each mouse received a single electroconvulsive shock, 100 V for 0.4 s, delivered through clips attached to the ears. The mice were individually returned to the lighted compartment 24 h after ECS treatment to measure the retention of the inhibitory avoidance response. The period the mice remained in the nonelectrical lighted compartment was measured for at least 5 min.

Stability Study. Stock solution of all compounds was prepared in acetonitrile at a concentration of 0.1 M and an appropriate volume was mixed with an aqueous buffer or biological media for stability study.

All stability experiments were carried out in aqueous buffer solutions at 37 ± 0.2 °C. The pH of the solution was maintained at the desired value by using appropriate buffer systems (sodium citrate/HCl and NaH_2PO_4/Na_2HPO_4). The total buffer concentration was 0.1 M. The ionic strength of each buffer solution was adjusted to 0.3 with sodium chloride. Degradation was initiated by the addition of the stock solution to a preheated, buffered solution to give a concentration of 1 mM. Aliquots of the solution were withdrawn at appropriate time intervals.

Male ddY mice weighing 20–30 g and male Wistar albino rats weighing 200–250 g were used to obtain plasma, brain, and liver homogenates. The liver and brain were homogenized at 0–5 °C in a glass–Teflon homogenizer to prepare 40% homogenate with a pH 7.4 isotonic phosphate buffer, then centrifuged at 2500*g* for 15 min, and the supernatant was used for the experiments. Degradation experiments were performed at 37 ± 0.2 °C and initiated by adding the stock solution to give a final concentration of 1 mM. At appropriate time intervals aliquots of the solution were withdrawn and acetonitrile was added to them to precipitate the protein. The supernatant was subjected to HPLC analysis.

Analysis. The derivatives were determined by the use of an HPLC system (LC-6A, Shimadzu Co., Kyoto, Japan) equipped with a variable-wavelength UV detector (UVIDEC 100-II, Japan Spectroscopic Co., Ltd., Tokyo, Japan) in a reverse-phase mode. Derivatives were monitored at 205 nm. The stationary phase used was a Cosmosil $5C_{18}$-P packed column (150 × 4.6 mm i.d., Nacalai Tesque Inc.). Mixtures of methanol/water (15:85 for 1, 40:60 for 3, 65:35 for 6, 80:20 for 7 85:15 for 8, v/v) were used as the mobile phase with a flow rate of 1.0 mL/min.

GABA regenerated from derivatives was determined by the use of an HPLC system equipped with a fluorescence spectromonitor (RF-530, Shimadzu Co.) after derivatization with dansyl chloride according to the method of Tapuhi et al.[34] Twenty microliters of 5-amino-*n*-valeric acid (internal standard, 2 mM) in a pH 9.5 lithium carbonate buffer (0.04 M) and 20 μL of dansyl chloride (5.56 mM) in acetonitrile were added to the sample (2 mL). After 30 min, the derivatization was terminated by adding 2% methylamine solution (2 mL). In all reactions, vials were wrapped with aluminum foil to exclude light. The stationary phase used was a Cosmosil $5C_{18}$-P packed column (150 × 4.6 mm i.d., Nacalai Tesque Inc.). Mixtures of methanol/0.01 M Tris HCl buffer, pH 7.4 (36:64, v/v), were used as the mobile phase with a flow rate of 1.0 mL/min. Excitation wavelength was 250 nm and emission wavelength was monitored at 470 nm.

Acknowledgment. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan. We wish to thank Machiyo Teraji and Minako Kawazu for their skilled technique.

Registry No. 1, 932-17-2; 2, 77015-41-9; 3, 22707-38-6; 4, 90942-80-6; 5, 51269-69-3; 6, 100400-78-0; 7, 33602-03-8; 8, 66283-26-9; 9, 2399-66-8; 10, 25083-02-7; 11, 130984-38-2; 12, 52558-70-0.

(34) Tapuhi, Y.; Schmidt, D. E.; Lindner, W.; Karger, B. L. *Anal. Biochem.* **1981**, *115*, 123.

Renin Inhibitory Peptides. Incorporation of Polar, Hydrophilic End Groups into an Active Renin Inhibitory Peptide Template and Their Evaluation in a Human Renin Infused Rat Model and in Conscious Sodium-Depleted Monkeys

Suvit Thaisrivongs,* Donald T. Pals, Donald W. DuCharme, Steve R. Turner, Garry L. DeGraaf, Judy A. Lawson, Sally J. Couch, and Mark V. Williams

Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, Michigan 49001. *Received May 31, 1990*

We previously reported that Boc-Pro-Phe-N-MeHis-Leuψ[$CHOHCH_2$]-Ile-Amp (1) is a potent and specific inhibitor of human renin in vitro. It was shown to resist degradation by selected proteases and a rat liver homogenate. It was shown to inhibit plasma renin activity and to reduce blood pressure in renin-dependent-animal models both by the intravenous and by the oral routes using dilute citric acid as vehicle. In an effort to discover compounds with improved pharmacological efficacy, we set out to modify the physical characteristics of this highly lipophilic renin inhibitor by incorporation of hydrophilic end groups. We report here a variety of water-solubilizing groups and the resulting structure–activity relationship of these compounds. They all maintain an extremely high level of enzyme inhibitory activity in vitro. Evaluation of these potent renin inhibitors in a human renin infused rat model suggests that some of these compounds exhibit improved pharmacological efficacy in vivo. This observation was further confirmed in the conscious sodium-depleted cynomolgus monkey. Importantly, the oral efficacy was demonstrated in a water vehicle in the absence of citric acid.

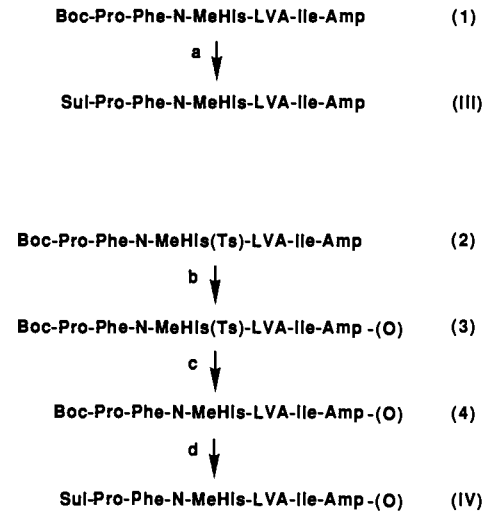
The renin–angiotensin system has been implicated in several forms of hypertension.[1] Renin catalyzes the first and rate-limiting step of the cascade by cleaving the substrate angiotensinogen to form the decapeptide angiotensin I. This intermediate is further hydrolyzed by angiotensin converting enzyme to yield the biologically active octapeptide angiotensin II. This octapeptide is one of the most potent vasoconstrictors known, and it also stimulates secretion of aldosterone and catecholamines, all of which lead to elevation of blood pressure. The uniquely high enzyme specificity of renin offers the potential of selective pharmacological intervention by compounds designed to be specific inhibitors.[2] Interest in the blockade of renin has led to rapid development of potent inhibitors by the design of substrate analogues. The most successful approach has been based on the concept of transition-state analogue of the catalytic mechanism of the aspartyl protease.[3]

(1) Davis, J. O. *Circ. Res.* **1977**, *40*, 439. Swales, J. D. *Pharmacol. Ther.* **1979**, *7*, 172.

(2) Peach, M. J. *Physiol. Rev.* **1977**, *57*, 313. Ondetti, M. A.; Cushman, D. W. *Annu. Rev. Biochem.* **1982**, *51*, 283. Haber, E. *N. Engl. J. Med.* **1984**, *311*, 1631.

0022-2623/91/1834-0633$02.50/0

Scheme I. Syntheses of Peptides III and IV[a]



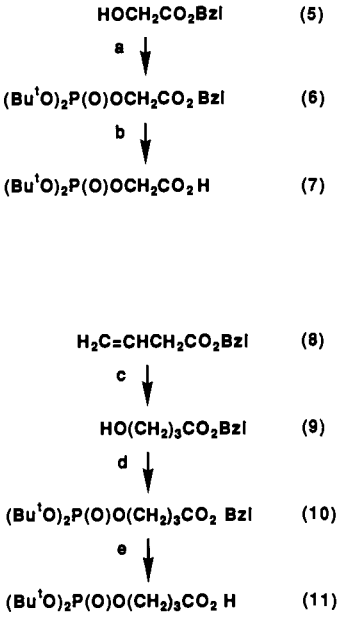
[a] (a) TFA, CH_2Cl_2; $Et_3NHO_3S(CH_2)_2N(CH_3)CO(CH_2)_6CO_2H$, Pr^i_2NEt, $(EtO)_2P(O)CN$; aqueous Na_2SO_4; (b) MCPBA, $NaHCO_3$; (c) 1-HOBT; (d) TFA, CH_2Cl_2; $Et_3NHO_3S(CH_2)_2N(CH_3)CO(CH_2)_6CO_2H$, Pr^i_2NEt, $(EtO)_2P(O)CN$; aqueous Na_2SO_4.

