2-[4-(2-Cyano-3-propargyl-1-guanidino)butyl]-6-phenyl-3(2*H*)-pyridazinone (79). To a solution of 4.9 g (0.02 mol) of 2-(4-aminobutyl)-6-phenyl-3(2*H*)-pyridazinone (**5**) in 50 mL of acetonitrile was added 3.1 g (0.02 mol) of 1-cyano-2-methyl-3-propargylisothiourea. The mixture was refluxed at 80 °C for 24 h. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in 100 mL of $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ solution and water and dried over Na_2SO_4. The dried solution was evaporated to give crude solid, which was recrystallized from acetonitrile to give 4.0 g (58%): IR (Nujol) 3280 (NH), 2150 (C≡N), 1650 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.4-2.0 (m, 4 H, $CH_2C_2H_4CH_2$), 3.0-3.3 (m, 3 H, CH_2NH and C≡C*H*), 3.92 (s, 2 H, $NHCH_2C{\equiv}CH$), 4.15 (t, 2 H, J = 7 Hz, CH_2NH), 6.98 (d, 1 H, J = 10 Hz, C_4 H), 7.1 (br, 1 H, NH), 7.3 (br, 1 H, NH), 7.5 (m, 3 H, Ar H), 7.8 (m, 2 H, Ar H), 7.94 (d, 1 H, J = 10 Hz, C_5 H).

Pharmacology. Gastric Antisecretory Activity. Gastric antisecretory activity was evaluated by the technique of Shay.[7] Male Wistar rats, weighing 150–200 g, were fasted for 24 h prior to the test in cages with wire-mesh floor to prevent coprophagy, but they were allowed water ad libitum. After fasting, the rats were divided into groups of six animals each. One group served as the control. A small midline incision was made, and the pylorus was ligated under ether anesthesia. The test compounds, dissolved or suspended in 1% carboxymethylcellulose solution, or the vehicle was administered intraduodenally to each group. Four hours after closing the abdomen, the stomach was extirpated under ether anesthesia, and the volume of accumulated gastric juice therein was measured. The gastric juice was titrated against 0.1 N NaOH to determine the concentration of free acid (at pH 3.0), and hourly outputs of free acid were calculated for each rat. In the first experiment, the test compounds were administered at a dose level of 100 mg/kg, and the results were represented as percent inhibition against control. In the next step, the selected test compounds from the first experiment were administered at several dose levels, and the ED_{50} values were calculated.[17]

Antiulcer Activity Induced by Stress. Ten male Wistar rats, weighing 200–220 g, per group were used. After oral administration of test compound, animals were immobilized in the stress cage and immersed in a water bath according to the method described by Takagi et al.[8] Seven hours later, the stomach was extirpated, and the length of lesions in the glandular portion was measured. The ulcer index (mm) was obtained by the summation of the length of the lesions. The ED_{50} values for antiulcer activity was calculated by the method of Litchfield and Wilcoxon.[17]

Anticholinergic Activity. Anticholinergic activity was determined by the guinea pig isolated ileum preparation suspended in Tyrode's solution aerated with 95% O_2/5% CO_2 at 30 °C. Cumulative dose–response curves for acetylcholine-induced contraction were determined in the absence or in the presence of test compounds (1×10^{-7} to 1×10^{-5} M) or atropine (3×10^{-7} to 1×10^{-4} M).

Histamine H_2-Receptor Antagonistic Activity. The histamine H_2-receptor antagonistic activity was determined by the guinea pig isolated right atrium preparation suspended in Krebs solution aerated with 95% O_2/5% CO_2 at 32 °C. Cumulative dose–response curves for histamine-induced positive chronotropic action were determined in the absence or in the presence of test compounds (1×10^{-7} to 1×10^{-5} M) or cimetidine (3×10^{-6} to 3×10^{-5} M).

(17) Lichfield, J. T.; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.

Antihypertensive Agents: Angiotensin Converting Enzyme Inhibitors. 1-[3-(Acylthio)-3-aroylpropionyl]-L-prolines

Francis J. McEvoy, Fong M. Lai, and J. Donald Albright*

American Cyanamid Company, Medical Research Division, Lederle Laboratories, Pearl River, New York 10965.
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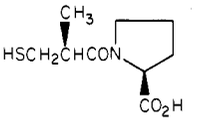
A series of 1-[3-(acylthio)-3-aroylpropionyl]-L-proline derivatives was synthesized. A number of these compounds are potent angiotensin converting enzyme (ACE) inhibitors that lowered blood pressure in aorta-coarcted renal hypertensive rats. The most active derivatives are 1-[3(*R*)-(acetylthio)-3-substituted-benzoyl)-2(*S*)-methylpropionyl]-L-prolines with an in vivo activity equivalent to SQ 14,225 (captopril). Structure–activity relationships are discussed. Changes in the configuration of the α-methyl group and the *S*-acetyl group affect the ACE activity. Coupling of 3-(substituted-benzoyl)-2-methylpropionic acids to L-proline via enol lactones is described.

Since the discovery of the renin–angiotensin system, there have been continued efforts to determine the role of the renin–angiotensin system in the regulation of blood pressure. One of the principal physiological functions of angiotensin converting enzyme (ACE) is to catalyze the removal of the terminal dipeptide from the decapeptide angiotensin I to give the octapeptide angiotensin II.[1-3] Angiotensin II is a potent peptide that causes vasoconstriction of blood vessels and is involved in regulation of blood pressure. ACE is also involved in the inactivation of bradykinin, a nonapeptide present in blood plasma, by successively removing two dipeptides. Bradykinin is a potent vasodilator and might be involved in the control of blood pressure.

Early work centered on competitive peptide antagonists of angiotensin II and peptide inhibitors of angiotensin converting enzyme. The peptide ACE inhibitor isolated from venom of *Bothrope jararaca* (SQ 20,881, teprotide)[4] was shown to lower blood pressure in humans and demonstrated that ACE inhibitors could effectively lower blood pressure.[5] In 1977 Ondetti reported[6] on a new class of ACE inhibitors (**1** and **2**) that contain only one amino acid and are orally active antihypertensive agents. One of these derivatives, **1** (SQ 14,225, captopril), is an effective antihypertensive drug.[7]



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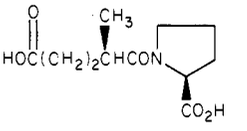

2

(1) Skeggs, L. T.; Marsh, W. H.; Kahn, J. R.; Shumway, N. P. *J. Exp. Med.* **1954**, *99*, 275–282.
(2) Skeggs, L. T.; Kahn, J. R.; Shumway, N. P. *J. Exp. Med.* **1956**, *103*, 295–300.
(3) Skeggs, L. T.; Kahn, J. R.; Shumway, N. P. *J. Exp. Med.* **1956**, *103*, 301–307.
(4) Ondetti, M. A. *Biochemistry*, **1971**, *19*, 4033–4039.
(5) Ondetti, M. A.; Cushman, D. W. *Annu. Rep. Med. Chem.* **1978**, *13*, 82.
(6) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science* **1977**, *196*, 441–443.
(7) Bravo, E. L.; Tarazi, R. C. *Hypertension* **1979**, *1*, 39–46.

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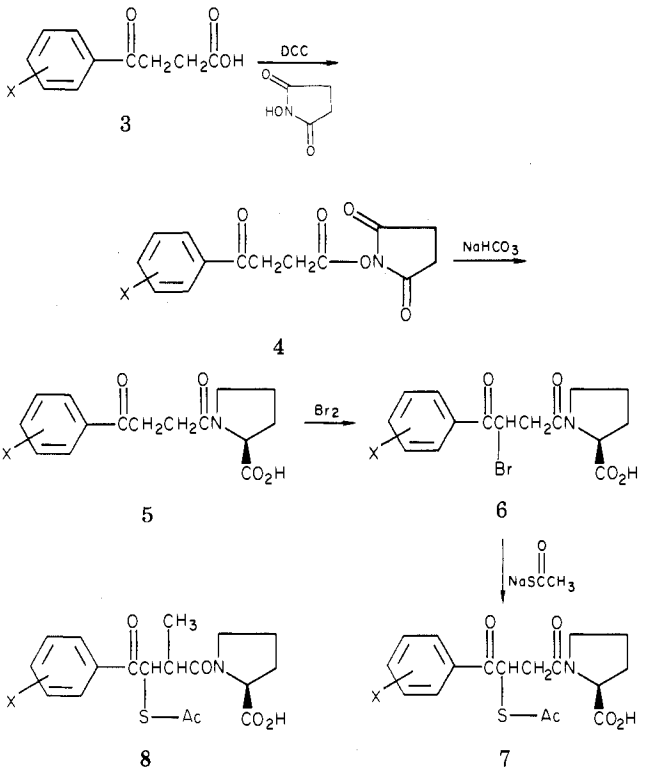
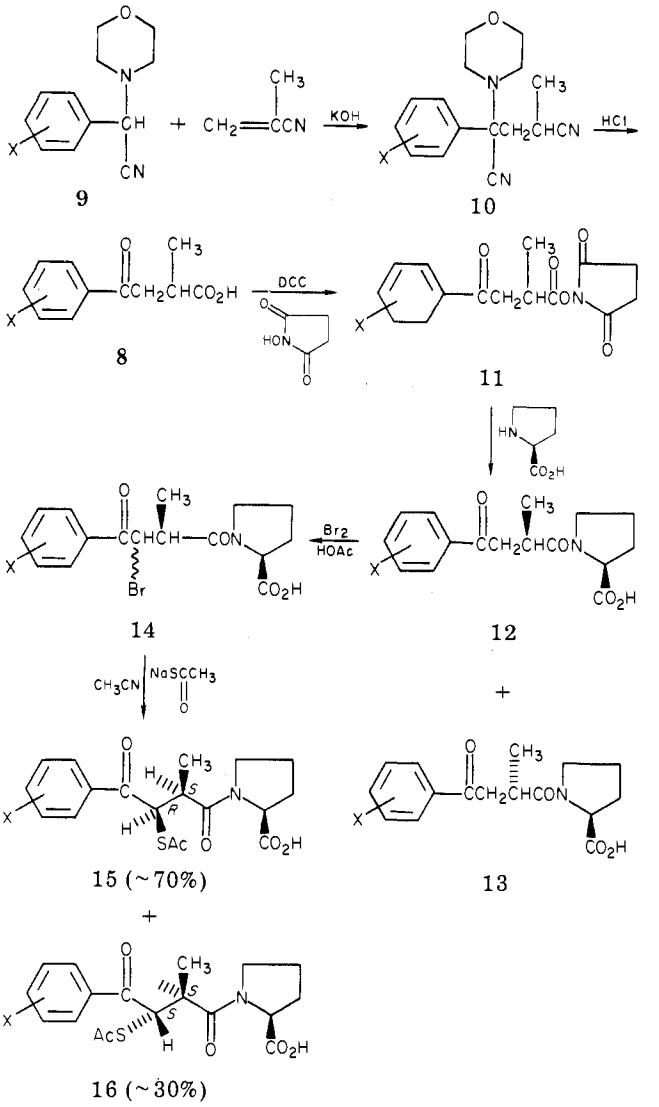
Scheme I



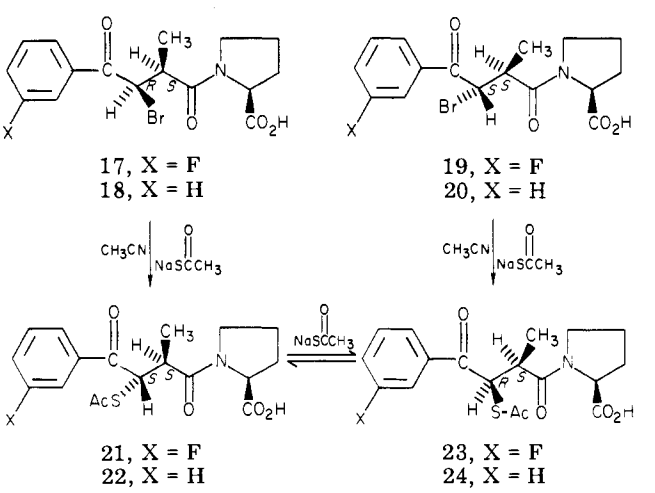
Scheme II



Chemistry and Biology. A program to synthesize ACE inhibitors was initiated, and our efforts centered on methods for coupling 3-benzoylpropionic acids, **3**, to L-proline (Scheme I). A number of peptide-coupling methods were studied, such as dicyclohexylcarbodiimide (DCC) and *N,N'*-carbonyldimidazole with the methyl or *tert*-butyl ester of L-proline. However, to avoid the necessity of deblocking the carboxylic function in a later step, coupling of *N*-hydroxysuccinimide esters, **4**, with L-proline was adopted as the preferred method[8] of preparing intermediates **5**. Bromination of **5**, followed by reaction of **6** (mixture of diastereomers) with sodium or potassium thioacetate, gave products **7**. The derivatives **7** showed potent inhibition of ACE activity in vitro but exhibited only moderate in vivo activity at 10 mg/kg in aorta-coarcted hypertensive rats. The products **7** are mixtures of diastereomers, and conventional chromatography failed to separate the diastereomers. Our attention was directed to the products **8** with an α-methyl group, since the α-methyl derivatives **1** and **2** showed enhanced activity over the demethyl analogues.[6]

