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Enantioselective hydrolysis of long chain α-amino acid esters by chiral sulfur-containing macrocyclic metallomicelles

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Abstract

A novel chiral lipophilic sulfur-containing macrocyclic ligand **5** with bis-pendant alcohols in the proximity of the coordination center has been synthesized. Its metal ion complexes have been investigated as catalysts for the enantioselective hydrolysis of long chain α-amino acid esters in aqueous comicellar solution with Brij35. Large rate accelerations (up to 220 times) and moderate enantioselectivities (up to 4.85 (k_S/k_R)) employing the macrocyclic **5**-Cu^{2+} have been observed, whereas the acyclic **3**-Cu^{2+} exhibits less reactivity and stereoselectivity. Taking the analogous ligand **4**, lacking the hydroxy groups leads to a dramatic rate decrease, and an inversion of enantioselectivity is observed. The pKa value of the hydroxyl bound to Cu^{2+} is determined to be pKa=7.2 under our micellar reaction conditions.

1. Introduction

Metallomicelles are currently receiving interest because of their catalytic ability to mimic hydrolytic metalloenzymes.[1–7] They are made up of either transition metal complexes of ligand surfactants or comicelles of lipophilic ligand complexes with nonfunctionalized surfactants. These artificial enzymes are effective in promoting the cleavage of carboxylic and phosphoric acid esters or amides: in most cases the ligands contain a hydroxyl group in close proximity to the metallocleft. The hydroxyl group is activated by transition metal ions such as Cu^{2+} or Zn^{2+} and acts as an effective nucleophile in a transacylation process. More recently, enantioselective cleavage of α-amino acid esters by chiral metallomicelles has been reported, and remarkable enantioselectivities have been observed.[8] Following our interest in chiral sulfur-containing ligands,[9] we have designed and synthesized a chiral lipophilic sulfur-containing macrocyclic ligand **5** with two alcohol pendants. According to CPK models, a metal ion bound to the metallocleft of ligand **5** is favorably positioned to activate the hydroxyl group for nucleophilic attack. Moreover, the metal ion is able to coordinate and activate the substrate. We report

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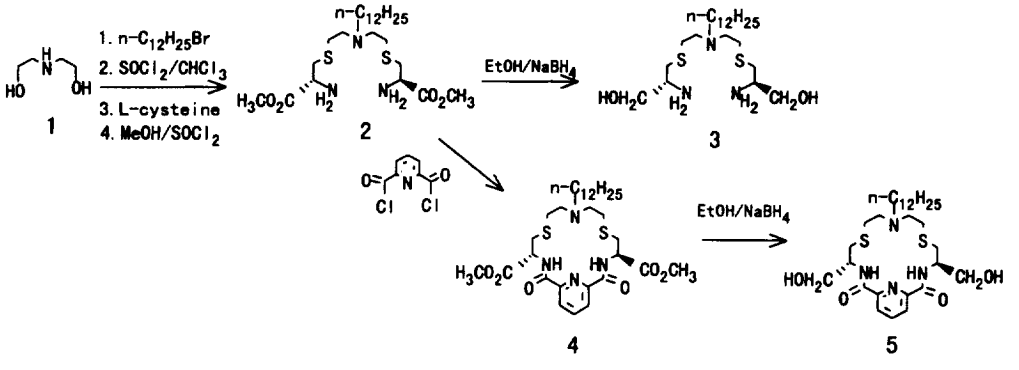
here the first example of chiral macrocyclic metallomicelles and their effect on the enantioselective cleavage of N-dodecanoylamino acid *p*-nitrophenyl esters. Macrocyclic ligand **4** lacks the hydroxymethyl group with the aim of defining the role of the hydroxy function. In order to study the structural effect of acyclic ligand and macrocyclic ligand on the catalytic activity and enantioselectivity, we have synthesized ligand **3** that is a more flexible open stucture.

2. Results and discussion

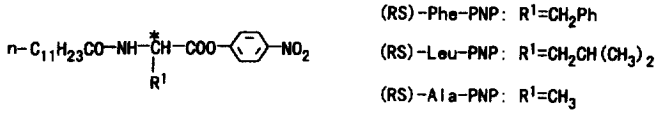
2.1. Ligands and substrates

Macrocyclic ligand **5** was synthesized according to the procedures outlined in Scheme 1 using L-cysteine and diethanolamine as the starting material. Reaction of diethanolamine with 1-bromododecane yielded N,N-bis(β-hydroxyethyl)-n-dodecylamine, and then converted N,N-bis(β-chloroethyl)-n-dodecylamine with $SOCl_2$. In the presence of NaOH and NaI, L-cysteine reacted with the long chain dichloroalkane to form a bridged bis-amino acid, and then converted to the diester derivative. Subsequently the diester reacted with 2,6-bis(chlorocarbonyl)pyridine by the high-dilution technique to give macrocyclic ligand **4**. Reduction eventually yielded macrocyclic ligand **5**. Model compound **3** was obtained by reducing compound **2** with $NaBH_4$.