Modification at the cleavage site to mimic the tetrahedral species of the peptidic bond has generated compounds with very high inhibitory potency in vitro.[4]

Many renin inhibitors have already been shown to lower blood pressure in renin-dependent models by intravenous administration and also by the oral route.[5] Efforts to discover inhibitors with longer duration of action and higher oral bioavailability continue.[6] We have initiated a program with the intention to address the stability of these peptidic compounds against proteolytic degradation[7-9] to discover compounds with increased duration of action in vivo. The feasibility of orally active renin inhibitory peptides from this approach could be demonstrated.[10] The present report describes a continuation of our work in this area with the intention to improve the efficacy of the original lead compound by additional structural modifications.

Examples of efforts to increase the aqueous solubility of renin inhibitors by adjusting the hydrophilicity of the end groups have been reported.[11] We were interested in changing the physical characteristics of our previously reported compound, Boc-Pro-Phe-N-MeHis-Leuψ-

Scheme II. Syntheses of Acids 7 and 11[a]



[a] (a) $(Bu^tO)_2PNEt_2$, 1*H*-tetrazole; MCPBA; (b) 5% Pd/C, H_2(g), EtOH; (c) Sia_2BH; H_2O_2, aqueous NaOH; (d) $(Bu^tO)_2PNEt_2$, 1*H*-tetrazole; MCPBA; (e) 5% Pd/C, H_2(g), EtOH.

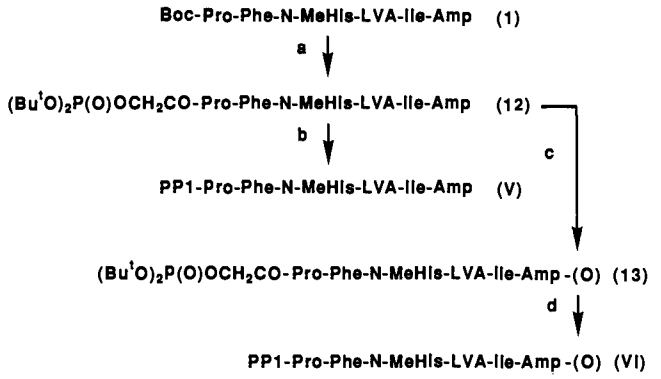
[$CHOHCH_2$]-Ile-Amp (1).[7] This peptide was shown to be highly selective for renin with potent in vitro activity (IC_{50} = 0.26 nM). It also was shown to be metabolically stable against proteolytic degradation by selected proteases and rat liver homogenate. It was demonstrated to lower blood pressure and inhibit plasma renin in conscious, sodium-depleted cynomolgus monkeys by the intravenous and oral routes.[10] This peptide is highly lipophilic with low aqueous solubility and requires diluted citric acid as vehicle. We report here our effort to increase hydrophilicity and water solubility of this peptide by selected modifications of polar end groups. It was hoped that changing the physical characteristics of this peptide could lead to a beneficial effect on the resulting pharmacological profile. Compounds that are active in vitro would be evaluated in a human renin infused rat model.[12] Finally, the observation would be confirmed in the conscious sodium-depleted cynomolgus monkey.

Chemistry[13]

Suleptanate-Containing Peptides. As shown in Scheme 1, the previously prepared peptide 1[7] was treated with trifluoroacetic acid in dichloromethane to remove the *tert*-butyloxycarbonyl protecting group. The corresponding free amine was condensed with suleptanic acid[14]

(3) Wolfenden, R. *Transition States of Biochemical Processes*: Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; p 555.
(4) Boger, J. *Annu. Rep. Med. Chem.* **1985**, *20*, 257.
(5) Greenlee, W. J. *Pharm. Res.* **1987**, *4*, 364.
(6) Luther, R. R.; Stein, H. H.; Glassman, H. N.; Kleinert, H. D. *Drug Res.* **1989**, *39*, 1.
(7) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1986**, *29*, 2088.
(8) Thaisrivongs, S.; Pals, D. T.; Harris, D. W.; Kati, W. M.; Turner, S. R. *J. Med. Chem.* **1987**, *30*, 536.
(9) Thaisrivongs, S.; Pals, D. T.; Turner, S. R.; Kroll, L. T. *J. Med. Chem.* **1988**, *31*, 1369.
(10) Pals, D. T.; Thaisrivongs, S.; Lawson, J. A.; Kati, W. M.; Turner, S. R.; Degraaf, G. L.; Harris, D. W.; Johnson, G. A. *Hypertension* **1986**, *8*, 1105.
(11) 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. *J. Med. Chem.* **1988**, *31*, 1918. 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, I.; Garren, K. W.; Hoffman, D. J.; Plattner, J. J. *J. Med. Chem.* **1990**, *33*, 1962 and references therein.
(12) Pals, D. T.; Lawson, J. A.; Couch, S. J., manuscript in preparation.
(13) The abbreviation ψ[x], indicating that x replaces the amide -CONH- unit, has been defined by IUPAC-IUB Joint Commission on Biochemical Nomenclature: *Eur. J. Biochem.* **1984**, *138*, 9. LVA is -Leuψ[$CHOHCH_2$]Val-; CVA is -Calψ-[$CHOHCH_2$]Val-; Cal is L-cyclohexylalanine; Sul is $NaO_3S(CH_2)_2N(CH_3)CO(CH_2)_6CO$-; Mba is 2(S)-methylbutylamine; Amp is 2-(aminomethyl)pyridine; Amp-(O) is 2-(aminomethyl)pyridine, *N*-oxide; PP1 is $(HO)_2P(O)OCH_2CO$; PP2 is $(HO)_2P(O)O(CH_2)_3CO$; β-Val is $H_2NC(CH_3)_2CO$; γ-Glu is $(NH_2)(CO_2H)CH(CH_2)_2CO$; TFA is trifluoroacetic acid; MCPBA is *m*-chloroperbenzoic acid; 1-HOBT is 1-hydroxybenzotriazole.
(14) Anderson, B. D.; Conradi, R. A.; Knuth, K. E. *J. Pharm. Sci.* **1985**, *74*, 365. Pearlman, B. A. Patent Application PCT International; WO 8900558 A1, 1989.

Scheme III. Syntheses of Peptides V and VI[a]



[a] (a) TFA, CH_2Cl_2; (Bu^{t}O)$_2$P(O)OCH$_2$CO$_2$H, C[=NPr^i]$_2$; (b) aqueous HCl; (c) MCPBA; (d) aqueous HCl.

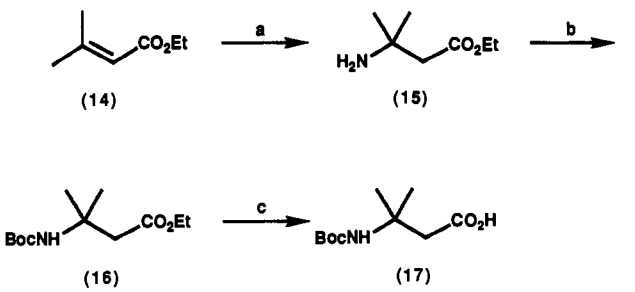
by using diethyl phosphorocyanate[15] as the coupling reagent. The isolated material was partitioned between aqueous Na_2SO_4 and 1-butanol to give the desired peptide III as the sodium salt. For the preparation of the *N*-oxide analogue IV, the previously prepared peptide **2** was oxidized with *m*-chloroperbenzoic acid to give the corresponding *N*-oxide peptide **3**. The tosyl protecting group was then removed with 1-hydroxybenzotriazole to give compound **4**. The *tert*-butyloxycarbonyl protecting group was removed with trifluoroacetic acid and the resulting amine was condensed with suleptanic acid by using diethyl phosphorocyanate. The desired peptide IV could then be isolated as the sodium salt as above. Peptides I and II were prepared in a similar manner as that described for the syntheses of peptides III and IV.

Phosphate-Containing Peptides. The required acids were prepared as shown in Scheme II. Benzyl glyoxalate (**5**) was treated with di-*tert*-butyl *N*,*N*-diethylphosphoramidite[16] to give initially the mixed phosphite. This was oxidized in situ with *m*-chloroperbenzoic acid to give phosphate ester **6**. Catalytic hydrogenolysis then removed the benzyl ester to give acid **7**, suitable for coupling to peptide substrates. For the preparation of the analogous acid **11**, benzyl 3-butenoate (**8**) was hydroborated to give hydroxylated compound **9**. Treatment with di-*tert*-butyl *N*,*N*-diethylphosphoroamidite, followed by oxidation with *m*-chloroperbenzoic acid, gave phosphate ester **10**. Hydrogenolysis of the benzyl ester then gave acid **11**.

As shown in Scheme III, the free amine from the *tert*-butyloxycarbonyl removal of peptide **1** was coupled to acid **7** with diisopropyl carbodiimide to give compound **12**. Deprotection of the di-*tert*-butyl ester was effected by hydrolysis in 4 M aqueous HCl to give the desired peptide V. For the preparation of the *N*-oxide analogue VI, compound **12** could be oxidized with *m*-chloroperbenzoic acid to give *N*-oxide **13**. This was then hydrolyzed with 4 M aqueous HCl to give the desired peptide VI. Peptides VII and VIII were then prepared in a similar manner using the above acid **11** in place of acid **7**.

