Mechanism of Enantioselective Ester Cleavage by Histidine-Containing Dipeptides at a Micellar Interface

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Chiral *p*-nitrophenyl esters derived from the amino acid phenylalanine are cleaved by histidine-containing dipeptides at a micellar interface. High enantioselectivities (up to k_L/k_D = 30.4 at 0 °C) are observed. Both the substrates and the catalysts contain an alternating sequence of hydrophobic and hydrophilic groups. Due to the need for hydration of the hydrophilic groups, the hydrophobic groups cannot dissolve completely into the micellar hydrocarbon phase. The kinetic data suggest that the micellar interface is capable of discriminating between transition states that have different hydrophilic and hydrophobic properties. One of the diastereomeric transition states is characterized by a hydrogen bond between the amide CO group of the ester and an NH group of the histidine-containing dipeptide. Upon formation of this hydrogen bond these polar CO and NH groups lose their hydrophilicity which allows the transfer of the adjacent apolar groups to the micellar hydrocarbon phase. The other diastereomeric transition state cannot form this hydrogen bond and the hydrophobic groups remain hydrated. Consequently, the latter transition state is of higher energy. The kinetic data reveal that it is important to prevent steric hinderance between the reactants in order to allow the unhindered formation of the hydrogen bond.

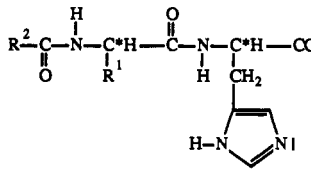
Introduction

In the past 10 years an increasing number of papers has been published dealing with reactions in aqueous surfactant solutions.[2] Many of these reactions occur at the micellar hydrocarbon–water interface. The adsorption of reactants at these micellar interfaces and their subsequent reaction resemble reactions that are controlled by enzymes. Large rate enhancements have been observed due to a concentration of the reactants in the micellar pseudophase.[2b] However, it is only until recently that high (enantio-) selectivities have been realized which are so characteristic of enzyme-controlled reactions. A number of these papers, in which remarkable enantioselectivities are reported, deal with the cleavage of chiral amino acid *p*-nitrophenyl esters by the imidazolyl moiety of histidine containing oligopeptides.[2b-l] Presently, the mechanism and origin of the observed enantioselectivities are not well understood. An understanding of the mode of adsorption of molecules at the micellar interface and of the factors that control the interaction between these adsorbed molecules may be important for synthetic and pharmaceutical applications.

In aqueous solution the imidazolyl moiety is an active catalyst in the hydrolysis of *p*-nitrophenyl esters. In the first step of this hydrolysis (A in Scheme I) the imidazolyl group is acylated. In the second, relatively slow step (B in Scheme I) the acetylated imidazolyl intermediate is hydrolyzed and the free imidazolyl moiety is regenerated. Upon increasing the substrate concentration typical Michaelis Menten kinetics is observed. At high substrate concentrations the catalyst is completely acylated and the rate is independent of the substrate concentration.

This paper describes the esterolytic activity and enantioselectivity of a number of imidazole-containing dipeptide derivatives dissolved in an aqueous micellar solution. The imidazolyl moiety is part of the chiral amino acid residue L-histidine at the C-terminal end (2-position) of the dipeptide. This C-terminal end is unprotected,

Chart I. Catalysts



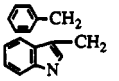
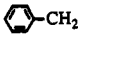
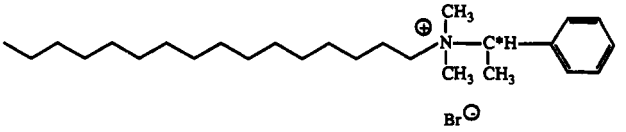
R^1	R^2	Abbreviation
CH_3	$CH_3(CH_2)_{11}O$	S_{12}-L-Ala-L-His
CH_3CH_2	"	S_{12}-L-But-L-His
$CH_3CH_2CH_2$	"	S_{12}-L-Norval-L-His
$(CH_3)_2CHCH_2$	"	S_{12}-L-Leu-L-His
	"	S_{12}-L or D-Phe-L-His
	"	S_{12}-L-Trp-L-His
	CH_3O	S_1-L-Phe-L-His
"	CH_3CH_2O	S_2-L-Phe-L-His
"	$CH_3(CH_2)_2O$	S_3-L-Phe-L-His
"	$CH_3(CH_2)_3O$	S_4-L-Phe-L-His
"	$CH_3(CH_2)_5O$	S_6-L-Phe-L-His
"	$CH_3(CH_2)_7O$	S_8-L-Phe-L-His
"	$CH_3(CH_2)_9O$	S_{10}-L-Phe-L-His
"	$CH_3(CH_2)_{11}O$	S_{12}-L-Phe-L-His
"	$CH_3(CH_2)_{15}O$	S_{16}-L-Phe-L-His
"		Z-L-Phe-L-His
"	CH_3	C_1-L-Phe-L-His
"	$CH_3(CH_2)_{10}$	C_{12}-L-Phe-L-His
"		F_5Z-L-Phe-L-His
"	p-$BrC_6H_4CH_2O$	p-BrZ-L-Phe-L-His
CH_3		Z-L-Ala-L-His

Chart II. *R*-Surf 16



(1) (a) University at Utrecht. (b) University at Nijmegen.

(2) (a) Meakin, B. J. *J. Pharm. Pharmacol.* **1971**, *23*, 25. (b) Ihara, Y.; Hosako, R.; Nango, M.; Kuroki, N. *J. Chem. Soc.* **1983**, *2*, 5. (c) Ueoka, R.; Murakami, Y. *Ibid.* **1983**, 219. (d) Ihara, Y.; Kunikiyo, N.; Kunimasa, T.; Kimura, Y.; Nango, M.; Kuroki, N. *Ibid.* **1983**, 1741. (e) Ohkubo, K.; Matsumoto, N.; Nagasaki, M.; Yamaki, K.; Ogata, H. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 214. (f) Moss, R. A.; Taguchi, T.; Bizzigotti, G. O. *Tetrahedron Lett.* **1982**, 1985. (g) Ueoka, R.; Matsumoto, Y.; Nagamatsu, T.; Hirohata, S. *Ibid.* **1984**, 1363. (h) Ueoka, R.; Matsumoto, Y.; Ihara, Y. *Chem. Lett.* **1984**, 1807. (i) Ueoka, R.; Moss, R. A.; Swarup, S.; Matsumoto, Y.; Strauss, G.; Murakami, Y. *J. Am. Chem. Soc.* **1985**, *107*, 2185. (j) Moss, R. A.; Chiang, Y. C. P.; Hui, Y. *J. Am. Chem. Soc.* **1984**, *106*, 7506. (k) Ueoka, R.; Matsumoto, Y.; Yoshino, T.; Hirose, T.; Kikuchi, J.; Murakami, Y. *Chem. Lett.* **1986**, 127. (l) Cho, I.; Kim, G. *J. Org. Chem.* **1988**, *53*, 5189; (m) Ueoka, R.; Matsumoto, Y.; Moss, R. A.; Swarup, S.; Sugii, A.; Harada, K.; Kikuchi, J.; Murakami Y.; *J. Am. Chem. Soc.* **1988**, *110*, 1588.

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Scheme I. Catalytic Cycle for the Imidazole-Catalyzed Hydrolysis of *p*-Nitrophenyl Esters

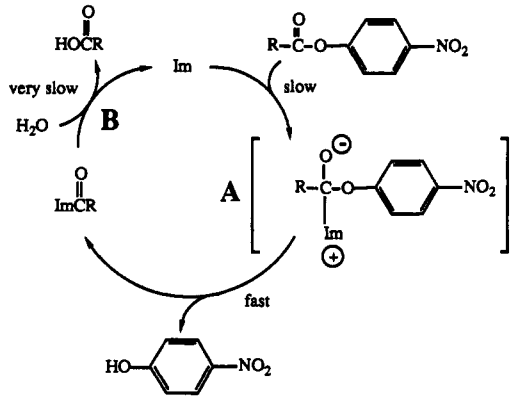
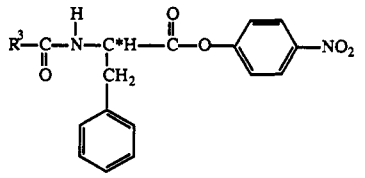
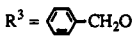


Chart III. Substrates



Abbreviations	
R^3 = 	Z-L- or D-Phe-ONP
$R^3 = CH_3(CH_2)_{10}$	C_{12}-L- or D-Phe-ONP

which implies that under the employed pH condition (pH = 7.3) the carboxyl moiety is deprotonated and in its anionic form. The amino acid residue at the N-terminal end is systematically varied and so is the nature of the N-protecting group which in most cases is of the alkyloxycarbonyl (carbamate) type. All catalysts including their abbreviations are listed in Chart I. The employed surfactant is the chiral cationic surfactant (*R*)-*N*,*N*-dimethyl-*N*-hexadecyl-*N*-(α-methylbenzyl)ammonium bromide (*R*-surf 16, see Chart II). The chiral substrates are N-protected *p*-nitrophenyl esters of the amino acid L- or D-phenylalanine (see Chart III). For comparison the achiral substrate *p*-nitrophenyl acetate (PNPA) is also included.

Attention is focused on the first step in the catalytic cycle, i.e. the acylation of the imidazolyl moiety. The enantioselectivity is expressed as the ratio of the rate constants obtained for this acylation step employing L- and D-substrate: k_L/k_D. The deacylation step of the acylated imidazolyl moiety, regenerating the free imidazolyl group, is not investigated. It is our aim to understand the cause of the observed enantioselectivities.

Results

Enantioselective Ester Cleavage by Histidine-Containing Dipeptides. In this section the effect is investigated of changing the nature of the amino acid residue at the N-terminal end of the L-histidine-containing dipeptide catalysts on the enantioselective hydrolysis of the long acyl chain substrates C_{12}-L/D-Phe-ONP. The N-protecting group in all dipeptides is the dodecyloxycarbonyl moiety (abbreviated as S_{12}). The amino acid residue that is varied contains a hydrocarbon side chain of varying hydrophobicity. The only exception is tryptophane, which has an amphiphilic side chain since the NH group of the indolyl ring can form a strong hydrogen bond with water. The hydrophobicity of the amino acid side chain increases from a methyl group in the L-alanyl residue up to a benzyl moiety in the L-phenylalanyl residue.

