

temperature for 20 min and then poured into H₂O (300 mL). The mixture was extracted with ether (3 × 100 mL), and the combined ethereal extracts were washed with H₂O and dried over MgSO₄. Filtration and evaporation of the solvent gave an oil. On trituration with petroleum ether (40–60 °C) the oil solidified, and crystallization from CHCl₃ gave the product 37: yield 1.0 g (62%); mp 140–141 °C. Anal. (C₁₀H₉ClNO₃S₃) C, H, N.

Compounds 33–36 and 38–42 (Table I) were all prepared by either method A or B.

5-(Phenylsulfonyl)thiophene-2-sulfonamide (43). A solution of 5-(phenylthio)thiophene-2-sulfonamide (8; 5.0 g, 0.018 mol) and 30% H₂O₂ (5.0 mL) in AcOH (50 mL) was heated on a steam bath for 1 h. H₂O was added to the hot solution until crystallization commenced and the mixture was then allowed to cool. The product was filtered off, washed with H₂O, dried, and crystallized from EtOAc/petroleum ether (60–80 °C) to give 43: yield 4.2 g (75%); mp 137.5–139 °C. Anal. (C₁₀H₉NO₄S₃) C, H, N. A polymorph of mp 170–172 °C was also obtained.

Compounds 44–60 and 62–67 (Table I) were all prepared by this method.

5-[4-(4-Aminophenyl)sulfonyl]thiophene-2-sulfonamide (61). A mixture of 5-[4-(isobutylamino)phenyl]sulfonyl]thiophene-2-sulfonamide (65; 1.8 g, 0.005 mol) and 15% aqueous HCl (20 mL) was heated under reflux until solution was complete (2 h). After cooling, the solution was neutralized by the addition of solid NaHCO₃, and the resultant precipitate was filtered off and crystallized from MeOH/H₂O to give 61: yield 0.9 g (61%); mp 195–196 °C. Anal. (C₁₀H₁₀N₂O₄S₃) C, H, N.

4-(Phenylsulfonyl)benzenesulfonamide (68). Diphenyl sulfide (18.6 g, 0.1 mol) was dissolved in CHCl₃ (50 mL) and

chlorosulfonic acid (11.7 g) was added dropwise with cooling to the solution over a period of 15 min. The solution turned deep violet and a vigorous evolution of HCl occurred. After the solution was stirred at room temperature for 30 min, the CHCl₃ was evaporated to give an oil. PCl₅ (21 g) was added in portions to this oil, and the mixture was warmed on the steam bath for 10 min. The resultant clear solution was then evaporated, the residual oil was dissolved in CHCl₃, and the solution was washed with H₂O and dried (MgSO₄). The residue obtained by filtration and evaporation of the filtrate was added in portions to a stirred solution of concentrated ammonia (150 mL), and the resultant solid was filtered off and dissolved in ether. The ether solution was dried (MgSO₄) and filtered, and the filtrate was evaporated. The residue was crystallized from EtOH/H₂O to give 4-(phenylthio)benzenesulfonamide: yield 12.0 g (45%); mp 138–140 °C (lit.¹⁸ mp 129–130 °C). 4-(Phenylthio)benzenesulfonamide (5.0 g, 0.02 mol) was added to 30% H₂O₂ (10 mL) in AcOH (50 mL), and the solution was heated on a steam bath for 1 h. The solution was then cooled in ice, and the resultant solid was filtered off and crystallized from EtOH/H₂O to give 68: yield 2.2 g (40%); mp 190–192 °C (lit.¹¹ mp 182 °C).

