3-[(2-Formamidoethyl)amino]-1-phenoxypropan-2-ol (4) was similarly prepared by treatment of 3-[*N*-(2-aminoethyl)-*N*-benzylamino]-1-phenoxypropan-2-ol[10] with methyl formate in *i*-PrOH at a reflux, followed by hydrogenolysis, and was crystallized from EtOAc: yield 15%; mp 107-109 °C.

3-[*N*-(2-Benzenesulfonamidoethyl)-*N*-methylamino]-1-phenoxypropan-2-ol Hydrogen Oxalate (84). Iodomethane (0.28 g, 0.002 mol) was added dropwise to a stirred mixture of 3-[(2-benzenesulfonamidoethyl)amino]-1-phenoxypropan-2-ol (**77**; 0.7 g, 0.002 mol), THF (10 mL), and an 80% dispersion of sodium hydride in oil (0.06 g, 0.002 mol). The mixture was stirred at room temperature for 1 h and then diluted with water and extracted with ether. The ether extract was dried and evaporated to dryness, and the residue was chromatographed on Merck Kieselgel 60F254 preparative TLC plates with $CHCl_3$/MeOH (9:1 v/v) as developing solvent. The band having R_f 0.5 was removed and extracted with methanol, and the methanol evaporated to dryness. The residue was crystallized as the hydrogen oxalate from EtOAc: yield 0.1 g (14%); mp 114-117 °C.

Pharmacology. β-Adrenoreceptor blocking potency was estimated in vivo with the previously described cat preparation.[11] The results given in Tables I-V are the estimated dose, infused over a period of 30 min, that would cause a 50% inhibition of the tachycardia produced by a submaximal dose of isoproterenol (0.2 μg/kg dosed iv). The estimated degree (percent) of blockade of the vasodepressor response at that dose level is also given. Three to five dose levels of each compound were used to calculate these estimates. The relative potencies in these two systems give an indication of selectivity for B_1 (cardiac) as opposed to β_2 (vascular) receptors. Mean log ED_{50}'s were calculated for each compound on the basis of two or three tests, and the standard errors of the means were computed. On average, these mean values had an error of 30%. Previous data[11] have shown that the error in the percent inhibition of the depressor response at the ED_{50} value for inhibition of isoproterenol-induced tachycardia is less than 5%.

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Derivatives of the Potent Angiotensin Converting Enzyme Inhibitor 5(*S*)-Benzamido-4-oxo-6-phenylhexanoyl-L-proline: Effect of Changes at Positions 2 and 5 of the Hexanoic Acid Portion

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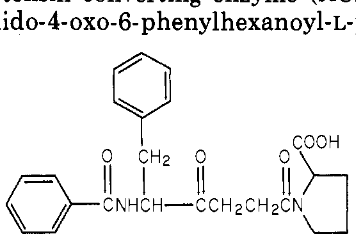
Several derivatives of the potent angiotensin converting enzyme inhibitor 5(*S*)-benzamido-4-oxo-6-phenylhexanoyl-L-proline (1) were synthesized and tested for converting enzyme inhibition activity and blood pressure lowering effects in rats. One compound, 5(*S*)-benzamido-2(*R*)-methyl-4-oxo-6-phenylhexanoyl-L-proline (**2a**), had an I_{50} against angiotensin converting enzyme of 1.0×10^{-9} M and is the most potent inhibitor prepared thus far in this class of compounds. Testing of **2a** orally at 30 mg/kg for inhibition of the angiotensin I induced blood pressure increase in conscious normotensive rats gave 100% inhibition that required 143 min before the angiotensin I blood pressure response returned to 70% of the pretreatment control response. In the conscious renal hypertensive rat, **2a** given orally at a dose of 3 mg/kg caused a lowering of blood pressure that reached its maximum of 40 mmHg 8 h following drug administration.

In a previous publication,[1] numerous derivatives of the potent angiotensin converting enzyme (ACE) inhibitor 5(*S*)-benzamido-4-oxo-6-phenylhexanoyl-L-proline[2] (1)



1

were described. These compounds were tested as ACE inhibitors both in vivo and in vitro and as antihypertensive agents in renal hypertensive rats. Many of these compounds were potent ACE inhibitors in vitro but much less potent in vivo.

In order to increase the in vivo activity of this class of ACE inhibitors, we tried two approaches. First, structural changes in 1 were made with the hope of increasing its ACE inhibiting activity and thus decreasing the amount of compound that must be absorbed orally to inhibit ACE in vivo. Considering that the tripeptide Phe-Ala-Pro had over 10 times the ACE inhibitory activity of our model tripeptide Phe-Gly-Pro,[2] we thought that a 2-methyl substitution in the hexanoyl chain of 1 would greatly increast its ACE inhibition. Therefore, compounds **2a-d** were synthesized to investigate the 2-methyl substitution effect.

A second method of increasing oral absorption of compounds is to increase their lipophilicity. We have synthesized a series of derivatives of 1, compounds **3a-4c**, with increased lipid character. These compounds were tested in vitro and in vivo as ACE inhibitors.

Chemistry. The synthetic pathway for the preparation of compounds **2a-4c** is shown in Scheme I. As described previously[1] using a modification of the Dakin-West reaction,[3] the oxazolone **5** was reacted with the desired acid

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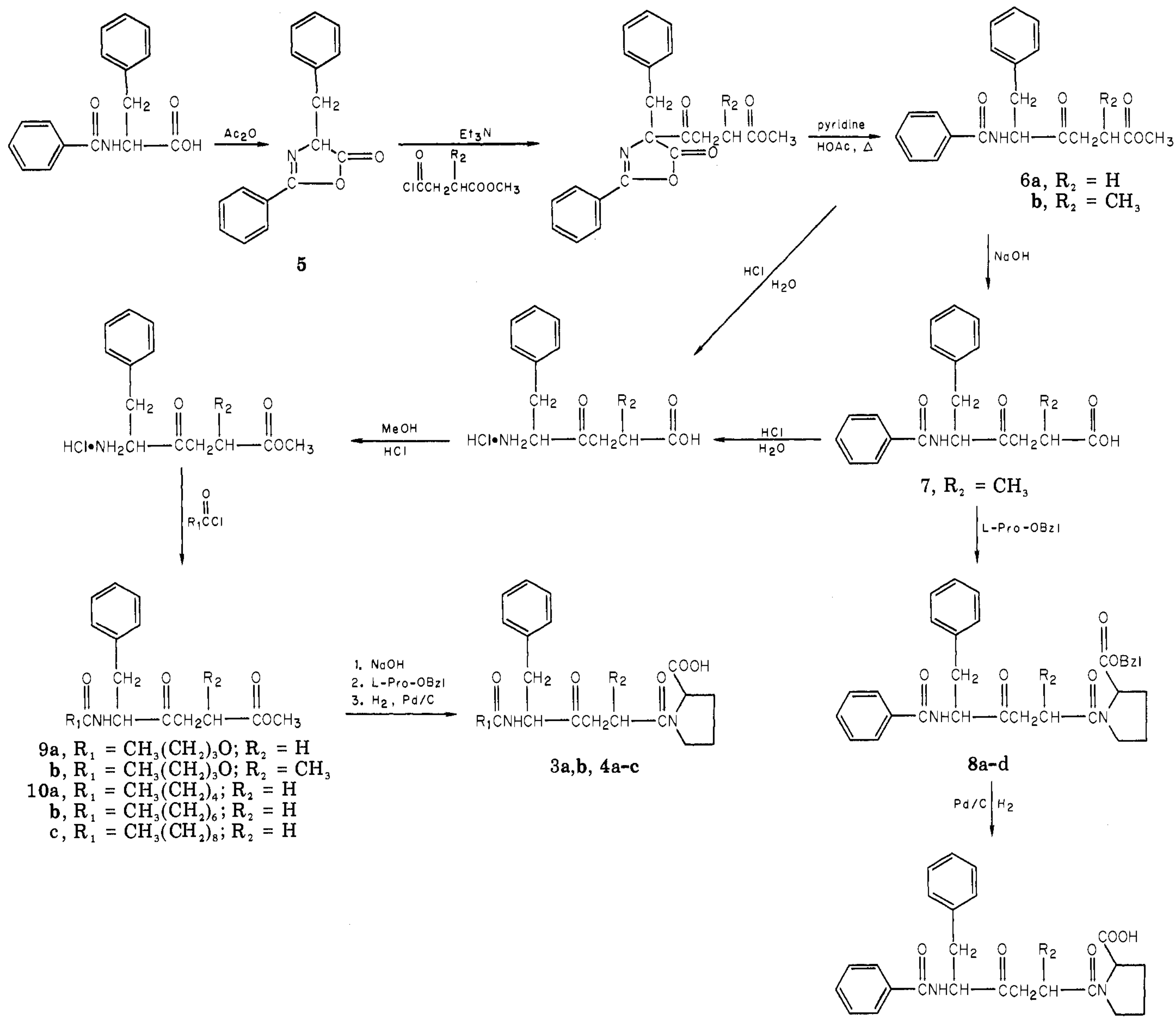
Scheme I



