Chiral Lipophilic Ligands. 1. Enantioselective Cleavage of α-Amino Acid Esters in Metallomicellar Aggregates

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Several chiral ligands (**1a,b**, **2a-d**), their marked lipophilic structure featuring a binding subunit comprising a 2-substituted pyridine, a tertiary amine, and a hydroxyl, have been synthesized and their complexes with Cu(II), Zn(II), or Co(II) ions investigated in homomicellar or comicellar aggregates as enantioselective catalysts of the cleavage of *p*-nitrophenyl esters of α-amino acids (Phe, Phg, Leu). Rate accelerations up to 3 orders of magnitude over the Cu(II) catalyzed hydrolysis and enantioselectivities ranging from 3.2 to 11.6 have been observed. In each case explored, the chiral ligand reacts faster with the enantiomeric substrate of opposite absolute configuration. Several pieces of evidence indicate that the effective cleavage process in micellar aggregates involves the following: (a) the formation of a ternary (ligand–metal ion–substrate) complex; (b) within such a complex, a nucleophilic attack of the ligand hydroxyl on the substrate to give a transacylation intermediate; and (c) the metal ion promoted hydrolysis of the transacylation intermediate with a relatively fast turnover of the catalyst. Such a mode of action does not operate outside or in the absence of micellar aggregates: in this case, the hydroxyl is displaced by water that acts as the nucleophile in a slower (less enantioselective) process. The enantioselectivity of the transacylation process appears to be little affected by the steric interaction between the substituents at the chiral center of the amino acid ester and of the ligand. We suggest that the enantioselectivity arises from a different hydration, due to steric reasons, of the diastereomeric complexes comprising the two enantiomers of the substrate. As a consequence, the relevance of the competing mechanisms of cleavage of the ester, the first one, faster, involving the hydroxyl and the second one, slower, involving a Cu(II)-bound water molecule, may be different. In the case of the less hydrated, more hydrophobic *R–S* or *S–R* complex the former, faster, mode of cleavage may be more relevant than in the case of the more hydrated, less hydrophobic, *S–S* or *R–R* complex.

Because of their ability to mimic some aspects of enzyme catalysis,[1] functional aggregates (micelles and vesicles) have been the focus of active investigation in order to (a) shed light on selected features of enzymatic rate acceleration and (b) achieve large rate accelerations of reactions under controlled experimental conditions. Aspects of micellar catalysis have been reviewed,[2] and a substantial agreement exists on the origin of the rate accelerations observed.

As an obvious extention of these investigations several research groups (Moss,[3] Ihara,[4] Ueoka,[5] Nolte,[6] and others) have explored enantioselectivity in micellar aggregates using chiral nonfunctional matrices, chiral functional surfactants, and chiral additives, in different compositions, in order to achieve high enantioselectivities. Among the reactions studied hydrolytic processes largely prevail.

Although in a few cases a rationale has been offered[3c,6] to explain the enantioselectivities observed, there are no general guidelines to predict the phenomena and the effects for any given system. However, some principles appear to be frequently operative: (a) the enantioselectivity does not come from a difference in binding of two enantiomers by the chiral aggregate, and (b) a multipoint interaction between the two reacting molecules enhances the enantioselectivity. A particular constraint due to the aggregate as well as a differentiation in the reaction loci[6] between the two enantiomers have been also suggested in order to account for a specific contribution of the aggregate in the enantioselection process.

Recently, functional surfactants made up of ligand amphiphiles or lipophilic ligands embedded in nonfunctional micellar matrices have been reported by ourselves[7] and other[8] research groups. They appear, as transition metal complexes, to be powerful catalysts of the cleavage of α-amino acid esters as well as of other carboxylic or phosphoric acid esters. Enantioselective cleavage by chiral metalloaggregates has been also reported by two

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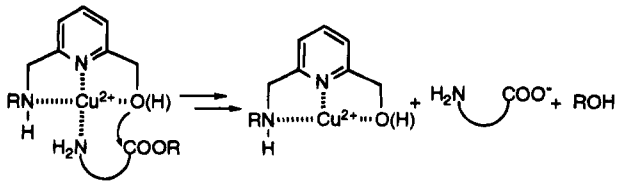
Scheme 1

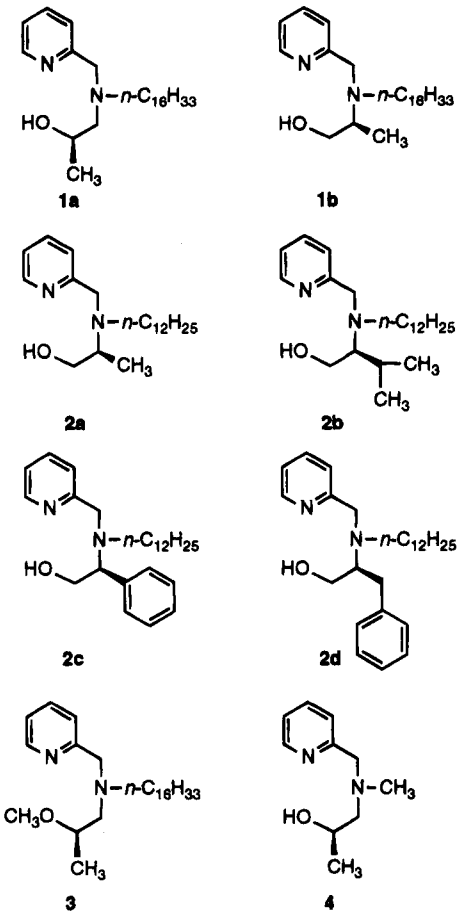


laboratories[8d,9] . In both cases, the substrates chosen were the *p*-nitrophenyl esters of N-protected α-amino acids, and enantioselectivities up to 15.3 have been reported.[9]. Following a preliminary communication,[10] we report here the full account of our work on the enantioselctive cleavage of some *p*-nitrophenyl esters of α-amino acids by chiral metalloaggregates. At variance with the work reported by the above-mentioned research groups our investigation explored the enantioselectivities using *esters with the free amino group*, i.e., substrates able to interact with the transition metal ion. We argued that such an interaction could reduce the degrees of freedom of the reacting species and introduce the multipoint contact which, as mentioned above, appears to exert a quite positive effect in terms of increasing the selectivity in the process. Previous work from our[7] and other laboratories[8c-f,j,k] showed that high rate acceleration in the cleavage of the esters of α-amino acids with good leaving groups is achieved in metalloaggregates in which the nucleophilic species is an alcoholic function coordinated to the transition metal ion (Scheme 1). This led us to design a series of chiral lipophilic ligands structurally similar to that reported in Scheme 1 and study their ability to act as enantioselective hydrolytic catalysts of the cleavage of activated, chiral α-amino esters.