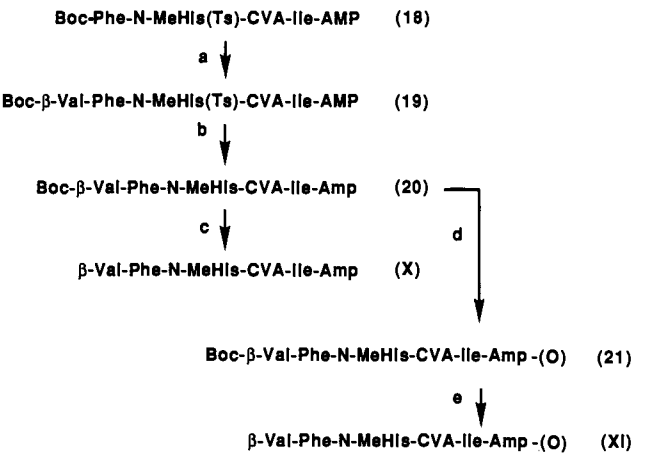
β-Valine-Containing Peptides. As shown in Scheme IV, heating of ethyl 2,2-dimethylacrylate (**14**) in ethanolic ammonia gave β-amino ester **15**. The amino function was then protected with the *tert*-butyloxycarbonyl group as in compound **16**. Hydrolysis of the ester with base afforded the desired acid **17**.

Scheme IV. Synthesis of Acid 17[a]



[a] (a) NH_3, EtOH, 80°C; (b) (Bu^{t}OCO)$_2$O; (c) THF, aqueous KOH.

Scheme V. Syntheses of Peptides X and XI[a]



[a] (a) TFA, CH_2Cl_2; BocNHC(CH$_3$)$_2$CH$_2$CO$_2$H, Pr$^i_2$NEt, (EtO)$_2$P(O)CN; (b) 1-HOBT; (c) TFA, CH_2Cl_2; (d) MCPBA; (e) TFA, CH_2Cl_2.

For the synthesis of peptide X as shown in Scheme V, trifluoroacetic acid treatment of compound **18**[17] removed the *tert*-butyloxycarbonyl protecting group. The resulting amine was coupled to acid **17** with diethyl phosphorocyanate to give compound **19**. The tosyl group was then removed with 1-hydroxybenzotriazole to give compound **20**. Finally, the *tert*-butyloxycarbonyl group was removed with trifluoroacetic acid and the desired peptide X was isolated as the tris(trifluoroacetate) salt. Peptide IX was also prepared in a similar manner as that described for the preparation of peptide X. For the synthesis of the *N*-oxide analogue XI, compound **20** was oxidized with *m*-chloroperbenzoic acid to give compound **21**. The *tert*-butyloxycarbonyl group was then removed with trifluoroacetic acid and peptide XI was isolated as the tris(trifluoroacetate) salt.

γ-Glutamic Acid Containing Peptides. As shown in Scheme VI, peptide **22**, prepared in a similar manner to that of the preparation of peptide **2**, was treated with trifluoroacetic acid to remove the *tert*-butyloxycarbonyl protecting group. The resulting amine was coupled to N^{α}-(*tert*-butyloxycarbonyl)-L-glutamic acid α-*tert*-butyl ester with diethyl phosphorocyanate to give compound **23**. The tosyl group was then removed with 1-hydroxybenzotriazole to give compound **24**. Finally treatment with trifluoroacetic acid gave the desired peptide XIII, which was isolated as the tris(trifluoroacetate) salt. Peptide XII

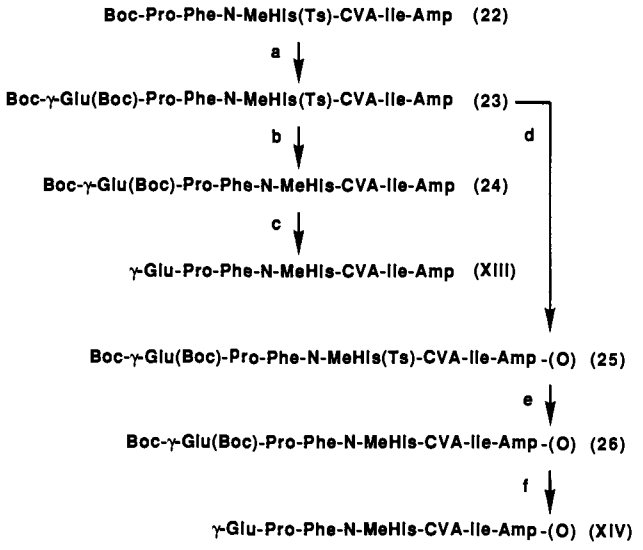
(15) Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595.
(16) Perich, J. W.; Johns, R. B. *Tetrahedron Lett.* **1988**, 2369. *Synthesis* **1988**, 142.
(17) For synthesis of the hydroxyethylene isostere insert, see: Hester, J. B.; Pals, D. T.; Saneii, H. H.; Sawyer, T. K.; Schostarez, H. J.; TenBrink, R. E.; Thaisrivongs, S. European Patent Application, Publication Number 0173481, 1986.

Table I. Human Renin Inhibitory Activities and Hypotensive Responses in the Human Renin Infused Rat Model[a]

			iv		po	
		IC_{50}, nM	δ_{max}	$T_{1/2}$	δ_{max}	$T_{1/2}$
I	Sul-Phe-N-MeHis-LVA-Ile-Amp	0.66	15	20	08	25
II	Sul-Phe-N-MeHis-LVA-Ile-Amp-(O)	1.2	28	15	18	34
III	Sul-Pro-Phe-N-MeHis-LVA-Ile-Amp	1.8	26	18	28	90
IV	Sul-Pro-Phe-N-MeHis-LVA-Ile-Amp-(O)	0.31	12	11	44	36
V	PP1-Pro-Phe-N-MeHis-LVA-Ile-Amp	1.6	28	21	47	39
VI	PP1-Pro-Phe-N-MeHis-LVA-Ile-Amp-(O)	2.5	38	28	30	56
VII	PP2-Pro-Phe-N-MeHis-LVA-Ile-Amp	0.45				
VIII	PP2-Pro-Phe-N-MeHis-LVA-Ile-Amp-(O)	1.9				
IX	β-Val-Phe-N-MeHis-CVA-Mba	0.41	30	49	28	54
X	β-Val-Phe-N-MeHis-CVA-Ile-Amp	0.29	29	82	45	>120
XI	β-Val-Phe-N-MeHis-CVA-Ile-Amp-(O)	0.43	34	>120	46	>120
XII	γ-Glu-Pro-Phe-N-MeHis-CVA-Mba	0.28	41	102	50	71
XIII	γ-Glu-Pro-Phe-N-MeHis-CVA-Ile-Amp	0.29	35	37	46	>120
XIV	γ-Glu-Pro-Phe-N-MeHis-CVA-Ile-Amp-(O)	0.32	37	69	58	81

[a] IC_{50} values represent the concentration of the inhibitors at which 50% of the enzymatic activity remains. δ_{max} values represent the magnitude of the maximal reduction of blood pressure in millimeters of Hg caused by administration of the inhibitors. T_{12} values represent the interval in minutes from the timepoint at which the maximal responses are observed to the time at which the blood pressures recover to half the maximal responses. Compounds were given both intravenously and orally in 0.1 M citric acid vehicle. See the Experimental Section for a description of the rat model.

Scheme VI. Syntheses of Peptides XIII and XIV[a]



[a] (a) TFA, CH_2Cl_2; Boc-L-Glu(α-OBu^t)-OH·DCHA, Pr^i_2NEt, $(EtO)_2P(O)CN$; (b) 1-HOBT; (c) TFA; (d) MCPBA; (e) 1-HOBT; (f) TFA.

was also prepared in a similar manner as that described for the preparation of peptide XIII. For the synthesis of the *N*-oxide analogue XIV, compound **23** was oxidized with *m*-chloroperbenzoic acid to give compound **25**. Removal of the tosyl group by 1-hydroxybenzotriazole gave compound **26**. The *tert*-butyloxycarbonyl groups were then removed with trifluoroacetic acid and peptide XIV was isolated as the tris(trifluoroacetate) salt.

Pharmacology

Inhibition of Human Renin in Vitro. Peptides I–XIV were evaluated as potential inhibitors of human plasma renin by utilizing a standard commercial kit. The IC_{50} values for their renin inhibitory activity are shown in Table I. When compared to the previously reported IC_{50} value of 0.26 nM for peptide 1,[7] these modified peptides retained very high renin inhibitory activities, most of which were in the subnanomolar range. These end groups possess different steric bulk and hydrophilicity from that of the parent compound 1. These results suggest that these terminal functionalities do not make specific contact with the enzyme surface for strong binding affinity. We have then selected some of these compounds for evaluation in a rat model for antihypertensive activities, especially by the oral route.

