was used as the positive reference standard. Two hours later, paw edema was induced by the subcutaneous injection into the plantar surface of the right hind paw of 0.1 mL of a 1.0% homogenized suspesnion of carrageenan.

Immediately, the swelling of the paw was measured by immersing it in a mercury plethysmomeric system to above the lateral mateolus. The mercury plethysmometer was constructed as follows: a glass cylinder 22 mm in diameter and 60 mm deep was connected at the bottom of the cylinder by a column of water to a Statham transducer (model P23BB), of pressure range 0–5 cm. The displacement was recorded electronically on a Beckman R511 recorder. Three hours later, the inflamed paw volume was measured again, and the change in displacement was recorded for each group. The percent inhibition of edema was calculated using the control group paw volume as 100% edema, i.e.

$$\frac{\Delta \text{ control group edema} - \Delta \text{ test group edema}}{\Delta \text{ control group edema}} \times 100 = \text{inhibn of edema}$$

Drug effects on paw volumes were evaluated by comparing the extent of edema with that produced in the corresponding control group. Using an analysis of variance test followed by a Newman–Keuls t-test.

Acknowledgment. We are greatly indebted to Messrs. A. Kover, K. Belfield, and P. Ehrlich and Mmes. J. A. Finley, T. E. Milgate, D. K. Kamp, and L. A. Trusso for their excellent technical assistance and Drs. T. A. Davidson and D. W. Payling for their intelligent suggestions and to E. L. Gooch for her excellent secretarial assistance.

Renin Inhibitors Containing New P_1–P_1' Dipeptide Mimetics with Heterocycles in P_1'

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A series of renin inhibitors containing new P_1-P_1' dipeptide mimetics are presented. The P_1-P_1' mimetics were obtained from (4*S*,5*S*)-3-(*tert*-butoxycarbonyl)-4-(cyclohexylmethyl)-5-[(ω-mesyloxy)alkyl]-2,2-dimethyloxazolidines **5b**, **9**, and **11b** by nucleophilic substitution of the mesylate groups with the sodium salts of mercapto- and hydroxyheterocycles. Removal of the protecting groups and stepwise acylations with amino acid derivatives provided renin inhibitors with a length of a tripeptide. Replacement of P_2 histidine by other amino acids maintained or enhanced renin inhibitory potency. By alteration of P_3 phenylalanine, compounds with IC_{50} values in the nanomolar range and stability against chymotrypsin were obtained. Finally, the effect of the C-terminal heterocycle on the renin inhibition was studied. Compound XVII was examined in vivo for its hypotensive effects. In salt-depleted cynomolgus monkeys, XVII inhibited plasma renin activity and lowered blood pressure after oral administration of a dose of 10 mg/kg.

Introduction

The renin–angiotensin system (RAS) is a complex enzymatic–hormonal system controlling electrolyte homeostasis, fluid volume, and arterial blood pressure by the production of the potent vasopressor and aldosteronogenic octapeptide angiotensin II. The great success of angiotensin converting enzyme inhibitors in the treatment of hypertension and congestive heart failure[1] provided the impetus to look for alternate approaches to interfering with the RAS, by inhibition of renin[2] and antagonism of angiotensin II at the receptor level.[3] Renin is the enzyme that catalyzes the first and rate-limiting step in the ultimate production of angiotensin II. The high substrate specificity of renin led to an intensive search for effective inhibitors. Most of the potent renin inhibitors have been developed by starting from peptides which correspond to the sequence around the cleavage site of angiotensinogen and by replacing the scissile Leu^{10}-Val^{11} dipeptide by nonhydrolyzable transition-state mimetics.

These inhibitors remain partly peptidic and their therapeutic efficacy is limited due to proteolytic instability, poor resorption, and rapid biliary excretion.[4] Therefore, we initiated a synthesis program to overcome these limitations by reducing the peptide character and molecular size of the inhibitors.

As one aspect of our renin inhibitor strategy, we chose to prepare modified compounds of small peptides, such as I and II,[5] containing the statine analogue ACHPA (4-

(1) (a) Ondetti, M. A.; Cushman, D. W. Enzymes of the Renin-Angiotensin System and their Inhibitors. *Annu. Rev. Biochem.* **1982**, *51*, 283–308. (b) Wyvratt, M. J.; Patchett, A. A. Recent Developments in the Design of Angiotensin-Converting Enzyme Inhibitors. *Med. Res. Rev.* **1985**, *5*, 483–531.

(2) For recent reviews see (a) Greenlee, W. Renin Inhibitors. *J. Med. Res. Rev.* **1990**, *10*, 173–236. (b) Greenlee, W. Renin Inhibitors. *J. Pharm. Res.* **1987**, *4*, 364–374. (c) Wood, J. M.; Stanton, J. L.; Hofbauer, K. G. Inhibitors of Renin as Potential Therapeutic Agents. *J. Enzyme Inhib.* **1987**, *1*, 169–185. (d) Ocain, T. D.; Abou-Gharbia, M. Renin-Angiotensin System (RAS) Dependent Antihypertensives: I. Renin Inhibitors. *Drugs Future* **1991**, *16*, 37–51. (e) Breipohl, G.; Geiger, R.; Henke, S.; Kleemann, H.-W.; Knolle, J.; Ruppert, D.; Schölkens, B. A.; Urbach, H.; Wagner, A.; Wegmann, H. Studies on Renin Inhibitors. *Spec. Publ. R. Soc. Chem.* **1988**, *65*, 101–127. (f) Corvol, P.; Chaureau, D.; Jeunemaitre, X.; Menard, J. Human Renin Inhibitor Peptides. *Hypertension* **1990**, *16*, 1–11.

(3) Timmermans, P. B. M. W. M.; Wong, P. C.; Chin, A. T.; Herblin, W. F. Non-peptide Angiotensin II Receptor Antagonists. *Trends Pharmacol. Sci.* **1991**, *12*, 55–62.

(4) Kleinert, H. D. Renin Inhibitors: Discovery and Development. *Am. J. Hypertens.* **1989**, *2*, 800–808.

(5) Raddatz, P.; Hölzemann, G.; Jonczyk, A.; Schmitges, C. J.; Minck, K.-O. Tripeptide Renin Inhibitors. European Patent Application EP 220 665, 1986.

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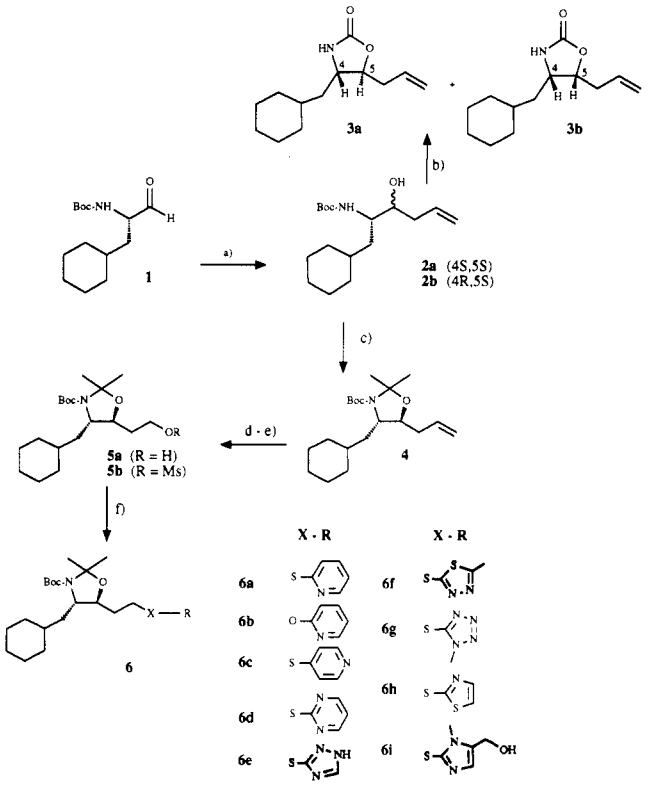
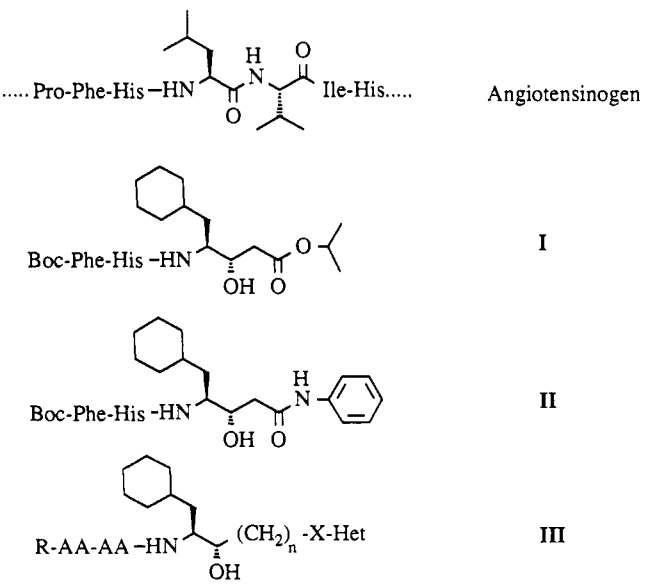
Scheme I.[a] Synthesis of the P_1-P_1' Mimetics with Ethylene Chain



[a] (a) Zn, Allyl bromide, NH_4Cl (sat.); (b) NaH, toluene; (c) $Me_2C(OMe)_2$, PTSA; (d) O_3, CH_2Cl_2; $NaBH_4$, MeOH; (e) CH_3SO_2Cl, NEt_3; (f) HX-R, NaH, DMF.

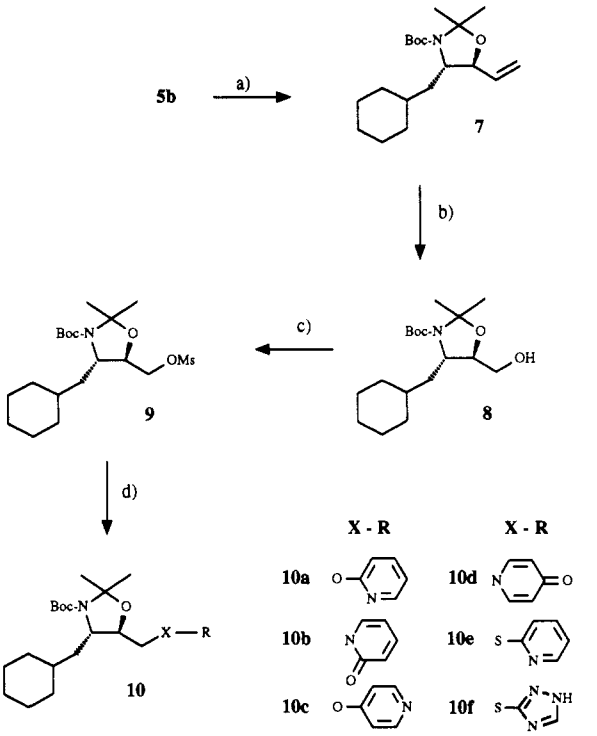
(*S*)-amino-5-cyclohexyl-3(*S*)-hydroxypentanoic acid) as the transition-state mimetic in P_1-P_1'.[6] We sought to reduce



the peptidic character of these inhibitors by incorporation of dipeptide mimetics for P_1-P_1' as represented generally by structure III.[7]

(6) Schlechter, J.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Comm.* **1967**, *27*, 157–162. P_n-P_n' refer to the side-chain position of the peptide substrate.

Scheme II.[a] Synthesis of the P_1-P_1' Mimetics with Methylene Chain



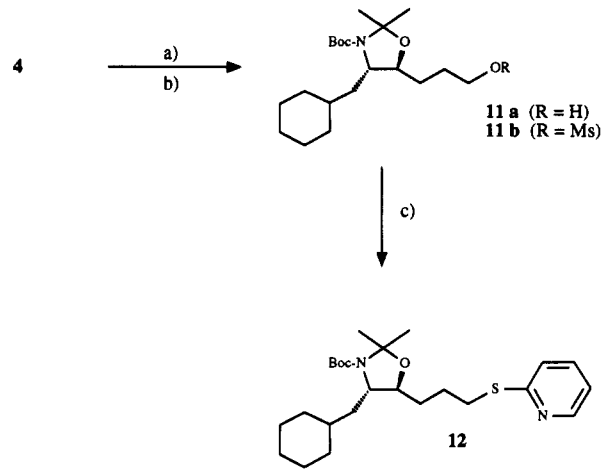
[a] (a) KOtBu, DMSO; (b) O_3, CH_2Cl_2; $NaBH_4$, MeOH; (c) CH_3SO_2Cl, NEt_3; (d) HX-R, NaH, DMF.

