institute of the City of Hope and from Dr. A. Craig of the Salk Institute.

Registry No. 1 (free base), 130883-26-0; 1·xHOAc, 134485-04-4; 2 (free base), 134457-18-4; 2·xHOAc, 134485-05-5; 3 (free base), 134457-19-5; 3·xHOAc, 134485-06-6; 4 (free base), 134457-20-8; 4·xHOAc, 134485-07-7; 5 (free base), 134457-21-9; 5·xHOAc, 134457-40-2; 6 (free base), 134457-22-0; 6·xHOAc, 134457-42-4; 7 (free base), 134457-23-1; 7·xHOAc, 134457-43-5; 8 (free base), 134457-24-2; 8·xHOAc, 134485-08-8; 9 (free base), 134457-25-3; 9·xHOAc, 134457-44-6; 10 (free base), 134457-26-4; 10·xHOAc, 134485-09-9; 11 (free base), 134457-27-5; 11·xHOAc, 134457-45-7; 12 (free base), 134457-28-6; 12·xHOAc, 134485-10-2; 13 (free base), 134457-29-7; 13·xHOAc, 134457-46-8; 14 (free base), 134457-30-0; 14·xHOAc, 134457-47-9; 14·xTFA, 134457-64-0; 15 (free base), 134457-48-0; 15·xHOAc, 134457-49-1; 16 (free base), 134457-31-1; 16·xHOAc, 134457-50-4; 17 (free base), 134457-32-2; 17·xHOAc, 134457-51-5; 18 (free base), 134457-33-3; 18·xHOAc, 134457-52-6; 19 (free base), 134457-34-4; 19·xHOAc, 134485-11-3; 20 (free base), 134457-35-5; 20·xHOAc, 134457-53-7; 21 (free base), 134457-54-8; 21·xHOAc, 134457-55-9; 22 (free base), 134457-36-6; 22·xHOAc, 134485-12-4; 23 (free base), 134457-56-0; 23·xHOAc, 134457-57-1; 24 (free base), 134457-58-2; 24·xHOAc, 134457-59-3; 25 (free base), 134485-03-3; 25·xHOAc, 134485-13-5; 26 (free base), 134457-37-7; 26·xHOAc, 134457-61-7; 27 (free base), 134457-38-8; 27·xHOAc, 134457-62-8; 28 (free base), 134457-39-9; 28·xHOAc, 134457-63-9; GnRH, 33515-09-2; BOC-Phe(4-NO_2)-OH, 33305-77-0; BOC-Phe(4-NH_2)-OH, 55533-24-9; BOC-Phe(4-NHFmoc)-OH, 114346-31-5; $(PhO)_2C{=}NCN$, 79463-77-7; $BuNH_2$, 109-73-9; $MeNH_2$, 74-89-5; *i*-$PrNH_2$, 75-31-0; $Me(CH_2)_5NH_2$, 111-26-2; c-$C_6H_{11}NH_2$, 108-91-8; BuSH, 109-79-5; 2-pyridinemethanamine, 3731-51-9; histamine, 51-45-6.

Supplementary Material Available: Table of MS characterization data of GnRH antagonists (1 page). Ordering information is given on any current masthead page.

Synthesis and Biological Activity of Angiotensin II Analogues Containing a Val-His Replacement, Valψ[CH($CONH_2$)NH]His

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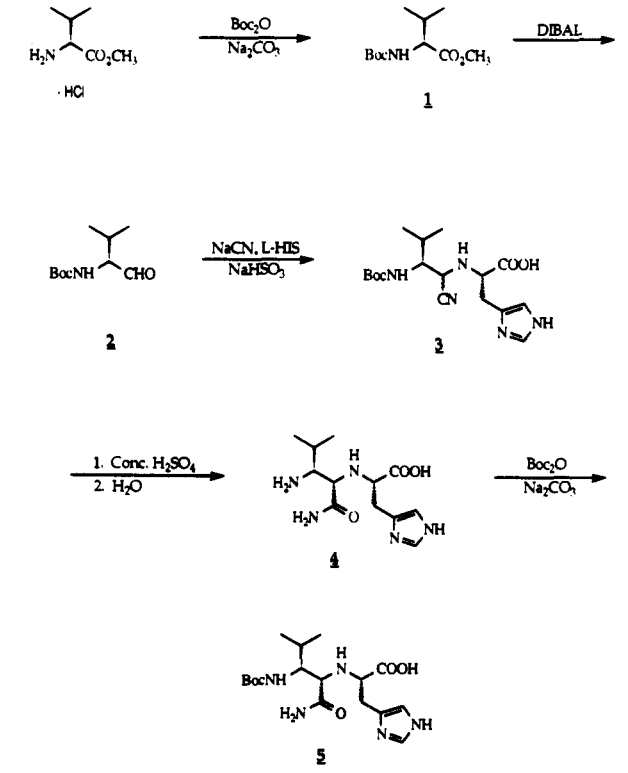
The dipeptide mimic Valψ[CH($CONH_2$)NH]His (4) was incorporated into angiotensin II (AII) analogues to provide an octapeptide saralasin derivative (29) as well as tetrapeptide analogue 19. Three C-terminal tetrapeptides (21, 25, and 28) were also prepared. All compounds were tested for their ability to displace ^{3}H-AII from rabbit adrenal gland homogenate and as antagonists of AII and AI on guinea pig ileum. The octapeptide analogue 29 was 700 times less active than the parent peptide 30. All the C-terminal fragments 19, 21, 25, and 28 have no measurable AII antagonist activity. Of the four tetrapeptide fragments, only 21 showed any appreciable binding activity.

Introduction

The octapeptide angiotensin II (AII) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) is a potent pressor agent that acts on smooth muscle to elicit its actions.[1] Over 400 analogues of angiotensin II have been synthesized to define the role of various amino acid substituents in the biological response of the parent hormone.[2] [Sar^1,Val^5,Ile^8]-AII (30) and [Sar^1,Val^5,Ala^8]-AII (saralasin)[3] (32) are two potent antagonists that have resulted from the replacement of the C-terminal Phe by aliphatic amino acids. These compounds, however, still retain transient agonist activity and short in vivo half-lives. Modification of the peptide bonds[4] to make them less susceptible toward hydrolysis while retaining the antagonist activity has not been very successful.[5] One such amide bond replacement has been the synthesis of methyleneamino peptide analogues.[6] One or two replacements of the amide bonds at the N-terminal of 32 by the methyleneamino group does not appreciably alter the activity of the hormone but the activity rapidly decreases as these replacements are extended toward the C-terminal.

In extending this strategy we have utilized a single amide bond replacement at a sterically sensitive region of an-

Scheme I



(1) Bumpus, F. M.; Khosla, M. C. In *Hypertension: Physiopathology and Treatment*; Genest, J., Koiw, E., Kuchel, O., Eds.; McGraw-Hill: New York, 1977; pp 183-201.
(2) Khosla, M. C.; Smeby, R. R.; Bumpus, F. M. *Handb. Exp. Pharmacol.* **1974**, *37*, 126-161.
(3) Pals, D. T.; Masucci, F. D.; Denning, G. S., Jr.; Sipos, F.; Fessler, D. C. *Circ. Res.* **1971**, *29*, 673.
(4) For a review of pseudo amide bonds, see: Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, pp 267-357.
(5) Moore, A. F.; Fulton, R. W. *Drug. Dev. Res.* **1984**, *4*, 331.
(6) Kaltenbronn, J. S.; Dejohn, D. E.; Hudspeth, J. P.; Humblet, C. C.; Lunney, E. A.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Tinne, F. J. In *Peptides: Proceedings of the Eleventh American Peptide Symposium*, La Jolla, CA, 1989; pp 969-970.

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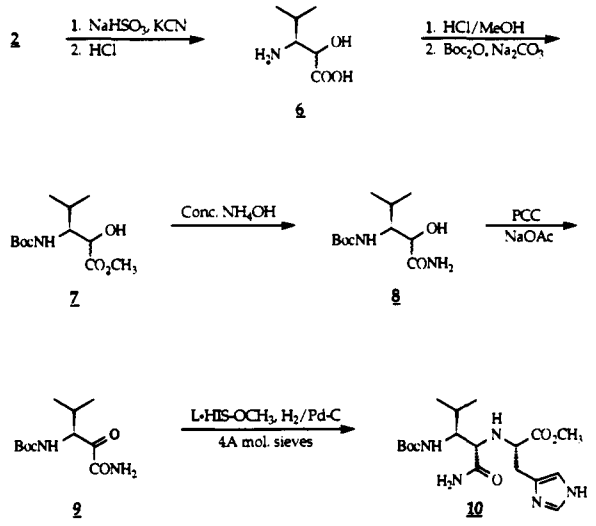
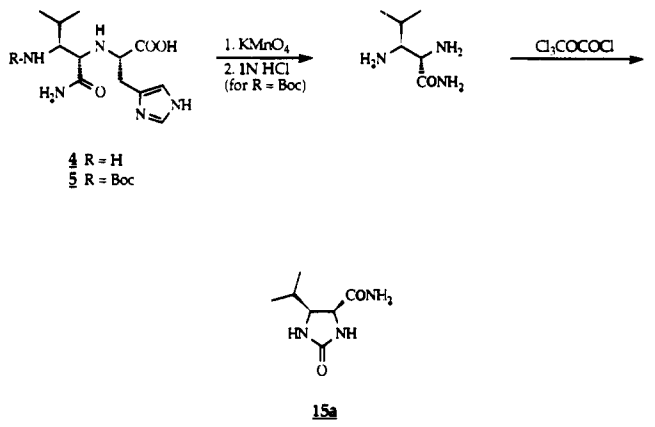
Scheme II



Scheme III



giotensin II, such as the Val-His residue,[7] by the synthesis of an analogue of **30** containing the Val-His mimic Valψ[CH(CONH$_2$)NH]His (**4**). Replacement of the Val-His sp^2 amide bond by an sp^3 carbon (bearing a carboxamide function) could provide conformational mobility for the histidine residue for interactions with other residues in the chain[8] while the carboxamide function could provide the hydrogen-bonding interactions present in the Val-His bond. Toward this goal, we synthesized this Val-His mimic, Valψ[CH(CONH$_2$)NH]His (**4**), and incorporated it into an octapeptide analogue of **30** (compound **29**) and the corresponding tetrapeptide (compound **19**). We also synthesized the C-terminal fragments of **30** (compounds **21**, **25**, and **28**), where Tyr[4] has been replaced by a benzyloxycarbonyl group and by (methoxyphenyl)propionyl and (hydroxyphenyl)propionyl groups (as *O*-methyltyrosine and tyrosine replacements). These compounds were tested for [^{3}H]angiotensin II binding activity (rabbit adrenal gland), angiotensin II antagonist activity (guinea pig ileum), and their ability to antagonize the action of angiotensin I (as ACE inhibitors).

