50% aqueous HCl (20 mL) and extracted with Et_2O. The extracts were combined and washed with 50% aqueous HCl (10 mL) and then H_2O and dried to give 3-[*cyano*-^{14}C]cyanophenol, which was used without further purification. This product was reduced with Ni/Al alloy and added in three equal portions (1.27 g of 50% dispersion) to a solution in 75% aqueous HCO_2H (2.8 mL), with heating under reflux for 6 h. The reaction mixture was then filtered, washed with 75% aqueous HCO_2H, and evaporated to dryness. The residue was extracted with Et_2O, and the extracts were dried over anhydrous $MgSO_4$. Evaporation afforded 3-hydroxy[*carbonyl*-^{14}C]benzaldehyde (310 mg, 2.5 mmol, 59 mCi).

The above product was mixed with piperidine (454 mg, 5.4 mmol) in EtOH (2.5 mL) and stirred for 45 min, followed by reduction using Pd/C catalyst (177 mg, 10% w/w) added in two portions during 5 h. The reaction mixture was filtered and the filtrate evaporated to dryness. The residue was purified by preparative TLC (SiO_2, EtOAc/MeOH/NH_4OH, 5:1:1) to give product containing unreacted aldehyde, which was subsequently removed by acid/base extraction to give **70** (203 mg, 1.1 mmol, 25 mCi, 27% overall radiochemical yield).

Tritiation of Unlabeled Materials. Compounds **7**, **12**, **16**, **17**, **26**, and **29–31** were tritiated by using the TR8 procedure by Amersham International plc, which entailed approximately 5 mg of each compound being treated with ^3H_2O of high isotopic abundance, generally under neutral conditions, in a suitable solvent such as CH_3CN or DMF. After tritiation, the compounds were diluted with unlabeled material and subjected to thin-layer chromatographic purification in a suitable system. The compounds were stored in EtOH at −25 °C.

Brain-Penetration Measurements. Brain penetration was estimated by using the method described previously.[70] An intravenous (iv) bolus of radiolabeled antagonist in saline was administered to urethane-anesthetized male rats and an iv infusion maintained for 2–3 h or until radioactivity in the blood reached a plateau. Steady-state blood concentrations in the range 1–10 μM were explored. In some cases (viz. compounds **4–6**, **8**, **30**, **37**, **41**, and **42**), concentrations as low as 2.6 nM were also examined and for mepyramine a 1000-fold range of concentrations was investigated (2.4 nM–2.38 μM). In no case was a concentration-dependent difference in the measured brain/blood ratio apparent. The rat was next exsanguinated and the brain removed. Brain tissues were weighed and solubilized in Soluene-100. Aliquots of this were scintillation counted with Dimilume-30 scintillant in the presence of glacial acetic acid to prevent chemiluminescence. Dose solutions and blood samples were counted in Pico-fluor 15 scintillant in a Searle Mk III liquid scintillation counter. Samples were quench corrected by using an automated external standard method. The extent of drug entry into brain was estimated as the ratio of radioactivity in brain to that in peripheral blood at the end of infusion, and the data are given in Table VII.

The proportion of parent antagonist in the peripheral circulation and in the brain, for most of the compounds studied, was determined by thin-layer chromatography (TLC). Plasma (5 or 10 μL) and ethyl acetate brain extract (50 μL) were applied to TLC plates (Merck Kieselgel 60F_{254nm}, 20 cm × 250 μm or Camlab combination layer Kieselgel/silica gel) under an atmosphere of N_2 and allowed to dry. Elution was performed in an appropriate solvent system (see Table VII), and the proportion of radioactivity associated with the parent antagonist was quantified with use of a Berthold linear analyzer. In all cases where significant metabolism occurred, metabolites were more polar than the parent compound, as judged by their chromatographic mobility. It has been assumed that none of the circulating metabolites entered the brain and that the steady state achieved in these experiments represents an equilibrium between parent compound in the blood and the brain. The use of total radioactivity for quantifying brain penetration could therefore result in an underestimate in brain/blood ratios by as much as twofold (for compounds **16** and **30**) due to the presence of radiolabeled metabolites in the blood.

Partition Coefficients and Ionization Constants. Partition coefficients were measured by a conventional shake-flask technique[71] at 37 °C. The concentrations of the compound in the aqueous phase before and after partitioning were determined spectrophotometrically. Buffer salts were used to control the pH of the aqueous phase. The pK_a values of the isocytosine group of **3** were determined spectrophotometrically at 37 °C. The pK_a values of **5**, **6**, and 1-(piperidin-1-ylmethyl)-3-methoxybenzene[72] were determined potentiometrically at 25 °C in 0.1 M KCl. All the partition coefficients and pK_a values are the means of at least two determinations.

Pharmacology. H_2 receptor histamine antagonist activity was determined in vitro in the histamine-stimulated guinea pig right atrium by the method described by Parsons et al.[51] Dose ratios (X) were calculated as the ratio of histamine concentrations required to produce half-maximal responses in the presence and absence of different concentrations (B) of antagonist, and dissociation constants (K_B) were derived from the equation $K_B = B/(X - 1)$.

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Renin Inhibitors Containing ψ[CH_2O] Pseudopeptide Inserts[1]

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Renin inhibitors **2–4** with the D-Lys renin inhibitory peptide (RIP) sequence, but containing Leuψ[CH_2O]Ala (**2**), Leuψ[CH_2O]Val (**3**), and Leuψ[CH_2O]Leu (**4**) at the P_1-P_1' site, were of a comparable potency to RIP. N-Terminal Boc-protected inhibitors containing Proψ[CH_2O]Phe in positions P_4-P_3 were potent inhibitors of renin, with Boc-Phe-Proψ[CH_2O]Phe-His-Leuψ[CH(OH)CH_2]Val-Ile-(2-aminomethyl)pyridine (**17**) having an IC_{50} of 1.6×10^{-9} M.

Renin is a proteolytic enzyme whose only known function is the conversion of angiotensinogen into angiotensin I. Angiotensin I is in turn converted to the potent vasoconstrictor angiotensin II by a converting enzyme.[2] The

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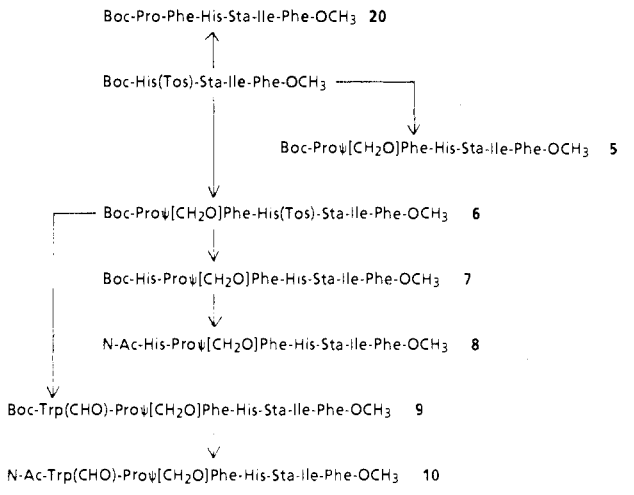
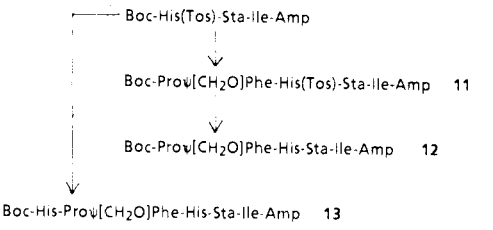
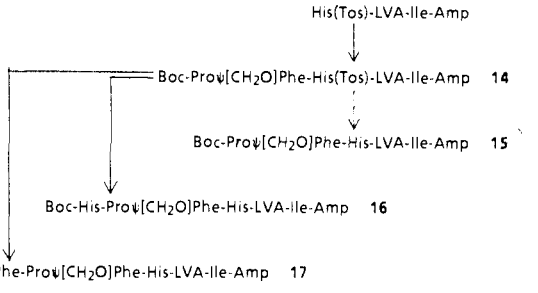
Scheme I



Scheme II



Scheme III



early blockade of the renin–angiotensin system and the substrate specificity of renin make the blockage of renin an exciting target for a mechanism-based approach to the control of blood pressure.

