

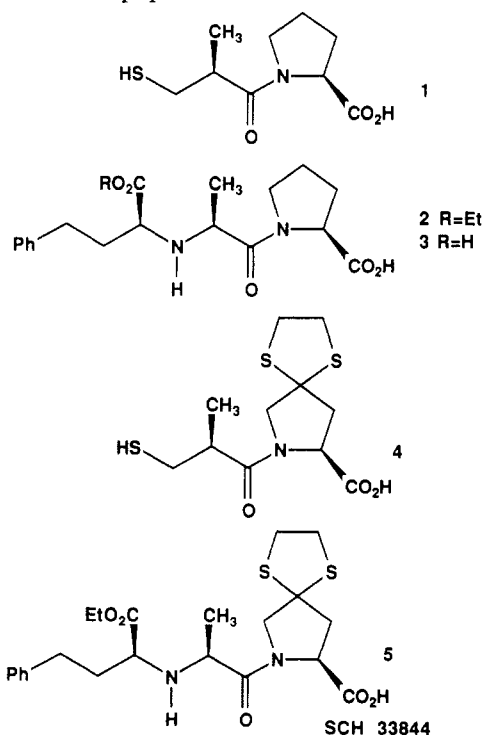
# Angiotensin Converting Enzyme Inhibitors: Spirapril and Related Compounds

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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<sub>50</sub> 16 µg/kg; spiraprilat IC<sub>50</sub> 0.8 nM, ID<sub>50</sub> 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.<sup>1</sup> 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.<sup>2</sup> Enalapril is an ethyl ester prodrug of the active inhibitor enalaprilat (3). Many analogues of enalapril have been reported.<sup>3</sup> Our investigation of the substitution of other novel amino acids for the proline in captopril led to the discovery of 4 and related compounds.<sup>4</sup> Incorporation into enalapril of the same novel amino acid found in 4 resulted in very potent ACE inhibitors: ester 5 (spirapril, SCH 33844)<sup>5-10</sup> and diacid 25 (spiraprilat, SCH 33861).<sup>11</sup> 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

