Synthesis and Biological Activity of Ketomethylene-Containing Nonapeptide Analogues of Snake Venom Angiotensin Converting Enzyme Inhibitors

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Two ketomethylene-containing nonapeptide analogues were synthesized to determine if ketomethylene analogues of the nonapeptide venom inhibitors of angiotensin converting enzyme (ACE) would have oral ACE inhibition activity. Both ketomethylene-containing nonapeptides **18** and **19** were potent inhibitors of rabbit lung ACE with I_{50}s of 3.4 and 8.0 nM, respectively, compared to 340 nM for their parent nonapeptide and 450 nM for captopril. Peptide **18** was rapidly cleaved by trypsin, but **19** was reasonably stable to all enzyme degradation systems tested with maximum degradation of 50% by pepsin in 3 h. Both **18** and **19** when given iv to normotensive rats were between 3 and 10 times more potent than captopril in inhibiting an angiotensin I induced blood pressure increase. Peptide **19** showed no ACE inhibition activity in unanesthetized normotensive rats when administered orally at doses of 10 or 100 mg/kg. Experiments were conducted to determine whether **19** is adsorbed from the gastrointestinal track following oral administration. These experiments indicated that **19** is adsorbed. It is concluded that the lack of oral activity of **19** is probably due to its rapid excretion, probably into the bile.

The ketomethylene-containing tri- and pentapeptide analogues **1** and **2** have been shown to be potent inhibitors of angiotensin converting enzyme (ACE), I_{50}s = 70 and 7.0 nM, respectively.[1,2] Neither of these compounds, however, have therapeutically useful blood pressure lowering activity when given orally or intravenously to renal hypertensive rats.[2,3] Numerous analogues of these compounds have been made in an attempt to improve their in vivo activities without success.[2-5]

Studies of radiolabeled analogues of **1** and **2** have shown that both of these compounds have poor bioavailability due to their rapid excretion mainly via the bile.[2,6] The important factors leading to biliary excretion of compounds with organic acid functionalities such as **1** and **2** appear to be mainly molecular weight. Klaassen et al.[7,8] report that compounds that are highly concentrated in the bile in rats are usually carboxylic acids with molecular weights of 300 or greater.

In more recent studies by Doyle et al.,[9] there appears to be a molecular weight limit above which biliary excretion of peptides is no longer a major excretion pathway. Doyle et al. examined the percent hepatic clearance of a series of gastrin and cholescystokinin fragment peptides in a perfused rat liver. They found that peptide fragments larger than seven amino acids were not cleared by the liver.

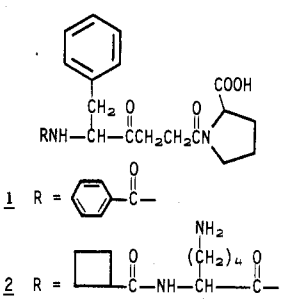
Considering this information, we decided to prepare some ketomethylene-containing analogues of the nonapeptide snake venom ACE inhibitors as possible orally active ACE inhibitors. We hoped that such peptide analogues would be large enough to avoid rapid clearance by the liver and would therefore possess oral antihypertensive activity of significant duration.

Table I. ACE Inhibition Activity of Selected Venom Peptide Analogues

structure	I_{50}, μg/mL
pGlu-Lys-Phe-Ala-Pro (BPP_{5a})	0.05
Cbc-Lys-Phe-Ala-Pro[a]	0.04
pGlu-Lys-Phe-Gly-Pro	0.1
pGlu-Nle-Phe-Ala-Pro	0.2
pGlu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (SQ20881)	1.1
pGlu-Tyr-Pro-Arg-Pro-Gln-Ile-Pro-Pro	2.6
pGlu-Trp-Pro-Gly-Pro-Gln-Ile-Pro-Pro	1.9
pGlu-Trp-Pro-Arg-Pro-Lys-Phe-Ala-Pro	0.05

[a] Cbc = cyclobutylcarbonyl. Underlined residues were used in designing peptides **3**, **18**, and **19**.

The parent nonapeptide for insertion of ketomethylene linkages was chosen on the basis of structure–ACE inhibition data previously published on snake venom inhibitors.[10] This data is shown in Table I. The parent peptide **3** that we chose is most similar to the last venom peptide in Table I. We replaced pGlu with the more stable cyclobutanecarboxylic acid group that we had previously used in our ketomethylene-containing pentapeptide analogue **2**. Tryptophan in the 2-position was replaced by



the more stable Tyr residue. Replacement of Arg with Gly was expected to cause little decrease in ACE inhibition activity while at the same time provide a greatly simplified residue for insertion of a ketomethylene group in the amino terminal end of the molecule to protect it against peptidase degradation. Replacement of Ala with Gly had been previously done successfully in **1** and **2** to provide a good site for insertion of a ketomethylene linkage for protection

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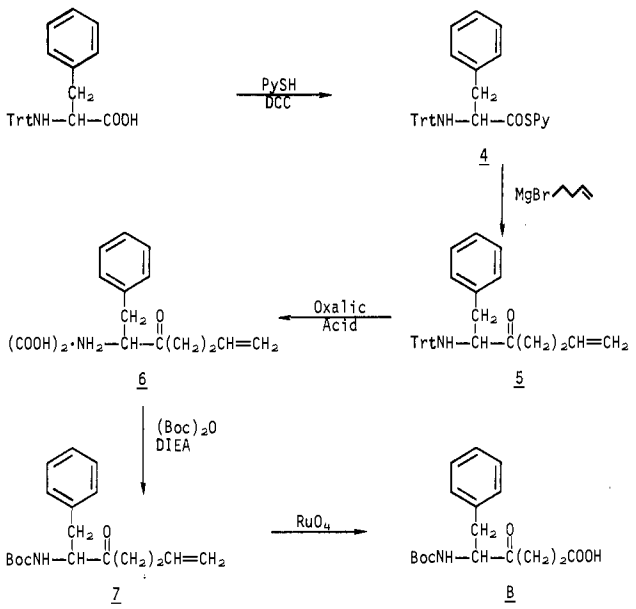
Cbc-Tyr-ProΨ($COCH_2$)Gly-Pro-Lys-Phe-Gly-Pro, 3

Cbc-Tyr-ProΨ($COCH_2$)Gly-Pro-Lys-PheΨ($COCH_2$)Gly-Pro, 18

Cbc-Tyr-ProΨ($COCH_2$)Gly-Pro-Nle-PheΨ($COCH_2$)Gly-Pro, 19

Figure 1. Nonapeptide analogues synthesized.

Scheme I



of the carboxy terminal end of the molecule from peptidase degradation. We prepared the ketomethylene-containing analogue **18** initially and then prepared the norleucine analogue of **18**, compound **19**, when the Lys–Phe bond was found to be unstable to cleavage by trypsin.