Evaluation in the Human Renin Infused Rat. We were interested in the evaluation of a large number of compounds in order to discover those with improved efficacy over the previously reported peptide 1. In order to achieve this goal, a rat model that provided a simple and rapid method for the in vivo evaluation of potential inhibitors of human renin was developed and validated.[12] Briefly, recombinant human renin[18] was infused into anesthetized, nephrectomized, ganglion-blocked, Sprague–Dawley rats. The magnitude of the human renin dependent blood pressure component was approximately 60 mmHg. For compounds in Table I, the intravenous dose was equimolar to 0.05 mg/kg of compound 1 and the oral dose was equimolar to 5.0 mg/kg of compound 1. These doses were located on the linear portion of dose–response curves of compound 1 and allowed changes in potency to be reflected in changes in blood pressure as a result of the inhibition of human renin. Potency was expressed as the maximum hypotensive effect (δ_{max}) in millimeters of Hg caused by an equimolar dose while the duration of effect was expressed as the duration ($T_{1/2}$) in minutes required for a 50% return of the blood pressure from its maximum effect. For compound 1, the intravenous dose of 0.05 mg/kg gave a maximal change in blood pressure of 25 mmHg and a $T_{1/2}$ value of 27 min, while the oral dose of 5 mg/kg caused a maximal change in blood pressure of 24 mmHg with a $T_{1/2}$ value of 60 min.

As shown in Table I, the highly water soluble suleptanates I–IV did not show improvement in the hypotensive responses over compound 1. For the phosphate-containing compounds V–VIII, we selected the peptides V and VI for testing as representative compounds in this class. They also did not show significant improvement in their hypotensive responses over compound 1. However, for the β-valine-containing peptides (IX–XI), we did observe improvement in the resulting hypotensive activity in the magnitude and/or duration of responses. We were particularly interested in the enhancement of the oral activity of compound X and XI over that of reference compound 1. The improvement in the hypotensive activity was also observed for the γ-glutamyl peptides XII–XIV. Encour-

(18) Poorman, R. A.; Palermo, D. P.; Post, L. E.; Murakami, K.; Kinner, J. H.; Smith, C. W.; Reardon, I.; Heinrickson, R. L. *Proteins: Structure, Function and Genetics* **1986**, 139.

aged by these results, we further evaluated peptides X–XIV in this human renin infused rat model by the oral route in 0.25% (carboxymethyl)cellulose in water vehicle. Compound 1 was not soluble in water and required 0.1 M citric acid as vehicle. These modified peptides, however, are water soluble and offered an opportunity to evaluate their hypotensive activity in the absence of citric acid, which might have acted as an adjuvant. The maximal magnitude of responses and the $T_{1/2}$ values for peptides X–XIV in 0.25% (carboxymethyl)cellulose in water vehicle given orally are shown below:

	δ_{max}, mmHg	$T_{1/2}$, min
X	36	50
XI	20	>120
XII	45	52
XIII	25	52
XIV	40	>120

This set of results suggested that compound XIV exhibited a significant improvement of the hypotensive response as compared to that of the lead compound 1. In order to further confirm this observation in another relevant model, we decided to evaluate compound XIV in the sodium-depleted monkey.

Evaluation in the Conscious Sodium-Depleted Monkey. Six male cynomolgus monkeys were sodium-depleted via daily intramuscular injections of furosemide, 0.5 mg/kg, for four days while on a sodium-free diet. Sodium depletion was confirmed via plasma renin activity measurements of blood samples obtained prior to each experiment. Compound XIV was administered orally in 5 mL/kg sterile water via nasogastric tube while the conscious animal was seated in a primate-restraining chair.

In our previous report, compound 1, at 50 mg/kg given orally in 0.1 M citric acid vehicle, elicited a significant hypotension in the sodium-depleted monkey that persisted for 5 h.[10] The hypotensive response was accompanied by a significant reduction in plasma renin activity. Oral administration of compound 1 at 15 mg/kg in 0.1 M citric acid, however, did not lead to an observable reduction in blood pressure. Compound XIV was given orally in sterile water at a dose of 20.4 mg/kg, which would be equimolar to that of compound 1 at 15 mg/kg, and also at a dose of 6.8 mg/kg (equimolar to 5 mg/kg of compound 1). The mean arterial pressure (MAP) responses and the plasma renin activity (PRA) for these two doses are shown in Figure 1. The hypotensive responses appeared to be dose dependent, and oral activity of compound XIV could be demonstrated at doses at which the lead compound 1 showed no observable hypotensive effect. Plasma renin activities were also inhibited in a dose-dependent manner with near total inhibition following the larger dose of compound XIV.

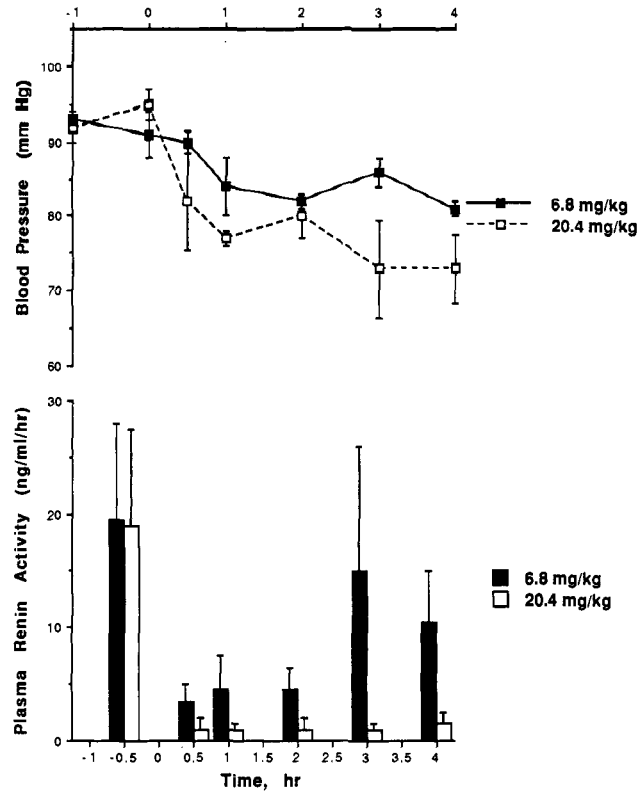


Figure 1. Effects of peptide XIV in the conscious sodium-depleted monkey. Compound XIV was given via nasogastric tube at doses of 6.8 and 20.4 mg/kg in sterile water. See the Experimental Section for a description of the monkey model.

Conclusion

In this study, we were interested in discovering renin inhibitory peptides with improved hypotensive activity in vivo over our previously reported compound 1. The potent renin inhibitory activity of compound 1 in vitro and its hypotensive efficacy by the oral route has been previously demonstrated. It is a highly lipophilic compound with very low water solubility and its hypotensive response was dependent on the use of citric acid as a vehicle. We have investigated a number of functional group modifications by focusing on increased hydrophilicity at the end groups. With these changes in the physical characteristics of the reference compound, we were interested in assessing the resulting pharmacological effects of the modified compounds with improved water solubility. We have studied the N-terminal groups of suleptanic acid, phosphoric acid, β-valine, and γ-glutamic acid. We have also looked at a set of C-terminal groups as in the alkyl amide (methylbutyl amide), the aminomethylpyridyl amide, and the corresponding *N*-oxide. The resulting modified peptides have much improved water solubility and could be tested in vivo in a water vehicle. They all retained high binding affinity to human renin, which suggested that the end groups were not involved in the binding to the active site of the enzyme. For a high volume in vivo assay, the anesthetized, nephrectomized, ganglion-blocked, recombinant human renin infused rats provided a rapid assessment of the hypotensive activities of these renin inhibitors. As compared to the reference compound 1, the suleptanic acid and the phosphoric acid containing peptides did not show improved in vivo activity. However, significant enhancement of the hypotensive activity could be demonstrated for peptides that contain β-valine and γ-glutamic acid at the N-terminal. In order to avoid a potential adjuvant effect of citric acid, some of these compounds were further tested in a vehicle of 0.25% (carboxymethyl)cellulose in water by the oral route. Compound XIV retained the largest hypotensive effect was further evaluated in conscious sodium-depleted monkeys. It was demonstrated that this compound elicited dose-dependent hypotensive effects at doses much lower than those previously used for the lead compound 1. The hypotensive effect was accompanied by a dose-dependent reduction in plasma renin activity. Significantly, oral activity of compound XIV could be demonstrated with a sterile water vehicle without the need for the citric acid vehicle which was necessary for activity of compound 1. The major obstacles for successful development of inhibitors of renin as potential pharmaceutical agents lie in the low oral bioavailability and also the rapid biliary clearance. The improvement of hypotensive activity for compound XIV over the previously reported

Table II. Physical Characteristics of Peptides

compds[a]	HPLC[b], k'	formula	FAB-HRMS calcd	FAB-HRMS found
I	5.1	$C_{51}H_{80}N_9O_{10}S$	1010.575	1010.583
II	8.2	$C_{51}H_{79}N_9O_{11}SNa$	1048.552	1048.554
III	6.7	$C_{56}H_{86}N_{10}O_{11}SNa$	1129.610	1129.614
IV	11.2	$C_{56}H_{86}N_{10}O_{12}SNa$	1145.605	1145.603
V	1.7	$C_{47}H_{71}N_9O_{11}P$	965.5010	965.5042
VI	1.4	$C_{47}H_{71}N_9O_{12}P$	984.4959	984.4945
VII	2.1	$C_{49}H_{75}N_9O_{11}P$	996.5323	996.5354
VIII	2.0	$C_{49}H_{75}N_9O_{12}P$	1012.527	1012.534
IX	8.9	$C_{41}H_{68}N_7O_5$	738.5282	735.5247
X	10.7	$C_{48}H_{74}N_9O_6$	872.5762	872.5787
XI	9.2	$C_{48}H_{74}N_9O_7$	888.5711	888.5736
XII	3.2	$C_{46}H_{73}N_8O_8$	865.5551	865.5506
XIII	9.7	$C_{53}H_{78}N_{10}O_9K$	1037.559	1037.557
XIV	6.6	$C_{53}H_{79}N_{10}O_{10}$	1015.599	1015.596

[a] ^{1}H NMR consistent with the structures. [b] See the Experimental Section for conditions; k' is the partition ratio.

compound 1 could be due to a greater volume of distribution, better oral bioavailability, and/or decreased clearance. We have interest in pursuing studies to further characterize the pharmacokinetics of these compounds and also in the additional enhancement of efficacy of modified peptides.