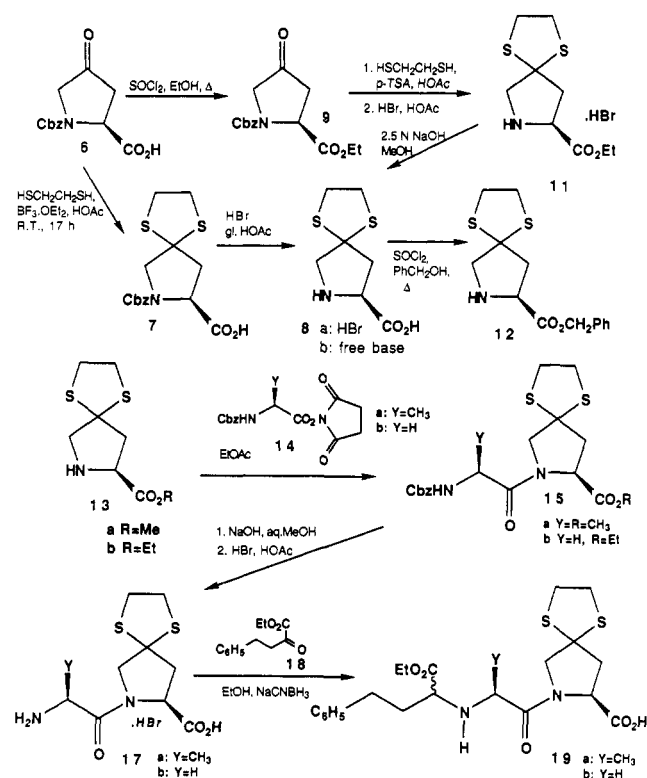
- (1) (a) Ondetti, M. A.; Rubin, B.; Cushman, D. W. *Science (Washington, D.C.)* **1977**, *196*, 441. (b) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.
- (2) Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Broeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.
- (3) (a) Suh, J. T.; Skiles, J. W.; Williams, B. E.; Youssefeyeh, R. D.; Jones, H.; Loev, B.; Neiss, E. S.; Schwab, A.; Mann, W. S.; Khandwala, A.; Wolf, P. S.; Wienryb, I. *J. Med. Chem.* **1985**, *28*, 57. (b) Greenlee, W. J. *Tetrahedron Lett.* **1982**, *23*, 3995. (c) Parsons, W. H.; Davidson, J. L.; Taub, D.; Aster, S. D.; Thorsett, E. D.; Patchett, A. A.; Ulm, E. H.; Lamont, B. I. *Biochem. Biophys. Res. Commun.* **1983**, *117*, 108. (d) Hayashi, K.; Nunami, K.-I.; Sakai, K.; Ozaki, Y.; Kato, J.; Kinashi, K.; Yoneda, N. *Chem. Pharm. Bull.* **1983**, *31*, 3553. (e) Urbach, H.; Henning, R. *Tetrahedron Lett.* **1984**, *25*, 1143. (f) Wyvratt, M. J.; Tristram, E. W.; Ikeler, T. J.; Lohr, N. S.; Joshua, H.; Springer, J. P.; Arison, B. H.; Patchett, A. A. *J. Org. Chem.* **1984**, *49*, 2816. (g) Wu, M. T.; Douglas, A. W.; Ondeyka, D. L.; Payne, L. G.; Ikeler, T. J.; Joshua, H.; Patchett, A. A. *J. Pharm. Sci.* **1985**, *74*, 352. (h) Andrews, P. R.; Carson, J. M.; Caselli, A.; Spark, M. J.; Woods, R. *J. Med. Chem.* **1985**, *28*, 393. (i) Hayashi, K.; Nunami, K.-I.; Sakai, K.; Ozaki, Y.; Kato, J.; Kinashi, K.; Yoneda, N. *Chem. Pharm. Bull.* **1985**, *33*, 2021. (j) Urbach, H.; Henning, R. *Tetrahedron Lett.* **1985**, *26*, 1839. (k) Roark, W. H.; Tinney, F. J.; Cohen, D.; Essenburg, A. D.; Kaplan, H. R. *J. Med. Chem.* **1985**, *28*, 1291. (l) Watthey, J. W. H.; Stanton, J. L.; Desai, M.; Babiaz, J. E.; Finn, B. M. *J. Med. Chem.* **1985**, *28*, 1511. (m) Slade, J.; Stanton, J. L.; Ben-David, D.; Mazzenga, G. C. *J. Med. Chem.* **1985**, *28*, 1517. (n) Johnson, A. L.; Price, W. A.; Wong, P. C.; Vavala, R. F.; Stump, J. M. *J. Med. Chem.* **1985**, *28*, 1596. (o) Stanton, J. L.; Watthey, J. W. H.; Desai, M. N.; Finn, B. M.; Babiarz, J. E.; Tomaselli, H. C. *J. Med. Chem.* **1985**, *28*, 1603. (p) Ksander, G. M.; Yuan, A. M.; Diefenbacher, C. G.; Stanton, J. L. *J. Med. Chem.* **1985**, *28*, 1606. (q) Blankley, C. J.; Kaltenbronn, J. S.; DeJohn, D. E.; Werner, A.; Bennett, L. R.; Bobowski, G.; Krolls, U.; Johnson, D. R.; Pearlman, W. M.; Hoeffle, M. L.; Essenburg, A. D.; Cohen, D. M.; Kaplan, H. R. *J. Med. Chem.* **1987**, *30*, 992. (r) Blacklock, T. J.; Shuman, R. F.; Butcher, J. W.; Shearin, W. E.; Budavari, J.; Grenda, V. J. *J. Org. Chem.* **1988**, *53*, 836. (s) Boyer, S. K.; Pfund, R. A.; Portmann, R. E.; Sedelmeier, G. H.; Wetter, H. F. *Helv. Chim. Acta* **1988**, *71*, 337.
- (4) Smith, E. M.; Swiss, G. F.; Neustadt, B. R.; Gold, E. H.; Sommer, J. A.; Brown, A. D.; Chiu, P. J. S.; Moran, R.; Sybertz, E. J.; Baum, T. *J. Med. Chem.* **1988**, *31*, 875.
- (5) (a) Swiss, G. F.; Yehaskel, A. S.; Bronnenkant, A.; Sunday, B.; Smith, E. M.; Witkowski, J. T.; Neustadt, B. R.; Gold, E. H.; Desiderio, D.; Sybertz, E. J.; Baum, T.; Kastner, B. Ahn, H.-S. *Abstracts, 19th Medicinal Chemistry Symposium, Tucson, AZ, June 1984, Poster 2.* (b) Leitz, F.; Crosio, D.; Nagabhushan, N.; Ernest, M.; Suri, J.; Symchowicz, S.; Patrick, J. *Fed. Proc.* **1987**, *46*, 1147, Abstract 4845.

