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Full Papers

Mechanism of enantioselective ester cleavage by histidine-containing peptides at a micellar interface. 2. Effect of changing peptide chain length[1]

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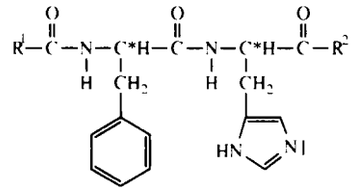
Abstract. Chiral *p*-nitrophenyl esters derived from the amino acid phenylalanine are cleaved by various histidine-containing tripeptides and higher oligopeptides as catalysts at a micellar interface. It is assumed that the oligopeptides adopt an internally hydrogen-bonded C_7 conformation when they dissolve into the micellar hydrocarbon phase. Chiral recognition is attributed to the formation of a hydrogen bond in the micellar hydrocarbon phase between one enantiomer of the ester and the peptide. It is important for the activity that the imidazolyl NH moiety of the His residue remains in the aqueous phase. The hydrophilic/hydrophobic balance, which determines the location of each of the amino acid residues of the peptide in the two-phase system, can be controlled by varying the length and stereochemistry of the peptide chain and by attaching hydrophobic groups. The most selective catalyst is a tripeptide with the structure $C_4H_9OC(O)$-L-Phe-L-His-L-Leu. It cleaves the *p*-nitrophenyl esters of N-protected L- and D-phenylalanine with an enantioselective of $k_L/k_D \approx 40$. Further lengthening of the peptide chain with L-Leu and L-Ala residues and lengthening of the N-protecting group of the catalyst decreases the enantioselectivity down to $k_L/k_D \approx 6$ for the pentapeptide $C_{12}H_{25}OC(O)$-L-Phe-L-His-(L-Leu)$_3$. The origin of these effects is discussed.

Introduction

Reactions in aqueous surfactant solutions are the subject of an increasing number of papers[2-12]. The kinetics of these reactions resemble to some extent the kinetics of enzyme-catalyzed reactions. Recently, high (enantio-)selectivities, characteristic of enzyme-controlled reactions, have been described in a number of papers on micellar-catalyzed reactions. These papers deal with the cleavage of chiral amino acid *p*-nitrophenyl esters by the imidazolyl moiety of histidine-containing oligopeptides[2-12]. Until recently, the origin of the observed enantioselectivities was not understood. In our previous paper we presented a detailed model that explains these phenomena[1]. In the present paper, this model is refined and chiral recognition is improved.

In aqueous solution, the imidazolyl moiety is an active catalyst in the hydrolysis of *p*-nitrophenyl esters. In the first step of this hydrolysis, the imidazolyl group is acylated. In the second, relatively slow step, the acylated imidazolyl intermediate is hydrolyzed and the free imidazolyl moiety is regenerated[1]. Upon increasing the substrate concentration, typical Michaelis–Menten kinetics are observed. At high substrate concentrations, the catalyst is completely acylated and the reaction rate is independent of substrate concentration.

This paper describes the esterolytic activity and enantioselectivity of a number of imidazolyl-containing oligopeptide derivatives dissolved in an aqueous micellar solution. These peptides are listed in Chart 1, including their abbreviations. The nomenclature and conventions used follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature[13,14]. The imidazolyl moiety is part of the chiral amino acid residue L-histidine at the 2-position of the oligopeptide. In our previous paper[1], we showed that, for a number of histidine-containing dipeptides, the highest enantioselectivity was obtained with L-phenylalanine in position 1. Consequently, in the higher oligopeptides that are presented here, this position 1 is always occupied by this amino acid residue. The C-terminal end is unprotected, which implies that, under the pH condition used (pH 7.3), the carboxyl moiety is deprotonated and in its anionic form. The peptide chain of the L-Phe-L-His derivative is extended up to a pentapeptide using various L- and D-amino acid residues and the effect of these residues on the esterolytic activity and enantioselectivity was studied. The N-protecting group (R^1CO) of the peptide chain is of the alkyloxycarbonyl type, which proved to be the most effective protecting group[1]. The surfactant used is the chiral cationic surfactant (*R*)-*N*-hexadecyl-*N*,*N*-dimethyl-(α-methylbenzyl)ammonium bromide (*R*-surf 16, see



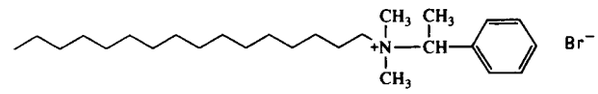
R^1	R^2	Abbreviation
$CH_3(CH_2)_{11}O$-	OH	S_{12}-L-Phe-L-His
"	NH-CH_2-COOH	S_{12}-L-Phe-L-His-Gly
"	NH-$CHCH_3$-COOH	S_{12}-L-Phe-L-His-L-Ala
"	NH-$CH(CH_2CH_3)$-COOH	S_{12}-L-Phe-L-His-L-But
"	NH-$CH(CH_2CH_2CH_3)$-COOH	S_{12}-L-Phe-L-His-L-Norval
"	NH-$CH(CH_2CH(CH_3)_2)$-COOH	S_{12}-L-Phe-L-His-L(or D)-Leu
"	NH-$CH(CH_2C_6H_5)$-COOH	S_{12}-L-Phe-L-His-L-Phe
"	NH-$CH(CH_2C_8H_6N)$-COOH	S_{12}-L-Phe-L-His-L-Trp
"	NH-$CH(CHCH_3OH)$-COOH	S_{12}-L-Phe-L-His-L-Thr
"	N-$CH(CH_2CH_2CH_2)$-COOH	S_{12}-L-Phe-L-His-L-Pro
"	NH-$CH(CH(CH_3)CH_2CH_3)$-COOH	S_{12}-L-Phe-L-His-L-Ileu
"	NH-$CH(C_6H_5)$-COOH	S_{12}-L-Phe-L-His-L-Phegly
"	NH-$CH(CH_2COOH)$-COOH	S_{12}-L-Phe-L-His-L-Asp
CH_3CH_2O-	NH-$CH(CH_2CH(CH_3)_2)$-COOH	S_2-L-Phe-L-His-L-Leu
"	NH-$CHCH_3$-COOH	S_2-L-Phe-L-His-L-Ala
$CH_3(CH_2)_3O$-	NH-$CH(CH_2CH(CH_3)_2)$-COOH	S_4-L-Phe-L-His-L-Leu
"	NH-$CHCH_3$-COOH	S_4-L-Phe-L-His-L-Ala
$CH_3(CH_2)_5O$-	NH-$CH(CH_2CH(CH_3)_2)$-COOH	S_6-L-Phe-L-His-L-Leu
"	NH-$CHCH_3$-COOH	S_6-L-Phe-L-His-L-Ala
$C_6H_5CH_2O$-	NH-$CH(CH_2CH(CH_3)_2)$-COOH	Z-L-Phe-L-His-L-Leu
$CH_3(CH_2)_{11}O$-	[NH-$CH(CH_2CH(CH_3)_2$-CO-]$_2$-OH	S_{12}-L-Phe-L-His-L-Leu-L (or D)-Leu
"	NH-$CHCH_3$CONHCH($CH_2CH(CH_3)_2$)-COOH	S_{12}-L-Phe-L-His-L-Ala-L-Leu
"	NH-CH_2-CONH-$CH(CH_2CH(CH_3)_2)$-COOH	S_{12}-L-Phe-L-His-Gly-L-Leu
"	NH-$CH(CH_2CH(CH_3)_2)$-CONH-$CHCH_3$-COOH	S_{12}-L-Phe-L-His-L-Leu-L-Ala
"	[NH-$CHCH_3$-CO-]$_2$-OH	S_{12}-L-Phe-L-His-L-Ala-L (or D)-Ala
"	[NH-$CH(CH_2CH(CH_3)_2)$-CO-]$_3$-OH	S_{12}-L-Phe-L-His-(L-Leu)$_3$
"	[NH-$CHCH_3$-CO-]$_3$-OH	S_{12}-L-Phe-L-His-(L-Ala)$_3$
$CH_3(CH_2)_{11}O$-	NH-CH_3	S_{12}-L-Phe-L-His-*N*MA
$CH_3(CH_2)_3O$-	NH-CH_3	S_4-L-Phe-L-His-*N*MA

Chart 1. Catalysts.

Chart 2). This surfactant was chosen to study the effect of a chiral centre in the surfactant headgroup on the enantioselectivity of the esterolytic reaction. The chiral substrates, which are shown in Chart 3, are *N*-acyl *p*-nitrophenyl esters of the amino acid L- or D-phenylalanine (C_n-Phe-ONp).

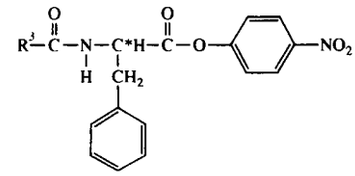
It is our aim to understand the cause of the observed enantioselectivity and to rationalize the effects on reactivity and selectivity upon structural changes in catalyst and/or substrate. Attention is, therefore, focussed on the first step in the catalytic cycle, *i.e.*, the acylation of the imidazolyl moiety. In this step, the enantioselectivity is expressed as the ratio of the rate constants obtained for this acylation step using L and D substrates: k_L/k_D. The deacylation step of the acylated imidazolyl moiety, regenerating the free imidazolyl group was not investigated.

Special attention was paid to the blank (background) reaction. It is generally assumed that the blank reaction in the presence and absence of the catalyst is identical. It will be shown that this assumption is valid only under certain conditions. If these conditions are not met, large errors can occur.



