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

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Received June 23, 1992

The synthesis of seven new, chiral 1,10-phenanthrolines (1–7) containing a 2-pyrrolidinemethanol or ephedrine substitutent at the α-position is reported. The catalytic activity of Zn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Cd^{2+} complexes of these ligands in the hydrolysis of *p*-nitrophenyl esters of picolinic acid (PNPP) and chiral N-protected amino acids was examined in water and micellar solution. The Zn^{2+} complex of the chiral, asymmetrically disubstituted (*S*)-1-[[9-[(*N*-methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidinemethanol (1) exhibits the highest activity toward PNPP. The highest enantioselectivity in the stereoselective hydrolysis of *p*-nitrophenyl *N*-dodecanoyl-D(L)-phenylalaninate (D(L)-C_{12}-Phe-PNP) is observed for 1-Co^{2+} solubilized in Brij 35 micelles ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 15.3). In a mixed micellar system of 1-Zn^{2+} with Brij 35 as the cosurfactant, hydrolysis of D-C_{12}-Phe-PNP predominates over that of the L-enantiomer ($k^{D}_{a,obs}/k_{a,obs}$ = 2.4), whereas with CTABr as the cosurfactant an inversion of enantioselectivity is observed ($k^{D}_{a,obs}/k^{L}_{a,obs}$ = 0.54).

The bidentate 1,10-phenanthroline nucleus is a strongly chelating agent for a variety of metal ions.[1] Therefore, it is an attractive building block for incorporation into host molecules where a ligated metal ion serves as a Lewis acid binding site and catalyst.[2-3] Metal–ion complexes of functionalized 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 metalloenzymes. In these models the metal ion is coordinated in a fixed position in the metallocleft 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 metalloenzymes may operate.

The catalytic role of the metal ion in the active site of carboxypeptidase A (CPA) has been the subject of several model studies.[4b,5] This Zn^{2+}-containing proteolytic enzyme catalyzes the hydrolysis of the C-terminal amino acid residues of peptides and the hydrolysis of corresponding esters. The function of the metal ion chelated at the active site of CPA is thought to be manifold: bringing the substrate and nucleophile together in a ternary complex, polarizing the substrate carbonyl bond, activating the nucleophile, and stabilizing the tetrahedral intermediate.[6,7] Although the protein matrix in which the metal ion is embedded will be of influence on substrate specificity and orientation, the coordination geometry of the metal ion is considered to be a major factor for the catalytic activity of the enzyme.[4b,7]

Several of the above-mentioned metal–ion features have been investigated in artificial hydrolytic metalloenzymes. Metallomicelles have been developed in order to improve the substrate binding properties of synzymes.[8-11] The adsorption 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 catalyzed by histidine containing di- and tripeptides in surfactant aggregates.[13] However, until now only a few model

(1) Holyer, R. H.; Hubbard, C. D.; Kettle, S. F. A.; Wilkins, R. G. *Inorg. Chem.* **1965**, *4*, 929.
(2) (a) Keipert, S. J.; Knobler, C. B.; Cram, D. J. *Tetrahedron* **1987**, *43*, 4861. (b) Chandler, C. J.; Deady, L. W.; Reiss, J. A. *J. Heterocycl. Chem.* **1986**, *23*, 1327. (c) Newkome, G. R.; Kiefer, G. E.; Puckett, W. E.; Vreeland, T. *J. Org. Chem.* **1983**, *48*, 5112. (d) Buhleier, E.; Vögtle, F. *Liebigs Ann. Chem.* **1977**, 1080.
(3) (a) Sigman, D. S. *Biochemistry* **1990**, *29*, 9097. (b) Gladiali, S.; Ghelucci, G.; Soccolini, F. *J. Organomet. Chem.* **1989**, *370*, 285. (c) Gladiali, S.; Pinna, L.; Delogu, G.; De Martin, S.; Zassinovich, G.; Mestroni, G. *Tetrahedron: Asymmetry* **1990**, *1*, 635.
(4) (a) Engbersen, J. F. J.; Koudijs, A.; van der Plas, H. C. *J. Org. Chem.* **1990**, *55*, 3647. (b) Fife, T. H.; Przystas, T. J.; Squillacote, V. L. *J. Am. Chem. Soc.* **1979**, *101*, 3017. (c) Sigman, D. S.; Wahl, G. M.; Creighton, D. J. *Biochemistry* **1972**, *11*, 2236. (d) Breslow, R.; Fairweather, R.; Keana, J. *J. Am. Chem. Soc.* **1967**, *89*, 2135.
(5) (a) Groves, J. T.; Chambers, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 630. (b) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 2251. (c) Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1985**, *107*, 1041. (d) Suh, J. *Bioorg. Chem.* **1990**, *18*, 345.
(6) (a) Dugas, H. *Bioorganic Chemistry*; Springer-Verlag: New York, 1988. (b) Breslow, R.; Schepartz, A. *Chem. Lett.* **1987**, 1.
(7) Hanzlik, R. P. *Inorganic Aspects of Biological and Organic Chemistry*; Academic Press: New York, 1976.
(8) (a) Tagaki, W.; Ogino, K. *Top. Curr. Chem.* **1985**, *128*, 143 and references cited therein. (b) Ogino, K.; Kashihara, N.; Fujita, T.; Ueda, T.; Isaka, T.; Tagaki, W. *Chem. Lett.* **1987**, 1303. (c) Tagaki, W.; Ogino, K.; Tanaka, O.; Machiya, K.; Kashihara, N.; Yoshida, T.; *Bull. Chem. Soc. Jpn.* **1991**, *64*, 74.
(9) (a) Fornasier, R.; Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Am. Chem. Soc.* **1989**, *111*, 224. (b) De Santi, G.; Scrimin, P.; Tonellato, U. *Tetrahedron Lett.* **1990**, *31*, 4791. (c) Scrimin, P.; Tecilla, P.; Tonellato, U. *J. Org. Chem.* **1991**, *56*, 161.
(10) (a) Gutsche, C. D.; Mei, G. C. *J. Am. Chem. Soc.* **1985**, *107*, 7964. (b) Gellman, S. H.; Petter, R.; Breslow, R. *J. Am. Chem. Soc.* **1986**, *108*, 2388. (c) Menger, F. M.; Gan, L. H.; Johnson, E.; Durst, D. H. *J. Am. Chem. Soc.* **1987**, *109*, 2800.
(11) (a) Weijnen, J. G. J.; Koudijs, A.; Engbersen, J. F. J. *J. Chem. Soc., Perkin Trans. 2* **1991**, 1121. (b) Weijnen, J. G. J.; Koudijs, A.; Schellekens, G. A.; Engbersen, J. F. J. *J. Chem. Soc., Perkin Trans. 2* **1992**, 829.
(12) Fendler, J. H.; Fendler, E. J. *Catalysis in Micellar and Macromolecular Systems*; Academic Press: New York, 1975.