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<sup>§</sup> Deceased Feb 1985.

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.

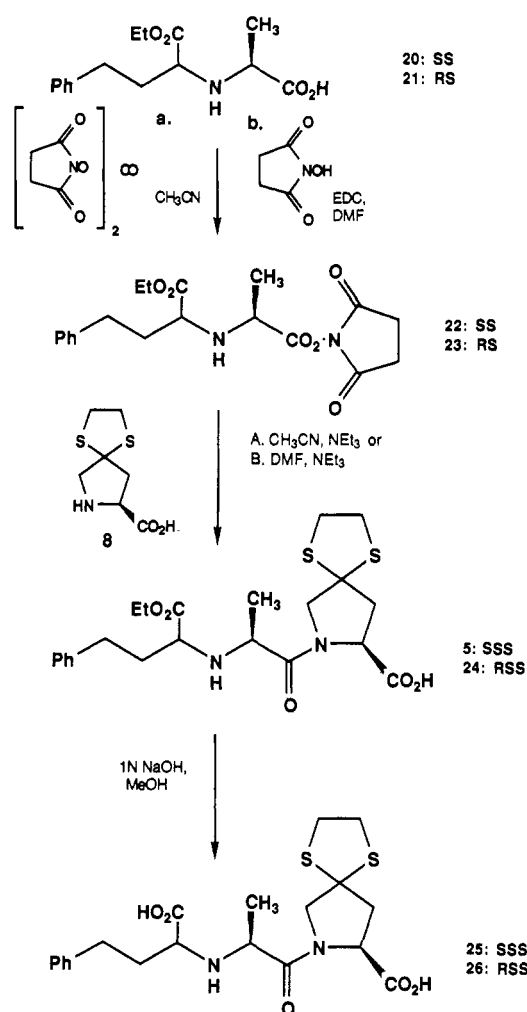
The compounds of the lysine series were prepared (Scheme III) from the ester-monoacid, which could be obtained via either of two routes.<sup>12</sup> Protection via benzyl ester and *N*-Cbz groups, which could be removed simultaneously with  $\text{HBr}$ , was advantageous. Coupling was achieved via the azide method.

## Results and Discussion

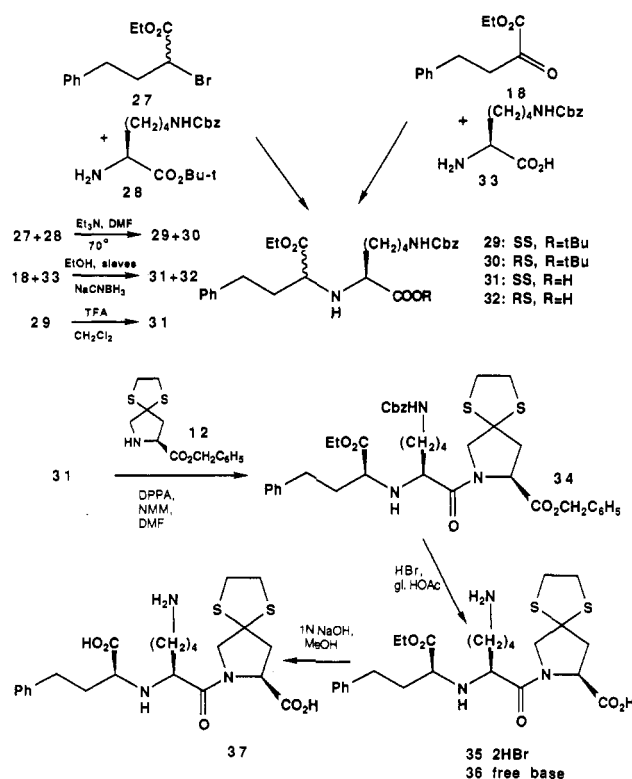
Compounds were tested for *in vitro* ACE-inhibitory activity by the procedure of Cushman and Cheung,<sup>13</sup> and