The early inhibitors of renin, pepstatin[3] and renin inhibitory peptide (RIP),[4] suffer from lack of potency, solubility, and duration of action.[5] RIP also suffers from rapid degradation by several common proteases, resulting in the need for continuous infusion to sustain a hypotensive response.[6] Working under the assumption that stability toward peptidases is a necessary (but not necessarily sufficient) condition for a therapeutic agent, we set about as a first step to modify the peptide backbone of a series of angiotensinogen-based inhibitors with the goal of obtaining potent renin inhibitors, which, due to the absence of certain susceptible peptide bonds, would no longer be rapidly degraded.

Szelke et al. prepared a potent inhibitor of renin wherein the amide bond at the cleavage site (Leu-Val in human substrate) was replaced with a secondary amine (CH_2NH).[7] This change from sp^2 to sp^3 carbon is thought to allow the carbon of the "reduced" peptide bond to assume the tetrahedral configuration of the transition state and thus increase significantly the binding energy. In addition, the pseudopeptide bond can no longer be cleaved by renin. We have chosen to modify the peptide backbone at the P_1-P_1' site (transition-state mimic) and at the P_4-P_3 (Pro-Phe) site with $\psi[CH_2O]$ pseudopeptide moieties. The peptide backbone modification at Pro-Phe would also be expected to confer some degree of stability to neighboring amide bonds.

Replacement of the peptide amide (CONH) bond with an "ether" (CH_2O) isostere results in a change from sp^2 geometry about the carbonyl carbon to sp^3 (tetrahedral) geometry in the ether isostere. This change can have rather dramatic effects on the positioning of residues distal to the modified bond. Thus, this modification, while conferring stability to the peptide backbone in that region, also has a high probability of destroying the fit of the

Table I. Inhibition of Renin by $\psi[CH_2O]$[10,11] Peptides[a]

compound	IC_{50}, μM
RIP, Pro-His-Pro-Phe-His-Phe-Phe-Val-Tyr-Lys[b]	7[c]
H-142, Pro-His-Pro-Phe-His-Leuψ[CH_2NH]Val-Ile-His-Lys[d]	0.01
1, Pro-His-Pro-Phe-His-Leuψ[CH_2NH]Val-Ile-His-D-Lys[e]	0.05
2, Pro-His-Pro-Phe-His-Leuψ[CH_2O]Ala-Ile-His-D-Lys	18
3, Pro-His-Pro-Phe-His-Leuψ[CH_2O]Val-Ile-His-D-Lys	1.7
4, Pro-His-Pro-Phe-His-Leuψ[CH_2O]Leu-Ile-His-D-Lys	1.8

[a] Compounds 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 phenylmethanesulfonyl 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. [b] See ref 4. [c] Average value for $n = 24$. [d] See ref 10. [e] Smith, C. W.; Saneii, H. H.; Sawyer, T. K.; Scahill, A.; Skala, G.; Pals, D. T., manuscript in preparation.

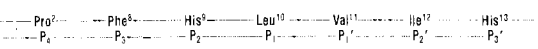


Figure 1. Human angiotensinogen[6–13] sequence with P, P′ positions.

(1) The unit inside the bracket is the unit substituting for the peptide amide (CONH) bond. IUPAC–IUB Joint Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **1984**, *138*, 9. Abbreviations are: Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; Boc, *tert*-butyloxycarbonyl; Ac, acetyl; LVA or Leuψ[CH(OH)CH_2]Val, (2*S*,4*S*,5*S*)-5-[(*tert*-butoxycarbonyl)-amino]-4-hydroxy-2-isopropyl-7-methyloctanoic acid; *t*-BDMS, *tert*-butyldimethylsilyl; Amp, 2-(aminomethyl)pyridine; DCC, dicyclohexylcarbodiimide; DCU, dicyclohexylurea; 1-HOBt, 1-hydroxybenzotriazole; Tos, tosyl; TBDMS, *tert*-butyldimethylsilyl; TFA, trifluoroacetic acid; CH_2Cl_2, dichloromethane; DMF, dimethylformamide; THF, tetrahydrofuran; MeOH, methanol; $CHCl_3$, chloroform; Et_2O, ether.

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Table II. Inhibition of Renin by Proψ[CH_2O]Phe Peptides[a]

compound	IC_{50}, nM
5, Boc-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3	2500
7, Boc-His-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3	440
8, *N*-Ac-His-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3	550
9, Boc-Trp(CHO)-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3	1000
10, *N*-Ac-Trp(CHO)-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3	330
12, Boc-Proψ[CH_2O]Phe-His-Sta-Ile-Amp	140
13, Boc-His-Proψ[CH_2O]Phe-His-Sta-Ile-Amp	100
15, Boc-Proψ[CH_2O]Phe-His-LVA-Ile-Amp	2.3
16, Boc-His-Proψ[CH_2O]Phe-His-LVA-Ile-Amp	3.0
17, Boc-Phe-Proψ[CH_2O]Phe-His-LVA-Ile-Amp	1.6
18, Boc-Pro-Phe-His-Sta-Ile-Amp	4.1
19, Boc-Pro-Phe-His-LVA-Ile-Amp	0.36
20, Boc-Pro-Phe-His-Sta-Ile-Phe-OCH_3	5.8

[a] See footnote *a*, Table I.

peptide to the enzyme. Indeed, ψ[CH_2O] pseudopeptides as transition state type inserts were uniformly disappointing. We have found, however, that the Pro^7-Phe^8 "ether" peptide bond modification does, in fact, stabilize the peptide to chymotrypsin degradation at both Pro^7-Phe^8 and Phe^8-His^9 and that these Proψ[CH_2O]Phe-containing peptides are potent inhibitors of renin.

Chemistry

Compounds **1–4** were assembled via the Merrifield solid phase method[8] according to the protocol of Table IV. Compounds **5** and **7–19** were synthesized in a stepwise fashion via solution techniques (Schemes I–III). The preparation of key intermediates Boc-Proψ[CH_2O]Phe-OH, Boc-Leuψ[CH_2O]Ala-OH, Boc-Leuψ[CH_2O]Val-OH, and Boc-Leuψ[CH_2O]Leu-OH was described elsewhere.[9]

Results

Table I lists three peptides containing ether pseudodipeptides as transition-state inserts (**2–4**). The Leuψ[CH_2O]Ala peptide **2** is somewhat less potent than RIP (renin inhibitory peptide), while the Leuψ[CH_2O]Val and Leuψ[CH_2O]Leu peptides (**3** and **4**) are somewhat better. In this series, the valine and leucine side chains at P_1' (Figure 1) provide a better fit than the alanine side chain. None of these three compounds compare well with the Leuψ[CH_2NH]Val peptide H-142 of Szelke[10] or with the D-Lys version of H-142, **1**.

