Design and Synthesis of a Potent and Specific Renin Inhibitor with a Prolonged Duration of Action in Vivo

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A structure–activity analysis of peptides containing backbone C^{α}-methyl and N^{α}-methyl modifications led to the discovery of potent renin inhibitors with high metabolic stability. In vitro, Boc-Pro-Phe-N^{α}-MeHis-Leuψ-[$CHOHCH_2$]Val-Ile-Amp (XII) is a potent inhibitor of human plasma renin with IC_{50} of 0.26 nM. It is a much weaker inhibitor of other aspartic proteases such as porcine pepsin or bovine cathepsin D (IC_{50} = 6 μM). It was shown not to be degraded by a rat liver homogenate preparation. In vivo, it inhibited plasma renin activity and lowered blood pressure of furosemide-treated cynomolgus monkeys. At a dose of 5 mg/kg iv, the pronounced hypotensive response persisted for greater than 3 h postinfusion.

The renin–angiotensin system has been implicated in several forms of hypertension.[1] Renin is an aspartyl protease that is produced mainly in the juxtaglomerular apparatus of the kidney.[2] It is a highly specific proteolytic enzyme and cleaves the circulating α-globulin angiotensinogen, produced by the liver, to form the decapeptide angiotensin I.[3] The N-terminal sequence of human angiotensinogen is shown in Figure 1, the cleavage site being the peptidic bond between amino acids 10 and 11.[4] Angiotensin I has no known biological activity, but it is converted to the octapeptide angiotensin II by the angiotensin-converting enzyme present in lungs and other organs as a result of the removal of the C-terminal dipeptide histidylleucine. Angiotensin II is a very potent vasoconstrictor and also stimulates the release of aldosterone from the adrenal gland. This mineralocorticoid induces sodium and water retention, leading to an increase in blood pressure.[3]

The antihypertensive activity of inhibitors of converting enzyme is not clear mechanistically due to its involvement in the kinin system. Renin, however, is an enzyme of high substrate specificity, and inhibitors of renin should effect the clear involvement of the renin–angiotensin system.[5] Interest in the blockade of renin has led to rapid development of potent inhibitors based on the angiotensinogen sequence. The most successful approach has been based upon the concept of a transition-state analogue[6] of the amide hydrolysis. Modifications at the cleavage site to mimic the tetrahedral species have generated analogues of the minimum substrate with high inhibitory potency in vitro.[7]

Many renin inhibitors have been shown to lower blood pressure during intravenous infusion. However, blood pressure usually recovers within minutes after stopping an infusion.[8] We initiated a program with the intention to overcome the problem of short biological half-life of these peptides. We chose to focus our attention on peptide backbone modifications that would lead to greater resistance of the resulting peptides toward enzymatic degradation. The present study demonstrates that such a simple approach can lead to the discovery of highly potent enzyme inhibitors with therapeutically useful biological half-life.

Table I. Inhibition of Human Plasma Renin, N^{α}-Methyl and C^{α}-Methyl Modifications with Phenylalanine at the P_2 Site

	no.	IC_{50}, μM
Boc–Phe–Phe-Sta-Ile-Amp	I	0.024
Boc–N^{α}-Me-Phe–Phe-Sta-Ile-Amp	II	0.34
Boc–C^{α}-Me-Phe–Phe-Sta-Ile-Amp	III	6.8
Boc–Phe–N^{α}-Me-Phe-Sta-Ile-Amp	IV	0.1
Boc–Phe–C^{α}-Me-Phe-Sta-Ile-Amp	V	>10

Table II. Inhibition of Human Plasma Renin, N^{α}-Methyl Modification with Histidine at the P_2 Site

	no.	IC_{50} μM
Boc–N^{α}-Me-Phe–His-Sta-Ile-Amp	VI	0.73
Boc–Phe–N^{α}-Me-His-Sta-Ile-Amp	VII	0.12

Chemistry. The building blocks for the dipeptidic isosteres[9] at the scissile site, 4(*S*)-[(*tert*-butyloxycarbonyl)amino]-3(*S*)-hydroxy-6-methyl-L-heptanoic acid (Boc-Sta-OH),[10] *N*-[2(*S*)-[(*tert*-butyloxycarbonyl)amino]-4-methylpentyl]-L-valine(Boc-Leuψ[CH_2NH]Val-OH),[11] and 4(*S*)-[(*tert*-butyldimethylsilyl)oxy]-5(*S*)-

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Table III. Inhibition of Human Plasma Renin, Comparison of Three Transition-State Analogue Inserts with N^{α}-Methylhistidine at the P_2 Site

	no.	IC_{50}, nM
Boc-Phe-N^{α}-Me-His—Sta—Ile-Amp	VII	120
Boc-Phe-N^{α}-Me-His—Leuψ[CH_2NH]Val—Ile-Amp	VIII	450
Boc-Phe-N^{α}-Me-His-Leuψ[$CHOHCH_2$]Val-Ile-Amp	IX	0.65
Boc-Pro-Phe-N^{α}-Me-His—Sta—Ile-Amp	X	190
Boc-Pro-Phe-N^{α}-Me-His—Leuψ[CH_2NH]Val—Ile-Amp	XI	68
Boc-Pro-Phe-N^{α}-Me-His-Leuψ[$CHOHCH_2$]Val-Ile-Amp	XII	0.26

1 5 10 11
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-His-
←P_3 P_2 P_1 P'_1 P'_2 P'_3→

Figure 1. Human angiotensinogen.