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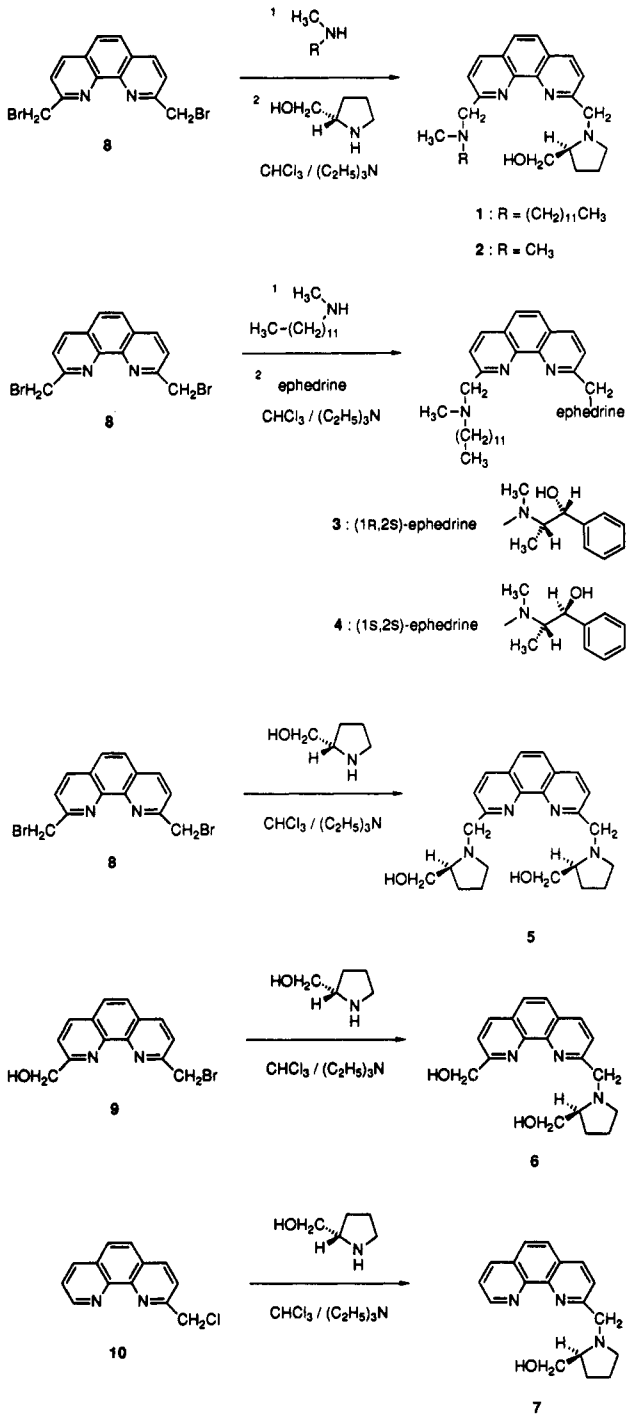
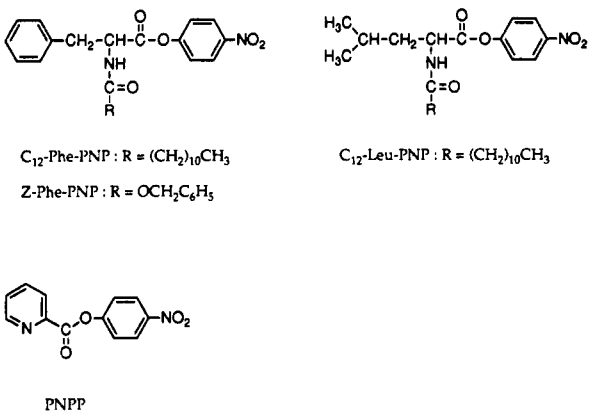
Scheme I



studies have been undertaken with respect to the enantioselectivity of hydrolytic metalloenzymes.[14]

In previous studies on the catalytic effects of metal–ion complexes of functionalized 1,10-phenanthrolines in the hydrolysis of carboxylic and phosphoric esters, we have demonstrated that the nature of the ligated metal ion

Scheme II



determines largely the catalytic efficiency.[11] In the present work 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 was investigated.[15] Seven new chiral 1,10-phenanthrolines were synthesized, which are all functionalized with a hydroxymethyl group in close proximity to the metallocleft and the catalytic activity and enantioselectivity in the cleavage of *p*-nitrophenyl esters of N-protected amino acids were studied.

Results

Ligands. The chiral 1,10-phenanthroline ligands **1–7** were prepared according to the procedures outlined in Scheme I. The asymmetrically disubstituted ligands **1–4** were obtained by coupling of 2,9-bis(bromomethyl)-1,10-phenanthroline[16] to 1 equiv of *N*-methyldodecylamine,[17] 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 of (*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-pyrrolidinemethanol group with one chiral center, or by the more flexible ephedrine group with two chiral centers. In all ligands a nucleophilic hydroxyl group is at the same distance to the metallocenter (except 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 sufficient 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^{2+} to **1–7** shifts the absorbance maximum from 272 to 276 nm, and a shoulder in the region of 295–300 nm appears. From the changes in the UV spectra it is shown that in the presence of 1 equiv of Zn^{2+} complexation of **1–7** is complete, indicating the high metal–ion affinity of these ligands.