Table II contains a series of peptides with Proψ[CH_2O]Phe in positions P_4-P_3. The compounds may be grouped into three categories: C-terminal Phe-OCH_3 with statine as the transition-state insert (compounds **5** and **7–10**), C-terminal (amidomethyl)pyridine with statine as the transition-state insert (compounds **12** and **13**), and C-terminal (amidomethyl)pyridine with Leuψ[CH(OH)-CH_2]Val as the transition-state insert (compounds **15–17**).

N-Terminal extension of compound **5** with histidine to give **7** results in a sixfold increase in IC_{50}. Replacing the Boc protecting group of **7** with acetyl (**8**) has no effect on inhibitory potency. However, when the P_3 residue is Trp(CHO), acetyl is preferred as the N-terminal protecting group (**9** vs **10**).

Substitution of the C-terminal Phe-OCH_3 of **5** with 2-(amidomethyl)pyridine gives a renin inhibitor (**12**) with an IC_{50} 18-fold greater. N-Terminal extension with histidine had no effect on inhibitory potency (compound **13**).

Table III. In Vitro Stability of **8** to Degradative Enzymes[a]

	% remaining at 60 min			
	carboxypeptidase Y	chymotrypsin	elastase	pepsin
8	3	100	81	100
RIP	2	3	13	16

[a] Enzymes were purchased from Sigma Chemical Co., St. Louis, MO, and were used without further purification. Each enzyme was dissolved in 50 mM Tris·HCl, pH 7.5 (chymotrypsin 25 units/mL; elastase and carboxypeptidase Y, 50 units/mL). Peptides (0.8 mg/mL) were dissolved in 10% DMSO in Tris buffer. Aliquots (5 μL) of enzyme solution were added to 0.25 mL of peptide solution and incubated at 37 °C for 0 or 60 min. Total incubation volume was 0.255 mL. The incubation was terminated by the addition of an equal volume of solvent B (described below). Stability of the peptides was determined by reverse phase HPLC with a 25 cm × 0.46 mm SynChrom RP-P column. A gradient of an increasing concentration of acetonitrile was used to elute the peptide and any resulting fragments. Solvent A consisted of 50 mM NaH_2PO_4, 1% H_3PO_4, 1% acetonitrile in water. Solvent B consisted of 12.5 mM NaH_2PO_4, 0.25% H_3PO_4 in acetonitrile–water (3:1). A Kontron Model 200 controller was programmed to deliver the solvents starting at 10% B and linearly increasing to 60% B over 15 min. This solvent concentration was held until 30 min before dropping back to 10% B over a 2-min period. The column was allowed to equilibrate for 13 min before another sample was injected. Samples were injected onto the column by a Kontron Model MSI-660 autosampler equipped with a 100-μL sample loop. Column effluent was monitored by a Beckman Model 165 dual-wavelength detector, which measured absorption at 204 and 280 nm. A Hewlett-Packard Model 3390A integrator received the signal from the 204-nm channel, and resulting integrated peak height was compared with the peak height from the 0 time control sample to determine peptide stability. Triplicate incubations were run with each peptide at each time period and each incubation mixture was assayed in duplicate.

Comparison of **12** with **18**, the unmodified inhibitor, indicates that the Proψ[CH_2O]Phe modification has produced a 35-fold diminution of inhibitory potency.

In the third group, statine has been replaced with the transition-state isostere Leuψ[CH(OH)CH_2]Val. This replacement results in a dramatic increase in IC_{50}. The Leuψ[CH(OH)CH_2]Val-containing inhibitor **15** is over 60 times more potent than the statine-containing compound **12**. Further N-terminal extension with histidine or phenylalanine (**16** and **17**) does not dramatically alter potency. Compound **15** differs in inhibitory potency from the unmodified Pro-Phe parent compound **19** by only a factor of 6, indicating a better overall fit of Leuψ[CH(OH)-CH_2]Val-containing renin inhibitors as compared to statine-containing inhibitors.

Compound **8** was subjected to carboxypeptidase Y, chymotrypsin, elastase, and pepsin (Table III). As expected, carboxypeptidase Y rapidly attacked **8** from the C-terminus. Chymotrypsin, which would normally hydrolyze the Pro-Phe or Phe-His bonds of substrate, was not effective in cleaving either the modified Proψ[CH_2O]Phe bond or the neighboring Phe–His bond.

Discussion

As can be seen from Table I, ψ[CH_2O] as a transition-state insert in compounds **3–5** was uniformly disappointing when compared with ψ[CH_2NH]-containing inhibitors. The hydrogen bond donating ability of the secondary amine of ψ[CH_2NH] is one obvious difference between the ψ[CH_2NH] insert and the ψ[CH_2O] insert; however, Szelke has shown that an inhibitor containing ψ[$CH_2N(SO_2Ph)$] is equipotent with the secondary amine parent compound.[10] Bond angles and bond lengths (Figure 2) for the ψ[CH_2O]-modified bond differ less from the peptide amide bond than does the ψ[CH_2NH]-modified bond. Thus, it appears that fairly subtle differences in bond lengths, in

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Table IV. Solid-Phase Synthesis Protocol for Compounds **2–4**

step	description	reagent[a]	time
1	acid prewash	50% (v/v) TFA–CH_2Cl_2	30 s
2	deblock	50% (v/v) TFA–CH_2Cl_2	1 min
3	deblock	50% (v/v) TFA–CH_2Cl_2	30 min
4–7	wash	CH_2Cl_2	15 s
8	base prewash	5% (v/v) Et_3N–CH_2Cl_2	30 s
9	neutralize	5% (v/v) Et_3N–CH_2Cl_2	1 min
10	neutralize	5% (v/v) Et_3N–CH_2Cl_2	4 min
11–14	wash	CH_2Cl_2	15 s
15		add 1.5–2.5 molar excess of Boc-amino acid and DCC; add CH_2Cl_2, stirr 30–60 min	
16–17	wash	EtOH	30 s
18	wash	CH_2Cl_2	15 s
19	wash	EtOH	15 s
20–21	wash	CH_2Cl_2	15 s

[a] Reagent volume is 10 mL/g of resin.

Table V. Amino Acid Analysis Ratios and Mass Spectral Data for Compounds **2–4**

compound	Pro	His	Phe	Ile	Lys	FAB MS: [M + H] m/z
2[a]	1.93	2.61	1.05	0.71	1.02	1183 (1183)
3	1.91	2.99	1.14	0.91	1.06	1211 (1211)
4	1.96	2.92	1.13	0.92	1.07	1225 (1225)

[a] A shoulder of unhydrolyzed Ile-His was observed.

angles, and in the degree of sp^3 character of the transition-state insert may dramatically affect the match of the insert to the proposed tight binding conformation. Both $\psi[CH_2O]$ and $\psi[CH_2NH]$ inserts may be classed as transition-state analogues according to the transition-state analogue-collected substrate concept of Rich.[11] The tighter binding inhibitors of Table II which contain statine or the hydroxyethylene insert at P1-P1′ benefit from the additional energy gained by returning bound water to the bulk solvent (collected substrate hypothesis).

