophilic attack by hydroxide ions is strongly hampered by the electrostatic repulsion of the negatively charged hydroxide ion and the anionic ester. Complexation of both substrate and nucleophile to a metal ion results in charge neutralisation of the anionic ligands and has a rate-accelerating effect (Fig. 2.3). The half-live of bis(*p*-nitrophenyl) phosphate (BNPP)

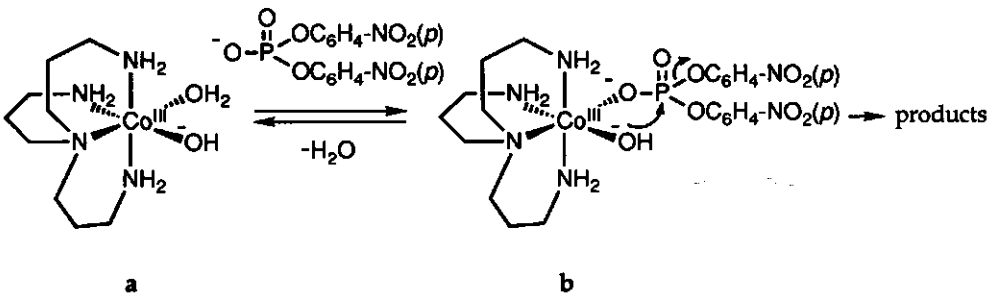


Figure 2.3 Co^{III}-tris(3-aminopropyl)amine catalysed hydrolysis of BNPP.

is about 100 years at neutral pH and 25 °C, but bound to the Co^{III} complex it is hydrolysed within a couple of seconds. This means that the Co^{III} complex induces a 10 billion-fold rate enhancement.[13]

Metal ions are superior to organic functional groups in terms of the variety of catalytic roles. As was seen from foregoing examples, the main catalytic roles of metal ions in hydrolytic reactions are activation of electrophiles, leaving group, and nucleophiles. Moreover, metal ions can act as a template whereby they can neutralise the negative charge of nucleophile and substrate. Unlike most of the organic catalytic groups, metal ions often perform several catalytic roles simultaneously.[9,14-16] For example, the repertoires of the metal ions in the hydrolysis of 2-(hydroxymethyl)-pyridine esters in Figure 2.2b include the template effect, the activation of the leaving group, the stabilisation of the tetrahedral intermediate, the enhanced ionisation of the coordinated water molecule, and the nucleophilic attack by the metal-bound hydroxide ion.[12]

2.2.2 Carboxypeptidase A and the Role of Zinc

The various catalytic functions of metal ions revealed from model studies as presented in the Figures 2.1-2.3, can be used as a guide to understanding the catalytic behaviour of hydrolytic metallo-enzymes. In these enzymes, metal ions become more effective by cooperation with organic catalytic groups. Since it is difficult to study the catalytic roles of the active site metal ions in detail with currently available tools, biomimetic models can provide valuable clues. Carboxypeptidase A (CPA), a Zn^{II}-containing metallo-exopeptidase, has become a paradigm case of hydrolytic metallo-enzymes. Both CPA and CPA models have been extensively studied in order to elucidate the enzyme mechanism. The biological function of CPA is the hydrolysis of C-

terminal amino acids from polypeptide substrates and it exhibits a preference toward those substrates possessing large, hydrophobic C-terminal side chains such as phenylalanine. Ester analogues of the amide substrates are also hydrolysed by CPA. The most crucial catalytic groups of CPA are the active site Zn^{II} ion and the Glu-270 carboxylate group. The Zn^{II} ion is bound to two imidazole nitrogens of His-69 and His-196 and to the carboxylate oxygen of Glu-72.[17] Maximum activity of CPA is manifested at pH = 7-8, which indicates that the carboxyl group of the catalytically active Glu-270 group is in the anionic form. The exact role of the carboxylate anion of Glu-270 has been the most crucial issue in the mechanistic study of CPA. In one of the most often proposed mechanisms, the Glu-270 carboxylate makes a nucleophilic attack on the polarised carbonyl group of the substrate leading to the formation of an anhydride intermediate (Fig. 2.4). However, only in the case of ester substrates accumulation of an intermediate is reported, which is presumed to be the acylated enzyme.[18] Cleavage of amide bonds requires a proton donor for the leaving amine group. Historically, the role of proton donor has been attributed to the phenol group of Tyr-248, but replacement of Tyr-248 by phenylalanine by site-directed mutagenesis gave no loss of activity toward peptides.[19] This means that if this mechanism is valid some other active site functional group must act as a proton donor. Model studies suggest that Zn^{II}-bound water may perform this function (Figs. 2.1 and 2.5).

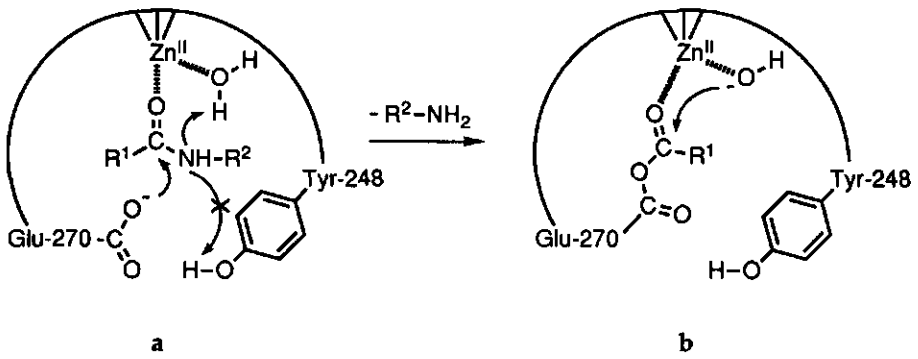


Figure 2.4 Proposed anhydride mechanism of CPA catalysis.

Recent results obtained from model compound **a** of Figure 2.5 support the anhydride mechanism.[20] In this model compound, efficient cooperation between metal ion and carboxylate group in the hydrolysis of the intramolecular amide linkage has been achieved. The M^{II}-bound water molecule acts as a general acid to protonate the leaving amine moiety. In the hydrolysis of amide **a**, the anhydride intermediate **b** accumulates, which indicates that the carboxylate group behaves as a nucleophile.

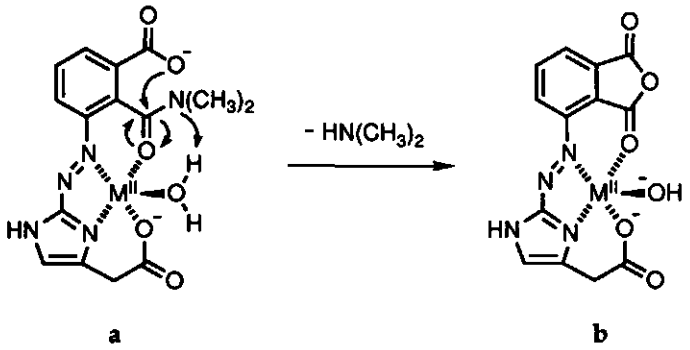


Figure 2.5 Cooperative catalysis by carboxyl group and metal ion, and general acid catalysis by metal-bound water in a CPA model.

In another widely proposed mechanism of CPA, the Glu-270 carboxylate group acts as a general base to assist the attack by water on the metal-ion coordinated carbonyl group of the substrate and forming a tetrahedral intermediate (Fig. 2.6). This converts Glu-270 into a carboxylic acid, which can subsequently act as a proton source for the leaving nitrogen atom, permitting a catalysed decomposition of the tetrahedral intermediate in the forward direction.[21]

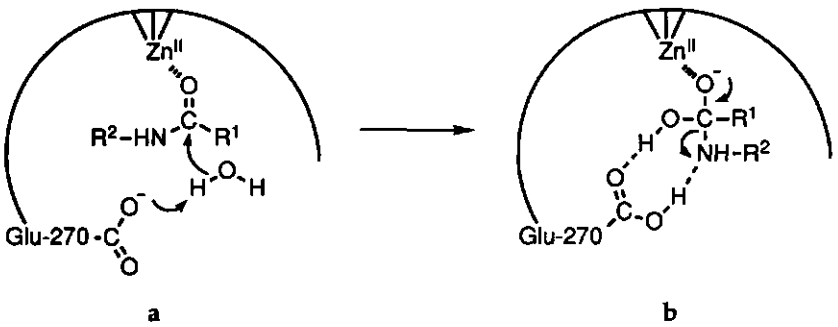


Figure 2.6 Proposed general base mechanism of CPA catalysis.

The results of recent X-ray crystallographic studies on unproductive and static complexes of CPA formed with pseudo-substrates and inhibitors, suggest that the Zn^{II} ion is probably not involved in the polarisation of the substrate carbonyl group prior to catalysis.[17] The mechanistic role of Zn^{II} may rather be to promote a water molecule, with general base assistance of Glu-270, to attack the carbonyl carbon atom, which is polarised by hydrogen bonding from Arg-127 (Fig. 2.7). The protonated Glu-270 residue

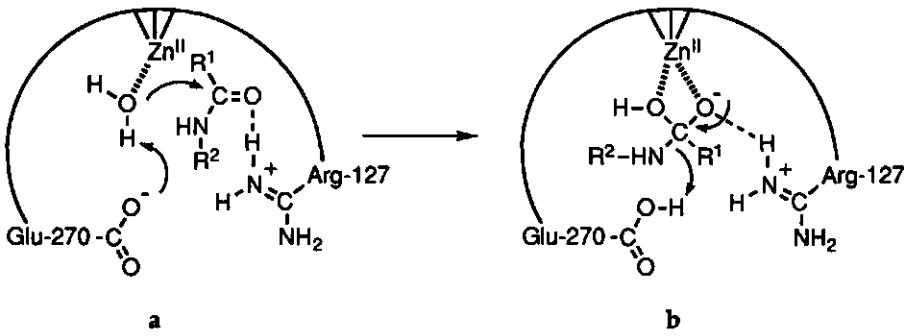


Figure 2.7 Proposed zinc-hydroxide mechanism of CPA catalysis.

acts as the proton donor for the leaving amine in the tetrahedral intermediate.

Although several lines of evidence have been found in support of the mechanisms presented in Figures 2.4, 2.6, and 2.7, none of the mechanisms have been conclusively established as the mechanism of CPA action.

2.3 Micellar and Vesicular Aggregates as Enzyme Models

Surfactants are amphiphilic molecules. That is, they possess a hydrophilic headgroup (ionic or neutral) and an apolar hydrocarbon chain. Amphiphiles associate in aqueous solutions and at interfaces of air/water or water/organic solvent to form a variety of structures.[22] Aggregation behaviour of amphiphiles depends on the chemical structure of the monomers, on the nature of the media, and on the method of preparation. Micelles in aqueous solutions are mostly spherical entities, having average diameters of 30-60 Å and contain 20-100 monomers.[23] The hydrophobic parts of the surfactants are directed toward the interior of the micelle whereas the polar headgroups are located at the micelle-water interface (Fig. 2.8a). Counterions of ionic surfactants which surround the micelles are subjected to two opposing forces: coulombic attraction and thermal agitation. The surfactant headgroups and associated counterions are found in the Stern layer, which is a compact region located at the rugged surface of the micelle.[24] Part of the counterions are bound within the shear surface, which is the outer edge of the Stern layer, and other ions are located in the Gouy-Chapman electrical double layer, where they can dissociate from the micelle and are free to exchange with ions in the bulk phase (Fig. 2.8b).[23]

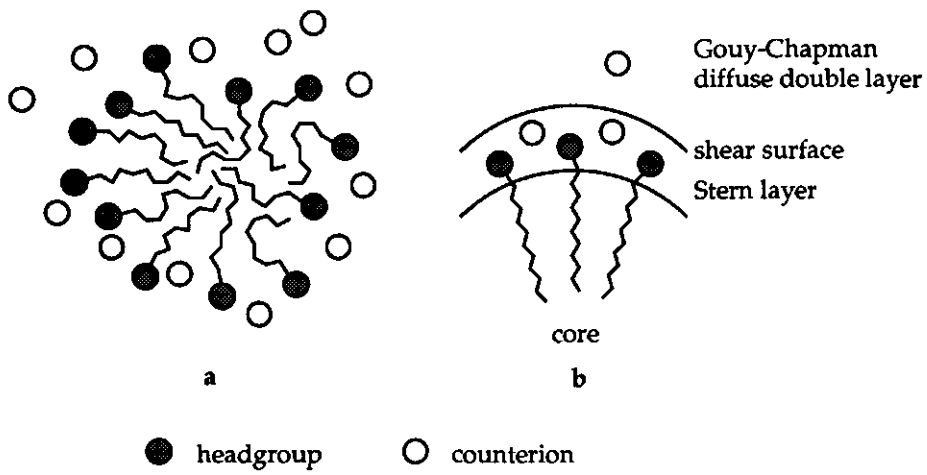
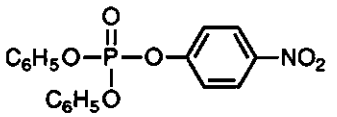


Figure 2.8 Two-dimensional representation of a spherical ionic micelle (**a**) and of a micellar section, giving the different regions of the micelle (**b**).

Micellisation of monomeric surfactants is observed when the surfactant concentration exceeds the critical micelle concentration (cmc). The tendency of surfactants towards self-association in water stems primarily from a favourable entropy change because of the liberation of water molecules from the aqueous-apolar interfaces. Micelles are dynamic entities; the time scale for the exchange of a single surfactant molecule between micelle and bulk solvent is in the order of microseconds and the stepwise dissolution of micelles to monomers and the subsequent reassociation occurs on the millisecond time scale. The amount of water in the micellar interior varies from surfactant to surfactant, but water is thought to penetrate the micellar pseudo-phase up to distances of at least seven carbon atoms.[24] The interior of the micelle is hydrocarbon-like and it is this difference in polarity between the interior and the surface that provides a micro-environment that resembles that of the binding sites of enzymes. Also, the non-covalent binding of substrates to micelles and the kinetics of micellar catalysis bear resemblance to enzymatic processes. Organic compounds, particularly apolar ones, can absorb onto or into micelles, thereby increasing their solubility relative to that in pure water and often altering their chemical reactivity.[25] In the case of ionic micelles, the comicellised substrate is exposed to high concentrations of counterions located in the water-micelle interface. For hydroxide catalysed reactions, for example, it is relevant that in the Stern layer of cationic micelles the concentration of hydroxide ions is higher than in the bulk solvent due to electrostatic effects, whereas in the case of anionic micelles the hydroxide ion concentration in the Stern layer is lower compared to the bulk solvent. For example, cationic CTABr micelles [$CH_3(CH_2)_{15}N^+(CH_3)_3$ Br^-] enhance

the rate of hydrolysis of the phosphate triester diphenyl *p*-nitrophenyl phosphate (DPPNPP, **1**) by maximally a factor of 10, whereas anionic micelles composed of SDS [$CH_3(CH_2)_{11}SO_3^-$ Na^+] inhibit this reaction (80 times).[26]



1

The term vesicle is used to describe spherical or ellipsoidal single- or multicompartimental closed bilayer structures composed of surfactants, and with water interspaced between them. Amphiphiles that form curved bilayer structures must have a truncated conic molecular shape. The double leaflet is formed by alignment of the bulky hydrocarbon chains in the apolar interior, whereas the relatively small headgroups protrude into the aqueous phase (Fig. 2.9). Vesicles are considerably larger than micelles, their diameters mainly ranging from 300-5000 Å.[27] The vesicular structure can be visualised by electron microscopy.[28] Various techniques have been developed for the preparation of vesicles, such as sonication of an aqueous solution in a bath or with a tip, and injection of an alcoholic surfactant solution into a rapidly stirred aqueous solution. The size and the structure of vesicles, *e.g.* unilamellar or multilamellar, depend on the method of preparation.[27,29]

The hydrophobic core of vesicles is considerably more rigid than that of micelles. Thermotropic phase transition of vesicles from the ordered gel phase to the liquid-crystalline phase involves an abrupt and large decrease in the packing density of the alkyl chains.[27,30] Vesicular aggregates are much more stable than micelles; a surfactant molecule may reside in the double layer for minutes or even hours, although molecular

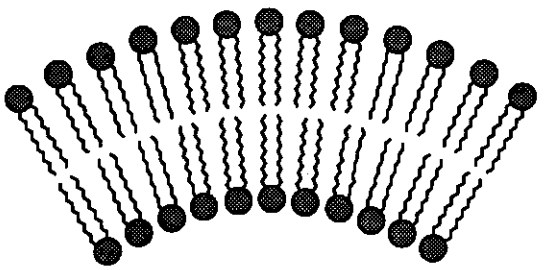


Figure 2.9 Cross section of a vesicular lamella showing the double leaflet.

motions within the bilayer structure do occur. Surfactants may undergo lateral diffusion, segmental and rotational motions, and transverse migration from one leaflet to the other (flip-flop). The intervesicular exchange rate and the molecular motions of surfactant monomers in the bilayer increase dramatically at temperatures above the phase transition temperature (T_c). At temperatures below T_c, vesicles are so stable that they can be concentrated by centrifugation and their size can be established by gel chromatography.[31]

The effect on the rate of a particular reaction caused by incorporation of the substrate into micelles or vesicles composed of commercially available surfactants is usually not very high, as was exemplified by the 10 times higher rate of hydrolysis of 1 in the presence of CTABr. Especially as models for the catalytic activity of enzymes, these surfactant aggregates are inadequate. A refinement towards biological models is the design and synthesis of functionalised amphiphiles.[32] These amphiphiles are tailor-made compounds containing reactive functional groups covalently bound to the molecular structure. Most of the functionalised surfactant aggregates have been tested in the hydrolysis of carboxylic esters and amides and phosphoric esters as model systems for hydrolytic enzymes. The functional groups of the surfactants are those present in the active sites of hydrolytic enzymes: sulphydryl,[31] hydroxyl,[33] and amino (imidazole, in particular) groups.[34] For example, mixed micelles composed of N^{α}-myristoyl-L-histidine (Fig. 2.10a) and CTABr have a much larger rate-enhancing effect on the hydrolysis of *p*-nitrophenyl esters than CTABr alone. The mechanism involves a rapid preliminary association of catalyst and substrate followed by nucleophilic attack by the imidazole group on the ester function and expulsion of the *p*-nitrophenolate ion. This results in acylation of the imidazole group, which is regenerated by hydrolysis of the acylated catalyst (Fig. 2.10b).[35] The role of the micelle is to bring substrate and catalyst together in a small volume, realising high concentrations out of dilute aqueous solutions. Moreover, the pK_a value of the nucleophile is lowered when solubilised in cationic micelles like CTABr, providing a high concentration of the more reactive nucleophile.

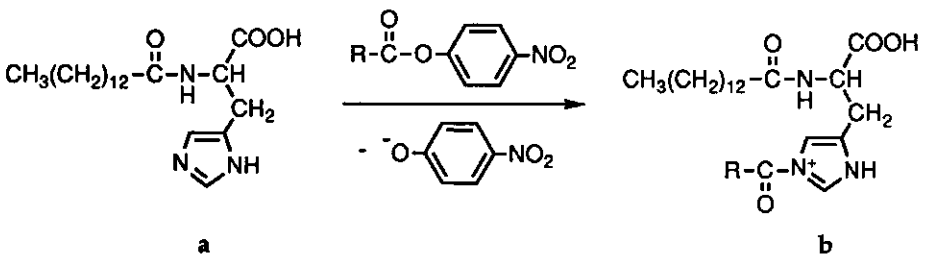
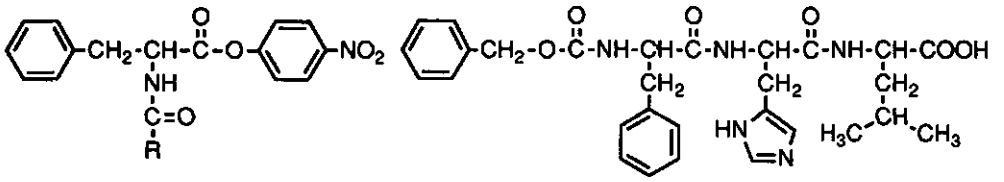


Figure 2.10 Mechanism of the imidazole catalysed hydrolysis of *p*-nitrophenyl esters.

Functionalised micelles and vesicles, containing chiral surfactants or di- and tripeptide catalysts, have attracted considerable attention because of their catalytic potential in enantioselective hydrolysis and as mimics for proteolytic enzymes. Remarkably high stereoselectivity was established in the hydrolysis of N-protected amino acid esters (2) catalysed by hydrophobic peptide-type histidine catalysts such as 3 in micellar,[36] vesicular,[37] and co-aggregate systems.[38] It was found that the composition of the surfactant aggregate system has a large effect on the stereoselectivity.



2 : R = $(CH_2)_{10}CH_3$

R = $OCH_2C_6H_5$

3

2.4 Metallo-Micelles and Metallo-Vesicles

As was pointed out in the previous section, functionalised micelles and vesicles have been studied as model systems for enzymes, because these systems possess the hydrophobic environment for substrate binding and provide the catalytically active groups. Moving along these lines, metallo-aggregates have been constructed to mimic hydrolytic metallo-enzymes.[39] As illustrated schematically in Figure 2.11, mixed metallo-micelles are composed of metal-ion complexes of amphiphilic ligands embedded in a micellar matrix. In addition to a long apolar chain the amphiphilic ligand contains a chelating headgroup. As chelating subunit aromatic nitrogen-containing heterocycles such as (bis)imidazole,[40] pyridine,[39,41] benzimidazole,[42] and 1,10-phenanthroline,[43] as well as non-aromatic ethylenediamino,[44] and triethylene-tetraamino [45] groups have been used. The nucleophilic group for catalysis is a hydroxyl group covalently bound to the ligand or a metal-bound hydroxide ion. For example, the imidazole moiety of **4** is the chelating subunit, the hydroxyl group is the nucleophilic function, and the alkyl chain acts as a hydrophobic anchor by which the catalyst is bound to the micelle.[40,46]

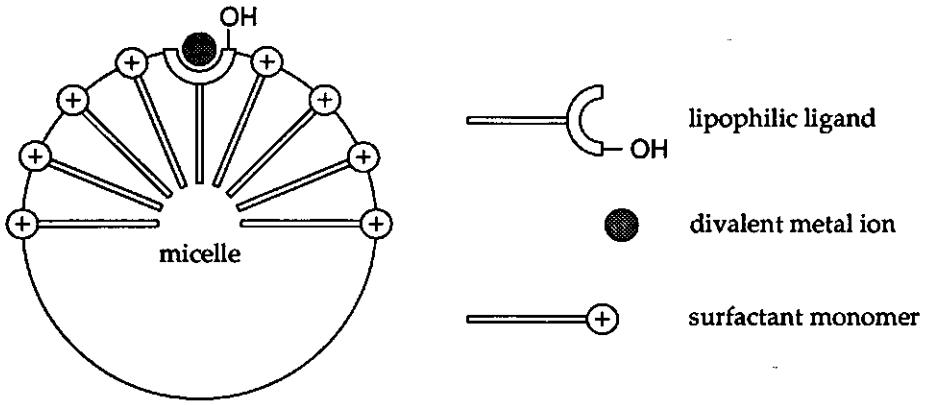
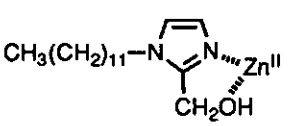


Figure 2.11 Schematic illustration of a mixed metallo-micelle composed of a lipophilic ligand-metal-ion complex and a cationic co-surfactant.

In homo-micelles, the metallo-amphiphilic monomers contain an ionic group covalently bound to the chelating subunit (**5**) or to the tail end of the alkyl chain (**6**, bolaform), so that it is not necessary to use a co-surfactant to solubilise the catalyst.[47]



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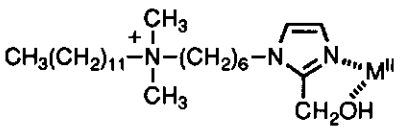

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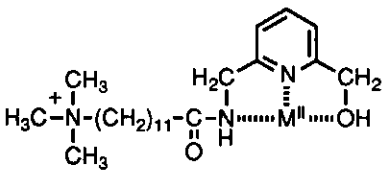


6

The catalytic behaviour of metallo-micelles has been studied in the hydrolysis of activated carboxylic and phosphoric esters. Four examples of metallo-micellar and metallo-vesicular reactions will now be discussed.

The lipophilic Zn^{II} complex **a** (Fig. 2.12) solubilised in CTABr micelles catalyses the hydrolysis of the metallophilic substrate *p*-nitrophenyl picolinate (PNPP, Fig. 2.2b). In the presence of 0.2 mM of **a** (Fig. 2.12), a 20 x 10^3-fold rate enhancement was observed.[40,48] The central feature in the metallo-amphiphile catalysed hydrolysis is the

formation of a reactive ternary complex composed of metal ion, ligand, and substrate.[40,47e,49] In addition to the template effect of the metal ion, Zn^{II} lowers the pK_a value of the ligand hydroxyl group and polarises the ester carbonyl group, which makes it more susceptible to nucleophilic attack. The acylated intermediate (**b**, Fig. 2.12) is subsequently hydrolysed to regenerate the free hydroxyl group and thus enabling turn-over. The metal-ion catalysed hydrolysis of the acylated intermediate is a relatively slow step compared to the transacylation step.[49,50]

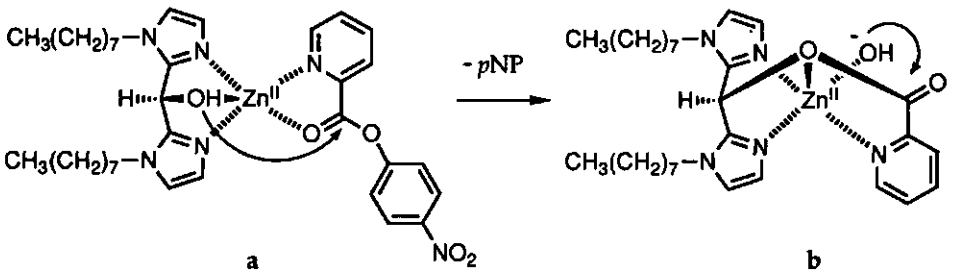
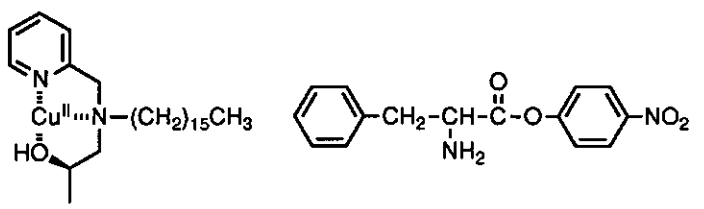


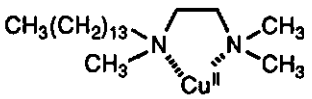
Figure 2.12 Mechanism of hydrolysis of PNPP catalysed by metallo-surfactants containing a covalently bound hydroxyl group.

In order to mimic the stereoselectivity of proteolytic metallo-enzymes, aggregates made of chiral metallo-amphiphiles have been investigated as catalyst for the enantioselective hydrolysis of chiral substrates.[51] In the highly ordered ternary complexes, the motional freedom of the ligand and substrate is restricted by the template effect of the metal ion. Chiral discrimination between substrate enantiomers was observed in micellar systems composed of the chiral metallo-surfactant R-7. In the presence of R-7 the S-enantiomer of substrate 8 is hydrolysed 14 times faster than the R-enantiomer.[51a] The origin of stereoselectivity must be either a higher affinity of R-7 for S-8 than for R-8, or a faster reaction of the ternary complex R-7-S-8 than that of the diastereomeric complex R-7-R-8.



7 8

Phosphate esters with good leaving groups have been applied as chemical warfare agents and as acetyl-cholinesterase inhibiting insecticides. Detoxification of these neurotoxins has been effected by the use of metallo-micelles.[44,52] The hydrolysis of DPPNPP (1) proceeds more than 10^5 times faster in the presence of micelles composed of the Cu^{II} containing amphiphile **9**.[44] The mode of action involves binding of the lipophilic substrate to the micelle by hydrophobic interactions, electrophilic activation of the P=O group by the Cu^{II} ion, and nucleophilic attack by the metal-bound hydroxide ion. Two aspects of metallo-micelles composed of **9** were remarkable: firstly the very low pK_a value of the copper-bound water molecule ($pK_a < 6$) and secondly the enhanced electrophilic activation of Cu^{II} toward substrates in the cationic Stern layer compared to non-micellar Cu^{II}-tetramethylethylenediamine complex.[44]



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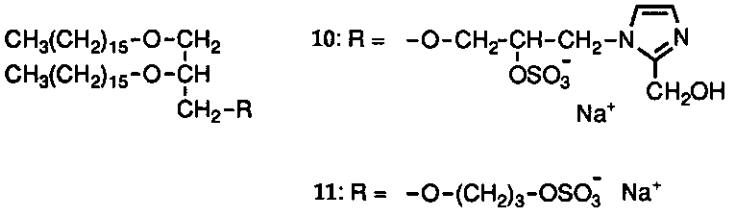
Recently functionalised vesicular systems have also been investigated as metallo-enzyme models.[53] Metallo-vesicles are better organised systems than metallo-micelles and may display several different features which can affect the catalytic activity, such as surface differentiation, limited neutral and ionic permeation, and phase-transition temperature. For example, when Cu^{II} ions are added to mixed metallo-vesicles composed of **10** and **11** below T_c, only the exovesicular side is active toward the substrate PNPP, because the ligand molecules (**10**) at the endovesicular side are devoid of metal ions.[53a]



11: R = $-O-(CH_2)_3-OSO_3^-$ Na^+

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Chapter 3

Carboxylic and Phosphate Ester Hydrolysis Catalysed by Bivalent Zinc and Copper Metallo-Surfactants*

3.1 Introduction

Micelles of functionalised 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 metallo-enzymes, only a few examples have been reported. These artificial enzymes are effective in promoting the cleavage of phosphate[3-5] and carboxylic esters.[2b-d,6]

Metallo-surfactants must contain a chelating headgroup for metal-ion fixation. We have chosen 2-substituted 1,10-phenanthroline or pyridine derivatives to meet this requirement. 1,10-Phenanthrolines can 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 metallo-enzyme reactions for carboxypeptidase A, NADH-alcohol dehydrogenase, and metallo-enzymes 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 2-hydroxymethyl-1,10-phenanthroline,[10] and the reduction of 2-pyridinecarboxaldehyde by 1,4-dihydronicotinamide which is covalently bound 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, functionalised 1,10-phenanthroline, and substrate. No studies of metal-ion catalysis of functionalised phenanthroline surfactant molecules have been published so far. In this chapter 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 *p*-

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* Adopted from: J. G. J. Weijnen, A. Koudijs and J. F. J. Engbersen, Carboxylic and Phosphate Ester Hydrolysis Catalysed by Bivalent Zinc and Copper Metallo-Surfactants, *J. Chem. Soc., Perkin Trans.* 2, 1991, 1121-1126.

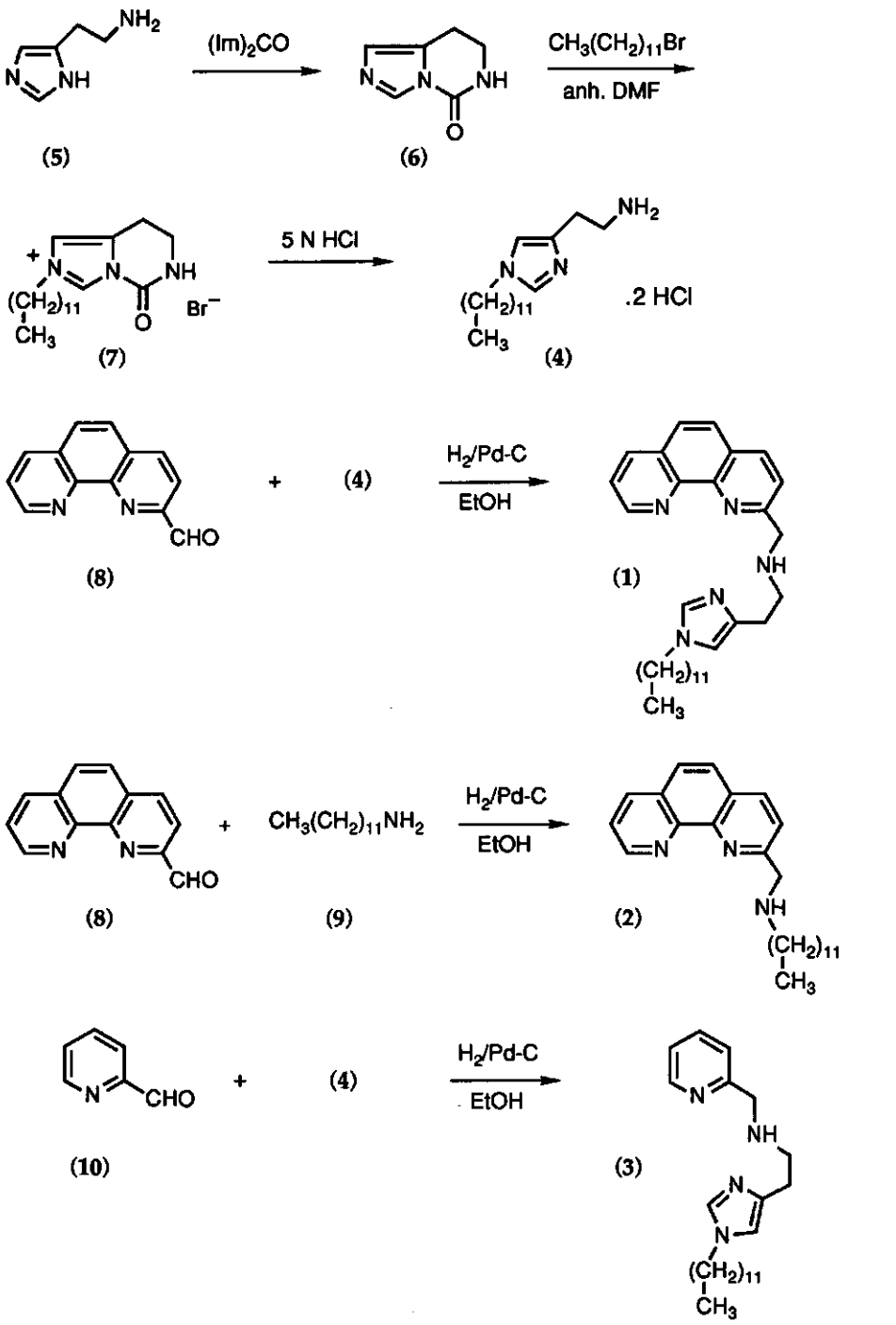
nitrophenyl picolinate (PNPP) and diphenyl *p*-nitrophenyl phosphate (DPPNPP) in mixed micelles in the presence of Zn^{II} and Cu^{II}. Ligands **1** and **3** possess, in addition to the metal-ion binding site, an imidazole group in order to test the possibility of bifunctional catalysis, *i.e.* electrophilic 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 that 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**.

3.2 Results and Discussion

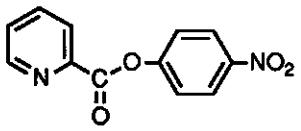
The lipophilic ligands **1**, **2**, and **3** were prepared following the synthetic pathways shown in Scheme 3.1. The regiospecific alkylation of histamine (**5**) at the N^{τ} position was accomplished by temporarily protecting the other two nitrogen atoms by reaction with 1,1'-carbonyldiimidazole which yields **6**.[12] Reaction of **6** with 1-bromododecane to give **7**, followed by hydrolysis yields N^{τ}-dodecylhistamine (**4**). 1,10-Phenanthroline-2-carboxaldehyde was obtained by a modified literature procedure from 1,10-phenanthroline. After cyanation of phenanthroline *via* oxidation with H_2O_2 and the Reissert reaction,[10,13,14] methyl 1,10-phenanthroline-2-carboxylate could be obtained directly in excellent yield by methanolysis. Subsequently this compound was reduced to the hydroxymethyl derivative 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** results in a characteristic absorbance change in the 270-300 nm region, as has been previously found for the binding of bivalent metal ions to 1,10-phenanthroline.[16] For **1** and **2** the absorbance maximum shifts from 267 to 273.5 nm and a shoulder at 295 nm appears. 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** does not induce significant changes in the absorbance spectrum. Therefore the binding of Zn^{II} to **3** could not be quantitatively determined from the spectrum.

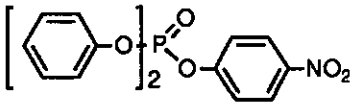
The esterolytic activity of the Zn^{II} complexes of the lipophilic ligands **1**, **2**, and **3** was tested with PNPP (Scheme 3.2) as substrate in the absence of co-surfactant, as well as in micellar systems admixed with the chemically inert cationic CTABr



Scheme 3.1 Synthesis of the ligands



PNPP



DPPNPP

Scheme 3.2 The substrates PNPP and DPPNPP

$[CH_3(CH_2)_{15}N(CH_3)_3Br]$ or neutral Brij 35 $[CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH]$ 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 3.1.

The rate data in Table 3.1 show that a low concentration (0.4 mM) of the metallo-surfactants induces a rate enhancement of *ca.* 50 times. 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 molar equivalents of co-surfactant significantly increases the catalytic activity of the metallo-surfactants. It should be noted that solutions containing only the co-surfactants exhibit almost no rate-enhancing effect. A possible explanation for the lower catalytic activity of the metallo-surfactants in the absence of co-surfactant might be the formation of less active cylindrical micelles with a

Table 3.1 Pseudo-first-order rate constants (k_{obs} / 10^{-3} s^{-1}) for the hydrolysis of PNPP, catalysed by Zn^{II} complexes of **1**, **2**, and **3** in the presence of various co-surfactants.[a]

	co-surfactant		
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 M *N*-ethylmorpholine-HBr buffer), [PNPP] = 4 x 10^{-5} M, [ligand] = 4 x 10^{-4} M, [CTABr] = 4 x 10^{-3} M, and [Brij 35] = 4 x 10^{-3} M.
[b] $[Zn^{II}]$ = 4 x 10^{-4} M.
[c] $[Zn^{II}]$ = 1.2 x 10^{-3} M.

larger aggregation number in these solutions, whereas in the presence of a co-surfactant globular micelles with a smaller aggregation number are 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 co-surfactant, thereby affecting the reactivity.[17]

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

In order to establish the affinity of PNPP for the metallo-surfactants, we measured the rate of hydrolysis as a function of the **2**-Zn^{II} and **3**-Zn^{II} concentration (Fig. 3.1). For **2**-Zn^{II} rapid saturation kinetics are observed indicating strong affinity of PNPP for this metal-ion-ligand complex, whereas the shape 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 ternary complex **3**-Zn^{II}-PNPP.

