Pharmacological Testing. All of the test compounds were evaluated for antihypertensive activity in conscious spontaneously hypertensive rats (14-24 weeks old), derived from the Japanese (Okamoto) strain. Animals with systolic blood pressure >180 mmHg (1 mmHg = 133 Pa) were considered to be hypertensive.

Systolic blood pressure was recorded by the tail-cuff method using a W+W B.P. recorder, Model No. 8005; each determination was the mean of at least six recordings. Blood pressure measurements were made prior to the oral administration of test compound and at intervals for up to 6 h postdose.

All compounds were administered (via an oral dosing needle placed in the esophagus) as a solution or suspension in 1% w/v methylcellulose solution.

With the use of the above procedure, vehicle alone typically had little or no effect on blood pressure apart from a slight reduction (by 5-10%) at 6 h postdose.

Acknowledgment. We wish to thank Milena Boschetti, Frances Hicks, Graham Moore and Roberto Rigolio for skilled technical assistance.

Substrate Analogue Renin Inhibitors Containing Replacements of Histidine in P_2 or Isosteres of the Amide Bond between P_3 and P_2 Sites

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Incorporation of β-alanine or γ-aminobutyric acid in position P_2 of ACHPA or LeuΨ[CHOHCH$_2$]Val-based tetrapeptides gave highly active renin inhibitors (compounds V, VI, and XVII) with high specificity for renin and a remarkable stability against chymotrypsin. Replacement of the amide bond between P_2 and P_3 by isosteres (ketomethylenes, hydroxyethylenes, and the corresponding thio-insertion analogues) led to compounds (VIII-XIII, XVIII, and XIX) with renin inhibitory activity in the nanomolar range. Oral activity was achieved by incorporation of polar functionalities at the N-terminus of β-alanine-containing tetrapeptides. One of these compounds (XXVIII) was chosen for further studies. This inhibitor demonstrated excellent efficacy and a long duration of action after intravenous and oral administration to cynomolgus monkeys.

Introduction

The search for orally active renin inhibitors as therapeutic agents for the treatment of hypertension and congestive heart failure continues to represent a challenging target for medicinal chemists.[1] Analogues of the angiotensinogen region flanking the bond split by renin have turned out to be very potent and specific inhibitors of renin. However, the high affinity of these angiotensinogen analogues for human renin is often associated with fast hydrolysis between P_3 and P_2 sites[2] by the intestinal serine protease chymotrypsin.

Since stability against proteolytic attack in the digestive tract is a requirement for orally active peptides,[3] we focused our synthetic efforts on angiotensinogen analogues that are resistant to chymotrypsin and that retain a high specificity and high inhibitory potency for human renin.

Proteolytic stability has been achieved by incorporation of *N*-Me-histidine in P_2,[4] by alteration of the phenylalanine residue in P_3,[5] and by isosteric replacement of the amide bond connecting the P_3 and P_2 sites.[6] The latter approach led to inhibitors with moderate in vitro activity.

In this paper our results of replacing histidine in P_2 and of the isosteric substitution of the amide bond between P_3 and P_2 sites are described. We expected that resistance to proteolytic degradation in the digestive tract would lead to longer duration of action after intravenous and oral administration.

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

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

(3) Humphrey, M. J.; Ringrose, P. S. Peptides and Related Drugs: A Review of Their Absorption, Metabolism, and Excretion. *Drug. Metab. Rev.* **1986**, *17*, 283-310.

(4) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. Design and Synthesis of a Potent and Specific Renin Inhibitor with a Prolonged Duration of Action in Vivo. *J. Med. Chem.* **1986**, *29*, 2088-2093.

(5) (a) 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.; 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. (b) Bühlmayer, P.; Caselli, A.; Fuhrer, W.; Göschke, R.; Rasetti, V.; Rüeger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. Synthesis and Biological Activity of Some Transition State Inhibitors of Human Renin. *J. Med. Chem.* **1988**, *31*, 1839-1846. (c) Iizuka, K.; Kamijo, T.; Harada, H.; Akahane, K.; Kubota, T.; Umeyama, H.; Ishida, T.; Kiso, Y. Orally Potent Human Renin Inhibitors Derived from Angiotensinogen Transition State: Design, Synthesis, and Mode of Interaction. *J. Med. Chem.* **1990**, *33*, 2707-2714.

(6) Kaltenbronn, J. S.; Hudspeth, J. P.; Lunney, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Stier, M. A.; Tinney, F. J.; Woo, P. K. W.; Essenburg, A. D. Renin Inhibitors Containing Isosteric Replacements of the Amide Bond Connecting the P_3 and P_2 Sites. *J. Med. Chem.* **1990**, *33*, 838-845.

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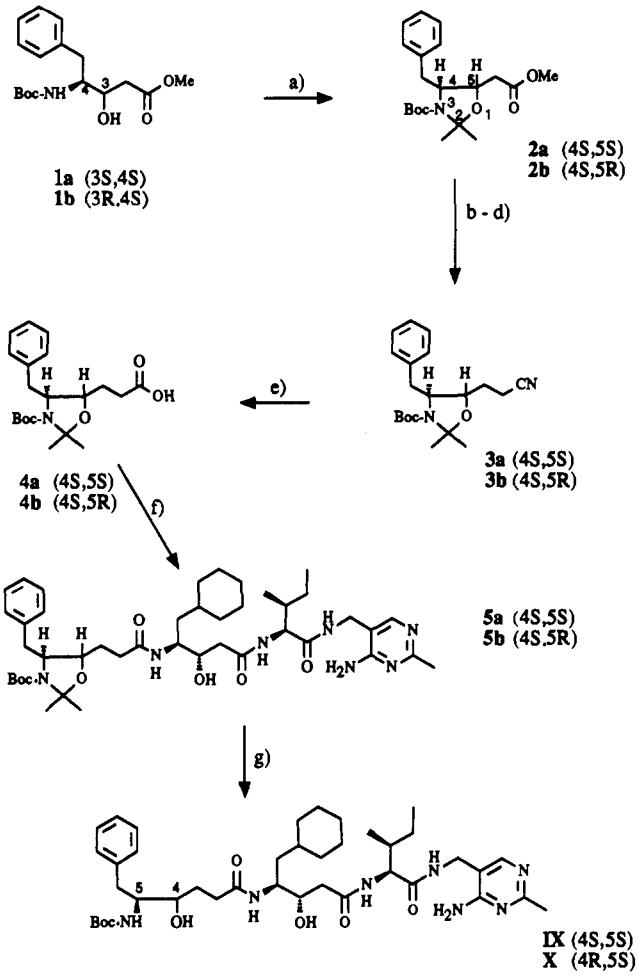
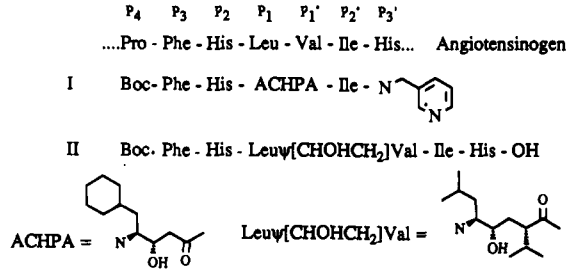
Scheme I.[a] Synthesis of Hydroxyethylene Isosteres and the Corresponding Inhibitors

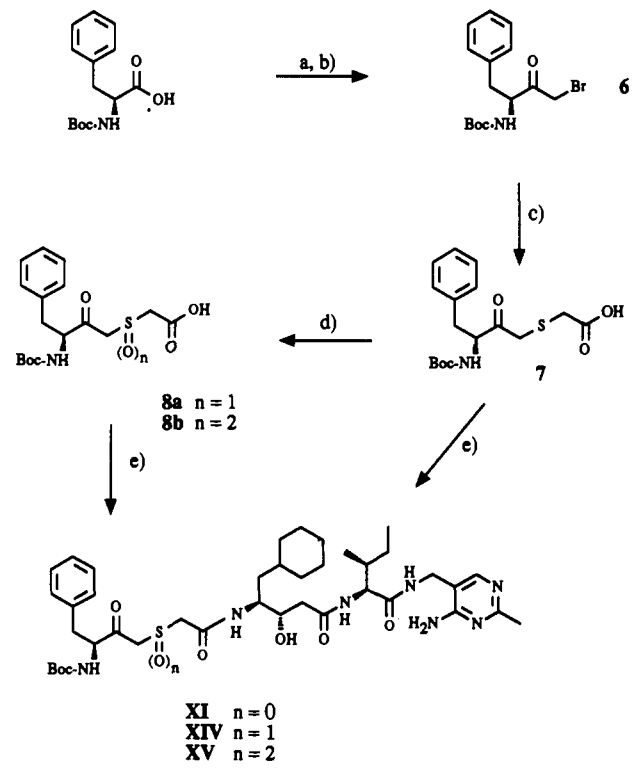


[a] (a) $Me_2C(OMe)_2$, PTSA; (b) Dibal; (c) CH_3SO_2Cl, NEt_3; (d) NaCN, DMSO; (e) KOH; (f) H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide, EDCI, HOBT; (g) PTSA, MeOH.

In our synthetic strategy we chose to prepare modified compounds based on the potent renin inhibitors I and II reported by Boger[7] and Szelke,[8] respectively.



Scheme II.[a] Synthesis of Ketomethylene Analogues and the Corresponding Inhibitors



[a] (a) Isobutyl chloroformate, CH_2N_2; (b) HBr (40%); (c) $HSCH_2CO_2H$, NaH; (d) $(HO_3C\text{-}C_6H_4CO_2)_2Mg6H_2O$, THF; (e) H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide, EDCI, HOBT.

Chemistry

The syntheses of the P_2-P_3 isosteres and the preparation of representative examples of the tetrapeptides are outlined in Schemes I–III. The P_1–P_1' isosteres BOC-ACHPA-OH[9] and BOC-LeuΨ[CHOHCH$_2$]Val-OH[10] were prepared by known procedures.

As shown in Scheme I BOC-PheΨ[CHOHCH$_2$]Gly-OH isostere **4a**, protected as acetonide, was obtained by starting from (3*S*,4*S*)-4-(*N*-BOC-amino)-3-hydroxy-5-phenylpentanoic acid methyl ester (**1a**). Protection of the hydroxyl and BOC-NH functions of **1a** led to acetonide **2a**. Reduction of the ester function with diisobutylaluminum hydride, followed by conversion of the alcohol into the mesylate and subsequent cyanide displacement, afforded nitrile **3a**, which after hydrolysis led to acid **4a**. Condensation of **4a** with ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide[11] using EDCI/HOBT and cleavage of the acetonide with PTSA in MeOH gave the modified peptide IX. Compound X was obtained by the same procedure starting from (3*R*,4*S*)-4-(*N*-BOC-amino)-3-hydroxy-5-phenylpentanoic acid methyl ester (**1b**). The BOC-PheΨ[COCH$_2$]Gly-OH isostere which was prepared by a method described by H.-E. Radunz et al.[12]

(7) (a) Boger, J.; Payne, L. S.; Perlow, D. S.; Martin, P.; Blaine, E. H.; Ulm, E. H.; Schorn, T. W.; LaMont, B.; Bock, M. G.; Freidinger, R. M.; Evans, B. E.; Veber, D. F. *Peptides: Structure and Function, Proceedings of the Ninth American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co.: Rockford, IL, 1985; p 747–750. (b) For synthesis of compound I, see: Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber, D. F. Renin Inhibitors Containing Hydrophilic Groups. Tetrapeptides with Enhanced Aqueous Solubility and Nanomolar Potency. *J. Med. Chem.* 1988, *31*, 1918–1923.