Scheme 1.

Macrocyclic ligands **5** and **4** are barely soluble in neutral water. Their clear solutions, free or as transition metal ion complexes, can be obtained only in the presence of micelles of inert surfactants such as Brij35 as the matrix of comicellar aggregates. The kinetic experiments were carried out using mixed micelles, composed of a metal ion complex and Brij35. Compound **3** is soluble in water and forms a micellar aggregate: the critical micellar concentration (cmc) is 4.4×10^{-4} M in MES buffer (0.05 M, pH 6.80).[8c] Scheme 2 shows the substrates investigated: they are all chiral, nonmetallophilic substrates, i.e., the N-protected *p*-nitrophenyl esters of R(S)-phenylalanine, R(S)-leucine and R(S)-alanine.



Scheme 2.

Table 1
Pseudo-first-order rate constants (k_R and k_S, s^{-1}) and enantioselectivities (k_S/k_R) for cleavage of the N-protected α-amino acid esters by mixed micellar systems composed of chiral metal complexes and Brij35

Entry	Ligand	M^{2+}	Substrate	$k_S/10^{-5}$	$k_R/10^{-5}$	k_S/k_R
1	none	none	R(S)-Phe-PNP	9.01	9.02	1.00
2	none	Cu^{2+}	R(S)-Phe-PNP	12.9	12.6	1.02
3	**5**	none	R(S)-Phe-PNP	12.8	10.8	1.19
4	**5**	Cu^{2+}	R(S)-Phe-PNP	2000	412	4.85
5	**5**	Zn^{2+}	R(S)-Phe-PNP	350	121	2.89
6	**5**	Co^{2+}	R(S)-Phe-PNP	89.2	61.3	1.46
7	**5**	Cu^{2+}	R(S)-Leu-PNP	1320	400	3.30
8	**5**	Cu^{2+}	R(S)-Ala-PNP	222	156	1.42
9	**3**	Cu^{2+}	R(S)-Phe-PNP	582	242	2.40
10	**4**	Cu^{2+}	R(S)-Phe-PNP	204	259	0.79

Conditions: 25 °C, pH 6.80 (0.05M MES buffer), [ligand]= 2.0×10^{-4}M, [substrate]= 2.0×10^{-5} M, $[M^{2+}]= 2.0\times10^{-4}$ M, [Brij35]=4.0×10^{-3}M

2.2. *Kinetics*

The rate of hydrolysis was followed by observing the release of *p*-nitrophenolate spectrophotometrically (400 nm) under pseudo-first-order conditions. The pseudo-first-order constants (k_R and k_S) for the cleavage of the substrates catalyzed by metal ion complexes comicellized with Brij35 are summarized in Table 1. The enantioselectivity of each system is indicated by the ratios k_S/k_R. From the data in Table 1, copper ion itself shows essentially no catalytic activity, whereas in the absence of metal ions, the ligands exhibit only a slight rate enhancement and enantioselectivity. Large rate enhancement and enantioselectivity (k_S/k_R) are observed in the presence of both ligand and metal ion. These indicate that catalytic activity is the result of synergistic cooperation of metal ion and ligand. The catalytic activity order of metal ion is $Cu^{2+}>Zn^{2+}>Co^{2+}$. Most strikingly, the enantioselectivity of **5**-M^{2+} is also very sensitive to the nature of the transition metal ion and the highest value (4.85) is found for **5**-Cu^{2+} (entries 4, 5 and 6). For all **5**-M^{2+} the (S)-substrate is hydrolyzed faster than its (R)-enantiomer. Entries 4 and 9 in Table 1 show that acyclic metallocatalyst **3**-Cu^{2+} are less reactive and enantoiselective compared with macrocyclic metallocatalyst **5**-Cu^{2+}. Though these data are far from impressive, they clearly indicate that the macrocyclic system is more efficient, as expected from the reduction in the degrees of freedom of the system. Taking the analgous ligand **4** and **5** as an exemplary case (compare entries 4 and 10), lacking the hydroxyl leads to a dramatic rate decrease, and an inversion of enantioselectivity is observed.[3,8c] These results imply that the free hydroxy group of the ligand and the rigidity of the ligand are mandatory in order to achieve both high rate accelerations and good enantioselectivities. Table 1 also indicates that increasing the size and lipophilicity of the side chain in the substrate (entries 4, 7 and 8) causes an increase in the rate and enantioselectivity.