Since peptides such as I and II are susceptible to the gastrointestinal enzyme chymotrypsin (which rapidly cleaves these peptides between the Phe and His residues in vitro[8]), we modified phenylalanine in P_3 and the adjacent protecting group in P_4 with the goal of achieving proteolytic stability. We synthesized peptide derivatives with phenylalanine surrogates previously described by

(7) For similar approaches see (a) Williams, P. D.; Perlow, D. S.; Payne, L. S.; Holloway, M. K.; Siegl, P. K. S.; Schorn, R. J. L.; Doyle, J. J.; Strouse, J. F.; Vlasuk, G. P.; Hoogsteen, K.; Springer, J. P.; Bush, B. L.; Halgren, T. A.; Richards, A. D.; Kay, J.; Veber, D. F. Renin Inhibitors Containing Conformationally Restricted P_1-P_1' Dipeptide Mimetics. *J. Med. Chem.* **1991**, *34*, 887–900. (b) Rosenberg, S. H.; Dellaria, J. F.; Kempf, D. J.; Hutchins, C. W.; Woods, K. W.; Maki, R. G.; deLara, E.; Spina, K. P.; Stein, H. H.; Cohen, J.; Baker, W. R.; Plattner, J. J.; Kleinert, H. D.; Perun, T. J. Potent, Low Molecular Weight Renin Inhibitors Containing a C-Terminal Heterocycle: Hydrogen Bonding at the Active Site. *J. Med. Chem.* **1990**, *33*, 1582–1590. (c) Luly, J. R.; Bolis, G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. New Inhibitors of Human Renin that Contain Novel Leu-Val Replacements. Examination of P_1 Site. *J. Med. Chem.* **1988**, *31*, 532–539. (d) Bolis, G.; Fung, A. K. L.; Greer, J.; Kleinert, H. D.; Marcotte, P. A.; Perun, T. J.; Plattner, J. J.; Stein, H. H. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Incorporating Transition-State, Nonpeptide Replacements at the Scissile Bond. *J. Med. Chem.* **1987**, *30*, 1729–1737. (e) Dellaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. Optimization and in Vivo Evaluations of a Series of Small, Potent, and Specific Renin Inhibitors Containing a Novel Leu-Val Replacement. *J. Med. Chem.* **1987**, *30*, 2137–2144.

(8) Plattner, J. J.; Marcotte, P. A.; Kleinert, H. D.; Stein, H. H.; Greer, J.; Bolis, G.; Fung, A. K. L.; Bopp, B. A.; Luly, J. R.; Sham, H. L.; Kempf, D. J.; Rosenberg, S. H.; Dellaria, J. F.; De, B.; Merits, I. and Perun, T. J. Renin Inhibitors. Dipeptide Analogues of Angiotensinogen Utilizing a Structurally Modified Phenylalanine Residue to Impart Proteolytic Stability. *J. Med. Chem.* **1988**, *31*, 2277–2288.

Scheme III.[a] Synthesis of the P_1-P_1' Mimetics with Propylene Chain



[a] (a) BH_3·THF; H_2O_2; (b) CH_3SO_2Cl, NEt_3; (c) HX-R, NaH, DMF.

Stanton[9] and Plattner.[8]

We also examined several amino acids as surrogates for histidine in P_2. Finally, we prepared a variety of C-terminally modified compounds (Table V) of which many are highly potent and selective for renin.

Chemistry

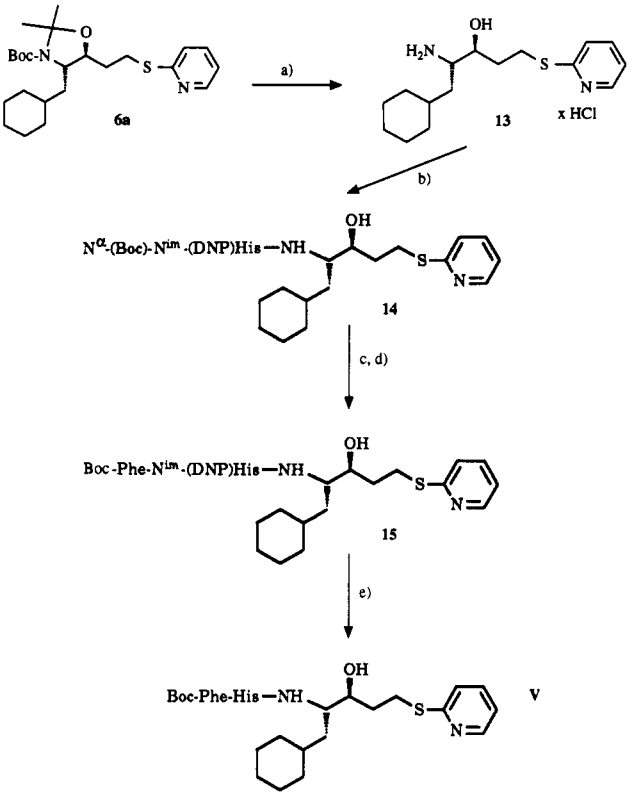
The syntheses of the P_1-P_1' dipeptide mimetics and the preparation of a representative example of a renin inhibitor are outlined in Schemes I–IV. As shown in Scheme I, the P_1-P_1' mimetics **6a–i**, protected as acetonides, were obtained starting from N^{α}-Boc-L-cyclohexylalaninal (1).[10] Treatment of this aldehyde with allyl bromide and zinc under Barbier-type reaction conditions led to the diastereomeric mixture of allyl alcohols **2a/2b** in a ratio of 62:38. Separation by silica gel column chromatography provided the pure diastereomers. Assignment of the C4 configuration of these diastereomers was possible by formation of the corresponding oxazolidinones **3a** and **3b**. The assignment of stereochemistry for these compounds is based on the vicinal coupling, $^3J_{4,5}$, between protons on the fourth and fifth carbons. We measured 5.8 Hz for isomer **3a** and 7.6 Hz for isomer **3b**, indicating the trans (threo) and cis (erythro) stereochemical arrangement. This leads to the 4*S*,5*S* and 4*S*,5*R* configurations for the diastereomers **3a/3b**, respectively. These findings are in good agreement with those reported by Castro[11] and Rich.[12] Protection of the hydroxyl and Boc-NH functions of the 4*S*,5*S* diastereomer **2a** led to the acetonide **4**. Ozonolysis of the double bond followed by reduction of the resulting aldehyde gave alcohol **5a** which was converted into mesylate **5b**. Nucleophilic displacement of the mesylate by the sodium salts of compounds HXR, shown in Scheme I, provided the protected P_1-P_1'-mimetics **6a–i**.

The route to the protected mimetics **10a–f** began with mesylate **5b** as shown in Scheme II. Treatment of this mesylate with the potassium salt of *tert*-butyl alcohol in a solvent mixture of DMSO and toluene afforded olefin **7**. Ozonolysis of the double bond and subsequent reduction of the resulting aldehyde gave alcohol **8** which was converted into mesylate **9**. Nucleophilic substitution of this mesylate by the sodium salts of 2-mercaptopyridine and 3-mercapto-1,2,4-triazole led to the protected mimetics **10e** and **10f**. However, the alkylation of 2-hydroxypyridine and 4-hydroxypyridine using sodium hydride as base led to mixtures of the O- and N-alkylated products **10a,b** and **10c,d**. Separation by silica gel chromatography provided the pure isomers. Proton NMR and UV spectra allowed clear distinction between pyridine and pyridone derivatives.

Scheme III shows the route leading to compound **12**, a higher homologue of compound **6a**. Hydroboration of olefin **4** with borane in THF and subsequent treatment with hydrogen peroxide (30%) in alkaline solution gave alcohol **11a**. The preparation of mesylate **11b** and the nucleophilic displacement were achieved as mentioned above.

Renin inhibitors of Tables I–V were prepared by standard procedures, as shown in Scheme IV for the synthesis of inhibitor V. Boc-ACHPA-OH[13] was prepared by

Scheme IV.[a] Preparation of the Peptide Derivatives



[a] Step 1: (a) 4 N HCl in dioxane, MeOH; (b) N^{α}-Boc-N^{im}-DNP-His, EDCI, HOBt, NMM; step 2: (c) 4 N HCl in dioxane; (d) Boc-Phe, EDCI, HOBt, NMM; step 3: (e) thiophenol, NEt_3, DMF.

(9) Bühlmayer, P.; Caselli, A.; Fuhrer, W.; Gösche, R.; Rasetti, V.; Rüeger, H.; Stanton, J. L.; Criscione, L. and Wood, J. M. Synthesis and Biological Activity of Some Transition State Inhibitors of Human Renin. *J. Med. Chem.* **1988**, *31*, 1839–1846.

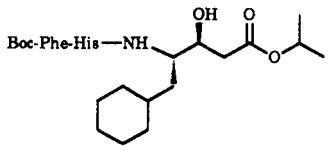
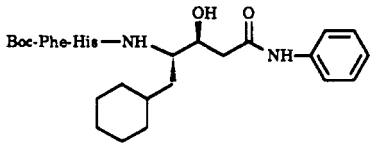
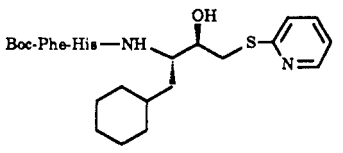
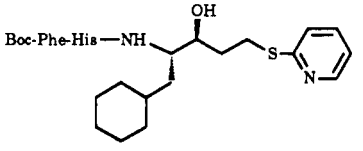
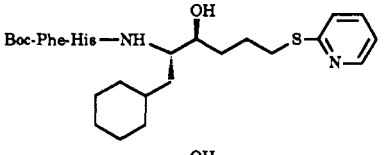
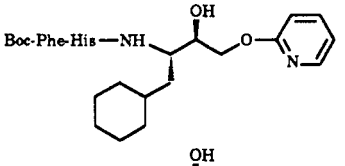
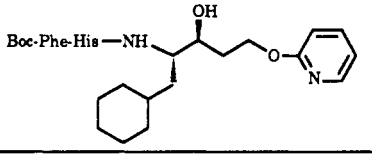
(10) Bayer, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, F. H.; Ulm, E.; Schorn, T. W.; LaMont, B. L.; Lin, T.-Y.; Kawa, M.; Rich, D. H.; Veber, D. F. Renin Inhibitors. Synthesis of Subnanomolar, Competitive, Transition-State Analogue Inhibitors Containing a Novel Analogue of Statine. *J. Med. Chem.* **1985**, *28*, 1779–1790.

(11) Dufour, M.-N.; Jouin, P.; Poncet, J.; Pantalon, A. and Castro, B. J. Synthesis and Reduction of α-Amino Ketones Derived from Leucine. *Chem. Soc. Perkin Trans. I* **1986**, *1895–1899*.

(12) Rich, D. H.; Sun, E. T. O. Synthesis of Analogues of the Carboxyl Protease Inhibitor Pepstatin. Effect of Structure on Inhibition of Pepsin and Renin. *J. Med. Chem.* **1980**, *23*, 27–33.

(13) (a) Raddatz, P.; Radunz, H.-E.; Schneider, G.; Schwartz, H. Reduktion mit Hefezellen, der Schlüsselschritt einer effizienten Synthese von (3S,4S)-4-Amino-3-hydroxypentansäuren. *Angew. Chem.* **1988**, *100*, 414–415. (b) For a review see Altenbach, H.-J. Statin-Synthesen. *Nachr. Chem. Tech. Lab.* **1988**, *36*, 756–758.

Table I. Effect of C-Terminal Ester or Amide Replacement and Effect of Chain Length on Renin Inhibition

		HPLC[a]	IC_{50},[b] nM
I		97.3	175
II		98.2	190
IV		98.7	150
V		97.9	130
VI		99.1	310
VII		96.9	140
VIII		98.5	750

[a] Percent purity as determined by reversed-phase HPLC analysis; see Experimental Section for details. [b] Human plasma renin assay, pH 5.5. IC_{50} values have an estimated error of ±20%.

known procedures. The synthesis of 2-benzyl-3-(*tert*-butylsulfonyl)propionic acid was achieved as described by Stanton et al.[9] The 3-phenyllactic acid derivatives of renin inhibitors XIII and XIV were prepared by a modified procedure described by J. J. Plattner et al.[8]

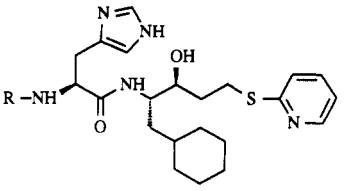
Results and Discussion

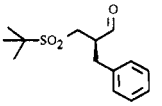
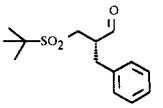
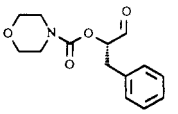
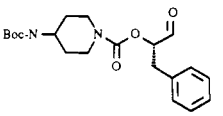
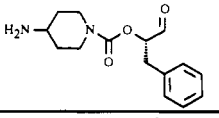
In Vitro Activity. The structures and in vitro activities of the renin inhibitors are listed in Tables I–V. Human plasma renin inhibition was measured at pH 5.5. Potencies are expressed as IC_{50} values for suppression of angiotensin I formation.