[(*tert*-butyloxycarbonyl)amino]-2(*S*)-isopropyl-7-methyloctanoic acid (Boc-Leuψ[CHO(TBS)CH_2]Val-OH),[12] were prepared by known procedures. The peptides were synthesized by standard solution method, and intermediates from each coupling were purified by chromatography on silica gel. The synthetic sequence of a representative example (peptide XII) is shown in Scheme I.[13] The purity of the final peptides I–XII was analyzed by reversed-phase analytical HPLC and the identity verified by high-resolution fast atom bombardment spectroscopy.

In Vitro Renin Inhibition. The amide bond between the amino acids at P_2 and P_3 sites appeared to be a likely point of cleavage by proteolytic enzymes in a number of potent renin inhibitors. We chose to examine the N^{α}-methylation and C^{α}-methylation[14] of the amino acids at these two positions to increase steric hindrance as possible means of imparting resistance to enzymatic degradation. We hoped to uncover some structural change that would not preclude the bioactive conformers as a consequence of the added conformational constraint.

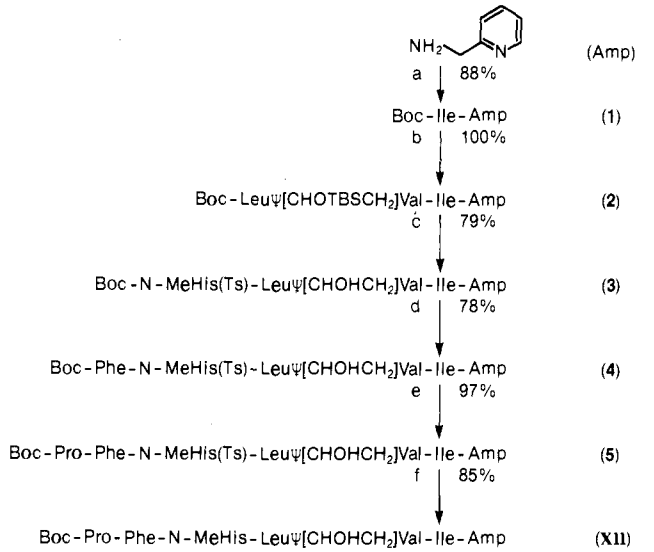
We first chose to work with phenylalanine at these two sites since the corresponding N^{α}-methyl C^{α}-methyl amino acids are readily available. The small peptide I[15] with IC_{50} of 2.4×10^{-8} M is a potent renin inhibitor and was chosen as a reference point. The individual phenylalanine was systematically substituted by the N^{α}-methyl and C^{α}-methyl amino acids to give peptides II–V as shown in Table I.

From the inhibitory potency of the resulting peptides, the N^{α}-methyl modification in peptides II and IV was clearly shown to be more suitable than the C^{α}-methyl modification in peptides III and V. We next changed the phenylalanine residue at P_2 site in peptides II and IV to a histidine residue. The resulting peptides VI and VII are shown in Table II. The N^{α}-methyl modification at P_2 site retained higher inhibitory potency.

This constraint might be a key to improved stability of renin inhibitors without substantial sacrifice of inhibitory potency.

We then examined a series of peptides with N^{α}-methylhistidine at the P_2 site and with three different dipeptide isosteres at the scissile site in search of highly potent renin inhibitors with increased resistance to enzymatic degradation. Peptides VII–XII were synthesized, and their renin inhibitory activities are displayed in Table III. In this series of compounds, it is clear that the hydroxyethylene isostere is far superior to statine or the reduced-bond isostere. Peptides IX and XII effect 50% inhibition of renin at subnanomolar concentration. It is to be noted that peptide XII is a potent renin inhibitor

Scheme I. Synthesis of Peptide XII[a]



[a] Key: (a) Boc-Ile-OH, DCC, HOBT; (b) TFA, CH_2Cl_2; Boc-Leuψ[CHOTBSCH$_2$]Val-OH, DEPC, Et_3N; (c) Et_2O, HCl(g); Boc-*N*-MeHis(Ts)-OH, DEPC, Et_3N; (d) TFA, CH_2Cl_2; Boc-Phe-OH, DEPC, Et_3N; (e) TFA, CH_2Cl_2; Boc-Pro-OH, DEPC, Et_3N; (f) HOBT, CH_3OH.