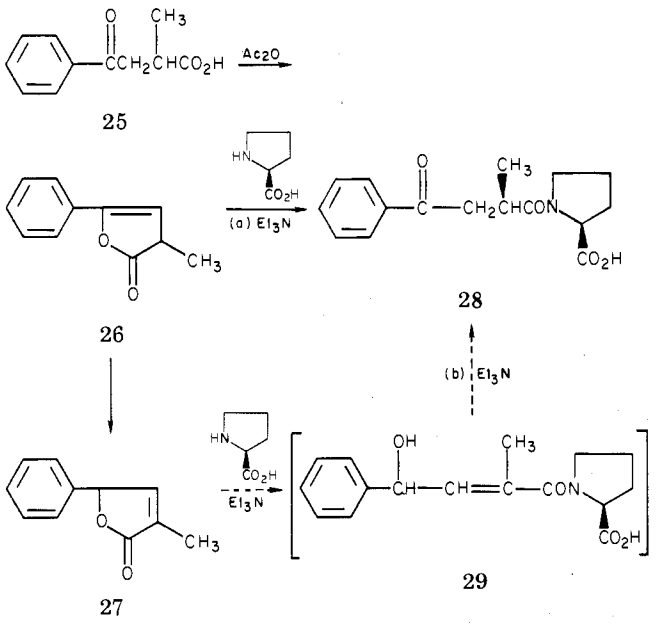
The intermediate 3-benzoyl-2-methylpropionic acids needed for the synthesis of derivatives **8** were prepared by 1,4 addition of α-phenyl-4-morpholineacetonitriles **9** to α-methylacrylonitrile, followed by hydrolysis of **10** (Scheme II). Development of this method for the preparation of diverse 3-aroylpropionic acids and 3-aroylpropionitriles has been reported.[9,10] Preparation of the O-Su activated ester of acids **8** and coupling with L-proline gave a mixture of diastereomers **12** and **13**. The desired isomer **12** in all cases studied could be crystallized from the reaction mixture and was brominated to give **14** (mixture of diastereomers). The mixture **14** was reacted with sodium or potassium thioacetate to give the products **15** (ca. 70%) and **16** (ca. 30%). The diastereomers of **15** and **16** where separated by chromatography, and in all cases studied, **15** crystallized while diastereomer **16** remained as a glass. The stereochemical assignments were established for **23** and **24** by

Scheme III

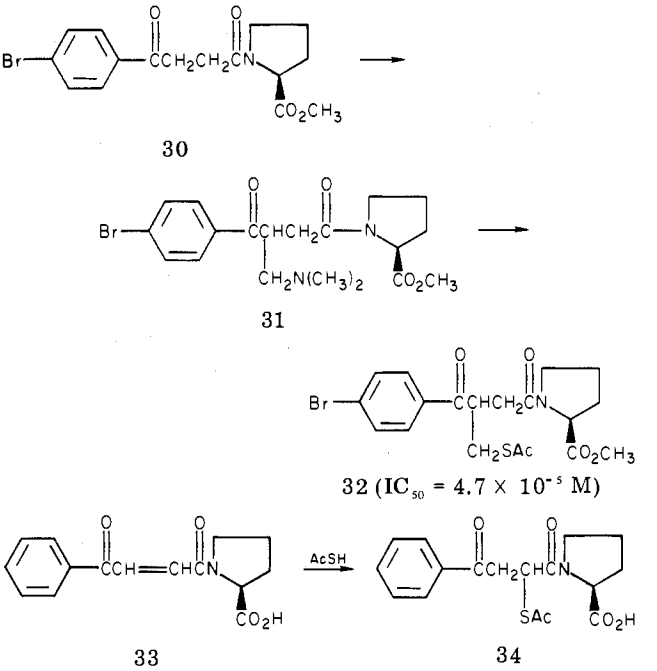


(8) Zimmerman, J. E.; Anderson, G. W. *J. Am. Chem. Soc.* **1967**, *89*, 7151.
(9) Albright, J. D.; McEvoy, F. J.; Moran, D. B. *J. Heterocycl. Chem.* **1978**, *15*, 881-892.
(10) McEvoy, F. J.; Albright, J. D. *J. Org. Chem.* **1979**, *44*, 4597-4603.
(11) Literature precedent exists for S_N2 displacement of *S*-Ac groups: Owen, L. M.; Rahman, M. B. *J. Chem. Soc. C* **1971**, 2432.

Scheme IV



Scheme V



X-ray analysis. In one case, diastereomers **17** and **19** were separated by chromatography (HPLC). ^1H NMR analysis indicated that the two bromo diastereomers, **17** and **19**, were formed in a ratio of approximately 3:1 (Br_2 in acetic acid). Reaction of each pure diastereomer with sodium thioacetate overnight gave a mixture of **21** (30%) and **23** (70%) (Scheme III). Rapid workup (after ca. 3 min) of the reaction of **17** with sodium thioacetate and analysis of the products by TLC showed that the reaction occurs by S_N2 displacement of bromine, since diastereomer **21** was the major product (ca. 70%). Even under such short reaction times, **21** was being equilibrated to a mixture of **21** and **23**. Bromination of **12** (X = H) in acetic acid gave a mixture of **18** and **20** (ca. 7:3) while bromination in dichloromethane gave almost exclusively diastereomer **18** (95+%), which crystallized from the reaction mixture. Reaction of pure diastereomer **18** with potassium thioacetate gave a mixture of **22** and **24**. The ratio of **22** and **24** formed depended on the solvent as well as the concentration of bromo derivative **18**. Debromination of the mixture of diastereomers (**18** and **20**) was observed in the displacement reactions, and up to 10% of the amide **28** was occasionally isolated. Debromination was the major reaction (58%) when a mixture of **18** and **20** (7:3) was stirred with potassium thioacetate and thioacetic acid in dichloromethane–acetic acid (11:2).

For scale up and ease of preparation, a method for coupling 3-benzoyl-2-methylpropionic acid (**25**) to L-proline without the use of peptide coupling reagents was developed. Cyclization of **25** with acetic anhydride has been reported[12] to give enol lactone **26** (on heating at 100 °C) or α,β-unsaturated lactone **27** (on heating at 140 °C) (Scheme IV).

Following the literature procedure, cyclization with acetic anhydride gave mixtures of **26** and **27**. Under identical conditions (temperature, time, and quantity of reagents, isolation), one batch of acid **25** gave exclusively enol lactone five times out of six runs. A recrystallized batch of acid **25** consistantly gave mixtures of **26** and **27**. Addition of HCl gas to the acetic anhydride cyclization of **25** changed the proportion of **26** (70%) and **27** (30%) formed in one run but gave exclusively **27** in another run. At present, the conditions for exclusive formation of **26** depends on some undetermined factors not understood. Enol lactone **26** is isomerized to **27** with triethylamine in acetonitrile.

Reaction of either pure enol lactone **26**, pure α,β-unsaturated lactone **27**, or mixtures of **26** and **27** with L-proline in acetonitrile gave **28** in the same isolated yields (ca. 25%). Condensation of **26** or a mixture of **26** and **27** with L-proline in 2-propanol gave better yields (40%) of **28**. Whether the reaction goes by establishing an equilibrium between **26** and **27**, followed by reaction of L-proline with enol lactone **26**, or whether **29** is formed and isomerized to **28** has not been established. It should be noted that the reaction involves crystallization of one diastereomer, **28**, from the mixture of two diastereomers, and the theoretical yield of **28** is only 50%.

Analogues with the acetylthio group in the α or γ position were synthesized. Michael addition of thioacetic acid to the 3-benzoylacryloyl derivative **33** gave the α-acetylthio analogue **34**. The Mannich base **31** was prepared from **30**. Quaternization of **31** with methyl iodide, followed by reaction with sodium thioacetate, afforded the analogue **32** (Scheme V).

Deacylation of the 3-acetylthio group was studied briefly. The free SH derivative **37** was obtained by reaction of **35** with *N*-methylpyridine-2-thione[13] to give intermediate **36**, which was cleaved with sodium hydroxide (Scheme VI).

Attempts to prepare **37** by reaction of bromo derivative **35** with sodium hydrogen sulfide or to deacylate **39** with hydroxylamine or ammonium hydroxide gave mixtures of products.

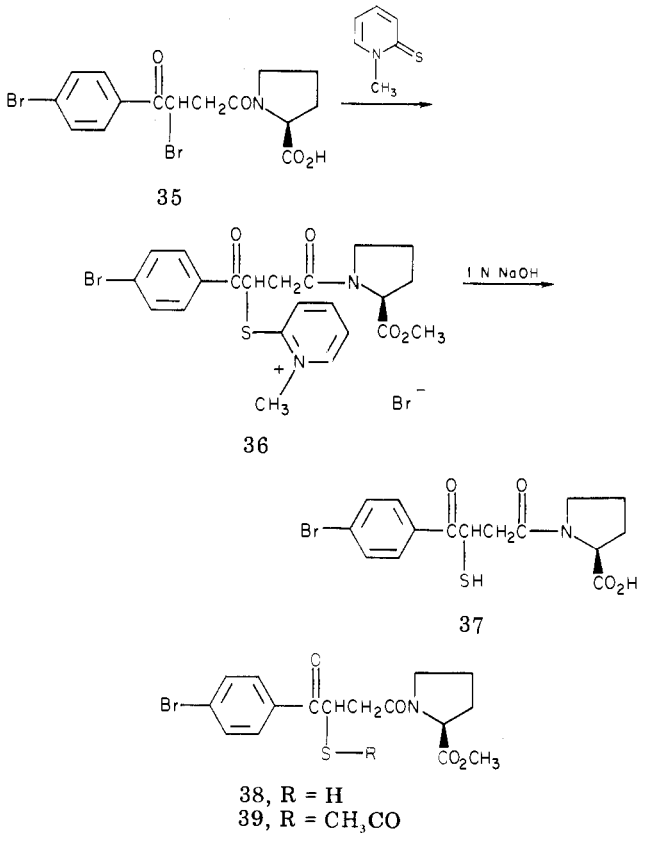
Because of the difficulty in preparing derivatives with a free SH group, no further efforts were made to prepare such analogues. The free SH derivatives may be the active species (formed in situ) in the in vitro ACE inhibitor tests.

In order to prepare a water-soluble salt, compound **24** was stirred with 1 equiv of aqueous sodium bicarbonate. The *S*-acetyl group was not cleaved, but epimerization of the *S*-acetyl group occurred to give approximately 20%

(12) Leclerc, G.; Wermuth, C. G.; Schreiber, J. *Bull. Soc. Chim. Fr.* **1967**, 1302–1307.

(13) Yamada, M.; Sotoya, K.; Sakakibora, T.; Takamoto, T.; Sudoh, R. *J. Org. Chem.* **1977**, *42*, 218.

Scheme VI



35

36

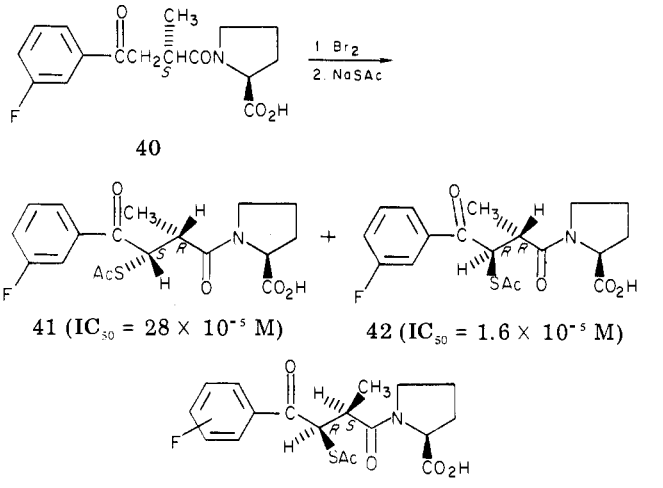
37

38, R = H
39, R = CH_3CO

of diastereomer **22** (as the Na salt). Addition of 1 equiv of aqueous sodium bicarbonate to a solution of **24** in ethanol also gave a mixture of **24** and **22** as the Na salts (ca. 7:3). Addition of imidazole or anhydrous ammonia to **24** in dichloromethane followed by removal of the solvent gave the imidazole and ammonium salts without epimerization of the *S*-acetyl function.