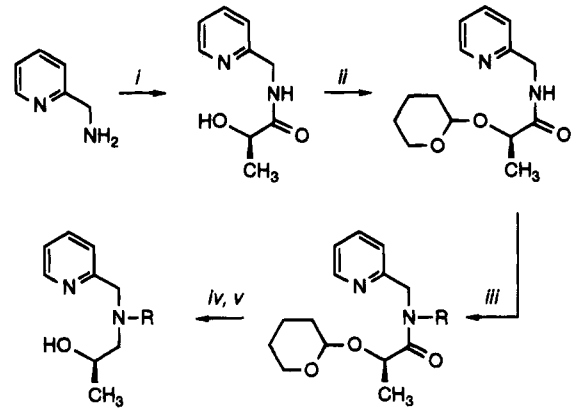
Results and Discussion

Synthesis of the Ligands and of the Substrates. The complete series of ligands is reported in Chart 1. Common features are the pivotal tertiary amine, the 2-pyridyl unit, and the alcoholic group. Linked to the nitrogen is a linear paraffinic chain (of 12 or 16 carbon atoms) or, in the case of ligand **4**, a methyl group. The chiral center is introduced in the carbon bearing the hydroxyl or in that close to it. Because of this, two different strategies were followed for the synthesis of the ligands. In the case of the ligands with the chiral carbon bearing the hydroxyl, lactic acid was converted into the amide of 2-(aminomethyl)pyridine, the alcohol protected with dihydropyran, and the amide alkylated. Reduction and deprotection eventually gave the desired ligand

Chart 1



Scheme 2[a]



[a] Key: (i) methyl lactate, $CHCl_3$, rt, 3 days; (ii) dihydropyran, PPTS, CH_2Cl_2, rt, 2 days; (iii) $C_{16}H_{33}Br$, NaH, THF, reflux, 24 h; (iv) HCl, MeOH, rt, 30 min; (v) $LiAlH_4$, THF, reflux, 6 h.

(Scheme 2). In the case of the ligands with the chiral carbon in a different position, the commercially available chiral 2-aminoethanol derivatives were reacted with the proper aldehyde, and the adduct was reduced and subsequently alkylated with 2-(chloromethyl)pyridine to give the expected products (Scheme 3).

Lipophilic ligands **1** and **2** are not dispersible in neutral water. Ligands **1a,b** and **2a,b** are soluble in slightly acidic water or as Cu(II) complexes and form micellar aggregates: their critical micellar concentrations (cmc) are reported in the Experimental Section. Under the same conditions, compounds **2c,d**, bearing a phenyl or benzyl group at the chiral carbon, are not appreciably soluble; clear solutions of these ligands, free or as

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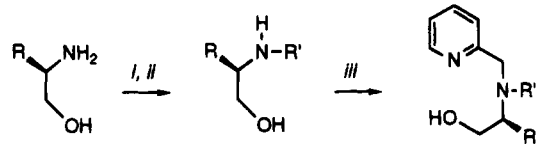
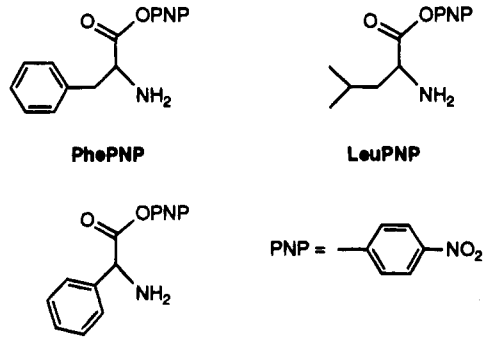
Scheme 3[a]



[a] Key: (i) n-$C_nH_{2n+1}CHO$, benzene, reflux, 3 h; (ii) $NaBH_4$, EtOH, rt, 12 h; (iii) 2-(chloromethyl)pyridine, i-Pr_2NEt, 2-propanol, 80 °C, 72 h.

Chart 2



transition metal complexes, could be obtained only in the presence of micelles of inert surfactants such as cetyltrimethylammonium bromide (CTABr) as the matrix of comicellar aggregates. In the case of Zn(II) or Co(II), the complexes with all the lipophilic ligands were only soluble as comicellar aggregates.

The limited solubility and the aggregation state of the complexes did not allow us to determine the association constants for the different ligands and metal ions employed. However, they may be roughly estimated from the values reported[11] for 2-(aminomethyl)pyridine and 2-[*N*,*N*-(dihydroxyethyl)amino]methyl]pyridine and by taking into account possible electrostatic effects in the case of cationic aggregates:[12] pK_M larger than 9 for Cu(II) and larger than 5 for Zn(II) and Co(II) can be taken as conservative values.

Chart 2 shows the substrates investigated: they are all *p*-nitrophenyl esters of the α-amino acids phenylalanine (PhePNP), phenylglycine (PhgPNP), and leucine (LeuPNP).

Kinetics. Since the early evidences reported by Kroll[13] in 1952, it is well known that the hydrolysis of the esters of α-amino acids is accelerated by the addition of transition metal ions, Cu(II) in particular. Upon addition of ligand (*R*)-**1a** to a Cu(II) solution at pH = 5.5 the rate of cleavage of each enantiomer of PhePNP is further accelerated. The observed acceleration, different for each enantiomer, depends on the amount of ligand added and reaches a maximun when [(*R*)-**1a**] ≈ 2[Cu(II)]. Such a kinetic profile is reported[14] in Figure 1. A similar behavior is observed for the other systems investigated.

The pseudo-first-order rate constants measured at pH = 5.5, [ligand] = 1.5×10^{-4} M, and [Cu(II)] = 8.3×10^{-5} M for the different substrates are reported in Table 1. It

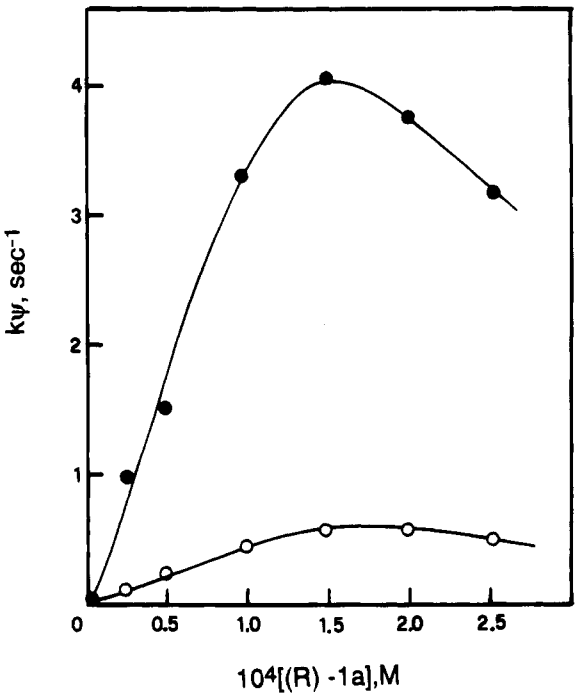


Figure 1. k_ψ *vs* (*R*)-**1a** concentration profiles for the hydrolysis of (*S*)-PhePNP (filled circles) and (*R*)-PhePNP (empty circles) in the presence of Cu(II) (8.3×10^{-5} M) in MES buffer, pH 5.5, 25 °C.