(13) (a) Ueoka, R.; Matsumoto, Y.; Moss, R. A.; Swarup, S.; Sugii, A.; Harada, K.; Kikuchi, J.; Murakami, Y. *J. Am. Chem. Soc.* **1988**, *110*, 1588 and references cited therein. (b) Cleij, M. C.; Drenth, W.; Nolte, R. J. M. *J. Org. Chem.* **1991**, *56*, 3883. (c) Ueoka, R.; Cho, M.; Matsumoto, Y.; Goto, K.; Kato, Y.; Harada, K.; Sugii, A. *Tetrahedron Lett.* **1990**, *31*, 5335.

(14) (a) Yamskov, I. A.; Berezin, B. B.; Belchich, L. A.; Davankov, V. A. *Makromol. Chem.* **1979**, *180*, 799. (b) Yamskov, I. A.; Berezin, B. B.; Belchich, L. A.; Davankov, V. A. *Eur. Polym. J.* **1979**, *15*, 1067. (c) Ogino, K.; Tomita, I.; Machiya, K.; Tagaki, W. *Chem. Lett.* **1982**, 1875. (d) Sakaki, S.; Nakano, Y.; Ohkubo, K. *Chem. Lett.* **1983**, 413. (e) Fornasier, R.; Scrimin, P.; Tonellato, U.; Zanta, N. *J. Chem. Soc., Chem. Commun.* **1988**, 716.

(15) Preliminary communication concerning part of this work: Weijnen, J. G. J.; Koudijs, A.; Engbersen, J. F. J. *J. Mol. Catal.* **1992**, *73*, L5.

(16) Chandler, C. J.; Deady, L. W.; Reiss, J. A. *J. Heterocycl. Chem.* **1981**, *18*, 599.

(17) Ralston, A. W.; Reck, R. A.; Harwood, H. J.; Dubrow, P. L. *J. Org. Chem.* **1948**, *13*, 186.

Table I. Pseudo-First-Order Rate Constants (k_{obs}) for the Hydrolysis of PNPP by Different Zn^{2+}–Ligand Complexes[a]

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

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

Kinetic Studies. 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 ester was followed spectrophotometrically at 400 nm. First we investigated the catalytic activity of the synthesized ligands in the hydrolysis of the metallophilic substrate *p*-nitrophenyl picolinate (PNPP, Scheme II). This ester has shown to be a useful substrate in studies of metal-ion-activated hydrolysis reactions, and catalytic data thus obtained could be used for comparison of catalytic efficiency. Pseudo-first-order rate constants for the hydrolysis of PNPP catalyzed by Zn^{2+} complexes of 1–7 are given in Table I. 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^{2+}- and ephedrine-containing ligands (3, 4) or the water-soluble 2-pyrrolidinemethanol ligands (2, 5, 6, and 7). The highest rate acceleration (6780×) is observed in the presence of 1–Zn^{2+}.

Next, we investigated the catalytic activities of metallocomplexes of 1-7 toward chiral, nonmetallophilic substrates, i.e., the N-protected *p*-nitrophenyl esters of D-(L)-phenylalanine and D(L)-leucine (Scheme II). Since these substrates are not sufficiently 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 nonionic 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^{2+}, Co^{2+}, Ni^{2+}, and Cd^{2+} in the absence of ligand show no catalytic activity toward *p*-nitrophenyl esters of N-protected amino acids, whereas addition of Cu^{2+} even retards the rate of spontaneous hydrolysis. Rate enhancements caused by nonmetalated 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 strong metal–ion binding site as is present in PNPP. The metal–ion activation of 1 is in the order of $Zn^{2+} > Co^{2+} > Cu^{2+}$ (Table II). For all 1-M^{2+} 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^{2+}. The ephedrine-containing metallosurfactants (3, 4) and the water-soluble 2-pyrrolidinemethanol metallocatalysts (2, 5, 6, and 7) are less reactive and stereoselective compared to 1-M^{2+}. 5–Co^{2+} hydrolyzes the L-substrate 4.4 times faster than the D-substrate; however, the catalytic activity of this metallocomplex is low. The results indicate that both hydrophobic interactions between substrate and metallocatalyst and rigidity of the ligand are important factors for the activity and enantioselectivity.

Table II. Apparent Second-Order Rate Constants ($k_{a,obs}$, $M^{-1}\ s^{-1}$) and Enantioselectivities for the Hydrolysis of D(L)-Z-Phe-PNP, Catalyzed by Different Metal Complexes[a]

catalyst	comicellar additive	$k^D_{a,obs}$	$k^L_{a,obs}$	$k^D_{a,obs}/k^L_{a,obs}$
1–Zn^{2+}	CTABr	37.8	27.3	1.4
1–Co^{2+}	CTABr	30.0	7.10	4.2
1–Cu^{2+}	CTABr	19.2	6.44	3.0
2–Zn^{2+}	Brij 35	0.25	0.22	1.1
3–Zn^{2+}	CTABr	3.27	2.63	1.2
3–Co^{2+}	CTABr	1.03	0.81	1.3
4–Zn^{2+}	CTABr	2.81	5.12	0.55
4–Co^{2+}	CTABr	0.80	1.24	0.65
5–Zn^{2+}	Brij 35	0.31	0.67	0.46
5–Co^{2+}	Brij 35	0.07	0.31	0.23
6–Zn^{2+}	Brij 35	0.34	0.29	1.2
7–Zn^{2+}	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^{2+}] = 5×10^{-4} M, and [D(L)-Z-Phe-PNP] = 5×10^{-5} M. The first-order rate constants measured in the absence of metallocatalyst (k_s) are $7.99 \times 10^{-5}\ s^{-1}$ and $7.56 \times 10^{-5}\ s^{-1}$ for the hydrolysis of D- and L-Z-Phe-PNP, respectively.