R (or *S*) - surf 16

Chart 2. Surfactant



C_n-L (or D) -Phe-ONP: $R^3 = CH_3(CH_2)_{n-2}$— ; (n = 2,4,7,12,16)

Chart 3. Substrates

Results

Catalytic activity and enantioselectivity of S_{12}-L-Phe-L-His-L-(or D-) X

In Table I, the catalyc activities and enantioselectivity of tripeptide catalysts towards C_{12}-Phe-ONp (L and D) in the presence of the surfactant *R*-surf 16 are listed. For comparison, the data of the dipeptide catalysts S_{12}-L-Phe-L-His and S_{12}-L-Ala-L-His are also included[1]. In general, introducing a third amino acid residue at the 3-position of the catalyst appears to have little effect on the observed selectivity. An exception is S_{12}-L-Phe-L-His-L-Leu (entry 7) which exhibits an enhanced selectivity of $k_L/k_D = 23$ when compared to the corresponding dipeptide catalyst (k_L/k_D 16.6). Increase in hydrophobicity and size of the amino acid residue in the 3-position has no marked effect, up to a certain limit (entries 3–7). However, with the very bulky Phe residue, the

rate and selectivity are considerably suppressed (entry 8) as if a critical point is passed. The catalysts with the very hydrophobic Ile and Phg residues in the 3-position, whose side chains are both branched at C^{β}, also exhibit reduced activity and enantioselectivity (entries 9 and 10). However, with the Trp residue in the 3-position, which is the largest residue tested, the rates and enantioselectivity are not affected to any large extent (entry 11). Neither a proline residue, whose side chain is covalently attached to the amide nitrogen atom of the peptide backbone, nor a hydrophilic threonyl residue has a large effect on the observed selectivity and activity (entries 12 and 13). From the results in Table I, it can be concluded that the reactivity of the imidazolyl group is reduced upon adding a third amino acid residue. The smallest reduction is observed for the S_{12}-L-Phe-L-His-L-Asp catalyst, which has two unprotected COO moieties in the 3-position. The activity of this catalyst is by far the highest of all tripeptide catalysts examined here, although, its selectivity is somewhat reduced. It seems that the presence of a carboxylate moiety in close proximity to the imidazolyl group promotes the activity of the latter. Changing the chirality of the Leu residue in the 3-position from L to D causes a dramatic drop in selectivity from 23 to 1.9 (entries 7 and 15). This loss of selectivity is the result of a six-fold reduction in activity towards the L substrate and a two-fold increase in activity towards the D substrate, *i.e.*, changing L-Leu into D-Leu has an opposite effect on the rates towards both enantiomers. Interestingly, the presence of the achiral amino acid residue Gly in the 3-position does not reduce the selectivity (compare entries 3, 7 and 15). The chirality of the surfactant molecules has no significant efffect on the rates and enantioselectivity (entries 7 and 16), a fact that was also observed previously for a number of dipeptide catalysts[1]. This means that no specific interactions (*e.g.*, π stacking of aromatic rings) exists between these surfactant molecules and the catalysts or substrate, resulting in chiral recognition. This can also be concluded from the fact that the enantioselective profile of the esterolytic reaction does not change when *R*-surf 16 is substituted by the surfactant cetyltributylammonium bromide[1]. The only apparent of the surfactant molecules is to form micelles that provide an interface at which the reaction can occur.

Table I Rate constants $[k_{a,obs}/(dm^3 \cdot mol^{-1} \cdot s^{-1})]$ *and enantioselectivity* (k_L/k_D) *in the cleavage of* C_{12}*-Phe-ONp (L and D) by comicelles of* S_{12}*-L-Phe-L-His-L- (or D-) X and* R*-surf 16*[a].

Catalyst	k_L	k_D	k_L/k_D
1: S_{12}-L-Ala-L-His	693	191	3.6
2: S_{12}-L-Phe-L-His	6012	363	16.6
3: S_{12}-L-Phe-L-His-Gly	2605	157	16.6
4: S_{12}-L-Phe-L-His-L-Ala	2420	145	16.7
5: S_{12}-L-Phe-L-His-L-Abu	2134	134	15.9
6: S_{12}-L-Phe-L-His-L-Nva	2224	136	16.4
7: S_{12}-L-Phe-L-His-L-Leu	2319	100	23.2
8: S_{12}-L-Phe-L-His-L-Phe	948	89	10.7
9: S_{12}-L-Phe-L-His-L-Ile	535	66	8.1
10: S_{12}-L-Phe-L-His-L-Phg	516	64	8.1
11: S_{12}-L-Phe-L-His-L-Trp	1866	102	18.3
12: S_{12}-L-Phe-L-His-L-Pro	1973	130	15.2
13: S_{12}-L-Phe-L-His-L-Thr	2002	129	15.5
14: S_{12}-L-Phe-L-His-L-Asp	3295	325	10.1
15: S_{12}-L-Phe-L-His-L-Leu	417	224	1.9
16: S_{12}-L-Phe-L-His-L-Leu[b]	2490	105	23.7

[a] Conditions: 0.08M Tris/HCl and 0.40M KCl; pH 7.3; *T* 25°C; solvent, acetonitrile/water, 3:97 (v/v); c_{cat} $5 \cdot 10^{-5}$M; c_{ester} $1 \cdot 10^{-5}$M; c_{surf} $2.5 \cdot 10^{-3}$M. [b] *S*-surf 16.

Fluorescence measurement on the S_{12}*-L-Phe-L-His-L-Trp catalyst*

Fluorescence measurements on the S_{12}-L-Phe-L-His-L-Trp catalyst under these reaction conditions show that the Trp side chain is in a relatively apolar environment. At an excitation wavelength of λ_{ex} 286 nm, the fluorescence emission maximum of the Trp side chain appears at λ_{em} 352 nm. This position is also observed in a 1,4-dioxane/water mixture of 55:45 (v/v), demonstrating that the degree of hydration of the indolyl moiety in this catalyst at the micellar interface is similar, *i.e.*, it is about 40–50% dehydrated. The same conclusion was derived from fluorescence measurements on the dipeptide catalyst S_{12}-L-Trp-L-His, *i.e.*, the indolyl group is partially dehydrated but is still in contact with bulk water[1]. Higher surfactant concentrations do not cause a further blue shift in the position of the emission maximum indicating that this catalyst is completely micellar-bound at the applied surfactant concentration[1].

Catalytic activity of S_n*-L-Phe-L-His-L-Leu and* S_n*-L-Phe-L-His-L-Ala catalysts*

In this section, the length, *n*, of the hydrocarbon chain of the S_n-L-Phe-L-His-L-Leu catalyst is varied from $n = 2$ to 12. The results obtained with the substrate C_{12}-Phe-ONP (L and D) are listed in Table II. In this Table, the data for the *N*-benzyloxycabonyl- (*Z*-) protected catalyst are also included. Upon increasing the length and thus the hydrophobicity of the N-protecting group from $n = 2$ to 12, the reaction rate strongly increases. This enhanced reactivity reflects the fact that an increasing fraction of the catalyst is bound to the micelle where the substrate resides. The enantioselectivity increases from S_2 to S_4 where it reaches a maximum ($k_L/k_D = 38.1$) and decreases upon further chain lengthening.

Table II Rate constants $[k_{a,obs}/(dm^3 \cdot mol^{-1} \cdot s^{-1})]$ *and enantioselectivity* (k_L/k_D) *in the cleavage of* C_{12}*-Phe-ONp (L and D) by comicelles of* S_n*-L-Phe-L-His-L-Leu* (n = 2, 4, 6, 12) *and* R*-surf 16*[a].

Catalyst	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$ (kcal/mol)
S_2-L-Phe-L-His-L-Leu	157	15	10.5	1.39
S_4-L-Phe-L-His-L-Leu	1257	33	38.1	2.16
S_6-L-Phe-L-His-L-Leu	1544	59	26.2	1.93
S_{12}-L-Phe-L-His-L-Leu	2319	100	23.2	1.86
Z-L-Phe-L-His-L-Leu	1284	32	40.1	2.19

[a] Conditions as described in Table I.

The activities and selectivity of Z- and S_4-protected L-Phe-L-His-L-Leu catalysts do not differ significantly. Such behaviour was also observed for Z-L-Phe-L-His and S_4-L-Phe-L-His dipeptide catalysts[1]. This indicates that the R^1 moieties butyl and benzyl are sterically and hydrophobically very similar in the present reaction[15].

Table III shows that a bell-shaped selectivity dependence on *n* is also observed for S_n-L-Phe-L-His-L-Ala ($n = 2, 4, 6, 12$). However, the enantioselectivity is approximately 25–30% lower than for the S_n-L-Phe-L-His-L-Leu catalysts. Although not investigated, it is likely that such bell-shaped selectivity curves will also be found for the other tripeptide catalysts listed in Table I. We reported[1] previously a similar but smaller effect in the S_n-L-Phe-L-His series, with a maximum of $k_L/k_D = 18.3$ at $n = 3$. This means that the relatively small effect on the selectivity observed when the catalyst is extended with a third amino residue (Table I) is rather fortuitous and probably only occurs in the S_{12}-

-protected series. In the S_4 series, the enantioselectivcity considerably increases from 18 to 38 by adding L-Leu as a third residue.

Table III Rate constants [$k_{a,obs}$/(dm^3 · mol^{-1} · s^{-1})] and enantioselectivity (k_L/k_D) in the cleavage of C_{12}-Phe-ONp (L and D) by comicelles of C_n-L-Phe-L-His-L-Ala (n = 2, 4, 6, 12) and R-*surf 16*[a].

Catalyst	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$ (kcal/mol)
S_2-L-Phe-L-His-L-Ala	128	14	9.1	1.31
S_4-L-Phe-L-His-L-Ala	1190	46	25.9	1.93
S_6-L-Phe-L-His-L-Ala	1749	89	19.7	1.76
S_{12}-L-Phe-L-His-L-Ala	2420	145	16.7	1.67

[a] Conditions as described in Table I.

Catalytic activity of S_{12}-L-Phe-L-His-L-Leu towards C_n-Phe-ONp

Table IV shows the activities and enantioselectivity obtained with the S_{12}-L-Phe-L-His-L-Leu catalyst towards the C_n-Phe-ONp substrates (n = 2, 4, 7, 12, 16). Increasing the length of the acyl chain increases both the activity and selectivity. The enhanced activity will be due to increased binding of the substrate to the micelle. Complete binding is achieved at approximately n = 12. The applied catalyst, S_{12}-L-Phe-L-His-L-Leu, is also completely bound to the micelle. Upon increasing n in the substrate, the selectivity approaches a limiting value of k_L/k_D = 23 at n = 12. A different effect on selectivity was observed for the S_n chain in the catalyst, which displays a clear optimum at S_4.

