Angiotensin Converting Enzyme Inhibitors: Spirapril and Related Compounds

Elizabeth M. Smith,*,† Gerald F. Swiss,† Bernard R. Neustadt,† Paul McNamara,† Elijah H. Gold,† Edmund J. Sybertz,‡ and Thomas Baum‡,§

Departments of Medicinal Chemistry and Pharmacology, Schering-Plough Corporation, Bloomfield, New Jersey 07003.
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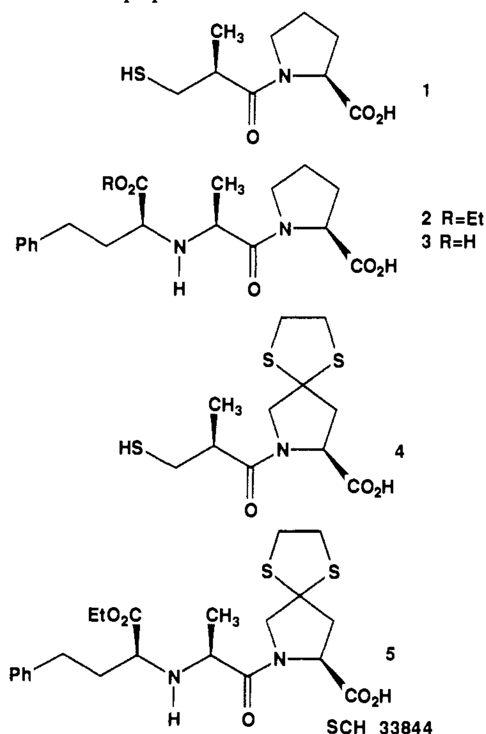
The synthesis of spirapril (**5**), spiraprilat (**25**), their *RSS* stereoisomers, and their glycyl (**18b**) and lysyl (**36**, **37**) analogues is described. These compounds were evaluated in vivo for inhibition of angiotensin converting enzyme (ACE), and selected compounds were evaluated for in vitro ACE inhibition (spirapril ID_{50} 16 μg/kg; spiraprilat IC_{50} 0.8 nM, ID_{50} 8 μg/kg). In anesthetized rats, iv, esters **5** and **36** are more potent than enalapril, and diacids **25** and **37** are more potent than enalaprilat in vitro. In the conscious rats, orally, **5** and enalapril (**2**) showed potent and sustained activity at doses of 0.03-1 and 0.1-1 mg/kg, respectively. From this work, spirapril was selected for clinical evaluation as an antihypertensive agent.

The design and development of captopril (**1**), the first nonpeptide orally active angiotensin converting enzyme (ACE) inhibitor for the treatment of hypertension, were disclosed in 1977.[1] This discovery resulted in the study of many compounds which differ from captopril by the replacement of the C-terminal proline with other amino acids. In 1980, enalapril (**2**), the first nonmercaptan ACE inhibitor, was disclosed.[2] Enalapril is an ethyl ester prodrug of the active inhibitor enalaprilat (**3**). Many analogues of enalapril have been reported.[3] Our investigation of the substitution of other novel amino acids for the proline in captopril led to the discovery of **4** and related compounds.[4] Incorporation into enalapril of the same novel amino acid found in **4** resulted in very potent ACE inhibitors: ester **5** (spirapril, SCH 33844)[5-10] and diacid **25** (spiralprilat, SCH 33861).[11] The synthesis and biological activity of these and several related compounds are described in this paper.



Chemistry. Three groups of compounds were prepared—the glycine, alanine, and lysine groups. The proline dithioketal acid and esters required for these were prepared as shown in Scheme I. For the glycine and alanine series, the desired dipeptide derivatives were prepared as mixtures of diastereomers by a final reductive alkylation step (Scheme I). Alternatively, compounds

† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Deceased Feb 1985.

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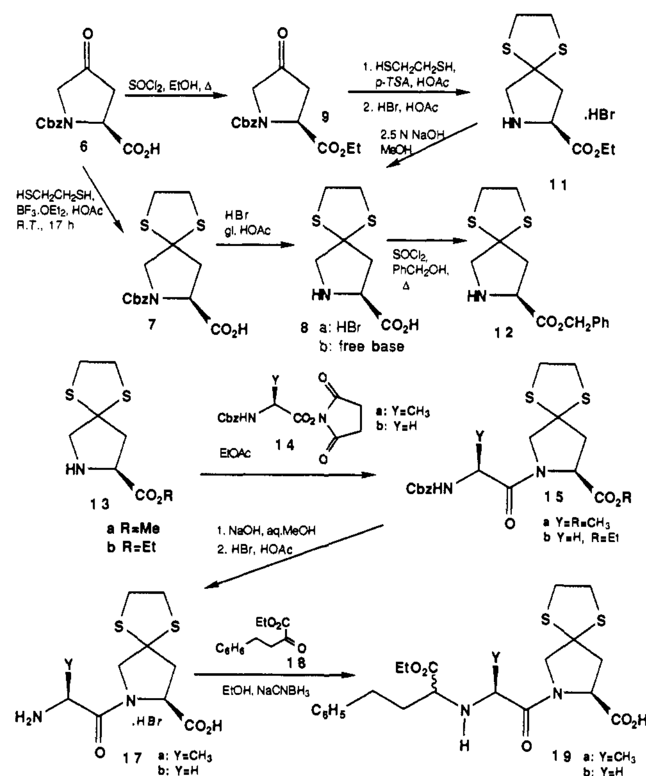
Scheme I



could be obtained as single diastereomers by separation of the diastereomers of the intermediate amino ester and subsequent coupling (Scheme II). The hydroxysuccinimide coupling method proved particularly useful for the coupling of the proline acid with the activated ester–monoacid.

The compounds of the lysine series were prepared (Scheme III) from the ester–monoacid, which could be obtained via either of two routes.[12] Protection via benzyl ester and N-Cbz groups, which could be removed simultaneously with HBr, was advantageous. Coupling was achieved via the azide method.