Table I shows that the C-terminal replacement of the ester or amide functions of the ACHPA-based tripeptides I and II by the (arylthio)methyl group led to the 2-mercaptopyridine derivative V with comparable renin inhibitory potency. However, the standard peptides I and II and the modified compound V display only moderate IC_{50} values in the range of 110–290 nM.

Inhibitor V provides a reference compound for evaluating the effect of chain length between the hydroxyl group and the pyridine ring on renin inhibition (Table I). Analogues IV and VII with one chain carbon atom shorter did not differ in inhibitory potency from V. The higher homologue VI displayed a 2.5-fold diminution of potency.

Table II. Effect of the N-Terminus on Renin Inhibition and Proteolytic Stability



	R	HPLC[a]	IC_{50},[b] nM	stability against chymotrypsin
V	Boc-Phe	98.7	130	$\tau_{1/2}$ = 45 min
IX		96.7	3600	ND[c]
X		98.8	46	stable[d]
XI		96.7	250	stable
XII		98.1	230	stable
XIII		97.8	33	stable

[a] Percent purity as determined by reversed-phase HPLC analysis; see Experimental Section for details. [b] Human plasma renin assay, pH 5.5. IC_{50} values have an estimated error of ±20%. [c] ND = not determined. [d] Stable means no detectable degradation after incubation with chymotrypsin for >8 h.

However, the oxygen analogue of V, compound VIII, showed a 5-fold drop in activity.

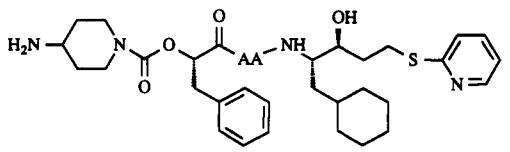
Replacements of phenylalanine in position P_3 (Table II) were made to enhance potency and to increase stability against degradation by chymotrypsin. Coupling of racemic 2-benzyl-3-(*tert*-butylsulfonyl)propionic acid with the deprotected dipeptide 14 led to the diastereomeric mixture of IX and X, which was separated by silica gel chromatography.

Compound X showed a 3-fold increase in renin inhibitory potency whereas the other diastereomer IX was about 28-fold weaker. The L-phenyllactic acid carbamates XI and XII were found to be almost 2-fold less active than reference peptide V. Removal of the Boc group of inhibitor XII led to XIII accompanied by a 4-fold increase in potency. Inhibitors V, X, XI, XII, and XIII were subjected to degradation by chymotrypsin. As expected, chymotrypsin did not attack the peptides containing phenylalanine surrogates in P_3, while the standard inhibitor V was rapidly cleaved between phenylalanine and histidine.

Compound XIII was chosen as a reference for a further structure–activity study summarized in Table III. Substitution of histidine in P_2 by norvaline and norleucine led to inhibitors XIV and XVI which were comparable to XIII in renin inhibition. Leucine and methionine in P_2 led to compound XV and XVIII which were about 4-fold weaker in potency. A further truncation of the side chain gave the alanine-containing analogue XIX which displayed a 23-fold drop in activity. Finally, the incorporation of S-methylcysteine in P_2 provided compound XVII with an IC_{50} value in the nanomolar range. The specificity of these

Table III. Effect of P_2 Substitutions on in Vitro Activity

P_2



	AA	HPLC[a]	IC_{50},[b] nM human plasma renin	IC_{50},[b] nM catepsin D	IC_{50},[b] nM pepsin
XIII	His	97.8	33	>10000	>10000
XIV	Nva	99.1	37	>10000	4500
XV	Leu	99.6	130	>10000	>10000
XVI	Nle	98.3	58	>10000	>10000
XVII	Cys(Me)	97.2	9.3	>10000	>10000
XVIII	Met	98.3	140	>10000	>10000
XIX	Ala	98.8	770	>10000	>10000

[a] Percent purity as determined by reversed-phase HPLC analysis; see Experimental Section for details. [b] IC_{50} values have an estimated error of ±20%.

modified inhibitors toward renin as compared with bovine cathepsin D and porcine pepsin, two related aspartic proteinases, is summarized in Table III. In all instances, these inhibitors demonstrated excellent enzyme specificity. Our findings of P_2 substitutions are in good agreement with data previously described by Guégan,[17] Dellaria,[7e] and Plattner,[7d] who showed that replacement of histidine by amino acid with hydrophobic side chains (Nle, Nva) led to inhibitors with comparable or slightly enhanced renin inhibitory activity without loss of selectivity.

The optimal inhibitor XVII exhibited an in vitro potency that is 20-fold improved compared to the ACHPA-containing compound II and an enhanced proteolytic stability against chymotrypsin. This compound provided a reference point for investigating the effect of the C-terminal heterocycle on renin inhibition (Table IV). Interestingly, the 4-pyridine (XX) and the 2-pyrimidine (XXI) derivatives suffered 4–5-fold losses in potency relative to the parent compound XVII.

Further variation of the C-terminal heterocycle led to the 5-membered ring derivatives XXII–XXV, which showed a comparable renin inhibitory potency to compound XVII. However, the thiazole- and imidazole-containing inhibitors XXII and XXV displayed a slightly enhanced potency with IC_{50} values of 5 and 2.6 nM, respectively.

Compounds XXVI to XXXI (Table V) belong to a series of inhibitors, which are formally lower homologues of XVII. Of the six compounds only thioether analogues XXVI and XXVII matched the inhibitor potency of XVII. The

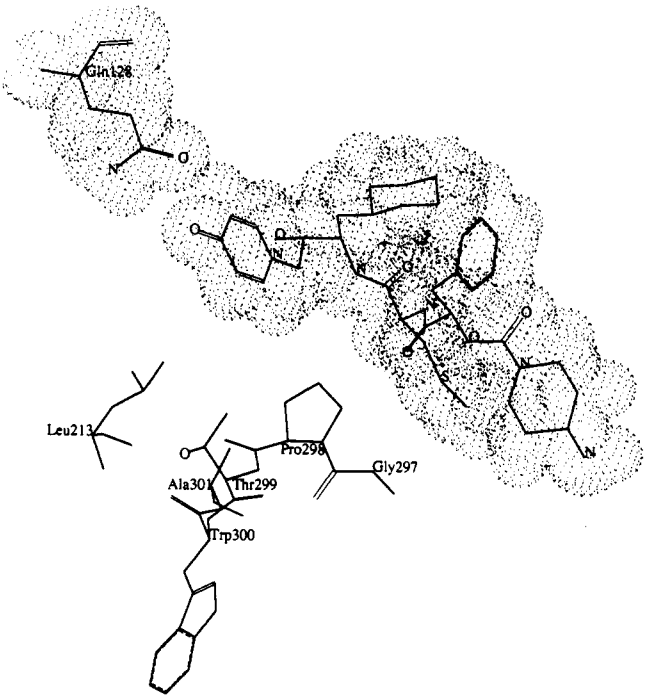


Figure 1. Compound XXXI is shown with selected residues of the S_1' site of the renin model. The oxygen of the pyridine ring interacts unfavorably with the oxygen of Gln 128. Strong electrostatic repulsion may explain the low activity of this compound. In contrast, compound XXIX has the pyridone oxygen in the ortho position where no electrostatic or steric repulsion is expected and shows good activity. (Only functionally important heteroatoms of the S_1' site are labeled).

analogues containing an oxygen atom (XXVIII) and the corresponding 2-pyridone derivative (XXIX) demonstrated a 4-fold decrease in potency.

A striking drop in activity was observed by comparing the pyridine derivative XXX and the pyridone XXXI. The 4-alkoxypyridine compound XXX is equipotent with its 2-alkoxypyridine counterpart XXVIII, whereas the incorporation of the 1,4-dihydro-4-oxopyridine (XXXI) is accompanied by a 80-fold loss of potency. The reason for this loss of activity is not immediately obvious.

Modeling Studies

Molecular graphics and model building was used to further clarify the structure–activity relationships for the C-terminal part of these inhibitors. An early model of human renin[14] based on the homologous aspartic protease penicillopepsin and a more recent model[15] based mainly on high resolution X-ray structures of endothiapepsin with and without inhibitors were used. The different inhibitors were model built into the protease following as far as possible the orientation and local conformation of the endothiapepsin inhibitors in their complexes.

Geometrically speaking, the six inhibitors of Table V fall into two classes. In compounds XXIX and XXXI, the pyridone ring is attached via a short CH_2 bridge to the secondary hydroxy group which interacts with the two aspartic acids of the active site. In inhibitors XXVI, XXVII, XXVIII, and XXX the heterocycle is attached via an additional S or O ether group. This additional group displaces the ring not only laterally relative to the shorter bridge but also introduces a different orientation by approximately 60°. However, inhibitors XXVI, XXVII XXVIII, XXIX, and XXX show no dramatic difference in activity.

In compounds XXIX and XXXI it is likely that the C-terminal ring system comes close to Gln 128. Strong

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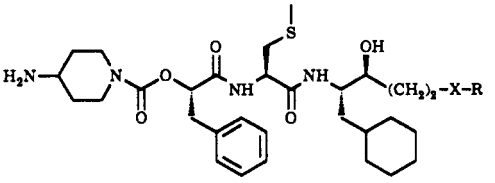
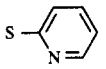
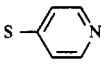
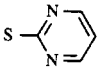
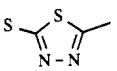
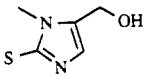
(15) (a) Sibanda, B. L.; Blundell, T.; Hobart, P. M.; Fogliano, M.; Bindra, J. S.; Dominy, B. W.; Chirgwin, J. M. Computer Graphics Modelling of Human Renin. *FEBS Lett.* **1984**, *174* (1), 102–111. (b) Morris, B. J.; Guss, J. M.; Hunter, W. N.; Constanzo, D. F. A Structural Analysis of Human Renin. *Clin. Exp. Pharmacol. Physiol.* **1985**, *12*, 299–304.

(16) Wood, J. M.; Gulati, N.; Michel, J.-B. and Hofbauer, K. G. Two-Kidney, One Clip Renal Hypertension in the Marmoset. *J. Hypertens.* **1986**, *4*, 251–254.

(17) Guégan, R.; Diaz, J.; Cazaubon, C.; Beaumont, M.; Carlet, C.; Clément, J.; Demarne, H.; Mellet, M.; Richaud, J.-P.; Segondy, D.; Vedel, M.; Gagnol, J.-P.; Roncucci, R.; Castro, B.; Corvol, P.; Evin, G.; and Roques, B. P. Pepstatin Analogues as Novel Renin Inhibitors. *J. Med. Chem.* **1986**, *29*, 1152–1159.

Table IV. Effect of the C-Terminal Heterocycle on in Vitro Activity



	X-R	HPLC[a]	IC_{50},[b] nM		
			human plasma renin	cathepsin D	pepsin
XVII		98.3	9.3	>10000	>10000
XX		97.9	53	>10000	>10000
XXI		98.3	41	>10000	>10000
XXII		97.8	5.0	>10000	4800
XXIII		96.7	16	>10000	10000
XXIV		98.1	34	>10000	9000
XXV		96.6	2.6	>10000	>10000

[a] Percent purity as determined by reversed-phase HPLC analysis; see Experimental Section for details. [b] IC_{50} values have an estimated error of ±20%.

steric and electrostatic repulsion of the *p*-oxygen in inhibitor XXXI and the amide oxygen in Gln 128 could be responsible for the very low potency of this compound (Figure 1). The *o*-oxygen in derivative XXIX does not interfere with any parts of Gln 128 and this might explain the relatively good inhibitory activity of this compound.