Table I. Rates ($k_{a,obs}$ (M^{-1} s^{-1})) and Enantioselectivies (k_L/k_D) of the Cleavage of C_{12}-Phe-ONP (L and D) by Comicelles of S_{12}-L-X-L-His and *R*-Surf 16 at 25 °C[a]

catalyst	k_L[b]	k_D[b]	k_L/k_D
S_{12}-L-Ala-L-His	391	109	3.6
S_{12}-L-But-L-His	537	129	4.2
S_{12}-L-Norval-L-His	1171	141	8.3
S_{12}-L-Leu-L-His	1675	136	12.3
S_{12}-L-Phe-L-His	3688	216	17.1
S_{12}-L-Phe-L-His[c]	3531	203	17.4
S_{12}-L-Trp-L-His	251	126	2.0

[a] Conditions: 0.08 M Tris·HCl buffer and 0.4 M KCl; pH = 7.3; acetonitrile/water = 3/97 (v/v); $c_{catalyst} = 5 \times 10^{-6}$ M; $c_{substrate} = 1 \times 10^{-5}$ M; $c_{surfactant} = 5 \times 10^{-3}$ M. [b] $k_L = k_{a,obs}$ for the L-enantiomer of the substrate and $k_D = k_{a,obs}$ for the D-enantiomer of the substrate. [c] In the presence of *S*-surf 16.

Table II. Rates of the Cleavage of PNPA by Comicelles of S_{12}-L-X-L-His and *R*-Surf 16[a]

catalyst	$k_{a,obs}$ (M^{-1} s^{-1})	catalyst	$k_{a,obs}$ (M^{-1} s^{-1})
S_{12}-L-Ala-L-His	5.0	S_{12}-L-Leu-L-His	5.8
S_{12}-L-But-L-His	5.1	S_{12}-L-Phe-L-His	9.0
S_{12}-L-Norval-L-His	6.1	S_{12}-L-Trp-L-His	5.3

[a] Conditions as described in Table I.

Table I shows the results for the hydrolysis of L- and D-C_{12}-Phe-ONP by comicelles of S_{12}-L-X-L-His and *R*-surf 16. The increase in hydrophobicity of the amino acid side chain of residue X from alanine up to phenylalanine is accompanied by an increase in selectivity from k_L/k_D = 3.6 up to 17.1. In the presence of S_{12}-L-Trp-L-His the selectivity is only k_L/k_D = 2.0. The enhancement of selectivity is almost exclusively due to the increasing rate of the L-substrate whereas the rate of the D-substrate is hardly affected. Apparently, the increase in rate of the L-substrate is due to an increased hydrophobically driven attractive interaction between catalyst and L-substrate. Such an interaction is absent with the D-substrate. The very low selectivity of the tryptophane containing catalyst is primarily due to the almost total collapse of activity toward the L-substrate. Such behavior indicates that the interaction with the substrate is not stabilized by the Trp side chain.

In Table I data for the S_{12}-L-Phe-L-His catalyst in the presence of *S*-surf 16 are also included. It is clearly seen that the chirality of the head group of this surfactant has no significant effect on the observed rates and enantioselectivity. The blank reactions in the absence of catalyst also display an insignificant enantioselectivity (k_L/k_D = 1.08 ± 0.05 in the presence of *R*-surf 16). These observations indicate that the chiral surfactant molecules *R*- or *S*-surf 16 do not recognize the chirality of the adsorbed catalyst, substrate, or transition state.

Cleavage of *p*-Nitrophenylacetate (PNPA) by Histidine-Containing Dipeptides. Table II shows the rates of cleavage of the achiral substrate *p*-nitrophenyl acetate (PNPA) in the presence of the micellar bound catalysts S_{12}-L-X-L-His. When Tables I and II are compared the most striking difference is the relatively low activity observed with PNPA. When the rates are compared with the C_{12}-D-Phe-ONP substrate a 20-fold reduction is observed. This reduced activity primarily arises from the low binding affinity of the less hydrophobic PNPA ester to the micelles. At the employed surfactant concentration the vast majority of the substrate resides in the aqueous phase.[3] Consequently, the rate enhancing effect of

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Table III. Rates ($k_{a,obs}$ (M^{-1} s^{-1})) and Enantioselectivities (k_L/k_D) of the Cleavage of C_{12}-Phe-ONP (L and D) by Comicelles of S_n-L-Phe-L-His and *R*-Surf 16 at 25 °C[a]

catalyst	k_L	k_D	k_L/k_D
S_1-L-Phe-L-His[b]	77	5.6	13.8
S_2-L-Phe-L-His[b]	172	10	17.2
S_3-L-Phe-L-His	512	28	18.3
S_4-L-Phe-L-His	1705	96	17.8
S_6-L-Phe-L-His	2957	171	17.3
S_8-L-Phe-L-His	4378	260	16.8
S_{10}-L-Phe-L-His	5440	324	16.8
S_{12}-L-Phe-L-His	5983	363	16.6
S_{16}-L-Phe-L-His	6460	389	16.6

[a] Conditions: 0.08 M Tris·HCl buffer and 0.4 M KCl; pH = 7.3; acetonitrile/water = 3:97 (v/v); $c_{cat} = 5 \times 10^{-5}$ M; $c_{sub} = 1 \times 10^{-5}$ M; $c_{surf} = 2.5 \times 10^{-3}$ M. [b] $c_{cat} = 25 \times 10^{-5}$ M.

bringing the catalyst and substrate together in the micelles is much smaller.

A clear correlation exists between the rates in Table II and the rates toward the D-substrate in Table I. They both increase to the same extent when the amino acid side chain in the catalyst becomes more hydrophobic. The rate enhancement is most pronounced for the phenylalanyl-containing catalyst whereas the rate drops for the Trp-containing catalyst.

Effect of the N-Protecting Group on the Enantioselectivity. The effect of changing the hydrophobicity of the *N*-(alkyloxycarbonyl) protecting group of the dipeptide catalyst S_n-L-Phe-L-His on the esterolytic activity and enantioselectivity toward the substrate C_{12}-Phe-ONP (L and D) is shown in Table III. Increasing the alkyl chain length S_n of the catalyst results in an enormous enhancement of the activities up to n = 10–12 after which a ceiling value is reached. The rate enhancement from S_2 to S_{12} is about 25-30 fold. The most remarkable aspect is that, in spite of the large rate differences, the selectivities are hardly affected and remain constant around k_L/k_D = 16–18, except for the change from S_1 to S_2, i.e. it is only the part of the hydrophobic alkyl chain S_n directly adjacent to the hydrophilic OC(O)NH group that affects the chiral recognition. The hydrophobic chain of the N-protecting group in the catalyst mainly functions as a hydrophobic anchor by which the catalyst is bound to the micelle. An increase in hydrophobicity of this chain will increase the fraction of catalyst that is bound to the micelles, where the very hydrophobic substrate resides, resulting in a rate enhancement. However, at S_{12}–S_{16} the catalyst approaches a situation in which it is completely micellar bound at the employed surfactant concentration. A further increase in hydrophobicity has no effect on the reaction rates.

The data in Table III show that the rate becomes negligibly small when the hydrophobicity of the N-protecting group is low as in the case of S_1-L-Phe-L-His. This indicates that only a very small fraction of the S_1-L-Phe-L-His catalyst is bound to the micelle. If it is assumed that S_{16}-L-Phe-L-His is completely micellar bound, this fraction can be calculated to be no more than 1–2%. This result is remarkable since the S_1-L-Phe-L-His catalyst still contains the very hydrophobic side chain of the phenylalanyl residue. Apparently, this side chain, which is located between two hydrophilic moieties, cannot dissolve into the micellar hydrocarbon phase and contribute to the binding strength of the catalyst to the micelle.

Table IV shows rate data for *N*-(benzyloxycarbonyl) (Z) and substituted *N*-(benzyloxycarbonyl)-protected The observed selectivities, k_L/k_D = 17–18, indicate that the mode of interaction of these catalysts with the micelle and with the C_{12}-Phe-ONP (L and D) substrates is identical to that of the *N*-(alkyloxycarbonyl) protected catalysts.

Table IV. Rates ($k_{a,obs}$ (M^{-1} s^{-1})) and Enantioselectivities (k_L/k_D) of the Cleavage of C_{12}-Phe-ONP (L and D) by Comicelles of R-L-Phe-L-His and *R*-Surf 16 at 25 °C[a]

catalyst	k_L	k_D	k_L/k_D
Z-L-Phe-L-His	1648	93	17.7
p-BrZ-L-Phe-L-His	2637	149	17.7
F_5Z-L-Phe-L-His	3067	179	17.1

[a] Conditions as described in Table III.

Table V. Rates ($k_{a,obs}$ (M^{-1} s^{-1})) and Enantioselectivities (k_L/k_D) of the Cleavage of Z-Phe-ONP (L and D) by Comicelles of R-L-Phe-L-His and *R*-Surf 16 at 25 °C[a]

catalyst	k_L	k_D	k_L/k_D
S_2-L-Phe-L-His	38.9	7.2	5.4
Z-L-Phe-L-His	297	61	4.9
F_5Z-L-Phe-L-His	694	130	5.3
S_{12}-L-Phe-L-His	1070	221	4.8

[a] Conditions as described in Table III.

Table VI. Rates ($k_{a,obs}$ (M^{-1} s^{-1})) and Enantioselectivities (k_L/k_D) of the Cleavage of Z-Phe-ONP and C_{12}-Phe-ONP (L and D) by Comicelles of His-Containing Dipeptides and *R*-Surf 16 at 25 °C[a]

	C_{12}-Phe-ONP			Z-Phe-ONP		
catalyst	k_L	k_D	k_L/k_D	k_L	k_D	k_L/k_D
S_{12}-D-Phe-L-His	1889	362	3.0	478	218	2.2
C_{12}-L-Phe-L-His	1451	251	5.8	378	225	1.7
C_1-L-Phe-L-His[b]	16	3	5	3.2	1.4	2.3
Z-L-Ala-L-His	358	89	4.0	149	59	2.5

[a] Conditions as described in Table 3. [b] $c_{cat} = 5 \times 10^{-4}$ M.

The activity of Z-L-Phe-L-His corresonds to the activity of S_4-L-Phe-L-His (Table III), indicating that the hydrophobicity of a Z moiety is identical with the hydrophobicity of an S_4 moiety. This would mean that a phenyl and a *n*-propyl group have equal hydrophobicities. This conclusion is in agreement with the almost equal free energies of transfer of benzene and propane from water to a liquid hydrocarbon at 25 °C. These energies amount to –4.6 and –4.9 kcal/mol, respectively.[4] It is also in agreement with the identical free energy changes for transfer of the amino acids phenylalanine (benzyl side chain) and norleucine (*n*-butyl side chain) from water to an organic solvent.[5]

The higher activities observed for the *p*-BrZ- and F_5Z-protected catalysts are in line with the fact that these moieties are more hydrophobic than the unsubstituted Z moiety. The activity of the F_5Z moiety in comparison with the Z moiety corresponds to an increased hydrophobicity of two methylene groups in an unbranched hydrocarbon chain. The substitution of the para hydrogen by a bromine atom corresponds to an increased hydrophobicity of approximately 1.7 CH_2 groups.