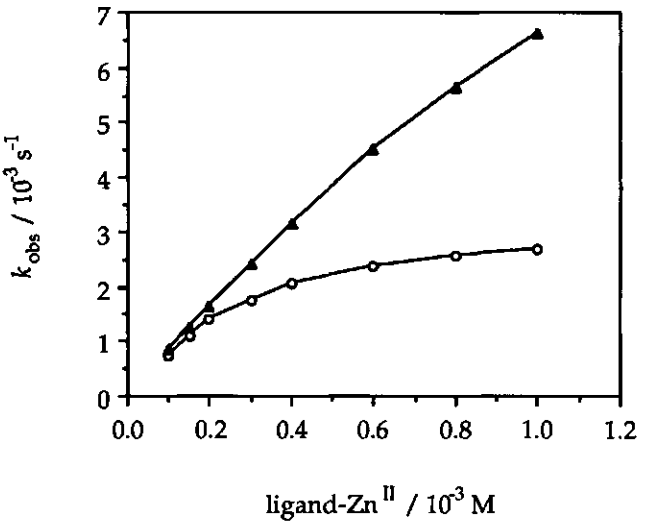


Figure 3.1 Plots of pseudo-first-order rate constants for the hydrolysis of PNPP as a function of ligand-Zn^{II} concentration at pH = 7.00 and 25 °C; [PNPP] = 4 x 10^{-5} M, [CTABr] = 4 x 10^{-3} M, [**2**] : [Zn^{II}] = 1 : 1 (o), and [**3**] : [Zn^{II}] = 1 : 3 (▲).

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

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

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

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

The rate-concentration profiles can be analysed quantitatively by assuming the rapid and reversible formation of a reactive ternary complex composed of ligand, Zn^{II}, and PNPP,[19] followed by a rate-determining hydrolysis step [eqns. (1) and (2)]. 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 described by eqn. (4) the association constant, K, and the catalytic rate constant, k_c, are obtained. These values for **2**-Zn^{II} and **3**-Zn^{II} are given in Table 3.2.

Table 3.2 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/M^{-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 M *N*-ethylmorpholine-HBr buffer), [CTABr] = 4 x 10^{-3} M.
[b] [**2**] : [Zn^{II}] = 1 : 1.
[c] [**3**] : [Zn^{II}] = 1 : 3.

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} ions in solution on the rate of hydrolysis. Figure 3.2 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 mM of Zn^{II}, which suggests only a 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 mM) of **2** and **3** is also depicted in Figure 3.2. At $[Zn^{II}] = 0$, 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 ions. Addition of Zn^{II} to **2** causes a rapid increase in the 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 of 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 to **2**.

In order to test the turn-over behaviour of the catalysts, we investigated the catalytic activity of the metallo-surfactants under conditions of [PNPP] > [ligand-Zn^{II}]. For all three ligand-Zn^{II} complexes, *p*-nitrophenolate was produced in quantitative yield (Fig. 3.3). The release of *p*-nitrophenolate shows no biphasic behaviour, indicating

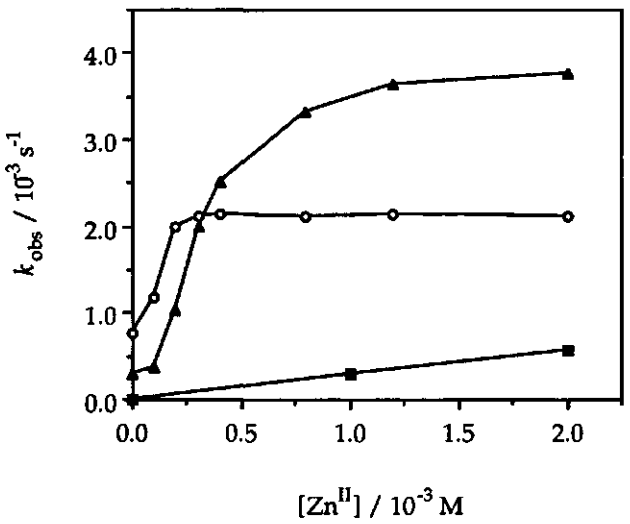


Figure 3.2 Plots of pseudo-first-order rate constants for the hydrolysis of PNPP as a function of $[Zn^{II}]$ under a fixed concentration of ligand at pH = 7.00 and 25 °C; [CTABr] = 4 x 10^{-3} M, [PNPP] = 4 x 10^{-5} M, no ligand (■), [**2**] = 4 x 10^{-4} M (o), and [**3**] = 4 x 10^{-4} M (▲).

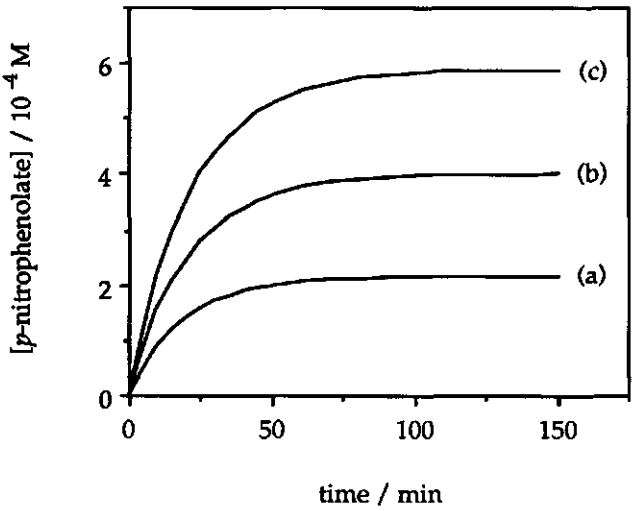


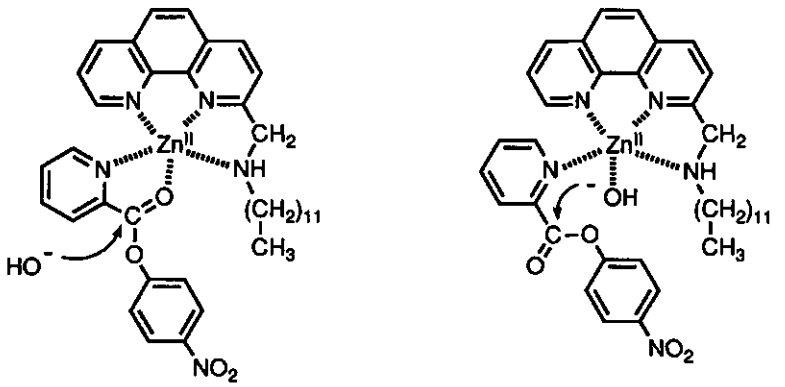
Figure 3.3 Time-courses for p-nitrophenolate release from PNPP [(a): 2 x 10^{-4} M, (b): 4 x 10^{-4} M, and (c): 6 x 10^{-4} M] as catalysed by **2** ([2] = 2 x 10^{-4} M) in the presence of 6 x 10^{-4} M $ZnBr_2$ (0.05 M N-ethylmorpholine-HBr buffer, pH = 7.00, and 25 °C). Ligands **1** and **3** show similar behaviour.

that the catalyst is rapidly regenerated during the 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 metallo-cleft 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 x 10^3, 1.77 x 10^3, and 6.12 x 10^3 M^{-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 x 10^2 M^{-1} s^{-1}. This points to the involvement of a hydroxide ion in the mechanism, whose action is catalysed by the presence of a 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



(a) (b)

Scheme 3.3 Proposed mechanism of the 2-Zn^{II} catalysed hydrolysis of PNPP.

ligand. Acylated intermediates have been found in catalysis by active hydroxyl,[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 3.3.

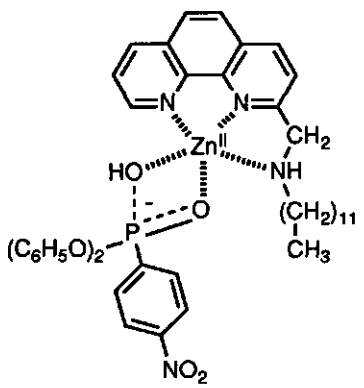
In Scheme 3.3 (a), binding of the pyridine moiety and the carbonyl group of the substrate to Zn^{II} results in electrophilic activation of the carbonyl bond and a consequent enhancement of external attack by a hydroxide ion. In Scheme 3.3 (b), substrate binding to the metal ion brings the ester group into close proximity with a Zn^{II}-bound hydroxide ion, enabling intramolecular nucleophilic attack by this Zn^{II}-bound hydroxide ion to the carbonyl group.

We also tested the catalytic activity of the metallo-surfactants in the hydrolysis of the phosphate triester DPPNPP (Scheme 3.2). Phosphate triesters are less sensitive to hydrolysis than carboxylic esters. It has been found that the hydrolysis of DPPNPP can be catalysed by micellar and non-micellar metal-ion complexes.[3,4] Table 3.3 gives the observed pseudo-first-order rate constants for hydrolysis of 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 metallo-surfactant-substrate complexes. The geometry of metal-ion coordination is an

Table 3.3 Pseudo-first-order rate constants (k_{obs} / 10^{-3} s^{-1}) for the hydrolysis of DPPNPP, catalysed by Zn^{II} and Cu^{II} complexes of **1**, **2**, and **3** in the presence of 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 M N-ethylmorpholine-HBr buffer), [CTABr] = 4 x 10^{-3} M, [ligand] = 4 x 10^{-4} M, and [DPPNPP] = 4 x 10^{-5} M.
[b] $[M^{II}] = 4 \times 10^{-4}$ M.
[c] $[M^{II}] = 1.2 \times 10^{-3}$ M.



Scheme 3.4 Proposed mechanism of the **2**-Zn^{II} catalysed hydrolysis of DPPNPP.

important factor in the catalytic activity of metallo-enzyme 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 probably 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} ion polarises the P=O bond (Scheme 3.4).

In conclusion, the present study clearly demonstrates that metallo-micelles made of **1**-Zn^{II}, **2**-Zn^{II}, and **3**-Zn^{II} in the presence of an inert co-surfactant 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 substrate PNPP binds more strongly to the phenanthroline metallo-surfactant **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 strong affinity for the metal ion are formed, as in the hydrolysis of PNPP. In the presence of excess of two equivalents of Zn^{II} to bind picolinate, the metallo-surfactant **2**-Zn^{II} retains its full catalytic activity. Moreover, the presence of an imidazole function in **1**-Zn^{II} does not have a marked effect on the catalysis relative to the activity of **2**-Zn^{II}.

3.3 Experimental Section

Materials and Methods

$ZnBr_2$ (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. ^1H NMR spectra were recorded on Bruker AC 200-E or Varian EM390 spectrometers. Coupling constants are in Hz. Mass spectral data were obtained on an AEI MS 902 spectrometer equipped with a VG ZAB console and using field desorption ionisation technique in the case of the 1,10-phenanthroline derivatives. 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**)

This compound was prepared by 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 to 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 mL) was refluxed for 0.5 h. The solution was cooled to 0-5 °C and made slightly acidic with 2% HCl (100 mL). After stirring for 0.5 h, the solution was neutralised with $NaHCO_3$. MeOH was removed under reduced pressure and the aqueous layer was extracted with $CHCl_3$ (3 x 100 mL). The organic layers were combined 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, H-8), 7.85 (2 H, s, H-5 and H-6), 8.25 (1 H, dd, H-7), 8.40 (2 H, d, H-3 and H-4) and 9.26 (1 H, dd, H-9).

5-Oxo-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine (6)

This compound 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, 7.81 mmol) and 1-bromododecane (9.66 g, 38.8 mmol) was heated overnight at 90 °C in dry DMF (100 mL). 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, *J* 6.0, CH_3), 1.25 [18 H, s, $(CH_2)_9CH_3$], 2.01 [2 H, m, $CH_2(CH_2)_9CH_3$], 3.22 (2 H, t, *J* 6.8, CH_2CH_2NH), 3.74 (2 H, t, *J* 6.8, CH_2NH), 4.48 [2 H, t, *J* 7.0, $NCH_2(CH_2)_{10}$], 7.72 (1 H, s, H-1) and 9.58 (1 H, s, H-3). This product was used for the synthesis of **4** without further purification.

N^{τ}-*Dodecylhistamine dihydrochloride* (4)

A solution of **7** (2.87 g, 7.44 mmol) in 5 N HCl (100 mL) 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, m, $CH_2(CH_2)_9CH_3$], 3.14 (2 H, t, *J* 6.4, $CH_2CH_2NH_2$), 3.36 (2 H, t, *J* 6.4, CH_2NH_2), 4.19 [2 H, t, *J* 7.2, $NCH_2(CH_2)_{10}$], 7.47 (1 H, s, H-5) and 8.58 (1 H, s, H-2); *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 (40 mL) was hydrogenated in a Parr apparatus (0.05 g 10% Pd-C) 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 [neutral Al_2O_3 (activity III), 0-10% MeOH/$CHCl_3$]. Ligand **1** (0.26 g, 29%) was obtained as a thick oil. δ_H ($CDCl_3$) 0.84 (3 H, t, *J* 6.4, CH_3), 1.20 [18 H, s, $(CH_2)_9CH_3$], 1.68 [2 H, m, $CH_2(CH_2)_9CH_3$], 2.86 (2 H, t, *J* 6.6, CH_2CH_2NH), 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, Phen-CH_2), 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, H-8), 7.72 and 7.77 (2 H, 2 d, *J* 9.7, H-5 and H-6), 7.79 (1 H, d, *J* 8.1, H-3), 8.19 (1 H, d, *J* 8.1, H-4), 8.23 (1 H, dd, *J* 1.7, 8.1, H-7) and 9.15 (1 H, dd, *J* 1.7, 4.4, H-9). FDMS: *m/z* 471.

N-*Dodecyl-2-aminomethyl-1,10-phenanthroline* (**2**)

A solution of dodecylamine (**9**, 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 (40 mL) 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. δ_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, m, $CH_2(CH_2)_9CH_3$], 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, Phen-CH_2), 7.60 (1 H, dd, *J* 4.4, 8.0, H-8), 7.72 and 7.78 (2 H, 2 d, *J* 9.7, H-5 and H-6), 7.78 (1 H, d, *J* 8.4, H-3), 8.20 (1 H, d, *J* 8.4, H-4), 8.22 (1 H, dd, *J* 1.7, 8.0, H-7) and 9.18 (1 H, dd, *J* 1.7, 4.4, H-9); FDMS: *m/z*: 377.

N^{α}-(2-*Pyridylmethyl*)-N^{τ}-*dodecylhistamine* (**3**)

A solution of **4** (1.0 g, 2.84 mmol), 2-pyridinecarboxaldehyde (**10**, 0.3 g, 2.84 mmol) and Et_3N (1.15 g, 11.4 mmol) in abs. EtOH (40 mL) was hydrogenated in a Parr apparatus (0.2 g 10% Pd-C) 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. δ_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, m, $CH_2(CH_2)_9CH_3$], 2.55 (1 H, br s, NH), 2.74 (2 H, t, *J* 6.5, CH_2CH_2NH), 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, Pyr-CH_2), 6.62 (1 H, s, Im-H5), 7.09 (1 H, m, H-5), 7.27 (2 H, m, Im-H2 and H-3), 7.57 (1 H, dt, *J* 1.8, 7.6, H-4) and 8.84 (1 H, dd, *J* 1.8, 4.6, H-6); *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} M of **1** or **2**, or 2×10^{-4} M 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 μL portion of 20 mM PNPP or 20 mM DPPNPP in CH_3CN into a 1-cm cuvette containing 2 mL of the buffer solution [containing 1% (v/v) EtOH and 0.6-1.4% (v/v) CH_3CN], the appropriate surfactant (4 mM), 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 under 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_∞-A_t) *vs.* time were always obtained for at least 3 half-lives. Kinetic runs, carried out in triplicate, gave rate constants with an uncertainty of less than ± 3%.

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Chapter 4

Functionalised 1,10-Phenanthroline Metallo-Catalysts as Models for Hydrolytic Metallo-Enzymes*

4.1 Introduction

Among the enzymes that have been mimicked in enzyme model studies, the Zn^{II}-containing metallo-protein carboxypeptidase A (CPA) has been particularly well studied.[1,2] The catalytic functions of the Zn^{II} ion in the active site of CPA have been discussed in chapter 2. 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} reduces the original peptidase activity, whereas substitution with Co^{II} leads to an even more active enzyme.[3]

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.[4,5] These systems exhibit intramolecular metal-ion catalysis, but there is no turn-over. Biomimetic models of hydrolytic metallo-enzymes 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 have attracted considerable attention as biomimetic hydrolytic metallo-catalysts. Homo and mixed metallo-micelles 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 metallo-enzyme models.

In chapter 3 we have demonstrated that lipophilic ligands containing the 1,10-phenanthroline moiety are strongly metal-ion chelating agents, forming metallo-micelles. These Zn^{II}- and Cu^{II}-containing metallo-surfactants are effective in the hydrolysis of carboxylic and phosphoric esters in homo and mixed micelles.[19]

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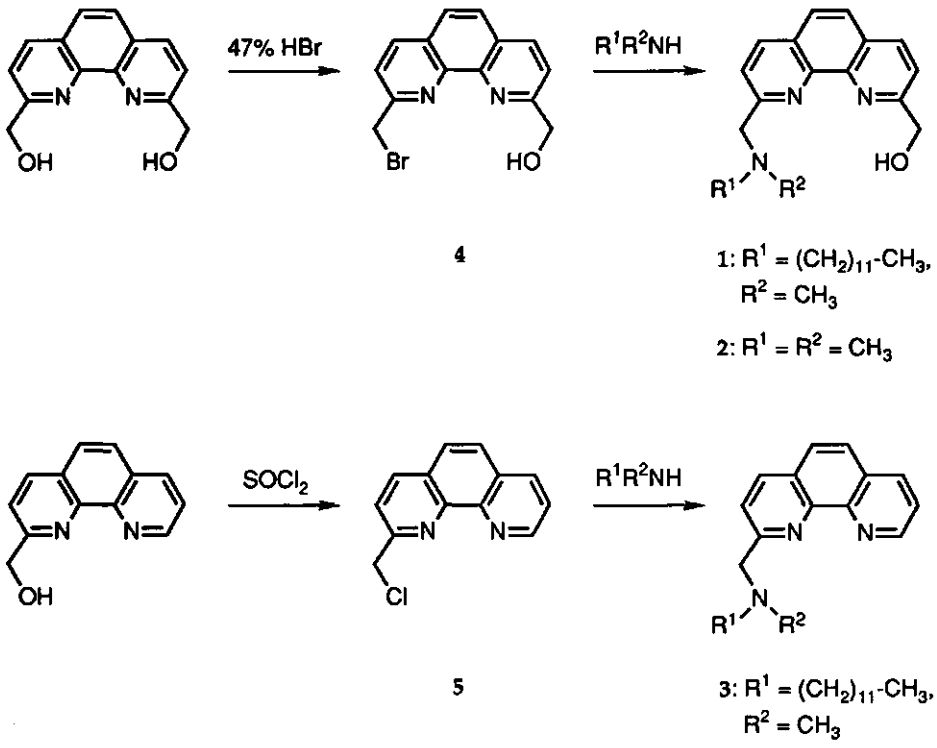
* Adopted from: J. G. J. Weijnen, A. Koudijs, G. A. Schellekens and J. F. J. Engbersen, Functionalised 1,10-Phenanthroline Metallo-Catalysts as Models for Hydrolytic Metallo-Enzymes, *J. Chem. Soc., Perkin Trans.* 2, 1992, 829-834.

In this chapter, 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 hydroxyl 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 metallo-cleft of **1** and **2** activates the hydroxyl group for nucleophilic attack. Ligand **3** lacks the hydroxymethyl group so that the catalytic activity of this compound can be used for comparison.

4.2 Results and Discussion

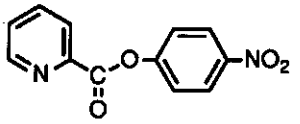
The 1,10-phenanthroline derivatives **1**, **2**, and **3** were prepared according to the synthetic pathways outlined in Scheme 4.1. The lipophilic ligand **1** and the water-soluble analogue **2**, were obtained by treatment of 2,9-bis(hydroxymethyl)-1,10-phenanthroline[24] with aqueous HBr to give the bromoalcohol **4**, followed by the coupling of **4** with *N*-methyldodecylamine or dimethylamine. Except for the earlier



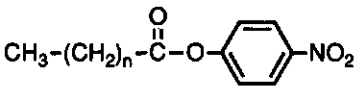
Scheme 4.1 Synthesis of the ligands **1**, **2**, and **3**

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 disubstituted 1,10-phenanthrolines. For the synthesis of **3**, 2-hydroxymethyl-1,10-phenanthroline[5b,19] was converted into 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

Scheme 4.2 The substrates PNPP, PNPO, and PNPD

The esterolytic activity of bivalent metal ions alone, non-metalated ligands, and complexes of ligands and metal ions toward the metallophilic substrate *p*-nitrophenyl picolinate (PNPP, Scheme 4.2), 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 metal-ion catalyst over substrate, in buffered micelles and in pure buffer solutions are shown in Tables 4.1 and 4.2, respectively.

These tables indicate that both in the presence and in the absence of the co-surfactant CTABr, addition of Zn^{II}, Co^{II}, Cd^{II}, and Ni^{II} causes only a slight rate enhancement.** The non-metalated 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**, co-micellised 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.

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

Table 4.1 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 M *N*-ethylmorpholine-HBr buffer), [CTABr] = 4 x 10^{-3} M, [ligand] = 5 x 10^{-4} M, [M^{II}] = 5 x 10^{-4} M, and [PNPP] = 5 x 10^{-5} M.
[b] In the absence of 1,10-phenanthroline ligand, $CdBr_2$ is insoluble in a CTABr micellar solution.

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**-M^{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 that for Cu^{II}-containing 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.[16d] 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 function is the nucleophilic group.

Table 4.2 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 M *N*-ethylmorpholine-HBr buffer), [**2**] = 5 x 10^{-4} M, [M^{II}] = 5 x 10^{-4} M, and [PNPP] = 5 x 10^{-5} M.

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 Figure 4.1, 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, whereas 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 slopes of the first parts of the graphs are 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 activates catalysis at this moiety. By this metal-ion hopping mechanism, the hydrolysis is truly catalytic in terms of the metal ion.

Figure 4.2 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 complex (ligand : metal ion) at low metal-ion

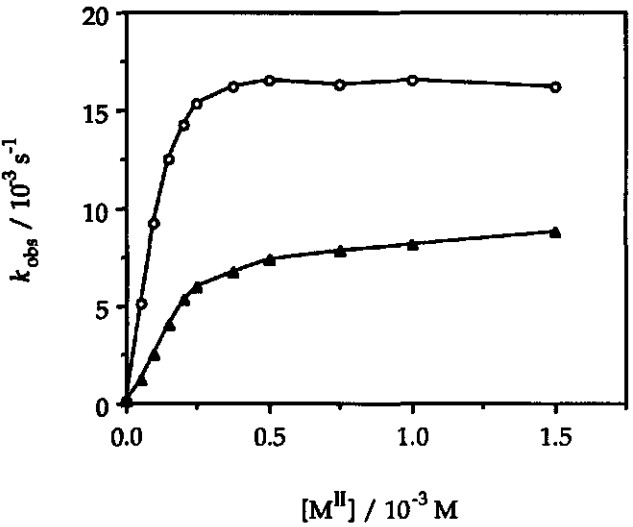


Figure 4.1 Plots of pseudo-first-order rate constants for the cleavage of PNPP in a mixed micellar system as a function of [Zn^{II}] (o) and [Co^{II}] (▲) under a fixed concentration of **1** at pH = 7.00 and 25 °C; [CTABr] = 4 x 10^{-3} M, [**1**] = 5 x 10^{-4} M, and [PNPP] = 5 x 10^{-5} M.

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 hydroxyl groups. Therefore, binding of the metallophilic substrate PNPP to the metal-ion catalyst 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.

Metal-ion complexes of **1** co-micellised 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 of **1** and **2**, there are also differences in the location of the complexes in the reaction medium. Table 4.3 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, whereas **2**-M^{II} remains largely in the bulk solvent due to the electrostatic repulsion between **2**-M^{II} and the cationic headgroups of the CTABr micelle.

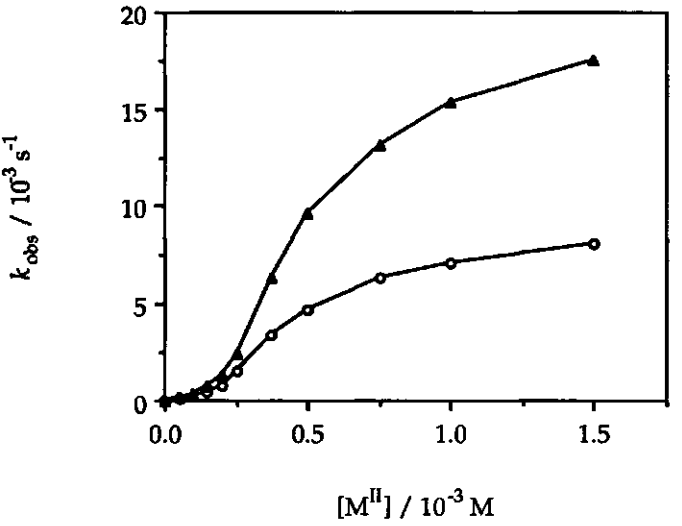


Figure 4.2 Plots of pseudo-first-order rate constants for the cleavage of PNPP as a function of [Zn^{II}] (o) and [Co^{II}] (▲) under a fixed concentration of **2** at pH = 7.00 and 25 °C; [**2**] = 5 x 10^{-4} M and [PNPP] = 5 x 10^{-5} M.

Next, we investigated the substrate specificity (Table 4.3). The hydrolysis of the lipophilic esters *p*-nitrophenyl octanoate (PNPO, Scheme 4.2) 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 complex plays an important role in a large rate enhancement. The water-soluble 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 metal-ion complex 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} co-micellised 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-metalated **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 metallo-surfactant with PNPP (association constant *K*), followed by pseudo-intramolecular acyl transfer (rate constant k_a) and subsequent

Table 4.3 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]

metallo-catalyst	co-micellar 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 M *N*-ethylmorpholine-HBr buffer), [CTABr] = 4 x 10^{-3} M, [**1**] = 5 x 10^{-4} M, [**2**] = 5 x 10^{-4} M, [M^{II}] = 5 x 10^{-4} M, [PNPP] = 5 x 10^{-5} M, [PNPO] = 5 x 10^{-5} M, and [PNPD] = 5 x 10^{-5} M.

hydrolysis of the acylated ligand (rate constant k_d) [eqns. (1) and (2)]. It is clear that the system cannot be termed catalytic if only one stoichiometric amount of PNP is released because the hydroxyl group of the catalyst is regenerated in a slow deacylation step. In Figure 4.3 the observed rate of release of PNP is plotted against the 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: $K = (1.08 \pm 0.05) \times 10^3$ M^{-1} and $k_a = (3.77 \pm 0.03) \times 10^{-2}$ s^{-1} [see also p. 28, eqn. (4)].

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

$$\text{- PNP}$$

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

Experiments under conditions of excess of substrate over **1**-Zn^{II} and **3**-Zn^{II} were performed in order to test the turn-over behaviour of the mixed micellar systems. As is

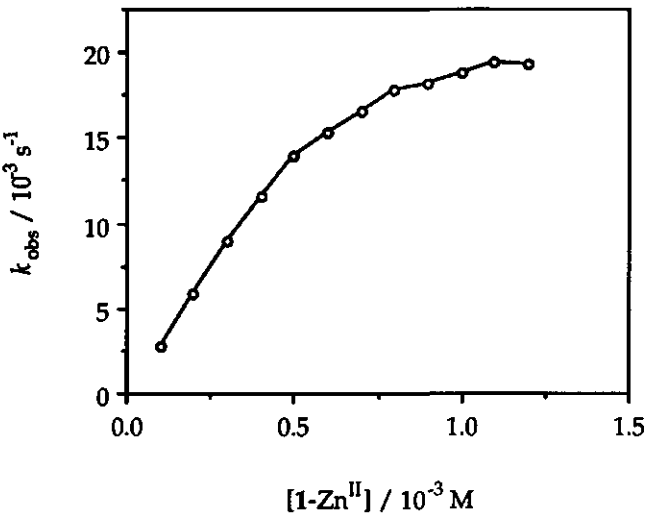


Figure 4.3 Pseudo-first-order rate constants for the cleavage of PNPP as a function of **1**-Zn^{II} concentration at pH = 7.00 and 25 °C; [CTABr] = 4 x 10^{-3} M, [PNPP] = 5 x 10^{-5} M, and [**1**] : [Zn^{II}] = 1.

shown in Figure 4.4, both reactions proceed beyond the stoichiometric conversion range. A striking difference between these systems is that **1**-Zn^{II} displays distinct biphasic kinetics whereas **3**-Zn^{II}, lacking the hydroxymethyl group, does not. In the case of **1**-Zn^{II}, after an initial burst release of PNP stoichiometrically equivalent to the amount of metallo-surfactant, the deacylation of the intermediate to regenerate the catalyst is the rate-determining step. The same biphasic behaviour is 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):

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

in which [**1**-Zn^{II}-PNPP]$_0$ is 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):

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

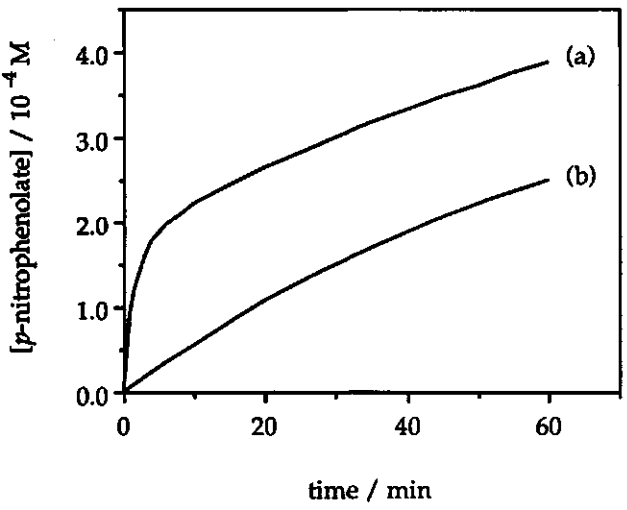


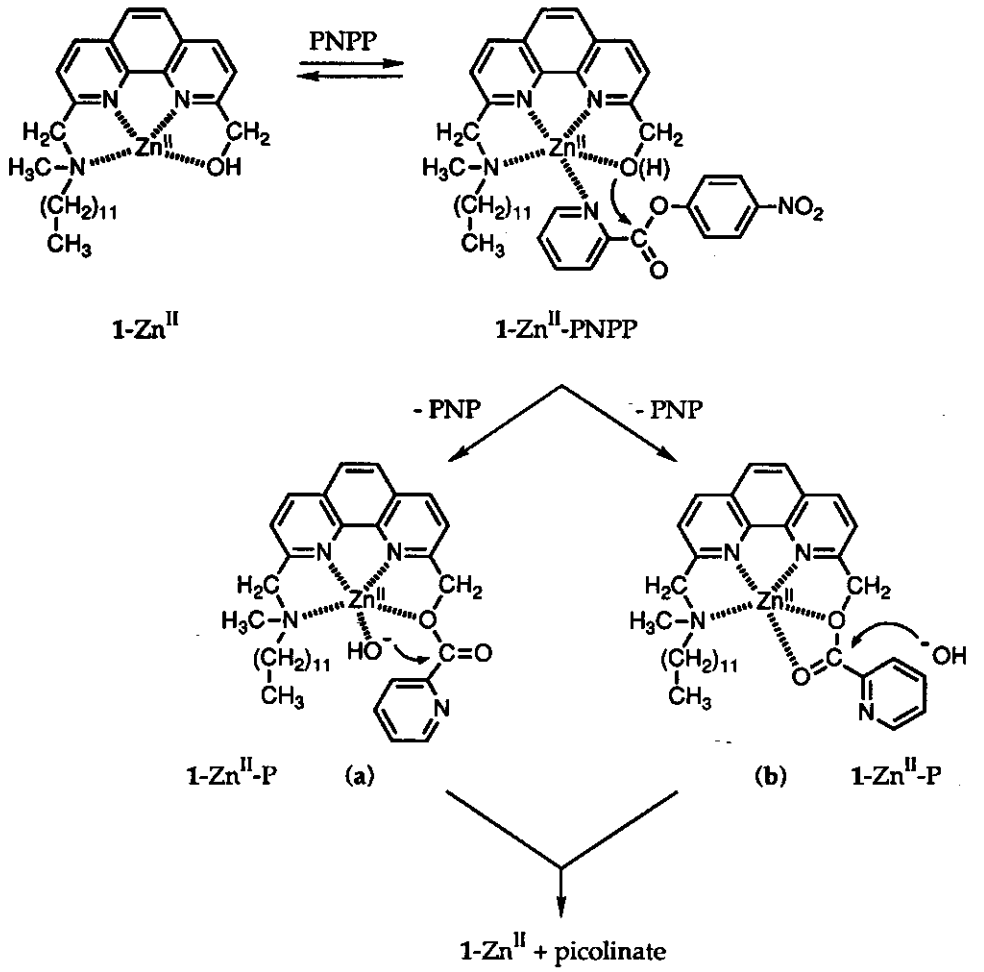
Figure 4.4 Time courses for *p*-nitrophenolate release from PNPP as catalysed by **1**-Zn^{II} (a) and **3**-Zn^{II} (b) at pH = 7.00 and 25 °C; [CTABr] = 4 x 10^{-3} M, [**1**] = 2 x 10^{-4} M, [**3**] = 2 x 10^{-4} M, [Zn^{II}] = 2 x 10^{-4} M, and [PNPP] = 6 x 10^{-4} M.

in which [**1**-Zn^{II}-PNPP]$_s$ 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 is shown in eqn. (5):

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

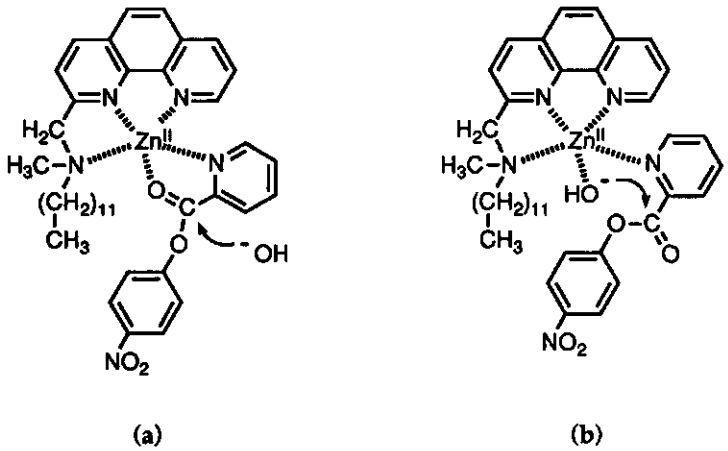
From the slope of the steady state section of the kinetic plot the k_d value is calculated; $k_d = (2.24 \pm 0.12) \times 10^{-4}$ s^{-1}.

The schematic representations of the mechanism of the hydrolysis of PNPP, catalysed by **1**-Zn^{II} and **3**-Zn^{II} are shown in Scheme 4.3 and Scheme 4.4, respectively. In Scheme 4.3 the 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 by the hydroxyl function. Another catalytic function of the Zn^{II} ion in the metallo-surfactant is to lower the pK_a value of the hydroxymethyl 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-



Scheme 4.3 Mechanism of the cleavage of PNPP catalysed by the metallo-surfactant 1-Zn^{II}.

intramolecular attack by the hydroxyl group on the carbonyl function of the activated ester,[7b,27] yields the intermediate transacylation product with simultaneous liberation of the 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 by metal-ion-coordinated OH^- on the ester group, or (b) attack by free OH^- on the ester



Scheme 4.4 Mechanism of the hydrolysis of PNPP catalysed by the metallo-surfactant 3-Zn^{II}.

group which is activated by coordination to the metal ion. In both mechanisms, the metal ion stabilises the expulsion of the leaving group. 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 metallo-surfactant 3-Zn^{II} operates *via* the zinc-hydroxide mechanism (Scheme 4.4) as was seen previously for the hydrolysis of PNPP catalysed by the Zn^{II} complex of *N*-dodecyl-2-aminomethyl-1,10-phenanthroline in chapter 3.[19]

In summary, the present study demonstrates that metal-ion complexes of **1** solubilised in an inert CTABr micellar matrix are efficient synzymes for the hydrolysis of PNPP, PNPO, and PNPD. Ligand **1** has a strong 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 metallo-surfactant 3-Zn^{II}, which lacks the nucleophilic hydroxyl 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.