Discussion

Chemical Syntheses. The strategy employed for the synthesis of the Val-His mimic **3** is shown in Scheme I. L-Valine methyl ester hydrochloride was transformed to the aldehyde **2**[9] in 82% yield, using a published procedure.[10] Strecker reaction of the aldehyde **2** with L-histidine afforded a 2:1 inseparable mixture of the amino nitriles **3** in 79% yield. This mixture appeared to be unstable in the presence of protic solvents and initial attempts to transform the nitriles **3** to the amide **4** by using alkaline peroxide conditions resulted in decomposition even under controlled pH conditions.[11] Treatment of the nitrile **3** with concentrated sulfuric acid[12] at 0–5 °C afforded the desired amide **4** in 36% yield. Spectroscopic analyses of the amide **4** indicated that the reaction afforded only a single diastereomer. On the basis of degradation and correlation with NMR and X-ray data, the dipeptide mimic **4** was assigned the *S,S,S* configuration (see below). The dipeptide mimic **4** was then protected as its t-Boc derivative **5** and incorporated into the target AII analogues (see below).

In an effort to obtain the other (*S,R,S*) diastereomer of the Val-His mimic **4**, we explored the reductive amination of the keto amide **9** with histidine methyl ester (Scheme II). Boc-valinal **2** was converted to the hydroxy amino acid **6** in 76% yield by using a procedure published for similar compounds.[13] The diastereomeric mixture of the hydroxy amino acids **6** was esterified with HCl/MeOH followed by N-protection using di-*tert*-butyl dicarbonate to afford the α-amino-protected hydroxy ester **7**. The ester **7** was transformed to the corresponding amide **8** by treatment with concentrated NH$_4$OH. The mixture of diastereomeric hydroxy amides **8** was then oxidized with PCC to the α-keto amide **9** in 33% yield. Reductive amination of the keto amide **9** with histidine methyl ester proceeded sluggishly under hydrogenation conditions, with 10% Pd–C as catalyst, to afford the Val-His analogue **10** in 41% yield.[14] Esterification of the Val-His mimic **4** with diazomethane and ^{1}H NMR correlation of the resulting ester with **10** indicated that the major diastereomer had the same stereochemistry as **4**. ^{13}C NMR analysis of the mixture of **10** indicated the possible presence of small amounts of other diastereomers, inseparable by column chromatography.

Stereochemical Assignment of the Val-His Mimic 4. The *S* absolute configuration of the newly formed chiral center was determined by degradation of either **4** or **5** with KMnO$_4$[15] to afford (*S,S*)-2,3-diamino-4-methylpentanamide, which was cyclized with trichloromethyl chloroformate[16] (diphosgene) to give cis imidazolidinone **15a** (Scheme III). The cis-(*S,S*) relative configuration was deduced from the ^{1}H NMR coupling constant ($J_{4,5}$ = 9.3

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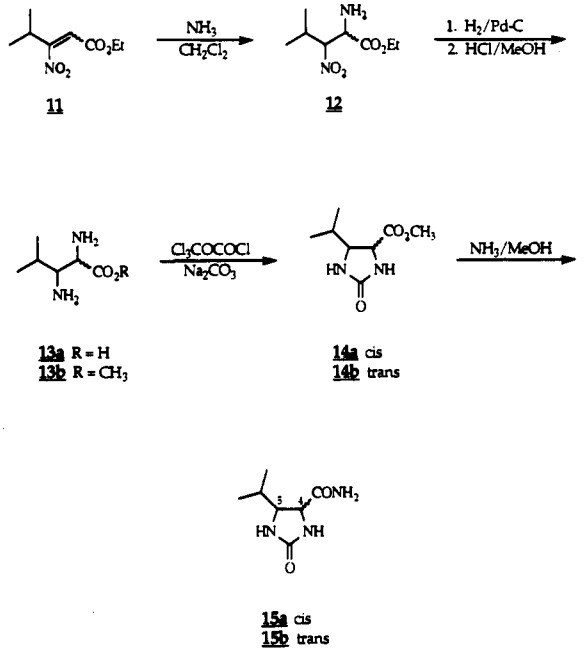
(10) Rich, D. H.; Sun, E. T.; Boparai, A. S. *J. Org. Chem.* **1978**, *43*, 3624.
(11) Several studies have been carried out on the stability of the Strecker adducts at various pH ranges: (a) Commeyras, A.; Taillades, J. *Tetrahedron* **1974**, *30*, 127. (b) Stout, D. M.; Black, L. A.; Matier, W. L. *J. Org. Chem.* **1983**, *48*, 5369.
(12) Turner, R.; Djerassi, C. *J. Am. Chem. Soc.* **1950**, *72*, 3081.
(13) Nishizawa, R.; Saino, T.; Takita, T.; Suda, H.; Aoyagi, T.; Umezawa, H. *J. Med. Chem.* **1977**, *20*, 510.
(14) Initial attempts to carry out the reductive amination of the keto amide **9** with NaBH$_3$CN resulted in the reduction of the carbonyl to the hydroxy amide **8** with very little reductive amination.
(15) Bates, H. A.; Magrath, J. J.; Kaushal, A. *J. Nat. Prod.* **1985**, *48*, 598 and references noted therein.
(16) Efraty, A.; Feinstein, I.; Wackerle, L.; Goldman, A. *J. Org. Chem.* **1980**, *45*, 4059.

Table I. Characterization Data for All Analogues[a]

compd	amino acid analysis								HPLC	
	Sar	Arg	Tyr	Val	His	Pro	Ile	Ala	k'	% purity
19						0.93	1.07		6.76[b]	>95
21				0.99	0.97	1.04	1.00		6.87[b]	>95
25				1.01	0.95	1.04	1.06		29.04[c]	>95
28				0.99	0.91	1.07	1.04		24.42[c]	>92
29	1.01	1.10	0.92	1.05		0.91	1.02		20.55[c]	>97
30	0.97	0.97	0.89	2.01	1.01	1.02	1.01		20.88[c]	>94
31	+[e]	0.97	1.00	1.08	0.95	1.03	0.84	1.10	9.62[d]	>99
32	+	0.93	0.94	2.11	1.01	1.03		0.99	13.51[d]	>99
33	+	0.99	0.99	1.09	0.97	0.98	1.99		22.47[c]	>97

[a] Abbreviations used are those recommended by the IUPAC-IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1972**, *247*, 977). [b] HPLC method 1. [c] HPLC method 2. [d] HPLC method 3. [e] Found but not quantitated.

Scheme IV



Hz), which was in good agreement with cis-substituted oxazolidinones.[17]

Authentic samples of racemic cis and trans imidazolidinones **15a** and **15b** were prepared via independent synthesis (Scheme IV). Ammonia reacted in a regiospecific conjugate manner with 3-nitroacrylate **11**[18] to give the nitro amino ester **12**. As expected, this conjugate addition proceeds via formation of the more stable anion intermediate, α to the nitro group rather than α to the ester group. Catalytic reduction (Pd–C) of the nitro group also caused hydrolysis of the ester to provide **13a**. Ester hydrolysis during catalytic reduction of 3-nitro esters, which we find to be general, appears to be caused by intramolecular attack of the hydroxylamine intermediate upon the ester, followed by reductive N–O cleavage. Esterification of **13a** gave volatile diamino ester **13b**, which was cyclized with trichloromethyl chloroformate to give a mixture of cis and trans imidazolidinones **14a** and **14b**, distinguished by $J_{4,5}$ of 8.2 and 3.7 Hz, respectively. When this mixture was treated with ammonia in methanol at room temperature, trans ester **14b** produced trans amide **15b** in less than 24 h. In contrast, the more hindered cis ester **14a** was consumed with a half-life of approximately 5 days, affording cis amide **15a** accompanied by epimerization to trans amide **15b**. The cis amide **15a** synthesized by this route was spectroscopically and chromatographically identical with that obtained by the degradation and cyclization transformations on **4** or **5**. The cis and trans amides displayed coupling constants $J_{4,5}$ of 9.3 and 4.5 Hz, respectively. The stereochemistry of **15b** was unambiguously

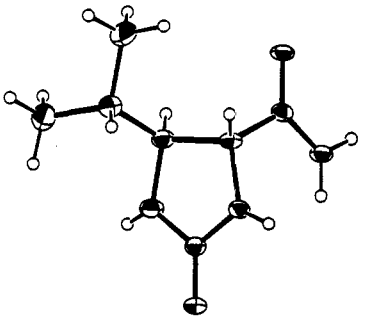
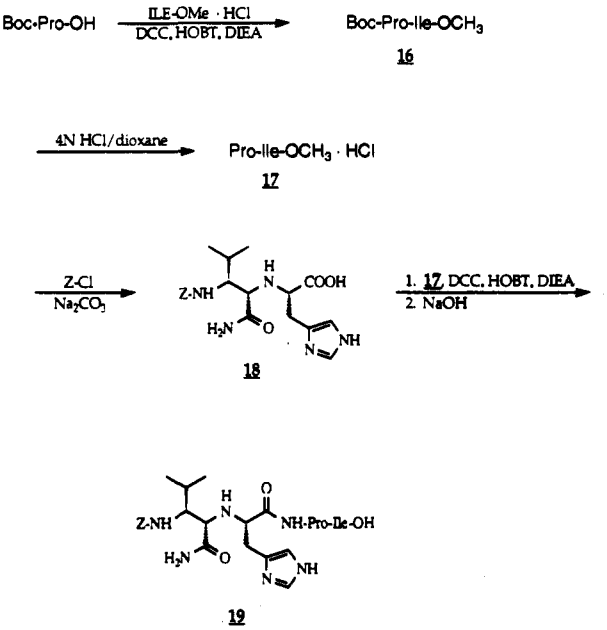
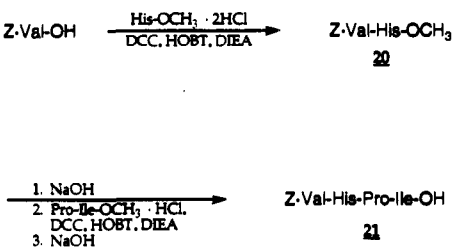


Figure 1. X-ray crystal structure of compound **15b**.