5

6a, R_2 = H
b, R_2 = CH_3

7, R_2 = CH_3

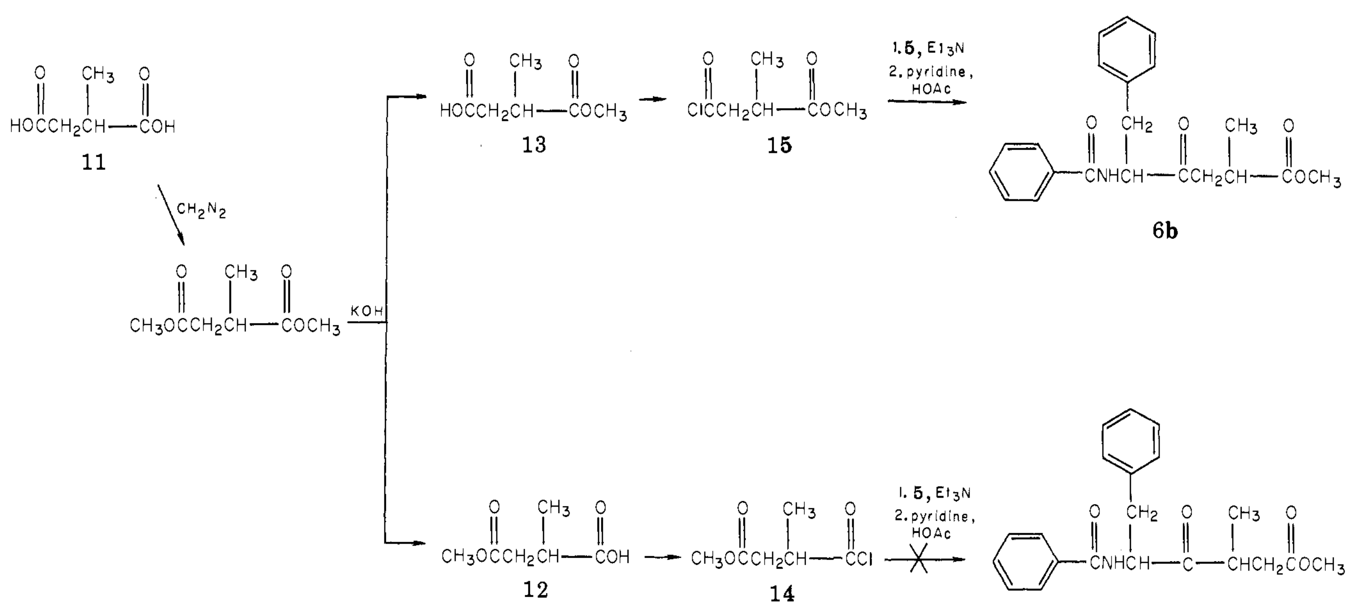
9a, R_1 = $CH_3(CH_2)_3O$; R_2 = H
b, R_1 = $CH_3(CH_2)_3O$; R_2 = CH_3
10a, R_1 = $CH_3(CH_2)_4$; R_2 = H
b, R_1 = $CH_3(CH_2)_6$; R_2 = H
c, R_1 = $CH_3(CH_2)_8$; R_2 = H

3a,b, 4a–c

8a–d

1, 2a–d

Scheme II



11

13

15

6b

12

14

chloride to yield methyl esters **6a** or **6b**.

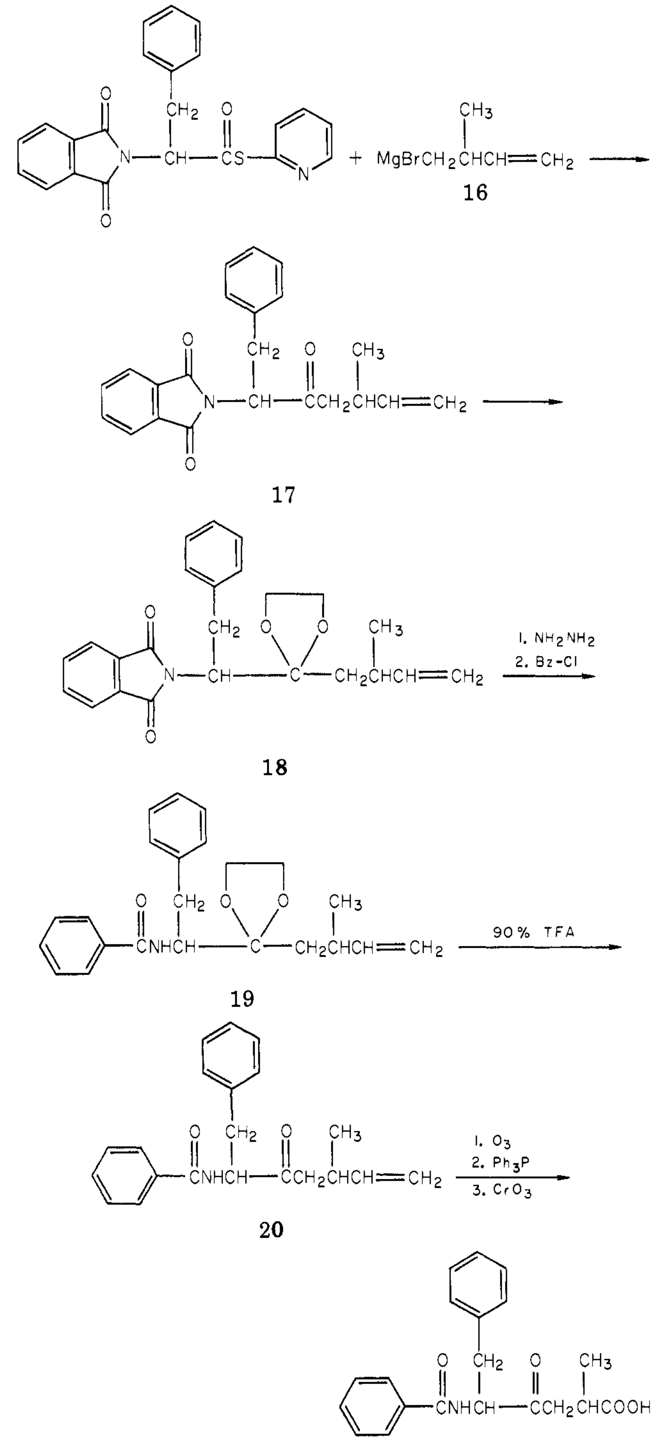
The acid chloride **15** (Scheme II) was obtained by two different methods. In the first method, partial saponification of dimethyl methylsuccinate yielded a mixture of **12** and **13**. A small amount of each of these acids was obtained pure by preparative HPLC. Both **12** and **13** were converted to their corresponding acid chlorides **14** and **15** and condensed with the oxazolone **5**. Compound **14** would not condense with **5** under normal reaction conditions, but compound **15** did condense in the usual manner to yield **6b** following decarboxylation. Evidently, the presence of the methyl group α to the acid chloride in **14** sterically hinders condensation with the oxazolone **5**. This lack of reactivity by **14** allowed us to synthesize **6b** using the 50:50 mixture of **12** and **13** without separating them.