With good transition-state inserts, distal perturbations of the peptide backbone are well tolerated. For the Pro$\psi[CH_2O]$Phe series of Table II, Leu$\psi[CH(OH)CH_2]$Val as the transition-state insert is critical for nanomolar range binding. This is in agreement with the results of Thaisrivongs et al.[12] but is in contrast to the results of Szelke,[13] who found no advantage of the hydroxyethylene isostere over statine. Also, N-terminal extension beyond the P_4-P_3 $\psi[CH_2O]$ insert does not add significantly to binding.

Summary

Renin inhibitors containing $\psi[CH_2O]$ pseudopeptide bonds were prepared and tested in vitro as renin inhibitors. Those peptides containing the "ether" pseudopeptide moiety at the P_1-P_1' positions were of a potency comparable to RIP. Those peptides containing the "ether" pseudopeptide moiety at the P_4-P_3 positions were found to be potent inhibitors of renin. When the P_4-P_3 "ether" pseudopeptide moiety was combined with the hydroxyethylene isostere at the P_1-P_1' positions, renin inhibitors with potency in the nanomolar range were obtained.

Experimental Section

General Remarks. Boc-amino acids were purchased from U.S. Biochemical Corp. and Peninsula Laboratories. DCC, Et_3N, and TFA are from Aldrich. 1-HOBt was obtained from Sigma. All solvents were Burdick and Jackson distilled from glass. DMF was stored over 4A molecular sieves, and THF was distilled from Na and benzophenone prior to use. *N*-Acetylimidazole was purchased from Vega Biochemicals.

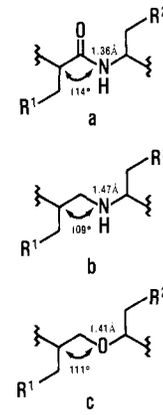

Figure 2. Bond angles and bond lengths for (a) normal amide bond, (b) $\psi[CH_2NH]$ replacement and (c) $\psi[CH_2O]$ replacement.

Mass spectra [found (calcd)] were performed by the Physical and Analytical Chemistry Department of The Upjohn Company and are gratefully acknowledged. ^1H NMR spectra were obtained on a Varian Model CFT-20 FT spectrometer operated at 80 MHz with use of tetramethylsilane as an internal standard. Chemical shifts cited are not necessarily comprehensive due to the complex nature of the spectra of the large peptides.

HPLC was performed with use of dual Altex 110A pumps at 1.5 mL/min, an LDC Spectro Monitor III detector at 225 nM, an Altex Model 420 programmer, and a Hewlett-Packard HP 3390A integrator. Column A is E. Merck Hibar LiChrosorb RP-18, 10 μM, 250 mm × 4.0 mm. Column B is Brownlee RP-18 Sheri-10, 10 μM, 250 mm × 4.0 mm. HPLC solvent systems are I, A, H_2O (0.2% TFA); and B, CH_3CN (0.2% TFA); and II; A, 50 mM NaH_2PO_4 (6.9 g/L), H_3PO_4 (1 mL/L), H_2O; and B, 12.5 mM NaH_2PO_4 (1.7 g/L), H_3PO_4 (0.25 mL/L), H_2O (250 mL/L), and CH_3CN (750 mL/L).

TLC (Analtech Uniplates) and chromatography (E. Merck silica gel 60) solvent systems are A, 4% MeOH–96% CH_2Cl_2; B, 4% MeOH–96% CH_2Cl_2–NH_4OH, saturated; C, 8% MeOH–92% CH_2Cl_2; D, 8% MeOH–92% CH_2Cl_2–NH_4OH, saturated.

Compounds 2–4. Compounds **2–4** were prepared by solid-phase methods by using the protocol of Table IV. HPLC compound **2**: column A, solvent I, 75% A–25% B; k', 7.1. Compounds **3** and **4**: column A; solvent I, 70% A–30% B; k', 2.3 (compound **3**); k', 4.4 (compound **4**). Amino acid analyses and mass spectra are listed in Table V.

General Method for Removal of *N*-*t*-Boc Group. The protected amino acid is stirred for 30 min in 3–15 mL of trifluoroacetic acid and CH_2Cl_2 (1:1, v/v). Trifluoroacetic acid and CH_2Cl_2 are then removed in vacuo, and the residue is partitioned

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between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The organic layers are filtered through Na_2SO_4 and taken to dryness to give the free amine.

General Procedure for Coupling with DCC. To a 1–1.5 molar excess of the acid, 1 molar equiv of the free amine, and 2–25 mL of CH_2Cl_2 (with dimethylformamide added when reactants are insoluble in CH_2Cl_2 alone) is added 1–1.5 equiv of DCC. After the mixture is stirred at room temperature under N_2 or argon for 30 min to overnight (follow by TLC), dicyclohexylurea is filtered off, and the filtrate is washed with saturated aqueous $NaHCO_3$. The organic layers are filtered through Na_2SO_4 and concentrated, and dicyclohexylurea is again removed by filtration (if necessary). The crude product is chromatographed on silica gel (10–30 g) to give product.

General Procedure for Workup of His(Tos) Deprotection Step. The solvents are removed in vacuo, and the residue is partitioned between CH_2Cl_2 or $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layers are filtered through Na_2SO_4 and taken to dryness.

Boc-Ile-Phe-OCH_3. To a solution of 1.34 g (13.2 mmol) of triethylamine in 100 mL of CH_2Cl_2 was added 2.80 g (13.0 mmol) of Phe-OCH_3·HCl. To this were added 3.00 g (13.0 mmol) of Boc-Ile-OH, 1.98 g (13.0 mmol) of 1-HOBt·H_2O, and 2.68 g (13.0 mmol) of DCC. After the mixture was stirred for 2 h, the reaction was worked up (extraction with 1 M citric acid and aqueous $NaHCO_3$) and chromatographed (gradient of 20% ethyl acetate to 40% ethyl acetate–hexane to give, after crystallization from ethyl acetate–hexane, 4.31 g (85%) of Boc-Ile-Phe-OCH_3, mp 121–122 °C. ^1H NMR ($CDCl_3$): δ 0.88 (d); 1.44 (s, Boc), 3.11 (d, J = 6), 3.70 (s, OCH_3), 3.80–4.0 (dd), 4.75–5.0 (m), 6.25 (br d, NH), 7.0–7.35 (m). Anal. ($C_{21}H_{32}N_2O_5$) C, H, N.

Boc-Sta-Ile-Phe-OCH_3. Boc-Ile-Phe-OCH_3 (713 mg, 1.8 mmol) was deprotected and coupled with Boc-Sta (500 mg, 1.8 mmol) in 50 mL of CH_2Cl_2 with use of 292 mg (1.9 mmol) of 1-HOBt·H_2O and 393 mg (1.9 mmol) of DCC. After workup and chromatography (4% MeOH–CH_2Cl_2), 0.98 g (98%) of product was obtained. R_f (4% MeOH–CH_2Cl_2): 0.28. FAB MS: [M + H] m/z 550 (550). ^1H NMR ($CDCl_3$): δ 0.83–0.95 (m), 1.44 (s, Boc), 3.09 (d, J = 6.2), 3.70 (s, OCH_3), 7.15–8.26 (m, aromatic). HPLC: column C, solvent I, 40% A–60% B; k', 6.3. Anal. ($C_{25}H_{42}N_4O_5$) H, N; C: calcd 63.36, found 63.81.