Scheme V

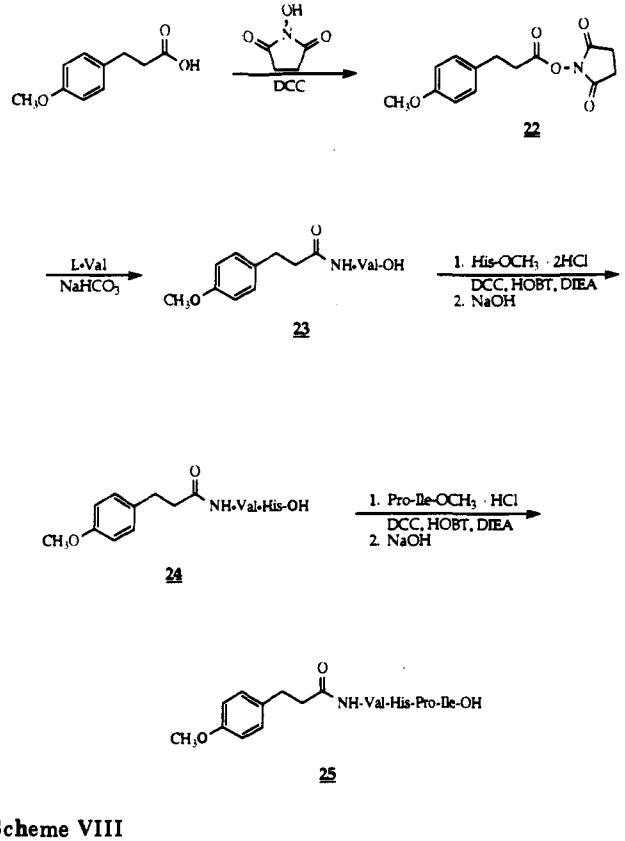


Scheme VI

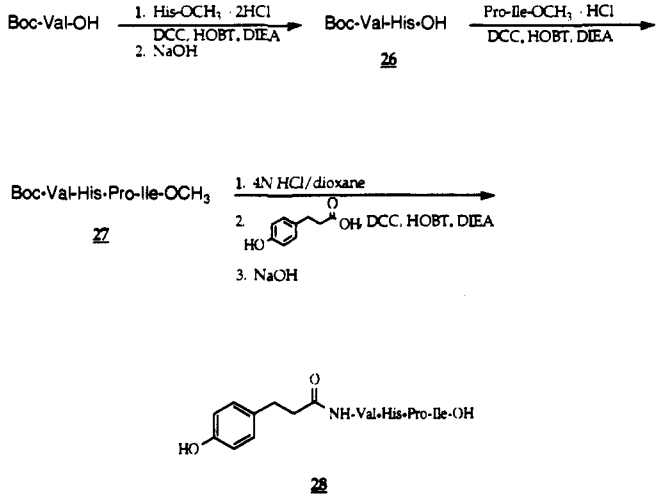


(17) (a) Johnson, R. L. *J. Med. Chem.* **1982**, *25*, 605. (b) Williams, T. M.; Crumbie, R.; Mosher, H. S. *J. Org. Chem.* **1985**, *50*, 91.
(18) Shin, C.; Yonezawa, Y.; Narukawa, H.; Nanjo, K.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 3595.

Scheme VII



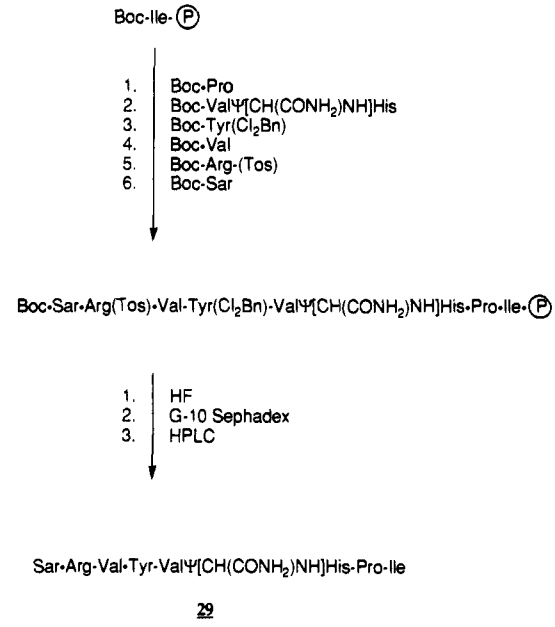
Scheme VIII



proven by X-ray crystallography as shown in Figure 1.[19]

Peptide Syntheses. The C-terminal tetrapeptides **19**, **21**, **25**, and **28** were synthesized by solution chemistry using DCC/HOBT coupling methods (Schemes V–VIII). In order to establish that there is no significant diastereomer contamination in the target compounds (from racemization at the histidine α carbon during HOBT-mediated coupling), HPLC analysis was carried out and the data are presented in Table I. The Valψ[CH(CONH$_2$)NH]His-AII analogue **29** and the corresponding standard [Sar^1,Val^5,Ile^8] **30** were assembled by using manual solid-phase peptide synthesis[20] starting from the commercially available Boc-Ile-polystyrene Merrifield resin (Bachem Inc., CA.; substitution 0.72 mequiv/g) (Scheme IX). Single coupling cycles (using 5 equiv of the amino acids including compound **4**, DIC, HOBT) were sufficient in all cases except when coupling with Boc-valine (three couplings necessary). All couplings were monitored by using the Kaiser ninhydrin test. The secondary amine function of the dipeptide mimic **4** appears to have no reactivity toward various amino acids during the solid-phase synthesis.[21] The peptides were cleaved from the resin by using the low/high HF procedure.[22]

Scheme IX



Peptide Purification and Physiochemical Characterization. The crude peptides were loaded on G-10 Sephadex and eluted with 10% acetic acid in water. After lyophilization, the peptides were further purified by semi-preparative reverse phase HPLC on an ES Industries M C18 (5-μm particle size) column (2.3 cm i.d. × 15 cm length) using a binary solvent system consisting of solvent A (50% acetonitrile–0.1% TFA in water) and solvent B (1% acetonitrile–0.1% TFA in water). A flow rate of 10 mL/min was used and the elution of the peptides monitored at 220 nm. The peptides were obtained by pooling of the desired fractions, removal of the acetonitrile on a rotary evaporator, and lyophilization. Analytical HPLC utilizing a Partisil 5 ODS-3 (5-μm particle size) column (4.6 mm i.d. × 25 cm length) was used to determine the k' and percent purities of the target compounds (Table I). Other chromatographic protocol included a flow rate of 1 mL/min, a standard binary solvent system [solvent A (0.1% TFA in water) and solvent B (0.1% TFA in acetonitrile)] and detection at 220 nm. The mobile-phase programs were as follows: method 1, 30–70% solvent B in a linear gradient (over 15 min) followed by 70–100% solvent B in a linear gradient (over 5 min); method 2, 0–60% solvent B in a linear gradient (over 40 min); method 3, 10–35% solvent B in a linear gradient (over 25 min). Amino acid analysis was carried out on all compounds and the data are presented in Table I.

Biology. All the target compounds were tested in duplicate at one concentration (300 ng/mL) for ability to

(19) X-ray crystallographic analysis provided by J. C. Huffman, Molecular Structure Center, Indiana University, Bloomington, IN.

(20) For a recent review of solid-phase peptide synthesis on Merrifield resin, see: Stewart, J. M., Young, J. D. In *Solid Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984.

(21) Yamada, T.; Motoyama, N.; Taniguchi, T.; Kazuta, Y.; Miyazawa, T.; Kuwata, S.; Matsumoto, K.; Sugiura, M. *Peptide Chem.* **1986**, 287.

(22) Tam, J. P.; Heath, W. F.; Merrifield, R. B. *J. Am. Chem. Soc.* **1983**, *105*, 6442.

displace ^{3}H-AII from rabbit adrenal gland homogenate (^{3}H-angiotensin II receptor binding assay). All compounds were also tested as antagonists of AII and AI on guinea pig ileum (guinea pig ileum assay). The results of the biological assays are presented in Table II. The data show that the results of ^{3}H-AII binding to rabbit adrenal gland homogenate were reasonably consistent with guinea pig ileum assays. The data for AI antagonist activity for all compounds except **31** are consistent with that of AII antagonism. The four octapeptide standards **30–33** display strong AII antagonist activity with IC_{50} values below 1 nM. The octapeptide mimic **29**, containing the Val-His replacement, was more than 700 times less active than the parent peptide analogue **30**. Amongst the smaller C-terminal fragments, only the tetrapeptide **21** displayed any appreciable binding activity (10% at 300 ng/mL). All the C-terminal fragments **19**, **21**, **25**, and **28** have no measurable AII antagonist activity, suggesting that additional residues from the parent hormone (such as Arg) may be necessary for biological potency.[23] Considering the sensitivity of the Val-His region, the biological activity of compound **29** is encouraging and is a starting point for further manipulation of mimics such as **4** in angiotensin II analogues. This will hopefully lead to a better understanding of how conformational changes brought about by modification of the peptide backbone affect the biological activity of hormones such as angiotensin II and its analogues.