2.3. Stoichiometry of the reactive complexes

In order to know the stoichiometry of the kinetically reactive complexes, the kinetic version of Job Plots was examined by plotting k_R and k_S as a function of molar fraction of the ligand (γ), keeping the total concentrations of ligand and metal ion constant. The results shown in Fig. 1 indicate that in the case of Cu^{2+} and ligand **5** the rate maxima are observed at γ=0.5, which correspond to a stoichiometry of ligand: Cu^{2+}=1:1. Ligand **5** forms stable complexes with Cu^{2+} as indicated by the sharp maxima in the Job plots.

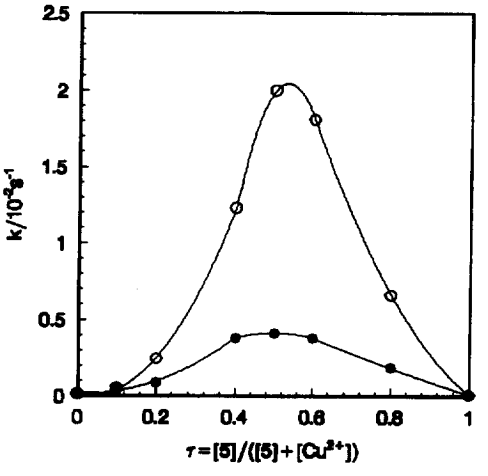


Fig. 1. Kinetic Job plots for the cleavage of (R)-Phe-PNP (filled circles) and (S)-Phe-PNP (empty circles) by ligand **5** and Cu^{2+} in MES buffer, pH 6.80, 25°C. ([5]+[Cu^{2+}])=4.0×10^{-4} M, [Brij35]=4.0×10^{-3} M, (R(S)-Phe-PNP)=2.0×10^{-5} M

2.4. pH–Rate profile

As said above, from the analysis of the data in Table 1, a free hydroxyl in the ligand structure is a requisite for higher rate acceleration and enantioselectivity in micellar systems. A pH–rate constant profile was determined for reactions of (S)-Phe-PNP with catalyst **5**-Cu^{2+}. The pH value was checked before and after any kinetic run and proved to be constant within ±0.05 pH unit. A plot of log k *versus* pH (Fig. 2) gave a sharp break at pH 7.2, which we take as the systematic pKa of the hydroxyl bound to Cu^{2+} under our micellar reaction conditions. A pKa=6.4 for a Cu^{2+} co-ordinated hydroxyl has been recently reported by Tonellato.[8c] It would be very interesting to know the pKa of the Cu^{2+}-bound water molecule, which should be responsible for the cleavage of R(S)-Phe-PNP by the macrocyclic ligand **4**. Regrettably solutions of the complex **4**-Cu^{2+} were unstable at pH>7.5. Up to that pH we did not observe any break in the log k_S *versus* pH profile.

3. Experimental

3.1. General methods and materials

Melting points were taken on a micro-melting apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 299.95 MHz, and chemical shifts in ppm are reported relative to internal Me_4Si. Mass spectra data were recorded on a Finnigan MAT 4510 spectrometer. Elemental analy-

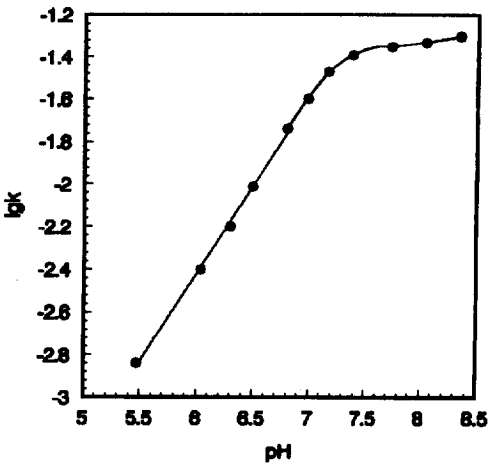


Fig. 2. Log k *versus* pH for the hydrolysis of (S)-Phe-PNP by Cu^{2+}. See Table 1 for other conditions

ses were performed with a Carlo Erba 1106 instrument. Optical rotations were taken on a WZZ-1 polarimeter. Kinetic runs were conducted on a Shimadzu UV-265FW spectrophotometer equipped with a thermostated cell compartment. $Zn(NO_3)_2 \cdot 6H_2O$, $Cu(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2$, and polyethylene glycol dodecyl ether (Brij35) were purchased from commercial sources and used without purification. The buffers were 2-morpholinoethanesulfonic acid (MES) (pH=5.0–6.8), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH=6.8–7.8), 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (EPPS) (pH=7.7–8.5). L-Cysteine hydrochloride was purchased from Sino-American Biotechnology Company for use without further purification. The following compounds were prepared according to literature procedures: 2,6-bis(chlorocarbonyl)pyridine,[10] the *p*-nitrophenyl esters of the N-protected α-amino acids.[11] Methylene dichloride was purified according to the standard method. All other chemicals and reagents were obtained commercially and used without further purification.

