as a triethylammonium salt were pooled and lyophilized to afford 24 mg (33%) of **7**: ^{1}H NMR (360 MHz, D_2O) δ 7.56 (s, 1 H, H-6), 6.90 (br s, 1 H, H-1'), 6.42 (br d, 1 H, J = 6.0 Hz, H-3'), 5.89 (br d, 1 H, J = 6.0 Hz, H-2'), 5.03 (br s, 1 H, H-4'), 3.98 (m, 2 H, H-5'), 3.14 (q, 6 H, CH_2 of NEt_3), 1.82 (s, 3 H, CH_3), 1.22 (t, 9 H, CH_3 of NEt_3); ^{31}P NMR (145.8 MHz, D_2O) 0.07 ppm.

1-(2,3-Dideoxy-5-triphospho-β-D-*glycero*-pent-2-enofuranosyl)thymine (8). Tributylamine (0.5 mL, 2.10 mmol) and a solution of tributylammonium pyrophosphate (1.0 g, 2.3 mmol) in dry DMF (4 mL) were added at 0 °C to a solution of phosphodichloridate **6** in trimethyl phosphate (0.6 mL) prepared as above from 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (1) (25 mg, 0.112 mmol). The reaction mixture was stirred for 3 h and then poured into a cold 1 M solution of triethylammonium bicarbonate (30 mL) and then concentrated in vacuo. The residue was triturated with diethyl ether (3 × 20 mL). The ether solutions were discarded, and the insoluble residue was purified by HPLC on a C_{18} column (Whatman 500 × 18; 0%-20% of methanol in 0.1 M ammonium formate). The fractions were analyzed on an Altech NH_2-bonded column using 2 M ammonium formate in 10% aqueous methanol. Lyophilization yielded the desired product **8** (8.0 mg, 13%) as a tris(triethylammonium) salt: ^{1}H NMR (360 MHz, D_2O) δ 7.57 (s, 1 H, H-6), 6.91 (br s, 1 H, H-1'), 6.49 (br d, 1 H, J = 6 Hz, H-3'), 5.89 (br d, 1 H, J = 6 Hz, H-2'), 5.07 (br s, 1 H, H-4'), 4.15 (m, 2 H, H-5'), 3.14 (q, 18 H, CH_2 of NEt_3), 1.84 (s, 3 H, CH_3), 1.22 (t, 27 H, CH_3 of NEt_3); ^{31}P NMR (145.8 MHz, D_2O) -9.93 (d, J = 20 Hz, γ-P), -10.77 (d, J = 20.3 Hz, α-P), -22.5 (br t, β-P) ppm. Smaller amounts of the corresponding tetra- and pentaphosphates were also isolated.

1-(2,3-Dideoxy-5-tetraphospho-β-D-*glycero*-pent-2-enofuranosyl)thymine (9): ^{1}H NMR (360 MHz, D_2O) δ 7.58 (s, 1 H, H-6), 6.91 (m, 1 H, H-1'), 6.51 (dm, 1 H, J = 6.3 Hz, H-3'), 5.88 (dm, 1 H, J = 5.9 Hz, H-2'), 5.07 (br s, 1 H, H-4'), 4.14 (m, 2 H, H-5'), 3.13 (q, 24 H, CH_2 of NEt_3), 1.84 (s, 3 H, CH_3), 1.23 (t, 36 H, CH_3 of NEt_3); ^{31}P NMR (145.8 MHz, D_2O) -10.17 (d, J = 17.5 Hz, δ-P), -10.91 (d, J = 16.2 Hz, α-P), -22.52 (br t, internal P) ppm.

1-(2,3-Dideoxy-5-pentaphospho-β-D-*glycero*-pent-2-enofuranosyl)thymine (10): ^{1}H NMR (360 MHz, D_2O) δ 7.58 (s, 1 H, H-6), 6.91 (br s, 1 H, H-1'), 6.52 (br d, 1 H, J = 6.0 Hz, H-3'), 5.88 (br d, 1 H, J = 6.0 Hz, H-2'), 5.08 (br s, 1 H, H-4'), 4.16 (m, 2 H, H-5'), 3.14 (q, 30 H, CH_2 of NEt_3), 1.84 (s, 3 H, CH_3), 1.22 (t, 45 H, CH_3 of NEt_3); ^{31}P NMR (145.8 MHz, D_2O) -10.15 (br s, ε-P), -10.87 (br s, α-P), -22.46 (br s, internal P) ppm.

Antiviral Assays. The anti-HIV/LAV activity was measured in cultures of CEM-F cells. The CEM cells were infected with approximately 30 $TCID_{50}$ (50% tissue culture infectious dose) of HIV (LAV strain). The cells were then incubated for 45 min 37 °C. The test compounds, in culture medium, were added at various concentrations to the infected cells and then incubated for a further 8 days. After 8 days the antiviral activity was evaluated in the culture media supernatant for p-24 gag protein by an enzyme capture assay (ELISA). The antiviral activity was expressed as the dose that inhibits 50% of the virus expression (ID_{50} in μM) as detected by the assay described.

The culture assay for granulocyte–macrophage CFU (GM-CFU) was performed by using the assay previously described.[22]

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Registry No. **1**, 3056-17-5; **2**, 50-89-5; **3**, 56822-33-4; **5**, 7481-90-5; **6**, 117269-77-9; **7**·NEt_3, 117556-48-6; **8**·3 NEt_3, 117404-75-8; **9**, 117269-78-0; **10**, 117269-79-1.

Potent Angiotensin II Antagonists with Non-β-Branched Amino Acids in Position 5

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Amino acids with lipophilic side chains that contain more than one functional group on the β-carbon, i.e. a β-branched hydrocarbon moiety, are required in position 5 of angiotensin II (AII) analogue with potent agonist activity. This requirement for agonist activity does not follow for AII analogues with potent antagonist activity. Straight-chain amino acids may be substituted into position 5 of [Sar^1,X^5,Ile^8]AII with retention or enhancement of antagonist activity, e.g. (X^5, pA_2 rabbit aorta) Phe, 9.15; Tyr, 9.6; His, 9.0; Arg, 9.0; Glu, 9.0; Nle, 8.85, compared to Ile, 9.1. β-Branched side chains can still enhance the antagonist activities of [Sar^1,X^5,Ile^8]AII analogues, e.g. X^5 = (βMe)Phe, pA_2 = 9.3. An X-ray crystal structure of the Boc-(βMe)Phe DCHA salt, prepared for the synthesis of [Sar^1,-(βMe)Phe^5,Ile^8]AII, revealed an *S,S* configuration of α- and β-carbon atoms. Contrary to previous literature reports, chemical nonequivalence of the δ-protons of Pro was observed in the ^{1}H NMR spectra of [Sar^1,X^5,Ile^8]AII analogues bearing both β-branched X^5 side chains (X^5 = Ile) and non-β-branched X^5 side chains (X^5 = Ala, His).