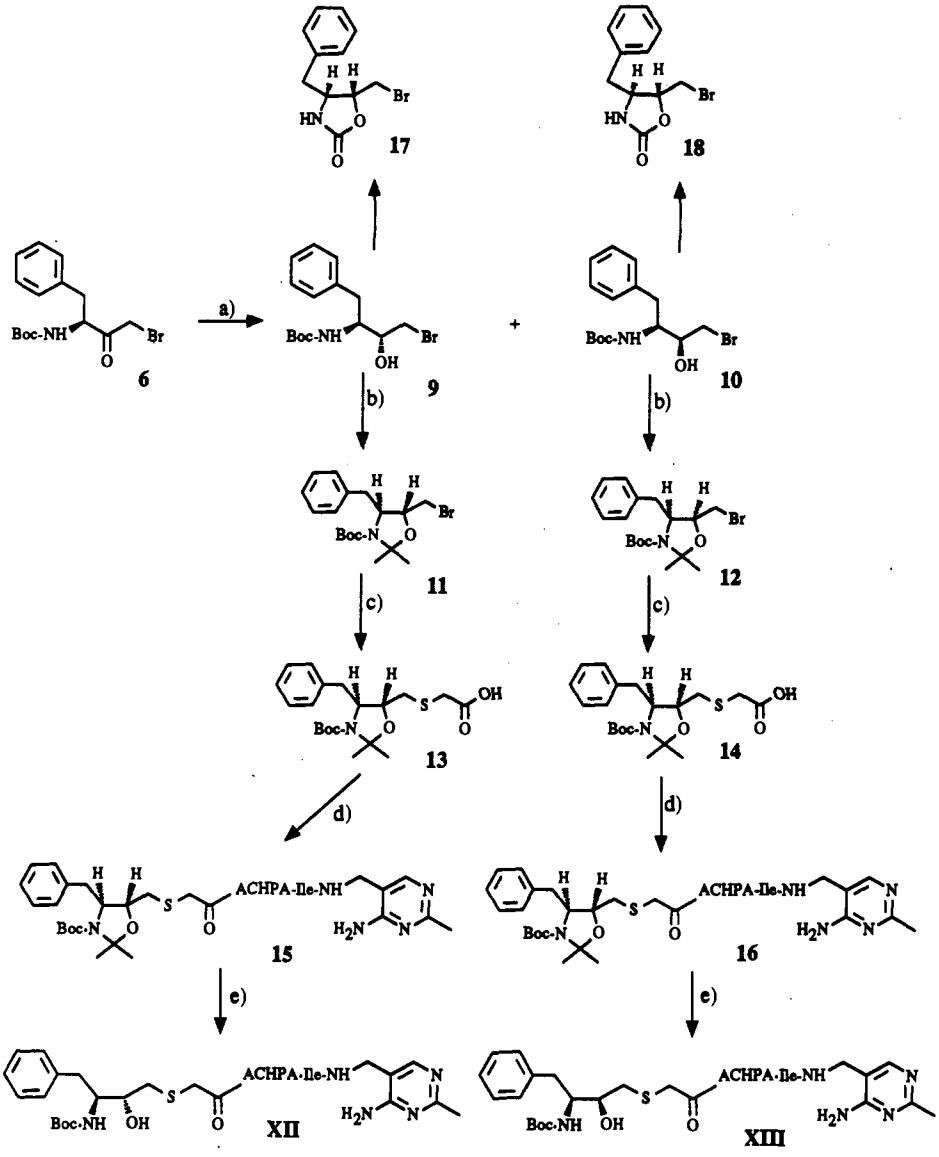
(8) Szelke, M.; Jones, D. H.; Hallett, A.; Atrash, B. Enzyme Inhibitors. International application published under the patent cooperation treaty WO 84/03044, 1984.

(9) (a) Raddatz, P.; Radunz, H. E.; Schneider, G.; Schwarz, H. *Angew. Chem.* 1988, *100*, 414–415. (b) For a review, see: Altenbach, H.-J.; Statin-Synthesen. *Nachr. Chem. Tech. Lab.* 1988, *36*, 756–758.

(10) For a review, see: Henning, R. *Nachr. Chem. Tech. Lab.* 1990, *38*, 460–463.

(11) (a) Raddatz, P.; Gante, J.; Schmitges, C. J.; Minck, K.-O.; Jonczyk, A.; Hölzemann, G. European patent application EP 249096, 1987. (b) Gante, J.; Kahlenberg, H. Synthesis of a Renin Inhibitor of the Azapeptide Type. *Liebigs Ann. Chem.* 1989, 1085–1087.

Scheme III.[a] Synthesis of Hydroxyethylene Analogues and the Corresponding Inhibitors



[a] (a) $LiAl(OtBu)_3H$, ether (9/10 = 5/1) or $NaBH_4$/MeOH (9/10 = 5/95); (b) $Me_2C(OMe)_2$, PTSA, (c) $HSCH_2CO_2H$, NaH; (d) H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide, EDCI, HOBT, (e) PTSA, MeOH.

was incorporated into the peptides VIII and XVIII.

Scheme II outlines the route leading to the BOC-PheΨ[$COCH_2S(O)_n$]Gly-OH isosteres. BOC-phenylalanine was converted to (3*S*)-3-[(*tert*-butoxycarbonyl)amino]-1-diazo-4-phenyl-2-butanone using a method described by Johnson.[13] Subsequent treatment with aqueous HBr (40%) afforded the (3*S*)-1-bromo-3-[(*tert*-butoxycarbonyl)amino]-4-phenyl-2-butanone (**6**). Nucleophilic displacement of the bromide by the bis-sodium salt of mercaptoacetic acid gave BOC-PheΨ[$COCH_2S$]Gly-OH isostere **7**. Oxidation of **7** with magnesium monoperoxyphthalate hexahydrate led to a mixture of sulfoxide **8a** and sulfone **8b** which was separated by chromatography on silica gel. Condensation of **7**, **8a**, and **8b** with ACHPA-Ile-*N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide using EDCI/HOBT gave the peptide derivatives XI, XIV, and XV. Coupling of isostere **7** with LeuΨ[$CHOHCH_2$]-Val-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-amide[11] led to the renin inhibitor XIX.

The route to the protected BOC-PheΨ[$CHOHCH_2S$]-Gly-OH isosteres **13** and **14** is shown in Scheme III. Reduction of (3*S*)-1-bromo-3-[(*tert*-butoxycarbonyl)-amino]-4-phenyl-2-butanone (**6**) with lithium tri-*tert*-butoxyaluminum hydride in ether gave a mixture of alcohols **9** and **10** in a ratio of 5/1. Using sodium borohydride in methanol as reducing reagent we observed a reversal of the diastereomeric ratio (**9**/**10** = 5/95). These observations are in accordance with data reported by Castro et al.[14] The assignment of the absolute stereochemistry of the diastereomers **9** and **10** was possible after conversion into the corresponding oxazolidinones **17** and **18** by removal of the BOC group and reaction with phosgene. The assignment of stereochemistry for **17** and **18** is based on the vicinal coupling, $^3J_{4,5}$, between protons on the fourth and fifth carbons. We measured 5.0 Hz for the trans (threo) isomer **17** and 8.0 Hz for the cis (erythro)

(12) Radunz, H.-E.; Reissig, H.-U.; Schneider, G.; Riethmüller, A. *Liebigs Ann. Chem.* **1990**, 705–707.

(13) Johnson, R. L.; Verschoor, K. Inhibition of Renin by Angiotensinogen Peptide Fragments Containing the Hydroxy Amino Acid Residue 5-Amino-3-hydroxy-7-methyloctanoic Acid. *J. Med. Chem.* **1983**, *26*, 1457–1462.

(14) Dufour, M.-N.; Jouin, P.; Poncet, J.; Pantalon, A.; Castro, B. J. Synthesis and Reduction of α-Amino Ketones Derived from Leucine. *Chem. Soc. Perkin Trans 1* **1986**, 1895–1899.

Table I. In Vitro Activities of Inhibitors with Replacement of Histidine in P_2

	P_2	IC_{50}, nM human renin	IC_{50}, nM cathepsin D	IC_{50}, nM pepsin
I	Boc-Phe-[His]-ACHPA-Ile-NH	2.9	5800	>10000
III	Boc-Phe-[$NHCH_2CO$]-ACHPA-Ile-NH	2.8	6500	40000
IV	Boc-Phe-[$NHCH_2CO$]-ACHPA-Ile-NH	2.5	13000	38500
V	Boc-Phe-[$NH(CH_2)_2CO$]-ACHPA-Ile-NH	1.5	7900	18500
VI	Boc-Phe-[$NH(CH_2)_3CO$]-ACHPA-Ile-NH	28.5	3200	>10000
VII	Boc-Phe-[Ala]-ACHPA-Ile-NH	0.53	28	950

isomer 18. These values are in excellent agreement with those reported by Castro[14] and Rich.[15]

Protection of the hydroxyl and BOC–NH functions of **9** and **10** led to acetonides 11 and 12. Nucleophilic displacement of the bromides with the bis-sodium salt of mercaptoacetic acid led to the protected BOC-PheΨ-[$CHOHCH_2S$]Gly-OH isosteres 13 and 14. The modified peptides XII and XIII were obtained after coupling with ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]amide and subsequent cleavage of the acetonides with PTSA in MeOH.

Peptide inhibitors of Tables I–IV were prepared by standard peptide-condensation reactions, coupling protected dipeptides (BOC-Phe-Gly-OH, BOC-Phe-β-Ala-OH, BOC-Phe-γ-amino butyric acid...) with ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide or LeuΨ[$CHOHCH_2$]Val-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide. The chemical data for the new compounds are summarized in Table V.

Results and Discussion

The structures and in vitro activities of the renin inhibitors are listed in Tables I–IV. Table I shows that replacement of histidine in P_2 of ACHPA-based tetrapeptides by glycine (III, IV) and β-alanine (V) led to compounds with renin inhibitory potency and specificity comparable to those of the standard peptide (I). Peptide VI with γ-aminobutyric acid in P_2 is about 10-fold less potent. The L-alanine derivative VII is about 3-fold more active than the β-alanine peptide V, but considerably less specific. The C-terminal (amidomethyl)pyridine can be replaced by [(4-amino-2-methyl-5-pyrimidinyl)methyl]-amide without loss of potency and specificity (peptides III, IV). For reasons of better solubility, we chose the latter heterocycle as C-terminus for further studies.

Incubation of the peptides shown in Table I with the digestive enzyme chymotrypsin revealed a rapid degradation of histidine (I), glycine (III, IV), and L-alanine (VII) containing compounds. Surprisingly, the β-alanine (V) and the γ-aminobutyric acid (VI) containing inhibitors demonstrated a remarkable resistance against chymotrypsin under these conditions (Figure 1).

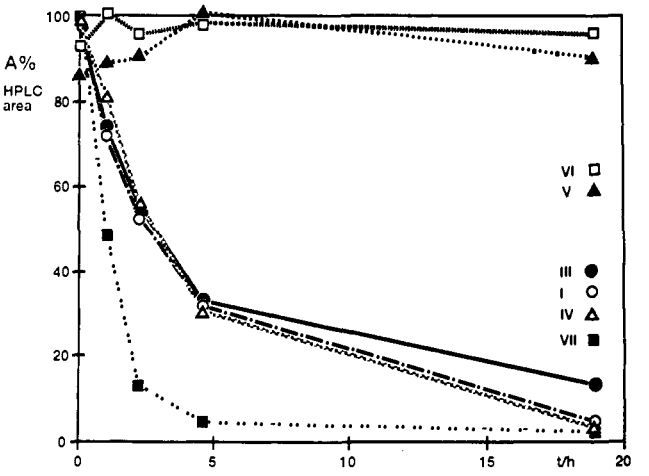


Figure 1. Chymotryptic degradation of renin inhibitors (structures shown in Table I).