Results and Discussion

Compounds were tested for in vitro ACE-inhibitory activity by the procedure of Cushman and Cheung,[13] and

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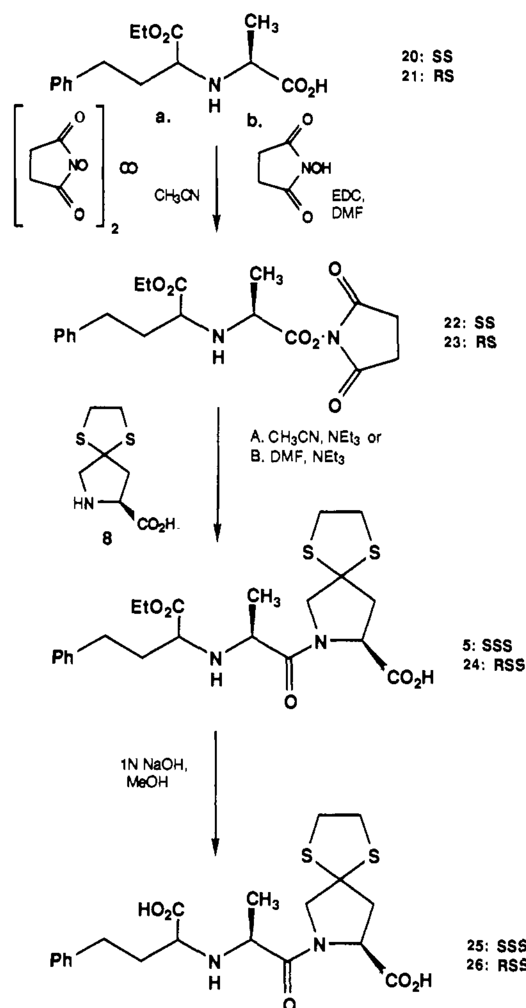
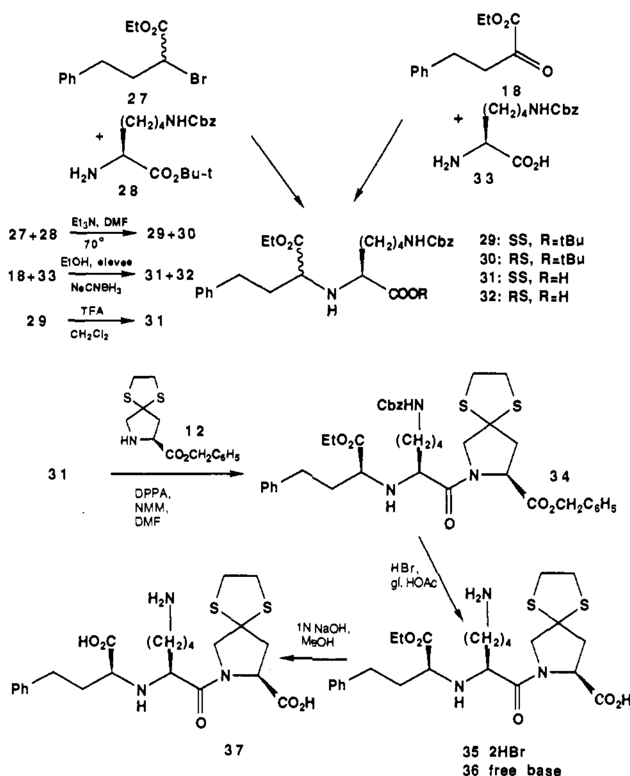
(12) The assignment of S,S stereochemistry to **31** is based on related work in which **31** and **32** were coupled with octahydroindole-2(S)-carboxylic acid. Since the product derived from **31** showed ACE-inhibitory activity far superior to that derived from **32**, the diester was assigned the S,S configuration.

Scheme II



Scheme III



the results are given in Table I. As in the comparison of enalapril (**2**) with enalaprilat (**3**), the monoesters spirapril

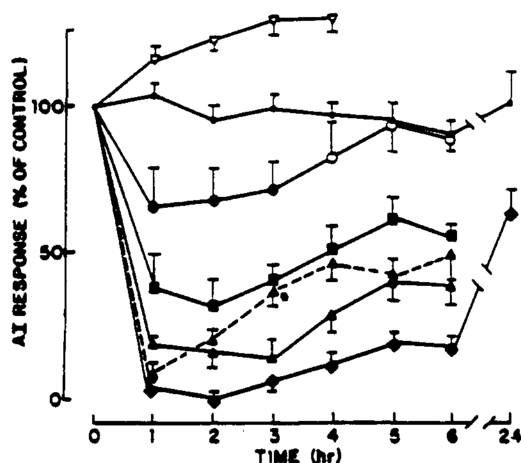


Figure 1. Angiotensin converting enzyme inhibitory activity of orally administered spirapril, spirapril hydrochloride, and spirapril diacid in conscious rats. AI (0.3 mg/kg) was injected iv before (time = 0) and at 30-min intervals after oral administration of test drug or placebo for 4–6 h. Only hourly data (mean ± SEM, expressed as percent of control) are presented. Four placebo-treated animals and all animals receiving 1 mg/kg of SCH 33844 also were challenged with AI 24 h after dosing. Dose code (mg/kg) is as follows (number of animals per group is in parentheses; closed symbol indicates $P < 0.05$ vs placebo; *$P < 0.05$ vs same dose of SCH 33844): ●—●, 0 (9); ○—○, 0.03 (6); □—□, 0.1 (8); △—△, 0.3 (6); ◇—◇, 1 (9); △- - -△, 0.3, HCl salt (5); ▽—▽, 1, diacid, (5).

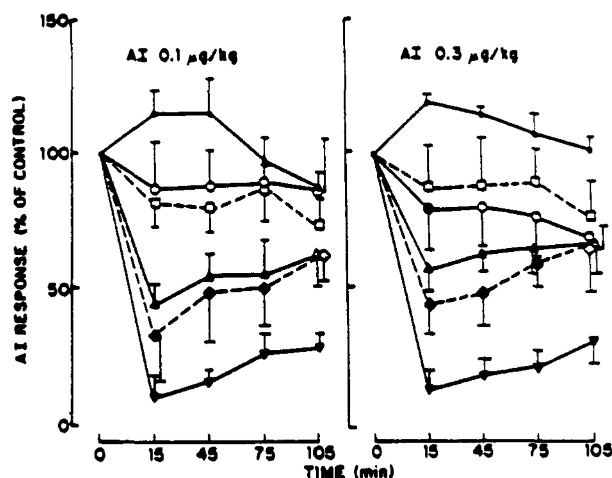


Figure 2. Effect of intravenous administration of spirapril and its diacid on pressor responses to angiotension I in anesthetized dogs. AI (0.1 and 0.3 µg/kg, iv) was injected before and at the indicated times after test drug or placebo (4% ethanol in saline). Data (mean ± SEM) after test drug are expressed as a percent of the control AI response. Dose code (µg/kg) is as follows (number of animals per group is in parentheses; closed symbols indicate that AI response is significantly different from that in the placebo group at $P < 0.05$, analysis of variance): ●, 0 (4); ○, 100 (4); △, 300 (4); ▽, 1000 (5); □, 10, diacid (4); ◇ 30, diacid (6).