4.3 Experimental Section

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 an 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 $CdBr_2 \cdot 4\ H_2O$ (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 mm Hg, 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 mL) 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 x 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography [SiO_2, 1% (v/v) $CH_3OH/CHCl_3$]. The bromoalcohol **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, H-3 and H-8), 7.76 and 7.82 (2 H, 2 d, *J* 8.9, H-5 and H-6), 8.24 and 8.26 (2 H, 2 d, *J* 8.3, H-4 and H-7); $\delta_C(CDCl_3)$ 32.78 (CH_2Br), 64.70 (CH_2OH), 120.49 and 123.35 (C-3 and C-8), 125.38 and 126.64 (C-5 and C-6), 127.61 and 127.79 (C-4a and C-6a), 136.81 and 137.57 (C-4 and C-7), 143.68 and 143.97 (C-10a and C-10b), 156.71 (C-2), and 160.23 (C-9); FDMS: *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 mL) 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 [neutral Al_2O_3 (activity III), 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, H-3 and H-8), 7.69 and 7.73 (2 H, 2 d, *J* 9.1, H-5 and H-6), 8.14 and 8.18 (2 H, 2 d, *J* 8.3, H-4 and H-7); $\delta_C(CDCl_3)$ 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 (C-3 and C-8), 125.41 and 125.51 (C-5 and C-6), 127.34 (C-4a and C-6a), 136.19 (C-4 and C-7), 144.30 and 144.65 (C-10a and C-10b), 160.17 and 160.93 (C-2 and C-9); FDMS: *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 mL), dimethylamine (650 mg, 14.4 mmol) dissolved in $CHCl_3$ (5 mL) was added. The reaction mixture was stirred at room temperature under a N_2 atmosphere for 3 h. 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 [neutral Al_2O_3 (activity III), 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, H-3 and H-8), 7.66 and 7.71 (2 H, 2 d, *J* 9.1, H-5 and H-6), 8.11 and 8.17 (2 H, 2 d, *J* 8.3, H-4 and H-7); $\delta_C(CDCl_3)$ 45.55 (CH_3), 65.25 and 65.87 (CH_2OH and $PhenCH_2N$), 120.19 and 122.24 (C-3 and C-8), 125.55 and 125.63 (C-5 and C-6), 127.46 (C-4a and C-6a), 136.33 (C-4 and C-7), 144.36 and 144.69 (C-10a and C-10b), 159.73 and 160.69 (C-2 and C-9); FDMS: *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 mL) was stirred at 0 °C for 2 h with the exclusion of moisture. Light petroleum (40-

60 °C, 150 mL) was added to the pale orange reaction mixture to precipitate the product as an oil. After decantation of light petroleum, cold diethyl ether (150 mL) was added and the oil solidified. The suspension was stirred at 0 °C for 20 min 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, H-8), 7.80 (2 H, s, H-5 and H-6), 7.91 (1 H, d, *J* 8.3, H-3), 8.26 (1 H, dd, *J* 1.7, 8.1, H-7), 8.30 (1 H, d, *J* 8.3, H-4), and 9.22 (1 H, dd, *J* 1.7, 4.4, H-9); $\delta_C(CDCl_3)$ 47.26 (CH_2), 122.16 and 122.88 (C-3 and C-8), 125.98 and 126.67 (C-5 and C-6), 127.66 and 128.70 (C-4a and C-6a), 135.92 and 137.05 (C-4 and C-7), 144.91 and 145.59 (C-10a and C-10b), 150.25 (C-9), and 156.92 (C-2); FDMS: *m/z* 228/230 (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 mL), was heated at 50 °C under a N_2 atmosphere for 16 h. After washing the reaction mixture 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 [neutral Al_2O_3 (activity III), $CHCl_3$]. 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, H-8), 7.71 and 7.77 (2 H, 2 d, *J* 9.1, H-5 and H-6), 7.91 (1 H, d, *J* 8.3, H-3), 8.19 (1 H, d, *J* 8.3, H-4), 8.21 (1 H, dd, *J* 1.8, 8.1, H-7), and 9.18 (1 H, dd, *J* 1.8, 4.4, H-9); $\delta_C(CDCl_3)$ 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 (C-3 and C-8), 125.45 and 126.07 (C-5 and C-6), 127.18 and 128.36 (C-4a and C-6a), 135.67 and 135.99 (C-4 and C-7), 145.02 and 145.59 (C-10a and C10b), 149.76 (C-9), and 160.61 (C-2); FDMS: *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 M solution of ester substrate into a 1-cm cuvette containing 2 mL 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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Chapter 5

Synthesis of Chiral 1,10-Phenanthroline Ligands and the Activity of Metal-Ion Complexes in the Enantioselective Hydrolysis of N-Protected Amino Acid Esters*

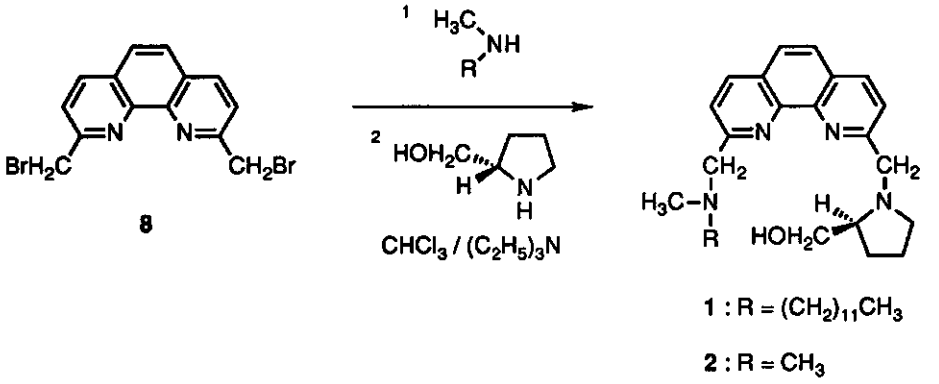
5.1 Introduction

Since the bidentate 1,10-phenanthroline nucleus is a strongly chelating agent for a variety of metal ions,[1] it is an attractive building block for host molecules in which a ligated metal ion serves as a Lewis acid binding site and catalyst.[2-3] Metal-ion complexes of functionalised 1,10-phenanthrolines have been used as catalysts in the oxidative cleavage of DNA,[3a] and in the enantioselective reduction of acetophenone.[3b,c] The complexing ability of the 1,10-phenanthroline ring has also been beneficially used in the development of biomimetic models for metallo-enzymes. In these models the metal ion is coordinated in a fixed position in the metallo-cleft of 2-substituted 1,10-phenanthrolines and is consequently in close proximity to the reaction site.[4] These biomimetic models provide insight into the mechanism by which metallo-enzymes may operate.[5-7]

Metallo-micelles have been developed in order to improve the substrate binding properties of artificial metallo-enzymes.[8-11] The absorption of apolar substrates into or onto these molecular assemblies and their subsequent reaction resemble enzymatic reactions,[12] and large rate accelerations are observed for the hydrolysis of carboxylic,[8,9,11] and phosphoric esters[10] in these systems.

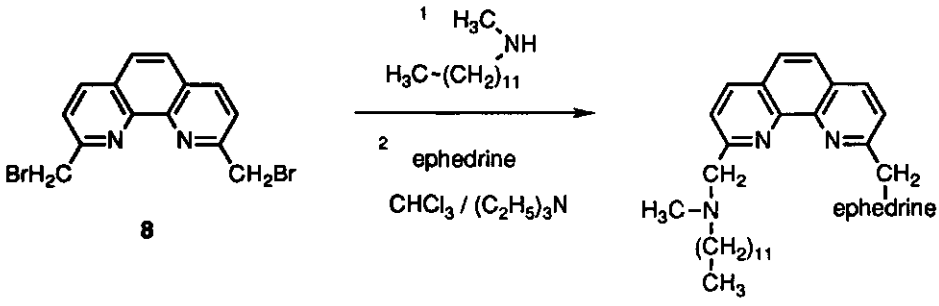
High stereoselectivity could be attained in the hydrolysis of *p*-nitrophenyl esters of N-protected amino acids catalysed by histidine containing di- and tripeptides in surfactant aggregates.[13] However, until now only a few model studies have been undertaken with respect to the enantioselectivity of hydrolytic metallo-enzymes.[14-16] The hydrolysis of amino acid esters catalysed by metal-ion complexes of D(L)-histidine,[14] and by polymers containing chiral amino acids,[15] proceeds with only moderate stereoselectivity; the highest enantioselectivity was observed in the case of

* Adopted from: J. G. J. Weijnen, A. Koudijs and J. F. J. Engbersen, Synthesis of Chiral 1,10-Phenanthroline Ligands and the Activity of Metal-Ion Complexes in the Enantioselective Hydrolysis of N-Protected Amino Acid Esters, *J. Org. Chem.*, 1992, 57, 7258-7265.

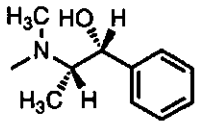


1 : R = $(CH_2)_{11}CH_3$

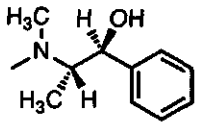
2 : R = CH_3

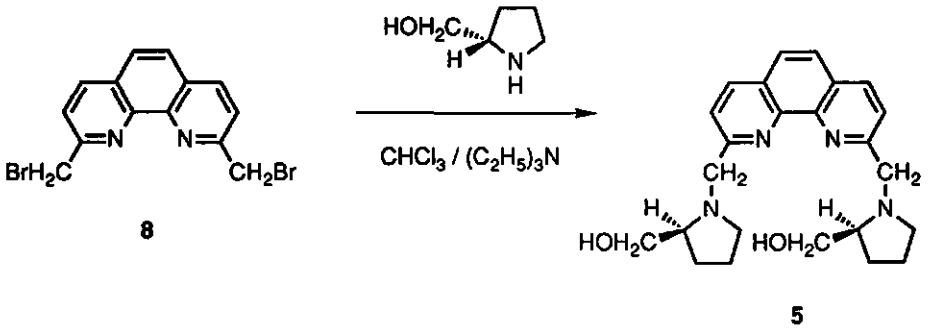


3 : (1R,2S)-ephedrine



4 : (1S,2S)-ephedrine





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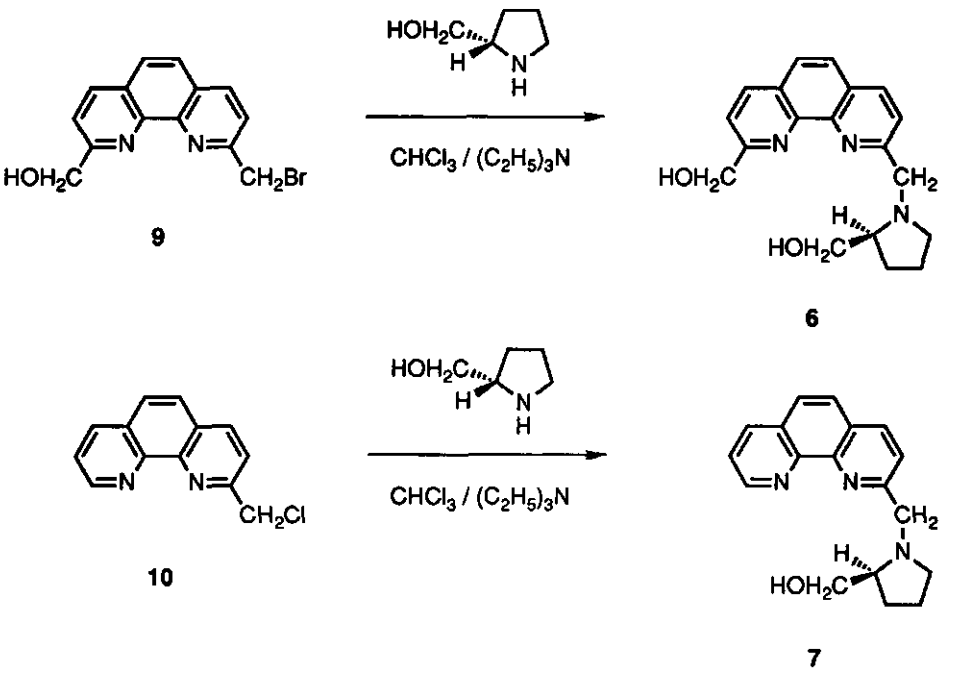


Scheme 5.1 Synthesis of the ligands **1-7**

Co^{II}-L-histidine as a catalyst (k^{L}_{obs}/k^{D}_{obs} = 3.49).[14b] Chiral homo and mixed metallomicelles gave a better enantioselectivity.[16] The largest enantioselectivity factor (14) was observed for the cleavage of unprotected *p*-nitrophenyl esters of phenylalanine by chiral Cu^{II}-chelating micelles.[16c]

In the chapters 3 and 4, we have demonstrated that the nature of the ligated metal ion determines largely the catalytic efficiency of M^{II} complexes of functionalised 1,10-phenanthrolines in the hydrolysis of carboxylic and phosphoric esters.[11] In this chapter, the factors controlling the magnitude and direction of the stereoselective hydrolysis of enantiomeric substrates, in the presence of chiral 1,10-phenanthroline ligands coordinated with bivalent metal ions, are investigated.[17] Seven new chiral 1,10-phenanthrolines are synthesised, which are all functionalised with a hydroxymethyl group in close proximity to the metallo-cleft, and the catalytic activity and enantioselectivity in the cleavage of *p*-nitrophenyl esters of N-protected amino acids are studied.

5.2 Results

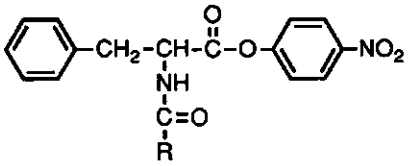
The chiral 1,10-phenanthroline ligands **1-7** were prepared according to the procedures outlined in Scheme 5.1. The asymmetrically disubstituted ligands **1-4** were obtained by coupling 2,9-bis(bromomethyl)-1,10-phenanthroline[18] to one equivalent of *N*-methyldodecylamine,[19] and without isolation of the unstable intermediate, the desired chiral amino alcohol was added to the reaction mixture. Compounds **5-7** were prepared by coupling (S)-2-pyrrolidinemethanol to the required 1,10-phenanthroline halide.

Ligands **1-7** contain a strongly metal-ion chelating moiety, composed of the 1,10-phenanthroline nucleus and one or two tertiary amino substituents at the 2 and 9 positions. Chirality in the ligands is introduced by the rigid 2-(hydroxymethyl)-pyrrolidine group with one chiral centre, or by the more flexible ephedrine group with two chiral centres. In all ligands, a nucleophilic hydroxyl group is at the same distance from the metallo-centre (except for the hydroxymethyl group at C9 of **6**).

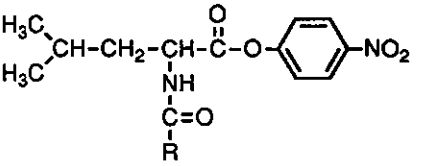
Since **1**, **3**, and **4** are only slightly soluble in water, their catalytic activity was studied in mixed micellar systems, composed of chemically inert surfactant molecules and metal-ion complexes of the lipophilic ligands. The water-soluble ligands **2**, **5**, **6**, and **7** were studied in pure buffer or, in case the substrate was not sufficiently soluble in water, in Brij 35 micelles.

Binding of metal ions to the 1,10-phenanthroline nucleus can be monitored spectrophotometrically.[1] Addition of Zn^{II} to **1-7** shifts the absorbance maximum from 272 to 276 nm and a shoulder appears in the region of 295-300 nm. From the changes in the UV spectra it is shown that in the presence of one equivalent of Zn^{II} complexation of **1-7** is complete, indicating the high metal-ion affinity of these ligands.

Kinetic experiments were performed in pure *N*-ethylmorpholine-HBr buffer or in buffered micelles at pH = 7.00 and 25 °C under pseudo-first-order conditions. The release of *p*-nitrophenolate from the substrate esters was followed spectrophotometrically at 400 nm. First, we investigated the catalytic activity of the synthesised ligands in the hydrolysis of the metallophilic substrate *p*-nitrophenyl picolinate (PNPP, Scheme 5.2). This ester has shown to be a useful substrate in studies of metal-ion activated hydrolysis reactions,[8,9,11] and catalytic data thus obtained could be used for comparison of catalytic efficiency. Pseudo-first-order rate constants for the hydrolysis of PNPP catalysed by Zn^{II} complexes of **1-7** are given in Table 5.1. Pure cationic CTABr micelles exhibit almost no rate-accelerating effect. Large rate enhancements of 250-1400 fold are obtained in the presence of equimolar Zn^{II} and ephedrine-containing ligands (**3** and **4**) or the water-soluble 2-(hydroxymethyl)pyrrolidine ligands (**2**, **5**, **6**, and **7**). The highest rate acceleration (6780 x) is observed in the presence of **1**-Zn^{II}.

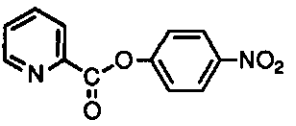


C_{12}-Phe-PNP : R = $(CH_2)_{10}CH_3$



C_{12}-Leu-PNP : R = $(CH_2)_{10}CH_3$

Z-Phe-PNP : R = $OCH_2C_6H_5$



PNPP

Scheme 5.2 The substrates C_{12}-Phe-PNP, Z-Phe-PNP, C_{12}-Leu-PNP, and PNPP

Next, we investigated the catalytic activities of metallo-complexes of **1-7** toward chiral, non-metallophilic substrates, *i.e.*, the N-protected *p*-nitrophenyl esters of D(L)-phenylalanine and D(L)-leucine (Scheme 5.2). Since these substrates are not sufficiently

Table 5.1 Pseudo-first-order rate constants (k_{obs}) for the hydrolysis of PNPP catalysed by different Zn^{II}-ligand complexes.[a]

catalyst	co-micellar additive	$k_{obs}/10^{-3}$ s^{-1}	k_{obs}/k_0
none	none	0.010	1
none	CTABr	0.023	2.3
1-Zn^{II}	CTABr	68.7	6870
2-Zn^{II}	none	2.52	252
3-Zn^{II}	CTABr	2.58	258
4-Zn^{II}	CTABr	3.22	322
5-Zn^{II}	none	2.74	274
6-Zn^{II}	none	14.20	1420
7-Zn^{II}	none	2.88	288

[a] Conditions: 25 °C, pH = 7.00 (0.01 M *N*-ethylmorpholine-HBr buffer), [CTABr] = 4 x 10^{-3} M, [ligand] = 5 x 10^{-4} M, [Zn^{II}] = 5 x 10^{-4} M, and [PNPP] = 5 x 10^{-5} M.

soluble in pure aqueous buffer, in experiments with the hydrophilic metal-ion complexes of **2**, **5**, **6**, and **7**, the substrate was dissolved in solutions of non-ionic Brij 35 micelles. Cationic micelles are not appropriate in this case, because of the electrostatic repulsion of these micelles with the positively charged metal-ion-ligand complex.[11b]

Zn^{II}, Co^{II}, Ni^{II}, and Cd^{II} in the absence of ligand show no catalytic activity toward *p*-nitrophenyl esters of N-protected amino acids, whereas addition of Cu^{II} even retards the rate of spontaneous hydrolysis. Rate enhancements caused by non-metalated ligands are relatively small and enantioselectivities are low. Metal-ion complexes of **1**, however, are efficient catalysts in the hydrolysis of D(L)-Z-Phe-PNP, although this substrate has no strongly metal-ion binding site as is present in PNPP. The metal-ion activation of **1** is in the order of $Zn^{II} > Co^{II} > Cu^{II}$ (Table 5.2). For all **1**-M^{II} complexes, hydrolysis of D-Z-Phe-PNP predominates over that of the L-enantiomer. The degree of enantioselectivity ($k^{D}_{a,obs}/k^{L}_{a,obs}$) is dependent on the nature of the metal ion, and the highest value (4.2) is found for **1**-Co^{II}. The ephedrine-containing metallo-surfactants

Table 5.2 The apparent second-order rate constants ($k_{a,obs}$, M^{-1} s^{-1}) and enantioselectivities ($k^{D}_{a,obs}/k^{L}_{a,obs}$) for the hydrolysis of D(L)-Z-Phe-PNP, catalysed by different metal-ion complexes.[a]

catalyst	co-micellar additive	$k^{D}_{a,obs}$	$k^{L}_{a,obs}$	$k^{D}_{a,obs}/k^{L}_{a,obs}$
1-Zn^{II}	CTABr	37.8	27.3	1.4
1-Co^{II}	CTABr	30.0	7.10	4.2
1-Cu^{II}	CTABr	19.2	6.44	3.0
2-Zn^{II}	Brij 35	0.25	0.22	1.1
3-Zn^{II}	CTABr	3.27	2.63	1.2
3-Co^{II}	CTABr	1.03	0.81	1.3
4-Zn^{II}	CTABr	2.81	5.12	0.55
4-Co^{II}	CTABr	0.80	1.24	0.65
5-Zn^{II}	Brij 35	0.31	0.67	0.46
5-Co^{II}	Brij 35	0.07	0.31	0.23
6-Zn^{II}	Brij 35	0.34	0.29	1.2
7-Zn^{II}	Brij 35	0.35	0.36	0.97

[a] Conditions: 25 °C, pH = 7.00 (0.01 M *N*-ethylmorpholine-HBr buffer), [CTABr] = 4×10^{-3} M, [Brij 35] = 4×10^{-3} M, [ligand] = 5×10^{-4} M, [M^{II}] = 5×10^{-4} M, and [D(L)-Z-Phe-PNP] = 5×10^{-5} M. The first-order rate constants measured in the absence of metallo-catalyst (k_s) are 7.99×10^{-5} s^{-1} and 7.56×10^{-5} s^{-1} for the hydrolysis of D- and L-Z-Phe-PNP, respectively.

(3 and 4) and the water-soluble 2-(hydroxymethyl)pyrrolidine metallo-catalysts (2, 5, 6, and 7) are less reactive and stereoselective compared to 1-M^{II}. 5-Co^{II} hydrolyses the L-substrate 4.4 times faster than the D-substrate; however, the catalytic activity of this metal-ion complex is low. The results indicate that both hydrophobic interactions between substrate and metal-ion catalyst and rigidity of the ligand are important factors for the activity and enantioselectivity.

The effect of the 1-Co^{II} concentration on the rate and enantioselectivity in the hydrolysis of D(L)-Z-Phe-PNP is depicted in Figure 5.1. The rate of hydrolysis of D- and L-Z-Phe-PNP increases linearly with [1-Co^{II}], and over the entire concentration range the D-enantiomer is hydrolysed faster than the L-enantiomer in a ratio of 4 : 1. No saturation kinetics are observed, indicating that the binding constant of D(L)-Z-Phe-PNP to 1-Co^{II} is low.

Substitution of the *N*-benzyloxycarbonyl protecting group of the substrate by the more hydrophobic *N*-dodecanoyl group, results in a substrate with a higher affinity for the micellar phase. Also for these substrates, the lipophilic ligand 1 is hardly active in the absence of a metal ion and the enantioselectivity is relatively low (Table 5.3). In the presence of a metal ion, however, the enantioselectivity of 1 toward D(L)-C_{12}-Phe-PNP is higher compared to that of D(L)-Z-Phe-PNP. The most remarkable feature in the data of Table 5.3 is that the nature of the metal ion determines not only the degree of enantioselectivity, but also the direction of enantioselectivity. 1-Co^{II} and 1-Cu^{II}

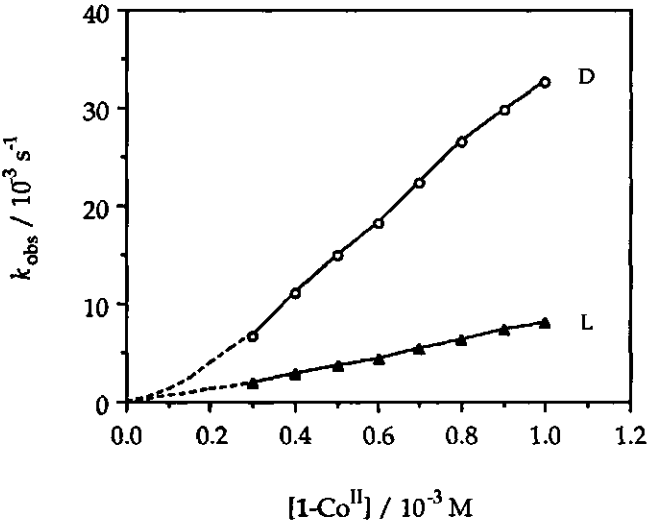


Figure 5.1 Pseudo-first-order rate constants for the hydrolysis of D- and L-Z-Phe-PNP as a function of 1-Co^{II} concentration in CTABr micelles at pH = 7.00 and 25 °C; [CTABr] = 4 x 10^{-3} M, [D(L)-Z-Phe-PNP] = 5 x 10^{-5} M, and [1] : [Co^{II}] = 1.

Table 5.3 The apparent second-order rate constants ($k_{a,obs}$, M^{-1} s^{-1}) and enantioselectivities ($k^D_{a,obs}/k^L_{a,obs}$) for the hydrolysis of D(L)-C_{12}-Phe-PNP and D(L)-C_{12}-Leu-PNP, catalysed by mixed micellar systems composed of **1**-M^{II} and CTABr.[a]

	C_{12}-Phe-PNP			C_{12}-Leu-PNP		
catalyst	$k^D_{a,obs}$	$k^L_{a,obs}$	$k^D_{a,obs}/k^L_{a,obs}$	$k^D_{a,obs}$	$k^L_{a,obs}$	$k^D_{a,obs}/k^L_{a,obs}$
1	1.26	0.64	2.0	1.70	1.88	0.90
1-Zn^{II}	37.3	68.8	0.54	21.1	40.1	0.53
1-Co^{II}	74.0	9.26	8.0	45.9	7.26	6.3
1-Cu^{II}	15.8	3.60	4.4	10.6	3.08	3.4
1-Ni^{II}	0.70	1.40	0.50	0.74	0.52	1.4

[a] Conditions: 25 °C, pH = 7.00 (0.01 M *N*-ethylmorpholine-HBr-buffer); [CTABr] = 4 x 10^{-3} M, [**1**] = 5 x 10^{-4} M, [M^{II}] = 5 x 10^{-4} M, [D(L)-C_{12}-Phe-PNP] = 5 x 10^{-5} M, and [D(L)-C_{12}-Leu-PNP] = 5 x 10^{-5} M. The first-order rate constants measured in the absence of metallo-catalyst (k_s) are 11.1 x 10^{-3} s^{-1} (D-C_{12}-Phe-PNP), 10.7 x 10^{-3} s^{-1} (L-C_{12}-Phe-PNP), 4.59 x 10^{-3} s^{-1} (D-C_{12}-Leu-PNP), and 4.69 x 10^{-3} s^{-1} (L-C_{12}-Leu-PNP).

hydrolyse D-C_{12}-Phe-PNP more rapidly than the L-enantiomer (8.0 and 4.4 times, respectively), whereas **1**-Zn^{II} gives an inversion of the enantioselectivity.

The dependence of the direction of enantioselectivity on the nature of the metal ion in **1**-M^{II} is not limited to D(L)-C_{12}-Phe-PNP as a substrate. With the N-protected aliphatic amino acid ester, D(L)-C_{12}-Leu-PNP, corresponding results are obtained (Table 5.3). Although the rate of hydrolysis and enantioselectivity of D(L)-C_{12}-Leu-PNP are somewhat lower compared to D(L)-C_{12}-Phe-PNP, hydrolysis of D-C_{12}-Leu-PNP predominates over that of the L-enantiomer for **1**-Co^{II} and **1**-Cu^{II}, whereas an inversion of stereoselectivity is found for **1**-Zn^{II}.

The activity of **1**-M^{II} incorporated in non-ionic Brij 35 micelles is shown in Table 5.4. In Brij 35, the rates of the spontaneous hydrolysis of D(L)-C_{12}-Phe-PNP and D(L)-C_{12}-Leu-PNP in the absence of a catalyst are negligible. Comparing the data in CTABr and in Brij 35 (Tables 5.3 and 5.4), the most striking differences are the higher degree of enantioselectivity for the **1**-Co^{II}/Brij 35 system and the absence of inversion of enantioselectivity in the **1**-Zn^{II}/Brij 35 system. These results indicate the important role of the micellar micro-environment on the stereochemical control of the **1**-M^{II} catalysed hydrolysis of long-chain esters. In contrast, the catalytic activity and stereoselectivity of

Table 5.4 The apparent second-order rate constants ($k_{a,obs}$, M^{-1} s^{-1}) and enantioselectivities ($k^D_{a,obs}/k^L_{a,obs}$) for the hydrolysis of D(L)-C_{12}-Phe-PNP and D(L)-C_{12}-Leu-PNP, catalysed by mixed micellar systems composed of **1**-M^{II} and Brij 35.[a]

	C_{12}-Phe-PNP			C_{12}-Leu-PNP		
catalyst	$k^D_{a,obs}$	$k^L_{a,obs}$	$k^D_{a,obs}/k^L_{a,obs}$	$k^D_{a,obs}$	$k^L_{a,obs}$	$k^D_{a,obs}/k^L_{a,obs}$
1-Zn^{II}	44.6	18.4	2.4	22.7	13.0	1.7
1-Co^{II}	77.4	5.07	15.3	28.4	4.14	6.9
1-Cu^{II}	16.1	5.59	2.9	11.9	8.00	1.5
1-Ni^{II}	1.74	1.00	1.7	1.24	0.83	1.5
1-Cd^{II}	1.75	3.24	0.54	1.27	1.72	0.74

[a] Conditions: 25 °C, pH = 7.00 (0.01 M *N*-ethylmorpholine-HBr-buffer); [Brij 35] = 4 x 10^{-3} M, [**1**] = 5 x 10^{-4} M, [M^{II}] = 5 x 10^{-4} M, [D(L)-C_{12}-Phe-PNP] = 5 x 10^{-5} M, and [D(L)-C_{12}-Leu-PNP] = 5 x 10^{-5} M. The first-order rate constants measured in the absence of metallo-catalyst (k_s) are 10.2 x 10^{-5} s^{-1} (D-C_{12}-Phe-PNP), 10.7 x 10^{-5} s^{-1} (L-C_{12}-Phe-PNP), 5.98 x 10^{-5} s^{-1} (D-C_{12}-Leu-PNP), and 6.27 x 10^{-5} s^{-1} (L-C_{12}-Leu-PNP).

1-M^{II} toward D(L)-Z-Phe-PNP and of **4**-M^{II} toward D(L)-C_{12}-Phe-PNP is hardly altered upon changing the co-surfactant CTABr into Brij 35. The metallo-surfactant **1**-Cd^{II}, which is not soluble in CTABr micelles, is only moderately active toward the ester substrates and shows a reverse stereoselectivity.

The pseudo-first-order rate constants of the hydrolysis of D- and L-C_{12}-Phe-PNP as a function of [**1**-Co^{II}] in Brij 35 are plotted in Figure 5.2. In the concentration range [**1**-Co^{II}] = 0.3-1.0 x 10^{-3} M, a linear dependence of k_{obs} *vs.* the concentration of metallo-catalyst is observed for both D- and L-C_{12}-Phe-PNP. Over the whole concentration range the D-enantiomer is hydrolysed 15 times faster than the L-enantiomer. The absence of saturation kinetics indicates that the degree of complex formation of metallo-surfactant and substrate is low.

The effect of variation of the concentration of M^{II} in the **1**-M^{II} catalysed hydrolysis of D-Z-Phe-PNP, at a fixed concentration of **1** is given in Figure 5.3. Initial addition of Zn^{II} to **1** leads to a fast increase in the reaction rate until the ratio reaches unity. Further increment of the Zn^{II} concentration has no effect. For Co^{II} a less pronounced saturation effect is found. Under the conditions of [Co^{II}] = [**1**], the pseudo-first-order rate constant

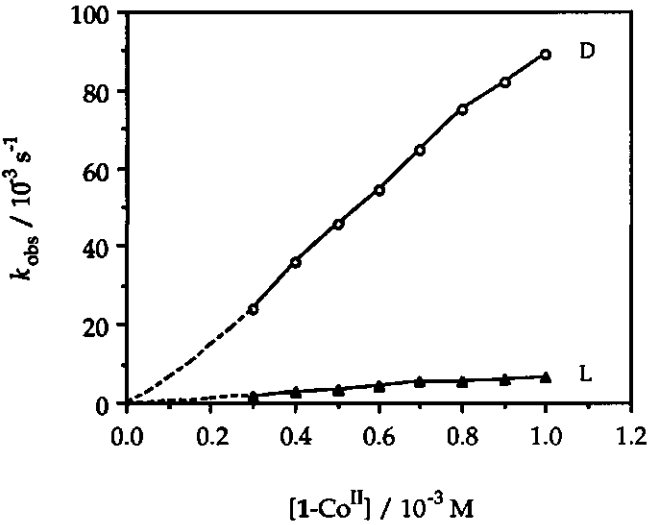


Figure 5.2 Pseudo-first-order rate constants for the hydrolysis of D- and L-C_{12}-Phe-PNP as a function of 1-Co^{II} concentration in Brij 35 micelles at pH = 7.00 and 25 °C; [Brij 35] = 4 x 10^{-3} M, [D(L)-C_{12}-Phe-PNP] = 5 x 10^{-5} M, and [**1**] : [Co^{II}] = 1.

has reached 60% of its maximum value. Increasing the Co^{II} concentration above stoichiometric amounts (*e. g.* [Co^{II}] : [**1**] = 3 : 1) results in a higher rate of hydrolysis for both enantiomers, but does not affect the enantioselective ratio.

The Cu^{II} titration curve is bell shaped with a maximum for [Cu^{II}] : [**1**] = 1. This implies that at low Cu^{II} concentration, Cu^{II} is mainly bound to **1** yielding the catalytically active species 1-Cu^{II}, whereas at a higher concentration the presence of an uncomplexed metal ion has a rate-retarding effect. In contrast to the former bivalent metal ions, addition of Ni^{II} shows only a moderate effect on the rate of hydrolysis.

5.3 Discussion

The Zn^{II} complex of the lipophilic ligand **1** solubilised in CTABr is a very effective catalyst in the hydrolysis of the metallophilic substrate PNPP. Mixed metallo-micelles containing the ephedrine ligands **3** and **4** are about 25 times less active compared to **1**, although for all three ligands the OH group is located at the δ-position in the side group of the 1,10-phenanthroline nucleus. According to CPK models, **1** has a rigid structure; when the nitrogen atom of 2-(hydroxymethyl)pyrrolidine is bound to the metal ion, the structure of the 2-(hydroxymethyl)pyrrolidine group is frozen and the OH group is in

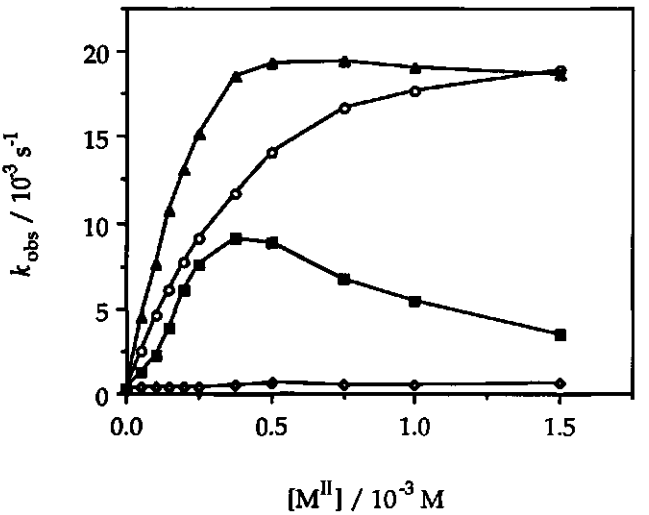


Figure 5.3 Pseudo-first-order rate constants for the hydrolysis of D-Z-Phe-PNP as a function of the metal-ion concentration under a fixed concentration of **1** in CTABr micelles at pH = 7.00 and 25 °C; [CTABr] = 4 x 10^{-3} M, [**1**] = 5 x 10^{-4} M, and [D-Z-Phe-PNP] = 5 x 10^{-5} M, (▲): Zn^{II}, (o): Co^{II}, (■): Cu^{II}, and (◇): Ni^{II}.

close proximity to the metal ion. For the ligands **3** and **4**, binding of the metal ion to the ephedrine nitrogen atom does not induce complete fixation of the side group, but rotation around the C1-C2 axis is still possible. The higher structural flexibility and the more sterically hindered secondary OH group of **3** and **4** are probably the origin of the lower activity of metal-ion complexes of these ligands compared to **1**-M^{II}.

The water-soluble Zn^{II} complexes of **2**, **5**, **6**, and **7**, all containing one or two 2-(hydroxymethyl)pyrrolidine groups, are also less active than the amphiphilic **1**-Zn^{II} catalyst. The higher catalytic activity observed in micellar complexes of ligand **1** compared to their non-micellar analogues may be ascribed to the following: (i) increased concentration of reactants in the micellar pseudo-phase by hydrophobic binding,[12] (ii) increased concentration of OH^- in the CTABr micellar interphase, resulting in a higher concentration of the ligand-oxido anion nucleophile,[8c,9c] (iii) enhanced electrophilicity of the catalytic metal ion due to the positive charge of the Stern layer,[10c] and (iv) formation of exclusively 1 : 1 complexes of the amphiphilic 1,10-phenanthroline ligands with metal ions in micelles, due to the steric requirements of the alkyl chains of the ligand in the micellar phase, whereas in the bulk water phase the water-soluble ligands may also form catalytically inactive 2 : 1 (ligand : M^{II}) complexes.[11b]

The Zn^{II} complexes of **2**, **5**, and **7** are all comparably active in the hydrolysis of PNPP. The similar activity of **2**-Zn^{II} and **7**-Zn^{II} indicates that the presence of the extra chelating (*N*,*N*-dimethylamino)methyl group of **2** has no influence on the rate of hydrolysis. Binding of PNPP to the metal ion of **5**-Zn^{II} is sterically hindered by the presence of two 2-(hydroxymethyl)pyrrolidine groups in the ligand, but apparently this effect is compensated by the extra nucleophilic hydroxyl group. The most active water-soluble catalyst turns out to be **6**-Zn^{II}, presumably because the hydroxymethyl group at the 9 position of the phenanthroline nucleus is in a favourable position for nucleophilic attack in the ternary complex with the substrate.

The effectiveness of the various metallo-catalysts toward D(L)-Z-Phe-PNP as the substrate shows the same pattern as toward PNPP. Metallo-surfactants **1**-M^{II} are highly active, whereas **3**-M^{II} and **4**-M^{II} are less effective. However, mixed metallo-micelles composed of **3**-M^{II} hydrolyse D-Z-Phe-PNP faster than L-Z-Phe-PNP, whereas in the case of the diastereomeric **4**-M^{II} an inversion of stereoselectivity is observed. The relatively low reactivity of the water-soluble metallo-complexes of **2**, **5**, **6**, and **7** toward hydrophobic substrates is caused by the incorporation of the substrate into the micellar phase, whereas the catalyst is distributed over the bulk and the micellar phase. Moreover, complex formation is weak due to the absence of hydrophobic interaction between metallo-catalyst and substrate.