is anticipated and emphasized that in all cases reported here and so far explored the two enantiomers reacting faster are the (*R*)-ligand and (*S*)-substrate (or *vice versa*). Thus, the enantioselectivity of each system is indicated by the ratios (enantioselectivity ratio, ER) k_R/k_S or k_S/k_R. The ER values are substantially the same when complementary enantiomers are used (*R*-ligand and *S*-substrate or *vice versa*; see entries 4 and 6) and range from 3.2 to 11.6 for the substrates of Chart 2 and the different micellar ligands. Table 1 also reports the ER observed for other systems: (*S*)-**3**, the O-methylated sibling of (*S*)-**1a**, the N-protected ester of the phenylalanine, CBZ-PhePNP, and the complexes with transition metal ions Zn(II) and Co(II) (under slightly different conditions). On browsing among the entries of Table 1 we notice that (a) changing from homomicellar to comicellar aggregates is of little relevance for the C_{16} system while it leads to enhanced ER in the case of the C_{12} ligands (compare entries 4 and 5 with entries 11 and 12, 13 with 14, and 15 with 16), (b) on moving from the ligand with the chiral center bearing the hydroxyl (**1a**) to that with the chiral center one carbon apart (**1b**) little change is observed in the ER (see entries 4 and 7), (c) no clear dependence of the ER on the bulkiness of the substituents is observed, (d) protection of the amino group of the amino ester leads to a substantial decrease of the ER (compare entry 4 with 10), (e) alkylation of the hydroxyl of the ligand leads to an almost complete disappearance of enantioselectivity (compare entry 5 with 26). These observations clearly indicate that the free hydroxyl of the ligand and the free amine of the substrate are mandatory in order to achieve both high rate accelerations and good enantioselectivities. The presence of the aggregates plays a crucial role: as a matter of fact, the ER values increase steadily until the cmc is reached, flattening thereafter (see Figure S1 of the supplementary material for ligand **2a** and PhePNP). This observation may explain the different behavior in homomicellar or comicellar aggregates of the longer chain

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(14) This figure should be taken as a revision of the figure reported in ref 10.

Table 1. Observed Rate Constants, k_S and k_R, (s^{-1}), for the Cleavage of α-Amino Acid *p*-Nitrophenyl Esters by the Different Ligands and Divalent Transition Metal Ions (M(II)). Conditions (unless Otherwise Stated): pH = 5.5 (0.05 M MES Buffer); 25 °C; [M(II)] = 8.3×10^{-5} M.

entry	ligand	additive[a]	M(II)	substrate	k_S	k_R	ER[b]
1	none	none	Cu(II)	PhePNP		6.7×10^{-2}	
2	none	none	Cu(II)	PhgPNP		0.11	
3	none	none	Cu(II)	LeuPNP		2.6×10^{-2}	
4	(*R*)-**1a**[c]	none	Cu(II)	PhePNP	4.04	0.55	7.4
5	(*R*)-**1a**[c]	CTABr	Cu(II)	PhePNP	2.90	0.33	8.8
6	(*S*)-**1a**[c]	none	Cu(II)	PhePNP	0.55	4.14	7.5
7	(*S*)-**1b**[c]	none	Cu(II)	PhePNP	0.37	3.52	9.5
8	(*R*)-**1a**[c]	none	Cu(II)	PhgPNP	3.05	0.30	10.2
9	(*R*)-**1a**[c]	none	Cu(II)	LeuPNP	0.60	0.10	6.0
10	(*R*)-**1a**[c]	none	Cu(II)	CBZPhePNP	7.5×10^{-4}	4.7×10^{-4}	1.6
11	(*S*)-**2a**[d]	none	Cu(II)	PhePNP	0.11	0.40	3.6
12	(*S*)-**2a**[e]	CTABr	Cu(II)	PhePNP	1.45	11.19	7.7
13	(*S*)-**2a**[d]	none	Cu(II)	PhgPNP	0.16	0.56	3.5
14	(*S*)-**2a**[e]	CTABr	Cu(II)	PhgPNP	1.08	7.62	7.1
15	(*S*)-**2a**[d]	none	Cu(II)	LeuPNP	5.6×10^{-2}	0.19	3.4
16	(*S*)-**2a**[e]	CTABr	Cu(II)	LeuPNP	0.36	2.23	6.2
17	(*S*)-**2b**[e]	CTABr	Cu(II)	PhePNP	1.20	8.26	6.9
18	(*S*)-**2b**[e]	CTABr	Cu(II)	PhgPNP	1.14	13.19	11.6
19	(*S*)-**2b**[e]	CTABr	Cu(II)	LeuPNP	0.38	2.39	6.3
20	(*R*)-**2c**[e]	CTABr	Cu(II)	PhePNP	15.96	2.01	7.9
21	(*R*)-**2c**[e]	CTABr	Cu(II)	PhgPNP	17.43	2.50	7.0
22	(*R*)-**2c**[e]	CTABr	Cu(II)	LeuPNP	4.24	0.73	5.8
23	(*R*)-**2d**[e]	CTABr	Cu(II)	PhePNP	18.05	3.52	5.1
24	(*R*)-**2d**[e]	CTABr	Cu(II)	PhgPNP	20.42	6.42	3.2
25	(*R*)-**2d**[e]	CTABr	Cu(II)	LeuPNP	5.96	1.50	4.0
26	(*S*)-**3**[c]	CTABr	Cu(II)	PhePNP	0.21	0.24	1.1
27	(*S*)-**4**[c]	none	Cu(II)	PhePNP	2.9×10^{-3}	7.3×10^{-3}	2.5
28	none	none	Zn(II)[f]	PhePNP		0.5	
29	(*S*)-**1a**[g]	CTABr	Zn(II)[f]	PhePNP	2.85	4.35	1.5
30	none	none	Zn(II)[h]	PhgPNP		3.0×10^{-2}	
31	(*S*)-**2a**	CTABr	Zn(II)[h]	PhgPNP	0.70	0.87	1.24
32	none	none	Co(II)[h]	PhgPNP		9.0×10^{-3}	
33	(*S*)-**2a**	CTABr	Co(II)[h]	PhgPNP	4.2×10^{-2}	0.18	4.3

[a] In all cases [CTABr] = 10[ligand]. [b] ER, enantioselectivity ratio, = k_S/k_R or k_R/k_S. [c] [Ligand] = 1.5×10^{-4} M. [d] [Ligand] = 2.5×10^{-4} M. [e] [Ligand] = 2.0×10^{-4} M. [f] pH = 6.25 (0.05 M MES); [$Zn(NO_3)_2$] = 8.0×10^{-4} M. [g] [Ligand] = 9.0×10^{-4} M. [h] pH = 7.0 (0.05 M HEPES).

ligand (**1a,** entries 4 and 5) compared with that of the shorter chain derivative (**2a,** entries 11 and 12; also entries 13–16). In fact, the concentrations of **2a** used for the kinetic measurements of Table 1 are very close to the cmc value (and, quite likely, under conditions of partial micellization); only comicellization with CTABr may ensure the complete formation of micellar aggregates. The role of micelles is quite important for another reason. Kinetic experiments, analogous to those reported in Figure 1, employing the hydrophilic (unable to form aggregates) ligand (*S*)-**4** led to the observation of rate *inhibition* (compared to the Cu(II)-catalyzed reaction) instead of acceleration: at the highest ligand concentration investigated, the observed rate constant is *3 orders of magnitude lower* than that obtained with micellar (*S*)-**1a**. Such an inhibition is also associated with a much lower ER. The above-mentioned kinetic effects are clearly illustrated in the rate–concentration profiles of Figure 2 where the rate constants have been reported in the logarithmic scale, in order to allow for the large difference in the values of the rate constants. This strikingly different behavior between the micellar and nonmicellar system has been already observed and reported by us[15] using the *p*-nitrophenyl ester of picolinic acid as a substrate and appears to be associated with some changes in the reaction mechanism (see below).