- (6) Sybertz, E. J.; Baum, T.; Ahn, H. S.; Desiderio, D. M.; Pula, K. K.; Tedesco, R.; Washington, P.; Sabin, C.; Smith, E.; Becker, F.; Kastner, B. *Arch. Int. Pharmacodyn.* 1987, 286, 216.
- (7) Baum, T.; Watkins, R. W.; Sybertz, E. J.; Ahn, H.-S.; Nelson, S.; Coleman, W.; Tedesco, R.; Pula, K. K.; Rivelli, M.; Sabin, C. *Arch. Int. Pharmacodyn.* 1987, 286, 230.
- (8) Monopoli, A.; Forlani, A.; Lazzaroni, A.; Milani, S.; Guzzon, V. *Arch. Int. Pharmacodyn.* 1987, 286, 246.
- (9) Hof, R. P.; Hof-Miyashita, A.; Evenou, J. P. *J. Cardiovasc. Pharmacol.* 1987, 10, 599.
- (10) (a) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spitzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnyak, G.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Neubeck, R.; Atwal, K. S.; Petrillo, E. W. *J. Med. Chem.* 1988, 31, 1148. (b) Spirapril and spiraprilat were prepared by routes complementary to those described here.
- (11) (a) Watkins, R. W.; Baum, T.; Cedeno, K.; Smith, E. M.; Yuen, P.-H.; Ahn, H.-S.; Barnett, A. *J. Ocular Pharmacol.* 1987, 3, 295. (b) Watkins, R.; Cedeno, K.; Tedesco, R.; Pula, K.; Barnett, A. *Fed. Proc.* 1987, 46, 1067, Abstract 4379.
- (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.