Boc-His(Tos)-Sta-Ile-Phe-OCH_3. Boc-Sta-Ile-Phe-OCH_3 (0.200 g, 0.364 mmol) was deprotected and coupled with Boc-His(Tos)-OH (0.44 mmol). After 2 h, an additional 0.09 g of DCC was added. After the mixture was stirred overnight, a drop of acetic acid was added to quench excess DCC, and the reaction was worked up and chromatographed (4% MeOH–CH_2Cl_2) to give 0.297 g (97%) of product. R_f (4% MeOH–CH_2Cl_2): 0.29. FAB MS: $[M + H]^+$ m/z 841 (841). ^1H NMR ($CDCl_3$): δ 0.73–0.94 (m), 1.42 (s, Boc), 2.43 (s, tosyl CH_3), 2.93 (d, J = 6), 3.10 (d, J = 6.1), 3.70 (s, OCH_3), 4.3 (m), 4.9 (m), 6.01 (d, NH), 6.56 (d, NH), 6.8–7.95 (m, aromatic).

Boc-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3 (5). Boc-His(Tos)-Sta-Ile-Phe-OCH_3 (87.9 mg, 0.093 mmol) was deprotected and coupled with Boc-Proψ[CH_2O]Phe-OH[9] (48.9 mg, 0.014 mmol) with use of 28.9 mg (0.014 mmol) of DCC. After the mixture was stirred for 1 h, 357 mg (0.233 mmol) of 1-HOBt was added. After the reaction mixture was stirred an additional 6 h, the reaction was worked up (extraction with ethyl acetate) and chromatographed (100% CH_2Cl_2 to 8% MeOH–CH_2Cl_2) to give 58.0 mg (68%) of **5**. R_f (solvent C): 0.33. FAB MS: [M + H] m/z 918 (918). ^1H NMR ($CDCl_3$): δ 0.81–0.87 (m), 1.43 (s, Boc), 3.69 (s, OCH_3), 7.22 (s, Ph). HPLC: column A, solvent I, 50% A–50% B; k', 11; column B, solvent II, 35% A–65% B; k', 6.9. Anal. ($C_{49}H_{71}N_7O_{10}$) C, H, N.

Boc-Proψ[CH_2O]Phe-His(Tos)-Sta-Ile-Phe-OCH_3 (6). Boc-His(Tos)-Sta-Ile-Phe-OCH_3 (297 mg, 0.355 mmol) was deprotected and coupled with Boc-Proψ[CH_2O]Phe-OH[9] (110 mg, 0.315 mmol) with use of DCC (65 mg, 0.315 mmol). After 2 h, an additional 70 mg of Boc-Proψ[CH_2O]Phe-OH and 40 mg of DCC were added. After the mixture was stirred overnight, the reaction was worked up and chromatographed (2% MeOH–CH_2Cl_2) to give 290 mg (76%) of **6**. R_f (solvent A): 0.29.

Boc-His-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3 (7). Compound **6** (171.7 mg, 0.16 mmol) was deprotected and coupled with Boc-His(Tos)-OH (0.202 mmol) with use of 41.7 mg (0.202 mmol) of DCC. After 3 h, the reaction was worked up and chromatographed (solvent A) to give 201 mg (92%) of Boc-His(Tos)-Proψ[CH_2O]Phe-His(Tos)-Sta-Ile-Phe-OCH_3 [R_f (solvent C): 0.51. FAB MS: [M + H] m/z 1363 (1363)].

This material was dissolved in 5 mL of THF and stirred with 1-HOBt (100 mg, 0.7 mmol) for 7 days, after which the solvent was removed in vacuo, and the resulting solid was washed with Et_2O (three times). The solid was partitioned with CH_2Cl_2 and saturated aqueous $NaHCO_3$, filtered through Na_2SO_4, and taken to dryness to give 119 mg (77%) of **7**. R_f (solvent C): 0.07. FAB MS: [M + H] m/z 1055 (1055). ^1H NMR ($CDCl_3$): δ 0.79–0.80 (m), 1.39 (s, Boc), 3.67 (s, OCH_3), 7.20, 7.21 (aromatic). HPLC: column A, solvent I, 55% A–45% B; k', 4.9; column B, solvent II, 50% A–50% B; k', 7.7. Anal. ($C_{55}H_{78}N_{10}O_{11}$·H_2O) C, H, N.

***N*-Ac-His-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3 (8).** Compound **7** (61.9 mg, 0.0587 mmol) was deprotected in the usual manner. The free amine (54.1 mg) was taken up in 3 mL of CH_2Cl_2 and stirred with 17.3 mg (0.17 mmol) of acetic anhydride and 18.7 mg (0.17 mmol) of *N*-acetylimidazole at room temperature for 2.5 h, after which it was partitioned with CH_2Cl_2, saturated aqueous $NaHCO_3$, and aqueous $NaHCO_3$–brine. The organic layers were filtered through Na_2SO_4, concentrated, and chromatographed (solvent D) to give 36.6 mg (64%) of **8**. No diacetylated material was indicated by TLC, MS, or HPLC. R_f (solvent D): 0.18. FAB MS: [M + H] m/z 997 (997). ^1H NMR ($CDCl_3$): δ 0.79–0.86 (m), 1.99, 1.96 (*N*-Ac), 3.65, 3.67 (OCH_3), 6.7–7.7 (m, aromatic). HPLC: column A, solvent I, 62.5% A–37.5% B; k', 5.1. Anal. ($C_{52}H_{72}N_{10}O_{10}$·H_2O) C, H, N.

Boc-Trp(CHO)-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3 (9). Compound **6** (113.6 mg, 0.106 mmol) was deprotected and coupled with 38.7 mg (0.116 mmol) of Boc-Trp(CHO) with use of 24.0 mg (0.116 mmol) of DCC. After 1.5 h, an additional 0.5 equiv each of Boc-Trp(CHO) and DCC were added. The reaction was stirred 2 h, concentrated, and filtered to remove DCU. The filtrate was chromatographed with 3% MeOH–CH_2Cl_2 to give 109.7 mg (81%) of Boc-Trp(CHO)-Proψ[CH_2O]Phe-His(Tos)-Sta-Ile-Phe-OCH_3. R_f (solvent C): 0.53.

This material was stirred overnight with 71 mg (0.52 mmol) of 1-HOBt in 5 mL of THF. After workup and chromatography (6% MeOH-CH_2Cl_2), 88.7 mg (92%) of **9** was obtained. R_f (solvent C): 0.30. FAB MS: [M + H] m/z 1132 (1132). ^1H NMR ($CDCl_3$): δ 0.75–0.90 (m), 1.37 (s, Boc), 3.69 (s, OCH_3), 6.7–7.5 (m, aromatic). HPLC: column A, solvent I, 43% A–57% B; k', 5.0; column B, solvent II, 30% A–70% B; k', 6.5. Anal. ($C_{61}H_{81}N_9O_{12}$) C, H, N.

***N*-Ac-Trp(CHO)-Proψ[CH_2O]Phe-His-Sta-Ile-Phe-OCH_3 (10).** Compound **9** (46 mg, 0.0406 mmol) was stirred for 15 min in 3 mL of TFA–CH_2Cl_2 (1:1). TFA and CH_2Cl_2 were then removed in vacuo, and 13.6 mg (0.134 mmol) of Et_3N and 4 mL of CH_2Cl_2 were added. After 5 min, 12.4 mg (0.122 mmol) of acetic anhydride and 13.4 mg (0.122 mmol) of *N*-acetylimidazole were added. The reaction was stirred for 4.5 h and was then partitioned with CH_2Cl_2 and aqueous $NaHCO_3$ (pH 8). The organic layers were filtered through Na_2SO_4, concentrated, and chromatographed (6% MeOH–CH_2Cl_2) to give 21 mg (48%) of **10**. R_f (solvent D): 0.26. FAB MS: [M + H] m/z 1074 (1074). HPLC: column A, solvent I, 52.5% A–47.5% B; k', 5.4. Anal. ($C_{58}H_{75}NgO_{11}$·H_2O) C, H, N.