(5) and **36** are significantly less potent in vitro ACE inhibitors than their corresponding diacids **25** and **37**. This result is in agreement with the observation of others with regard to a variety of ACE inhibitors bearing the carboxyalkyl group.[2] In all cases, it has been demonstrated that, for enzyme inhibition, the carboxyalkyl group must be in the acid form. The in vitro data also show that spiraprilat (**25**) and **37** are comparable to each other in ACE-inhibitory potency and that they are significantly more potent than captopril (**1**) and somewhat more potent than enalaprilat (**3**) in the direct enzyme inhibition. These differences in in vitro enzyme inhibitory activity indicate that the $S_{2'}$ subsite of ACE can readily accommodate the 4-spirothioketal ring on the proline portion of the molecule with possible increase in binding energy.

In vivo ACE-inhibitory activity of compounds was determined by intravenous administration in the anesthetized rat (Table I), and oral administration in the conscious rat (Figure 1).[6] The enhanced in vitro potency of spiraprilat (**5**) and **37** vs captopril (**1**) is further reflected in the 4–9-fold increase in in vivo ACE inhibition of spirapril (**5**), spiraprilat (**25**), **36**, and **37** vs captopril (**1**). Furthermore, spirapril (**5**) and **36** are respectively about 4 and 6 times more potent than enalapril (**2**), and diacids spiraprilat (**25**) and **37** are respectively about 2 and 1.5 times more potent than enalaprilat (**3**) in the in vivo assay. Thus, addition of the spiroketal group appears to enhance the pharmacokinetic properties of compounds **5**/**36** and **25**/**37** relative to their unsubstituted analogues **2** and **3**. As seen with enalapril[2] and its analogues, the glycine analogue **18b** and the *RSS* diastereomer **26** are both much less potent than spirapril and spiraprilat, respectively.

The ACE-inhibitory activity described above allowed selection of spirapril and spiraprilat as appropriate candidates for further study. Consequently, the ACE-inhibitory activity was determined upon oral administration in conscious rats. Spirapril demonstrated potent and

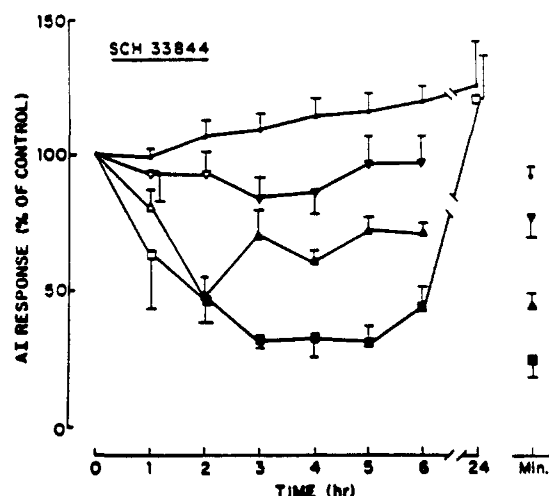


Figure 3. Effect of oral administration of spirapril on pressor responses to angiotensin I in conscious dogs. AI (0.1 µg/kg, iv) was injected before (time = 0) and at hourly intervals after oral administration of the test drug or placebo. Four placebo-treated animals and all dogs receiving 3 mg/kg SCH 33844 were challenged with AI 24 h after dosing. The minimum response to AI (min) regardless of time is at right. Data (mean ± SEM) expressed as percent of control. Dose code (mg/kg) is as follows (number of animals per group is in parentheses; closed symbol indicates $P < 0.05$ vs placebo): ●, placebo (13); ▽, 0.1 (5); △, 1 (6); □, 3 (5).

sustained oral activity at doses of 0.03–1.0 mg/kg (Figure 1)[6] [enalapril (0.1–1 mg/kg)].[14] At the higher dose, significant inhibition was observed for 24-h postdose. Spiraprilat, in contrast, was not active by the oral route (1 mg/kg).

The ACE-inhibitory activity was next studied in dogs. Activity was determined on intravenous administration in the anesthetized dog, and by oral administration to the

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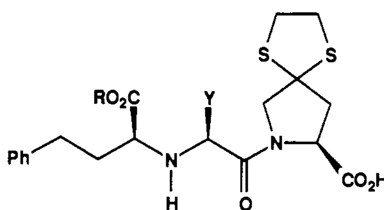
Table I. In Vitro and in Vivo Inhibition of Angiotensin Converting Enzyme



compd	R	Y	stereochemistry	ACE inhibition in vitro IC_{50}, nM	ACE inhibition in vivo ID_{50}, μg/kg[a]
19a	Et	CH_3	*RSS,SSS* (1:1)	92 (5)[b]	
spirapril (**5**)	Et	CH_3	*SSS*	67 (14)[b]	16
spiraprilat (**25**)	H	CH_3	*SSS*	0.8 (14)[b] 0.9 (3.5)[b]	8
26	H	CH_3	*RSS*		800
36	Et	$(CH_2)_4NH_2$	*SSS*	200 (21)[b]	10
37	H	$(CH_2)_4NH_2$	*SSS*	0.5 (25)[b]	10
19b	Et	H	*RS,SS* (1:1)		221
enalapril (**2**)	Et	CH_3	*SSS*	1273	57
enalaprilat (**3**)	H	CH_3	*SSS*	2.6 (3.5)[b]	15
lisinopril	H	$(CH_2)_4NH_2$	*SSS*	1.2[c]	
captopril (**1**)				12.9[d]	71 ± 25

[a] Because of the low number of animals on which these ID_{50} values are based, it is difficult to ascribe a precise value of reliability. However, under the conditions of the experiment described above, captopril and enalapril, two standard ACE inhibitors of two different structural classes, possess ID_{50} values associated with low variability; e.g., the ID_{50} for captopril is 71 ± 25 (mean ± SEM). [b] IC_{50} value of captopril run concurrently. [c] Reference 2g. [d] This range of IC_{50} for captopril (0.0035–0.025 μM) was obtained over a period of almost 2 years, starting with greater values and getting smaller with time. The variation is most likely due to progressive changes in the batches of crude enzyme employed.