In contrast, the longer S and O ether bridges in XXVI, XXVII, XXVIII, and XXX accomodate the C-terminal ring system in a different environment. Pro 298, Thr 299, and Leu 213 form this rather hydrophobic binding pocket. The C-terminus of the inhibitors appears to be closely packed against Pro 298 and Thr 299 (Figure 2a). However, opposite the nitrogen of the pyridine group is a strong steric constraint. The close packing in this compartment suggests that substitutions in the para position of the heterocycle would lead to a further decrease in potency because of the steric constraints imposed by the loop Pro 293–Thr 300. The most active compound (XXVI) differs in several respects from the O analogue XXVIII. It avoids an unfavorable electrostatic interaction of the bridge oxygen present in XXVIII with the carbonyl oxygen of Gly 34 (Figure 2b). Semiempirical calculations using AMPAC showed us that the bridge sulfur is positively charged compared to the negatively charged oxygen. The sulfur further changes the charge distribution and the local dipole moment in the ring system significantly. As the rotational barrier present in the O ether XXVIII disappears in the S ether XXVI, the ring system has more freedom for adaptation to its environment. Additionally, the larger sulfur atom (compared to oxygen in XXVIII) may lead to a slightly different orientation of the C-terminal heterocycle resulting in an optimized hydrophobic interaction with the S_1' site.

The molecular modeling was performed using the program SYBYL of Tripos Associates, Inc., on a PS 390 of Evans and Sutherland. AMPAC was obtained from Quantum Chemistry Program Exchange.

Enzyme Selectivity

All inhibitors of Tables IV and V were tested for activity against bovine cathepsin D and porcine pepsin. All exhibited IC_{50} values greater than 10000 nM with cathepsin D. Compounds XXII, XXIV, XXVI, and XXVIII did inhibit porcine pepsin albeit with very weak activity. These results indicate the high specificity of the inhibitors for renin in comparison to related aspartic proteinases.

Enzymatic Stability

Some representative compounds were studied in a chymotryptic digestion assay. As shown in Figures 4 and 5 only those of the peptides presented here containing the L-Phe-L-His peptide bond were cleavable by chymotrypsin. Replacement of the α-amino group of phenylalanine either by oxygen or by the sulfonylmethyl group resulted as expected in enzymatically stable derivatives X, XI, and XIII. These findings are in good agreement with results described by Plattner[8] and Stanton.[9] The substitution of histidine with *S*-methylcysteine in combination with the phenyllactic acid analogue of phenylalanine yielded compounds XVII, XXV, and XXVIII, which are also not degradable by chymotrypsin.

In Vivo Activity

Compound XVII was chosen to investigate the in vivo properties of this new series of renin inhibitors. As shown in Figure 3, inhibitor XVII was given orally at a dose of 10 mg/kg to conscious, sodium-depleted cynomolgus monkeys. Plasma renin activity (PRA) and mean arterial blood pressure (BP) were monitored as a function of time.

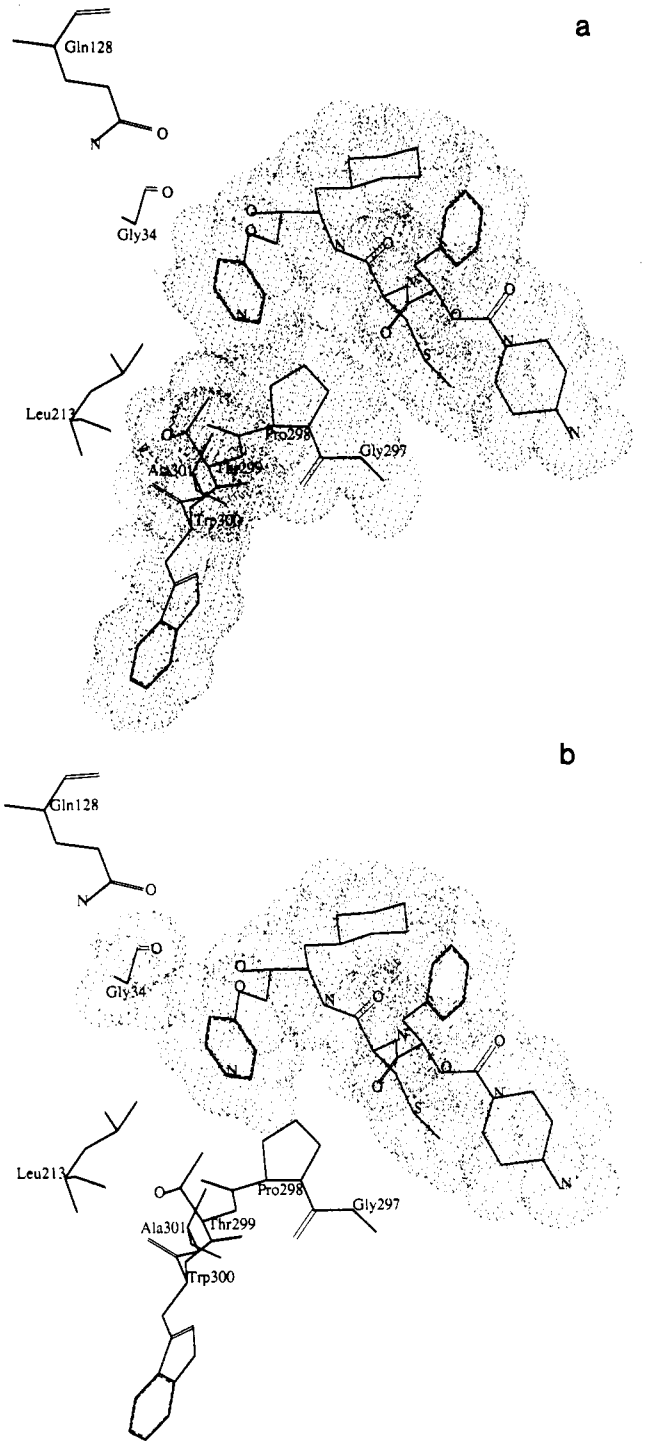


Figure 2. (a) Compound XXX is shown with selected residues of the S_1' site of the renin model. The pyridine ring is surrounded by mainly hydrophobic residues. (Only functionally important heteroatoms of the S_1' site are labeled). (b) The interaction of compound XXX with Gly 34 is highlighted. The unfavorable electrostatic interaction of the carbonyl oxygen of Gly 34 with the ether oxygen (both are negatively charged) may explain the somewhat lesser activity of compound XXX compared to compound XXVI which has a positively charged sulfur in the equivalent position.

The administered dose produced a hypotensive response of 16 mmHg accompanied by a 73% drop in PRA. Both effects lasted for more than 180 min.

Conclusion

A new series of renin inhibitors containing P_1-P_1' mimetics with various heterocycles in P_1' was developed.

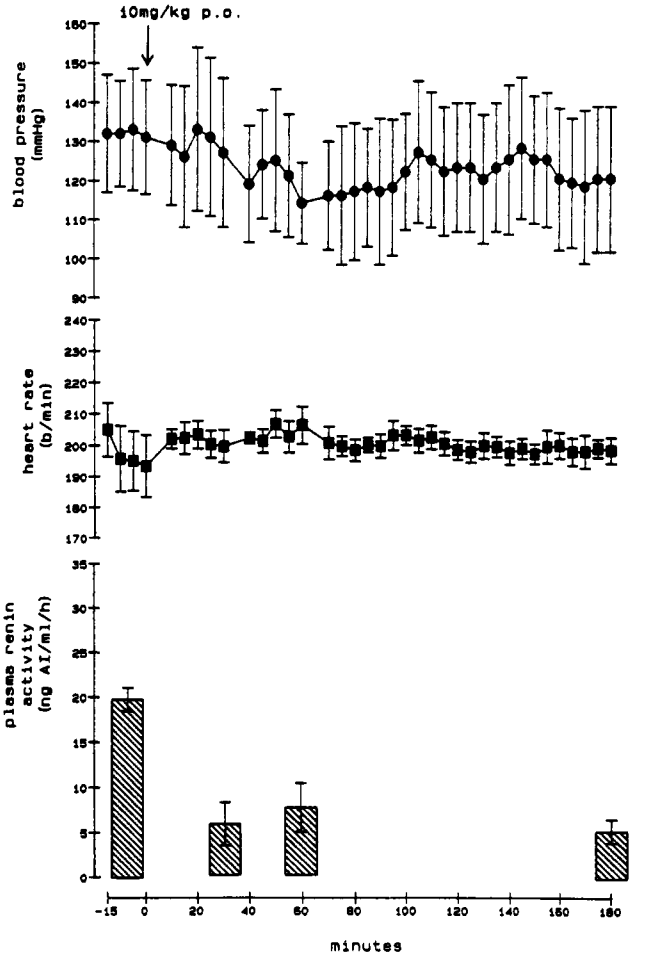


Figure 3. Effects of a 10 mg/kg oral dose of inhibitor XVII in salt-depleted cynomolgus monkeys. Results are shown as mean ± SEM of four animals. Blood pressure = systolic arterial blood pressure, AI = angiotensin I.

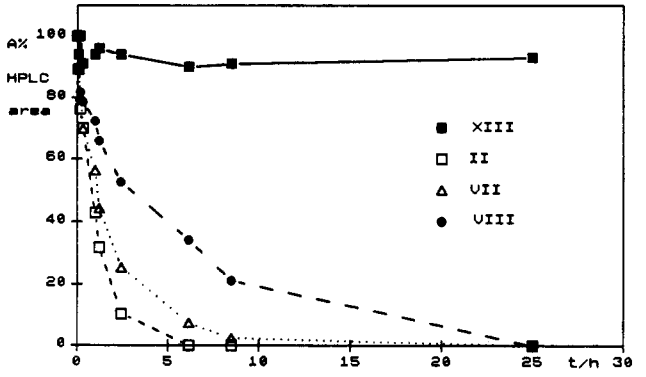


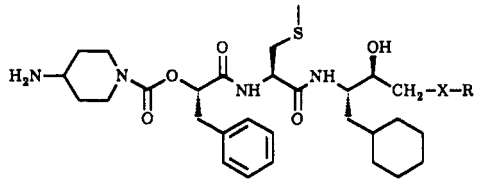
Figure 4. Chymotryptic degradations of renin inhibitors (structures shown in Tables I, II, and III).

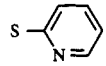
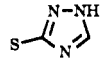
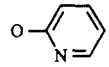
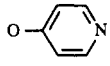
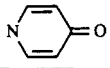
Structure–activity studies provided compounds with inhibitory activity in the nanomolar range. Modifications in the P_3 position lead to inhibitors with stability against the intestinal protease chymotrypsin. These inhibitors are highly selective for renin over the two related aspartic proteinases cathepsin D and pepsin. Preliminary in vivo studies with compound XVII demonstrated a good efficacy after oral administration. Further studies are currently underway to exploit the potential of these novel renin inhibitors.

Experimental Section

Melting points were determined with a Mettler FP 62 melting point apparatus and are uncorrected. Specific rotations were

Table V. Effect of the C-Terminal Heterocycle on in Vitro Activity



	X-R	HPLC[a]	IC_{50},[b] nM: human plasma renin	IC_{50},[b] nM: cathepsin D	IC_{50},[b] nM: pepsin
XXVI		99.5	8.5	>10000	4000
XXVII		98.6	15.5	>10000	>10000
XXVIII		96.7	42	>10000	2700
XXIX		97.8	41	>10000	>10000
XXX		98.7	56	>10000	>10000
XXXI		97.6	4400	>10000	>10000

[a] Percent purity as determined by reversed-phase HPLC analysis; see Experimental Section for details. [b] IC_{50} values have an estimated error of ±20%.

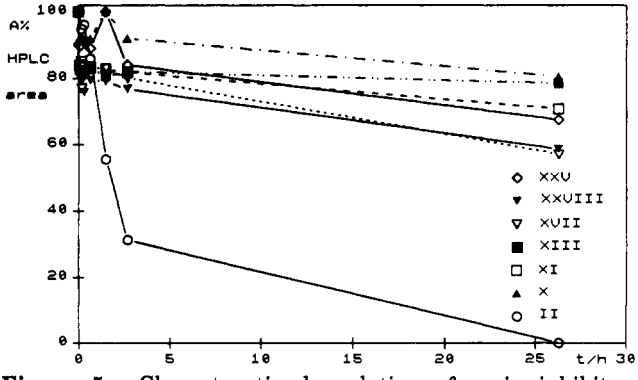


Figure 5. Chymotryptic degradation of renin inhibitors (structures shown in Tables I–V).

measured with a Perkin Elmer 241 MC polarimeter. IR, NMR, and mass spectra are in agreement with the structures cited and were recorded on a Bruker 85 IFS 48 IR spectrophotometer, a Bruker AC 200, WM 250 or AM 500 (TMS as internal standard), and a Vacuum Generator VG 70-70 or 70-250 at 70 eV, respectively. Microanalyses were obtained with a Perkin-Elmer 240B CHN analyzer. Thin-layer chromatography (TLC) was carried out on precoated silica gel F_{254} plates with a layer thickness of 0.25 mm from E. Merck, Darmstadt. Visualization was done with UV and I_2. Analytical HPLC data were obtained by using an E. Merck-Hitachi instrument (L-6200 pump, L-4250 detector, D-2500 integrator) equipped with a Lichrospher 60 RP-select B reverse-phase column (250 × 4 mm, E. Merck) at ambient temperature with CH_3CN and NaH_2PO_4 buffer (0.05 m, pH 6) as eluents, and detection at 210 nm.