Table IV Rate constants [$k_{a,obs}$/(dm^3 · mol^{-1} · s^{-1})] and enantioselectivity (k_L/k_D) in the cleavage of C_n-L- (and D-) Phe-ONp (n = 2, 4, 7, 12, 16) by comicelles of S_{12}-L-Phe-L-His-L-Leu and R-*surf 16*[a].

Ester	k_L	k_D	k_L/k_D	$\Delta\Delta G^{\neq}$ (kcal/mol)
C_2-Phe-ONp	328	43	7.6	1.20
C_4-Phe-ONp	669	75	8.9	1.29
C_7-Phe-ONp	1559	90	17.3	1.69
C_{12}-Phe-ONp	2319	100	23.2	1.86
C_{16}-Phe-ONp	2320	106	21.9	1.83

[a] Conditions as described in Table I.

Activity and enantioselectivity of tetra- and pentapeptide catalysts

Table V shows how the activity and enantioselectivity of the esterolytic reaction is affected when the peptide chain of the catalyst is further extended with L and/or D residues. For comparison, the data of the corresponding di- and tripeptide catalysts are also included. Increasing the peptide chain length of the S_{12}-L-Phe-L-His catalyst with L-Ala or L-Leu residues causes a dramatic drop in activity. The effect is considerably larger for the more hydrophobic L-Leu residue (entries 1–4) than for the smaller L-Ala residue (entries 1, 5–7). The enantioselectivity remains unaffected up to the tripeptides or even slightly increases (for the S_{12}-L-Phe-L-Leu catalyst), as noted above. Upon further chain lengthening, the enantioselectivity also rapidly decreases.
The tetrapeptide catalysts with L-Leu-L-Ala and L-Ala-L-Leu in the 3- and 4-positions (entries 8 and 9) display intermediate activities and enantioselectivity in comparison

Table V Rate constants [$k_{a,obs}$/(dm^3 · mol^{-1} · s^{-1})] and enantioselectivity (k_L/k_D) in the cleavage of C_{12}-Phe-ONp (L and D) by comicelles of His-containing oligopeptides and R-*surf 16*[a].

Catalyst	k_L	l_D	k_L/k_D
1: S_{12}-L-Phe-L-His	6012	363	16.6
2: S_{12}-L-Phe-L-His-L-Leu	2319	100	23.2
3: S_{12}-L-Phe-L-His-L-Leu-L-Leu	156	33	4.7
4: S_{12}-L-Phe-L-His-L-Leu-L-Leu-L-Leu	89	15	5.9
5: S_{12}-L-Phe-L-His-L-Ala	2420	145	16.7
6: S_{12}-L-Phe-L-His-L-Ala-L-Ala	809	77	10.5
7: S_{12}-L-Phe-L-His-L-Ala-L-Ala-L-Ala	349	46	7.6
8: S_{12}-L-Phe-L-His-L-Leu-L-Ala	345	48	7.2
9: S_{12}-L-Phe-L-His-L-Ala-L-Leu	577	62	8.5
10: S_{12}-L-Phe-L-His-L-Leu-D-Leu	333	26	12.8
11: S_{12}-L-Phe-L-His-L-Ala-D-Ala	1510	69	21.9

[a] Conditions as described in Table I.

to the catalysts with L-Leu-L-Leu and L-Ala-L-Ala in these positions.
Interestingly, the tetrapeptide catalyst with L-Leu-D-Leu (entry 10) in the 3- and 4-positions exhibits much higher enantioselectivity than the catalyst with the equally hydrophobic L-Leu-L-Leu sequence (entry 3). A similar effect is observed for the tetrapeptide catalysts with L-Ala-L-Ala and L-Ala-D-Ala in the 3- and 4-positions (entries 6 and 11). These differences must be caused by a difference in the shape of the diastereomeric catalysts.

Catalytic activity of S_4- and S_{12}-L-Phe-L-His methylamide

Table VI shows the activities and selectivity obtained with S_4- and S_{12}-L-Phe-L-His-NMA. In these catalysts, the carboxyl groups are protected by methylamine. The activities and enantioselectivity are strongly reduced compared to the corresponding di- or tripeptide catalysts which have an unprotected carboxyl group (see Tables I, II and III). This almost total collapse of the observed rates and severe suppression of the enantioselectivity cannot be attributed to a steric effect of the NMA-protecting group, as this group is smaller than any of the amino acid residues in the 3-position of the tripeptide catalysts. The catalytic activity of S_{12}-L-Phe-L-His-NMA, for instance, should be compared with the S_{12}-L-Phe-L-His-Gly catalyst (Table I, entry 3). The structural difference is that, in the latter catalyst, one hydrogen atom of the NMA moiety is replaced by a carboxylate group. These data indicate that the proximity of the carboxylate group increases the activity of the imidazolyl moiety.

Table VI Rate constants [$k_{a,obs}$/(dm^3 · mol^{-1} · s^{-1})] and enantioselectivity (k_L/k_D) in the cleavage of C_{12}-Phe-ONp (L and D) by comicelles of S_4/S_{12}-L-Phe-L-His-NMA and R-*surf 16*[a].

Catalyst	k_L	k_D	k_L/k_D
S_4-L-Phe-L-His-NMA	62	8	7.8
S_{12}-L-Phe-L-His-NMA	149	26	5.7

[a] Conditions as described in Table I.

Solvent isotope effect and apparent pK_a of the imidazolyl moiety

Table VII shows the solvent deuterium kinetic isotope effect for the cleavage of C_{12}-Phe-ONp by various histidine-

containing catalysts. The kinetic isotope effects do not differ significantly for both enantiomers of the substrate. All values of k_H/k_D are in the range 1.1–1.5, which indicates that proton transfer does not take place in the rate-determining step, *i.e.*, the reaction is not general base-catalyzed[16].

Table VII Solvent kinetic isotope effects for the cleavage of C_{12}-Phe-ONp (L and D) by comicelles of His-containing peptides and R-*surf 16*[a].

Catalyst	k_H/k_D (L)	k_H/k_D (D)
S_{12}-L-Phe-L-His	1.1	1.2
S_{12}-L-Phe-L-His-L-Leu	1.5	1.5
S_{12}-L-Phe-L-His-L-Asp	1.4	1.5
S_{12}-L-Phe-L-His-L-Leu-L-Leu	1.1	1.2

[a] Conditions as described in Table I.

In Table VIII, the apparent pK_a values are given of two catalysts that are bound to micelles of *R*-surf 16. The pK_a values of the S_2 compounds were measured in the absence of surfactant. It was impossible to measure the pK_a of the S_{12}-protected catalyst in the absence of surfactant because self-aggregation occurred. Comparison of the pK_a values in the presence and absence of surfactant gives the effect of micellar adsorption on the pK_a. The pK_a (ImH^+) of 6.9 measured in the absence of surfactant is a normal value for N-protected histidine[17]. The apparent pK_a (ImH^+) values of the micellar-bound catalysts are approximately 0.5 pK_a unit lower, which is to be expected for cationic micelles[18,19]. Due to the positive charge of the micellar surface, protonation of the imidazolyl group is impeded (field effect). The positively charged surface will also attract hydroxide ions from the solution, *i.e.*, the local hydroxide ion concentration (and hence the pH) at the micellar surface is higher than in the bulk aqueous phase. Consequently, the imidazolyl group appears to deprotonate at a lower pH.

Table VIII Apparent pK_a values of the imidazolyl moieties in various catalysts in the presence (S_{12}) and absence (S_2) of R-*surf 16*[a].

Catalyst	pK_a (ImH^+)
S_2-L-Phe-L-His[b]	6.9
S_2-L-Phe-L-His-L-Leu[b]	6.9
S_{12}-L-Phe-L-His	6.4
S_{12}-L-Phe-L-His-L-Leu	6.3

[a] Conditions: 0.40M KCl; c_{cat} $2 \cdot 10^{-4}$M; c_{surf} $1 \cdot 10^{-2}$M. [b] In the absence of surfactant.

The blank reaction

In order to calculate the catalytic activity of a catalyst (k_{cat}) from the observed reaction rate (rate constant k_{obs}), the magnitude of the blank or background reaction (k_{bl}) must be known ($k_{cat} = k_{obs} - k_{bl}$). The blank reaction rate is determined in the absence of catalyst, and it is assumed that, in the presence of the catalysis, this blank reaction is unaffected. However, it will be demonstrated that this assumption is only valid under certain conditions.