conscious dog using the method described under Experimental Section. An intravenous dose–response study (Figure 2)[6] on spirapril allowed determination of the ID_{50} value as 0.3 mg/kg. Duration exceeded the 105-min observation period. In this assay, spiraprilat at a dose of 0.03 mg/kg showed comparable efficacy to 0.3 mg/kg of spirapril, indicating less efficient esterase-induced activation in the dog than in the rat. Oral activity was determined in conscious dogs (Figure 3),[6] with a minimum effective dose for spirapril of 0.3 mg/kg. Duration was greater than 6 h at 1.0 mg/kg, but no effect was seen at 24 h. Thus, a consistent profile of ACE inhibition was observed in both the rat and the dog.

On the basis of the ACE-inhibitory activity, as well as in-depth pharmacological evaluation, spirapril and spiraprilat were recommended as candidates for clinical evaluation in the treatment of hypertension and are in advanced clinical evaluation.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. The NMR spectra were recorded on Varian instruments: FT80 H (79 MHz), CFT-20 (79 MHz), EM-390 (90 MHz), and a T60A (60 MHz); IR spectra were recorded on a Perkin-Elmer 180 grating instrument and a Nicolet MX-1 Fourier transform IR instrument; the EI mass spectra were determined with a Finnigan MAT CH-5 instrument and the rotations with a Rudolf Autopol at ambient temperature. Microanalyses were performed by Physical–Analytical Chemistry Department of Schering-Plough Corp.

Rotations: $[\alpha]^{26}_D$ (solvent, concentration). Solvents: C, chloroform; E, ethanol; H, water; M, methanol.

Chemistry. Starting materials were purchased or prepared by literature methods: *N*-[(Phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline (**6**),[15] *N*-[(phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline methyl ester (neutralization of the HBr salt using 1 N NaOH and extraction with EtOAc),[4] 1,4-dithia-7-azaspiro[4.4]nonane-8-(*S*)-carboxylic acid methyl ester HBr (**13a**),[4] *N*-[(phenylmethoxy)carbonyl]-(*S*)-alanine hydroxysuccinimide ester (**14a**) (Sigma), *N*-[(phenylmethoxy)carbonyl]glycine hydroxysuccimide ester (**14b**),[16] 2-bromo-4-phenylbutyric acid ethyl ester (**27**) (Parish), 4-phenyl-2-oxobutyric acid ethyl ester (**18**)[2] (also available from Fluka), *N*-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanine (**20**),[17] *N*-[1(*R*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanine (**21**),[18] and N^ϵ-[(phenylmethoxy)carbonyl]-(S)-lysine *tert*-butyl ester (**28**) (Bachem). Abbreviation: EDC, 3-ethyl-1-[(dimethylamino)propyl]carbodiimide hydrochloride.

7-[(Phenylmethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]-nonane-8(*S*)-carboxylic Acid (7). *N*-[(Phenylmethoxy)-carbonyl]-4-oxo-(*S*)-proline (**6**) (1.31 g, 0.005 mol), boron trifluoride etherate (0.62 mL), and ethanedithiol (0.42 mL) in glacial HOAc (10 mL) were stirred at room temperature for 17 h. The reaction was poured into ice water and extracted with EtOAc. The EtOAc solution was extracted with saturated $NaHCO_3$ solution. The basic solution was made acidic with concentrated HCl, and the acidic solution was extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated under reduced pressure to give the title compound (**7**) as a colorless foam (1.3 g). This was dissolved in absolute EtOH (5 mL) and treated with cyclohexylamine (0.36 g) in absolute EtOH (1.0 mL). The mixture was diluted with Et_2O (60 mL), and the resulting white precipitate was filtered to yield the cyclohexylamine salt of **7**, as a white solid (1.36 g): mp 207–208 °C; $[\alpha]^{26}_D$ −3.6° (M, *c* 0.3). Anal. ($C_{21}H_{30}N_2O_4S_2$) C, H, N.

1,4-Dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Hydrobromide (8a). *N*-[(Phenylmethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid (**7**) (16.31 g, 0.048 mol) in glacial HOAc (25 mL) was treated with 29% HBr in glacial HOAc (40 mL) at room temperature for 3 h. The reaction mixture was poured into Et_2O (300 mL) and filtered to give a tan solid (12.3 g). The tan solid (6.0 g) was dissolved in hot EtOH (100 mL), and Darco (6.0 g) was added. The mixture was filtered and the filtrate recycled through the Darco. The EtOH solution was concentrated to 25 mL to give a white precipitate, which was filtered to give **8a** as a white solid (3.22 g), $[\alpha]^{26}_D$ +10.7° (C–M, 1:1, *c* 0.5). Anal. ($C_7H_{11}NO_2S_2\cdot HBr$) C, H, N.

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N-[(Phenylmethoxy)carbonyl]-4-oxo-(S)-proline Ethyl Ester (9). A solution of N-[(phenylmethoxy)carbonyl]-4-oxo-(S)-proline (6) (13.0 g, 0.034 mol) dissolved in absolute EtOH (220 mL) was treated with thionyl chloride (8 mL), and the resulting mixture was heated under reflux for 20 h. The reaction mixture was concentrated under reduced pressure, and the residue was placed on a column of silica gel (300 mL) and eluted with hexane–EtOAc 4:1, to yield **9** as a pale yellow oil (6.8 g), $[\alpha]^{26}_D$ −1.7° (E, c 0.5). Anal. ($C_{15}H_{17}NO_5 \cdot {}^1/_8CHCl_3$) C, H, N.

N-[(Phenylmethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Ethyl Ester (10). A solution of N-[(phenylmethoxy)carbonyl]-4-oxo-(S)-proline ethyl ester (**9**) (6.5 g, 0.022 mol) in glacial HOAc (100 mL) was treated with 1,2-ethanedithiol (3 mL) and p-toluenesulfonic acid (0.66 g). The reaction mixture was stirred under reflux for 44 h, cooled, and then added dropwise to saturated $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2, and the dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give a residue which was placed on a column of silica gel (300 g) and eluted with hexane–EtOAc, 4:1. Fractions containing the title compound were concentrated under reduced pressure to give **10** as a pale yellow oil (3.86 g), $[\alpha]^{26}_D$ −21.0° (E, c 0.3). Anal. ($C_{17}H_{21}NO_4S_2$) C, H, N.