(2*S*,3*S*)-2-(*tert*-Butoxycarboxamido)-1-cyclohexyl-5-hexen-3-ol (2a) and (2*S*,3*R*)-2-(*tert*-Butoxycarboxamido)-1-cyclohexyl-5-hexen-3-ol (2b). To a solution of *N*-Boc-L-cyclohexylalaninal (1) (12.75 g, 50 mmol) in dry THF (10 mL) were added 3-bromopropene (14.5 g, 120 mmol), a saturated aqueous solution of NH_4Cl (50 mL), and zinc dust (7.8 g, 120 mmol). The resulting suspension was stirred at ambient temperature for 2 h. The suspension was poured into 1 N aqueous HCl solution (250 mL) and extracted three times with EtOAc. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The diastereomeric mixture was separated by flash chromatography (12% EtOAc/hexane), giving 5.8 g of **2a** (38% yield) as colorless liquid and 3.8 g of **2b** (25% yield) as a white solid. **2a**: $[\alpha]^{20}_D$ −30.3° (*c* 0.81, MeOH); ^{1}H-NMR (DMSO-d_6) δ 6.08 (d, *J* = 9.2 Hz, 1 H), 5.78 (m, 1 H), 5.03 (m, 2 H), 4.47 (d, *J* = 6.4 Hz, 1 H), 3.52 (m, 1 H), 3.38 (m, 1 H), 2.13 (t, *J* = 7.2 Hz, 2 H), 1.5–1.85 (m, 6 H), 1.37 (s, 9 H), 1.25 (m, 5 H), 0.91 (m, 2 H); FAB MS (M^+ + H) 298; TLC R_f = 0.35 (12% EtOAc/hexane). Anal. ($C_{17}H_{31}NO_3$) C, H, N. **2b**: mp 91–2 °C; $[\alpha]^{20}_D$ −17.4° (*c* 1, MeOH); ^{1}H-NMR (DMSO-d_6) δ 6.38 d, *J* = 9 Hz, 1 H), 5.72 (m, 1 H), 4.99 (m, 2 H), 4.48 (d, *J* = 6.2 Hz, 1 H), 3.35 (m, 2 H), 1.5–2.2 (m, 8 H), 1.41 (s, 9 H), 1.22 (m, 5 H), 0.89 (m, 2 H); FAB MS (M^+ + H) 298; TLC R_f = 0.27 (12% EtOAc/hexane). Anal. ($C_{17}H_{31}NO_3$) C, H, N.

(4*S*,5*S*)-5-Allyl-4-(cyclohexylmethyl)oxazolidin-2-one (3a). To a solution of compound **2a** (297 mg, 1 mmol) in 5 mL dry DMF was added NaH (36 mg, 1.2 mmol) at 0 °C. After being stirred for 14 h at room temperature, the reaction mixture was poured into aqueous NaCl solution. The resulting mixture was extracted with EtOAc, and the organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (30% EtOAc/hexane) yielding 185 mg (83% yield) of **3a** as a white solid: mp 66–7 °C; $[\alpha]^{20}_D$ −80.3° (*c* 1.03, MeOH); ^{1}H-NMR (DMSO-d_6) δ 7.78 (s, 1 H), 5.75 (m, 1 H), 5.16 (m, 2 H), 4.11 (q, *J* = 5.8 Hz, 1 H), 3.48 (q, *J* = 5.8 Hz, 1 H), 2.38 (t, *J* = 5.5 Hz, 2 H), 1.5–1.75 (m, 6 H), 1.05–1.35 (m, 5 H), 0.93 (m, 2 H), $J_{H4,H5}$ = 5.8 Hz; FAB MS (M^+ + H) 224; TLC R_f = 0.35 (20% EtOAc/hexane). Anal. ($C_{13}H_{21}NO_2$) C, H, N.

(4*S*,5*R*)-5-Allyl-4-(cyclohexylmethyl)oxazolidin-2-one (3b). The title compound was obtained from **2b** as described for **3a** as a white solid (193 mg, 87% yield): mp 103–4 °C; $[\alpha]^{20}_D$ −20.6° (*c* 0.99, MeOH); ^{1}H-NMR (DMSO-d_6) δ 7.72 (s, 1 H), 5.81 (m, 1 H), 5.17 (m, 2 H), 4.05 (q, *J* = 7.6 Hz, 1 H), 3.82 (m, 1 H), 2.33 (t, *J* = 5.5 Hz, 2 H), 1.5–1.75 (m, 6 H), 1.1–1.4 (m, 5 H), 0.92 (m, 2 H), $J_{H4,H5}$ = 7.6 Hz; FAB MS (M^+ + H) 224; TLC R_f = 0.27 (20% EtOAc/hexane). Anal. ($C_{13}H_{21}NO_2$) C, H, N.

(4*S*,5*S*)-5-Allyl-3-(*tert*-butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyloxazolidine (4). Homoallyl alcohol **2a** (16 g, 53.8 mmol) was stirred in a solution of 20 mL of dimethoxypropane, 100 mL of CH_2Cl_2, and 200 mg of *p*-toluenesulfonic acid for 14 h at ambient temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel by elution with hexane/MTBE (95/5) yielding 15 g (83%) of 4 as a white solid: mp 68–9 °C; $[\alpha]^{20}_D$ +7.3° (*c* 0.97, MeOH); ^{1}H-NMR (DMSO-d_6) δ 5.75 (m, 1 H), 5.12 (m, 2 H), 3.89 (dt, J_d = 2.5 Hz, J_t = 7.2 Hz, 1 H), 3.65 (m, 1 H), 2.30 (t, *J* = 7.2 Hz, 2 H), 1.5–1.85 (m, 6 H), 1.53 (s, 3 H), 1.47 (s, 12 H), 1.23 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H − Boc) 238; TCL R_f = 0.27 (2% EtOAc/hexane). Anal. ($C_{20}H_{35}NO_3$) C, H, N.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyloxazolidine-5-ethanol (5a). Ozone was passed into a solution containing the allyl compound 4 (33.7 g, 100 mmol) in 500 mL of CH_2Cl_2 at −78 °C until the color turned light blue. Excess O_3 was blown out with O_2, and the ozonide was cleaved by adding Me_2S (50 mL). The reaction mixture was kept stirring at room temperature overnight. The solvent was removed under reduced pressure. The crude aldehyde (35 g) was stirred in 150 mL MeOH with 2 g sodium borohydride at 0 °C for 3 h. The reaction mixture was neutralized with 1 N aqueous HCl, and the solvent was removed under reduced pressure. The residue was extracted with EtOAc, and the extract was dried (Na_2SO_4) and evaporated. The crude product was purified by flash chromatography (5% MTBE/CH_2Cl_2), giving 25.3 g (74%) of alcohol **5a** as a white solid: mp 64–5 °C; $[\alpha]^{20}_D$ +6.1° (*c* 0.99, MeOH); ^{1}H-NMR (DMSO-d_6) δ 4.45 (t, *J* = 7.2 Hz, 1 H), 3.93 (m, 1 H), 3.62 (m, 1 H), 3.48 (q, *J* = 7.2 Hz, 2 H), 1.5–1.85 (m, 8 H), 1.54 (s, 3 H), 1.43 (s, 12 H), 1.23 (m, 5 H), 0.94 (m, 2 H); FAB MS (M^+ + H) 342; TLC R_f = 0.26 (20% MTBE/hexane). Anal. ($C_{19}H_{35}NO_4$) C, H, N.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-5-[2-(mesyloxy)ethyl]-2,2-dimethyloxazolidine (5b). Alcohol **5a** (23.8 g, 70 mmol) was dissolved in 200 mL of CH_2Cl_2

at 0 °C. Addition of triethylamine (10.1 g, 100 mmol) was followed by methanesulfonyl chloride (11.5 g, 100 mmol). After stirring at room temperature for 6 h, the mixture was washed twice with 1 N aqueous HCl and saturated $NaHCO_3$ solutions each. The organic layer was dried (Na_2SO_4) and evaporated. The residue was crystallized from hexane/MTBE yielding 25.2 g (86%) of mesylate **5b**: mp 76–7 °C; $[\alpha]^{20}_D$ –6.4° (*c* 0.98, MeOH); ^{1}H-NMR (DMSO-d_6) δ 4.32 (t, *J* = 7.1 Hz, 2 H), 3.95 (m, 1 H), 3.68 (m, 1 H), 3.17 (s, 3 H), 1.95 (m, 2 H), 1.5–1.75 (m, 6 H), 1.54 (s, 3 H), 1.43 (s, 12 H), 1.22 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 420; TLC R_f = 0.59 (5% MTBE/CH_2Cl_2). Anal. ($C_{20}H_{37}NO_6S$) C, H, N, S.

General Procedure for the Preparation of the Protected Dipeptide Mimetics 6a–i, 10a–e, and 12a,b. To a 0 °C suspension of sodium hydride (1.35 g, 45 mmol) in 100 mL of dry DMF was added dropwise a solution of 2-mercaptopyridine (5 g, 45 mmol) in 20 mL of DMF. After stirring for 30 min at ambient temperature, mesylate **5b** (6.3 g, 15 mmol) was added. After further stirring for 14 h, the reaction mixture was poured into water and neutralized with 1 N aqueous HCl. The aqueous phase was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. Purification was accomplished by flash chromatography (5–20% EtOAc/hexane or 5–30% MTBE/hexane).