Experimental Section

General Methods. Melting points were determined on a Thomas-Hoover capillary melting point apparatus in open capillaries and are uncorrected. IR spectra were obtained on a Perkin-Elmer 1720 FTIR spectrophotometer. Proton NMR spectra were obtained on a Varian XL-300 spectrometer and chemical shifts are expressed in parts per million (δ) downfield from tetramethylsilane (TMS) as internal standard. Thin-layer chromatography (TLC) was carried out on E. Merck glass plates precoated with silica gel 60 F-254 with spots visualized by UV light, iodine, ninhydrin, phosphomolybdic acid, or chlorine–starch–iodide. Elemental analyses were performed by Berlex Laboratories (Cedar Knolls, NJ) or Microlit Laboratory (West Caldwell, NJ). Amino acid analyses were run by Peninsula Laboratories (Belmont, CA). Mass spectra were obtained on a Kratos MS25 using FAB ionization techniques.

(1*RS*,2*S*)-*N*-[1-Cyano-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-methylbutyl]-L-histidine (3). A solution of *N*-Boc-valinal (**2**)[9] (5.5 g, 19.9 mmol) in methanol (10 mL) was added to a solution of sodium bisulfite (2.1 g, 19.9 mmol) in water (25 mL), and the resulting solution was stirred for 1 h. This was followed by the addition of a solution of L-histidine (3.1 g, 19.9 mmol) in 1 N NaOH (20 mL). After 1 h, NaCN (0.98 g, 19.95 mmol) was added and the mixture was stirred at room temperature for 18 h. The solution was adjusted to pH ≈ 7 with 2 N HCl. After evaporation to dryness, the solid was washed with a mixture of ethanol and methanol (1:1, 3 × 60 mL). The washings were combined and stripped to dryness. The solid was purified on silica gel (75:22:3 CH_2Cl_2:MeOH:H_2O; R_f = 0.3) to give the desired product (9.9 g, 79%) as a 2:1 mixture of diastereomers. **Major**: ^{1}H NMR (DMSO-d_6) δ 0.70 (d, 3 H), 0.78 (d, 3 H), 1.39 (s, 9 H), 1.90 (m, 1 H), 2.95 (dd, 1 H), 3.10 (dd, 1 H), 3.70 (m, 2 H), 6.90 (d, 1 H), 7.43 (s, 1 H), 9.02 (s, 1 H). **Minor**: ^{1}H NMR (DMSO-d_6) δ 0.63 (d, 3 H), 0.79 (d, 3 H), 1.41 (s, H), 1.73 (m, 1 H), 2.83 (dd, 1 H), 3.03 (dd, 1 H), 3.45 (ddd, 1 H), 3.56 (s, 1 H), 3.88 (d, 1 H), 7.03 (d, 1 H), 7.38 (s, 1 H), 8.97 (s, 1 H).

(1*S*,2*S*)-*N*-[2-Amino-1-(aminocarbonyl)-3-methylbutyl]-L-histidine (4). Concentrated sulfuric acid (30 mL) was cooled to −20 °C and added dropwise to the solid nitrile **3** (7.8 g, 24.65 mmol), under nitrogen. After the addition was complete, the foaming solution was kept at −20 °C for 2 h. After the initial foaming had subsided, the reaction was kept at 5 °C (refrigerator) for 3 days. The reaction was then poured into ice–water (100 mL) and the solution adjusted to pH ≈ 7 by careful addition of solid Na_2CO_3. The aqueous solution was evaporated to afford a white residue, which was dissolved in 10 mL of methanol and chromatographed on silica gel (7:3 1-propanol: concentrated NH_4OH; R_f = 0.27) to afford 2.5 g (36%) of the product **4** as a white fluffy solid: mp 135–142 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.88 (d, 3 H), 0.97 (d, 3 H), 1.83 (m, 1 H), 2.59 (dd, 1 H), 2.96 (dd, 1 H), 3.08 (dd, 1 H), 3.21 (d, 1 H), 3.30 (d, 1 H), 6.84 (br s, 1 H), 7.21 (br s, 1 H), 7.54 (br s, 1 H), 7.72 (br s, 1 H); ^{13}C NMR (CD_3OD) δ 181.0, 175.5, 135.8, 135.1, 118.7, 64.2, 62.0, 59.9, 32.0, 29.5, 20.2, 19.5; IR (KBr) 3400, 1678, 1580, 1480, 1396, 1106 cm^{-1}; MS (FAB) $(M + H)^+$ 284. Anal. ($C_{12}H_{25}N_5O_3$) C, H, N.

(1*S*,2*S*)-*N*-[1-(Aminocarbonyl)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-methylbutyl]-L-histidine (5). A mixture of *N*-[1-(aminocarbonyl)-2-amino-3-methylbutyl]-L-histidine (**4**) (1.7 g, 6.0 mmol) and Na_2CO_3 (0.65 g, 6.1 mmol) in water (10 mL) was stirred at 30 °C for 20 min, followed by the addition of methanol (80 mL). The mixture was cooled in an ice bath and di-*tert*-butyl dicarbonate (6.7 g, 30.0 mmol) was added. After being stirred at 5 °C for 4 h, concentrated NH_4OH (6 mL) was added and the reaction stirred for 2 h. The mixture was kept at 5 °C for 15 h and evaporated to dryness. The solid residue was chromatographed on silica gel (water:methanol:CH_2Cl_2 1:7:12, R_f = 0.31) to give the desired product **5** (1.12 g, 48%) as a white solid: ^{1}H NMR (DMSO-d_6, TFA added) δ 0.78 (d, 3 H), 0.83 (d, 3 H), 1.38 (s, 9 H), 1.90 (m, 1 H), 2.79 (dd, 1 H), 2.86 (dd, 1 H), 3.14 (d, 1 H), 3.32 (dd, 1 H), 3.44 (m, 1 H), 6.22 (d, 1 H), 6.94 (s, 1 H), 7.16 (s, 1 H), 7.29 (s, 1 H), and 7.87 (s, 1 H). Anal. ($C_{17}H_{29}N_5O_5 \cdot 1.3H_2O$) C, H, N.

(2*RS*,3*S*)-3-Amino-2-hydroxy-4-methylpentanoic Acid (6). Boc-valinal (**2**)[9] (4.00 g, 19.9 mmol) was dissolved in EtOAc (30 mL) and water (20 mL) at 0 °C and $NaHSO_3$ (2.32 g, 22.3 mmol), was added to the solution. After 30 min of stirring at 0 °C, KCN (1.44 g, 22.1 mmol) was added. After 4.5 h at 0 °C, the organic layer was separated, the aqueous layer was extracted with EtOAc, and the combined organic layers were dried ($MgSO_4$). Evaporation of the solvent and drying under high-vacuum afforded 4.75 g (105%) of the cyanohydrin as a colorless gum, which was shown by NMR to be a mixture of diastereomers. The crude cyanohydrin (1.10 g, 4.82 mmol) was dissolved in a mixture of dioxane (8 mL) and concentrated HCl (8 mL). After the gas evolution and exothermic reaction subsided, the mixture was refluxed for 16 h, then cooled to 20 °C, and extracted with ether. The aqueous phase was evaporated to dryness and the residue was redissolved in water (7 mL) and decolorized with activated charcoal. Evaporation of the water followed by chromatography on silica gel (9:1 1-propanol:water; R_f = 0.40) afforded 0.61 g (76%) of a mixture of diastereomeric amino acids **6**: ^{1}H NMR (DMSO-d_6, TFA added) δ 0.99 (m, 6 H), 1.97 (m, 1 H), 3.15 (m, 1 H), 4.23 (d, 0.5 H), 4.44 (d, 0.5 H), 10.40 (br s, 1 H).

Methyl (2*RS*,3*S*)-3-[[(1,1-dimethylethoxy)carbonyl]amino]-2-hydroxy-4-methylpentanoate (7). A suspension of the diastereomeric mixture of the amino acids **6** (11.1 g, 75.5 mmol) in MeOH (100 mL) was saturated with HCl while cooling in an ice bath. The reaction mixture became homogeneous and then cloudy as the HCl was added. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue dissolved in MeOH (500 mL). Di-*tert*-butyl dicarbonate (20.0 g, 91.6 mmol) followed by Na_2CO_3 (9.0 g, 91.6 mmol) was added, and the mixture was stirred for 18 h at room temperature. After evaporation of the volatile materials, the residue was partitioned between CH_2Cl_2 (200 mL) and water (300 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 200 mL), the combined organic layers were dried ($MgSO_4$), and the solvent was removed to afford a white solid. Chromatography on silica gel (1:1 Et_2O:hexane; R_f = 0.11) afforded 6.45 g (33%) of **7**. ***R*,*S* diastereomer (7a)**: mp 103–104 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 (d, 3 H), 1.03 (d, 3 H), 1.41 (s, 9 H), 1.89 (m, 1 H), 3.11 (d, 1 H), 3.69 (t, 1 H), 3.79 (s, 3 H), 4.36 (dd, 1 H), 4.73 (d, 1 H); IR (KBr) 3480, 3350, 2980, 1745, 1700, 1530 cm^{-1}. Anal. ($C_{12}H_{23}NO_5$) C, H, N. ***S*,*S* diastereomer (7b)**: R_f = 0.21; mp 49–50 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 6 H), 1.45 (s, 9 H), 1.91 (m, 1 H), 3.29 (d, 1 H), 3.78 (m, 1 H), 3.79 (s, 3 H), 4.30 (t, 1 H), 4.81 (d, 1 H); IR (KBr) 3380, 2940, 2860, 1730, 1700 cm^{-1}. Anal. ($C_{12}H_{23}NO_5$) C, H, N.

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Table II. Potency of Angiotensin II Antagonists Measured on Guinea Pig Illeum Tissue Bath and in [^{3}H]Angiotensin II Binding Assay to Rabbit Adrenal Gland Homogenate

compd	structure	% [^{3}H]AII displacement (300 ng/mL)[a]	guinea pig ileum IC_{50} (nM) (AII antagonism)	guinea pig ileum IC_{50} (nM) (antagonist of AI action)
	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe (AII)	c		
19	Z-Valψ[CH($CONH_2$)NH]His-Pro-Ile	0	>15 000	>15 000
21	Z-Val-His-Pro-Ile	10	>15 000	>15 000
25	*p*-MeO-phenylpropionyl-Val-His-Pro-Ile	0	>15 000	>15 000
28	*p*-OH-phenylpropionyl-Val-His-Pro-Ile	0	>15 000	>15 000
29	Sar-Arg-Val-Tyr-Valψ[CH($CONH_2$)NH]His-Pro-Ile	40	330	345
30[b]	Sar-Arg-Val-Tyr-Val-His-Pro-Ile	100	0.45	0.51
31[b]	Sar-Arg-Val-Tyr-Ile-His-Pro-Ala	100	5.8	0.56
32[b]	Sar-Arg-Val-Tyr-Val-His-Pro-Ala	100	0.066	0.048
33[b]	Sar-Arg-Val-Tyr-Ile-His-Pro-Ile	100	0.21	0.17

[a] All compounds tested in duplicate. [b] These peptides are standards. [c] IC_{50} = 7 nM.