1,4-Dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Ethyl Ester Hydrobromide (11). N-[(Phenylmethoxy)carbonyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic acid ethyl ester (**10**) (7.5 g, 0.02 mol) was treated with 20% HBr in glacial HOAc (20 mL) at 0–5 °C and then warmed to room temperature and stirred for 2 h. The reaction mixture was poured into 1.8 L of cold Et_2O to give a pale yellow precipitate. The mixture was filtered, the solid was dissolved in EtOH, and the ethanolic solution was concentrated under reduced pressure to give 11 as a tan oily foam (6.3 g), which was used in the next step.

1,4-Dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid (8b). A solution of 1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic acid ethyl ester HBr (11) (6.3 g, 0.0194 mol) in MeOH (100 mL) at 0–5 °C was treated with 2.5 N NaOH (17 mL) and stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure to give a white solid, which was placed on a column of silica gel (900 mL) and eluted with $CHCl_3$–2-propanol–7% NH_4OH, 1:1:1, to give **8b** as a white foam (3.64 g), $[\alpha]^{26}_D$ −17.6° (H, c 0.4). Anal. ($C_7H_{11}NO_2S_2 \cdot {}^1/_2H_2O$) C, H, N.

1,4-Dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Benzyl Ester (12). 1,4-Dithia-7-azasprio[4.4]nonane-8(S)-carboxylic acid hydrobromide (**8a**) (10.0 g, 0.035 mol) in benzyl alcohol (150 mL) at 0–5 °C was treated with thionyl chloride (50 mL), warmed to 95–100 °C and stirred at that temperature for 3 h, cooled, and then poured into Et_2O (3.5 L). The Et_2O was decanted, and the brown residue was washed with Et_2O and then dissolved in CH_2Cl_2. The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give an amber brown residue, which was partitioned between Et_2O (800 mL) and 1 N NaOH (140 mL). The aqueous solution was extracted with additional Et_2O (300 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to give **12** as an amber oil (5.81 g), which was used without further purification.

7-{N-[(Phenylmethoxy)carbonyl]-(S)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Methyl Ester (15a). A solution of 1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic acid methyl ester (**13a**) (0.40 g, 0.0018 mol) in EtOAc (200 mL) was treated with N-[(phenylmethoxy)carbonyl]-(S)-alanine hydroxysuccinimide ester (**14a**) (0.65 g, 0.002 mol) and then stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure and then placed on a column of silica gel and eluted with $CHCl_3$–EtOHAc, 3:1. The fractions containing the title compound were concentrated under reduced pressure to give **15a** as a yellow oil (0.20 g), $[\alpha]^{26}_D$−14.8° (E, c 0.4). Anal. ($C_{19}H_{24}N_2O_5S_2$) H, N; C: calcd, 53.75; found, 53.30.

In a similar manner **13b** (1.91 g) afforded **15b** (3.31 g) as a pale yellow oil, $[\alpha]^{26}_D$ −15.9° (E, c 0.4), after elution from a column of silica gel (300 g) with hexane–EtOAc, 4:1. Anal. $C_{19}H_{24}N_2O_5S_2 \cdot 0.4CHCl_3$) C, H, N.

7-{N-[(Phenylmethoxy)carbonyl]-(S)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid (16a). A solution of 1-{N-[(phenylmethoxy)carbonyl]-(S)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic acid methyl ester (**15a**) (0.56 g, 0.0013 mol) in MeOH (100 mL) was treated with 2.5 N NaOH (10 mL) and stirred at room temperature for 18 h. The reaction mixture was concentrated under reduced pressure, the residue was dissolved in H_2O, and the solution was extracted with EtOAc. The aqueous solution was acidified with 20% HCl solution, and the acidic solution was extracted with EtOAc. The dried ($MgSO_4$) EtOAc solution was concentrated under reduced pressure to give a residue which was placed on a column of silica gel (200 mL) and eluted with glacial HOAc–$CHCl_3$, 4:96. The fractions containing the product were concentrated under reduced pressure to give **16a** as a brown oil (0.20 g), $[\alpha]^{26}_D$ −15.8° (E, c 0.3). Anal. ($C_{18}H_{22}N_2O_5S_2 \cdot 0.5H_2O$) C, H; N: calcd, 6.68; found, 5.42.

In an analogous manner, **15b** (3.00 g) was hydrolyzed to **16b** as a colorless oil (2.69 g), $[\alpha]^{26}_D$ −7.9° (E, c 0.2). Anal. ($C_{17}H_{20}N_2O_5S_2 \cdot {}^3/_4H_2O$) C, H, N.

7-[(S)-Alanyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Hydrobromide (17a). 7-{N-[(Phenylmethoxy)carbonyl]-(S)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8-(S)-carboxylic acid (**16a**) (0.17 g, 0.0004 mol) was dissolved in 20% HBr in glacial HOAc (10 mL) and stirred at room temperature for 2 h. The reaction mixture was added dropwise to cold Et_2O to give **17a** as a precipitate, which was collected and used in the next step.

In a similar manner, **16b** (2.6 g) was converted to **17b** as an off-white foam (1.76 g), $[\alpha]^{26}_D$ −18.7° (E, c 0.5). MS, m/e 342 (M^+).

7-{N-[1(R,S)-(Ethoxycarbonyl)-3-phenylpropyl]-(S)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid (19a). A solution of 7-[(S)-alanyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic acid hydrobromide (**17a**) (0.86 g, 0.0024 mol) in anhydrous MeOH (100 mL) was treated with 4-phenyl-2-oxobutyric acid ethyl ester (0.5 g, 0.0024 mol) and 3-Å molecular sieves (10 mL) and was stirred at room temperature for 18 h. The mixture was filtered, the filtrate was treated with sodium cyanoborohydride (0.33 g), and the resulting mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated under nitrogen at room temperature, and oil was dissolved in 10% HCl (pH 1), and the mixture was stirred for 1 h. The solution was made bacic (pH 8) with dilute NaOH. The resulting solution was adsorbed on XAD-2 resin. The resin was washed with H_2O and then with MeOH. The fractions containing the product were concentrated under reduced pressure to give an oily residue (0.87 g), which was placed on a column of silica gel (100 mL) and eluted with $CHCl_3$–2-propanol–7%NH_4OH, 1:1:1 (organic layer). The fractions containing the product were concentrated under reduced pressure to give **19a** as an oil (0.17 g), $[\alpha]^{26}_D$ −25.7° (E, c 0.4). Anal. ($C_{22}H_{30}N_2O_5S_2 \cdot {}^1/_4NH_3 \cdot {}^1/_2H_2O$) C, H, N.