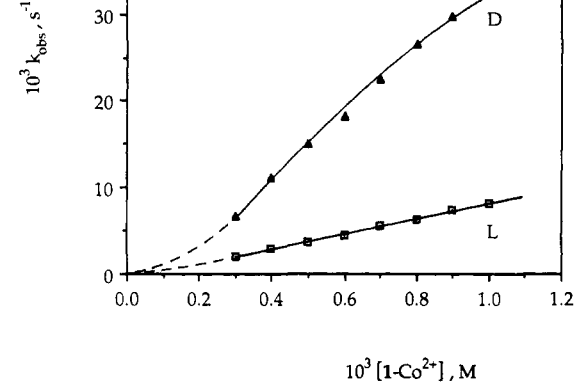


Figure 1. Pseudo-first-order rate constants for the hydrolysis of D- and L-Z-Phe-PNP as a function of 1–Co^{2+} concentration in CTABr micelles at pH = 7.00 and 25 °C: [CTABr] = 4×10^{-3} M, [D(L)-Z-Phe-PNP] = 5×10^{-5} M, and [1]:[Co^{2+}] = 1.

The effect of the 1–Co^{2+} concentration on the rate and enantioselectivity in the hydrolysis of D(L)-Z-Phe-PNP is depicted in Figure 1. The rate of hydrolysis of D- and L-Z-Phe-PNP linearly increases with [1–Co^{2+}], and over the entire concentration range the D-enantiomer is hydrolyzed faster than the L-enantiomer in a ratio of 4:1. No saturation kinetics is observed, indicating that the binding constant of D(L)-Z-Phe-PNP to 1–Co^{2+} is low.

Substitution of the *N*-(benzyloxycarbonyl) protecting group of the substrate into 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 metal ion and the enantioselectivity is relatively low (Table III). In the presence of 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 III is that the nature of the metal ion determines not only the degree of enantioselectivity but also the direction of enantioselectivity. 1–Co^{2+} and 1–Cu^{2+} hydrolyze D-C_{12}-Phe-PNP more rapidly than the L-enantiomer (8.0 and 4.4 times, respectively) whereas 1–Zn^{2+} gives an inversion of the enantioselectivity.

The dependence of the direction of enantioselectivity on the nature of the metal ion in 1-M^{2+} is not limited to

Table III. Apparent Second-Order Rate Constants ($k_{a,obs}$, M^{-1} s^{-1}) and Enantioselectivity ($k^D_{a,obs}/k^L_{a,obs}$) for the Hydrolysis of D(L)-C_{12}-Phe-PNP and D(L)-C_{12}-Leu-PNP, Catalyzed by Mixed Micellar Systems Composed of 1-M^{2+} 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^{2+}	37.3	68.8	0.54	21.1	40.1	0.53
1-Co^{2+}	74.0	9.26	8.0	45.9	7.26	6.3
1-Cu^{2+}	15.8	3.60	4.4	10.6	3.08	3.4
1-Ni^{2+}	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] = 4×10^{-3} M, [1] = 5×10^{-4} M, [M^{2+}] = 5×10^{-4} M, [D(L)-C_{12}-Phe-PNP] = 5×10^{-5} M, [D(L)-C_{12}-Leu-PNP] = 5×10^{-5} M. The first-order rate constants measured in the absence of metallocatalyst (k_s) are 11.1×10^{-3} s^{-1} (D-C_{12}-Phe-PNP), 10.7×10^{-3} s^{-1} (L-C_{12}-Phe-PNP), 4.59×10^{-3} s^{-1} (D-C_{12}-Leu-PNP), and 4.69×10^{-3} s^{-1} (L-C_{12}-Leu-PNP).

Table IV. Apparent Second-Order Rate Constants ($k_{a,obs}$, M^{-1} s^{-1}) and Enantioselectivity ($k^D_{a,obs}/k^L_{a,obs}$) for the Hydrolysis of D(L)-C_{12}-Phe-PNP and D(L)-C_{12}-Leu-PNP, Catalyzed by Mixed Micellar Systems Composed of 1-M^{2+} 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^{2+}	44.6	18.4	2.4	22.7	13.0	1.7
1-Co^{2+}	77.4	5.07	15.3	28.4	4.14	6.9
1-Cu^{2+}	16.1	5.59	2.9	11.9	8.00	1.5
1-Ni^{2+}	1.74	1.00	1.7	1.24	0.83	1.5
1-Cd^{2+}	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] = 4×10^{-3} M, [1] = 5×10^{-4} M, [M^{2+}] = 5×10^{-4} M, [D(L)-C_{12}-Phe-PNP] = 5×10^{-5} M, [D(L)-C_{12}-Leu-PNP] = 5×10^{-5} M. The first-order rate constants measured in the absence of metallocatalyst (k_s) are 10.2×10^{-5} s^{-1} (D-C_{12}-Phe-PNP), 10.7×10^{-5} s^{-1} (L-C_{12}-Phe-PNP), 5.98×10^{-5} s^{-1} (D-C_{12}-Leu-PNP), and 6.27×10^{-5} s^{-1} (L-C_{12}-Leu-PNP).

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 were obtained. 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^{2+} and 1-Cu^{2+} while an inversion of stereoselectivity is found for 1-Zn^{2+}.

The activity of 1-M^{2+} incorporated in nonionic Brij 35 micelles is shown in Table IV. 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 catalyst are neglectably low. Comparing the data in CTABr and Brij 35 (Tables III and IV) the most striking differences are the higher degree of enantioselectivity for the 1-Co^{2+}/Brij 35 system and the absence of inversion of enantioselectivity in the 1-Zn^{2+}/Brij 35 system. These results indicate the important role of the micellar microenvironment on the stereochemical control of the 1-M^{2+}-catalyzed hydrolysis of long-chain esters. In contrast, the catalytic activity and stereoselectivity of 1-M^{2+} toward D(L)-Z-Phe-PNP and of 4-M^{2+} toward D(L)-C_{12}-Phe-PNP is hardly altered upon changing the cosurfactant CTABr into Brij 35. The metallosurfactant 1-Cd^{2+}, 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^{2+}] in Brij 35 are plotted in Figure 2. In the concentration range