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-(2-pyridylthio)ethyl]oxazolidine (6a) was obtained as a white solid (4.95 g, 76% yield): mp 76–7 °C; $[\alpha]^{20}_D$ –22.9° (*c* 1.04, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.41 (dd, *J* = 1.9, 5.5 Hz, 1 H), 7.62 (dd, *J* = 1.9, 7.5 Hz, 1 H), 7.30 (d, *J* = 7.5 Hz, 1 H), 7.12 (dt, J_d = 1.5 Hz, J_t = 5.5 Hz, 1 H), 4.03 (m, 1 H), 3.65 (m, 1 H), 3.09 (m, 2 H), 1.95 (m, 2 H), 1.5–1.75 (m, 6 H), 1.52 (s, 3 H), 1.48 (s, 12 H), 1.15 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 435; TLC R_f = 0.33 (10% MTBE/hexane). Anal. ($C_{24}H_{38}N_2O_3S$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-(2-pyridyloxy)ethyl]oxazolidine (6b) was obtained from **5b** and 2-hydroxypyridine as a white solid (4.6 g, 75% yield): mp 111–2 °C; $[\alpha]^{20}_D$ +10.6° (*c* 1.03, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.13 (dd, *J* = 1.9, 5.5 Hz, 1 H), 7.64 (dt, J_d = 1.9 Hz, J_t 7.6 Hz, 1 H), 6.91 (dt, J_d = 1.5 Hz, J_t = 5.5 Hz, 1 H), 6.75 (d, *J* = 7.6 Hz, 1 H), 4.27 (m, 2 H), 4.02 (m, 1 H), 3.72 (m, 1 H), 1.95 (m, 2 H), 1.55 (s, 3 H), 1.5–1.75 (m, 6 H), 1.45 (s, 12 H), 1.15 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 419; TLC R_f = 0.16 (10% MTBE/hexane). Anal. ($C_{24}H_{38}N_2O_4$) C, H, N.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-(4-pyridylthio)ethyl]oxazolidine (6c) was obtained from **5b** and 4-mercaptopyridine as a white solid (3.6 g, 55.6% yield): mp 67–8 °C; $[\alpha]^{20}_D$ –20.4° (*c* 1.01, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.38 (dd, *J* = 1, 7.2 Hz, 2 H), 7.27 (dd, *J* = 1, 7.2 Hz, 2 H), 4.02 (m, 1 H), 3.62 (m, 1 H), 3.15 (m, 2 H), 1.87 (q, *J* = 7.5 Hz, 2 H), 1.5–1.75 (m, 6 H), 1.53 (s, 3 H), 1.47 (s, 12 H), 1.15 (m, 5 H), 0.92 (m, 2 H); FAB MS (M^+ + H) 435; TLC R_f = 0.15 (20% EtOAc/hexane). Anal. ($C_{24}H_{38}N_2O_3S$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-(2-pyrimidinylthio)ethyl]oxazolidine (6d) was obtained from **5b** and 2-mercaptopyrimidine as a white solid (4.1 g, 62.8% yield): mp 85–6 °C; $[\alpha]^{20}_D$ –21.6° (*c* 0.99, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.63 (d, *J* = 5.3 Hz, 2 H), 7.23 (t, *J* = 5.3 Hz, 1 H), 3.97 (m, 1 H), 3.68 (m, 1 H), 3.15 (m, 2 H), 1.93 (q, *J* = 7.2 Hz, 2 H), 1.5–1.75 (m, 6 H), 1.53 (s, 3 H), 1.43 (s, 12 H), 1.12 (m, 5 H), 0.95 (m, 2 H); FAB MS (M^+ + H) 436; TLC R_f = 0.41 (30% MTBE/hexane). Anal. ($C_{23}H_{37}N_3O_3S$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-(1*H*-1,2,4-triazol-3-ylthio)ethyl]oxazolidine (6e) was obtained from **5b** and 3-mercapto-1*H*-1,2,4-triazole as a slightly yellow solid (2.6 g, 41% yield): mp 134–5 °C; $[\alpha]^{20}_D$ –25.0° (*c* 0.98, MeOH); ^{1}H-NMR (DMSO-d_6) δ 14.0 (br s, 1 H), 8.41 (s, 1 H), 3.95 (m, 1 H), 3.62 (m, 1 H), 3.22 (m, 2 H), 1.93 (q, *J* = 7.1 Hz, 2 H), 1.55–1.85 (m, 6 H), 1.52 (s, 3 H), 1.45 (s, 12 H), 1.23 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 425; TLC R_f = 0.22 (20% EtOAc/hexane). Anal. ($C_{21}H_{36}N_4O_3S$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-[(2-methyl-1,3,4-thiadiazol-5-yl)thio]ethyl]oxazolidine (6f) was obtained from **5b** and 5-mercapto-2-methyl-1,3,4-thiadiazole as a white solid (4.4 g, 64% yield): mp 81–1 °C; $[\alpha]^{20}_D$ –25.0° (*c* 1, MeOH); ^{1}H-NMR (DMSO-d_6) δ 3.96 (m, 1 H), 3.63 (m, 1 H), 3.25 (m, 2 H), 2.71 (s, 3 H), 2.03 (q, *J* = 7.2 Hz, 2 H), 1.5–1.75 (m, 6 H), 1.53 (s, 3 H), 1.47 (s, 12 H), 1.17 (m, 5 H), 0.95 (m, 2 H); FAB MS (M^+ + H) 456; TLC R_f = 0.38 (20% EtOAc/hexane). Anal. ($C_{22}H_{37}N_3O_3S_2$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-[(1-methyl-1*H*-tetrazol-5-yl)thio]ethyl]oxazolidine (6g) was obtained from **5b** and 5-mercapto-1-methyl-1*H*-tetrazole as a white solid (5.7 g, 86% yield): mp 79–80 °C; $[\alpha]^{20}_D$ –15.6° (*c* 0.98, MeOH); ^{1}H-NMR (DMSO-d_6) δ 3.96 (m, 1 H), 3.92 (s, 3 H), 3.64 (m, 1 H), 3.27 (m, 2 H), 2.03 (q, *J* = 7.1 Hz, 2 H), 1.5–1.83 (m, 6 H), 1.53 (s, 3 H), 1.44 (s, 12 H), 1.17 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 440; TLC R_f = 0.35 (20% EtOAc/hexane). Anal. ($C_{21}H_{37}N_5O_3S$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[2-(2-thiazolylthio)ethyl]oxazolidine (6h) was obtained from **5b** and 2-mercaptothiazole as a white solid (4.95 g, 75% yield): mp 75–6 °C; $[\alpha]^{20}_D$ –27.1° (*c* 1.02, MeOH); ^{1}H-NMR (DMSO-d_6) δ 7.78 (d, *J* = 3.5 Hz, 1 H), 7.72 (d, *J* = 3.5 Hz, 1 H), 3.99 (m, 1 H), 3.66 (m, 1 H), 3.27 (m, 2 H), 1.97 (q, *J* = 7.2 Hz, 2 H), 1.5–1.8 (m, 6 H), 1.53 (s, 3 H), 1.43 (s, 12 H), 1.13 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 441; TLC R_f = 0.25 (10% MTBE/hexane). Anal. ($C_{22}H_{36}N_2O_3S_2$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-5-[2-[(5-(hydroxymethyl)-1-methyl-2-imidazolyl)thio]ethyl]-2,2-dimethyloxazolidine (6i) was obtained from **5b** and 5-(hydroxymethyl)-2-mercapto-1-methylimidazole as a slightly yellow solid (4.2 g, 60% yield): mp 79–80 °C; $[\alpha]^{20}_D$ –26.8 °C (*c* 1.05, MeOH); ^{1}H-NMR (DMSO-d_6) δ 6.83 (s, 1 H), 5.03 (t, *J* = 5 Hz, 1 H), 4.40 (d, *J* = 5 Hz, 2 H), 3.98 (m, 1 H), 3.61 (m, 1 H), 3.55 (s, 3 H), 3.05 (m, 2 H), 1.85 (q, *J* = 7.2 Hz, 2 H), 1.5–1.75 (m, 6 H), 1.52 (s, 3 H), 1.43 (s, 12 H), 1.12 (m, 5 H), 0.92 (m, 2 H); FAB MS (M^+ + H) 468; TLC R_f = 0.32 (5% MeOH/CH_2Cl_2). Anal. ($C_{24}H_{41}N_3O_4S$) C, H, N, S.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[(2-pyridyloxy)methyl]oxazolidine (10a) and (4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-5-[(1,2-dihydro-2-oxo-1-pyridyl)methyl]-2,2-dimethyloxazolidine (10b) were obtained from **9** and 2-hydroxypyridine as white solids. **10a**: (2.3 g, 38% yield); mp 125–6 °C; ^{1}H-NMR (DMSO-d_6) δ 8.15 (dd, *J* = 1.9, 5.3 Hz, 1 H), 7.72 (dt, J_d = 1.9 Hz, J_t = 6.7 Hz, 1 H), 7.0 (dt, J_d = 1.5 Hz, J_t = 5.3 Hz, 1 H), 6.78 (d, *J* = 8.3 Hz, 1 H), 4.41 (t, *J* = 3.5 Hz, 2 H), 4.25 (m, 1 H), 3.91 (m, 1 H), 1.5–1.70 (m, 6 H), 1.52 (s, 3 H), 1.46 (s, 3 H), 1.42 (s, 9 H), 1.15 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 405; TLC R_f = 0.22 (10% MTBE/hexane); UV_{max} 214, 271 nm (*c* 4mg/100 mL MeOH). Anal. ($C_{23}H_{36}N_2O_4$) C, H, N. **10b**: (1.1 g, 19% yield); mp 144–5 °C; ^{1}H-NMR (DMSO-d_6) δ 7.65 (dd, *J* = 2, 6.5 Hz, 1 H), 7.43 (dt, J_d = 2 Hz, J_t = 6.5 Hz, 1 H), 6.42 (dd, *J* = 1.3, 9.2 Hz, 1 H), 6.24 (dt, J_d = 1.4 Hz, J_t = 6.5 Hz, 1 H), 4.18 (br s, 2 H), 3.76 (m, 2 H), 1.5–1.70 (m, 6 H), 1.56 (s, 3 H), 1.42 (s, 12 H), 1.1–0.9 (m, 7 H); FAB MS (M^+ + H) 405; TLC R_f = 0.35 (40% hexane/EtOAc); UV_{max} 229, 304 nm (*c* 4 mg/100 mL MeOH). Anal. ($C_{23}H_{36}N_2O_4$) C, H, N.

(4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[(4-pyridyloxy)methyl]oxazolidine (10c) and (4*S*,5*S*)-3-(*tert*-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[(1,4-dihydro-4-oxo-1-pyridyl)methyl]oxazolidine (10d) were obtained from **9** and 4-hydroxypyridine. **10c**: (2.4 g, 39% yield); mp 116–7 °C; ^{1}H-NMR (DMSO-d_6) δ 8.41 (d, *J* = 5.5 Hz, 2 H), 6.97 (d, *J* = 5.5 Hz, 2 H), 4.23 (m, 1 H), 4.12 (m, 2 H), 3.92 (m, 1 H), 1.55–1.85 (m, 6 H), 1.53 (s, 3 H), 1.48 (s, 3 H), 1.45 (s, 9 H), 1.18 (m, 5 H), 0.94 (m, 2 H); FAB MS (M^+ + H) 405; UV_{max} 218, 272 nm (*c* 4 mg/100 mL MeOH). Anal. ($C_{23}H_{36}N_2O_4$) C, H, N. **10d**: (1.33 g, 22% yield); mp 196 °C; ^{1}H-NMR (DMSO-d_6) δ 7.68 (d, *J* = 8.5 Hz, 2 H), 6.07 (d, *J* = 8.5 Hz, 2 H), 4.13 (m, 1 H), 3.96 (m, 2 H), 3.75 (m, 1 H), 1.60 (s, 6 H), 1.5–1.75 (m, 6 H), 1.44 (s, 9 H), 1.18 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 405; UV_{max} 231, 306 nm (*c* 4 mg/100 mL MeOH). Anal. ($C_{23}H_{36}N_2O_4$) C, H, N.

(4S,5S)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[(2-pyridylthio)methyl]oxazolidine (10e) was obtained from **9** and 2-mercaptopyridine as a white solid (3.94 g, 62.5% yield): mp 123–4 °C; $[\alpha]^{20}_D$ +49.6° (*c* 1.03, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.42 (dd, *J* = 1.5, 5.5 Hz, 1 H), 7.63 (dt, J_d = 1.9 Hz, J_t = 7.5 Hz, 1 H), 7.32 (d, *J* = 7.5 Hz, 1 H), 7.15 (dd, *J* = 1.9, 7.5 Hz, 1 H), 4.05 (m, 1 H), 3.88 (m, 1 H), 3.38 (m, 2 H), 1.5–1.85 (m, 6 H), 1.52 (s, 3 H), 1.44 (s, 12 H), 1.12 (m, 5 H), 0.92 (m, 2 H); FAB MS (M^+ + H) 421; TLC R_f = 0.32 (5% EtOAc/hexane). Anal. ($C_{23}H_{36}N_2O_3S$) C, H, N, S.

(4S,5S)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[(1*H*-1,2,4-triazol-3-ylthio)methyl]oxazolidine (10f) was obtained from **9** and 3-mercapto-1*H*-1,2,4-triazole as a slightly yellow solid (3.4 g, 55% yield): mp 168–9 °C; $[\alpha]^{20}_D$ +58.3° (*c* 0.87, MeOH); ^{1}H-NMR (DMSO-d_6) δ 14.05 (br s, 1 H), 8.45 (s, 1 H), 4.08 (m, 1 H), 3.87 (m, 1 H), 3.25 (br s, 2 H), 1.5–1.85 (m, 6 H), 1.57 (s, 3 H), 1.44 (s, 12 H), 1.13 (m, 5 H), 0.92 (m, 2 H); FAB MS (M^+ + H) 411; TLC R_f = 0.24 (40% EtOAc/hexane). Anal. ($C_{20}H_{34}N_4O_3S$) C, H, N, S.

(4S,5S)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-[3-(2-pyridylthio)propyl]oxazolidine (12) was obtained from **11b** and 2-mercaptopyridine as a white solid (4.65 g, 69% yield): mp 74–5 °C; $[\alpha]^{20}_D$ –5.4° (*c* 0.91, MeOH); ^{1}H-NMR (DMSO-d_6) δ 8.41 (dd, *J* = 1.9, 5.5 Hz, 1 H), 7.62 (dt, J_d = 1.5 Hz, J_t = 7.5 Hz, 1 H), 7.26 (d, *J* = 7.5 Hz, 1 H), 7.11 (dd, *J* = 1.5, 5.5 Hz, 1 H), 3.82 (m, 1 H), 3.58 (m, 1 H), 3.15 (t, *J* = 7.2 Hz, 2 H), 1.5–1.75 (m, 10 H), 1.51 (s, 3 H), 1.49 (s, 9 H), 1.47 (s, 3 H), 1.15 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 449; TLC R_f = 0.20 (7.5% MTBE/hexane). Anal. ($C_{25}H_{40}N_2O_3S\cdot 0.5H_2O$) C, H, N, S.