(2*RS*,3*S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-2-hydroxy-4-methylpentanamide (8). The diastereomers of **7** (5.3 g) were treated with concentrated NH_4OH (350 mL). After 18 h, the solvent was evaporated to afford the amide as a white solid (4.9 g, 98% yield). ***R*,*S* diastereomer**: ^{1}H NMR ($CDCl_3$) δ 0.99 (d, 3 H), 1.02 (d, 3 H), 1.44 (s, 9 H), 2.24 (m, 1 H), 3.39 (t, 1 H), 4.30 (br s, 1 H), 5.26 (m, 2 H), 5.94 (br s, 1 H), 6.92 (br s, 1 H). ***S*,*S* diastereomer**: ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 3 H), 1.03 (d, 3 H), 1.43 (s, 9 H), 2.14 (m, 1 H), 3.51 (t, 1 H), 4.16 (br m, 1 H), 5.94 (br s, 1 H), 6.90 (br s, 1 H).

(*S*)-3-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-methyl-2-oxopentanamide (9). A mixture of the hydroxy amide **8** (4.6 g, 18.7 mmol), NaOAc (1.56 g, 19 mmol), and powdered 4-Å molecular sieves (2.3 g) was suspended in CH_2Cl_2 (100 mL). Pyridinium chlorochromate (7.18 g, 33.3 mmol) was added over 1.5 h, and the mixture was stirred at 20 °C for 18 h. The mixture was poured into ether (400 mL) and filtered. The precipitate was extracted with hot EtOAc (3 × 100 mL) and the solvent was evaporated. Chromatography on silica gel (45:55 ether:hexanes; R_f = 0.40) afforded the keto amide **9** (1.5 g, 33%) as a white solid: mp 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 0.82 (d, 3 H), 1.03 (d, 3 H), 1.44 (s, 9 H), 2.38 (m, 1 H), 4.99 (dd, 1 H), 5.12 (br s, 1 H), 5.65 (br s, 1 H), 6.80 (br s, 1 H); IR (KBr) 3355, 3196, 2969, 1738, 1690, 1646, 1525, 1300 cm^{-1}. Anal. ($C_{11}H_{20}N_2O_4$) C, H, N.

(1*S*,2*S*)-*N*-[1-(Aminocarbonyl)-2-[[(1,1-dimethylethoxy)carbonyl]amino]-3-methylbutyl]-L-histidine Methyl Ester (10). Keto amide **9** (480 mg, 1.96 mmol) and His-OMe (2.00 g, 11.8 mmol) dissolved in dry MeOH (20 mL) were hydrogenated for 2 weeks over 10% Pd–C (1.0 g) and 3-Å powdered molecular sieves (2 g). The mixture was filtered through Celite, and the solvent was evaporated. The residue was dissolved in water (25 mL) and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ gave a light yellow foam (630 mg), which was purified by chromatography on silica gel (9:1 CH_2Cl_2:MeOH; R_f = 0.48) to give the product **10** (319 mg, 41% yield) as a crude amorphous white solid. ^{1}H NMR and ^{13}C NMR indicated the possibility that the mixture contained four diastereomers in the approximate ratio 80:12:6:2. The stereochemistry of the major isomer **10** was assigned by correlating the ^{1}H spectrum of **10** with that of an authentic sample of **10** independently prepared by treating compound **4** with diazomethane.

Ethyl 2-Amino-4-methyl-3-nitropentanoate (12). Ethyl 4-methyl-3-nitro-2-pentenoate (**11**)[18] (12.95 g, 69.3 mmol) was dissolved in CH_2Cl_2 (160 mL), saturated with ammonia at 0 °C (0.75 M, 120 mmol), and kept in a pressure bottle at 20 °C for 17 h. The precipitate was removed and the solvent was evaporated from the filtrate to afford the crude product as an amber oil (14.35 g). The oil was dissolved in ether (140 mL), and the red precipitate was removed. The filtrate was extracted with 1 M HCl (75 mL) and water (2 × 10 mL). The aqueous phases were combined and made basic with saturated Na_2CO_3 to pH 10 and extracted with CH_2Cl_2. The CH_2Cl_2 layers were dried, and the solvent was removed to afford the product as a very light yellow oil (7.18 g, 50%), which appeared to be unstable to acid or on silica gel: ^{1}H NMR ($CDCl_3$) δ 0.95–1.06 (m, 6 H), 1.27 (2 t, 3 H), 1.74 (br s, 2 H), 2.41 (m, 0.6 H), 2.58 (m, 0.4 H), 3.82 (d, 0.6 H), 3.99 (d, 0.4 H), 4.20 (m, 2 H), 4.54 (m, 1 H); IR (neat) 3400, 2975, 1738, 1547. 1467 cm^{-1}. Anal. ($C_8H_{16}N_2O_4$) C, H, N.

Methyl 2,3-Diamino-4-methylpentanoate (13b). Nitro amino ester **12** (6.1 g, 29.9 mmol) was dissolved in ethanol (65 mL) and hydrogenated at 50 psi over 10% Pd–C (650 mg) for 7 days. The precipitate was collected by centrifugation, rinsed with ethanol, and then extracted with water. Evaporation of the water afforded 3.77 g of the diamino acid **13a** as a light yellow residue. The residue (3.75 g, 25.7 mmol) was treated with 2.5 M HCl_g in MeOH (30 mL) at 50 °C for 4 days. The solvent was evaporated to afford the crude dihydrochloride salt of **13b** (5.41 g, 90% yield). Half of the salt was basified with saturated Na_2CO_3 and extracted ten times with $CHCl_3$. The combined $CHCl_3$ layers were dried and evaporated to afford the diastereomeric mixture of **13b** as a yellow oil (0.595 g, 29%). An analytical sample was prepared by Kugelrohr distillation (90–110 °C, 1.2 Torr): ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 3 H), 0.97 (d, 6 H), 0.99 (d, 3 H), 1.39 (br m, 8 H), 1.67 (m, 1 H), 1.85 (m, 1 H), 2.60 (t, 1 H), 2.71 (dd, 1 H), 3.47 (d, 1 H, J = 6.1 Hz), 3.56 (d, 1 H, J = 3.8 Hz), 3.74 (t, 3 H), 3.75 (t, 3 H); ^{13}C NMR ($CDCl_3$) 176.0, 175.5, 60.6, 59.5, 57.5, 56.5, 51.8, 51.5, 30.8, 29.9, 20.1, 20.1, 18.5, 17.2; IR (neat) 3381, 2957, 1734, 1438, 1201, 1173 cm^{-1}; FAB MS (M + H)$^+$ 161. Anal. ($C_7H_{16}N_2O_2$) C, H, N.

Methyl *cis*- and *trans*-5-(1-Methylethyl)-2-oxo-4-imidazolidinecarboxylate (14a and 14b). The crude diamino ester dihydrochloride **13b** (2.1 g, 9.0 mmol) was dissolved in water at 0 °C, containing Na_2CO_3 (900 mg, 8.49 mmol). *In an efficient hood*, trichloromethyl chloroformate (0.80 mL, 1.31 g, 663 mmol) and Na_2CO_3 (1.4 g, 13.2 mmol) dissolved in water (6 mL) were added alternately in portions over 30 min. The mixture was allowed to warm to 20 °C and adjusted to pH 8 with Na_2CO_3. The precipitate was collected by filtration, rinsed with water, and dried to afford the product (1.05 g) as a 1:1 mixture of isomers: mp 188–192 °C. Additional product (0.20 g) was isolated by extraction of the filtrate with $CHCl_3$ (75% total yield). An analytical sample was purified by chromatography on silica gel (90:10 $CHCl_3$:EtOH) to afford a mixture of the cis and trans isomers **14a** (R_f = 0.30) and **14b** (R_f = 0.35): mp 200–202 °C: ^{1}H NMR ($CDCl_3$) δ 0.98 (m, 12 H), 1.74 (m, 1 H), 1.84 (m, 1 H), 3.68 (m, 1 H), 3.70 (m, 1 H), 3.78 (s, 3 H), 3.79 (s, 3 H), 3.99 (d, 1 H, J = 3.66 Hz, trans isomer), 4.37 (d, 1 H, J = 8.22 Hz, cis isomer), 5.12 (s, 1 H), 5.17 (s, 1 H), 5.38 (s, 1 H), 5.48 (s, 1 H); IR (KBr) 3221, 2966, 1745, 1708, 1454, 1205, 1180 cm^{-1}. Anal. ($C_{18}H_{14}N_2O_3$·0.15H_2O) C, H, N.

***cis*- and *trans*-5-(1-Methylethyl)-2-oxo-4-imidazolidinecarboxamide (15a and 15b).** A 1:1 mixture of methyl esters **14a** and **14b** (200 mg, 1.07 mmol) was dissolved in MeOH (15 mL). The solution was saturated with NH_3 at 0 °C (4 M) and sealed in a pressure bottle at room temperature for 19 days. The reaction was followed by observing the ^{1}H NMR of aliquots at various times. The trans ester was completely converted into the trans amide within 24 h, while 90% of the cis ester remained unchanged. The ratio of the cis ester **14a**:cis amide **15a**:trans amide **15b** was 7:13:80 after 6 days and 0:10:90 after 19 days. The crystals of the pure trans amide **15b** (50 mg, 27% yield) were collected by filtration. The solvent was evaporated from the supernatant to afford a 7:1 mixture of trans and cis amides (132 mg, 72% yield). Recrystallization from hot water afforded pure trans amide **15b**, while the mother liquor contained a 1:1 mixture of cis and trans amides, inseparable by chromatography on silica gel (50:50

$CHCl_3$:EtOH; R_f = 0.55). Cis amide **15a** was also obtained by recrystallization from water. The trans configuration of **15b** was determined by ^1H NMR and X-ray crystallography. **Cis isomer 15a**: mp 250–254 °C; ^1H NMR (D_2O) δ 0.94 (m, 6 H), 1.89 (m, 1 H), 3.90 (dd, 1 H, J = 6.3, 9.4 Hz), 4.49 (d, 1 H, J = 9.3 Hz). **Trans isomer 15b**: mp 262–263 °C; ^1H NMR (DMSO-d_6) δ 0.94 (m, 6 H), 1.89 (m, 1 H), 3.66 (t, 1 H), 4.06 (d, 1 H, J = 4.5 Hz).