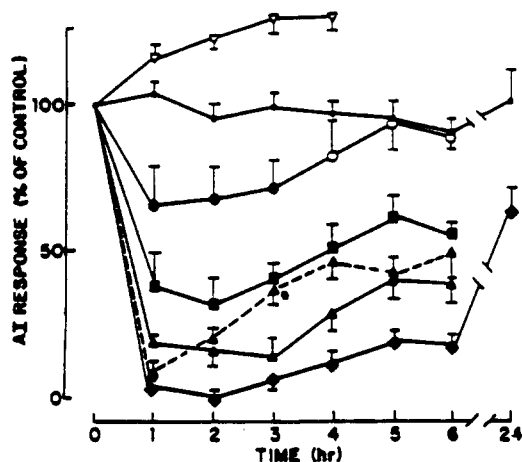
Scheme II



Scheme III



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

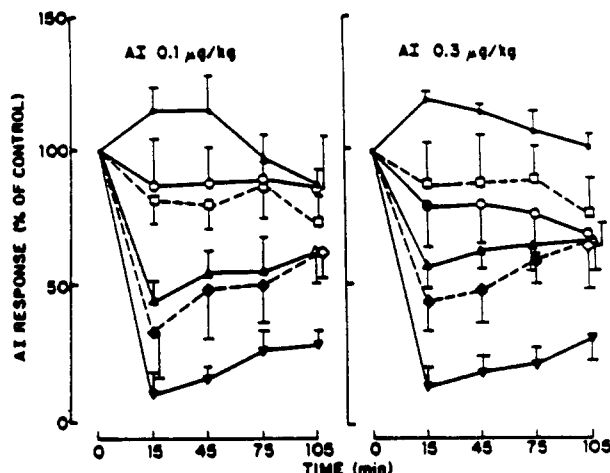


**Figure 1.** Angiotensin converting enzyme inhibitory activity of orally administered spirapril, spirapril hydrochloride, and spirapril diacid in conscious rats. AI (0.3 mg/kg) was injected iv before (time = 0) and at 30-min intervals after oral administration of test drug or placebo for 4–6 h. Only hourly data (mean  $\pm$  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):  $\bullet$ — $\bullet$ , 0 (9);  $\circ$ — $\circ$ , 0.03 (6);  $\square$ — $\square$ , 0.1 (8);  $\Delta$ — $\Delta$ , 0.3 (6);  $\diamond$ — $\diamond$ , 1 (9);  $\Delta$ — $\Delta$ , 0.3, HCl salt (5);  $\nabla$ — $\nabla$ , 1, diacid, (5).

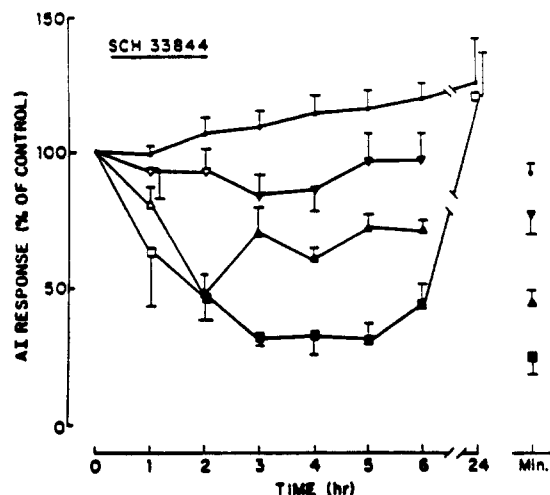
(5) and 36 are significantly less potent in vitro ACE inhibitors than their corresponding diacids 25 and 37. This result is in agreement with the observation of others with regard to a variety of ACE inhibitors bearing the carboxyalkyl group.<sup>2</sup> In all cases, it has been demonstrated that, for 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).<sup>6</sup> 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<sup>2</sup> 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 2.** Effect of intravenous administration of spirapril and its diacid on pressor responses to angiotensin I in anesthetized dogs. AI (0.1 and 0.3  $\mu$ g/kg, iv) was injected before and at the indicated times after test drug or placebo (4% ethanol in saline). Data (mean  $\pm$  SEM) after test drug are expressed as a percent of the control AI response. Dose code ( $\mu$ g/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):  $\bullet$ , 0 (4);  $\circ$ , 100 (4);  $\Delta$ , 300 (4);  $\nabla$ , 1000 (5);  $\square$ , 10, diacid (4);  $\diamond$ , 30, diacid (6).



**Figure 3.** Effect of oral administration of spirapril on pressor responses to angiotensin I in conscious dogs. AI (0.1  $\mu$ g/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  $\pm$  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):  $\bullet$ , placebo (13);  $\nabla$ , 0.1 (5);  $\Delta$ , 1 (6);  $\square$ , 3 (5).

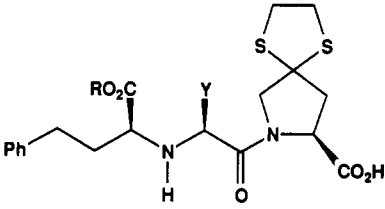
sustained oral activity at doses of 0.03–1.0 mg/kg (Figure 1)<sup>6</sup> [enalapril (0.1–1 mg/kg)].<sup>14</sup> At the higher dose, significant inhibition was observed for 24-h postdose. Spiraprilat, in contrast, was not active by the oral route (1 mg/kg).

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

(13) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1673.

(14) Sybertz, E. J.; Baum, T.; Ahn, H. S.; Nelson, S.; Eynon, E.; Desiderio, D. M.; Pula, K.; Becker, F.; Sabin, C.; Moran, R.; Vandervliet, G.; Kastner, B.; Smith, E. J. *Cardiovasc. Pharmacol.* **1983**, *5*, 643.