Table V presents the activity and enantioseletivity of comicelles composed of R-L-Phe-L-His and *R*-surf 16 towards the chiral substrates Z-Phe-ONP (L and D). Again, the enantioselectivities are not significantly affected by the degree of hydrophobicity of the N-protecting group in the catalysts. The rate enhancement is approximately 25–30-fold when the hydrophobicity of the N-protecting group increases from S_2 to S_{12}. This rate enhancement is identical with the one observed for the long acyl chain substrates C_{12}-Phe-ONP. The selectivities, however, are markedly lower, suggesting a less favorable fit of the former substrates and the catalyst in the transition state. The

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(5) (a) Nozaki, Y.; Tanford, C. *J. Biol. Chem.* **1971**, *246*, 2211. (b) Tanford, C. *The hydrophobic effect: formation of micelles and biological membranes*, 2nd ed.; Wiley-Interscience: New York, 1980; p 23.

reduced selectivities in comparison with the C_{12}-Phe-ONP substrates are mainly due to a reduced activity toward the L-substrate.

In Table VI the activities and enantioselectivities of some additional L-histidine-containing catalysts toward the substrates C_{12}-Phe-ONP and Z-Phe-ONP are given. The activities obtained for S_{12}-D-Phe-L-His (entry 1) should be compared with those of the diastereomeric catalyst S_{12}-L-Phe-L-His in Tables III and V. The enantioselectivity appears to depend strongly on the configuration of the phenylalanyl residue in the catalyst. The selectivity k_L/k_D = 17 toward C_{12}-Phe-ONP observed with the L-Phe-L-His catalyst is reduced to only k_L/k_d = 3 with the D-Phe-L-His catalyst. The reduction in selectivity is exclusively due to a 5–6-fold decrease in activity towards the L-substrate. The activity toward the D-substrate is not affected to a significant extent. A very similar, although smaller, effect is observed for the Z-Phe-ONP substrate. These results again demonstrate the intimate involvement of the Phe residue of the catalyst in the chiral recognition of the substrate.

Entry 2 of Table VI shows the results of the esterolysis of C_{12}-Phe-ONP by C_{12}-L-Phe-L-His. The data should be compared with those for the S_{12}-L-Phe-L-His catalyst in Table III. The difference between the two catalysts is that the former is protected with a dodecanoyl group and the latter with a dodecyloxycarbonyl group. Both protecting groups have similar hydrophobic parts. The C_{12}-L-Phe-L-His catalyst exhibits a much lower selectivity (k_L/k_D = 5.8) than the S_{12}-L-Phe-L-His catalyst (k_L/k_D = 17). This reduction in selectivity is mainly due to a 4-fold decrease in reaction rate of the L-substrate, whereas the rate of the D-substrate is only slightly affected. This suggests that the carbamate moiety in the S_{12}-protected catalyst is involved in the interaction of the catalyst with the L-substrate in the transition state. Replacing the carbamate moiety by an amide group apparently reduces this interaction. Note that in the previous section it was observed that the opposite occurs for the substrate, i.e. with the C_{12}-protected substrate (amide) higher enantioselectivities and activities are obtained than with the Z-protected substrate (carbamate).

Entry 3 of Table VI gives the results obtained with the *N*-acetyl-L-Phe-L-His catalyst. Both the activities and enantioselectivities are very low. The activity is even lower than that of S_1-L-Phe-L-His (Table III), indicating that the interaction of the catalyst with the micelles is very weak. Most of the catalyst will reside in the aqueous phase. If the activities of C_1-L-Phe-L-His and C_{12}-L-Phe-L-His (Table VI, entries 2 and 3) toward the substrate C_{12}-Phe-ONP are compared, it can be concluded that no more than 1% of the former catalyst is bound to the micelles.

Entry 4 of Table VI shows the activity and enantioselectivity of Z-L-Ala-L-His toward the esters C_{12}-Phe-ONP and Z-Phe-ONP. The enantioselectivity toward C_{12}-Phe-ONP (k_L/k_D = 4.0) is nearly identical with the one observed with the much more hydrophobic catalyst S_{12}-L-Ala-L-His (Table I, k_L/k_D = 3.6) only the rates are reduced due to the lower binding to the micelles. A similar behavior was observed for the catalysts Z-L-Phe-L-His and S_{12}-L-Phe-L-His (Tables IV and III). It is of interest to compare the activities of the Z-L-Ala-L-His and the S_1-L-Phe-L-His catalysts (Tables VI and III). On the basis of the structural formula both catalysts can be classified as equally hydrophobic. The methyl and benzyl moiety have changed their positions in these catalysts. However, the catalytic activity of Z-L-Ala-L-His is much higher (up to 15-fold for the D-substrate), indicating that a much larger fraction of this catalyst is bound to the micelles. The benzyl moiety in Z-L-Ala-L-His is not located between two hydrophilic groups and can efficiently interact with (dissolve into) the hydrocarbon phase of the micelles.

Table VII. Rates ($k_{a,obs}$ (M^{-1} s^{-1})) and Enantioselectivities (k_L/k_D) of the Cleavage of C_{12}-Phe-ONP by Comicelles of S_{12}-L-Phe-L-His and *R*-Surf 16 at Various Temperatures[a]

T (°C)	k_L	k_D	k_L/k_D
5	456	15	30.4
15	785	32	24.5
25	1106	63	17.6
35	1217	113	10.8
45	1229	181	6.8
50	1249	218	5.7

[a] Conditions as described in Table I; $c_{surf} = 10^{-2}$ M.

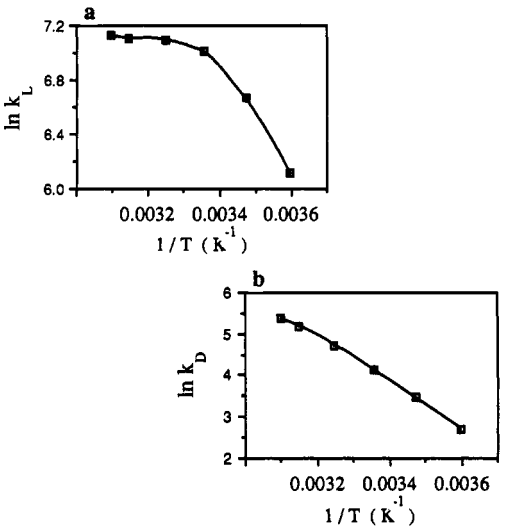

Figure 1. (a) ln k_L as a function of $1/T$. (b) ln k_D as a function of $1/T$ (data from Table VII).

Effect of Temperature. The effect of the temperature on the catalytic activity of S_{12}-L-Phe-L-His toward C_{12}-Phe-ONP in the presence of *R*-surf 16 is shown in Table VII. A relatively high surfactant concentration is chosen (10^{-2} M) in order to be sure that both catalyst and substrate remain completely bound to the micelles over the entire temperature range. Above 25 °C, the activity toward the L-substrate is severely suppressed, i.e. the activity remains almost constant although the temperature is raised. Similar behavior is observed for enzymes that often display a maximum activity at a certain temperature. This phenomenon is not observed for the catalytic activity toward the D-substrate which increases regularly above 25 °C. In Figure 1, parts a and b, ln k is plotted against $1/T$. With the D-substrate an almost linear plot is obtained, but with the L-substrate a strong deviation from linearity above 25 °C is observed. It is clear that this behaviour will strongly reduce the enantioselectivity (see Table VII). These results indicate that upon raising the temperature a favorable interaction that exists between the catalyst and the L-substrate, is severely reduced. Such an interaction does not exist with the D-substrate.

Fluorescence Measurements on the Tryptophane-Containing Catalyst. The position of the fluorescence spectrum of Trp strongly depends on the polarity of the microenvironment. The emission spectrum of Trp-containing probes in a solution of micelles not only furnishes information about the average polar surroundings of micellar bound molecules but also gives information about the heterogeneity or distribution of the probe between the aqueous and micellar phase.[6a-d]

Table VIII. Fluorescence Maximum of S_{12}-L-Trp-L-His Dissolved in Dioxane–Water Mixtures[a]

solvent mixture[b]	$E_T(30)$ (kcal/mol)[c]	λ_F (nm)[d]
0	63.1	360
10	61.1	359
20	58.7	357
30	57.1	354
40	55.5	352
50	53.9	351
60	52.3	349
70	50.7	348
80	49.1	346
90	46.8	343
100	36.1	333

[a] T = 25 °C; $c_{probe} = 2 \times 10^{-5}$ M. [b] Volume percentage dioxane in water. [c] Values taken from ref 17 or from data in this reference by linear interpolation. [d] Wavelength of maximum fluorescence intensity (±1 nm) at an excitation wavelength of 286 nm.

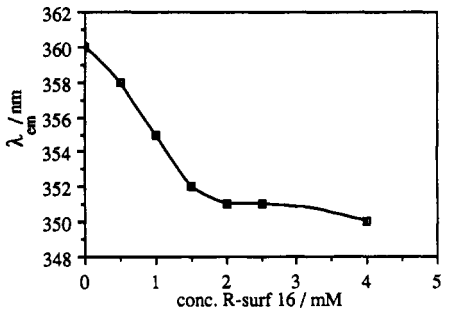

Figure 2. The positions of the fluorescence emission maximum of S_{12}-L-Trp-L-His in a surfactant solution of *R*-surf 16. λ_{ex} = 286 nm; 0.08 M Tris·HCl; 0.40 M KCl; pH = 7.3; $c_{probe} = 2 \times 10^{-5}$ M.

The probe S_{12}-L-Trp-L-His was added to a solution of *R*-surf 16 under reaction conditions. Subsequently, a fluorescence spectrum was recorded. For comparison, the fluorescence emission spectrum of the Trp-containing catalyst was also measured in various 1,4-dioxane–water mixtures. The position of the emission maximum in these mixtures is presented in Table VIII, together with the corresponding solvent polarities expressed by the $E_T(30)$ parameter.[6e] Upon increasing the surfactant concentration the emission maximum of the catalyst gradually shifts from 360 nm (surfactant free buffer solution) to 351 nm (completely micellar bound catalyst at approximately 2 mM, Figure 2). The emission maximum of the completely micellar bound catalyst corresponds to a polarity of 54 kcal/mol on the $E_T(30)$ scale or to a 50%/50% (v/v) 1,4-dioxane–water mixture. This clearly indicates that the indolyl moiety is not completely dehydrated but remains exposed to the aqueous phase.