Structure–Activity Relationships. In Table II are listed the 1-(3-benzoylpropionyl)-L-proline intermediates, and in Table I activity data (in vitro ACE inhibition) are given for the various analogues synthesized. In one series, the diastereomer **40** was separated as the dicyclohexylamine salt and purified. In order to establish the effect that the configuration of the methyl has on ACE activity, compound **40** was converted to products **41** and **42**, which were separated by chromatography (Scheme VII). As anticipated from prior studies,[6] these derivatives **41** and **42** (*R* configuration at α-CH_3) exhibited markedly less inhibition of angiotensin converting enzyme, as shown by IC_{50} values (Table I), than **21** and **23** (*S* configuration at α-CH_3). It is interesting to note that on comparison of the pair of derivatives (**41** vs. **42** and **21** vs. **23**), the compounds with the *S* configuration at the S-Ac carbon are less potent. The most potent compounds have the *S* configuration at the C-CH_3 carbon and the *R* configuration at the S-Ac carbon. Derivatives with the opposite configurations (*R*, *S*) at these two centers are the least potent analogues. Variations of substituents in the phenyl ring produced analogues with some significant differences in ACE inhibitory activity (in vitro); however, derivatives **23**, **24**, and **43** were essentially equipotent when tested (in vivo) in aorta-coarcted, hypertensive rats. (Figure 1). Changing the substituents on the phenyl ring or changing the aryl group in these 1-[3-(acetylthio)-3-aroylpropionyl]-L-proline derivatives altered the activity a maximum of 20-fold. In vitro, the demethyl compounds **44** and **50** are approximately equipotent with the analogues **43** and **23** containing

Scheme VII



40

41 (IC_{50} = 28 × 10^{-5} M)

42 (IC_{50} = 1.6 × 10^{-5} M)

23, X = *m*-F (IC_{50} = 0.62 × 10^{-7} M)
24, X = H (IC_{50} = 0.55 × 10^{-7} M)
43, X = *p*-Br (IC_{50} = 0.98 × 10^{-7} M)

the 2-methyl substituent. However, the 2-methyl derivatives **43** and **23** showed significantly greater potency in vivo than **44** and **50** (Table III). Derivatives without the 2-methyl group were invariably less potent in vivo. The 2-methyl substituent may inhibit metabolic cleavage of the amide bond in addition to providing a better fit to the active site of the enzyme.

Comparison of the *S*-acetyl derivatives **46**, **44**, and **48** with the corresponding *S*-benzoyl derivatives **45**, **47**, and **49** shows that these analogues were approximately equipotent (in vitro). All the *S*-benzoyl derivatives exhibited lower activity than the *S*-acetyl compounds when tested in vivo (aorta-coarcted, renal hypertensive rats).

Greater activity (in vitro and in vivo) was observed with compounds **22**, **23**, **42**, and **43**, in which the *S*-acetyl group has the *R* configuration, than with the corresponding compounds **21**, **24**, **41**, and **52**, in which the *S*-acetyl group has the *S* configuration.

NMR and X-ray. Diastereomers **28** and **76** with a 2-methyl group could be distinguished from each other by their ^1H NMR spectra on the basis of the chemical shift of the methyl group. Of special interest was the ^{13}C NMR spectrum of **28** and **76**, which showed that crystalline **28** is a 9:1 mixture of rotomers (around the prolyl–amide bond), while **76** (gum) was a 3:2 mixture of rotomers. Above 150 °C (Me_2SO), the ^{13}C NMR spectrum of **28** and **76** showed single peaks for each of carbon atoms. On cooling to room temperature, the original spectrum was generated and exhibited multiple ^{13}C peaks characteristic of rotomers. Thus, the configuration of the 2-methyl group in diastereomers **28** and **76** has a profound effect on the population of rotomers. The complexity of the ^1H NMR spectra of **28** and **76** also indicated the presence of rotomers. Above 150 °C, peaks in the ^1H NMR spectrum coalesce to give a simpler spectrum, which returns to its original complexity at room temperature.

The derivative **24** showed the absence of rotomers, as evidenced by its ^{13}C NMR spectrum (Me_2SO), while diastereomer **22** is a mixture of rotomers in solution at room temperature.

Structures **23**, **24**, and **41** were established by X-ray analysis. Thus, the 2-methyl group in both **23** and **24** has the *S* configuration, and the 3-acetylthio group has the *R* configuration. The X-ray analysis also showed that in both **23** and **24** the amide carbonyl is oriented toward the carboxylic acid function (trans amide). The X-ray analysis

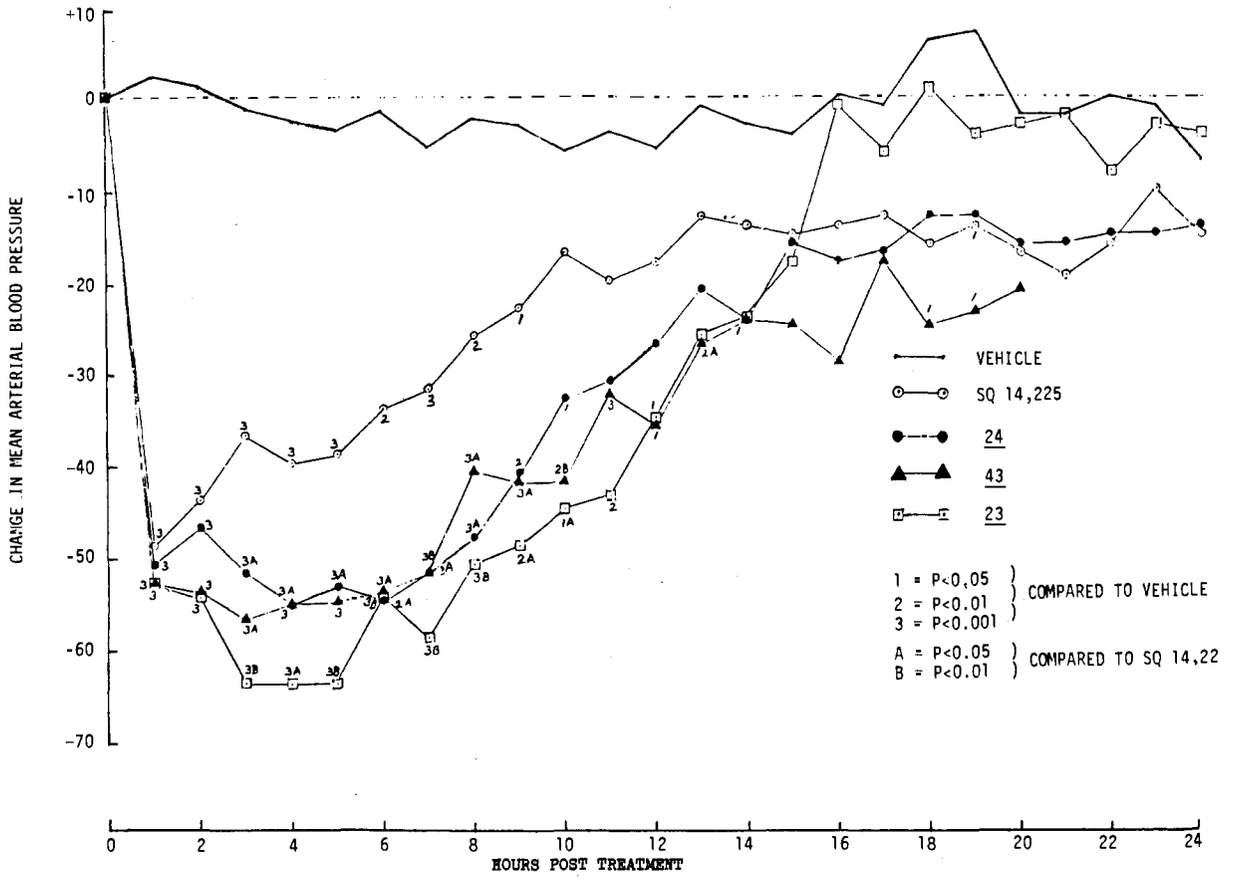


Figure 1. Dose–response curves. Antihypertensive activity was measured in conscious, unrestrained, aorta-coarcted, hypertensive rats. Only rats with mean arterial blood pressure (MABP) equal to or greater than 150 mmHg on the 4th to 6th day following coarctation were used. Direct arterial blood pressure was measured through an indwelling carotid artery cannula that was implanted 24 h before the experiment. The number of rats (n) used for each average MABP plotted up to 14 h was as follows: controls, 5–7; SQ 14,225, 8–12; compound **24**, 8; compound **43**, 8; compound **23**, 5–8. Data points beyond 14 h are based on average MABP values of three to eight animals. Compounds were administered orally at 10 mg/kg. The numbers 1, 2, and 3 indicate significant difference at $p < 0.5$, 0.01, 0.001, respectively, compared to vehicle controls.

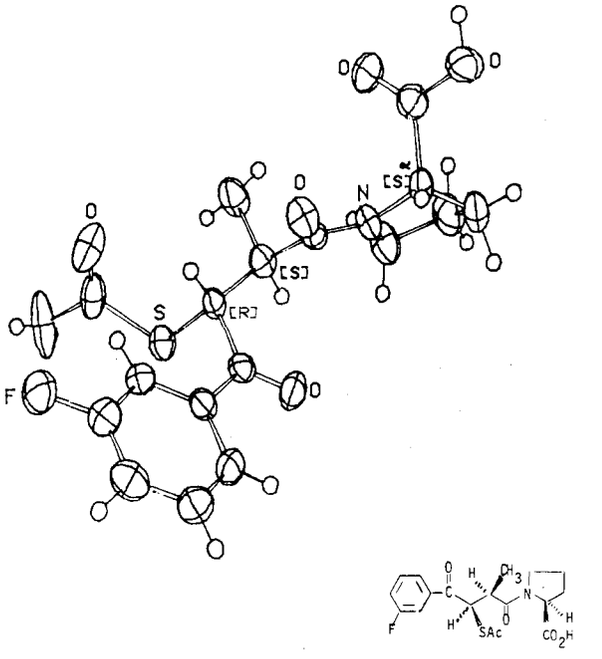


Figure 2. X-ray plot of 1-[3(R)-(acetylthio)-3-(3-fluorobenzoyl)-2(S)-methylpropionyl]-L-proline (**23**).

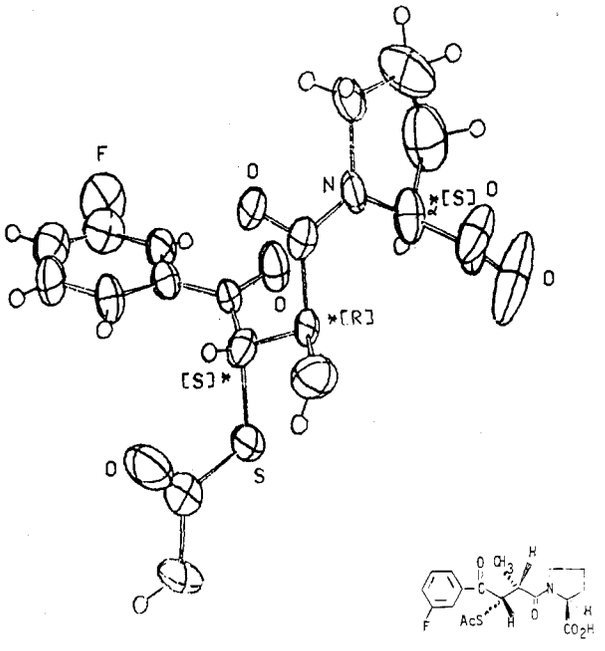
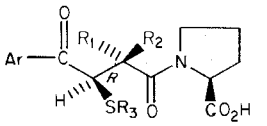


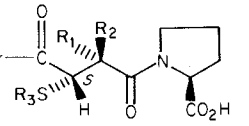
Figure 3. X-ray plot of 1-[3(S)-(acetylthio)-(3-fluorobenzoyl)-2(R)-methylpropionyl]-L-proline (**41**).

of structure **41** (R configuration of CH_3; S configuration of S-Ac) shows the amide carbonyl is oriented away from the carboyxlic acid function (cis amide). Thus, the orientation of the amide carbonyl and the carboxylic acid function are in a gauche conformation with the amide carbonyl, either directed toward (cis) or away (trans) from the carboxylic acid group. It has not been determined whether the cis or trans conformation of the amide car-

Table I. Physical Properties of 1-[3-(Acylthio)-3-aroylpropionyl]-L-proline and in Vitro ACE Activity