The divalent metal ions Co^{II}, Cu^{II}, and particularly Zn^{II} bind strongly to the 1,10-phenanthroline ligands and have a large effect on the rate of hydrolysis as is shown in Figure 5.3. In the case of Co^{II}, the kinetic titration curve can be described by eqn. (1), where k_{max} is the maximum rate constant under saturation conditions and K_M is the equilibrium constant for the formation of the metal-ion complex [eqn. (2)]. From a least-

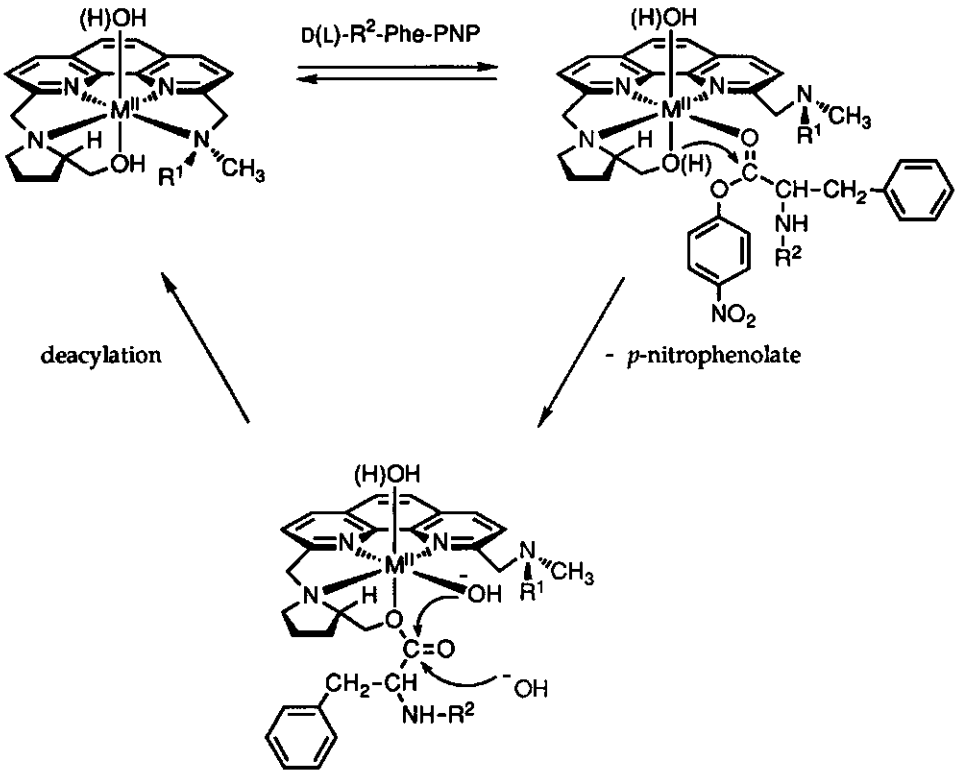
$$k_{obs} = \frac{k_{max} K_M [M^{II}]}{1 + K_M [M^{II}]} \qquad (1)$$

$$K_M = \frac{[\mathbf{1}\text{-}M^{II}]}{[\mathbf{1}] [M^{II}]} \qquad (2)$$

squares analysis of the double reciprocal plot of k_{obs}^{-1} *vs.* $[Co^{II}]^{-1}$, K_M (2.07×10^3 M^{-1}) and k_{max} (26.4×10^{-3} s^{-1}) were established. In the case of **1**-Zn^{II}, the slope of the first part of the graph is too steep to be analysed by eqn. (1), which points to a metal-ion hopping mechanism.[11b] For Co^{II}, this metal-ion hopping mechanism cannot be excluded and therefore the K_M value must be considered as an approximate maximum value. This means that, for example, under the conditions of [**1**] = $[Co^{II}]$ = 0.5 mM maximally 39%

of the ligand is complexed with Co^{II}. The percentage of complexed ligand decreases as the concentrations of metal ion and ligand decrease [eqn. (2)] as is indicated by the dashed lines in the Figures 5.1 and 5.2. The reaction under these conditions proceeds by non-first-order kinetics, and therefore no rate constants have been measured in this concentration range. In the case of the Zn^{II} ions, which bind more strongly, the plots of k_{obs} *vs.* the concentration of lipophilic 1,10-phenanthroline complexes show a linear relationship at low concentration.[11]

A possible mechanism of the 1-M^{II} catalysed hydrolysis of *p*-nitrophenyl esters is schematically represented in Scheme 5.3. This scheme is based on an octahedral geometry of the metal ion, although for Zn^{II}, Co^{II}, Cu^{II}, Ni^{II}, and Cd^{II} several alternatives are possible.[20] For the formation of the reactive ternary complex, one of the ligand-metal-ion bonds must be substituted. This may occur by replacement of the *N*-alkylmethylamino moiety by the carbonyl group of the substrate, as is indicated in Scheme 5.3. These ligand-exchange processes are very fast (10^4-10^8 s^{-1}) compared to the



Scheme 5.3 Mechanism of the 1-M^{II} catalysed hydrolysis of *p*-nitrophenyl esters of N-protected phenylalanine.

rate of hydrolysis.[7] CPK models show that the nitrogen atom of the $R^1(CH_3)NCH_2$ group of the ligand is rather sterically hindered to coordinate to the metal ion, and therefore the function of this group will be primarily to incorporate the ligand into the micellar phase and to direct the approaching substrate. The carbonyl group of the substrate and the nucleophilic hydroxymethyl group should preferably be coordinated to the metal ion in a perpendicular fashion as was previously pointed out for substitutionally inert Co^{III} complexes by Chin *et al.*[21] The pK_a value of the hydroxymethyl group is reduced by coordination to the metal ion, providing a high concentration of effective nucleophile at neutral pH. The binding constant of the pre-equilibrium complexation of 1-M^{II} with the substrate is low since no saturation kinetics are observed in the plots of k_{obs} *vs.* [1-Co^{II}] for D(L)-Z-Phe-PNP (Fig. 5.1) and D(L)-C_{12}-Phe-PNP (Fig. 5.2). The low affinity of the metallo-surfactant to the substrate is due to the absence of a strongly metallophilic moiety in D(L)-Z-Phe-PNP and D(L)-C_{12}-Phe-PNP. In contrast, the binding constants of metal-ion complexes of lipophilic 1,10-phenanthroline derivatives with the metallophilic substrate PNPP are relatively high (K = 1-2 x 10^3 M).[11]

In the ternary complex, nucleophilic attack by the metal-ion activated hydroxyl group on the carbonyl function of the substrate, results in the expulsion of *p*-nitrophenolate and the formation of an acylated intermediate (Scheme 5.3). This intermediate is hydrolysed by attack of a free or metal-ion-bound hydroxide ion to the carbonyl group. In this process the presence of the metal ion stabilises the expulsion of the 2-pyrrolidinemethanolate moiety.[5b]

As a consequence of the weak binding affinity of the amino acid esters to the metallo-catalyst, the enantioselectivity in the hydrolysis is mainly caused by differences in Gibbs free energy of the diastereomeric transition states in the intracomplex transacylation step. In this ternary complex, the motional freedom of the hydroxyl group of the ligand and the substrate is restricted by the template effect of the metal ion. In addition, the coordination geometry of the metal ion in the ternary complex has a large effect on the reaction rate,[8b,9b,11b] as well as on the degree and direction of stereoselectivity. In CTABr micelles, 1-Co^{II} hydrolyses D-C_{12}-Phe-PNP faster than the L-enantiomer, whereas in the case of 1-Zn^{II} an inversion of enantioselectivity is observed. A reverse direction of chiral induction upon changing the metal ion was reported before in the hydrogenation of prochiral alkenes catalysed by chiral Rh^I and Ru^{II} diphosphine complexes.[22] This effect was attributed to a different coordination geometry of Rh^I and Ru^{II} and to a different reaction pathway. In our case, we have no evidence that 1-Co^{II} and 1-Zn^{II} operate *via* a different mechanism. In neutral Brij 35, the enantioselectivity induced by 1-Co^{II} is higher than in the cationic CTABr micelles, whereas no inversion of enantioselectivity is observed for 1-Zn^{II}. This implies that the

coordination geometry of the ternary complex is sensitive to the micro-environment of the micellar interphase.

Although the catalytic unit of **1** is equal to that of **2** (only the alkyl substituent R^1 is different), not only the activity of **1**-M^{II} is higher, but there is also a higher degree of stereoselectivity observed. Consequently, hydrophobic interaction of substrate and ligand in the ternary complex is favourable for a high degree of stereoselectivity,[23] since this introduces an extra orientation requirement between catalyst and substrate. The importance of this factor is illustrated by changing the *N*-benzyloxycarbonyl protecting group of the substrates (R^2) into the more hydrophobic dodecanoyl group, which results in a higher rate of conversion and a larger extent of stereoselectivity.

5.4 Conclusion

Mixed metallo-micelles containing the lipophilic ligand **1** are effective synzymes in the hydrolysis of the metallophilic substrate PNPP and the amino acid esters D(L)-Z-Phe-PNP, D(L)-C_{12}-Phe-PNP, and D(L)-C_{12}-Leu-PNP. In the ternary complex, the substrate is non-covalently bound to the metallo-surfactant by different binding forces. Hydrophobic interaction between alkyl chains of substrate (R^2) and ligand (R^1) in the apolar core of the micelle, and coordination of ligand headgroup and carbonyl group of the substrate to the metal ion at the micellar interphase, yield a highly oriented ternary complex. In this complex, the coordination mode of the metal ion and the rigidity of the functionalised headgroup largely determine the rate and degree of stereoselectivity.

5.5 Experimental Section

General Methods

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 200-E spectrometer operating at 200.1 MHz and chemical shifts are reported relative to internal $(CH_3)_4Si$. ^{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 an 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. The temperature was controlled at 25 ± 0.1 °C.

Materials

$ZnBr_2$, $CuBr_2$, $CoBr_2$, $NiBr_2$, $CdBr_2 \cdot 4\ H_2O$, *N*-ethylmorpholine, CTABr, and Brij 35 were purchased from commercial sources and used without purification. The following compounds were prepared and purified according to literature procedures: *p*-nitrophenyl picolinate (PNPP),[24] *N*-methyldodecylamine,[19] *p*-nitrophenyl *N*-dodecanoyl-D(L)-phenylalaninate [D(L)-C_{12}-Phe-PNP], *p*-nitrophenyl *N*-(benzyloxycarbonyl)-D(L)-phenylalaninate [D(L)-Z-Phe-PNP], and *p*-nitrophenyl *N*-dodecanoyl-D(L)-leucinate [D(L)-C_{12}-Leu-PNP].[25]

General Procedure for the Synthesis of the Chiral Asymmetrically Disubstituted 1,10-Phenanthroline Ligands **1-4**

To a cold solution (0 °C) of 2,9-bis(bromomethyl)-1,10-phenanthroline (**8**),[18] (366 mg, 1.0 mmol) and $(C_2H_5)_3N$ (101 mg, 1.0 mmol) in freshly distilled $CHCl_3$ (15 mL), the dialkylamine (*N*-methyldodecylamine or *N,N*-dimethylamine, 1.0 mmol) dissolved in $CHCl_3$ (5 mL)) was added dropwise. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred at this temperature for a further 4 h. To the reaction mixture was added a solution of the chiral amino alcohol [(S)-2-pyrrolidinemethanol or ephedrine, 1.3 mmol] and $(C_2H_5)_3N$ (151 mg, 1.5 mmol) dissolved in $CHCl_3$ (5 mL). After being stirred for another 16 h at room temperature, the reaction mixture 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 residue was purified by column chromatography [neutral Al_2O_3 (activity III), 0.25% (v/v) $CH_3OH/CHCl_3$].

(S)-1-[[9-[(N-Methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidinemethanol (**1**)

This compound was obtained from the coupling reaction of **8** to *N*-methyldodecylamine and (S)-2-pyrrolidinemethanol as a pale yellow oil (220 mg, 44%): ^{1}H NMR ($CDCl_3$) δ 0.83 [t, J = 6.5 Hz, 3 H, $(CH_2)_{11}CH_3$], 1.20 [s, 18 H, $(CH_2)_9CH_3$], 1.55 [m, 2 H, $NCH_2CH_2(CH_2)_9$], 1.80 [m, 4 H, $(CH_2)_2CH$], 2.29 (s, 3 H, NCH_3), 2.49 [t on m, J = 7.5 Hz, 3 H, $NCH_2(CH_2)_{10}$ and $CH_{2a}(CH_2)_2CH$], 2.79 (m, 1 H, $CHCH_2OH$), 3.10 [m, 1 H, $CH_{2b}(CH_2)_2CH$], 3.49 (dd, J = 3.4, 11.8 Hz, 1 H, $CH_{2a}OH$), 3.70 (dd, J = 2.9, 11.8 Hz, 1 H, $CH_{2b}OH$), 4.01 [s, 2 H, $CH_2N(CH_3)C_{12}H_{25}$], 4.03 (d, J = 14.5 Hz, 1 H, PhenCH_{2a}-2-pyrrolidinemethanol), 4.38 (d, J = 14.5 Hz, 1 H, PhenCH_{2b}-2-pyrrolidinemethanol), 5.13 (br s, 1 H, OH), 7.60 and 7.79 (2d, J = 8.2 Hz, 2 H, Phen H-3 and H-8), 7.70 (s, 2 H, Phen

H-5 and H-6), and 8.16 (d, J = 8.2 Hz, 2 H, Phen H-4 and H-7); ^{13}C NMR ($CDCl_3$) δ 13.85 [$(CH_2)_{11}CH_3$], 22.38, 27.15, 29.05, 29.34, and 31.61 [$(CH_2)_{10}CH_3$], 22.90 and 26.94 (CH_2CH_2CH), 42.36 (NCH_3), 55.12 [$CH_2(CH_2)_2CH$], 57.86 [$CH_2(CH_2)_{10}CH_3$], 60.69 and 62.37 (CH_2OH and PhenCH_2-2-pyrrolidinemethanol), 63.91 [$CH_2N(CH_3)C_{12}H_{25}$], 66.08 ($CHCH_2OH$), 122.07 and 122.24 (Phen C-3 and C-8), 125.35 and 126.65 (Phen C-5 and C-6), 127.37 (Phen C-4a and C-6a), 136.12 and 136.27 (Phen C-4 and C-7), 144.78 and 144.96 (Phen C-10a and C-10b), 159.54 and 160.28 (Phen C-2 and C-9); FDMS: m/z 505 (MH^+).

(S)-1-[[9-[(N,N-Dimethylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidine-methanol **(2)**

Ligand **2** was obtained from the coupling reaction of **8** to *N,N*-dimethylamine and (S)-2-pyrrolidinemethanol as a pale yellow oil (165 mg, 47%): ^{1}H NMR ($CDCl_3$) δ 1.79 [m, 4 H, $(CH_2)_2CH$], 2.34 (s, 6 H, CH_3), 2.44 [m, 1 H, $CH_{2a}(CH_2)_2CH$], 2.78 (m, 1 H, $CHCH_2OH$), 3.07 [m, 1 H, $CH_{2b}(CH_2)_2CH$], 3.49 (dd, J = 3.4, 11.7 Hz, 1 H, $CH_{2a}OH$), 3.69 (dd, J = 3.0, 11.7 Hz, 1 H, $CH_{2b}OH$), 3.95 [s, 2 H, $CH_2N(CH_3)_2$], 4.01 (d, J = 14.5 Hz, 1 H, PhenCH_{2a}-2-pyrrolidinemethanol), 4.36 (d, J = 14.5 Hz, 1 H, PhenCH_{2b}-2-pyrrolidinemethanol), 5.29 (br s, 1 H, OH), 7.59 and 7.72 (2d, J = 8.2 Hz, 2 H, Phen H-3 and H-8), 7.68 (s, 2 H, Phen H-5 and H-6), and 8.14 (d, J = 8.2 Hz, 2 H, Phen H-4 and H-7); ^{13}C NMR ($CDCl_3$) δ 22.99 and 26.95 (CH_2CH_2CH), 45.49 (NCH_3), 55.19 [$CH_2(CH_2)_2CH$], 60.69 and 62.27 (CH_2OH and PhenCH_2-2-pyrrolidinemethanol), 65.71 [$CH_2N(CH_3)_2$], 66.10 ($CHCH_2OH$), 122.31 (Phen C-3 and C-8), 125.54 and 125.73 (Phen C-5 and C-6), 127.40 (Phen C-4a and C-6a), 136.39 (Phen C-4 and C-7), 144.90 (Phen C-10a and C-10b), 159.52 and 159.63 (Phen C-2 and C-9); FDMS: m/z 351 (MH^+).

(1R,2S)-N-[[9-[(N-Methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]ephedrine **(3)**

This compound was obtained as a pale yellow oil (300 mg, 53%) from the coupling reaction of **8** to *N*-methyldodecylamine and (1R,2S)-ephedrine: ^{1}H NMR ($CDCl_3$) δ 0.82 [t, J = 6.4 Hz, 3 H, $(CH_2)_{11}CH_3$], 1.07 (d, J = 6.7 Hz, 3 H, $CHCH_3$), 1.19 [s, 18 H, $(CH_2)_9CH_3$], 1.53 [m, 2 H, $NCH_2CH_2(CH_2)_9$], 2.25 and 2.31 (2s, 6 H, 2 NCH_3), 2.45 [t, J = 7.6 Hz, 2 H, $NCH_2(CH_2)_{10}$], 2.96 (m, 1 H, $CHCH_3$), 3.94 [d, J = 14.5 Hz, 1 H, $CH_{2a}N(CH_3)C_{12}H_{25}$], 4.02 [d, J = 14.5 Hz, 1 H, $CH_{2b}N(CH_3)C_{12}H_{25}$], 4.11 (d, J = 15.2 Hz, 1 H, PhenCH_{2a}-ephedrine), 4.23 (d, J = 15.2 Hz, 1 H, PhenCH_{2b}-ephedrine), 5.01 (d, J = 4.1 Hz, 1 H, $CHOH$), 5.24 (br s, 1 H, OH), 7.26 (m, 5 H, C_6H_5), 7.44 and 7.72 (2d, J = 8.3 Hz, 2 H, Phen H-3 and H-8), 7.65 (s, 2 H, Phen H-5 and H-6), 8.06 and 8.12 (2d, J = 8.3 Hz, 2 H, Phen H-4 and H-7); ^{13}C NMR ($CDCl_3$) δ 9.68 ($CHCH_3$), 13.91 [$(CH_2)_{11}CH_3$], 22.47, 26.79, 27.28, 29.13, 29.43, and 31.69 [$(CH_2)_{10}CH_3$], 39.86 (CH_3NCH), 49.65

($CH_3NC_{12}H_{25}$), 54.33 [$CH_2(CH_2)_{10}CH_3$], 60.50 and 61.02 (2 PhenCH_2N), 64.95 ($CHCH_3$), 73.90 (CHOH), 121.73 and 122.19 (Phen C-3 and C-8), 125.28, 125.68, 126.07, 126.43, and 127.66 (Phenyl C-2, C-3, and C-4, Phen C-5 and C-6), 127.36 (Phen C-4a and C-6a), 135.99 and 136.30 (Phen C-4 and C-7), 143.20 (Phenyl C-1), 144.84 and 145.11 (Phen C-10a and C-10b), 160.63 and 161.89 (Phen C-2 and C-9); FDMS: *m/z* 569 (MH^+).

(1S,2S)-N-*[[9-[(N-Methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]ephedrine* (4)

This ligand was obtained as a pale yellow oil (210 mg, 37%) from the coupling reaction of **8** to *N*-methyldodecylamine and (1S,2S)-ephedrine: ^{1}H NMR ($CDCl_3$) δ 0.84 [t, J = 6.8 Hz, 3 H, $(CH_2)_{11}CH_3$], 0.90 (d, J = 6.6 Hz, 3 H, $CHCH_3$), 1.21 [s, 18 H, $(CH_2)_9CH_3$], 1.58 [m, 2 H, $NCH_2CH_2(CH_2)_9$], 2.29 and 2.37 (2s, 6 H, 2 NCH_3), 2.48 [t, J = 7.7 Hz, 2 H, $NCH_2(CH_2)_{10}$], 2.87 (m, 1 H, $CHCH_3$), 3.36 (br s, 1 H, OH), 3.99 [s, 2 H, $CH_2N(CH_3)C_{12}H_{25}$], 4.12 (d, J = 14.8 Hz, 1 H, PhenCH_{2a}-ephedrine), 4.31 (d, J = 14.8 Hz, 1 H, PhenCH_{2b}-ephedrine), 4.39 (d, J = 9.7 Hz, 1 H, CHOH), 7.29 (m, 5 H, C_6H_5), 7.71 and 7.89 (2d, J = 8.3 Hz, 2 H, Phen H-3 and H-8), 7.75 (s, 2 H, Phen H-5 and H-6), 8.18 and 8.27 (2d, J = 8.3 Hz, 2 H, Phen H-4 and H-7); ^{13}C NMR ($CDCl_3$) δ 7.78 ($CHCH_3$), 13.92 [$(CH_2)_{11}CH_3$], 22.46, 26.96, 27.27, 29.12, 29.41, and 31.68 [$(CH_2)_{10}CH_3$], 36.41 (CH_3NCH), 42.36 ($CH_3NC_{12}H_{25}$), 57.99 [$CH_2(CH_2)_{10}CH_3$], 60.01 and 64.23 (2 PhenCH_2N), 65.62 ($CHCH_3$), 74.92 (CHOH), 121.60 and 122.46 (Phen C-3 and C-8), 125.61, 125.88, 127.17, 127.51, and 127.99 (Phenyl C-2, C-3, and C-4, Phen C-5 and C-6), 127.63 (Phen C-4a and C-6a), 136.24 and 136.75 (Phen C-4 and C-7), 141.64 (Phenyl C-1), 145.09 (Phen C-10a and C-10b), 159.83 and 160.04 (Phen C-2 and C-9); FDMS: *m/z* 569 (MH^+).

(S)-1-[[9-[((S)-2-(Hydroxymethyl)pyrrolidinyl)methyl]-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidinemethanol (5)

To a solution of **8** (366 mg, 1.0 mmol) and $(C_2H_5)_3N$ (220 mg, 2.2 mmol) in $CHCl_3$ (20 mL) at 0 °C, (S)-2-pyrrolidinemethanol (220 mg, 2.2 mmol) dissolved in $CHCl_3$ (5 mL) was added dropwise. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred for a further 3 h. 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 [neutral Al_2O_3 (activity III), 1% (v/v) $CH_3OH/CHCl_3$]. Ligand **5** (386 mg, 95%) was obtained as a pale red powder, m.p. 152 °C (decomp.): ^{1}H NMR ($CDCl_3$) δ 1.83 [m, 8 H, $(CH_2)_2CH$], 2.48 (m, 2 H, $NCH_{2a}CH_2$), 2.81 (m, 2 H,

C*H*CH_2OH), 3.12 (m, 2 H, NC$H_{2b}CH_2$), 3.52 (dd, J = 3.9, 12.0 Hz, 2 H, CH_{2a}OH), 3.70 (dd, J = 2.9, 12.0 Hz, 2 H, CH_{2b}OH), 3.99 (d, J = 14.2 Hz, 2 H, PhenCH_{2a}N), 4.42 (d, J = 14.2 Hz, 2 H, PhenCH_{2b}N), 4.60 (br s, 2 H, OH), 7.58 (d, J = 8.2 Hz, 2 H, Phen H-3), 7.73 (s, 2 H, Phen H-5), and 8.18 (d, J = 8.2 Hz, 2 H, Phen H-4); ^{13}C NMR ($CDCl_3$) δ 22.91 and 26.87 (CH_2CH_2CH), 55.45 (NCH_2CH_2), 60.82 and 62.56 (CH_2OH and PhenCH_2N), 66.17 ($CHCH_2OH$), 122.50 (Phen C-3), 125.62 (Phen C-5), 127.43 (Phen C-4a), 136.36 (Phen C-4), 144.92 (Phen C-10b), and 159.44 (Phen C-2); FDMS: m/z 407 (MH^+).

(S)-1-[[9-(Hydroxymethyl)-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidinemethanol (6)

To a solution of 2-(bromomethyl)-9-(hydroxymethyl)-1,10-phenanthroline (9),[11b] (606 mg, 2.0 mmol) and $(C_2H_5)_3N$ (220 mg, 2.2 mmol) in $CHCl_3$ (20 mL), (S)-2-pyrrolidinemethanol (220 mg, 2.2 mmol) dissolved in $CHCl_3$ (5 mL) was added dropwise. After being stirred at room temperature for 3 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 [neutral Al_2O_3 (activity III), 1% (v/v) $CH_3OH/CHCl_3$]. Ligand 6 (594 mg, 92%) was obtained as a pale yellow thick oil which solidified at -20 °C: ^{1}H NMR ($CDCl_3$) δ 1.80 [m, 4 H, (CH_2)$_2$CH], 2.72 (m, 1 H, NC$H_{2a}CH_2$), 3.03 (m, 1 H, CHCH_2OH), 3.23 (m, 1 H, NC$H_{2b}CH_2$), 3.70 (dd, J = 6.0, 11.8 Hz, 1 H, CHCH_{2a}OH), 3.85 (dd, J = 2.8, 11.8 Hz, 1 H, CHCH_{2b}OH), 4.18 (d, J = 15.5 Hz, 1 H, PhenCH_{2a}N), 4.39 (d, J = 15.5 Hz, 1 H, PhenCH_{2b}N), 5.01 (d, J = 16.7 Hz, 1 H, PhenCH_{2a}OH), 5.09 (d, J = 16.7 Hz, 1 H, PhenCH_{2b}OH), 7.44 and 7.49 (2d, J = 8.3 Hz, 2 H, Phen H-3 and H-8), 7.73 (s, 2 H, Phen H-5 and H-6), 8.15 and 8.19 (2d, J = 8.3 Hz, 2 H, Phen H-4 and H-7); ^{13}C NMR ($CDCl_3$) δ 22.61 and 26.98 (CH_2CH_2CH), 55.20 (NCH_2CH_2), 60.10, 63.24, and 64.86 (PhenCH_2OH, $CHCH_2OH$, and PhenCH_2N), 66.28 ($CHCH_2OH$), 120.28 and 122.48 (Phen C-3 and C-8), 125.37 and 125.76 (Phen C-5 and C-6), 127.38 (Phen C-4a and C-6a), 136.35 and 136.71 (Phen C-4 and C-7), 143.98 and 144.45 (Phen C-10a and C-10b), 158.87 and 160.13 (Phen C-2 and C-9); FDMS: m/z 324 (MH^+).

(S)-1-[(1,10-Phenanthrolin-2-yl)methyl]-2-pyrrolidinemethanol (7)

To a stirred solution of 2-chloromethyl-1,10-phenanthroline (10),[11b] (457 mg, 2.0 mmol) and $(C_2H_5)_3N$ (220 mg, 2.2 mmol) in $CHCl_3$ (30 mL), (S)-2-pyrrolidinemethanol (220 mg, 2.2 mmol) dissolved in $CHCl_3$ (5 mL) was added dropwise. The reaction mixture was kept at room temperature for 16 h. 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 [neutral Al_2O_3

(activity III), 1% (v/v) $CH_3OH/CHCl_3$]. Ligand 7 (486 mg, 83%) was obtained as a yellow oil which solidified at -20 °C: ^1H NMR ($CDCl_3$) δ 1.77 [m, 4 H, $(CH_2)_2CH$], 2.46 (m, 1 H, $NCH_{2a}CH_2$), 2.80 (m, 1 H, $CHCH_2OH$), 3.05 (m, 1 H, $NCH_{2b}CH_2$), 3.43 (dd, J = 3.0, 11.2 Hz, 1 H, $CH_{2a}OH$), 3.58 (dd, J = 3.5, 11.2 Hz, 1 H, $CH_{2b}OH$), 3.93 (br s, 1 H, OH), 4.10 (d, J = 14.9 Hz, 1 H, $PhenCH_{2a}N$), 4.33 (d, J = 14.9 Hz, 1 H, $PhenCH_{2b}N$), 7.50 (dd, J = 4.4, 8.1 Hz, 1 H, Phen H-8), 7.62 and 7.67 (2d, J = 8.8 Hz, 2 H, Phen H-5 and H-6), 7.69 (d, J = 8.3 Hz, 1 H, Phen H-3), 8.11 (d, J = 8.3 Hz, 1 H, Phen H-4), 8.12 (dd, J = 1.8, 8.1 Hz, 1 H, Phen H-7), and 9.10 (dd, J = 1.8, 4.4 Hz, 1 H, Phen H-9); ^{13}C NMR ($CDCl_3$) δ 23.16 and 27.05 (CH_2CH_2CH), 54.95 (NCH_2CH_2), 60.95 and 62.02 (CH_2OH and $PhenCH_2N$), 66.08 ($CHCH_2OH$), 122.26 and 122.68 (Phen C-3 and C-8), 125.90 and 126.06 (Phen C-5 and C-6), 127.35 and 128.55 (Phen C-4a and C-6a), 135.86 and 136.42 (Phen C-4 and C-7), 145.01 and 145.48 (Phen C-10a and C-10b), 149.86 (Phen C-9), and 158.99 (Phen C-2); FDMS: m/z 294 (MH^+).

Kinetic Studies

CTABr and Brij 35 micellar solutions were prepared in *N*-ethylmorpholine-HBr buffer pH = 7.00. Each kinetic run was initiated by injecting an acetonitrile solution (0.01 M) of substrate ester into a 1-cm cuvette, containing 2 mL of buffered micellar solution and the desired concentrations of metal ion and ligand. Pseudo-first-order rate constants for the hydrolysis of substrate ester were determined by monitoring the release of *p*-nitrophenolate at 400 nm, under the conditions of excess of catalyst over substrate. Reactions were generally followed for at least 10 half-lives. Pseudo-first-order rate constants were obtained from linear plots of ln (A_∞ - A_t) *vs.* time for at least 3 half-lives. Kinetic runs, carried out in triplicate, gave rate constants with an uncertainty of less than 3%.

The apparent second-order rate constants ($k_{a,obs}$) were calculated from $k_{a,obs} = (k_{complex} - k_s)/[complex]_0$, where $k_{complex}$ and k_s refer to the observed pseudo-first-order rate constants for the hydrolysis of the ester substrates in the presence and absence of ligand-metal-ion complex, respectively.

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Chapter 6

Hydrolysis of *p*-Nitrophenyl Esters of Picolinic Acid and N-Protected Amino Acids by Metallo-Enzyme Models in Vesicular Assemblies*

6.1 Introduction

Biological membranes are involved in most of the fundamental functions of cells, such as compartmentalisation, photochemical solar energy conversion, selective uptake of solutes, signal transduction, and maintenance of transmembrane potentials. Stable bilayer membranes formed from a variety of synthetic amphiphiles have been the subject of extensive model studies to obtain insight into the structure, physiochemical properties, and functioning of biological membranes.[1] Particularly, non-protein mixed vesicles composed of long-chain dialkyl surfactants and functionalised amphiphatic molecules have been applied in mimicking membrane-mediated processes.[2] The highly ordered dialkyl bilayer membranes provide a unique micro-environment for reactions.[3] In biomimetic chemistry, functionalised vesicles have been used as enzyme models for clarifying the origin of catalytic effects and specificity of native enzymes. In the last decade, several reports have appeared on the hydrolysis of carboxylic and phosphoric esters by amphiphilic imidazole,[4] thiol,[5] and hydroxamate nucleophiles.[6] High stereoselectivity was observed in the deacylation of long-chain substrate esters, such as *p*-nitrophenyl *N*-dodecanoyl-D(L)-phenylalaninate with histidine-containing di- and tripeptide catalysts, and stereochemical control could be established by changing the composition of the co-aggregates.[7]

In chapter 5, we have demonstrated that chiral 1,10-phenanthroline derivatives in micelles are effective catalysts for the enantioselective hydrolysis of *p*-nitrophenyl esters of N-protected amino acids.[8] The direction and magnitude of enantioselective catalysis was remarkably dependent on the metal-ion species and on the micellar co-surfactant.

Metallo-vesicles are composed of a ligand amphiphile, with the metal ion coordinated to the hydrophilic headgroup which extrudes into the aqueous phase.[9] As model systems for hydrolytic metallo-enzymes, Cu^{II}-containing metallo-vesicles have been investigated as catalysts in the hydrolysis of *p*-nitrophenyl picolinate (PNPP).[10]

* Adopted from: J. G. J. Weijnen, A. Koudijs, P. G. J. A. Tap and J. F. J. Engbersen, Hydrolysis of *p*-Nitrophenyl Esters of Picolinic Acid and N-Protected Amino Acids by Metallo-Enzyme Models in Vesicular Assemblies, *Recl. Trav. Chim. Pays-Bas*, accepted for publication.

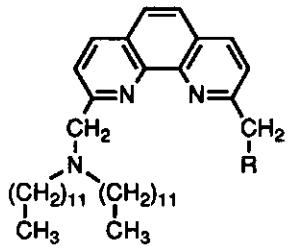
However, no studies have been yet undertaken with respect to the enantioselectivity of hydrolytic metallo-vesicles. This chapter describes the catalytic activity and enantioselectivity of four new amphiphilic ligands toward PNPP and *p*-nitrophenyl esters of N-protected leucine [D(L)-Z-Leu-PNP and D(L)-C_{12}-Leu-PNP] in a vesicular matrix in the presence of divalent metal ions. It was expected that these co-aggregates would provide solvolytic matrices of pertinent hydrophobicity with proper fluidity for the substrate absorption, while maintaining a suitable micro-environment for catalysis.

6.2 Results and Discussion

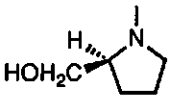
The lipophilic ligands **1-4** (Scheme 6.1), which contain the strongly chelating 1,10-phenanthroline moiety and two long alkyl chains, were prepared analogously to the ligands described in the chapters 4 and 5.[8b,11] The rigid 2-(hydroxymethyl)pyrrolidine moiety of ligand **2** contains one chiral centre, whereas the more flexible ephedrine ligands (**3** and **4**) possess two chiral centres. All ligands are essentially insoluble in aqueous media, even in the presence of metal ions. Stable vesicles containing **1-4** are obtained by embedding the ligands in a chemically inert membrane matrix of the amphiphile *N*-dodecyl-*N*,*N*-dimethyl-1-octadecanaminium bromide ($C_{18}C_{12}$, Scheme 6.1). The formation of closed vesicular aggregates was verified by electron microscopy. We chose $C_{18}C_{12}$ as co-surfactant because in water $C_{18}C_{12}$ forms optically clear vesicular solutions,[12] which is a requisite for following spectrophotometrically the catalytic activity of the metal-ion-ligand complexes. The rates of hydrolysis were determined by monitoring the release of *p*-nitrophenolate at 400 nm from the substrate esters (Scheme 6.1). The vesicular co-aggregates composed of $C_{18}C_{12}$ and ligand-metal-ion complexes displayed good reproducibility in kinetic experiments.

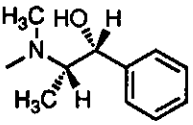
Table 6.1 gives the pseudo-first-order rate constants (k_{obs}) for the hydrolysis of the metallophilic ester PNPP and the lipophilic ester PNPO under the conditions of excess of metal-ion-ligand complex over substrate. The ammonium bilayer composed of $C_{18}C_{12}$ shows only a small rate-enhancing effect in the hydrolysis of PNPP (entries 1 and 2). The metal ions Zn^{II}, Co^{II}, and Ni^{II} in the absence of ligand as well as non-metalated **1** exhibit only a moderate rate-enhancing effect on the hydrolysis of PNPP.[13] However, large rate augmentations (of up to 1600 times in the case of **1**-Co^{II}), require the presence of equimolar amounts of metal ion and ligand. For the lipophilic ligand **1** incorporated into $C_{18}C_{12}$ vesicles, the activity order of metal ions is: $Co^{II} > Zn^{II} > Ni^{II}$. Addition of excess of metal ion to **1** (*e.g.* $[M^{II}] : [1] = 3 : 1$) only slightly increases the rate of hydrolysis, demonstrating the high affinity of divalent metal ions for ligand **1**.

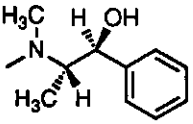
ligands:

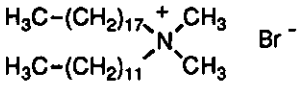


1: R = OH

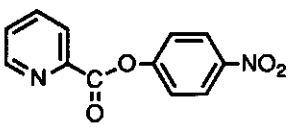
2: R =  (S)-2-pyrrolidinemethanol

3: R =  (1R,2S)-ephedrine

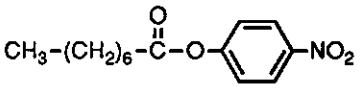
4: R =  (1S,2S)-ephedrine

co-surfactant: 

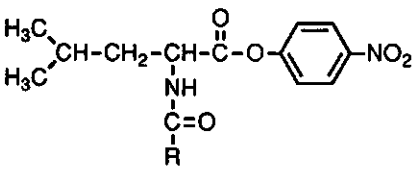
$C_{18}C_{12}$

substrates: 

PNPP



PNPO



C_{12}-Leu-PNP : R = $(CH_2)_{10}CH_3$

Z-Leu-PNP : R = $OCH_2C_6H_5$

Scheme 6.1 Ligands, co-surfactant, and substrates

Table 6.1 Pseudo-first-order rate constants ($k_{obs}/10^{-5}$ s^{-1}) for the hydrolysis of PNPP in mixed vesicular systems.[a]

entry	catalyst	co-vesicular additive	substrate	$k_{obs}/10^{-5}$ s^{-1}
1	none	none	PNPP	1.0
2	none	$C_{18}C_{12}$	PNPP	3.9
3	**1**	$C_{18}C_{12}$	PNPP	42.0
4	**1**-Zn^{II}	$C_{18}C_{12}$	PNPP	962
5	**1**-Co^{II}	$C_{18}C_{12}$	PNPP	1560
6	**1**-Ni^{II}	$C_{18}C_{12}$	PNPP	300
7	none	$C_{18}C_{12}$ [b]	PNPO	1.1
8	**1**-Zn^{II}	$C_{18}C_{12}$	PNPO	89.3
9	**1**-Co^{II}	$C_{18}C_{12}$	PNPO	325

[a] Conditions: 25 °C, pH = 7.00 (0.01 M *N*-ethylmorpholine-HBr buffer), $[C_{18}C_{12}] = 4 \times 10^{-3}$ M, $[\mathbf{1}] = 5 \times 10^{-4}$ M, $[M^{II}] = 5 \times 10^{-4}$ M, [PNPP] = 5×10^{-5} M, and [PNPO] = 5×10^{-5} M.
[b] PNPO is insoluble in buffer in the absence of $C_{18}C_{12}$.

The lipophilic ester PNPO, which lacks a metal-ion coordinating site, is also readily hydrolysed by **1**-M^{II}, which indicates the importance of substrate binding to the catalyst by hydrophobic interactions.

The k_{obs} values represent the rate constants for the transesterification of *p*-nitrophenyl esters by **1**-M^{II}, in which the hydroxyl group of **1** is acylated and concomitantly *p*-nitrophenolate is released. Development of an efficient catalytic process dictates that the metal-ion promoted hydrolysis of the acylated intermediate, to regenerate the free hydroxyl function, should be fast. We tested the turn-over behaviour of **1**-Zn^{II} under the conditions of excess of substrate over metallo-catalyst. As is shown in Figure 6.1, the release of *p*-nitrophenolate obeys dichotomic kinetics, which indicates that the acylation step is fast and the deacylation step is relatively slow. The amount of *p*-nitrophenolate obtained by extrapolating the steady-state line to time = 0 corresponds to the initial concentration of **1**-Zn^{II}.** This indicates that all nucleophilic metallo-complexes, located at both the extravesicular side and the endovesicular side are

** In the steady-state of the reaction, after *ca.* 45 min, the production of *p*-nitrophenolate does not follow complete zero-order kinetics (*i.e.* a straight line in Fig. 6.1 above 45 min) because picolinate is produced, which inhibits the acylation step by binding to the metallo-catalyst.