Chemistry

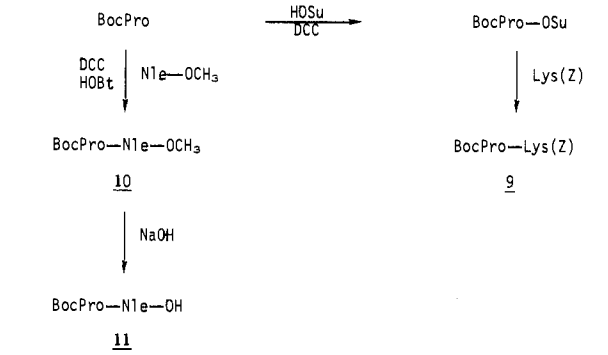
The synthesis of **3** was by standard solid-phase synthesis techniques as detailed in the Experimental Section. Peptide analogues **18** and **19** were also prepared by solid-phase synthesis by sequentially coupling four dipeptides. The syntheses of the dipeptides required to prepare **18** and **19** are shown in Schemes I–IV.

In Scheme I, the *S*-pyridinyl ester of Trt-L-Phe, **4**, is converted to the ketomethylene analogue **5** by condensation with the Grignard reagent of 4-bromobutene.[11,12] The trityl group is then exchanged for the Boc amino protecting group to yield the more stable derivative **7**. Oxidation of the olefin with RuO_4 to the desired acid functionality proceeded in good yield to give the ketomethylene dipeptide analogue **8**.

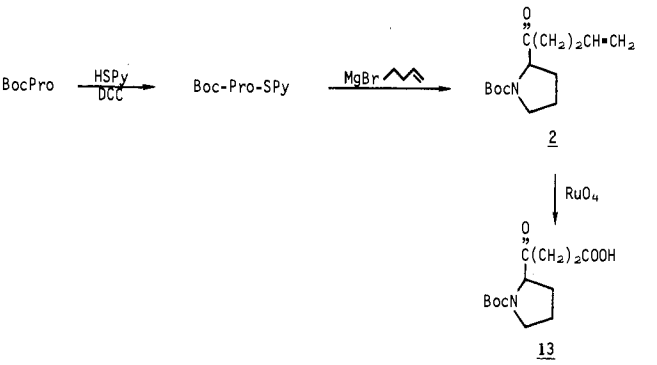
The other ketomethylene dipeptide analog **13** (Scheme III) was prepared in a similar manner to **8**. The Boc amino protecting group can be used on L-Pro in this case during the Grignard condensation step since there is no proton on the amino group to be eliminated and promote the formation of the oxazolone derivative.

The syntheses of **18** and **19** were conducted, starting with commercially obtained Boc-L-Pro-Merrifield resin. Scheme V outlines the synthetic steps used to prepare these two ketomethylene-containing nonapeptide ana-

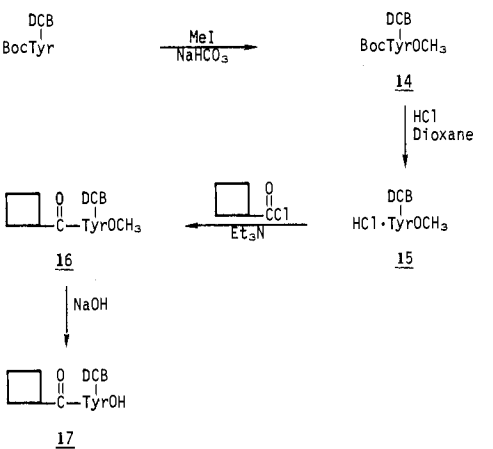
Scheme II



Scheme III



Scheme IV



DCB = dichlorobenzyl

logues. The dipeptides were coupled by using special coupling conditions designed to prevent the ketone groups from interacting with the terminal amino groups. The methods used for solid-phase syntheses of **18** and **19** gave excellent yields of 62% and 56%, respectively, of purified ketomethylene-containing peptides.

Biological Results and Discussion

Table II shows the rabbit lung ACE inhibition results with peptides **1–3**, **18**, and **19** compared to captopril. Both **18** and **19** are potent inhibitors of ACE in vitro with I_{50}s over 100 times more potent than either captopril or the parent peptide **3**. The relative differences between the ACE inhibition activity of **3** and **18** is similar to the difference we observed between **1** and its parent peptide Bz-Phe-Gly-Pro.[1]

These peptides were tested for their stability in several enzyme systems as shown in Table III. Peptides **3** and **18** were very unstable to trypsin. Unlike our previous ketomethylene-containing pentapeptide analogue **2**,[2] the

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Table II. ACE Inhibition and Analytical Data on Peptide Analogues[a]

compd	amino acid analysis ratios						HPLC k' (% CH_3CN)	ACE inhibn:[d] I_{50}, μM
	Pro	Gly	Tyr	Phe	Lys	Nle		
3	2.83	2.03	1.05	1.04	1.05		1.47 (23)	0.34
18			0.98		0.79[b]		1.92 (27)	0.0034
19			0.98			0.78[b]	2.30 (35)	0.0080
1								0.016
2								0.0060
captopril								0.45

[a] Additional analytical data for **18** and **19** are given in the Experimental Section. [b] Amino acids attached to the amino terminal side of ketomethylene dipeptides are frequently not totally cleaved during 24-h acid hydrolysis.[13] [c] HPLC was conducted on a Vydac TP21854 column (4.6 × 250 mm) with the noted percentage of CH_3CN in H_2O + 0.1% TFA as the eluting solvent at a rate of 1.0 mL/min. Ultraviolet visualization was at a wavelength of 220 nm. [d] The ACE inhibition assay was conducted using rabbit lung ACE purchased from Sigma by using the procedure described in ref 1. I_{50} values are the average of two or more experiments. I_{50} values for **1**, **2**, and captopril differ from those reported in ref 1 and 2 because of the different source of ACE used.

Table III. Time Required for Given Percent Degradation of Peptide Analogues

compd	trypsin			chymotrypsin			pepsin			rat intestinal homogenate			rat stomach homogenate		
	50%	100%	none	50%	100%	none	50%	100%	none	50%	100%	none	50%	100%	none
3	<5 min	20 min		22 h					24 h	20 min	40 min		>1 h	5 h	
18	5 min	1 h		22 h					20 h	1 h	5 h				
19			24 h			24 h	3 h			7 h	23 h		5 h		

Table IV. Inhibition of the Angiotensin I Induced Blood Pressure Increase in Normotensive Anesthetized Rats