(4S,5S)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyl-5-vinyloxazolidine (7). To a 0 °C solution of mesylate **5b** (42 g, 100 mmol) in 200 mL of toluene was added dropwise over a period of 15 min a solution of *t*-BuOK (22.4 g, 200 mmol) in 200 mL of DMSO. After stirring for 30 min at room temperature, the reaction mixture was poured into water and neutralized with 1 N aqueous HCl. The aqueous phase was extracted three times with EtOAc. The combined phases were washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (hexane) giving olefin **7** as a colorless oil (25.3 g, 78% yield): $[\alpha]^{20}_D$ +16.2° (*c* 1, MeOH); ^{1}H-NMR (DMSO-d_6) δ 5.95 (ddd, *J* = 7, 10.3, 17.2 Hz, 1 H), 5.5 (dd, *J* = 10.3, 17.2 Hz, 2 H), 4.30 (dd, *J* = 3.9, 7 Hz, 1 H), 3.71 (m, 1 H), 1.5–1.80 (m, 6 H), 1.57 (s, 3 H), 1.44 (s, 12 H), 1.15 (m, 5 H), 0.92 (m, 2 H); FAB MS (M^+ + H) 324; TLC R_f = 0.35 (2% EtOAc/hexane). Anal. ($C_{19}H_{33}NO_3$) C, H, N.

(4S,5R)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyloxazolidine-5-methanol (8). The title compound was prepared in 56% yield from **7** as described for **5a**: mp 87–8 °C; $[\alpha]^{20}_D$ +25.1° (*c* 1, MeOH); ^{1}H-NMR (DMSO-d_6) δ 4.85 (t, *J* = 5.3 Hz, 1 H), 3.78 (m, 2 H), 3.37 (t, *J* = 5.5 Hz, 2 H), 1.50–1.85 (m, 6 H), 1.48 (s, 3 H), 1.42 (s, 12 H), 1.15 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 328; TLC R_f = 0.35 (5% MTBE/CH_2Cl_2). Anal. $C_{18}H_{33}NO_4$) C, H, N.

(4S,5R)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-5-[(mesyloxy)methyl]-2,2-dimethyloxazolidine (9). The title compound was prepared from **8** (18 g, 55 mmol) as described for **5b** to give a white solid (19 g, 85% yield): mp 62–3 °C; $[\alpha]^{20}_D$ +6.1° (*c* 0.94, MeOH); ^{1}H-NMR (DMSO-d_6) δ 4.22 (dd, *J* = 5.5, 1.2 Hz, 2 H), 4.15 (m, 1 H), 3.85 (m, 1 H), 3.25 (s, 3 H), 1.5–1.85 (m, 6 H), 1.53 (s, 3 H), 1.42 (s, 12 H), 1.13 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 406; TLC R_f = 0.52 (20% EtOAc/hexane). Anal. ($C_{19}H_{35}NO_6S$) C, H, N, S.

(4S,5S)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-2,2-dimethyloxazolidine-5-propanol (11a). To a solution of olefin **4** (50.6 g, 150 mmol) in dry THF (100 mL) under an atmosphere of N_2 was added an 1 N solution of BH_3 in THF (60 mL, 60 mmol). After stirring at ambient temperature for 1 h, water (15 mL), 3 N aqueous NaOH (20 mL), and 30% H_2O_2 (20 mL) were added. The mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with ether (250 mL), washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (35% MTBE/hexane) to give **11a** as a white solid (39.1 g, 73% yield): mp 65–6 °C; $[\alpha]^{20}_D$ +5.5° (*c* 0.91, MeOH); ^{1}H-NMR (DMSO-d_6) δ 4.38 (t, *J* = 5.3 Hz, 1 H), 3.8 (m, 1 H), 3.58 (m, 1 H), 3.41 (q, *J* = 7.2 Hz, 2 H), 1.5–1.75 (m, 10 H), 1.48 (s, 3 H), 1.41 (s, 12 H), 1.15 (m, 5 H), 0.92 (m, 2 H); FAB MS (M^+ + H) 356; TLC R_f = 0.2 (35% MTBE/hexane). Anal. ($C_{20}H_{37}NO_4$) C, H, N.

(4S,5S)-3-(tert-Butoxycarbonyl)-4-(cyclohexylmethyl)-5-[3-(mesyloxy)propyl]-2,2-dimethyloxazolidine (11b). The title compound was prepared from **11a** as described for **5b**: mp 74–5 °C; $[\alpha]^{20}_D$ +5.3° (*c* 1.1, MeOH); ^{1}H-NMR (DMSO-d_6) δ 4.25 (t, *J* = 7.2 Hz, 2 H), 3.84 (m, 1 H), 3.62 (m, 1 H), 3.15 (s, 3 H), 1.5–1.85 (m, 10 H), 1.52 (s, 3 H), 1.46 (s, 12 H), 1.15 (m, 5 H), 0.93 (m, 2 H); FAB MS (M^+ + H) 434; TLC R_f = 0.28 (35% MTBE/hexane). Anal. ($C_{21}H_{39}NO_6S$) C, H, N, S.

General Procedure for Amino Acid Coupling To Obtain Compound I–XXXI. (2S,3S)-2-(Boc-Phe-His-Amino)-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol (V). Step 1. A solution of **6a** (1.73 g, 4 mmol) in 10 mL of MeOH and 30 mL of 4 N HCl in dioxane was stirred for 1 h at ambient temperature. The solvents were removed under reduced pressure and the solid HCl salt **13** (1.25 g, 96% yield) was obtained by trituration in ether. To a 0 °C solution of N^{α}-(*tert*-butoxycarbonyl)-N^{im}-(2,4-dinitrophenyl)-L-histidine (1.68 g, 4 mmol) in dry DMF (30 mL) were added *N*-methylmorpholine (1.62 g, 12 mmol), and HCl salt **13** (1.25 g), 1-hydroxybenzotriazole (0.6 g, 4 mmol), and *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.77 g, 4 mmol). After stirring for 14 h at room temperature, the mixture was poured into a saturated $NaHCO_3$ solution. The precipitate was extracted into CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by flash chromatography (2–10% MeOH/CH_2Cl_2). The product **14** was obtained as a yellow foam (2.2 g, 79% yield).

Step 2. A solution of the product from step 1 (2.2 g, 3.16 mmol) was stirred with 30 mL of 4 N HCl in dioxane for 1 h at room temperature. After evaporation under reduced pressure, the residue was triturated with ether to give a yellow solid (1.95 g, 92% yield). To a 0 °C solution of N^{α}-(*tert*-butoxycarbonyl)-L-phenylalanine (0.84 g, 3.16 mmol) in dry DMF (30 mL) were added *N*-methylmorpholine (1.28 g, 9.5 mmol), the HCl salt (2.2 g), 1-hydroxybenzotriazole (0.47 g, 3.16 mmol), and *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.61 g, 3.16 mmol). After stirring at ambient temperature, the mixture was poured into a saturated $NaHCO_3$ solution. The precipitate was extracted into CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness to give an orange solid which was purified by flash chromatography (2–10% MeOH/CH_2Cl_2). The product **15** was obtained as a yellow solid (1.94 g, 73% yield).

Step 3. The product from step 2 (1.9 g, 2.25 mmol) was dissolved in dry DMF (20 mL), and thiophenol (3 mL) and NEt_3 (0.03 mL) were added. After stirring for 5 h at room temperature, the reaction mixture was poured into water. The precipitate was extracted into CH_2Cl_2. The combined extracts were washed twice with 1 N aqueous NaOH, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography (5–10% MeOH/CH_2Cl_2). Trituration in MTBE/ether gave V as an amorphous slightly yellow powder (1.05 g, 73% yield): HPLC 98.3%; FAB MS (M^+ + H) 679; $[\alpha]^{20}_D$ –37.1° (*c* 0.98/MeOH). Anal. ($C_{36}H_{50}N_5O_5S\cdot 0.5H_2O$) C, H, N, S.

Boc-Phe-His-ACHPA-*O*-iPr (I). This compound was prepared by procedures as described for V, but using Boc-ACHPA-*O*-iPr instead of **6a**: HPLC 97.6%; FAB MS (M^+ + H) 642; $[\alpha]^{20}_D$ –32.9° (*c* 1.02/MeOH). Anal. ($C_{34}H_{51}N_5O_7$) C, H, N.

Boc-Phe-His-ACHPA-NHC$_6$H$_5$ (II). This compound was obtained by procedures similar to those for the synthesis of V, but using Boc-ACHPA-NHC$_6$H$_5$ instead of **6a**: HPLC 98.2%; FAB MS (M^+ + H) 675; $[\alpha]^{20}_D$ –27.5° (*c* 0.92/MeOH). Anal. ($C_{37}H_{50}N_6O_6$) C, H, N.

(2S,3S)-3-(Boc-Phe-His-Amino)-4-cyclohexyl-1-(2-pyridylthio)-2-butanol (IV). The title compound was prepared by using **10e** instead of **6a** in step 1 of the general procedures: HPLC 98.7%; FAB MS (M^+ + H) 665; $[\alpha]^{20}_D$ –10.6° (*c* 0.96/MeOH). Anal. ($C_{35}H_{48}N_6O_5S\cdot H_2O$) C, H, N, S.

(2S,3S)-2-(Boc-Phe-His-Amino)-1-cyclohexyl-6-(2-pyridylthio)-3-hexanol (VI). The title compound was prepared by using **12** instead of **6a** in step 1 of the general procedures: HPLC 99.1%; FAB MS (M^+ + H) 694; $[\alpha]^{20}_D$ –29.2° (*c* 0.87/

MeOH). Anal. ($C_{37}H_{52}N_6O_5S$) C, H, N, S.

(2*S*,3*S*)-2-(Boc-Phe-His-Amino)-4-cyclohexyl-1-(2-pyridyloxy)-2-butanol (VII). The title compound was prepared by using **10a** instead of **6a** in step 1 of the general procedures: HPLC 99.3%; FAB MS (M^+ + H) 649; $[\alpha]^{20}_D$ −20.9° (*c* 1.0/MeOH). Anal. ($C_{35}H_{48}N_6O_6$) C, H, N.

(2*S*,3*S*)-2-(Boc-Phe-His-Amino)-1-cyclohexyl-5-(2-pyridyloxy)-3-pentanol (VIII). The title compound was prepared by using **6b** instead of **6a** in step 1 of the general procedures: HPLC 98.5%; FAB MS (M^+ + H) 663; $[\alpha]^{20}_D$ −31.7° (*c* 0.97/MeOH). Anal. ($C_{36}H_{50}N_6O_6 \cdot 0.5H_2O$) C, H, N.