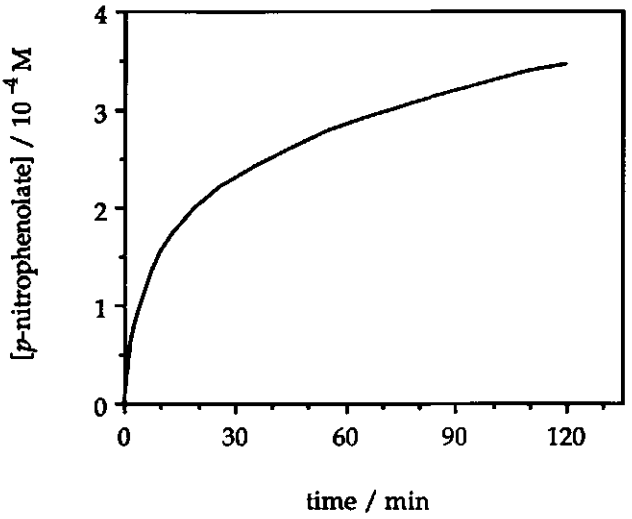


Figure 6.1 Time course for *p*-nitrophenolate release from PNPP as catalysed by **1**-Zn^{II} in $C_{18}C_{12}$ vesicles under the condition of [substrate] > [metallo-catalyst] at pH = 7.00 and 25 °C; $[C_{18}C_{12}] = 4 \times 10^{-3}$ M, [**1**] = 2×10^{-4} M, $[Zn^{II}] = 2 \times 10^{-4}$ M, and [PNPP] = 6×10^{-4} M.

involved in the hydrolysis of PNPP. Consequently, fast permeation of Zn^{II} and PNPP across the lipid bilayer must occur, because metal ion and substrate are added to the vesicular solution after sonication. Neutral *p*-nitrophenyl esters can readily diffuse across the vesicular membrane at rates faster than the metallo-surfactant catalysed hydrolysis,[1b,10b] whereas the transport of the metal ion is probably facilitated by the ligand, which acts as an ionophore. In this process, the metal ion binds to the ligand at the extravesicular side of the bilayer and, subsequently, transverse migration of the metallo-complex to the interior leaflet takes place.[10b,14]

Next, we investigated the effect of the temperature on the rate of metallo-surfactant catalysed hydrolysis of PNPP over the temperature range 2.5 - 45 °C. Figure 6.2 shows the Arrhenius plots of the catalysed hydrolysis of PNPP performed by the co-aggregate systems **1**-$Zn^{II}/C_{18}C_{12}$ and **1**-$Co^{II}/C_{18}C_{12}$. The Arrhenius plots show an inflection point at 24 °C for both the **1**-Zn^{II} and the **1**-Co^{II} catalysed reactions, which is referred to as the kinetic-phase transition temperature. In general, inflection points in Arrhenius plots of homo-vesicles and mixed vesicular systems correspond reasonably well with the crystalline-gel to liquid-crystalline phase-transition temperature (T_c) determined by

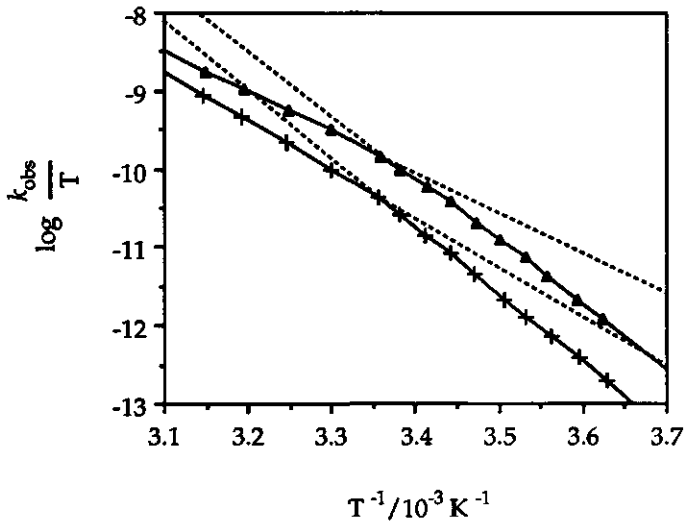


Figure 6.2 Arrhenius plots for the hydrolysis of PNPP as catalysed by **1**-Zn^{II} (+) and **1**-Co^{II} (▲) in $C_{18}C_{12}$ vesicles at pH = 7.00 and 25 °C; $[C_{18}C_{12}] = 4 \times 10^{-3}$ M, [**1**] = 5×10^{-4} M, $[Zn^{II}] = 5 \times 10^{-4}$ M, $[Co^{II}] = 5 \times 10^{-4}$ M, and [PNPP] = 5×10^{-5} M.

various physical techniques, including differential scanning calometry.[3,15-17,***] At temperatures above T_c, the fluidity of the vesicular membrane increases and more importantly, also the micro-environment at the vesicular interface, where hydrolysis takes place, changes.[17]

The catalytic activities of Zn^{II} complexes of the chiral ligands **2**-**4** in co-aggregate systems toward PNPP are reported in Table 6.2. The 2-(hydroxymethyl)pyrrolidine substituted metallo-surfactant **2**-Zn^{II} is a very effective catalyst in the esterolysis of PNPP. Entries 1 and 2 show that the catalytic activity is dependent on the nature of the co-surfactant aggregate. In the presence of $C_{18}C_{12}$ vesicles, the activity of **2**-Zn^{II} is 3.8 times higher than that in CTABr (cetyltrimethylammonium bromide) micelles, which indicates that the micro-environment of the vesicular assemblies is more effective in esterolysis than that of the micelles. Mixed metallo-vesicles of the ephedrine-containing ligands **3** and **4** (entries 3 and 4) are less active than **2**-Zn^{II}, although the nucleophilic hydroxyl group is at the same distance from the metallo-centre. The lower activity of **3** and **4** is attributed to the higher flexibility of the ephedrine moiety compared to the

*** For $C_{18}C_{12}$ vesicles T_c (29 °C) could only be measured when the sonicated solution was slowly cooled to -50 °C. However, the T_c values of frozen samples of dialkylammonium bilayers are higher than those of the corresponding sonicated samples.[16]

Table 6.2 Pseudo-first-order rate constants ($k_{obs}/10^{-5}$ s^{-1}) for the hydrolysis of PNPP catalysed by different Zn^{II}-ligand complexes.[a]

entry	catalyst	additive	$k_{obs}/10^{-5}$ s^{-1}
1	2-Zn^{II}	$C_{18}C_{12}$	15700
2	2-Zn^{II}	CTABr	4170
3	3-Zn^{II}	$C_{18}C_{12}$	120
4	4-Zn^{II}	$C_{18}C_{12}$	955

[a] Conditions: 25 °C, pH = 7.00 (0.01 M *N*-ethylmorpholine-HBr buffer), [$C_{18}C_{12}$] = 4 x 10^{-3} M, [ligand] = 5 x 10^{-4} M, [Zn^{II}] = 5 x 10^{-4} M, and [PNPP] = 5 x 10^{-5} M.

more rigid 2-(hydroxymethyl)pyrrolidine group and to the lower nucleophilicity of the more hindered secondary OH group in **3** and **4**. It is noteworthy that the activity of **4**-Zn^{II} is 8 times higher than that of **3**-Zn^{II}, as the only difference between the diastereomeric ligands **3** and **4** is the configuration of C1 of the ephedrine moiety (Scheme 6.1). The metallo-complex **2**-Zn^{II} is also more active toward PNPP than **1**-Zn^{II} (entry 4, Table 6.1). This may be explained by the different activities of the nucleophilic hydroxyl groups in both catalysts. Activation of the hydroxyl group demands ligation to the metal ion, which is strongly bound to the 1,10-phenanthroline nitrogen atoms. According to CPK models, coordination of the OH group to the metal ion in **1**-Zn^{II} requires the formation of a strained and distorted five-membered chelate ring, whereas in the case of **2**-Zn^{II} a less strained seven-membered ring can be formed. This highlights the fact that the catalytic activity of these metallo-surfactants is ruled by a subtle combination of metal-ion activation, orientation of ligand and substrate, and the micro-environment of the reaction site.[8]

The activity and enantioselectivity of metallo-complexes of **2-4** in vesicular systems toward the chiral substrate *p*-nitrophenyl *N*-(benzyloxycarbonyl)-D(L)-leucinate [D(L)-Z-Leu-PNP] are given in Table 6.3. In the absence of ligands, addition of Zn^{II}, Co^{II}, Cu^{II}, and Ni^{II} has no effect on the rate of hydrolysis of D(L)-Z-Leu-PNP, because presumably, the substrate is mainly incorporated into the bilayer system, whereas the metal ions reside in the bulk aqueous phase. The kinetic data in Table 6.3 show that the rate enhancements by the non-metalated ligands are relatively small and that the enantioselectivities are low. However, in the presence of equimolar amounts of ligand and metal ion, a large rate-accelerating effect in the hydrolysis of D(L)-Z-Leu-PNP is observed, despite the fact that D(L)-Z-Leu-PNP does not possess a metal-ion binding

Table 6.3 The apparent second-order rate constants ($k_{a,obs}/M^{-1}\ s^{-1}$)[a] and enantioselectivities ($k^L_{a,obs}/k^D_{a,obs}$) for the hydrolysis of D(L)-Z-Leu-PNP catalysed by metal-ion complexes in vesicular assemblies.[b]

entry	catalyst	$k^L_{a,obs}$	$k^D_{a,obs}$	$k^L_{a,obs}/k^D_{a,obs}$
1	**2**	1.19	0.95	1.25
2	**2**-Zn^{II}	126.0	97.2	1.59
3	**2**-Co^{II}	77.1	44.3	1.74
4	**2**-Cu^{II}	13.0	21.4	0.61
5	**2**-Ni^{II}	1.67	1.72	0.97
6	**3**-Zn^{II}	2.71	3.99	0.68
7	**4**-Zn^{II}	41.4	43.3	0.96
8	**4**-Co^{II}	9.72	21.9	0.44

[a] $k_{a,obs} = (k_{obs} - k_s)/[complex]_0$, where k_{obs} and k_s denote the observed pseudo-first-order rate constants in the presence and absence of ligand-metal-ion-complex, respectively, and $[complex]_0$ indicates the initial metallo-complex concentration. k_s = $8.4 \times 10^{-5}\ s^{-1}$ for both enantiomers of [D(L)-Z-Leu-PNP].
[b] Conditions as described in Table 6.2, [D(L)-Z-Leu-PNP] = 5×10^{-5} M.

site near the reacting ester bond. For ligand **2** the activation by metal ions is in the order of $Zn^{II} > Co^{II} > Cu^{II} > Ni^{II}$. The degree and direction of the enantioselectivity caused by **2**-M^{II} are dependent on the metal-ion species. In the case of **2**-Zn^{II} and **2**-Co^{II}, the rate of hydrolysis of L-Z-Leu-PNP predominates over that of D-Z-Leu-PNP, whereas for **2**-Cu^{II} and **2**-Ni^{II} an inversion of enantioselectivity is observed: the D-enantiomer is hydrolysed faster than the L-enantiomer. The ephedrine-containing metallo-surfactants **3**-M^{II} and **4**-M^{II} are less reactive than **2**-M^{II}, but **4**-Co^{II} exhibits the highest enantioselectivity toward D(L)-Z-Leu-PNP; the D-enantiomer is hydrolysed 2.2 times faster than the L-enantiomer.

Substitution of the *N*-benzyloxycarbonyl group of D(L)-Z-Leu-PNP by the *N*-dodecanoyl group enhances the hydrophobicity of the N-protecting group and increases the fraction of substrate bound to the vesicles. Table 6.4 presents the second-order rate constants and enantioselectivities of metallo-complexes of **2**-**4** toward D(L)-C_{12}-Leu-PNP in surfactant aggregates. Among the metallo-complexes, **2**-Zn^{II} shows the highest activity and enantioselectivity toward the ester substrate. The enantioselectivity of the hydrolysis of the lipophilic D(L)-C_{12}-Leu-PNP by **2**-Zn^{II} in $C_{18}C_{12}$ vesicles

Table 6.4 The apparent second-order rate constants ($k_{a,obs}/M^{-1}\ s^{-1}$)[a] and enantioselectivities ($k^L_{a,obs}/k^D_{a,obs}$) for the hydrolysis of D(L)-C_{12}-Leu-PNP catalysed by different metal-ion complexes.[b]

entry	catalyst	additive	$k^L_{a,obs}$	$k^D_{a,obs}$	$k^L_{a,obs}/k^D_{a,obs}$
1	2-Zn^{II}	CTABr	71.9	23.2	3.10
2	2-Zn^{II}	$C_{18}C_{12}$	254	71.1	3.57
3	2-Zn^{II}	$C_{18}C_{12}$ [c]	81.9	23.8	3.44
4	2-Zn^{II}	$C_{18}C_{12}$/cholesterol [d]	234	64.8	3.61
5	2-Co^{II}	$C_{18}C_{12}$	74.0	73.8	1.00
6	2-Cu^{II}	$C_{18}C_{12}$	16.2	23.8	0.68
7	3-Zn^{II}	$C_{18}C_{12}$	7.74	4.64	1.67
8	4-Zn^{II}	$C_{18}C_{12}$	30.3	30.1	1.01

[a] $k_{a,obs} = (k_{obs} - k_s)/[complex]_0$, $k_s = 5.24 \times 10^{-3}\ s^{-1}$ for both enantiomers of [D(L)-C_{12}-Leu-PNP] and $[complex]_0 = 5 \times 10^{-4}$ M.
[b] Conditions as described in Table 6.2, [D(L)-C_{12}-Leu-PNP] = 5×10^{-5} M.
[c] 14 °C
[d] [cholesterol] = 8×10^{-4} M

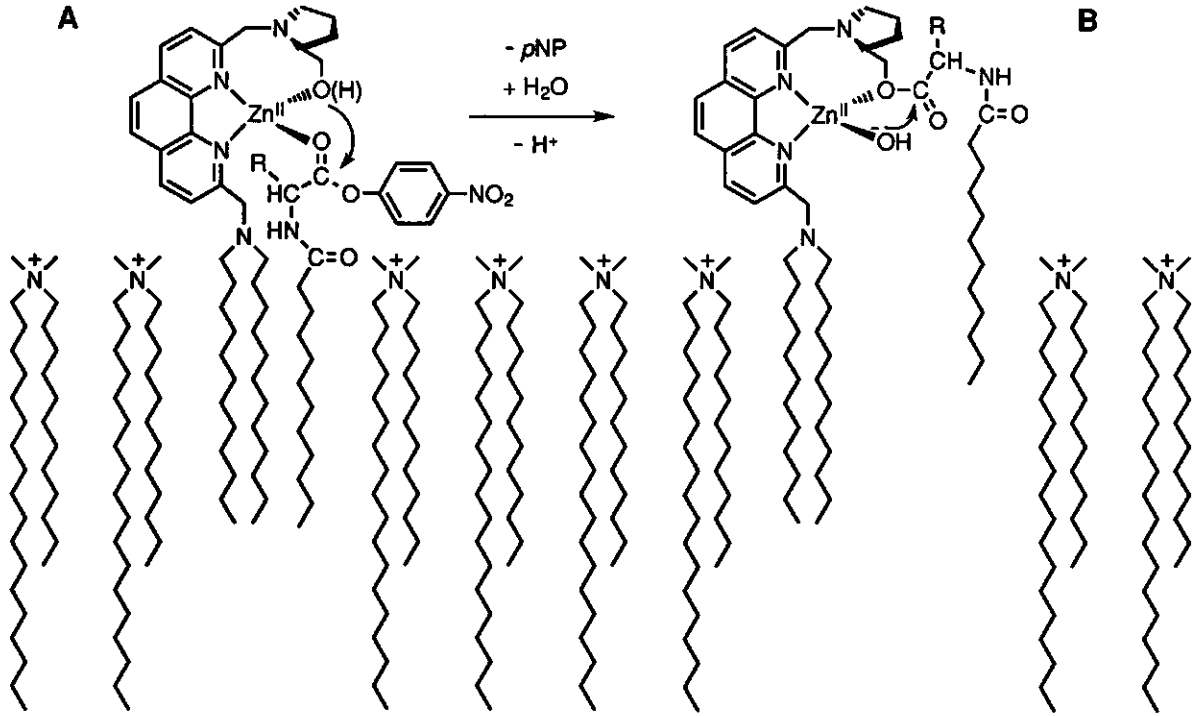
($k^L_{a,obs}/k^D_{a,obs}$ = 3.57, Table 6.4, entry 2) is considerably larger compared to the hydrolysis of D(L)-Z-Leu-PNP ($k^L_{a,obs}/k^D_{a,obs}$ = 1.59, Table 6.3, entry 2). In contrast, no chiral discrimination is observed in the 2-Co^{II} catalysed hydrolysis of D(L)-C_{12}-Leu-PNP (Table 6.4, entry 5), whereas 2-Cu^{II} shows the same stereoselective behaviour toward both Z- and C_{12}-substituted leucine ester substrates (Table 6.4, entry 6 and Table 6.3, entry 4). The enantioselective hydrolysis catalysed by 2-M^{II}, 3-M^{II}, and 4-M^{II} is not restricted to the aliphatic amino acid ester D(L)-C_{12}-Leu-PNP, but appears to have a wider scope. With the substrate D(L)-C_{12}-Phe-PNP, in which the isobutyl residue of D(L)-C_{12}-Leu-PNP is substituted by a benzyl group, corresponding results are obtained.

In CTABr micelles, the activity of 2-Zn^{II} is 3.3 times lower than in $C_{18}C_{12}$ vesicles, but the enantioselectivity remains about the same (Table 6.4, entries 1 and 2). Lowering the temperature of the mixed vesicular system composed of 2-Zn^{II} and $C_{18}C_{12}$ to 14 °C, which is below the inflection point in the Arrhenius plot, results in a proportional reduction of the rate of hydrolysis of both enantiomers (entry 3). It is known that the presence of cholesterol affects the fluidity of dialkylammonium membranes and may increase the activity of membrane-bound catalysts.[18] In the catalytic system 2-Zn^{II}/$C_{18}C_{12}$ addition of cholesterol, up to 0.8 mM, has no influence on the catalytic

efficiency and stereoselectivity (entry 4). The absence of effects on the enantioselectivity by temperature variation, cholesterol addition, and substitution of the vesicular matrix into the micellar matrix suggests that catalysis takes place in the aqueous interface of the surfactant aggregate and that the fluidity of the membrane matrix plays a minor role.

A schematic representation of the **2**-Zn^{II} catalysed hydrolysis of long-chain amino acid esters in vesicles is shown in Scheme 6.2. The apolar hydrocarbon parts of the amphiphilic catalyst and substrate are embedded in the lipophilic core of the dialkylammonium bilayer, whereas the polar parts protrude into the aqueous pseudo-phase. Upon complexation with metal ions, the tendency of the headgroup of the catalyst to remain in the aqueous pseudo-phase is enhanced due to the increased hydrophilicity and the electrostatic repulsion between the membrane surface and the chelated headgroup. The ternary complex of substrate and metallo-catalyst is formed by coordination of the carbonyl group of the substrate to the metal ion and the association of the hydrophobic parts of substrate and ligand into the apolar core of the membrane. Since no differences in affinity of the metallo-surfactants for both enantiomers of the substrate in the ground state were observed,[8b] the enantioselectivity must originate from dissimilarities in free energies of the transition states of both diastereomeric complexes. In the ternary complex, the metal ion activates the hydroxyl group of the ligand for intracomplex nucleophilic attack on the carbon atom of the polarised C=O bond (A, Scheme 6.2). The metal ion freezes the motional freedom of nucleophile and substrate in the ternary complex and consequently, the coordination geometry of the metal ion determines the catalytic efficiency and enantioselectivity. For example, it is most likely that the metal ion in the ternary complex of **2**-Zn^{II} adopts a tetrahedral geometry,[19a] and **2**-Zn^{II} catalyses the hydrolysis of L-C_{12}-Leu-PNP more rapidly than the D-enantiomer. In contrast, **2**-Cu^{II} in which the metal ion probably has a square-planar coordination mode,[19] is less active and shows an inverse preference for the enantiomeric substrates. The metal-ion catalysed deacylation step of the transacylation product to regenerate the catalyst (B, Scheme 6.2), is relatively slow.[11,20] The function of the metal ion in this process is the activation of the nucleophile (H_2O or OH^-) and the stabilisation of the hydroxymethyl leaving group in the transition state, although kinetic equivalent mechanistic pathways cannot be excluded.

In summary, the results presented in this chapter reveal that metallo-complexes of **1-4** incorporated into the $C_{18}C_{12}$ vesicular matrix are efficacious catalysts in the hydrolysis of the metallophilic substrate PNPP and of the N-protected amino acid esters D(L)-Z-Leu-PNP and D(L)-C_{12}-Leu-PNP. The substrate is hydrolysed by the acylation-deacylation mechanism with metallo-surfactants located at both the exo- and



Scheme 6.2 Mechanism of 2-Zn^{II} catalysed hydrolysis of C_{12}-Leu-PNP in one leaflet of a $C_{18}C_{12}$ membrane.

endovesicular side of the bilayer membrane. The chelated headgroups of the amphiphilic catalysts reside in the aqueous interface, whereas the alkyl chains are anchored in the membrane matrix. The rate of hydrolysis and the enantioselectivity caused by the metallo-surfactants are dependent on the position of the nucleophilic hydroxyl moiety in the ligand headgroup and on the mode of complexation of the metal ion. In the case of 2-Zn^{II} catalysed hydrolysis of D(L)-C_{12}-Leu-PNP, the composition of the matrix has no influence on the enantioselectivity, but the rate of hydrolysis is about 3.5 times higher in metallo-vesicles compared to metallo-micelles.

6.3 Experimental Section

General Methods

^{1}H NMR spectra were recorded on a Bruker AC 200-E spectrometer operating at 200.1 MHz and the chemical shifts are reported relative to internal $(CH_3)_4Si$. Proton-decoupled ^{13}C NMR spectra and multiplicity analysis (by DEPT) were recorded on the same spectrometer operating at 50.3 MHz. ^{13}C NMR shifts were measured relative to $CDCl_3$. Mass spectral data were recorded on an AEI MS 902 spectrometer equipped with a VG ZAB console using field desorption ionisation technique. Electron micrographs were taken on a JEOL 1200EX transmission electron microscope by means of positive-staining with uranyl acetate. Kinetic measurements were run on a Beckman DU-7 spectrophotometer equipped with a thermostatted cell compartment and a kinetic device.

Materials

$ZnBr_2$ (Janssen Chimica), $CuBr_2$ (Baker), $CoBr_2$ and $NiBr_2$, (Alfa Products), *N*-ethylmorpholine (Janssen Chimica), and CTABr (Merck) were used without further purification. The following compounds were prepared and purified following literature procedures: *N*-dodecyl-*N*,*N*-dimethyl-1-octadecanaminium bromide,[12] *p*-nitrophenyl picolinate (PNPP),[21] *p*-nitrophenyl *N*-(benzyloxycarbonyl)-D(L)-leucinate [D(L)-Z-Leu-PNP], and *p*-nitrophenyl *N*-dodecanoyl-D(L)-leucinate [D(L)-C_{12}-Leu-PNP].[22]

2-[(N,N-Didodecylamino)methyl]-9-(hydroxymethyl)-1,10-phenanthroline (1)

To a stirred solution of 2-(bromomethyl)-9-(hydroxymethyl)-1,10-phenanthroline[11] (606 mg, 2.0 mmol) and Et_3N (250 mg, 2.47 mmol) in 60 mL of freshly distilled $CHCl_3$,

didodecylamine (707 mg, 2.0 mmol) dissolved in 20 mL of $CHCl_3$ was added dropwise. After stirring under N_2 at 40 °C for 16 h, the reaction mixture 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 residue was purified by column chromatography [neutral Al_2O_3 (activity III), 0.25% (v/v) $CH_3OH/CHCl_3$]. Ligand **1** (0.97 g, 84%) was obtained as a white, waxy solid. ^1H NMR ($CDCl_3$): δ 0.82 (t, 6H, *J* 6.3 Hz, CH_3), 1.19 [s, 36H, $(CH_2)_9CH_3$], 1.43 [m, 4H, $CH_2(CH_2)_9CH_3$], 2.45 (t, 4H, *J* 7.2 Hz, CH_2CH_2N), 4.03 (s, 2H, $PhenCH_2N$), 5.09 (s, 2H, CH_2OH), 7.61 and 7.90 (2d, 2H, *J* 8.2 Hz, H-3 and H-8), 7.63 and 7.68 (2d, 2H, *J* 8.9 Hz, H-5 and H-6), 8.10 and 8.13 (2d, 2H, *J* 8.2 Hz, H-4 and H-7). ^{13}C NMR ($CDCl_3$): δ 13.88 (CH_3), 22.45, 26.77, 27.26, 29.11, 29.40, 31.67 [$(CH_2)_{10}CH_3$], 54.34 [$NCH_2(CH_2)_{10}$], 60.97 ($PhenCH_2N$), 65.21 (CH_2OH), 120.19 and 122.52 (C-3 and C-8), 125.41 and 125.78 (C-5 and C-6), 127.49 (C-4a and C-6a), 136.19 and 136.45 (C-4 and C-7), 144.84 and 144.93 (C-10a and C-10b), 160.10 and 161.68 (C-2 and C-9). FDMS: *m/z* 575 (M^+).

General Procedure for the Synthesis of Ligands **2-4**

To a stirred solution of 2,9-bis(bromomethyl)-1,10-phenanthroline[23] (366 mg, 1.0 mmol) and Et_3N (101 mg, 1.0 mmol) in freshly distilled $CHCl_3$ (20 mL), didodecylamine (353 mg, 1.0 mmol) dissolved in $CHCl_3$ (20 mL) was added dropwise. After 6 h at room temperature, the amino alcohol [(S)-2-pyrrolidinemethanol or ephedrine, 1.5 mmol] and Et_3N (151 mg, 1.5 mmol) dissolved in $CHCl_3$ (5 mL) were added to the reaction mixture. The solution was stirred for an additional 16 h and then washed with water containing 5% (w/v) $NaHCO_3$ and 2% (w/v) EDTA, dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by column chromatography (neutral Al_2O_3, activity III, $CHCl_3$).

(S)-1-[[9-[(N,N-Didodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidine-methanol **(2)**

This ligand was obtained as a pale yellow, waxy solid (340 mg, 52%). ^1H NMR ($CDCl_3$): δ 0.84 (t, 6H, *J* 6.4 Hz, CH_3), 1.21 [s, 36H, $(CH_2)_9CH_3$], 1.48 [m, 4H, $CH_2(CH_2)_9CH_3$], 1.84 [m, 4H, $(CH_2)_2CH$], 2.54 [m, 5H, $NCH_2(CH_2)_{10}$ and $CH_{2a}(CH_2)_2CH$], 2.82 (m, 1H, $CHCH_2OH$), 3.15 [m, 1H, $CH_{2b}(CH_2)_2CH$], 3.50 (dd, 1H, *J* 3.2, 11.6 Hz, $CH_{2a}OH$), 3.69 (dd, 1H, *J* 2.9, 11.6 Hz, $CH_{2b}OH$), 4.09 [s, 2H, $CH_2N(C_{12}H_{25})_2$], 4.14 (d, 1H, *J* 14.9 Hz, $PhenCH_{2a}$-2-pyrrolidinemethanol), 4.35 (d, 1H, *J* 14.9 Hz, $PhenCH_{2b}$-2-pyrrolidine-methanol), 7.63 and 7.92 (2d, 2H, *J* 8.2 Hz, H-3 and H-8), 7.68 and 7.73 (2d, 2H, *J* 9.7 Hz, H-5 and H-6), 8.16 and 8.17 (2d, 2H, *J* 8.2 Hz, H-4 and H-7). ^{13}C NMR ($CDCl_3$): δ 13.88

(CH_3), 22.44, 26.72, 27.27, 29.11, 29.40, 31.67 [$(CH_2)_{10}CH_3$], 23.03 and 27.12 [$(CH_2)_2CH$], 54.31 [$NCH_2(CH_2)_{10}$], 55.28 [$CH_2(CH_2)_2CH$], 60.77 and 62.27 (CH_2OH and $PhenCH_2$-2-pyrrolidinemethanol), 61.09 [$CH_2N(C_{12}H_{25})_2$], 66.28 ($CHCH_2OH$), 122.17 and 122.40 (C-3 and C-8), 125.25 and 125.82 (C-5 and C-6), 127.42 (C-4a and C-6a), 136.10 and 136.35 (C-4 and C-7), 144.76 and 145.07 (C-10a and C-10b), 159.31 and 161.73 (C-2 and C-9). FDMS: *m/z* 659 (MH^+).

(1R,2S)-N-*[[9-[(N,N-Didodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]ephedrine* (3)

This ligand was obtained as a white waxy solid (260 mg, 36%). ^1H NMR ($CDCl_3$): δ 0.84 [t, 6H, *J* 6.4 Hz, $(CH_2)_{11}CH_3$], 1.09 (d, 3H, *J* 6.8 Hz, $CHCH_3$), 1.20 [s, 36H, $(CH_2)_9CH_3$], 1.47 [m, 4H, $CH_2(CH_2)_9CH_3$], 2.36 (s, 3H, NCH_3), 2.48 [t, 4H, *J* 7.1 Hz, $NCH_2(CH_2)_{10}$], 3.00 (m, 1H, $CHCH_3$), 4.04 [d, 1H, *J* 12.9 Hz, $CH_{2a}N(C_{12}H_{25})_2$], 4.11 [d, 1H, *J* 12.9 Hz, $CH_{2b}N(C_{12}H_{25})_2$], 4.14 (d, 1H, *J* 15.4 Hz, $PhenCH_{2a}$-ephedrine), 4.27 (d, 1H, *J* 15.4 Hz, $PhenCH_{2b}$-ephedrine), 4.63 (br s, 1H, OH), 5.03 (d, 1H, *J* 4.1 Hz, CHOH), 7.29 (m, 5H, C_6H_5), 7.50 and 7.88 (2d, 2H, *J* 8.3 Hz, Phen H-3 and H-8), 7.67 and 7.71 (2d, 2H, *J* 9.1 Hz, Phen H-5 and H-6), 8.11 and 8.15 (2d, 2H, *J* 8.3 Hz, Phen H-4 and H-7). ^{13}C NMR ($CDCl_3$): δ 9.66 ($CHCH_3$), 13.91 [$(CH_2)_{11}CH_3$], 22.47, 26.81, 27.28, 29.13, 29.43, 31.69 [$(CH_2)_{10}CH_3$], 39.79 (NCH_3), 54.33 [$NCH_2(CH_2)_{10}$], 60.46 and 61.03 ($PhenCH_2N$), 64.93 ($CHCH_3$), 73.93 (CHOH), 121.72 and 122.16 (Phen C-3 and C-8), 125.27 and 125.66 (Phen C-5 and C-6), 126.08, 126.40, and 127.64 (Phenyl C-2, C-3, and C-4), 127.35 (Phen C-4a and C-6a), 135.97 and 136.27 (Phen C-4 and C-7), 143.30 (Phenyl C-1), 144.84 and 145.10 (Phen C-10a and C-10b), 160.67 and 161.91 (Phen C-2 and C-9). FDMS: *m/z* 722 (M^+).

(1S,2S)-N-*[[9-[(N,N-Didodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]ephedrine* (4)

Ligand 4 was obtained as a white waxy solid (260 mg, 36%). ^1H NMR ($CDCl_3$): δ 0.84 [t, 6H, *J* 6.6 Hz, $(CH_2)_{11}CH_3$], 0.89 (d, 3H, *J* 6.7 Hz, $CHCH_3$), 1.20 [s, 36H, $(CH_2)_9CH_3$], 1.49 [m, 4H, $CH_2(CH_2)_9CH_3$], 2.37 (s, 3H, NCH_3), 2.51 [t, 4H, *J* 7.4 Hz, $NCH_2(CH_2)_{10}$], 2.88 (m, 1H, $CHCH_3$), 4.09 [s, 2H, $CH_2N(C_{12}H_{25})_2$], 4.13 (d, 1H, *J* 13.8 Hz, $PhenCH_{2a}$-ephedrine), 4.33 (d, 1H, *J* 13.8 Hz, $PhenCH_{2b}$-ephedrine), 4.39 (d, 1H, *J* 9.6 Hz, CHOH), 5.32 (br s, 1H, OH), 7.31 (m, 5H, C_6H_5), 7.74 (s, 2H, Phen H-5 and H-6), 7.89 and 7.90 (2d, 2H, *J* 8.3 Hz, Phen H-3 and H-8), 8.18 and 8.26 (2d, 2H, *J* 8.3 Hz, H-4 and H-7). ^{13}C NMR ($CDCl_3$): δ 7.73 ($CHCH_3$), 13.90 [$(CH_2)_{11}CH_3$], 22.45, 27.01, 27.24, 29.12, 29.41, 31.67 [$(CH_2)_{10}CH_3$], 36.49 (NCH_3), 54.35 [$NCH_2(CH_2)_{10}$], 60.10 and 61.24 ($PhenCH_2N$), 65.63 ($CHCH_3$), 74.91 (CHOH), 121.13 and 121.94 (Phen C-3 and C-8), 125.28 and 125.90 (Phen C-5 and C-6), 127.13, 127.45, and 127.93 (Phenyl C-2, C-3, and C-4, and Phen C-4a

and C-6a), 136.00 and 136.58 (Phen C-4 and C-7), 141.69 (Phenyl C-1), 144.94 and 145.18 (Phen C-10a and C-10b), 159.68 and 162.28 (Phen C-2 and C-9). FDMS: *m/z* 722 (M^+).

Kinetic Studies

Vesicle solutions were prepared by injection of an ethanolic solution of 0.4 M $C_{18}C_{12}$/0.05 M ligand into 0.01 M *N*-ethylmorpholine-HBr buffer pH = 7.00 at 50 °C, followed by sonication for 6-15 min at 75 W and 50 °C (Sonics & Materials Inc.). After addition of the metal ion, the vesicle solutions were allowed to cool slowly to the desired temperature. Each kinetic run was initiated by adding an acetonitrile solution of the substrate ester into a 1-cm cuvette containing 2 mL of the mixed vesicular solution. Pseudo-first-order rate constants for the hydrolysis of substrate ester were determined by monitoring the release of *p*-nitrophenolate at 400 nm, under the conditions of excess of catalyst over substrate. Reactions were generally followed for at least 10 half-lives. Pseudo-first-order rate constants were obtained from linear plots of ln (A_∞ - A_t) *vs.* time for at least 3 half-lives. Kinetic runs carried out in triplicate gave rate constants with an uncertainty of less than 3%.

6.4 References

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Chapter 7

Catalytic Hydrolysis of Phosphate Esters by Metallo-Complexes of 1,10-Phenanthroline Derivatives in Micellar Solution*

7.1 Introduction

The chemistry of nucleophilic displacement reactions of phosphorus (V) centres has been studied extensively due to its relevance to biological systems. Many enzymes which catalyse phosphoryl group transfer reactions require divalent metal ions for their activity.[1] In order to study the role of metal ions in the hydrolysis of phosphate esters, model systems for metallo-phosphatases have been designed and tested for catalytic activity. The functions of the metal ion in biomimetic models are to bring together the substrate and nucleophile through formation of a *cis*-coordination complex, partially screen the negative charge of mono- and diesters, polarise the phosphoryl group, activate the nucleophile, and stabilise the leaving group.[2-5] Studies of various metal-ion-ligand complexes have revealed that the coordination geometry of the metal-ion complex is an important factor for the activity of these catalysts.[5a] One of the functions of the ligand is to keep the metal ion in solution under alkaline conditions by preventing formation of metal hydroxide precipitates. Additionally, the ligand effects a stronger binding affinity of the metal-ion-ligand complex for substrate molecules compared to the non-ligated metal ion, despite the reduced number of available binding sites for the substrate.[6] Moreover, water bound to a ligated metal ion has a lower pK_a value than water bound to a non-ligated metal ion,[6a,7] and consequently, the nucleophilicity of the H_2O-M^{II}-ligand complex is higher.

As substrate for metallo-enzyme models, various phosphate esters and anhydrides have been employed, such as activated phosphate esters,[2,8] biologically important molecules such as ATP,[9] DNA and RNA strands,[10] and man-made phosphorus (V) toxins.[7] The hydrolysis of activated phosphate triesters is of considerable interest because of their relevance to the detoxification of acetylcholinesterase-inhibiting insecticides and chemical warfare agents.[6a,11] In order to bring about binding of the neutral phosphate triesters to the enzyme model in close proximity to the metallo-

* Adopted from: J. G. J. Weijnen and J. F. J. Engbersen, Catalytic Hydrolysis of Phosphate Esters by Metallo-Complexes of 1,10-Phenanthroline Derivatives in Micellar Solution, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 351-357.

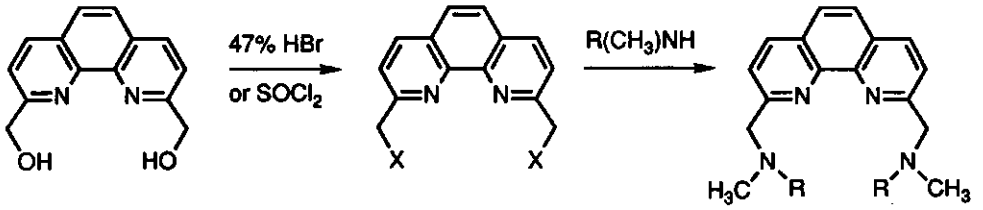
catalytic centre, the metal-ion chelating unit was covalently bound to cyclodextrins,[12] polymers,[13] and amphiphiles in micelles.[3,6b,7,14]

In the chapters 3-6, we have demonstrated that metal-ion complexes of functionalised 1,10-phenanthroline ligands are effective catalysts in the hydrolysis of carboxylic and phosphoric esters.[6b,15] In this chapter, we report on the synthesis of the water-soluble ligand **C_1Phen** (Scheme 7.1), which contains the strongly chelating 1,10-phenanthroline nucleus and its amphiphilic counterpart **C_{12}Phen**. The esterolytic activity of these ligands in the presence of metal ions toward phosphate esters has been studied. The efficacy of esterolysis has been improved by incorporation of the metallo-catalyst and substrate into micelles. Since micelles exhibit similarities in structure and catalytic properties to enzymes, these surfactant aggregates have often been used to mimic biological systems.[16] We have also tried to improve the substrate-catalyst-binding affinity, which is a key feature in enzyme mimetic processes. Introduction of a metallophilic binding site near the reactive group has been shown to enhance reactivity due to increased formation of the reactive ternary complex.[17] However, a potential drawback of such an approach is that reaction products may also bind strongly to the metallo-catalyst, thereby reducing the turn-over capacity of the catalyst, as was found in the hydrolysis of phosphate mono- and diesters by substitutionally inert Co^{III} complexes.[2] In order to study the effect of the introduction of an additional binding site in the substrates, we have prepared phosphate triesters with a metal-ion binding moiety near the P=O bond (Scheme 7.2: **2a**, **2b**, and **3**) and have compared their reactivities with the non-chelating *p*-nitrophenyl phosphate triesters.