In recent years, potent antagonists to angiotensin II have been developed through a variety of alterations in position 1 (aspartic acid)[1] and eight (phenylalanine).[2] Several of these analogues have been shown to lower blood pressure in humans with high plasma renin levels, including [Sar^1,Ala^8]AII (saralasin)[3] and [Sar^1,Ile^8]AII.[4]

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(1) The abbreviations for natural amino acids and nomenclature for peptide structures follow the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977. Unnatural amino acids used in this study have been given the following abbreviations: Cpg, L-cyclopentylglycine; (βMe)Phe, β-methylphenylalanine; Boc-ON, 2-[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile; Chg, L-cyclohexylglycine; (SMe)Pen, *S*-methyl-L-penicillamine; Phg, L-phenylglycine; (pNO$_2$)Phe, L-*p*-nitrophenylalanine; Peg, L-phenylethylglycine, (OMe)Thr, *O*-methyl-L-threonine.

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Table I. Position-5 Analogues of Asn[1]-AII

β-branched amino acids			other alkyl amino acids		
	in vivo rat blood pressure[a]			in vivo rat blood pressure[a]	
ref	amino acid	AII-like[b]	ref	amino acid	AII-like[b]
5	Ile[5] (human) 1	100%	6	Ala[5] 7	3%
5	α-Ile[5] 2	100%	6	Abu[5] 8	16%
6	Val[5] 3	100%	7	Leu[5] 9	25%
5	Cpg[5] 4	89%	6	Nle[5] 10	21%
5	Chg[5] 5	114%	8	Tyr[5] 11	0%
5	(OMe)Thr[5] 6	118%	8	Arg[5] 12	0%
			9	Pro[5] 13	10%

[a] Agonist AII-like activity was measured in vivo in the rat blood pressure assay described in references listed in the table. [b] AII-like activity is expressed as percent activity relative to that of AII.

The bulk of angiotensin structure–activity studies have delineated the requirements for agonist activity.[2] For example, previous studies on the structural requirements for high pressor activity of angiotensin II (AII) analogues highlight the importance of the fifth position side chain structure. Pressor activities of the analogues in Table I[5-9] led to the conclusion[9] that the fifth position side chain should be lipophilic and should contain more than one functional group on the β-carbon, i.e., a β-branched hydrocarbon moiety. By contrast, the structural requirements for potent antagonists have yet to be elaborated fully.

Here we present data that indicate that the requirement for lipophilic β-branched amino acid in the fifth position is not a necessity for antagonists. Our results demonstrate that the SAR of AII antagonists can differ strikingly from those of the AII agonists.

Results and Discussion

Table II displays the structures and activities of five analogues to [Sar[1],Ile[8]]AII, **14**, which contain more than one functional group on the β-carbon of the fifth position amino acid. All are potent analogues both in vivo and in vitro. Analogues **15**,[10] **18**, and **19** are more potent in vivo than [Sar[1],Ile[8]]AII. The fifth residue in analogues **17** and **21** bear three groups on the β-carbon, but these analogues are not superior to the antagonist analogues in Table II bearing two groups. Also listed in Table II are two agonist analogues **16** and **20**, which will be discussed later in this section.

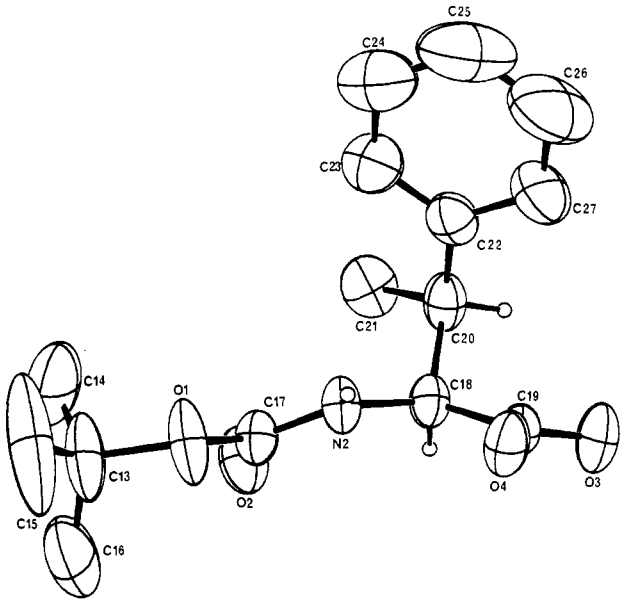

Figure 1. X-ray crystal structure of BOC-(*S,S*)-β-methylphenylalanine dicyclohexylamine salt. The DCHA group is not shown. The hydrogen atoms are not shown except for the α and β carbons. Phi -141.8, Psi 173.8, X1 (β-Me) -67.6, X1 (β-Ph) 59.2, X2 -93.4.

Analogues of [Sar[1],Ile[8]]AII containing fifth position residues that lack a β-branched hydrocarbon side chain are shown in Table III. The diminished activity of [Sar[1],Ala[5],Ile[8]]AII, **22**, compared to more lipophilic analogues parallels agonist requirements. The high activities of the antagonist analogues **23–31**, however, dramatically contrast the agonist requirements of Table I. Whereas Tyr[5] or Arg[5] substitutions eliminate all agonist activity in [Asn[1]]AII (Table I), the same substitutions enhance in vitro antagonist activity in [Sar[1],Ile[8]]AII. All seven analogues, **23–31**, lack β-branched substituents in position 5, yet display excellent in vitro antagonist activity. Analogue **26**, [Sar[1],His[5],Ile[8]]AII, is twice as potent in vivo as [Sar[1],Ile[8]]AII. Although a β-branched amino acid is necessary for agonist activity, these analogues demonstrate that a β-branched amino acid is not necessary for antagonist activity.

Clearly, the position 5 structural requirements for agonist activity are much more stringent than the requirements for antagonist activity. A narrower range of both substituents and side-chain flexibility is tolerated in position 5 for agonist molecules than for antagonist molecules. These results suggest that the antagonists can bind to the receptor in a greater variety of ways than an agonist; i.e. the agonist must bind in a particular fashion to effect cellular stimulation.