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Novel Synthesis of (S)-1-[5-(Benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline and Analogues: Potent Angiotensin Converting Enzyme Inhibitors

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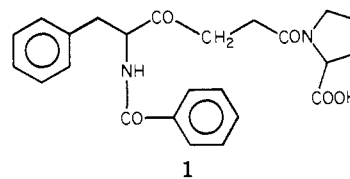
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A new approach was developed for the synthesis of (S)-1-[5-(benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline (1) and 23 analogues. The δ-(acylamino)-γ-keto acid intermediates were obtained by a modified Dakin-West reaction using 3-carbomethoxypropionyl chloride. Acylation of L-proline and recrystallization of the mixture of diastereomers gave the optically pure title compound in three reaction steps. The in vitro angiotensin converting enzyme (ACE) inhibitory activity of 1 was confirmed. Some of the novel analogues (6, 11, 13, and 17) were also found to be potent inhibitors of ACE in vitro with an IC₅₀ of 1.4–8.8 × 10⁻⁹ M (IC₅₀ for captopril = 0.9 × 10⁻⁸ M). In vivo these compounds (6, 11, 17, and 18) were much less active than captopril, especially by the oral route. Against angiotensin I (AI) challenge in normotensive conscious rats, 1 and 6 produced <50% inhibition at 30 mg/kg po but 57 to 82% inhibition at 3 mg/kg iv. Inhibition by both routes lasted <1 h. In renal hypertensive rats, 1 and 15 of its analogues failed to produce significant blood pressure lowering effects, in contrast to the marked effects of captopril. Near maximum inhibition of AI was achieved by continuous intravenous infusions of 1 and 20, suggesting that limited oral activity may be due to degradation and/or clearance.

Angiotensin converting enzyme (ACE) inhibitors hold great promise in the treatment of hypertension.¹ Recently a very potent ACE inhibitor (1), an analogue of the tripeptide Bz-Phe-Gly-Pro, was disclosed² in which the NH

of the amide portion of Phe-Gly was replaced by a methylene group.

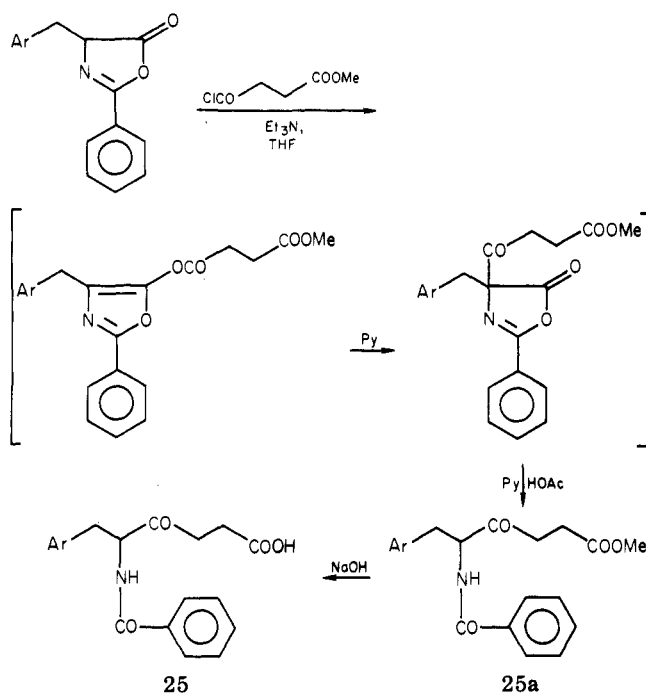


1

In the original synthesis² the key intermediate, (S)-δ-(benzoylamino)-γ-oxobenzenehexanoic acid, was obtained via a lengthy procedure in 11% yield. In order to explore the structure-activity relationship of this series, a more

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Scheme I



practical synthesis was required. Thus, a modification of the Dakin-West reaction³ was utilized as shown in Scheme I.

Chemistry. The required oxazolones are readily obtained from the acylalanines by treatment with Ac_2O ⁴ or with dicyclohexylcarbodiimide (DCC)⁵ or by catalytic hydrogenation of the corresponding unsaturated oxazolones⁶ obtained from the reaction of aldehydes and hippuric acid. The Dakin-West reaction and the base hydrolysis to the racemic acylamino keto acids of type 25 usually proceeded in excellent yields. Acylation of L-proline with 25 gave a mixture of the *R*, *L* and *S*, *L* diastereomers of 1. Repeated recrystallization from EtOAc gave pure *S*, *L* diastereomer, identical in every respect with an authentic sample.² For the final acylation procedure, two methods were used. The acylation of L-proline benzyl ester in the presence of hydroxybenzotriazol (HBT) and DCC, followed by catalytic debenzoylation, as described,² gave after repeated recrystallization from EtOAc a 24.6% yield of optically pure 1. Alternatively, the imidazolide method was somewhat more convenient^{7,8} but gave a somewhat lower yield (20%) of optically pure 1.