Table IV. Comparative Inhibition of Selected Proteases

	IC_{50}, M		
	human renin	porcine pepsin	bovine cathepsin D
pepstatin	6.0×10^{-6}	1.0×10^{-8}	2.8×10^{-8}
XII	2.6×10^{-10}	6.8×10^{-6}	6.3×10^{-6}

(12) Hester, J. B., Jr.; Emmert, D. E., manuscript in preparation.
(13) For simplicity, the 2-pyridylmethylamine end group is abbreviated as Amp.
(14) Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, p 267.
(15) For a related compound such as Boc-Phe-Phe-Sta-Leu-$NHCH_2Ph$, see: Evans, B. E.; Rittle, K. E.; Bock, M. G.; Bennett, C. D.; DiPardo, R. M.; Boger, J.; Poe, M.; Ulm, E. H.; LaMont, B. I.; Blaine, E. H.; Fanelli, G. M.; Stabilito, I. I.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1755.

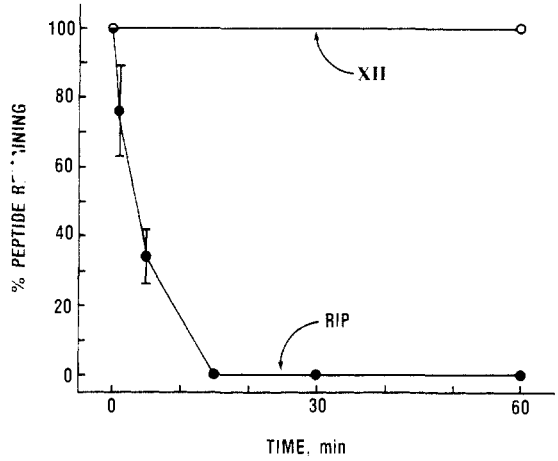


Figure 2. Degradation of peptides in a rat liver homogenate preparation. Peptide XII and RIP were added to buffer (pH 7.5) and were incubated at 37 °C with the supernatant from a centrifuged (30000*g*, 30 min) rat liver homogenate. The incubations were terminated at the times indicated, and the breakdown of each peptide was monitored by HPLC. Peak heights were used to determine the percent peptide remaining.

at physiological pH ($IC_{50} = 3.9 \times 10^{-10}$ M at pH 7.4). We elected to pursue peptide XII and to demonstrate its usefulness as a hypotensive agent in vivo.

Protease Specificity. A high degree of enzyme specificity is desirable for a potentially useful therapeutic agent. The methods to demonstrate enzyme specificity have been utilized in a related work.[16] We chose two other aspartyl proteases to study the inhibitory specificity. Pepstatin,[17] as shown in Table IV, is a general aspartyl protease inhibitor and exhibited poor selectivity. In contrast, peptide XII was more than 4 orders of magnitude less effective against pepsin and cathepsin D than against renin.

Metabolic Stability. In the absence of in vivo metabolic studies, we adopted a simple method that might offer some indication of the survival of a compound from proteolytic degradation. The metabolic stability was evaluated in vitro via a rat liver homogenate preparation. As shown in Figure 2, we could demonstrate that a renin inhibitor such as RIP[18] was rapidly degraded by proteolytic enzymes under this condition. In contrast, peptide XII remained unchanged in the rat liver homogenate.

Hypotensive Efficacy. The in vivo consequence of the apparent in vitro metabolic stability of peptide XII was explored via experiments in conscious sodium-depleted cynomolgus monkeys. The methods have been described previously in connection with a study on RIP.[19] In the present experiments, peptide XII at 5 mg/kg in 5 mL of 0.1 M citric acid and 5 mL of 5% dextrose or the vehicle alone was infused intravenously over 17 min. The results, shown in Figure 3, illustrate that peptide XII evoked a pronounced hypotension in conscious sodium-depleted cynomolgus monkeys that persisted for greater than 3 h postinfusion. The plasma renin activity (ng of angiotensin I/mL per h) during the control period was 18.5 ± 3.5 in the animals receiving vehicle and was 15.4 ± 3.2 in the animals receiving peptide XII. The plasma renin activity

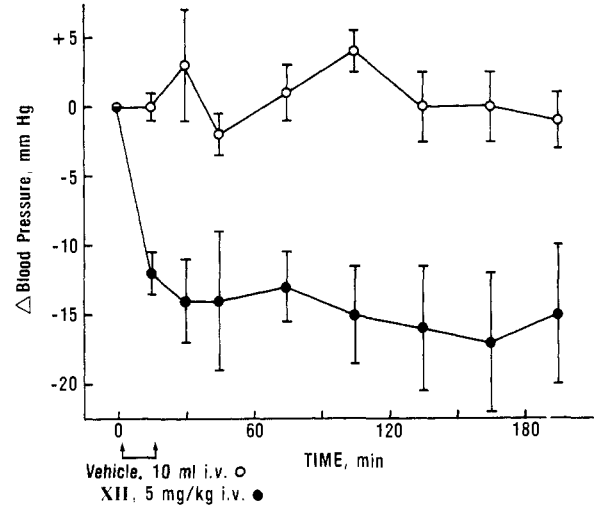


Figure 3. Hypotensive effect of renin inhibitors in sodium-depleted cynomolgus monkeys. Sodium depletion was accomplished by omitting the daily intravenous administration of saline and substituting the intravenous administration of furosemide for 7 days prior to a study. Blood pressure was recorded continuously from the arterial catheter via a Statham pressure transducer and a Grass polygraph. Peptide XII at 5 mg/kg in 5 mL of 0.1 M citric acid and 5 mL of 5% dextrose or the vehicle alone was infused intravenously over 17 min.