Cationic micelles greatly accelerate hydrolysis of the adsorbed substrates. This effect is due to the attraction of hydroxide ions from the solution, *i.e.*, the local hydroxide ion concentration or local pH at the micellar surface is higher than the pH in the bulk aqueous solution. In spite of the presence of a buffer, the micellar surface is not buffered[18,19]. Most catalysts used are anionic amphiphilic molecules that bind tightly to the micelles. Consequently, the micellar charge density is reduced and fewer hydroxide ions will be bound. This results in a reduction in the rate of spontaneous ester hydrolysis. In order to investigate this effect, we used the catalytically inactive model compound *N*-(dodecyloxycarbonyl)-L-phenylalanine sodium salt (S_{12}-L--Phe-ONa) at a concentration similar to that of our catalysts and determined the reduction of the blank hydrolysis (Δk_{bl}) of C_{12}-D-Phe-ONp at various concentrations of added KCl (pH 7.3, 0.02 mol/dm^3 Tris/HCl). The results are shown in Figure 1a. The observed reduction in rate constant is expressed in the same units as the catalytic rate constants in the Tables and their magnitudes can be directly compared. At $c_{KCl} = 0$, the addition of the model compound causes a reduction of k_{bl} from 1112 to 1012 $dm^3 \cdot mol^{-1} \cdot s^{-1}$. In the presence of 0.4 mol·dm^{-3} KCl, the blank reaction is reduced from $k_{bl} = 25$ to $k_{bl} = 22$ $dm^3 \cdot mol^{-1} \cdot s^{-1}$. At low KCl concentrations, many hydroxide ions are bound to the micelles. Consequently, binding of the negatively charged model compound replaces many hydroxide ions and the blank reaction rate is considerably reduced. At high salt concentrations ($c_{Cl^-} > 0.3$ mol/dm^3), the micelles only bind Cl^- ions at their surface which are partially replaced by the model compound. The blank reaction rates now remain almost unaffected. It should be noted that the reduction of the blank reaction rate at low KCl concentration ($\Delta k_{bl} = -100$ $dm^3 \cdot mol^{-1} \cdot s^{-1}$) becomes of similar magnitude as or is even larger than the catalytic reaction rates towards the D substrates (k_D is approximately 100 $dm^3 \cdot mol^{-1} \cdot s^{-1}$). This means that if we calculate $k_D = k_{obs} - k_{bl}$, with the assumption that k_{bl} is unaffected by the presence of the catalyst, we severely underestimate the magnitude of k_D since we use a value for k_{bl} that is too large. The underestimation of k_L, which generally has a much larger value than Δk_{bl}, is of minor importance. The result is that the enantioselectivity of a

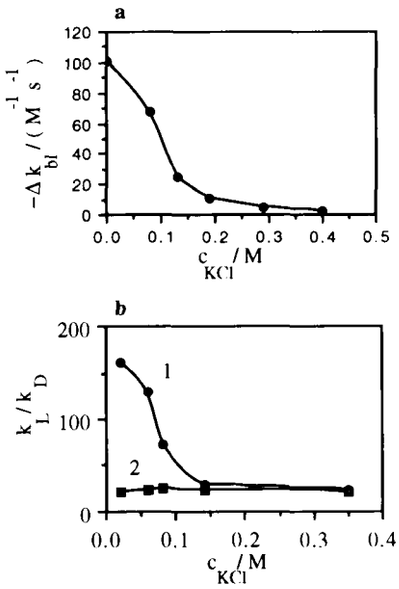


Figure 1. a) The reduction of the blank reaction of C_{12}-D--Phe-ONp by S_{12}-L-Phe-ONa ($5 \cdot 10^{-5}$M) as a function of the KCl concentration. c_{surf} 2.5 mM; c_{ester} $1 \cdot 10^{-5}$M; c_{Tris} 0.02M; pH 7.3. b) The enantioselectivity (k_L/k_D) of the reaction between the S_{12}-L-Phe-L-His-L-Leu catalyst and C_{12}-Phe-ONp as a function of the KCl concentration. Conditions as described for Figure 1a; c_{cat} $5 \cdot 10^{-5}$M. 1) Uncorrected blank reaction. 2) Blank reaction corrected with the aid of Figure 1a.

catalyst (k_L/k_D) is severely overestimated. This artifact is shown in Figure 1b for the S_{12}-L-Phe-L-His-L-Leu catalyst as a function of the added amount of KCl. It can be concluded that the assumption k_{bl} (in the presence of catalyst) = k_{bl} (in the absence of catalyst) in the present system is only valid at high ionic strength ($c_{Cl^-} > 0.3$ mol/dm^3). High enantioselectivity has been reported due to the above described artifact[9,11,20,21]. The experiments described in this paper were carried out in the presence of 0.4 mol/dm^3 KCl, which means that the calculated enantioselectivity values are reliable.

Non-ionic molecules that adsorb to the micelles and cause an increase in surfactant headgroup–headgroup distance will also reduce the charge density at the micellar interface. Consequently, fewer OH^- ions will be bound, resulting in a reduction in the blank reaction rate.

Discussion

A micellar solution is a two-phase system consisting of an aqueous phase and a hydrocarbon-like micellar pseudophase (Figure 2a). When our peptide catalysts dissolve into the micellar hydrocarbon phase, the polar amide groups will be dehydrated, which is very unfavorable. To compensate for this dehydration, the amide groups will try to form internal hydrogen bonds. Since a completely internally hydrogen-bonded conformation is not possible for our peptides, the resulting folded peptide chain has an amphiphilic character. To what extent the peptide chain will dissolve into the micellar hydrocarbon phase will be determined by the hydrophilic/hydrophobic balance of the peptide chain. Peptides shift their preference to the apolar phase if they adopt the internally hydrogen bonded C_7- or γ-turn conformation[22]. A seven-membered ring including the H atom is formed (Figure 2b). In the following, we assume that our small peptide catalysts also adopt this C_7 conformation, when they dissolve into the micellar hydrocarbon phase, forming as many intramolecular hydrogen bonds as possible (Figure 2b).

As explained in our first paper[1], the NH group of the Phe residue in position 1 of the catalyst forms an intermolecular hydrogen bond with the amide CO moiety of the L substrate, when the imidazolyl moiety of the His residue attacks the ester group to form the tetrahedral intermediate (Figure 2c, see also Figure 3a). This hydrogen bond contributes to the stability of the transition state when it is formed in the apolar micellar hydrocarbon phase. This stabilizing hydrogen bond is not formed in the diastereomeric intermediate with the D substrate. Consequently, the reaction rate towards the L substrate is much higher. When residue 1 in the catalyst is less hydrophobic, the transfer of the NH moiety to the micellar hydrocarbon phase is incomplete and the hydrogen bond with the L ester becomes less favorable, resulting in a reduction in the reaction rate (compare entries 1 and 2 in Table I). This feature was discussed in our previous paper[1].

In the tetrahedral intermediate, a positive charge has developed on the imidazolyl moiety which is not present in the ground state (Figures 2c and 3). In order to stabilize this positive charge, the imidazolyl moiety requires a polar environment: the imidazolyl NH group must remain in the aqueous phase. In order to prevent the imidazolyl moiety from dissolving into the micellar hydrocarbon phase, the imidazolyl NH moiety must be structurally connected to the very hydrophilic carboxylate moiety. In liquid water at room temperature, such a connection is possible if the distance between the two groups (d) does not exceed the space required for two hydrogen-bond water molecules, or approximately 7–8 Å (Figure 2c)[23,24]. If this distance is larger, the hydration of the imidazolyl and that of the carboxylate moiety become independent. The imidazolyl moiety can dissolve in the apolar micellar hydrocarbon phase and become a less effective catalyst. This carboxylate-imidazolyl interaction in combination with the above proposed hydrogen bonds affords a satisfying explanation for our observations.

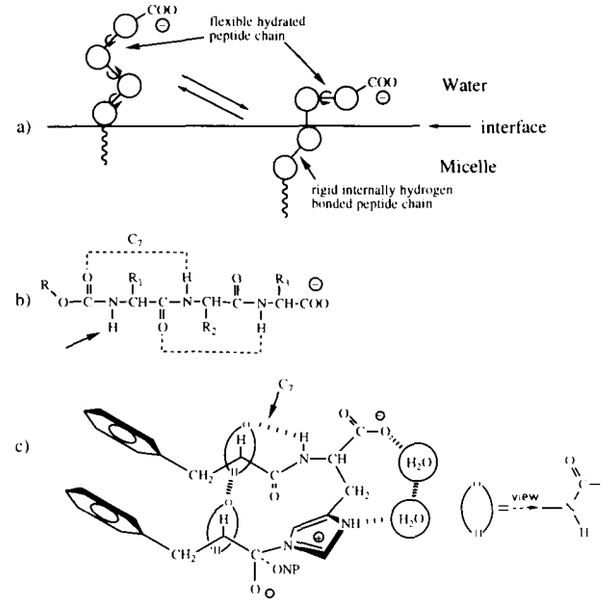


Figure 2. a) Schematic representation of a peptide catalyst adsorbed at the micellar interface. The amide groups of the peptide chain in the aqueous phase are hydrated, resulting in a flexible chain. The amide groups in the micellar hydrocarbon phase are internally hydrogen-bonded, resulting in a rigid conformation. b) Structural formula of a peptide catalyst that has adopted the 2^7 helix. The internal C_7 hydrogen bonds are shown as dotted lines. The arrow indicates the NH group that is not internally hydrogen bonded and a potential hydrogen-bond donor. c) The tetrahedral intermediate formed by the S_n-L-Phe-L-His catalyst and the C_n-L-Phe-ONp substrate. Hydrogen bonds are shown as dotted lines.

Figure 3 shows the tetrahedral intermediates of a number of catalysts, which have adopted the C_7 conformation. This Figure further indicates whether the imidazolyl group falls within the hydration mantle of the C-terminal carboxylate group ($d < 8$ Å) or not ($d > 8$ Å). The distance d is determined with the aid of CPK space-filling models.

Figure 3a gives the intermediate for the reaction with the S_n-L-Phe-L-His catalyst. For both the L and D substrates, the imidazolyl NH moiety falls within the hydration mantle of the carboxylate group. Consequently, the activity towards both substrates is relatively high. However, due to the additional hydrogen bond with the L substrate, the activity towards this enantiomer is higher (k_L/k_D = 16.6; Table I, entry 2).