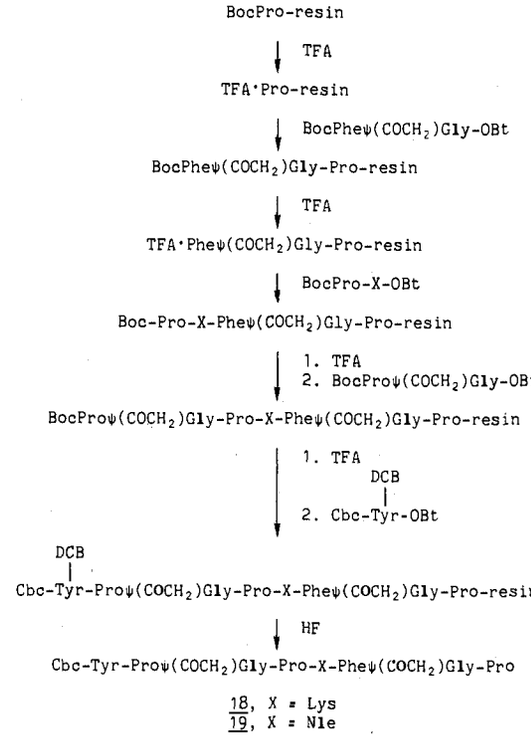
compd	dose, μg/kg iv	N	% inhibition over time, min					
			1.5	5	10	30	60	75
18	1	4	24.3	0	0	0	0	
	10	4	39.5	23.5	14.5	7.8	0	
	30	4	79.5	45.0	31.5	17.3	15.3	12.5
19	1	4	26.3	25.6	24.3	22.4		
	10	4	64.3	27.5	14.5	0		
	30	4	88.5	59.8	37.0	30.8	16.0	0
captopril	3	4	18.7	8.3	0			
	30	4	78.3	60.5	8.3	0		
	100	4	71.5	83.5	51.1	27.3	1.8	0

Lys–Phe bond in **3** and **18** is easily cleaved by trypsin. The stability of this bond in **2** must be at least partially a result of the cyclobutane carboxyl group that is attached to the amino terminus of **2**. Replacement of Lys in **18** with Nle to yield **19** maintained ACE inhibition activity as expected from the data presented in Table I, and it also yielded a peptide analogue that is completely stable to trypsin. At the same time, the Nle–Phe bond in **19** is now a site for degradation by pepsin. Peptide analogue **19**, however, has a half-life of 5 h in rat stomach homogenate and 7 h in rat intestinal homogenate. We felt this was a long enough time for a significant level of **19** to be absorbed if it was given to rats orally.

Table IV shows the effects of intravenously administered **18** and **19** on the angiotensin I induced blood pressure rise seen in normotensive rats. Both **18** and **19** appear to be between 3 and 10 times more potent than captopril on a molar basis in this assay. When peptide **19** was given orally to unanesthetized normotensive rats at 10 or 100 mg/kg, no inhibition of the angiotensin-induced blood pressure rise was seen. Thus even though **19** appeared to be sufficiently stable to peptidase degradation to be given orally in rats, it shows no activity by this route.

To investigate the fate of **19** following oral administration in rats, we performed two experiments. First we monitored the penetration of **19** over time through tied off pieces of rat intestine. We monitored penetration at 1 and 2 h and found 54% and 85% penetration, respectively, of intact **19**. We also examined the contents of the rat stomach and intestine 2.5 h following oral dosing with **19** and found 6.9% and 0%, respectively, of the administered **19** still remaining. These two experiments together with the degradation results of **19** by rat stomach and intestinal homogenates indicate that orally administered **19** is absorbed mostly intact from the gastrointestinal tract within

Scheme V. Synthesis of Ketomethylene-Containing Nonapeptide Analogues



2.5 h following administration. The lack of oral activity of **19** as in the case of **1** and **2** is most probably due to its rapid excretion, probably via the bile.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover Uni-Melt instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter. Mass spectra were taken on a Reibermag Model R-10-10-C mass spectrometer using desorption chemical ionization. NMR spectra were taken on either a JEOL FX 90Q, a Varian EM390, or a Varian XL-400 spectrometer. Thin-layer chromatography was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Column chromatography was performed with Woelm dry column silica gel from ICN. Evaporations were performed at 40 °C under house vacuum on a Büchi rotavapor. Elemental analyses were conducted by Galbraith Labs, Knoxville, TN, or Stanford University, Palo Alto, CA. Analytical HPLC was carried out on a Waters 6000A HPLC with a 4.6 mm × 30 cm column containing Vydac TP21854 reverse-phase packing material, with UV visualization at 220 nm with a Schoeffel GM 770 UV spectrometer. Reverse-phase analytical columns were eluted with the appropriate percentage of acetonitrile in water with 0.1% trifluoroacetic acid. Solid-phase peptide synthesis was carried out on a Beckman 990C peptide synthesizer. Some of the compounds could not be totally freed of solvent even on heating under reduced pressure. The elemental analyses of these compounds have been recorded with solvent present. The existence of solvents of crystallization was confirmed by ^{1}H NMR whenever possible.

***N*-(Triphenylmethyl)-L-phenylthioalanine *S*-2-Pyridinyl Ester (4).** A solution of *N*-(triphenylmethyl)-L-phenylalanine[14] (20.0 g, 49.0 mmol) and 2-mercaptopyridine (5.45 g, 49.0 mmol) was stirred in EtOAc in an ice bath under argon, and dicyclohexylcarbodiimide (10.1 g, 49.0 mmol) in EtOAc (10 mL) was added. The mixture was stirred at ice-bath temperature for 30 min and at room temperature for 16 h. The mixture was then cooled in an ice bath and filtered. The filtrate was evaporated to a yellow gum. This gum was purified on silica gel (300 g) in a 600-mL fritted-glass funnel. The material was loaded onto the silica gel with a minimum amount of ether and eluted by pulling low vacuum with a step gradient of 300-mL portions of the following solvents in order: four of petroleum ether (35–60 °C bp) and 16 of 2:1 petroleum ether (35–60 °C bp)–ether. Separate fractions were collected of each 300-mL elution, and fractions 7–20 containing purified product were combined and evaporated to a yellow solid foam, **4**: 20.6 g (84%); R_f 0.50 (hexane–EtOAc, 4:1); ^{1}H NMR ($CDCl_3$) δ 2.70 (m, 2 H), 3.87 (dt, 1 H, J = 8 and 6 Hz), 7.20 (m, 20 H), 7.35 (m, 3 H), 8.50 (dd, 1 H, J = 5 and 1 Hz). This material was used in the next reaction without further purification.

