Synthesis of Analogues of the Carboxyl Protease Inhibitor Pepstatin. Effect of Structure on Inhibition of Pepsin and Renin[1]

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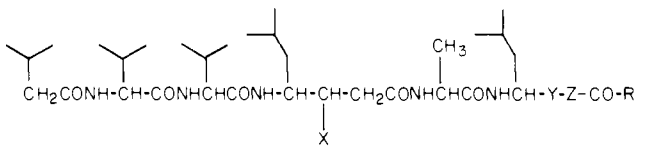
Analogues of the carboxyl protease inhibitor, pepstatin, were synthesized from optically pure forms of *N*-(*tert*-butoxycarbonyl)-4-amino-3-hydroxy-6-methylheptanoic acid (Boc-Sta), and the inhibition of pepsin and renin was determined. In addition, the new amino acid (3*S*,4*S*)-4-amino-3-hydroxy-5-phenylpentanoic acid [AHPPA] was synthesized and the stereochemistry of the 3 and 4 positions established. The tripeptides isovaleryl-L-valyl-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoyl-L-alanine isoamylamide [Iva-Val-(3*S*,4*S*)-Sta-Ala-NH^{i}C$_5$H$_{11}$] and Iva-Val-(3*S*,4*S*)-AHPPA-Ala-NH^{i}C$_5$H$_{11}$ were found to be potent inhibitors of pepsin with $K_i = 1 \times 10^{-9}$ and 0.9×10^{-9} M, respectively. Changing the chirality of the (3*S*)-hydroxy group to 3*R* or shortening the peptide chain diminished binding to pepsin over 100-fold. Three structural requirements necessary for potent inhibition of pepsin are proposed.

Pepstatin, isovaleryl-L-valyl-L-valyl-(3*S*,4*S*)-4-amino-3-hydroxyl-6-methylheptanoyl-L-alanyl-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid (1; Chart I)[2] is one of several enzyme inhibitors discovered by Umezawa[3] as a result of efforts to identify naturally occurring inhibitors of therapeutically important enzymes. Pepstatin is a specific inhibitor of carboxyl proteases. Pepstatin binds to the active site of these enzymes,[4,5] and the dissociation constant for the enzyme–pepstatin complex (4.57×10^{-11} M for pepsin)[6] is unusually small. Pepstatin contains a novel amino acid in residue positions 3 and 5, called statine (Sta), which appears to be important to inhibition.

Information relating the structure of pepstatin to its specificity for carboxyl proteases would increase our knowledge of mechanisms of inhibition of tight-binding enzyme inhibitors and could facilitate the development of new inhibitors of renin or cathepsin D, two carboxyl proteases implicated in hypertension and inflammation, respectively. Several pepstatin analogues have been synthesized.[7-9] One of these, Iva-Val-Sta-Ala-Sta, which corresponds to deletion of one valyl residue from pepstatin is a potent inhibitor, but smaller statine-containing peptides have not been studied systematically. We report here the synthesis of pepstatin analogues modified in the C-terminal tetrapeptide region of the molecule and studies of the inhibition of pepsin and renin produced by these analogues. The synthesis of a new statine-like amino acid is also described. Our results help define the structural features needed for potent inhibition of pepsin and renin.

Chart I. Structures of Pepstatin and Related Derivatives[a]

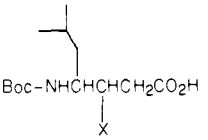


1 (pepstatin), X = (*S*)-OH; Y = (*S*)-CHOH; Z = CH_2; R = OH
19 (5-dehydropepstatin), X = (*S*)-OH; Y-Z = CH=CH; R = OH
20 (pepstatone A), X = (*S*)-OH; Y = CO; Z = CH_3
21 (dideoxypepstatin), X = H; Y = Z = CH_2; R = OH

[a] Numbers below amino acids designate residue number described in text.

Results

Synthesis of Amino Acids. The synthesis of optically pure Boc-(3*S*,4*S*)-Sta (**2a**), Boc-(3*R*,4*S*)-Sta (**2b**), and



2a, X = (*S*)-OH
b, X = (*R*)-OH
3, X = H

Boc-dSta (**3**) have been described.[10,11] To test certain biological hypotheses, we also synthesized the new amino acid 4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoic acid (**7a**, AHPPA; Scheme I) according to a modification of the procedure used to synthesize statine.[10]

N-(*tert*-Butyloxycarbonyl)-L-phenylalaninal (**4**) was obtained in 97% yield by reduction of Boc-L-Phe methyl ester with diisobutylaluminum hydride. Because aldehyde derivatives of protected amino acids and peptides can racemize readily,[12] a sample of aldehyde **4** was reduced with sodium borohydride to *N*-(*tert*-butoxycarbonyl)-L-phenylalaninol. The rotation of the alcohol was nearly

(1) Abstracted in part from the Ph.D. Thesis of Eric T. O. Sun, submitted to the University of Wisconsin—Madison, May 1979. Abbreviations used follow IUPAC–IUP tentative rules as described in *J. Biol. Chem.*, **247**, 977 (1972). Additional abbreviations used are: DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DMF, dimethylformamide; HOBt, 1-hydroxybenzotriazole; Boc, *tert*-butyloxycarbonyl; Sta, 4-amino-3-hydroxy-6-methylheptanoic acid (statine); AHPPA, 4-amino-3-hydroxy-5-phenylpentanoic acid; dSta, 4(*S*)-amino-6-methylheptanoic acid (deoxystatine).
(2) H. Umezawa, T. Aoyagi, H. Morishima, M. Matsuzaki, H. Hamada, and T. Takeuchi, *J. Antibiot.*, **23**, 259 (1970).
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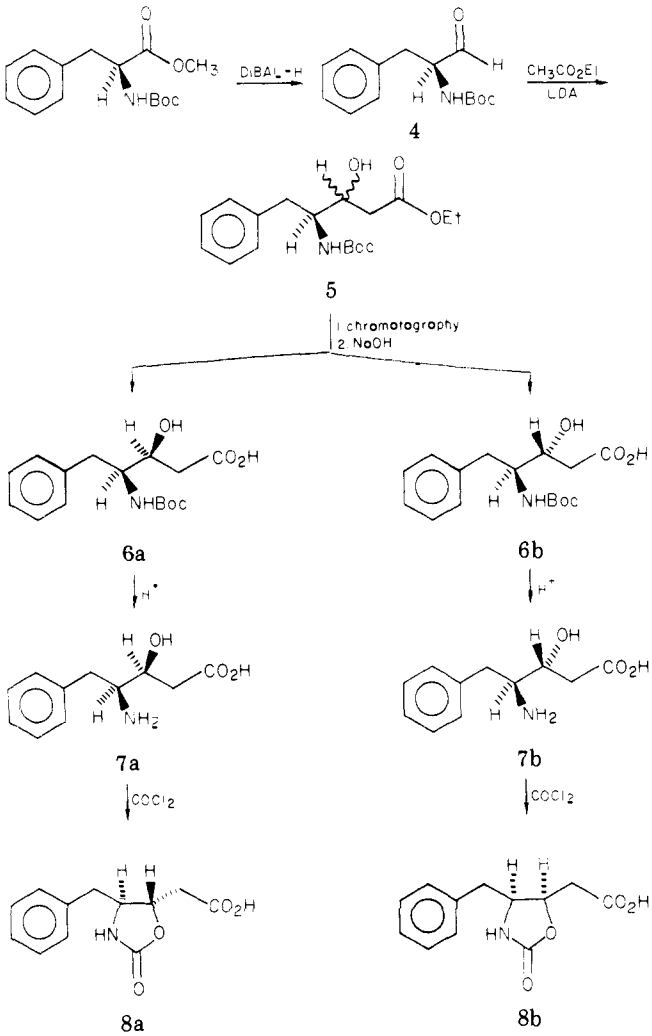
Table I. Optical Rotations and Melting Points of AHPPA Derivatives[a]

no.	compd	$[\alpha]^{24}_D$, deg	mp, °C
5a	Boc-(3*S*,4*S*)-AHPPA-OEt	−35.9 (c 1.0, CH_3OH)	88-89
5b	Boc-(3*R*,4*S*)-AHPPA-OEt	−14.2 (c 1.0, CH_3OH)	140-140.5
6a	Boc-(3*S*,4*S*)-AHPPA-OH	−37.0 (c 1.1, CH_3OH)	148-148.5
6b	Boc-(3*R*,4*S*)-AHPPA-OH	−16.1 (c 1.1, CH_3OH)	187.5
7a	H-(3*S*,4*S*)-AHPPA-OH	−24.8 (c 0.44, H_2O)	193
7b	H-(3*R*,4*S*)-AHPPA-OH	−42.7 (c 0.12, H_2O)	170