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



compd	R	Y	stereochemistry	ACE inhibition	
				in vitro IC <sub>50</sub> , nM	in vivo ID <sub>50</sub> , µg/kg <sup>a</sup>
19a	Et	CH <sub>3</sub>	RSS,SSS (1:1)	92 (5) <sup>b</sup>	
spirapril (5)	Et	CH <sub>3</sub>	SSS	67 (14) <sup>b</sup>	16
spiraprilat (25)	H	CH <sub>3</sub>	SSS	0.8 (14) <sup>b</sup> 0.9 (3.5) <sup>b</sup>	8
26	H	CH <sub>3</sub>	RSS		800
36	Et	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	SSS	200 (21) <sup>b</sup>	10
37	H	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	SSS	0.5 (25) <sup>b</sup>	10
19b	Et	H	RS,SS (1:1)		221
enalapril (2)	Et	CH <sub>3</sub>	SSS	1273	57
enalaprilat (3)	H	CH <sub>3</sub>	SSS	2.6 (3.5) <sup>b</sup>	15
lisinopril	H	(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>	SSS	1.2 <sup>c</sup>	
captopril (1)				12.9 <sup>d</sup>	71 ± 25

<sup>a</sup> Because of the low number of animals on which these ID<sub>50</sub> 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<sub>50</sub> values associated with low variability; e.g., the ID<sub>50</sub> for captopril is 71 ± 25 (mean ± SEM). <sup>b</sup> IC<sub>50</sub> value of captopril run concurrently. <sup>c</sup> Reference 2g. <sup>d</sup> This range of IC<sub>50</sub> 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)<sup>6</sup> on spirapril allowed determination of the ID<sub>50</sub> 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),<sup>6</sup> 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: [α]<sub>D</sub><sup>25</sup> (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),<sup>15</sup> *N*-[(phenylmethoxy)carbonyl]-4-oxo-(*S*)-proline methyl ester (neutralization of the HBr salt using 1 N NaOH and extraction with EtOAc),<sup>4</sup> 1,4-dithia-7-azaspiro[4.4]nonane-8-(*S*)-carboxylic acid methyl ester HBr (13a),<sup>4</sup> *N*-[(phenylmethoxy)carbonyl]-(*S*)-alanine hydroxysuccinimide ester (14a) (Sigma),

*N*-[(phenylmethoxy)carbonyl]glycine hydroxysuccinimide ester (14b),<sup>16</sup> 2-bromo-4-phenylbutyric acid ethyl ester (27) (Parish), 4-phenyl-2-oxobutyric acid ethyl ester (18)<sup>2</sup> (also available from Fluka), *N*-[1(*S*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanine (20),<sup>17</sup> *N*-[1(*R*)-(ethoxycarbonyl)-3-phenylpropyl]-(*S*)-alanine (21),<sup>18</sup> and *N*-[(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<sub>3</sub> solution. The basic solution was made acidic with concentrated HCl, and the acidic solution was extracted with EtOAc. The dried (MgSO<sub>4</sub>) 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<sub>2</sub>O (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; [α]<sub>D</sub><sup>26</sup> −3.6° (M, c 0.3). Anal. (C<sub>21</sub>H<sub>30</sub>N<sub>2</sub>O<sub>4</sub>S<sub>2</sub>) 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<sub>2</sub>O (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), [α]<sub>D</sub><sup>26</sup> +10.7° (C–M, 1:1, c 0.5). Anal. (C<sub>7</sub>H<sub>11</sub>NO<sub>2</sub>S<sub>2</sub>·HBr) C, H, N.

(16) Anderson, G. Fr. Pat. 1,406,785, 1965, *Chem. Abstr.* **1965**, 63, 13413f.

(17) Gold, E. H.; Neustadt, B. R.; Smith, E. M. U. S. Pat. 4 470 972, 1984.

(18) Kaltenbronn, J. S.; DeJohn, D.; Krolls, U. *Org. Prep. Proced. Int.* **1983**, 15, 35.

(15) Patchett, A. A.; Witkop, B. J. *Am. Chem. Soc.* **1957**, 79, 185.