5-Oxo-7-phenyl-6(*S*)-[(triphenylmethyl)amino]-1-heptene (5). The 4-bromobutene Grignard reagent was prepared with use of oven- and then flame-dried glassware under an argon atmosphere. The 4-bromobutene (9.15 mL, 90.6 mmol) was added dropwise at a rate fast enough to maintain reflux to a mixture of Mg turnings (2.21 g, 90.6 mmol) in anhydrous Et_2O containing an iodine crystal. Following this addition, the mixture was refluxed for an additional 1 h and then was allowed to cool to room temperature. This Grignard reagent was added slowly to a stirred solution of **4** (20.6 g, 41.2 mmol) in dry tetrahydrofuran (300 mL) cooled in a dry ice–acetone bath. After 61 mmol of Grignard reagent had been added, the cooling was changed to an ice bath, and the reaction was monitored by TLC while another 10 mmol of Grignard reagent was added. Then 10% aqueous NH_4Cl solution (400 mL) was added. The resulting solution was extracted twice with EtOAc (100 mL + 50 mL). The combined EtOAc extracts were washed with 1 N NaOH (300 mL) and saturated NaCl solution (100 mL) successively. The EtOAc layer was dried (Drierite) and evaporated to a yellow syrup, 19.0 g. This syrup was adhered to silica gel (70 g) and poured onto a short, fat column of silica gel (300 g) in a 600-mL fritted-glass funnel. This column was eluted first with petroleum ether (bp 30–65 °C, 5 × 300 mL) and then successively with the following mixtures of petroleum ether–ether: 20:1, 4 × 300 mL; 13:1, 4 × 300 mL; and (10:1, 4 × 300 mL). Fractions (300 mL each) 7–9 were combined and evaporated to **5** as a clear oil: 15.2 g (82.6%); R_f 0.85 (hexane–EtOAc, 4:1); ^{1}H NMR ($CDCl_3$) δ 1.00–1.90 (m, 4 H), 2.88 (dd, 2 H, J = 7 and 5 Hz), 3.16 (brd, 1 H, J = 10 Hz), 3.60 (m, 1 H), 4.65 (brd, 1 H, J = 7 Hz), 4.85 (s, 1 H), 5.40 (m, 1 H), 7.20 (m, 15 H), 7.35 (m, 5 H); mass spectrum, m/e 445 (M^+). This sample was used without further purification to prepare **6**.

6(*S*)-Amino-5-oxo-7-phenyl-1-heptene Oxalate Salt (6). A solution of **5** (15.2 g, 34.1 mmol) in acetic acid–water (5:1, 200 mL) was stirred vigorously with oxalic acid (3.25 g, 36.1 mmol) for 5 h at room temperature. The reaction mixture was then mixed with H_2O (450 mL) and filtered. The mixture was partially evaporated at 1 mmHg at 50 °C for 30 min to remove most of the acetic acid, and it was then lyophilized to a white solid. This solid was triturated in ether and collected by filtration and dried as **6**, 9.70 g (97%), mp 112–116 °C (softens), 178–190 °C (melts, gas evolves). This sample was used in the next reaction without further purification. An analytical sample was obtained by two recrystallizations of a 300-mg sample from tetrahydrofuran to yield white crystalline **6**: 67 mg; mp 106–108 °C; ^{1}H NMR (methanol-d_4) δ 2.20 (m, 4 H), 3.15 (m, 2 H), 4.42 (m, 1 H), 5.60 (m, 1 H), 7.20 (s, 5 H). Anal. ($C_{15}H_{19}NO_5 \cdot H_2O$) C, H, N.

6(*S*)-[(Butyloxycarbonyl)amino]-5-oxo-7-phenyl-1-heptene (7). Crude **6** (9.5 g, 32.4 mmol) was slurried in HPLC grade CH_2Cl_2 (250 mL) and mixed with di-*tert*-butyl dicarbonate (11.2 mL, 48.6 mmol). This mixture was stirred in an ice bath, and triethylamine (6.8 mL, 48.8 mmol) was added. This mixture was stirred at ice-bath temperature for 30 min and at room temperature for 15 h. The mixture was then washed successively with ice-cold 2 N HCl (200 mL), saturated $NaHCO_3$ solution (250 mL), and saturated NaCl solution (250 mL). The organic layer was dried (Drierite) and evaporated to a yellow syrup, which crystallized on standing. The crystals were slurried in hexane, cooled to 5 °C, collected by filtration, and dried as white crystalline **7**: 7.25 g; mp 77–78 °C. Purification of the mother liquor on silica gel (80 g) with a gradient elution with hexane to 20% ether in hexane yielded more **7**: 0.600 g (total yield 79.8%); mp 77–78 °C; R_f 0.70 (hexane–EtOAc, 4:1); ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9 H), 2.38 (m, 4 H), 2.97 (m, 2 H), 4.50 (m, 1 H), 4.93 (m, 3 H), 5.60 (m, 1 H), 7.16 (m, 5 H). Anal. ($C_{18}H_{25}NO_3$) C, H, N.

5(*S*)-[(Butyloxycarbonyl)amino]-6-phenyl-4-oxohexanoic Acid (8). A solution of **7** (1.00 g, 3.30 mmol) in acetone (70 mL) was stirred at room temperature, and a yellow solution containing $NaIO_4$ (5.30 g, 24.8 mmol) and 51% RuO_2 (50 mg) in H_2O (30 mL) was added. The reaction mixture was stirred vigorously for 20 min. Then, 2-propanol (5 mL) was added, and stirring was continued for 10 min. Celite (5 g) was added to the reaction mixture, and it was filtered through a Celite pad into an aqueous solution containing 2% aqueous $NaHCO_3$ solution (100 mL). The filter cake was washed with $CHCl_3$ (30 mL), and the two-phase filtrate was extracted with $CHCl_3$ (100 mL). The aqueous layer was stirred with ice (50 g) and $CHCl_3$ (100 mL) and acidified to pH 5. The $CHCl_3$ layer was separated, and the aqueous layer was reextracted with $CHCl_3$ (50 mL). The two pink $CHCl_3$ extracts were combined and washed with an aqueous solution of 2% Na_2SO_3 (100 mL) that had been adjusted to pH 4.0 with 2 N HCl. The colorless organic layer was then washed with saturated aqueous NaCl solution (100 mL), dried (anhydrous Na_2SO_4), and evaporated to white solid **8**: 0.719 g (71.2%), mp 111–112 °C; ^{1}H NMR ($CDCl_3$) 1.40 (s, 9 H), 2.65 (m, 4 H), 3.05 (m, 2 H), 4.53 (brs, 1 H), 5.13 (m, 1 H), 7.20 (s, 5 H), 8.23 (brs, 1 H). Anal. ($C_{17}H_{23}NO_5$) C, H, N.

N^α-[*N*-(*tert*-Butyloxycarbonyl)-L-prolyl]-N^γ-(benzyloxycarbonyl)-L-lysine (9). A DMF (30 mL) solution of *N*-(*tert*-butyloxycarbonyl)-L-proline succinimido ester[15] (1.90 g, 6.08 mmol), N^γ-(benzyloxycarbonyl)-L-lysine[2] (1.70 g, 6.08 mmol), and triethylamine (0.85 mL, 6.11 mmol) was stirred at ice-bath temperature for 30 min and at room temperature for 15 h. Then, an additional (0.85 mL, 6.11 mmol) of triethylamine was added,

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and the reaction mixture was stirred for an additional 4 days at which time the solution was clear. The reaction mixture was then evaporated at 40 °C at 1-mmHg vacuum to a pale yellow syrup. This syrup was dissolved in EtOAc (50 mL) and washed successively with ice-cold 2 N HCl (50 mL), H_2O (50 mL), and saturated NaCl (40 mL). The EtOAc layer was dried (Na_2SO_4) and evaporated to a white solid foam, 2.88 g. This foam was triturated in hexane, and the resulting amorphous white solid was collected by filtration and dried as **9**: 2.69 g (92.8%); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9 H), 1.10–2.35 (m, 10 H), 3.15 (m, 2 H), 3.40 (brs, 2 H), 4.27 (brs, 1 H), 4.55 (m, 1 H), 5.03 (s, 2 H), 7.28 (s, 5 H); mass spectrum, m/z 478 ($M^+ + H$), 378 ($M^+ - t$-Boc + H_2). Anal. ($C_{24}H_{35}N_3O_7 \cdot 0.5H_2O$) C, H, N.