It was assumed that crystallization of the mixture of diastereomers of the analogues gave the desired *S*, *L* diastereomer as in the case of 1. When the final product was amorphous or an oil, the diastereomeric mixture was tested.

The methyl ester 2 and amide 3 of 1 were prepared from optically pure 1. The oxime 4 was obtained from 1 and

hydroxylamine. Compound 6 was obtained from 9 by catalytic debenzoylation in the presence of Pd/C in MeOH. The 3-pyridinyl compound 13, in contrast to all the others, was quite water soluble. The key intermediate 28 was obtained from crude 2-phenyl-4-(3-pyridinylmethyl)-5-(4*H*)-oxazolone, which was prepared from 2-phenyl-4-(3-pyridinylmethylene)-5(4*H*)-oxazolone⁹ by hydrogenation in THF with 20% Pd/C as catalyst.

Acid hydrolysis of 25 gave the α -amino ketone hydrochloride 34. N-Acylation was accomplished readily to give 30, but acylation with benzyl chloroformate apparently gave a stable mixed anhydride. This acylation worked well, however, when the methyl ester of 34 was used. Mild hydrolysis of 29a gave 29, which was converted to 15 using DCC and HBT as described for the preparation of 1.² Catalytic debenzoylation of 15 gave 14.

The tetrahydro-2-furanylcarbonyl compound 18 was obtained from the 2-furanylcarbonyl compound 17 by hydrogenation in MeOH with Pd/C as catalyst.

The final products are tabulated in Table I and the intermediates are shown in Table II.

Biological Results and Discussion

In Vitro Results. It has been previously shown² that 1 fits the hypothetical active site of angiotensin converting enzyme proposed by Ondetti et al.,¹ and it is not surprising that the structural requirements for inhibitory activity in this portion of the molecule agree with previous findings. Thus, a free carboxyl group on the terminal amino acid is required for maximum activity (1, 7, 13, 20, and 23). Since an unpurified enzyme preparation was utilized in this assay, it appears likely that the activity shown by the corresponding esters (2, 8, 9, 12, and 19) and amide 3 can be attributed to partial hydrolysis. The pyrrolidino compound 24 was void of activity.

Conversion of the ketone (1) to an oxime (4) greatly lowers the activity.

The importance of additional binding sites on the converting enzyme located at some distance beyond the projected site of the zinc ion is demonstrated by this series of compounds. Essential requirements for optimal activity in this series appear to be an *aromatic* 5-(acylamino) moiety (1 vs. 14 or 16) and an aromatic group in the 6 position, as shown by the lack of activity of 10. Substitution on this aryl group seems to have little effect on activity (1 vs. 6, 11), and even the pyridyl compound 13 shows comparable activity.

In Vivo Results. The in vivo ACE inhibitory activity of 1 and its analogues was evaluated in conscious normotensive rats ($N = 2$ for each compound). In this test, the compounds were given orally and then challenged with angiotensin I (AI) until the responses to AI returned to the predose values. The test compounds were then administered intravenously in the same rats and the AI challenges were repeated. Compound 1 at 30 mg/kg po produced only a 48% inhibition of AI, and the inhibition lasted only 49 min (time required for the AI response to return to 70% of the pretreatment response). Compound 1 at 3 mg/kg administered intravenously as a single bolus injection produced only a 57% inhibition of AI, whereas captopril at 0.3 mg/kg produced 83% inhibition. Compounds 6, 11, 13, 17, and 18 were all less effective orally than the parent compound, but when given intravenously, they produced significant (64–84%) inhibition of AI (Table III).