Figure 3b shows the intermediate for the L substrate with the S_n-L-Phe-L-His-L-Leu catalysts. Transfer of the Leu residue to the micellar phase is facilitated because of dehydration of its NH group is compensated for by hydrogen bonding to the Phe C=O. In the micellar phase, the Leu NH moiety forms a hydrogen bond with the L substrate and chiral recognition will improve (up to k_L/k_D = 40; Table II). These two internally hydrogen-bonded groups are located on both sides of the His residue (see also Figure 2b in which

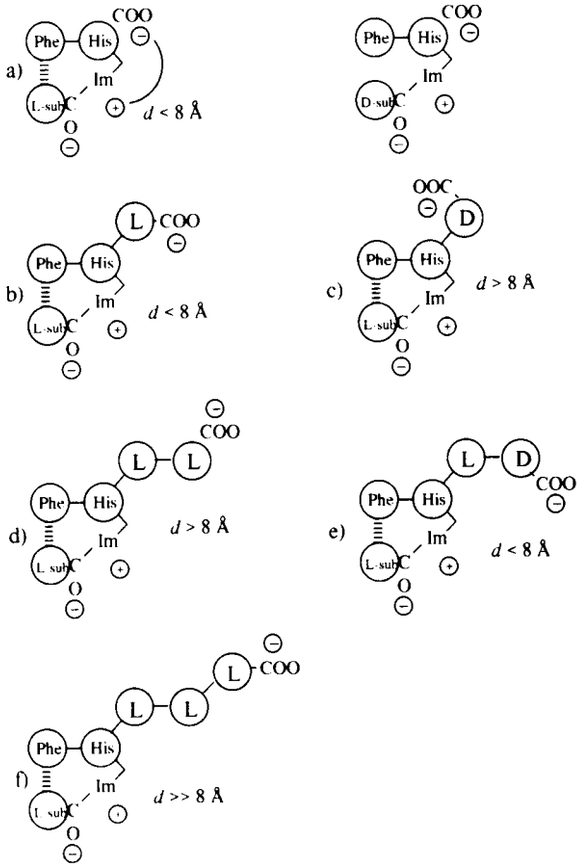


Figure 3. Schematic representation of the tetrahedral intermediates formed by the L *substrate and a)* S_n*-*L*-Phe-*L*-His (the intermediate with the* D *substrate is also shown); b)* S_n*-*L*-Phe-*L*--His-*L*-Leu; c)* S_n*-*L*-Phe-*L*-His-*D*-Leu; d)* S_n*-*L*-Phe-*L*-His-*L*-X--*L*-X; e)* S_n*-*L*-Phe-*L*-His-*L*-X-*D*-X; f)* S_n*-*L*-Phe-*L*-His-*L*-X-*L*--X-*L*-X. The distance between the carboxylate group and the imidazolyl NH moiety is given by* d. *All catalysts are shown in the* 2^7*-helical conformation.*

R^2 = CH_2-Im) and their dehydration will cause a more hydrophobic environment for the imidazolyl moiety.

Consequently, the reaction rate decreases if compared to the corresponding dipeptide (Table I, entries 2 and 7 and Table II, entry 4). The distance between the imidazolyl NH moiety and the carboxylate group has increased but is still smaller than 8 Å. This will prevent the imidazolyl moiety from completely dissolving into the micellar hydrocarbon phase. However, if the third residue in the catalyst becomes too hydrophobic (Phe, Ile, Phg; Table I, entries 8–10), the imidazolyl moiety will tend to dissolve completely into the micellar hydrocarbon phase and the reaction rate will be suppressed further. The Trp residue does not promote the transfer of the imidazolyl moiety to the micellar hydrocarbon phase because the hydrophilic NH group of the indolyl moiety prefers to remain hydrated (Table I, entry 11). A similar resistance of the Trp residue to dissolve into the micellar hydrocarbon phase was also observed in our previous paper with Trp in the 1-position[1].

Figure 3c gives the intermediate for the S_n-L-Phe-L-His--D-Leu catalysts. Here, the imidazolyl NH group just falls outside the hydration mantle of the carboxylate moiety ($d > 8$ Å) and the imidazolyl moiety will tend to dissolve into the micellar hydrocarbon phase. Consequently, the activity towards the L substrate and the enantioselectivity are strongly reduced (Table I, entry 15).

Figure 3d shows the intermediate for the L substrate and the S_n-L-Phe-L-His-L-X-L-Y catalysts. The imidazolyl NH moieties fall clearly outside the hydration mantle of the carboxylate group and the imidazolyl moieties will tend to dissolve into the micellar hydrocarbon phase. Consequently, the activities towards the L substrate and the enantioselectivities are strongly reduced. The effect is much larger for the more hydrophobic amino acid residues (L-X-L-Y = L-Leu-L-Leu; Table V, entry 3) than for the less hydrophobic residues (L-X-L-Y = L-Ala-L-Ala; Table V, entry 6). The effect is intermediate when L-X-L-Y = L-Leu-L-Ala or L-Ala-L-Leu.

Figure 3e shows the tetrahedral intermediates for the L substrate and the S_n-L-Phe-L-His-L-X-D-X catalysts. Changing the terminal residue in the catalyst from L into D brings the imidazolyl NH moiety just within the hydration mantle of the carboxylate group ($d < 8$ Å). Consequently, both the activity towards the L substrate and the enantioselectivity increase (compare Table V, entries 3 and 6 with entries 10 and 11). However, it should be noted that particularly when X = Leu, the reaction rates are low. This indicates that the hydrophobic/hydrophilic balance is such that the imidazolyl moiety tends to dissolve into the micellar hydrocarbon phase despite the proximity of the carboxylate group.

Figure 3f shows the tetrahedral intermediate or the L substrate and the S_n-L-Phe-L-His-L-X-L-X-L-X catalysts. Increasing the peptide chain of the catalyst with a 5th residue further increases the distance between the carboxylate residue and the imidazolyl moiety ($d >> 8$ Å). If X becomes more hydrophobic, the tendency for the imidazolyl group to dissolve into the micellar hydrocarbon phase increases and the reaction rates decrease.

The S_n-L-Phe-L-His-NMA catalysts (Table VI) lack the very hydrophilic carboxylate group. Consequently, the imidazolyl moiety can easily dissolve into the micellar hydrocarbon phase. This causes a large decrease in activity and enantioselectivity.

Tables II and III show that, in order to obtain maximum enantioselectivity, S_n in the catalysts must have optimum hydrophobicity. This behaviour is typical for an amphiphilic transition state in a two-phase hydrocarbon/water system. A shift in the hydrophobic/hydrophilic balance of a transition state affects its distribution between the two phases (which part dissolves into the micellar hydrocarbon phase and which part will remain in the aqueous phase), as already shown above. Increasing the hydrophobicity from S_2 to S_4 promotes transfer of the NH moiety of the Phe residue to the micellar hydrocarbon phase. Consequently, the enantioselectivity increases. If the S_n group becomes more hydrophobic, the effect levels off more for the L than for the D enantiomer, inasmuch as the transition state of the former is less hydrophilic because of its additional hydrogen bond. Consequently, the enantioselectivity is reduced.

Optimum hydrophobicity is not observed for the C_n group of the ester (Table IV). The hydrophobicity of C_n of the ester affects its distribution between the aqueous phase and the micellar hydrocarbon phase. The more hydrophobic C_n becomes, the greater the tendency of the ester to dissolve into the micellar hydrocarbon phase. At a certain point, the ester is completely dissolved into the micellar hydrocarbon phase. In the micellar hydrocarbon phase, the affinity of the amide CO group of the L enantiomer of the ester to form a hydrogen bond with the NH group of the Phe residue in the catalyst is at its maximum. Consequently, the enantioselectivity increases up to a limit value.

Experimental

General remarks

Thin-layer chromatography (TLC) was performed on silica gel (Merck DC-Plastikrolle, Kieselgel 60 F_{254}) and detection was

effected by ultraviolet light or iodine. Column chromatography was performed with silica gel (Merck Kieselgel 60, 230–400 mesh). Solvents and reagents were of analytical grade. The preparation of the surfactant solutions with and without catalyst is described elsewhere[1]. The kinetic measurements were carried out as described in Ref. 1. Elemental analyses were made from the final products and were all in good agreement with the calculated values.

Synthesis

N-*(Dodecycloxycarbonyl)-(L-phenylalanyl)-L-histidine (S_{12}-L-Phe-L--His-OH);* N-*(dodecyloxycarbonyl)-L-alanyl-L-histidine (S_{12}-L-Ala-L--His-OH).* The syntheses of these compounds are described in our previous paper[1].

N-*(Dodecycloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L- (or D-) X-OMe (S_{12}-L-Phe-L-His-X-OMe).* H-X-OMe-HCl (X = amino acid) was coupled to *N*-(dodecycloxycarbonyl)-(L-phenylalanyl)-L-histidine in DMF with the aid of 1,3-dicyclohexylcarbodiimide according to a published method[25,26]. This method can cause some racemization of the histidyl residue but the diastereomers can be removed by chromatography. The crude product was purified by column chromatography (Silica 60, eluent chloroform/methanol, 15:1 v/v) and recrystallized from acetone. TLC: R_f 0.3 (silica chloroform/methanol, 10:1 v/v) for all compounds. Traces of diastereomers that were formed by racemization of the histidyl residue showed an R_f value on TLC around 0.25. These impurities were absent in the purified final products. IR (KBr): 1745 (CO ester), 1690 (CO carbamate), 1650 (CO amide) cm^{-1}. For individual compounds, ^1H NMR ($CDCl_3$), $[\alpha]_D^{20}$/(*c* 1.0, methanol), m.p./°C and yield/% are:

S_{12}-L-Phe-L-His-Gly-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 3.1 (2 × d (dist), 2 × 2H, CH_2 (His) and CH_2 (Phe)); 3.7 (s, 3H, OCH_3); 3.85 (s, 2H, CH_2 (Gly)); 3.95 (t, 2H, CH_2O); 4.3–4.9 (m, 2H, CH (Phe) and CH (His)); 5.5 (d, 1H, NH (carbamate)); 6.75 and 7.45 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH); 7.3 and 7.7 (2 × d, 2 × 1H, 2 × NH (amide)); 8.7 (s (br), 1H, NH (Im)) ppm; −22.0°; 150.0; 56.

S_{12}-L-Phe-L-His-L-Ala-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 1.45 (d, 3H, CH_3 (Ala)); 3.1 (2 × d (dist), 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 4.0 (t, 2H, CH_2O); 4.2–4.8 (m, 3H, 3 × CH); 5.5 (d, 1H, NH (carbamate)); 6.8 and 7.5 (2 × s, 2 × 1H, 2 × CH (Im)); 7.3 (s, 5H, ArH); 7.7 and 7.8 (2 × d, 2 × 1H, 2 × NH); 9.0 (s (br), 1H, NH (Im)) ppm; −31.8°; 149.4; 64.