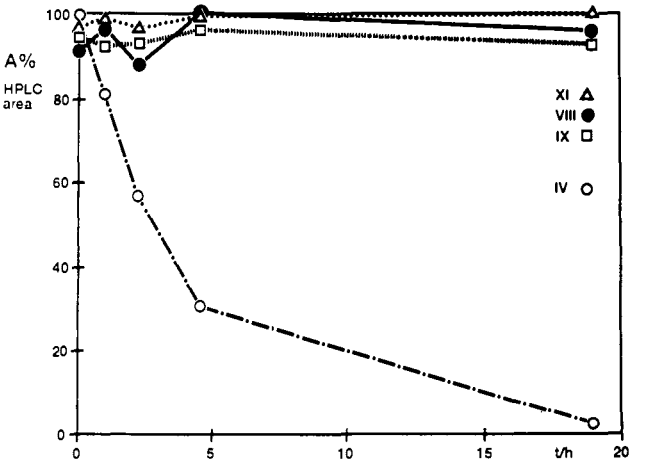
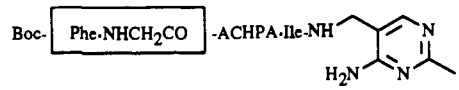
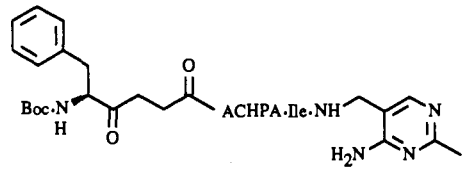
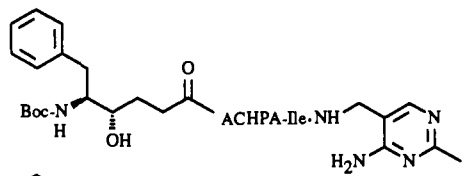
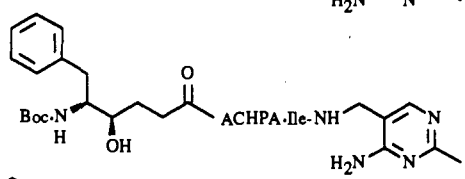
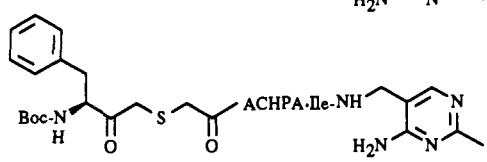
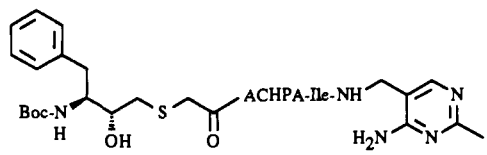
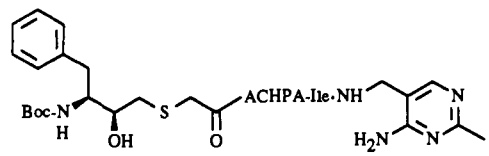
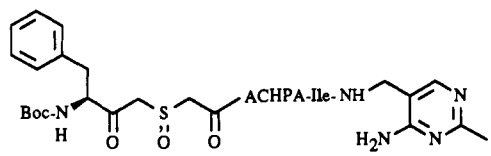
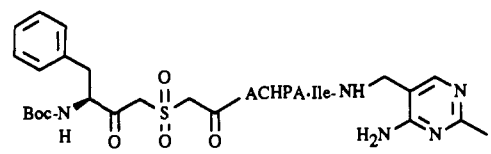


Figure 2. Chymotryptic degradation of renin inhibitors (structures shown in Table II).

Table II contains a series of peptides with isosteres of the amide bond between P_3 and P_2. The Boc-PheΨ-[$COCH_2$]Gly derivative VIII is 3–4-fold less active than the standard peptide IV with glycine in P_2. However, the hydroxyethylene-containing peptides IX and X match the high potency shown by IV. The thio-insertion analogue (XI) of compound VIII did not differ in inhibitory potency from IV, while the corresponding hydroxy derivatives XII and XIII showed a 2–3-fold diminution of potency. The

(15) 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.

Table II. In Vitro Activities of Inhibitors with Amide Bond Isosteres in P_2-P_3

	P_3 P_2	IC_{50}, nM		
		human renin	cathepsin D	pepsin
IV		2.5	13000	38500
VIII		8.5	>10000	>10000
IX		4.2	>10000	>10000
X		1.2	>10000	10000
XI		2.1	28000	53000
XII		6.3	>100000	>100000
XIII		5.5	73000	>100000
XIV		70	>10000	>10000
XV		240	>10000	>10000

oxidized derivatives XIV and XV displayed a dramatic increase in IC_{50} (30- and 100-fold drop in activity, respectively).

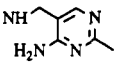
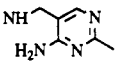
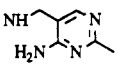
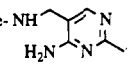
Compounds VIII, IX, and XI were subjected to chymotrypsin degradation (Figure 2). As expected, chymotrypsin did not attack the P_2-P_3-isostere-containing peptides, while the standard peptide IV was rapidly cleaved between phenylalanine and glycine.

Table III contains a class of inhibitors with the LeuΨ-[$CHOHCH_2$]Val isostere in position P_1-P_1'. In comparison to peptide II, which has a histidine in P_2, the glycine (XVI), β-alanine (XVII), Boc-PheΨ[$COCH_2$]Gly (XVII), and Boc-PheΨ[$COCH_2S$]Gly (XIX) derivatives possess a slightly reduced activity.

All compounds of Table III were tested for sensitivity to chymotryptic attack (Figure 3). The rapid degradation of the histidine (II) and glycine (XVI) containing peptides is in good agreement with the results of Tables I and II. As expected, the incorporation of β-alanine (XVII) in P_2 and the introduction of the amide bond isosteres Boc-PheΨ[$COCH_2$]Gly (XVIII) and Boc-PheΨ[$COCH_2S$]Gly (XIX) led to peptides with dramatically increased stability against enzymatic attack.

Our results suggest that the incorporation of β-alanine in the P_2 site of renin inhibitors is the easiest way to circumvent the degradation by chymotrypsin. We expected that these metabolically stable peptides would demonstrate an enhanced gastrointestinal stability and absorption

Table III. In Vitro Activities of Inhibitors with LeuΨ[$CHOHCH_2$]Val in P_1-P_1' and Modifications of P_2

	P_3 P_2	IC_{50}, nM		
		human renin	cathepsin D	pepsin
II	Boc-Phe- His -LeuΨ[$CHOHCH_2$]Val-Ile-His-OH	2.4	nd	nd
XVI	Boc-Phe- $NHCH_2CO$ -Leuψ[$CHOHCH_2$]Val-Ile-NH 	5.4	3900	1400
XVII	Boc-Phe-$NH(CH_2)_2CO$ -Leuψ[$CHOHCH_2$]Val-Ile-NH 	4.1	4700	830
XVIII	Boc-Phe-ψ[$COCH_2$]Gly-Leuψ[$CHOHCH_2$]Val-Ile-NH 	5.1	8000	12000
XIX	Boc-Phe-ψ[$COCH_2S$]Gly-Leuψ[$CHOHCH_2$]Val-Ile-NH 	5.4	8800	6000

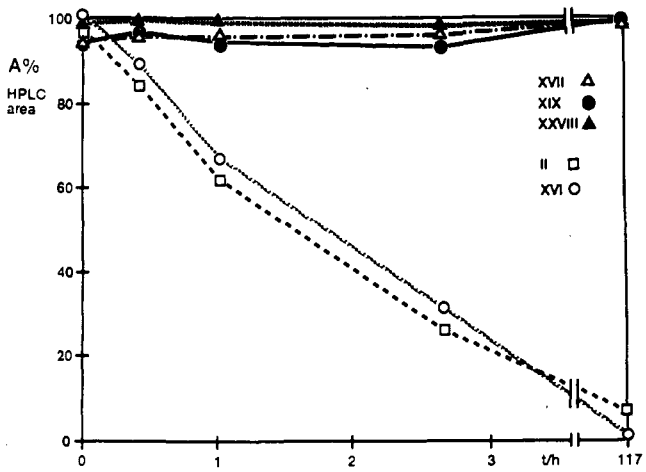


Figure 3. Chymotryptic degradation of renin inhibitors (structures shown in Table III).

leading to oral activity with prolonged duration of action. Therefore, the ACHPA-based tetrapeptide V was chosen for in vivo studies and was given orally (30 mg/kg) to salt-depleted monkeys.

Disappointingly, we did not observe a decrease of systolic and arterial blood pressure, and plasma renin activity was only reduced by about 25% (Table VI). Lipophilic compounds like inhibitor V are known to suffer from poor bioavailability and rapid elimination by the liver.[16] Increasing the polarity and water solubility of these lipophilic peptides seems to be an approach to circumvent these limitations.[17] Incorporation of polar functionalities at the N-terminus of renin inhibitor V led to a series of hydrophilic peptides, shown in Table IV.

The Boc group can be replaced by a variety of functionalities without loss of potency and specificity. However, removal of the Boc group led to compound XXX with a 12-fold diminished activity. All the other inhibitors in Table IV showed IC_{50} values in the nanomolar and subnanomolar range. The morpholinocarbonyl group proved to be the best replacement of the Boc group in terms of potency (compound XXIV was 5-fold more active than inhibitor V), but it provides a compound with less polarity and hydrophilicity compared to peptides with basic or charged N-termini. Three of the compounds listed in Table IV (compound XXII, XXIII, and XXVIII) with higher hydrophilicity as measured by partition coefficient (Table VI) were selected for further in vivo studies. Table VI shows the efficacy of these renin inhibitors after oral administration of 30 mg/kg to salt-depleted monkeys. Compound XXII, with 4-(dimethylamino)butyric acid as replacement for the Boc group, reduced the systolic blood pressure by 6.9 ± 2.5% for about 120 min. Maximum decrease of PRA was 44.4 ± 12% after 60 min and 41.3 ± 7.4% after 180 min.

Compound XXIII with a permanent positive charge at the N-terminus decreased the systolic pressure by 10.6 ± 3.9% for 130 min. PRA was inhibited by 83.2 ± 3.4% and 78.3 ± 8.5% after 60 and 180 min, respectively. Renin inhibitor XXVIII, which posses an N-terminal (4-aminopiperidino)carbonyl residue similar to the morpholinocarbonyl group, was the most potent compound of this series. Blood pressure was reduced by 24.7 ± 8.7%. This effect lasted for more than 180 min. PRA was lowered by 90.5 ± 7.9% and 89.3 ± 7.3% after 30 and 180 min, respectively.

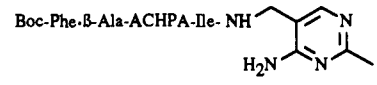
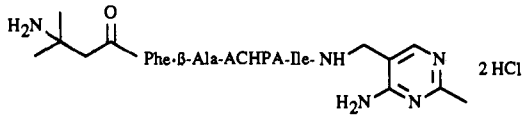
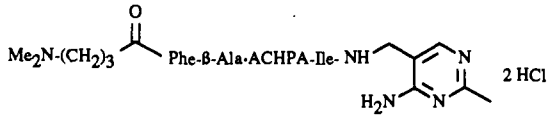
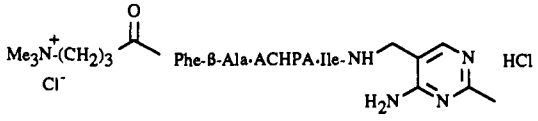
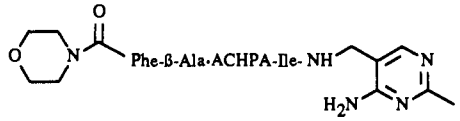
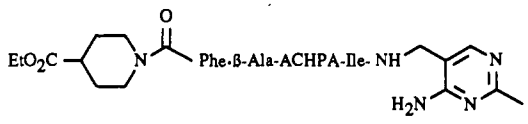
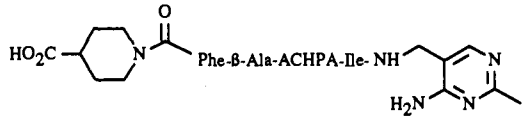
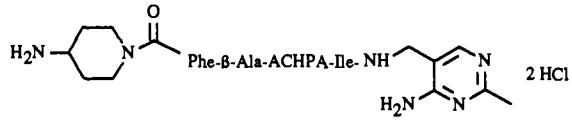
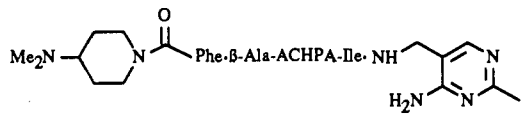
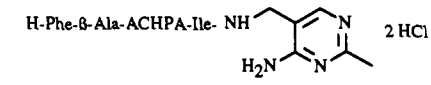
The effects of intravenous administration of XXVIII to salt-depleted cynomolgus monkeys are shown in Figure 4. A 0.01 mg/kg bolus injection of XXVIII produced a hypotensive response of 10 mmHg accompanied by a 75% drop in PRA. The increase of the iv dose to 0.03 mg/kg led to a more pronounced reduction in blood pressure and PRA. At a dose of 1 mg/kg we observed a dramatic fall of blood pressure in the range of 30 mmHg, which lasted for more than 180 min. PRA was suppressed by 98% and 80% after 60 and 180 min, respectively. The heart rate remained unaffected at all doses administered.