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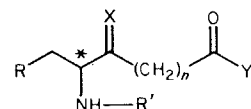
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Table I. Chemical and Pharmacological Data of Title Compound and Analogues



compd	R	R'	X	n	Y ^a	mp, °C	recrystn solv	formula	anal.	optical config at C*	[α] ²³ _D (c, solv), ^b deg	IC ₅₀ ^c
1	Ph	PhCO	O	2	A	155-157 ^d	EtOAc	C ₂₄ H ₂₆ N ₂ O ₅	C, H, N	S	-81.2 ^e	3.2 × 10 ⁻⁹
2	Ph	PhCO	O	2	B	~40		C ₂₅ H ₂₈ N ₂ O ₅	C, H, N	S	-35.8	8.2 × 10 ⁻⁸
3	Ph	PhCO	O	2	C	204-205	<i>i</i> -PrOH	C ₂₄ H ₂₇ N ₃ O ₄	C, H, N	S	-123	1.5 × 10 ⁻⁷
4	Ph	PhCO	NOH	2	A	~100	EtOAc/ <i>i</i> -Pr ₂ O	C ₂₄ H ₂₇ N ₃ O ₅	C, H, N	S	-57.1	1.2 × 10 ⁻⁶
5	Ph	PhCO	O	2	D	83-101		C ₂₄ H ₂₆ N ₂ O ₆ ^f	C, H, N	SR	-0.98 (1, MeOH)	5.4 × 10 ⁻⁷
6	4-HO-Ph	PhCO	O	2	A	113-116		C ₂₄ H ₂₆ N ₂ O ₆	C, H, N	SR	-131.0	4.7 × 10 ⁻⁹
7	4-Bzl-O-Ph	PhCO	O	2	A	70-78		C ₃₁ H ₃₂ N ₂ O ₆ ^h	C, H, N	SR	-56.6	1 × 10 ⁻⁸
8	4-Bzl-O-Ph	PhCO	O	2	E	47-50		C ₃₅ H ₄₀ N ₂ O ₆	C, H, N	SR	-32.6	8.6 × 10 ⁻⁶
9	4-Bzl-O-Ph	PhCO	O	2	F	52-55		C ₃₈ H ₃₈ N ₂ O ₆	C, H, N	SR	-36.2 (0.45, CHCl ₃)	8.6 × 10 ⁻⁸
10	H	PhCO	O	2	A	60-63		C ₁₈ H ₂₀ N ₂ O ₅ ^g	C, H, N	SR	-38.6 (1, MeOH)	3 × 10 ⁻⁷
11	3,4-(MeO) ₂ -Ph	PhCO	O	2	A	152-153	EtOH	C ₂₆ H ₃₀ N ₂ O ₇	C, H, N	S	-65.4	1.4 × 10 ⁻⁹
12	3-pyridyl	PhCO	O	2	F	35-45		C ₃₀ H ₃₁ N ₃ O ₅	C, H, N	SR	-37.4	1.5 × 10 ⁻⁸
13	3-pyridyl	PhCO	O	2	A	80-100		C ₂₃ H ₂₅ N ₃ O ₅ ^g	C, H, N	SR	-58.4	5.7 × 10 ⁻⁹
14	Ph	H, HCl	O	2	A	100-120		C ₁₇ H ₂₃ N ₂ O ₄ Cl ^h	C, H, N	SR	-52.0 (1.1, H ₂ O)	2.5 × 10 ⁻⁵
15	Ph	BzlOCO	O	2	A	67-69		C ₄₃ H ₆₃ N ₃ O ₆ ⁱ	H, N; C ⁱ	SR	-4.08	2.4 × 10 ⁻⁷
16	Ph	CH ₃ CO	O	2	A	156-157	EtOAc	C ₁₅ H ₂₄ N ₂ O ₅	C, H, N	S	-85.6	3.3 × 10 ⁻⁷
17	Ph		O	2	A	159-161	EtOAc	C ₂₂ H ₂₄ N ₂ O ₆	C, H, N	S	-85.3	1.4 × 10 ⁻⁹
18	Ph		O	2	A	~40		C ₂₂ H ₂₈ N ₂ O ₆ ^g	C, H, N	S	-77.5	2 × 10 ⁻⁸
19	Ph	2-MePhCO	O	2	F	38-41		C ₃₂ H ₃₄ N ₂ O ₅ ^k	C, H, N	SR	-48.0	1.6 × 10 ⁻⁶
20	Ph	2-MePhCO	O	2	A	150-158		C ₂₅ H ₂₈ N ₂ O ₅	C, H, N	SR	-75.0	4.6 × 10 ⁻⁸
21	Ph	PhCO	O	3	A	40-50		C ₂₅ H ₂₈ N ₂ O ₅ ^k	C, H, N	SR	-29.3	4.2 × 10 ⁻⁸
22	Ph	PhCO	O	2	G	175-178	<i>i</i> -PrOH	C ₃₅ H ₃₄ N ₂ O ₅	C, H, N	S	+21.1	1.8 × 10 ⁻⁷
23	Ph	PhCO	O	2	H	65-70		C ₂₈ H ₂₈ N ₂ O ₅ ^k	C, H, N	S	+47.2	2.8 × 10 ⁻⁸
24	Ph	PhCO	O	2	I	162-163	<i>i</i> -PrOH	C ₂₃ H ₂₆ N ₂ O ₃	C, H, N	SR	0	inactive
captopril (Squibb)												0.9 × 10 ⁻⁸