***N*-[*N*-(*tert*-Butyloxycarbonyl)-L-prolyl]-L-norleucine Methyl Ester (10).** A solution of *N*-(*tert*-butyloxycarbonyl)-L-proline (4.03 g, 18.7 mmol), L-norleucine methyl ester[16] (1.87 g, 12.5 mmol), and 1-hydroxybenzotriazole hydrate (1.91 g, 12.5 mmol) in CH_2Cl_2 (30 mL) was stirred in an ice bath while dicyclohexylcarbodiimide (2.83 g, 13.2 mmol) in CH_2Cl_2 (10 mL) was added. The reaction mixture was stirred at ice-bath temperature for 30 min and at room temperature for 16 h. The mixture was then cooled in an ice bath and filtered. The filtrate was washed successively with ice-cold 0.2 N HCl (50 mL), 1.0 N NaOH (50 mL), H_2O (50 mL), and saturated NaCl solution (50 mL). The organic layer was dried (Drierite) and evaporated to a semisolid syrup, 5.02 g. This syrup was triturated in cold EtOAc (5 mL), and dicyclohexylurea was removed by filtration. The filtrate was evaporated to a clear oil, which crystallized on standing. The crystals were triturated in hexane and collected and dried as white needles, **10**: 2.35 g; mp 61–63 °C; R_f 0.67 (3% CH_3OH in $CHCl_3$). The filtrate was evaporated to a pale yellow syrup. TLC indicated that this syrup contained quite a bit of Boc-Pro-OBt. Therefore, this syrup was dissolved in CH_3OH (20 mL), and saturated aqueous $NaHCO_3$ solution was added dropwise until the solution remained cloudy for 10 min (45 min total time required). The mixture was concentrated in vacuo to approximately 10 mL and then was partitioned between ether (50 mL) and 0.2 N NaOH (30 mL). The ether layer was washed successively with H_2O (15 mL) and saturated aqueous NaCl solution (15 mL) and dried (Drierite). After evaporation, the pale yellow syrup 1.76 g that was obtained was seeded with **10** and triturated with hexane. The white crystals that formed were collected by filtration and dried to yield more **10**: 1.06 g (total yield 79.7%); mp 62–64 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 6 Hz), 1.31 (m, 4 H), 1.51 (s, 9 H), 1.65–2.40 (m, 6 H), 3.42 (m, 2 H), 3.73 (s, 3 H), 4.30 (m, 1 H), 4.55 (m, 1 H). Anal. ($C_{17}H_{30}N_2O_5$) C, H, N.

***N*-[*N*-(*tert*-Butyloxycarbonyl)-L-prolyl]-L-norleucine (11).** A solution of **10** (2.00 g, 5.84 mmol) was stirred in MeOH (25 mL), and 1 N NaOH (8.5 mL) was added dropwise over an 8-min period. The reaction solution was stirred at room temperature for 3 h and was then partitioned between petroleum ether (bp 35–60 °C, 100 mL) and H_2O (60 mL). The H_2O layer was separated and stirred with ice and $CHCl_3$ (50 mL) while it was acidified to pH 5 by addition of 2 N HCl. The $CHCl_3$ layer was separated, washed with saturated aqueous NaCl solution (50 mL), and dried (Na_2SO_4). Evaporation of the dried $CHCl_3$ solution yielded **11** as a pale yellow syrup: 1.09 g (56.8%); R_f 0.36 ($CHCl_3$, CH_3OH, CH_3COOH, 25:1.2:0.1); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, J = 6 Hz), 1.27 (m, 4 H), 1.47 (s, 9 H), 1.57–2.35 (m, 6 H), 3.43 (m, 2 H), 4.27 (m, 1 H), 4.50 (m, 1 H), 6.88 (brs, 2 H). Anal. ($C_{16}H_{28}N_2O_5 \cdot 0.5H_2O$) C, H, N.

***N*-(Butyloxycarbonyl)-2(*S*)-(1-oxo-4-pentenyl)pyrrolidine (12).** The 4-magnesiobromobutene (233 mmol) was prepared by the procedure described in the preparation of compound **5**. This Grignard reagent was added in a slow stream to a solution of *N*-(butyloxycarbonyl)-L-thioproline *S*-2-pyridinyl ester[17] (28.8 g, 13.5 mmol) in dry tetrahydrofuran (400 mL) with stirring in a −5 °C bath. The reaction was monitored by TLC, R_f(product) 0.50 (30% EtOAc in petroleum ether, bp 30–60 °C), and Grignard addition was stopped after approximately half of it had been added. The reaction was then mixed with 10% aqueous NH_4Cl solution (600 mL). The resulting solution was extracted twice with EtOAc (150 mL + 75 mL). The combined EtOAc extracts were washed with 0.1 N NaOH (450 mL) and saturated aqueous NaCl solution (150 mL) successively. The EtOAc layer was dried (Drierite) and evaporated to give 26.4 g of a yellow syrup. This syrup was purified on a short, fat column of silica gel (700 g), eluting with a step gradient of 1–20% EtOAc in petroleum ether (bp 30–60 °C) to give 10.6 g of **12** as a yellow syrup. Preparative HPLC of the impure fractions was conducted on a silica gel cartridge on a Waters 500 HPLC. Elution with 15% EtOAc in petroleum ether and pooling of the purified fractions gave an additional 5.19 g of **12** (total yield 66.6%): ^{1}H NMR ($CDCl_3$) δ 1.43 (d, 9 H, J = 3 Hz), 1.87 (m, 4 H), 2.43 (m, 4 H), 3.50 (m, 2 H), 4.29 (m, 1 H), 4.90, 5.00, 5.10 (3 s, 2 H), 5.73 (m, 1 H);[1] mass spectrum, m/z 252 ($M - H^-$). Anal. ($C_{14}H_{23}NO_3$) C, H, N.