***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),  $[\alpha]_D^{26} -1.7^\circ$  (E, *c* 0.5). Anal. ( $C_{15}H_{17}NO_5 \cdot \frac{1}{8}CHCl_3$ ) 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),  $[\alpha]_D^{26} -21.0^\circ$  (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),  $[\alpha]_D^{26} -17.6^\circ$  (H, *c* 0.4). Anal. ( $C_7H_{11}NO_2S_2 \cdot \frac{1}{2}H_2O$ ) C, H, N.

**1,4-Dithia-7-azaspiro[4.4]nonane-8(*S*)-carboxylic Acid Benzyl Ester (12).** 1,4-Dithia-7-azaspiro[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),  $[\alpha]_D^{26} -14.8^\circ$  (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,  $[\alpha]_D^{26} -15.9^\circ$  (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),  $[\alpha]_D^{26} -15.8^\circ$  (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),  $[\alpha]_D^{26} -7.9^\circ$  (E, *c* 0.2). Anal. ( $C_{17}H_{20}N_2O_5S_2 \cdot \frac{3}{4}H_2O$ ) 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),  $[\alpha]_D^{26} -18.7^\circ$  (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 basic (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),  $[\alpha]_D^{26} -25.7^\circ$  (E, *c* 0.4). Anal. ( $C_{22}H_{30}N_2O_5S_2 \cdot \frac{1}{4}NH_3 \cdot \frac{1}{2}H_2O$ ) 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):  $[\alpha]_D^{26} -39.0^\circ$  (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),  $[\alpha]_D^{26} -29.5^\circ$  (E, *c* 0.2) [lit.<sup>10</sup>  $[\alpha]_D^{26} -21.3^\circ$  (pyr, *c* 1.1)]. Anal. ( $C_{22}H_{30}N_2O_5S_2 \cdot \frac{1}{2}H_2O$ ) 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<sub>2</sub>O (100 mL) and then brine (100 mL). The dried (MgSO<sub>4</sub>) 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<sub>2</sub>O (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<sub>2</sub>O (20 mL) and then brine (20 mL). The dried (MgSO<sub>4</sub>) 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),  $[\alpha]^{26}_D -26.3^\circ$  (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;  $[\alpha]^{26}_D -35.3^\circ$  (M, c 0.5). Anal. (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>·1/2NH<sub>3</sub>·1/6CHCl<sub>3</sub>) 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<sub>3</sub>CN (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<sub>3</sub>CN and Et<sub>2</sub>O 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<sub>3</sub>CN (5 mL) and cooled for 10 h to give 5·HCl as a white solid (0.4 g): mp 192–194 °C dec;  $[\alpha]^{26}_D -11.2^\circ$  (E, c 0.4) [lit.<sup>10</sup>  $[\alpha]^{26}_D 2.0^\circ$  (M, c 1.5)]. Anal. (C<sub>22</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>·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<sub>2</sub>O and placed on a Bio-Rad AG 50W-X2, 100-mesh (hydrogen form), column. The column was eluted with H<sub>2</sub>O (20 × 200 mL) followed by 3% C<sub>5</sub>H<sub>5</sub>N in H<sub>2</sub>O to give **25** as a white solid (6.22 g): mp 163–165 °C;  $[\alpha]^{26}_D +4.1^\circ$  (E, c 0.4). Anal. (C<sub>20</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>·1/2H<sub>2</sub>O) C, H, N.

In an analogous manner, **24** was hydrolyzed to give **26** as a white solid (0.67 g): mp 134–136 °C;  $[\alpha]^{26}_D -27.0^\circ$  (E, c 0.5). Anal. (C<sub>20</sub>H<sub>26</sub>N<sub>2</sub>O<sub>5</sub>S<sub>2</sub>·1/2H<sub>2</sub>O) C, H, N.