^a A = L-proline; B = L-proline methyl ester; C = L-prolinamide; D = 4-hydroxy-L-proline; E = L-proline *tert*-butyl ester; F = L-proline benzyl ester; G = L-phenylalanine benzyl ester; H = L-phenylalanine; I = pyrrolidine. ^b c, 1, CHCl₃, unless stated otherwise. ^c Molar concentration required for 50% inhibition. ^d Literature² mp 156-160 °C. ^e Literature² rotation [α]²¹_D -83.2° (c 0.98, CHCl₃). ^f 0.125 CHCl₃. ^g 0.5H₂O. ^h H₂O. ⁱ 1.5-dicyclohexylamine. C: calcd, 71.24; found, 71.73. ^k 0.25H₂O.

Table IV. Summary of the 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	base line	mean aortic blood pressure, ^b mmHg	
				max effect	change
1	30	4	190 ± 6	178 ± 6	-12 (at 8 h)
2	30	4	153 ± 3	142 ± 2 ^c	-11 (at 10 h)
2	3	4	199 ± 10	165 ± 3 ^c	-34 (at 2 h)
5	30	3	188 ± 10	168 ± 10	-20 (at 4 h)
6	30	3	195 ± 9	172 ± 12	-23 (at 5 h)
7	30	3	200 ± 11	195 ± 11	-5 (at 8 h)
11	30	4	179 ± 7	174 ± 6	-5 (at 2 h)
12	30	3	191 ± 1	159 ± 10 ^c	-32 (at 8 h)
13	30	3	204 ± 8	184 ± 3	-20 (at 6 h)
15	30	3	197 ± 7	175 ± 4	-22 (at 10 h)
16	30	3	202 ± 8	176 ± 10	-26 (at 8 h)
17	30	3	185 ± 13	184 ± 13	-1 (at 1 h)
18	30	4	188 ± 7	163 ± 5 ^c	-23 (at 8 h)
19	10	3	195 ± 9	175 ± 12	-20 (at 6 h)
20	30	3	203 ± 5	188 ± 2 ^c	-15 (at 5 h)
22	30	3	187 ± 5	182 ± 8	-5 (at 6 h)
captopril	0.3	4	189 ± 3	170 ± 7 ^c	-19 (at 10 h)
	3.0	4	192 ± 7	99 ± 7 ^c	-93 (at 6 h)
	30	4	186 ± 4	85 ± 1 ^c	-101 (at 6 h)

^a Vehicle employed: 4% gum acacia in 2 mL of distilled H₂O/kg 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, 4-6 df).

po produced only a small reduction in mean aortic blood pressure (Table IV). Compounds 2, 12, 18 and 20 produced somewhat greater responses, but the reductions in pressures were limited, although statistically significant ($p < 0.05$). By comparison, captopril produced a marked, dose-related decrease in blood pressure at doses less than 3.0 mg/kg. These results show that the oral efficacy of compound 1 previously reported² is limited both in magnitude and duration.