7.2 Results and Discussion

The 1,10-phenanthroline derivatives **C_1Phen** and **C_{12}Phen** were prepared according to the procedures outlined in Scheme 7.1. The bis(chloromethyl) derivative **5** was obtained from 2,9-bis(hydroxymethyl)-1,10-phenanthroline by a modified procedure using $SOCl_2$.[18] This compound is a potentially interesting building block for metallo-receptors. The phosphate triesters (Scheme 7.2) were synthesised by reaction of diphenyl or diethyl chlorophosphate with the appropriate phenol derivative. In the cases of **2a**, **2b**, and **3**, phosphorylation takes place exclusively at the O position of 5-nitro-2-pyridinol and 5-nitro-8-quinolinol, as was established by ^{1}H NMR, ^{13}C NMR, IR, and UV spectroscopy.[19]

Since the metallo-complexes **C_{12}Phen**-M^{II} dispersed in water do not form clear and stable micellar solutions, their catalytic properties were studied using mixed micelles,



5: X = Cl

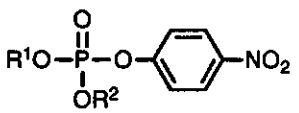
6: X = Br

C_1Phen: R = CH_3

C_{12}Phen: R = $(CH_2)_{11}CH_3$

Scheme 7.1 Synthesis of the ligands **C_1Phen** and **C_{12}Phen**

composed of **C_{12}Phen-M^{II}** and an inert co-surfactant. As a co-surfactant we selected Brij 35 [$C_{12}H_{25}(OCH_2CH_2)_{23}OH$], because this neutral surfactant shows no effect on the rate of hydrolysis of **1b**.[3] Cationic CTABr [$CH_3(CH_2)_{15}N(CH_3)_3Br$] micelles catalyse the hydrolysis of **1b**, whereas anionic micelles of sodium dodecyl sulphate and the uncharged surfactant Igepal show an inhibiting effect.[20] **C_1Phen-M^{II}** is well-soluble in

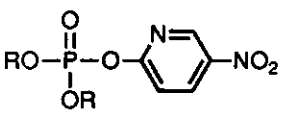


1a: $R^1 = R^2 = CH_2CH_3$

1b: $R^1 = R^2 = C_6H_5$

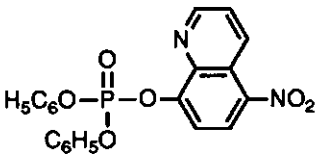
1c: R^1 = H, $R^2 = C_6H_4NO_2(p)$

1d: $R^1 = R^2$ = H



2a: R = CH_2CH_3

2b: R = C_6H_5



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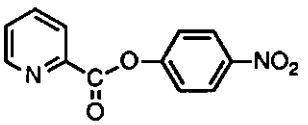


4

Scheme 7.2 Substrates

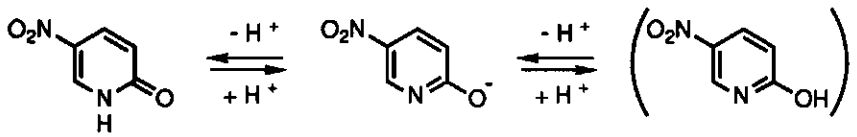
water and the activity of **C_1Phen-M^{II}** has been studied in aqueous buffer solution in the absence of micelles. The rates of hydrolysis were determined spectrophotometrically by monitoring the release of 4-nitrophenolate (400 nm), 5-nitro-2-pyridinolate (355 nm), or 5-nitro-8-quinolinolate (449 nm) from the phosphate esters.

The effect of micellar aggregation on the catalytic activity of the phenanthroline metallo-catalysts can be estimated by comparison of the reactions in micelles with similar reactions in non-micellar media. Using this technique, the catalytic activity of **C_1Phen-M^{II}** in an aqueous buffer solution and that of the lipophilic analogue **C_{12}Phen-M^{II}** in Brij 35 micelles were compared in the hydrolysis of the water-soluble phosphate triesters **1a** and **2a** (Scheme 7.2). Table 7.1 shows representative pseudo-first-order rate constants obtained under conditions of excess of metallo-catalyst over the phosphate triesters. The rate of spontaneous hydrolysis (k_0 value) of **2a** is higher than that of **1a**. This seems to be unexpected in view of the higher basicity of the leaving group for **2a**,[21] as the pK_a values of 5-nitro-2-pyridinol and 4-nitrophenol are 8.04 and 7.15, respectively. However, this apparent deviation may be rationalised by the fact that the pK_a value of 5-nitro-2-pyridinol is predominantly based upon protonation to and from the nitrogen of the cyclic amide tautomer [Schemes 7.3 (a) and 7.3 (**b**)], whereas upon leaving group expulsion the incipient negative charge is on the oxygen. The pK_a value for the hypothetical equilibrium of proton transfer from and to the oxygen atom of 5-nitro-2-pyridinol would be expected to be lower than that of 4-nitrophenol due to

Table 7.1 Pseudo-first-order rate constants (k_{obs} / 10^{-5} s^{-1}) for the hydrolysis of **1a** and **2a**, catalysed by metal-ion complexes of **C_1Phen** and **C_{12}Phen**.[a]

catalyst	co-micellar additive	**1a**	**2a**
none	none	1.7	4.9
C_1Phen-Zn^{II} (1 mM)	none	2.9	11.8
C_1Phen-Co^{II} (1 mM)	none	5.9	20.0
C_1Phen-Ni^{II} (1 mM)	none	3.3	9.9
C_{12}Phen-Zn^{II} (0.5 mM)	Brij 35	11.9	30.2
C_{12}Phen-Co^{II} (0.5 mM)	Brij 35	89.7	88.6
C_{12}Phen-Ni^{II} (0.5 mM)	Brij 35	125	95.7

[a] Conditions: 75 °C, pH = 7.35 (0.01 M *N*-ethylmorpholine-HBr buffer), **[C_1Phen]** : [M^{II}] = 1, **[C_{12}Phen]** : [M^{II}] = 1, **[1a]** = 5 x 10^{-5} M, and [2a] = 5 x 10^{-5} M.



(a) (b) (c)

Scheme 7.3 Tautomerism of 5-nitro-2-pyridinol

the additional electron-withdrawing effect of the pyridine nitrogen [Schemes 7.3 (**b**) and 7.3 (**c**)].

The data in Table 7.1 show that the presence of 1 mM of **C_1Phen**-M^{II} induces a modest increase of 1.7-4.1 fold in the rates of hydrolysis, whereas in the case of the micellar analogue **C_{12}Phen**-M^{II} (0.5 mM) the rate-accelerating effect is one order of magnitude larger (6.2-74 fold). This implies that the rate acceleration caused by **C_{12}Phen**-M^{II} results from a micellisation effect superimposed upon metal-ion catalysis. In Brij 35 solution, substrates **1a** and **2a** are partitioned between the bulk aqueous phase and the micellar pseudo-phase, whereas **C_{12}Phen**-M^{II} is almost completely in the micellar pseudo-phase. The catalyst **C_{12}Phen**-Ni^{II} co-micellised in Brij 35, is the most active species toward **1a** and **2a** at 75 °C (Table 7.1) and also toward the relatively stable phosphate diester bis(4-nitrophenyl) phosphate (**1c**). The initial pseudo-first-order rate constant for the hydrolysis of **1c**, catalysed by 0.5 mM **C_{12}Phen**-Ni^{II} is 2.87×10^{-4} s^{-1} at 75 °C and pH = 7.35. This is 7000 times faster than the spontaneous rate of hydrolysis of 4.1×10^{-8} s^{-1}, extrapolated from data of Trogler *et al.*[5] Since the kinetics are complicated by further slow hydrolysis of the intermediate reaction product 4-nitrophenyl phosphate (**1d**) ($k_{obs} = 0.79 \times 10^{-4}$ s^{-1}), detailed analysis of this catalytic activity has not been performed.

Substitution of the ethyl groups of the substrates by the more hydrophobic phenyl groups results in substrates with a higher affinity for the micellar phase and, consequently, a higher catalytic effect for **C_{12}Phen**-M^{II} should be observed. Table 7.2 shows the pseudo-first-order rate constants for the hydrolysis of the diphenyl phosphate triesters **1b**, **2b**, and **3** at 25 °C and 75 °C. Mixed micelles composed of Brij 35 and **C_{12}Phen** in the absence of metal ions enhance the rate of hydrolysis 25-50 times (entries 3 and 9). This ligand contains four basic nitrogen centres, which may enlarge the local pH at the interface. In the presence of metal ions, however, the nitrogen atoms of **C_{12}Phen** are coordinated to M^{II} and a different catalytic mechanism of hydrolysis occurs. At 25 °C, addition of 1 equivalent of Ni^{II} to this system has only a small rate-

Table 7.2 Pseudo-first-order rate constants (k_{obs} / 10^{-5} s^{-1}) for the hydrolysis of **1b**, **2b**, and **3** catalysed by mixed micellar systems composed of C_{12}**Phen**-M^{II} or C_1**Phen**-M^{II} and Brij 35.[a]

		T = 25 °C [b]		
entry	catalyst	**1b**	**2b**	**3**
1	none	0.28	4.18	0.65
2	C_1**Phen**-Zn^{II}	5.55	25.7	4.13
3	C_{12}**Phen**	14.7	51.1	13.0
4	C_{12}**Phen**-Zn^{II}	66.7	248	41.5
5	C_{12}**Phen**-Co^{II}	167	424	88.4
6	C_{12}**Phen**-Ni^{II}	21.5	52.3	14.0
		T = 75 °C [c]		
entry	catalyst	**1b**	**2b**	**3**
7	none	8.29	82.3	8.07
8	C_1**Phen**-Zn^{II}	147	652	136
9	C_{12}**Phen**	186	567	162
10	C_{12}**Phen**-Zn^{II}	3270	10200	2720
11	C_{12}**Phen**-Co^{II}	3020	7430	2710
12	C_{12}**Phen**-Ni^{II}	1130	2580	781

[a] Conditions: [Brij 35] = 4 x 10^{-3} M, [C_1**Phen**] = 5 x 10^{-4} M, [C_{12}**Phen**] = 5 x 10^{-4} M, [M^{II}] = 5 x 10^{-4} M, [**1b**] = 5 x 10^{-5} M, [**2b**] = 5 x 10^{-5} M, and [**3**] = 5 x 10^{-5} M.
[b] pH = 8.00 (0.01 M *N*-ethylmorpholine-HBr buffer)
[c] pH = 7.35 (0.01 M *N*-ethylmorpholine-HBr buffer)

enhancing effect (entry 6), but in the presence of C_{12}**Phen**-Zn^{II} and C_{12}**Phen**-Co^{II} considerably larger rate enhancements are obtained (entries 4 and 5). For example, 0.5 mM C_{12}**Phen**-Co^{II} reduces the half-life of **1b** from 69 hours to 6.9 minutes.** The lower activity of the water-soluble C_1**Phen**-Zn^{II} (entries 2 and 8) compared to C_{12}**Phen**-Zn^{II} (entries 4 and 10) is the result of the low concentration of C_1**Phen**-Zn^{II} in the micellar

** Cu^{II} complexes have been extensively studied as catalysts in the hydrolysis of phosphate triesters,[5b,7,11b,11c,13,14] since the pK_a value of Cu^{II}-bound water is about 2 units lower than that of Zn^{II}- and Co^{II}-bound water.[4a] However, addition of Cu^{II} to C_{12}Phen gives a slow and continuous increase in absorbance in the 320-500 nm region, which makes this system unusable in the spectrophotometric monitoring of hydrolysis of the phosphate esters.

pseudo-phase, because this metallo-catalyst is distributed over the bulk solvent and the Brij 35 micelles. In the absence of ligands, Co^{II} and Ni^{II} show no catalytic activity toward **1b**, **2b**, and **3**, although **2b** and **3** possess a metal-ion binding site near the scissile P-O bond. The activity of non-ligated Zn^{II} could not be measured due to precipitation of zinc hydroxide at this pH.

The catalytic effect of **C_{12}Phen**-Ni^{II} (k_{obs}/k_0) on the rate of hydrolysis at 75 °C is higher than that at 25 °C, despite the less favourable ternary complex formation at higher temperatures. This result indicates that the hydrolysis step of the **C_{12}Phen**-Ni^{II}-substrate ternary complex has the highest activation barrier in the series of **C_{12}Phen**-M^{II}-substrate complexes.

The dependence of the rate of hydrolysis of **2b** on the metal-ion concentration in the presence of a constant concentration of **C_{12}Phen** (0.5 mM) is shown in Figure 7.1. At $[M^{II}] = 0$, addition of EDTA (0.5 mM) has no effect, indicating that the k_{obs} value at $[M^{II}] = 0$ represents the catalysis of **C_{12}Phen** essentially free of metal ions. Addition of metal ions increases the rate of hydrolysis to a saturation level at equimolar amounts of M^{II} and **C_{12}Phen**. Further addition of M^{II} has only a small enhancing effect. These k_{obs} *vs.* $[M^{II}]$ plots indicate that exclusively 1 : 1 complexes of **C_{12}Phen** and M^{II} are formed and that these are the catalytically active species. In contrast, under the conditions of equimolar concentrations of ligand and metal ion, the water-soluble ligand **C_1Phen**

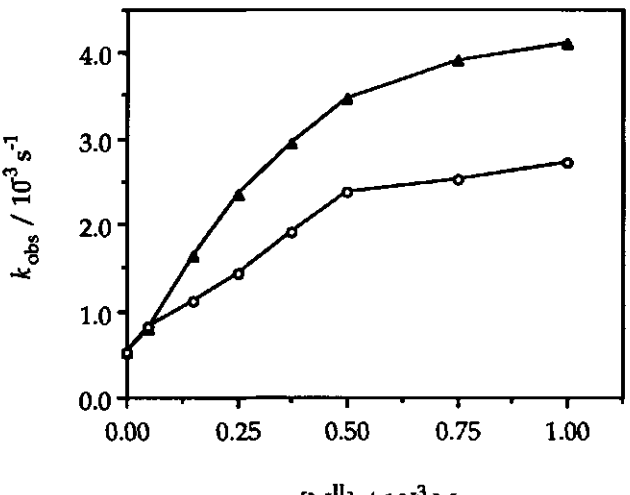


Figure 7.1 Plots of pseudo-first-order rate constants for the cleavage of **2b** in mixed micellar systems as a function of $[Zn^{II}]$ (o) and $[Co^{II}]$ (▲) under a fixed concentration of **C_{12}Phen** at pH = 8.00 and 25 °C; [Brij 35] = 4 x 10^{-3} M, **[C_{12}Phen]** = 5 x 10^{-4} M, and [**2b**] = 5 x 10^{-5} M.

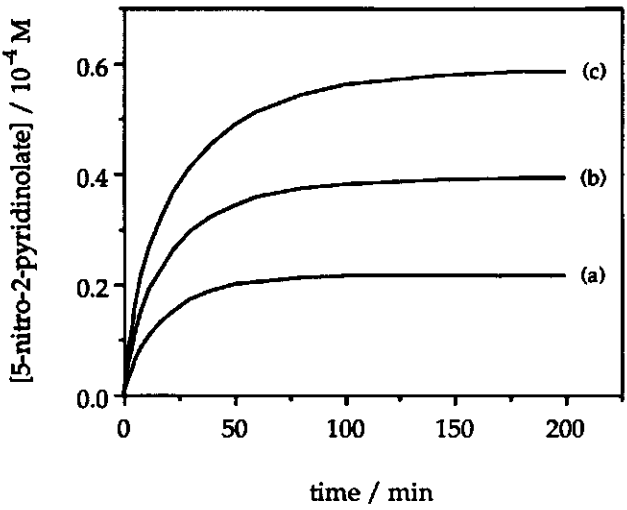


Figure 7.2 Time courses for 5-nitro-2-pyridinolate release from **2b** as catalysed by **C_{12}Phen**-Co^{II} at pH = 8.00 and 25 °C; [Brij 35] = 4 x 10^{-3} M, [**C_{12}Phen**] = 2 x 10^{-4} M, [Co^{II}] = 2 x 10^{-4} M, [**2b**] = 2 x 10^{-4} M (a), 4 x 10^{-4} M (b), and 6 x 10^{-4} M (c).

forms the active 1 : 1 complexes **C_1Phen**-M^{II}, as well as the catalytically inactive 2 : 1 complexes (**C_1Phen**)$_2$-M^{II}.[5b,11c,15a] Formation of these 2 : 1 complexes is sterically not possible for the lipophilic ligand **C_{12}Phen** due to the alignment of the alkyl chains of **C_{12}Phen** in the hydrophobic core of the micelles.[6b,15]

A true catalytic system should exhibit turn-over behaviour where the catalyst accomplishes the conversion of more than stoichiometric amounts of substrate. Figure 7.2 shows the time courses for the release of 5-nitro-2-pyridinolate catalysed by **C_{12}Phen**-Co^{II} (0.2 mM) in the presence of excess of **2b** (0.2, 0.4, and 0.6 mM). Under these conditions, quantitative yields of 5-nitro-2-pyridinolate are produced without concomitant substrate or product inhibition, indicating that the catalyst is rapidly regenerated during the hydrolysis. Metallo-catalyst **C_{12}Phen**-Co^{II} shows a similar turn-over behaviour in the hydrolysis of **1b** and **3**.

In order to establish whether the nitrogen atoms of the 5-nitro-2-pyridyl and 5-nitro-8-quinolyl moieties contribute to the binding affinity of **2b** and **3** for metallo-surfactants, we measured the rate of hydrolysis of these substrates as a function of the **C_{12}Phen**-Zn^{II} concentration, and compared this with the behaviour of **1b**. In Figure 7.3, it is shown that at higher concentrations of **C_{12}Phen**-Zn^{II} saturation kinetics are observed for the phosphate triesters, a kinetic feature which is consistent with a mechanism involving pre-equilibrium complexation of **C_{12}Phen**-Zn^{II} with the

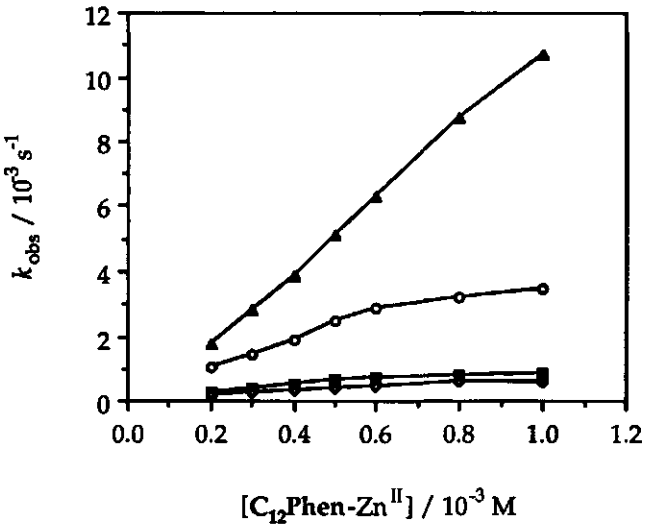


Figure 7.3 Pseudo-first-order rate constants for the cleavage of **1b** (■), **2b** (o), **3** (◇), and **4** (▲) as a function of C_{12}**Phen**-Zn^{II} concentration at pH = 8.00 and 25 °C; [Brij 35] = 4 x 10^{-3} M, [**1b**] = 5 x 10^{-5} M, [**2b**] = 5 x 10^{-5} M, [**3**] = 5 x 10^{-5} M, [**4**] = 5 x 10^{-5} M, and [C_{12}**Phen**] : [Zn^{II}] = 1.

$$C_{12}\text{Phen-}Zn^{II} + S \underset{}{\overset{K}{\rightleftharpoons}} C_{12}\text{Phen-}Zn^{II}\text{-}S \xrightarrow{k_{cat}} C_{12}\text{Phen-}Zn^{II} + P \qquad (1)$$

$$S \xrightarrow{k_0} P \qquad (2)$$

substrates, followed by intracomplex phosphoryl transfer [eqn. (1)]. The association constant (K) between C_{12}**Phen**-Zn^{II} and phosphate triester and the rate constant for decomposition of the ternary complex (k_{cat}) were determined from least-squares analysis of the double reciprocal plots of $(k_{obs}\text{-}k_0)^{-1}$ *vs.* [C_{12}**Phen**-Zn^{II}] [see also p. 28, eqn. (4)]. As can be seen from the results presented in Table 7.3, the association constants of **1b**, **2b**, and **3** are of the same order of magnitude. This indicates that no extra binding affinity is caused by the nitrogen in the leaving group. The catalytic rate constant, k_{cat}, is 5 times larger for **2b** than for **1b** and **3**, reflecting the increased leaving ability of the nitropyridinolate group. Compared to the uncatalysed reaction of **1b** (k_0), the hydrolysis in the ternary complex C_{12}**Phen**-Zn^{II}-**1b** proceeds 8700 times faster.

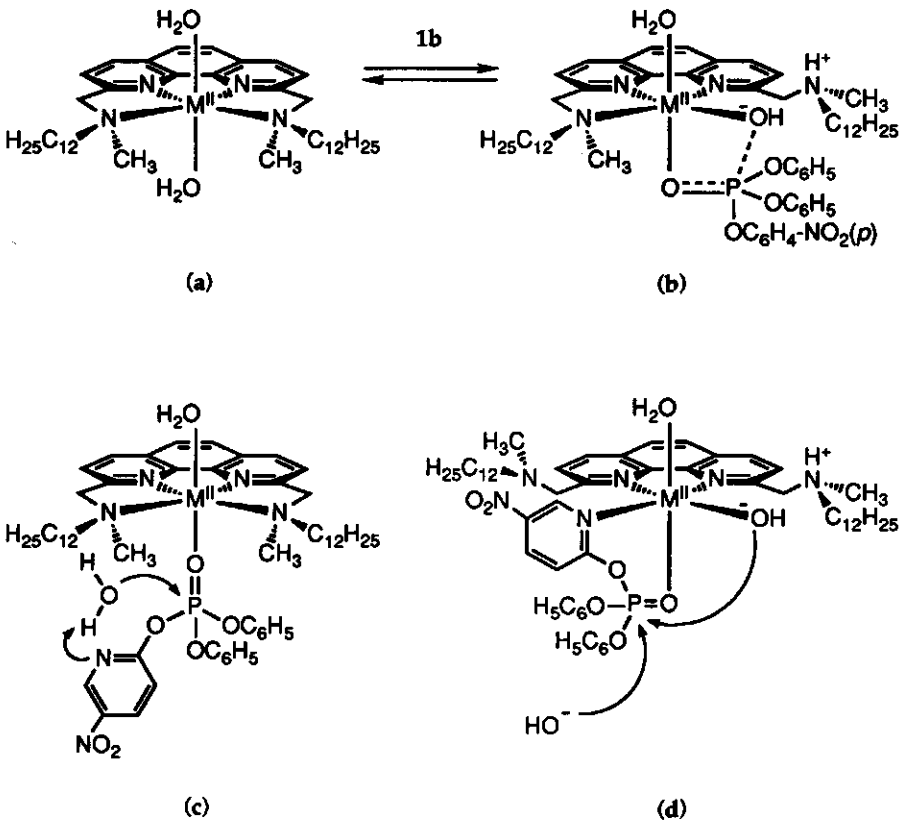
Table 7.3 Association constants (K) and catalytic rate constants (k_{cat}) for the hydrolysis of **1b**, **2b**, and **3** in the presence of C_{12}**Phen**-Zn^{II}.[a]

substrate	K/M^{-1}	$k_{cat}/10^{-3}\ s^{-1}$
1b	655 ± 33	2.44 ± 0.12
2b	469 ± 37	11.9 ± 0.06
3	314 ± 6	2.83 ± 0.09

[a] Conditions as described in Figure 7.2.

In order to compare the catalytic efficiency of C_{12}**Phen**-Zn^{II} on the hydrolysis of phosphate triesters with that of carboxylic esters, we have also measured the catalytic activity toward the metallophilic substrate 4-nitrophenyl picolinate (**4**, Scheme 7.2). Figure 7.3 shows that the rate of hydrolysis of the water-soluble substrate **4** increases linearly with [C_{12}**Phen**-Zn^{II}] without leveling off at high catalyst concentration. This points to a low binding affinity of **4** to C_{12}**Phen**-Zn^{II}. From the slope, an apparent second-order rate constant k_2 = 11.1 $M^{-1}\ s^{-1}$ is calculated. Furthermore, the linear dependence of the hydrolysis rate of **4** upon increasing catalyst concentration indicates that higher concentrations of C_{12}**Phen**-Zn^{II} do not give rise to loss of activity and, consequently, rules out possible dimerisation of the metallo-surfactants or transformation of globular micelles into less active cylindrical micelles.[22]

In the Schemes 7.4 (**a**) and 7.4 (**b**), a possible mechanism for the C_{12}**Phen**-M^{II} catalysed phosphate triester hydrolysis is proposed. This mechanism is based on an octahedral metal-ion complex, although for Zn^{II}, Co^{II}, and Ni^{II} alternative coordination geometries are possible.[23] For the formation of the reactive ternary complex with the substrate, one of the ligand-M^{II} bonds has to be substituted by the phosphoryl-oxygen-M^{II} bond. Most likely, upon binding of the phosphate triester, the *N*-methyldodecylamino group dissociates from the metal ion [Scheme 7.4 (**b**)], because CPK models show that binding of this group is sterically strained when the metal ion is strongly chelated to the 1,10-phenanthroline nucleus.[15b] These kinds of ligand-exchange processes are very fast compared to the rate of hydrolysis.[24] In the catalytic process, the metal ion can play a dual role in effecting hydrolysis by electrophilic activation of the phosphoryl bond and by providing the *cis*-coordinated hydroxyl nucleophile for attack on the phosphorus atom [Scheme 7.4 (**b**)].[3] The *N*-methyldodecylamino group may act as an intramolecular base to deprotonate the metal-ion-coordinated water molecule. Isotopic-labelling experiments have shown that the hydrolysis of phosphate triesters



Scheme 7.4 Proposed mechanisms for the C_{12}**Phen**-M^{II} catalysed hydrolysis of phosphate triesters.

occurs predominantly by P-O bond cleavage and not by C-O bond cleavage.[11c] Decomposition of the trigonal-bipyramidal intermediate is attended with expulsion of the leaving group with the lowest pK_a value from the apical position. The resulting phosphate diester, which is not a strong ligand,[6a] is readily replaced by water ligands and the catalyst re-enters the catalytic cycle without suffering from product inhibition.

In the cases of substrates **2b** and **3**, besides the mechanism depicted in Scheme 7.4 (b), two alternative and kinetically equivalent mechanisms can be considered, which give an additional role to the nitrogen atom in the leaving group. In Scheme 7.4 (c), catalysis occurs by activation of the phosphoryl bond by the metal ion and the pyridine (or quinoline) nitrogen acts as an intramolecular general base in the nucleophilic attack by water on the phosphorous atom. However, a reason which makes this mechanism not very likely, is the low basicity of the ring nitrogen caused by the strongly electron-

withdrawing 5-nitro substituent in the pyridine and quinoline groups.[25] Similarly, for the metal-ion promoted hydrolysis of 5-nitro-8-quinolyl picolinate, no intramolecular base catalysis by the quinoline nitrogen was observed.[26] A variant of the mechanism in which **2b** or **3** bind bidentately to the metal ion followed by attack by coordinated or free hydroxide [Scheme 7.4 (**d**)] can also be ruled out, since this mechanism would involve a relatively high association constant between catalyst and substrate. As shown in Table 7.3, the association constant of C_{12}**Phen**-Zn^{II} with **2b** or **3** is even slightly lower than that of **1b**. Therefore it is concluded that for all phosphate triesters **1a**, **1b**, **2a**, **2b**, and **3**, the most likely mechanism of hydrolysis catalysed by C_{12}**Phen**-M^{II} is represented by Scheme 7.4 (**b**). It was expected that a ternary complex, as depicted in Scheme 7.4 (**d**), would be part of the hydrolytic pathway of **2b** or **3**, since the carbonyl analogues did show affinity for metallo-complexation. For example, we found that the hydrolysis of 5-nitro-8-quinolyl acetate is catalysed by 1 mM C_1**Phen**-Zn^{II} with a factor 16.7, whereas under the same conditions the hydrolysis of 4-nitrophenyl acetate is enhanced only 7.4 times. However, for the pentavalent phosphate triesters, the bulkiness of the aryl moieties and of the alkyl chains of the catalyst apparently hampers the correct orientation in the ternary complexes necessary for catalytic activity.

In summary, the results presented in this chapter show that Zn^{II}, Co^{II}, and Ni^{II} complexes of C_{12}**Phen** solubilised in an inert Brij 35 micellar matrix are efficient catalysts in the hydrolysis of the phosphate triesters **1a**, **1b**, **2a**, **2b**, and **3**. The phosphate diester **1c** and the phosphate monoester **1d** are also readily hydrolysed by these catalytic systems. Turn-over behaviour has been demonstrated using mixed micelles of Brij 35 and C_{12}**Phen**-Co^{II} as an example. The presence of a metallophilic nitrogen atom in the leaving group of phosphate triesters **2b** and **3** does not increase the catalytic activity of the metallo-catalysts. Rate enhancements of 600 times are observed when both substrate and metallo-catalyst are concentrated into the micellar pseudo-phase. By increasing the concentration of metallo-catalyst in the system, rate accelerations of up to 8700 times can be realised.

7.3 Experimental Section

General Methods

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 200-E spectrometer operating at 200.1 MHz and the chemical shifts are reported relative to internal $(CH_3)_4Si$. Proton-decoupled ^{13}C NMR spectra and multiplicity analysis (by DEPT) were recorded on the same spectrometer operating at 50.3 MHz. ^{13}C NMR shifts

were measured relative to $CDCl_3$. ^{31}P NMR spectra were recorded on a Bruker CXP 300 spectrometer at 121.5 MHz and chemical shifts are reported relative to external 85% H_3PO_4. Infrared spectra were taken on a JASCO A-100 infrared spectrophotometer. Mass spectral data were obtained on an AEI MS 902 spectrometer equipped with a VG ZAB console and using field desorption ionisation technique in the case of the 1,10-phenanthroline derivatives. Kinetic runs were recorded on a Beckman DU-7 spectrophotometer with a temperature-controlled cell compartment and kinetic device or on a Hewlett-Packard 8452 A Diode Array spectrophotometer. The pK_a values of 4-nitrophenol (400 nm), 5-nitro-2-pyridinol (355 nm), and 5-nitro-8-quinolinol (449 nm) were evaluated from the equation $pK_a = pH + \log [(\varepsilon_m-\varepsilon_B^-)/(\varepsilon_{HB}-\varepsilon_m)]$, in which ε_{HB} is the extinction coefficient of the completely protonated species, ε_B^- of the anionic species, and ε_m of those at intermediate pH.

Materials

$ZnBr_2$ (Janssen Chimica), $CoBr_2$ and $NiBr_2$ (Alfa Products), *N*-ethylmorpholine (Janssen Chimica), **1c** (Sigma), Brij 35 (Aldrich), CTABr and **1d** (Merck) were purchased from commercial sources and used without further purification. *N*-methyldodecylamine,[27] b.p. 96-98 °C/2 mm Hg, **4**, m.p. 148-156 °C (decomp.) (lit.[17b] 144-146 °C), and **1b**, m.p. 48-49 °C (lit.[28] 49-51 °C) were prepared and purified according to literature procedures. The water used in the kinetic experiments was Milli-Q purified water. Acetonitrile and ethanol were of spectrophotometric grade.

2,9-Bis(chloromethyl)-1,10-phenanthroline (**5**)

2,9-Bis(hydroxymethyl)-1,10-phenanthroline[29] (3.0 g, 12.5 mmol) was dissolved in cold (0 °C) $SOCl_2$ (30 mL). The solution was stirred for 2 h at 0 °C with the exclusion of moisture. After addition of light petroleum (b.p. 40-60 °C, 150 mL), the product was precipitated as a solid and was filtered off by suction. The residue was dissolved in $CHCl_3$ (150 mL) and washed with water (100 mL) containing 5% (w/v) $NaHCO_3$ and 5% Na_2CO_3. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, $CHCl_3$) and pure **5** (2.7 g, 78%) was obtained as a white solid; m.p. 182-184 °C (decomp.) [lit.[18] 178-180 °C (decomp.)]. The spectral data confirm those reported by Newkome *et al.*[18]

2,9-Bis[(N,N-dimethylamino)methyl]-1,10-phenanthroline (**C_1Phen**)

To a solution of **5** (1.39 g, 5 mmol) in freshly distilled $CHCl_3$ (60 mL), dimethylamine (4.5 g, 100 mmol) dissolved in $CHCl_3$ (50 mL) was added dropwise. After stirring under N_2 at room temperature for 16 h, the reaction mixture 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 residue was purified by column chromatography [neutral Al_2O_3 (activity III), $CHCl_3$]. **C_1Phen** (1.40 g, 95%) was obtained as a pale yellow solid; m.p. 150-152 °C. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 12H, CH_3), 4.01 (s, 4H, CH_2), 7.71 (s, 2H, H-5), 7.85 (d, 2H, *J* 8.3 Hz, H-3), and 8.19 (d, 2H, *J* 8.3 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 45.55 (CH_3), 65.90 (CH_2), 122.16 (C-3), 125.66 (C-5), 127.51 (C-4a), 136.36 (C-4), 145.08 (C-10b), and 159.79 (C-2). FDMS: *m/z* 294 (M^+).

2,9-Bis[(N-methyldodecylamino)methyl]-1,10-phenanthroline (**C_{12}Phen**)

N-Methyldodecylamine (1.59 g, 8 mmol) dissolved in $CHCl_3$ (15 mL) was added, dropwise, to a stirred solution of **6**,[29] (1.46 g, 4 mmol) and $(C_2H_5)_3N$ (1.0 g, 10 mmol) in $CHCl_3$ (60 mL). The reaction mixture was stirred under N_2 for 16 h and after work-up and purification as described for **C_1Phen**, 2.12 g **C_{12}Phen** (3.52 mmol, 88%) was obtained as a white, waxy solid. ^{1}H NMR ($CDCl_3$): δ 0.84 [t, 6H, *J* 6.4 Hz, $(CH_2)_{11}CH_3$], 1.21 [s, 36H, $(CH_2)_9CH_3$], 1.55 [m, 4H, $CH_2(CH_2)_9CH_3$], 2.30 (s, 6H, CH_3N), 2.48 (t, 4H, *J* 7.4 Hz, CH_2CH_2N), 4.05 (s, 4H, $PhenCH_2N$), 7.70 (s, 2H, H-5), 7.86 (d, 2H, *J* 8.4 Hz, H-3), and 8.17 (d, 2H, *J* 8.4 Hz, H-4). ^{13}C NMR ($CDCl_3$): δ 13.89 [$(CH_2)_{11}CH_3$], 22.45, 27.15, 27.27, 29.12, 29.41, 31.68 [$(CH_2)_{10}CH_3$], 42.41 (CH_3N), 58.03 [$NCH_2(CH_2)_{10}$], 64.12 ($PhenCH_2N$), 122.24 (C-3), 125.56 (C-5), 127.44 (C-4a), 136.22 (C-4), 145.07 (C-10b), and 160.39 (C-2). FDMS: *m/z* 602 (M^+).

Diethyl 5-nitro-2-pyridyl phosphate (**2a**)

To a suspension of 5-nitro-2-pyridinol (2.80 g, 20 mmol) and $(C_2H_5)_3N$ (2.43 g, 24 mmol) in Et_2O (150 mL), diethyl chlorophosphate (3.45 g, 20 mmol) dissolved in Et_2O (25 mL) was added dropwise. After stirring at room temperature for 2 h, the precipitated $(C_2H_5)_3N{\cdot}HCl$ was removed by filtration. The filtrate was extracted with water containing 5% (w/v) $NaHCO_3$ and 5% (w/v) Na_2CO_3, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2, $CHCl_3$). The phosphate triester **2a** was obtained as a pale yellow oil. Yield: 3.89 g (14.1 mmol, 70%). ^{1}H NMR ($CDCl_3$): δ 1.34 (dt, 6H, *J* 1.1, 7.0 Hz, CH_3), 4.29 (dq, 4H, *J* 7.0, 8.1 Hz, CH_2), 7.10 (d, 1H, *J* 9.0 Hz, H-3), 8.48 (ddd, 1H, *J* 1.0,

3.0, 9.0 Hz, H-4), and 9.10 (d, 1H, *J* 3.0 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 15.78 (d, *J* 6.6 Hz, CH_3), 65.18 (d, *J* 5.7 Hz, CH_2), 113.23 (d, *J* 8.0 Hz, C-3), 135.26 (s, C-4), 141.51 (s, C-5), 144.43 (s, C-6), and 160.83 (d, C-2). ^{31}P NMR ($CDCl_3$): δ -7.73 (quintet, *J* 8.1 Hz). MS [70 eV, *m/z* (%)]: 276 (6), 221 (23), 155 (100), 150 (35), 140 (44), and 127 (49). High-resolution mass: *m/z* 276.0510; calcd. for $C_9H_{13}N_2O_6P$: 276.0511.

Diphenyl 5-nitro-2-pyridyl phosphate (**2b**)

This substrate was prepared by the procedure given for **2a** using diphenyl chlorophosphate (1.34 g, 5 mmol) and 5-nitro-2-pyridinol (0.70 g, 5.0 mmol). The phosphate triester **2b** was obtained as a white solid, m.p. 84-86 °C. Yield: 1.43 g (3.84 mmol, 77%). ^1H NMR ($CDCl_3$): δ 7.14 (d, 1H, *J* 9.1 Hz, 5N2P H-3), 7.26 (m, 10 H, C_6H_5), 8.51 (ddd, 1H, *J* 1.0, 2.9, 9.1 Hz, 5N2P H-4), and 9.14 (d, 1H, *J* 2.9 Hz, 5N2P H-6). ^{13}C NMR ($CDCl_3$): δ 113.37 (d, *J* 8.5 Hz, 5N2P C-3), 119.94 (d, *J* 4.1 Hz, Ph C-2), 125.71 (s, Ph C-4), 129.72 (s, Ph C-3), 135.50 (s, 5N2P C-4), 142.01 (s, 5N2P C-5), 144.50 (s, 5N2P C-6), 150.04 (d, *J* 7.4 Hz, Ph C-1), and 160.23 (d, 5N2P C-2). ^{31}P NMR ($CDCl_3$): δ -19.35 (s). MS [70 eV, *m/z* (%)]: 372 (0.6), 279 (1.7), 278 (2.5), 264 (100), and 263 (60). High-resolution mass: *m/z* 372.0511; calcd. for $C_{17}H_{13}N_2O_6P$: 372.0511.

Diphenyl 5-nitro-8-quinolyl phosphate (**3**)

This substrate was prepared from diphenyl chlorophosphate (2.15 g, 8.0 mmol) and 5-nitro-8-quinolinol (1.52 g, 8.0 mmol) by following the same procedure as given for **2a**. The crude product was recrystallised twice from abs. EtOH. Phosphate triester **3** (2.81 g, 83%) was obtained as pale yellow needles; m.p. 107 °C. ^1H NMR ($CDCl_3$): δ 7.28 (m, 10H, C_6H_5), 7.57 (dd, 1H, *J* 1.5, 8.7 Hz, 5N8Q H-7), 7.64 (dd, 1H, *J* 4.1, 8.9 Hz, 5N8Q H-3), 8.35 (d, 1H, *J* 8.7 Hz, 5N8Q H-6), 8.88 (dd, 1H, *J* 1.5, 4.1 Hz, 5N8Q H-4), and 9.03 (dd, 1H, *J* 1.5, 8.9 Hz, 5N8Q H-2). ^{13}C NMR ($CDCl_3$): δ 117.30 (d, 5N8Q C-7), 120.34 (d, *J* 4.4 Hz, Ph C-2), 122.82 (s, 5N8Q C-4a), 124.74 and 125.44 (2s, 5N8Q C-3 and C-6), 125.73 (s, Ph C-4), 129.83 (s, Ph C-3), 132.12 (s, 5N8Q C-4), 140.39 (d, 5N8Q C-8a), 142.06 (s, 5N8Q C-5), 150.50 (d, *J* 7.6 Hz, Ph C-1), 151.30 (s, 5N8Q C-2), and 151.69 (d, 5N8Q C-8). ^{31}P NMR ($CDCl_3$): δ -17.63 (s). MS [70 eV, *m/z* (%)]: 329 (59), 300 (100), 190 (65), and 160 (30). High-resolution mass: *m/z* 329.0325 (M - C_6H_5O); calcd. for $C_{15}H_{10}N_2O_5P$: 329.0327.