[a] AHPPA is an abbreviation for 4-amino-3-hydroxy-5-phenylpentanoic acid.

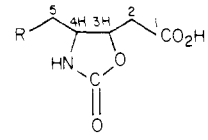
Scheme I. Synthesis of 4-Amino-3-hydroxy-5-phenylpentanoic Acid Derivatives



identical with an authentic sample obtained by reduction of Boc-L-Phe methyl ester so that less than 4% racemization occurred during the synthesis of 4. Without purification, aldehyde 4 was reacted with lithium–ethyl acetate to give esters **5a,b** in 55% yield. The diastereomers were separated by chromatography and saponified to obtain the Boc amino acids **6a** and **6b**. Removal of the Boc group gave the free amino acids **7a** and **7b**. The diastereomers of AHPPA and derivatives **5** and **6** could be distinguished by melting point and optical rotation (Table I).

To assign the absolute stereochemistry of the AHPPA diastereomers **7a,b**, the oxazolidinones **8a,b** were prepared by reaction with phosgene and their NMR spectra obtained (Table II). The assignment of stereochemistry for **8a** and **8b** is based on the vicinal coupling, $^3J_{3,4}$, between protons on the third and fourth carbons. It has been shown from studies of the 2-oxazolidinone derivatives of 2-amino-3-hydroxy acids that the vicinal coupling constant ($^3J_{2,3}$) for the cis (erythro) isomer is 9.6 ± 0.6 Hz and that

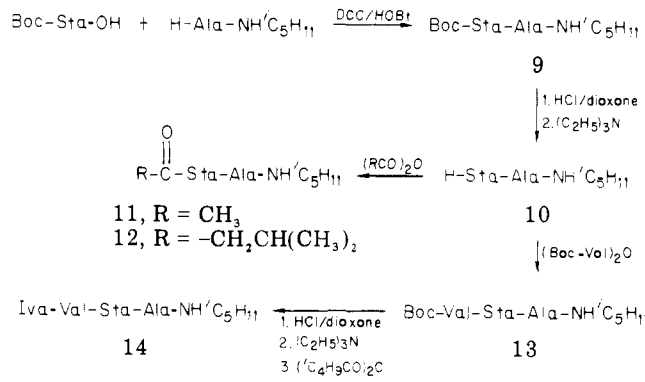
Table II. Chemical Shifts and Coupling Constants of 2-Oxazolidinone Derivatives[a]



2-oxazolidine from	H_{C-3}, δ (ppm)	J_{3-4}, Hz
(3*S*,4*S*)-Boc-AHPPA-OEt (5a; R = Ph)	4.7	5.0
(3*R*,4*S*)-Boc-AHPPA-OEt (5b; R = Ph)	5.1	8.8
(3*S*,4*S*)-statine (2a; R = *i*-Pr)	4.5	5.0
(3*R*,4*S*)-statine (2b; R = *i*-Pr)	5.1	8.8

[a] AHPPA is an abbreviation for 4-amino-3-hydroxy-5-phenylpentanoic acid.

Scheme II. Synthesis of Pepstatin Analogues



$^3J_{2,3}$ for the trans (threo) isomer is 5.0 ± 1 Hz.[13] The data in Table II indicate that the higher R_f AHPPA diastereomer **5a** is converted to the *threo*-carbamate **8a** so that the stereochemistry is 3*S*,4*S*. The lower R_f AHPPA diastereomer **5b** is converted to the *erythro*-carbamate **8b** so that the stereochemistry of **5b** is 3*R*,4*S*. To confirm that this method is valid for establishing the stereochemistry of these amino acid derivatives, oxazolidinones were prepared from (3*S*,4*S*)-statine and (3*R*,4*S*)-statine. The chemical shifts and coupling constants for the C-3 proton of the oxazolidinone derivatives from statine are in excellent agreement (Table II) with those obtained from **8a** and **8b**.

Synthesis of Pepstatin Analogues. Pepstatin analogues in which the C-terminal β-hydroxypropionic acid group [$CH(OH)CH_2CO_2H$] had been deleted were synthesized by the stepwise procedure outlined in Scheme II. The key intermediate dipeptide **9** first was prepared using optically pure (3*S*,4*S*)-Boc-Sta. Later the physical properties of peptide amide **9a** (3*S*,4*S* diastereomer; Table III) were found to differ from those of the diastereomeric peptide **9b** [(3*R*,4*S*)-statine], permitting separation of both diastereomers **9a,b** by column chromatography over silica

(13) S. Futagawa, T. Inui, and T. Shiba, *Bull. Chem. Soc. Jpn.*, **46**, 3308 (1973).

Table III. Optical Rotations and Melting Points of Synthetic Statine- and AHPPA-Containing Peptides

no.	compd[a]	$[\alpha]^{22}_D$, deg	mp, °C
9a	Boc-(*S*,*S*)-Sta-Ala-NHR	−33.9 (c 1.0, CH_3OH)	130-131
9b	Boc-(*R*,*S*)-Sta-Ala-NHR	−22.5 (c 1.0, CH_3OH)	151-152
11	Ac-(*S*,*S*)-Sta-Ala-NHR		68-70
12a	Iva-(*S*,*S*)-Sta-Ala-NHR	−47.7 (c 0.6, CH_3OH)	114-116
12b	Iva-(*R*,*S*)-Sta-Ala-NHR	−29.4 (c 0.5, CH_3OH)	178-180
13a	Boc-Val-(*S*,*S*)-Sta-Ala-NHR	−43.4 (c 0.4, CH_3OH)	173.5-174.5
13b	Boc-Val-(*R*,*S*)-Sta-Ala-NHR	−43.0 (c 0.46, CH_3OH)	212
14a	Iva-Val-(*S*,*S*)-Sta-Ala-NHR	−61.1 (c 0.5, CH_3OH)	243-245
14b	Iva-Val-(*R*,*S*)-Sta-Ala-NHR	−54.6 (c 0.4, CH_3OH)	252.5
15	Boc-(3*S*,4*S*)-AHPPA-Ala-NHR	−28.3 (c 1.0, CH_3OH)	113
16	Boc-Val-(*S*,*S*)-AHPPA-Ala-NHR	−49.4 (c 0.43, CH_3OH)	191.5-192.5
17	Iva-Val-(*S*,*S*)-AHPPA-Ala-NHR	−58.8 (c 0.40, CH_3OH)	237 dec
18	Iva-Val-dSta-Ala-NHR	−36.8 (c 0.068, CH_3OH)	261

[a] R = $-CH_2CH_2CH(CH_3)_2$; AHPPA, 4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoic acid; dSta = 4(*S*)-amino-6-methylheptanoic acid.