Compound 1 and its analogues, like captopril, exert their maximum AI antagonistic effects in the normotensive rat within 30 min following oral dosing, whereas in renal hypertensive rats the antihypertensive effects do not fully develop until 2 to 8 h posttreatment. Unlike captopril, however, these new compounds produced only limited effects, even at 30 mg/kg po. It is possible that this limited effect is not related to ACE inhibition. Compounds 1 and 16 were administered to spontaneously hypertensive rats at 100 mg/kg po. Compound 1 was without effect, but 16 produced a limited but significant ($p < 0.05$) blood pressure lowering (172 ± 3 mmHg base line to 151 ± 6 mmHg after 6 h). Since captopril has been shown to produce similar effects in the SHR, an additional mechanism of action for these compounds is not supported.

Experimental Section

Melting points were determined in a Thomas-Hoover capillary melting point apparatus or a Mel-Temp apparatus. Infrared (IR) data were recorded on a Beckman IR-9 or IR-7 prism grating instrument on a Digilab FTS-14 interferometer. Nuclear magnetic resonance measurements (NMR) were made on a Bruker WH-90 pulsed Fourier transform instrument. IR and NMR were compatible with the assigned structures. Homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on precoated TLC sheets (silica gel 60 F 254, Merck), using principally the solvent system HOAc-MeCN-toluene (1:9:10). The TLC of the compounds described in Table I were homogeneous single spots when visualized with UV and/or I₂ vapors. Micro-analytical results that are reported by symbols of the elements were within ±0.4% of theory unless otherwise indicated.

Methyl δ-(Benzoylamino)-γ-oxobenzenehexanoate (25a). Triethylamine (75 mL, 0.55 mol) was added dropwise with stirring and cooling in an ice bath to a solution of 2-phenyl-4-(phenylmethyl)-5(4*H*)-oxazolone⁴ (125 g, 0.5 mol) and 3-carbomethoxypropionyl chloride (75 g, 0.5 mol) in THF (1000 mL). After 2 h the ice bath was removed and the suspension was allowed to stand

overnight. The filtrate was evaporated at reduced pressure at <50 °C to give a quantitative yield of acylated oxazolone [TLC (*i*-Pr₂O) *R*_f 0.51]. The crude product was dissolved in pyridine (600 mL), warmed to 80 °C, and HOAc (450 mL) was added in one lot (CO₂ evolution). The solution was heated on a steam bath with stirring for 1 h and was evaporated at reduced pressure to give 184 g of crude ester 25a, which crystallized on standing. The resulting solid was slurried in *i*-Pr₂O (500 mL) and washed (2 × 50 mL of *i*-Pr₂O) to yield 132.5 g (78%) of 25a, mp 90-95 °C, sufficiently pure for the next step. An analytical sample from *i*-Pr₂O had mp 102-103 °C; TLC (*i*-Pr₂O) *R*_f 0.13. Anal. (C₂₀H₂₁NO₄) C, H, N.

δ-(Benzoylamino)-γ-oxobenzenehexanoic Acid (25). A solution of the crude 25a (132.5 g, 0.391 mol) in 1000 mL of THF and 900 mL of aqueous NaOH (0.5 N) was warmed to 40 °C and then allowed to stand for 20 h at ambient temperature. THF was evaporated at reduced pressure at <40 °C, and the solution was acidified to pH 4 with dilute HCl. The product was collected by filtration, washed with H₂O, and recrystallized from EtOH to give 96.4 g (76%) of analytically pure 25, mp 182-184 °C. Anal. (C₁₉H₁₉NO₄) C, H, N.

(S)-1-[5-(Benzoylamino)-1,4-dioxo-6-phenylhexyl]-L-proline (1). To a slurry of 25 (65 g, 0.2 mol) in 1000 mL of THF was added at 20 °C 1,1'-carbonyldiimidazole (40 g, 0.247 mol). The reaction mixture was warmed for 1 h at 40 °C, the solution was cooled in ice, and *N,O*-bis(trimethylsilyl)-L-proline⁸ (60 g, 0.23 mol) was added and refluxed for 5 h. After the solvent was evaporated, the residue was partitioned between CHCl₃ and H₂O and then acidified to pH 4 with dilute HCl. The organic layer was washed with H₂O and evaporated, and the product crystallized 3 times from EtOAc to give optically pure product: yield 16.9 g (20%); mp 155-157 °C. Anal. (C₂₄H₂₆N₂O₅) C, H, N.