values in the vehicle-treated animals were 20.4 ± 2.4 at 30 min, 22.4 ± 2.2 at 105 min, and 18.7 ± 2.0 at 195 min while the values in the animals treated with peptide XII were essentially zero at these time intervals. In contrast, previous studies[19] had demonstrated that the infusion of 40 mg/kg of RIP (approximately 5 times the molar concentration of peptide XII) over 20 min caused a pronounced hypotension that persisted for less than 15 min postinfusion. The results of these studies suggested that the apparent metabolic stability of peptide XII contributed to its prolonged duration of action relative to that observed with RIP. A more detailed description of the pharmacology of peptide XII will be reported elsewhere.[20]

Discussion

A recent report[21] described CGP29287 as a potent and specific renin inhibitor with a prolonged duration of action. The structural feature that was suggested to be responsible for its increased half-life in vivo is the protecting groups at both terminals of the peptide.

It should be emphasized that in our investigation the N^{α}-methyl group at P_2 site is not the only element that confers metabolic stability to the peptides. The *tert*-butyloxycarbonyl group, the 2-methylpyridyl amide functionality, and the dipeptide isostere also contribute to the metabolic stability of peptide XII. The sphere of influence of any element is not necessarily exclusive of one another, and most probably vary with any particular peptide under consideration and also with the environment to which the peptide is subjected. If one focuses on the metabolic instability of the backbone at the peptidic bond between P_2 and P_3 sites, we hope to have demonstrated that there exist potent renin inhibitors in which a stabilizing element, N-methylation of the amide nitrogen, was introduced. We should also bear in mind that a stable compound under physiological condition is not necessarily sufficient to elicit

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the desired pharmacological response. In this study, we were able to demonstrate the hypotensive response with a prolonged duration of action for a renin inhibitor with apparent metabolic stability.

Summary

This report gives an account of the chemical development of a potent renin inhibitor, Boc-Pro-Phe-N^{α}-Me-His-Leuψ[CHOHCH$_2$]Val-Ile-Amp (XII). N^{α}-Methyl modification on the peptide backbone at P_2 site could result in peptides that retain high inhibitory potency. Peptide XII showed metabolic stability as suggested by its resistance to degradation by a rat liver homogenate preparation. It showed high specificity for renin as compared with other representative aspartyl proteases. It elicited the expected pharmacological response by inhibiting renin in vivo and lowered blood pressure of sodium-depleted cynomolgus monkeys. It also showed reasonable biological half-life; and at a dose of 5 mg/kg iv, the hypotensive response did not diminish after 3 h.

Experimental Section

Chemistry. Mass spectra, infrared spectra, ultraviolet spectra, optical rotations, melting points, and combustion analyses were obtained by the Physical and Analytical Chemistry Department of the Upjohn Co. ^{1}H NMR spectra were recorded at 80 MHz with a Varian Model CFT-20 Fourier transform spectrometer. Chemical shifts were reported as δ units relative to tetramethylsilane as internal standard.

Thin-layer chromatography was conducted with Analtech 0.25-mm glass plates precoated with silica gel GF. For column chromatography, E. Merck silica gel 60, 230–400 mesh, was used. All solvents for chromatography were Burdick and Jackson reagent grade distilled in glass.

Tetrahydrofuran was distilled under argon from sodium metal in the presence of benzophenone. Dichloromethane was distilled from phosphorus pentoxide. Triethylamine was distilled from calcium hydride. Diethylphosphoryl cyanide was freshly distilled before use.

Peptides I–V and VII–XII were analyzed on a Perkin-Elmer Series 4 liquid chromatograph with a Kratos Spectroflow 773 detector (254 nm) and a Perkin-Elmer LCI-100 integrator using a Brownlee RP-18, 10-μm, 25 cm × 4.6 mm analytical column at a flow rate of 1.5 mL/min. The mobile phase for peptides I–V was an isocratic mixture of 75% methanol and 25% aqueous phosphate pH 3 buffer. The mobile phase for peptides VII, IX, X, and XII was an isocratic mixture of 90% methanol and 10% aqueous phosphate pH 3 buffer. The mobile phase for peptides VIII and XI was an isocratic mixture of 70% acetonitrile and 30% aqueous phosphate pH 3 buffer, with 1% tetrabutylammonium dihydrogen phosphate.

Peptide VI was analyzed on an Altex system with Model 110A pumps, a Model 420 mixer with an LDC Spectromonitor III detector (225 nm), and a Hewlett-Packard 3390A integrator using a 10-μm Bondapak phenyl analytical column at a flow rate of 1.5 mL/min. The mobile phase was an isocratic mixture of 32.5% acetonitrile and 67.5% water, and 0.2% trifluoroacetic acid.