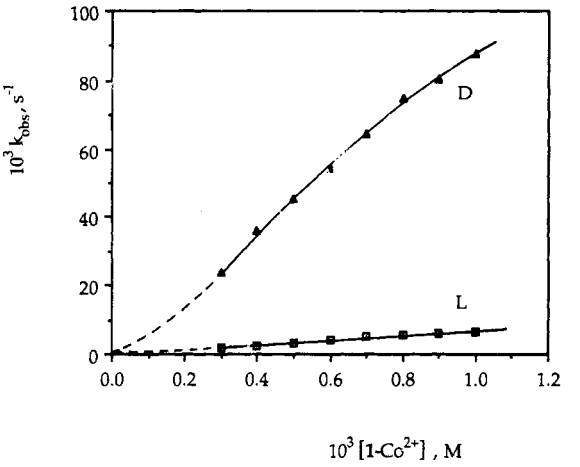


Figure 2. Pseudo-first-order rate constants for the hydrolysis of D- and L-C_{12}-Phe-PNP as a function of 1-Co^{2+} concentration in Brij 35 micelles at pH = 7.00 and 25 °C: [Brij 35] = 4×10^{-3} M, [D(L)-C_{12}-Phe-PNP] = 5×10^{-5} M and [1]:[Co^{2+}] = 1.

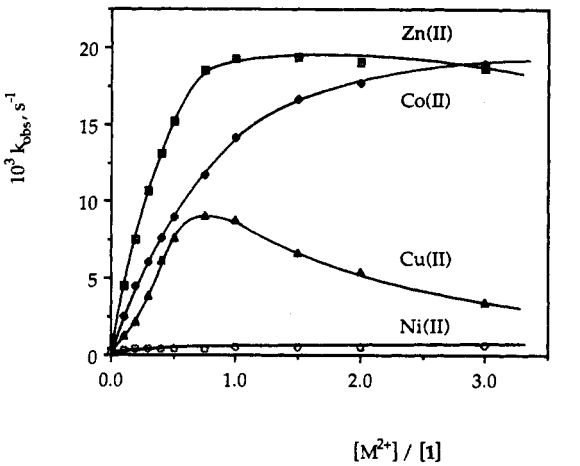


Figure 3. Pseudo-first-order rate constants for the hydrolysis of D-Z-Phe-PNP as a function of the metal ion concentration under fixed concentration of 1 in CTABr micelles at pH = 7.00 and 25 °C: [CTABr] = 4×10^{-3} M, [1] = 5×10^{-4} M, and [D-Z-Phe-PNP] = 5×10^{-5} M.

[1-Co^{2+}] = 0.3–1.0 $\times 10^{-3}$ M, a linear dependance of k_{obs} vs the concentration of metallocatalyst is observed for both D- and L-C_{12}-Phe-PNP. Over the total concentration range the D-enantiomer is hydrolyzed 15 times faster than the L-enantiomer. The absence of saturation kinetics indicates that the degree of complex formation of metallosurfactant and substrate is low.

The effect of variation of the concentration of M^{2+} in the 1-M^{2+}-catalyzed hydrolysis of D-Z-Phe-PNP at fixed concentration of 1 is given in Figure 3. Initial addition of Zn^{2+} to 1 leads to a fast increase in the reaction rate until the ratio reaches unity. Further increment of the Zn^{2+} concentration has no effect. For Co^{2+} a less pronounced saturation effect is found. Under the conditions of [Co^{2+}] = [1], the pseudo-first-order rate constant has reached 60% of its maximal value. Increasing the Co^{2+} concentration above stoichiometric amounts (e.g., [Co^{2+}]:[1] = 3:1) results in a higher rate of hydrolysis for both enantiomers, but does not affect the enantioselective ratio.

The Cu^{2+} titration curve is bell shaped with a maximum for [Cu^{2+}]:[1] = 1. This implies that at low Cu^{2+} concentration, Cu^{2+} is mainly bound to 1 yielding the catalytic active species 1-Cu^{2+} while at higher concentration the presence of uncomplexed metal ion has a rate-retarding effect. In contrast to the former bivalent metal ions, ad-

dition of Ni^{2+} shows only a moderate effect on the rate of hydrolysis.

Discussion

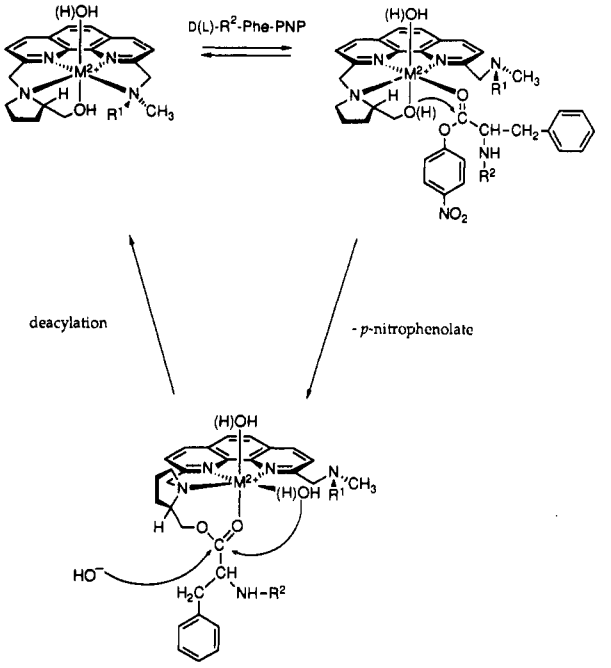
The Zn^{2+} complex of the lipophilic ligand **1** solubilized in CTABr is a very effective catalyst in the hydrolysis of the metallophilic substrate PNPP. Mixed metallomicelles containing the ephedrine ligands **3** and **4** are about 25 times less active compared to **1**, although for all three ligands 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-pyrrolidinemethanol is bound to the metal ion, the structure of the 2-pyrrolidinemethanol group is frozen and the OH group is in 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^{2+}.

The water-soluble Zn^{2+} complexes of **2**, **5**, **6**, and **7**, all containing one or two 2-pyrrolidinemethanol groups, are also less active than the amphiphilic **1**–Zn^{2+} catalyst. The higher catalytic activity observed in micellar complexes of ligand **1** compared to their nonmicellar analogs may be ascribed to the following: (i) increased concentration of reactants in the micellar pseudophase 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 lipophilic 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 catalytic inactive 2:1 complexes.[11b]

The Zn^{2+} complexes of **2**, **5**, and **7** are all comparably active in the hydrolysis of PNPP. The similar activity of **2**–Zn^{2+} and **7**–Zn^{2+} 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^{2+} is sterically hindered by the presence of two 2-pyrrolidinemethanol 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^{2+}, presumably because the hydroxymethyl group at the 9 position of the phenanthroline nucleus is in a favorable position for nucleophilic attack in the ternary complex with the substrate.