S_{12}-L-Phe-L-His-L-Abu-OMe: δ 0.9 (2 × t, 2 × 3H, 2 × CH_3); 1.3 (m, 22H, $(CH_2)_{10}$ and CH_2 (But)); 3.1 (2 × d (dist), 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 4.0 (t, 2H, CH_2O); 4.2–4.8 (m, 3H, 3 × CH); 5.5 (d, 1H, NH (carbamate)); 7.7 and 8.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.25 (s, 5H, ArH); 7.6 and 7.8 (2 × d, 2 × 1H, 2 × NH); 8.2 (s (br), 1H, NH (Im)) ppm; −25.8°; 139.4; 61.

S_{12}-L-Phe-L-His-L-Nva-OMe: The ^1H NMR spectrum of this compound closely resembles the spectrum of the *S_{12}-L-Phe-L--His-L-But-OMe* compound; −23.0°; 112.8; 66.

S_{12}-L-Phe-L-His-L-Leu-OMe: δ 0.9 (m, 9H, 3 × CH_3); 1.3 (m, 23H, $(CH_2)_{10}$ and CH_2CH (Leu)); 3.1 (2 × d (dist); 2 × 2H, CH_2 (Phe) and CH_2 (Hist)); 2.65 (s, 3H, OCH_3); 4.0 (t, 2H, CH_2O); 4.2–4.9 (m, 3H, 3 × CH), 5.4 (d, H, NH (carbamate)); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH); 7.5 and 7.8 (2 × d, 2 × 1H, 2 × NH); 8.0 (s (br), 1H, NH (Im)) ppm; −19.5°; 84.0; 68.

S_{12}-L-Phe-L-His-L-Phe-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 3.0 (m, 6H, 3 × $\underline{CH_2}CH$); 3.65 (s, 3H, OCH_3); 4.0 (t, 2H, OCH_2); 4.2–4.9 (m, 3H, 3 × CH); 5.5 (d, 1H, NH (carbamate)); 7.2 (s, 10H, 2 × ArH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 6.8 and 7.8 (2 × d, 2 × 1H, 2 × NH); 8.4 (s (br), 1H, NH (Im)) ppm; −22.4°; 128.7; 54.

S_{12}-L-Phe-L-His-L-Trp-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 3.0 (m, H, 2 × $\underline{CH_2}CH$); 3.2 (d, 2H, CH_2 (Trp)); 3.65 (s, 3H, OCH_3); 3.9 (t, 2H, CH_2O); 4.2–4.9 (m, 3H, 3 × CH); 5.6 (d, 1H, NH (carbamate)); 6.7 and 7.3 (2 × s, 2 × 1H, 2 × CH (Im)); 6.8–7.7 (m, 10H, ArH and 5 × CH (indolyl)); 7.4 and 7.7 (2 × d, 2 × 1H, 2 × NH); 9.1 (s, 1H, NH (indole)) ppm; −21.5°; 109.7; 58.

S_{12}-L-Phe-L-His-L-Ile-OMe: δ 0.9 (m, 9H, 3 × CH_3); 1.3 (m, 23H, $(CH_2)_{10}$ and $CHCH_2$ (Ile)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.65 (s, 3H, OCH_3); 3.95 (t, 2H, CH_2O); 4.2–5.0 (m, 3H, 3 × CH); 5.6 (d, 1H, NH (carbamate)); 6.65 and 7.35 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, C_6H_5); 7.7 (2 × d, 2 × 1H, 2 × NH); 8.1 (s (br), 1H, NH (Im)) ppm; −8.6°; 79.8; 51.

S_{12}-L-Phe-L-His-L-Pro-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 2.0 (m, H, 2 × CH_2 (Pro)); 3.0 (2 × d (dist), 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 3.95 (t, 2H, CH_2O); 4.4 (m, 2 × 1H, 2 × CH); 4.9 (q (dist), 1H, CH); 5.6 (d, 1H, NH (carbamate)); 6.8 and 7.5 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH); 7.4 (d, 1H, NH); 7.8 (s (br), 1H, NH (Im)) ppm; −39.0°; 57.7; 51.

S_{12}-L-Phe-L-His-L-Asp(OMe)-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 2.8 (d, 2H, CH_2 (Asp)); 3.0 (m, 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.7 and 3.8 (2 × s, 2 × 3H, 2 × OCH_3); 4.0 (t, 2H, CH_2O); 4.2–4.9 (m, 3H, 3 × CH); 5.7 (d, 1H, NH (carbamate)); 6.8 and 7.5 (2 × s, 2 × 1H, 2 × CH (Im)); 7.25 (s, 5H, ArH); 7.7 (2 × d, 2 × 1H, 2 × NH); 8.0 (s (br), 1H, NH (Im)) ppm; −24.8°; 128.8; 55.

S_{12}-L-Phe-L-His-L-Thr-OMe: ^1H NMR ($CDCl_3$ and CD_3OD, 1:1 (v/v)): δ 0.9 (t, 3H, CH_3); 1.3 (m, 23H, $(CH_2)_{10}$ and CH_3 (Thr)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 4.0 (m, 3H, CH_2O and $\underline{CH}OH$ (Thr)); 4.2–5.0 (m, 3 × 1H, 3 × CH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −13.3°; 92.2; 51.

S_{12}-L-Phe-L-His-L-Leu-OMe: The ^1H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L--Leu-OMe*; +3.9°; 121.4; 60.

N-*(Dodecycloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L- (or D-) X-OH (S_{12}-L-Phe-L-His-L- (or D-) X-OH).* Deprotection of the corresponding methyl esters was performed in aqueous ethanol using NaOH. The crude product was recrystallized from acetone. The final yields amounted to 70%. IR (KBr): 1685 cm^{-1} (CO carbamate); 1650 cm^{-1} (broad; CO amide) unless noted otherwise. ^1H NMR (methanol-d_4); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C.

S_{12}-L-Phe-L-His-Gly-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 3.0 (2 × d (dist), 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.0 (s (br), 2H, CH_2 (Gly)); 4.2–4.8 (m, 2H, 2 × CH); 6.9 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −1.4°; 205.9.

S_{12}-L-Phe-L-His-L-Ala-OH: δ 0.9 (t, 3H, CH_3), 1.3 (m, 23H, $(CH_2)_{10}$ and CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.2–4.8 (m, 3H, 3 × CH); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im)); 7.1 (s, 5H, ArH) ppm; +5.6°; 206.1.

S_{12}-L-Phe-L-His-L-Abu-OH: The ^1H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L--Ala-OH* with the exception: δ 0.9 (2 × t, 2 × 3H, 2 × CH_3) ppm; +4.1; 196.1. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 cm^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Nva-OH: The ^1H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L--Abu-OH*; +4.1°; 195.7°C. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 cm^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Leu-OH: (m, 9H, 3 × CH_3); 1.3 (m, 23H, $(CH_2)_{10}$ and CH_2CH (Leu)); 3.0 (2 × d (dist), 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.2–4.8 (m, 3H, 3 × CH); 7.1 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.1 (s, 5H, ArH) ppm; −0.2°; 185.5. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 m^{-1} (CO amide).

S_{12}-L-Phe-L-His-D-Leu-OH: The ^1H NMR spectrum of this compound closely resembles the spectrum of the diastereomeric *S_{12}-L-Phe-L-His-L-Leu-OH* compound; −5.2°; 167.2. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 cm^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Phe-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 3.0 (m, 6H, 3 × CH_2); 3.9 (t, 2H, CH_2O); 4.2–4.9 (m, 3H, 3 × CH); 7.0 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 10H, 2 × ArH) ppm; +7.5°; 169.6 IR (KBr): 1685 cm^{-1} (CO carbamate); 1645 cm^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Trp-OH: δ 0.9 (t, 3H, CH_3; 1.3 (m, 20H, $(CH_2)_{10}$); 3.0 (m, 6H, 3 × CH_2); 3.9 (t, 2H, CH_2O); 4.2–4.8 (m, 3H, 3 × CH); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH); 6.8–7.7 (m, 5H, 5 × CH (indolyl)) ppm; +3.4°; 146.4. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 m^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Ile-OH: The ^1H NMR spectrum of this compound closely resembles the spectrum of the *S_{12}-L-Phe-L--His-L-Leu-OH* compound; +2.5°; 166.5. IR (KBr): 1685 cm^{-1} (CO carbamate); 1640 cm^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Phg-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 3.0 (2 × d (dist); 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.2–4.7 (m, 2H, 2 × CH); 5.5 (s, 1H, CH (Phg); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im); 7.2 and 7.3 (2 × s, 2 × 5H,

$2 \times C_6H_5$) ppm; +21.6; 197.0. IR (KBr): 1685 cm^{-1} (CO carbamate); 1645 cm^{-1} (CO amide).

S_{12}-L-Phe-L-His-L-Thr-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 23H, $(CH_2)_{10}$ and CH_3 (Thr)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 4.0 (m, 3H, CH_2O and CHOH); 4.2–4.8 (m, 3H, 3 × CH); 7.0 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; +1.5°; 174.5.

S_{12}-L-Phe-L-His-L-Pro-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 2.0 (m, 4H, 2 × CH_2 (Pro)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 3.65 (t, 2H, CH_2 (Pro)); 4.2–4.7 (m, 3H, 3 × CH); 7.0 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −18.2°; 145.9.

S_{12}-L-Phe-L-His-L-Asp-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 2.6 (d (dist), 2H, CH_2 (Asp)); 3.0 (m, 4H, CH (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O), 4.1–4.8 (m, 3H, 3 × CH); 7.1 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; +6.8°; 215.0.

N-*(Dodecyloxycarbonyl)-(L-phenylalanyl)-L-histidine methylamide (S_{12}-L-Phe-L-His-NMA);* N-*(Butyloxycarbonyl)-(L-phenylalanyl)-L-histidine methylamide (S_4-L-Phe-L-His-NMA).* These compounds were prepared by coupling methylamine to the corresponding dipeptides (see Ref. 1 for the synthesis of these dipeptides) with the aid of 1,3-dicyclohexylcarbodiimide in DMF according to a published method[25,26]. The crude product was purified by column chromatography (Silica 60: $CHCl_3/CH_3OH$, 10:1 v/v) and subsequent recrystallization from acetone. The final yields amounted to approximately 40%. TLC: R_f 0.35 (Silica, $CHCl_3/CH_3OH$, 10:1 v/v) for both compounds.