The steric role of a β-branched position 5 residue may still be important for antagonists, nevertheless. The fifth position amino acid in analogue **19** [Sar[1](β-Me)-Phe[5],Ile[8]]AII (Table II) contains both a β-branch and an aromatic group. This analogue is more potent in vitro and in vivo than either analogue **14** [Sar[1],Ile[8]]AII or analogue **23** [Sar[1],Phe[5],Ile[8]]AII. Similarly, the fifth position amino acid in analogue **21** [Sar[1],Dtc[5],Ile[8]]AII is a proline-like version of (SMe)Pen in analogue **17**. Unlike proline, Dtc contains two methyl groups on the β-carbon atom. Like the other β-branched amino acids, the Dtc analogue **21** displays enhanced in vitro activity over the proline analogue **33**. As seen in Table II the agonist analogue **20** bearing Dtc[5] substitution is also superior to the agonist

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Table II. Position-5 Analogues of [Sar^1,X^5,Y^8]-AII That Bear a β-Branched Amino Acid

	[Sar^1,X^5,Y^8]-AII		biological activities			
			in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
analogue	X	Y	AII-like[c]	pA_2/(% rel Ile^5 α^E)	AII-like[d]	ID_{50}[e]
14	Ile	Ile	1.0	9.1 (100)	10.2 ± 0.94[f]	10.0 ± 0.71[f]
15[g]	(OMe)Thr	Ile	0	9.7 (399)	7.5 ± 1.12	2.5 ± 0.16
16	(SMe)Pen	Phe	80 ± 10		60 ± 8	
17	(SMe)Pen	Ile	0	9.0 (79)	12.5 ± 1.1	20.0 ● 0.71
18	Phg	Ile	0	9.0 (79)	8.5 ± 1.02	5.5 ± 0.72
19	(β-Me)Phe	Ile	0	9.3 (158)	10.0 ± 1.0	7.5 ± 0.42
20[h]	Dtc	Phe	250 ± 18		100 ± 13	
21	Dtc	Ile	0	9.0 (79)	11.4 ± 0.95	25.0 ± 3.2

[a] Agonist, "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta assay according to the method of Rioux et al.[28] [b] Residual "AII-like" activity and antagonist activity, ID_{50}, were measured in vivo, in the rat blood pressure assay described by Regoli et al.[29] [c] AII-like activity in vitro is expressed as percent activity relative to that of AII. [d] AII-like activity in vivo is expressed by the mmHg of blood pressure increase produced by a 1-μg bolus intravenous injection of compound. [e] ID_{50} in ng/rat per min (using 250-g rats). [f] Mean standard error of at least five tests. [g] Previously described in ref 10. [h] The Dtc^5 agonist **20** demonstrated a prolonged effect in vitro (the rabbit aorta strip remains contracted over control values for over 1 h despite repeated washings. Increased duration was not observed for **20** in vivo and none of the other analogues displayed increased duration relative to Ile^5 in vitro or in vivo.

Table III. Position-5 Analogues of [Sar^1,Ile^8]AII That Lack a β-Branched Amino Acid

	[Sar^1,X^5,Ile^8]AII,	biological activities			
		in vitro rabbit aorta[a]		in vivo rat blood pressure[b]	
analogue	X	AII-like[c]	pA_2/(% rel Ile^5)	AII-like[d]	ID_{50}[e]
22	Ala	0	7.5 (2.5)	5.0 ± 0.81[f]	50 ± 6.52[f]
23	Phe	0	9.15 (112)	7.5 ± 0.31	100 ± 7.1
24	Tyr	0	9.6 (316)	2.5 ± 0.42	50 ± 5.5
25	(pNO_2)Phe	0	8.7 (40)	7.5 ± 0.57	100 ± 8.9
26	His	0	9.0 (79)	10.0 ± 0.75	5 ± 0.67
27	Lys	0	8.7 (40)	10.0 ± 0.95	50 ± 4.5
28	Arg	0	9.0 (79)	10.0 ± 0.81	15 ± 2.3
29	Glu	0	9.0 (80)	2.0 ± 0.25	100 ± 7.1
30	Nle	0	8.85 (56)	20.0 ● 1.2	20 ± 1.56
31	Pro	0	8.7 (40)	7.0 ± 1.0	20 ● 3.5
32	Peg	0	7.75 (4.5)	10.0 ± 0.8	20 ± 2.7
33	Trp	0	7.25 (1.4)	7.5 ± 0.35	50 ± 4.5
compared to:					
14	Ile	1.0	9.1 (100)	10.2 ± 0.94	10 ± 0.71

[a] Agonist "AII-like" activity and antagonist activity, pA_2, were measured in the in vitro rabbit aorta strip assay according to the method of Rioux et al.[28] [b] Residual "AII-like" activity and antagonist activity, ID_{50}, were measured in vivo, in the rat blood pressure assay described by Regoli et al.[29] [c] AII-like activity in vitro is expressed as percent activity relative to AII. [d] AII-like activity in vivo is expressed by the mmHg of blood pressure increase produced by a 1-μg bolus intravenous injection of compound. [e] ID_{50} in ng/rat per min (using 250-g rats). [f] Mean standard error of at least five tests.

analogue **13** bearing proline in position 5. These results suggest that the conformational constraint of proline on the torsion angle Φ-5 is acceptable in either agonist or antagonist analogues, but that an attendant steric constraint on the torsion angle Φ-5 by the β-methyls is also important.

Fermandjian and co-workers[11,12] have observed differences between the NMR spectra of AII analogues containing β-branched and non-β-branched side chains in position 5. In particular they have observed a chemical shift nonequivalence of the β-protons of proline in both agonist and antagonist analogues bearing β-branched position 5 side chains.