Table IV. Inhibition of Pepsin and Renin by Pepstatin Analogues[a]

no.	compd	pepsin K_i, M	renin IC_{50}, M
1	Iva-Val-Val-Sta-Ala-Sta	4.57×10^{-11}	3.2×10^{-7}
11	Ac-Sta-Ala-$NH^iC_5H_{11}$	3×10^{-5}	$>3.3 \times 10^{-4}$
12a	Iva-Sta-Ala-$NH^iC_5H_{11}$	3.5×10^{-7}	$>3.3 \times 10^{-4}$
14a	Iva-Val-(3*S*,4*S*)-Sta-Ala-$NH^iC_5H_{11}$	1.1×10^{-9}	1.4×10^{-4}
14b	Iva-Val-(3*R*,4*S*)-Sta-Ala-$NH^iC_5H_{11}$	3×10^{-6}	
17	Iva-Val-(3*S*,4*S*)-AHPPA-Ala-$NH^iC_5H_{11}$	0.9×10^{-9}	1.4×10^{-4}
18	Iva-Val-dSta-Ala-$NH^iC_5H_{11}$	$>6 \times 10^{-6}$	
22	Iva-Val-Sta	$\sim 10^{-6}$	$>3.3 \times 10^{-4}$
23	Iva-Val-Val-Sta	$\sim 10^{-6}$	$>3.3 \times 10^{-4}$

[a] AHPPA, 4-amino-3-hydroxy-5-phenylpentanoic acid; dSta, 4(*S*)-amino-6-methylheptanoic acid; Iva, isovaleryl.

gel (TLC R_f (B) 0.31 for **9a**, 0.38 for **9b**). As a result, it was convenient to synthesize pure **9a** and **9b** from the mixture **2a**,**b**.

After removing the *tert*-butoxycarbonyl protecting group from **9a**, the hydrochloride salt was neutralized with triethylamine, and dipeptide **10** was acetylated with acetic anhydride in DMF to give the *N*-acetyl analogue 11. When the *N*-isovaleryl derivative **12** was prepared by reaction of dipeptide **10** with 1 equiv each of isovaleric acid and DCC, peptide **12** was obtained only in a low yield (24%). However, when preformed isovaleric acid anhydride (2 equiv) was used as the acylating agent, an excellent yield (90%) of **12** was obtained. Therefore, the symmetrical anhydride method was used throughout the remainder of our work for preparing the longer peptides listed in Table III. This method generally gave good to excellent yields of products.

Peptides in which the Sta residue had been replaced by another amino acid were synthesized in a stepwise manner employing the procedure described above for synthesizing statine-containing peptides. Tripeptide **17** [Iva-Val-(3*S*,4*S*)-AHPPA-Ala-$NH^iC_5H_{11}$], which is analogous to analogue **14a** except that (3*S*,4*S*)-AHPPA has replaced (3*S*,4*S*)-statine, was synthesized from Boc-(3*S*,4*S*)-AHPPA (**6a**). In a similar fashion, the deoxystatine-containing peptide **18** was synthesized by using Boc-dSta (**3**)[10,11] in place of Boc-Sta (**2a**).

Biological Results. The potency of the synthetic pepstatin analogues as inhibitors of carboxyl proteases was measured in two assay systems, and the results are presented in Table IV. Pepsin inhibition was measured using the synthetic heptapeptide substrate Phe-Gly-His-Phe-(NO_2)-Phe-Ala-Phe-OMe as previously described.[11] Inhibition was measured at five substrate and three inhibitor concentrations, and inhibition constants (K_i) were calculated as described previously for each analogue.[11]

Inhibition of renin was measured using hog kidney renin and the radiolabeled synthetic substrate, H-Ile-His-Pro-Phe-His-Leu-[^{14}C]Leu-Val-Tyr-Ser-OH. The procedure is based on a double enzyme incubation.[18] In the first incubation, renin acted upon the Leu–Leu bond of the synthetic decapeptide to release the labeled tetrapeptide, [^{14}C]Leu-Val-Tyr-Ser. The amino terminal [^{14}C]leucyl residue was hydrolyzed in a subsequent incubation after addition of leucine aminopeptidase at a pH which precluded further renin activity. Unhydrolyzed substrate was removed by adsorption and the radioactivity remaining in the supernatant served as the measure of renin activity. The inhibition results, expressed as IC_{50} values (Table IV), typically were obtained by plotting data from four inhibitor concentrations. The highest concentration tested was 3.3×10^{-4} M.

Discussion

The decision to modify the C-terminal residue of pepstatin was based on reports from Umezawa's laboratories that the C-terminal residue could be chemically modified without destroying tight binding to pepsin. For example, 5-dehydropepstatin (**19**; Chart I), which corresponds to removal of the hydroxyl group by dehydration of the fifth residue of pepstatin, is a good pepsin inhibitor,[4] as is pepstatone A (**20**) which lacks both the hydroxyl group and the carboxyl group in the fifth residue.[7] Within the limits of the assay employed to test inhibition of pepsin, compounds **19** and **20** were found to be equipotent with pepstatin as pepsin inhibitors. Thus, neither the hydroxyl nor the carboxyl group in the fifth residue of pepstatin is essential for tight-binding inhibition.

Therefore, we synthesized a series of pepstatin analogues in which this C-terminal residue was further simplified by replacing the C-terminal statine residue with an *N*-isoamyl amide group. This substituent corresponds to replacing the $-CH(OH)CH_2CO_2H$ group of statine with a hydrogen atom. As is clear from the data (Table IV), this structural simplification is compatible with good inhibition of pepsin. Compounds **14a** and **17** are only about 20-fold weaker pepsin inhibitors than is pepstatin. In addition, compound **14a** was found to be a readily reversible, competitive in-

hibitor of pepsin and because of its simpler kinetic properties was chosen as the standard for further structure–activity correlations in the pepstatin series.

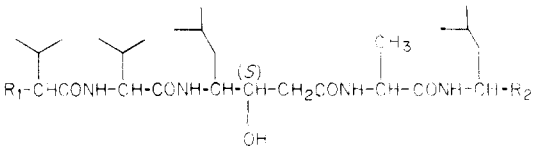
Previous work had shown that one or more of the hydroxyl groups in pepstatin was needed for tight-binding inhibition of pepsin. Dideoxypepstatin (**21**), which corresponds to the deletion of the hydroxyl groups in both statine residues in positions 3 and 5 of pepstatin, is at least 2000-fold weaker than pepstatin in inhibiting pepsin.[11] This figure was based on an early estimate of the pepstatin inhibition constant ($K_i = 10^{-10}$ M).[7] More recent data indicate that the dissociation constant for the pepsin–pepstatin complex is as low as 4.57×10^{-11} M,[6] in which case dideoxypepstatin would be ~4000-fold weaker than pepstatin as a pepsin inhibitor. In contrast to the weak inhibitor produced by deletion of both hydroxyl groups, removal of only a single hydroxyl group in the fifth residue of pepstatin (e.g., **19** and **20**) gave good pepsin inhibitors.[7] The implication of these findings is that the hydroxyl group in the third residue of pepstatin is essential to the tight-binding inhibition.

To test this hypothesis, we chose to synthesize analogues of peptide **14a** in which the hydroxyl group was systematically varied. Our results (Table IV) show that changing the hydroxyl chirality from *S* to *R* (as in compound **14b**) or deletion of the hydroxyl group (as in compound **18**) produced analogues which were about 1000-fold weaker pepsin inhibitors than reference compound **14a**. This difference in potency is roughly the same order of magnitude as the difference in inhibitory potencies between pepstatin (**1**) and dideoxypepstatin (**21**). The two sets of data point to the importance of a 3(*S*)-hydroxyl group in the third residue of pepstatin as a contributor to tight-binding inhibition.