5-[*N*-(Butyloxycarbonyl)-2(*S*)-pyrrolidinyl]-5-oxopentanoic Acid (13). A solution of **12** (10.0 g, 39.5 mmol) in acetone (535 mL) was stirred in an ice bath. A mixture of RuO_2 (263 mg, 1.97 mmol) and $NaIO_4$ (42.1 g, 197 mmol) in water (275 mL) was heated on a steam bath until all solids had dissolved. This solution was cooled in an ice bath until the RuO_4 began to precipitate and was then added to the above acetone solution over a 5-min period. The mixture became so thick that stirring was stopped, and more acetone (100 mL) was added to allow stirring to resume. After being stirred in an ice bath for 1 h, the mixture was filtered through Celite, and the filtrate was stirred with 2-propanol (6 mL) for 10 min. The filtrate was then saturated with NaCl and extracted with $CHCl_3$ (2 × 1 L). The $CHCl_3$ extracts were combined, washed with saturated NaCl solution (500 mL), dried (Na_2SO_4), and evaporated to a brown syrup, 13.6 g. This syrup was mixed with $CHCl_3$ (200 mL) and stirred with a solution containing $NaHCO_3$ (10.0 g, 0.12 mol) in water (400 mL). After stirring for 5 min, the layers were separated, and the aqueous layer was stirred with $CHCl_3$ (400 mL) in an ice bath, and 2 N HCl (65 mL) containing 35 g of ice was added. The $CHCl_3$ layer was separated, washed with saturated NaCl solution (200 mL), and dried (Na_2SO_4). The dried $CHCl_3$ solution was evaporated to a light pink syrup, 11.0 g. This syrup was crystallized from EtOAc–hexane to yield crystalline **13**: 5.31 g, mp 89–90 °C. More **13** was obtained from the mother liquor: 1.71 g (total yield 65.6%); R_f 0.70 (MeOH–$CHCl_3$–AcOH, 1:9:0.1); $[\alpha]^{20}_D$ −65.0° (c 4.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.42 (d, 9 H, J = 3 Hz), 1.92 (m, 4 H), 2.70 (m, 4 H), 3.49 (m, 2 H), 4.30 (m, 1 H), 9.52 (brs, 1 H); mass spectrum, m/z 270 ($M - H^-$). Anal. ($C_{13}H_{21}NO_5$) C, H, N.

***N*-(Butyloxycarbonyl)-*O*-[(2,6-dichlorophenyl)methyl]-L-tyrosine Methyl Ester (14).** A mixture of *N*-(butyloxycarbonyl)-*O*-[(2,6-dichlorophenyl)methyl]-L-tyrosine (5.0 g, 11.3 mmol) and $NaHCO_3$ (1.91 g, 22.7 mmol) in dry dimethylformamide (50 mL) was mixed with methyl iodide (3.5 mL, 56.5 mmol) and stirred at room temperature for 18 h. The mixture was then mixed with H_2O (150 mL) and extracted with EtOAc (100 mL). The EtOAc layer was washed with H_2O (100 mL), dried (Drierite), and evaporated to a yellow solid, 4.96 g. This solid was slurried in refluxing hexane–ether (1:10, 110 mL) and cooled to 5 °C for 15 h, and pale yellow fluffy crystals were collected by filtration and dried as **14**: 3.51 g, mp 110–112 °C. More **14** (0.645 g) was obtained from the mother liquor for a total yield of 80.9%: ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9 H), 3.03 (d, 2 H, J = 5 Hz), 3.72 (s, 3 H), 4.60 (m, 1 H), 5.25 (s, 2 H), 7.00 (m, 4 H), 7.29 (m, 3 H). Anal. ($C_{22}H_{25}Cl_2NO_5$) C, H, N.

***O*-[(2,6-Dichlorophenyl)methyl]-L-tyrosine Methyl Ester Hydrochloride (15).** Compound **14** (4.00 g, 8.80 mmol) was dissolved and stirred in dioxane (15 mL, passed through alumina) that had been saturated with gaseous HCl. After 1.25 h, the mixture was evaporated to a white solid. This solid was triturated in ether and collected by filtration as white solid **15**: 3.34 g (86%); mp 182 °C with gas evolution; R_f 0.60 (10% CH_3OH in $CHCl_3$). Anal. ($C_{17}H_{18}Cl_3NO_3$) C, H, N.

***N*-(Cyclobutylcarbonyl)-*O*-[(2,6-dichlorophenyl)methyl]-L-tyrosine Methyl Ester (16).** A mixture of **15** (3.30 g, 8.45 mmol) and triethylamine (2.90 mL, 20.8 mmol) in CH_2Cl_2 (90 mL) was stirred in an ice bath, and cyclobutanecarboxylic acid chloride (1.36 mL, 12.7 mmol) was added. The mixture was stirred at ice-bath temperature for 30 min and at room temperature for 2.5 h. At this time, the mixture was washed successively with 0.3 N NaOH (90 mL), H_2O (90 mL), 2 N HCl (90 mL), and H_2O

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(90 mL). The organic layer was dried (Na_2SO_4) and evaporated to a white solid, 3.83 g. This solid was triturated in refluxing ether (70 mL), and a white solid was collected by filtration and dried as pure 16: 2.83 g; mp 138–139 °C. The mother liquor was cooled in the refrigerator for 15 h, and more 16 was obtained as cottonlike white crystals: 0.305 g (total yield 84.8%); mp 138–140 °C; ^1H NMR ($CDCl_3$) δ 2.15 (m, 6 H), 3.10 (m, 3 H), 3.70 (s, 3 H), 4.85 (m, 1 H), 5.25 (s, 2 H), 5.75 (m, 1 H), 6.95 (m, 4 H), 7.25 (m, 3 H). Anal. ($C_{22}H_{23}Cl_2NO_4$) C, H, N.

***N*-(Cyclobutylcarbonyl)-*O*-[(2,6-dichlorophenyl)-methyl]-L-tyrosine (17).** To a stirred solution of 16 (3.00 g, 6.87 mmol) in CH_3OH (30 mL) was added 1 N NaOH (10 mL, 10 mequiv) dropwise over a 5-min period. The mixture was then mixed with THF (9.0 mL), and after 30 min, the clear solution was acidified with 2 N HCl to pH 5. The CH_3OH and THF were then removed by evaporation, and the resulting white solid slurry was extracted with $CHCl_3$ (50 mL + 2 × 20 mL). The $CHCl_3$ extracts were combined and washed with saturated NaCl solution (50 mL). The organic layer was dried (anhydrous Na_2SO_4) and evaporated to a white solid foam, 3.12 g. The foam was crystallized from $CHCl_3$ (3 mL) and ether (20 mL) to white crystalline 17: 2.68 g (92.4%); mp 172–174 °C. Anal. ($C_{21}H_{21}Cl_2NO_4$) C, H, N.

Synthesis of Cbc-Tyr-Pro-Gly-Pro-Lys-Phe-Gly-Pro (3). This nonapeptide analogue was prepared with Boc-Pro-O-Merrifield resin (1.0 g, 0.73 mequiv, Peninsula Labs, Belmont, CA) as starting material with use of programs 1 and 2 on a Beckman 990C peptide synthesizer. These programs gave double coupling (2 h each) with DCC in CH_2Cl_2 of the appropriate Boc amino acids with side-chain protection on Tyr and Lys consisting of 2,6-dichlorobenzyl and *m*-chlorobenzyloxycarbonyl, respectively. After removal of the Boc group of tyrosine and neutralization, cyclobutanecarboxylic acid chloride (25-fold excess) in CH_2Cl_2 (8 mL) was added followed by 25 equiv of diisopropylethylamine, and coupling was allowed to occur over a 2-h period. After this time a negative Kaiser test[18] was achieved.