To the contrary, we observed nonequivalent δ-protons of proline in the NMR spectra of the antagonist analogues [Sar^1,His^5,Ile^8]AII, **26**, and [Sar^1,Ala^5,Ile^8]AII, **22**, bearing non-β-branched position 5 side chains (Figure 3). The downfield δ-proton signal in the spectrum of [Sar^1,Ile^8]AII, **14**, overlapped the sarcosine *N*-methyl signal (Figure 2), but could be readily assigned in the 2D COSY spectrum (Figure 3). This result suggests that a similar assignment problem may have occurred in the spectra of the non-β-branched position 5 analogues described by Fermandjian et al.[11,12] These analogues contained aspartic acid in position 1, the α-CH signal of which is known to vary with

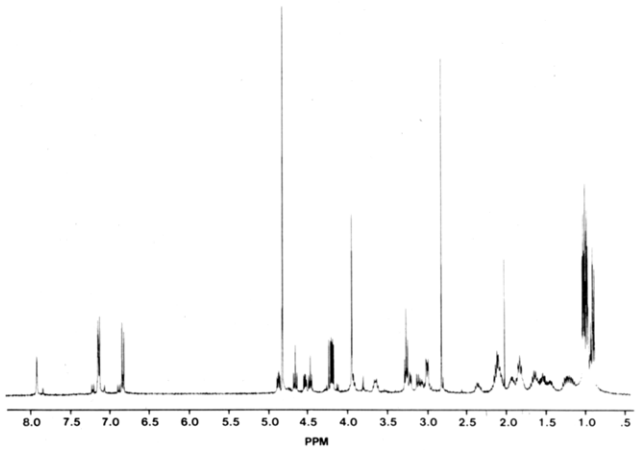

Figure 2. 360-MHz ^{1}H NMR of [Sar^1,Ile^8]AII (**14**), in D_2O, pH 7.4. The sarcosine NCH_3 (δ 3.92 ppm) partially overlaps the downfield of two proline H-δ signals (δ 3.89, 3.79 ppm).

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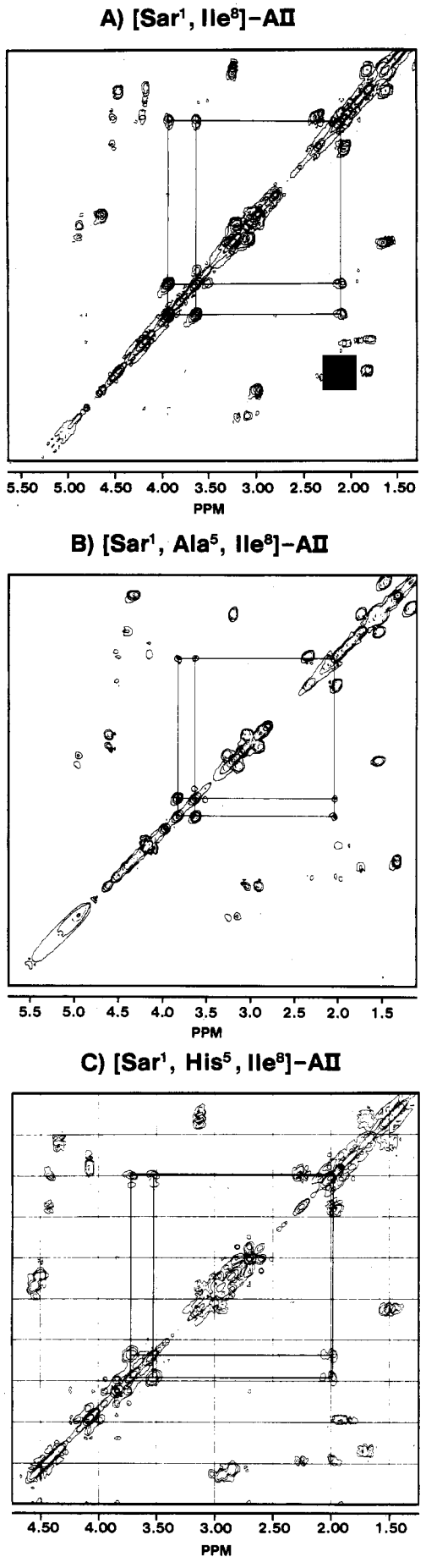

Figure 3. (A) Partially assigned COSY spectra for [Sar¹,Ile⁸]AII (**14**), (B) [Sar¹,Ala⁵,Ile⁸]AII (**22**), and (C) [Sar¹,His⁵,Ile⁸]AII (**26**) (label each figure). The upfield expansions clearly show the diastereomeric proline δ protons (δ 3.6 and 3.9 ppm).

pH in the region of the β-protons of proline,[13] potentially obscuring their assignment.

This lack of correlation between position 5 bulk and proline δ-proton nonequivalence in the NMR, however, does not diminish the importance of the steric influence of the position 5 side chain on bioactive conformation. A more thorough evaluation of the conformational influences of position 5 residues in angiotensin analogues will be the subject of a subsequent paper.

Analogues **23–25**, **27**, and **29** are weaker antagonists in vivo than the parent Ile⁵ analogue despite potent in vitro activity. Analogue **32** is surprisingly potent in vivo despite weak in vitro activity. Explanations for these anomalies are being pursued. One must bear in mind that, when comparing in vivo and in vitro activities of peptide analogues, alterations in side-chain structure could alter the pharmacodynamic properties of the peptide, increase or decrease susceptibility to in vivo peptidase degradation normally suffered by the native peptide, or even invite novel degradation by peptidases for which the native peptide is not a normal substrate.

Although the antagonists in Table III bear substituents that would reduce agonist activity in [Phe⁸]AII analogues, none of the potent molecules displayed dramatically lower residual agonist activities relative to [Sar¹,Ile⁸]AII, **14**.

The data presented here demonstrate that the AII antagonist SAR can be different from the AII agonist SAR. Other variations between AII agonist and antagonist SARs remain the subject of future reports.

Experimental Section

Routine NMR spectra were performed on a Varian EM 360 90-MHz spectrometer. High-resolution spectra were performed with a Bruker Instruments WM 360 spectrometer operating at 302 K. Optical rotations were performed on a Perkin-Elmer polarimeter.

Amino acids, *tert*-butyloxycarbonyl amino acids, and peptide reagents were obtained from Bachem Fine Chemicals, Inc., Protein Research Foundation, or Chemical Dynamics Corp. and were used without further purification. Thin-layer chromatography (TLC) was performed on Brinkman precoated silica gel plates (SIL-G-25). The spots were visualized by ninhydrin or Pauly reagent. Boc-Phg,[14] Boc-(pNO$_2$)Phe,[15] and Boc-Peg[16] were prepared from the commercially available amino acids by literature procedures.

(*tert*-Butyloxycarbonyl)-*S*-methyl-L-penicillamine, Boc-(SMe)Pen. A suspension of penicillamine (5 g, 33.5 mmol) in 0.4 N Ba(OH)$_2$ (165 mL, 33.5 mmol) was chilled on an ice water bath. Dimethyl sulfate (4.4 g, 35.2 mmol) was added dropwise over a period of ~0.5 h. The mixture was stirred for 24 h as it warmed to room temperature. A solution of 1 N H_2SO_4 (33.5 mL, 33.5 mmol) was added to the reaction mixture to precipitate $BaSO_4$. The suspension was cooled and centrifuged at 6000 rpm for 10 min. The pH of the supernatant solution was adjusted to pH 6 with concentrated ammonium hydroxide and lyophilized to a white amorphous powder. NMR (D_2O/DCl) indicated the presence of *S*-methylpenicillamine. The powder gave a single spot on TLC (butanol/acetic acid/water, 4:1:5 upper phase, R_f = 0.39). The lyophilized powder was used without further purification.