3.2. *N,N-Bis[((R)-2-amino-2-methoxycarbonylethylthio)ethyl]-n-dodecylamine trihydrochloride* 2

A mixture of diethanolamine **1** 18.3 g (0.174 mol), 1-bromododecane 43.6 g (0.175 mol) and K_2CO_3 30.0 g (0.217 mol) was heated at 170°C for 7 h, C_2H_5OH was then added. The mixture was filtered and washed with C_2H_5OH. The organic phase was evaporated to leave a dark brown oil, which was purified by column chromatography (silica, $CHCl_3$:CH_3OH 10:1) to give N,N-bis(β-hydroxyethyl)-n-dodecylamine as a pale yellow waxy solid (yield 70%). MS (m/z): 273 (M^+, 40).

N,N-Bis(β-hydroxyethyl)-n-dodecylamine 4.0 g (14.7 mmol) in $CHCl_3$ (50 mL) was cooled to 0°C. Thionyl chloride (11 mL) was then added dropwise. After the addition the reaction mixture was refluxed for 4 h, then brought down to room temperature and left to stand overnight. The solvent was evaporated. The residue was treated with a solution of sodium carbonate (10%) until the pH was 8.0, then extracted with $CHCl_3$ (3×50 mL). The organic layer was then separated, dried over $MgSO_4$, filtered and evaporated to yield N,N-bis(β-chloroethyl)-n-dodecylamine as a pale yellow oil (yield 97%). MS (m/z): 310 (M^+, 100).

L-Cysteine hydrochloride 4.1 g (26 mmol) and NaI 0.5 g were dissolved in a solution of 25 mL water and 60 mL ethanol, and NaOH 2.08 g (52 mmol) was then addd slowly with stirring. N,N-Bis(β-chloroethyl)-n-dodecylamine 4.0 g (12.9 mmol) was added dropwise over 30 min and continued for 4 h at 60°C. The mixture was cooled in an ice bath. The precipitate was filtered off and sequentially washed with water, ethanol, ether, and then dried to gain a bridged amino acid (yield 65%) as a white solid.

The bridged amino acid 4.0 g (8.35 mmol) was suspended in 50 mL of methanol and cooled to 0°C in an ice bath. $SOCl_2$ 2.0 mL was added dropwise with stirring. The mixture was stirred at the same temperature for 1 h and then at 60°C for another 4 h. The solvent was evaporated under vacuum to give the ester of bridged amino acid hydrochloride **2** (yield 98%).

3.3. (4R,14R)-9-Dodecyl-4,14-dimethoxycarbonyl-6,12-dithia-3,9,15,18-tetraazabicyclo[15.3.1]heneicosa-1(18),17(19),20-triene-2,16-dione 4

Compound **2** 1.5 g (2.4 mmol) and K_2CO_3 2.2 g were added to 500 mL dry CH_2Cl_2, and the mixture was stirred for 4 h. 2,6-Bis(chlorocarbonyl)pyridine 0.5 g (2.4 mmol) in 50 mL CH_2Cl_2 was added drop by drop. When the reaction was complete as monitored by TLC, water (10 mL) was added. The organic layer was separated, and dried over $MgSO_4$. The filtered solution was evaporated under reduced pressure, and the raw product was chromatographed on silica gel (ethyl acetate:petroleum=2:1) to give a white solid (yield 50%); m.p. 61–63°C; $[\alpha]_D$=+22.5 (c=1.0, CH_3OH). ^1H NMR($CDCl_3$) δ in ppm: 0.87 (t, J=6.6 Hz, 3H, CH_2CH_3), 1.24 (m, 18H, $(CH_2)_9CH_3$), 1.40 (m, 2H, $(CH_2)_9CH_2CH_2N$), 2.36 (m, 4H, $2SCH_2CH_2N$), 2.63 (m, 6H, $3NCH_2$), 3.04 (dd, J=4.8, 4.2 Hz, 2H, $2SCH_{2a}CHNH$), 3.10 (dd, J=4.8, 4.2 Hz, 2H, $2SCH_{2b}CHNH$), 3.78 (S, 6H, $2OCH_3$), 5.01 (m, 2H, 2CH), 8.05 (t, J=8.1 Hz, 1H, *Py*H4), 8.36 (d, J=7.8 Hz, 2H, *Py*H3 and *Py*H5), 8.78 (d, J=8.4 Hz, 2H, 2NH). Anal. calcd for $C_{31}H_{50}N_4O_6S_2$: C, 58.28, H, 7.89, N, 8.77; found: C, 58.12, H, 7.99, N, 8.52. MS (m/z): 639 (M^+, 20).