4(*S*)-[(*tert*-Butyloxycarbonyl)amino]-3(*S*)-hydroxy-6-methylheptanoic acid (Boc-Sta-OH) was prepared according to D. H. Rich et al.[10] *N*-[2(*S*)-[(*tert*-Butyloxycarbonyl)amino]-4-methylpentyl]-L-valine (Boc-Leuψ[CH$_2$NH]Val-OH) was prepared in a similar manner to that of M. Szelke et al.[11] 4(*S*)-[(*tert*-Butyldimethylsilyl)oxy]-5(*S*)-[(*tert*-butyloxycarbonyl)amino]-2-(*S*)-isopropyl-7-methyloctanoic acid (Boc-Leuψ[CHO(TBS)-CH$_2$]Val-OH) was prepared according to Hester and Emmert.[12] N^{α}-(*tert*-Butyloxycarbonyl)-N^{α}-methyl-L-phenylalanine was prepared according to Cheung and Benoiton.[22] DL-C^{α}-Methylphenylalanine was purchased from Research Organics.

N^{α}-(*tert*-Butyloxycarbonyl)-N^{α}-methyl-N^{im}-tosyl-L-histidine. A 0.75-g portion of 50% NaH in oil (15.6 mmol) was washed with three 7-mL portions of pentane under argon. The residue was suspended in 5 mL of dry tetrahydrofuran. To this stirred mixture was added 1.59 g (3.88 mmol) of N^{α}-(*tert*-butyloxycarbonyl)-N^{im}-tosyl-L-histidine in 5 mL of tetrahydrofuran, followed by 2.0 mL (32 mmol) of methyl iodide. After being stirred at room temperature for 16 h, the resulting mixture was slowly added to a stirred solution of 20 mL of cold 1 M aqueous $NaHSO_4$. The aqueous phase was extracted with several portions of ethyl acetate. The combined organic phase was dried ($MgSO_4$). Concentration of this solution gave 1.18 g (2.79 mmol, 72%) of N^{α}-(*tert*-butyloxycarbonyl)-N^{α}-methyl-N^{im}-tosyl-L-histidine: ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9 H, $(CH_3)_3CCO$), 2.43 (s, 3 H, CH_3Ar), 2.76 (s, 3 H, CH_3N), 7.09 and 7.98 (2 s, 2 × 1 H, imidazole hydrogens), 7.37 and 7.81 (2 d, 2 × 2 H, J = 9Hz, phenyl hydrogens); IR ($CHCl_3$) 2950, 2920, 2870, 2850, 1730, 1690, 1630 cm^{-1}; UV (EtOH) λ_{max} (ϵ) 225 (11500), 228 (12100), 223 (11400), 275.5 (751); $[\alpha]_D$ −22° ($CHCl_3$, c 0.36); MS, for $C_{19}H_{25}N_3O_6SK$ calcd 462.1101, found 462.1114. Anal. ($C_{19}H_{25}N_3O_6S$) C, H, N, S.

General Procedure A. Removal of the *tert*-Butyloxycarbonyl Group with Dry HCl. Dry HCl gas was passed into a solution of the substrate in ether. The resulting mixture was allowed to stir at room temperature for an addition 20 min, and then the volatile components were removed with a stream of nitrogen. The resulting solid residue was dried in vacuo.

General Procedure B. Removal of the *tert*-Butyloxycarbonyl Group with Trifluoroacetic Acid. A solution of the substrate in equal volumes of dichloromethane and trifluoroacetic acid was allowed to stir at room temperature for $^1/_2$–2 h. The reaction mixture was then concentrated and the residue treated with excess aqueous $NaHCO_3$. The aqueous phase was extracted with several portions of dichloromethane. The combined organic phase was dried ($MgSO_4$) and then concentrated to give the free amine.

General Procedure C. Coupling Reaction with Dicyclohexylcarbodiimide/1-Hydroxybenzotriazole. To a stirred solution of the acid component and the amine component in dichloromethane was added a slight excess of 1-hydroxybenzotriazole, followed by a slight excess of dicyclohexylcarbodiimide. After being stirred at room temperature for 10–24 h, the resulting mixture was filtered and the filtrate washed with saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was then purified by chromatography on silica gel.

General Procedure D. Coupling Reaction with 2-Chloro-1-methylpyridinium Iodide. A mixture of the acid component, the amine component, a slight excess of 2-chloro-1-methylpyridinium iodide, and an excess of triethylamine in dichloromethane was allowed to stir at reflux for 2–12 h. The cooled reaction mixture was diluted with dichloromethane and then washed with saturated aqueous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was then purified by chromatography on silica gel.

General Procedure E. Coupling Reaction with Diethoxyphosphoryl Cyanide. To a stirred solution of the acid component and the amine component in dichloromethane was added a slight excess of triethylamine, followed by slow addition of a slight excess of diethoxyphosphoryl cyanide. After being stirred at room temperature for 2–12 h, the reaction mixture was diluted with dichloromethane and then washed with saturated aqeous $NaHCO_3$. The organic phase was dried ($MgSO_4$) and then concentrated. The residue was then purified by chromatography on silica gel.

General Procedure F. Removal of the *p*-Tolylsulfonyl Group from Histidine. A solution of the peptide substrate and 3–5 equiv of 1-hydroxybenzotriazole in methanol was allowed to stir at room temperature for 12–24 h. The reaction mixture was then concentrated and the residue purified by chromatography on silica gel.