Experimental Section

Solvents for chromatography were from Burdick & Jackson and used as received. Reagents were from commercial sources and used without further purification unless otherwise noted. Tetrahydrofuran was distilled from sodium/benzophenone. Diethyl ether was Mallinckrodt anhydrous grade. Dichloromethane for reaction solvent was dried over 4A molecular sieves. Dimethylformamide was Aldrich gold-label grade. Diisopropylethylamine was distilled from calcium hydride. Diethyl phosphorocyanate was distilled before use. Thin-layer chromatography was performed on Merck precoated plates (silica gel 60, F-254). Column chromatography used 70–230 mesh silica gel 60 and, for flash chromatography, 230–400 mesh grade.

Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM-300 (300-MHz) instrument, in deuterated chloroform. Mass spectra were performed by Physical and Analytical Chemistry of the Upjohn Company. High-pressure liquid chromatography was performed on a Perkin-Elmer Series IV liquid chromatograph operating at 1.5 mL/min using a Brownlee RP-18 10μm column, with UV monitoring by a Kratos Spectroflow 773 detector, at 225 and 254 nm. A Perkin-Elmer LCI-100 integrator was used for peak data. Solvent A is methanol. Solvent B is aqueous phosphate buffer, pH 3. Solvent C is 10% acetonitrile in water with 0.1% trifluoroacetic acid, and solvent D is 10% water in acetonitrile with 0.1% trifluoroacetic acid. Solvent system for peptides I and III is 65% solvent A and 35% solvent B. Solvent system for peptides II and IV is 55% solvent A and 45% solvent B. Solvent system for peptides V–VIII is 90% solvent A and 10% solvent B. Solvent system for peptide IX is 25% solvent C and 75% solvent D. Solvent system for peptide X and XI is a linear gradient from 50% solvent C and 50% solvent D to 20% solvent C and 80% solvent D in 30 min. Solvent system for peptides XII–XIV is a linear gradient from 40% solvent C and 60% solvent D to 10% solvent C and 90% solvent D in 30 min. Physical characteristics for peptides I–XIV are summarized in Table II.

***N*-(*N*-Methyltaurine) *N'*-[*N*-[*N*-[*N*-[*N*$^\alpha$-(L-Prolyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine] Octanedioic Acid Diamide Sodium Salt (III).** A solution of 195 mg (0.21 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (1)[7] in 1 mL of 1:1 trifluoroacetic acid/dichloromethane was allowed to stir for 1 h. This was slowly added to a solution of 1 g of $NaHCO_3$ in 20 mL of water and the resulting mixture was extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated to give 177 mg of the amine.

To a stirred solution of this amine and 0.07 mL (0.4 mmol) of diisopropylethylamine in 1 mL of dichloromethane was added 0.35 mL (0.23 mmol) of a 0.652 M solution of suleptanic acid, triethylamine salt, in acetonitrile,[14] followed by 0.04 mL (0.26 mmol) of diethyl phosphorocyanate. After stirring for 18 h, the concentrated mixture was chromatographed on silica gel with 10%–20% methanol in dichloromethane. This material was partitioned between 20 mL of 10% aqueous Na_2SO_4 and 20 mL of 1-butanol. The organic phase was dried (Na_2SO_4) and then concentrated. The residue was dissolved in water and then lyophilized to give 233 mg (0.206 mmol, 98%) of *N*-(*N*-methyltaurine) N'-[N-[N-[N-[N$^\alpha$-(L-prolyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine] octanedioic acid diamide sodium salt (III). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 1129.614.

***N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5-(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (3).** To a stirred solution of 302 mg (0.29 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (2)[7] in 1.5 mL of dichloromethane was added 40 mg (0.48 mmol) of $NaHCO_3$ and 76 mg (ca. 0.4 mmol) of 80%–90% *m*-chloroperbenzoic acid. After 1 h, the mixture was partitioned between dichloromethane and 10% aqueous $NaHSO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica gel with 15% methanol in ethyl acetate to give 230 mg (0.21 mmol, 72%) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (3). The ^{1}H NMR spectrum was consistent with the structure. FAB-MS: (M + H) at *m/z* 1101.

***N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4-(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (4).** A solution of 230 mg (0.21 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (3) and 113 mg (0.84 mmol) of 1-hydroxybenzotriazole in 2 mL of methanol was allowed to stir for 4 h. The concentrated mixture was chromatographed on silica gel with 5%–7% methanol (saturated with ammonia) in dichloromethane to give 160 mg (0.17 mmol, 80%) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (4). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 946.5756, calcd 946.5766.

***N*-(*N*-Methyltaurine) *N'*-[*N*-[*N*-[*N*-[*N*$^\alpha$-(L-Prolyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine] Octanedioic Acid Diamide Sodium Salt (IV).** A solution of 160 mg (0.17 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (4) in 1 mL of 1:1 trifluoroacetic acid/dichloromethane was allowed to stir for 1 h. This was slowly added to a solution of 1 g of $NaHCO_3$ in 20 mL of water and the resulting mixture was extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated to give 127 mg of the amine.

To a stirred solution of 96 mg (0.11 mmol) of this amine and 0.035 mL (0.2 mmol) of diisopropylethylamine in 1 mL of dichloromethane was added 0.185 mL (0.12 mmol) of a 0.652 M solution of suleptanic acid, triethylamine salt in acetonitrile, followed by 0.02 mL (0.13 mmol) of diethyl phosphorocyanate. After stirring for 18 h, the concentrated mixture was chromatographed on silica gel with 10%–20% methanol in dichloromethane. This material was partitioned between 20 mL of 10% aqueous

Na_2SO_4 and 20 mL of 1-butanol. The organic phase was dried (Na_2SO_4) and then concentrated. The residue was dissolved in water and then lyophilized to give 113 mg (0.1 mmol, 90%) of *N*-(*N*-methyltaurine) *N'*-[*N*-[*N*-[*N*-[*N*-(L-prolyl-L-phenylalanyl)-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine] octandioic acid diamide sodium salt (IV). The ^1H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 1145.603.

(Benzyloxycarbonyl)methyl Di-*tert*-butyl Phosphate (6). To a stirred mixture of 500 mg (3.0 mmol) of benzyl glyoxalate (**5**) and 422 mg (6.0 mmol) of 1*H*-tetrazole in 3 mL of dry tetrahydrofuran under argon was added 0.84 mL (3.0 mmol) of di-*tert*-butyl *N*,*N*-diethylphosphoramidite.[16] After 2 h, the reaction mixture was cooled in an ice bath and then a solution of 570 mg (ca 3.3 mmol) of 80%–90% *m*-chloroperbenzoic acid in 4 mL of dichloromethane was slowly added. The resulting mixture was then partitioned between 10% aqueous $NaHSO_3$ and dichloromethane. The organic phase was dried (Na_2SO_4) and then concentrated. The residue was chromatographed on silica gel with 8% acetone in dichloromethane to give 978 mg (2.7 mmol, 90%) of (benzyloxycarbonyl)methyl di-*tert*-butyl phosphate (**6**). ^1H NMR ($CDCl_3$): δ 1.48 (s, 18 H), 4.55 (d, J = 9 Hz, 2 H), 5.23 (d, J = 8 Hz, 2 H), 7.4 (m, 5 H), HRMS: (M + H) at *m/z* 359.1652, calcd 359.1623.

Carboxymethyl Di-*tert*-butyl Phosphate (7). A mixture of 575 mg (1.6 mmol) of (benzyloxycarbonyl)methyl di-*tert*-butyl phosphate (**6**) and 50 mg of 5% palladium on carbon in 5 mL of ethanol was shaken under 60 psi of hydrogen for 18 h. It was then filtered through Celite with ethanol washings. The filtrate was concentrated to provide 293 mg (1.1 mmol, 70%) of carboxymethyl di-*tert*-butyl phosphate (**7**). ^1H NMR ($CDCl_3$): δ 1.49 (s, 9 H), 1.51 (s, 9 H), 4.54 (d, J = 10 Hz, 2 H). This material decomposed slowly when allowed to stand neat at room temperature.