A

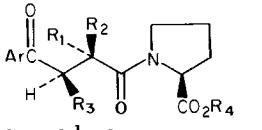


B

no.	Ar	R_1	R_2	R_3	diastereomer[c]	IC_{50},[g] M × 10^{-7} (n)	mp, °C	procedure	$[\alpha]^{23}_D$, deg (c, C_2H_5OH)	yield, %	formula	anal.[o]
21	3-FC_6H_4	H	CH_3	CH_3CO	B	2.33 (2)	glass[j]	VII[p]	−147 ± 1 (0.83)	34	$C_{18}H_{20}FSNO_5$	H, N, F; C;[s] S[t]
22	C_6H_5	H	CH_3	CH_3CO	B	0.78 (2)	glass[j]	VII[p]	−91 ± 1 (1.08)	10	$C_{18}H_{21}SNO_5 \cdot 0.20CCl_4$	C, H, N, S; Cl[u]
23	3-FC_6H_4	H	CH_3	CH_3CO	A[d,f]	0.617 (3)	157-159[j,k]	VII[p]	+251 ± 1 (0.81)	12	$C_{18}H_{20}FSNO_5$	C, H, N, S, F
24	C_6H_5	H	CH_3	CH_3CO	A[e]	0.549 (4)	160-161[j,k]	VII[p]	+276 ± 1 (0.71)	13	$C_{18}H_{21}SNO_5$	C, H, N, S
37	4-BrC_6H_4	H	H	H	A + B	3.52 (3)	glass[j]	VII[p]	−50 ± 1 (1.1)	14	$C_{15}H_{16}BrSNO_4$	H, N, S; C;[v] Br[w]
41	3-FC_6H_4	CH_3	H	CH_3CO	B[d]	2800 (1)	91-93[j,l]	VII[p]	−325 ± 2 (0.65)	5	$C_{18}H_{20}FSNO_5 \cdot 0.5CH_2Cl_2$	C, H, N, F; Cl[x]
42	3-FC_6H_4	CH_3	H	CH_3CO	A	160 (2)	glass[j]	VII[p]	+18 ± 1 (1.08)	31	$C_{18}H_{20}FSNO_5$	H, N, F; C;[y] S[z]
43	4-BrC_6H_4	H	CH_3	CH_3CO	A	0.985 (2)	137-138[j,k]	VII[p]	+211 ± 1 (0.92)	35	$C_{18}H_{20}BrSNO_5$	C, H, N, S, Br
44	4-BrC_6H_4	H	H	CH_3CO	A + B	1.31 (3)	glass[j]	VII	−42 ± 1 (0.921)	95	$C_{17}H_{18}BrSNO_5 \cdot 0.5CH_2Cl_2 \cdot H_2O$	C, H, N; S;[aa] Br;[bb] Cl[cc]
45	C_6H_5	H	H	C_6H_5CO	A + B	5.13 (3)	glass[j]	VIII	−30 ± 1 (0.965)	35	$C_{22}H_{21}SNO_5 \cdot 0.5CH_2Cl_2$	C, H, N, S; Cl[dd]
46	C_6H_5	H	H	CH_3CO	A + B	4.55 (3)	glass[j]	VII	−62 ± 1 (1.02)	41	$C_{17}H_{19}SNO_5 \cdot 0.25CCl_4 \cdot H_2O$	C, H, S; N;[ee] Cl[ff]
47	4-BrC_6H_4	H	H	C_6H_5CO	A + B	1.15 (3)	glass[j]	VIII	−21 ± 1 (0.834)	27	$C_{22}H_{20}BrSNO_5 \cdot 0.25CH_2Cl_2$	C, H, N, Br, Cl; S[gg]
48	4-FC_6H_4	H	H	CH_3CO	A + B	4.73 (3)	glass[j]	VII		27	$C_{17}H_{18}FSNO_5 \cdot H_2O \cdot 0.5(CH_3)_2CO$	C, N, S, F; H[hh]
49	4-FC_6H_4	H	H	C_6H_5CO	A + B	3.53 (3)	glass[j]	VIII	−55 ± 1 (0.96)	37	$C_{22}H_{20}FSNO_5$	C, H, N, S, F
50	3-FC_6H_4	H	H	CH_3CO	A + B	0.317 (3)	glass[j]	VIII	−44 ± 1 (1.13)	42	$C_{17}H_{18}FSNO_5 \cdot 0.5CH_2Cl_2$	C, H, N, F; S[ii]
51	3-FC_6H_4	H	H	C_6H_5CO	A + B	17.6 (3)	glass[j]	VIII	−34 ± 1 (1.09)	58	$C_{22}H_{20}FSNO_5 \cdot 0.25CH_2Cl_2$	H, N, S, F; C[jj]
52	4-BrC_6H_4	H	CH_3	CH_3CO	B	1.33 (2)	glass[j]	VII[p]	−69 ± 1 (0.98)	13	$C_{18}H_{20}BrSNO_5 \cdot 0.25CCl_4$	C, H, N, Br; S;[kk] Cl[ll]
53	3-F-4-$CH_3OC_6H_3$	H	H	CH_3CO	A + B	6.0 (2)	glass	VII		98	$C_{18}H_{20}FSNO_6 \cdot {}^1/_3CCl_4$	H, N, S, F; C[mm]
54[a]	4-Ph-C_6H_4	H	H	CH_3CO	A + B	0.86 (2)	173-176[j,m]	VII	−297 ± 1 (0.84)	23	$C_{23}H_{23}SNO_5$	C, H, N, S
55[a]	4-ClC_6H_4	H	H	CH_3CO	A + B	0.83 (3)	glass[j]	VII		78	$C_{17}H_{18}ClSNO_5$	H, N, S, Cl; C[nn]
56[a]	3-$CF_3C_6H_4$	H	H	CH_3CO	A + B	10.7 (2)	glass	VII		95	$C_{18}H_{18}F_3SNO_5$	C, H, N; S;[oo] F[pp]
57	4-ClC_6H_4	H	CH_3	CH_3CO	A + B	3.35 (3)	glass	VII	−56 ± 1 (0.86)	94	$C_{18}H_{20}ClSNO_5$	C, H, N; S;[qq] Cl[rr]
58	4-(4-ClC_6H_4O)C_6H_4	H	H	CH_3CO	A + B	3.58 (3)	glass	VII	−175 ± 1 (1.42)	90	$C_{23}H_{22}ClSNO_6 \cdot 0.5CH_2Cl_2$	C, H, N; S;[ss] Cl[tt]
59[b]	4-*t*-BuC_6H_4	H	H	CH_3CO	A + B	1.83 (3)	glass	VII	−100 ± 1 (1.55)	95	$C_{21}H_{27}SNO_5 \cdot 0.5CH_2Cl_2$	C, N, S; C;[uu] Cl[vv]
60[b]	5-indanyl	H	H	CH_3CO	A + B	2.18 (2)	glass	VII	−109 ± 1 (1.03)	89	$C_{20}H_{23}SNO_5 \cdot 0.25CH_3COSH$	C, H, N, S
61	2-naphthyl	H	H	CH_3CO	A + B	1.3 (2)	glass[j]	VII	−36 ± 1 (0.96)	50	$C_{21}H_{21}SNO_5 \cdot 0.4CCl_4$	H, N, S; C[ww]
62	1-naphthyl	CH_3	H	CH_3CO	B[h]	830 (2)	180-183[j,n]	VII	−338 ± 3 (0.36)	12	$C_{22}H_{23}SNO_5$	H, N, S; C[xx]
63	C_6H_5	H	CH_3		A[i]	r	glass[j]	VIIIq	+233 ± 2 (0.48)	24	$C_{20}H_{21}SN_3O_4 \cdot CCl_4$(0.3%)	C, H, N, S
SQ 14,225						0.156						

[a] Compounds synthesized by D. B. Moran. [b] Compounds synthesized by Dr. R. I. Trust. [c] Proportions of diastereomers A + B were not precisely determined; however, available evidence (^{1}H NMR) indicated approximately 50% of each diastereomer. [d] Structures were established by X-ray analysis. Determined by Dr. F. M. Lovell and N. A. Perkinson. [e] Structure determined by X-ray analyses by Molecular Structure Corp. [f] Originally crystallized to give low-melting conformer (mp 114–116 °C). Structures of both crystalline forms were established by X-ray analysis. [g] The IC_{50} is the molar concentration of compound that inhibits angiotensin converting enzyme (in vitro) by 50%; average of *n* determinations. Values determined by Dr. F. Lai and co-workers. [h] The *R* configuration of the CH_3 group was assigned on the basis of the large negative rotation. The *S* configuration of the CH_3 group was assigned on the basis of the large negative rotation. The *S* configuration of the S-Ac group was assigned on the basis that only diastereomers with *S,R* configurations were crystalline. [i] One diastereomer isolated by chromatography. Structure assigned on basis of large positive rotation. [j] Purified by chromatography on silica gel column with ethyl acetate–hexane (3:1) containing 2% acetic acid. [k] Recrystallized from ethyl acetate–hexane. [l] Recrystallized from CH_2Cl_2–petroleum ether (30–60 °C). [m] Recrystallized from ether–CH_2Cl_2–hexane. [n] Recrystallized from ethyl acetate. [o] Analysis for compounds that were not crystalline showed retention of solvent (CH_2Cl_2, CCl_4, acetone, etc.) from which the glass was obtained even after drying at room temperature under high vacuum. Satisfactory ^{1}H NMR spectra were obtained, and thin-layer chromatography showed one spot. [p] CH_3CN used as solvent in reaction with thioacetic acid. [q] Solvent $CHCl_3$. [r] 20% inhibition at 10^{-4} molar concentration. [s] C: calcd, 56.6; found, 55.9. [t] S: calcd, 8.4; found, 7.4. [u] Cl: calcd, 7.2; found, 7.8. [v] C: calcd, 46.6; found, 47.3. [w] Br: calcd, 20.7; found, 20.0. [x] Cl: calcd, 7.2; found, 7.8. [y] C: calcd, 56.7; found, 54.1. [z] S: calcd, 8.4; found, 7.6. [aa] S: calcd, 6.5; found, 4.8. [bb] Br: calcd, 16.4; found, 22.9. [cc] Cl: calcd, 7.2; found, 6.5. [dd] Cl: calcd, 7.7; found, 6.2. [ee] N: calcd, 3.3; found, 3.9. [ff] Cl: calcd, 8.3; found, 6.4. [gg] S: calcd, 6.3; found, 4.7. [hh] H: calcd, 5.8; found, 4.7. [ii] S: calcd, 8.2; found, 5.6. [jj] C: calcd, 59.1; found, 59.7. [kk] S: calcd, 6.7; found, 6.0. [ll] Cl: calcd, 7.3; found, 7.8. [mm] C: calcd, 49.4; found, 47.6. [nn] C: calcd, 53.2; found, 51.3. [oo] S: calcd, 7.7; found, 9.4. [pp] F: calcd, 13.7; found, 12.8. [qq] S: calcd, 8.1; found, 9.0. [rr] Cl: calcd, 8.9; found, 9.6. [ss] S: calcd, 6.2; found, 7.1. [tt] Cl: calcd, 13.7; found, 12.0. [uu] C: calcd, 57.6; found, 55.6. [vv] Cl: calcd, 7.9; found, 6.1. [ww] C: calcd, 55.7; found, 55.1. [xx] C: calcd, 63.9; found, 63.0.

Table II. Physical Properties of 1-(3-Aroylpropionyl)-L-prolines



no.	Ar	R_1	R_2	R_3	R_4	mp, °C	procedure (yield, %)	recrystn solvent	formula	anal.	$[\alpha]^{23}_D$, deg (*c*, C_2H_5OH)
18	C_6H_5	H	CH_3	Br	H	112–118[c]	*d* (95)		$C_{16}H_{18}BrNO_4 \cdot HBr \cdot H_2O$	C, H, N; Br[e]	−9 ± 1 (0.851)
28	C_6H_5	H	CH_3	H	H	218–220	V (40)	MeOH–H_2O	$C_{16}H_{19}NO_4$	C, H, N	−24 ± 1 (1.24)
30	4-BrC_6H_4	H	H	H	CH_3	84–86	II (72) III (44)	acetone–hexane	$C_{16}H_{18}BrNO_4$	C, H, N, Br	
32	4-BrC_6H_4	H	H	CH_2(S-Ac)	CH_3	glass	*d* (65)		$C_{19}H_{22}BrSNO_5$	C, H, N, S, Br	
34	C_6H_5	H	S-Ac[a]	H	H	149–150	*d* (30)	EtOAc–hexane	$C_{17}H_{19}SNO_5$	C, H, N, S	−151 ± 1 (0.96)
40	3-FC_6H_4	CH_3	H	H	H	107–108	II (12)	EtOAc–hexane	$C_{16}H_{18}FNO_4$	C, H, N, F	−104 ± 1 (0.91)
64	4-BrC_6H_4	H	H	H	*t*-Bu	70–71	III (40)	acetone–hexane	$C_{19}H_{24}BrNO_4$	C, H, N, Br	
65	4-ClC_6H_4	H	H	H	H	137–138	II (54)	acetone–hexane	$C_{15}H_{16}ClNO_4$	C, H, N, Cl	
66	4-BrC_6H_4	H	H	H	H	143–144	IV (60) II (68)	acetone–hexane	$C_{15}H_{16}BrNO_4$	C, H, N, Br	−44 ± 1 (0.97)
67	C_6H_5	H	H	H	H	95–97	II (53)	acetone–hexane	$C_{15}H_{17}NO_4 \cdot 0.5H_2O$	C, H, N	
68	4-FC_6H_4	H	H	H	H	127–129	II (48)	acetone–hexane	$C_{15}H_{16}FNO_4$	C, H, N, F	
69	3-FC_6H_4	H	CH_3	H	H	184–186	II (14)	CH_3CN	$C_{16}H_{18}FNO_4$	C, H, N, F	−19 ± 2 (0.56)
70	3-FC_6H_4	H	H	H	H	85–89	II (73)	hexane–ether	$C_{15}H_{16}FNO_4$	C, H, N, F	−61 ± 1 (1.72)
71	4-BrC_6H_4	H	CH_3	H	H	168–169	II (10)	EtOAc–hexane	$C_{16}H_{18}BrNO_4$	C, H, N, Br	−9 ± 1 (1.13)
72	2-naphthyl	H	H	H	H	116–118	II (50)	CH_2Cl_2–hexane	$C_{19}H_{19}NO_4$	C, H, N	−53 ± 1 (1.00)
73	3-F,4-$CH_3OC_6H_3$	H	H	H	H	180–182	II (55)	MeOH	$C_{16}H_{18}FNO_5$	C, H, N, F	
74	4-Ph-C_6H_4	H	H	H	H	131–133	II (85)	CH_2Cl_2–ether–hexane	$C_{21}H_{21}NO_4$	C, H, N	
75	3-$CF_3C_6H_4$	H	H	H	H	gum	II (88)		$C_{16}H_{16}F_3NO_4$	C, H, N, F	
76	C_6H_5	CH_3	H	H	H	gum[b]	II		$C_{16}H_{19}NO_4$	C, H, N	−97 ± 5 (0.23)
77	4-*t*-BuC_6H_4	H	H	H	H	glass	II (79)		$C_{19}H_{25}NO_4$	C, H, N	−99 ± 1 (0.26)
78	4-(4-ClC_6H_4O)C_6H_4	H	H	H	H	glass	II (70)		$C_{21}H_{20}ClNO_5$		
79	5-indanyl	H	H	H	H	89–91	II (68)	ether–EtOAc	$C_{18}H_{21}NO_4$	C, H, N	−118 ± 1 (0.71)
80	1-naphthyl	H	CH_3	H	H	160–162	II (8.7)	EtOAc–hexane	$C_{20}H_{21}NO_4$	C, H, N	−28 ± 1 (1.06)
81	C_6H_5	H	CH_3	S-Ac	CH_3	104–106	*d* (29)	acetone–hexane	$C_{19}H_{23}SNO_5$	C, H, N, S	+249 ± 1 (0.85)
82	C_6H_5	H	CH_3	H	CH_3	92–94	*d* (56)	acetone–hexane	$C_{17}H_{21}NO_4$	C, H, N	−37 ± 1 (0.91)

[a] Assigned *R* configuration but stereochemistry not established. [b] Separated from diastereomer 25 by HPLC. NMR (^{13}C) showed 3:2 mixture of rotomers (proline–amide bond): ^{13}C NMR of 28 showed a 9:1 mixture of rotomers. [c] Crystallizes with 1 mol of HBr and H_2O. Could not be crystallized from CH_2Cl_2 in the absence of HBr. [d] See Experimental Section for procedure. [e] Br: calcd, 34.3; found, 31.6.