Preparation of Peptide XII as a Representative Example. *N*-[N^{α}-(*tert*-Butyloxycarbonyl)-L-isoleucyl]-2-pyridylmethylamine (1). According to general procedure C, 9.25 g (40 mmol) of N^{α}-(*tert*-butyloxycarbonyl)-L-isoleucine, 4.2 mL (40.7 mmol) of 2-(aminomethyl)pyridine, 5.95 g (44 mmol) of 1-hydroxybenzotriazole, and 8.66 g (42 mmol) of dicyclohexylcarbodiimide in 40 mL of dichloromethane afforded 11.34 g (35.3 mmol, 88%) of compound 1 after chromatography on silica gel with ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3 H, J = 7 Hz),

(22) Cheung, S. T.; Benoiton, N. L. *Can. J. Chem.* 1977, *55*, 906.

0.93 (d, 3 H, J = 7 Hz), 1.42 (s, 9 H), 4.05 (dd, 1 H, J = 6, 9 Hz), 4.57 (d, 2 H, J = 5 Hz), 5.12 (br d, 1 H, J = 9 Hz), 7.21 (m, 2 H), 7.65 (m, 1 H), 8.54 (br d, 1 H, J = 5 Hz).

***N*-[*N*$^{\alpha}$-[4(*S*)-[(*tert*-Butyldimethylsilyl)oxy]-5(*S*)-[(*tert*-butyloxycarbonyl)amino]-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (2).** According to general procedure B, 11.3 g (35.2 mmol) of compound 1 in 25 mL of dichloromethane and 25 mL of trifluoroacetic acid afforded 7.76 g (35.1 mmol, 100%) of L-isoleucyl-2-pyridylmethylamine after the aqueous phase was continuously extracted with dichloromethane for 1 day and the resulting residue chromatographed on silica gel with 3% methanol and 3% methanol (saturated with ammonia) in dichloromethane.

According to general procedure E, 4.457 g (10.0 mmol) of Boc-Leuψ[CHO(TBS)CH$_2$]Val-OH, 2.434 g (11.0 mmol) of L-isoleucyl-2-pyridylmethylamine, 1.53 mL (11.0 mmol) of triethylamine, and 1.69 mL (11.0 mmol) of diethylphosphoryl cyanide in 60 mL of dichloromethane afforded 6.50 g (10.0 mmol, 100%) of compound **2** after chromatography on silica gel with 20% hexane in ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 0.11 (s, 6 H), 0.91 (m, 18 H), 1.43 (s, 9 H), 7.20 (m, 2 H), 7.64 (m, 1 H), 8.54 (br d, 1 H, J = 5 Hz).

***N*-[*N*-[*N*-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\alpha}$-methyl-*N*^{im}-tosyl-L-histidyl]-5(*S*)-amino-4(*S*)-hydroxy-2(*S*)-isopropyl-7-methyloctanoyl]-L-isoleucyl]-2-pyridylmethylamine (3).** According to general procedure A, 6.50 g (10.0 mmol) of compound **2** in 350 mL of ether saturated with dry HCl afforded a white solid amine hydrochloride.

According to general procedure E, 5.505 g (13.0 mmol) of *N*$^{\alpha}$-(*tert*-butyloxycarbonyl)-*N*$^{\alpha}$-methyl-*N*^{im}-tosyl-L-histidine, the above amine hydrochloride, 4.3 mL (31 mmol) of triethylamine, and 2.0 mL (13.0 mmol) of diethylphosphoryl cyanide in 120 mL of dichloromethane afforded 6.62 g (7.88 mmol, 79%) of compound **3** after chromatography on silica gel with 5% methanol in ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 0.87 (m, 18 H), 1.37 (s, 9 H), 2.41 (s, 3 H), 2.74 (s, 3 H), 8.50 (br d, 1 H, J = 5 Hz).

***N*-[*N*-[*N*-[*N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-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 (4).** According to general procedure B, 6.62 g (7.88 mmol) of compound **3** in 20 mL of dichloromethane and 20 mL of trifluoroacetic acid afforded 5.81 g of the free amine.

According to general procedure E, 4.166 g (15.7 mmol) of *N*$^{\alpha}$-(*tert*-butyloxycarbonyl)-L-phenylalanine, 5.81 g (7.85 mmol) of the above amine, 2.19 mL (15.7 mmol) of triethylamine, and 2.35 mL (15.3 mmol) of diethylphosphoryl cyanide in 100 mL of dichloromethane afforded 6.03 g (6.11 mmol, 78%) of compound **4** after chromatography on silica gel with 5% methanol in ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 0.85 (m, 18 H), 1.35 (s, 9 H), 2.42 (s, 3 H), 2.79 and 2.90 (s, 3 H. 2 rotamers), 8.50 (br d, 1 H, J = 5 Hz).

***N*-[*N*-[*N*-[*N*$^{\alpha}$-[(*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 (5).** According to general procedure B, 6.03 g (6.11 mmol) of compound **4** in 20 mL of dichloromethane and 20 mL of trifluoroacetic acid afforded 5.4 g of the free amine.