Discussion

If amphiphilic molecules like our catalysts and substrates are added to an aqueous micellar solution, the apolar hydrocarbon parts of these molecules will dissolve into the micellar hydrocarbon phase, whereas the polar hydrophilic parts will tend to remain in the aqueous phase. However, the hydrophobic groups in the catalyst or substrate that are in close proximity to hydrophilic groups will not be able to dissolve into the micellar hydrocarbon phase without causing dehydration of these hydrophilic groups,

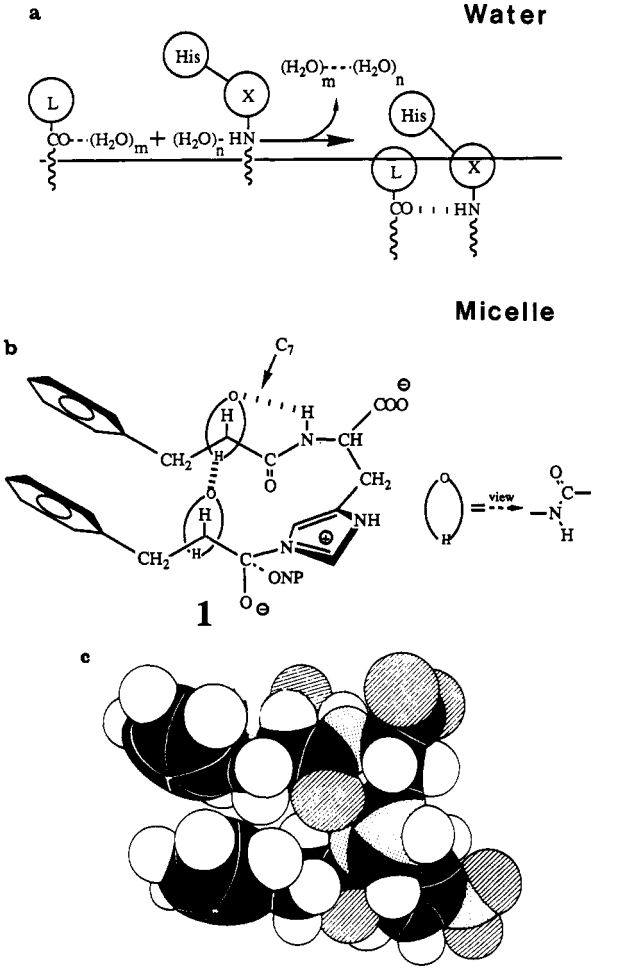

Figure 3. (a) The formation of a hydrogen bond at the micellar interface between the catalyst and the L-enantiomer of the substrate. (b) Schematic representation of the tetrahedral intermediate 1 that is formed from the S_n-L-Phe-L-His catalyst and the C_{12}-L-Phe-ONP ester. The hydrophobic parts of the N-protecting groups are not shown. The hydrogen bonds between the L-Phe and L-His residue (C_7) and between both L-Phe residues are represented by dotted lines. (c) A CPK space-filling model of 1.

i.e., they must remain in the aqueous phase. The apolar amino acid side chain of the amino acid residue X adjacent to the His residue in the catalyst and the amino acid side chain of the substrate, which are both located between two hydrophilic groups, will remain exposed to the aqueous phase when these molecules are adsorbed to the micellar interface (Figure 3a). Also the CH_2 groups of the hydrophobic alkyl chain of S_n in the catalyst that are adjacent to the hydrophilic OC(O)NH group will remain exposed to the aqueous phase, the rest of this alkyl chain is free to dissolve into the micellar hydrocarbon phase. The hydrophilic–hydrophobic conflict can be solved in the following way: if the polar hydrated groups form hydrogen bonds with each other, they lose their hydrophilicity and are free to dissolve in an apolar environment which allows the transfer of the adjacent hydrophobic groups to the apolar micellar phase (Figure 3a).

When dipeptides are dissolved in apolar solvents, they adopt the so-called C_7 conformation.[7d] This conformation is characterized by an intramolecular hydrogen bond be-

(6) (a) Lakowicks, J. R. *J. Biol. Chem.* **1983**, *258*, 5519. (b) Permyakov, M. *Biophys. Chem.* **1983**, *18*, 145. (c) Irace, G. *Biochemistry* **1981**, *20*, 792. (d) Shinitzky, M. *Biochemistry* **1977**, *16*, 982. (e) The intramolecular charge-transfer process of Trp can be best compared with a solvent polarity parameter based on a similar intramolecular charge-transfer process, e.g. the $E_T(30)$ parameter. For a discussion see: Kosower, E. M.; Dodiuk, H.; Kanety, H. *J. Am. Chem. Soc.* **1978**, *100*, 4179.

(7) (a) Ramachandran, *J. Mol. Biol.* **1963**, *7*, 95. (b) Bricas, E., Ed. *Peptides 1968*; North-Holland Publishing Company: Amsterdam, 1968. (c) Walton, A. G. *Polypeptides and Protein Structure*; Elsevier: New York, 1981. (d) Udenfriend, S.; Meienhofer, J. *The Peptides, Analysis, Synthesis, Biology*; Academic Press Inc.: New York, 1985; p 84.

tween the adjacent amide groups. A seven-membered ring is formed. If our S_n-L-Phe-L-His catalyst attains the C_7 conformation, one side of the amino acid side chain of Phe can adsorb to the micellar hydrocarbon interface. The CO moiety of the N-protecting group and the NH moiety of the His residue form the intramolecular hydrogen bond, and the NH and CO moieties of the Phe residue remain hydrated. In this position the amino acid side chain of the Phe residue lies flat on the micellar interface, and the alkyl chain of the N-protecting group points to the center of the micelle.

Figure 3, parts b and c, gives a top view on the micellar interface of the tetrahedral intermediate 1 that is proposed for the reaction between the S_n-L-Phe-L-His catalyst and the C_{12}-L-Phe-ONP substrate. This intermediate closely resembles the transition state. The NH group of the Phe residue of the catalyst forms an intermolecular hydrogen bond with the amide CO group of the L-substrate. This allows the adjacent apolar groups to be dissolved in the micellar hydrocarbon phase. The larger these apolar groups are (from X = Ala to X = Phe, and from S_1 to S_2) the more stable this transition state will be. In 1, the *p*-nitrophenyl moiety is adsorbed to the micellar interface. The proposed conformation is $(\phi,\psi,\chi^1,\chi^2)_{phe,catalyst} = -100°$, 70°, g^-, 90°; $(\phi,\chi^2,\chi^2)_{His} = -120°$, g^-, 90°; $(\phi,\chi^1,\chi^2)_{Phe,substrate} = -100°$, g^-, 90° (ϕ is the torsional angle around the N–C^α bond, ψ around the C^α–CO bond, χ^1 around C^α–C^β, and χ^2 around C^β–C^γ).[7,8] These are all low energy conformations.

N-Acetyl-L-Phe-*N*-methylamide[9,10] can be considered as the structural element of the Phe residue in the S_n-L-Phe-L-His catalysts and the L-substrates. In the crystalline state this molecule has the conformation $\phi,\chi^1,\chi^2 = -106.7°$, g^-, 89.8°. This is essentially identical with the conformation in the proposed transition state 1. In addition a hydrogen bond is present between the molecules that is similar to the proposed hydrogen bond in 1.

Model building shows that the D-enantiomer of the substrate cannot form a tetrahedral intermediate with an L,L-catalyst, in which an intermolecular hydrogen bond is formed that allows the transfer of all hydrophobic groups to the micellar hydrocarbon phase.

If the Phe residue of the catalyst is changed from L into D, the interaction of the D-substrate with the D-Phe residue in the D,L-catalyst can be identical with and just as favorable as the interaction between the L-substrate and S_{12}-L-Phe-L-His, shown in 1. The kinetic data, however, show that in comparison to the L,L-catalyst the D,L-catalyst exhibits no enhanced activity toward the D-substrate. Model building shows that the imidazolyl moiety cannot reach the ester group.

The side chain of the Trp residue is amphiphilic. Consequently, it is not favorable to transfer this side chain to the micellar hydrocarbon phase when 1 is formed, unless the L-substrate can form a hydrogen bond with the NH part of the indolyl moiety. Since this is not the case, the transition state will not be stabilized. The kinetic data in Table I for S_{12}-L-Trp-L-His show that the rate toward the L-substrate even falls below the rate observed with the Ala-containing catalyst.

Steric Effects. Upon formation of the tetrahedral intermediate 1, the N-protecting groups of the catalyst and substrate approach each other very closely. The hydrophobic parts of these N-protecting groups, which are dissolved in the micellar hydrocarbon phase should preferentially point away from each other. This is the case when the catalyst is N-protected with an alkyloxycarbonyl moiety (S_n-L-Phe-L-His; see Figure 4a which shows the low energy *Z* conformation[10]) and the L-substrate with an acyl moiety (C_{12}-L-Phe-ONP; see Figure 4a). If the substrate is N-protected with an alkyloxycarbonyl moiety or the catalyst with an acyl moiety, the formation of the hydrogen bond and thus the transition state with the L-substrate is hindered (Figure 4, parts b and c, respectively). When the catalyst is protected with an acyl moiety and the L-substrate with an alkyloxycarbonyl moiety, the steric hindrance is the largest (Figure 4d). The experimental data show that the reduced enantioselectivities are almost exclusively due to a reduced activity toward the L-substrate, i.e. only the formation of the transition state with the L-substrate is suppressed.

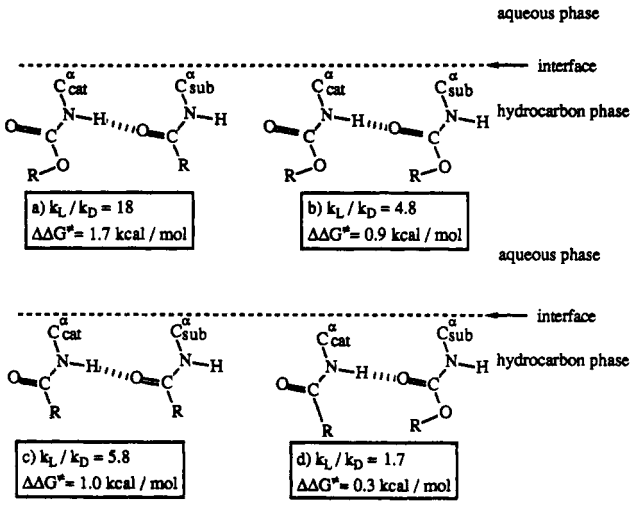

Figure 4. Steric effects of the N-protecting groups in 1.