Inhibitor XXVIII was given orally at 10 mg/kg to four salt-depleted cynomolgus monkeys, and the results are shown in Figure 5. The blood pressure decrease of 30 mmHg was accompanied by a 71% drop in PRA. Both effects persisted longer than 180 min, which demonstrated the high efficacy of compound XXVIII after oral administration.

(16) (a) Greenfield, J. C.; Cook, K. J.; O'Leary, I. A. Disposition, Metabolism, and Excretion of U-71038, a Novel Renin Inhibitor Peptide in Rat. *Drug Metab. Distrib.* **1989**, *17*, 518–525. (b) DeGasparo, M.; Cumin, F.; Nussberger, J.; Guyenne, T. T.; Wood, J. M.; Menard, J. Pharmacological Investigations of a New Renin Inhibitor in Normal Sodium-unrestricted Volunteers. *Br. J. Clin. Pharmacol.* **1989**, *27*, 587–596.

(17) (a) Rosenberg, S. H.; Woods, K. W.; Sham, H. L.; Kleinert, H. D.; Martin, D. L.; Stein, H.; Cohen, J.; Egan, D. A.; Bopp, B.; Merits, J.; Garren, K. W.; Hoffman, D. J.; Plattner, J. J. Water-Soluble Renin Inhibitor. Design of a Subnanomolar Inhibitor with a Prolonged Duration of Action. *J. Med. Chem.* **1990**, *33*, 1962–1969. (b) Bundy, G. L.; Pals, D. T.; Laweson, J. A.; Couch, S. J.; Lipton, M. F.; Mauragis, M. A. Potent Renin Inhibitory Peptides Containing Hydrophilic End Groups. *J. Med. Chem.* **1990**, *33*, 2276–2283. (c) Greenlee, W. J.; ten Broeke, J.; de Laszlo, S.; Chakravarty, P. K.; Camara, V. J.; Fitch, K.; Sarnella, C.; Patchett, A. A.; Williams, P. D.; Perlow, D. S.; Veber, D. F.; Lynch, R. J.; Doyle, J. J.; Sham, T.; Strouse, J. F.; Siegl, P. K. S. *Peptides: Chemistry, Structure and Biology, Proceedings of the Eleventh American Peptide Symposium*; Rivier, J. E., Marshall, G. R., Eds.; ESCOM Science Publishers B. V.: Leiden, Netherlands, 1990; p 411–412.

Table IV. In Vitro Activities of Inhibitors with Modifications of P_4

	P_4	IC_{50}, nM		
		human renin	cathepsin D	pepsin
V		1.5	7900	18500
XX		1.8	>10000	>10000
XXI		4.5	>10000	>10000
XXII		4.7	>10000	>10000
XXIII		8.0	>10000	>10000
XXIV		0.28	>10000	>10000
XXV		0.41	3150	4200
XXVI		1.15	>10000	>10000
XXVII		0.65	5900	>10000
XXVIII		0.76	8500	>10000
XXIX		0.58	10000	>10000
XXX		19.5	>10000	>10000

Conclusions

Replacement of histidine by β-alanine in the P_2-position and the incorporation of peptidomimetics for the P_2-P_3 sites of ACHPA- and LeuΨ[CHOHCH$_2$]Val-based tetrapeptides lead to renin inhibitors with high potency and remarkable stability against the intestinal protease chymotrypsin.

These inhibitors are highly selective for renin over the two related aspartic proteinases cathepsin D and pepsin.

Oral activity of β-alanine-containing inhibitors can be achieved by incorporation of polar and hydrophilic residues at the N-terminus. After intravenous and oral administration to cynomolgus monkeys, one of these compounds (XXVIII) demonstrates excellent efficacy and a duration of action significantly longer than found with inhibitors containing α-amino acids in the P_2-position.[7b] These results make this renin inhibitor a promising candidate for the treatment of hypertension and congestive heart failure.

Experimental Section

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

Table V. Chemical Data for Renin-Inhibiting Compounds

no.[a]	HPLC[b] % purity	formula[c]	no.[a]	HPLC[b] % purity	formula[c]
I	92.1	$C_{43}H_{62}N_8O_7 \cdot 1.5H_2O$	XVI	96.4	$C_{40}H_{64}N_8O_7$
III	95.3	$C_{39}H_{58}N_8O_7$	XVII	98.4	$C_{41}H_{66}N_8O_7$
IV	99.3	$C_{39}H_{60}N_8O_7 \cdot H_2O$	XVIII	98.2	$C_{41}H_{65}N_7O_7$
V	97.9	$C_{40}H_{62}N_8O_7 \cdot 0.6H_2O$	XIX	96.9	$C_{41}H_{65}N_7O_7S$
VI	97.1	$C_{41}H_{64}N_8O_7$	XXI	96.3	$C_{41}H_{67}Cl_2N_9O_6$
VII	96.5	$C_{40}H_{62}N_8O_7$	XXII	92.4	$C_{41}H_{67}Cl_2N_9O_6$[f]
VIII	97.8	$C_{40}H_{61}N_7O_7 \cdot 0.5H_2O$	XXIII	98.4	$C_{42}H_{69}Cl_2N_9O_6$
IX	92.3	$C_{40}H_{63}N_7O_7$	XXIV	99.6	$C_{40}H_{61}N_9O_7$
X	96.7	$C_{40}H_{63}N_7O_7 \cdot H_2O$[d]	XXV	95.5	$C_{44}H_{67}N_9O_8$
XI	97.6	$C_{40}H_{61}N_7O_7S$[e]	XXVI	97.4	$C_{42}H_{63}N_9O_6$[g]
XII	97.5	$C_{40}H_{63}N_7O_7S$	XXVII	97.1	$C_{46}H_{72}N_{10}O_8 \cdot H_2O$
XIII	96.3	$C_{40}H_{63}N_7O_7S$	XXVIII	98.3	$C_{41}H_{66}Cl_2N_{10}O_6 \cdot H_2O$
XIV	98.5	$C_{40}H_{61}N_7O_8S$	XXIX	90.6	$C_{43}H_{66}N_{10}O_6 \cdot 3H_2O$[h]
XV	93.8	$C_{40}H_{61}N_7O_9S \cdot H_2O$	XXX	96.2	$C_{35}H_{56}Cl_2N_8O_5$

[a] See Tables I–IV for structures. [b] The purity of the compounds was examined by HPLC at 210 nm on a reverse-phase column (Lichrosorb RP-8, 7 μm, 250 × 4 mm, or Lichrospher 60 RP-Select B, 5 μm, 250 × 4 mm, E. Merck) with CH_3CN and NaH_2PO_4 buffer (0.05 M, pH 6) as eluents. [c] Analyses for C, H, N, Cl, S were ±0.4% of the expected values (for formula shown) unless otherwise noted. [d] N: calcd, 12.70; found 12.50. [e] C: calcd, 61.26; found 61.60. [f] Cl: calcd 8.31; found 8.65. [g] H: calcd, 7.74; found 7.50. [h] H: calcd, 8.54; found 8.35.

Table VI. In Vivo and in Vitro Monkey Data for Selected Renin Inhibitors

no.	IC_{50}, nM, human plasma pH 5.5	log P,[b] pH 7.4	oral efficacy[a] maximum % of syst blood pressure change[c]	oral efficacy[a] duration of syst blood pressure decrease	oral efficacy[a] maximum % of PRA change[c]	oral efficacy[a] % of PRA change after 180 min
V	1.5	2.88			−25.1 ± 3.6	−21.6 ± 10.3
XXII	4.7	1.76	−6.9 ± 2.5	~120 min	−44.4 ± 12.1	−41.3 ± 7.4
XXIII	8.0	1.53	−10.6 ± 3.9	~130 min	−83.2 ± 3.4	−78.3 ± 8.5
XXVIII	0.76	1.83	−24.7 ± 8.7	>180 min	−90.5 ± 7.9	−89.3 ± 7.3

[a] 30 mg/kg administered to salt-depleted cynomolgus monkeys (n = 4). [b] Octanol/water. [c] From T = 0 base-line value; mean ± SE.

tively. Microanalyses were obtained with a Perkin-Elmer 240B CHN analyzer. Thin-layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} plates with a layer thickness of 0.25 mm from E. Merck (Darmstadt, Germany). Visualization was done with UV and I_2. Yields are not optimized.

(4*S*,5*S*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidine-5-acetic Acid Methyl Ester (2a). To a solution of 20 g (61.8 mmol) of (3*S*,4*S*)-4-[(*tert*-butoxycarbonyl)amino]-3-hydroxy-5-phenylpentanoic acid methyl ester (**1a**) in 150 mL of CH_2Cl_2 were added 32 mL of dimethoxypropane and 0.75 g of *p*-toluenesulfonic acid. After stirring for 14 h, the solvent was removed under reduced pressure. The residue was chromatographed on silica gel using hexane/EtOAc (9/1) as eluent. Crystallization from hexane gave 17.2 g (77%) of white crystals: mp 50 °C; $[\alpha]^{20}_D$ 9.6° (*c* 1, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.25 (m, 5 H), 4.23 (q, *J* = 7 Hz, 1 H), 3.63 (m, 1 H), 3.5 (s, 3 H), 3.05 (m, 2 H), 2.41 (m, 2 H), 1.48 (s, 15 H). Anal. ($C_{20}H_{29}NO_5$) C, H, N.

(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidine-5-acetic Acid Methyl Ester (2b). This compound was prepared in 74% yield from **1b** as described for **2a**: mp 122–123 °C; $[\alpha]^{20}_D$ −55.4° (*c* = 1.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.26 (m, 5 H), 4.42 (q, *J* = 7 Hz, 1 H), 4.25 (m, 1 H), 3.45 (s, 3 H), 2.73 (m, 2 H), 2.60 (q, *J* = 9 Hz, 2 H), 1.45 (s, 9 H), 1.32 (s, 6 H). Anal. ($C_{20}H_{29}NO_5$) C, H, N.