According to general procedure E, 1.97 g (9.15 mmol) of *N*$^{\alpha}$-(*tert*-butyloxycarbonyl)-L-proline, 5.4 g (6.1 mmol) of the above amine, 1.28 mL (9.18 mmol) of triethylamine, and 1.36 mL (8.87 mmol) of diethylphosphoryl cyanide in 75 mL of dichloromethane afforded 6.45 g (5.95 mmol, 97%) of compound **5** after chromatography on silica gel with 7% methanol in ethyl acetate: ^{1}H NMR ($CDCl_3$) δ 0.88 (m, 18 H), 1.43 (s, 9 H), 2.41 (s, 3 H), 2.77 and 2.93 (s, 3 H, 2 rotamers), 8.50 (br d, 1 H, J = 5 Hz).

***N*-[*N*-[*N*-[*N*$^{\alpha}$-[(*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 (XII).** According to general procedure F, 6.45 g (5.95 mmol) of compound **5** and 3.22 g (23.8 mmol) of 1-hydroxybenzotriazole in 70 mL of methanol afforded 4.678 g (5.03 mmol, 85%) of peptide XII after chromatography on silica gel with 5% methanol (saturated with ammonia) in dichloromethane: ^{1}H NMR ($CDCl_3$) δ 0.85 (m, 18 H), 1.42 (s, 9 H), 2.76 and 2.93 (s, 3 H. 2 rotamers), 8.50 (br d, 1 H, J = 5 Hz).

Table V

peptides	HPLC[a] k'	formula	FAB-MS calcd	FAB-MS found
I	5.03	$C_{43}H_{60}N_6O_7$	773.4601	773.4597
II	8.11	$C_{44}H_{62}N_6O_7$	787.4758	787.4722
III	6.87	$C_{44}H_{62}N_6O_7$	787.4758	787.4744
IV	5.50	$C_{44}H_{62}N_6O_7$	787.4758	787.4744
V	7.18	$C_{44}H_{62}N_6O_7$	787.4758	787.4759
VI	5.60	$C_{41}H_{60}N_8O_7$	777.4663	777.4652
VII	5.31	$C_{41}H_{60}N_8O_7$	777.4663	777.4652
VIII	2.44	$C_{44}H_{67}N_9O_6$	818.5292	818.5301
IX	6.45	$C_{45}H_{68}N_8O_7$	833.5289	833.5269
X	5.90	$C_{46}H_{67}N_9O_8$	874.5190	874.5172
XI	3.17	$C_{49}H_{74}N_{10}O_7$	915.5820	915.5795
XII	10.05	$C_{50}H_{75}N_9O_8$	930.5816	930.5789

[a] See HPLC conditions at the beginning of the Experimental Section. k' = partition ratio.

Other peptides reported here were synthesized in a similar manner. The physical characteristics for the final products I–XII are listed in Table V.

Biology. Inhibition of Human Plasma Renin. Compounds I–XII 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 (phenylmethyl)sulfonyl fluoride, 25 μL of maleate buffer (pH 6.0), and 10 μL of an appropriate concentration of inhibitor 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 inhibitor tubes were compared to control tubes to estimate percent inhibition. The inhibition results were expressed as IC_{50} values, which were obtained by plotting three to four inhibitor concentrations on semilog graph paper and estimating the concentration producing 50% inhibition.

Protease Specificity. Inhibition of pepsin was determined as described[17] using porcine pepsin (Sigma), porcine hemoglobin (Sigma), and 0.02 M KCl–HCl buffer (pH 2). The compounds were dissolved in the buffer, and after incubation for 25 min at 37 °C with the enzyme and substrate, perchloric acid was added and the absorbance of the acid-soluble fractions measured at 280 nm. The percent inhibition was estimated from the net absorbance of inhibited assays in relation to uninhibited control assays. A plot of percent inhibition vs. log inhibitor concentration was constructed with the IC_{50} defined as the inhibitor concentration causing 50% inhibition.

Bovine cathepsin D (Sigma) inhibition was measured via a slightly modified literature procedure.[23] The compounds were dissolved in 0.2 M acetate buffer (pH 3.2) as was the porcine hemoglobin (Sigma) substrate. Incubations for 30 min at 37 °C were terminated in ice, and 1.7 M perchloric acid was added to all mixtures. Extinction at 280 nm was measured and the IC_{50} determined as above.

Metabolic Stability. Sprague–Dawley rats were sacrificed by decapitation and the livers removed. The rat livers were chilled on ice and homogenized in 10 volumes (w/v) of 50 mM Tris-HCl, pH 7.5. The homogenate was centrifuged at 30000g for 30 min and the supernatant removed. RIP[18] and peptide XII were dissolved in 10% Me_2SO–90% 50 mM Tris-HCl, pH 7.5, to yield concentrations of 0.125 mM. Aliquots (50 μL) of the liver homogenate supernatant were added to 200 μL of each solution of inhibitor. Incubations were carried out at 37 °C for varying periods of time and were terminated via addition of 250 μL of 0.2% trifluoroacetic acid (TFA) in 80% acetonitrile–20% H_2O. The breakdown of each peptide was monitored by HPLC separation of the parent peptide from any products of degradation that may have formed during incubation. Thus, 100 μL of each incubate was injected into a 25 cm × 4.5 mm SynChrom RP-P column and eluted with a linear gradient of 90% buffer A (0.1% TFA in H_2O) and 10% buffer B (0.1% TFA in 75% acetonitrile, 25% H_2O) to 25% buffer A and 75% buffer B at a flow rate of

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1.5 mL/min over 35 min. Peak heights were used to determine the percent peptide remaining and were plotted as a function of incubation time.