Boc-His(Tos)-Sta-Ile-Amp. Boc-Sta-Ile-Amp[12] (0.73 g, 1.52 mmol) was deprotected and coupled with Boc-His(Tos)-**OH** (2 mmol) in 20 mL of CH_2Cl_2 with use of DCC (2 mmol). After workup (ethyl acetate extraction) and chromatography [6% MeOH (NH_3)–EtOAc], 52.7 mg (45%) of product was obtained. R_f [6% MeOH (NH_3)–EtOAc]: 0.35. ^1H NMR ($CDCl_3$): δ 0.70–0.97 (m), 1.43 (s, Boc), 1.84 (s), 2.43 (s, tosyl CH_3), 2.93 (d, J = 5.7), 4.57 (d, J = 5.3), 6.10 (br d, NH), 6.9–8.5 (m, aromatic).

Boc-Proψ[CH_2O]Phe-His(Tos)-Sta-Ile-Amp (11). Boc-His(Tos)-Sta-Ile-Amp (183.5 mg, 0.238 mmol) was deprotected and coupled with Boc-Proψ[CH_2O]Phe-OH[9] (108.2 mg, 0.31 mmol) with use of DCC (63.9 mg, 0.31 mmol). After 2 h, the reaction was worked up and chromatographed (6% MeOH–CH_2Cl_2–NH_4OH, saturated) to give 240 mg (100%) of **11**. ^1H NMR ($CDCl_3$): δ 0.68–0.99 (m), 1.42 (s, Boc), 2.40 (s, tosyl CH_3), 7.0–8.5 (m, aromatic).

Boc-Proψ[CH_2O]Phe-His-Sta-Ile-Amp (12). A solution of 54.1 mg (0.054 mmol) of **11** in 4 mL of CH_2Cl_2 and 1 mL of THF was stirred overnight with 80 mg (0.54 mmol) of 1-HOBt. Workup

and chromatography (6% MeOH–CH_2Cl_2–NH_4OH, saturated) gave 37.7 mg (82%) of **12**. R_f (solvent D): 0.38. FAB MS: [M + H] m/z 847 (847). ^1H NMR ($CDCl_3$): δ 0.78–0.99 (m), 1.42 (s, Boc), 4.54 (m), 6.72 (br s), 7.22 (s), 8.46 (m). HPLC: column A, solvent I, 52.5% A–47.5% B; k', 3.1; column B, solvent II, 45% A–55% B; k', 6.6. Anal. ($C_{45}H_{66}N_8O_8 \cdot H_2O$) C, H, N.

Boc-His-Proψ[CH_2O]Phe-His-Sta-Ile-Amp (13). Compound 11 (136 mg, 0.136 mmol) was deprotected and coupled to Boc-His(Tos)-OH (0.190 mmol) with use of 39.2 mg (0.190 mmol) of DCC. After workup and chromatography (6% MeOH–CH_2Cl_2), 146.6 mg (83%) of Boc-His(Tos)-Proψ[CH_2O]Phe-His(Tos)-Sta-Ile-Amp was obtained. R_f (solvent D): 0.57.

This material was stirred for 2 days with 260 mg (1.7 mmol) of 1-HOBt in 15 mL of THF. Workup and chromatography (solvent D) gave 50.5 mg of monotosylated material [R_f (solvent D): 0.30] and 116.5 mg of **13**. R_f (solvent D): 0.04. NMR indicated two peaks for the Boc group in a ratio of 2:1 so the material was rechromatographed. Early, middle, and late fractions showed no change in the ratio of peaks at δ 1.39 and 1.36. FAB MS: [M + H] m/z 984 (984). ^1H NMR ($CDCl_3$): δ 0.65–1.0 (m), 1.39, 1.36 (sh, Boc), 6.65–8.5 (aromatic). HPLC: column A, solvent I, 65% A–35% B; k', 3.1; column B, solvent II, 40% A–60% B; k', 3.5.

Boc-His(Tos)-Leuψ[CH(O-*t*-BDMS)CH_2]Val-Ile-Amp. Boc-His(Tos)-OH (60 mg, 0.146 mmol) and Leuψ[CH(O-*t*-BDMS)CH_2]Val-Ile-Amp (0.097 mmol) (obtained from the TFA–CH_2Cl_2 deprotection of Boc-Leuψ[CH(*O*-*t*-BDMS)CH_2]-Val-Ile-Amp[12]) were coupled with use of 30 mg (0.146 mmol) of DCC. After workup and chromatography (solvent B), 79.3 mg (87%) of product was obtained as an oil. R_f (solvent B): 0.38. ^1H NMR ($CDCl_3$): δ 0.07 (s), 0.88–0.95 (m), 1.40 (s, Boc), 2.42 (s, tosyl CH_3), 5.95 (br d, NH), 6.20 (br d, NH), 6.65 (br d, NH), 7.05–8.5 (m).

His(Tos)-Leuψ[CH(OH)CH_2]Val-Ile-Amp. A solution of 79 mg (0.084 mmol) of Boc-His(Tos)-Leuψ[CH(*O*-*t*-BDMS)-CH_2]Val-Ile-Amp in 10 mL of TFA–CH_2Cl_2 (1:1) was stirred for 4 h at room temperature. After workup and chromatography (6% MeOH–CH_2Cl_2–NH_4OH saturated), 51.3 mg (84%) of His-(Tos)-LVA-Ile-Amp was obtained. R_f (solvent C): 0.40. ^1H NMR ($CDCl_3$): δ 0.87–0.95 (m), 1.32–1.91 (m), 2.44 (s, tosyl CH_3), 3.4–3.8 (m), 4.53 (d), 6.45 (br d, NH), 7.09–7.92 (m), 8.48 (br d). HPLC: column C, solvent II, 65% A–35% B; k', 9.8.

Boc-Proψ[CH_2O]Phe-His(Tos)-Leuψ[CH(OH)CH_2]Val-Ile-Amp (14). To 122 mg (0.168 mmol) of His(Tos)-Leuψ[CH-(OH)CH_2]Val-Ile-Amp and 112 mg (0.320 mmol) of Boc-Proψ-[CH_2O]Phe-OH[9] in 10 mL of CH_2Cl_2 was added 66 mg (0.320 mmol) of DCC. After the mixture was stirred for 2 h, excess DCC was quenched with approximately 10 μL of acetic acid. The reaction was worked up and chromatographed (solvent A) to give 173.5 mg (98%) of **14**. R_f (solvent D): 0.51.

Boc-Proψ[CH_2O]Phe-His-Leuψ[CH(OH)CH_2]Val-Ile-Amp (15). To 16.8 mg (0.0159 mmol) of **14** in 3 mL of CH_2Cl_2 and 1 mL of THF was added 70 mg (0.52 mmol) of 1-HOBt. After the mixture was stirred overnight, the reaction was worked up and chromatographed (6% MeOH–CH_2Cl_2–NH_4OH, saturated) to give 8.7 mg (61%) of **15**. R_f (solvent D): 0.38. FAB MS: [M + H] m/z 903 (903). ^1H NMR ($CDCl_3$): δ 0.79–0.96 (m), 1.42 (s, Boc), 6.7–8.5 (m, aromatic). HPLC: column B, solvent II, 20% A–80% B; k', 4.8. Anal. ($C_{49}H_{74}N_8O_8 \cdot {}^1/_2H_2O$) C, H, N.