Diethyl 4-nitrophenyl phosphate (**1a**)

This substrate was prepared analogously to **2a** using diethyl chlorophosphate and 4-nitrophenol and distilled *in vacuo* (0.25 Torr at 130 °C). This procedure is less laborious than the known literature method.[30]

Kinetic Studies

Solutions were prepared in *N*-ethylmorpholine-HBr buffer pH = 7.00 (25 °C), pH = 8.00 (25 °C), or pH = 7.35 (75 °C). In the case of **C_{12}Phen**, solubilisation requires sonication of a suspension of the ligand in a buffer solution containing the co-surfactant Brij 35. After thermal equilibration, kinetic runs were initiated by injecting a 0.02 M solution of the ester substrate into a 1-cm cuvette containing 2 mL of 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 4-nitrophenolate (400 nm), 5-nitro-2-pyridinolate (355 nm), or 5-nitro-8-quinolinolate (449 nm) was monitored for at least 10 half-lives. Observed pseudo-first-order rate constants were obtained either by linear plots of ln (A_∞ - A_t) *vs.* time for at least 3 half-lives or by fitting the absorbance *vs.* time with a standard exponential model [$A_t = A_\infty + (A_0 - A_\infty) \times e^{-kt}$] by non-linear least-squares treatment. Kinetic runs, carried out under the conditions of excess of catalyst over substrate, showed good first-order behaviour and were run at least in triplicate to give an uncertainty of ± 3%.

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Chapter 8

Synthesis of Metal-Chelating α-Amino Acids and Bis-α-Amino Acid Derivatives and Enzymatic Resolution with Aminopeptidase from *Pseudomonas Putida* *

8.1 Introduction

In addition to the heterocyclic protein amino acids proline, histidine, and tryptophan, amino acids containing heterocyclic ring systems are widely encountered in nature. They appear as free amino acids or as constituents of peptides with divergent physiological properties, such as anti-biotic or anti-tumor activity.[1] Besides the versatile class of naturally occurring heterocyclic amino acids, there is a growing interest in the synthesis of chiral unnatural amino acids which have a heterocyclic unit as building blocks for polypeptides.[2] However, as for synthetic metal-chelating amino acids only a few examples have appeared so far.[3] There is an increasing demand for optically pure metal-binding amino acids in the area of *de novo* metallo-protein synthesis,[4] as ligand-exchange complex in enantioselective chromatography,[5] and as synthon for chiral models of metallo-enzymes or metallo-receptors.[6]

In this chapter we describe the synthesis and enzymatic resolution of DL-β-(2-pyridyl)-α-alanine amide (DL-**5a**) and DL-β-(1,10-phenanthrolin-2-yl)-α-alanine amide (DL-**5b**). The pyridine residue of the amino acid **3a** is a moderately chelating ligand, whereas the bidentate 1,10-phenanthroline nucleus of **3b** is a strongly chelating agent for a variety of metal ions.[7] The catalytic activities of metallo-enzymes are known to be dependent on the metal-ion species in the active site, because the coordination geometry of the enzyme-metal-substrate complex determines largely the efficiency of the catalytic process.[8] Therefore, for the synthesis of model systems, building blocks are required that can complexate various metal ions. Since **3a** is also a synthon in the preparation of compounds of medical interest, it is crucial that it is available in enantiomerically pure form.[2a,b] Stereoselective enzymatic hydrolysis of derivatives of

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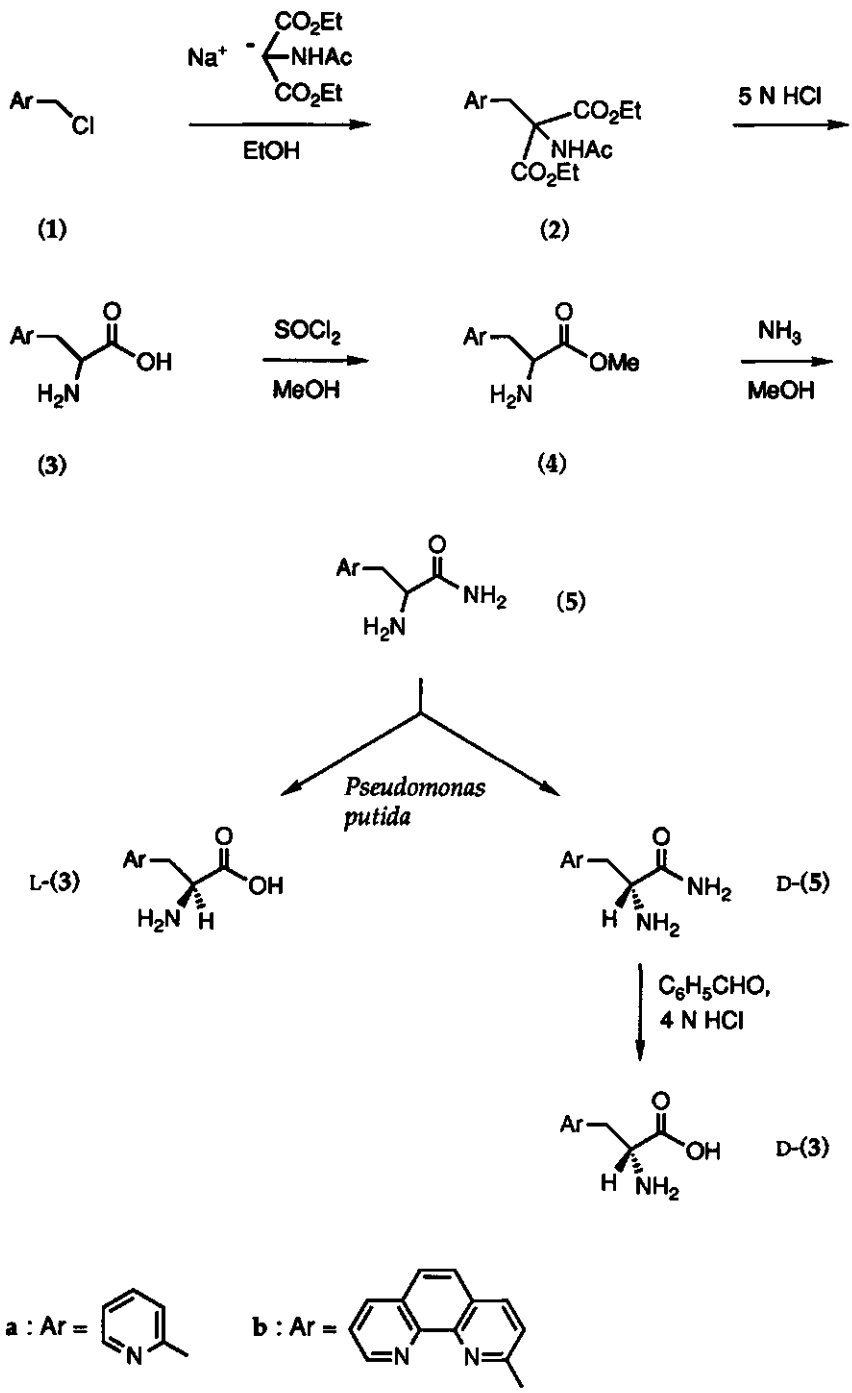
3a have been reported, namely the ethyl ester of N^{α}-acetyl-**3a** with α-chymotrypsin[2a] and N^{α}-acetyl-**3a** with Hog renal acylase I.[2b]

The enzymatic kinetic resolution of the racemic mixtures of α-amino acid amides was accomplished by the method developed at DSM, *i.e.* asymmetric enzymatic hydrolysis catalysed by the L-specific aminopeptidase from *Pseudomonas putida*.[9] The aminopeptidase couples a high degree of stereoselectivity with a broad substrate specificity. The enzyme is indifferent to the size of the alkyl or aryl residue of α-H-amino acid amides. The effect of the presence of two alanyl amide moieties in the same substrate molecule on the activity and selectivity of the aminopeptidase has not been investigated before. Optically pure bis-alanines are potentially interesting building blocks due to the presence of two chiral amino acid units, which can be utilised in *e.g.* interchain linking of peptides. Only a few examples of aromatic bis-alanine amino acids are known.[10] However, bis-amino acids which contain a metal-ion binding site have, to the best of our knowledge, not been described so far. We have synthesised in good yield the metal-chelating 2,9-bis(alanin-3-yl amide)-1,10-phenanthroline (**10b**) and the test substrate 1,4-bis(alanin-3-yl amide)benzene (**10a**) and have subjected these substrates to the aminopeptidase of *P. putida*.

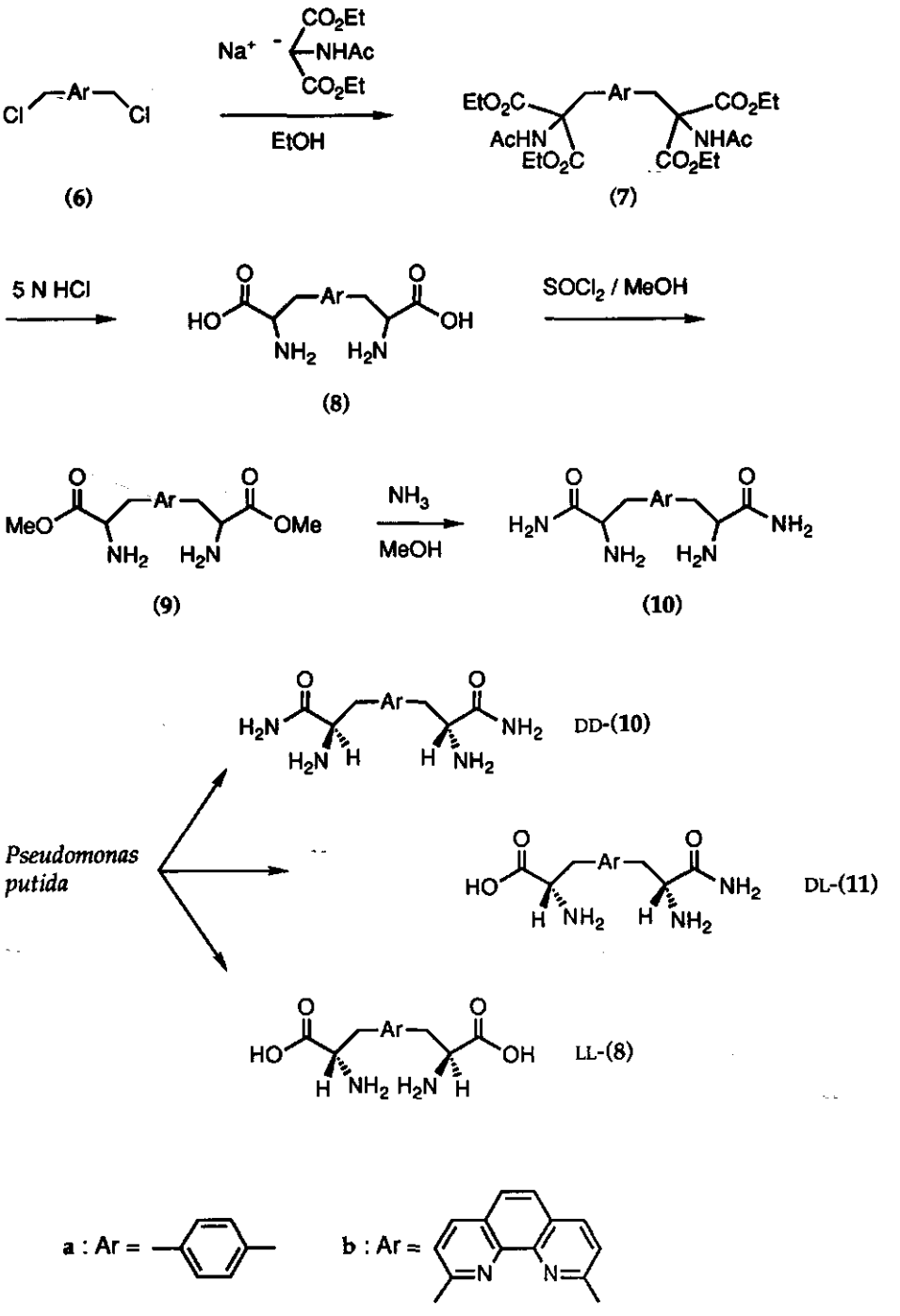
8.2 Results and Discussion

The general method employed for the synthesis of the racemic amino acid amides **5a**, **5b**, **10a**, and **10b** consisted of condensing the appropriate aryl halide with the sodium salt of diethyl acetamidomalonate, followed by complete hydrolysis of the resulting compounds to the amino acids (Schemes 8.1 and 8.2). Subsequently, the amino acids were esterified and converted into the amides. The mono amino acid amides **5a** and **5b** were then subjected to the L-specific aminopeptidase from *P. putida* to give a mixture of the L-amino acid (L-**3a**, L-**3b**) and the unchanged D-amino acid amide (D-**5a**, D-**5b**).[9] In the absence of enzyme, no hydrolysis of the amino acid amides at pH = 8 was observed after prolonged time. Addition of one equivalent of benzaldehyde to the enzymatic hydrolysate resulted in the formation of the insoluble Schiff base of the D-amino acid amide, which was then separated. After complete acid hydrolysis of the Schiff base of the D-amino acid amide, the D-amino acid was obtained without racemisation. The L-amino acid was isolated from the aqueous reaction mixture. In the case of **3a**, both stereoisomers were obtained enantiomerically pure (e.e. > 99%) as was judged by HPLC.[11]

The enzymatic resolution of DL-**5b** was performed identically to that of DL-**5a**.



Scheme 8.1 Synthesis and enzymatic resolution of mono-alanine derivatives



Scheme 8.2 Synthesis and enzymatic resolution of bis-alanine derivatives

However, the rate of enzymatic hydrolysis of DL-**5b** was slower than that of DL-**5a** because the former was poorly soluble in water. Moreover, the strongly chelating amino acid amide **5b** is able to remove the Mn^{II} ion from the active site of the aminopeptidase, which leads to inactivation of the enzyme.[12] The bidentate 1,10-phenanthroline ligand is a known inhibitor of metallo-aminopeptidases.[12,13] Although a larger amount of enzyme and a longer reaction time were used to hydrolyse DL-**5b**, the conversion was only approximately 20%. This resulted in a lower optical purity of D-**3b** (e.e. = 26%) than for the enantiomerically pure L-**3b** (e.e. > 95%), as determined by HPLC on a Crownpack CR (+) column.

The synthesised compounds **8a**, **8b**, **9a**, **9b**, **10a**, and **10b** were all 1 : 1 diastereomeric mixtures of the DD- and LL-enantiomers and the meso-compound (DL), as observed by HPLC analysis.[14a] In most cases, the diastereomeric mixtures were not separable on tlc, except for **8b**, which showed two equally intensive spots on silicagel using $CHCl_3$ / MeOH / concentrated ammonium hydroxide = 60 : 45 : 20 for development. However, large scale separation of the diastereomers was not possible. Moreover, the aliphatic protons CH_2CH and the aromatic H-3 in the ^1H NMR spectrum as well as the aliphatic CH in the ^{13}C NMR spectrum gave distinctive signals originating from the racemic compound and the meso-compound of **8b**, **9b**, and **10b**. In the case of the *p*-xylene amino acid derivatives (**8a**, **9a**, and **10a**), no distinction was observed between the diastereomers on tlc or in the NMR spectra.

Next, we investigated the enzymatic activity of *P. putida* for the stereoselective cleavage of the bis-alanine amides **10a** and **10b**. Since separation of the bis-alanine derivatives into the diastereoisomers (meso and LL/DD) by physical methods turned out to be impossible, the catalytic activity of the aminopeptidase was tested on the diastereomeric mixtures of **10a** and **10b**. When the bis-amino acid amide **10a** was subjected to the activity of the aminopeptidase of *P. putida*, tlc analysis showed that apart from the starting material **10a** (Rf = 0.59) and the bis-amino acid **8a** (Rf = 0.29), a third product was formed (Rf = 0.46). The relative intensities of the spots were 1 : 1 : 2, respectively. The compound with the intermediate Rf value is most likely the D-amino acid amide L-amino acid **11a**. After work-up of the reaction mixture (addition of 1 equivalent of benzaldehyde, extraction with $CHCl_3$, and acid hydrolysis of the Schiff base of the amide at room temperature to give the free amide), both the isolated bis-amide **10a** and the bis-acid **8a** were contaminated with the acid-amide **11a**, which was difficult to separate. The enantiomeric excess of **8a** and **10a** was determined by HPLC analysis.[14] The chiral bis-acid **8a** had an e.e. value of 76% (LL) and also contained 10% of the meso-bis-acid. The e.e. of the bis-amide **10a** could not be determined: the meso-compound and one enantiomer co-eluted. However, in the enzymatic reaction the (HPLC) isolated enantiomer DD-**10a** remained in excess.

Analogously to **10a**, resolution of the 1,10-phenanthroline bis-amide **10b** yielded three products as was shown by tlc. The three spots were assigned to DD-**10b** (Rf = 0.77), the D-amide-L-acid **11b** (Rf = 0.61), and LL-**8b** (Rf = 0.44). However, the work-up of the enzymatic hydrolysate was hampered by the high amount of bacterial remnant, and no HPLC system was found which could separate all of the stereoisomers **8b**, **10b**, and **11b**.

In conclusion, metal-ion-chelating mono-alanine derivatives containing pyridine or 1,10-phenanthroline ring systems, and bis-alanine derivatives with 1,4-phenyl or 2,9-(1,10-phenanthroline) linker moieties were obtained in high yield by the malonic ester condensation method. The bis-alanine amides **10a** and **10b** were converted by the aminopeptidase of *P. putida*, giving the products **8a(b)** and **11a(b)** apart from the starting material, but the large amount of biocatalyst gave difficulties in the work-up procedure. Enzymatic resolution of the mono-alanine amides **5a** and **5b** was successful and the amino acids **3a** and **3b** were obtained in good enantiomeric excess.

8.3 Experimental Section

General Methods

Melting points are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 200-E spectrometer operating at 200.1 MHz and chemical shifts are reported relative to internal $(CH_3)_4Si$. Proton-decoupled ^{13}C NMR spectra and multiplicity analysis (by DEPT) were recorded on the same spectrometer operating at 50.3 MHz. ^{13}C NMR shifts were measured relative to $CDCl_3$. Mass spectral data were obtained on an AEI MS 902 spectrometer equipped with a VG ZAB console and using field desorption ionisation technique in the case of the 1,10-phenanthroline derivatives. 2-(Chloromethyl)pyridine hydrochloride (**1a**),[15] 2-(chloromethyl)-1,10-phenanthroline hydrochloride (**1b**),[16a] and 2,9-bis(chloromethyl)-1,10-phenanthroline (**6b**)[16b] were prepared and purified according to methods reported earlier.

Diethyl acetamido (2-pyridylmethyl)malonate (**2a**)

To a solution of sodium (1.40 g, 61 mmol) in absolute ethanol (100 mL) under nitrogen atmosphere, diethyl acetamidomalonate (13.3 g, 61.2 mmol) was added. After stirring at room temperature for 30 min, **1a** (5.0 g, 30.5 mmol) was added and the reaction mixture was refluxed for 6 h. The precipitated NaCl was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (150

mL), washed with water (150 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, eluting with $CH_3OH/CHCl_3$ (1:20) to give 8.0 g (25.9 mmol, 85%) of **2a**; m.p. 93 °C (lit.[15] m.p. 92.5-93 °C). ^1H NMR ($CDCl_3$): δ 1.25 (t, 6H, *J* 7.1 Hz, CH_2CH_3), 1.92 (s, 3H, $COCH_3$), 3.81 (s, 2H, Pyr-CH_2), 4.26 (q, 4H, *J* 7.1 Hz, CH_2CH_3), 6.80 (br s, 1H, NH), 7.07 (m, 2H, H3 and H5), 7.54 (dt, 1H, H4), 8.39 (dd, 1H, H6). ^{13}C NMR ($CDCl_3$): δ 13.69 (CH_2CH_3), 22.71 ($COCH_3$), 39.87 (Pyr-CH_2), 62.33 (CH_2CH_3), 65.99 (CH_2C), 121.50 (C5), 124.12 (C3), 135.91 (C4), 148.71 (C6), 156.23 (C2), 167.54 and 168.87 (2 x C=O). High-resolution mass: *m/z* 308.1371; calcd. for $C_{15}H_{20}N_2O_5$: 308.1372. MS [70 eV, *m/z* (%)]: 308 (M^+, 3), 235 (81), 193 (100), 119 (29), 93 (53).

DL-β-*(2-Pyridyl)*-α-*alanine* (**3a**)

A solution of 7.5 g (24.3 mmol) of **2a** in 150 mL of 6 N HCl was heated under reflux for 8 h. The solvent was evaporated under reduced pressure, the residue was dissolved in a small volume of water, and percolated through a column of the cation exchange resin Dowex AC-50W-X8 (H^+-form). The column was washed with water, then eluted with 2.5% (w/v) ammonia, and the ninhydrin-positive fractions were combined and evaporated under reduced pressure to give 3.3 g (19.9 mmol, 82%) of the free amino acid **3a**; m.p. 207-217 °C (dec.) (lit.[15] m.p. 200-208 °C). ^1H NMR (D_2O): δ 2.92 (dd, 1H, *J* 8.1, 14.7 Hz, CH_{2a}), 3.09 (dd, 1H, *J* 5.2, 14.7 Hz, CH_{2b}), 3.75 (dd, 1H, *J* 5.2, 8.1 Hz, CH_2CH), 7.09 (dd, 1H, *J* 4.4, 7.7 Hz, H5), 7.10 (d, 1H, *J* 7.7 Hz, H3), 7.56 (dt, 1H, *J* 1.7, 7.7 Hz, H4), 8.22 (dd, 1H, *J* 1.7, 4.4 Hz, H6). ^{13}C NMR (D_2O/DMSO-d6): δ 42.84 (CH_2), 56.39 (CH_2CH), 124.21 (C5), 125.85 (C3), 139.61 (C4), 150.03 (C6), 157.24 (C2), 173.98 (C=O). High-resolution mass: *m/z* 166.0742; calcd. for $C_8H_{10}N_2O_2$: 166.0742. MS [70 eV, *m/z* (%)]: 166 (M^+, 3), 121 (73), 94 (27), 93 (100), 92 (12).

DL-β-*(2-Pyridyl)*-α-*alanine methyl ester di-hydrochloride* (**4a**)

To a mixture of 5.0 g of **3a** (30.1 mmol) in 100 mL of dry CH_3OH, 6 mL $SOCl_2$ was added dropwise. After 16 h at room temperature, the reaction mixture was concentrated under reduced pressure to give 7.0 g (27.7 mmol, 92%) of **4a**; m.p. 200-202 °C (dec.). NMR spectra were recorded of the free base of **4a**, obtained by treatment of the reaction product with a mixture of aqueous $NaHCO_3$ and $CHCl_3$. ^1H NMR ($CDCl_3$): δ 1.64 (s, 2H, NH_2), 2.87 (dd, 1H, *J* 8.0, 14.1 Hz, CH_{2a}), 3.10 (dd, 1H, *J* 4.7, 14.1 Hz, CH_{2b}), 3.54 (s, 3H, CH_3), 3.83 (dd, 1H, *J* 4.7, 8.0 Hz, CH_2CH), 6.97 (dd, 1H, *J* 4.6, 7.7 Hz, H5), 7.00 (d, 1H, *J* 7.7 Hz, H3), 7.44 (dt, 1H, *J* 1.8, 7.7 Hz, H4), 8.36 (dd, 1H, *J* 1.8, 4.6 Hz, H6). ^{13}C NMR ($CDCl_3$): δ 42.25 (CH_2), 51.64 (CH_3), 53.99 (CH_2CH), 121.27 (C5), 123.51

(C3), 136.01 (C4), 149.00 (C6), 157.60 (C2), 175.23 (C=O). High-resolution mass: *m/z* 180.0899; calcd. for $C_9H_{12}N_2O_2$: 180.0899. MS [70 eV, *m/z* (%)]: 180 (M^+, 10), 165 (4), 121 (100), 119 (5), 93 (86).

DL-β-(2-Pyridyl)-α-alanine amide (**5a**)

A solution of 5.0 g (19.8 mmol) of **4a** in 175 mL of dry CH_3OH, which was saturated with NH_3, was kept at room temperature for 16 h. The solvent was evaporated under reduced pressure and the residue was recrystallised from C_2H_5OH/CH_3OH to give 2.8 g (16.9 mmol, 86%) of **5a** as white crystals; m.p. 174-175 °C. ^1H NMR (DMSO-d6): δ 3.25 (m, 2H, CH_2), 4.16 (t, 1H, *J* 6.4 Hz, CH_2CH), 7.27 (dd, 1H, *J* 4.5, 7.6 Hz, H5), 7.33 (d, 1H, *J* 7.6 Hz, H3), 7.51 (s, 1H, $CONH_{2a}$), 7.75 (dt, 1H, *J* 1.7, 7.6 Hz, H4), 7.90 (br s, 2H, $CHNH_2$), 8.01 (s, 1H, $CONH_{2b}$), 8.49 (dd, 1H, *J* 1.7, 4.5 Hz, H6). ^{13}C NMR (DMSO-d6): δ 42.59 (CH_2), 56.82 (CH_2CH), 127.24 (C5), 129.02 (C3), 141.98 (C4), 153.85 (C6), 160.94 (C2), 174.84 (C=O). High-resolution mass: *m/z* 165.0903; calcd. for $C_8H_{11}N_3O$: 165.0902. MS [70 eV, *m/z* (%)]: 165 (M^+, 0.2), 148 (2.3), 122 (11), 121 (100), 94 (22), 93 (27).

Diethyl acetamido [(1,10-phenanthrolin-2-yl)methyl]malonate (**2b**)

According to the procedure described for **2a**, starting from 0.87 g (37.8 mmol) sodium, 8.21 g (37.8 mmol) diethyl acetamidomalonate, and 5.0 g (18.9 mmol) **1b** in 200 mL of dry ethanol (reaction time 1.5 h), 6.2 g (15.2 mmol, 80%) of **2b** was obtained as a crystalline compound; m.p. 126-127 °C. ^1H NMR ($CDCl_3$): δ 1.29 (t, 6H, *J* 7.1 Hz, CH_2CH_3), 2.06 (s, 3H, $COCH_3$), 4.10 (s, 2H, Phen-CH_2), 4.35 (m, 4H, CH_2CH_3), 7.46 (d, 1H, *J* 8.1 Hz, H3), 7.60 (dd, 1H, *J* 4.3, 8.1 Hz, H8), 7.74 (s, 2H, H5 and H6), 7.78 (br s, 1H, NH), 8.14 (d, 1H, *J* 8.1 Hz, H4), 8.21 (dd, 1H, *J* 1.8, 8.1 Hz, H7), 9.09 (dd, 1H, *J* 1.8, 4.3 Hz, H9). ^{13}C NMR ($CDCl_3$): δ 13.75 (CH_2CH_3), 22.80 ($COCH_3$), 40.90 (Phen-CH_2), 62.41 (CH_2CH_3), 66.63 (CH_2C), 122.76 and 124.06 (C3 and C8), 126.08 (C5 and C6), 127.01 and 128.59 (C4a and C6a), 135.58 and 136.15 (C4 and C7), 145.17 and 145.66 (C10a and C10b), 149.79 (C9), 156.75 (C2), 167.66 and 169.41 (2 x C=O). FDMS: *m/z* 409 (M^+).

DL-β-(1,10-Phenanthrolin-2-yl)-α-alanine (**3b**)

A solution of 6.2 g (15.2 mmol) of **2b** in 100 mL of 6 N HCl was refluxed for 7 h, to give after work-up and purification as described for **3a**, the amino acid **3b** in a yield of 3.23 g (12.1 mmol, 80%); m.p. 278-282 °C (dec.). ^1H NMR (D_2O/DMSO-d6): δ 3.40 (dd, 1H, *J* 8.1, 15.7 Hz, CH_{2a}), 3.57 (dd, 1H, *J* 4.6, 15.7 Hz, CH_{2b}), 4.28 (dd, 1H, *J* 4.6, 8.1 Hz, CH_2CH), 7.46 (d, 1H, *J* 8.3 Hz, H3), 7.57 (s, 2H, H5 and H6), 7.60 (dd, 1H, *J* 4.3, 8.1 Hz,

H8), 8.10 (d, 1H, *J* 8.3 Hz, H4), 8.19 (d, 1H, *J* 8.1 Hz, H7), 8.81 (d, 1H, *J* 4.3 Hz, H9). ^{13}C NMR (D_2O/DMSO-d6): δ 43.00 (CH_2), 55.76 (CH_2CH), 125.19 and 125.62 (C3 and C8), 127.84 and 128.01 (C5 and C6), 128.99 and 130.46 (C4a and C6a), 139.08 and 139.47 (C4 and C7), 145.33 (C10a and C10b), 151.12 (C9), 158.48 (C2), 173.69 (C=O). FDMS: *m/z* 268 (MH^+).

DL-β-*(1,10-Phenanthrolin-2-yl)*-α-*alanine methyl ester* (**4b**)

Following the same procedure as described for **4a**, 3.93 g (14.7 mmol) of **3b** was esterified in a solution of 200 mL of dry CH_3OH and 5 mL of $SOCl_2$ to afford 3.6 g (12.8 mmol, 87%) of the free ester **4b** as a pale yellow oil. ^1H NMR ($CDCl_3$): δ 2.20 (s, 2H, NH_2), 3.37 (dd, 1H, *J* 8.7, 13.8 Hz, CH_{2a}), 3.65 (dd, 1H, *J* 4.9, 13.8 Hz, CH_{2b}), 3.71 (s, 3H, CH_3), 4.19 (dd, 1H, *J* 4.9, 8.7 Hz, CH_2CH), 7.52 (d, 1H, *J* 8.3 Hz, H3), 7.56 (dd, 1H, *J* 4.3, 8.2 Hz, H8), 7.67 and 7.72 (2d, 2H, *J* 9.1 Hz, H5 and H6), 8.13 (d, 1H, *J* 8.3 Hz, H4), 8.18 (dd, 1H, *J* 1.8, 8.2 Hz, H7), 9.14 (dd, 1H, *J* 1.8, 4.3 Hz, H9). ^{13}C NMR ($CDCl_3$): δ 43.44 (CH_2), 51.90 (CH_3), 54.66 (CH_2CH), 122.61 and 123.48 (C3 and C8), 125.80 and 126.14 (C5 and C6), 127.03 and 128.60 (C4a and C6a), 135.78 and 136.19 (C4 and C7), 145.73 (C10a and C10b), 149.99 (C9), 158.54 (C2), 175.19 (C=O). FDMS: *m/z* 282 (MH^+).

DL-β-*(1,10-Phenanthrolin-2-yl)*-α-*alanine amide* (**5b**)

A solution of 5.0 g (17.7 mmol) of **4b** in 350 mL of dry CH_3OH, which was saturated with NH_3, was kept at room temperature for 60 h. The solvent was evaporated under reduced pressure and the residue was chromatographed on a column of neutral Al_2O_3 (activity III). Elution with $CH_3OH/CHCl_3$ (0:100 → 10:90) gave after evaporation 3.46 g of pure **5b** (13.0 mmol, 73%) as a pale yellow solid; m.p. 205 °C (dec.). ^1H NMR (DMSO-d6): δ 3.11 (dd, 1H, *J* 8.5, 14.1 Hz, CH_{2a}), 3.42 (dd, 1H, *J* 4.8, 14.1 Hz, CH_{2b}), 3.81 (dd, 1H, *J* 4.8, 8.5 Hz, CH_2CH), 6.92 (br s, 1H, $CONH_{2a}$), 7.54 (br s, 1H, $CONH_{2b}$), 7.67 (d, 1H, *J* 8.2 Hz, H3), 7.74 (dd, 1H, *J* 4.3, 8.1 Hz, H8), 7.91 and 7.96 (2d, 2H, *J* 8.9 Hz, H5 and H6), 8.37 (d, 1H, *J* 8.2 Hz, H4), 8.45 (dd, 1H, *J* 1.8, 8.1 Hz, H7), 9.09 (dd, 1H, *J* 1.8, 4.3 Hz, H9). ^{13}C NMR (DMSO-d6): δ 43.58 (CH_2), 55.17 (CH_2CH), 123.15 and 124.08 (C3 and C8), 125.86 and 126.50 (C5 and C6), 126.85 and 128.51 (C4a and C6a), 136.19 and 136.30 (C4 and C7), 144.78 and 145.30 (C10a and C10b), 149.80 (C9), 159.90 (C2), 176.92 (C=O). FDMS: *m/z* 267 (MH^+).

α,α'-Bis(diethyl acetamidomalonyl)-p-xylene (**7a**)

To a solution of 3.2 g (139.1 mmol) of sodium in 100 mL of absolute ethanol under nitrogen, 25.0 g (115.0 mmol) diethyl acetamidomalonate was added. After stirring at room temperature for 30 min, 10.1 g (57.5 mmol) *α,α'*-dichloro-*p*-xylene (**6a**) was added. The reaction mixture was stirred for an additional 10 min and then concentrated under reduced pressure. The residue was taken up in $CHCl_3$ (500 mL) and washed with water (3 x 200 mL). The organic layer was dried (Na_2SO_4), evaporated under reduced pressure, and the residue was recrystallised from toluene to give 30.2 g (56.3 mmol, 98%) of pure **7a**; m.p. 219 °C. ^{1}H NMR ($CDCl_3$): δ 1.29 (t, 12H, *J* 7.1 Hz, CH_2CH_3), 2.03 (s, 6H, $COCH_3$), 3.61 (s, 4H, C_6H_4-CH_2), 4.26 (q, 8H, *J* 7.1 Hz, CH_2CH_3), 6.54 (s, 2H, NH), 6.91 (s, 4H, C_6H_4). ^{13}C NMR ($CDCl_3$): δ 14.03 (CH_2CH_3), 23.04 ($COCH_3$), 37.44 (C_6H_4-CH_2), 62.66 (CH_2CH_3), 67.14 (CH_2C), 129.85 (C2 and C3), 134.21 (C1), 167.48 and 169.07 (2 x C=O). High-resolution mass: *m/z* 536.2351; calcd. for $C_{26}H_{36}N_2O_{10}$: 536.2360.

1,4-Bis(alanin-3-yl)benzene di-hydrochloride (**8a**)

A solution of 27.0 g (50.4 mmol) of **7a** in 500 mL of a mixture of 12 N HCl and EtOH (1 : 1) was heated under reflux for 16 h. After evaporation of the solvent under reduced pressure, the residue was dissolved in 6 N HCl and the reaction mixture was refluxed for an additional 3 h. The solution was concentrated under reduced pressure and the residue was washed with acetone to give 15.7 g (48.3 mmol, 96%) of **8a**.[10a] ^{1}H NMR (DMSO-d6): δ 3.13 (m, 4H, CH_2), 4.10 (m, 2H, CH_2CH), 7.28 (s, 4H, C_6H_4), 8.52 (br s, 4H, NH_2). ^{13}C NMR (DMSO-d6): δ 35.12 (CH_2), 53.14 (CH_2CH), 129.61 (C2 and C3), 133.85 (C1), 171.01 (C=O). FDMS: *m/z* 252 (M^+).

1,4-Bis(alanin-3-yl methyl ester)benzene (**9a**)

To a stirred mixture of 5.0 g (15.4 mmol) of **8a** in 100 mL of dry CH_3OH, 2.3 mL (31.5 mmol) of $SOCl_2$ at 0 °C was added dropwise. After 16 h at 40 °C, the reaction mixture was concentrated under reduced pressure and the residue was taken up in 150 mL of saturated aqueous $NaHCO_3$. The aqueous solution was extracted with $CHCl_3$ (3 x 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to give 2.4 g (8.6 mmol, 56%) of **9a** as a waxy solid. ^{1}H NMR ($CDCl_3$): δ 1.51 (br s, 4H, NH_2), 2.84 (dd, 2H, *J* 8.6, 13.0 Hz, CH_{2a}), 3.11 (dd, 2H, *J* 5.7, 13.0 Hz, CH_{2b}), 3.74 (s on dd, 8H, *J* 5.7, 8.6 Hz, CH_2CH and CH_3), 7.15 (s, 4H, C_6H_4). ^{13}C NMR ($CDCl_3$): δ 40.69 (CH_2), 51.95 (CH_3), 55.79 (CH_2CH), 129.47 (C2 and C3), 135.79 (C1), 175.42 (C=O). FDMS: *m/z* 280 (M^+).

1,4-Bis(alanin-3-yl amide)benzene (**10a**)

A solution of 2.0 g (7.1 mmol) of **9a** in 100 mL of saturated methanolic NH_3 was set aside at room temperature for 60 h. The solvent was evaporated under reduced pressure and the residue was washed with $CHCl_3$ to give 1.7 g (6.8 mmol, 96%) of **10a** as a white solid. ^1H NMR (DMSO-d6): δ 1.58 (br s, 4H, CHNH_2), 2.55 (dd, 2H, *J* 8.6, 13.7 Hz, CH_{2a}), 2.89 (dd, 2H, *J* 5.7, 13.7 Hz, CH_{2b}), 3.32 (dd, 2H, *J* 5.7, 8.6 Hz, CH_2CH), 6.99 (br s, 2H, $CONH_{2a}$), 7.14 (s, 4H, C_6H_4), 7.32 (br s, 2H, $CONH_{2b}$). ^{13}C NMR (D_2O): δ 36.73 (CH_2), 54.43 (CH_2CH), 130.55 (C2 and C3), 133.84 (C1), 171.68 (C=O). FDMS: *m/z* 250 (M^+).

2,9-Bis[(diethyl acetamidomalonyl)methyl]-1,10-phenanthroline (**7b**)

To a solution of 1.6 g (14.4 mmol) of *t*-BuOK in 100 mL of dry THF, 3.1 g (14.4 mmol) of diethyl acetamidomalonate was added. After stirring at room temperature for 30 min, 2.0 g (7.2 mmol) of **6b** was added and the reaction mixture was heated under reflux for 4 h. The solvent was evaporated under reduced pressure and the residue was taken up in 200 mL of $CHCl_3$. The organic layer was washed with water (3 x 100 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO_2; $CH_3OH/CHCl_3$ 1:20) to give 4.0 g (6.3 mmol, 88%) of **7b** as a solid; m.p. 126-128 °C. ^1H NMR ($CDCl_3$): δ 1.27 (t, 12H, *J* 7.2 Hz, CH_2CH_3), 2.16 (s, 6H, $COCH_3$), 4.03 (s, 4H, Phen-CH_2), 4.34 (q, 8H, *J* 7.2 Hz, CH_2CH_3), 7.48 (d, 2H, *J* 8.6 Hz, H3), 7.75 (s, 2H, H5), 7.97 (s, 2H, NH), 8.17 (d, 2H, *J* 8.6 Hz, H4). ^{13}C NMR ($CDCl_3$): δ 13.99 (CH_2CH_3), 23.02 ($COCH_3$), 41.33 (Phen-CH_2), 62.68 (CH_2CH_3), 66.61 (CH_2C), 124.33 (C3), 126.15 (C5), 127.60 (C4a), 136.37 (C4), 145.26 (C10b), 156.68 (C2), 167.80 and 169.75 (2 x C=O). FDMS: *m/z* 638 (M^+).