Although it is clear that a 3(*S*)-hydroxyl group in residue 3 is an important contributor to tight-binding inhibition of pepsin, it is also evident that the length of the acyl group on the amino group of statine contributes substantially to inhibition (Table IV). Thus, as the chain length, and hydrophobic character, of the *N*-acyl group is extended from an acetyl group (**11**) to an isovaleryl-Val group (**14a**) in compounds which contain the correct (3*S*,4*S*)-Sta residue, the dissociation constant decreases 10000-fold. However, when the isobutyl side chain of the statine residue in **14a** is replaced by a more hydrophobic benzyl group, as in the AHPPA analogue **17**, the dissociation constant for pepsin inhibition does not decrease significantly. This shows that addition of hydrophobic groups to the pepstatin analogues does not always lead to tighter-binding pepsin inhibitors and that the position of the hydrophobic group must be important. The AHPPA analogue **17** is a slightly better inhibitor of pepsin and this shows that the larger benzyl group does not diminish binding.

Variations in the C-terminal dipeptide portion of pepstatin were not studied systematically in the present work beyond the simplification of the statine residue to an isoamyl amide group already described. Several pepstatin analogues are known in which the C-terminal dipeptide unit, Ala-Sta, was removed (e.g., **22** and **23**), and suggestions have been made that these are good pepsin inhibitors because their IC_{50} values are similar to pepstatins. However, analysis of the reported inhibition data establishes that **22** and **23** are approximately 1000- to 2000-fold weaker pepsin inhibitors than **14a** or **17** (Table IV). It has been shown that under conditions where $IC_{50} \geq 100[E_t]$, $IC_{50} = K_i(1 + S/K_m)$.[17] Therefore the inhibition constants for analogues **22** and **23** are about 10^{-6} to 10^{-7} M,[8,9] so that these compounds are at least 10000-fold weaker inhibitors of pepsin than pepstatin even though their IC_{50} values are about the same. The discrepancy between IC_{50} and K_i arises because pepstatin is a tight-binding inhibitor ($K_i = 4.57 \times 10^{-11}$ M) of pepsin. Comparisons of the inhibitory potencies of analogues of tight-binding inhibitors using IC_{50} values are meaningful only when the enzyme concentration is known and is substantially lower than the apparent IC_{50} of the analogue. This follows from the fact that the IC_{50} of an inhibitor cannot be less than one-half the enzyme concentration when $[E]_t = 100K_i$.[14-16] In most pepsin assays the enzyme concentration is between 10^{-8} to 10^{-6} M. Under these conditions, the IC_{50} for pepstatin would be approximately one-half the enzyme concentration and this IC_{50} would not reflect the very small K_i for the pepstatin–pepsin binding.

Chart II. Comparisons of Structures of Tight-Binding Pepstatin Analogues 14a with Pepstatin[a]



14a, R_1 = H; R_2 = H
1, R_1 = Iva-NH-; R_2 = –CH(OH)CH_2CO_2H

[a] Numbers below amino acids designate residue number in text.

The effect of structure of pepstatin on inhibition of hog kidney renin was studied using compounds **1**, **12a**, **14a**, and **17** (Table IV). Although the limited number of compounds precludes a detailed interpretation of the structure–activity relationships on this enzyme, it is clear that the modifications of pepstatin studied here diminish inhibition of renin far more than they diminish inhibition of pepsin. Thus, compounds **14a** and **17** are about 450-fold weaker inhibitors of renin than is pepstatin, in contrast to the 20- to 25-fold difference when pepsin inhibition is measured. As was found for pepsin, replacement of the isobutyl side chain of statine in **14a** with the benzyl group (**17**) did not change the IC_{50} on renin.

In summary, our data indicate that for good pepsin inhibition a pepstatin analogue must have a 3(*S*)-hydroxyl group in the third residue, a substituent occupying space equivalent to the first residue in pepstatin, and a C-terminal group extending from the statine residue. These requirements are illustrated in Chart II, where the structures of pepstatin and **14a** are compared. The isovaleryl group in **14a** corresponds to a deaminovaline residue which can be postulated to bind to the same site on pepsin that the valine in residue 1 of pepstatin binds to. This appears to be an important binding site, since removal of this group, as in compound **12a**, causes a 1000-fold decrease in binding to pepsin.

In a similar fashion, it is clear that some groups in amino acid residues 4 and 5 augment inhibition because Iva-Val-Val-Sta (**23**) is a 1000-fold weaker inhibitor of pepsin than **14a** and over 10000-fold weaker than pepstatin with which it shares an identical N-terminal sequence. The minimum size of the C-terminal group has not been determined, but the high potency of **14a**, which contains the

(14) J. F. Morrison, *Biochim. Biophys. Acta*, **185**, 269 (1969).
(15) P. J. F. Henderson, *Biochem. J.*, **127**, 321 (1972).
(16) S. Cha, *Biochem. Pharmacol.*, **24**, 2177 (1975).
(17) Y.-C. Cheng and W. H. Prusoff, *Biochem. Pharmacol.*, **22**, 3099 (1973).

Ala-NH^{i}C$_5$H$_{11}$ group, establishes that the β-hydroxypropionic acid portion of the Ala-Sta structure is not essential for good pepsin inhibition.

Finally, our results indicate that this minimal structure for pepsin inhibition embodied in **14a** in not sufficient to strongly inhibit renin. The greater potency of the analogue phenoxyacetyl-Val-Sta-Ala-Sta relative to Iva-Val-Sta-Ala-Sta[7] suggests that further extension of the N-terminal portion of the peptide chain in **14a** may lead to improved renin inhibitors. A detailed analysis of the kinetics of pepstatin inhibition of pepsin will be reported separately.

Experimental Section

Melting points were determined with a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were determined on a Perkin-Elmer Model 241 automatic polarimeter (1,000-dm cell). Proton nuclear magnetic resonance spectra were recorded on Varian Model EM-390, Bruker HX-90E-pulse Fourier transform NMR interfaced with a Nicolet 1080 computer and disc unit, and Bruker WH270 spectrometers. Chemical shifts were reported as δ units (ppm) relative to tetramethylsilane or 3-(trimethylsilyl-d_4)propionic acid, sodium salt, as internal standards. Low-resolution mass spectra were determined on a Finnigan Model 1015 mass spectrometer. High-resolution mass spectra were determined on AEI MS 902 C. Infrared spectra were determined on a Perkin-Elmer Model 257 recording spectrophotometer. TLC solvent systems used were: A, ethyl acetate–benzene (20:80); B, methanol–chloroform (8:92); C, methanol–chloroform (10:90); D, chloroform–methanol–acetic acid (31:2:0.5); E, butanol–acetic acid–water (4:1:1).

General Procedure A. Removal of the *tert*-Butoxycarbonyl Group. Boc amino acid or peptide (1 mmol) in a solution of ~4 N HCl in dioxane (3–5 mL) was stirred at room temperature for 30 min. After the removal of excess reagent under reduced pressure, the solid residue was triturated with ether several times to give a solid and dried in vacuo for a minimum of 8 h. All peptide hydrochlorides were prepared by this method.

The Boc group also could be removed by stirring in 25% trifluoroacetic acid in methylene chloride for 30 min at room temperature.