Compound **13** could also be synthesized in 35% yield by alkylation of the dianion of methyl succinate with methyl iodide as described by Kofron and Wideman.[4] This method is preferred for small-scale synthesis of **6b** from **13**.

Compound **6b** was saponified, and the resulting acid **7** was condensed with L-proline benzyl ester with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole as condensing reagents. The tripeptide analogue obtained was a mixture of four diastereomers, **8a–d**. These diastereomers were separated by the use of preparative HPLC on a radially compressed silica gel cartridge with elution by ethyl acetate–petroleum ether (30–60 °C)–2-propanol (75:25:4). In order to identify which two diastereomers had the *R* configuration for the asymmetric carbon substituted by the methyl group, we repeated the synthesis of **6b** using β-carbomethoxybutyric acid chloride[5] obtained from (*R*)-(+)-2-methylsuccinic acid[6] (**11**, Scheme II). Final identification of the absolute configuration of the four diastereomeric benzyl esters was achieved by preparation of two more of the diastereomers by a synthetic route (Scheme III) that maintains the *S* configuration for the phenylalanine asymmetric center. This synthesis is similar to that used to make the Bz-Phe-Gly ketomethylene intermediate for the synthesis of **1**.[2] An olefin is used as a synthon for the desired carboxylic acid group in Scheme III. The Grignard reaction of the olefin **16** with the 2-pyridyl thioester of *N*-phthaloyl-L-phenylalanine[2] gave a 50% yield of crystalline **17**. The phthalimido ketoolefin **18** is converted to the benzamido ketoolefin **19** as shown in Scheme III. Ozonolysis of **20**, followed by reaction with triphenylphosphine and then chromic acid, gave the acid **21** in 69% yield. Optically active **7**, 5(*R*,*S*)-benzamido-2-(*R*)-methyl-4-oxo-6-phenylhexanoic acid, and **21**, 5(*S*)-benzamido-2(*R*,*S*)-methyl-4-oxo-6-phenylhexanoic acid, were condensed with L-proline benzyl ester as in Scheme I. The optically active acid **7** led to **8a** and **8b** with optical configurations at C5 and C2, respectively, of the hexanoic acid chain of *S* and *R* for **8a** and *R* and *R* for **8b**. The optically active acid **21** yielded **8a** and **8c** with optical configurations at C5 and C2 of *S* and *R* for **8a** and *S* and *S* for **8c**. The remaining diastereomer **8d** isolated from the original synthesis of **8a–d** was assigned the *R*,*S* stereochemistry.

Hydrogenolysis of **8a–d** with 10% palladium on carbon in acetic acid yielded **2a–d**, respectively, whose physical properties are listed in Table II.

Scheme III



The benzamido esters **6a** and **6b** were converted by acid hydrolysis and then esterification to the corresponding amine hydrochloride esters. These esters were acylated with the desired acyl or acyloxy chloride to yield **9a–10c**, whose physical properties are listed in Table I. The final L-proline-coupled products **3a–4c** obtained from these intermediates (**9a–10c**) are described in Table II.

Biological Results and Discussion

Table II lists the I_{50} for compounds **2a–4c** against guinea pig serum angiotensin converting enzyme. Compound **2a** is three times more potent than **1** as a converting enzyme inhibitor. This is not unexpected, since **2a** has the same stereostructure as the tripeptide inhibitor L-phenyl-

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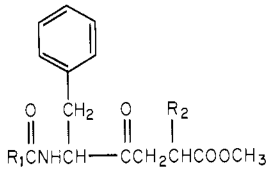
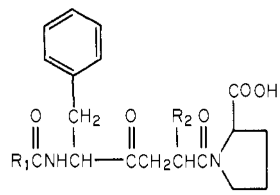
Table I. Acylamino Keto Ester Intermediates



compd	R_1	R_2	mp, °C	recrystn solv	yield, %	formula	anal.
6b	Ph	CH_3	97-98.5	Et_2O	35[a]	$C_{21}H_{23}NO_4$	C, H, N
9a	$CH_3(CH_2)_3O$	H	56-57	pet. ether	62[b]	$C_{18}H_{25}NO_5$	C, H, N
9**b**	$CH_3(CH_2)_3O$	CH_3	oil		82[c]	$C_{19}H_{27}NO_5$	C, H, N
10a	$CH_3(CH_2)_4$	H	73-74	Et_2O	55[c]	$C_{19}H_{27}NO_4$	C, H, N
10**b**	$CH_3(CH_2)_6$	H	53-54	hexane	75[c]	$C_{21}H_{31}NO_4$	C, H, N
10c	$CH_3(CH_2)_8$	H	53-54	hexane	74[c]	$C_{23}H_{35}NO_4$	C, H, N

[a] Yield based on oxazolone. [b] Yield based on amine hydrochloride acid. [c] Yield based on methyl ester amine hydrochloride.

Table II. Chemical and Pharmacological Data on Title Compound and Analogues



compd	R_1	R_2	mp, °C	recrystn solv	optical confign at C5	optical confign at C2	$[\alpha]^{23}_D$ (c, solv), deg	formula	anal.	I_{50},[g] M
1	Ph	H	151-153	EtOAc	*S*		−134 (1.0, EtOH)			3.2×10^{-9}
2a	Ph	CH_3	glass		*S*	*R*	−102 (1.0, 95% EtOH)	$C_{25}H_{28}N_2O_5$[a]	C, H, N	1.0×10^{-9}
2**b**	Ph	CH_3	glass		*R*	*R*	+25 (1.0, 95% EtOH)	$C_{25}H_{28}N_2O_5$[b]	C, H, N	8.2×10^{-9}
2c	Ph	CH_3	glass		*S*	*S*	−113 (1.1, EtOH)	$C_{25}H_{28}N_2O_5$[c]	C, H, N	4.6×10^{-8}
2d	Ph	CH_3	glass		*R*	*S*	+9.8 (1.0, EtOH)	$C_{25}H_{28}N_2O_5$[d]	C, H, N	3.2×10^{-6}
3a	$CH_3(CH_2)_3O$	H	85-95	H_2O	*R,S*			$C_{34}H_{53}N_3O_6$[e]	C, H, N	2.1×10^{-7}
3b	$CH_3(CH_2)_3O$	CH_3	syrup		*R,S*	*R,S*		$C_{23}H_{32}N_2O_6$[f]	C, H, N	2.9×10^{-8}
4a	$CH_3(CH_2)_4$	H	138-139	EtOAc	*S*		−91.4 (1.1, EtOH)	$C_{23}H_{32}N_2O_5$	C, H, N	4.7×10^{-8}
4b	$CH_3(CH_2)_6$	H	137-138	ether	*S*		−83.4 (1.0, EtOH)	$C_{25}H_{36}N_2O_5$	C, H, N	6.5×10^{-7}
4c	$CH_3(CH_2)_8$	H	140-141	EtOAc	*S*		−80.1 (1.0, EtOH)	$C_{27}H_{40}N_2O_5$	C, H, N	6.5×10^{-6}
captopril (Squibb)										9×10^{-9}

[a] $0.100CHCl_3$. [b] $0.125CHCl_3$. [c] $0.111CHCl_3$. [d] $0.285CHCl_3$. [e] Dicyclohexylamine salt. [f] $0.05CHCl_3$. [g] Molar concentration required for 50% inhibition of guinea pig serum angiotensin converting enzyme.