Conclusions

From the data presented in this paper the following conclusions can be drawn. (1) The amphiphilic catalysts and substrates are adsorbed to and oriented at the micellar interface. (2) Not all the hydrophobic parts of the catalyst and the substrate can dissolve completely into the micellar hydrocarbon phase due to the need for hydration of the hydrophilic groups. (3) Of the two diastereomeric transition states, only the transition state with the L-enantiomer of the ester has a hydrogen bond between the ester and the catalyst. (4) The hydrogen bonded groups lose their hydrophilicity, which allows the transfer of these groups and the adjacent hydrophobic moieties to the micellar hydrocarbon phase. This stabilizes the transition state. (5) Steric hinderance between the N-protecting groups of the catalyst and the L-substrate opposes the formation of this stabilizing hydrogen bond. In the present case, the least steric hinderance occurs when the catalyst is protected by an alkyloxycarbonyl group and the substrate by an acyl group.

Experimental Section

General Remarks. Thin layer chromatography (TLC) was performed on silica (Merck DC-Plastikrolle, Kieselgel 60 F_{254}), and detection was effected by ultraviolet light or iodine. Column chromatography was performed with silica (Merck Kieselgel 60, 230–400 mesh). Solvents and reagents were of analytical grade.

(8) The nomenclature and conventions used follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature see *Biochemistry* **1970**, *9*, 3471.

(9) (a) Harada, Y. *Biochemistry* **1977**, *B33*, 244. (b) Ichikawa, T. *Ibid.* **1977**, *B25*, 1824.

(10) Harada, Y. *Acta Crystallogr.* **1974**, *B30*, 726.

(11) Benedetti, E.; Pedone, C.; Toniolo, C.; Dudek, M.; Nemethy, G.; Scheraga, H. A. *Int. J. Pept. Protein Res.* **1983**, *21*, 163.

Synthesis. (Alkyloxycarbonyl)-L-(or D)-Amino Acids (S_n-L-(or D)-X-OH), (Benzyloxycarbonyl)-L-Phe-OH, (Benzyloxycarbonyl)-L-Ala-OH, (2,3,4,5,6-Pentafluorobenzyloxycarbonyl)-L-Phe-OH, (*p*-Bromobenzyloxycarbonyl)-L-Phe-OH. These compounds were prepared by coupling of the chloroformates with the corresponding amino acids according to a literature method.[12] The yields amounted to 60–70%. The IR spectra (NaCl, KBr, or neat) of all compounds showed bands at 3300 (NH carbamate), 1720 (COOH), 1685 cm^{-1} (C=O carbamate), unless noted otherwise. TLC: R_f = 0.3–0.4 (chloroform–methanol, 4/1, v/v) for all compounds. The compounds had the following physical properties: ^1H NMR ($CDCl_3$); $[\alpha]^{20}_D$ (c = 1.0, methanol) unless noted otherwise; mp, °C.

S_{12}-L-Ala-OH: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 1.45 (d, 3 H, CH_3), 4.05 (t, 2 H, CH_2O), 4.3 (2 q, 1 H, CH), 5.5 (d, 1 H, NH), 10.9 (s, 1 H, COOH) ppm; –12.7°; 68.8.

S_{12}-L-But-OH (from α-aminobutyric acid): δ 0.9 (t, 3 H, CH_3), 1.0 (t, 3 H, $CHCH_2CH_3$), 1.3 (m, 20 H, $(CH_2)_{10}$), 1.65 (m, 2 H, $CHCH_2CH_3$), 4.0 (t, 2 H, CH_2O), 4.25 (2 t, 1 H, CH), 5.4 (d, 1 H, NH), 10.5 (s, 1 H, COOH) ppm; –10.8°; 70.5.

S_{12}-L-Norval-OH: δ 0.9 (t, 6 H, 2 CH_3), 1.3 (m, 24 H, $(CH_2)_{10}$ and $CHCH_2CH_2CH_3$), 4.0 (t, 2 H, CH_2O), 4.2 (2 t, 1 H, CH), 5.1 (d, 1 H, NH), 10.4 (s, 1 H, COOH) ppm; –9.8°; 55.4.

S_{12}-L-Leu-OH: δ 0.9 (t, 3 H, $(CH_2)_{10}$-CH_3), 0.9 (2 d, 6 H, $CH(CH_3)_2$), 1.3 (m, 23 H, $(CH_2)_{10}$ and $CH_2CH(CH_3)_2$), 4.0 (t, 2 H, CH_2O), 4.05 (2 t, 1 H, CH), 5.4 (d, 1 H, NH), 10.7 (s, 1 H, COOH) ppm; –8.1°; oil.

S_{12}-L-Phe-OH: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$, 3.15 (d (dist), 2 H, CH_2Ph); 4.0 (t, 2 H, CH_2), 4.65 (2 t, 1 H, CH), 5.2 (d, 1 H, NH), 7.2 (s, 5 H, ArH), 11.4 (s (br), 1 H, COOH) ppm; –2.8°, oil.

S_{12}-L-Trp-OH: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 3.25 (d (dist), 2 H, CH_2-indolyl), 4.0 (t, 2 H, CH_2O), 4.6 (2 t, 1 H, CH); 5.25 (d, 1 H, NH), 6.7–7.7 (m, 5 H, indolyl), 8.1 (s (br), 1 H, NH (indolyl)), 8.8 (s (br), 1 H, COOH) ppm; –3.2°; 123.2.

Z-L-Phe-OH: δ 3.05 (d (dist), 2 H, $CHCH_2Ph$), 4.45 (2 t (dist), 1 H, CH), 4.9 (s, 2 H, CH_2O), 5.45 (d, 1 H, NH), 7.1 and 7.15 (2 s, 2 × 5 H, 2 Ph), 11.0 (s, 1 H, COOH) ppm; +5.1° (c = 1.0, glacial acetic acid); 88.1.

***p*-BrZ-L-Phe-OH**: δ 3.1 (d (dist), 2 H, $CHCH_2Ph$), 4.5 (2 t (dist), 1 H, CH), 5.0 (s, 2 H, CH_2O), 5.3 (d, 1 H, NH), 7.1 (s, 5 H, C_6H_5), 6.9 and 7.3 (s d, 2 × 2 H, C_6H_4Br), 10.1 (s (br), 1 H, COOH) ppm; –9.2°; 108.7.

F_5Z-L-Phe-OH: δ 3.3 (d (dist), 2 H, CH_2), 4.6 (2 t (dist), 1 H, CH), 5.1 (s, 2 H, CH_2), 5.2 (d, 1 H, NH), 7.2 (m, 5 H, Ph), 9.45 (s, 1 H, COOH) ppm; –9.4°; 134.8; IR (KBr) 1705 (w) and 1660 cm^{-1} (s) (C=O carbamate).

Z-L-Ala-OH: δ 1.45 (d, 3 H, CH_3), 4.3 (2 q (dist), 1 H, CH), 4.9 (s, 2 H, CH_2O), 5.2 (d, 1 H, NH), 7.1 (s, 5 H, Ph), 10.5 (s, 1 H, COOH) ppm; –14.2°; 83.2.

S_1-L-Phe-OH: δ 3.15 (d (dist), 2 H, CH_2), 3.6 (s, 3 H, CH_3), 4.5 (2 t, 1 H, CH), 5.2 (d, 1 H, NH), 7.2 (s, 5 H, Ph), 10.4 (s, 1 H, COOH) ppm; –4.7°; oil.

S_2-L-Phe-OH: δ 1.15 (t, 3 H, CH_3), 3.0 (d (dist), 2 H, CH_2Ph), 4.0 (q, 2 H, CH_2), 4.5 (2 t, 1 H, CH), 5.3 (d, 1 H, NH), 7.2 (s, 5 H, Ph), 10.2 (s (br), 1 H, COOH) ppm; –4.0°; oil. The ^1H NMR and IR spectra of S_3,S_4,S_6,S_8,S_{10}- and S_{16}-L-Phe-OH (all viscous oils) are all very similar to the spectrum of the S_{12}-L-Phe-OH compound. $[\alpha]^{20}_D$: –3.8°, –3.5°, –3.4°, –3.2°, –3.0°, and –2.5°, respectively.

L-Histidine Methyl Ester Dihydrochloride. This compound was a commercial product from Aldrich.

(Alkyloxycarbonyl)-L-X-L-histidine Methyl Ester (S_n-L-X-L-His-OMe), (Benzyloxycarbonyl)-L-phenylalanyl-L-histidine Methyl Ester (Z-L-Phe-L-His-OMe), (2,3,4,5,6-pentafluorobenzyloxycarbonyl)-L-phenylalanyl-L-histidine Methyl Ester (F_5Z-L-Phe-L-His-OMe), (Benzyloxycarbonyl)-L-alanyl-L-histidine Methyl Ester (Z-L-Ala-L-His-OMe), (*p*-Bromobenzyloxycarbonyl)-L-phenylalanyl-L-histidine Methyl Ester (*p*-BrZ-L-Phe-L-His-OMe). Coupling of the oxycarbonyl-protected amino acids with the methyl ester of L-histidine was accomplished using 1,6-dicyclohexylcarbodiimide according to a method described in literature.[13] The crude products were purified by column chromatography (Silica 60; chloroform–methanol, 20/1, v/v) and recrystallization from ethyl acetate–hexane. The yields amounted to 60–70%. IR (KBr): 3300 (NH amide and carbamate), 1732 (C=O ester), 1695 (C=O carbamate), 1645 cm^{-1} (C=O amide), unless noted otherwise. TLC (Silica): R_f = 0.4 (chloroform–methanol, 4/1, v/v) for all compounds. ^1H NMR ($CDCl_3$); $[\alpha]^{20}_D$ (c = 1.0, methanol); mp °C.

S_{12}-L-Ala-L-His-OMe: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 1.45 (d, 3 H, CH_3), 3.15 (d (dist), 2 H, CH_2-Im), 3.7 (s, 3 H, OCH_3), 4.0 (t, 2 H, CH_2O), 4.2 (2 q, 1 H, CH (Ala)), 4.75 (2 t (dist), 1 H, CH (His), 5.7 (d, 1 H, NH (carbamate)), 6.7 and 7.45 (2 s, 2 × 1 H, 2 CH (Im)), 7.55 (d, 1 H, NH (amide)), 10.1 (s, br), 1 H, NH (Im)) ppm; –9.0°; 118.6.

S_{12}-L-But-L-His-OMe; δ 0.9 (t, 3 H, $(CH_2)_{10}CH_3$), 1.0 (t, 3 H, CH_3), 1.3 (m, 22 H, $(CH_2)_{10}$ and $CHCH_2CH_3$), 3.1 (d (dist), 2 H, CH_2 (His)), 3.65 (s, 3 H, OCH_3), 4.0 (t, 2 H, CH_2O), 4.1 (2 t (dist), 1 H, CH (But)), 4.8 (2 t (dist), 1 H, CH (His)), 5.7 (d, 1 H, NH (carbamate)), 6.7 and 7.45 (2 s, 2 × 1 H, 2 CH (Im)), 7.8 (d, 1 H, NH (amide)), 9.2 (s (br), 1 H, NH (Im)) ppm; –9.5°; 107.8.