S_{12}-L-Phe-L-His-NMA: ^1H NMR ($CDCl_3$): δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 2.55 (s, 3H, NCH_3); 3.0 (2 × d (dist); 2 × 2H, CH_2 (Phe) and CH_2 (His)); 4.3 and 4.5 (2 × t, 2 × 1H, 2 × CH); 7.25 (s, 5H, ArH); 6.8 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)) ppm; $[\alpha]_D^{20}$ −13.8° (*c* 1.0, methanol); m.p. 142.5°C. IR (KBr): 1690 (CO carbamate); 1650 (CO amide) cm^{-1}.

S_4-L-Phe-L-His-NMA: ^1H NMR ($CDCl_3$): δ 0.9 (t, 3H, CH_3); 1.3 (m, 4H, $(CH_2)_2$); 2.55 (s, 3H, NCH_3); 3.0 (2 × d (dist); 2 × 2H, CH_2 (Phe) and CH_2 (His)); 4.3 and 4.5 (2 × t, 2 × 1H, 2 × CH); 7.25 (s, 5H, ArH); 6.8 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)) ppm; $[\alpha]_D^{20}$ −15.8° (*c* 1.0, methanol); m.p. 111.1°C. IR (KBr): 1690 (CO carbamate); 1650 (CO amide) cm^{-1}.

N-*(Alkyloxycarbonyl)-(L-phenylalanyl)-L-hystidyl-L-leucine methyl ester (S_n-L-Phe-L-His-L-Leu-OMe);* N-*(Benzyloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L-leucine methyl ester (Z-L-Phe-L-His-L-Leu-OMe);* N-*(Alkyloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L-alanine methyl ester (S_n-L-Phe-L-His-L-Ala-OMe).* These compounds were prepared and purified as described for the *S_{12}-L-Phe-L-His-L-Leu-OMe* and *S_{12}-L-Phe-L-His-L-Ala-OMe* compounds. The final yields amounted to 60–70%. IR (KBr): 1745 (CO ester); 1695 (CO carbamate); 1650 (CO amide) cm^{-1} unless noted otherwise. ^1H NMR ($CDCl_3$); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C.

S_2-L-Phe-L-His-L-Leu-OMe: δ 1.0 (m, 9H, 3 × CH_3); 1.5 (m, 3H, CH_2CH (Leu)); 3.1 (2 × d (dist), 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.65 (s, 3H, OCH_3); 4.0 (q, 2H, CH_2O); 4.2–4.9 (m, 3H, 3 × CH); 5.4 (d, 1H, NH (carbamate)); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH); 7.2 and 7.5 (2 × d, 2 × 1H, 2 × NH); 7.9 (s (br), 1H, NH (Im)) ppm; −28.0°; 48.5.

S_4-L-Phe-L-His-L-Leu-OMe: The ^1H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L-Leu-OMe*; −22.2°; 51.7.

S_6-L-Phe-L-His-L-Leu-OMe: The ^1H NMR spectrum closely resembles the spectrum of *S_{12}-L-Phe-L-His-L-Leu-OMe*; −21.8°; 72.0.

Z-L-Phe-L-His-L-Leu-OMe: δ 0.9 (2 × d, 2 × 3H, 2 × CH_3 (Leu)); 1.5 (m, 3H, CH_2CH (Leu)); 3.1 (2 × d (dist); 2 × 2H, CH_2 (Phe) and CH_2 (His)); 3.6 (s, 3H, OCH_3); 4.2–5.0 (m, 3 × 1H, 3 × CH); 4.9 (s, 2H, CH_2O); 5.8 (d, 1H, NH (carbamate)); 6.7 and 7.7 (2 × s, 2 × 1H, 2 × CH (Im)); 7.1 and 7.2 (2 × s, 2 × 5H, 2 × ArH); 7.7 and 7.9 (2 × d, 2 × 1H, 2 × NH); 8.2 (s (br), 1H, NH (Im)) ppm; −17.2°; 51.5.

S_2-L-Phe-L-His-L-Ala-OMe: δ 1.0 (t, 3H, CH_3); 1.45 (d, 3H, CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 4.0 (q, 2H, CH_2O); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −38.2°; 111.1. IR (KBr): 1690 cm^{-1} (CO carbamate).

S_4-L-Phe-L-His-L-Ala-OMe: δ 1.0 (m, 7H, CH_3 $(CH_2)_2$); 1.45 (d, 3H, CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 4.0 (t, 2H, CH_2O); 4.2–4.9 (m, 3H, 3 × CH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −33.8°; 124.2. IR (KBr): 1690 cm^{-1} (CO carbamate).

S_6-L-Phe-L-His-L-Ala-OMe: The ^1H NMR and IR spectra of this compound closely resemble the spectra of *S_{12}-L-Phe-L-His-L-Ala-OMe*; −32.0°; 138.2.

N-*(Dodecyloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L-X-L- (or D-) Y-OMe (X and Y are amino acid residues).* The tetrapeptides were prepared from the corresponding tripeptides and the methyl ester of Y in DMF with the aid of 1,3-dicyclohexylcarbodiimide according to a published method[25,26]. The crude products were purified as described for the corresponding tripeptide methyl esters. The final yields amounted to 50–60%. TLC: R_f 0.35 (Silica, $CHCl_3/CH_3OH$, 10:1 v/v) for all compounds. ^1H NMR ($CDCl_3/CD_3OD$, 1:1 v/v) unless noted otherwise; $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C; yield/%; IR (KBr): CO ester, CO carbamate, CO amide/cm^{-1}.

S_{12}-L-Phe-L-His-L-Leu-L-Leu-OMe: ^1H NMR ($CDCl_3$): δ 0.9 (m, 15H, 5 × CH_3); 1.1–1.9 (m, 26H, $(CH_2)_{10}$ and 2 × CH_2CH (Leu)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.55 (s, 3H, OCH_3); 3.9 (t, 2H, CH_2O); 4.1–4.7 (m, 4H, 4 × CH); 5.5 (d, 1H, NH (carbamate)); 6.6 and 7.3 (2 × s, 2 × 1H, 2 × CH (Im)); 7.1 (m, 5H, ArH); 7.0–7.5 (m, 3H, 3 × NH); 8.7 (s (br), 1H, NH (Im)) ppm; −15.3°; 70.6; 62; 1740, 1690, 1645.

S_{12}-L-Phe-L-His-L-Ala-L-Leu-OMe: δ 0.9 (m, 9H, 3 × CH_3); 1.3 and 1.5 (m, 26H, $(CH_2)_{10}$ and $CHCH_2$ (Leu) and CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.6 (s, 3H, OCH_3); 3.9 (t, 2H, CH_2O); 4.2–4.8 (m, 4H, 4 × CH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.1 (s, 5H, ArH) ppm; −30.0°; 75.0; 60; 1745, 1690, 1650.

S_{12}-L-Phe-L-His-L-Ala-L-Ala-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 26H, $(CH_2)_{10}$ and 2 × CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.6 (s, 3H, OCH_3); 3.9 (t, 2H, CH_2O); 4.2–4.8 (m, 4H, 4 × CH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −32.5°; 86.2; 55; 1745, 1690, 1650.

S_{12}-L-Phe-L-His-L-Leu-L-Ala-OMe: δ 0.9 (m, 9H, 3 × CH_3); 1.3 (m, 29H, $(CH_2)_{10}$ and CH_3 (Ala) and 2 × CH_3 (Leu)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 4.0 (t, 2H, CH_2O); 4.1–4.8 (m, 4H, 4 × CH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −28.7°; 79.2; 57; 1740, 1690, 1645.

S_{12}-L-Phe-L-His-L-Leu-D-Leu-OMe: The ^1H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L-Leu-L-Leu-OMe*; −0.9°; 65.8; 49; 1745, 1690, 1650.

S_{12}-L-Phe-L-His-L-Ala-D-Ala-OMe: The ^1H NMR and IR (KBr) spectra of this compound closely resemble the spectra of *S_{12}-L-Phe-L-His-L-Ala-L-Ala-OMe*; −5.8°; 83.0; 51.

N-*(Dodecyloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L-leucyl-L-leucyl-L-leucine methyl ester (S_{12}-L-Phe-L-His-L-Leu-L-Leu-L-Leu-OMe);* N-*(dodecyloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L-alanyl-L-alanyl-L-alanine methyl ester (S_{12}-L-Phe-L-His-L-Ala-L-Ala-L-Ala-OMe).* These compounds were prepared from the corresponding tetrapeptides *S_{12}-L-Phe-L-His-L-Leu-L-Leu-OH* and *S_{12}-L-Phe-L-His-L-Ala-L-Ala-OH* and H-L-Leu-OMe · HCl and H-L-Ala-OMe · HCl with the aid of 1,3-dicyclohexylcarbodiimide in DMF according to a published method[25,26]. The crude products were purified as described for the tripeptide methyl esters. The final yields amounted to approximately 55%. TLC: R_f 0.35 (Silica, $CHCl_3/CH_3OH$, 10:1 v/v) for both compounds. ^1H NMR ($CDCl_3/CD_3OD$, 1:1 v/v); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C; IR (KBr): CO ester, CO carbamate, CO amide/cm^{-1}.

S_{12}-L-Phe-L-His-L-Leu-L-Leu-L-Leu-OMe: δ 0.9 (m, 21H, 7 × CH_3); 1.3 and 1.6 (m, 29H, $(CH_2)_{10}$ and 3 × CH_2CH (Leu)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.7 (s, 3H, OCH_3); 3.9 (t, 2H, CH_2O); 4.1–4.7 (m, 5H, 5 × CH); 6.75 and 7.45 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −22.9°; 97.8; 1745, 1690, 1650.