Table III. Effects of ACE Inhibitors against Angiotensin I in Conscious, Normotensive Rats

compd	dose, mg/kg	route[a]	no. tested	antagonism of max inhibn, %	AI[b] recovery time,[c] min
1	30	po	2	48	49
	3	iv	2	57	34
2a	30	po	1	100	143
	3	iv	1	100	73
2c	30	po	1	71	>180
	3	iv	1	74	71
captopril	0.3	po	5	69	170
	3	po	5	95	>240

[a] Vehicle employed for po: 4% gum acacia in 2 mL of distilled H_2O/kilogram of body weight. [b] Angiotensin I (0.32 μg/kg iv) was administered 2 or 3 times before and every 5-10 min after each drug treatment. The postdrug AI responses were compared to the average of the predrug AI responses. [c] Recovery time = time in minutes required for the AI response to return to 70% of the pretreatment control response.

alanyl-L-alanyl-L-proline. As mentioned earlier, this latter tripeptide has been found[2] to be over 10 times more potent than the tripeptide Phe-Gly-Pro that served as the model for 1. The other diastereomers of 2, **2b–d**, as well as compounds **3a–4c**, were less potent than 1 as converting enzyme inhibitors.

Table III shows the ability of 1, **2a**, and **2c** to inhibit the blood pressure increase produced by iv injection of angiotensin I in rats. Both compounds **2a** and **2c** were better inhibitors with longer duration than 1. The fact that **2c**, a poorer in vitro ACE inhibitor than 1, is a better in vivo ACE inhibitor than 1, indicates that **2c** is either more easily absorbed orally or is metabolically more stable that 1 in vivo.

Table IV shows the effect of **2a–4c** on blood pressure in renal hypertensive rats. Only compound **2a** causes any significant decrease in blood pressure. This effect does not appear to be dose related, since a dose increase from 3 to

Table IV. Effects of Certain ACE Inhibitors on Blood Pressure in the Conscious Renal (1 Clip/2 Kidney) Hypertensive Rat

compd	dose,[a] mg/kg po	no. tested	mean aortic blood pressure,[b] mmHg: base line	max effect	change
1	30	4	190 ± 6	178 ± 6	−12 (at 8 h)
2a	3	6	209 ± 5	169 ± 9[c]	−40 (at 8 h)
	30	5	197 ± 5	159 ± 3[c]	−38 (at 4 h)
2b	30	4	188 ± 7	166 ± 6	−22 (at 4 h)
2c	30	3	189 ± 10	173 ± 9	−16 (at 3 h)
2d	30	3	183 ± 15	163 ± 15	−20 (at 8 h)
3a	30	4	175 ± 7	160 ± 9	−15 (at 10 h)
3b	30	3	188 ± 6	176 ± 3	−12 (at 10 h)
4a	30	4	193 ± 14	191 ± 10	−2 (at 6 h)
4b	30	2	182 ± 10	167 ± 8	−15 (at 8 h)
4c	30	4	197 ± 9	176 ± 15	−21 (at 6 h)
captopril	3	4	192 ± 7	99 ± 7[c]	−93 (at 1 h)

[a] Vehicle employed: 4% gum acacia in 2 mL of distilled H_2O/kilogram of body weight. [b] All values are the mean ± 1 SEM. [c] Values significantly different from the comparable base-line value $p < 0.05$ (two-tailed probability, 2-14 df).

30 mg/kg does not lower blood pressure any further, although the time of onset appears to be shortened. Considering that **2a** given orally in rats at 30 mg/kg causes an immediate (after 5–10 min) 100% inhibition of the conversion of iv administered angiotensin I to angiotensin II, the maximum effect on blood pressure that is seen in renal hypertensive rats from 4 to 8 h after oral administration of **2a** may not be related to plasma converting enzyme inhibition.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-melt and are uncorrected. Optical rotations were measured using a Perkin-Elmer 141 automatic polarimeter. Mass spectra were taken on an LKB 9000 GC–MS spectrometer. ^{1}H NMR spectra were taken with a Varian EM390 spectrometer. ^{13}C NMR spectra were taken on a Varian XL-100 FT (25.17 MHz) spectrometer. Thin-layer chromatography was carried out on Uniplates from Analtech coated with 250 μm of silica gel GF. Evaporations were performed at 40 °C under house vacuum on a Büchi rotavapor unless otherwise stated. Elemental analyses were conducted by Eric Meier, Stanford University, Palo Alto, CA. Analytical high-performance LC was carried out on a Waters ALC-201 HPLC, with a Radialpak B column in an RCM 100 unit with UV visualization at 260 nm with a Schoeffel GM770 UV spectrometer. Preparative high-performance LC was performed using the Waters Prep LC/System 500 with silica gel cartridges. Some of the noncrystalline compounds could not be totally freed of solvent even on heating under reduced pressure. The elemental analyses of these compounds have been recorded with solvent present. The existence of solvents of crystallization was confirmed by ^{1}H NMR whenever possible.

Methyl 5-Benzamido-2-methyl-4-oxo-6-phenylhexanoate (6b). Method A. Dimethyl methylsuccinate (25.0 mL, 168 mmol) was dissolved in methanol and stirred in an ice bath while a solution of potassium hydroxide (9.56 g, 170 mmol) in methanol was added dropwise over a 1-h period. This mixture was left at 5 °C in the refrigerator for 50 h. The resulting clear solution was evaporated to a syrup. The syrup was poured into a separatory funnel containing $CHCl_3$ (400 mL) and H_2O (400 mL). After shaking, the $CHCl_3$ layer was separated, and the aqueous layer was reextracted with $CHCl_3$ (150 mL). The aqueous layer was stirred in an ice bath and acidified with concentrated HCl to pH 2. The resulting mixture was extracted twice with $CHCl_3$ (2 × 300 mL). The $CHCl_3$ extracts were combined, dried (Na_2SO_4), and evaporated to a clear oil: yield 14.7 g (60.0%). Analytical HPLC of this oil in 1% acetic acid–1% 2-propanol in hexane at a flow rate of 3.0 mL/min (visualized with a refractive index detector) showed it to be a mixture of 53% **12** (elution time 7.5 min) and 47% **13** (elution time 8.5 min). Small amounts of pure **12** and **13** could be obtained by preparative HPLC and were characterized by comparison of their ^{1}H NMR spectra with literature values.[4]

The clear oily mixture of **12** and **13** (14.7 g, 100 mmol) was stirred with dry benzene (25.0 mL) and oxalyl chloride (10.3 mL, 120 mmol) under a nitrogen atmosphere at 35 °C for 3 h.[4] The mixture was then evaporated on a rotary evaporator at 30 °C. The resulting residue was reevaporated three times from benzene (3 × 30 mL) to yield an orange oil. This oil was distilled at 1.0 mmHg vacuum, and the distillate coming over at 52–54 °C was collected as a mixture of acid chlorides **14** and **15**: yield 14.3 g; ^{1}H NMR shows no acid present.

This distillate was combined with the acid chloride mixture obtained from an earlier run of the same reaction sequence to yield 17.8 g (108 mmol). This mixture was combined with 2-phenyl-4-(phenylmethyl)-5(4*H*)-oxazolone (**5**;[1] 27.2 g, 108 mmol) and stirred with dry tetrahydrofuran (80 mL) under nitrogen in an ice bath while triethylamine (16.7 mL, 120 mmol) in dry tetrahydrofuran (80 mL) was added over a 20-min period. The mixture was stirred in an ice bath for 1 h and at room temperature for 16 h. The mixture was filtered and evaporated to a yellow syrup: yield 42.5 g.

This syrup was stirred with dry pyridine (195 mL) in a 90 °C oil bath, and acetic acid (142 mL) was added. The oil bath was heated to 100 °C, and the reaction mixture was stirred in this bath for 1 h. The mixture was then evaporated at 1 mmHg at 50 °C to an orange syrup. This syrup was evaporated twice from toluene (2 × 250 mL) to help remove pyridine. The resulting orange syrup was dissolved in EtOAc (600 mL) and washed successively with 2 N HCl (500 mL), saturated $NaHCO_3$ solution (400 mL), and H_2O (500 mL). The EtOAc layer was dried (Drierite) and evaporated to yield crude **6b** as an orange syrup: yield 27.0 g.