Mechanism. To ascertain the main mechanistic aspects of the process, the following experiments were performed in order to define the stoichiometry of the active complex and the esterolytic species.

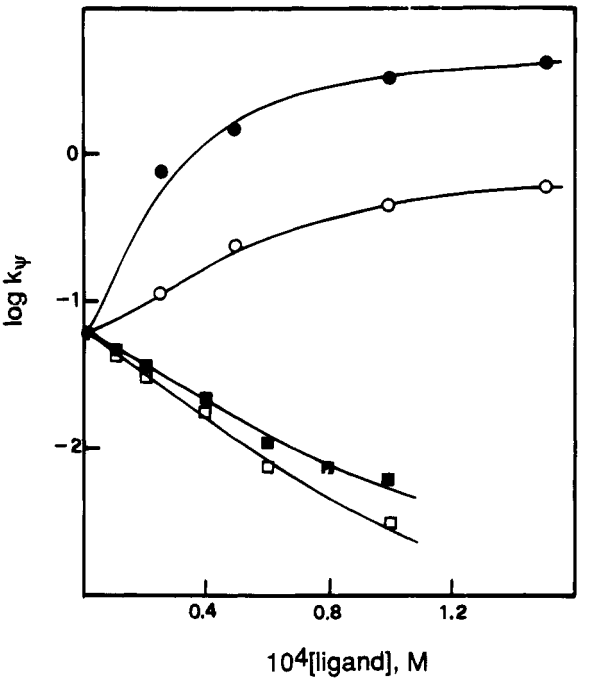

Figure 2. Log k_ψ *vs* (*S*)-**1a** (circles) and (*S*)-**4** (squares) concentration profiles for the hydrolysis of (*R*)-PhePNP (filled symbols) and (*S*)-PhePNP (empty symbols) in the presence of Cu(II) (8.3×10^{-5} M) in MES buffer, pH 5.5, 25 °C.

(A) **The Stoichiometry of the Active Complex.**

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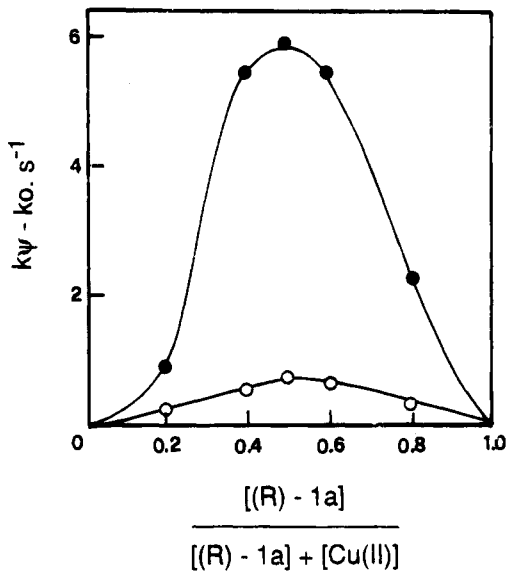


Figure 3. Kinetic Job's plot for the cleavage of (*S*)-PhgPNP (filled circles) and (*R*)-PhgPNP (empty circles) by (*R*)-**1a** and Cu(II) in MES buffer, pH 6.3, 25 °C, [(*R*)-**1a**] + [Cu(II)] = 4.1 × 10^{-5} M. All experiments with [CTABr] = 4.1 × 10^{-3} M.

This information was obtained from the kinetic version of the classic Job's plot[16] where the [ligand = L]/[Cu(II)] ratio is continously changed while keeping the total concentration of two species constant. The results obtained with the system (*R*)-**1a**·Cu(II) and PhgPNP are reported in Figure 3; they clearly point to the 1:1 complex ligand/metal ion as the most active species. This is in line with the observation that the plots k_ψ *vs* [L] at a given [Cu(II)] (see Figure 1) go through a maximum at [L] = ca. 2[Cu(II)]. With further increasing ligand concentration, the formation of L_2Cu or higher complexes favorably competes for that of the *productive* ternary complex L·Cu·substrate, and as a consequence, a rate decrease is observed. Furthermore, when the [L]/[Cu(II)] ratio is changed, the ER value reaches a maximum when the concentration of the two species are the same and decreases, albeit only slightly, when [L] > [Cu(II)] (see Figure 4 for the system (*R*)-**1a**·Cu(II) and PhgPNP).

(B) **The Esterolytic (Nucleophilic) Species.** As said above, from the analysis of the data of Table 1 a free hydroxyl in the ligand structures is needed for higher rate acceleration and enantioselection in micellar systems. The involvement of this species is further highlighted by two sets of experiments. First, using an excess of substrate over the metal complex, in the case of ligand (*R*)-**1a**·Cu(II) and PhgPNP, we observed "burst" kinetics (see Figure S2 of the supplementary material). These are clearly diagnostic of the fast formation of an intermediate (associated with the release of an amount of *p*-nitrophenol equal to the amount of metal complex) followed by a slower hydrolysis of the intermediate to restore the catalytic species.[17] The catalytic cycle is remarkably effective: the turnover number may be estimated close to 3 (mol per mol) per min. In line with previous findings, we attribute to the intermediate the structure of the transacylation product II (see Scheme 4). Second, kinetics performed employing (*R*)-**1a** with

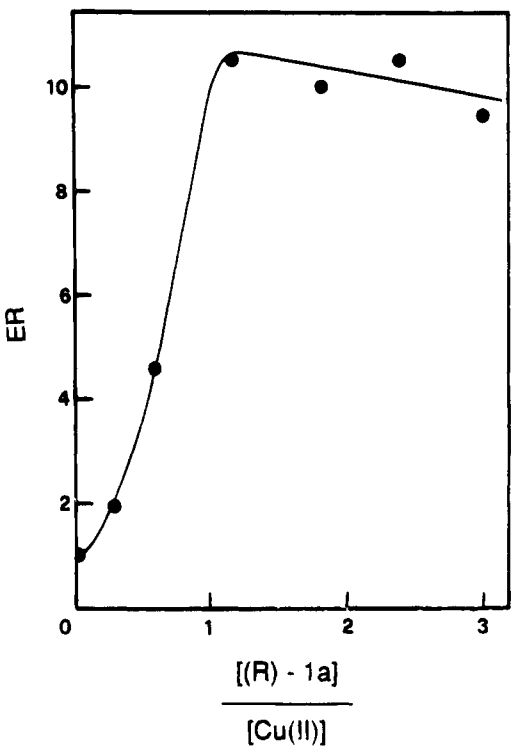
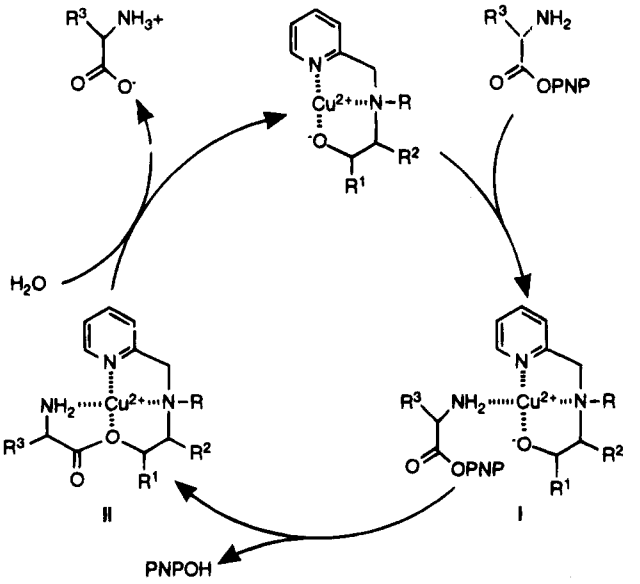


Figure 4. ER *vs* [(*R*)-**1a**]/[Cu(II)] in the hydrolysis of PhgPNP in MES buffer, pH 5.5, 25 °C. Values determined at [Cu(II)] = 8.3 × 10^{-5} M and increasing ligand concentration.