Boc-His-Proψ[CH_2O]Phe-His-Leuψ[CH(OH)CH_2]Val-Ile-Amp (16). Compound **14** (70.5 mg, 0.0667 mmol) was deprotected and coupled with Boc-His(Tos)-OH (0.125 mmol) in 5 mL of CH_2Cl_2 with use of 25.8 mg (0.125 mmol) of DCC. After 3 h, the reaction was worked up and chromatographed (solvent B) to give 62.4 mg (65%) of Boc-His(Tos)-Proψ[CH_2O]Phe-His(Tos)-Leuψ[CH(OH)CH_2]Val-Ile-Amp and 24.7 mg that was contaminated with a small amount of DCU. R_f (solvent D): 0.46.

This material was stirred for 4 days with 70 mg (0.52 mmol) of 1-HOBt in 3 mL of THF and 3 mL of CH_2Cl_2. After workup and chromatography (solvent D), 22.9 mg (51%) of **16** was obtained. R_f (solvent D): 0.22. FAB MS: [M + H] m/z 1040 (1040). ^1H NMR ($CDCl_3$): δ 0.83–0.94 (m), 1.40, 1.36 (sh, Boc), 6.8 (br d, (NH), 6.7–8.55 (m, aromatic). HPLC: column B, solvent II, 60% A–40: B; k', 9.3. Anal. ($C_5H_{81}N_{11}O_9$) C, H; N: calcd 14.81, found 14.34.

Boc-Phe-Proψ[CH_2O]Phe-His-Leuψ[CH(OH)CH_2]Val-Ile-Amp (17). Compound **14** (72.8 mg, 0.0689 mmol) was deprotected and coupled with Boc-Phe-OH (32 mg, 0.12 mmol) in 5 mL of CH_2Cl_2 with use of 24.9 mg (0.12 mmol) of DCC. After workup and chromatography (solvent B), 69.3 mg (84%) of Boc-Phe-Proψ[CH_2O]Phe-His(Tos)-Leuψ[CH(OH)CH_2]Val-Ile-Amp was obtained. R_f (solvent D): 0.45. ^1H NMR ($CDCl_3$): δ 1.38 (s, Boc), 2.38 (s, tosyl CH_3).

The above material was stirred for 6 days with 100 mg (0.74 mmol) of 1-HOBt in 5 mL of THF and 2 mL of CH_2Cl_2. After workup and chromatography (solvent D), 35.3 mg (59%) of **17** was obtained. R_f (solvent D): 0.38. FAB MS: [M + H] m/z 1050 (1050). ^1H NMR ($CDCl_3$): δ 0.78–0.96 (m), 1.38 (s, Boc), 5.50 (br d, NH), 6.7–8.52 (m). HPLC: column B, solvent II, 35% A–65% B; k', 7.0. Anal. ($C_{58}H_{83}N_9O_9 \cdot H_2O$) C, H, N.

Boc-Pro-Phe-His-Sta-Ile-Amp (18). Boc-His(Tos)-Sta-Ile-Amp (75.2 mg, 0.098 mmol) was deprotected and coupled with Boc-Phe-OH (31.1 mg, 0.117 mmol) in 5 mL of CH_2Cl_2 with use of DCC (24.2 mg, 0.117 mmol). The reaction was worked up and chromatographed (6% MeOH–CH_2Cl_2–NH_4OH, saturated) to give 88.4 mg (99%) of Boc-Phe-His(Tos)-Sta-Ile-Amp [R_f (6% MeOH–CH_2Cl_2–NH_4OH, saturated): 0.37]. This material was then stirred for 2 days with 155 mg (1.1 mmol) of 1-HOBt in 25 mL of THF and 5 mL of CH_2Cl_2. After workup ($CHCl_3$) and chromatography (solvent C), 56.7 mg (77%) of Boc-Phe-His-Sta-Ile-Amp was obtained. R_f (6% MeOH–CH_2Cl_2–NH_4OH, saturated): 0.15. FAB MS: $[M + H]^+$ m/z 763 (763). HPLC: column B, solvent I, 68% A–32% B; k', 3; column C, solvent II, 60% A–40% B; k', 9.3.

Boc-Phe-His-Sta-Ile-Amp (18.8 mg, 0.0246 mmol) was deprotected and coupled with Boc-Pro-OH (5.8 mg, 0.0271 mmol) in 5 mL of CH_2Cl_2 with 4.9 μL (0.032 mmol) of diethyl cyanophosphonate and 4.5 μL (0.032 mmol) of triethylamine. After workup and chromatography (solvent C), 5.0 mg (24%) of **18** was obtained. R_f (solvent C): 0.36. FAB MS: $[M + H]^+$ m/z [M + H]$^+$ m/z 860.5048 (860.5034). ^1H NMR ($CDCl_3$ + MeOH-d_4): δ 0.58–1.00 (m), 1.42 (s, Boc), 6.77 (br s), 7.19–8.45 (aromatic). HPLC: column C, solvent II, 55% A–45% B; k', 6.8.

Boc-Pro-Phe-His-Leuψ[CH(OH)CH_2]Val-Ile-Amp (19). To Boc-Pro-Phe-OH (215 mg, 0.59 mmol) and His(Tos)-LVA-Ile-Amp (332 mg, 0.457 mmol) in 4 mL of CH_2Cl_2 were added 100 μL (0.57 mmol) of diisopropylethylamine and 91 μL (0.59 mmol) of diethyl cyanophosphonate. After the mixture was stirred overnight, the crude material was chromatographed with use of a 3% MeOH to 5% MeOH–CH_2Cl_2 gradient to give 361 mg (74%) of Boc-Pro-Phe-His(Tos)-Leuψ[CH(OH)CH_2]Val-Ile-Amp. R_f (10% MeOH–CH_2Cl_2): 0.37. This material was stirred overnight in 3 mL of methanol with 182 mg (1.4 mmol) of 1-HOBt. After workup and chromatography (3% to 6% to 12% MeOH/NH_3–CH_2Cl_2), 244 mg (79%) of **19** was obtained. FAB MS: $[M + H]^+$ m/z 916.5634 (916.5660). HPLC: column B, 90% MeOH–10% solvent II, A; t_R, 13 min; 254 nM.

Boc-Pro-Phe-His-Sta-Ile-Phe-OCH$_3$ (20). His (Tos)-Sta-Ile-Phe-OCH_3 (105 mg, 0.125 mmol) was coupled with Boc-Pro-Phe-OH (67.8 mg, 0.187 mmol) in 3 mL of CH_2Cl_2 with triethylamine (23 μL, 0.162 mmol) and diethyl cyanophosphonate (25 μL, 0.162 mmol). After being stirred for 2 h, the reaction mixture was partitioned with CH_2Cl_2 and aqueous $NaHCO_3$. The crude product was chromatographed (4% MeOH–CH_2Cl_2) to give 131.5 mg (89%) of Boc-Pro-Phe-His (Tos)-Sta-Ile-Phe-OCH_3. This material was stirred overnight in 2 mL of methanol and 2 mL of CH_2Cl_2 with 75 mg (0.552 mmol) of 1-HOBt. After workup and chromatography (6% MeOH–CH_2O_2, NH_4OH, saturated), 100.5 mg (98%) of **20** was obtained. FAB MS: [M + H] m/z 931 (931). HPLC: column B, solvent II, 30% A–70% B; k', 7.5. Anal. ($C_{49}H_{70}N_8O_{10} \cdot {}^1/_2H_2O$) C, H, N.