Benzyl 4-Hydroxybutanoate (9). To a stirred solution of 25 mL (25 mmol) of 1 M borane in tetrahydrofuran at −10 °C under argon was added a solution of 3.53 g (50.3 mmol) of 2-methyl-2-butene in 8 mL of dry tetrahydrofuran over 15 min. The resulting solution was allowed to warm to 0 °C. After 90 min, 4.41 g (25 mmol) of benzyl 3-butenoate (**8**) was added over 20 min, and the resulting mixture was stirred for additional 30 min. It was then treated with 1 mL of water, followed by a mixture of 8.3 mL of 30% hydrogen peroxide and 8.3 mL of 3 M NaOH. The resulting mixture was extracted with several portions of diethyl ether. The organic phase was then washed with aqueous Na_2SO_3 and then dried ($MgSO_4$). Concentration of the organic layer and chromatography of the resulting residue on silica gel with 50% ether in pentane to ether afforded 2.21 g (11.4 mmol, 45%) of benzyl 4-hydroxybutanoate (**9**). ^1H NMR ($CDCl_3$): δ 1.91 (quin, J = 6.5 Hz, 2 H), 2.5 (d, J = 6.5 Hz, 2 H), 3.69 (t, J = 6.5 Hz, 2 H), 5.13 (s, 2 H), 7.4 (m, 5 H). Anal.: C, 68.08; H, 7.31 (calcd C, 68.02; H, 7.27).

3-(Benzyloxycarbonyl)propyl Di-*tert*-butyl Phosphate (10). To a stirred mixture of 777 mg (4.0 mmol) of benzyl 4-hydroxybutanoate (**9**) and 560 mg (8.0 mmol) of 1*H*-tetrazole in 4 mL of dry tetrahydrofuran under argon was added 1.15 mL (4.1 mmol) of di-*tert*-butyl *N*,*N*-diethylphosphoramidite. After 18 h, the reaction mixture was cooled in an ice bath and then a solution of 812 mg (ca. 4 mmol) of 80%–90% *m*-chloroperbenzoic acid in 6 mL of dichloromethane was slowly added. The resulting mixture was then partitioned between 10% aqueous $NaHSO_3$ and dichloromethane. The organic phase was dried (Na_2SO_4) and then concentrated. The residue was chromatographed on silica gel with 5%–10% acetone in dichloromethane to give 1.46 g (3.78 mmol, 94%) of (benzyloxycarbonyl)propyl di-*tert*-butyl phosphate (**10**). ^1H NMR ($CDCl_3$): δ 1.47 (s, 9 H), 1.51 (s, 9 H), 2.0 (m, 2 H), 2.51 (t, J = 7 Hz, 2 H), 4.0 (dq, J = 8, 25 Hz, 2 H), 5.12 (s, 2 H), 7.4 (m, 5 H). HRMS: (M + H) at *m/z* 387.1944, calcd 387.1936.

3-Carboxypropyl Di-*tert*-butyl Phosphate (11). A mixture of 155 mg (0.4 mmol) of (benzyloxycarbonyl)propyl di-*tert*-butyl phosphate (**10**) and 15 mg of 5% palladium on carbon in 2 mL of ethanol was shaken under 60 psi of hydrogen for 18 h. It was then filtered through Celite with ethanol washings. The filtrate was concentrated to provide 93 mg (0.31 mmol, 78%) of 3-carboxypropyl di-*tert*-butyl phosphate (**11**). ^1H NMR ($CDCl_3$): δ 1.47 (s, 9 H), 1.53 (s, 9 H), 2.0 (m, 2 H), 2.5 (m, 2 H), 4.0 (m, 2 H). This material decomposed slowly when allowed to stand neat at room temperature.

***N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[[(Di-*tert*-butylphosphono)oxy]acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5-(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (12).** To a stirred solution of 83 mg (0.10 mmol) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-(L-prolyl-L-phenylalanyl)-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine and 54 mg (0.20 mmol) of carboxymethyl di-*tert*-butyl phosphate (**7**) in 0.6 mL of dichloromethane was added 19 μL (0.12 mmol) of diisopropylcarbodiimide. After 18 h, the reaction mixture was chromatographed directly on silica gel with 4%–6% methanol (saturated with ammonia) in dichloromethane to provide 55.3 mg (0.05 mmol, 50%) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[[(di-*tert*-butylphosphono)oxy]acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**12**). The ^1H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 1080.622, calcd 1080.626.

***N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[(Phosphonooxy)acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (V).** A solution of 20.2 mg (18.7 μmol) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[[(di-*tert*-butylphosphono)oxy]acetyl]-L-propyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl]-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**12**) in 0.5 mL of 4 M aqueous HCl was allowed to stand for 1 h, then the mixture was concentrated. The residue was evaporated twice from ethanol. The resulting residue was then dissolved in water and the solution was lyophilized to provide 18.5 mg (17.8 μmol, 95%) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[phosphonooxy)acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (V). The ^1H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 965.5042.

***N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[[(Di-*tert*-butylphosphono)oxy]acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5-(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (13).** A solution of 48.1 mg (0.045 mmol) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[[(di-*tert*-butylphosphono)oxy]acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (**12**) and 10 mg (ca. 0.05 mmol) of 80%–90% *m*-chloroperbenzoic acid was stirred for 2 h. The reaction mixture was then quenched with a few drops of aqueous Na_2SO_3. The solvents were evaporated and the residue was chromatographed on silica with 4%–6% methanol (saturated with ammonia) in dichloromethane to afford 31.7 mg (0.03 mmol, 65%) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[[(di-*tert*-butylphosphono)oxy]acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-1-oxo-2-pyridylmethylamine (**13**). The ^1H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 1096.621, calcd 1096.621.

***N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[(Phosphonooxy)acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4-(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (VI).** A solution of 47 mg (43 μmol) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[[(di-*tert*-butylphosphono)oxy]acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (**13**) in 1 mL of 4 M aqueous HCl was allowed to stand for 1 h, and then concentrated. The residue was evaporated twice from ethanol. The resulting residue was then dissolved in water and the solution lyophilized to provide 41 mg (30 μmol, 91%) of *N*-[*N*-[*N*-[*N*$^{\alpha}$-[*N*-[*N*-[(phosphonooxy)acetyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (VI). The ^1H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 984.4945.

Ethyl 3-Amino-3-methylbutanoate (15). A solution of 0.64 g (5 mmol) of ethyl dimethacrylate in 10 mL of ethanol in a

thick-walled glass vessel, which was sealable by a Teflon Ace-thread plug, was cooled to −15 °C in an ice/salt bath and saturated with gaseous ammonia. The tube was sealed and heated behind a safety shield to 80 °C for 22 h. The tube was then cooled and the solution was purged with nitrogen. Removal of the remaining solvent under reduced pressure afforded 0.645 g (4.45 mmol, 89%) of ethyl 3-amino-3-methylbutanoate (**15**). ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 6 H), 1.30 (t, J = 7 Hz, 3 H), 1.65 (s, 2 H), 2.39 (s, 2 H), 4.15 (q, J = 7 Hz, 2 H).

Ethyl 3-[(Butyloxycarbonyl)amino]-3-methylbutanoate (16). To a stirred solution of 645 mg (4.4 mmol) of ethyl 3-amino-3-methylbutanoate (**15**) in 10 mL of dichloromethane was added 970 mg (4.4 mmol) of di-*tert*-butyl dicarbonate. After stirring for 18 h, the solution was concentrated, and the residue chromatographed on silica with 10% ethyl acetate in hexane to afford 880 mg (3.56 mmol, 81%) of ethyl 3-[(butyloxycarbonyl)amino]-3-methylbutanoate (**16**). ^{1}H NMR ($CDCl_3$): δ 1.26 (*t*, J = 7 Hz, 3 H), 1.38 (s, 6 H), 1.44 (s, 9 H), 2.67 (s, 2 H), 4.13 (q, J = 7 Hz, 2 H), 4.9 (s, 1 H).

3-[(Butyloxycarbonyl)amino]-3-methylbutanoic Acid (17). To a stirred solution of 491 mg (2.0 mmol) of ethyl 3-[(butyloxycarbonyl)amino]-3-methylbutanoate (**16**) in 4 mL of tetrahydrofuran was added 2.2 mL of 1 M KOH. The mixture was stirred vigorously for 18 h, then tetrahydrofuran was evaporated. The remaining aqueous phase was washed once with dichloromethane and then acidified. The resulting mixture was extracted with dichloromethane, and the organic phase dried ($MgSO_4$) and then concentrated to afford 358 mg (1.64 mmol, 82%) of 3-[(butyloxycarbonyl)amino]-3-methylbutanoic acid (**17**). ^{1}H NMR ($CDCl_3$): δ 1.41 (s, 6 H), 1.46 (s, 9 H), 2.75 (s, 2 H).

***N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5-(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (19).** A solution of 100 mg (0.1 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-(*tert*-butyloxycarbonyl)-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5-(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**18**)[17] in 1 mL of 1:1 trifluoroacetic acid/dichloromethane was allowed to stir for 45 min. The reaction mixture was added to 1 g of $NaHCO_3$ in 15 mL of water and the resulting mixture was extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated to give 90.4 mg of the amine.

To a stirred solution of 90.4 mg (0.10 mmol) of *N*-[*N*-[*N*-(*N*$^\alpha$-L-phenylalanyl-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl)-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine from above and 26 mg (0.12 mmol) of 3-[(butyloxycarbonyl)amino]-3-methylbutanoic acid (**17**) in 0.75 mL of dichloromethane was added 20 μL (0.11 mmol) of diisopropylethylamine and 18 μL (0.12 mmol) of diethyl phosphorocyanate. The mixture was stirred for 18 h and then chromatographed on silica with 3%−5% methanol in dichloromethane to afford 117 mg (0.1 mmol, 100%) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-*N*-(*tert*-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**19**). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at m/z 1126.638, calcd 1126.637.