Scheme 4



(*S*)-PhgPNP and (*S*)-**2a** with (*R*)-PhePNP at different pHs showed a clear break point at pH = 7.4 for the system bearing a secondary alcoholic function and at pH = 6.6 for the system bearing a primary alcoholic function (see Figure 5). We associated these different break points with the systematic pK_a of the alcoholic functions coordinated to the Cu(II) ion of the two micellar aggregates studied. The different pK_a values are in accord with the higher acidity of the primary alcohol with respect to the secondary one and are in line with those, ranging from 6.5 to 7.8, reported[7c,8e,f] for other hydroxyl-functionalized complexes with Cu(II).

All these evidences are in accord with the mechanism outlined in Scheme 4. This mechanism involves (a) the formation of a ternary complex between Cu(II), the

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(17) Williams, R. J. P. *J. Mol. Catal., Rev. Issue* **1986**, 1. Hanzlik, R. P. *Inorganic Aspects of Biological and Organic Chemistry*; Academic Press: New York, 1973; pp 128–132.

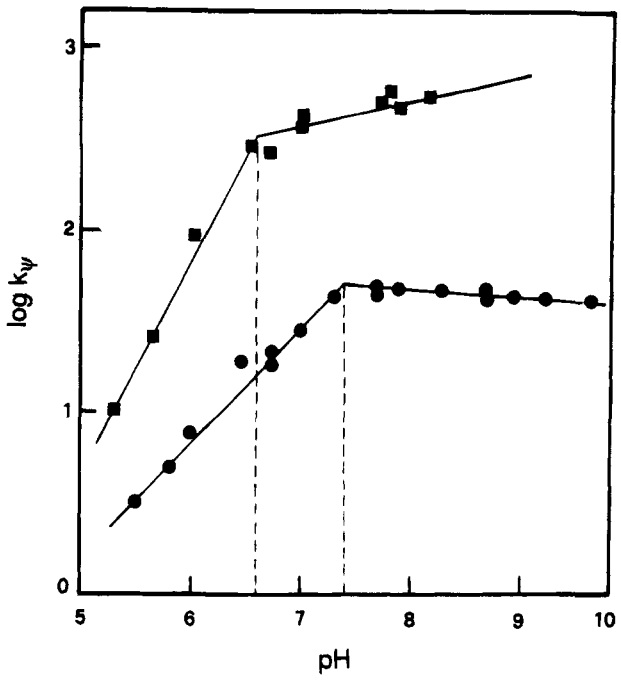

Figure 5. Log k_ψ *vs* pH for the hydrolysis of (*S*)-PhgPNP in the presence of (*R*)-**1a** (1.0×10^{-4} M) and Cu(II) (8.3×10^{-5} M) (circles) and for the hydrolysis of (*R*)-PhePNP in the presence of (*S*)-**2a** (1.0×10^{-4} M) and Cu(II) (1.0×10^{-4} M) (squares).

lipophilic ligand, and the substrate (I), (b) a pseudo-intramolecular nucleophilic attack of the (activated) ligand hydroxyl leading to the release of *p*-nitrophenol and the formation of the transacylation intermediate (II),[18] and (c) the Cu(II)-catalyzed hydrolysis of the intermediate (II) to release the amino acid and regenerate the catalytic species, thus defining the catalytic cycle. After several catalytic cycles, inhibition is expected (and observed) as the amino acid produced competes with the ester for the formation of a ternary complex. We here emphasize that *the catalytic cycle* outlined in Scheme 4 *is effective only for the micellar aggregates.*

In the absence of aggregates, the hydroxyl is no longer the main nucleophilic species: in fact, when the hydrophilic ligand **4** or its O-methylated analog and Cu(II) as the metal ion were employed, no appreciable difference in rate was observed. In such cases, we still assume that the reaction proceeds *via* the formation of a ternary complex; however, within such a complex there is a favorable competition of a bound water molecule over the hydroxyl as the effective nucleophile for esterolysis. We assume that such a competition is present also in the case of aggregates (as a matter of fact, the mechanism of Scheme 4 indicates a bound water molecule as the nucleophile for the hydrolysis of the transacylation intermediate), though in this case, the ligand hydoxyl largely prevails. We speculate that this peculiar behavior of the micellized ligands is due to a geometry of complexation, present only in the micellar system,[15] which allows the hydroxyl to be bound to the metal ion and to effectively compete with water molecules. Outside the aggregates, the weakly bound hydroxyl is easily displaced

(18) We have recently reported firm evidence (Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Org. Chem.* **1994**, *59*, 18) that in the case of similar, achiral metallomicellar systems and *p*-nitrophenyl and other activated amino acid esters, the rate-determining step of the transacylation process is the formation of the tetrahedral intermediate which rapidly collapses to products, the analogs of II of Scheme 4.

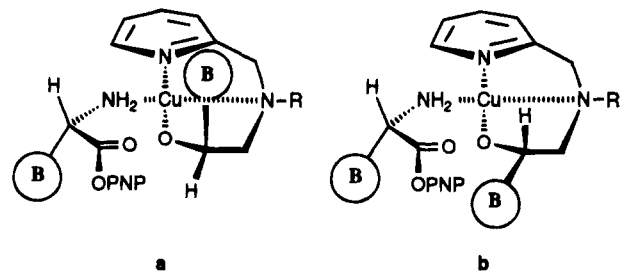

Figure 6. Proposed ternary complexes ligand **1a**–Cu(II)–substrate for the most reactive couple ligand substrate (a) and for the less reactive couple ligand substrate (b).

by a water molecule; such a weak interaction is indicated by the close similarity between the the binding constant with Cu(II) of 2-aminopyridine and 2-[[*N*,*N*-(dihydroxyethyl)amino]methyl]pyridine.[11] This is only one possible, though quite compelling, explanation.

Enantioselectivity. The enantioselectivity values of Table 1 have been evaluated from rate data measured under pseudo-first-order conditions using excess catalyst over the substrate by following the appearance of *p*-nitrophenol. They are obviously related to the cleavage of the ester, *i.e.*, to the (trans)acylation step (Scheme 4) leading to **II**. The subsequent deacylation is also diasteroselective and characterized by a very modest ER value (ca. 2, in the case of **1a**·Cu(II) and Phg as evaluated by turnover experiments).