General Procedure B. Coupling Reactions Using Dicyclohexylcarbodiimide/1-Hydroxybenzotriazole.[19] The amino hydrochloride (1 mmol, unless otherwise specified; prepared by general procedure A) was dissolved in methylene chloride or DMF (5 mL) and neutralized at 0 °C with triethylamine (139 μL, 1 mmol). Boc amino acid (1.0 mmol) and HOBt (1.5 mmol) were added, followed by a solution of DCC (206 mg, 1 mmol) in methylene chloride (5 mL). The reaction mixture was allowed to stir at 4 °C overnight (12–14 h, unless otherwise specified) and at room temperature for 2–3 h. DCU was filtered, and the filtrate was evaporated under reduced pressure and elevated temperature. The residue was dissolved in ethyl acetate or 1-butanol, washed successively with cold 1 N HCl and 1 N NaOH, and dried ($MgSO_4$ or Na_2SO_4). The peptide was purified by silica gel column chromatography (gradient elution: $CHCl_3$ to 10% CH_3OH in $CHCl_3$) and crystallized from chloroform or ethyl acetate–Skellysolve B, unless otherwise specified.

General Procedure C. Preparation of Symmetrical Anhydride. Boc amino acid or carboxylic acid (2 equiv) and DCC (1 equiv) in methylene chloride were stirred at 0 °C for 40 min. The reaction mixture was cooled for 20–30 min (−30 °C) and filtered to remove DCU. The filtrate was used immediately without further purification.

General Procedure D. Coupling Reactions and N-Acylation Reactions via Symmetrical Anhydride. A solution of the hydrochloride of deprotected peptide (1 mmol, unless otherwise specified) in DMF (5 mL) was cooled to 0 °C and neutralized with triethylamine (139 μL, 1 mmol). After the addition of a solution of the symmetrical anhydride (2 equiv) in CH_2Cl_2, stirring was continued at 4 °C for about 40 h and then at room temperature for 3 h. The solvent was removed under reduced pressure and elevated temperature, and the crude reaction mixture was dissolved in ethyl acetate or 1-butanol, washed with cold 1 N HCl and saturated $NaHCO_3$, and dried ($MgSO_4$ or Na_2SO_4). The solvent was removed under reduced pressure and elevated temperature to give a solid residue, which was purified by silica gel chromatography (gradient elution: 2% CH_3OH in $CHCl_3$ to 10% CH_3OH in $CHCl_3$) and by crystallization.

General Procedure E. Saponification of *N*-(*tert*-Butoxycarbonyl) Amino Acid Esters in Aqueous Dioxane.[20] A stirred solution of Boc amino acid ester (2 mmol) in aqueous dioxane (4 mL of dioxane/2 mL of H_2O) was maintained at pH 10 with 2 N NaOH for 30 min. The reaction mixture was washed once with ether and then acidified to pH 2–3 with cold 1 N hydrochloric acid. The aqueous layer was extracted with ethyl acetate. The organic layer was dried ($MgSO_4$) and evaporated to give Boc amino acid.

General Procedure F. Ion-Exchange Chromatography of Amino Acid Hydrochloride. The amino acid hydrochloride (or TFA salt; <300 mg) was dissolved in a minimum amount of eluting buffer and chromatographed over AG50W-X8 at room temperature, using as eluting solvent a pH 5.0, 0.1 N buffer solution of pyridine–acetic acid–water (16:10:1974 mL). Fractions of 4.0 mL were collected at a flow rate of 0.51 mL min^{-1}; generally, the amino acid was eluted from the column after about 32 fractions. The presence of amino acid was detected by ninhydrin. The appropriate fractions were combined and then concentrated under reduced pressure and elevated temperature (40 °C). Lyophilization of the concentrated aqueous solution gave the amino acid as a white powder.

Heptapeptide substrate Phe-Gly-His-Phe(NO_2)-Phe-Ala-Phe(OMe) was prepared as described.[11] Pepsin assay and determination of dissociation constants on pepsin have been described.[11]

***N*-(*tert*-Butoxycarbonyl)-L-phenylalaninal (4).** The title compound was prepared in 97% yield (crude) by the procedure described for the synthesis of *N*-(*tert*-butoxycarbonyl)-L-leucinal:[10] NMR ($CDCl_3$) δ 1.4 (s, 9 H), 2.9 (2 H), 4.2 (1 H), 5.3 (1 H, NH), 7.3 (s, 5 H), 9.6 (s, 1 H).

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-3(*RS*)-hydroxy-5-phenylpentanoic Acid Ethyl Ester (5a,b; Boc-AHPPA-OEt).** The diastereomeric mixture was prepared in 55–60% yield from *N*-(*tert*-butoxycarbonyl)-L-phenylalaninal (**4**) using the procedure described for preparing Boc-Sta-OEt.[10]

Chromatography of mixture **5a,b** over silica gel with a gradient of 10 to 50% ethyl acetate in benzene separated **5a** from **5b**.

(3*S*,4*S*)-Boc-AHPPA-OEt (5a): mp 88–89 °C (ether–Skellysolve B); TLC R_f (A) 0.27; $[\alpha]^{24}_D$ −36° (*c* 1.0, CH_3OH); NMR ($CDCl_3$) δ 1.22 (t, 3 H, *J* = 7 Hz), 1.39 (s, 9 H), 2.41–2.57 (m, 2 H, $-CH_2CO-$), 2.88 (d, 2 H, *J* = 7 Hz, benzylic), 3.49–4.25 [m, 5 H, includes δ 4.14 (q, *J* = 7 Hz) and 3.5 (br s), exchangeable, OH] 4.93 (d, 1 H, *J* = 10 Hz, NH), 7.25 (s, 5 H); MS *m/e* (relative intensity) 338 (M + 1, 0.20), 337 (0.11). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

(3*R*,4*S*)-Boc-AHPPA-OEt (5b): mp 140–140.5 °C (ether–Skellysolve B); TLC R_f (A) 0.19; $[\alpha]^{24}_D$ −14.2° (*c* 1.0, CH_3OH); NMR ($CDCl_3$) δ 1.26 (t, 3 H, *J* = 7 Hz), 1.34 (s, 9 H), 2.49–2.57 (m, 2 H, $-CH_2CO-$), 2.8–2.95 (m, 2 H, benzylic), 2.54–4.29 [m, 5 H, includes δ 4.18 (q, *J* = 7 Hz) and 3.55, exchangeable, OH], 4.57 (d, 1 H, *J* = 8.5 Hz, NH), 7.25 (s, 5 H); MS *m/e* (relative intensity) 338 (M + 1, 0.04), 264 (3.2), 237 (0.33), 236 (0.86), 192 (9), 190 (10), 176 (1), 174 (7), 164 (20), 147 (5), 146 (33), 121 (10), 120 (33), 119 (27), 100 (41), 99 (15), 91 (28), 57 (100).

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoic Acid (6a).** The title compound was prepared from **5a** by general procedure E: mp 148–148.5 °C ($CHCl_3$); TLC R_f (D) 0.28; $[\alpha]^{24}_D$ −37° (*c* 1.1, CH_3OH); NMR ($CDCl_3$–methanol-d_4, 10:1) δ 1.39 (s, 9 H), 2.41–2.55 (m, 2 H), 2.88 (d, 2 H, *J* ≈ 7 Hz, benzylic), 3.62–3.83 (m, 1 H), 3.91–4.07 (m, 1 H), 7.25 (s, 5 H). Anal. ($C_{16}H_{23}NO_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-3(*R*)-hydroxy-5-phenylpentanoic Acid (6b).** The title compound was prepared

(18) Another assay for renin activity employing aminopeptidase M coupled with renin has been described. M. Roth and A. Reinharz, *Helv. Chim. Acta*, **49**, 1903 (1966).

(19) W. König and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).

(20) R. Steulmann and H. Klostermeyer, *Justus Liebigs Ann. Chem.*, 2245 (1975).

from **5b** by general procedure E: mp 187.5 °C (CH_3OH–$CHCl_3$); TLC R_f (D) 0.22; $[\alpha]^{24}_D$ −16° (*c* 1.1, CH_3OH); NMR ($CDCl_3$–methanol-d_4, 10:1) δ 1.32 (s, 9 H), 2.47–2.56 (m, 2 H), 2.75–3.1 (m, 2 H), 3.7–4.1 (m, 2 H, C-3 and C-4 protons), 7.24 (s, 5 H).