(S)-1-[5-(Benzoylamino)-4-(hydroxyimino)-1-oxo-6-phenylhexyl]-L-proline (4). A solution of 1 (6.3 g, 0.015 mol) and H₂NOH-HCl (1.2 g, 0.0173 mol) in pyridine (50 mL) was allowed to stand at room temperature for 20 h, warmed for 0.5 h on a steam bath, and evaporated. The product was taken up in CHCl₃ and washed with H₂O. The crude product, 5.7 g, was crystallized from EtOAc/*i*-Pr₂O to give 3.9 g (60%) of white powder, mp ~100 °C. Anal. (C₂₄H₂₇N₃O₅) C, H, N.

δ-Amino-γ-oxobenzenehexanoic Acid Hydrochloride (34). A solution of 25 (32.5 g, 0.1 mol) in HOAc (500 mL), concentrated HCl (500 mL), and H₂O (250 mL) was refluxed for 6 h. After the solvent was evaporated, the hydrochloride was crystallized from MeCN to give 22.8 g (89%) of 34, mp 130-132 °C. Anal. (C₁₂H₁₃NO₃Cl) C, H, N.

δ-(Acetylamino)-γ-oxobenzenehexanoic Acid (32). To a solution of 34 (12.8 g, 0.05 mol) in H₂O (100 mL) and HOAc (500

mL) was added NaOAc (5 g, 0.06 mol) followed by the slow addition of Ac₂O (25 mL, 0.266 mol) at ice-bath temperature. After 2 h the reaction mixture was allowed to warm to room temperature and allowed to stand for 60 h. The residue after evaporation of solvent was taken up in H₂O and acidified to pH 4 with aqueous KHSO₄. The product solidified on scratching; it was washed with H₂O and recrystallized from EtOAc to give 8.5 g (65%) of **32**, mp 130–131 °C. Anal. (C₁₄H₁₇NO₄) C, H, N.

Methyl δ-[[Phenylmethoxy]carbonyl]amino]-γ-oxobenzenehexanoate (29a). A solution of **34** (1 g, 3.9 mmol) in MeOH (30 mL) was saturated with gaseous HCl, allowed to stand for 18 h, and evaporated. The residue was again dissolved in MeOH and treated with HCl. Evaporation gave an oil. The crude ester was dissolved in H₂O (30 mL) and benzyl chloroformate (1.5 g, 8.8 mmol) was added. The pH of the solution was brought to 6.5 with Na₂CO₃ solution. The reaction proceeded rapidly at this pH and was maintained there by adding Na₂CO₃ solution. At the end of 1 h the precipitated gum was taken up in Et₂O and dried (MgSO₄), and the Et₂O was evaporated. The residue was recrystallized from hexane/petroleum ether to give white needles of **29a**: yield 1 g (71%); mp 64–65 °C. Anal. (C₂₁H₂₃NO₅) C, H, N.

δ-[[Phenylmethoxy]carbonyl]amino]-γ-oxobenzenehexanoic Acid (29). A solution of **29a** (0.5 g, 1.25 mmol) in MeOH (10 mL) and 2 N NaOH (1 mL, 2 mmol) was allowed to stand at 25 °C for 10 min and was then diluted with H₂O (10 mL) and acidified with 2 N HCl (1 mL). MeOH was evaporated at reduced pressure; the product was collected, washed with H₂O, and recrystallized from EtOAc to give 0.45 g (94%) of **29**, mp 116–117 °C. Anal. (C₂₀H₂₁NO₅) C, H, N.

Biological Methods. The *in vitro* ACE inhibitory activity was determined by a radioassay procedure reported previously.¹¹ Activity is reported as the IC₅₀, which is the approximate 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 Me₂SO and diluting to the desired concentration with a pH 8 buffer of Hepes (Calbiochem), 0.1 mol NaCl, and 0.6 mol of Na₂SO₄ in H₂O.

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 with 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 access 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 an 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 40 units of heparin per 24-h period) to prevent clot formation in 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.¹²

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