***N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (20).** A solution of 117 mg (0.10 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**19**) and 53 mg (0.39 mmol) of 1-hydroxybenzotriazole in 1 mL of methanol was allowed to stir for 18 h. The reaction mixture was concentrated and the residue chromatographed on silica gel with 2%−5% methanol (saturated with ammonia) in dichloromethane to afford 74.7 mg (0.08 mmol, 80%) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**20**). The ^{1}H NMR spectrum was consistent with structure. HRMS: (M + H) at m/z 972.6314, calcd 972.6286.

***N*-[*N*-[*N*-[*N*$^\alpha$-(β-L-Valyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine Tris(trifluoroacetate) (X).** A solution of 23.0 mg (23.7 μmol) of N-[N-[N-[N$^\alpha$-[N-[*N*-(tert-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**20**) in 0.5 mL of 1:1 trifluoroacetic acid/dichloromethane was allowed to stir for 1 h. The solution was then added slowly into 10 mL of rapidly stirred 1:2 diethyl ether/hexane. The mixture was centrifuged and decanted. The residual solid was washed twice with 1:2 diethyl ether/hexane and then dried to afford 26 mg (0.02 mmol, 90%) of *N*-[*N*-[*N*-[*N*$^\alpha$-(β-L-valyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine tris(trifluoroacetate) (X). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at m/z 872.5787.

***N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (21).** A solution of 49.5 mg (50.9 μmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5-(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**20**) and 11.8 mg (58 μmol) of *m*-chloroperbenzoic acid in 1 mL of dichloromethane was stirred at room temperature for 3 h. The reaction mixture was then chromatographed directly on silica with 4%−7% methanol (saturated with ammonia) in dichloromethane to provide 37.3 mg (37.7 μmol, 74%) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5-(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (**21**). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at m/z 988.6219, calcd 988.6235.

***N*-[*N*-[*N*-[*N*$^\alpha$-(β-L-Valyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine Tris(trifluoroacetate) (XI).** A solution of 35.9 mg of (36.3 μmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-β-L-valyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4-(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (**21**) in 0.5 mL of 1:1 trifluoroacetic acid/dichloromethane was allowed to stir for 1 h and was then added slowly 10 mL of rapidly stirred 1:2 diethyl ether/hexane. The mixture was centrifuged and then decanted. The residual solid was washed twice with 1:2 diethyl ether/hexane and then dried to afford 39 mg (32 μmol, 87%) of *N*-[*N*-[*N*-[*N*$^\alpha$-(β-L-valyl-L-phenylalanyl)-*N*$^\alpha$-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4-(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine tris(trifluoroacetate) (XI). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at m/z 888.5736.

***N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (23).** A solution of 0.85 g (0.756 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-[*N*-[*N*-(*tert*-butyloxycarbonyl)-L-prolyl]-L-phenylalanyl]-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**22**)[17] in 4 mL of 1:1 trifluoroacetic acid/dichloromethane was allowed to stir for 45 min. The reaction mixture was added to 3 g of $NaHCO_3$ in 50 mL of water and the resulting mixture was extracted with dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated to give 0.775 g of the amine.

To a stirred solution of 0.775 g (0.756 mmol) of *N*-[*N*-[*N*-[*N*$^\alpha$-(L-prolyl-L-phenylalanyl)-*N*$^\alpha$-methyl-*N*^{im}-tosyl-L-histidyl]-5-(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine from above and 0.367 g (0.757 mmol) of *N*$^\alpha$-(*tert*-butyloxycarbonyl)-L-glutamic acid α-*tert*-butyl ester dicyclohexylamine salt in 4 mL of dichloromethane was added 140 μL (0.8 mmol) of diisopropylethylamine, followed by 120 μL (0.78 mmol) of diethyl phosphorocyanate. The reaction

mixture was stirred for 18 h and then concentrated. The residue was chromatographed on silica with ethyl acetate to 5% methanol in ethyl acetate to afford 0.887 g (0.67 mmol, 89%) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-N^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**23**). The ^{1}H NMR spectrum was consistent with the structure. FAB-MS: (M + H) at *m/z* 1309.

***N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-propyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (24).** A solution of 420 mg (0.32 mmol) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-N^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**23**) and 194 mg (1.3 mmol) of 1-hydroxybenzotriazole in 3 mL of methanol was allowed to stir for 18 h. The reaction mixture was concentrated and the resulting residue was chromatographed on silica with 6%–8% methanol (saturated with ammonia) in dichloromethane to afford 289 mg (0.28 mmol, 88%) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**24**). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 1155.715, calcd 1155.718.

***N*-[*N*-[*N*-[*N*-[*N*-(γ-L-Glutamyl-L-prolyl)-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (XIII).** A solution of 219 mg (0.19 mmol) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**24**) in 1 mL of trifluoroacetic acid was allowed to stir for 1 h and was then added slowly into 10 mL of rapidly stirred 1:2 diethyl ether/hexane. The mixture was centrifuged and then decanted. The residual solid was washed twice with 1:2 diethyl ether/hexane and then dried to afford 291 mg of *N*-[*N*-[*N*-[*N*-[*N*-(γ-L-glutamyl-L-prolyl)-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (XIII). The ^{1}H NMR spectrum was consistent with the structure. FAB-MS: (M + H) at *m/z* 999.

***N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-α-*tert*-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-N^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (25).** A solution of 467 mg (0.356 mmol) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-N^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl]-2-pyridylmethylamine (**23**) and 90 mg (0.42 mmol) of 85% *m*-chloroperbenzoic acid in 2 mL of dichloromethane was stirred for 18 h. The resulting mixture was partitioned between aqueous Na_2SO_3 and dichloromethane. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was chromatographed on silica with 7% methanol (saturated with ammonia) in dichloromethane to afford 342 mg (0.26 mmol, 72%) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-N^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)-methylamine (**25**). The ^{1}H NMR spectrum was consistent with the structure. FAB-MS: (M + H) at *m/z* 1325.

***N*-[*N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-Butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)-methylamine (26).** A solution of 142 mg (0.11 mmol) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-N^{im}-tosyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (**25**) and 67 mg (0.44 mmol) of 1-hydroxybenzotriazole in 1 mL of methanol was allowed to stir for 18 h. The reaction mixture was concentrated and the residue chromatographed on silica with 8% methanol (saturated with ammonia) in dichloromethane to afford 110 mg (0.09 mmol, 85%) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)-methylamine (**26**). The ^{1}H NMR spectrum was consistent with the structure. HRMS: (M + H) at *m/z* 1171.712, calcd 1171.713.

***N*-[*N*-[*N*-[*N*-[*N*-(γ-L-Glutamyl-L-prolyl)-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (XIV).** A solution of 150 mg (0.13 mmol) of *N*-[*N*-[*N*-[*N*-[*N*-[*N*-(*tert*-butyloxycarbonyl)-α-*tert*-butyl-γ-L-glutamyl]-L-prolyl]-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (**26**) in 1 mL of trifluoroacetic acid was allowed to stir for 1 h and was then added slowly into 10 mL of rapidly stirred 1:2 diethyl ether/hexane. The mixture was centrifuged and then decanted. The residual solid was washed twice with 1:2 diethyl ether/hexane and then dried to afford 182 mg (0.13 mmol, 100%) of *N*-[*N*-[*N*-[*N*-[*N*-(γ-L-glutamyl-L-prolyl)-L-phenylalanyl]-N^{α}-methyl-L-histidyl]-5(*S*)-amino-6-cyclohexyl-4(*S*)-hydroxy-2(*S*)-isopropylhexanoyl]-L-isoleucyl](1-oxo-2-pyridyl)methylamine (XIV). The ^{1}H NMR spectrum was consistent with the structure. FAB-MS: (M + H) at *m/z* 1015.

Biology. Inhibition of Human Plasma Renin. These peptides were assayed for plasma renin inhibitory activity as follows. Lyophilized human plasma with 0.1% EDTA was obtained commercially (New England Nuclear). The angiotensin I generation step utilized 250 μL of plasma, 2.5 μL of phenylmethanesulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of peptide in a 1% Tween 80 in water vehicle. Incubation was for 90 min at 37 °C. Radioimmunoassay for angiotensin I was carried out with a commercial kit (Clinical Assays). Plasma renin activity values for tubes with peptide were compared to those for control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values, which were obtained by plotting three to four peptide concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

Human Renin Infused Rat Model. Sprague–Dawley rats weighing approximately 200 g were anesthetized with methoxyflurane and bilaterally nephrectomized. Approximately 18 h later, each animal was reanesthetized with dialurethane (100 mg/kg ip), tracheostomized, and bilaterally vagotomized. One carotid artery and both jugular veins were catheterized for the recording of blood pressure and for intravenous infusions, respectively. In some animals that had fasted for 24 h prior to nephrectomy, an infant feeding tube was passed into the stomach through the mouth for the oral administration of compounds. Mecamylamine at 1.25 mg/kg iv was utilized to elicit ganglionic blockade. An intravenous infusion of recombinant human renin[18] was administered at 2.4 GU/kg per min for 10 min and was followed by a sustained infusion at 0.6 GU/kg per min for the 2-h duration of the experiment (GU = Goldblatt unit). The renin inhibitory compounds were dissolved in 0.1 M citric acid and infused intravenously at 0.05 mL/min for 10 min or administered into the stomach as a 5 mL/kg bolus. In some experiments, compounds were administered into the stomach in a 0.25% (carboxymethyl)cellulose in water vehicle.