4(*S*)-Amino-3(*S*)-hydroxy-5-phenylpentanoic Acid (7a). The title compound was prepared from Boc-AHPPA **6a** (50 mg, 0.16 mmol) by general procedure A. After purification by ion-exchange chromatography (general procedure F, eluting solvent was 1 N pyridine–acetate buffer), there was obtained 27 mg (80%) of AHPPA **7a**: mp 193 °C (H_2O–EtOH); $[\alpha]^{24}_D$ −24° (*c* 0.44, H_2O); NMR (D_2O) δ 2.49–2.61 (m, 2 H), 2.73–3.29 (m, 2 H), 3.45–3.68 (m, 1 H), 3.99–4.19 (m, 1 H), 7.4 (s, 5 H).

4(*S*)-Amino-3(*R*)-hydroxy-5-phenylpentanoic Acid (7b). The title compound was prepared from Boc-AHPPA **6b** (50 mg, 0.16 mmol) by general procedure A. After purification by ion-exchange chromatography (general procedure F, eluting solvent was 1 N pyridine–acetate buffer), there was obtained 9.3 mg (due to mechanical loss) of AHPPA **7b**: mp 170 °C (H_2O–EtOH); $[\alpha]^{24}_D$ −42.7° (*c* 0.12, H_2O); NMR (D_2O) δ 2.49–2.57 (m, 2 H), 2.68–3.29 (m, 2 H), 3.58–3.78 (m, 1 H), 4.25–4.45 (m, 1 H), 7.40 (s, 5 H).

2-Oxazolidinonecarboxylic Acid Derivatives of γ-Amino-β-hydroxy Acids. The amino acid (20 mg) was dissolved in 1 M potassium hydroxide solution (6 mL) and was cooled to 5 °C. A solution of 10% phosgene in toluene (10 mL) was added. After stirring for 1 h, the aqueous layer was separated, and the toluene layer was washed three times with 1 N KOH. The combined aqueous layers were acidified using concentrated hydrochloric acid and extracted with ethyl acetate. The organic extracts were dried (Na_2SO_4), concentrated under reduced pressure, and dried in vacuo. The residue was dissolved in methanol-d_4 and the solution analyzed by NMR. Irradiation of the multiplets due to α-methylene caused the C-3 proton multiplet to collapse to a doublet; the coupling constant of this doublet gave $J_{3,4}$. The oxazolidinone derivatives **8a,b** were converted to higher R_f [R_f (D) 0.5] compounds on treatment with diazomethane.

2-Oxazolidinone derivative of 4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoic acid (8a): TLC R_f (D) 0.24; NMR (CD_3OD) δ 2.56 (m, 2 H), 2.91 (d, 2 H, *J* = 7 Hz), 3.78–3.98 (m, 1 H), 4.58–4.78 (m, 1 H), 7.28 (s, 5 H).

2-Oxazolidinone derivative of 4(*S*)-amino-3(*R*)-hydroxy-5-phenylpentanoic acid (8b): TLC R_f (D) 0.28; R_f (E) 0.12; NMR (CD_3OD) δ 2.1–2.99 (m, 4 H), 4.11–4.35 (m, 1 H), 4.98–5.2 (m, 1 H), 7.26 (s, 5 H).

2-Oxazolidinone derivative of (4*S*)-amino-(3*S*)-hydroxyl-6-methylheptanoic acid: TLC R_f (D) 0.24; NMR (CD_3OD) δ 0.95 (dd, 6 H, *J* = 6 and 6 Hz), 1.1–1.9 (m, 3 H), 2.73 (d, 2 H, *J* = 7 Hz), 3.56–3.76 (m, 1 H), 4.44–4.64 (m, 1 H).

2-Oxazolidinone derivative of (4*S*)-amino-(3*R*)-hydroxyl-6-methylheptanoic acid: TLC R_f (D) 0.28; R_f (B) 0.12; NMR (CD_3OD) δ 0.95 (dd, 6 H, *J* = 5 and 6 Hz), 1.1–1.9 (m, 3 H), 2.71–2.84 (dd, 2 H, *J* = 7 and 7 Hz), 3.89–4.15 (m, 1 H), 4.95–5.20 (m, 1 H).

***N*-(*tert*-Butoxycarbonyl)-L-alanyl Isoamylamide.** To a methylene chloride solution (10 mL) of Boc-L-Ala (1.89 g, 10 mmol) and HOBt (2.3 g, 15 mmol) chilled in ice–water was added isoamylamine (870 mg or 1.16 mL), followed by a solution of DCC (2.06 g, 10 mmol) in methylene chloride (10 mL). The reaction mixture was stirred at 4 °C overnight. After filtering DCU, the methylene chloride was removed under reduced pressure. The residue was redissolved in ethyl acetate, washed with cold 1 N HCl and 1 N NaOH, dried ($MgSO_4$), and evaporated to give a solid. Trace amounts of DCU were removed by filtration of a $CHCl_3$ solution of the amide through silica gel, eluting with 1% CH_3OH in $CHCl_3$. The amide was eluted from the silica gel with EtOH–$CHCl_3$ (50:50) and crystallized from chloroform–hexane: yield 2.06 g (80%); mp 75–76 °C; $[\alpha]^{24}_D$ −25.4° (*c* 1.1, CH_3OH); NMR ($CDCl_3$) δ 0.90 (d, 6 H, *J* = 6 Hz), 1.15–2.0 [m, 15 H, includes δ 1.36 (d, *J* = 7 Hz) and 1.44 (d)], 3.24 (q, 2 H, *J* = 7 Hz, $-HNCH_2-$), 4.17 (quintet, 1 H, *J* = 7 Hz), 5.41 (br d, 1 H, *J* = 8 Hz, NH), 6.63 (br t, 1 H, NH); MS *m/e* (relative intensity) 258 (M^+, 0.06), 202 (0.46), 185 (2.35). Anal. ($C_{13}H_{26}N_2O_3$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (9a).** The title compound was prepared by general procedure B from HCl·L-Ala-$NH^iC_5H_{11}$ (1.15 mmol) and Boc-Sta **2a** (316 mg, 1.15 mmol) was isolated: yield after column chromatography was 74%; mp 130–131 °C (EtOAc–Skellysolve B); TLC R_f (B) 0.31; $[\alpha]^{24}_D$ −34° (*c* 1.0, CH_3OH); NMR ($CDCl_3$) δ 0.90 (d, 12 H, *J* = 6 Hz), 1.07–2.0 (m, 18 H, includes δ 1.42, s), 2.37–2.54 (m, 2 H, $-CH_2CO$–Sta), 3.24 (q, 2 H, *J* ≈ 7 Hz), 3.40–3.80 (m, 1 H), 3.84–4.30 (m, 2 H), 4.29 (br, 1 H, exchangeable), 4.45 (quintet, 1 H, *J* = 7 Hz), 4.94 (d, 1 H, *J* = 10 Hz, exchangeable), 6.71–7.12 (m, 2 H, exchangeable); MS *m/e* (relative intensity) 415 (M^+, 0.36). Anal. ($C_{21}H_{41}N_3O_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-3(*R*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (9b).** The title compound was prepared by general procedure B from HCl·AlaNH$^iC_5H_{11}$ and Boc-Sta **2b** and was isolated in 74% yield: mp 151–152 °C ($CHCl_3$–Skellysolve B); TLC R_f (B) 0.38; $[\alpha]^{24}_D$ −23° (*c* 1.0, CH_3OH); NMR ($CDCl_3$) δ 0.92 (dd, 12 H, *J* = 6 and 6 Hz), 1.15–2.0 [m, 18 H, includes δ 1.44 (s)], 2.34–2.44 (m, 2 H), 3.24 (q, 2 H, *J* = 7 Hz), 3.41–4.1 (m, 2 H), 4.33–4.83 [m, 3 H, includes quintet at δ 4.45 (*J* = 7 Hz)], 6.45–7.1 (m, 2 H); MS *m/e* (relative intensity) 415 (M^+, 0.14). Anal. ($C_{21}H_{41}N_3O_5$) C, H, N.