S_{12}-L-Norval-L-His-OMe: δ 0.9 (2 t, 2 × 3 H, 2 CH_3), 1.3 (m, 24 H, $(CH_2)_{10}$ and $(CH_2)_2$), 3.1 (d (dist), 2 H, CH_2 (His)), 3.65 (s, 3 H, OCH_3), 4.0 (t, 2 H, CH_2O), 4.15 (2 t (dist), 1 H, CH (Norval), 4.8 (2 t, 1 H, CH (His)), 5.8 (d, 1 H, NH (carbamate)), 6.7 and 7.45 (2 s, 2 × 1 H, 2 CH (Im)), 7.7 (d, 1 H, NH (amide)), 9.5 (s, br), 1 H, NH (Im)) ppm; –9.4°; 110.9.

S_{12}-L-Leu-L-His-OMe: δ 0.9 (m, 9 H, 3 CH_3), 1.25 (m, 23 H, $(CH_2)_{10}$ and CH_2CH (Leu)), 3.1 (d, 2 H, CH_2 (His)), 3.65 (s, 3 H, OCH_3), 4.0 (t, 2 H, CH_2O), 4.15 (2 t (dist), 1 H, CH (Leu)), 4.8 (2 t, 1 H, CH (His)), 5.5 (d, 1 H, NH (carbamate)), 6.7 and 7.45 (2 s, 2 × 1 H, 2 CH (Im)), 7.45 (d, 1 H, NH (amide)), 8.9 (s (br), 1 H, NH (Im)) ppm; –16.8°; 104.8.

S_{12}-L-Phe-L-His-OMe: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 3.1 (2 d (dist), 2 × 2 H, CH_2(Phe) and CH_2(His)), 3.65 (s, 3 H, OCH_3), 3.95 (t, 2 H, CH_2O), 4.3–5.1 (m, 2 × 1 H, CH (Phe) and CH (His)), 6.7 and 7.45 (2 s, 2 × 1 H, 2 CH (Im)), 7.2 (s, 5 H, Ph), 7.55 (d, 1 H, NH (amide)), 8.9 (s (br), 1 H, NH (Im)) ppm; –7.4°; 118.2.

S_{12}-D-Phe-L-His-OMe: the ^1H NMR spectrum is identical with the spectrum of the S_{12}-L-Phe-L-His-OMe diastereomer; +1.0°; 121.3.

S_{12}-L-Trp-L-His-OMe: δ 0.95 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 3.1 (m, 2 × 2 H, CH_2 (Trp) and CH_2 (His)), 3.6 (s, 3 H, OCH_3), 4.0 (t, 2 H, CH_2O), 4.6 (m, 2 H, CH (Trp) and CH (His)), 5.65 (d, 1 H, NH (carbamate)), 6.55 and 7.35 (2 s, 2 × 1 H, 2 CH (Im)), 6.8–7.7 (m, 5 H, 5 CH (indolyl)), 7.55 (d, 1 H, NH (amide)), 8.7 (s (br), 1 H, NH (Im)), 8.95 (s (br), 1 H, NH (indolyl)) ppm; –7.9°; 113.5.

Z-L-Phe-L-His-OMe: δ 2.4–3.3 (m, 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.55 (s, 3 H, OCH_3), 4.3–5.0 (m, 2 × 1 H, CH (Phe) and CH (His)), 5.05 (s, 2 H, CH_2 (Z)), 6.7 and 7.55 (2 s, 2 × 1 H, 2 CH (Im)), 5.6 (d, 1 H, NH (carbamate)), 7.0 and 7.25 (2 s, 2 × 5 H, 2 Ph), 7.7 (d, 1 H, NH (amide)), 8.9 (s (br), 1 H, NH (Im)) ppm; –8.8°; 101.3.

F_5Z-L-Phe-L-His-OMe (methanol-d_4/DMSO-d_6): δ 2.7–3.3 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.55 (s, 3 H, OCH_3), 4.0–4.5 (2 t (dist), 2 × 1 H, CH (Phe) and CH (His)), 5.05 (s, 2 H, CH_2 (Z)), 7.18 (s, 5 H, Ph), 7.1 and 8.2 (2 s, 2 × 1 H, 2 CH (Im)) ppm; –14.9°; 151.5; IR (KBr) 1709 cm^{-1} (C=O carbamate).

***p*-BrZ-L-Phe-L-His-OMe**: δ 2.4–3.3 (m, 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.6 (s, 3 H, OCH_3), 4.3–5.0 (m, 2 × 1 H, CH (Phe) and CH (His)), 5.1 (s, 2 H, CH_2O), 6.7 and 7.6 (2 s, 2 × 1 H, 2 CH (Im)), 5.5 (d, 1 H, NH (carbamate)), 7.1 (s, 5 H, C_6H_5), 7.0 and 7.4 (2 d, 2 × 2 H, C_6H_4Br), 7.8 (d, 1 H, NH (amide)), 9.1 (s, 1 H, NH (Im)) ppm; –12.7°; 128.0.

Z-L-Ala-L-His-OMe: δ 1.4 (d, 3 H, CH_3), 3.1 (d (dist), 2 H, CH_2 (His)), 3.7 (s, 3 H, OCH_3), 4.25 (2 q, 1 H, CH (Ala)), 4.8 (2 t, 1 H, CH (His)), 5.7 (d, 1 H, NH (carbamate)), 6.6 and 7.35 (2 s, 2 × 1 H, 2 CH (Im)), 7.7 (d, 1 H, NH (amide), 9.7 (s (br), 1 H, NH (Im)) ppm; –10.4°; 114.2.

S_1-L-Phe-L-His-OMe: δ 3.05 (m, 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.4 (s, 3 H, CH_3 (S_1)), 3.6 (s, 3 H, OCH_3), 4.4–5.1 (m, 2 × 1 H, CH (Phe) and CH (His)), 5.7 (d, 1 H, NH (carbamate)), 6.65

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and 7.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.15 (s, 1 H, Ph); 7.75 (d, 1 H, NH (amide)), 9.0 (s (br), 1 H, NH (Im)) ppm; −6.6°; 69.0.

S_2-L-Phe-L-His-OMe: δ 1.1 (t, 3 H, CH_3 (S_2)), 3.05 (m, 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.6 (s, 3 H, OCH_3), 3.9 (q, 2 H, CH_2O), 4.45 (2 t (dist), 1 H, CH (Phe)), 4.8 (2 t (dist), 1 H, CH (His)), 5.9 (d, 1 H, NH (carbamate)), 6.6 and 7.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.5 (s, 5 H, Ph), 7.5 (d, 1 H, NH (amide)), 1.04 (s (br), 1 H, NH (Im)) ppm; −7.6°; 82.0.

The ^1H NMR spectra of the S_3, S_4, S_6, S_8, S_{10}, and S_{16} compounds are all very similar to the spectrum of S_{12}-L-Phe-L-His-Ome. $[\alpha]^{20}_D$: −8.4°, −8.2°, −8.1°, −8.0°, −7.6°, and −7.1°, respectively. Mp (°C): 80.7, 92.4, 101.0, 109.9, 114.4, and 124.1, respectively.

(Alkyloxycarbonyl)-L-(or D)-X-L-histidine (S_n-L- (or D)-X-L-His-OH), (Benzyloxycarbonyl)-L-phenylalanine-L-histidine, (Benzyloxycarbonyl)-L-alanyl-L-histidine, (2,3,4,5,6-Pentafluorobenzyloxycarbonyl)-L-phenylalanyl-L-histidine, (*p*-Bromobenzyloxycarbonyl)-L-phenylalanyl-L-histidine. Deprotection of the methyl esters was performed in aqueous ethanol using NaOH. The crude material was recrystallized from hot acetone. The yield after crystallization amounted to 65%. IR (KBr): 3300 (NH amide and carbamate), 1685 (C=O carbamate), 1650 cm^{-1} (C=O amide), unless noted otherwise. ^1H NMR (methanol-d_4); $[\alpha]^{20}_D$ (c = 1.0, methanol); mp, °C:

S_{12}-L-Ala-L-His-OH: δ 0.95 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 1.4 (d, 3 H, CH_3 (Ala)), 3.1 (d, 2 H, CH_2(His)), 3.95 (t, 2 H, CH_2O), 4.3 (t, 1 H, CH (Ala)), 4.5 (t, 1 H, CH (His)), 7.25 and 8.5 (2 s, 2 × 1 H, 2 CH (Im)) ppm; +17.3°; 148.3. Anal. Calcd for $C_{22}H_{38}N_4O_5$: C, 60.25; H, 8.73; N, 12.78; O, 18.24. Found: C, 60.35; H, 8.78; N, 12.78; O, 18.29.

S_{12}-L-But-L-His-OH (But = α-aminobutyric acid): δ 0.95 (t, 3 H, CH_3), 1.0 (t, 3 H, CH_3 (But)), 1.3 (m, 22 H, $(CH_2)_{10}$ and CH_2 (But)), 3.1 (d, (dist), 2 H, CH_2 (His)), 3.95 (t, 2 H, CH_2O); 4.2 (t, 1 H, CH (But)), 4.5 (t, 1 H, CH (His)), 7.25 and 8.5 (2 s, 2 × 1 H, 2 CH (Im)) ppm; +16.3°; 131.5. Anal. Calcd for $C_{23}H_{40}N_4O_5$: C, 61.04; H, 8.91; N, 12.38; O, 17.68. Found: C, 61.14; H, 8.90, N, 12.40; O, 17.65.

S_{12}-L-Norval-L-His-OH: δ 0.9 (2 t, 2 × 2 H, 2 CH_3), 1.3 (m, 24 H, $(CH_2)_{10}$ and $(CH_2)_2$ (Norval)), 3.1 (d, 2 H, CH_2 (His)), 4.0 (t, 2 H, CH_2O), 4.2 (t, 1 H, CH (Norval)), 4.5 (t, 1 H, CH (His)), 7.3 and 8.5 (2 s, 2 × 1 H, 2 CH (Im)) ppm; +16.4°; 136.9. Anal. Calcd for $C_{24}H_{42}N_4O_5$: C, 61.78; H, 9.07; N, 12.01; O, 17.14. Found: C, 61.81; H, 9.07; N, 12.10; O, 17.16.