Triethylamine (6.89 g, 36.4 mmol) was added to a mixture of crude *S*-methylpenicillamine and Boc-ON (8.96 g, 36.4 mmol) in 150 mL of 50% aqueous acetone.

After 2 days, TLC ($CHCl_3$/MeOH/AcOH, 95:4:1) showed less than 1% starting material remaining. The volume of solution

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was reduced to one-half by rotary evaporation. Approximately 20 mL of 10% aqueous Na_2CO_3 was added. The aqueous solution was washed with 2 × 100 mL of EtOAc. Following acidification to pH 2 with 3.0 N HCl, the aqueous mixture was washed with 2 × 100 mL of EtAc. The organic layers were combined and dried over anhydrous Na_2SO_4 (5 g) and then dried to an oil by rotary evaporation.

The oil appeared homogeneous by TLC (R_f = 0.64, $CHCl_3$/MeOH/AcOH, 95:4:1). ^1H NMR (MeOH-d_4) of the oil was correct for Boc-*S*-methylpenicillamine: 4.05 (s, 1 H), 1.43 (s, 9 H), 1.38 (s, 3 H), 1.30 ppm (s, 3 H).

Dicyclohexylamine (7.3 g, 40 mmol) was added to a solution of the crude Boc-*S*-methylpenicillamine in ether (250 mL). The mixture was stored overnight in a refrigerator followed by storage at room temperature for 2 days. Colorless crystals of Boc-*S*-methylpenicillamine DCHA salt were filtered from solution washing with ether and dried in vacuo, 8.3 g, 55% overall yield; mp 152.5–154 °C; TLC ($CHCl_3$/MeOH/AcOH, 95:4:1); R_f = 0.80; $[\alpha]^{24}_D$ = −12.2° (*c* = 1.0, MeOH). Anal. ($C_{23}H_{44}N_2O_4S$) C, H, N, S.

(*tert*-Butyloxycarbonyl)-L-5,5-dimethylthiazolidine-4-carboxylic Acid, Boc-Dtc. A suspension of penicillamine (5 g, 33.5 mmol) and 37% aqueous formaldehyde (5.23 mL, 85 mmol) in water (25 mL) was adjusted to pH 5 with pyridine (ca. 2.5 mL). After several hours a clear solution formed with stirring. After the solution chilled to 0° C for 3 h, pyridine (6.2 mL, 77 mmol) and ethanol were added (25 mL). After cooling at 0 °C overnight, the solution was filtered to remove a white crystalline solid. The solid was recrystallized in 10% $NaHCO_3$/AcOH (4.4 g, 80%): mp 195–197 °C; TLC ($CHCl_3$/MeOH/AcOH, 85:10:5) R_f = 0.18; ^1H NMR (D_2O/DCl) δ 4.55 (s, 2 H), 4.35 (s, 1 H), 1.65 (s, 3 H), 1.40 (s, 3 H) ppm. The material was used without further purification.

Triethylamine (6.97 mL, 50.3 mmol) was added to a suspension of 5,5-dimethylthiazolidine-4-carboxylic acid (5.47 g, 33.5 mmol) and Boc-ON (9.05 g, 36.8 mmol) in 50% aqueous acetone (60 mL). After stirring overnight, the solution no longer contained starting acid by TLC ($CHCl_3$/MeOH/AcOH, 85:10:5). The solution was concentrated by rotary evaporation. The resulting aqueous solution was extracted with ether (50 mL), acidified to pH 2 with 3 N HCl, and extracted with ethyl acetate (2 × 50 mL). The ethyl acetate layers were combined, washed with H_2O (25 mL each), until the water layer was neutral following extraction, and rotary evaporated to a solid. The solid was recrystallized from ether/hexane as a white crystalline powder (6.6 g, 75%): 95% pure by TLC ($CHCl_3$/MeOH/AcOH, 85:10:5) R_f = 0.78; NMR (CD_3OD) δ 4.6 (s, 2 H), 4.1 (s, 1 H), 1.6 (s, 3 H), 1.3 (m, 12 H).

The crude product (3 g) was applied to a column of silica gel (170 g, 40–140 mesh) in a $CHCl_3$/MeOH (90:5) mixture. After elution of 500 mL of the $CHCl_3$/MeOH (90:5) mixture, the product eluted in an elution mixture $CHCl_3$/MeOH/AcOH (80:10:10) (500 mL). The eluant was rotary evaporated, yielding a solid which was crystallized from ether/hexane to give a white crystalline solid (2 g, 66% recovery): mp 126.5–127.5 °C; $[\alpha]^{24}_D$ = −76.2° (*c* = 1.0 in EtOH).[17] Anal. ($C_{11}H_{19}N_1O_4S_1$) C, H, N, S.

(*tert*-Butyloxycarbonyl)-(*S*,*S*)-β-methylphenylalanine, Boc-(βMe)Phe. Trifluoroacetic anhydride (1.10 mL, 8.35 mmol) was added to a mixture of diastereomerically pure, racemic (βMe)Phe (1.5 g, 6.95 mmol) in anhydrous TFA (6 mL) chilled on an ice water bath. After 1 h the mixture was rotary evaporated to an oil. An aqueous solution (30 mL) of the oil was neutralized with 2 N NaOH. A precipitate (unreacted amino acid) was filtered from the filtered solution. The filtrate was acidified to pH 3 with 3 N HCl and washed with ethyl acetate (2 × 30 mL). The ethyl acetate layers were washed with H_2O (30 mL), combined, and rotary evaporated to a white solid.

An aqueous solution (50 mL) of the solid was neutralized to pH 7.2 with 2 N NaOH and warmed to 39 °C. Carboxypeptidase A (4 mg) was added with stirring to the solution. After 2 days the mixture was acidified to pH 5 with 3 N HCl. Powdered charcoal (0.5 g) was added and the mixture was filtered through Celite (1 g). The filtrate was acidified to pH 3 with 3 N HCl and washed with EtOAc (3 x 50 mL). The aqueous solution was neutralized with 2 N NaOH and concentrated by rotary evaporation until a precipitate began to appear. The mixture was chilled overnight. The precipitate was filtered and dried in vacuo to give L-(βMe)Phe as a white crystalline powder (0.55 g, 74%): mp 201.5–203 °C; TLC (nBuOH/AcOH/H_2O, 4:1:5) R_f = 0.48; $[\alpha]^{24}_D$ = −23.0° (*c* = 1.006 in H_2O). Anal. ($C_{10}H_{13}NO_2$·HCl·H_2O) C, H, N.