Saponification of **6b** to its corresponding acid was carried out in tetrahydrofuran (200 mL) with 0.5 N NaOH (175 mL). After the solution was stirred for 4 h at room temperature, the tetrahydrofuran was evaporated, and the aqueous residue was extracted with $CHCl_3$ (450 mL). The $CHCl_3$ layer was back-extracted with H_2O (150 mL). The two aqueous layers were combined and extracted again with $CHCl_3$ (100 mL). The aqueous layer was acidified to pH 4 with concentrated HCl (8.0 mL) and then extracted twice with $CHCl_3$ (200 and 100 mL). The two $CHCl_3$ extracts were combined, dried (Na_2SO_4), and evaporated to yield the crude acid **7** as a yellow foam: yield 15.3 g. This crude foam was used in the synthesis of **8a–d**.

Method B. Using the method of Kofron and Wideman,[4] we first condensed 2.5 L of ammonia in a three-neck round-bottomed flask and stirred it mechanically at −78 °C while lithium wire (215 cm, 1.32 mol) was added in 10- to 20-cm portions. The blue solution was warmed to ammonia reflux temperature, and a few crystals of $Fe(NO_3)_2 \cdot 9H_2O$ were added. The reaction mixture became medium gray and was again cooled to −78 °C. A solution of methyl succinate (59.0 g, 447 mmol) in anhydrous ether (800 mL) was added under nitrogen pressure in 10 min. The reaction temperature was allowed to increase to ammonia reflux temperature and stirred for 1 h. A solution of methyl iodide (63.4 g, 447 mmol) in anhydrous ether (600 mL) was added. The mixture was then stirred at ammonia reflux temperature for 5 h. The reaction mixture was then cautiously quenched with 79 g (1.46 mol) of ammonium chloride. The ammonia was allowed to evaporate overnight. The resulting residue was mixed with H_2O (1.5 L) and washed with $CHCl_3$ (3 × 1 L). The aqueous phase was filtered to remove a black residue, and the filtrate was acidified to pH 3 with concentrated HCl. The acidified aqueous layer was decolorized by addition of $NaHSO_3$ and then was extracted with $CHCl_3$ (3 × 1 L). These extracts were combined, dried ($MgSO_4$), and concentrated to yield crude methyl 2-methylsuccinate (**13**; 24.0 g, 36.7%): ^1H NMR chemical shifts agreed with literature values for this compound.

Using the procedures described in method A, we converted this acid to its acid chloride [21.0 g, bp 42–46 °C (0.75 mmHg)], which was subsequently condensed with 2-phenyl-4-(phenylmethyl)-5-(4*H*)-oxazolone (32.1 g, 128 mmol) and decarboxylated to yield 40.3 g of crude **6b** as an orange syrup. This syrup was purified by preparative silica gel HPLC with 15% EtOAc in petroleum ether (35–60 °C bp) as the eluting solvent. The first 2.4 L of effluent was discarded, and the next 3.8 L of effluent was combined and concentrated to a yellow semisolid residue: yield 25.0 g; R_f 0.40 [30% EtOAc in petroleum ether (35–60 °C)]. This residue was crystallized from ether to white crystalline **6b**: 16.1 g (35.0% based on oxazolone); mp 97–98.5 °C. Anal. ($C_{21}H_{23}NO_4$) C, H, N.

5-Benzamido-2-methyl-4-oxo-6-phenylhexanoic Acid (7). The crystallized **6b** was combined with its mother liquor to yield 24.0 g (68.0 mmol) of crude **6b**, which was saponified as described in method A to yield 16.7 g of crude **7** as an off-white solid foam. This foam was crystallized from water–ethanol (2:1) to white powdery crystals: yield 13.4 g (58.0%); mp 132–134 °C. Anal. ($C_{20}H_{21}NO_4$) C, H, N.

5-Benzamido-2-methyl-4-oxo-6-phenylhexanoyl-L-proline Benzyl Ester (8a–d). A mixture of crude 5-benzamido-2-methyl-4-oxo-6-phenylhexanoic acid (from saponification of **6b** obtained by method A; 15.3 g, 45.1 mmol), 1-hydroxybenzotriazole hydrate (6.73 g, 45.1 mmol), L-proline benzyl ester hydrochloride (10.9 g, 45.1 mmol), and CH_2Cl_2 (400 mL) were stirred in an ice bath, and triethylamine (6.30 mL, 45.1 mmol) was added, followed by dicyclohexylcarbodiimide (9.31 g, 45.1 mmol) in CH_2Cl_2 (100 mL). Stirring was continued at ice-bath temperature for 1 h and then at room temperature for 20 h. The mixture was then cooled in an ice bath, and dicyclohexylurea (DCU) was removed by filtration. The filtrate was washed successively with ice-cold 2 N HCl (400 mL), 0.2 N NaOH (400 mL), and H_2O (400 mL). The organic layer was dried (Drierite) and evaporated to an orange foam: yield 20.3 g. The foam was mixed with EtOAc (20 mL), cooled to 5 °C, and filtered to remove more DCU. The filtrate was evaporated to a very crude mixture of **8a–d** as an orange solid foam: yield 19.5 g. This crude mixture of diastereomeric proline esters was separated by repeated preparative liquid chromatography on silica gel with elution by 4% 2-propanol in EtOAc-petroleum ether (35–60 °C) (3:1). The following final amounts of each diastereomer (98% pure by HPLC) obtained are listed according to their elution order on analytical HPLC in EtOAc–hexane (1:1, 3.0 mL/min): **8a**, 1.00 g, elution time 4.8 min; **8b**, 0.866 g, elution time 5.4 min; **8c**, 0.730 g, elution time 7.6 min; **8d**, 0.633 g, elution time 8.5 min. The ^1H NMR spectra of **8a–d** are greatly complicated by cis–trans isomerization about the proline amide bond as discussed previously,[2] but they are listed below for further characterization purposes. **8a**: ^1H NMR ($CDCl_3$) δ 7.75 (2 H, dd, J = 3 and 9 Hz, benzoyl CH's), 7.43 (2 H, s, benzoyl CH's), 7.28 (5 H, s, benzyl CH's), 7.22 (5 H, s, phenyl CH's), 5.13 and 5.08 (2 H, 2 s, intensity ratio 1:1, benzyl CH_2), 4.95 (1 H, m), 4.50 (1 H, t, J = 4 Hz), 3.67 (2 H, m), 3.15 (4 H, m), 1.85 (4 H, m, proline CH_2's), 1.05 (3 H, d, J = 6 Hz, CH_3). **8b**: ^1H NMR ($CDCl_3$) δ 7.67 (2 H, dd, J = 3 and 9 Hz, benzoyl CH's), 7.40 (2 H, s, benzoyl CH's), 7.33 (5 H, s, benzyl CH's), 7.20 (5 H, s, phenyl CH's), 6.63 (1 H, d, J = 6 Hz, NH), 5.15 and 5.12 (2 H, 2 s, intensity ratio 1:1, benzyl CH_2), 4.93 (1 H, m), 4.60 (1 H, t, J = 4 Hz), 3.70 (2 H, m), 3.47–2.90 (4 H, m), 2.07 (4 H, m, proline CH_2's), 1.13 (3 H, d, J = 6 Hz, CH_3). **8c**: ^1H NMR ($CDCl_3$) δ 7.73 (2 H, dd, J = 3 and 9 Hz, benzoyl CH's), 7.41 (2 H, s, benzoyl CH's), 7.27 (5 H, s, benzyl CH's), 7.20 (5 H, s, phenyl CH's), 7.00 (1 H, d, J = 7 Hz, NH), 5.17 and 5.03 (1 H, 2 s, intensity ratio 2:5, benzyl CH_2), 4.90 (1 H, m), 4.50 (1 H, t, J = 4 Hz), 4.03–3.36 (2 H, m), 3.13 (4 H, m), 2.00 (4 H, m, proline CH_2's), 1.07 and 0.85 (3 H, 2 d, J = 6 Hz, intensity ratio 2.3:1, CH_3). **8d**: ^1H NMR ($CDCl_3$) δ 7.67 (2 H, dd, J = 3 and 9 Hz, benzoyl CH's), 7.42 (2 H, s, benzoyl CH's), 7.30 (5 H, d, rotomers, benzyl CH's), 7.20 (5 H, s, phenyl CH's), 6.67 (1 H, d, J = 7 Hz, NH), 5.17 and 5.10 (1 H, 2 s, intensity ratio 1:2, benzyl CH_2), 4.90 (1 H, m), 4.53 (1 H, m), 4.03–2.93 (6 H, m), 2.06 (4 H, m, proline CH_2's), 1.13 and 0.97 (3 H, 2 d, J = 6 Hz, intensity ratio 5:3, CH_3).