***cis*-5-(1-Methylethyl)-2-oxo-4-imidazolidinecarboxamide by Degradation of 4.** Dipeptide mimic 4 (45 mg, 0.159 mmol) was dissolved in water (0.5 mL), and $KMnO_4$ (20 mg, 0.127 mmol) dissolved in water (1.0 mL) was added dropwise with stirring. After 2 h, the precipitate was removed by centrifugation. The supernatant was lyophilized, dissolved in water (0.5 mL) containing NH_4OH (4 drops), applied to a 1-mL column of Dowex AG1X8 resin (OH^- form, 20–50 mesh), and eluted with 5 mL of water. The eluent was lyophilized to give a yellow solid (57 mg), which was purified by chromatography on silica gel (70:30 1-propanol:concentrated NH_4OH) to give impure diamino amide (10 mg). This was dissolved in D_2O (0.4 mL) and placed in an NMR tube at 0 °C. Trichloromethyl chloroformate (0.02 mL, 33 mg, 0.16 mmol) was added (*in an efficient hood*) and the tube was capped. After 10 min, Na_2CO_3 (60 mg, 0.56 mmol) was added. After 18 h, ^1H NMR of the crude product indicated cis imidazolidinone, with no detectable trans imidazolidinone. The solvent was evaporated and the residue was extracted with methanol (2 × 4 mL). Soluble material was suspended in EtOH–$CHCl_3$ (50:50) and chromatographed on silica gel (50:50 EtOH:$CHCl_3$) to afford pure cis imidazolidinone **15a** (1 mg, 4% yield), spectroscopically identical with that prepared from **14a**.

***cis*-5-(1-Methylethyl)-2-oxo-4-imidazolidinecarboxamide by Degradation of 5.** Compound **5** (10.2 mg, 0.027 mmol) was dissolved in water (0.1 mL), and $KMnO_4$ (4.4 mg, 0.028 mmol) dissolved in water (0.22 mL) was added dropwise with stirring over 40 min. After 40 min, the precipitate was removed by centrifugation. The supernatant was made basic with 1 M NaOH and extracted four times with $CHCl_3$. The combined organic phase was dried and the solvent evaporated to yield the Boc diamino amide (0.9 mg, 14% yield). The Boc group was removed by treatment with 1 M HCl (0.5 mL) for 1.5 h. Treatment with trichloromethyl chloroformate as above provided **15a**.[24]

L-Boc-Pro-Ile-OCH_3[25] (16). To a solution of Boc proline (10.75 g, 50 mmol), L-isoleucine methyl ester hydrochloride (9.09 g, 50 mmol), hydroxybenzotriazole (7.66 g, 50 mmol), and diisopropylethylamine (8.7 mL, 50 mmol) in 30 mL of THF, cooled to 0 °C, was added dicyclohexylcarbodiimide (10.82 g, 52.5 mmol), and the reaction was stirred for 1 h at 0 °C and then another 1 h at room temperature. The solution was filtered to remove the dicyclohexylurea. EtOAc (500 mL) was added to the filtrate and the combined solution was washed with saturated $NaHCO_3$ solution (200 mL), 10% citric acid solution (200 mL), and saturated NaCl (200 mL) and dried ($MgSO_4$), and the solvent was evaporated to afford 9.7 g (57%) of compound **16** as a white solid: mp 67–68 °C; ^1H NMR ($CDCl_3$) δ 0.87 (d, 3 H), 0.90 (t, 3 H), 1.1–1.4 (m, 2 H), 1.47 (s, 9 H), 1.8–2.2 (m, 5 H), 3.2–3.6 (m, 2 H), 3.7 (s, 3 H), 4.2–4.6 (m, 2 H), 6.5 (br m, 0.5 H), 7.5 (br m, 0.5 H); IR (KBr) 3344, 2971, 2879, 1744, 1674, 1402, 1367, 1211, 1168 cm^{-1}. Anal. ($C_{17}H_{30}N_2O_5$) C, H, N.

***N*-[1-(Aminocarbonyl)-2-[(benzyloxycarbonyl)amino]-3-methylbutyl]-L-histidine (18).** To a solution of dipeptide mimic 4 (1.0 g, 3.53 mmol), in 10 mL of water was added Na_2CO_3 (1.06 g, 10 mmol), and the reaction mixture was cooled to 0 °C. Benzyl chloroformate (682 mg, 571 μL, 4 mmol) was added in three portions over 15-min intervals. The reaction mixture was stirred at 0 °C for 3 h. Another 100 μL of benzyl chloroformate was added to the reaction and the reaction stirred an additional 1 h. Water (50 mL) was then added to the reaction followed by 25 mL of ether. The aqueous layer was separated and washed with Et_2O (25 mL). The water was removed on a rotory evaporator at 35 °C to afford 3 g of the crude product. The entire mixture was chromatographed on silica gel and eluted with CH_2Cl_2:MeOH:concentrated NH_4OH (15:5:1, R_f = 0.31) to afford 400 mg (30%) of the Z amino acid **18** as a white solid: mp 130–134 °C; ^1H NMR (DMSO-d_6) δ 0.82 (d, 3 H), 0.85 (d, 3 H), 1.95–1.99 (m, 1 H), 2.70 (dd, 1 H), 2.85 (dd, 1 H), 3.10 (d, 1 H), 3.20 (ddd, 1 H), 3.56–3.59 (m, 1 H), 4.99 (d, 1 H), 5.03 (d, 1 H), 6.79 (s, 1 H), 6.93 (d, 1 H), 7.12 (br s, 1 H), 7.24 (br s, 1 H), 7.30–7.41 (m, 5 H), 7.53 (s, 1 H).

2-[[2-[(Benzyloxycarbonyl)amino]-1-(aminocarbonyl)-3-methylbutyl]amino]-L-histidyl-L-prolyl-L-isoleucine (19). To a solution of the Z amino acid **18** (330 mg, 0.79 mmol) in 7 mL of DMF and 2 mL of CH_2Cl_2, cooled to 0 °C, were added hydroxybenzotriazole (122 mg, 0.80 mmol), dicyclohexylcarbodiimide (165 mg, 0.80 mmol), diisopropylethylamine (110 mg, 0.85 mmol), and prolyl-isoleucine methyl ester hydrochloride (**17**)[25] (obtained from treating the t-Boc compound **16** (291 mg, 0.85 mmol) with 5 mL of 4 N HCl in dioxane for 1 h followed by evaporation of the solvent and drying under high vacuum). The reaction was allowed to warm to room temperature and stirred for 20 h. The volatile materials were removed on the rotory evaporator to afford a viscous oil. The material was treated with 5 mL of 1 N NaOH at 0 °C for 15 min. The solvent was evaporated and the crude solid was purified on silica gel, eluting with CH_2Cl_2:MeOH:concentrated NH_4OH (15:5:1), to afford 410 mg (83%) of the product **19** as a white foam: R_f = 0.38 (I_2, Pauly positive); ^1H NMR (DMSO–TFA added) δ 0.87–0.94 (m, 12 H), 1.10–1.68 (m, 8 H), 3.18–3.58 (m, 3 H), 3.81 (d, 1 H), 3.88–3.98 (m, 1 H), 4.29 (d, 1 H), 4.39 (t, 1 H), 4.58–4.66 (m, 1 H), 5.01 (d, 1 H), 5.11 (d, 1 H), 7.21–7.37 (m, 5 H), 7.62 (br s, 1 H), 8.02 (br s, 0.5 H), 9.17 (br s, 1 H); IR (KBr) 3409, 2964, 1669, 1518, 1455, 1405 cm^{-1}. Anal. ($C_{31}H_{45}N_7O_7 \cdot 2H_2O$) C, H, N.

Z-Val-His-OCH_3 (20).[26] To a suspension of L-histidine methyl ester dihydrochloride (4.12 g, 17 mmol) in 30 mL of THF was added HOBt (3.05 g, 19.9 mol), Z-valine (5.0 g, 19.9 mmol), and diisopropylethylamine (4.4 g, 5.92 mL, 34 mmol) and the cloudy solution cooled to 0 °C. To this solution was added DCC (4.1 g, 19.9 mmol), and the reaction was stirred at 0 °C for 1 h and then at room temperature for an additional 1 h. The precipitated dicyclohexylurea was filtered and 50 mL of EtOAc was added to the reaction mixture. The organic layer was washed with saturated $NaHCO_3$ (2 × 100 mL) and dried ($MgSO_4$), and the solvent was evaporated. After drying over P_2O_5 (high vacuum), 5.9 g (80%) of the dipeptide **20** was obtained as a white solid: ^1H NMR (DMSO-d_6) δ 0.78 (d, 3 H), 0.85 (d, 3 H), 1.85–1.90 (m, 1 H), 3.12 (dd, 1 H), 3.20 (dd, 1 H), 3.60 (s, 3 H), 3.80 (d, 1 H), 4.62–4.80 (m, 1 H), 5.01 (d, 1 H), 5.18 (d, 1 H), 7.30–7.40 (m, 5 H), 7.42 (s, 1 H), 8.50 (d, 1 H), 9.00 (s, 1 H); HRMS (FAB) calcd for $C_{20}H_{27}N_4O_5$ m/z 403.1981, found 403.1966.