**N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(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<sup>ε</sup>-[(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<sub>2</sub>O (1.5 L), and the organic layer was washed with H<sub>2</sub>O (4 × 200 mL). The dried (MgSO<sub>4</sub>) 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<sup>α</sup>-[1(R)-(ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine tert-butyl ester (**30**) as a pale yellow oil (2.79 g)  $[\alpha]^{26}_D -5.4^\circ$  (M, c 0.3); anal. (C<sub>30</sub>H<sub>42</sub>N<sub>2</sub>O<sub>6</sub>) C, H, N and N<sup>α</sup>-[1(S)-(ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine tert-butyl ester (**29**) as a pale yellow oil (3.24 g)  $[\alpha]^{26}_D -2.6^\circ$  (M, c 0.4); anal. (C<sub>30</sub>H<sub>42</sub>N<sub>2</sub>O<sub>6</sub>) C, H, N.

**N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine (31).** N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(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<sub>3</sub>–2-propanol–7% NH<sub>4</sub>OH, 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;  $[\alpha]^{26}_D +6.1^\circ$  (E, c 0.3). Anal. (C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>) C, H, N.

**N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine (31) and N<sup>α</sup>-[1(R)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine (32).** A solution of N<sup>ε</sup>-[(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<sub>2</sub>O (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<sub>2</sub>O (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<sub>3</sub>–EtOAc, 4:1 (3 L), and then CHCl<sub>3</sub>–2-propanol–7% NH<sub>4</sub>OH, 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<sub>3</sub>–2-propanol–7% NH<sub>4</sub>OH, 1:1:1 (organic), as eluant, to give N<sup>α</sup>-[1(S)-(ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine (**31**) as a white solid (0.68 g)  $[\alpha]^{26}_D +6.1^\circ$  (E, c 0.25); anal. (C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>) C, H, N and N<sup>α</sup>-[1(R)-(ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysine (**32**) as a white solid (0.74 g)  $[\alpha]^{26}_D +1.1^\circ$  (E, c 0.3); anal. (C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>6</sub>) C, H, N.

**7-[N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(phenylmethoxy)carbonyl]-(S)-lysyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Benzyl Ester (34).** N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(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<sub>3</sub> solution, H<sub>2</sub>O added, and the mixture extracted with Et<sub>2</sub>O. The combined organic layers were dried (MgSO<sub>4</sub>) 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),  $[\alpha]^{26}_D -18.4^\circ$  (M, c 0.7). Anal. (C<sub>40</sub>H<sub>49</sub>N<sub>3</sub>O<sub>7</sub>S<sub>2</sub>) C, H, N.

**7-[N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-(S)-lysyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid Dihydrobromide (35).** N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-N<sup>ε</sup>-[(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<sub>2</sub>O (2 × 250 mL) to give **35** as a tan gray solid (1.29 g), which was used without further purification.

**7-[N<sup>α</sup>-[1(S)-(Ethoxycarbonyl)-3-phenylpropyl]-(S)-lysyl]-1,4-dithia-7-azaspiro[4.4]nonane-8(S)-carboxylic Acid (36).** 7-[N<sup>α</sup>-[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<sub>2</sub>O (900 mL), H<sub>2</sub>O-pyridine, 96:4 (4000 mL), and then H<sub>2</sub>O-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),  $[\alpha]_D^{26}$  -21.3 (E, c 0.4). Anal. (C<sub>25</sub>H<sub>37</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>) C, H, N.

7-[N<sup>α</sup>-[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<sup>α</sup>-[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<sub>2</sub>O (900 mL) followed by H<sub>2</sub>O-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),  $[\alpha]_D^{26}$  +7.5° (E, c 0.3). Anal. (C<sub>25</sub>H<sub>33</sub>N<sub>3</sub>O<sub>5</sub>S<sub>2</sub>·1/2H<sub>2</sub>O) 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.<sup>13</sup> 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.<sup>6</sup> 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<sub>50</sub> (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 anesthetized and prepared for caudal arterial and venous jugular catheter implantations as described previously.<sup>6,14</sup> 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.<sup>6</sup> 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).<sup>6</sup> Anesthesia was maintained by a mixture of methoxyflurane and oxygen. The omocervical artery and omocervical vein were cannulated as described previously.<sup>14</sup> 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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