The completed peptide was cleaved from the resin by stirring with 10% anisole in anhydrous HF at 0–5 °C for 1 h. The HF was removed by vacuum, and the peptide + resin was washed with ether (3 × 15 mL). The peptide was extracted with trifluoroacetic acid and evaporated. The residue was triturated with ether, and the resulting white powder was collected by filtration and washed with ether. After drying, it was lyophilized from 0.5% aqueous acetic acid to 454 mg of crude product as a white solid. The crude product was purified by gel filtration on a Sephadex LH-20 column (2.5 × 83 cm) eluting with 0.1% aqueous acetic acid. The desired product was obtained following lyophilization of the appropriate fractions as a white fluffy solid 3, 198 mg (28.8%).

Synthesis of Cbc-Tyr-Proψ($COCH_2$)Gly-Pro-Lys-Pheψ-($COCH_2$)Gly-Pro (18). The synthesis was performed with use of the Beckman 990C programs 1 and 2 except that the neutralization steps were replaced with washes of 80% 2-propanol in CH_2Cl_2, and the coupling reagents and dipeptide analogues to be coupled were added manually. The synthesis started with Boc-Pro-*O*-Merrifield resin (1.2 g, 1.06 mequiv, Peninsula Labs, Belmont, CA). After deprotection and washing, a solution of 8 (0.487 g, 1.58 mmol) in DMF (1.0 mL) was mixed with 1-hydroxybenzotriazole (HOBt) (0.242 g, 1.58 mmol) and CH_2Cl_2 (2.0 mL) and stirred in an ice bath while DCC (0.359 g, 1.74 mmol) in CH_2Cl_2 (2.0 mL) was added. This mixture was stirred at room temperature for 10 min, and then diisopropylethylamine (DIEA) (2.75 mL, 1.58 mmol) was added, and the resulting mixture was filtered and added to the peptide synthesis vessel that contained TFA·Pro-*O*-Merrifield resin. The dipeptide mixture was rinsed in with CH_2Cl_2 (3.0 mL), and more CH_2Cl_2 was added until a reaction volumn of approximately 10 mL was achieved. The coupling reaction was conducted for 2 h, and a Kaiser test[18] at this point indicated that the coupling was not complete. A recoupling with 8 (0.161 g, 0.500 mmol) and the same coupling reagents as above for 2 h gave a negative Kaiser test. The peptide-resin was then acetylated for 45 min with acetic anhydride (1.0 mL, 11 mmol) and pyridine (0.1 mL, 1.24 mmol) in CH_2Cl_2 (9 mL).

After the deprotection and washing steps, the next dipeptide, 9 (1.00 g, 2.07 mmol), was coupled for 5 h by using the same procedure and ratio of coupling and neutralization reagents as described for the coupling of 8. A negative Kaiser test was obtained, and the peptide-resin was acetylated as described above.

After the deprotection and washing steps, the next dipeptide, 13 (0.543 g, 2.00 mmol), was coupled for 17 h by using the same procedure and ratio of coupling and neutralization reagents as described for the coupling of 8. A second coupling for 5 h with 0.250 g (0.921 mmol) of 13 and the same procedure and ratio of coupling and neutralizing reagents was needed to give a negative Kaiser test. The peptide-resin was then acetylated as described previously.

After the deprotection and washing steps, the final dipeptide, 17 (0.845 g, 2.00 mmol), was coupled for 17 h by using the same procedure and ratio of coupling and neutralizing reagents as described for the coupling of 8. A negative Kaiser test was obtained at this point.

The peptide was cleaved from the resin and isolated by using the method described for the isolation of Cbc-Tyr-Pro-Gly-Pro-Lys-Phe-Gly-Pro. The crude peptide, 1.21 g, obtained was purified in $^1/_2$ portions on a LH-20 Sephadex column (2.5 × 90 cm) using 30% acetic acid in water as the eluting solvent. The fractions were examined by HPLC, and the pure fractions (>95%) were combined and lyophilized to a white powder, 18: 616 mg (61.9%); ^{13}C NMR (CD_3OD) δ (line height, mm) 19.0 (30), 23.5 (34), 25.5 (25), 25.6 (47), 25.7 (36), 25.9 (29), 26.3 (33), 27.6 (25), 28.8 (28), 29.0 (35), 29.1 (38), 30.4 (38), 30.6 (25), 32.0 (21), 34.6 (22), 35.3 (26), 36.9 (28), 38.2 (22), 40.3 (33), 40.6 (33), 54.1 (45), 60.4 (24), 60.9 (35), 61.4 (28), 66.5 (27), 116 (86), 127 (41), 128 (19), 129 (110), 130 (83), 131 (76), 138 (16), 158 (17), 172 (15), 173 (17), 173 (14), 174 (16), 175 (12), 176 (12), 176 (8), 177 (11), 209 (14), 210 (11). Anal. ($C_{50}H_{67}N_7O_{11}\cdot CF_3COOH\cdot 2H_2O$) C, H, N.

Synthesis of Cbc-Tyr-Proψ($COCH_2$)Gly-Pro-Nle-Pheψ-($COCH_2$)Gly-Pro (19). The synthesis started with Boc-Pro-*O*-Merrifield resin (0.6 g, 0.528 mequiv, Peninsula Labs, Belmont, CA) and was carried out by using the modified procedure for washings and couplings described for the preparation of 18. The dipeptides required for this synthesis were coupled until a negative Kaiser test[18] was obtained in the following order and manner: Dipeptide 5 (0.254 g, 0.792 mmol) required a single coupling for 17 h. The peptide-resin was acetylated at this point by using the procedure described in the preparation of 18. Dipeptide 11 (0.347 g, 1.056 mmol) required a single coupling for 13 h. Dipeptide 13 required two couplings of 0.286 g (1.056 mmol) for 20 h and 0.136 g (0.500 mmol) for 4 h. Dipeptide 17 required two couplings of 0.446 g (1.056 mmol) for 22 h and 0.211 g (0.500 mmol) for 1.5 h.

The peptide was cleaved from the resin and isolated in crude form by using the procedure described for Cbc-Tyr-Pro-Gly-Pro-Lys-Phe-Gly-Pro isolation. The crude peptide, 0.464 g, was purified by elution through a LH-20 Sephadex (145 g) column with 30% acetic acid in water. The fractions that correspond to >95% purity by HPLC were combined and lyophilized to white powdery 19, 0.273 g (55.9%). Anal. ($C_{50}H_{66}N_6O_{11}\cdot 2H_2O$) C, H, N.

Enzymatic Degradation Studies of Peptide Analogues. Trypsin, chymotrypsin, and pepsin were obtained from Sigma Chemical Co.

Trypsin. This assay is described in ref 2.