Compounds **8a–d** were converted by hydrogenolysis to **2a–d**, respectively. In addition to the analytical data given in Table II, **2a–d** gave the expected ^1H NMR, ^{13}C NMR, IR, mass spectra, and CD spectra.

5-Benzamido-2(*R*)-methyl-4-oxo-6-phenylhexanoyl-L-proline Benzyl Ester (8a and 8b). A mixture of 2-methylsuccinic acid (76.2 g, 577 mmol) and strychnine (193 g, 577 mmol) was recrystallized five times to give a salt of constant rotation: yield 52.8 g; $[\alpha]^{23}_D$ +14.3° (c 1.0, EtOH). This salt was dissolved in H_2O (900 mL), and 2 N HCl (63 mL) was added. The aqueous mixture was extracted with ether (5 × 1 L). The ether extracts were combined, dried (Na_2SO_4), and evaporated to a white solid (*R*)-(+)-2-methylsuccinic acid: yield 10.5 g; $[\alpha]^{23}_D$ +10.3° (c 4.8, H_2O) [lit.[7] $[\alpha]^{20}_D$ +11.7° (c 3.1, H_2O)].

The (*R*)-(+)-2-methylsuccinic acid (10.5 g, 79.5 mmol) in ether (300 mL) was treated with diazomethane in ether (2.5 L) that had been generated by adding nitrosomethylurea (52.0 g, 571 mmol) to a mixture of ether (2.5 L) and 10% NaOH (1.30 L, 3.25 mol) at 0 °C. The reaction mixture was dried ($MgSO_4$) and concentrated to a pale yellow oil. This oil was dissolved in pentane (50 mL) and filtered. The filtrate was washed with 2 N $NaHCO_3$ (3 × 50 mL), dried ($MgSO_4$), and concentrated to yield dimethyl (*R*)-(+)-2-methylsuccinate: yield 6.28 g (49.4%); ^1H NMR ($CDCl_3$) δ 3.74 (6 H, s, 2 OCH_3), 2.35–3.25 (3 H, m, $CHCH_2$), 1.30 (3 H, d, J = 6 Hz, CH_3).

Dimethyl (*R*)-(+)-2-methylsuccinate was converted to crude **6b** by method A, and, after saponification, the acid **7** was condensed with L-proline benzyl ester as described for the synthesis of **8a–d**. Analytical HPLC of the product in EtOAc–hexane (1:1, 3.0 mL/min) showed two major benzyl ester products corresponding to **8a**, elution time 4.9 min, and **8b**, elution time 5.6 min. Identification of the two products was confirmed by coinjection with **8a** and **8b** obtained from the previous separation of the **8a–d** mixture.

General Method A: Used for the Synthesis of 9a,b and 10a–c. Methyl 5-[(*n*-Butyloxycarbonyl)amino]-4-oxo-6-phenylhexanoate (9a). 5-Benzamido-4-oxo-6-phenylhexanoic acid[2] (**6a**; 1.00 g, 3.07 mmol) was refluxed in a mixture of H_2O (15 mL), concentrated HCl (45 mL), and acetic acid (25 mL) for 24 h. The mixture was evaporated at 60 °C (azeotroping with toluene) to an orange residue. This residue was dissolved in acetonitrile (10 mL) and added to stirring ether (75 mL) to remove benzoic acid. After the solution was cooled to 5 °C, the ether was decanted, and the remaining residue was again dissolved in acetonitrile (10 mL) and added to ether (125 mL). After cooling to 5 °C, the ether was again decanted. The residue was dissolved in acetonitrile and evaporated to an orange gummy foam as the crude amine hydrochloride: yield 0.620 g (78%). This foam was stirred with HCl-saturated methanol for 18 h. After evaporation, the crude methyl ester was obtained as an orange gum. This gum was stirred in H_2O (20 mL), and *n*-butyl chloroformate (0.603 mL, 4.74 mmol) was added. Using a pH meter, we maintained the aqueous solution at pH 6.5 by addition of 0.2 N Na_2CO_3 over a 1-h period. The mixture then was extracted with ether (70 mL). The ether extract was dried (Drierite) and evaporated to an orange oil: yield 0.568 g. This oil was crystallized from petroleum ether (35–60 °C, 30 mL) to off-white cottony needles of **9a**: yield 0.492 g; mp 56–57 °C. Anal. ($C_{18}H_{25}NO_5$) C, H, N.

General Method B: Used for the Synthesis of 3a,b and 4a–c. 5-[(*n*-Butyloxycarbonyl)amino]-2-methyl-4-oxo-6-phenylhexanoyl-L-proline (3b). Methyl 5-[(*n*-butyloxycarbonyl)amino]-2-methyl-4-oxo-6-phenylhexanoate (6.73 g, 19.2 mmol) was stirred in MeOH (140 mL), and 1 N NaOH (28 mL) was added. After 10 min of stirring, the mixture was diluted with H_2O (280 mL) and acidified with 2 N HCl to pH 3. The MeOH was evaporated and the remaining aqueous–oil mixture was extracted with $CHCl_3$ (175 mL). The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to a yellow syrup (6.11 g, 94.9%). A portion of this syrup (3.52 g, 10.5 mmol) was combined with L-proline benzyl ester hydrochloride (2.54 g, 10.5 mmol), CH_2Cl_2 (60 mL), and triethylamine (1.40 mL, 10.5 mmol), this mixture was stirred in an ice bath, and dicyclohexylcarbodiimide (2.17

(7) M. Naps and I. B. Johns, *J. Am. Chem. Soc.*, **62**, 2450 (1940).

g, 10.5 mmol) in CH_2Cl_2 (10 mL) was added. The mixture was stirred in an ice bath for 30 min and at room temperature overnight (18 h). The mixture was then cooled in an ice bath and filtered. The filtrate was diluted to 100 mL with $CHCl_3$ and washed successively with 2 N HCl (100 mL), 0.3 N NaOH (100 mL), and H_2O (100 mL). The $CHCl_3$ layer was dried (Drierite) and evaporated to a yellow syrup: yield 6.36 g. This syrup was purified by preparative HPLC with 30% EtOAc in petroleum ether (35–60 °C) with 1% 2-propanol as the eluting solvent. The fractions that corresponded to product R_f 0.40 (2:1 $CHCl_3$–ether) were combined and evaporated to a pale yellow syrup: yield 3.28 g (59.7%). This syrup (3.28 g, 6.28 mmol), acetic acid (100 mL), and 10% palladium on carbon (3.28 g) were stirred under 1 atm of hydrogen gas at room temperature for 16 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated (azeotroping with EtOH) to a clear syrup. This syrup was mixed with H_2O (200 mL) and basified to pH 9 by the addition of 1 N NaOH (13 mL). The aqueous mixture was extracted twice with $CHCl_3$ (200 and 100 mL). The two $CHCl_3$ extracts were combined and extracted with H_2O (100 mL). This H_2O extract was combined with the previous aqueous layer and mixed with $CHCl_3$ (200 mL) in a separatory funnel. The aqueous layer was acidified to pH 3 with 2 N HCl. After shaking, the $CHCl_3$ layer was separated, and the aqueous layer was reextracted with $CHCl_3$ (100 mL). The two $CHCl_3$ extracts were combined, dried (Na_2SO_4), and evaporated to **3b**, a clear gum: yield 2.42 g (89.3% from benzyl ester intermediate); MS, m/e 432 (M^+). Anal. ($C_{23}H_{32}N_2O_6$·0.5$CHCl_3$) C, H, N.