Di-*tert*-butyl carbonate (0.45 g, 2.0 mmol) was added to a solution of L-(βMe)Phe (0.20 g, 0.9 mmol) and Et_3N (0.19 mL, 1.35 mmol) in 50% aqueous nBuOH (10 μL). After the solution was allowed to stir overnight, starting amino acid could no longer be detected by TLC ($CHCl_3$/MeOH/AcOH, 95:4:1). The solution was concentrated to 5 mL by rotary evaporation and lyophilized to an oil. DCHA (215 mL, 1.08 mmol) was added to a concentrated solution of the oil in MeOH. The DCHA salt was precipitated from solution with Et_2O and recrystallized from MeOH/Et_2O as white needles (294 mg, 71%): mp 194–195.5 °C; TLC ($CHCl_3$/MeOH/AcOH, 95:4:1) R_f = 0.71; $[\alpha]^{24}_D$ = +9.34° (*c* = 1.03 in MeOH). Anal. ($C_{27}H_{42}N_2O_4$) C, H, N.

Although the commercially available D,L-(βMe)Phe was determined to be diastereomerically pure by melting point and chromatographic analysis, the configuration of the β-carbon was not known (personal communication, Sigma Chemical Co.). An X-ray crystal structure of Boc-(βMe)Phe DCHA, Figure 1, revealed that the β-carbon is of the *S* absolute configuration.

X-ray Structure Determination of Boc-(*S*,*S*)-β-Methylphenylalanine Dicyclohexylamine Salt. Crystals of (β-Me)Phe were grown from 2-propanol. The crystal used for data collection was a colorless acicula of approximate dimensions 0.40 × 0.40 × 0.45 mm and was mounted with epoxy on a glass filter. Unit cell constants for the orthorhombic $P2_12_12_1$ lattice, derived from a least-squares fit of the angular settings for 25 reflections with 30° ≤ 2θ(Mo) ≤ 35° measured on the diffractometer, are *a* = 9.542 (3) Å, *b* = 16.179 (5) Å, *c* = 17.842 (7) Å, *Z* = 4, *V* = 2757.5 Å^3. The calculated density is 1.100 g cm^{-3}, μ = 0.659 cm^{-1} and *F*(000) = 1000. Intensities for 4431 independent reflections were in the range 2° ≤ 2θ ≤ 60° were measured at 293 (2) K on an Enraf-

Table IV. Positional Parameters and Their Estimated Standard Deviations[a]

atom	*x*	*y*	*z*	*B*, Å^2
O1	0.4630 (2)	1.0766 (1)	0.5344 (1)	5.62 (5)
O2	0.6706 (2)	1.1321 (1)	0.4987 (1)	5.00 (4)
O3	0.9352 (2)	0.8635 (1)	0.5298 (1)	5.48 (5)
O4	0.7049 (2)	0.8490 (1)	0.5428 (1)	4.53 (4)
N1	1.2116 (2)	0.8090 (1)	0.5073 (1)	3.19 (4)
N2	0.6551 (2)	1.0075 (1)	0.5571 (1)	3.19 (4)
C1	1.2498 (3)	0.8652 (2)	0.4431 (2)	3.39 (5)
C2	1.1496 (3)	0.8501 (2)	0.3798 (2)	4.73 (7)
C3	1.1825 (4)	0.9082 (2)	0.3137 (2)	6.27 (9)
C4	1.3323 (4)	0.8993 (2)	0.2881 (2)	6.50 (9)
C5	1.4326 (4)	0.9123 (2)	0.3535 (2)	6.01 (8)
C6	1.4011 (3)	0.8543 (2)	0.4187 (2)	4.48 (6)
C7	1.2983 (3)	0.8130 (2)	0.5771 (1)	3.09 (5)
C8	1.3066 (3)	0.9004 (2)	0.6075 (2)	4.06 (6)
C9	1.3896 (4)	0.9018 (2)	0.6811 (2)	5.47 (8)
C10	1.3259 (4)	0.8453 (2)	0.7391 (2)	5.93 (8)
C11	1.3149 (4)	0.7581 (2)	0.7095 (2)	5.88 (8)
C12	1.2356 (4)	0.7543 (2)	0.6336 (2)	4.52 (7)
C13	0.3805 (3)	1.1516 (2)	0.5191 (2)	6.56 (9)
C14	0.3881 (5)	1.1729 (2)	0.4368 (3)	7.9 (1)
C15	0.2362 (4)	1.1261 (3)	0.5440 (4)	13.3 (2)
C16	0.4295 (6)	1.2219 (3)	0.5654 (3)	9.6 (1)
C17	0.6031 (3)	1.0773 (2)	0.5275 (2)	3.54 (5)
C18	0.8015 (3)	0.9848 (1)	0.5534 (2)	3.31 (5)
C19	0.8150 (3)	0.8907 (2)	0.5408 (2)	3.65 (5)
C20	0.8850 (3)	1.0124 (2)	0.6227 (2)	3.88 (6)
C21	0.8954 (4)	1.1070 (2)	0.6256 (2)	5.08 (7)
C22	0.8277 (3)	0.9758 (2)	0.6942 (2)	4.25 (6)
C23	0.7307 (4)	1.0159 (2)	0.7387 (2)	5.54 (8)
C24	0.6808 (5)	0.9837 (3)	0.8034 (2)	7.3 (1)
C25	0.7251 (7)	0.9079 (3)	0.8269 (2)	9.7 (1)
C26	0.8210 (8)	0.8649 (3)	0.7833 (2)	10.4 (2)
C27	0.8705 (5)	0.8990 (2)	0.7186 (2)	7.3 (1)

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $^4/_3[a^2B(1,1) + b^2B(2,2) + c^2B(3,3) + ab(\cos\gamma)B(1,2) + ac(\cos\beta)B(1,3) + bc(\cos\alpha)B(2,3)]$.