(4*S*,5*S*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidine-5-propionitrile (3a). To a solution of 35 g (96 mmol) of ester **2a** in 150 mL of THF at 0 °C was added dropwise 250 mL (250 mmol) of a 1 M solution of Dibal in toluene. After stirring for 1 h at 0 °C, the mixture was poured into an aqueous solution of sodium potassium tartrate. Ether was added, and the mixture was shaken. The organic layer was separated, dried (Na_2SO_4), and evaporated to yield 29.8 g of the corresponding alcohol as an oil. Without further purification the crude alcohol (89 mmol) was dissolved in 200 mL of CH_2Cl_2 at 0 °C. Addition of 10.1 g (100 mmol) of triethylamine was followed by 11.5 g (100 mmol) of methanesulfonyl chloride. After 4 h of stirring at 0 °C, the mixture was washed twice with both a 1 N HCl solution and a saturated aqueous $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and evaporated. The remaining crude mesylate (34 g) was dissolved in 200 mL of DMSO and heated to 50 °C with 12 g (245 mmol) of NaCN for 24 h. For workup the mixture was diluted with water and extracted with ether. The combined ether extracts were washed three times with water, dried (Na_2SO_4), and evaporated to give 25.5 g of crude nitrile **3a**. Crystallization from hexane gave 20.2 g (61%) of the product: mp 86–87 °C; $[\alpha]^{20}_D$ 4.6° (*c* 1.04, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.26 (m, 5 H), 3.92 (m, 1 H), 3.75 (m, 1 H), 2.95 (m, 2 H), 2.38 (m, 2 H), 1.60 (m, 2 H), 1.48 (s, 9 H), 1.45 (s, 6 H). Anal. ($C_{20}H_{28}N_2O_3$) C, H, N.

(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidine-5-propionitrile (3b). This compound was prepared in 65% yield from ester **2b** as described for nitrile **3a**: mp 59–60 °C; $[\alpha]^{20}_D$ −36.4° (*c* 1.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.28 (m, 5 H), 4.18 (m, 1 H), 4.07 (m, 1 H), 2.75 (m, 2 H), 2.40 (m, 2 H), 1.85 (m, 1 H), 1.70 (m, 1 H), 1.48 (s, 9 H), 1.25 (s, 6 H). Anal. ($C_{20}H_{28}N_2O_3$) C, H, N.

(4*S*,5*S*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidine-5-propanoic Acid (4a). Nitrile **3a** (5 g, 14.5 mmol) dissolved in 50 mL of methanol was heated under reflux for 8 h with 50 mL of an aqueous 30% KOH solution. After cooling to room temperature, the mixture was poured into a 1 N HCl solution and extracted with CH_2Cl_2. Washing of the combined extracts with saturated $NaHCO_3$ solution gave after drying (Na_2SO_4) and evaporation a white gum which crystallized from ether: 4.5 g (85%); mp 94 °C; $[\alpha]^{20}_D$ 1.9° (*c* 1.06, MeOH); ^{1}H NMR (DMSO-d_6) δ 12.05 (s, br, 1 H), 7.25 (m, 5 H), 3.87 (q, *J* = 7 Hz, 1 H), 3.70 (q, *J* = 7 Hz, 1 H), 2.90 (m, 2 H), 2.20 (m, 2 H), 1.55 (m, 2 H), 1.48 (s, 15 H). Anal. ($C_{20}H_{29}NO_5$) C, H, N.

(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyloxazolidine-5-propanoic Acid (4b). This compound was prepared in 87% yield from nitrile **3b** as described for acid **4a**: mp 102–103 °C; $[\alpha]^{20}_D$ −38.4° (*c* 1.1, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.02 (s, br, 1H), 7.27 (s, 5 H), 4.07 (m, 2 H), 2.75 (m, 2 H), 2.10 (m, 2 H), 1.65 (m, 2 H), 1.48 (s, 9 H), 1.25 (s, 6 H). Anal. ($C_{20}H_{29}NO_5$) C, H, N.

***N*$_\alpha$-[*N*-[3-[(4*S*,5*S*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-5-oxazolidinyl]propanoyl]ACHPA]Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (5a).** To a −5 °C cold stirred solution of 1.09 g (3 mmol) of compound **4a** and 610 mg (6 mmol) of *N*-methylmorpholine (NMM) in 30 mL of dry DMF were added successively 1.56 g (3 mmol) of H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide di-

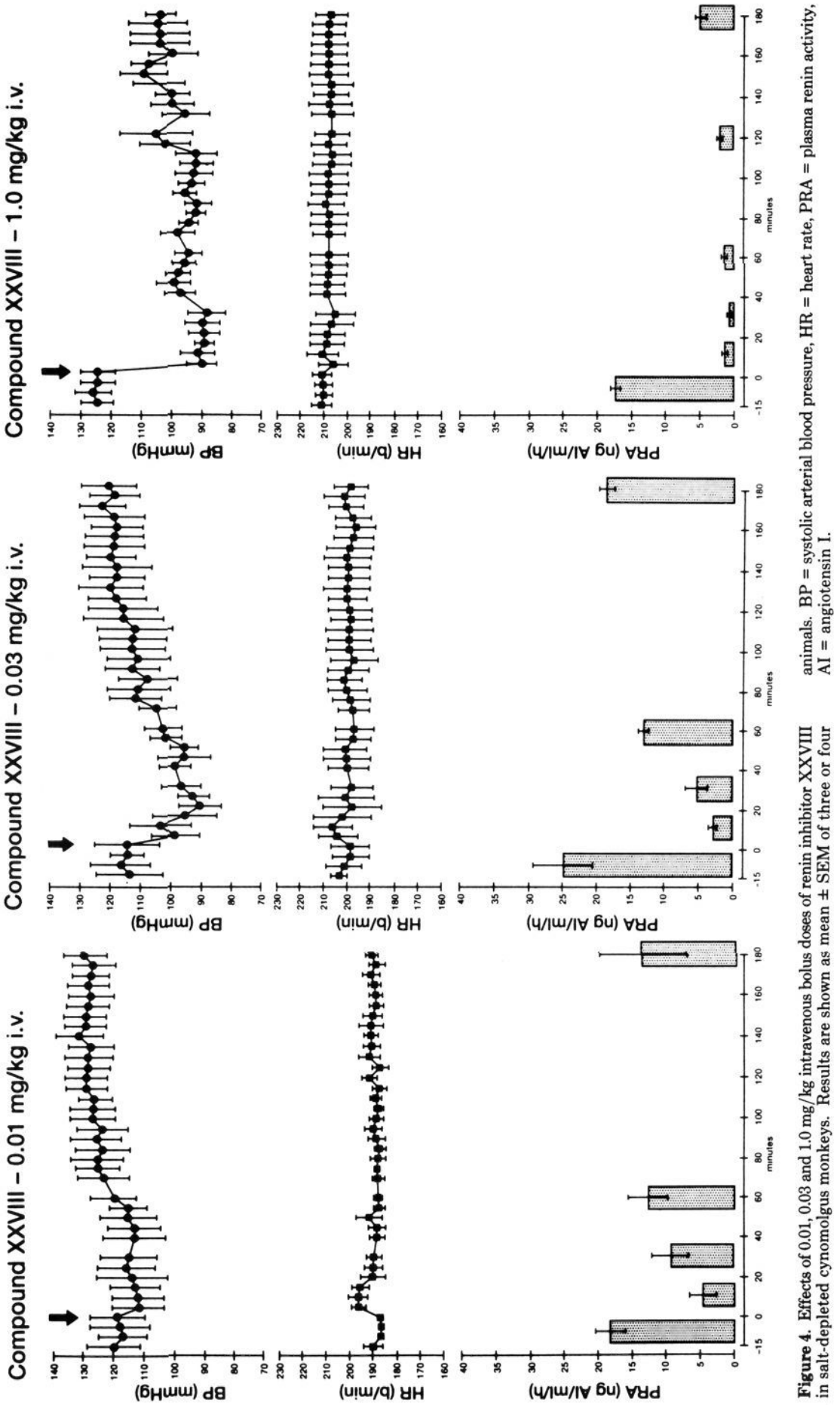


Figure 4. Effects of 0.01, 0.03 and 1.0 mg/kg intravenous bolus doses of renin inhibitor XXVIII in salt-depleted cynomolgus monkeys. Results are shown as mean ± SEM of three or four animals. BP = systolic arterial blood pressure, HR = heart rate, PRA = plasma renin activity, AI = angiotensin I.

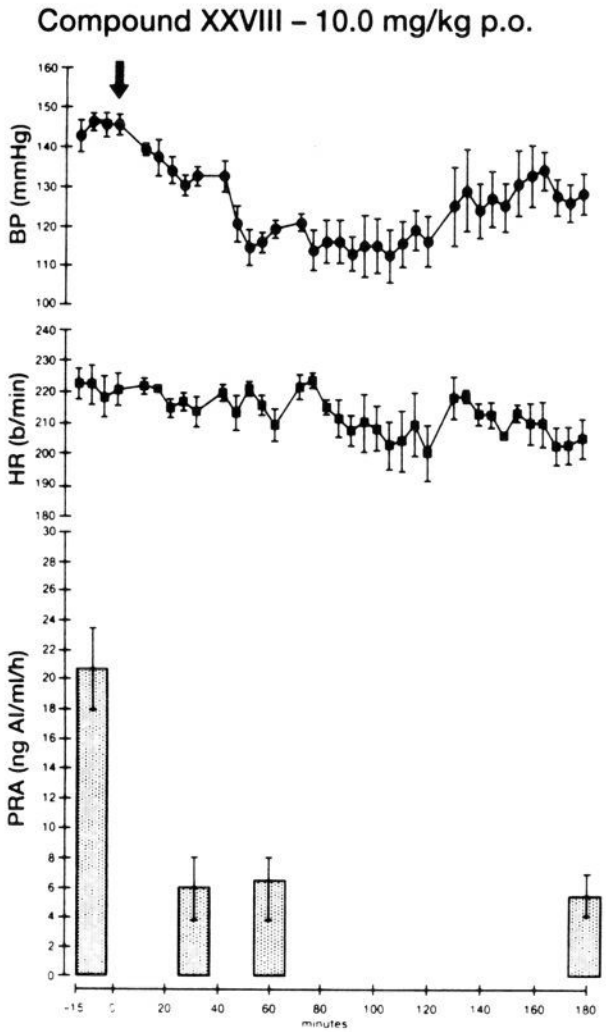


Figure 5. Effects of a 10 mg/kg oral dose of inhibitor XXVIII in salt-depleted cynomolgus monkeys. Results are shown as mean ± SEM of four animals. Blood pressure = systolic arterial blood pressure, AI = angiotensin I.

hydrochloride,[11] 500 mg (3.3 mmol) of 1-hydroxybenzotriazole (HOBT), and 630 mg (3.3 mmol) *N*-ethyl-*N'*-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI). After stirring for 14 h at room temperature, the mixture was poured into a saturated aqueous $NaHCO_3$ solution. The precipitate was extracted into CH_2Cl_2. The combined extracts were washed with brine. After drying (Na_2SO_4) and evaporation to dryness, the residue was chromatographed on silica gel. Elution with EtOAc/MeOH (95/5) yielded 1.53 g (64%) of a white solid: $[\alpha]^{20}_D$ −19.8° (*c* 0.95, MeOH); FAB MS *m/e* 795 ($M^+ + H$). Anal. ($C_{43}H_{67}N_7O_7$) C, H, N.

N_α-[*N*-[3-[(4*S*,5*R*)-4-Benzyl-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-5-oxazolidinyl]propanoyl]ACHPA]Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (5b). Intermediate **4b** (3.64 g, 10 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (5.22 g, 10 mmol) were coupled by using the procedure described for **5a**. Purification of the crude product by chromatography on silica gel eluting with CH_2Cl_2/EtOAC/MeOH (85/10/5) gave 4.86 g (61%) of **5b**: $[\alpha]^{20}_D$ −37.2° (*c* 0.34, MeOH); FAB MS *m/e* 795 ($M^+ + H$). Anal. ($C_{43}H_{67}N_7O_7$) C, H, N.