Chymotrypsin. Chymotrypsin (37 units) was dissolved in cold 0.001 N HCl (50 mL). A solution containing peptide (0.5–1.0 μmol) and Tris buffer (0.1 M, pH 7.8, 0.580 mL/μmol of peptide) was stirred at room temperature, and 0.050 mL/μmol of peptide of the above chymotrypsin solution was added. The mixture was stirred and monitored by TLC as previously described for the trypsin assay.[2]

Pepsin. The peptide (0.5–1.0 μmol) was dissolved in 0.300 mL of H_2O containing an equal equivalent of 1 N NaOH. This solution was neutralized with 2 N HCl and then was mixed with 0.600 mL of 0.04 M $NaHCO_2$ buffer (pH 2.0). Pepsin (760 units) was dissolved in $NaHCO_2$ buffer (0.04 M, 0.120 mL, pH 2.0). The two solutions were then mixed, and the degradation study was carried out at 37 °C and monitored by TLC as described previously for the trypsin assay.[2] A peptide, Asp-Arg-Gly-Phe-Tyr-Phe-Asn-Lys-Pro-Thr-Gly-TyrGly-Ser-Ser-Ser-Arg-Arg-Ala

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prepared in our laboratory was cleaved by pepsin at a 50% level in 10 min by using this assay.

Rat Stomach Homogenate. A fresh rat stomach was homogenized in physiological saline (2 mL). The peptides (0.7 μmol) were dissolved in 30 μL of 0.1 N NaOH and mixed with 90 μL of 0.25% methyl Cellosolve in H_2O. The solution was partially neutralized with 27 μL of 0.1 N HCl. This solution was mixed with 150 mg of rat stomach homogenate and incubated at 37 °C. Degradation was monitored by TLC as described previously for trypsin.[2]

Rat Intestinal Homogenate. Rat small intestine was removed immediately after the rat was sacrificed and was minced in Krebs Ringer solution (10 mL/1 g of intestine). Peptide samples (0.2–0.5 μmol) were mixed with homogenate (1.0 mL of homogenate/1.0 μmol of peptide) and then shaken in a 37 °C bath. Degradations were monitored by TLC as described previously for trypsin.[2]

Angiotensin I Converting Enzyme Inhibition Assay in Normotensive Rats. The testing of compounds for ACE inhibition in rats was performed by Pharmakon Research Internation Inc., Waverly, PA. The testing methods that were employed are described below.

For testing of the inhibitors by the intravenous route, first, male Sprague–Dawley normotensive rats (290-540 g) were anesthetized with sodium pentobarbital at 50 mg/kg, ip, and supplemented as necessary. A patent airway was maintained by tracheal intubation for spontaneous respiration. The jugular vein was catherized with PE50 tubing for intravenous administration of angiotensin I and test compound. The carotid artery was catherized with PE50 tubing for measurement of pulsatile blood pressure. The blood pressure was measured with a Statham P23Db pressure transducer and Grass Model 7 polygraph. Angiotensin I at 175 ng/kg in distilled water was administered intravenously following iv injection of 1 mL/kg of the test compound vehicle to determine the control blood pressure response to angiotensin I. The test compound dissolved in distilled water or 5% sodium bicarbonate solution was then given iv at the desired dose. Blood pressure was then monitored while angiotensin I at 175 ng/kg in distilled water was given iv at 1.5, 5, 10, 15, 20, 30, 45, 60, and 75 min or until the blood pressure response to angiotensin had returned to normal. An ID_{50} dose was calculated as the inhibitor dose that decreased the rise in blood pressure expected for 175 ng/kg of iv angiotensin I by 50%.

For the testing of inhibitors by the oral route, male Sprague–Dawley normotensive rats (250–320 g) were cannulated for blood pressure and heart rate monitoring by the method of Weeks and Jones.[19] Prior to administration of the test compound, a control pressor response to angiotensin I at 350 ng/kg ia was determined. The test compound was dissolved in 5% sodium bicarbonate at the desired dose and administered orally. Angiotensin I was readministered to each rat at 10, 20, 30, 60, 90, 120, 150, 180, 210, 240, and 300 min and every 30 min for 3 more h, and blood pressure was monitored.

Peptide Penetration of Rat Intestine. Three 1.5-in. sections of freshly isolated rat intestine were rinsed inside and out with Krebs Ringer and then tied off at one end. Then, 0.2 mL of Krebs Ringer solution containing 1.0 mg of peptide was added to the inside of each of the intestinal sections through a blunt intubation tube. The second end of the intestinal sections were tied off, and they were incubated at 37 °C in 5 mL of Krebs Ringer for 0.5 h. The sections were then transferred to fresh 5-mL solutions of Krebs Ringer and incubated at 37 °C. Additional transfers and incubations were made at 1, 2, and 2.5 h. At the end of the incubation period, the intestinal sections were cut up and extracted with Krebs Ringer (5.0 mL). Concentrations of intact peptides in each incubation solution were measured by HPLC peak integration of a 20-λ injection compared to 20 λ of a peptide solution (1.0 mg in 5.2 mL of Krebs Ringer). A 4.6 mm × 25 cm Vydac TP21854 column was used and eluted with 35% CH_3CN in H_2O + 0.1% TFA.

The leakiness of tied intestinal sections like those used in these assays was assessed by pouring a nonpenetrating high molecular weight blue dye into them and tying off the ends and incubating them in Krebs Ringer (5 mL) for 3 h. There was no penetration of dye into the Krebs Ringer during this time period.

Assay of 19 in Rat Stomach and Intestine 2.5 h following Oral Dosing. Peptide **19** (5.3 mg) was mixed with 0.25% methyl Cellosolve in H_2O (2.5 mL). A 1.5-mL portion of this mixture was given to each rat (starved 72 h) by intubation into the stomach. Control rats (starved 72 h) were given 1.5 mL of 0.25% methyl Cellosolve in H_2O. After 2.5 h, the stomach and upper and lower small intestine were removed and homogenized first in 0.1 M NaH_2PO_4 buffer (pH 8.0, 5.0 mL) and then in EtOH (5.0 mL). After centrifugation, each supernatant was examined by HPLC (described in rat intestinal penetration assay) compared to a standard solution of **19** to obtain a percent recovery value.

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Clofazimine Analogues Active against a Clofazimine-Resistant Organism

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Clofazimine analogues active against a strain of *Mycobacterium smegmatis* 607 made resistant to the antileprosy agent have been synthesized. Activity (i.e., ≤2 μg/mL causing complete inhibition of growth) requires that there be a basic nitrogen in the "rimino" side chain and that the spacer distance between this nitrogen and the imino nitrogen be at least three carbon atoms. The nitrogen may be primary, secondary, or tertiary and may be part of an open chain or enclosed in a ring compound. Provided that the criteria of basicity and spacer distance are satisfied, all are active in vitro against both the sensitive and resistant strains. Substitution elsewhere in the molecule had little effect on the activity. The compounds have been shown to have growth inhibitory activity against human-derived *Mycobacterium leprae* in murine macrophages in culture.

Resistance to antileprosy agents such as rifampicin and dapsone has already been established,[1-5] and more recently, a case of clofazimine-resistant leprosy has been reported.[6] Clofazimine originated in the MRC Laboratories, and we have since 1977, under the aegis of WHO, been seeking clofazimine analogues that would be active against clof-

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