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

Scheme III



The divalent metal ions Co^{2+}, Cu^{2+}, and particularly Zn^{2+} bind strongly to the 1,10-phenanthroline ligands and have a large effect on the rate of hydrolysis as shown by Figure 3. In the case of Co^{2+} the kinetic titration curve can be described by eq 1 where k_{max} is the maximum rate

$$k_{obs} = \frac{k_{max}K_M[M^{2+}]}{1 + K_M[M^{2+}]} \quad (1)$$

$$K_M = \frac{[1\text{–}M^{2+}]}{[1][M^{2+}]} \quad (2)$$

constant under saturation conditions and K_M is the equilibrium constant for the formation of the metal ion complex (eq 2). From a least-squares analysis of the double reciprocal plot of k_{obs}^{-1} vs $[Co^{2+}]^{-1}$, K_M (2.07×10^3 M^{-1}) and k_{max} (26.4×10^{-3} s^{-1}) were established. In case of **1**–Zn^{2+} the slope of the first part of the graph is too steep to be analyzed by eq 1 which points to a metal–ion hopping mechanism.[11b] For Co^{2+} 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] = $[Co^{2+}]$ = 0.5 mM maximally 39% of the ligand is complexed with Co^{2+}. The percentage of complexed ligand decreases as the concentration of metal ion and ligand decreases (eq 2) as is indicated by the dashed lines in Figures 1 and 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 case of the stronger binding Zn^{2+} ions, 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^{2+}-catalyzed hydrolysis of *p*-nitrophenyl esters is schematically represented in Scheme III. This scheme is based on an octahedral geometry of the metal ion although for Zn^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Cd^{2+} several alternatives are possible.[18] For the formation of the reactive ternary complex one of the lig-

(18) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 3rd Ed.; Wiley: New York, 1972.

and-metal-ion bonds must be substituted. This may occur by replacement of the *N*-alkylmethylamine moiety by the carbonyl group of the substrate as indicated in Scheme III. These ligand-exchange processes are very fast (10^4–10^8 s^{-1}) compared to the rate of hydrolysis.[7] CPK models show that the amino 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 incorporating the ligand into the micellar phase and directing the approaching substrate. The carbonyl group of the substrate and the nucleophilic hydroxymethyl group should be preferably coordinated to the metal ion in a perpendicular fashion as was previously pointed out for substitutionally inert Co^{3+} complexes by Chin et al.[19] 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 preequilibrium complexation of 1–M^{2+} with the substrate is low since no saturation kinetics is observed in the plots of k_{obs} vs [1–Co^{2+}] for D(L)-Z-Phe-PNP (Figure 1) and D(L)-C_{12}-Phe-PNP (Figure 2). The low affinity of the metallosurfactant 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 constrast, the binding constants of lipophilic metallocomplexes of 1,10-phenanthroline with the metallophilic substrate PNPP are relatively high (K = 1–2 × 10^3 M).[11]

In the ternary complex, nucleophilic attack of the metal-ion-activated hydroxyl group on the carbonyl function of the substrate results in expulsion of *p*-nitrophenolate and formation of an acylated intermediate. This intermediate is hydrolyzed by attack of free or metal-ion-bound hydroxide ion to the coordinated ester group. In this process the presence of the metal ion stabilizes the expulsion of the 2-pyrrolidinemethanolate moiety.[5b] As a consequence of the weak binding affinity of the amino acid substrates to the metallocatalyst, 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. Next to this, 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^{2+} hydrolyzes D-C_{12}-Phe-PNP faster than the L-enantiomer, while in case of 1–Zn^{2+} 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 catalyzed by chiral Rh^+ and Ru^{2+} diphosphine complexes.[20] This effect was attributed to a different coordination geometry of Rh^+ and Ru^{2+} and to a different reaction pathway. In our case we have no evidence that 1–Co^{2+} and 1–Zn^{2+} operate via a different mechanism. In neutral Brij 35 the enantioselectivity induced by 1–Co^{2+} is higher than in the cationic CTABr micelles while there is no inversion of enantioselectivity observed for 1–Zn^{2+}. This implies that the coordination geometry of the ternary complex is sensitive to the microenvironment 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^{2+} is higher, but also the degree of stereoselectivity is larger. Consequently, hydrophobic interaction of substrate and ligand in the ternary complex is favorable for a high degree of stereoselectivity,[21] 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.

Conclusion

Mixed metallomicelles 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 noncovalently bound to the metallosurfactant 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 head group and carbonyl group of substrate to the metal ion at the micellar interphase yields a highly oriented ternary complex. In this complex the coordination mode of the metal ion and the rigidity of the functionalized head group largely determines the rate and degree of stereoselectivity.

Experimental Section

General Methods. NMR spectra were recorded at 200.1 MHz for ^1H and 50.3 MHz for ^{13}C NMR.

Materials. $ZnBr_2$, $CuBr_2$, $CoBr_2$, $NiBr_2$, $CdBr_2 \cdot 4H_2O$, *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),[22] *N*-methyldodecylamine,[17] *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).[23]

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**)[16] (366 mg, 1.0 mmol) and $(C_2H_5)_3N$ (101 mg, 1.0 mmol) in freshly distilled $CHCl_3$ (15 mL) was added, dropwise, the dialkylamine (*N*-methyldodecylamine or *N*,*N*-dimethylamine, 1.0 mmol) dissolved in 5 mL of $CHCl_3$. 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 amino alcohol ((*S*)-2-pyrrolidinemethanol or ephedrine, 1.3 mmol) and $(C_2H_5)_3N$ (151 mg, 1.5 mmol) in 5 mL of $CHCl_3$. 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 (Al_2O_3, 0.25% (v/v) $CH_3OH/CHCl_3$).