(3*S*)-1-Bromo-3-[(*tert*-butoxycarbonyl)amino]-4-phenyl-2-butanone (6). BOC-Phe-OH (185.5 g, 0.7 mol) and 70.8 g (0.7 mol) of NMM were dissolved in 1.5 L of EtOAc and cooled to −20 °C. Isobutyl chloroformate (100.4 g, 0.7 mol) was added at such a rate that the temperature never exceeded −10 °C. After 10 min at −10 °C, NMM HCl was filtered off, and the solution of the mixed anhydride poured into a solution of diazomethane (ca. 1.4 mol) in 3 L of ether. The reaction was stirred for 3 h at room temperature. The solvent was removed under reduced pressure to give a yellow oil, which was taken up in ether and washed with water and saturated $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4) and evaporated to yield the crude diazo ketone (210 g). The diazo ketone was dissolved in 500 mL of dioxane, and 150 mL of 47% aqueous HBr was added at 0 °C. The mixture was stirred for 1 h at 0 °C. The pH of the reaction mixture was adjusted to 5 by slow addition of $NaHCO_3$, and the dioxane was evaporated. The remaining aqueous solution was extracted with EtOAc. The extracts were washed with brine, dried (Na_2SO_4), and evaporated. Crude bromo ketone **6** was crystallized from ether/petroleum ether (bp 60–70 °C), yielding 177 g (74%) of light yellow crystals: mp 104–105 °C; $[\alpha]^{20}_D$ −49.1° (*c* 1.1, MeOH); ^1H NMR (DMSO-d_6) δ 7.28 (m, 5 H), 4.45 (s, 2 H), 4.22 (m, 1 H), 3.15 (dd, *J* = 5.3 and 14 Hz, 1 H), 2.75 (dd, *J* = 10.5 and 14 Hz, 1 H), 1.32 (s, 9 H). Anal. ($C_{15}H_{20}BrNO_3$) C, H, Br, N.

2-[[(3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-4-phenylbutyl]thio]acetic Acid (7). To a solution of 6.48 g (120 mmol) of NaOMe in 120 mL of MeOH was added a solution of 5.9 g (60 mmol) of mercaptoacetic acid in 50 mL of MeOH. After stirring for 30 min at room temperature, 20.5 g (60 mmol) of **6** was added. After stirring for 1 h, the solution was poured into water, containing 120 mL of 1 N HCl, and the aqueous phase was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was crystallized from MTB ether: 18.6 g (87%) of product **7** as white crystals; mp 122 °C; $[\alpha]^{20}_D$ −47.2° (*c* 1.06, MeOH); ^1H NMR (DMSO-d_6) δ 12.58 (s, 1 H), 7.26 (m, 5 H), 4.35 (m, 1 H), 3.68 (m, 2 H), 3.25 (s, 2 H), 3.05 (dd, *J* = 3.5 and 14 Hz, 1 H), 2.72 (dd, *J* = 3.5 and 14 Hz, 1 H), 1.45 (s, 9 H); TLC R_f = 0.51 (20% MeOH, 2% H_2O/CH_2Cl_2). Anal. ($C_{17}H_{23}NO_5S$) C, H, N. S.

2-[[(3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-4-phenylbutyl]sulfinyl]acetic Acid (8a) and 2-[[(3*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-4-phenylbutyl]-sulfonyl]acetic Acid (8b). Compound **7** (3.53 g, 10 mmol) was dissolved in 100 mL of THF and 16.3 g (33 mmol) of magnesium monoperoxyphthalate was added. After stirring for 1 h at room temperature, the mixture was poured into water, containing 50 mL of 1 N HCl. The solution was extracted with CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (8/2), yielding 1.3 g (33%) of **8a** and 1.8 g (47%) of **8b** as white solids.

8a: mp 139–40 °C; ^1H NMR (DMSO-d_6) δ 13.5 (s, 1 H), 7.25 (m, 5 H), 4.25 (d, *J* = 6 Hz, 2 H), 4.22 (m, 1 H), 4.0 (dd, *J* = 3.5 and 14 Hz, 1 H), 3.78 (dd, *J* = 7 and 14 Hz, 1 H), 3.2 (m, 1 H), 2.7 (m, 1 H), 1.35 (s, 9 H); TLC R_f = 0.15 (20% MeOH, 2% H_2O/CH_2Cl_2). Anal. ($C_{17}H_{23}NO_6S$) C, H, N, S.

8b: mp 111–112 °C; ^1H NMR (DMSO-d_6) δ 13.2 (s, 1 H), 7.25 (m, 5 H), 4.76 (s, 2 H), 4.37 (s, 2 H), 4.25 (m, 1 H), 3.12 (dd, *J* = 3.5 and 14 Hz, 1 H), 2.68 (dd, *J* = 10 and 14 Hz, 1 H), 1.35 (s, 9 H); TLC R_f = 0.26 (20% MeOH, 2% H_2O/CH_2Cl_2). Anal. ($C_{17}H_{23}NO_7S$) C, H, N, S.

(2*R*,3*S*)-1-Bromo-3-[(*tert*-butoxycarbonyl)amino]-4-phenyl-2-butanol (9). Bromo ketone **6** (5 g, 14.5 mmol) was added gradually to a stirred solution of 4 g (16 mmol) of lithium tri-(*tert*-butoxyaluminum hydride in 150 mL of ether. After 30 min of stirring at room temperature, the mixture was neutralized with 1 N aqueous HCl and concentrated. The residue was extracted with EtOAc, and the extract was dried (Na_2SO_4) and evaporated to yield the crude product. The diastereoisomeric ratio of **9**/**10** = 5/1 as evaluated by HPLC at 210 nm on a reversed-phase column (Lichrospher 60 RP-Select B, 5 μm, 250 × 4 mm, E. Merck) with CH_3CN and NaH_2PO_4 buffer (0.05 M, pH 6) as eluents (70:30). The crude product was chromatographed on silica gel by elution with hexane/EtOAc (72/25) yielding 3.5 g (70%) of alcohol **9** and 0.55 g (11%) of alcohol **10**.

9: mp 86–87 °C; $[\alpha]^{20}_D$ −13.7° (*c* 1.03, MeOH); ^1H NMR (DMSO-d_6) δ 7.25 (m, 5 H), 6.48 (d, *J* = 9 Hz, 1 H), 3.85 (m, 1 H), 3.62 (m, 1 H), 3.5 (dd, *J* = 3.5 and 10 Hz, 1 H), 3.33 (dd, *J* = 7 and 10 Hz, 1 H), 2.80 (dd, *J* = 5 and 14 Hz, 1 H), 2.66 (dd, *J* = 10 and 14 Hz, 1 H), 1.25 (s, 9 H); TLC R_f = 0.46 (30% MTB/hexane). Anal. ($C_{15}H_{22}BrNO_3$) C, H, Br, N.

(2S,3S)-1-Bromo-3-[(tert-butoxycarbonyl)amino]-4-phenyl-2-butanol (10). Sodium borohydride (0.6 g, 16 mmol) was added gradually at 0 °C to a stirred solution of 5 g (14.5 mmol) of bromo ketone **6** in 50 mL of MeOH. The reaction mixture was stirred further for 1 h, then neutralized with 1 N aqueous HCl, and the solvent removed under reduced pressure. The residue was extracted with EtOAc, and the extract was dried (Na_2SO_4) and evaporated to yield 5 g of the crude product. The diastereomeric ratio of **9**/**10** = 5/95 as determined by HPLC. Pure alcohol **10** was obtained by crystallization from diisopropyl ether (3.8 g, 76%). **10**: mp 149–50 °C; $[\alpha]^{20}_D$ −3.4° (*c* 1.04, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 5 H), 6.67 (d, *J* = 9 Hz, 1 H), 3.62 (m, 3 H), 3.37 (m, 1 H), 3.0 (dd, *J* = 3.5 and 14 Hz, 1 H), 2.57 (dd, *J* = 10 and 14 Hz, 1 H), 1.28 (s, 9 H); TLC R_f = 0.32 (30% MTB/hexane). Anal. ($C_{15}H_{22}BrNO_3$) C, H, Br, N.

(4S,5R)-4-Benzyl-5-(bromomethyl)-3-(tert-butoxycarbonyl)-2,2-dimethyloxazolidine (11). Compound **9** (3.36 g, 9.76 mmol) was stirred in a solution of 5 mL of 2-dimethoxypropane, 50 mL of CH_2Cl_2, and 50 mg of *p*-toluenesulfonic acid for 14 h at room temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel by elution with hexane/EtOAc (9/1), yielding 2.93 g (78%) of product **11** as white crystals: mp 104–105 °C; $[\alpha]^{20}_D$ 2.5° (*c* 1.01, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 5 H), 4.14 (q, *J* = 7 Hz, 1 H), 3.98 (m, 1 H), 3.35 (m, 2 H), 3.22 (dd, *J* = 3.5 and 14 Hz, 1 H), 2.95 (m, 1 H), 1.58 (s, 6 H), 1.55 (s, 9 H). Anal. ($C_{18}H_{26}BrNO_3$) C, H, Br, N.

(4S,5S)-4-Benzyl-5-(bromomethyl)-3-(tert-butoxycarbonyl)-2,2-dimethyloxazolidine (12). The title compound was prepared in analogy to **11** from **10** with the following modification. The reduction was stirred for 36 h at room temperature. Purification of the crude product was by chromatography on silica gel by elution with hexane/EtOAc (85/15), yielding 3.45 g (88%) of the product **12** as an oil: $[\alpha]^{20}_D$ −37.7° (*c* 0.95, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.25 (s, 5 H), 4.35 (m, 1 H), 4.23 (m, 1 H), 3.62 (dd, *J* = 10 and 14 Hz, 1 H), 3.40 (m, 1 H), 2.74 (dd, *J* = 7 and 14 Hz, 1 H), 2.68 (m, 1 H), 1.51 (s, 9 H), 1.24 (s, 6 H). Anal. ($C_{18}H_{26}BrNO_3$) C, H, Br, N.

2-[[[(4S,5R)-4-Benzyl-2,2-dimethyl-5-oxazolidinyl]methyl]thio]acetic Acid (13). To a solution of 324 mg (6 mmol) of NaOMe in 20 mL of MeOH was added a solution of 285 mg (3 mmol) of mercaptoacetic acid in 5 mL of MeOH. After stirring for 30 min at room temperature, 1.15 g (3 mmol) of compound **11** and 100 mg of KI were added. After stirring for 36 h at room temperature, the solution was poured into water, containing 6 mL of 1 N aqueous HCl. The solution was extracted three times with CH_2Cl_2. The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel by elution with CH_2Cl_2/MeOH/H_2O (90/10/1). There was obtained 320 mg (27%) of compound **13** as a white solid: mp 138–139 °C; $[\alpha]^{20}_D$ 11.0° (*c* 0.38, MeOH); ^{1}H NMR (DMSO-d_6) δ 12.53 (s, br, 1 H), 7.26 (m, 5 H), 4.06 (q, *J* = 4.8 Hz, 1 H), 3.92 (m, 1 H), 3.09 (dd, *J* = 3.5 and 14 Hz, 1 H), 3.05 (s, 2 H), 2.87 (dd, *J* = 7 and 14 Hz, 1 H), 2.57 (d, *J* = 7 Hz, 2 H), 1.48 (s, 15 H). Anal. ($C_{20}H_{29}NO_5S$) C, H, N, S.

2-[[[(4S,5S)-4-Benzyl-2,2-dimethyl-5-oxazolidinyl]methyl]thio]acetic Acid (14). The title compound was prepared in 41% yield from **12** as described for **13**: $[\alpha]^{20}_D$ −39.8° (*c* 0.95, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.34 (s, br, 1 H), 7.24 (m, 5 H), 4.23 (m, 2 H), 3.25 (s, 2 H), 2.82 (dd, *J* = 7 and 10 Hz, 1 H), 2.63 (m, 3 H), 1.48 (s, 9 H), 1.23 (s, 6 H). Anal. ($C_{20}H_{29}NO_5S$) C, H, N, S.

N_α-[N-[2-[[[(4S,5R)-4-Benzyl-3-(tert-butoxycarbonyl)-2,2-dimethyl-5-oxazolidinyl]methyl]thio]acetyl]ACHPA]Ile N-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (15). To a stirred solution of 792 mg (2 mmol) of compound **13** and 404 mg (4 mmol) of NMM in 50 mL of dry DMF, cooled to −5 °C, were added 1.04 g (2 mmol) of H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride, 305 mg (2 mmol) HOBT, and 385 mg (2 mmol) of EDCI. After stirring for 14 h at room temperature, the mixture was poured into a saturated aqueous $NaHCO_3$ 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 chromatographed on silica gel by elution with EtOAc/MeOH (9/1). There was obtained 1.25 g (76%) of product **15** as a white solid: FAB MS *m/e* 827 (M^+ + H). Anal. ($C_{43}H_{67}N_7O_7S$) C, H, N, S.