2-Methyl-3-butenylmagnesium Bromide (16). A solution of triphenylphosphine (105 g, 400 mmol) in dry DMF (900 mL) under nitrogen in a 2-L, one-neck, round-bottom flask cooled in ice was stirred vigorously while bromine (20.6 mL, 400 mmol) was added dropwise via an addition funnel. Then 2-methyl-3-buten-1-ol (41.6 mL, 400 mmol) was added dropwise via an addition funnel. The ice bath was removed, and the mixture was stirred for 16 h. The mixture was distilled at 20 mmHg until 120 mL of distillate had collected. The distillate was poured onto ice (100 g) and was extracted with pentane (2 × 50 mL). The organic extracts were washed with water (2 × 100 mL), combined, dried ($MgSO_4$), and evaporated in vacuo to give 37.3 g of the bromide (63%) as a colorless liquid: bp 111–114 °C (760 mmHg) (lit.[8] 110–112 °C); ^1H NMR ($CDCl_3$) δ 5.73 (1 H, ddd, J = 6, 9, and 16 Hz), 5.07 (1 H, d, J = 16 Hz), 5.05 (1 H, d, J = 9 Hz), 3.33 (2 H, d, J = 6 Hz), 2.53 (1 H, septet, J = 6 Hz), 1.13 (3 H, d, J = 6 Hz).

Conversion of the bromide to its Grignard derivative **16** required addition of the bromide to 1 equiv of magnesium turnings in anhydrous ether. Heating under nitrogen at reflux for 5 h yielded the desired magnesium bromide derivative **16**.

3-Methyl-5-oxo-7-phenyl-6(*S*)-phthalimido-1-heptane (17). A solution of **16** prepared from 0.910 mL (9.60 mmol) of 4-bromo-3-methyl-1-butene and 2.30 g (9.60 mmol) of magnesium turnings in anhydrous ether (10 mL) was added via syringe to a stirred solution of *N*-phthaloyl-L-phenylalanine 2-mercaptopyridyl thioester[2] (1.24 g, 3.20 mmol) in dry THF (300 mL) under nitrogen, maintained between 0 and 5 °C. The ice bath was removed, and the mixture was stirred for 15 min. The mixture was poured into ice-cold saturated ammonium chloride solution (500 mL) and was extracted with ethyl acetate (2 × 500 mL). The organic extracts were washed successively with 5% sodium hydroxide solution (500 mL), saturated sodium bicarbonate solution (500 mL), and saturated sodium chloride solution (500 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to give 1.20 g of pale yellow oil. This was recrystallized from ethyl acetate/hexane to give 548 mg of **17** (49%): mp 69–70 °C; IR ($CHCl_3$) 3020 (w), 2970 (w), 2940 (w), 1780 (m), 1720 (s), 1650 (w), 1620 (w), 1500 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.72 (4 H, m), 7.11 (5 H, s) 5.73 (1 H, ddd, J = 6, 10, 17 Hz), 4.94 (3 H, m), 3.46 (2 H, m), 2.77 (1 H, m), 2.47 (2 H, m), 1.00 and 0.98 (3 H, 2 d, J = 7 Hz); mass spectrum, m/e 347 (M^+), 320 (M − C_2H_3), 250 (M − C_6H_9O). Anal. ($C_{22}H_{21}NO_3$) C, H, N.

2-(2-Methyl-3-butenyl)-2-[1(*S*)-phthalimido-2-phenylethyl]-1,3-dioxolane (18). A mixture of **17** (2.50 g, 7.20 mmol), dry methanol (150 mL), 2-methoxy-1,3-dioxolane (6.50 mL), and *p*-toluenesulfonic acid monohydrate (250 mg) was heated under reflux in a nitrogen atmosphere for 6 days. After cooling to room temperature, the mixture was partially evaporated in vacuo to 15 mL. This was poured onto ice (50 g) and was extracted with diethyl ether (2 × 50 mL). The organic extracts were washed successively with saturated sodium bicarbonate solution (50 mL) and saturated sodium chloride solution (50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to give 2.90 g pale yellow oil. This was purified by column chromatography on 200 g of silica gel (90–200 mesh), eluting with acetone–hexane (1:4) to give 2.14 g of **18** (76%) as a colorless gummy solid: mp 122–128 °C; R_f 0.46 (acetone–hexane, 1:4); IR ($CHCl_3$) 2970 (m), 2900 (m), 1780 (m), 1705 (s), 1640 (w), 1610 (w), 1500 (w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.64 (4 H, m), 7.13 (5 H, s), 5.79 (1 H, m), 4.93 (2 H, m), 4.59 and 4.56 (1 H, 2 dd, J = 4 and 13 Hz), 4.05 (4 H, m), 3.83 (1 H, t, J = 13 Hz), 3.13 (1 H, dd, J = 4 and 13 Hz), 2.46 (1 H, m), 2.00 (2 H, m), 1.07 and 1.03 (3 H, 2 d, J = 7 Hz); mass spectrum, m/e 392 (M + H^+), 322 (M − C_5H_9). Anal. ($C_{24}H_{25}NO_4$) C, H, N.

2-(2-Methyl-3-butenyl)-2-[1(*S*)-benzamido-2-phenylethyl]-1,3-dioxolane (19). A mixture of **18** (1.90 g, 4.85 mmol), absolute ethanol (70 mL), and 97% hydrazine (1.00 mL) was heated under reflux in a nitrogen atmosphere for 15 h. After cooling to room temperature, the mixture was partially evaporated in vacuo to 15 mL. This was poured into ice-cold 5% sodium hydroxide solution (50 mL) and was extracted with chloroform (3 × 50 mL). The organic extracts were washed with water (50 mL), combined, dried (K_2CO_3), and evaporated in vacuo to give 1.20 g of crude amine as a colorless oil. This was dissolved in dry pyridine (70 mL) under nitrogen, and benzoyl chloride (0.800 mL, 6.60 mmol) was added via syringe while stirring and maintaining the temperature between 0 and 5 °C. The ice bath was removed 30 min following completion of the addition, and the mixture was stirred at room temperature for 48 h. The mixture was evaporated in vacuo and reevaporated twice following the addition of toluene (2 × 100 mL). The residue was dissolved in chloroform (50 mL) and washed successively with 1 N hydrochloric acid (50 mL), 0.1 M sodium carbonate solution (50 mL), and water (50 mL). The aqueous layers were washed with chloroform (2 × 50 mL) and the combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to give 1.70 g of yellow oil. This was purified by column chromatography on 150 g of silica gel (90–200 mesh), eluting with ethyl acetate–hexane (1:3) to give 1.33 g of a pale yellow solid. This was crystallized from ethyl acetate/hexane to give 1.12 g of **19** (63%): mp 97–100 °C; R_f 0.27 (ethyl acetate–hexane, 1:3); IR ($CHCl_3$) 3400 (m), 3040 (m), 2970 (m), 2940 (m), 2870 (m), 1660 (s), 1600 (m), 1580 (m), 1505 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.40 (5 H, m), 7.18 (5 H, s), 6.14 and 6.03 (1 H, 2 s), 5.83 (1 H, ddd, J = 7, 9, and 17 Hz), 4.95 (3 H, m), 4.01 (4 H, m), 3.24 (1 H, dd, J = 4 and 14 Hz), 2.62 (1 H, dd, J = 11 and 14 Hz), 2.52 (1 H, m), 1.77 (2 H, m), 1.02 (3 H, d, J = 7 Hz); mass spectrum, m/e 366 (M + H^+) 296 (M − C_5H_9). Anal. ($C_{23}H_{27}NO_3$) C, H, N.