Table III. Oral Hypotensive Activity in Aorta-Coarcted Renal Hypertensive Rats

compd	dose,[a] mg/kg, po	Δ MABP,[b] mmHg at 0.5 h	Δ MABP,[b] mmHg at 2.0 h	n[c]	IC_{50}, M (n)
21	20	−24.75 ± 4.15****	−28.00 ± 5.12****	4	2.33×10^{-7} (2)
22	10	−23.00 ± 5.56***	−23.17 ± 5.17***	6	7.8×10^{-8} (2)
23	20	−17.50 ± 4.67***		6	6.17×10^{-8} (3)
24	20	−22.43 ± 3.61****	−17.14 ± 3.06****	7	5.49×10^{-8} (4)
37	20	−8.80 ± 3.61	−12.00 ± 2.10***	5	3.52×10^{-7} (3)
43	20	−27.14 ± 4.34****	−31.33 ± 3.23****	7	9.85×10^{-8} (2)
44	20	−18.33 ± 5.65*	−10.38 ± 5.57	9	1.31×10^{-7} (3)
45	20[d]	−1.00 ± 7.37	−22.67 ± 11.57**[d]	3	5.13×10^{-7} (3)
46	20	−19.25 ± 5.86***	−5.25 ± 3.42	4	4.55×10^{-7} (3)
47	20	−10.00 ± 4.62	−14.33 ± 6.84*	3	1.15×10^{-7} (3)
48	20	−15.75 ± 5.36*	−14.67 ± 6.69**	4	4.73×10^{-7} (3)
49	20	−12.00 ± 7.81	−6.50 ± 16.50	3	3.53×10^{-7} (3)
50	20	−17.00 ± 4.81*	−5.43 ± 4.52	8	3.17×10^{-8} (3)
51	20	−41 ± 13.83***	3.75 ± 2.39	4	1.76×10^{-6} (3)
52	20	−23.50 ± 7.53**	−26.67 ± 8.33***	4	1.33×10^{-7} (2)
53	20	−12.67 ± 1.45**	−11.00 ± 5.86*	3	6.0×10^{-7} (2)
55	20	−17.33 ± 6.23*	−11.67 ± 7.66	6	8.25×10^{-8} (3)
56	20	−17.50 ± 4.56**	−0.83 ± 6.76	6	1.07×10^{-6} (2)
57	20	−24.00 ± 10.9*	−30.50 ± 7.49***	2	3.65×10^{-7} (3)
59	20	−30.00 ± 5.31***	−19.25 ± 3.25****	4	1.83×10^{-7} (3)
60	20	−18.50 ± 6.50**	−30.00 ± 15.00***	2	2.18×10^{-7} (2)
61	20	−17.33 ± 4.40**	−6.67 ± 3.15*	6	1.33×10^{-7} (2)
SQ 14,225	30	−27.66 ± 8.19***	21.67 ± 3.33***	3	1.56×10^{-8} (9)
control[e]		−2.33 ± 2.14	+3.5 ± 2.87	6	

[a] Compounds administered by gavage; 20 mg in vehicle consisting of 0.10 mL of ethanol, 0.10 mL of polyethylene glycol, 1 drop of Tween-80, and mixture diluted to 10 mL with saline. [b] Values are means ± standard error; unpaired Student's t test. * = $p < 0.05$; ** = $p < 0.02$; *** = $p < 0.01$; **** = $p < 0.001$. [c] n = number of animals. [d] At 1.5 h, a second dose was administered ip; MABP reduction at 0.5 h after ip dose. [e] Administered vehicle without test compound.

bonyl and the carboxy function as observed in the crystalline structures is also the preferred conformation in solution.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are not corrected. Samples for analysis were dried in vacuo at 20–25 °C for 16–24 h. ^1H NMR spectra were determined with a Varian A-100 spectrometer, and chemical shifts (δ) relative to internal tetramethylsilane were as expected. Solvents were removed under reduced pressure by the use of a rotary evaporator. Analtech silica gel GF plates (250 μm) were used for thin-layer chromatography (TLC). Silica gel (60–200 mesh) grade H (Davidson Chemical) was used for column chromatography. Jabin Yvon Chromatospec Prep column was packed with silica gel (60H) purchased from E. M. Reagents.

3-(3-Fluorobenzoyl)propionic Acid. To a solution of 25.5 g (0.116 mol) of α-(3-fluorophenyl)-4-morpholineacetonitrile[9] in 230 mL of THF was added 7 mL of 30% KOH in ethanol. To the mixture was added 9.1 mL (0.14 mol) of acrylonitrile in 55 mL of THF. The mixture was stirred for 18 h, and the solvent was removed. The residue was extracted with ether and filtered, and the filtrate was concentrated to give 35 g of oil. A mixture of the oil, 165 mL of acetic acid, and 19 mL of H_2O was refluxed for 1 h, and the solvent was removed. The residue was partitioned between H_2O and CH_2Cl_2. The organic layer was washed with H_2O, saturated $NaHCO_3$ solution, and saline and dried ($MgSO_4$). The solvent was removed, and the residue was triturated with hexane to give 18.5 g of waxy solid. A mixture of the solid and 100 mL of 6 N HCl was refluxed for 1 h. Chilling and filtering gave 17.4 g of solid. The solid was dissolved in 100 mL of CH_2Cl_2, and 100 mL of hexane was added. Chilling and filtering gave 11.5 g (50%) of yellow needles, mp 97–99 °C. Anal. ($C_{10}H_9O_3F$) C, H, F.

3-[4-(4-Chlorophenoxy)benzoyl]propionic Acid. A mixture of 9.8 g (0.05 mol) of 3-(4-fluorobenzoyl)propionic acid, 6.5 g (0.05 mol) of 4-chlorophenol, and 13.8 g of K_2CO_3 in N,N-dimethylacetamide was stirred and heated at 135 °C for 16 h under argon. The mixture was cooled, poured into 1 L of H_2O and filtered through diatomaceous earth. The filtrate was acidified with concentrated HCl, diluted to 2 L, and filtered. The solid was washed with water and recrystallized from ethanol–H_2O (1:1) to give 10 g of crystals, mp 148–150 °C.

Resolution of 3-Benzoyl-2-methylpropionic Acid. A. To a hot solution of 19.2 g (0.01 mol) of 3-benzoyl-2-methylpropionic acid in 50 mL of ethyl acetate was added 1.3 mL (0.01 mol) of (−)-α-methylbenzylamine. After the flask was scratched and the solution was cooled slowly, crystals separated. The mixture was allowed to stand at room temperature for 18 h and filtered. The solid was washed with ethyl acetate (ca. 5 mL) to give 1.42 g of crystals, mp 124–127 °C. Recrystallization from ethyl acetate gave 1.10 g of white crystals, mp 133–135 °C. The solid was suspended in ethyl acetate, and the mixture was shaken with 1 N HCl. The organic layer was separated, washed with NaCl solution, and dried ($MgSO_4$). The solvent was removed in vacuo to give 0.67 g of crystals. Recrystallization from acetone–hexane gave 0.57 g of white crystals: mp 122–124 °C; $[\alpha]^{23}_D$ −31 ± 1° (c 1.079, EtOH).

The orignal mother liquor (from crystallization of the amine salt) was washed with 1 N HCl and NaCl solution and dried ($MgSO_4$). The solvent was removed in vacuo, and the solid was recrystallized from acetone–hexane to give 0.67 g of white crystals: mp 124–126 °C; $[\alpha]^{23}_D$ +24 ± 1° (c 1.058, EtOH).

B. To a hot solution of 50.0 g (0.26 mol) of 3-benzoyl-2-methylpropionic acid in 600 mL of ethyl acetate was added 23.6 g (0.26 mol) of L-2-amino-1-butanol. After standing at room temperature (3 days), the mixture was filtered to give 28.5 g of white crystals, mp 88–90 °C. The filtrate was concentrated to one-half volume, chilled, and filtered to give 0.8 g of white crystals.

A sample of amine salt (5 g) was recrystallized (3 times) from ethyl acetate until constant melting point and rotation were obtained [3.5 g, mp 95–96 °C; $[\alpha]^{23}_D$ −26 ± 1° (c 1.074, EtOH)].

The 3.5-g sample was suspended in ethyl acetate and shaken with 1 N HCl. The ethyl acetate layer was separated, washed with NaCl solution, and dried ($MgSO_4$). The solvent was removed to give white crystals, which were recrystallized from acetone to give 1.97 g of white crystals: mp 123–125 °C; $[\alpha]^{23}_D$ −33 ± 1° (c 1.044, EtOH). Anal. ($C_{11}H_{12}O_3$) C, H.

Procedure I. Succinimido 3-Benzoyl-2-methylpropionate. To a mixture of 5.76 g (0.003 mol) of 3-benzoyl-2-methylpropionic acid and 3.45 g (0.003 mol) of N-hydroxysuccinimide in 60 mL of dioxane was added 6.18 g (0.003 mol) of N,N-dicyclohexylcarbodiimide. The mixture was stirred at room temperature for 18 h, cooled, and filtered. The filtrate was concentrated to dryness. The residue was triturated with hexane plus a trace of ether to give 7.8 g (90%) of white crystals, mp 98–106 °C. Recrystallization

from CH_2Cl_2–hexane gave 5.73 g (65%) of white crystals, mp 118–120 °C. Other intermediate activated esters were prepared by this procedure and used without further purification.

Procedure II. 1-[3-(4-Chlorobenzoyl)propionyl]-L-proline (65). To a solution of 2.30 g of L-proline and 3.36 g of sodium bicarbonate in 50 mL of water was added a mixture of 5.96 g of succinimido 3-(4-chlorobenzoyl)propionate in 60 mL of ethanol. The mixture was stirred at room temperature for 18 h and concentrated to one-half volume. The cooled mixture was acidified to pH 2 with concentrated HCl and extracted with ether. The ether extracts were dried ($MgSO_4$), and the solvent was removed to give 5.3 g of gum. Trituration with hexane containing a small amount of ether gave 4.88 g of tan crystals, mp 128–133 °C. Recrystallization (twice) from acetone–hexane gave 3.37 g (54%) of crystals, mp 137–138 °C.

Procedure III. 1-[3-(4-Bromobenzoyl)propionyl]-L-proline Methyl Ester (30). A stirred solution of 5.14 g (0.02 mol) of 3-(4-bromobenzoyl)propionic acid and 2.78 mL (0.02 mol) of triethylamine in 50 mL of THF was cooled to −15 °C (CH_3OH–ice–salt bath), and 1.9 mL (0.02 mol) of ethyl chlorocarbonate in 10 mL of THF was added dropwise. The mixture was stirred at −10 °C for 30 min. A solution of 3.32 g (0.02 mol) of L-proline methyl ester hydrochloride in 50 mL of CH_2Cl_2 was treated with 2.78 mL (0.02 mol) of triethylamine for 15 min, and the mixture was filtered. The filtrate was added dropwise to the solution of mixed anhydride while maintaining the temperature below −5 °C. The mixture was stirred for 30 min and allowed to warm to room temperature over a period of 1 h. The mixture was poured into 300 mL of water and extracted with CH_2Cl_2 (emulsion). The CH_2Cl_2 extract was washed with saturated $NaHCO_3$ solution, H_2O, and 1 N HCl solution. The extract was dried ($MgSO_4$), and the solvent was removed to give an oil. The oil was crystallized from petroleum ether (bp 30–60 °C) to give 3.32 g (44%) of white crystals, mp 80–83 °C. Recrystallization from acetone–hexane gave 2.1 g of white crystals, mp 84–86 °C.