Z-Val-His-Pro-Ile-OH (21). To the Z-Val-His-OCH_3 (**20**) (3.0 g, 6.8 mmol) in 10 mL of MeOH was added 20 mL of 1 N NaOH, and the reaction was stirred at room temperature for 30 min. The pH of the solution was adjusted to ca. 6.5 by the dropwise addition of 2 N HCl and the solvent removed to afford a white solid. To the solid dissolved in 20 mL of DMF were added Pro-Ile-OCH_3·HCl (**17**) (obtained from treating the t-Boc compound **16** (1.7 g, 5 mmol) with 10 mL of 4 N HCl in dioxane for 1 h followed by evaporation of the solvent and dyring under high vacuum), HOBt (1.04 g, 6.80 mmol), diisopropylethylamine (0.6 g, 5 mmol), and DCC (1.4 g, 6.8 mmol). The cloudy reaction was stirred at room tempertaure for 16 h. After evaporation of the solvents, the residue was directly treated with 10 mL of MeOH and 20 mL of 1 N NaOH solution for 30 min at room temperature. The volatile materials were removed and the crude residue was chromatographed on silica gel with CH_2Cl_2:MeOH:concentrated NH_4OH (15:5:1, R_f = 0.36) to afford 1.35 g (45%) of the product **21** as a white solid: ^1H NMR (DMSO-d_6) δ 0.75–0.90 (m, 12 H), 1.20–1.50 (m, 2 H), 3.22–3.32 (m, 1 H), 3.50–3.70 (m, 1 H), 3.86 (dd, 1 H), 4.15 (d, 1 H), 4.40–4.50 (m, 1 H), 4.60–4.70 (m, 1 H), 5.03 (br s, 2 H), 6.86 (s, 1 H), 7.29–7.40 (m, 6 H), 7.50 (s, 1 H), 8.10 (d, 1 H), 8.18 (d, 1 H).

1-[3-(4-Methoxyphenyl)-1-oxopropoxy]-2,5-pyrrolidinedione (22). To a solution of 3-(4-methoxyphenyl)propanoic acid (5 g, 27.8 mmol) and *N*-hydroxysuccinimide (3.2 g, 27.8 mmol) in 50 mL of dioxane and 50 mL of THF, cooled to 0 °C, was added DCC (5.72 g, 27.75 mmol), and the reaction was stirred at 0 °C

(24) Stereochemistry of these isomers was established by independent syntheses.

(25) Eur. Pat. Appl. EP 34259, August, 1981.

(26) Schwyzer, R.; Iselin, B.; Kappeler, B.; Riniker, B.; Rittel, W.; Zuber, H. *Helv.* **1958**, *41*, 1287.

for 30 min and then placed in the refrigerator overnight. After 16 h, the solution was filtered to remove the dicyclohexylurea and evaporated to afford an oil. Trituration with petroleum ether afforded 8.6 g of a white solid. Recrystallization from boiling 2-propanol afforded 5.9 g (77%) of **22** as white needles: mp 95–96 °C; ^{1}H NMR ($CDCl_3$) δ 2.75–2.90 (m, 6 H), 2.98 (dt, 2 H), 3.79 (s, 3 H), 6.84 (d, 2 H), 7.15 (d, 2 H); IR (KBr) 3447, 1814, 1786, 1731, 1515 cm^{-1}. Anal. ($C_{14}H_{15}NO_5 \cdot 0.1H_2O$) C, H, N.

***N*-[3-(4-Methoxyphenyl)propionyl]-L-Val-OH (23).** To a solution of L-valine (2.34 g, 20 mmol) in 20 mL of water was added $NaHCO_3$ (3.36 g, 40 mmol) followed by a solution of the succinimide active ester **22** (5.0 g, 18.03 mmol) in 20 mL of dimethoxyethane. The reaction was stirred at room temperature for 4 h. Water (30 mL) was then added to the reaction mixture and the solution acidified to pH 2 with the dropwise addition of 2 N HCl. The precipitate formed was collected and washed with cold water and dried under vacuum (P_2O_5) to afford 2.9 g (58%) of the product **23** as a white solid: mp 142–143 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3 H), 2.10 (m, 1 H), 2.45–2.57 (m, 2 H), 2.90 (t, 2 H), 3.77 (s, 3 H), 4.60 (dd, 1 H), 6.81 (d, 2 H), 8.7 (br s, 1 H); IR (KBr) 3327, 2964, 2935, 2837, 1703, 1614, 1514, 1246, 1177 cm^{-1}.

***N*-[3-(4-Methoxyphenyl)propionyl]-L-Val-L-His (24).** *N*-[3-(4-Methoxyphenylpropionyl]-L-valine (**23**) (2.0 g, 7.15 mmol) was coupled with histidine methyl ester (see compound **20**) and hydrolyzed to the corresponding acid, as described for compound **21**, to afford 1.9 g (64%) of the compound **24** as a white solid: mp; ^{1}H NMR (DMSO-d_6) δ 0.69 (d, 3 H), 0.79 (d, 3 H), 1.96–2.01 (m, 1 H), 2.28–2.46 (m, 2 H), 2.65–2.80 (t, 2 H), 2.90 (dd, 1 H), 2.96 (dd, 1 H), 3.70 (s, 3 H), 4.15 (dd, 1 H), 4.30 (dd, 1 H), 6.75 (s, 1 H), 6.80 (d, 2 H), 7.10 (d, 2 H), 7.60 (s, 1 H), 7.92 (d, 1 H), 8.40 (d, 1 H); HRMS (FAB) calcd for $C_{21}H_{29}N_4O_5$ *m/z* 417.2138, found 417.2150.

***N*-[3-(4-Methoxyphenyl)propionyl]-L-Val-His-Pro-Ile-OH (25).** *N*-[3-(4-Methoxyphenyl)propionyl]-L-Val-L-His (**24**) (833 mg, 2 mmol) was coupled with Pro-Ile-OCH_3 and the resulting tetrapeptide hydrolyzed to the corresponding carboxylic acid (as described for compound **21**) to afford 420 mg (35%) of the compound **25** as a white solid: mp 182–184 °C; ^{1}H NMR (DMSO-d_6, TFA added) δ 0.78 (d, 3 H), 0.81 (d, 3 H), 0.83–0.93 (m, 6 H), 1.19–1.48 (m, 2 H), 1.78–2.16 (m, 6 H), 2.40–2.59 (m, 2 H), 2.78 (br t, 2 H), 2.94–3.18 (m, 2 H), 3.53–3.59 (m, 1 H), 3.72 (s, 3 H), 4.13 (d, 1 H), 4.26 (d, 1 H), 4.54 (dd, 1 H), 4.86 (dd, 1 H), 6.83 (d, 2 H), 7.14 (d, 2 H), 7.44 (s, 1 H), 9.02 (s, 1 H); FAB MS (M + H)$^+$ 627.

Boc-Val-His-OH (26). Boc-valine (5.43 g, 25 mmol) was coupled with histidine methyl ester and hydrolyzed to the corresponding acid, as described for compound **21**, to afford 7.2 g (86%) of the compound **26** as a white solid: ^{1}H NMR (DMSO-d_6) δ 0.79 (d, 3 H), 0.85 (d, 3 H), 1.40 (s, 9 H), 1.91–2.00 (m, 1 H), 2.92 (dd, 1 H), 2.96 (d, 1 H), 4.02 (dd, 1 H), 4.21 (ddd, 1 H), 6.79 (s, 1 H), 6.81 (d, 1 H), 7.55 (s, 1 H), 7.94 (d, H); HRMS (FAB) calcd for $C_{16}H_{27}N_4O_5$ *m/z* 355.1982, found 355.2002.

***N*-[3-(4-Hydroxyphenyl)propionyl]-L-Val-His-Pro-Ile (28).** Boc-Val-His-OH (**26**) (2.0 g, 5.64 mmol) was coupled with Pro-Ile-OCH_3, as described for compound **21**, to afford 3.0 g (94%) of the tetrapeptide **27** as a white solid. The entire material was treated with 5 mL of 4 N HCl in dioxane for 1 h. Evaporation of the solvent and drying (high vacuum) afforded a white foam. To the foam dissolved in 10 mL of DMF were added 3-(4-hydroxyphenyl)propionic acid (748 mg, 4.5 mmol), HOBt (689 mg, 4.5 mmol), and DCC (927 mg, 4.5 mmol), and the reaction was stirred at room temperature for 16 h. The reaction mixture was filtered to remove the dicyclohexylurea and evaporated to afford an oil, which was directly treated with 15 mL of MeOH followed by 15 mL of 1 N NaOH solution for 1 h. The solution was then acidified to pH 6.5 by the dropwise addition of 2 N HCl. The solution was evaporated to dryness and the residue chromatographed on silica gel (CH_2Cl_2:MeOH:concentrated NH_4OH 15:5:1) to afford 1.4 g (51%) of the product. Further purification of 600 mg of material by gel filtration on Sephadex G-10 afforded 420 mg of an analytically pure sample of **28**: ^{1}H NMR (DMSO-d_6) δ 0.75–0.89 (m, 12 H), 1.14–1.50 (m, 2 H), 1.72–2.16 (m, 6 H), 2.32–2.44 (m, 2 H), 2.64–2.76 (m, 2 H), 2.92–3.12 (m, 2 H), 3.45–3.80 (m, 2 H), 4.10 (d, 2 H), 6.64 (d, 2 H), 6.98 (d, 2 H), 7.38 (s, 3 H), 7.88 (d, 0.5 H), 8.26 (d, 1 H), 8.94 (s, 1 H); FAB MS (M + H)$^+$ 613.

Sar-Arg-Val-Tyr-Valψ[CH_2($CONH_2$)NH]His-Pro-Ile (29) was prepared by using solid-phase peptide synthesis[20] as described earlier under Peptide Syntheses. Amino acid analyses and HPLC data are presented in Table I. HRMS (FAB):calcd for $C_{46}H_{74}N_{14}O_{10}$ *m/z* 983.5791, found 983.5774.