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Table V. Peptide Analytical Data[a]

	[Sar¹,X⁵,Y⁸]AII		amino acid analysis[b]								TLC R_f			HPLC solvent		HPLC %
no.	X	Y	Sar	Arg	Val	Tyr	X	His	Pro	Y	A	B	C	K′	% CH_3CN	purity
14	Ile	Ile	(+)[c]	0.99	0.97	0.97	1.01*[d]	1.07	0.99	1.01*	0.18	0.66	0.74	2.8	20	≥98
15	(OMe)Thr	Ile	(+)	1.00	1.00	1.00	(+)	1.02	1.00	0.98	0.28	0.33	0.53	11.02	17	≥98
16	(SMe)Pen	Phe	(+)	1.04	1.02	1.03	(+)	0.97	0.90	1.03	0.12	0.31	0.46	3.9	23	>98
17	(SMe)Pen	Ile	(+)	1.02	1.01	1.01	(+)	0.96	0.99	1.01	0.20	0.44	0.53	3.7	20	≥98
18	Phg	Ile	(+)	1.01	1.02	1.01	(+)	1.01	0.98	0.97	0.17	0.43	0.49	3.1	22	97
19	(βMe)Phe	Ile	(+)	1.01	0.90	0.96	(+)	1.04	1.07	1.01	0.16	0.53	0.57	4.9	20	≥98
20	Dtc	Phe	(+)	1.00	1.03	1.00	(+)	0.95	1.02	1.01	0.18	0.43	0.50	5.1	20	>98
21	Dtc	Ile	(+)	1.01	1.03	0.99	(+)	0.97	1.01	0.99	0.16	0.44	0.58	3.6	20	>98
22	Ala	Ile	(+)	1.02	1.01	1.02	0.98	1.01	0.98	0.99	0.13	0.29	0.41	1.8	20	≥98
23	Phe	Ile	(+)	1.00	1.02	1.00	0.99	1.01	0.99	1.01	0.16	0.63	0.62	3.2	25	≥98
24	Tyr	Ile	(+)	0.98	0.99	1.00*	1.00*	1.03	1.03	0.97	0.19	0.47	0.72	2.1	20	≥98
25	(βNO_2)Phe	Ile	(+)	1.01	1.01	1.00	(+)	0.97	1.02	0.96	0.21	0.65	0.55	10.0	15	≥98
26	His	Ile	(+)	1.02	1.03	0.97	0.97*	0.97*	1.05	0.99	0.03	0.11	0.39	3.8	10	98
27	Lys	Ile	(+)	1.00	1.01	1.01	1.00	1.01	1.00	0.98	0.05	0.15	0.47	9.4	85	98
28	Arg	Ile	(+)	1.01*	1.01	0.98	1.01*	1.03	0.98	0.98	0.06	0.15	0.37	3.3	10	95
29	Glu	Ile	(+)	1.01	1.00	1.00	1.00	1.03	1.00	0.96	0.15	0.37	0.43	5.3	10	>98
30	Nle	Ile	(+)	1.02	1.02	1.02	1.00	1.00	0.96	0.98	0.15	0.30	0.58	3.6	20	93
31	Peg	Ile	(+)	1.00	1.00	1.00	(+)	1.01	0.99	1.00	0.22	0.58	0.52	5.6	20	95
32	Trp	Ile	(+)	1.01	1.01	0.99	0.90	1.01	1.00	0.97	0.20	0.58		4.3	20	96

[a] See Text for details of analytical procedures. [b] Amino acid analysis expressed in molar ratios of the amino acids in the peptides. [c] (+) = amino acid present in roughly 1 molar equiv (in cases where quantitation is difficult). [d] * = amino acid present in two positions. Value expressed is one-half the experimental value.

Nonius CAD-4 diffractometer, using Mo Kα (λ = 0.71073 Å) radiation, a W-20 scan technique, and variable-scan speeds.

Data were corrected for Lorentz and polarization effects but not for absorption. Three intensity standards measured every 3 h showed less than a 0.5% variation from their means. The structure was solved by using MULTAN 80.[18] Non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen positions were suggested from difference Fourier maps; those associated with C18 and C20 as well as with hydrogen-bonding interactions were refined. The remainder of the hydrogens were treated as fixed with fixed isotropic thermal parameters. The refinements (on F) were done by full-matrix least-squares techniques.

The refinement converged to values of the conventional crystallographic residuals $R = 0.047$ and $R_w = 0.059$ for 2488 observations with $I > 3\sigma(I)$. The weights, w, were assigned as $4(F_o)^2/\sigma^2(I)$ with $\sigma(I)$ defined as $[\sigma(I)^2 + (0.05F_o)^2]^{1/2}$. The largest parameter shift was 0.06 times its esd in the final cycle. The maximum positive and negative features in a final difference map were of heights 0.151 and 0.161 e A^{-3}, respectively. The "goodness of fit" was 1.59 based on 319 variables. An extinction coefficient, of the type defined by Zacharsasen[19] refined to 7.43 (1) × 10^{-7}. All programs were from the Enraf-Nonius SDP. Neutral atom scattering factors were as incorporated in the SDP. Table IV lists the positional parameters for the crystal structure (Figure 1).

Peptide Synthesis and Purification. All peptides were prepared by the solid-phase method on Beckman 990-B peptide synthesizers.[20,21] The C-terminal residue was esterified to a chloromethylated copolymer of styrene and 2% divinylbenzene (Bio-Rad) via a cesium salt procedure.[22] The degree of substitution was determined by amino acid analysis of a hydrolysate obtained by treating the amino acid-resin with HCl/PrOH (1:1) at 120 °C for 3 h.[23] Routine deprotection of Boc-amino protecting groups was accomplished with 30% TFA in CH_2Cl_2 and neutralization with 10% TEA in CH_2Cl_2. Coupling of each amino acid was performed with a 2.5 molar excess of (*tert*-butyloxycarbonyl)amino acid and DCC in CH_2Cl_2 with completeness of reaction monitored by the ninhydrin test.[24] In most cases coupling was complete after 2 h. If the ninhydrin test remained positive, a recoupling cycle was performed. After the last coupling and deprotection, the peptide was cleaved from resin by treatment with anhydrous HF containing 50% (v/v) anisole at 0 °C for 60 min. After vacuum evaporation of HF, the resin was rinsed with Et_2O to remove anisole and then rinsed with glacial HOAc and filtered. The filtrate was diluted with water and lyophilized to a powder of crude peptide material.

The crude peptides were purified to homogeneity either by (a) partitioning through 200 transfers of counter-current distribution in nBuOH/AcOH/H_2O (4:1:5), (b) by partition chromatography[25] on Sephadex G-15 in nBuOH/HOAc/H_2O (4:1:5), or (c) reversed-phase semipreparative HPLC[26] on a Whatman C^{18} column using the appropriate solvent mixture of CH_3CN/0.1 N NH_4OAc, pH 4. The volumes of chromatographic fractions containing pure peptide were reduced by partial rotary evaporation and dried to powders by lyophilization to constant weight.