Procedure IV. 1-[3-(4-Bromobenzoyl)propionyl]-L-proline (66). To a mixture of 27.3 g (0.077 mol) of the *N*-hydroxysuccinimide ester of 3-(4-bromobenzyl)propionic acid and 13.0 g (0.078 mol) of L-proline methyl ester hydrochloride in 150 mL of acetonitrile was added 21.8 mL (0.156 mol) of triethylamine. The mixture was stirred at room temperature for 18 h and concentrated to dryness in vacuo. The residue was dissolved in CH_2Cl_2, and the solution was washed with 1 N HCl and dried ($MgSO_4$). The solvent was removed, and the residue was triturated with hexane to give 23.6 g of tan crystals, mp 74–78 °C. Recrystallization from acetone–hexane gave 16.6 g (59%) of white crystals, mp 83–85 °C.

A mixture of 2.64 g of 1-[3-(4-bromobenzoyl)propionyl]-L-proline methyl ester (as prepared above), 26 mL of 1 N NaOH, and 5 mL of THF was stirred at room temperature for 48 h. The mixture was extracted with ethyl acetate, and the aqueous layer was poured into cold 1 N HCl. The mixture was extracted with CH_2Cl_2, and the extract was dried ($MgSO_4$) and concentrated. The residue (2.0 g) was triturated with ether to give 1.55 g (60%) of white crystals, mp 135–139 °C. Recrystallization from hexane gave 1.35 g of crystals, mp 138–139 °C.

(*S*)- and (*R*)-1-[3-(3-Fluorobenzoyl)-2-methylpropionyl]-L-proline (40 and 69). To a solution of 6.21 g (0.054 mol) of L-proline and 9.07 g of $NaHCO_3$ in 220 mL of H_2O was added a slurry of 16.6 g (0.054 mol) of succinimido 3-(3-fluorobenzoyl)-2-methylpropionate in 220 mL of ethanol. The mixture was stirred at room temperature for 18 h and filtered. The filtrate was concentrated in vacuo to one-half volume, chilled, and filtered through diatomaceous earth. The clear filtrate was stirred in an ice bath and acidified to pH 4 with concentrated HCl. The mixture was extracted with CH_2Cl_2, and the extract was washed with H_2O and saline and dried ($MgSO_4$). The solvent was removed in vacuo to give 11.8 g of a gum. The gum was dissolved in 10 mL of ethyl acetate and 19 mL of hexane, and 0.2 mL of acetic acid was added. Chilling gave 3.18 g of crystals, mp 176–180 °C. Recrystallization from 50 mL of acetonitrile gave 2.34 g of white crystals mp 184–186 °C (*R*-diastereomer **69**). The mother liquors from crystallization of the *R*-diastereomer **69** were placed on a silica gel column and chromatographed with ethyl acetate–hexane (1:1) containing 2% acetic acid. The main cut (3.7 g of gum) was dissolved in 110 mL of acetone, and 2.2 mL of dicyclohexylamine was added. After standing at room temperature, the mixture was filtered to give 4.5 g of white crystals, mp 92–104 °C. The batch was combined with 3.5 g from a similar run and recrystallized from 300 mL of acetone (2×) to give 6.8 g of white crystals, mp 97–103 °C. The solid was dissolved in 50 mL of H_2O, and concentrated HCl was added. The mixture was filtered, and the solid was washed with H_2O, ether, and ethyl acetate. The combined filtrates were extracted repeatedly with ethyl acetate. The extracts were concentrated in vacuo and dissolved in a mixture of 20 mL of MeOH, 20 mL of H_2O, and 4 mL of 1 N NaOH. The solution was passed through a column of 50 g of IR-120 resin [previously washed with 1 N HCl, H_2O, and MeOH–H_2O (1:1)]. Cuts containing solid were combined, and the solvent was removed. The solid was dissolved in CH_2Cl_2, and the solution was washed with H_2O and saline and dried ($MgSO_4$). Removal of the solvent gave 3.3 g of solid. Trituration with hexane and a trace of ether gave 3.1 g of white crystals, mp 100–103 °C. Recrystallization (2×) from ethyl acetate–hexane gave 1.5 g of crystals: mp 107–108 °C; $[\alpha]^{25}_D$ −104 ± 1° (c 0.909, EtOH) (*S*-diastereomer **40**).

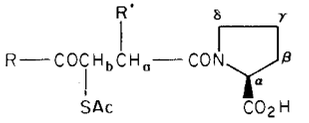
(*R*)-1-[3-(3-Fluorobenzoyl)-2-methylpropionyl]-L-proline (69) Dicyclohexylamine Salt. To a solution of 307 mg (0.001 mol) of (*R*)-1-[3-(3-fluorobenzoyl)-2-methylpropionyl]-L-proline in 10 mL of acetone was added 0.22 mL of dicyclohexylamine. The mixture was allowed to stand for 2 h at room temperature and was filtered to give 384 mg (79%) of white plates: mp 164–166 °C; $[\alpha]^{25}_D$ −15 ± 1° (c 0.866, EtOH). Anal. ($C_{28}H_{41}O_4N_2F$) C, H, N, F.

Procedure V. (*R*)-1-(3-Benzoyl-2-methylpropionyl)-L-proline (28). A slurry of 457.4 g (2.54 mol) of 3-benzoyl-2-methylpropionic acid in 635 mL of acetic acid and 480 mL (5.08 mol) of acetic anhydride was heated on a steam bath until solution was complete. After the solution was heated on a steam bath for an additional 3 h, the solvent was removed in vacuo. Toluene (500 mL) was added (2×), and the solvent was removed in vacuo. The residual oil was a mixture of enol lactone and α,β-unsaturated lactone (ca. 70:30), as determined by the ^1H NMR spectrum. The residue was dissolved in 1.4 L of hot 2-propanol and added to a slurry of 321 g (2.79 mol) of L-proline and 353 mL (2.54 mol) of triethylamine in 2 L of 2-propanol. The mixture was stirred and refluxed for 18 h. The mixture was chilled and 2.5 L of 1 N HCl was added slowly. The mixture (containing crystals) was diluted with 4.0 L of H_2O, chilled for 3 h, and filtered. The solid was slurried with 300 mL of CH_2Cl_2 (3×) and filtered to give 287 g (40%) of white crystals, mp 218–220 °C. A 60-g sample was recrystallized by dissolving in 900 mL of 95% CH_3OH, filtering, and diluting the filtrate with 100 mL of H_2O. Chilling and filtering gave 49.1 g of white crystals: mp 220–222 °C; $[\alpha]^{23}_D$ −57 ± 1° (c 0.817, acetic acid); $[\alpha]^{23}_D$ −16 ± 1° (c 1.24, Me_2SO).

(*S*)-1-(3-Benzoyl-2-methylpropionyl)-L-proline (76). To a solution of 5.76 g (0.03 mol) of 3-benzoyl-2-methylpropionic acid [partially resolved; $[\alpha]^{23}_D$ −30 ± 1° (c 1.0, EtOH)] and 3.55 g of *N*-hydroxysuccinimide in 65 mL of dioxane was added 6.19 g (0.03 mol) of *N*,*N*-dicyclohexylcarbodiimide. The mixture was stirred at room temperature for 4.5 h and filtered. The filtrate was concentrated in vacuo, and the residue was triturated with hexane to give 8.55 g (99%) of white crystals, mp 91–100 °C. To a solution of 5.18 g (0.045 mol) of L-proline and 7.38 g of $KHCO_3$ in 50 mL of water was added a slurry of 8.55 g (0.03 mol) of activated ester in 20 mL of acetonitrile. The mixture was stirred at room temperature for 18 h and filtered. The chilled filtrate was acidified with 6 N HCl and extracted with ethyl acetate. The extract was washed with NaCl solution and dried ($MgSO_4$), and the solvent was removed in vacuo. The residual gum (7.67 g) was chromatographed on two μ-Porosil (Waters) columns (30 cm × 4.6 mm) in series with formic acid (90%)–acetonitrile–CH_2Cl_2 (1.5:12:87.5, v/v) at flow rate of 1.5 mL/min. The sample was dissolved in formic acid–acetonitrile, and peaks were detected with UV at 270 nm. The retention time for elution of the *S* isomer was 776 s, while the *R* isomer had a retention time of 879 s. The pure *S* isomer was obtained as a glass, which could not be crystallized: $[\alpha]^{26}_D$ −97 ± 5° (c 0.23, EtOH). The ^{13}C NMR spectrum showed the *S* isomer to be a mixture (ca. 60:40) of cis and trans rotomers (amide carbonyl).

Procedure VI. General Method for Preparation of 1-[3-Bromo-3-aroylpropionyl]-L-prolines. 1-[3-Bromo-3-(4-fluorobenzoyl)propionyl]-L-proline. To a solution of 10.26 g

Table IV. ^{1}H NMR Spectral Data[a]



compd	R'(CH_3)	β γ CH_2CH_2	SAc	H_a (R')	δ CH_2	α CH	H_b	R	CO_2H	R_f[d]
21	1.24 (d, 3 H, J = 6.8)	2.18 (m, 4 H)	2.32 (s, 3 H)	3.30 (m, 1 H)	3.67 (2 H)	4.55 (m, 1 H)	5.42 (d, 1 H)	7.0–8.0 (m, 4 H)	8.69 (s, 1 H)	0.52
22	1.24 (d, 3 H, J = 7.8)	2.14 (m, 4 H)	2.32 (s, 3 H)	3.4 (m, 1 H)	3.68 (m, 2 H)	4.60 (m, 1 H)	5.50 (d, 1 H, J = 10.7)	7.3–8.1 (m, 5 H)	10.54 (s, 1 H)	0.47
23	1.30 (d, 3 H, J = 7.0)	2.12 (m, 4 H)	2.38 (s, 3 H)	3.22 (m, 1 H)	3.70 (m, 2 H)	4.42 (m, 1 H)	5.48 (d, 1 H, J = 10.0)	7.1–7.9 (m, 4 H)	9.50 (s, 1 H)	0.50
24	1.32 (d, 3 H, J = 7.1)	2.15 (m, 4 H)	2.37 (s, 3 H)	3.22 (m, 1 H)	3.70 (m, 2 H)	4.45 (m, 1 H)	5.59 (d, 1 H, J = 10.7)	7.3–8.1 (m, 5 H)	10.0 (s, 1 H)	0.48
37		2.10 (m, 4 H)		3.10 (m, 2 H)	3.65 (m, 2 H)	4.50 (m, 1 H)	4.70 (m, 1 H)	7.4–8.0 (m, 4 H)	8.55 (s, 1 H)	0.38
41	1.29 (d, 3 H, J = 7.0)	2.06 (m, 4 H)	2.42 (s, 3 H)	3.28 (m, 1 H)	3.52, 3.98 (2 m, 2 H)	4.51 (m, 1 H)	5.50 (d, J = 10.0), 5.32 (s, CH_2Cl_2) (1 H)	7.1–7.9 (m, 4 H)	9.79 (s, 1 H)	0.52
42	1.22 (d, 3 H, J = 6.7)	2.08 (m, 4 H)	2.33 (s, 3 H)	3.50 (m, 1 H)	3.60 (m, 2 H)	4.65 (m, 1 H)	5.50 (d, 1 H, J = 9.6)	7.0–8.0 (m, 4 H)	9.44 (s, 1 H)	
43	1.30 (d, 3 H, J = 7.1)	2.10 (m, 4 H)	2.38 (s, 3 H)	3.20 (m, 1 H)	3.70 (m, 2 H)	4.40 (m, 1 H)	5.50 (d, 1 H, J = 10.7)	7.4–8.0 (m, 4 H)	9.55 (s, 1 H)	0.47
44		2.08 (m, 4 H)	2.34 (s, 3 H)	2.80, 3.20 (m, 2 H)	3.60 (m, 2 H)	4.48 (m, 1 H)	5.52 (m, 1 H), 5.32 (s, 1 H, CH_2Cl_2)	7.5–8.0 (m, 4 H)	10.06 (s, 1 H)	
45		2.04 (m, 4 H)		2.90, 3.40 (m, 2 H)	3.62 (m, 2 H)	4.54 (m, 1 H)	5.86 (m, 1 H) 5.32 (s, 1 H, CH_2Cl_2)	7.1–8.1 (m, 10 H)	10.12 (s, 1 H)	0.39
46		2.08 (m, 4 H)	2.34 (s, 3 H)	2.80, 3.20 (m, 2 H)	3.62 (m, 2 H)	4.50 (m, 1 H)	5.64 (m, 1 H)	7.4–8.1 (m, 5 H)	9.88 (s, 1 H)	
47		2.08 (m, 4 H)		2.85, 3.40 (m, 2 H)	3.62 (m, 2 H)	4.52 (m, 1 H)	5.80 (m, 1 H), 5.32 (s, CH_2Cl_2) (1 H)	7.3–8.10 (m, 9 H)	9.32 (s, 1 H)	0.46
48		2.10 (m, 4 H), 2.12 [$(CH_3)_2CO$]	2.30 (s, 3 H)	2.76, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.50 (m, 1 H)	5.60 (m, 1 H)	7.10, 8.04 (2 m, 4 H)	9.63 (s, 1 H)	
49		2.10 (m, 4 H)		2.85, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.50 (m, 1 H)	5.82 (m, 1 H)	6.9–8.2 (m, 9 H)	8.90 (s, 1 H)	0.43
50		2.10 (m, 4 H)	2.36 (s, 3 H)	2.80, 3.40 (m, 2 H)	3.64 (m, 2 H)	4.50 (m, 1 H)	5.60 (m), 5.32 (s, CH_2Cl_2) (1 H)	7.1–8.0 (m, 4 H)	9.22 (s, 1 H)	
51		2.06 (m, 4 H)		2.90, 3.50 (m, 2 H)	3.64 (m, 2 H)	4.50 (m, 1 H)	5.80 (m), 5.32 (s, CH_2Cl_2) (1 H)	7.1–8.0 (m, 9 H)	8.48 (s, 1 H)	0.46
52[b]	1.23, 1.26 (2 d, 2:1, 3 H, J = 6.8)	2.15 (m, 4 H)	2.32 (s, 3 H)	2.9–3.5 (m, 1 H)	3.65 (m, 2 H)	4.55 (m, 1 H)	5.40 (d, 1 H, J = 8.0)	7.4–8.0 (m, 4 H)	9.05 (s, 1 H)	
53		2.10 (m, 4 H)	2.33 (s, 3 H)	2.75, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.45 (m, 1 H)	5.60 (m, 1 H)	7.0 (m, 1 H), 7.75 (m, 2 H) 3.92 (s, OCH_3)	8.42 (s, 1 H)	
54		2.00 (m, 4 H)	2.34 (s, 3 H)	2.80, 3.40 (m, 2 H)	3.50 (m, 2 H)	4.52 (m, 1 H)	5.66 (m, 1 H)	7.2–8.1 (m, 9 H)	8.84 (s, 1 H)	0.33