***N*-Acetyl-4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (11).** To a solution of HCl·(*S,S*)-Sta-Ala-$NH^iC_5H_{11}$ (70 mg, 0.2 mmol) in dry DMF (3 mL) was added triethylamine (28 μL, 0.2 mmol), followed by acetic anhydride (100 μL). The mixture was stirred at room temperature for 2.5 h before water was added. After stirring for an additional 30 min, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with 1 N HCl, saturated sodium bicarbonate, and dried ($MgSO_4$). After silica gel column chromatography [gradient elution: 2% CH_3OH in $CHCl_3$ to 15% CH_3OH in $CHCl_3$], 40 mg of the acetylated peptide 11 was obtained (56% yield); upon trituration with ether, a solid was obtained: mp 68–70 °C; TLC R_f (C) 0.35; R_f (E) 0.49; NMR ($CDCl_3$) δ 0.90 (d, 12 H, *J* = 6 Hz), 1.16–1.9 [m, 9 H, includes δ 1.37 (d, *J* = 7 Hz)], 2.02 (s, 3 H, CH_3CO), 2.34–2.41 (m, 2 H), 3.24 (q, 2 H, *J* ≃ 7 Hz), 3.84–4.17 (m, 3 H, one hydrogen exchangeable), 4.42 (quintet, 1 H, *J* = 7 Hz, C-2 proton of Ala), 5.84 (br d, 1 H, *J* = 10 Hz, NH), 6.48 (br, 1 H, NH), 6.88 (br, 1 H, *J* ≃ 8 Hz); MS *m/e* 357.2623 (calcd for $C_{18}H_{35}N_3O_4$, *m/e* 357.2627).

Isovaleryl-4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (12). The title compound was prepared by general procedure D from HCl·(*S,S*)-Sta-L-Ala-$NH^iC_5H_{11}$ (351 mg, 1 mmol) and isovaleric anhydride. The yield after column chromatography was 380 mg (95%): mp 114–116 °C ($CHCl_3$–hexane); TLC R_f (C) 0.50; R_f (E) 0.70; $[\alpha]^{24}_D$ −47.7° (*c* 0.6, CH_3OH); NMR ($CDCl_3$) δ 0.87–0.99 (m, 18 H), 1.06–2.23 (m, 12 H, includes δ 2.07–2.14 (m), $-CH_2CO-$ of Iva], 2.34–2.46 (m, 2 H), 3.24 (q, 2 H, *J* ≃ 7 Hz), 3.82–4.17 (m, 2 H), 4.5 (quintet, 1 H, *J* = 7 Hz), 4.69 (br s, 1 H), 6.2 (d, 1 H, *J* ≃ 10 Hz, NH), 7.15 (br t, 1 H, *J* ≃ 6 Hz), 7.41 (br d, 1 H); MS *m/e* (relative intensity) 399 (M^+, 1.5). Anal. ($C_{21}H_{41}N_3O_4$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-valyl-4(*S*)-amino-3(*S*)-hydroxyl-6-methylheptanoyl-L-alanyl Isoamylamide (13a).** The title compound was prepared by general procedure D from HCl·(*S,S*)-Sta-Ala-$NH^iC_5H_{11}$ (0.301 mmol) and Boc-L-Val (1.2 mmol). The yield of **13a** after column chromatography was 94%: mp 173.5–174.5 °C ($CHCl_3$–ether–hexane); TLC R_f (B) 0.40; $[\alpha]^{24}_D$ −43.4° (*c* 0.4, CH_3OH); NMR ($CDCl_3$) δ 0.85–1.1 (m, 18 H), 1.2–2.6 [m, 21 H, includes δ 1.44 (s) and 1.36 (d, *J* = 7 Hz)], 3.24 (q, 2 H, *J* = 7.5 Hz), 3.40–4.18 (m, 3 H), 4.36 (quintet, 1 H, *J* = 7 Hz), 4.86 (br d, 1 H, exchangeable), 5.14 (d, 1 H, *J* = 7 Hz), 6.70 (br t, 1 H), 7.32–7.67 (br, 2 H); MS *m/e* (relative intensity) 515 (M^+, 0.80).

***N*-(*tert*-Butoxycarbonyl)-L-valyl-4(*S*)-amino-3(*R*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (13b).** The title compound was prepared by general procedure D from Boc-L-Val and HCl·(3*R*,4*S*)-Sta-Ala-$NH^iC_5H_{11}$ and was isolated in 90% yield: mp 212 °C (CH_3OH); TLC R_f (B) 0.41; $[\alpha]^{24}_D$ −43° (*c* 0.4, CH_3OH); NMR ($CDCl_3$–methanol-d_4, 1:1) δ 0.87–1.02 (m, 18 H), 1.2–2.46 [m, 21 H, includes δ 1.37 (d, *J* = 7 Hz) and 1.46 (s)], 3.21 (t, 2 H, *J* = 7.5 Hz), 3.56–4.02 [m, 3 H, includes δ 4.30 (q, 1 H, *J* = 7 Hz)]. Anal. ($C_{26}H_{50}N_4O_6 \cdot H_2O$) C, H, N.

Isovaleryl-L-valyl-4(*S*)-amino-3(*S*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (14a). The title compound was prepared by general procedure D from HCl·Val-(*S,S*)-Sta-Ala-$NH^iC_5H_{11}$ (0.233 mmol) and isovaleric anhydride. The yield of 14a after column chromatography was 77%: mp 243–245 °C (CH_3OH–EtOAc); TLC R_f (C) 0.54; R_f (E) 0.80; $[\alpha]_D$ −61° (*c* 0.5,

CH_3OH); NMR ($CDCl_3$–methanol-d_4, 1:1) δ 0.87–1.06 (m, 24 H), 1.27–1.87 [m, 9 H, includes δ 1.36 (d, J = 7 Hz)], 1.87–2.42 (m, 6 H), 3.22 (t, 2 H, J = 7 Hz), 3.77–4.47 (m, 4 H); MS m/e (relative intensity) 499 (M^+, 0.48). Anal. ($C_{26}H_{50}N_4O_5$) C, H, N.