S_{12}-L-Leu-L-His-OH: δ 0.9 (m, 9 H, 3 CH_3), 1.3 (m, 23 H, $(CH_2)_{10}$ and CH_2CH (Leu)); 3.1 (d (dist), 2 H, CH_2 (His)), 4.0 (t, 2 H, CH_2O), 4.15 (t, 1 H, CH (Leu)), 4.5 (t, 1 H, CH (His)), 7.25 and 8.5 (2 s, 2 × 1 H, 2 CH (Im)) ppm; +13.0°; 129.8. Anal. Calcd for $C_{25}H_{44}N_4O_5$: C, 62.47; H, 9.23; N, 11.66; O, 16.64. Found: C, 62.51; H, 9.23; N, 11.71; O, 16.68.

S_{12}-L-(or D)-Phe-L-His-OH: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 3.15 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 4.0 (t, 2 H, CH_2O), 4.45 (2 t, 2 × 1 H, CH (Phe) and CH (His)), 7.2 and 8.5 (2 s, 2 × 1 H, 2 CH (Im)), 7.25 (s, 5 H, Ph) ppm. L,L derivative: +17.8°; 146.1. D,L derivative: +24.7°; 143.5. Anal. Calcd for $C_{28}H_{42}N_4O_5$: C, 65.35; H, 8.22; N, 10.89; O, 15.54. Found (L,L): C, 65.31; H, 8.31, N, 10.92; O, 15.58; (D,L): C, 65.39; H, 8.23; N, 10.89; O, 15.61.

S_{12}-L-Trp-L-His-OH: δ 0.9 (t, 3 H, CH_3), 1.3 (m, 20 H, $(CH_2)_{10}$), 3.1 (2 d (dist), 2 × 2 H, CH_2 (Trp) and CH_2 (His)), 4.0 (t, 2 H, CH_2O), 4.5 (m, 2 H, CH (Trp) and CH (His)), 7.25 and 8.45 (2 s, 2 × 1 H, 2 CH (Im)), 6.8–7.8 (m, 5 H, 5 CH (indolyl)) ppm; +27.8°; 134.0. Anal. Calcd for $C_{30}H_{43}N_5O_5$: C, 65.08; H, 7.83; N, 12.65; O, 14.45. Found: C, 65.18; H, 7.75; N, 12.65; O, 14.51.

F_5Z-L-Phe-L-His-OH: δ 2.7–3.3 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 4.0–4.5 (2 t (dist), 2 × 1 H, CH (Phe) and CH (His)), 5.05 (s, 2 H, CH_2O), 7.18 (s, 5 H, Ph), 7.3 and 8.5 (2 s, 2 × 1 H, 2 CH (Im)) ppm; +12.6°; 187.0; IR (KBr) 1708 cm^{-1} (C=O carbamate). Anal. Calcd for $C_{23}H_{19}N_4O_5F_5$: C, 52.48; H, 3.64; N, 10.64; O, 15.20. Found: C, 52.38; H, 3.69; N, 10.69; O, 15.16.

Z-L-Phe-L-His-OH: δ 3.1 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 4.1–4.6 (2 t, 2 × 1 H, CH (Phe) and CH (His)), 4.95 (s, 2 H, CH_2O), 7.15 and 8.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.15 (s (br), 10 H, 2 Ph) ppm; +16.5°; 205.7. Anal. Calcd for $C_{23}H_{24}N_4O_5$: C, 63.29; H, 5.54; N, 12.84; O, 18.33. Found: C, 63.29; H, 5.59; N, 12.94; O, 18.39.

***p*-BrZ-L-Phe-L-His-OH**: δ 3.1 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 4.1–4.6 (m, 2 × 1 H, 2 CH), 5.0 (s, 2 H, CH_2O), 7.2 (s, 5 H, C_6H_5), 7.2 and 8.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.0 and 7.3 (2 d, 2 × 2 H, C_6H_4Br) ppm; +14.9°; 191.6. Anal. Calcd for $C_{23}H_{23}N_4O_5Br$: C, 53.59; H, 4.50; N, 10.87; O, 15.52; Br, 15.52. Found: C, 53.71; H, 4.52; N, 10.99; O, 15.49; Br, 15.62.

Z-L-Ala-L-His-OH: δ 1.45 (d, 3 H, CH_3), 3.1 (d (dist), 2 H, CH_2 (His)), 4.3 (t, 1 H, CH (Ala)), 4.5 (t, 1 H, CH (His)), 4.95 (s, 2 H, CH_2O), 7.2 and 8.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.2 (s, 5 H, Ph) ppm; +14.8°; 198.7. Anal. Calcd for $C_{17}H_{20}N_4O_5$: C, 56.67; H, 5.59; N, 15.55; O, 22.20. Found: C, 56.72; H, 5.59; N, 15.58; O, 22.12.

S_1-L-Phe-L-His-OH: δ 3.0 (m, 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.5 (s, 3 H, CH_3O), 4.0–4.7 (2 t (dist), 2 × 1 H, CH (Phe) and CH (His)), 7.2 and 8.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.2 (s, 5 H, Ph) ppm; +20.1°; 100.7. Anal. Calcd for $C_{17}H_{20}N_4O_5$: C, 56.67; H, 5.59; N, 15.55; O, 22.20. Found: C, 56.74; H, 5.62; N, 15.45; O, 22.20.

S_2-L-Phe-L-His-OH: δ 1.15 (t, 3 H, CH_3), 2.7–3.3 (m, 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.95 (q, 2 H, CH_2O), 4.15–4.55 (m, 2 H, CH (Phe) and CH (His)), 7.1 and 8.35 (2 s, 2 × 1 H, 2 CH (Im)), 7.2 (s, 5 H, Ph) ppm; +21.0°; 104.9. Anal. Calcd for $C_{18}H_{22}N_4O_5$: C, 57.75; H, 5.92; N, 14.96; O, 21.37. Found: C, 57.79; H, 5.99; N, 14.86; O, 21.38.

The ^1H NMR spectra of the S_3, S_4, S_6, S_8, S_{10}, and S_{16} compounds were all very similar to the spectrum of S_{12}-L-Phe-L-His-OH. $[\alpha]^{20}_D$: +19.9°, +19.7°, +18.5°, +18.5°, +18.0°, +15.9°, respectively. Mp (°C): 110.1, 127.9, 135.9, 138.3, 141.2, 151.0, respectively. S_3: Anal. Calcd for $C_{19}H_{24}N_4O_5$: C, 58.75; H, 6.23; N, 14.42; O, 20.60. Found: C, 58.85; H, 6.29; N, 14.48; O, 20.74. S_4: Anal. Calcd for $C_{20}H_{26}N_4O_5$: C, 59.69; H, 6.51; N, 13.92; O, 19.88. Found: C, 59.78; H, 6.61; N, 13.82; O, 19.99. S_6: Anal. Calcd for $C_{22}H_{30}N_4O_5$: C, 61.38; H, 7.02; N, 13.01; O, 18.58. Found: C, 61.41; H, 7.22; N, 13.00; O, 18.49. S_8: Anal. Calcd for $C_{24}H_{34}N_4O_5$: C, 62.86; H, 7.47; N, 12.22; O, 17.45. Found: C, 62.71; H, 7.57; N, 12.22; O, 17.66. S_{10}: Anal. Calcd for $C_{26}H_{38}N_4O_5$: C, 64.18; H, 7.87; N, 11.51; O, 16.44. Found: C, 64.31; H, 7.92; N, 11.30; O, 16.46. S_{16}: Anal. Calcd for $C_{32}H_{50}N_4O_5$: C, 67.34; H, 8.83; N, 9.82; O, 14.02. Found: C, 67.19; H, 8.83; N, 9.89; O, 13.89.

L-Phenylalanyl-L-histidine Methyl Ester Dihydrobromide (H-L-Phe-L-His-OMe·2HBr). (Benzyloxycarbonyl)-L-phenylalanyl-L-histidine methyl ester was deprotected with HBr in glacial acetic acid according to a literature method.[14] This product was used without further purification. The yield amounted to 98%. IR (KBr): 1740 (C=O ester), 1680 cm^{-1} (C=O amide). ^1H NMR (methanol-d_4): δ 3.1–3.6 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.75 (s, 3 H, OCH_3), 4.4 (t, 1 H, CH (Phe)), 4.8 (t, 1 H, CH (His)), 7.3 (s, 5 H, Ph), 7.4 and 8.75 (2 s, 2 × 1 H, 2 CH (Im)) ppm.

***N*-Dodecanoyl-L-phenylalanyl-L-histidine Methyl Ester (C_{12}-L-Phe-L-His-OMe) and *N*-Acetyl-L-phenylalanyl-L-histidine Methyl Ester (C_1-L-Phe-L-His-OMe).** H-L-Phe-L-His-OMe·2HBr was acylated with dodecanoic anhydride or acetic anhydride according to a literature method.[15] The crude product was dissolved in 50 mL of methanol and stirred overnight at 20 °C. The solvent was evaporated, and the crude product was subjected to column chromatography (Silica 60; chloroform–methanol, 9/1, v/v). The product was recrystallized from ethyl acetate–petroleum ether. The final yield amounted to 78%. C_{12}-L-Phe-L-His-OMe: $[\alpha]^{20}_D$ −7.9° (c = 1.0, methanol). Mp: 120.3 °C. IR (KBr): 3300 (NH amide), 1734 (C=O ester), 1650 cm^{-1} (C=O amide). TLC (Silica): R_f = 0.45 (chloroform–methanol, 4/1, v/v). ^1H NMR (chloroform-d_1): δ 0.9 (t, 3 H, CH_3), 1.3 (m, 18 H, $(CH_2)_9$), 2.2 (t, 2 H, CH_2CO), 3.1 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 3.65 (s, 3 H, OCH_3), 4.3–5.0 (m, 2 × 1 H, CH (Phe) and CH (His)), 6.75 and 7.55 (2 s, 2 × 1 H, 2 CH (Im)), 7.2 (s, 5 H, Ph), 7.7 (2 d, 2 × 1 H, 2 NH (amide)), 8.9 (s (br), 1 H, NH (Im)) ppm.

C_1-L-Phe-L-His-OMe: $[\alpha]^{20}_D$ −9.1° (c = 1.0, methanol). Mp: 88.3 °C. ^1H NMR (methanol-d_4): δ 2.0 (s, 3 H, CH_3), 3.1 (m, 4 H, CH_2 (Phe) and CH_2 (His)), 3.7 (s, 3 H, OCH_3), 4.3–4.9 (2 t (dist), 2 × 1 H, 2 CH), 6.7 and 7.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.15 (s, 5 H, C_6H_5) ppm.