In an analogous manner, **17b** (1.27 g) was coupled with 18 (2.00 g) to provide **19b** as a white solid (0.048 g): $[\alpha]^{26}_D$ −39.0° (E, c 0.5); MS, m/e 334 ($M^+ - 18$).

7-{N-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-(S)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid (5). Method A. A solution of N-[1(S)-(ethoxycarbonyl)-3-phenylpropyl]-(S)-alanine (**20**) (4.00 g, 0.014 mol) in CH_3CN (320 mL) was treated with N,N-disuccinimidyl carbonate (3.68 g) and pyridine (1.16 mL) and stirred at room temperature for 44 h. This reaction mixture, containing N-[1(S)-(ethoxycarbonyl)-3-phenylpropyl]-(S)-alanine hydroxysuccinimide ester (**22**), was treated with 1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic acid (**8b**) (2.59 g, 0.0126 mol) and triethylamine (2.0 mL) and stirred at room temperature for 6 h. Additional CH_3CN (250 mL) was added, and the mixture was stirred for 18 h. Additional triethylamine (2.0 mL) and H_2O (50 mL) were added, and the resulting mixture was stirred for 24 h. The mixture was concentrated under reduced pressure, and the residue was placed on a column of silica gel (2 L) and was eluted with $CHCl_3$–2-propanol–7%NH_4OH, 1:1:1 (organic layer), to give **5** as a white foam (0.93 g), $[\alpha]^{26}_D$ −29.5° (E, c 0.2) {lit.[10] $[\alpha]^{26}_D$ −21.3° (pyr, c 1.1)}. Anal. ($C_{22}H_{30}N_2O_5S_2 \cdot {}^1/_2H_2O$) C, H, N. A mixture (1.61 g) of the title compound and N-[1(S)-(ethoxycarbonyl)-3-phenylpropyl]-(S)-alanine was also obtained.

Method B. A mixture of N-[1(S)-(ethoxycarbonyl)-3-phenylpropyl]-(S)-alanine (**20**) (8.98 g, 0.032 mol) and N-hydroxysuccinimide (4.42 g, 0.0384 mol) in dry DMF (64 mL) was treated with 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide

hydrochloride (7.3 g, 0.0384 mol) and stirred at room temperature for 18 h. The reaction mixture was poured into EtOAc (320 mL) and washed with H_2O (100 mL) and then brine (100 mL). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to obtain **22** as an oil. This oil was treated with 1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid hydrobromide (**8a**) (9.16 g, 0.032 mol) in DMF (155 mL) and cooled to 0–5 °C. Triethylamine (8.9 mL, 0.064 mol) was added dropwise to the stirred solution over 5 min, and stirring was continued at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure to 50 mL, diluted with H_2O (150 mL), and adjusted to pH 4 with 1 N HCl. The acidic solution was extracted with EtOAc (4 × 100 mL), and the combined extracts were washed with H_2O (20 mL) and then brine (20 mL). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give crude **5** as a brown oil (15.1 g). The crude product (6.4 g) in MeOH (15 mL) was placed on Sephadex LH-20 (2.5 × 60 cm column) and eluted with MeOH. The desired fractions were combined and the solvent was evaporated under reduced pressure to give **5** as a white foam (4.6 g), $[\alpha]^{26}_D$ −26.3° (EtOH).

In a similar manner, **21** (2.26 g) afforded **23** as a pale yellow oil (3.40 g), which was coupled to **8b** (2.02 g) to give **24** as a white foam (0.95 g): mp 50–53 °C; $[\alpha]^{26}_D$ −35.3° (M, *c* 0.5). Anal. ($C_{22}H_{30}N_2O_5S_2 \cdot {}^1/_2NH_3 \cdot {}^1/_8CHCl_3$) C, H, N.

7-{*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Hydrochloride (5·HCl). The brown oil from method B (2.7 g, 0.0058 mol) was dissolved in CH_3CN (18 mL), treated with concentrated HCl (0.5 mL, 0.006 mol), and kept at 5 °C for 10 h. The crystals were removed by filtration and washed with cold CH_3CN and Et_2O to give **5**·HCl as pink crystals (1.8 g), mp 176–178 °C dec. This salt (0.5 g) was recrystallized from hot MeOH (1.0 mL) and CH_3CN (5 mL) and cooled for 10 h to give **5**·HCl as a white solid (0.4 g): mp 192–194 °C dec; $[\alpha]^{26}_D$ −11.2° (E, *c* 0.4) {lit.[10] $[\alpha]^{26}_D$ 2.0° (M, *c* 1.5)}. Anal. ($C_{22}H_{30}N_2O_5S_2 \cdot HCl$) C, H, N.

7-{*N*-[1(*S*)-Carboxy-3-phenylpropyl]-(*S*)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid (25). 7-{*N*-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid (**5**) (7.91 g, 0.017 mol) in MeOH (50 mL) at 0–5 °C was treated with 2.5 N NaOH (25.0 mL) and stirred at room temperature for 20 h. The MeOH was removed under reduced pressure, and the residue was dissolved in H_2O and placed on a Bio-Rad AG 50W-X2, 100-mesh (hydrogen form), column. The column was eluted with H_2O (20 × 200 mL) followed by 3% C_5H_5N in H_2O to give **25** as a white solid (6.22 g): mp 163–165 °C; $[\alpha]^{26}_D$ +4.1° (E, *c* 0.4). Anal. ($C_{20}H_{26}N_2O_5S_2 \cdot {}^1/_2H_2O$) C, H, N.

In an analogous manner, **24** was hydrolyzed to give **26** as a white solid (0.67 g): mp 134–136 °C; $[\alpha]^{26}_D$ −27.0° (E, *c* 0.5). Anal. ($C_{20}H_{26}N_2O_5S_2 \cdot {}^1/_2H_2O$) C, H, N.