The analysis of Table 1 reveals two critical points for the achievement of high enantioselectivities: the presence of the free amino group in the substrate and of the hydroxyl in the micellar catalyst. These requirements restrict the possible geometries of the ternary complex (I, see Scheme 4) to those in which the ester of the substrate faces the ligand hydroxyl. Accordingly, the two ternary complexes that may be envisaged for the diastereomeric complexes are those depicted in Figure 6 (for the *S*–*R* and *S*–*S* enantiomers, respectively). As said above, in each case investigated the k_R/k_S (or k_S/k_R) ratios being greater than k_S/k_S (or k_R/k_R), the more reactive diasteromeric complex is the one comprising the enantiomers of opposite absolute configuration at the chiral carbons (Figure 6a). From a simple scrutiny of such geometrical arrangement, where the substituents at the chiral carbons reside on the opposite side with respect to the coordination plane of Cu(II), one tends to speculate that a lesser steric interaction between the bulky groups at the chiral carbons than in the other distereomeric complex may play an important role. Initially, this was our mode of reasoning. However, the data of Table 1 offer little (if any) support to this hypothesis. In fact, the ER values do not depend on the factors which are expected to strongly influence a (direct) steric interaction between the bulky groups (indicated as B in Figure 6), *i.e.*, the position of the chiral center in the ligand and the bulkiness of the substituent at the chiral carbon of the amino ester and of the ligand. Moreover, in the *R*–*S* (or *S*–*R*) ternary complexes with ligands **2**, where the chiral carbon is close to the nitrogen atom, the substituents reside on the same side of the coordination plane. We suggest that the (modest) steric effect is compounded with different solvation requirements of the two diastereomeric complexes. As a consequence, the two complexes may have different binding costants with the aggregate or may reside, within the aggregate, in regions with a different degree of water penetration. As discussed

above, water may effectively compete with the hydroxyl of the ligand by displacing it and may react as the nucleophile in a kinetically slower process. Such a competition, which prevails in a nonmicellar environment, may also occur in the most hydrated regions of the micellar aggregate for the more hydrophilic ternary complex. Notably, this competitive reaction would be less stereoselective in accordance with the data obtained with the water soluble ligand **4** (see entry 27 of Table 1). The experiments performed do not provide any indication on whether enantioselectivity is due to different binding constants or to an intrinsecally different process for the two enantiomers: the plots k_ψ *vs* concentration of the ligand–metal ion complexes were virtually linear in the accessible concentration intervals, thus hampering any reliable determination of the binding constants (see Figure S3 of the supplementary material). However, support to an important entropic contribution to the enantioselection process comes from the slight dependence of the ER from temperature (see Figure S4 of the supplementary material), though these data should be taken with caution as the temperature may also affect the micellization and binding processes.

Conclusion

From the data presented here we have shown how chiral metallomicelles may act as enantioselective catalysts of the cleavage of α-amino acid esters. Though the enantioselectivities observed are moderate, the present study of relatively simple models allowed to identify some of the parameters relevant to the enantioselection. Particularly striking is the role played by the aggregate in governing the involvement of the hydroxyl of the ligand as the nucleophile and hence enhancing the enantioselectivity. This switching-on of the built-in nucleophile (the ligand's hydroxyl) by aggregation has no precedent in micellar catalysis and, as we have already suggested,[15] may be related to a peculiar binding mode of the metal ion resembling the so-called entatic state[17] (a strained, unusual binding geometry) in metalloenzymes. The sp^3 hybridization (*i.e.*, not planar) of the central nitrogen of the ligand appears crucial: preliminary experiments with different ligands having a definitely planar coordination geometry point to a lower enantioselectivity.[19] We speculate that moving to a more ordered aggregate like that of a vesicular membrane where the reaction loci are better defined than in micelles could lead to higher enantioselectivities: experiments in this direction are in progress.

Experimental Section

General Methods. Surface tension measurements to evaluate the cmc of the aggregates were performed with a Kruss Type 8451 tensiometer. Microanalyses were performed by the Laboratorio di Microanalisi of our department. The enantiomeric purity of all the ligands synthesized was checked by ^{1}H-NMR using the shift reagent (*R*)-(−)-9-anthryltrifluoroethanol. In all cases no racemization was detected.

Materials. $Cu(NO_3)_2$, $Zn(NO_3)_2$, and $CoCl_2$ were analytical-grade products. Metal ion stock solutions were titrated against EDTA following standard procedures.[20] The buffer components were used as supplied by the manufacturers: 2-morpholinoethanesulfonic acid (MES), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS), and 2-(cyclohexylamino)ethanesulfonic acid (CHES). *n*-Hexadecyltrimethylammonium bromide (CTABr) was an analytical-grade commercial product. The *p*-nitrophenyl esters of the α-amino acids used as substrates were prepared using literature methods.[21]

(*R*)- and (*S*)-[[N-*n*-Hexadecyl-*N*-(2-hydroxy-1-propyl)-amino]methyl]pyridine (1a). A solution of methyl (*R*)- or (*S*)-lactate (2.0 g, 19.2 mmol) and 2-(aminomethyl)pyridine (3.7 g, 34.2 mmol) in 50 mL of chloroform was stirred at rt for 3 days. The solvent was then evaporated, and the crude was purified by column chromatography (SiO_2, ethyl acetate/MeOH (4:1)) yielding 3.15 g (91%) of *N*-(2-pyridylmethyl)lactamide that was crystallized from $CHCl_3$/hexane: mp 79–80 °C; $[\alpha]_D$ = +16.3 (*c* = 1, EtOH) for the *R* enantiomer and $[\alpha]_D$ = −16.8 (*c* = 1, EtOH) for the *S* enantiomer. The above amide (2.1 g, 11.6 mmol) was dissolved in 100 mL of CH_2Cl_2, and to this solution were added 3,4-dihydro-2*H*-pyran (3.9 g, 46.5 mmol) and pyridinium *p*-toluenesulfonate (0.4 g, 1.6 mmol). After the mixture was stirred for 2 days at room temperature the organic solvent was extracted with brine (3 × 50 mL), dried over Na_2SO_4, and evaporated to afford a crude that was purified by column chromatography (SiO_2, ethyl acetate/MeOH (4:1)) yielding 1.2 g (39%) of *N*-(2-pyridylmethyl)-*O*-(2-pyranyl)-lactamide as a clear oil.

To a solution of this O-protected amide (1.2 g, 4.5 mmol) in 50 mL of dry THF were added NaH (0.20 g of 60% dispersion in mineral oil) and 1-bromohexadecane (1.5 g, 5.0 mmol). After the mixture was heated at reflux for 24 h the slurry was filtered over Celite and the organic solvent evaporated giving a crude product that was first purified by column chromatography (SiO_2, ethyle acetate/toluene (1:1)) and then dissolved in 50 mL of MeOH containing 0.25 mL of concd HCl. After 15 min of stirring at room temperature the solvent was evaporated to give 1.08 g (58%) of *N*-*n*-hexadecyl-*N*-(2-pyridylmethyl)lactamide as a white solid: mp 104–105 °C, $[\alpha]_D$ = −1.90 (*c* = 1, EtOH) for the *R* enantiomer and $[\alpha]_D$ = +1.95 (*c* = 1, EtOH) for the *S* enantiomer.