N_α-[N-[2-[[[(4S,5S)-4-Benzyl-3-(tert-butoxycarbonyl)-2,2-dimethyl-5-oxazolidinyl]methyl]thio]acetyl]ACHPA]Ile N-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (16). Intermediate **14** (396 mg, 1 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methylpyrimidinyl)methyl]amide hydrochloride (522 mg, 1 mmol) were coupled by using the procedure described for **15**. The crude product was chromatographed on silica gel by elution with EtOAc/MeOH (93/7). There was obtained 410 mg (49%) of product **16** as a white solid: FAB MS *m/e* 827 (M^+ + H). Anal. ($C_{43}H_{67}N_7O_7S$) C, H, N, S.

(4S,5R)-4-Benzyl-5-(bromomethyl)-2-oxazolidinone (17). Compound **9** (344 mg, 1 mmol) was stirred with 10 mL of 4 N HCl in dioxane for 1 h at room temperature. After evaporation under reduced pressure, the residue was dissolved in a solution of 10% phosgene in toluene (30 mL) and refluxed for 1 h. After evaporation under reduced pressure, the residue was chromatographed on silica gel by elution with CH_2Cl_2/MeOH/H_2O (92/3/5). There was obtained 195 mg (72%) of oxazolidinone **17** as an oil: $[\alpha]^{20}_D$ −57.6 (*c* 0.69, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.93 (s, 1 H), 7.29 (m, 5 H), 4.43 (q, *J* = 5 Hz, 1 H), 3.81 (qd, *J* = 5 and 1 Hz, 1 H), 3.57 (dd, *J* = 4.2 and 12 Hz, 1 H), 3.43 (dd, *J* = 5.1 and 12 Hz, 1 H), 2.84 (d, *J* = 6 Hz, 2 H).

(4S,5S)-4-Benzyl-5-(bromomethyl)-2-oxazolidinone (18). The title compound was prepared from **10** in the same manner as that of **17**. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH/H_2O (92/3/5). There was obtained 183 mg (68%) of compound **18** as a white solid: mp 110–111 °C; $[\alpha]^{20}_D$ −43.3° (*c* 0.87, MeOH); ^{1}H NMR (DMSO-d_6) δ 7.74 (s, 1 H), 7.28 (m, 5 H), 4.85 (qd, *J* = 1 and 8 Hz, 1 H), 4.18 (m, 1 H), 3.76 (d, *J* = 6.5 Hz, 2 H), 2.96 (dd, *J* = 5 and 14 Hz), 2.69 (dd, *J* = 9.2 and 14 Hz, 1 H).

BOC-Phe-Gly-ACHPA-Ile N-(3-Pyridylmethyl)amide (III). To a stirred solution of 322 mg (1 mmol) of BOC-Phe-Gly-OH and 202 mg (2 mmol) of *N*-NMM in 30 mL of dry DMF, cooled to −5 °C, were added 491 mg (1 mmol) of H-ACHPA-Ile 3-(pyridylmethyl)amide dihydrochloride, 153 mg (1 mmol) of HOBT, and 192 mg (1 mmol) of EDCI. After stirring for 14 h at room temperature, the mixture was poured into a saturated aqueous $NaHCO_3$ 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 chromatographed on silica gel by elution with CH_2Cl_2/MeOH (95/5). There was obtained 485 mg (67%) of product III as a white solid: $[\alpha]^{20}_D$ −10.6° (*c* 1.5, MeOH); FAB MS *m/e* 724 (M^+ + H). Anal. ($C_{39}H_{58}N_6O_7$) C, H, N.

BOC-Phe-Gly-ACHPA-Ile N-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (IV). Boc-Phe-Gly-OH (322 mg, 1 mmol) and H-ACHP-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-amide dihydrochloride (522 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (95/5). There was obtained 520 mg (69%) of product IV as a white solid: $[\alpha]^{20}_D$ −11.6° (*c* 0.81, MeOH); FAB MS *m/e* 752 (M^+). Anal. ($C_{39}H_{60}N_8O_7 \cdot H_2O$) C, H, N.

BOC-Phe-β-Ala-ACHPA-Ile N-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (V). BOC-Phe-β-Ala-OH (673 mg, 2 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1044 mg, 2 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1). There was obtained 1170 mg (76%) of product V as a white solid: $[\alpha]^{20}_D$ −9.2° (*c* 1.0, MeOH); FAB MS *m/e* 768 (M^+ + H). Anal. ($C_{40}H_{62}N_8O_7 \cdot 0.6H_2O$) C, H, N.

BOC-Phe-N-$(CH_2)_3CO$-ACHPA-Ile N-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (VI). BOC-Phe-N-$(CH_2)_3CO_2H$ (700 mg, 2 mmol) and H-ACHPA-Ile N-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1044 mg, 2 mmol) were coupled by using the procedure described for **III**. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (85/15). Compound VI was obtained in 68% yield: $[\alpha]^{20}_D$ −6.1° (*c* 0.99, MeOH); FAB MS *m/e* 782 (M^+ + H). Anal. ($C_{41}H_{64}N_8O_7$) C, H, N.

BOC-Phe-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (VII). BOC-Phe-Ala-OH (1010 mg, 3 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1565 mg, 3 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MTB/MeOH (85/10/5), providing compound VII in 61% yield: $[\alpha]^{20}_D$ −26.3° (*c* 1.0, MeOH); FAB MS *m/e* 768 ($M^+ + H$). Anal. ($C_{40}H_{62}N_8O_7$) C, H, N.

BOC-PheΨ[COCH$_2$]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (VIII). BOC-PheΨ-[COCH$_2$]Gly-OH (321 mg, 1 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (522 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1), providing title compound VIII in 59% yield: $[\alpha]^{20}_D$ −11.1° (*c* 0.95, MeOH); FAB MS *m/e* 752 ($M^+ + H$). Anal. ($C_{40}H_{61}N_7O_7 \cdot 0.5H_2O$) C, H, N.

BOC-PheΨ[(*S*)-CHOHCH$_2$]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (IX). Compound **5a** (300 mg, 0.38 mmol) was stirred with a solution of 100 mg of *p*-toluenesulfonic acid in 20 mL of MeOH for 36 h at room temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1). There was obtained 200 mg (70%) of product IX as a white solid: $[\alpha]^{20}_D$ −25.1° (*c* 0.98, MeOH); FAB MS *m/e* 754 ($M^+ + H$). Anal. ($C_{40}H_{63}N_7O_7$) C, H, N.

BOC-PheΨ[(*R*)-CHOHCH$_2$]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (X). The title compound was prepared in 75% yield from **5b** as described for IX: $[\alpha]^{20}_D$ 5.2° (*c* 1.0, MeOH); FAB MS *m/e* 754 ($M^+ + H$). Anal. ($C_{40}H_{63}N_7O_7 \cdot H_2O$) C, H, N.

BOC-PheΨ[COCH$_2$S]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XI). BOC-PheΨ-[COCH$_2$S]Gly-OH (353 mg, 1 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (522 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (93/7), providing title compound XI in 76% yield: $[\alpha]^{20}_D$ −6.1° (*c* 0.95, MeOH); FAB MS *m/e* 785 ($M^+ + H$). Anal. ($C_{40}H_{61}N_7O_7S$) C, H, N, S.

BOC-PheΨ[(*R*)-CHOHCH$_2$S]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XII). Compound **15** (550 mg, 0.65 mmol) was stirred with a solution of 150 mg of *p*-toluenesulfonic acid in 20 mL of MeOH for 48 h at room temperature. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (95/5). There was obtained 385 mg (75%) of product XII as a white solid: $[\alpha]^{20}_D$ −10.1° (*c* 0.85, MeOH); FAB MS *m/e* 786 (M^+). Anal. ($C_{40}H_{63}N_7O_7S$) C, H, N.

BOC-PheΨ[(*S*)-CHOHCH$_2$S]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XIII). The title compound was prepared in 73% yield from **16** as described for XII: $[\alpha]^{20}_D$ −13.8° (*c* 0.83, MeOH); FAB MS *m/e* 786 (M^+). Anal. ($C_{40}H_{63}N_7O_7S$) C, H, N.

BOC-PheΨ[COCH$_2$SO]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XIV). BOC-PheΨ-[COCH$_2$SO]Gly-OH (770 mg, 2 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1044 mg, 2 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1). The title compound was obtained in 83% yield: FAB MS *m/e* 800 ($M^+ + H$). Anal. ($C_{40}H_{61}N_7O_8S$) C, H, N, S.

BOC-PheΨ[COCH$_2$SO$_2$]Gly-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XV). BOC-PheΨ-[COCH$_2$SO$_2$]Gly-OH (386 mg, 1 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (522 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MTB/MeOH (85/10/5). The title compound was obtained in 42% yield: FAB MS *m/e* 816 ($M^+ + H$). Anal. ($C_{40}H_{61}N_7O_9S \cdot H_2O$) C, H, N, S.

BOC-Phe-Gly-LeuΨ[CHOHCH$_2$]Val-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XVI). BOC-Phe-Gly-OH (322 mg, 1 mmol) and LeuΨ[CHOHCH$_2$]Val-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide[11] (465 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1), providing compound XVI in 68% yield: $[\alpha]^{20}_D$ −33.4° (*c* 1.01, MeOH); FAB MS *m/e* 769 (M^+). Anal. ($C_{40}H_{64}N_8O_7$) C, H, N.

BOC-Phe-β-Ala-LeuΨ[CHOHCH$_2$]Val-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XVII). BOC-Phe-β-Ala-OH (336 mg, 1 mmol) and LeuΨ[CHOHCH$_2$]Val-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide (465 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1), providing compound XVII in 64% yield: $[\alpha]^{20}_D$ −2.0° (*c* 1.02, MeOH); FAB MS *m/e* 784 ($M^+ + H$). Anal. ($C_{41}H_{66}N_8O_7$) C, H, N.

BOC-PheΨ[COCH$_2$]Gly-LeuΨ[CHOHCH$_2$]Val-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XVIII). BOC-PheΨ[COCH$_2$]Gly-OH (321 mg, 1 mmol) and LeuΨ-[CHOHCH$_2$]Val-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]amide (465 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MTB/MeOH (80/10/5), providing compound XVII in 63% yield: $[\alpha]^{20}_D$ −41.7° (*c* 0.94, MeOH); FAB MS *m/e* 770 ($M^+ + H$). Anal. ($C_{41}H_{65}N_7O_7$) C, H, N.

BOC-PheΨ[COCH$_2$S]Gly-LeuΨ[CHOHCH$_2$]Val-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XIX). BOC-PheΨ[COCH$_2$S]Gly-OH (353 mg, 1 mmol) and LeuΨ-[CHOHCH$_2$]Val-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]amide (465 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/EtOAc/MeOH (85/10/5). There was obtained 510 mg (64%) of title compound XIX. $[\alpha]^{20}_D$ −44.0° (*c* 1.01, MeOH); FAB MS *m/e* 801 ($M^+ + H$). Anal. ($C_{41}H_{65}N_7O_7S$) C, H, N, S.