Conscious Salt-Depleted Monkey Model. Six male cynomolgus monkeys were sodium-depleted via daily intramuscular injections of furosemide, 0.5 mg/kg, for four days while on a sodium-free diet. On the day of the experiment, each animal was lightly anesthetized with ketamine and polyethylene catheters were fixed in a femoral artery and vein. The animal was then placed in a primate-restraining chair and allowed to recover from the anesthesia for at least 4 h. Arterial blood samples were obtained before and at intervals after the oral administration of compound XIV for the estimation of plasma renin activity. Plasma renin activity was determined with standard radioimmunoassay technique at pH 7.4 using the Gamma Coat (^{125}I) Plasma Renin Activity Radioimmunoassay Kit from Baxter Healthcare Corp. Each animal was administered either 6.8 or

20.4 mg/kg of compound XIV in 5 mL/mg sterile water intragastrically through the nasal route with an infant feeding tube.

Registry No. 1, 103336-05-6; 2, 130985-34-1; 3, 130985-35-2; 4, 128657-34-1; 5, 52709-42-9; 6, 130985-36-3; 7, 130985-37-4; 8, 86170-45-8; 9, 91970-62-6; 10, 130985-38-5; 11, 130985-39-6; 12, 130985-40-9; 13, 130985-41-0; 14, 638-10-8; 15, 85532-42-9; 16, 130985-42-1; 17, 129765-95-3; 18, 130985-43-2; 19, 130985-44-3; 20, 130985-45-4; 21, 130985-46-5; 22, 130985-47-6; 23, 130985-48-7; 24, 130985-49-8; 25, 130985-50-1; 26, 130985-51-2; I, 131064-22-7; II, 131064-23-8; III, 131100-22-6; III·Na, 129272-65-7; IV, 131064-24-9; IV·Na, 129272-73-7; V, 130985-52-3; VI, 130985-53-4; VII, 130985-54-5; VIII, 130985-55-6; IX, 130985-56-7; X, 130985-57-8; XI, 130985-58-9; XII, 130985-59-0; XIII, 131064-25-0; XIII·K, 130985-60-3; XIV, 130985-61-4; suleptanic acid, 121807-13-4; di-*tert*-butyl-*N*,*N*-diethylphosphoramidite, 117924-33-1; N^{α}-(*tert*-butyloxycarbonyl)-L-glutamic acid α-*tert*-butyl ester, 24277-39-2.

Design of Potent Oxytocin Antagonists Featuring D-Tryptophan at Position 2

George Flouret,* William Brieher, and Kevin Mahan

Department of Physiology, Northwestern University Medical School, Chicago, Illinois 60611

Laird Wilson, Jr.

Department of Obstetrics and Gynecology, University of Illinois Medical Center, Chicago, Illinois 60612.
Received June 25, 1990

We prepared nine analogues (1–9) of MCPA-D-Phe-Phe-Ile-Asn-Cys-Pro-Arg-Gly-NH_2, [MCPA[1], D-Phe[2], Phe[3], Ile[4], Arg[8]]oxytocin (MCPA = β-mercapto-β,β-pentamethylenepropionic acid), a potent antagonist of the rat uterotonic action of oxytocin (OT). We replaced D-Phe with D-Trp and made [MCPA[1],D-Trp[2],Phe[3],Ile[4],Arg[8]]OT (1), which had OT pA_2 of 7.51, somewhat higher than that of the D-Phe[2] antagonist which has OT pA_2 = 7.35 in our rat uterotonic assay. Both compounds are equipotent as antagonists of [Arg[8]]vasopressin in the rat antidiuretic assay, with pA_2 = 8.1. Other substitutions gave [MCPA[1],D-Trp[2],4-Cl-Phe[3],Ile[4],Arg[8]]OT (2), OT pA_2 7.44; [MCPA[1],D-Trp[2],Phe[3],Ile[4],3,4-dehydro-Pro[7],Arg[8]]OT (3), OT pA_2 = 7.42; [MCPA[1], D-Trp[2],Phe[3],Arg[8]]OT (4), OT pA_2 = 7.58; [MCPA[1],D-Trp[2],Phe[3],Arg[8],Gly[9]-NHEt]OT (5), OT pA_2 = 7.49; [MCPA[1],D-Trp[2],Ile[4],Arg[8]]OT (6), OT pA_2 = 7.46; [MCPA[1],D-Trp[2],Val[4],Arg[8]]OT (7), OT pA_2 = 7.58; [MCPA[1],D-Trp[2],Thr[4],Arg[8]]OT (8), OT pA_2 = 7.48; and finally, [MCPA[1],D-Trp[2],Arg[8]]OT (9), which was a more potent and more selective OT antagonist, with OT pA_2 = 7.77 in the uterotonic assay and ADH $pA_2 < 5.9$ in the antidiuretic assay and hence is an important lead for the design of OT antagonists.

Introduction

In recent years, reversible antagonists of the uterotonic action of OT (oxytocin) have been sought as inhibitors of preterm labor[1] and as pharmacological tools in assessing the possible role of OT in labor. However, the main thrust in the synthesis of neurohypophyseal analogues has been in the development of potent antagonists of arginine vasopressin (AVP), the antidiuretic hormone, in the hope of developing new therapeutic agents selective either as as diuretics to counteract edema or as antivasopressors to treat hypertension. Unfortunately, these antagonists, in general, are not specific and act as reversible antagonists of AVP or OT, showing diuretic, antivasopressor, and antioxytocic actions.[2]

The use of D-tryptophan to substitute OT amino acids has been infrequent. The analogue [D-Trp[2]]OT has been prepared[3] and reported to be an inhibitor of OT uterotonic action, although Hruby and Smith report that no potency was specified.[4] Analogues of OT with lipophilic amino acids at position 2, among them D-tryptophan, combined with substitutions of a basic amino acid at position 8 and a β-mercaptopropionic acid at position 1 resulted in strong OT antagonists in the rat uterotonic assay.[5]

We decided to prepare OT antagonists featuring substitutions with D-tryptophan and to study the feasibility of enhancing their potency and specificity as antagonists to OT uterotonic action. In designing the desired antagonists we took advantage of the knowledge that (a) replacement at position 1 with β-mercapto-β,β-pentamethylenepropionic acid (MCPA)[6,36] instead of β-mercaptopropionic acid has led to stronger antagonists of OT[6,7] and (b) the analogues [MCPA[1],D-Phe[2],Phe[3],Ile[4],Arg[8]]OT and [MCPA[1],D-Phe[2],Phe[3],Gln[4],Arg[8]]OT were among the most potent antagonists of OT in the rat uterotonic assay.[8] We prepared [MCPA[1],D-Trp[2],Phe[3],Ile[4],Arg[8]]OT (ANTAG) as our initial antagonist. We tested this compound as antagonist of OT in the rat oxytocic bioassay[9] in the presence of magnesium salts, in an assay measuring the area under the peak resulting from a uterine contraction.[10] We found that ANTAG was a potent antagonist, as good or somewhat better than its D-Phe[2] counterpart, which we also prepared as a reference compound. Hence, we prepared additional analogues attempting to bring the sequence of the antagonists closer to that of the hormone itself, in the hope of attaining higher affinity for OT receptors, and hence higher potency, as well as higher specificity of action on the uterus rather than the kidney.

(1) Turnbull, A. C. *Br. J. Obstet. Gynaecol.* **1987**, *94*, 1009.
(2) Manning, M.; Sawyer, W. H. *J. Lab. Clin. Med.* **1989**, *114*, 617.
(3) Kaurov, O.; Martinov, V. F.; Mikhailov, Y. D.; Auna, Z. P. *Zh. Obsch. Khim.* **1972**, *42*, 1654.
(4) Hruby, V. J.; Smith, C. W. In *The Peptides*; Udenfriend, S., Meienhofer, J., Eds.; Academic Press: Orlando, FL, 1987; Vol 8, p 77.
(5) Melin, P.; Trojnar, J.; Johansson, B.; Vilhardt, H.; Åkerlund, M. *J. Endocr.* **1986**, *111*, 125.
(6) Nestor, J. J., Jr.; Ferger, M. F.; du Vigneaud, V. *J. Med. Chem.* **1975**, *18*, 284.
(7) Bankowski, K.; Manning, M.; Seto, J.; Haldar, J.; Sawyer, W. H. *Int. J. Pep. and Protein Res.* **1980**, *16*, 382.
(8) Manning, M.; Olma, A.; Klis, W. A.; Seto, J.; Sawyer, W. H. *J. Med. Chem.* **1983**, *26*, 1607.
(9) Schild, H. O. *Br. J. Pharmacol.* **1947**, *2*, 189.
(10) Wilson, L., Jr.; Parsons, M. T.; Ouano, L.; Flouret, G. *Am. J. Obstet. Gynecol.* **1990**, *163*, 195.

0022-2623/91/1834-0642$02.50/0