S_{12}-L-Phe-L-His-L-Ala-L-Ala-L-Ala-OMe: δ 0.9 (t, 3H, CH_3); 1.3 (m, 29H, $(CH_2)_{10}$ and 3 × CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.6 (s, 3H, OCH_3); 3.9 (t, 2H, CH_2O); 4.2–4.8 (m, 5H, 5 × CH); 6.7 and 7.4 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −36.2°; 87.4; 1745, 1690, 1650.

N-*(Alkoxycarbonyl)-L-(phenylalanyl)-L-histidyl-L-leucine (S_n-L-Phe-L-His-L-Leu-OH);* N-*(benzyloxycarbonyl)-L-(phenylalanyl)-L-histidyl-L-leucine (Z-L-Phe-L-His-L-Leu-OH);* N-*(alkoxycarbonyl)-L-(phenylalanyl)-L-histidyl-L-alanine (S_n-L-Phe-L-His-L-Ala-OH).* The corresponding methyl esters were deprotected as described for the *S_{12}-L-Phe-L-His-L-X-OH* compounds. The final yields after recrys-

tallization from acetone amounted to approximately 70%. ^{1}H NMR (CD_3OD); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C; IR (KBr): CO carbamate, CO amide/cm $^{-1}$.

S_2-L-Phe-L-His-L-Leu-OH: δ 1.0 (m, 9H, 3 × CH_3); 1.3 (m, 3H, CH_2CH (Leu)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 4.0 (q, 2H, CH_2O); 4.2–4.8 (m, 3H, 3 × CH); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im)); 7.15 (s, 5H, ArH) ppm; −2.9°; 258.7 (dec.); 1685, 1645.

S_4-L-Phe-L-His-L-Leu-OH: The ^{1}H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L--Leu-OH*; −7.2°; 238.7; 1685, 1645.

S_6-L-Phe-L-His-L-Leu-OH: The ^{1}H NMR and IR spectra of this compound closely resemble the spectra of *S_4-L-Phe-L-His-L-Leu--OH*; −0.8°; 190.3.

Z-L-Phe-L-His-L-Leu-OH: δ 1.0 (2 × d, 6H, 2 × CH_3); 1.7 (m, 3H, CH_2CH (Leu)); 3.1 (m, 4H, CH_2 (Phe) andCH_2 (His)); 4.0–4.8 (m, 3H, 3 × CH); 5.0 (s, 2H, CH_2O); 7.1 and 7.2 (2 × s, 2 × 5H, 2 × ArH); 6.9 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); −1.5°; 224.6; 1690, 1645.

S_2-L-Phe-L-His-L-Ala-OH: δ 1.0 (t, 3H, CH_3); 1.4 (d, 3H, CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (q, 2H, CH_2O); 4.1–4.8 (m, 3H, 3 × CH); 7.0 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; +6.4°; 255.1 (dec.); 1685, 1650.

S_4-L-Phe-L-His-L-Ala-OH: The ^{1}H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L--Ala-OH*; +5.9°; 254.0 (dec.); 1685, 1650.

S_6-L-Phe-L-His-L-Ala-OH: The ^{1}H NMR and IR spectra of this compound closely resemble the spectra of *S_4-L-Phe-L-His-L-Ala--OH*; +5.8°; 236.2.

N-(Dodecyloxycarbonyl)-L-(phenylalanyl)-L-histidyl-L- (or D-) X-L-Y--OH (S_{12}-L-Phe-L-His-L- (or D-) X-L-Y-OH). The corresponding methyl esters were deprotected as described for the S_{12}-L-Phe-L--His-L-X-OH compounds. The final yields after recrystallization from acetone/methanol amounted to approximately 75%. ^{1}H NMR (CD_3OD); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C; IR (KBr): CO carbamate, CO amide/cm $^{-1}$.

S_{12}-L-Phe-L-His-L-Leu-L-Leu-OH: δ 0.9 (m, 15H, 5 × CH_3); 1.3 (m, 26H, $(CH_2)_{10}$ and 2 × CH_2CH (Leu)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.1–4.7 (m, 4H, 4 × CH); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im)); 7.15 (s, 5H, ArH) ppm; −19.8°; 190.6; 1685, 1650.

S_{12}-L-Phe-L-His-L-Leu-D-Leu-OH: The ^{1}H NMR spectrum of this compound closely resembles the spectrum of *S_{12}-L-Phe-L-His-L--Leu-L-Leu-OH*; −3.9°; 200.6; 1685, 1645.

S_{12}-L-Phe-L-His-L-Leu-L-Ala-OH: δ 0.9 (m, 9H, 3 × CH_3); 1.3 (m, 26H, $(CH_2)_{10}$ and CH_2CH (Leu) and CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t. 2H, CH_2O); 4.1–4.7 (m, 4H, 4 × CH); 7.0 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −14.5°; 189.5; 1690, 1645.

S_{12}-L-Phe-L-His-L-Ala-L-Leu-OH: δ 0.9 (m, 9H, 3 × CH_3); 1.3 (m, 26H, $(CH_2)_{10}$ and CH_2CH (Leu) and CH_3 (Ala)); 3.0 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.1–4.7 (m, 4H, 4 × CH); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im)); 7.2 (s, 5H, ArH) ppm; −14.2°; 195.2; 1690, 1645.

S_{12}-L-Phe-L-His-L-Ala-L-Ala-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 20H, $(CH_2)_{10}$); 1.4 (2 × d, 2 × 3H, 2 × CH_3 (Ala)); 3.1 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.95 (t, 2H, CH_2O); 4.2–4.8 (m, 4H, 4 × CH); 7.1 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.15 (s, 5H, ArH) ppm; −11.5°; 231.7; 1685, 1645.

S_{12}-L-Phe-L-His-L-Ala-D-Ala-OH: The ^{1}H NMR and IR (KBr) spectra of this compound closely resemble the spectra of *S_{12}-L--Phe-L-His-L-Ala-L-Ala-OH*; +3.4°; 222.4.

N-(Dodecycloxycarbonyl)-(L-phenylalanyl)-L-histidyl-L-leucyl-L-leucyl--L-leucine (S_{12}-L-Phe-L-His-L-Leu-L-Leu-L-Leu-OH); *N-(dodecyclocarbonyl)-(L-phenylalanyl)-L-histidyl-L-alanyl-L-alanyl-L-alanine (S_{12}--L-Phe-L-His-L-Ala-L-Ala-L-Ala-OH).* The corresponding methyl esters were deprotected as described for the *S_{12}-L-Phe-L-His-L--X-OH* compounds. The final yields after recrystallization from acetone amounted to aprroximately 70%. ^{1}H NMR (CD_3OD); $[\alpha]_D^{20}$/(*c* 1.0, methanol); m.p./°C; IR (KBr): CO carbamate, CO amide/cm $^{-1}$.

S_{12}-L-Phe-L-His-L-Leu-L-Leu-L-Leu-OH: δ 0.9 m, (21H, 7 × CH_3); 1.3 and 1.7 (m, 29H, $(CH_2)_{10}$ and 3 × CH_2CH (Leu)); 3.1 (m, 4H, CH_2 (Phe) and CH_2 (His)); 3.9 (t, 2H, CH_2O); 4.1–4.7 (m, 5H, 5 × CH); 7.0 and 8.0 (2 × s, 2 × 1H, 2 × CH (Im)); 7.15 (s, 5H, ArH) ppm; −16.5°; 186.0; 1685, 1650.

S_{12}-L-Phe-L-His-L-Ala-L-Ala-L-Ala-OH: δ 0.9 (t, 3H, CH_3); 1.3 (m, 29H, $(CH_2)_{10}$ and 3 × CH_3 (Ala)); 3.1 (m, 4H, CH_2 (Phe) and CH_2 (His)); 4.2–4.8 (m, 5H, 5 × CH); 7.1 and 8.1 (2 × s, 2 × 1H, 2 × CH (Im)); 7.15 (s, 5H, ArH) ppm; −22.7°; 236.0; 1685, 1645.

N-Acyl-L- (or D-) phenylalanine 4-nitrophenyl ester [(C_n-L- (or D-) Phe--ONp (n = 2, 4, 7, 12, 16)]. The enantiomeric substrates were prepared and purified as described for the C_{12}-L- (or D-) Phe-ONp substrates in our previous paper[1]. ^{1}H NMR ($CDCl_3$); IR (KBr): CO ester, CO amide, NO_2 (3 ×)/cm $^{-1}$; $[\alpha]_D^{20}$/(*c* 1.0, $CHCl_3$); m.p./°C.

C_2-L- (or D-) Phe-ONp: δ 2.1 (s, 3H, CH_3CO); 3.2 (d, 2H, CH_2); 5.0 (2 × t, 1H, CH); 5.8 (d, 1H, NH); 7.0 and 8.1 (2 × d, 2 × 2H, $ArNO_2$); 7.1 (m, 5H, ArH) ppm; 1755, 1635, 1525, 1490, 1347; L: −18.5°; 139.7; D: +18.2°; 138.4.

C_4-L- (or D-) Phe-ONp: δ 0.95 (t, 3H, CH_3); 1.3 (m, 2H, CH_2); 2.2 (t, 2H, CH_2CO); 3.2 (d, 2H, CH_2); 5.0 (2 × t, 1H, CH); 5.9 (d, 1H, NH); 7.0 and 8.1 (2 × d, 2 × 2H, $ArNO_2$); 7.1 (m, 5H, ArH) ppm; IR (KBr): as described for the C_{12}-L- (or D-) Phe-ONp compounds; L: −14.2°; 131.5; D: +14.0°; 130.1.

C_7-L- (or D-) Phe-ONp: The ^{1}H NMR and IR spectra of these compounds closely resemble the spectra of *C_{12}-L- (or D-) Phe-ONp*; L: −12.7°; 119.0; D: +12.1°; 119.7.

C_{16}-L- (or D-) Phe-ONp: The ^{1}H NMR and IR spectra of these compounds closely resemble the spectra of *C_{12}-L- (or D-) Phe-ONp*; L: −9.6°; 101.5; D: +9.4°; 102.0.

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