(*S*)-1-[[9-[(*N*-Methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]-2-pyrrolidinemethanol (1) was obtained from the coupling reaction of **8** to *N*-methyldodecylamine and (*S*)-2-pyrrolidinemethanol as a pale yellow oil (220 mg, 44%): ^1H 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, $(CH_2)_2CHCH_2OH$), 2.29 (s, 3 H, NCH_3), 2.49 (t on m, J = 7.5 Hz, 3 H, $NCH_2(CH_2)_{10}$, and $CH_{2a}(CH_2)_2CHCH_2OH$), 2.79 (m, 1 H, $CHCH_2OH$), 3.10 (m, 1 H, $CH_{2b}(CH_2)_2CHCH_2OH$), 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

(19) Chin, J.; Banaszczyk, M.; Jubian, V. *J. Chem. Soc., Chem. Commun.* **1988**, 735.

(20) Kawano, H.; Ikariya, T.; Ishii, Y.; Saburi, M.; Yoshikawa, S.; Uchida, Y.; Kumobayashi, H. *J. Chem. Soc., Perkin Trans 1* **1989**, 1571.

(21) Moss, R. A.; Lee, Y. S.; Lukas, T. J. *J. Am. Chem. Soc.* **1979**, *101*, 2499.

(22) Sigman, D. S.; Jorgensen, C. T. *J. Am. Chem. Soc.* **1972**, *94*, 1724.

(23) (a) Ingles, D. W.; Knowles, J. R. *Biochem. J.* **1967**, *104*, 369. (b) Bodanszky, M.; du Vigneaud, V. *J. Am. Chem. Soc.* **1959**, *81*, 5688.

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), 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, 31.61 ($(CH_2)_{10}CH_3$), 22.90 and 26.94 ($CH_2CH_2CHCH_2OH$), 42.36 (NCH_3), 55.12 ($CH_2(CH_2)_2CHCH_2OH$), 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-7a), 136.12 and 136.27 (Phen C-4 and C-7), 144.78 and 144.96 (Phen C-1b 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-pyrrolidinemethanol (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, $(CH_2)_2CHCH_2OH$), 2.34 (s, 6 H, CH_3), 2.44 (m, 1 H, $CH_{2a}(CH_2)_2CHCH_2OH$), 2.78 (m, 1 H, $CHCH_2OH$), 3.07 (m, 1 H, $CH_{2b}(CH_2)_2CHCH_2OH$), 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), 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 ($CH_2CH_2CHCH_2OH$), 45.49 (NCH_3), 55.19 ($CH_2(CH_2)_2CHCH_2OH$), 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-7a), 136.39 (Phen C-4 and C-7), 144.90 (Phen C-1b and C-10b), 159.52 and 159.63 (Phen C-2 and C-9); FDMS m/z 351 (MH^+).

(1*R*,2*S*)-*N*-[[9-[(*N*-Methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]ephedrine (3) was obtained as a pale yellow oil (300 mg, 53%) from the coupling reaction of **8** to *N*-methyldodecylamine and 1*R*,2*S*-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 ($CH_3NC_{12}H_{25}$), 49.65 (CH_3NCH), 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, C-4, C-5, and C-6, Phen C-5 and C-6), 127.36 (Phen C-4a and C-7a), 135.99 and 136.30 (Phen C-4 and C-7), 143.20 (Phenyl C-1), 144.84 and 145.11 (Phen C-1b and C-10b), 160.63 and 161.89 (Phen C-2 and C-9); FDMS m/z 569 (MH^+).

(1*S*,2*S*)-*N*-[[9-[(*N*-Methyldodecylamino)methyl]-1,10-phenanthrolin-2-yl]methyl]ephedrine (4) was obtained as a pale yellow oil (210 mg, 37%) from the coupling reaction of **8** to *N*-methyldodecylamine and (1*S*,2*S*)-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, C-4, C-5, and C-6, Phen C-5 and C-6), 127.63 (Phen C-4a and C-7a), 136.24 and 136.75 (Phen C-4 and C-7), 141.64 (Phenyl C-1), 145.09 (Phen C-1b 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 20 mL of $CHCl_3$ was added, dropwise at 0 °C, (*S*)-2-pyrrolidinemethanol (220 mg, 2.2 mmol) dissolved in 5 mL of $CHCl_3$. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred at this temperature for a further 3 h. The solution was washed with water containing 5% $NaHCO_3$ (w/v) and 2% EDTA (w/v), dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by column chromatography (Al_2O_3, 1% (v/v) $CH_3OH/CHCl_3$). Ligand **5** (386 mg, 95%) was obtained as a pale red powder, mp 152 °C dec: ^{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, $CHCH_2OH$), 3.12 (m, 2 H, $NCH_{2b}CH_2$), 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), 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), 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 20 mL of $CHCl_3$ was added, dropwise, (*S*)-2-pyrrolidinemethanol (220 mg, 2.2 mmol) dissolved in 5 mL of $CHCl_3$. The reaction mixture was stirred for 3 h at room temperature. The solution was washed with water containing 5% $NaHCO_3$ (w/v) and 2% EDTA (w/v). Evaporation of the dried (Na_2SO_4) $CHCl_3$ layer yielded the crude product which was purified by column chromatography (Al_2O_3, 1% (v/v) $CH_3OH/CHCl_3$). Ligand **6** (594 mg, 92%) was obtained as a pale yellow waxy oil which solidified at ~20 °C; ^{1}H NMR ($CDCl_3$) δ 1.80 (m, 4 H, $(CH_2)_2CH$), 2.72 (m, 1 H, $NCH_{2a}CH_2$), 3.03 (m, 1 H, $CHCH_2OH$), 3.23 (m, 1 H, $NCH_{2b}CH_2$), 3.70 (dd, J = 6.0, 11.8 Hz, 1 H, $CH_{2a}OH$), 3.85 (dd, J = 2.8, 11.8 Hz, 1 H, $CH_{2b}OH$), 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 = 15.5 Hz, 1 H, PhenCH_{2a}OH), 5.09 (d, J = 15.5 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-7a), 136.35 and 136.71 (Phen C-4 and C-7), 143.98 and 144.45 (Phen C-1b 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 30 mL of $CHCl_3$ was added, dropwise, (*S*)-2-pyrrolidinemethanol (220 mg, 2.2 mmol) dissolved in 5 mL of $CHCl_3$. The reaction mixture was kept for 16 h at room temperature. The solution was washed with water containing 5% $NaHCO_3$ (w/v) and 2% (w/v) EDTA, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by column chromatography (Al_2O_3, 1% (v/v) $CH_3OH/CHCl_3$). Ligand **7** (486 mg, 83%) was obtained as a yellow oil which solidified at −20 °C: ^{1}H 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-7a), 135.86 and 136.42 (Phen C-4 and C-7), 145.01 and 145.48 (Phen C-1b 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 ($A_\infty - A_t$) vs time for at least 3 half-lives. Kinetic runs carried out in triplicate gave rate constants with 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 constant for the hydrolysis of the ester substrates in the presence and absence of ligand–metal–ion complex, respectively.