Isovaleryl-L-valyl-4(*S*)-amino-3(*R*)-hydroxy-6-methylheptanoyl-L-alanyl Isoamylamide (14b). The title compound was prepared by general procedure D from HCl·Val-(3*R*,4*S*)-Sta-Ala-$NH^iC_5H_{11}$ and isovaleric anhydride and was isolated in 70% yield: mp 252.5 °C (CH_3OH); TLC R_f (C) 0.54; $[\alpha]^{24}_D$ −55° (*c* 0.4, CH_3OH); NMR ($CDCl_3$–methanol-d_4, 1:1) δ 0.87–1.04 (m, 24 H), 1.26–1.85 [m, 9 H, includes δ 1.37 (d, J = 7 Hz)], 1.86–2.38 (m, 6 H), 3.22 (t, 2 H, J = 7 Hz), 3.70–4.48 (m, 4 H); MS m/e (relative intensity) 499 (M^+, not observed), 413 (0.06), 385 (1.05). Anal. ($C_{26}H_{50}N_4O_5·H_2O$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoyl-L-alanyl Isoamylamide (15).** The title compound was prepared in 80% yield from (3*S*,4*S*)-Boc-AHPPA (**6a**; 313 mg, 1.01 mmol) and HCl·Ala-$NH^iC_5H_{11}$ (210 mg, 1.08 mmol) by general procedure B: mp 113 °C ($CHCl_3$–ether–Skelly B); $[\alpha]^{24}_D$ −28° (*c* 1.0, CH_3OH); NMR ($CDCl_3$) δ 0.88 (d, 6 H, J = 6 Hz), 1.25–2.04 [m, 15 H, includes δ 1.35 (s)], 2.3–2.48 (m, 2 H), 2.87 (d, 2 H, J = 7 Hz), 3.19 (q, 2 H, $J \simeq$ 7 Hz), 3.52–4.14 (m, 2 H), 4.42 (quintet, 1 H, $J \simeq$ 7 Hz), 4.66 (br s, OH), 5.16 (br d, 1 H, $J \simeq$ 10 Hz, NH), 6.78–7.35 [7 H, includes δ 7.18 (s)]; MS m/e (relative intensity) 449 (M^+, 0.25). Anal. ($C_{24}H_{39}N_3O_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-L-valyl-4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoyl-L-alanyl Isoamylamide (16).** The title compound was prepared in 90% yield from Boc-L-Val (1.2 mmol) and HCl·(3*S*,4*S*)-AHPPA-Ala-$NH^iC_5H_{11}$ (0.301 mmol) by general procedure D: mp 191.5–192.5 °C (CH_3OH–EtOAc); $[\alpha]^{24}_D$ −49.4° (c 0.4, CH_3OH); NMR ($CDCl_3$–methanol-d_4, 1:1) δ 0.72–0.93 (m, 12 H), 1.31–2.1 [m, 16 H, includes δ 1.46 (s)], 2.28–2.4 (m, 2 H), 2.87–2.97 (m, 2 H), 3.21 (t, 2 H, J = 7 Hz), 3.66–4.41 (m, 4 H), 7.21 (s, 5 H); MS m/e (relative intensity) 549 (M, 0.33). Anal. ($C_{29}H_{48}N_4O_6$) C, H, N.

Isovaleryl-L-valyl-4(*S*)-amino-3(*S*)-hydroxy-5-phenylpentanoyl-L-alanyl Isoamylamide (17). The title compound was prepared in 93% yield by general procedure D: mp 237 °C dec (CH_3OH–EtOAc); $[\alpha]^{24}_D$ −59° (*c* 0.4, CH_3OH); NMR ($CDCl_3$–methanol-d_4, 1:1) δ 0.76–0.99 (m, 18 H), 1.15–2.14 [m, 10 H, includes δ 1.35 (d, J = 7 Hz) and 2.1 (m), $-CH_2CO$ of **Iva**], 2.25–2.38 (m, 2 H), 2.86–2.95 (m, 2 H), 3.21 (t, 2 H, $J \simeq$ 7.5 Hz), 3.83–4.41 (4 H), 7.22 (s, 5 H); MS m/e (relative intensity) 534 (M, 0.07).

A sample of 17 (~1 mg) was hydrolyzed in 6 N HCl–propionic acid for 3 h at 130 °C. The hydrolysate after being dried was analyzed for amino acid composition. Anal. Found: Ala, 0.94; Val, 1.0; AHPPA, 1.11. Anal. ($C_{29}H_{48}N_4O_5$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-4(*S*)-amino-6-methylheptanoyl-L-alanyl Isoamylamide.** The title compound was prepared in 74% yield from HCl·Ala-$NH^iC_5H_{11}$ (0.579 mmol) and Boc-dSta (150 mg, 0.579 mmol) by general procedure B: mp 141–143 °C; $[\alpha]^{24}_D$ −10.8° (*c* 1.01, CH_3OH); NMR ($CDCl_3$) δ 0.89 (d, 12 H, J = 6 Hz), 1.15–2.43 [m, 22 H, includes δ 1.43 (s) and 2.21 (m), $-CH_2CO$ of dSta], 3.2 (q, 2 H, $J \simeq$ 7 Hz), 3.57 (br m, 1 H), 4.42 (br, d, 1 H, $J \simeq$ 10 Hz), 4.45 (quintet, 1 H, J = 7 Hz), 6.49 (d, 1 H, J = 8 Hz), 7.09 (br, 1 H); MS m/e (relative intensity) 400 (1.46), 399 (M^+, 2.78).

***N*-(*tert*-Butoxycarbonyl)-L-valyl-4(*S*)-amino-6-methylheptanoyl-L-alanyl Isoamylamide.** The title compound was prepared in 88% yield from HCl·dSta-Ala-$NH^iC_5H_{11}$ (140 mg, 0.351 mmol) and Boc-L-Val (1.4 mmol) by general procedure D: mp 203–205 °C ($CHCl_3$–Skellysolve B); $[\alpha]^{24}_D$ −27.3° (*c* 0.5, CH_3OH); NMR ($CDCl_3$) δ 0.84–1.01 (m, 18 H), 1.15–2.46 [m, 23 H, includes δ 1.42 (s) and 2.13 (m), $-CH_2CO$ of dSta], 3.2 (q, 2 H, $J \simeq$ 7 Hz), 3.62–4.07 (m, 2 H), 4.39 (quintet, 1 H, J = 7 Hz), 5.59 (d, 1 H, J = 8 Hz, NH), 6.65 (d, 1 H, J = 10 Hz, NH), 6.95 (d, 1 H, J = 8 Hz, NH), 7.55 (br t, 1 H, NH).

Isovaleryl-L-valyl-4(*S*)-amino-6-methylheptanoyl-L-alanyl Isoamylamide (18). The title compound was prepared in 74% yield by general procedure D: mp 261.5 °C (CH_3OH); $[\alpha]_D$ −36.8° (*c* 0.068, CH_3OH); MS m/e (relative intensity) 484 (0.95), 483 (1.55), 482 (0.54). Anal. ($C_{26}H_{50}N_4O_5$) C, H, N.

Assay of Hog Kidney Renin. Renin was obtained from Nutritional Biochemicals, no. 10105. Each tube contained 0.02 μmol of substrate [Ile-His-Pro-Phe-His-Leu-[^{14}C]Leu-Val-Tyr-Ser-OHAc, 7200 dpm (prepared by R. Strachan, Merck Sharp & Dohme Research Laboratories)] and 0.04 mg of renin. Renin was dissolved in 0.05 M citrate phosphate, pH 5.7; substrate was dissolved in methanol and diluted with buffer before addition to assay. Inhibitors were dissolved in Me_2SO and diluted to 20% Me_2SO in buffer before addition to assay. Each component was added in 0.1 mL; total 0.3 mL.

Incubations with renin were carried out for 30 min at 30 °C. The renin reaction was stopped by the addition of 1.0 mL of a leucine aminopeptidase solution in 0.5 M Tris-HCl, pH 10. This solution was prepared 60 min before use by mixing 1.15 mL of 0.1 M $MnCl_2$ with 114.8 mL of 0.5 M Tris-HCl, followed by the addition of 0.15 mL of leucine aminopeptidase (Boering-Mounheim, no. 15006, 100 units/mg, 5 mg/mL). After an additional 30-min incubation, unhydrolyzed substrate was adsorbed by the addition of 1.0 mL of a 40% settled volume suspension of benzoylated DEAE-cellulase (Cellex-BD#, Bio-Rad). The tubes were shaken for 10 min and centrifuged. A 1.0-mL aliquot of the supernatant was added to 10 mL of PCS scintillation cocktail (Amersham Searle) for determination of [^{14}C]leucine released.

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