Homogeneity of each peptide was determined by the following methods, (results are shown in Table V): (a) Amino acid analysis of 72-h acid hydrolysate (6 N HCl, 110 °C) performed on a Beckman Model 120C analyzer. (b) Analytical TLC on silica gel plates with solvent systems A, nBuOH/AcOH/H_2O (4:1:5); B, nBuOH/AcOH/H_2O/EtOAc (1:1:1:1); C, nBuOH/AcOH/H_2O/pyridine (15:3:12:10), visualizing spots with Pauly reagent.[21] (c) Analytical reversed-phase HPLC on C_{18} silica gel column using the appropriate CH_3CN 0.1 N NH_4OAc (pH 4) mixture, following elution by uv (250 nm detection).

Analytical data for all peptides are listed in Table V.

NMR Studies. Solutions for NMR analysis were prepared by dissolving ca. 5 mg of the peptide in 0.5 mL of D_2O, (99.9% D) and then adjusting the pH to 7.4 with 0.1 M DCl/NaOD. NMR spectra were measured with a Bruker Instruments WM360 spectrometer operating at 302 K. 1D spectra were obtained with suppression of the residual HOD peak by using an inversion-recovery pulse sequence with τ = 2.1 s. Chemical shifts are

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referred to the HOD signal at δ 4.79.

2D-COSY-45 spectra were measured with HOD suppression via low-power irradiation, gated off during the acquisition time, using the Bruker COSYHG (90-DO-45-AQT) automated sequence. 256 K spectra were measured over a 4000-Hz spectral window for each experiment. Thirty-two scans were accumulated for each spectrum. The double Fourier transformation was carried out with sine-bell apodization in both dimensions and zero-filling in the T-1 dimension, leading to a final matrix size of 256 W for the absolute-value spectrum.

2D NOE (NOESY) spectra were measured by using the same size data matrix and spectral window as in the COSY experiments, also with low-power irradiation of the solvent peak gated off during the acquisition period. When a mixing time of 0.5 s was employed, no cross-peaks due either to NOEs or chemical exchange were observed.

Each 360-MHz NMR spectrum in D_2O for [Sar^1,Ile^8]AII (**14**) (Figure 2), [Sar^1,Ala^5,Ile^8]AII (**22**), and [Sar^1,His^5,Ile^8]AII (**26**) shows the coexistence of a major and minor species, whose exchange is slow on the NMR time scale. This is most clearly evident in the aromatic signals due to tyrosine and histidine. The relative proportions of major and minor species are variable, from near 10:1 in **14** to 2:1 in **22**. The chemical exchange between the species is demonstrated by the observation of reversible broadening and coalescence of the major and minor peaks due to tyrosine and histidine on heating the sample to 363 °C. The minor peaks have been ascribed to the *cis*-proline amide isomer[27] in the case of AII, *N*-acetyl-AII, and [Sar^1,Ala^8]AII.

Partial spectral assignments for these compounds were made via 2D-COSY experiments (Figure 3). The assignments for the proline δ protons at δ 3.6 and 3.69 ppm are thus unambiguous, by correlation to the γ protons in **19**, **22**, and **26**. The δ protons from the major and minor species can be readily distinguished in all spectra. All three compounds exhibit significant diastereomerism for the proline δ protons in the major species ($\Delta\delta$ = 0.2 ppm for **22**, **26** and 0.3 ppm for **14**). The dispersion between the proline δ protons is reduced in the minor species for **22** and **14** ($\Delta\delta$ = 0.1 ppm) and equal to that in the major species for **26**. The spectral overlap between the downfield signal of the proline δ protons with the α proton signals due to sarcosine could make assignment of the separate proline signals problematic without the advantages obtained from the COSY experiments.

Bioassays. All compounds were tested for agonistic and antagonistic activities on an in vitro preparation of the rabbit aorta as described by Rioux et al.[28] and in vivo on the rat blood pressure assay, according to the technique described by Regoli et al.[29] The pharmacological parameters of α^E (intrinsic activity, expressed in percent of that of [Sar^1,Ile^5]angiotensin II) and of apparent affinity (pA_2), as defined and used by Ariens[30] and by Van Rossum,[31] were utilized to characterize the in vitro activities of the compounds. AII antagonists bearing sarcosine in position 1 are slowly reversible antagonists depressing both the slope and maximum of the AII dose–response curve in vitro at high doses but not at low doses.[32] The "pA_2" values reported here for comparative purposes were determined at low doses in the range of competitive inhibition, and consequently may be overestimated.[33] The residual angiotensin-like activity of the compounds in vivo was evaluated by measuring the pressor effects of bolus intravenous injections of 1 μg, and the antagonistic effect was calculated from dose–response curves measured before and during the infusion of each compound. Antagonism in vivo is expressed by the ID_{50} in ng/rat per min (using 250-g rats) according to Regoli et al.[32]

Registry No. **14**, 37827-06-8; **15**, 63146-94-1; **16**, 117940-32-6; **17**, 117940-33-7; **18**, 117940-34-8; **19**, 117940-35-9; **20**, 117940-36-0; **21**, 117940-37-1; **22**, 117918-12-4; **23**, 117918-13-5; **24**, 117918-14-6; **25**, 117918-15-7; **26**, 117918-16-8; **27**, 117918-17-9; **28**, 117918-18-0; **29**, 117918-19-1; **30**, 117918-20-4; **31**, 117918-21-5; **32**, 117940-38-2; **33**, 117918-22-6; Pen, 1113-41-3; (SMe)Pen, 100217-05-8; BOC-(SMe)Pen, 112898-22-3; BOC-(SMe)Pen·DCHA, 112898-23-4; Dtc, 72778-00-8; BOC-Dtc, 117918-23-7; DL-*erythro*-(βMe)Phe, 25488-24-8; CF_3CO-DL-*erythro*-(βMe)Phe, 117918-24-8; L-*erythro*-(βMe)Phe, 25488-25-9; BOC-L-*erythro*-(βMe)Phe, 90731-57-0; BOC-L-*erythro*-(βMe)Phe·DCHA, 117918-25-9; BOC-Phg, 2900-27-8; BOC-(pNO_2)Phe, 33305-77-0; BOC-Peg, 100564-78-1.

Supplementary Material Available: Tables of hydrogen atom coordinates, anisotropic thermal parameters, and metric values for (βMe)Phe (9 pages). Ordering information is given on any current masthead page.

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