Registry No. **2**, 112374-66-0; **3**, 112374-67-1; **4**, 112374-68-2; **5**, 103352-62-1; **6**, 112374-69-3; **7**, 103352-66-5; **7** (deprotected), 112374-77-3; **8**, 103352-67-6; **9**, 112374-70-6; **9** (N^{im}-monotosyl derivative), 112374-78-4; **10**, 112374-71-7; **11**, 112374-72-8; **12**, 103352-64-3; **13**, 103352-65-4; **13** (N^{im}-monotosyl derivative), 112456-05-0; **14**, 103336-11-4; **15**, 112374-73-9; **16**, 103352-60-9; **17**, 103352-61-0; **17** (N^{im}-monotosyl derivative), 112374-81-9; **18**, 112398-40-0; **19**, 112374-74-0; **19** (N^{im}-monotosyl derivative), 112374-83-1; **20**, 112374-75-1; **20** (N^{im}-monotosyl derivative), 112374-85-3; BOC-Ile-OH, 13139-16-7; Phe-OCH_3·HCl, 7524-50-7; BOC-Ile-Phe-OCH_3, 97641-59-3; BOC-Sta-OH, 58521-49-6;

BOC-Sta-Ile-Phe-OCH_3, 103372-21-0; BOC-His(Tos)-OH, 35899-43-5; BOC-His(Tos)-Sta-Ile-Phe-OCH_3, 103372-22-1; BOC-Proψ[CH_2O]Phe-OH, 103336-10-3; BOC-His(Tos)-Proψ-[CH_2O]Phe-His(Tos)-Sta-Ile-Phe-OCH_3, 112374-76-2; BOC-Trp(CHO)-OH, 47355-10-2; BOC-Sta-Ile-Amp, 103372-24-3; BOC-His(Tos)-Sta-Ile-Amp, 103372-25-4; BOC-His(Tos)-Proψ-[CH_2O]Phe-His(Tos)-Sta-Ile-Amp, 112374-79-5; BOC-Leuψ[CH-(O-*t*-BDMS)CH_2]Val-Ile-Amp, 103372-32-3; Leuψ[CH(O-*t*-BDMS)CH_2]Val-Ile-Amp, 103406-77-5; BOC-His(Tos)-Leuψ-[CH(O-*t*-BDMS)CH_2]Val-Ile-Amp, 103372-33-4; His(Tos)-Leuψ-[CH(OH)CH_2]Val-Ile-Amp, 103372-31-2; BOC-His(Tos)-Proψ-[CH_2O]Phe-His(Tos)-Leuψ[CH(OH)CH_2]Val-Ile-Amp, 112374-80-8; BOC-Phe-OH, 13734-34-4; BOC-Phe-His(Tos)-Sta-Ile-Amp, 112374-82-0; BOC-Phe-His-Sta-Ile-Amp, 97920-11-1; BOC-Ao-OH, 15761-39-4; BOC-Pro-Phe-OH, 52071-65-5; His(Tos)-Sta-Ile-Phe-OCH_3, 112374-84-2; renin, 9015-94-5.

Design of Potent Cyclic Gonadotropin Releasing Hormone Antagonists†

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In order to improve the biological potency of cyclic gonadotropin releasing hormone (GnRH) antagonists, we have synthesized analogues, the conformations of which were restrained through internal side chain/side chain amide bridges linking aspartic acid or glutamic acid and L-2,3-diaminopropionic acid or L-ornithine. A disulfide bridge linking L-cysteine residues was also introduced. Residues belonging to the bridge spanned from position 4 to positions 9 or 10. Two series of analogues were synthesized and are characterized by residues at positions 1 [Ac-D-3-(2′-naphthyl)alanine], 2 [D-(4-chlorophenyl)alanine or D-(4-fluorophenyl)alanine], 3 [D-3-(3′-pyridyl)alanine or D-tryptophan], 5 (arginine or tyrosine), and 6 [D-3-(3′-pyridyl)alanine or D-arginine], respectively. These substitutions were selected in an effort to optimize high biopotency for inhibition of luteinizing hormone secretion, minimization of histamine release activity, and high (relative) hydrophilicity. The most potent analogues in the antiovulatory assay were *cyclo*(4–10)[Ac-DNal^1,DCpa^2,DPal^3,(Asp^4 or Glu^4),Arg^5,DPal]^6,Dpr^{10}]GnRH (compounds **5** and **7**), which were fully active at ca. 12.5 μg/rat in the first series, and *cyclo*(4–10)[Ac-DNal^1,DFpa^2,DTrp^3,Asp^4,DArg^6,Dpr^{10}]GnRH (compound **12**), which was fully active at 2.5 μg/rat in the second.

Several techniques, ranging from binding studies and structure–activity relationships (SAR) to conformational study of the ligand using both theoretical and spectroscopic approaches, are available to gain an understanding of the nature of the interaction of a ligand with its receptor. However, when the structure of the receptor is unknown (as in the case for releasing factors) and the ligands are relatively small linear peptides with virtually unlimited conformational freedom, any conclusion concerning the nature of their interaction is speculative at best. In order to gain insight into the nature of the interactions of GnRH (the structure of which is pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) and its pituitary receptor, extensive SAR studies using receptor binding assays, in vitro and in vivo assays, and some spectroscopic measurements have been carried out by us and others.[1] Potent and long-acting GnRH agonists were prepared and, because of the desensitizing effects resulting from long term administration, have been used in clinical studies where suppression of sex steroid secretion is desired.[2] However, the administration of these agonists to humans is complicated by the fact that an early stimulatory phase exists, which is never desired.

The potential of GnRH antagonists as contraceptive agents was realized even prior to the elucidation of GnRH's structure, and an extensive program sponsored by the National Institutes of Health Contraceptive Development Branch was initiated in 1972. Some 15 years and approximately 3000 analogues later, extremely potent and long-acting GnRH antagonists have been designed, and preliminary data from clinical investigators are very encouraging. Despite this, little was known regarding the role of the backbone and side-chain conformations or flexibility of GnRH when interacting with its receptor. In order to address this problem, we applied an integrated approach combining theoretical techniques, including molecular dynamics, energy minimization, template forcing and interactive graphics[3] with peptide synthesis,[4] NMR spectroscopy,[5] receptor binding studies,[6] and several biological

† Abbreviations: The abbreviations for the amino acids are in accord with the recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9–37). The symbols represent the L-isomer except when indicated otherwise. In addition: Dpr, 2,3-diaminopropionic acid; Nal, 3-(2′-naphthyl)alanine; Pal, 3-(3′-pyridyl)alanine; Cpa, (4-chlorophenyl)alanine; Fpa, (4-fluorophenyl)alanine; MeLeu, *N*-methylleucine; Boc, *tert*-butoxycarbonyl; Z, benzyloxycarbonyl; GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; AOA, antiovulatory assay; DCC, *N,N′*-dicyclohexylcarbodiimide; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid; EDT, ethanedithiol; DMF, dimethylformamide; TEA, triethylamine; sc, subcutaneous.

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