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***N*-Dodecanoyl-L-phenylalanyl-L-histidine (C_{12}-L-Phe-L-His-OH) and *N*-Acetyl-L-phenylalanyl-L-histidine (C_1-L-Phe-L-His-OH).** The methyl esters were deprotected and purified as described for the (dodecyloxycarbonyl)-L-phenylalanyl-L-histidine methyl ester. The yield amounted to 68%.

C_{12}-L-Phe-L-His-OH: $[\alpha]^{20}_D$ +18.5° (c = 1.0, methanol). TLC (Silica): R_f = 0.3 (ethanol). Mp: 149.9 °C. IR (KBr): 3300 (NH amide), 1650 cm^{-1} (C=O amide). ^1H NMR (methanol-d_4): δ 0.9 (t, 3 H, CH_3), 1.3 (m, 18 H, $(CH_2)_9$), 2.15 (t, 2 H, CH_2O), 2.7–3.3 (2 d (dist), 2 × 2 H, CH_2 (Phe) and CH_2 (His)), 4.1–4.8 (2 t (dist), 2 × 1 H, CH (Phe) and CH (His)), 7.2 and 8.3 (2 s, 2 × 1 H, 2 CH (Im)), 7.2 (s, 5 H, Ph) ppm. Anal. Calcd for $C_{27}H_{40}N_4O_4$: C, 66.92; H, 8.32; N, 11.56; O, 13.21. Found: C, 67.05; H, 8.34; N, 11.66.

C_1-L-Phe-L-His-OH: $[\alpha]^{20}_D$ +34.4° (c = 1.0, methanol). Mp: 240 °C dec. ^1H NMR (methanol-d_4): δ 2.1 (s, 3 H, CH_3), 3.2 (2 d (dist), 2 × 2 H, 2 CH_2), 4.4–4.9 (2 t (dist), 2 × 1 H, 2 CH), 7.3 and 8.4 (2 s, 2 × 1 H, 2 CH (Im)), 7.25 (s, 5 H, C_6H_4) ppm. Anal. Calcd for $C_{17}H_{20}N_4O_4$: C, 59.29; H, 5.85; N, 16.27; O, 18.58. Found: C, 59.01; H, 5.96; N, 16.02; O, 18.81.

***N*-Dodecanoyl-L-(or D)-phenylalanine 4-Nitrophenyl Ester (C_{12}-L-(or D)-Phe-ONP).** These compounds were prepared by deprotection of the Z-protected compounds[14] and subsequent acylation.[15] The final yield amounted to 45%.

C_{12}-L-Phe-ONP: $[\alpha]^{20}_D$ = 10.8° (c = 1.0, chloroform); mp 108.4 °C. Anal. Calcd for $C_{27}H_{36}N_2O_5$: C, 69.21; H, 7.74; N, 5.98. Found: C, 69.31; H, 7.84; N, 5.99.

C_{12}-D-Phe-ONP: $[\alpha]^{20}_D$ +10.7° (c = 1.0, chloroform); mp 108.6 °C. Anal. Found: C, 69.33; H, 7.78; N, 5.98. IR (KBr): 3320 (NH amide); 2920 (CH stretch), 1755 (C=O ester), 1635 (C=O amide), 1525 and 1490 (NO_2 stretch assym); 1347 cm^{-1} (NO_2 stretch sym). ^1H NMR ($CDCl_3$): δ 0.9 (t, 3 H, CH_3), 1.3 (m, 18 H, $(CH_2)_9$), 2.2 (t, 2 H, CH_2O), 3.2 (d, 2 H, CH_2Ph), 5.0 (2 t, 1 H, CH), 5.8 (d, 1 H, NH), 7.0 and 8.1 (2 d, 2 × 2 H, $C_6H_4NO_2$), 7.1 (m, 5 H, C_6H_5) ppm.

***(R)*- and *(S)*-*N*,*N*-Dimethyl-*N*-hexadecyl-*N*-(α-methylbenzyl)ammonium Bromide (*R*- and *S*-Surf 16).** These compounds were prepared according to a method described in literature.[16] The final yield after crystallization amounted to 44%. *R* compound: mp 112.0 °C; $[\alpha]^{20}_D$ +19.9° (c = 0.5, methanol). Anal. Calcd for $C_{26}H_{48}NBr$: C, 68.70; H, 10.64; N, 3.22; Br, 17.58. Found: C, 68.78; H, 10.62; N, 3.19; Br, 17.69. *S* compound: mp 112.3 °C; $[\alpha]^{20}_D$ −19.7° (c = 0.5, methanol). Anal. Found: C, 68.72; H, 10.66; N, 3.20; Br, 17.70. ^1H NMR ($CDCl_3$): δ 0.9 (t, 3 H, CH_3), 1.25 (m, 28 H, $(CH_2)_{14}$), 1.85 (d, 3 H, CH_3), 3.2 (2 s, 2 × 3 H, 2 NCH_3), 3.5 (t, 2 H, CH_2N), 5.4 (q, 1 H, CH), 7.2 (m, 5 H, C_6H_5) ppm.

Preparation of the Surfactant Solutions. Tris(hydroxymethyl)aminomethane (Baker) and potassium chloride (Baker) were dissolved in double distilled water. The concentrations amounted to 0.08 and 0.40 M, respectively. The pH was adjusted to 7.3 with the aid of concentrated HCl (Merck). The surfactant was dissolved in this buffer to a concentration of 2.5 or 5.0 × 10^{-3} M. The buffered surfactant solution was divided into two parts. One part was used to obtain the blank reaction rates in the absence of catalyst. To the other part the finely powdered catalyst was added (5 × 10^{-5} M) and the solution was stirred for 2 h at 40 °C in a sealed flask. The thus obtained solution was used to determine the reaction rates in the presence of catalyst. It was checked separately that sonication of the buffered catalyst-surfactant solutions had no effect on the observed rates. Therefore, sonication was ommited.

Kinetic Measurements. A UV cell containing 2.9 mL of the buffered surfactant solution (with or without catalyst) was placed in a UV/vis spectrophotometer thermostated at 25 °C. The reaction was started upon the injection of the substrate, which was dissolved in 0.1 mL of acetonitrile (Baker). The final substrate concentration amounted to 1.0 × 10^{-5} M. The release of the 4-nitrophenolate ion was followed by the increase in absorption at 400 nm. The rate data obeyed a pseudo-first-order rate profile, and the rate constants were calculated as the average value obtained from four repeated runs. The second-order rate constants, $k_{a,obs}$, were obtained with the aid of the following formula: $k_{a,obs} = k_m - k_{bl}$, in which k_m is the second-order rate constant measured in the presence of catalyst and k_{bl} is the second-order rate constant measured in its absence (blank or background reaction). The estimated error in $k_{a,obs}$ amounts to 5%.

Registry No. $S_{12}Cl$, 24460-74-0; ZCl, 501-53-1; *p*-BrZCl, 5798-78-7; F_5ZCl, 53526-74-2; S_1Cl, 79-22-1; S_2Cl, 541-41-3; Ala, 56-41-7; But, 1492-24-6; Nva, 6600-40-4; Leu, 61-90-5; Phe, 63-91-2; Trp, 73-22-3; S_{12}-Ala-OH, 133230-62-3; S_{12}-But-OH, 133230-63-4; S_{12}-Nva-OH, 133230-64-5; S_{12}-Leu-OH, 133230-65-6; S_{12}-Phe-OH, 133230-66-7; S_{12}-Trp-OH, 133230-67-8; Z-Phe-OH, 1161-13-3; *p*-BrZ-Phe-OH, 40297-81-2; F_5Z-Phe-OH, 133230-68-9; Z-Ala-OH, 1142-20-7; S_1-Phe-OH, 41844-91-1; S_2-Phe-OH, 19887-32-2; His-OMe·2HCl, 7389-87-9; S_{12}-Ala-His-OME, 133230-69-0; S_{12}-But-His-OMe, 133230-70-3; S_{22}-Nva-His-OMe, 133230-71-4; S_{12}-Leu-His-OMe, 133230-72-5; S_{12}-Phe-His-OMe, 133230-73-6; S_{12}-D-Phe-His-OMe, 133230-74-7; S_{12}-Trp-His-OMe, 133230-75-8; Z-Phe-His-OMe, 16689-13-7; F_5Z-Phe-His-OMe, 133230-76-9; *p*-BrZ-Phe-His-OMe, 133230-77-0; Z-Ala-His-OMe, 32303-82-5; S_1-Phe-His-OMe, 133230-78-1; S_2-Phe-His-OMe, 133230-79-2; S_{12}-Ala-His-OH, 133230-80-5; S_{12}-But-His-OH, 133230-81-6; S_{22}-Nva-His-OH, 133230-82-7; S_{12}-Leu-His-OH, 133230-83-8; S_{12}-Phe-His-OH, 133230-84-9; S_{12}-D-Phe-His-OH, 133230-85-0; S_{12}-Trp-His-OH, 133230-86-1; F_5Z-Phe-His-OH, 133230-87-2; Z-Phe-His-OH, 20806-38-6; *p*-Brz-Phe-His-OH, 133230-88-3; Z-Ala-His-OH, 79458-92-7; S_1-Phe-His-OH, 133230-89-4; S_2-Phe-His-OH, 114346-47-3; Phe-His-OMe·2HBr, 18779-16-3; $(H_3C(CH_2)_{10}CO)_2O$, 645-66-9; C_{12}-Phe-His-OMe, 79416-20-9; C_1-Phe-His-OMe, 133230-90-7; C_{12}-Phe-His-OH, 90703-14-3; C_1-Phe-His-OH, 123580-40-5; C_{12}-Phe-ONP, 75531-11-2; C_{12}-D-Phe-ONP, 75531-12-3; *R*-surf 16, 50640-92-1; *S*-surf 16, 50640-91-0; S_3-Phe-OH, 85590-63-2; S_4-Phe-OH, 75048-11-2; S_6-Phe-OH', 133230-91-8; S_8-Phe-OH, 133230-92-9; S_{10}-Phe-OH, 133230-93-0; S_{16}-Phe-OH, 133230-94-1; S_3-Phe-His-OMe, 133230-95-2; S_4-Phe-His-OMe, 133230-96-3; S_6-Phe-His-OMe, 133230-97-4; S_8-Phe-His-OMe, 133230-98-5; S_{10}-Phe-His-OMe, 133230-99-6; S_{16}-Phe-His-OMe, 133231-00-2; S_3-Phe-His-OH, 133231-01-3; S_4-Phe-His-OH, 133231-02-4; S_6-Phe-His-OH, 133231-03-5; S_8-Phe-His-OH, 133231-04-6; S_{10}-Phe-His-OH, 133231-05-7; S_{16}-Phe-His-OH, 133231-06-8; Z-Phe-ONP, 2578-84-9; Z-D-Phe-ONP, 2578-85-0.

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