N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine *tert*-Butyl Ester (29). A solution of 2-bromo-4-phenylbutyric acid ethyl ester (**27**) (29.0 g, 0.0915 mol) and N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine *tert*-butyl ester (**28**) (20.0 g, 0.06 mol) in DMF (80 mL) and triethylamine (62 mL) was heated under reflux at 70 °C for 44 h, then concentrated under reduced pressure. The resulting amber oil was treated with Et_2O (1.5 L), and the organic layer was washed with H_2O (4 × 200 mL). The dried ($MgSO_4$) organic layer was concentrated under reduced pressure to give a light amber oil (37.54 g). This oil (12.51 g) was chromatographed (Waters Prep 500, silica gel, two cartridges), eluting with hexane–EtOAc, 3:1. The fractions containing the product were concentrated under reduced pressure to give N^α-[1(*R*)-(ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine *tert*-butyl ester (**30**) as a pale yellow oil (2.79 g) {$[\alpha]^{26}_D$ −5.4° (M, *c* 0.3); anal. ($C_{30}H_{42}N_2O_6$) C, H, N} and N^α-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine *tert*-butyl ester (**29**) as a pale yellow oil (3.24 g) {$[\alpha]^{26}_D$ −2.6° (M, *c* 0.4); anal. ($C_{30}H_{42}N_2O_6$) C, H, N}.

N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (31). N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine *tert*-butyl ester (**29**) (5.17 g, 0.0098 mol) was treated with trifluoroacetic acid (30 mL) at 0–5 °C and stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure, and the residue was placed on a column of silica gel (1.2 L) and eluted with $CHCl_3$–2-propanol–7% NH_4OH, 1:1:1 (organic layer). The fractions containing the product were concentrated under reduced pressure to give **31** as a white solid (3.07 g): mp 114–115 °C; $[\alpha]^{26}_D$ +6.1° (E, *c* 0.3). Anal. ($C_{26}H_{34}N_2O_6$) C, H, N.

N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (31) and N^α-[1(*R*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (32). A solution of N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (4.80 g, 0.017 mol) and 4-phenyl-2-oxobutyric acid ethyl ester (**18**) (7.2 g, 0.035 mol) in absolute EtOH (500 mL) and 3-Å molecular sieves (50 mL) was stirred at room temperature for 18 h. The reaction mixture was treated with sodium cyanoborohydride (3.0 g) and was stirred at room temperature for 24 h. H_2O (15 mL) was added, and the resulting mixture was stirred at room temperature for 10 days. The reaction mixture was concentrated under reduced pressure and then made acidic with concentrated HCl (ice added). After 2 h, the reaction mixture was added to XAD-2 resin and the resin eluted with H_2O (1.5 L), followed by absolute EtOH (1 L). The EtOH solution was concentrated under reduced pressure to give a white semisolid, which was placed on a silica gel column (5 × 70 cm) and eluted with $CHCl_3$–EtOAc, 4:1 (3 L), and then $CHCl_3$–2-propanol–7% NH_4OH, 1:1:1 (organic), to give a pale yellow oil (2.50 g). This oil was chromatographed on a silica gel column (5 × 70 cm), using $CHCl_3$–2-propanol–7% NH_4OH, 1:1:1 (organic), as eluant, to give N^α-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (**31**) as a white solid (0.68 g) {$[\alpha]^{26}_D$ +6.1° (E, *c* 0.25); anal. ($C_{26}H_{34}N_2O_6$) C, H, N} and N^α-[1(*R*)-(ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (**32**) as a white solid (0.74 g) {$[\alpha]^{26}_D$ +1.1° (E, *c* 0.3); anal. ($C_{26}H_{34}N_2O_6$) C, H, N}.

7-{N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Benzyl Ester (34). N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysine (**31**) (3.07 g, 0.0065 mol) and 1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid benzyl ester (**12**) (4.00 g) in anhydrous DMF (35 mL) at 0–5 °C was treated with *N*-methylmorpholine (1.4 mL) in DMF (9 mL), followed by diphenyl phosphorazidate (2.7 mL) in DMF (9 mL). The reaction mixture was stirred at room temperature for 18 h and then poured into saturated aqueous $NaHCO_3$ solution, H_2O added, and the mixture extracted with Et_2O. The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure to give a yellow oil (8.15 g), which was placed on a column of silica gel (2 L) and eluted with EtOAc–hexane, 1:1. The fractions containing the product were concentrated under reduced pressure to give an oil (3.50 g). This oil together with the product (2.48 g) from a repeat of this reaction were combined and placed on a column of silica gel (1.6 L) which was eluted with EtOAc–hexane, 2:1, to give **34** as a pale yellow oil (4.25 g), $[\alpha]^{26}_D$ −18.4° (M, *c* 0.7). Anal. ($C_{40}H_{49}N_3O_7S_2$) C, H, N.

7-{N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Dihydrobromide (35). N^α-{[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-N^ϵ-[(phenylmethoxy)carbonyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid benzyl ester (**34**) (1.54 g, 0.00206 mol) in glacial HOAc (3 mL) at 0–5 °C was treated with 15% HBr in glacial HOAc (15 mL) and was warmed to room temperature. After 2 h, additional 15% HBr in glacial HOAc (10 mL) was added, and the resulting solution was stirred at room temperature for 3 h. The reaction mixture was concentrated under reduced pressure, and additional 15% HBr in glacial HOAc was added. The resulting mixture was stirred at room temperature for 18 h and then concentrated under reduced pressure to give a residue which was washed with Et_2O (2 × 250 mL) to give **35** a tan gray solid (1.29 g), which was used without further purification.

7-{N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid (36). 7-{N^α-[1(*S*)-(Ethoxycarbonyl)-3-phenylpropyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid dihydrobromide (**35**) (1.55 g) was adsorbed on Bio-Rad AG 50W-X2, 100-mesh (hydrogen form), and placed on a column of resin. The

resin was eluted with H_2O (900 mL), H_2O–pyridine, 96:4 (4000 mL), and then H_2O–pyridine–absolute EtOH, 76:4:20 (2000 mL). The fractions containing the product were concentrated under reduced pressure to give **36** as a pale yellow foam (1.0539 g), $[\alpha]^{26}_D$ −21.3 (E, *c* 0.4). Anal. ($C_{25}H_{37}N_3O_5S_2$) C, H, N.