(3-Amino-3-methylbutyryl)-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide Dihydrochloride (XX). To a stirred solution of 460 mg (2.1 mmol) of 3-BOC-amino-3-methylbutyric acid and 404 mg (4 mmol) of NMM in 40 mL of dry DMF, cooled to −5 °C, were added 1480 mg (2 mmol) of H-Phe-β-Ala-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride, 310 mg (2 mmol) of HOBT, and 384 mg (2 mmol) of EDCI. After stirring for 14 h at room temperature, the mixture was poured into a saturated aqueous $NaHCO_3$ 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 crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (95/5). The BOC-protected product was obtained in 64% yield. Removal of the BOC-group was achieved by stirring 900 mg of the protected peptide with 40 mL of 4 N HCl in dioxane for 1 h at room temperature. After evaporation under reduced pressure, the residue was triturated with dry ether. There was obtained 830 mg (95%) of product XX: $[\alpha]^{20}_D$ −4.9° (*c* 1, MeOH); FAB MS *m/e* 767 ($M^+ + H$). Anal. ($C_{40}H_{65}Cl_2N_9O_6$) C, H, Cl, N.

(6-Aminohexanoyl)-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide Dihydrochloride (XXI). 6-(BOC-amino)hexanoic acid (970 mg, 4.2 mmol) and H-Phe-β-Ala-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)-methyl]amide dihydrochloride (2.96 g, 4 mmol) were coupled by using the procedure described for the preparation of XX. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MTB/MeOH (80/10/5) providing 1800 mg (49%) of the BOC protected product. A 1.5-g portion of the protected peptide was stirred with 40 mL of 4 N HCl in dioxane for 1 h at room temperature. After evaporation under reduced pressure, the residue was triturated with dry ether. The product was obtained as a white solid (1.35 g, 93%): $[\alpha]^{20}_D$ 3.4° (*c* 1.02, MeOH); FAB MS *m/e* 781 ($M^+ + H$). Anal. ($C_{41}H_{67}Cl_2N_9O_6$) C, H, Cl, N.

[4-(Dimethylamino)butyryl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide Dihydrochloride (XXII). 4-(Dimethylamino)butyric acid (190 mg, 1.2 mmol) and H-Phe-β-Ala-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide (819 mg, 1.2 mmol) were coupled by using the procedure described for the preparation of XX. The crude product was chromatographed on silica gel by elution with

CH_2Cl_2/MeOH/H_2O (85/15/5). The product was dissolved in 10 mL of EtOH, and 0.94 mL of 1 N aqueous HCl was added. The solvent was removed under reduced pressure, and the residue was triturated with dry ether. The title compound was obtained as a white solid (380 mg, 47%): $[\alpha]^{20}_D$ -8.7° (c 1.06, MeOH); FAB MS m/e 781 (M^+ + H). Anal. ($C_{41}H_{67}Cl_2N_9O_6$) C, H, Cl, N.

[4-(Trimethylammonio)butyryl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide Chloride Hydrochloride (XXIII). *N*-(3-Carboxypropyl)trimethylammonium chloride (280 mg, 1.52 mmol) and H-Phe-β-Ala-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1.13 g, 1.52 mmol) were coupled by using the procedure described for the preparation of XX. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH/H_2O (80/20/2), yielding 480 mg (38%) of the product. The product was dissolved in 10 mL of EtOH, and 0.49 mL of 1 N aqueous HCl was added. The solvent was removed under pressure, and the residue triturated with dry ether to give 475 mg of the title compound XXIII: $[\alpha]^{20}_D$ 3.8° (c 1.04, MeOH); FAB MS m/e 794 (M^+ of the cation). Anal. ($C_{42}H_{69}Cl_2N_9O_6$) C, H, Cl, N.

(Morpholinocarbonyl)-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XXIV). (Morpholinocarbonyl)-Phe-β-Ala-OH (349 mg, 1 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (520 mg, 1 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1). There was obtained 520 mg (67%) of XXIV: $[\alpha]^{20}_D$ -8.1° (c 1.95, MeOH); FAB MS m/e 781 (M^+ + H). Anal. ($C_{40}H_{61}N_9O_7$) C, H, N.

[[4-(Ethoxycarbonyl)piperidino]carbonyl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XXV). [[4-(Ethoxycarbonyl)piperidino]carbonyl]-Phe-β-Ala-OH (3.23 g, 7.7 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (4.01 g, 7.7 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/EtOAc/MeOH (80/10/5), giving 4.2 g (64%) of compound XXV: $[\alpha]^{20}_D$ -13.1° (c 2.08, MeOH); FAB MS m/e 851 (M^+ + H). Anal. ($C_{44}H_{67}N_9O_8$) C, H, N.

[(4-Carboxypiperidino)carbonyl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XXVI). Compound XXV (510 mg, 0.6 mmol) was stirred with a solution of 0.35 mL of 2 N NaOH in 5 mL of dioxane for 6 h at room temperature. The mixture was poured into water, containing 0.8 mL of 1 N aqueous HCl, and extracted with CH_2Cl_2. The extracts were washed with brine, dried (Na_2SO_4), and evaporated. There was obtained 485 mg (98%) of the product XXVI as a white solid: $[\alpha]^{20}_D$ -8.6° (c 1.97, MeOH); FAB MS m/e 823 (M^+ + H). Anal. ($C_{42}H_{63}N_9O_8$) C, H, N.

[[4-(BOC-amino)piperidino]carbonyl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XXVII). [[4-(BOC-amino)piperidino]carbonyl]-Phe-β-Ala-OH (920 mg, 2 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1043 mg, 2 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH (9/1), providing 1100 mg (62%) of product XXVII: $[\alpha]^{20}_D$ -12.2° (c 1, MeOH); FAB MS m/e 894 (M^+ + H). Anal. ($C_{46}H_{72}N_{10}O_8 \cdot H_2O$) C, H, N.

[(4-Aminopiperidino)carbonyl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XXVIII). Compound XXVII (770 mg, 0.86 mmol) was stirred with 20 mL of 4 N HCl in dioxane for 1 h at room temperature. After evaporation under reduced pressure, the residue was triturated with ether. There was obtained 730 mg (98%) of product XXVIII as a hygroscopic white solid: $[\alpha]^{20}_D$ -11.2° (c 1.01, MeOH); FAB MS m/e 793 (M^+ + H). Anal. ($C_{41}H_{66}Cl_2N_{10}O_6 \cdot H_2O$) C, H, Cl, N.

[[4-(Dimethylamino)piperidino]carbonyl]-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide (XXIX). [[4-(Dimethylamino)piperidino]carbonyl]-Phe-β-Ala-OH (820 mg, 2.1 mmol) and H-ACHPA-Ile *N*-[(4-amino-2-methyl-5-pyrimidinyl)methyl]amide dihydrochloride (1043 mg, 2 mmol) were coupled by using the procedure described for III. The crude product was chromatographed on silica gel by elution with CH_2Cl_2/MeOH/H_2O (80/10/1), providing 860 mg (52%) of product XXIX. $[A]^{20}_D$ -3.6° (c 1.84, MeOH); FAB MS m/e 822 (M^+ + H). Anal. ($C_{43}H_{68}N_{10}O_6 \cdot 3H_2O$) C, H, N.

H-Phe-β-Ala-ACHPA-Ile *N*-[(4-Amino-2-methyl-5-pyrimidinyl)methyl]amide Dihydrochloride (XXX). Compound V (1 g, 1.31 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. There was obtained 950 mg (98%) of product XXX as a white solid: $[\alpha]^{20}_D$ 10.9° (c 0.097, MeOH); FAB MS m/e 668 (M^+ + H). Anal. ($C_{35}H_{56}Cl_2N_8O_5$) C, H, Cl, N.

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: 100 μL of plasma, 76 mM maleate buffer, pH 5.5, 7.2 mM EDTA, 1% DMSO, 8.3 mM 8-hydroxyquinoline. 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 munits 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 room temperature (24 °C) with bovine α-chymotrypsin (45 munits/mg) in 0.05 M Tris buffer containing 0.02 M $CaCl_2$ and adjusted to pH 7.4 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 concentrations of 0.5 mg/mL peptide, 0.375 mg/mL chymotrypsin, and 25% (v/v) formamide. 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 toward chymotrypsin was examined by HPLC at 254/220 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 as percent remaining HPLC area at 254 nm and plotted against time in Figures 1–3.

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.[18] 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 heart rate 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

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

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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Antitumor Properties of 2(1*H*)-Pyrimidinone Riboside (Zebularine) and Its Fluorinated Analogues

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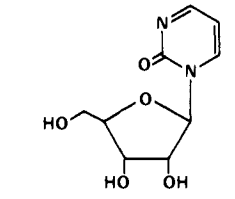
2(1*H*)-Pyrimidinone riboside (zebularine, **1b**) and its 5-fluoro (**6b**) and 2′-*ara*-fluoro (**7b**) analogues have been synthesized and evaluated in vivo as antitumor agents. Zebularine provides increase in life span (ILS) values of ca. 70% against intraperitoneal (ip) murine B16 melanoma and 50% against P388 leukemia. This compound is active when administered either ip or orally against ip or subcutaneously implanted L1210 leukemia, producing ILS values of about 100% at an optimum dose of 400 mg/kg. **1b** is also active (60% ILS) against *ara*-C-resistant L1210. The analogous unsubstituted purine riboside nebularine (**2**) has modest activity against P388 leukemia (60% ILS). While 2′-*ara*-fluorozebularine (**7b**) is only marginally active (40% ILS) at high doses against L1210 leukemia, 5-fluoro analogue **6b** is more active than zebularine and is ca. 100 times more potent. Although the activity of **6b** is about the same as that of **1b** against P388 leukemia, greater potency also is realized in this model. Zebularine is a strong inhibitor of cytidine deaminase, but in contrast to tetrahydrouridine, **1b** is acid-stable. In an attempt to use this property to advantage in oral administration, **1b** and *ara*-C have been orally coadministered to mice with ip L1210 leukemia. When zebularine is given in divided doses, up to a 2-fold increase in activity is realized, relative to treatment with the same dose of *ara*-C alone.

Earlier biochemical investigations with 1-β-D-ribofuranosyl-1,2-dihydropyrimidin-2-one (**1b**, zebularine, 2-(1*H*)-pyrimidinone riboside), established this compound as an inhibitor of cytidine deaminase (CDA).[1-4] The transition-state hypothesis[1] that lead to our initial work on **1b** as a CDA inhibitor has recently been examined in detail.[5] Although **1b**, with a 2 μM K_i, is about 10 times less potent than tetrahydrouridine (THU) as an inhibitor of mouse kidney CDA, it is still a good inhibitor of this enzyme. The stability of **1b** relative to THU in an acid-induced furanose to pyranose deactivation reaction[6] makes it a potentially useful adjuvant for oral combination studies with drugs which are substrates for CDA.

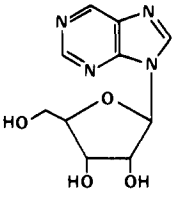
Zebularine (**1b**) was synthesized and evaluated as a bacteriostat in the early 1970s.[7,8] Our observation[1] that **1b** was cytotoxic to cultured L1210 leukemia cells, as well as active in vivo against P388 leukemia, prompted us to examine further the antitumor properties of this compound and two of its analogues. For comparison, data are included for nebularine (**2**), the purine counterpart of **1b**.

Chemistry

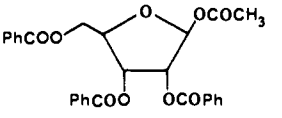
Zebularine (**1b**) was prepared by deblocking its tri-*O*-benzoyl derivative **1a** which had been synthesized by the method of Niedballa and Vorbruggen[9] utilizing the sugar intermediate **3** (Scheme I). 5-Fluorozebularine (**6b**) has been prepared by a number of methods, including the direct fluorination of **1a**.[10] Our route to **6b** utilized 5-fluoro-2(1*H*)-pyrimidinone (**4c**), which was prepared by the



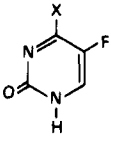
1b



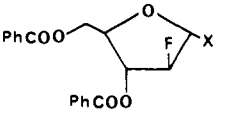
2



3



4a, X = OH
b, X = SH
c, X = H



5a, X = OCOPh
b, X = Br

treatment of 5-fluorouracil (**4a**) with P_2S_5 followed by reduction with Raney nickel.[11] The tin chloride catalyzed

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