This compound (1.08 g, 2.7 mmol) was dissolved in dry THF, and $LiAlH_4$ (0.16 g, 4.2 mmol) was added. After the mixture was heated at reflux for 6 h under a nitrogen atmosphere, 10 mL of a 10% solution of NaOH was cautiously added. The precipitate was filtered over a Celite pad and washed thoroughly with THF, and the combined organic washings were evaporated. The solid left was suspended in water and extracted with $CHCl_3$ (3 × 50 mL). From the evaporation of the dried (Na_2SO_4) organic phase was obtained a crude that was purified first by column chromatography (SiO_2, ethyl acetate) and then by crystallizing it as the hydrochloride from acetone and HCl. The salt was filtered, washed with Et_2O, dissolved in $CHCl_3$, and treated with a saturated solution of $NaHCO_3$ (3 × 20 mL). The evaporation of the dried (Na_2SO_4) organic solvent afforded 0.21 g (20%) of pure **1a** as an oil: $[\alpha]_D$ = −48.0 (*c* = 2, $CHCl_3$) and $[\alpha]_D$ = +52.0 (*c* = 2, $CHCl_3$), respectively, for the *R* and *S* enantiomer. ^{1}H-NMR δ ($CDCl_3$): 0.81 (t, *J* = 6.7 Hz, 3H), 1.02 (d, *J* = 7.5 Hz, 3H), 1.17 (m, 26H), 1.70 (m, 2H), 2.45 (m, 4H), 3.64 (d, *J* = 14.8 Hz, 1H), 3.75 (m, 1H), 3.89 (d, *J* = 14.8 Hz, 1H), 7.10 (m, 1H), 7.28 (d, *J* = 7.73 Hz, 1H), 7.65 (dt, *J* = 1.83 and 7.73 Hz, 1H), 8.52 (m, 1H). Anal. Calcd for $C_{25}H_{46}N_2O$ × 2HCl: C, 64.77; H, 10.43; N, 6.04. Found: C, 64.33, H, 10.59; N, 5.94.

General Procedure for the Synthesis of Ligands 1b and 2. To a solution of the proper commercially available amino alcohol (5.1 mmol) in 50 mL of benzene was added the proper aldehyde (hexadecyl aldehyde[22] for **1b** or dodecyl aldehyde for ligands **2**). The solution was heated at reflux for 3 h distilling azeotropically the water formed during the reaction. The solvent was then evaporated and the residue dissolved in 50 mL of EtOH containing $NaBH_4$ (0.38 g, 10.2 mmol). After the mixture was stirred at room temperature overnight, the solvent was evaporated and the residue was treated with water and extracted with $CHCl_3$ (3 × 50 mL). The evaporation of the dried (Na_2SO_4) afforded pure *N*-alkylamino alcohols.

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(21) Schnabel, E. *Ann.* **1964**, *673*, 171.
(22) Corey, E. J.; Suggs, W. J. *Tetrahedron.Lett.* **1975**, 2647.

To a solution of the above amino alcohol (4.9 mmol) in 15 mL of 2-propanol were added 2-(chloromethyl)pyridine hydrochloride (0.97 g, 5.9 mmol) and *N,N*-diisopropylethylamine (4.5 mL, 26.3 mmol). This solution sealed in a screw-top pressure vial was heated at 80 °C for 72 h. The reaction mixture was then diluted with 100 mL of $CHCl_3$ and washed with a solution of 10% $NaHCO_3$ (3 × 50 mL). The evaporation of the dried (Na_2SO_4) organic solvent afforded a crude that was purified by column chromatography (SiO_2, $CHCl_3/CH_3OH$, 50:1 for **1b**, **2a**, and **2c** or toluene/ethyl acetate (7:3) for **2b** and **2d**).

(*S*)-2-[[*N*-*n*-Hexadecyl-*N*-(1-methyl-2-hydroxyethyl)-amino]methyl]pyridine (1b): 0.80 g (42%), yellowish oil, $[\alpha]_D$ = +52.0 (*c* = 0.5, $CHCl_3$). ^{1}H-NMR δ ($CDCl_3$): 0.90 (m, 6H), 1.23 (m, 28 H), 2.60 (m, 2H), 3.10 (m, 1H), 3.41 (m, 2H), 3.50 (d, *J* = 14.95 Hz, 1H), 3.91 (d, *J* = 14.95 Hz), 7.18 (m, 1H), 7.27 (d, *J* = 7.33 Hz, 1H), 7.70 (dt, *J* = 1.83 and 7.33 Hz, 1H), 8.55 (m, 1H). Anal. Calcd for $C_{25}H_{46}N_2O$: C, 76.86; H, 11.87; N, 7.17. Found: C, 75.92, H, 11.95; N, 7.10.

(*S*)-2-[[*N*-*n*-Dodecyl-*N*-(1-methyl-2-hydroxyethyl)-amino]methyl]pyridine (2a): 0.96 g (59%), mp 39–41 °C, $[\alpha]_D$ = +55.4 (*c* = 0.5, $CHCl_3$). ^{1}H-NMR δ ($CDCl_3$): 0.90 (m, 6H), 1.29 (m, 20 H), 2.52 (m, 2H), 3.05 (m, 1H), 3.38 (m, 2H), 3.49 (d, *J* = 14.65 Hz, 1H), 3.89 (d, *J* = 14.65 Hz, 1H), 7.15 (m, 1H), 7.31 (d, *J* = 7.63 Hz, 1H), 7.63 (dt, *J* = 1.83 and 7.63 Hz, 1H), 8.53 (m, 1H). Anal. Calcd for $C_{21}H_{38}N_2O$: C, 75.4; H, 11.45; N, 8.37. Found: C, 75.12, H, 11.6; N, 8.22.

(*S*)-2-[[*N*-*n*-Dodecyl-*N*-(1-isopropyl-2-hydroxyethyl)-amino]methyl]pyridine (2b): 0.94 g (53%), yellowish oil, $[\alpha]_D$ = +4.6 (*c* = 0.6, $CHCl_3$). ^{1}H-NMR δ ($CDCl_3$): 0.84 (t, *J* = 6.7 Hz, 3H), 0.86 and 1.00 (2d, *J* = 6.71 Hz, 6H), 1.23 (m, 20 H), 1.80 (m, 1H), 2.55 (dt, *J* = 4.27 and 10.1 Hz, 1H), 2.71 (t, *J* = 7.32 Hz, 2H), 3.36 (t, *J* = 10.38 Hz, 1H), 3.65 (dd, *J* = 4.2 and 10.38 Hz, 1H), 3.82 (d, *J* = 15.26 Hz, 1H), 4.05 (d, *J* = 15.26 Hz, 1H), 7.17 (m, 2H), 7.62 (dt, *J* = 1.22 and 7.32 Hz, 1H), 8.54 (m, 1H). Anal. Calcd for $C_{23}H_{42}N_2O$: C, 76.19; H, 11.68; N, 7.73. Found: C, 75.94, H, 11.91; N, 7.63.

(*R*)-2-[[*N*-*n*-Dodecyl-*N*-(1-phenyl-2-hydroxyethyl)-amino]methyl]pyridine (2c): 0.44 g (23%), yellowish oil, $[\alpha]_D$ = −57.0 (*c* = 0.5, $CHCl_3$). ^{1}H-NMR δ ($CDCl_3$): 0.88 (t, 3H, *J* = 6.7 Hz), 1.21 (m, 18H), 1.39 (m, 2H), 2.53 (m, 2H), 3.47 (d, *J* = 15.26 Hz, 1H), 3.72 (m, 1H), 4.04 (m, 2H), 4.15 (d, *J* = 15.26 Hz, 1H), 7.18 (m, 1H), 7.31 (m, 6H), 7.63 (dt, *J* = 1.83 and 7.63 Hz, 1H), 8.58 (m, 1H). Anal. Calcd for $C_{26}H_{40}N_2O$: C, 78.74; H, 10.17; N, 7.06. Found: C, 78.45, H, 10.36; N, 6.89.