(2*S*,3*S*)-2-[[2-Benzyl-3-(*tert*-butylsulfonyl)propionyl]-His-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol (IX and X). The title compounds were prepared by using 2-benzyl-3-(*tert*-butylsulfonyl)propanoic acid instead of Boc-Phe in step 2 of the general procedures. The diastereomeric mixture was separated by flash chromatography (5% MeOH/CH_2Cl_2). IX: HPLC 96.7%; FAB MS (M^+ + H) 699; $[\alpha]^{20}_D$ −51.6° (*c* 1.01/MeOH). Anal. ($C_{36}H_{51}N_5O_5S_2 \cdot H_2O$) C, H, N, S. X: HPLC 98.8%; FAB MS (M^+ + H) 699; $[\alpha]^{20}_D$ −11.9° (*c* 0.92/MeOH). Anal. ($C_{36}H_{51}N_5O_5S_2$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[(Morpholinocarbonyl)oxy]-3-phenylpropionyl]-His-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol (XI). The title compound was prepared by using 2-[(morpholinocarbonyl)oxy]-3-phenylpropanoic acid instead of Boc-Phe in step 2 of the general procedure: HPLC 96.7%; FAB MS (M^+ + H) 693; $[\alpha]^{20}_D$ −39.7° (*c* 1.09/MeOH). Anal. ($C_{36}H_{48}N_6O_6S \cdot 0.3H_2O$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[[(4-Boc-amino)piperidino]carbonyl]oxy]-3-phenylpropionyl]-His-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol (XII). The title compound was prepared by using 2-[[[(4-Boc-amino)piperidino]carbonyl]-oxy]-3-phenylpropanoic acid instead of Boc-Phe in step 2 of the general procedure: HPLC 98.1%; FAB MS (M^+ + H) 807; $[\alpha]^{20}_D$ −41.4° (*c* 0.91/MeOH). Anal. ($C_{42}H_{59}N_7O_7S$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-His-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Tris(trifluoroacetate) (XIII). A solution of compound XII (806 mg, 1 mmol) in CH_2Cl_2 (10 mL) and TFA (10 mL) was stirred for 1 h at room temperature. The solvents were removed under reduced pressure, and product XIII (1030 mg, 98% yield) was obtained as TFA salt as a white solid. HPLC 97.8%; FAB MS (M^+ + H) 706; $[\alpha]^{20}_D$ −48.3° (*c* 0.95/MeOH). Anal. ($C_{37}H_{51}N_7O_5S \cdot 3CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Nva-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XIV). This compound was prepared in analogy to XIII by using Boc-norvaline instead of N^{α}-Boc-N^{im}-DNP-histidine in step 1 of the general procedure. HPLC 99.1%; FAB MS (M^+ + H) 668; $[\alpha]^{20}_D$ −46.3° (*c* 0.98/MeOH). Anal. ($C_{36}H_{53}N_5O_5S \cdot 2CF_3COOH \cdot 0.5H_2O$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Leu-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XV). This compound was prepared as described for XIII by using Boc-leucine in step 1 of the general procedure: HPLC 99.6%; FAB MS (M^+ + H) 682; $[\alpha]^{20}_D$ −50.5° (*c* 0.92/MeOH). Anal. ($C_{37}H_{55}N_5O_5S \cdot 2CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Nle-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XVI). The title compound was prepared as described for XIII by using Boc-norleucine in step 1 of the general procedure: HPLC 98.3%; FAB MS (M^+ + H) 682; $[\alpha]^{20}_D$ −39.1° (*c* 1.05/MeOH). Anal. ($C_{37}H_{55}N_5O_5S \cdot 2CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XVII). This compound was prepared as described for XIII by using *N*-Boc-*S*-methylcysteine in step 1 of the general procedure: HPLC 97.2%; FAB MS (M^+ + H) 687; $[\alpha]^{20}_D$ −33.4° (*c* 0.78/MeOH). Anal. ($C_{35}H_{51}N_5O_5S_2 \cdot 2CF_3CO_2H$) C, H, F, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Met-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XVIII). This compound was prepared by using Boc-methionine in step 1 of the general procedure: HPLC 98.3%; FAB MS (M^+ + H) 701; $[\alpha]^{20}_D$ −39.2° (*c* 0.97/MeOH). Anal. ($C_{36}H_{53}N_5O_5S_2 \cdot 2CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Ala-amino]-1-cyclohexyl-5-(2-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XIX). This compound was prepared by using Boc-alanine in step 1 of the general procedure: HPLC 98.8%; FAB MS (M^+ + H) 640; $[\alpha]^{20}_D$ −43.8° (*c* 1.02/MeOH). Anal. ($C_{34}H_{49}N_5O_5S \cdot 2CF_3CO_2H \cdot 0.5H_2O$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-(4-pyridylthio)-3-pentanol Bis(trifluoroacetate) (XX). This compound was prepared as described for XIII by using **6c** in step 1 of the general procedures: HPLC 97.9%; FAB MS (M^+ + H) 687; $[\alpha]^{20}_D$ −35.7° (*c* 1.12/MeOH). Anal. ($C_{35}H_{51}N_5O_5S_2 \cdot 2CF_3CO_2H$) C, H, F, N, S.

(2*S*,3*S*)-2-[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-(2-pyrimidinylthio)-3-pentanol Bis(trifluoroacetate) (XXI). This compound was prepared as described for XIII by using **6d** in step 1 of the general procedures: HPLC 98.3%; FAB MS (M^+ + H) 687; $[\alpha]^{20}_D$ −38.8° (*c* 0.95/MeOH). Anal. ($C_{34}H_{50}N_6O_5S_2 \cdot 2CF_3CO_2H$) C, H, F, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-(2-thiazolylthio)-3-pentanol Bis(trifluoroacetate) (XXII). This compound was prepared as described for XIII by using **6h** in step 1 of the general procedures: HPLC 97.8%; FAB MS (M^+ + H) 693; $[\alpha]^{20}_D$ −47.1° (*c* 0.50/MeOH). Anal. ($C_{33}H_{49}N_5O_5S_3 \cdot 2CF_3CO_2H \cdot H_2O$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-[(2-methyl-1,3,4-thiadiazol-5-yl)thio]-3-pentanol Bis(trifluoroacetate) (XXIII). This compound was prepared as described for XIII by using **6f** in step 1 of the general procedures: HPLC 96.7%; FAB MS (M^+ + H) 708; $[\alpha]^{20}_D$ −46.7° (*c* 0.79/MeOH). Anal. ($C_{33}H_{50}N_6O_5S_3 \cdot 2CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-[(1-methyl-1*H*-tetrazol-5-yl)thio]-3-pentanol Trifluoroacetate (XXIV). This compound was prepared for XIII using **6g** in step 1 of the general procedures: HPLC 98.1%; FAB MS (M^+ + H) 692; $[\alpha]^{20}_D$ −48.6° (*c* 0.78/MeOH). Anal. ($C_{32}H_{50}N_8O_5S_2 \cdot CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-2-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-1-cyclohexyl-5-[[5-(hydroxymethyl)-1-methyl-2-imidazolyl]thio]-3-pentanol Bis(trifluoroacetate) (XXV). This compound was prepared described for XIII by using **6i** in step 1 of the general procedures: HPLC 96.6%; FAB MS (M^+ + H) 720; $[\alpha]^{20}_D$ −38.0° (*c* 0.97/MeOH). Anal. ($C_{35}H_{54}N_6O_6S_2 \cdot 2CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-3-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-4-cyclohexyl-1-(2-pyridylthio)-2-butanol Bis(trifluoroacetate) (XXVI). This compound was prepared as described for XIII by using **10e** instead of **5a** in step 1 of the general procedures: HPLC 99.5%; FAB MS (M^+ + H) 672; $[\alpha]^{20}_D$ −26.0° (*c* 0.89/MeOH). Anal. ($C_{34}H_{49}N_5O_5S_2 \cdot 2CF_3CO_2H$) C, H, F, N, S.

(2*S*,3*S*)-3-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-4-cyclohexyl-1-(4*H*-1,2,4-triazol-3-ylthio)-2-butanol Bis(trifluoroacetate) (XXVII). This compound was prepared as described for XIII by using **10f** instead of **5a** in step 1 of the general procedures: HPLC 98.6%; FAB MS (M^+ + H) 662; $[\alpha]^{20}_D$ −35.9° (*c* 0.90/MeOH). Anal. ($C_{31}H_{47}N_7O_5S_2 \cdot CF_3CO_2H \cdot 0.25H_2O$) C, H, N, S.

(2*S*,3*S*)-3-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-4-cyclohexyl-1-(2-pyridyloxy)-2-butanol Bis(trifluoroacetate) (XXVIII). This compound was prepared as described for XIII using **10a** instead of **6a** in step 1 of the general procedures: HPLC 96.7%; FAB MS (M^+ + H) 656; $[\alpha]^{20}_D$ −36.1° (*c* 0.97/MeOH). Anal. ($C_{34}H_{49}N_5O_6S \cdot 2CF_3CO_2H$) C, H, N, S.

**(2*S*,3*S*)-3-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-4-cyclohexyl-1-

(1,2-dihydro-2-oxo-1-pyridyl)-2-butanol Trifluoroacetate (XXIX). This compound was prepared as described for XIII by using **10b** instead of **6a** in step 1 of the general procedures: HPLC 97.8%; FAB MS (M^+ + H) 656; $[\alpha]^{20}_D$ −72.1° (*c* 0.87/MeOH). Anal. ($C_{34}H_{49}N_5O_6S \cdot CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-3-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-4-cyclohexyl-1-(4-pyridyloxy)-2-butanol Bis(trifluoroacetate) (XXX). This compound was prepared as described for XIII by using **10c** instead of **6a** in step 1 of the general procedures: HPLC 98.7%; FAB MS (M^+ + H) 656; $[\alpha]^{20}_D$ −31.7° (*c* 0.88/MeOH). Anal. ($C_{34}H_{49}N_5O_6S \cdot 2CF_3CO_2H$) C, H, N, S.

(2*S*,3*S*)-3-[[(2*S*)-2-[[(4-Aminopiperidino)carbonyl]-oxy]-3-phenylpropionyl]-Cys(Me)-amino]-4-cyclohexyl-1-(1,4-dihydro-4-oxo-1-pyridyl)-2-butanol Trifluoroacetate (XXXI). This compound was prepared as described for XIII by using **10d** instead of **6a** in step 1 of the general procedures: HPLC 97.6%; FAB MS (M^+ + H) 656; $[\alpha]^{20}_D$ −68.7° (*c* 0.91/MeOH). Anal. ($C_{34}H_{49}N_5O_6S \cdot CF_3CO_2H$) C, H, N, S.

Biological Methods. In Vitro Enzyme Inhibition. The renin IC_{50} data were obtained with human EDTA plasma, utilizing the endogenous renin and angiotensinogen. Test compounds were dissolved in DMSO and diluted so that prior to addition to the assay system the solutions were 10% in DMSO. At least three different concentrations of the inhibitor that bracketed the IC_{50} were used for determining the IC_{50}. The final incubation mixture (750 μL) contained the following: plasma, 100 μL; maleate buffer, pH 5.5, 0.1 M; EDTA, 7.2 mM; DMSO, 1%; 8-hydroxyquinoline, 8.3 mM. Samples were incubated at 37 °C for 2 h and then placed on ice; an aliquot was analyzed for angiotensin I by radioimmunoassay utilizing a commercial kit (NEN Research). The percent inhibition of the reaction was determined, and the IC_{50} was calculated.

The pepsin and cathepsin D IC_{50} values were determined by incubating hemoglobin with 20 units of porcine pepsin at pH 1.8 for 10 min at 35.5 °C and with 100 milliunits of bovine cathepsin D at pH 3.2 for 20 min at 37 °C, respectively. Hemoglobin is degraded by these enzymes to liberate peptides soluble in trichloroacetic acid. The concentration of the peptides was determined by their absorbance at 280 nm. The concentration of the inhibitor that inhibited peptide liberation (= pepsin or cathepsin D activity) by 50% was calculated.

Degradation by Chymotrypsin. The enzymatic degradation of the synthetic peptides was performed at 30 °C in a thermostated Merck-Hitachi sample vial rack with bovine α-chymotrypsin (45 milliunits per milligram) in 0.05 M Tris-buffer containing 0.02 M $CaCl_2$ and adjusted to pH 7.8 with HCl. Because of poor solubility, all peptides were dissolved in formamide. Aliquots of the solutions of peptides in organic solvent and enzyme in aqueous buffer were mixed in autosampler vials to give final concentration of 0.5 mg/mL peptide and 25% (vol/vol) formamide. Chymotrypsin was used with 0.375 mg/mL in experiment of Figure 5 and 0.75 mg/mL in experiment of Figure 4. The content of each vial was acidified with 5% TFA in 80% 2-propanol at desired stop times and analyzed by HPLC. The vials for time t_0 contained no enzyme.

The stability of the synthetic compounds towards chymotrypsin was examined by HPLC at 254 and 215 nm on a reversed-phase column (Lichrosorb RP-8, 7 μm, 250 × 4 mm, E. Merck) in 0.3% trifluoroacetic acid at 1 mL/min with a gradient of 2-propanol (1–80%) for 60 min. The remaining amount of undegraded peptides was expressed in % remaining HPLC area at 254 nm and plotted against time. $\tau_{1/2}$ was calculated from these plots.

In Vivo Activity. Female cynomolgus monkeys (Macaca fascicularis) weighing 3–4 kg were used. The animals were housed under constant temperature and lighting conditions and provided with food consisting of a cereal mixture, barley germ, bread, fruit, and vegetables. The animals were treated daily with furosemide, 2 mg/kg im, beginning on the fourth day before an experiment. On the day of the experiment the animals were treated with the final dose of furosemide together with haloperidol, 0.3 mg/kg im for sedation. About 1.5 h after the last treatment, the monkeys were restrained in a chair and blood pressure (BP) and heart rate (HR) were measured by the tail cuff method (Blood-Pressure-Monitor, TSE, Kronberg) as described by Wood et al.[16]) for conscious marmosets. In detail, a pneumatic cuff (18–20 mm i.d.) and a piezoelectric pressure sensor were positioned on the tail of the monkeys. Blood pressure and HR were measured every 5 min and were allowed to stabilize before drug administration. Following this, test substances were applied orally and BP and HR were measured every 5 min. Blood samples for the measurement of plasma renin activity (PRA) were collected before and after administration of the compounds as indicated. The blood samples were taken by direct puncture of the saphenous vein.

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