2,9-Bis(alanin-3-yl)-1,10-phenanthroline (**8b**)

A solution of 5.46 g (8.56 mmol) of **7b** in 200 mL of 6 N HCl was heated under reflux for 6 h. After work-up and purification as described for **3a**, the free amino acid **8b** was obtained in a yield of 2.57 g (7.3 mmol, 85%); m.p. > 300 °C (dec.). ^1H NMR (D_2O): δ 3.65 (m, 4H, CH_2), 4.38 (m, 2H, CH_2CH), 7.64 (d, 2H, *J* 8.5 Hz, H3), 7.80 (s, 2H, H5), 8.33 (d, 2H, *J* 8.5 Hz, H4). ^{13}C NMR (D_2O/DCl): δ 35.85 (CH_2), 51.42 and 51.62 (CH_2CH), 126.67 (C3), 127.38 (C5), 128.95 (C4a), 136.77 (C4), 142.82 (C10b), 155.23 (C2), 172.44 and 172.56 (C=O). FDMS: *m/z* 354 (M^+).

2,9-Bis(alanin-3-yl methyl ester)-1,10-phenanthroline (**9b**)

Using the same method as for **9a**, 3.0 g (8.47 mmol) of **8b** dissolved in 60 mL of dry CH_3OH containing 4 mL (54.8 mmol) of $SOCl_2$ was converted into 2.43 g (6.36 mmol, 75%) of **9b**. ^1H NMR ($CDCl_3$): δ 2.85 (br s, 4H, NH_2), 3.42 (m, 2H, CH_{2a}), 3.68 (m, 2H, CH_{2b}), 3.76 and 3.77 (2s, 6H, CH_3), 4.35 (m, 2H, CH_2CH), 7.53 (d, 2H, *J* 8.2 Hz, H3), 7.75 (s, 2H, H5), 8.19 (d, 2H, *J* 8.2 Hz, H4). ^{13}C NMR ($CDCl_3$): δ 42.76 and 42.88 (CH_2), 52.15 (CH_3), 54.29 and 54.35 (CH_2CH), 123.61 (C3), 125.82 (C5), 127.31 (C4a), 136.34 (C4), 145.41 (C10b), 158.63 (C2), 175.73 (C=O). FDMS: *m/z* 382 (M^+).

2,9-Bis(alanin-3-yl amide)-1,10-phenanthroline (**10b**)

A solution of 4.0 g (10.5 mmol) of **9b** in 400 mL of dry CH_3OH, which was saturated with NH_3, was kept at room temperature for 2 days. The reaction mixture was concentrated under reduced pressure and the residue was recrystallised from CH_3OH/Et_2O to give 2.65 g (7.53 mmol, 72%) of **10b** as pale yellow crystals; m.p. 209 °C (dec.). ^1H NMR (DMSO-d6): δ 3.11 (dd, 2H, *J* 8.1, 14.3 Hz, CH_{2a}), 3.34 (br s, 4H, $CHNH_2$), 3.39 (dd, 2H, *J* 5.0, 14.3 Hz, CH_{2b}), 3.76 (m, 2H, CH_2CH), 7.06 (br s, 2H, $CONH_{2a}$), 7.65 (d, 2H, *J* 8.2 Hz, H3), 7.74 (br s, 2H, $CONH_{2b}$), 7.90 (s, 2H, H5), 8.37 (d, 2H, *J* 8.2 Hz, H4). ^{13}C NMR (DMSO-d6): δ 42.56 (CH_2), 51.36 (CH_2CH), 124.26 (C3), 126.24 (C5), 127.41 (C4a), 137.71 (C4), 143.15 (C10b), 156.71 (C2), 169.93 (C=O). FDMS: *m/z* 352 (M^+).

Resolution of DL-β-*(2-Pyridyl)-α-alanine amide* (DL-**5a**)

A solution of DL-**5a** (4.60 g, 27.9 mmol) in water (100 mL) was adjusted to pH = 8.1 with 4 N KOH and then treated with 4.60 g of permeabilised whole cells of *P. putida* containing aminopeptidase. After incubation at 37 °C for 46 h, the biocatalyst was removed by filtration and centrifugation. After addition of benzaldehyde (3.1 mL, 30.5 mmol), the reaction mixture was stirred at room temperature for 2.5 h and the insoluble Schiff base (2.04 g, 8.1 mmol) of the unreacted D-**5a** was filtered off. The filtrate was evaporated under reduced pressure and the residue was washed with CH_3OH to give 1.7 g (10.2 mmol, 73%) of the amino acid L-**3a**. The Schiff base of D-**5a** (2.02 g, 8.0 mmol) was dissolved in 50 mL of 4 N HCl and heated at 80 °C for 4 h. The solvent was evaporated under reduced pressure and the residue was applied to a Dowex-50W column. The column was washed with water and then eluted with 2 N ammonia. The ninhydrin-positive fractions were pooled and evaporated to dryness to give 0.94 g (5.66 mmol, 41%) of D-**3a**. The amino acids D-**3a** and L-**3a** were shown to be 99.7% and 99.3%

enantiomerically pure respectively, by chiral HPLC.[11] ^{1}H NMR and ^{13}C NMR spectroscopic data of D-**3a** and L-**3a** were identical to those of DL-**3a**.

Resolution of DL-β-*(1,10-Phenanthrolin-2-yl)-α-alanine amide* (DL-**5b**)

The amide DL-**5b** (7.25 g, 27.3 mmol) was suspended in 320 mL of water and treated with 16.4 g of permeabilised cells of *P. putida* at 37 °C for 8 days. Benzaldehyde (2.33 mL, 22.9 mmol) was added and the reaction mixture was stirred for 3 h. After extraction with $CHCl_3$, the organic layer was washed with aqueous $NaHCO_3$, dried (Na_2SO_4), and evaporated under reduced pressure to give 2.30 g (6.5 mmol, 48%) of the Schiff base of D-**5b**. The water layer was acidified with HCl to dissolve L-**3b** and then filtrated to remove the biocatalyst. The filtrate was neutralised with KOH, concentrated under reduced pressure, and the residue was percolated through a column of the cation exchange resin Dowex-50W (H^+ form). The column was washed with water and eluted with 2.5% (w/v) ammonia. The appropriate fractions were combined and evaporated to dryness to give 1.2 g (4.50 mmol, 33%) of L-**3b**. The Schiff base of D-**5b** (2.19 g, 6.2 mmol) was suspended in 100 mL of 4 N HCl and stirred at 80 °C for 3 h. The reaction mixture was extracted with $CHCl_3$ and the water layer was concentrated under reduced pressure. After passage over an ion-exchange Dowex-50W (H^+ form) column (as described for L-**3b**), the eluent was evaporated to dryness and the residue was washed with CH_3OH and acetone to give 1.15 g, (4.3 mmol, 32%) of D-**3b**. The amino acids D-**3b** and L-**3b** were shown to be 26% and > 95% enantiomerically pure respectively, by chiral HPLC [Crownpak CR (+)]. ^{1}H NMR and ^{13}C NMR spectroscopic data of D-**3b** and L-**3b** were identical to those of DL-**3b**.

8.4 References and Notes

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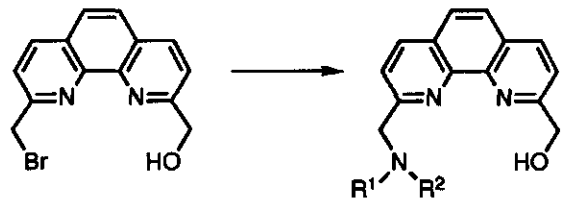
Chapter 9

Concluding Remarks

In this thesis, the study of metallo-amphiphiles as model systems for hydrolytic metallo-enzymes is described. As metal-ion chelating subunit 1,10-phenanthroline was used, since this moiety has a high affinity for a variety of metal ions. For the synthesis of these amphiphiles, the 1,10-phenanthroline nucleus has to be provided with a long apolar chain and a nucleophilic (chiral) group in the vicinity of the metallo-cleft. The 2 and 9 positions of 1,10-phenanthroline are well-spaced and chemically manipulable to provide sites for attachment of the alkyl chains and the catalytically active groups. The intermediates in the synthesis of monosubstituted 1,10-phenanthroline ligands (having the substituent on the 2 position) and symmetrically disubstituted derivatives, could be prepared according to the literature procedures with some modifications. For example, methyl 1,10-phenanthroline-2-carboxylate was obtained in one step from 2-cyano-1,10-phenanthroline instead of the three-step synthesis described in the literature, and 2,9-bis(chloromethyl)-1,10-phenanthroline was prepared by a modified procedure from the corresponding hydroxymethyl compound in good yield by the use of $SOCl_2$. Analogously to the bis-chloromethyl compound, 2-chloromethyl-1,10-phenanthroline was prepared, which was a new compound. Asymmetrically disubstituted 1,10-phenanthrolines were hitherto unknown in the literature. In these ligands the apolar chain and the nucleophilic (chiral) group are placed on either side of the 1,10-phenanthroline nucleus. For the synthesis of these ligands, two different strategies were followed (Scheme 9.1). In the cases of ligands with a hydroxymethyl group on the 9 position and a tertiary aminomethyl group on the 2 position (**2a-c**), 2-bromomethyl-9-hydroxymethyl-1,10-phenanthroline (**2**), which was obtained in three steps from neocuproine, reacted with the desired amine.[1] The synthesis of asymmetrically disubstituted 1,10-phenanthroline derivatives with two different tertiary aminomethyl groups on the 2 and 9 positions (**5a-c**, **6a,b**, and **7a,b**), was performed from 2,9-bis-(bromomethyl)-1,10-phenanthroline (**3**). Addition of one equivalent of the dialkylamine to **3**, gave the intermediate **4** in the reaction mixture apart from the disubstituted product and the starting material. Intermediate **4** was not isolated because of its instability, but was allowed to react with the chiral amino alcohol (S)-2-pyrrolidinemethanol or ephedrine to yield **5a-c** or **6a,b/7a,b**, respectively.[2]

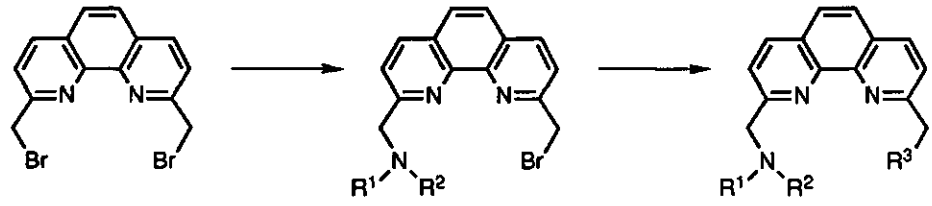


1 2

2a: $R^1 = (CH_2)_{11}CH_3$, $R^2 = CH_3$

2b: $R^1 = R^2 = CH_3$

2c: $R^1 = R^2 = (CH_2)_{11}CH_3$

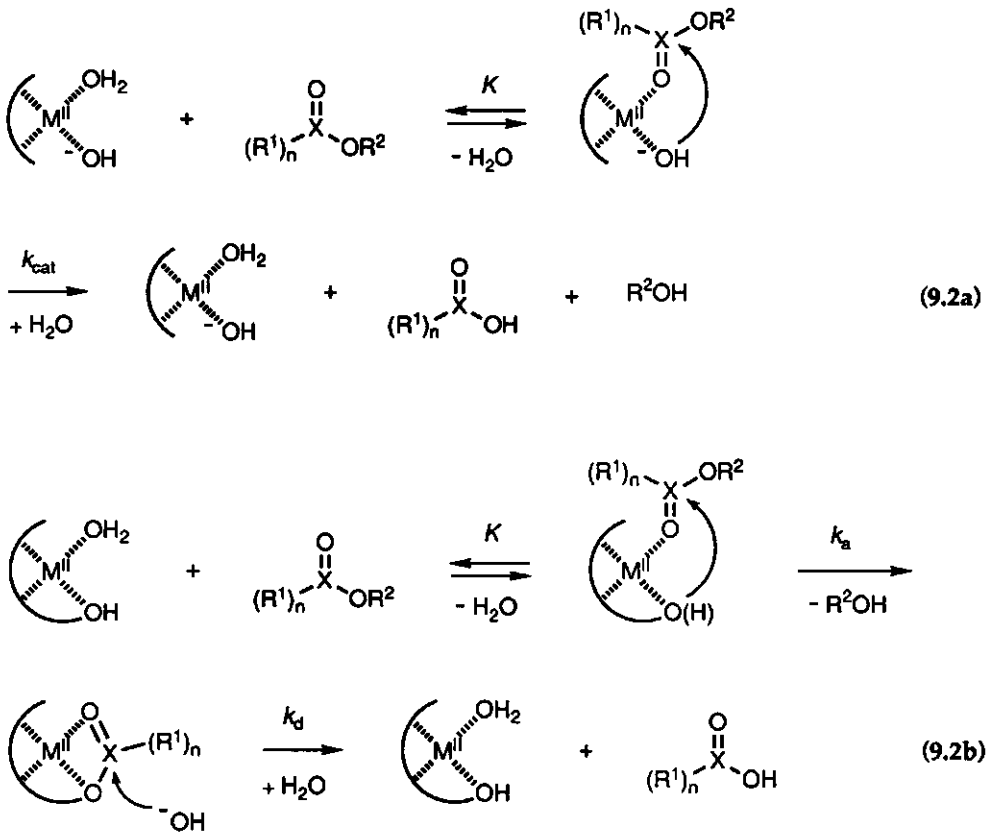


3 4 X

X	R^1	R^2	R^3
5a	$(CH_2)_{11}CH_3$	CH_3	(S)-(hydroxymethyl)pyrrolidinyl
5b	CH_3	CH_3	(S)-(hydroxymethyl)pyrrolidinyl
5c	$(CH_2)_{11}CH_3$	$(CH_2)_{11}CH_3$	(S)-(hydroxymethyl)pyrrolidinyl
6a	$(CH_2)_{11}CH_3$	CH_3	(1R,2S)-ephedrinyl
6b	$(CH_2)_{11}CH_3$	CH_3	(1S,2S)-ephedrinyl
7a	$(CH_2)_{11}CH_3$	$(CH_2)_{11}CH_3$	(1R,2S)-ephedrinyl
7b	$(CH_2)_{11}CH_3$	$(CH_2)_{11}CH_3$	(1S,2S)-ephedrinyl

Scheme 9.1 Synthesis of asymmetrically disubstituted 1,10-phenanthroline derivatives.

An alternative way of introducing a chiral centre in the 1,10-phenanthroline ligands was the enzymatic resolution of alanine and bis-alanine derivatives of 1,10-phenanthroline by the aminopeptidase from *Pseudomonas putida*. However, it appeared that 1,10-phenanthroline inactivated the enzyme, probably by removing the metal ion from the active site of the aminopeptidase. Therefore, an excess amount of enzyme was needed for the resolution, which strongly hampered the work-up of the reaction mixture and the isolation of products. As a consequence, only S-β-(1,10-phenanthrolin-2-yl)-α-alanine could be isolated as optically pure compound.



Scheme 9.2 General reaction mechanisms of the hydrolysis of activated carboxylic esters (X = C, R^1 = aryl or alkyl, and n = 1) and phosphate esters (X = P, R^1 = OC_2H_5 or OC_6H_5, and n = 2) catalysed by metallo-complexes.

Aggregates of metallo-amphiphiles show a high catalytic activity in the hydrolysis of activated carboxylic and phosphate esters and give rise to substantial enantioselectivity in the cleavage of chiral substrates. The results and discussions presented in the chapters 3-8 are summarised by referring to two generalised mechanisms of catalysis, as shown in Scheme 9.2. The central catalytic feature of these mechanisms is the formation of a reactive ternary complex composed of metal ion, ligand, and substrate. In the cases of ligands lacking a hydroxymethyl group covalently attached to the chelating subunit, the subsequent step is the nucleophilic attack by a hydroxide ion, probably activated by coordination to the metal ion, on the polarised X=O binding (**9.2a**). Ligands containing a hydroxymethyl group in close proximity to the metal ion, follow a different reaction pathway. The hydroxyl group is coordinated

to the metal ion and is involved as an activated nucleophile in the transesterification process (**9.2b**). Under pseudo-first order conditions, metallo-catalysts with a hydroxymethyl group are more active than ligands lacking this group. However, in the transesterification process the acylated intermediate has to be hydrolysed in order to regenerate the nucleophilic hydroxyl group, and this relatively slow deacylation step determines the overall reaction rate.[1] These hydroxymethyl-containing ligands are not applicable in the hydrolysis of phosphate triesters, because after phosphorylation of the hydroxyl group, the metallo-catalyst precipitates from the micellar solution.

From this study, it has become apparent that the bidentate 1,10-phenanthroline nucleus is a very suitable chelating subunit in metallo-catalysts, because 1,10-phenanthroline has a high affinity for various metal ions.[3] The high affinity of 1,10-phenanthroline for metal ions was established from the changes in the absorbance spectrum of the ligand upon addition of metal ions and from the kinetic titration curves. Especially Zn^{II}, and to a slightly lower extent, Co^{II} and Cu^{II} have a high affinity for 1,10-phenanthroline. Water-soluble ligands lacking a long apolar chain are able to form catalytically inactive 2 : 1 (ligand : M^{II}) complexes at low metal-ion concentration. In the cases of lipophilic 1,10-phenanthroline ligands in mixed micellar and vesicular solutions, almost all ligands are occupied by metal ions in the presence of one equivalent of metal ion, despite the electrostatic repulsion between M^{II} and the positively charged surfaces of CTABr micelles or $C_{18}C_{12}$ vesicles. Moreover, in aggregates 1 : 1 complexes are formed exclusively, which are the catalytically active species. Catalytically inactive 2 : 1 (ligand : M^{II}) complexes are not formed. The reason is that the coordination around the metal ion requires that two 1,10-phenanthroline units have to bind to the metal ion in a more or less perpendicular orientation. This geometry would render it impossible for both apolar chains of the $(ligand)_2$-metal-ion complex to incorporate into the micellar core. The high affinity of the ligands for metal ions and the absence of formation of 2 : 1 complexes in micellar solution facilitate the kinetic analysis because, given the concentration of ligand and metal ion, the concentration of the catalytically active 1 : 1 complexes is known and the contribution of free metal ion, non-metalated ligand, and complexes of a higher order to the catalysis is negligible.[1] In the cases of pyridine- and imidazole-containing lipophilic ligands, a much more complicated kinetic analysis is required, due to the existence of metal-ion complexes with various numbers of ligands (M^{II}-L_n). Each complex has its own equilibrium constant of formation ($K_M = [M^{II}\text{-}L_n]/[M^{II}][L]^n$) and its own association constant with the substrate ($K = [M^{II}\text{-}L_n\text{-}S]/[M^{II}\text{-}L_n][S]$, and the complexes can be productive or non-productive.[4] Moreover, pyridine and imidazole ligands require the presence of more than one equivalent of M^{II} to attain full catalytic activity.

Another advantage of strong complexation of metal ions by the 1,10-phenanthroline ligands is the substantial increase of the proton dissociation of the ligand hydroxyl group, generating a more effective nucleophile at neutral pH.

The binding of substrate to the metallo-catalyst is an important factor in the rate enhancement. The hydrolysis of PNPP is catalysed by both micellar and water-soluble metallo-complexes, due to the metallophilic character of this ester. In contrast, lipophilic carboxylic esters and phosphate triesters lacking a metal-ion chelating moiety near the reacting X=O bond, are only rapidly hydrolysed by lipophilic metallo-catalysts. These substrates associate with the lipophilic metallo-catalyst in micelles by hydrophobic interactions. In the case of phosphate triesters, introduction of a metallophilic nitrogen atom in the leaving group does not increase the affinity of these substrates for the metallo-catalysts. Water-soluble metallo-catalysts, which preferentially reside in the bulk aqueous solution, are only slightly active toward lipophilic substrates incorporated in micelles, because there is hardly any interaction between catalyst and substrate. Moreover, in the enantioselective hydrolysis of *p*-nitrophenyl esters of N-protected amino acids, the alignment of alkyl chains of substrate and metallo-catalyst in the apolar core of the micelle has a direct effect on the stereoselectivity. Non-micellar catalysts, which lack this extra orientation requirement of substrate and catalyst toward each other, are less stereoselective.

Remarkable changes in catalytic efficiency and enantioselectivity have been observed in metallo-complex catalysed hydrolysis reactions upon changing the metal ion. For example, the order of ligand activation for **2a** is: $Zn^{II} > Co^{II} > Cd^{II} > Ni^{II}$, and **5a**-$Co^{II}$ in CTABr micelles hydrolyses D-C_{12}-Phe-PNP 8 times faster than L-C_{12}-Phe-PNP, whereas in the case of **5a**-Zn^{II} an inversion of enantioselectivity is observed ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 0.54). This has been attributed to the geometry of metal-ion coordination. Generally, the effects of variation of the metal ion in metallo-complexes are unaccountable and unpredictable, and also in the case of these metallo-complexes no general rule can be derived from the experimental results. Moreover, the catalytic efficiency and enantioselectivity are dependent on the micro-environment of the metallo-catalyst.[2] For example, **5a**-Zn^{II} in neutral Brij 35 micelles hydrolyses D-C_{12}-Phe-PNP more rapidly than the L-enantiomer ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 2.4), whereas **5a**-Zn^{II} in cationic CTABr gives an inversion of the enantioselectivity ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 0.54). The affinity of **5a**-Co^{II} for *p*-nitrophenyl esters of N-protected phenylalanine is low, due to the absence of a metal-ion binding site in the substrate. Moreover, no differences in the affinity of **5a**-Co^{II} for both enantiomers are observed. This implies that the enantioselectivity in the hydrolysis is mainly caused by differences in Gibbs free energy of the transacylation step of both diastereomeric complexes. Metal-ion complexes of the ephedrine-containing ligands **6a,b** are less active and stereoselective than metal-ion

complexes of the 2-(hydroxymethyl)pyrrolidine ligand **5a**, although the nucleophilic hydroxyl group is at the same distance from the metallo-cleft and these former ligands possess an additional chiral centre. The lower activity and stereoselectivity of **6a,b** are attributed to the higher flexibility of the ephedrine moiety compared to the 2-(hydroxymethyl)pyrrolidine group and the lower nucleophilicity of the more hindered secondary OH group in **6a,b**.

Mixed metallo-vesicles composed of **5c**-Zn^{II} and the cationic surfactant $C_{18}C_{12}$ exhibit the same stereoselective behaviour toward D(L)-C_{12}-Leu-PNP than **5c**-Zn^{II} in cationic CTABr micelles. The fluidity of the vesicular bilayer has no effect on the degree of stereoselectivity, because the reaction takes place in the aqueous interface.[5]

A generally observed phenomenon is that the properties of metal ions are modified by complexation with ligands. In the hydrolysis of PNPP catalysed by free metal ion, no saturation kinetics are observed, suggesting a weak binding affinity of M^{II} to PNPP.[6] However, the hydrolysis of PNPP catalysed by metal-ion complexes of lipophilic 1,10-phenanthroline ligands in micelles does show saturation kinetics.[1,7] This implies that PNPP has a stronger binding affinity for metal-ion ligand complexes compared to non-ligated metal ions, despite the reduced number of available binding sites for the substrate.

The metallo-micelles and metallo-vesicles described in this thesis exhibit some of the major characteristics of enzyme catalysis, such as reversible and non-covalent substrate binding, acceleration, and specificity. However, enzyme catalysis is still superior to our model systems. For further refinement of synthetic catalysts which mimic metallo-enzymes, further development of the knowledge of the catalytic role of the metal ion and the synthetic ability to combine catalytic features of metal ions and organic catalytic groups is essential.

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Summary

In this thesis metal-ion complexes of functionalised 1,10-phenanthroline derivatives have been studied as model systems for hydrolytic metallo-enzymes. Amphiphilic metallo-complexes incorporated into micelles or vesicles and water-soluble complexes in pure aqueous buffer solutions, have been found catalytically active in the hydrolysis of activated (chiral) carboxylic and phosphate esters. The effect of changing the ligand structure and the metal ion on the activity and enantioselectivity of the complexes has been investigated.

After a general introduction and a description of the aim and contents of the thesis in chapter 1, an overview is given in chapter 2 of the catalytic roles that metal ions perform in hydrolytic reactions and of the application of micelles and vesicles as biomimetic systems, with emphasis on functionalised metallo-aggregates.

Mixed micellar systems containing Zn^{II} and Cu^{II} complexes of lipophilic ligands with the 1,10-phenanthroline or pyridine group as chelating moiety and a pendant N^{τ}-alkylated imidazole group (ligands **1** and **3**, chapter 3), are efficient catalysts in the hydrolysis of *p*-nitrophenyl picolinate (PNPP) and diphenyl *p*-nitrophenyl phosphate (DPPNPP). The lipophilic 1,10-phenanthroline ligands **1** and **2** have a higher affinity for metal ions than the pyridine ligand **3**. In the presence of one equivalent of metal ions, almost all 1,10-phenanthroline ligand sites are occupied by M^{II}, whereas for the pyridine ligand this amounts to only about 50%. Kinetic studies of the hydrolysis of PNPP strongly indicate that catalysis proceeds by preliminary 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.

The catalytic role of a hydroxymethyl group covalently bound to the 1,10-phenanthroline ligand in the vicinity of the reaction centre and the effect of incorporation of the ligand into micelles are discussed in chapter 4. The Zn^{II} complex of the lipophilic ligand bearing the hydroxymethyl group (ligand **1**) is 25 times more active in the hydrolysis of PNPP than the lipophilic metallo-complex lacking this group (ligand **3**). Under turn-over conditions, the hydroxymethyl-containing metallo-catalyst displays a kinetically biphasic behaviour, characteristic for an acylation-deacylation mechanism. The acylation of the hydroxymethyl group is 133 times faster than the deacylation step. Lipophilic 1,10-phenanthroline derivatives in mixed micelles are able to form only 1 : 1 complexes with bivalent metal ions, whereas the water-soluble ligand

2 can both form 1 : 1 and 2 : 1 (ligand : M^{II}) complexes. Only 1 : 1 complexes appear to be catalytically active.

Metal-ion complexes of a lipophilic 1,10-phenanthroline ligand containing the (S)-2-(hydroxymethyl)pyrrolidine function at the α-position (ligand **1**, chapter 5) are highly active and enantioselective in the hydrolysis of *p*-nitrophenyl esters of N-protected phenylalanine. The direction and magnitude of enantioselective catalysis are remarkably dependent on the nature of the metal ion and the co-surfactant. The Co^{II} complex in Brij 35 micelles exhibits the highest degree of enantioselectivity: $k^{D}_{a,obs}/k^{L}_{a,obs}$ = 15.3 toward the substrate D(L)-C_{12}-Phe-PNP. In mixed micellar systems composed of the Zn^{II} complex and Brij 35 as co-surfactant, hydrolysis of the D-enantiomer predominates over that of the L-enantiomer ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 2.4), whereas with CTABr as the co-surfactant an inversion of enantioselectivity is observed ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 0.54). Enantioselective hydrolysis is an important effect of the hydrophobic interaction between substrate and catalyst, since water-soluble ligands containing a (S)-2-(hydroxymethyl)pyrrolidine group (ligands **5**, **6**, and **7**) are less active and less stereoselective. Moreover, lipophilic 1,10-phenanthroline ligands with chiral ephedrine functions (ligands **3** and **4**) show a lower activity and stereoselectivity.

Metal-ion complexes of functionalised 1,10-phenanthroline ligands having two long alkyl chains and a nucleophilic hydroxymethyl (ligand **1**, chapter 6), (S)-2-(hydroxymethyl)pyrrolidine (ligand **2**), or ephedrine group (ligands **3** and **4**) at the α-position, incorporated in $C_{18}C_{12}$ vesicles, are catalytically active toward PNPP and show activity and enantioselectivity toward *p*-nitrophenyl esters of N-protected leucine as the substrate. In mixed metallo-vesicles, the amphiphilic ligand is anchored in the core of the bilayer membrane by the alkyl chains, whereas the chelated headgroup protrudes into the aqueous interface. The metallo-complexes appear to be active in both the exo- and endovesicular side of the bilayer and the fluidity of the vesicle membrane has no influence on the enantioselectivity.

The catalytic activity of metal-ion complexes of 1,10-phenanthroline with two long alkyl chains at the 2 and 9 positions (**C_{12}Phen**) in Brij 35 micelles toward various phosphate triesters, diesters, and monoesters is described in chapter 7. In the presence of Co^{II} and Zn^{II} complexes the rate of hydrolysis of DPPNPP is increased by factors of 600 and 240, respectively. The metallo-complexes exhibit turn-over behaviour without loss of activity. Saturation kinetics provide evidence for preliminary formation of ligand-M^{II}-phosphate ester complexes, which decay to products. Kinetic studies indicate that phosphate triesters containing a metal-ion binding site in the leaving group are hydrolysed by the same mechanism as DPPNPP.

In chapter 8 an outline is given of the synthesis and enzymatic resolution of mono-alanine amides containing pyridine (**5a**) or 1,10-phenanthroline (**5b**) side chains and of

bis-alanine amides with 1,4-phenyl (**10a**) or 2,9-(1,10-phenanthroline) (**10b**) linker moieties. Resolution of the bis-alanine derivatives using aminopeptidase from *Pseudomonas putida* requires an excess amount of enzyme due to substrate and product inhibition. The large amount of biocatalyst which, is necessary for the reaction, strongly hampers the work-up procedure, preventing the isolation of satisfactory amounts of enantiomerically pure product. In the cases of the mono-alanine derivatives, enzymatic resolution is successful and treatment of the racemic amino acid amides with the aminopeptidase yields the L-amino acid and the unchanged D-amino acid amide, which can easily be separated.

Samenvatting

In dit proefschrift worden metaalioncomplexen van gefunctionaliseerde 1,10-fenanthroline-derivaten bestudeerd als modelsystemen voor hydrolytische metalloenzymen. Amfifiele metaalioncomplexen, geïncorporeerd in micellen of vesicles, en wateroplosbare complexen in gebufferde oplossingen, vertonen activiteit in de hydrolyse van geactiveerde (zowel chirale als achirale) carbonzure esters en fosfaat esters. Het effect van veranderingen in de ligandstructuur en van verwisseling van het metaalion op de katalytische activiteit en enantioselectiviteit van de complexen werd bestudeerd.

In hoofdstuk 1 wordt een algemene inleiding over enzymmodellen gegeven en worden de doelstelling en de inhoud van het proefschrift beschreven.

In hoofdstuk 2 wordt een overzicht gegeven van de katalytische rol die metaalionen spelen in hydrolyse reacties en wordt de enzym-micel analogie bediscussieerd. Hierbij ligt de nadruk op de metallomicellen als modelsystemen voor hydrolytische metalloenzymen.

De synthese van lipofiele liganden met 1,10-fenanthroline of pyridine als chelerend deel en een N^{τ}-gealkyleerde imidazoolgroep (liganden **1** en **3**) is beschreven in hoofdstuk 3. Gemengde micellaire systemen, die bestaan uit deze liganden en een inerte cosurfactant in aanwezigheid van Zn^{II} of Cu^{II}, vertonen een hoge mate van activiteit in de hydrolyse van *p*-nitrofenylpicolinaat (PNPP) en difenyl-*p*-nitrofenylfosfaat (DPPNPP). De lipofiele 1,10-fenanthroline-liganden **1** en **2** hebben een grotere affiniteit voor metaalionen dan de pyridine-ligand **3**. In aanwezigheid van één equivalent metaalion zijn vrijwel alle bindingsplaatsen van de 1,10-fenanthroline-liganden bezet door M^{II}, terwijl onder dezelfde omstandigheden slechts 50% van de pyridine-liganden zijn gecomplexeerd. Resultaten van kinetische metingen duiden op de vorming van een ternair complex van metaalion, ligand en substraat, voorafgaande aan de katalytische stap. Deze synzymen werken via het metaalion-hydroxide mechanisme en vertonen turn-over zonder dat de katalytische activiteit afneemt.

In hoofdstuk 4 worden de katalytische functie van een hydroxymethyl-groep die covalent gebonden is aan de 1,10-fenanthroline-kern in de nabijheid van het metaalion en het effect van de incorporatie van de ligand in micellen besproken. De amfifiele ligand met de covalent gebonden hydroxymethyl-groep (ligand **1**) in aanwezigheid van Zn^{II}, zet het substraat PNPP 25 maal sneller om dan het amfifiele metaalioncomplex zonder deze groep (ligand **3**). Onder turn-over condities vertonen metaalion-katalysatoren met een covalent gebonden hydroxymethyl-groep (**1** en **2**) twee-fase

kinetiek, wat karakteristiek is voor een acylatie-deacylatie mechanisme. De acylering van de hydroxymethyl-groep verloopt 133 maal sneller dan de deacylering. In gemengde micellen vormen lipofiele 1,10-fenanthroline-derivaten uitsluitend 1 : 1 complexen met bivalente metaalionen, terwijl wateroplosbare liganden zowel 1 : 1 als 2 : 1 (ligand : M^{II}) complexen vormen. Alleen de 1 : 1 complexen vertonen katalytische activiteit.

Metaalioncomplexen van een lipofiele 1,10-fenanthroline-ligand met een (S)-2-(hydroxymethyl)pyrrolidine-groep op de α-plaats (ligand **1**, hoofdstuk 5) vertonen een hoge activiteit en een hoge mate van enantioselectiviteit in de hydrolyse van *p*-nitrofenylesters van N-beschermd fenylalanine. De mate en de richting van de enantioselectieve katalyse door de metallosurfactant worden bepaald door het soort metaalion en de cosurfactant. In Brij 35 micellen geeft het Co^{II}-complex de hoogste mate van enantioselectiviteit te zien in de hydrolyse van D(L)-C_{12}-Phe-PNP ($k^D_{a,obs}/k^L_{a,obs}$ = 15.3). In gemengde micellaire systemen, bestaande uit het Zn^{II}-complex en Brij 35, wordt de D-enantiomeer sneller omgezet dan de L-enantiomeer ($k^D_{a,obs}/k^L_{a,obs}$ = 2.4), terwijl met CTABr als cosurfactant inversie optreedt in de enantioselectiviteit ($k^D_{a,obs}/k^L_{a,obs}$ = 0.54). Omdat wateroplosbare liganden met een 2-(hydroxymethyl)-pyrrolidine-groep (liganden **5**, **6**, en **7**) een lagere activiteit en stereoselectiviteit vertonen, kan worden geconcludeerd dat de hydrofobe interactie tussen substraat en katalysator van groot belang is voor de enantioselectiviteit. Lipofiele 1,10-fenanthroline-liganden met een flexibele, chirale efedrine groep (liganden **3** en **4**) zijn minder actief en stereoselectief dan de lipofiele ligand met de stare (S)-2-(hydroxymethyl)pyrrolidine-groep (**1**).

Metaalioncomplexen van gefunctionaliseerde 1,10-fenanthroline-liganden met twee lange alkylketens en een nucleofiele hydroxymethyl- (ligand **1**, hoofdstuk 6), (S)-2-(hydroxymethyl)pyrrolidine- (ligand **2**) of efedrine-groep (liganden **3** en **4**) op de α-positie, geïncorporeerd in $C_{18}C_{12}$ vesicles, zijn katalytisch actief in de hydrolyse van PNPP en vertonen activiteit en enantioselectiviteit in de hydrolyse van *p*-nitrofenyl-esters van N-beschermd leucine. In gemengde metallovesicles zijn de alkylketens van de amfifiele ligand verankerd in het apolaire deel van de dubbellaag, terwijl de kopgroep met het gebonden metaalion de waterlaag uitsteekt. De metaalion-ligand-complexen zijn zowel actief aan de exo- als aan de endovesiculaire zijde van de dubbellaag en de vloeibaarheid van het vesicle membraan heeft geen invloed op de enantioselectiviteit.

De katalytische activiteit van metaalioncomplexen van 1,10-fenanthroline met twee lange alkylketens op de 2- en 9-positie (**C_{12}Phen**) in Brij 35 micellen in de hydrolyse van fosfaattriësters, -diësters en -monoësters is beschreven in hoofdstuk 7. Co^{II}- en Zn^{II}-complexen van ligand **1** versnellen de hydrolyse van DPPNPP respectievelijk met een

factor 600 en 240. De metaalioncomplexen vertonen turn-over gedrag, waarbij geen verlies van activiteit wordt gevonden. Omdat er verzadigingskinetiek optreedt, kan worden geconcludeerd dat het substraat eerst associeert met de metallosurfactant tot een ternair complex en dan wordt omgezet tot de producten. Kinetisch onderzoek wijst erop dat fosfaattriësters met een bindingsplaats voor metaalionen in de vertrekkende groep, op dezelfde manier worden gehydrolyseerd als DPPNPP.

In hoofdstuk 8 wordt de synthese en enzymatische resolutie beschreven van mono-alanine-amides met een pyridine- (**5a**) of 1,10-fenanthroline-groep (**5b**) als zijketen en bis-alanine-amides met een 1,4-fenyl- (**10a**) of 2,9-(1,10-fenanthroline)-groep (**10b**) als tussenstuk. Ten gevolge van substraat- en productinhibitie is voor resolutie van de bis-alanine-derivaten met het L-specifieke aminopeptidase van *Pseudomonas putida* een overmaat van het enzym vereist. De grote hoeveelheid biokatalysator, die nodig is om de reacties te laten verlopen, bemoeilijkt de opwerking van de reactiemengsels waardoor niet voldoende enantiomeer zuiver product kan worden geïsoleerd. Voor de mono-alanine-derivaten verloopt de enzymatische resolutie wel succesvol. Behandeling van de racemische aminozuuramides met het aminopeptidase levert het L-aminozuur en het niet-omgezette D-aminozuuramide op, die van elkaar gescheiden kunnen worden.

Curriculum Vitae

Johannes Gerardus Jozef Weijnen (John) werd geboren op 1 maart 1963 te Geleen. In 1981 behaalde hij het diploma Atheneum-B aan de Albert Schweitzer Scholengemeenschap te Geleen. Na een jaar hoger laboratoriumonderwijs aan de Zuidlimburgse Laboratoriumschool Sittard te hebben gevolgd, werd in 1982 begonnen met de studie Moleculaire Wetenschappen aan de toenmalige Landbouwhogeschool in Wageningen. Tijdens de doctoraalfase werden de afstudeervakken Organische Chemie en Toxicologie gevolgd en de praktijktijd Organische Chemie werd doorgebracht bij de sectie Bio-Organische Chemie van DSM-Research. Het doctoraalexamen werd afgelegd in maart 1988. Aansluitend was hij 9 maanden werkzaam als toegevoegd onderzoeker aan een samenwerkingsproject van DSM-Research en de vakgroep Organische Chemie van de Landbouwuniversiteit Wageningen. In de periode van 1 januari 1989 tot 1 januari 1993 was hij als assistent in opleiding verbonden aan bovengenoemde vakgroep, alwaar het in dit proefschrift beschreven onderzoek, onder leiding van dr. J. F. J. Engbersen, werd uitgevoerd.

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