Acknowledgment. The authors wish to thank Mr. C. Teunis (Wageningen Agricultural University) and Mr. R. H. Fokkens (University of Amsterdam) for carrying out the mass analyses and Mr. A. van Veldhuizen (Wageningen Agricultural University) for recording the NMR spectra.

Supplementary Material Available: Proton NMR spectra for compounds 1–7 (7 pages). This material is contained in many 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.

Chloroperoxidase-Catalyzed Asymmetric Synthesis: Enantioselective Reactions of Chiral Hydroperoxides with Sulfides and Bromohydration of Glycals

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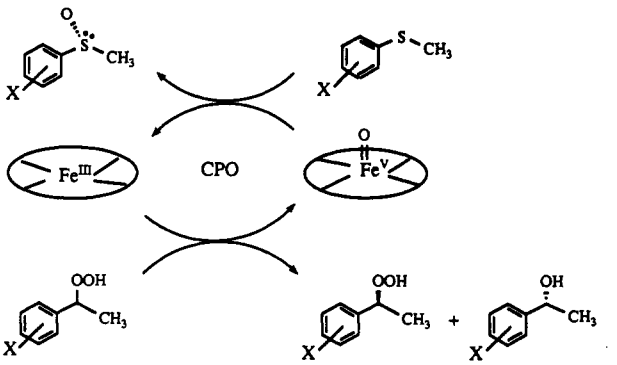
Received August 18, 1992

This paper describes the use of chloroperoxidase (CPO) from *Caldariomyces fumago* in the oxidation of sulfides to prepare (*R*)-sulfoxides with excellent ee (97–100%) and yield (66–92%) using H_2O_2 as oxidant. When racemic 1-phenylethyl hydroperoxides were used in the oxidation of sulfides, the corresponding (*R*)-alcohol generated from the oxidant and the unreacted (*S*)-hydroperoxide were recovered with high enantiomeric purity. The enantioselectivity in the enzymatic asymmetric oxidation was found to depend on the concentrations of the substrate and enzyme. Chloroperoxidase was also used in the regioselective bromohydration of certain saccharide glycals with KBr and H_2O_2 to give the corresponding 2-deoxy-2-bromo saccharides.

Introduction

Metal-assisted catalytic asymmetric oxidation is a subject of current interest in synthetic organic chemistry. Several practical methods based on biological[1] or abiological[2] catalysts have been developed. We report here the enantioselective asymmetric oxidation of sulfides catalyzed by chloroperoxidase (CPO, EC 1.11.1.10) from *Caldariomyces fumago* using hydrogen peroxide or chiral hydroperoxides as oxidation reagents. In the latter case, the enzyme is highly selective for the (*R*)-enantiomer of chiral hydroperoxides, generating (*R*)-alcohols and unreacted (*S*)-hydroperoxides. We also report the enzyme-catalyzed selective bromohydration of certain glycals to give 2-deoxy-2-bromo saccharides.

Scheme I. Chloroperoxidase (CPO) Catalyzation of Enantioselective Oxidation of Aryl Methyl Sulfides with Chiral Hydroperoxides



Chloroperoxidase from *Caldariomyces fumago* is a heme-containing glycoprotein.[3] Recent studies on the

(1) Fu, H.; Newcomb, M.; Wong, C.-H. *J. Am. Chem. Soc.* **1991**, *113*, 5878 and references cited therein. For a general reference, see: Davies, H. G.; Greene, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformations in preparative chemistry*; Academic Press: New York, 1989; pp 169–219. Abushanab, E.; Reed, D.; Suzuki, F.; Sih, C. J. *Tetrahedron Lett.* **1978**, *37*, 3415. Katopodis, A. G.; Smith, H. A.; May, S. W. *J. Am. Chem. Soc.* **1988**, *110*, 897. Baldwin, J. E.; Abraham, E. P. *Nat. Prod. Rep.* **1988**, *5*, 129. Buist, P. H.; Marecak, D. M. *J. Am. Chem. Soc.* **1992**, *114*, 5073. Colonna, S.; Gaggero, N.; Carrea, G.; Pasta, P. *J. Chem. Soc., Chem. Commun.* **1992**, 357.

(2) For examples of practical use, see: Zhang, W.; Loebach, J. L.; Wilson, S. R.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1990**, *112*, 2801. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765 and references cited therein. Rebiere, F.; Samuel, O.; Ricard, L.; Kagan, H. B. *J. Org. Chem.* **1991**, *56*, 5991. Phillips, M. L.; Berry, D. M.; Panetta, J. A. *J. Org. Chem.* **1992**, *57*, 4047. Zhao, S. H.; Samuel, O.; Kagan, H. B. *Tetrahedron* **1987**, *43*, 4135. Davis, F. A.; Reddy, R. T.; Han, W.; Carroll, P. J. *J. Am. Chem. Soc.* **1992**, *114*, 1428. For a review, see: Kogan, H. B.; Minoun, M.; Mark, C.; Schuring, V. *Angew. Chem., Int. Ed. Engl.* **1979**, 485.

(3) Thomas, J. A. *J. Biol. Chem.* **1970**, *245*, 12, 3129.

0022-3263/92/1957-7265$03.00/0