7-{N^{α}-[1(*S*)-Carboxy-3-phenylpropyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid (37). At 0 °C, 7-{N^{α}-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-lysyl}-1,4-dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic acid dihydrobromide (**35**) (1.64 g) was dissolved in 1 N NaOH (15 mL), and the resulting solution was warmed to room temperature and stirred for 16 h. 1 N HCl (10 mL) was added to the reaction mixture, followed by addition of Bio-Rad AG 50W-X2, 100-mesh (hydrogen form), and the resulting mixture was placed on a Bio-Rad AG 50W-X2, 100-mesh, column. The column was eluted with H_2O (900 mL) followed by H_2O–pyridine, 96:4 (2200 mL). The fractions containing the product were concentrated under reduced pressure to give **37** as a white solid (1.18 g), $[\alpha]^{26}_D$ +7.5° (E, *c* 0.3). Anal. ($C_{23}H_{33}N_3O_5S_2 \cdot {}^1/_2H_2O$) C, H; N: calcd, 8.32; found, 7.90.

Biology. In Vitro ACE-Inhibitory Activity. The in vitro inhibitory activity was determined by the method of Cushman and Cheung.[13] The crude ACE was prepared as described in ref 4.

In Vivo ACE-Inhibitory Activity. Anesthetized Rats. Male Sprague-Dawley rats (280–365 g) (two to five) were anesthetized with inactin (100 mg/kg, ip) or dial urethane.[6] The carotid artery and jugular vein were cannulated for blood pressure measurement and iv injection of drugs, respectively. Rectal temperature was maintained at 37 °C with a thermostatically controlled heating pad. Systemic blood pressure was recorded from the carotid artery cannula.

After a 20–30-min stabilization period, angiotensin I (AI, 1 μg/kg, dissolved in saline) was injected iv twice in a volume of 100 μL/kg at 5-min intervals. Data analysis is based on the second response. AI was injected again 5 min after each increasing dose (cumulative totals of 3, 10, and 30 μg/kg, iv) of the test drug. The test drug was administered iv over 3 min in a volume of 0.1 mL/kg at intervals of 10–15 min between doses. The drug (spirapril and spiraprilat) was dissolved in EtOH and diluted to appropriate concentrations with physiological saline solution such that the highest EtOH concentration was 12%.

Pressor responses to AI after each dose of inhibitor were expressed as a percent of the control response and plotted against the log dose of inhibitor. An ID_{50} (dose necessary to inhibit AI response by 50%) was derived from this regression line. Values were obtained for individual animals and averaged.

Conscious Rats. Male Sprague-Dawley rats (270–345 g) were anesthesized and prepared for caudal arterial and venous jugular catheter implantations as described previously.[6,14] Animals were studied on that day or were returned to their cages, fasted overnight, and studied on the next day. Animals were placed into plastic restrainers where they remained for the duration of the experiment. In one series, after a 30-min stabilization period, animals were challenged with AI and II (in volumes of 100 μL/kg followed by a 100-μL flush) twice at 5–10-min intervals during a control period. Test drugs were then administered orally (via a feeding needle) in 0.4% aqueous methylcellulose vehicle in a volume of 2 mL/kg. AI and AII challenges were repeated at 30-min intervals for the next 6 h.

Another series of experiments assessed the ability of increasing doses of AI to overcome the inhibitory effect of spirapril. After 30-min stabilization, animals were challenged with a single dose of AII (0.3 μg/kg, iv). AI then was administered iv in increasing doses, starting with 0.003 μg/kg and increasing by factors of 3 or 3.3 at 5–10-min intervals until a maximum pressor response was achieved (usually 50–60 mmHg at doses of 1 or 3 μg/kg). Animals then were dosed orally with spirapril (3 or 10 mg/kg) or placebo and AII and AI challenges repeated 1 h later. The dose of AI was increased by factors of 3 or 3.3 until a maximum pressor response was achieved. Animals were fasted overnight prior to experimentation.

Anesthetized Dogs. Mongrel dogs of either sex (12.8–21.4 kg) were anesthetized with pentobarbital sodium (30 mg/kg, iv, followed by a continuous iv infusion of 4 mg/k/h).[6] Animals were artificially ventilated with room air at a rate of 12/min and volume of 15 mL/kg. Decamethonium bromide (0.25 mg/kg, iv) was administered to facilitate artificial respiration. Two forelimb veins were cannulated for administration of drug and anesthetic. Animals were maintained at 37 °C with a thermostatically controlled heating pad. Blood pressure was recorded from a cannulated brachial artery. After a 20–30-min stabilization period, AI (0.1 μg/kg), AII (0.1 μg/kg), and bradykinin (0.1 μg/kg) were injected rapidly iv in volumes of 0.1 mL/kg at intervals of 5–10 min between doses. This sequence was repeated during the control period. Data analyses are based on the second series of injections. A single dose of the test compound or vehicle then was administered iv over 5 min, and the sequence of peptide challenges was repeated at 15, 45, 75, and 105 min after termination of the drug or vehicle infusion.

Conscious Dogs. Conditioned mongrel dogs of either sex (13–22.7 kg) were anesthetized with thiopental sodium (20 mg/kg, iv).[6] Anesthesia was maintained by a mixture of methoxyflurane and oxygen. The omocervical artery and omocervical vein were cannulated as described previously.[14] Experiments were performed no sooner than 1 day after the training session and 2 days after surgery.

On the day of the experiment, animals were placed in a sling and allowed a 30-min stabilization period. AI (0.1 μg/kg) and AII (0.056 μg/kg) were injected iv during the control period at intervals of approximately 5 min. Thirty minutes later, a second series of control challenges was performed. Data analyses are based on the second series. Animals then were dosed orally with placebo or spirapril. Peptide challenges were repeated at hourly intervals for 6 h. Animals were removed from the slings for approximately 40 min and 2 h after dosing and returned in time for the 3-h challenges. In some animals, AI and AII challenges also were elicited 24 h after drug administration.

Blood pressure was measured from the omocervical arterial catheter. Drugs injected iv were dissolved in saline and administered rapidly in volumes of 0.05–0.056 mL/kg, followed by 2-mL saline flush. Drugs administered orally were given in gelatin capsules. Empty capsules served as placebos. Dogs were fasted overnight prior to oral administration of test drug.

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