55		2.10 (m, 4 H)	2.34 (s, 3 H)	2.80, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.46 (m, 1 H)	5.60 (m, 1 H)	7.40, 7.90 (2 d, 4 H)	8.68 (s, 1 H)	0.33
56		2.10 (m, 4 H)	2.34 (s, 3 H)	2.82, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.46 (m, 1 H)	5.62 (m, 1 H)	7.5–8.3 (m, 4 H)	8.96 (s, 1 H)	0.33
57[c]	1.18, 126 (2 d, 1:2) (3 H)	2.10 (m, 4 H)	2.28, 2.34 (2 s, 1:2) (3 H)	3.20 (m, 1 H)	3.64 (m, 2 H)	4.50 (m, 1 H)	5.44 (m, 1 H)	7.35–7.90 (m, 4 H)	6.90 (s, 1 H)	0.49
58		2.15 (m, 4 H)	2.33 (s, 3 H)	2.75, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.50 (m, 1 H)	5.60 (m), 5.32 (s, CH_2Cl_2) (1 H)	6.8–8.1 (m, 8 H)	8.41 (s, 1 H)	
59		2.10 (m, 4 H)	2.31 (s, 3 H)	2.75, 3.40 (2 m, 2 H)	3.60 (m, 2 H)	4.50 (m, 1 H)	5.70 (m), 5.32 (s, CH_2Cl_2) (1 H)	7.45, 7.95 (2 d, 4 H), 1.30 (9 H, *t*-Bu)	9.21 (s, 1 H)	0.40
60		2.05 (m, 4 H)	2.33 (s), 2.31 (CH_3COSH) (3 H)	2.8–3.40 (m, 2 H)	3.60 (m, 2 H)	4.50 (m, 1 H)	5.70 (m, 1 H)	7.30 (d), 7.80 (m) (3 H), 2.10 (m), 2.90 (t) (6 H, CH_2)	9.35 (s, 1 H)	0.34
61		2.15 (m, 4 H)	2.34 (s, 3 H)	2.84, 3.40 (m, 2 H)	3.60 (m, 2 H)	4.51 (m, 1 H)	5.85 (m, 1 H)	7.5–8.1 (m, 7 H)	8.58 (s, 1 H)	
62	1.29 (d, 3 H, $J = 7.0$)	2.15 (m, 4 H)	2.19 (s, 3 H)	3.1–3.8 (m, 1 H)	3.1–3.8 (m, 2 H)	4.50 (m, 1 H)	5.59 (d, 1 H, $J = 10.7$)	7.3–8.2 (m, 7 H)		0.43
63	1.40 (d, 3 H, $J = 7.0$)	2.10 (m, 4 H)		3.40 (m, 1 H)	3.8 (m, 2 H)	4.50 (m, 1 H)	6.20 (d, 1 H, $J = 10$)	7.0–8.7 (m, 8 H)	9.36 (s, 1 H)	

[a] Spectra were determined with a Varian A-100 spectrometer in $CDCl_3$, and chemical shifts (δ) are relative to internal tetramethylsilane. Coupling constants are in hertz. [b] A 2:1 mixture of rotomers (prolyl amide bond). [c] A 1:2 mixture of diastereomers (A and B, Table I). [d] Thin-layer chromatography on Analtech silica gel GF plates (250 μm) with ethyl acetate–hexane (75:25) containing 2% acetic acid.

(0.035 mol) of 1-[3-(4-fluorobenzoyl)propionyl]-L-proline in 100 mL of HOAc containing 3 drops of 30% HBr in HOAc was added dropwise 5.6 g (0.035 mol) of bromine in 20 mL of HOAc. The mixture was stirred at room temperature for 18 h and concentrated to one-third volume. The solution was poured into ice and water and extracted with CH_2Cl_2. The extract was washed with H_2O and saturated NaCl solution and dried ($MgSO_4$). The solvent was removed in vacuo to give 12.2 g of glass. Chromatography 1.5 × 20 in. column) over silica gel (60–200 mesh) with ethyl acetate–hexane (75:25) containing 2% acetic acid gave 7.3 g of glass [one spot by TLC (silica gel); chromatography solvent system]; ^1H NMR supports the structure. In cases where the crude product was one spot by TLC and a satisfactory ^1H NMR spectrum was obtained, the product was not chromatographed but used directly in the next step. The ^1H NMR spectrum of derivatives with a 2-methyl group showed the presence of both diastereomers in a ratio of ca. 7:3. The mixture was used in reactions with anions of thioacetic acid and thiobenzoic acid.

1-[3-Benzoyl-3(*R*)-bromo-2(*S*)-methylpropionyl]-L-proline (18). To a partial solution of 11.56 g (0.04 mol) of (*S*)-1-(3-benzoyl-2-methylpropionyl)-L-proline in 90 mL of CH_2Cl_2 was added a solution of 7.29 g (0.044 mol) of bromine in 10 mL of CH_2Cl_2. Into the mixture was bubbled HBr gas for 60 s. The solid dissolved (20 min), and the mixture was allowed to stand (18 h). The mixture was filtered, and the solid was washed with CH_2Cl_2 and petroleum ether (30–60 °C). The solid was sucked dry under a $CaCl_2$ drying tube to give 13.3 g (70%) of white crystals: mp 112–118 °C; $[\alpha]^{26}_D$ −9 ± 1° (c 0.851, EtOH).

Procedure VII. 1-[3-(Acetylthio)-3-(4-fluorobenzoyl)-propionyl]-L-proline (48). To a solution of 3.7 g (0.01 mol) of 1-[3-bromo-3-(4-fluorobenzoyl)propionyl]-L-proline in 37 mL of ethanol was added 1.37 g (0.012 mol) of potassium thioacetate. The mixture was stirred at room temperature for 18 h, and the solvent was removed in vacuo. The residue was partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with water and saturated NaCl solution and dried ($MgSO_4$). The solvent was removed in vacuo to give a glass (3.15 g). The glass was dissolved in ethyl acetate–hexane (75:25) containing 2% acetic acid and chromatographed on a 1 × 18 in. column of silica gel (60–200 mesh). Elution with the same solvent mixture (50-mL cuts; HBV 100 mL) gave 1.44 g of **48** from cut 2. The glass was dissolved in acetone and hexane added until turbid. The solvent was removed to give a yellow glass. The ^1H NMR spectrum shows acetone as solvate. TLC on noncrystalline compounds in Table I showed one spot [silica gel; ethyl acetate–hexane (75:25) + 2% acetic acid].

Procedure VIII. 1-[3-(Benzoylthio)-3-(4-fluorobenzoyl)-propionyl]-L-proline (49). To 0.44 g (0.011 mol) of NaH (60% oil dispersion) washed with hexane was added dropwise a solution of 1.60 g (0.011 mol) of thiobenzoic acid in 25 mL of ethanol. The mixture was stirred for 1 h, and 3.7 g (0.01 mol) of 1-[3-bromo-3-(4-fluorobenzoyl)propionyl]-L-proline was added. The mixture was stirred at room temperature for 18 h, and the solvent removed in vacuo. The residue was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract was washed with saturated NaCl solution and dried ($MgSO_4$), and the solvent was removed to give 3.81 g of white glass. The glass was chromatographed (1 × 15 in. column) over silica gel (60–200 mesh) with ethyl acetate–hexane (75:25) containing 2% acetic acid (100 mL HBV, 20-mL cuts). Fractions 4–6 were combined and rechromatographed to give 1.6 g of white glass [one spot by TLC (ethyl acetate–hexane, 75:25, containing 2% acetic acid]: $[\alpha]^{23}_D$ −55 ± 1° (c 0.958, EtOH).

1-[3(*R*)-(Acetylthio)-3-benzoyl-2(*S*)-methylpropionyl]-L-proline (24). To a partial solution of 280.6 g (0.97 mol) of (*R*)-1-(3-benzoyl-2-methylpropionyl)-L-proline in 2.5 L of acetic acid was added 170 g (1.06 mol) of bromine. The mixture was gassed with HBr for 2 min and stirred at room temperature for 2 h. The mixture was gassed again with HBr and was stirred for 2 h at room temperature. The pale yellow solution was concentrated in vacuo to a syrup. The residue was poured into 2 L of ice–H_2O and extracted with two 1-L portions of CH_2Cl_2. The CH_2Cl_2 extract was washed with two 1-L portions of H_2O and with 1 L of saturated NaCl solution. The extract was dried ($MgSO_4$) and filtered. To the filtrate was added 165 g (1.45 mol) of potassium thioacetate (exotherm-moderated with cold H_2O bath). The mixture was stirred at room temperature for 18 h and washed

with two 1-L portions of H_2O and 1 L of NaCl solution. The solution was dried ($MgSO_4$), and the solvent was removed in vacuo to give 389 g of a gum. The gum was dissolved in 500 mL of hot ether and allowed to cool to room temperature and stand for 18 h. The mixture was filtered to give 24.5 g of white crystals (9%), mp 190–204 °C of (*R*)-1-(3-benzoyl-2-methylpropionyl)-L-proline. The filtrate was concentrated in vacuo to give 350 g of gum. The gum was divided into three parts and each was chromatographed on a Jobin Yvon Chromatospac Prep column with ethyl acetate–hexane (75:25) containing 2% acetic acid as solvent. The column was packed with 1.5 kg of silica gel (60H), and samples were placed on the column with 100 mL of eluting solvent. Cuts containing desired isomer (front running) were combined, and the solvent was removed. The residue (combined from three columns) was crystallized from ether to give 54.8 g of crystals, mp 155–161 °C. Intermediate cuts (from three columns) (182 g) were rechromatographed (two columns) to give 21.4 g of crystals. The intermediate cuts from the latter two columns were combined to give 149 g of gum. The gum (7.5-g portions) was chromatographed on a Waters Prep 500 HPLC column with hexane–ethyl acetate–formic acid (60:38:2) (flow rate 250 mL/min) as solvent. Peaks were detected by UV (300 nm) and *R,S* diastereomer (t_R = 2.5 min) was separated from *S,S* diastereomer (t_R = 3.5 min) to give 22.9 g of crystals.

The total crystalline product (99.1 g) was dissolved in a minimum amount (800 mL) of warm (60 °C) ethyl acetate and filtered, and the filtrate was diluted with boiling hexane (1 L). The solution was allowed to cool slowly to room temperature (6 h) and chilled overnight. Filtration gave 85.7 g of white crystals: mp 161–163 °C; $[\alpha]^{23}_D$ +276 ± 1° (c 0.709, EtOH).

1-[2-(Acetylthio)-3-benzoylpropionyl]-L-proline (34). A. To a solution of 26.4 g (0.15 mol) of benzoylacrylic acid and 17.25 g (0.15 mol) of *N*-hydroxysuccinimide in 165 mL of dioxane was added 30.9 g (0.15 mol) of *N,N*-dicyclohexylcarbodiimide in 120 mL of dioxane. The mixture was stirred at room temperature for 18 h and filtered. The filtrate was concentrated to dryness, and the residual oil was triturated with hexane to give 46 g of waxy solid. The solid was dissolved in 250 mL of CH_2Cl_2, and hexane (250 mL) was added until the solution was turbid. Chilling and filtering gave 18.2 g (45%) of yellow crystals, mp 96–99 °C. Dilution of mother liquors with hexane (100 mL) gave an additional 7.73 g of yellow crystals, mp 97–100 °C.