6(*S*)-Benzamido-3-methyl-5-oxo-7-phenyl-1-heptene (20). Compound **19** (900 mg, 2.46 mmol) in 90% aqueous trifluoroacetic acid (9 mL) was stirred in a nitrogen atmosphere at room temperature for 16 h. The mixture was poured onto ice (50 g) and was extracted with chloroform (3 × 50 mL). The organic extracts were washed successively with saturated sodium bicarbonate solution (50 mL) and saturated sodium chloride solution (50 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated in vacuo to give 999 mg of white solid. This was crystallized from ethyl acetate/hexane to give 723 mg of **20** (92%): mp 120–124 °C; IR ($CHCl_3$) 3400 (m), 3050 (m), 2990 (m), 2950 (m), 1710 (m), 1650 (s), 1600 (m), 1580 (m), 1500 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.73 (2 H, dd, J = 3 and 8 Hz), 7.42 (3 H, m), 7.21 (5 H, s), 6.91 and 6.83 (1 H, 2 s), 5.70 (1 H, ddd, J = 7, 9, and 16 Hz), 4.97 (3 H, m), 3.17 (2 H, d, J = 7 Hz), 2.73 (1 H, m), 2.51 (2 H, m), 0.97 (3 H, d, J = 6 Hz); mass spectrum, m/e 321 (M^+). High-resolution mass spectrum, found M^+ 321.1754; $C_{21}H_{23}NO_2$ requires M^+ 321.1729. Anal. ($C_{21}H_{23}NO_2$) C, H, N.

5(*S*)-Benzamido-2-methyl-4-oxo-6-phenylhexanoic Acid (21). Ozonized oxygen was bubbled through a solution of **20** (2.30 g, 7.17 mmol) in dry dichloromethane (25 mL) at −78 °C until a blue color appeared. Argon was then bubbled through until the solution became colorless, and the mixture was evaporated

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in vacuo to give the crude ozonide as a white foam. This was dissolved in absolute ethanol (200 mL) in a nitrogen atmosphere, and triphenylphosphine (3.80 g, 14.3 mmol) was added. The mixture was stirred for 3 days at room temperature and evaporated in vacuo. The white semisolid residue was dissolved in acetone (400 mL) and cooled in ice. To the stirred solution was added a solution of chromium trioxide (3.59 g, 35.9 mmol) in 35% sulfuric acid (120 mL) at such a rate that the temperature was maintained below 10 °C. The ice bath was removed, and the mixture was stirred for 10 min. The mixture was poured onto ice (500 g) and was extracted with diethyl ether (2 × 500 mL). The organic extracts were washed successively with water (200 mL) and saturated sodium bicarbonate solution (2 × 200 mL). The combined basic layers were acidified to pH 1 with 5 N hydrochloric acid and were extracted with ethyl acetate (3 × 200 mL). The organic extracts were washed successively with water (2 × 200 mL) and saturated sodium chloride solution (200 mL). The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated in vacuo to give 1.65 g of **21** (69%) as a white solid, which could be crystallized from chloroform/diethyl ether to give fine crystals: mp 121–124 °C; ^{1}H NMR ($CDCl_3$) δ 10.92 (1 H, s), 7.41 (5 H, m), 7.20 (5 H, s), 6.95 and 6.87 (1 H, 2 s), 5.04 (1 H, dd, J = 5 and 7 Hz), 3.14 (2 H, m), 2.93 (2 H, m), 2.50 (1 H, m), 1.18 and 1.13 (3 H, 2 d, J = 6 Hz). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

This acid **21** was condensed with L-proline benzyl ester as described for the synthesis of **8a–d**. Analytical HPLC of the product in EtOAc–hexane (1:1, 3.0 mL/min) showed two benzyl ester products that were identified as **8b** and **8c** by coelution with authentic **8b** and **8c** obtained from the previous separation of the **8a–d** mixture.

Biological Methods. The in vitro ACE inhibitory activity was determined by a radioassay procedure reported previously.[9] Activity is reported as the I_{50}, which is the approximate value molar concentration of test compound causing a 50% inhibition of the control converting-enzyme activity.

The test solutions were prepared by dissolving 2–5 mg of test compound in 1 mL of Me_2SO and diluting to the desired concentration with a pH 8 buffer of 0.05 mol of Hepes (Calbiochem), 0.1 mol of NaCl, and 0.6 mol of Na_2SO_4 in H_2O.

AI Challenge Test in the Conscious Rat. The oral and intravenous efficacy of test compounds to inhibit the conversion of AI to AII was evaluated in conscious normotensive rats. For this test, male albino rats (CD strain; Charles River; Wilmington, MA) weighing 300 to 387 g were surgically prepared withn an aortic cannula (for blood pressure monitoring) and a vena caval cannula (for intravenous drug injection) as described below. AI (0.32 μg/kg iv) was administered before and at 5- to 10-min intervals following intravenous or oral test drug administration. ACE inhibitors block the pressor effect of AI by interfering with its conversion to AII, which is the active pressor agent.

At the time of testing, the rats in their individual cages were transferred to the test room, and their aortic cannulae were connected to pressure transducers (P23Gb or P23De; Gould Statham, Hato Ray, Puerto Rico). Systolic, diastolic, and mean aortic blood pressures and heart rate were obtained from the pressure signal (Gould Brush couplers) and displayed on a strip chart recorder (Model 260, Gould Brush; Cleveland, OH).

Blood Pressure and Heart Rate Test in the Conscious Rat. Hypertension of renal origin was produced in rats by placing a silver clip (0.2-mm gap) around the left renal artery near the aorta and leaving the contralateral kidney intact. Four-week-old male albino rats (CD strain, Charles River; Wilmington, MA) were clipped soon after arrival, and the hypertension was allowed to develop for 3–4 weeks. The rats were then cannulated for blood pressure monitoring as described below. Only rats with pulsatile mean aortic blood pressures of >160 mmHg were used. At the time of cannulation the rats weighed 280 to 320 g. The rats were given free acess to a standard lab chow (5012, Purina; Richmond, IN) and tap water and were maintained on a 12-h dark/12-h light cycle.

Two to four days prior to testing, rats were surgically implanted with chronic polyethylene cannulae. Each rat was anesthetized intramuscularly with 20 mg/kg of Telazol (tiletamine hydrochloride/zolazepam hydrochloride, 1:1), and the descending aorta and vena cava were exposed via a midline incision. For blood pressure monitoring, cannulae consisting of a PE 100 (0.86-mm i.d.) body and a PE 50 (0.58-mm i.d.) tip were inserted into undersized puncture hole below the renal arteries. The cannulae are anchored to the psoas muscle, passed subcutaneously along the midline of the back, and externalized between the scapulae. Following surgery, each rat was given 30 000 units of penicillin subcutaneously (penicillin G procaine sterile suspension; Parke-Davis, Detroit, MI). The rats were then fitted with a harness–spring–swivel assembly designed to protect the cannula and to provide the rat relative freedom of movement. The aortic cannula of each rat was connected to a pressure transducer (P23Gb, Statham; Hato Rey, Puerto Rico) and an infusion pump (Sage Model 234-7, Orion Research, Cambridge, MA) by means of PE 100 tubing. While on test, each animal received a continuous slow infusion of heparinized saline solution (approximately 400 μL or in 40 units of heparin per 24-h period) to prevent clot formation iin the blood pressure monitoring cannula. As required, a PE 20 (0.38-mm i.d.) cannula was inserted directly into the vena cava and externalized as described for the aortic cannula. The intravenous cannulae are plugged when not being used.

One-minute running average values of heart rate and aortic blood pressure (mean, systolic, and diastolic) for each rat were recorded every 30th min by means of a computer-assisted data capture scheme as previously described.[10]

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