(*R*)-2-[[*N*-*n*-Dodecyl-*N*-(1-benzyl-2-hydroxyethyl)-amino]methyl]pyridine (2d): 0.45 g (23%), yellowish oil, $[\alpha]_D$ = −13.6 (*c* = 0.5, $CHCl_3$). ^{1}H-NMR δ ($CDCl_3$): 0.88 (t, 3H, *J* = 6.7 Hz), 1.24 (m, 20H), 2.43 (dd, *J* = 9.16 and 13.12 Hz, 1H), 2.64 (m, 2H), 2.99 (dd, *J* = 4.88 and 13.12 Hz, 1H), 3.14 (m, 1H), 3.46 (m, 2H), 3.55 (d, *J* = 14.95 Hz, 1H), 4.07 (d, *J* = 14.95 Hz, 1H), 4.71 (bs, 1H), 7.22 (m, 7H), 7.64 (dt, *J* = 1.83 and 7.63 Hz, 1H), 8.55 (m, 1H). Anal. Calcd for $C_{27}H_{42}N_2O$: C, 78.97; H, 10.31; N, 6.82. Found: C, 78.52, H, 10.50; N, 6.63.

(*S*)-[[*N*-*n*-Hexadecyl-*N*-(2-methoxy-1-propyl)amino]-methyl]pyridine (3). To a suspension of NaH (50 mg of a 60% dispersion in mineral oil) in 10 mL of dry THF, kept at 0 °C under a nitrogen atmosphere, were slowly added first a solution of (*S*)-**1a** (0.3 g, 0.7 mmol) in 20 mL of THF and then CH_3I (0.1 g, 0.7 mmol). The resulting slurry was stirred at room temperature for 4 h, and then 20 mL of water was added. The organic solvent was evaporated, and the remaining water was extracted with $CHCl_3$ (3 × 20 mL). The evaporation of the dried (Na_2SO_4) organic solvent afforded a crude that was purified by column chromatography (SiO_2, $CHCl_3$/MeOH (10:1)) to give 0.21 g (67%) of pure **3** as an oil: $[\alpha]_D$ = −6.9 (*c* = 0.15, CH_3OH). ^{1}H-NMR δ (CD_3OD): 0.94 (t, *J* = 6.7 Hz, 3H), 1.15 (d, *J* = 6.1 Hz, 3H), 1.33 (m, 26H), 1.52 (m, 2H), 2.57 (m, 4H), 3.36 (s, 3H), 3.52 (m, 1H), 3.78 (d, *J* = 14.9 Hz, 1H), 3.86 (d, *J* = 14.9 Hz, 1H), 7.34 (m, 1H), 7.67 (d, *J* = 7.93 Hz, 1H), 7.86 (dt, *J* = 1.83 and 7.93 Hz, 1H), 8.47 (m, 1H). Anal. Calcd for $C_{26}H_{48}N_2O$: C, 77.17; H, 11.96; N, 6.92. Found: C, 76.94, H, 12.03; N, 6.72.

(*S*)-[[*N*-Methyl-*N*-(2-hydroxy-1-propyl)amino]methyl]-pyridine (4). (*S*)-*N*-Methyl-2-hydroxypropylamine[23] (0.50 g, 5.6 mmol), 2-(chloromethyl)pyridine hydrochloride (1.0 g, 8.4 mmol), and *N,N*-diisopropylethylamine (1.4 mL, 8.4 mmol) were dissolved in 50 mL of 2-propanol. The reaction mixture was sealed in a pressure vial and heated at 80 °C for 24 h. The solvent was then evaporated and the crude dissolved in 50 mL of CH_3OH containing 2 g of Na_2CO_3. The slurry was stirred at 50 °C for 1 h and then filtered over a Celite pad. The organic solvent was evaporated and the oil left dissolved in $CHCl_3$ and eluted through a short column of basic alumina. The evaporation of the $CHCl_3$ gives 0.2 g (20%) of pure **4** as an oil: $[\alpha]_D$ = +69.1 (*c* = 0.24, $CHCl_3$). ^{1}H-NMR δ ($CDCl_3$): 1.13 (d, *J* = 6.10 Hz, 3H), 2.38 (s, 3H), 2.43 (m, 2H), 3.70 (d, *J* = 14.0 Hz), 3.89 (d, *J* = 14.0 Hz, 1H), 3.90 (m, 1H), 7.20 (m, 1H), 7.39 (d, *J* = 7.63 Hz, 1H), 7.69 (dt, *J* = 1.83 and 7.63 Hz, 1H), 8.56 (m, 1H). Anal. Calcd for $C_{10}H_{16}N_2O$: C, 66.64; H, 8.95; N, 15.54. Found: C, 66.30, H, 9.01; N, 15.38.

Cmc Measurements. Critical micellar concentrations for the ligands soluble in acidic water were determined kinetically from the break points in the plots k_ψ against ligand concentrations (obtained in the standard conditions, see Figure 1). The values obtained are as follows: **1a**, 3 × 10^{-5} M; **1b**, 2 × 10^{-5} M; **2a**, 5 × 10^{-4} M; **2b**, 3 × 10^{-4} M. For the last two ligands cmc values were also determined in MES buffer (0.05 M, pH 5.5) by surface tension measurements giving values of 3 × 10^{-4} M and 1.5 × 10^{-4} M, respectively.

Kinetic Studies. Slower reactions were followed on a spectrophotometer equipped with a thermostated cell holder and faster reactions on an Applied Photophysics SF.17MV stopped flow spectrometer. Solutions of the ligands, metal ions, and additives were prepared in MES (0.05 M, pH 5.5) buffer. Reaction temperature was maintained at 25 ± 1 °C. Slower reactions were started by addition of 20 μL of a solution 1–2 × 10^{-3} M of substrate in CH_3CN to 2 mL of solution of ligand and additives, and faster reactions were started by mixing equal volumes of a solution of (2–4) × 10^{-5} M of substrate in water at pH 3.5 (HNO_3) with the solution of ligands and additives and buffer. In both cases no changes in pH were observed during the kinetic runs. The final concentration of substrate was (1–2) × 10^{-5} M, and the kinetics follow in each case a first-order low up to 90% of reaction. This is true also at low ligand concentrations, and this may be explained with a much lower concentration of ternary complex with respect to the total concentration of substrate.[7d] The rate constants were obtained by nonlinear regression analysis of the absorbance *vs* time data (using the software package Enzfitter (Leatherbarrow, R. J. *Enzfitter*; Elsevier: Amsterdam, 1987) or the software package SF.17MV provided with the stopped flow work station), and the fit error on the rate constant was always less than 1%. To measure the pH dependence of the hydrolysis rate the following buffers were used: MES, 5.3 < pH < 6.7; HEPES, 6.7 < pH < 7.7; EPPS, 7.7 < pH < 8.7; CHES, 8.7 < pH < 9.7. Rate constants at borderline pH values were measured in both buffers to rule out any rate dependence on the buffer nature.

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Supplementary Material Available: Figures S1–S4 reporting the kinetic profiles mentioned in the text and full ^{1}H-NMR and analytical data for all new compounds described in the Experimental Section (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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