X-ray Crystallography. A colorless crystal of **15b** ($C_7H_{13}N_2O_3$, MW = 171.20) with appropriate dimensions of 0.25 × 0.25 × 0.25 mm was affixed to the end of a glass fiber on a goniometer head using silicone grease and transferred to the goniostat where it was cooled to −155 °C for characterization and data collection. The diffractometer utilized for data collection was designed and constructed locally. A Picker four-circle goniostat equipped with a Furnas Monochromator (HOG) crystal and Picker X-ray generator is interfaced to a Z80 microprocessor, which is controlled by a RS232 Serial port on an IBM PC microcomputer. The structure was solved by a combination of direct methods (MULTAN 78) and Fourier techniques. All hydrogen atoms were clearly visible in a difference Fourier synthesis phased on the non-hydrogen parameters. All hydrogen atoms were refined isotropically and non-hydrogen atoms anisotropically in the final cycles. Crystal parameters were as follows: cell dimensions: a = 9.270 (2) Å, b = 16.038 (3) Å, c = 11.704 (2) Å; space group, *Pnaa*; molecules/unit cell, 4; linear absorption coefficient = 38.30 cm^{-1}. Refinement parameters were as follows: number of reflections, 1910; nonzero reflections, 1530; R index, 0.005; GOF, 1.317; $R(F)$ = 0.0389; $R_w(F)$ = 0.0450. Tables 1–5 in the supplementary material contain the final fractional coordinates, temperature parameters, bond distances, and bond angles.

[^{3}H]Angiotensin II Receptor Binding Assay. Experimental details for the [^{3}H]angiotensin II receptor binding assays (performed by Pan Labs, Seattle, WA) have been previously described.[27]

Guinea Pig Ileum Assay. Segments of guinea pig ileum were suspended in 10-mL organ baths and bathed in physiological salt solution (Kreb's) at 30 °C. Different tissues were used for each agonist. Isotonically recorded responses to the contractile effects of approximate ED_{70} doses of AI or AII (10 ng/mL) were noted prior to and in presence of various concentrations of test compound (5-min incubations). If an antagonist effect was noted the bath was washed and the recovery of the sensitivity of the tissue to agonist contractile activity was evaluated. In some cases, at various doses of test compound, either no or only partial recovery was seen upon repeated washing. Where no recovery was seen, separate tissues were used to assess the effects of lower doses of the test compounds. Each tissue served as its own control and the percent inhibition of the response to agonist was calculated.

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Registry No. **2**, 79069-51-5; (1*R*,2*S*)-**3**, 134359-68-5; (1*S*,2*S*)-**3**, 134453-08-0; **4**, 134359-69-6; **5**, 134359-70-9; (2*R*,3*S*)-**6**, 134359-71-0; (2*S*,3*S*)-**6**, 134359-90-3; (2*R*,3*S*)-**7**, 134359-72-1; (2*S*,3*S*)-**7**, 134359-91-4; (2*S*,3*S*)-8, 134359-73-2; (2*S*,3*S*)-8, 134359-92-5; **9**, 134359-74-3; **10**, 134359-75-4; (*E*)-**11**, 52194-39-5; (*Z*)-**11**, 52194-44-2; (*R**,*R**)-(±)-**12**, 134359-76-5; (*R**,*S**)-(±)-**12**, 134359-93-6; (*R**,*R**)-(±)-**13a**, 134359-77-6; (*R**,*S**)-(±)-**13a**, 134359-94-7; (*R**,*R**)-(±)-**13b**, 134359-95-8; (*R**,*S**)-(±)-**13b**, 134359-96-9; (*R**,*R**)-(±)-**13b**·2HCl, 134359-97-0; (*R**,*S**)-(±)-**13b**·2HCl, 134359-98-1; (±)-**14a**, 134359-78-7; (±)-**14b**, 134359-99-2; (±)-**15a**, 134359-79-8; **15a** (absolute), 134453-09-1; (±)-**15b**, 134360-00-2; **16**, 80897-78-5; **17**, 80897-79-6; **18**, 134359-80-1; **19**, 134359-81-2; **20**, 16876-04-3; **21**, 134359-82-3; **22**, 131450-45-8; **23**, 134359-83-4; **24**, 134359-84-5; **25**, 134359-85-6; **26**, 134359-86-7; **27**, 134359-87-8; **28**, 134359-88-9; **29**, 134359-89-0; H-His-OH, 71-00-1; H-His-OMe, 1499-46-3; $ClCOOCCl_3$, 503-38-8; (2*S*,3*S*)-$H_2NCH(i\text{-}Ar)CH(NH_2)CONH_2$, 134360-01-3; BOC-Pro-OH, 15761-39-4; H-Ile-OMe·HCl, 18598-74-8; ZCl, 501-53-1; Z-Val-OH, 1149-26-4; H-His-OMe·2HCl, 7389-87-9; *p*-$MeOC_6H_4CH_2CH_2COOH$, 1929-29-9;

(27) Cox, H. M.; Munday, A.; Poat, A. *Biochem. Pharmacol.* **1984**, *33*, 4057.

H-Val-OH, 72-18-4; BOC-Val-OH, 13734-41-3; *p*-$HOC_6H_4CH_2CH_2COOH$, 501-97-3; BOC-Tyr(Cl_2Bn)-OH, 40298-71-3; BOC-Arg(Tos)-OH, 13836-37-8; BOC-Sar-OH, 13734-36-6; angiotensin II, 11128-99-7.

Supplementary Material Available: Tables 1–5 containing final fractional coordinates, temperature parameters, bond distances, and bond angles (9 pages). Ordering information is given on any current masthead page.

Conformationally Restricted Polysubstituted Biphenyl Derivatives with Angiotensin II Receptors Antagonist Properties

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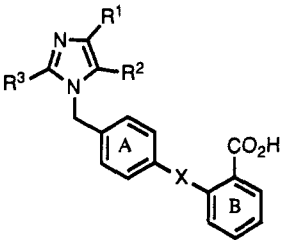
The synthesis and in vitro activity of new nonpeptide angiotensin II antagonists is presented. Compared to previously reported biphenyl compounds, the new analogues **8** and **9** have reduced conformational freedom derived from steric hindrance. Methyl 4′-methy-2′,6′-dimethoxy[1,1′-biphenyl]-2-carboxylate **4** has been synthesized by a Von Pechmann condensation of orcinol with oxocyclohexane-2-carboxylate followed by dehydrogenation. This scheme provided the carbon skeleton of the biphenyl potentially substituted on the 2-, 2′-, 4′-, and 6′-positions. Elaboration of the subsituents led to a biphenyl derivative used to alkylate a 2-*n*-butyl-4-chloro-5-(hydroxymethyl)imidazole. After coupling with the imidazole two regioisomers were separated and identified by ^{1}H NMR. NOESY experiments were useful to establish regiochemistry of the final products that have angiotensin II blocking activity. Their affinity for angiotensin II receptors was established in a binding assay experiment and in an isolated organ test. The presence of 2′,6′-dimethoxy substituent on the biphenyl moiety of the antagonist was found to significantly decrease affinity for the receptor.

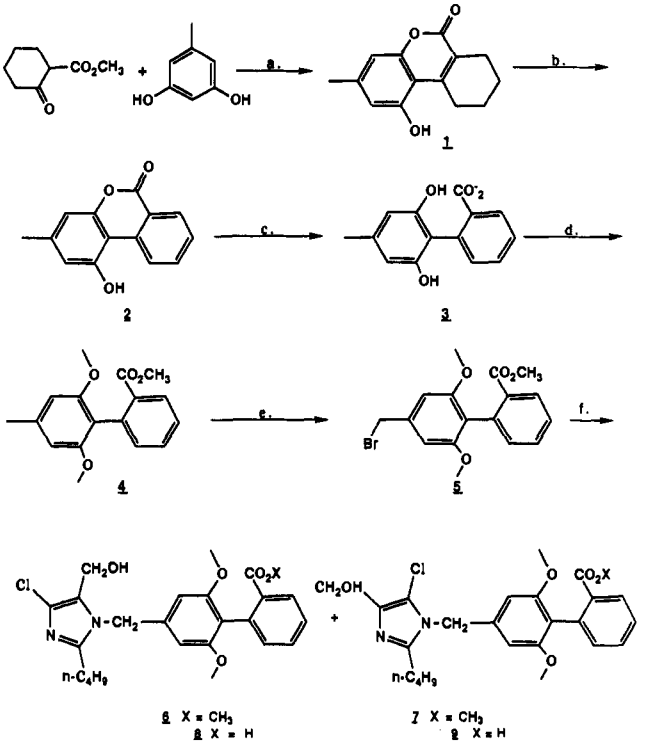
Recently, a series of *N*-(benzamidobenzyl)imidazole (formula I; X = CONH) and related compounds endowed with AII receptor antagonistic properties have been reported.[1] SAR studies revealed that the presence of ortho



FORMULA I

substituent on the benzamide ring (ring B) produces an enhancement of affinity toward the receptor. This observation has been related to steric hindrance resulting in added conformational rigidity favoring the active conformation.[1] The report that imidazoles alkylated with a biphenyl-2-carboxylic acid[2] (formula I; X = single bond) were also receptor antagonists of the peptidic hormone angiotensin II prompted us to explore the effect of ortho substituents at positions 2′ and 6′ of the A ring in biphenyl derivatives. Such substitution would introduce steric interactions between the 2,2′- and 6,6′-positions, which would hinder rotation of the phenyl rings with respect to each other and favor a twisted conformation for the biphenyl moiety. This phenomenon has been amply documented in many related cases.[3] We decided to probe the effect of such structural changes on the biological activity of the AII antagonist series. Many syntheses of biaryls rely on the coupling of two aromatic precursors.[4] Widely useful procedures involve, i.a., Ullman,[5] Gomberg-Bachmann,[6]

Scheme I[a]



[a] Reagents and conditions: (a) $H_2SO_4/POCl_3$, 25 °C, 24 h; (b) Pd/C, 300 °C, 40 min; (c) NaOH/H_2O, MeOH, reflux, 3 h; (d) Dimethyl sulfate, 25 °C, 20 h (32% yield); (e) NBS, AIBN, CCl_4, reflux, 3 H; (f) imidazole; tButOK, DMF; 2.5 N NaOH.

Meyers,[7] and Snieckus[8] reactions and each of these have advantages and limitations. Other methods have also been

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0022-2623/91/1834-2410$02.50/0