3.4. (4R,14R)-9-Dodecyl-4,14-dihydroxymethyl-6,12-dithia-3,9,15,18-tetraazabicyclo[15.3.1]heneicosa-1(18),17(19),20-triene-2,16-dione 5

Compound **4** (1.3 mmol) was dissolved in 60 mL of EtOH containing $NaBH_4$ 0.3 g (7.9 mmol). After it was stirred at room temperature overnight, the mixture was refluxed for 4 h. The solvent was evaporated and the residue was treated with water and extracted with $CHCl_3$ (5×30 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica, $CHCl_3$:CH_3OH=100:5) to give a white solid (yield 36%); m.p. 172–174°C; $[\alpha]_D$=+7.1 (c=1.0, $CHCl_3$). ^1H NMR ($CDCl_3$) δ in ppm, 0.88 (t, J=6.6 Hz, 3H, CH_3), 1.23 (m, 18H, $(CH_2)_9CH_3$), 1.39 (m, 2H, $NCH_2CH_2(CH_2)_9$), 2.40 (t, J=7.8 Hz, 4H, $2SCH_2CH_2N$), 2.65 (m, 6H, $3NCH_2$), 2.78 (m, 4H, $2SCH_2CHNH$), 2.95 (m, 2H, $2NHCHCH_2OH$), 3.86 (m, 4H, $2CH_2OH$), 4.35 (m, 2H, 2OH), 7.98 (t, J=7.8 Hz, 1H, *Py*H4), 8.27 (d, J=7.8 Hz, 2H, *Py*H3 and *Py*H5), 8.65 (d, J=7.5 Hz, 2H, 2NH). Anal. calcd for $C_{29}H_{50}O_4N_4S_2$: C, 59.76, H, 8.65, N, 9.61; found: C, 59.59, H, 8.80, N, 9.49. MS (m/z): 584 (M^++1, 100).

3.5. N,N-Bis[((R)-2-amino-3-hydroxylpropylthio)ethyl]-n-dodecylamine 3

This compound was synthesized by following the same procedure as followed in the synthesis of ligand **5**, using compound **2**. The crude product was purified by column chromatography (silica, $CHCl_3$:CH_3OH:$NH_3 \cdot H_2O$=7:1:0.15) to give a white solid (yield 40%); m.p. 49–51°C; $[\alpha]_D$=−20.1 (c=1.0, $CHCl_3$). ^1H NMR($CDCl_3$) δ in ppm, 0.88 (t, J=6.9 Hz, 3H, CH_3), 1.26 (S, 18H, $(CH2)_9CH_3$), 1.42 (m, 2H, $NCH_2CH_2(CH_2)_9$), 2.45 (m, 4H, $2NH_2$), 2.64 (m, 6H, $3NCH_2$), 2.75 (m, 2H, 2CH), 3.01 (m, 2H, 2OH), 3.26 (s, 8H, $4SCH_2$), 3.46 (dd, J=6.3, 4.5 Hz, 2H, $2CH_{2a}OH$), 3.50 (dd, J=6.0, 4.2 Hz, 2H, $2CH_{2b}OH$). Anal. calcd for $C_{22}H_{49}O_2N_3S_2$: C, 58.49, H, 10.93, N, 9.30; found: C, 58.24, H, 11.11, N, 9.18. MS (m/z): 452 (M^+, 30).

3.6. Kinetic studies

Solutions of the ligands, metal ions and Brij35 were prepared in the proper buffer (0.05 M). Reaction temperature was mantained at 25±1°C. Kinetics were typically started by injecting an acetonitrile solution (0.01 M) of substrate ester into a 1 cm cuvette containing 2.5 mL of buffered micellar solution and the desired concentrations of metal ion and ligand. Pseudo-first-order rate constants (k_R and k_S) for the hydrolysis of substrate ester were determined by monitoring the release of *p*-nitrophenolate at 320 nm (pH 5.0–6.3) or 400 nm (pH 6.3–8.5) for at least five half-lives, and obtained by linear plots of $\ln(A_\infty - A_t)$ *versus* time. The rate constants for each reaction were determined three times from three separate runs with an uncertainty of less than 5%.

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