To a solution of 7.71 g (0.067 mol) of L-proline and 11.26 g of $NaHCO_3$ in 240 mL of H_2O was added a slurry of 18.2 g (0.067 mol) of the above activated ester in 240 mL of ethanol. The mixture was stirred (23 °C) for 18 h and concentrated to one-half volume. The mixture was chilled, acidified with concentrated HCl, and extracted with CH_2Cl_2. The extract was washed with H_2O and saturated NaCl solution and dried ($MgSO_4$). The solvent was removed, and the residue was triturated with 50 mL of ethyl acetate–hexane (75:25) containing 2% acetic acid to give 10.1 g (57%) of off-white crystals, mp 122–124 °C. To 2.57 g (0.01 mol) of 1-(3-benzoylacryloyl)-L-proline was added 0.71 mL (0.02 mol) of thioacetic acid. An additional 0.71 mL of thioacetic acid was added as well as 30 mL of CCl_4. The mixture was stirred for 1 h, and the solvent was removed. The residual gum was repeatedly dissolved in CH_2Cl_2, and the solvent was removed to give 3.0 g of a glass. Chromatography over silica gel (1 × 15 in. column) (60–200 mesh) with ethyl acetate–hexane (75:25) containing 2% acetic acid gave a glass [one spot by TLC (ethyl acetate–hexane, 75:25, + 2% acetic acid). The glass was dissolved in CH_2Cl_2, and the solvent was removed in vacuo (1.56 g off-white glass). The ^1H NMR spectrum showed a mixture of two diastereomers solvated with CH_2Cl_2. Anal. ($C_{17}H_{19}SNO_5 \cdot 0.1CH_2Cl_2$) H, N, S, C; Cl: calcd, 1.98; found, 1.0.

B. To a stirred suspension of 3.86 g (0.015 mol) of 1-(3-benzoylacryloyl)-L-proline in 45 mL of CCl_4 was added 2.13 mL (0.03 mol) of thioacetic acid. The mixture was stirred at room temperature for 2 h, and the solvent was removed in vacuo. Toluene was added repeatedly, and the solvent was removed to give an amber gum. The gum was dissolved in 10 mL of ethyl acetate and 10 mL of hexane, and 0.4 mL of acetic acid was then added. Cooling and filtering gave 2.11 g (40%) of white crystals, mp 133–140 °C. The crystals were dissolved in 20 mL of warm ethyl acetate, and 20 mL of hexane was then added. Chilling gave 1.60 g of white crystals, mp 149–150 °C. Recrystallization from ethyl acetate–hexane gave 1.36 g (30%) of **34** as white crystals: mp 149–150 °C; $[\alpha]^{25}_D$ −151 ± 1° (c 0.962, EtOH).

1-[3-(4-Bromobenzoyl)-4-(acetylthio)butyryl]-L-proline Methyl Ester (32). A solution of 1.22 g (0.015 mol) of dimethylamine hydrochloride in 0.87 mL of 37% aqueous formaldehyde was allowed to stand at room temperature for 30 min. To the mixture was added 6.0 mL (0.064 mol) of acetic anhydride. To the warm solution was added 3.68 g (0.01 mol) of 1-[3-(4-bromobenzoyl)propionyl]-L-proline methyl ester, and the mixture was heated on a steam bath for 3 h. The solvent was removed, and 50 mL of acetone was added. The mixture was heated, and the solvent was removed. The residue was partitioned between H_2O and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2, chilled, and made alkaline with 5 N NaOH. The mixture was extracted with CH_2Cl_2, and the extract was washed with H_2O and dried ($MgSO_4$). The solvent was removed to give 2.34 g (55%) of a gum (^1H NMR supports the structure). To a solution of 2.37 g (5.6 mmol) of crude Mannich base in 50 mL of ether was added 1.1 mL (17 mmol) of methyl iodide. The mixture was refluxed for 18 h and filtered. The solid was washed with ether and petroleum ether (30–60 °C) to give 1.94 g (61%) of white solid. The product from two runs (3.5 g, 0.0060 mol) and 1.18 g (0.012 mol) of NaSAc in 85 mL of CH_3CN was stirred for 18 h at room temperature. Acetic acid (1 mL) was added, and the solvent was removed. The residue was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 layer was washed with H_2O, dried ($MgSO_4$), and concentrated. The residue was chromatographed (twice) on silica gel (60–200 mesh) columns (1 × 15 in.) with ethyl acetate–hexane (1:1) containing 2% acetic acid as solvent. Cuts containing product were combined to give 1.5 g (65%) of a glass (one spot by TLC).

1-[3-(4-Bromobenzoyl)-3-mercaptopropionyl]-L-proline (37). To a solution of 4.5 g (0.010 mol) of 1-[3-(4-bromobenzoyl)-3-bromopropionyl]-L-proline methyl ester in 50 mL of isopropyl alcohol was added 1.25 g (0.10 mol) of 1-methyl-2-(1*H*)pyridinethione.[13] The mixture was refluxed for 18 h, and the solvent was removed in vacuo. The dark gum (5.2 g) was stirred with 50 mL of water, and the mixture was extracted with $CHCl_3$. The extract was concentrated in vacuo to give 3.65 g of gum. This gum was stirred with 50 mL of 1 N NaOH at room temperature for 3 h. The mixture was extracted with $CHCl_3$. The aqueous layer was acidified with HCl and extracted with $CHCl_3$. The extract was dried ($MgSO_4$), and the solvent was removed to give a glass, which was triturated with hexane to give 1.1 g of tan amorphous solid. Purification by paritition chromatography on Celite with heptane–ethyl acetate–CH_3OH–H_2O (55:45:15:1) gave 0.539 g of tan glass (^1H NMR supports the structure): TLC (silica gel; ethyl acetate–hexane 75:25, plus 2% acetic acid) showed the material to be approximately 90% pure. Attempts to crystallize the glass were unsuccessful.

1-[3(*R*)-(Acetylthio)-3-benzoyl-2(*S*)-methylpropionyl]-L-proline Methyl Ester (81). To a solution of 3.63 g (0.01 mol) of **24** in 50 mL of CH_2Cl_2 was added dropwise a solution of 1.79 g (0.012 mol) of 3-methyl-1-*p*-tolyltriazene in 50 mL of CH_2Cl_2 over 15 min. After stirring at room temperature for 1 h, the mixture was washed with 1 N HCl and dried ($MgSO_4$). The solvent was removed to give 3.10 g of a gum. The gum was chromatographed over silica gel with 5% MeOH in CH_2Cl_2 as eluent. The solid from cuts 1 and 2 were crystallized from hexane to give 2.5 g of white crystals, mp 89–91 °C. Recrystallization (twice) from acetone–hexane gave 0.90 g of white crystals: mp 104–106 °C; $[\alpha]^{30}_D$ +249 ± 1° (c 0.85, EtOH).

(*R*)-1-(3-Benzoyl-2-methylpropionyl)-L-proline Methyl Ester (82). A. To a partial solution of 2.89 g (0.01 mol) of (*R*)-1-(3-benzoyl-2-methylpropionyl)-L-proline in 30 mL of CH_2Cl_2 was added 2.24 g (0.015 mol) of 3-methyl-1-*p*-tolyltriazene. After the vigorous evolution of gas (30 min), the mixture was stirred for an additional 4 h. The solution was washed with 1 N HCl, saturated NaCl solution, and saturated $NaHCO_3$ solution. The solution was dried ($MgSO_4$), and the solvent was removed to give 2.69 g (89%) of crystals. Trituration with hexane containing a trace of ether gave 2.02 g (66%) of white crystals, mp 92–94 °C. Recrystallization from acetone–hexane gave 1.72 g (56%) of white needles: mp 92–94 °C; $[\alpha]^{26}_D$ −37 ± 1° (c 0.910, EtOH).

B. To a suspension of 5.78 g (0.02 mol) of (*R*)-1-(3-benzoyl-2-methylpropionyl)-L-proline in 60 mL of CH_3OH was added 0.2 mL of concentrated H_2SO_4. The mixture was stirred and refluxed

for 6 h. The solution was cooled, and 0.95 g of sodium acetate was added. The solvent was removed, and the residue was partitioned between Et_2O and H_2O. The Et_2O layer was washed with H_2O, 1 N NaOH, and NaCl solution, dried ($MgSO_4$), and filtered. Chilling the filtrate gave 3.0 g (48%) of white crystals: mp 90–92 °C; $[\alpha]^{26}_D$ −37 ± 1° (c 1.30, EtOH). The filter cake ($MgSO_4$) was washed with CH_2Cl_2, and the washings were combined with the Et_2O mother liquors. The solvent was removed, and the residue was triturated with hexane to give an additional 2.37 g (39%) of crystals, mp 91–93 °C.

Measurement of Antihypertensive Effects. In vitro inhibition of angiotensin converting enzyme (ACE) was measured by the method of Cushman and Cheung[14] with benzoylglycylhistidylleucine as substrate. For in vivo studies in aorta-coarcted, hypertensive rats, hypertension was induced by complete ligation of the aorta between the origin of the two renal arteries according to the method of Rojo-Ortega and Genest,[16] with a minor modification of the surgical procedure.[17]

Preparation of Crude Angiotensin Converting Enzyme. Five grams of rabbit lung acetone powder obtained from Pel-Freez Biologicals, Inc., Rogers, AR, was blended with 50 mL of phosphate buffer (50 mM, pH 8.3) and centrifuged at 4 °C. The clear supernatant was kept in the refrigerator and used as an enzyme source for all the IC_{50} determinations reported in this study.

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Registry No. **9** (X = *m*-F), 66549-02-8; **18**, 84234-91-3; **21B**, 75442-75-0; **22B**, 75442-71-6; **23A**, 75493-88-8; **24A**, 75381-07-6; **25**, 1771-65-9; (±)-**25**, 84143-16-8; (+)-**25**, 84143-17-9; (−)-**25**, 84143-18-0; (−)-**25** (*R*)-2-amino-1-butanol, 84143-19-1; (−)-**25** (*S*)-α-methylbenzylamine, 84143-20-4; **26**, 15121-74-1; angiotensin converting enzyme, 15121-75-2; **28**, 75381-03-2; **29**, 84143-21-5; **30**, 75381-21-4; **31**, 84143-22-6; **31** methyl iodide, 84143-23-7; **32**, 84143-24-8; (*R*)-**32**, 84234-92-4; (*R*)-**34**, 84234-93-5; (*S*)-**34**, 84234-94-6; **35**, 75381-29-2; **36**, 75381-24-7; **37A**, 84234-95-7; **37B**, 84234-96-8; **40**, 75442-72-7; **41B**, 75442-80-7; **42A**, 75442-79-4; **43A**, 75382-15-9; **44A**, 84234-97-9; **44B**, 84234-98-0; **45A**, 84234-99-1; **45B**, 84235-00-7; **46A**, 84235-01-8; **46B**, 84235-02-9; **47A**, 84235-03-0; **47B**, 84235-04-1; **48A**, 84235-05-2; **48B**, 84235-06-3; **49A**, 84235-07-4; **49B**, 84235-08-5; **50A**, 84235-09-6; **50B**, 84235-10-9; **51A**, 84235-11-0; **51B**, 84235-12-1; **52B**, 75442-82-9; **53A**, 84235-13-2; **53B**, 84235-14-3; **54A**, 84235-15-4; **54B**, 84235-16-5; **55A**, 84235-17-6; **55B**, 84235-18-7; **56A**, 84235-19-8; **56B**, 84235-20-1; **57A**, 75442-88-5; **57B**, 75442-87-4; **58A**, 84235-21-2; **58B**, 84235-22-3; **59A**, 84235-23-4; **59B**, 84235-24-5; **60A**, 84235-25-6; **60B**, 84235-26-7; **61A**, 84235-27-8; **61B**, 84235-28-9; **62B**, 84235-29-0; **63A**, 75382-16-0; **64**, 75381-20-3; **65**, 75381-13-4; **66**, 75381-28-1; **67**, 75381-11-2; **68**, 75381-12-3; **69**, 75442-76-1; **69** dicyclohexylamine, 84143-25-9; **70**, 75381-95-2; **71**, 75382-13-7; **72**, 75382-01-3; **73**, 75381-68-9; **74**, 75381-87-2; **75**, 75381-55-4; (*S*)-**76**, 75381-04-3; (*R*)-**76**, 75381-03-2; **77**, 75381-52-1; **78**, 75381-91-8; **79**, 75382-34-2; **80**, 75381-42-9; **81**, 84143-26-0; **82**, 84143-27-1; acrylonitrile, 107-13-1; 3-(3-fluorobenzoyl)propionic acid, 69797-46-2; 3-[4-(4-chlorophenoxy)benzoyl]propionic acid, 57148-29-5; 4-chlorophenol, 106-48-9; (−)-α-methylbenzylamine, 2627-86-3; *N*-hydroxysuccinimide, 6066-82-6; succinimido 3-benzoyl-2-methylpropionate, 75380-99-3; succinimido 3-(4-chlorobenzoyl)propionate, 75381-22-5; L-proline, 147-85-3; 3-(4-bromobenzyoyl)propionic acid, 6340-79-0; L-proline methyl ester hydrochloride, 2133-40-6; succinimido 3-(4-bromobenzoyl)propionate, 75381-22-5; succinimido 3-(3-fluorobenzoyl)-2-methylpropionate, 84143-28-2; 1-[3-bromo-3-(4-fluorobenzoyl]-L-proline, 75381-14-5; potassium thioacetate, 10387-40-3; thiobenzoic acid, 98-91-9; benzoylacrylic acid, 583-06-2; succinimido benzoylacrylate, 75381-00-9; 1-(3-benzoylacryloyl)-L-proline, 75381-01-0; dimethylamine hydrochloride, 506-59-2; formaldehyde, 50-00-0; 1-methyl-2(1*H*)pyridinethione, 2044-27-1; (*R*)-2-amino-1-butanol, 5856-63-3; sodium thioacetate, 34832-35-4; angiotensin converting enzyme, 9015-82-1; 3-(4-fluorobenzoyl)propionic acid, 366-77-8.