Hypotensive Efficacy. Male cynomolgus monkeys weighing 4–6 kg were anesthetized, and polyvinyl catheters were implanted under sterile conditions in the abdominal aorta and the thoracic vena cava via an external iliac artery and vein respectively. At least 1 week was allowed for recovery from surgery before initiation of an experiment. The monkeys were fed a quantity of Standard SKF Monkey Diet deleting sodium (ICN Nutritional Biochemicals, Cleveland, OH) adequate to furnish potassium at 1 mequiv/kg per day. Intravenous 0.9% saline adequate to provide sodium at 1 mequiv/kg per day was administered to maintain the animals in a sodium-replete state. Sodium depletion was accomplished by omitting the daily intravenous administration of saline and substituting the intravenous administration of furosemide (1 mg/kg body weight per day) for 7 days prior to a study. Drinking water was allowed ad libitum. Experiments were carried out while the monkeys were seated in primate restraining chairs (Plas-Labs, Lansing, MI). Blood pressure was recorded continuously from the arterial catheter via a Statham pressure transducer and a Grass polygraph. Heart rate was continuously recorded from a Grass tachograph triggered by the arterial pressure pulse. Each animals received a bolus injection of saralasin (Peninsula Laboratories, Inc., Belmont, CA) at 1 mg/kg iv at least 5 h prior to the infusion of peptide XII to elucidate the magnitude of the renin-dependent blood pressure component. Blood samples were drawn at intervals, and plasma renin activity was determined via standard radioimmunoassay techniques at pH 7.4 using the Gamma Coat [^{125}I] Plasma Renin Activity Radioimmunoassay Kit (Travenol-Genentech Diagnostics, Cambridge, MA).

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Neuroleptics from the 4a,9b-*cis*- and 4a,9b-*trans*-2,3,4,4a,5,9b-Hexahydro-1*H*-pyrido[4,3-*b*]indole Series. 2

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Compounds derived from 4a,9b-*trans*-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole are consistently efficacious in displacing [^{3}H]spiroperidol from striatal dopamine receptors in vitro. Derivatives bearing substituents at position 2, particularly those derived from butyrophenone moieties, are exceptionally potent in vivo. Compounds from the corresponding 4a,9b-cis series are substantially less potent in both in vivo and in vitro assays of neuroleptic activity. Although the cis and trans derivatives have, in some conformations, similar basic nitrogen atom to aromatic ring separations of about 5.1 Å, the distance at which the basic nitrogen atom lies above or below the plane of the aromatic ring differs substantially between the two series. Consideration of these results in terms of this and earlier work indicates that the out-of-plane distance for the basic nitrogen in neuroleptic molecules may range from about 0 to about 0.90 Å but may be optimized at about 0.55 Å.

A major objective of medicinal chemistry in recent years has been the elucidation of mechanisms by which naturally occurring substances interact with pharmacologically relevant sites in in vivo systems. Such an understanding would permit the design of synthetic compounds that could interact with the same sites to potentiate or antagonize the action of the naturally occurring substance in hopes of producing a therapeutic response. Since many naturally occurring substances are conformationally flexible and capable in theory of interacting in any one of multiple molecular conformations, chemists have sought compounds of defined geometry that are also potently active in specific biological assays as investigational tools.[2] Specificity of an optically active enantiomer for a receptor yields valuable information about the spatial and conformational requirements of the receptor, especially in those cases in which the disfavored enantiomer is substantially less potent.

Current theories maintain that schizophrenia derives from alterations in the impulse flow in dopaminergic neurons in the central nervous system[3] and that patients can be treated by the administration of dopamine (DA) receptor blocking agents. Several recently developed neuroleptic agents, including the optically active (+)-butaclamol (I)[4] and Ro-22-1319 (II)[5] are potent DA receptor blockers in in vivo and in vitro model systems, are capable of interacting in only one enantiomeric form, and are derived from relatively rigid frameworks. A third structure that possesses these properties of potent activity, optical specificity, and structural rigidity at the DA receptor is (−)-apomorphine (III)[6] which is, however, an agonist at the receptor. We became interested in two novel series of chiral hexahydro-γ-carbolines, the trans derivatives represented by IV and the cis derivatives represented by V, as a result of our earlier work in the achiral tetrahydro-γ-carboline series[7,8] that culminated in the discovery of flutroline (VI), a compound that has demonstrated clinical antipsychotic activity in man.[9,10]

The hexahydro series IV and V were of interest to us for several reasons: First, these compounds would serve

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